Proceedings of the XI International Symposium on Biological Control of Weeds

Canberra, Australia, 27 April–2 May 2003

Edited by J.M. Cullen, D.T. Briese, D.J. Kriticos, W.M. Lonsdale, L. Morin and J.K. Scott

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Contents

Abstracts

Abstracts

Preface

Venue and delegates

The XI International Symposium on Biological Control of Weeds was held from 28 April to 2 May 2003, at the Manning Clark Centre, Australian National University, Canberra, Australia. One hundred and seventy five registered delegates from 60 organisations attended, from Argentina, Australia, Brazil, Canada, Chile, France, Italy, Japan, Mexico, New Zealand, Nigeria, Norway, Papua New Guinea, Russia, Senegal, Slovak Republic, South Africa, Switzerland, UK and USA.

Opening ceremony

The official opening of the conference took place on Monday 28 April 2003. A "Welcome to Country" ceremony was performed by local Ngunnawal elder, Mrs Agnes Shea, in the Manning Clark Centre.

Sponsors

The organizing committee thanks the following agencies for their generous contributions to the symposium and to the publication of the proceedings: the Grains Research and Development Corporation (GRDC), the Rural Industries Research and Development Corporation (RIRDC), Meat and Livestock Australia (MLA) and the Australian Centre for International Agricultural Research (ACIAR).

Symposium program structure

The formal program was made up of 11 sessions of 50 oral presentations and 122 posters, grouped into 5 themes, moving from theory through to impact. Posters were a central part of the proceedings, viewed in a dedicated session before lunch each day. The schema of the organisation of the symposium into themes and sessions, with their associated chairs and keynotes, is tabulated below.

Session chair		Keynote speakers and talks	
Theme: Biocontrol theory and new approaches			
Bruce Auld	Biocontrol theory and new approaches	Don Strong	Into the future for biocontrol
Theme: Target and agent selection			
Richard Groves	Ecology in target selection	Peter McEvoy	Role of ecology in selecting target species and agents for biological control
Sathyamurthy Raghu	Ecology in exploration and agent selection		
Theme: Risk analysis			
Toni Withers	Host specificity procedures	Michael Singer	Defining and distinguishing properties of interacting plants and insects
Alan Watson	Dealing with risk	Andy Sheppard	Ecological risk benefit assessment for biological control introductions – a world view
Marion Seier	Non-target effects	Bob Pemberton	Biological control safety: science with temporal and cultural contexts

Session chair		Keynote speakers and talks	
Theme: Integration and management			
Helen Spafford Jacob	Integration of biocontrol with other control methods	Quentin Paynter	Integrated weed management: could we be doing better?
John Ireson	Release and redistribution strategies		
Lynley Hayes	Technology transfer	Martin Hill	The transfer of appropriate technology, key to the successful biological control of five aquatic weeds in Africa
Theme: Evaluation			
Judy Myers	Population ecology in the measurement of biocontrol effectiveness		
Dennis Isaacson	Community- and landscape-scale approaches to evaluating impact		
Matthew Cock	Economic and social indicators of biocontrol impact	Ernest Delfosse	What is "success" in biological control?

Because posters were a high profile component of the symposium, we decided to award a prize for the best poster in each theme. The winners were selected by our keynote speaker Professor Don Strong from a short list of three per theme prepared by David Briese and Mark Lonsdale. They were as follows:

Liz Dovey – Weeds in the Pacific: the situation and the challenge

S.M. Boyetchko, G. Peng, K. Sawchyn, K. Byer and **R. Charudattan** – Evaluation of variable temperature regimes on bioherbicidal activity of non-indigenous fungal pathogens for biological control of green foxtail

Louise Morin and **Mireille Jourdan** – Biological control of saffron thistle with fungi: limited prospects

Paul D. Pratt and **Ted D. Center** – Indirect impacts of herbivory of *Oxyops vitiosa* on the reproductive performance of the invasive tree *Melaleuca quinquenervia*

A.J. Willis, L. Morin, P.H.R. Moore and **R.H. Groves** – Potential for population recovery of an endangered native plant by controlling bridal creeper with rust.

Five workshops were also held in association with the conference. These were: Glynn Maynard, Assessment of biological risk factors associated with the use of exotic organisms in containment facilities; Rachel McFadyen, Where biocontrol is heading in the 21st century; Yvonne Buckley, Modelling woody weeds – comparing approaches and results; Alec McClay, Centres of origin – do they exist, can we identify them, does it matter? John Wilson and John Hoffmann, Agents that reduce seed production – essential ingredient or fools' folly? Summaries of the last two are included in this volume.

In addition, the International Bioherbicide Group held its sixth workshop, Bioherbicides: the next generation. This is also summarised in this volume.

Close of conference

The closing address was given by Dr Jim Cullen, retiring this year from CSIRO, and was entitled "Synopsis: The long and winding road". This was an overview of the presentations and is to be found in this volume.

Mid-symposium tours

These took place on Wednesday, 28th April 2003. The tours were organized by David Briese and offered a choice of two trips. One took participants through areas to the west of Canberra burnt by the devastating bushfires in January 2003. The other trip headed east toward the south coast of New South Wales, arguably one the most beautiful stretches of coastline in the world. Both trips enabled delegates to learn more of Australia and recharge their batteries for the final two days of the symposium.

Conference dinner

This was held on the evening of Thursday, 1 May 2003, in the Members' Dining Hall at Old Parliament House. The speaker for the evening was the Right Honourable John Kerin, the former Minister for Primary Industries and Treasurer the Government of Australia, who regaled guests with recollections from a life in politics, many years of which he had spent in the very building where delegates were eating.

Committees and support

The organizing committee comprised David Briese, Sharon Corey, Jim Cullen, Louise Morin, Mark Lonsdale (Chair) and Kate Smith, with Tracey Cootes providing support to the Chair. The scientific program committee comprised David Briese (convenor), Tim Heard, Mic Julien, Darren Kriticos, Louise Morin, John Scott and Andy Sheppard. Joel Armstrong, Ruth Aveyard, John Lester, Mick Neave, Matt Smyth, Anthony Swirepik, Peter Turner and Andi Walker helped organise entertainment and logistics. Conference administration was provided by Consec Conference Management and Secretariat Services, Canberra, and the publication of the proceedings by Ed Highley of Clarus Design Pty Ltd, Canberra.

Next meeting

The symposium attendees agreed that the next meeting would be held in Montpellier, France in 2007.

Mark Lonsdale
CSIRO Entomology
December 2003

Theme 1:

Biocontrol Theory and New Approaches

Evolving weeds and biological control

Donald R. Strong[1]

Summary

Founder events in colonization, hybridization, and interactions with native species and agents, as well as strong natural selection in their new environments, can result in a mix of genotypes in an invasive species very different from those of the propagules or the population of origin. Some populations of invasive *Spartina* spp. in Pacific estuaries have been separated from the specialist planthopper *Prokelisia marginata* for many generations, while virtually no native *Spartina* populations in Atlantic and Gulf coast estuaries of NA, have experienced this separation. We found lower resistance and tolerance among six *Spartina* populations that have been long-separated from the planthopper than in six *Spartina* populations that had been consistently exposed to it.

Spartina alterniflora genotypes varied more in their ability to resist and support planthoppers in a population that had been separated from the herbivore for many generations (in Willapa Bay, WA) than in one that had been consistently exposed to the planthopper (in San Francisco Bay, CA). In the former, some plant genotypes experienced >50% shoot mortality while others experienced none. In contrast, no genotype in the latter experienced >20% shoot mortality. Population growth rates of the herbivore paralleled this pattern among cordgrass genotypes from the two populations.

One Willapa Bay genotype of *S. alterniflora* lacked resistance to the planthopper while being quite tolerant of high herbivore densities that developed upon it. Plant genotypes with this combination of traits could result in self-defeating biological control. These tolerant genotypes could foster herbivores and increase in frequency at the expense of the vulnerable genotypes. The presence of tolerant genotypes suggests the need for complementary chemical and/or mechanical control. Attention to the frequency and nature of genetic variation in vulnerability to natural enemies on target species is germane to both the science and the practice of biological control.

Keywords: cordgrass, resistance, self-defeating biocontrol, *Spartina*, tolerance, weed evolution.

Introduction

Biological control is an applied discipline within the larger new science of invasive species. While genetic change during invasions has not been well studied, the underlying ecological processes of dispersal and isolation after colonization are just those that facilitate allopatric speciation and form the rationale of the modern synthesis of evolutionary theory (Mayr 1970). Paleontology also gives evidence of rapid change during invasions (Vermeij 1996).

Plant populations vary greatly in their resistance to and tolerance of natural enemies (Strauss and Agrawal 1999), and successive invasive species from different parts of the native range can hybridize. These processes create a large array of unusual genotypes (Ayres *et al.* 1999); thus we should expect ample raw material for evolution in weeds as a matter of course. Furthermore, large selection gradients are generated by the lack of natural enemies and different competitive regimes in new environments. These factors make invasive plants prime candidates for rapid evolution (Thompson 1998). Understanding the evolution of weeds is pertinent to safe and effective biocontrol.

Our research has focused upon an idea suggested by the evolution of invasive *Spartina* species that we term "the potential for self-defeating biological control". The rationale is that natural selection for herbivore resistance and tolerance (vulnerability) are relaxed during spread of an invasive plant before agents are released (see e.g. Blossey & Nötzold 1995). This could lead to evolution of increased genetic variance in vulnerability to herbivores as recombination generates

[1] Section of Evolution and Ecology and the Bodega Marine Laboratory, University of California, Davis, California, USA 95616 <drstrong@ucdavis.edu>.

new genotypes as the invasive population grows rapidly. The high genetic variance would yield a high variance in phenotypes in the weedy population, with a broad range of vulnerability to agents. In this situation, introduced agents would eliminate vulnerable genotypes and leave a target population upon which the natural enemy had little effect. Without complementary control of the less vulnerable genotypes by some other means, natural selection could lead to self-defeating biological control. The invulnerable population would then proliferate.

Spartina as invasive species

Spartina species are perennial, wind pollinated, obligately outcrossing grasses in their native ranges. As the largest and most productive saltmarsh plants at high latitudes, *Spartina* species (cordgrasses), have large ecological and economic effects in the estuaries where they invade (Garcia Rossi *et al.* (2004), Ayres *et al.* 1999).

Invasive populations of *S. alterniflora* (and *S. anglica*) that have not recently (or perhaps ever) been in contact with the specialist planthopper *Prokelisia marginata* appeared to have lower mean resistance and tolerance to herbivory by this planthopper than native populations that have been exposed to it continuously (Daehler & Strong 1997, Wu *et al.* 1999). Preliminary observation suggested that the variation in vulnerability was also higher in populations of cordgrass that lacked a recent history of association with this herbivore. The ideal experimental design was precluded when some strains of our cordgrass were destroyed. Nevertheless, we were able to compare a population of *S. alterniflora* that had been released from herbivory when introduced to Willapa Bay, Washington State, USA about 100 years ago with a population introduced to San Francisco Bay about 25 years ago that has never been separated from this planthopper. Both populations are outside *S. alterniflora*'s native range (Daehler & Strong 1996). The planthopper is a native of San Francisco Bay, with native Californian cordgrass, *S. foliosa* as its host (Denno *et al.* 1996).

Spartina anglica is a new species created in the 19th century in England by hybridization between *S. alterniflora*, introduced from North America, and *S. maritima*, native to Europe (Raybould *et al.* 1991). Specialist insect herbivores of *Spartina* are native to Atlantic and Gulf coast estuaries of North America. The most abundant herbivores are planthoppers in the genus *Prokelisia* spp. (Denno *et al.* 1996, Heady & Wilson 1990). Others include the stem-boring cecidomyid fly *Calamomyia alterniflorae* (Gagne 1981) and the scale insect *Haliaspis spartinae* (Strong *et al.* 1984, Liu & Howell 1994). Thus, *S. anglica* comprises the *S. maritima* genome, which has never experienced *P. marginata*, and that of *S. alterniflora*, which evolved with this planthopper. We have no knowledge of any population of *S. anglica* being subject to herbivory by *P. marginata*, while the *S. alterniflora* part of its hybrid has an ancient, but recently interrupted interaction with the planthopper.

Spartina anglica was deliberately introduced to parts of Britain, Europe, China, New Zealand, Australia, Tasmania, and Puget Sound, Washington State, USA, and is now considered a serious weed in these countries. *P. marginata* has been introduced to Willapa Bay, WA for biological control of *S. alterniflora* (Grevstad *et al.* 2003).

Methods

Studying cordgrass vulnerability to *P. marginata* presents technical challenges common with sap-feeding insects, such as many species of Homoptera, upon long-lived plants. *P. marginata* is tiny, and the amount of vascular fluid removed by each insect is small and difficult to quantify (Walling 2000). Numbers of planthoppers upon the plant build through a series of generations over the growing season. Both oviposition wounds and sap removed by feeding planthoppers are potentially harmful to the plant, though we failed to find evidence that plant diseases are transmitted by these planthoppers (Davis *et al.* 2002).

We tested the effect of the planthopper upon cordgrasses in greenhouse assays. We measured effects of *P. marginata* upon vegetative growth and survival, but not on seed set. First, we asked how contact history between cordgrass populations and the planthopper affected the suitability of host plants for insects (resistance). Second, we asked how contact history affected the ability of cordgrass to withstand the herbivore (tolerance). Finally, we asked if a history of separation from the herbivore could affect within-population variance of vulnerability to it.

To understand resistance, we contrasted six populations of *Spartina alterniflora* and *S. anglica* that have been separated from *Prokelisia marginata* for many generations with six native populations of *S. alterniflora* that have never grown apart from this herbivore. We examined oviposition rate, nymphal emergence from eggs, rate of nymphal development to adults, and planthopper population growth rate of the insects and five plant traits – biomass, length of shoots, shoot number and mortality, leaf number, and plant mortality. To understand resistance independently of tolerance, we measured oviposition, nymphal emergence, and nymphal development to adults at planthopper densities so low that the herbivore did not degrade the plant. The most sensitive indicator of *P. marginata* damage to *Spartina* sp. is distinctive chlorosis of leaf tips, and the density of insects used in the resistance experiments was less than that causing chlorosis. Tolerance was measured as the plant trait value when grown with *P. marginata* divided by the trait value of replicate plants grown without it. In nature, planthopper colonies build up through several generations during the growing season, and we measured

increase in planthopper density for a test of the interaction of cordgrass and *P. marginata*. Planthoppers increased to densities that harmed the cordgrass, which means that population increase is a measure that combines resistance and tolerance. Planthopper density was divided by stem length, to adjust for different plant sizes among cordgrass populations. Details of the methods are in Daehler *et al.* (1996), Wu *et al.* (1999), and Garcia Rossi *et al.* (2004).

Results

Suitability of host plants for insects

Resistance to *Prokelisia marginata* was greater in cordgrass populations that have never been separated from it than in those populations that have evolved in separation from this herbivore. Development of second-instar nymphs to adults was higher on long-separated plants (72%) than on never-separated plants (50%, test of *a priori* hypothesis, $t = 8.0$, df = 4, $P = 0.0013$, Figure 1). Nymphal developmental on the seven native populations of *S. alterniflora* from the Atlantic coast was 53% and was 46% from San Francisco Bay (never separated from the plant hopper). This was in contrast to plants with the opposite plant–insect contact history (development on Willapa Bay *S. alterniflora,* 71%; on *S. anglica* from Puget Sound, 69%; Tasmania, 74%; and Victoria, Australia 74%). Planthopper mortality did not differ by plant–insect contact history (22% on long-separated provenances, 22.4% on never-separated natives, $t = 0.1$, df = 4, $P = 0.92$), therefore the distinction was due to developmental rate rather than survival.

The rate of nymphal emergence was twice as high on cordgrasses from populations separated from the planthopper for many generations than on the cordgrasses from populations with the opposite plant–insect contact history. An average of 9.7 nymphs emerged per each founding male–female pair on Willapa Bay *S. alterniflora*, significantly higher than the 4.6 nymphs emerging per male–female pair on cordgrasses from Virginia ($t = 3.7$, df = 71, $P < 0.001$, based on log-transformed data). Oviposition rates over 30 days did not differ as a function of contact history, and female planthoppers laid, on average, 31.2 eggs ($t = 1.9$, df = 6, $P = 0.11$) on plants from Willapa Bay and San Francisco Bay cordgrass.

Ability to withstand herbivory

The tolerance of *Prokelisia marginata* was greater in cordgrass populations of long-standing associations with the planthopper than in those that have been separated from it for many generations. Native *Spartina alterniflora* from Virginia grew better under herbivore pressure than did the Willapa population of *S. alterniflora* and populations of *S. anglica* (Figure 2). The advantage of the Virginia plants was greatest for biomass ($F_{4,20} = 4.4$; $P = 0.01$) and leaf number ($F_{4,20} = 4.9$; $P = 0.006$), but advantage over plants that have been long-separated from the insect was also substantial for shoot number ($F_{4,20} = 2.5$; $P = 0.07$) and shoot length ($F_{4,20} = 2.6$; $P = 0.07$). The mean effect on

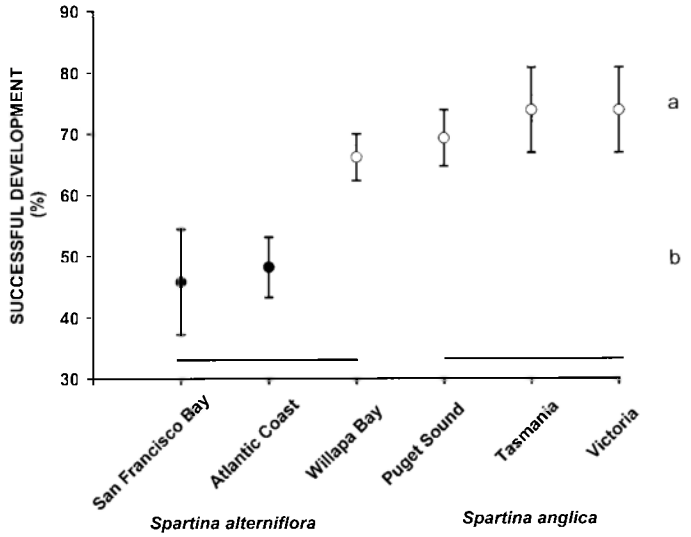

Figure 1. Development of *Prokelisia marginata* nymphs to adults during a 12-day experimental period, as a function of contact history. By *a priori* contrast, development on the cordgrass that has never grown apart from the planthopper (b) was lower than on cordgrasses that had grown apart from the herbivore for many generations (a). Redrawn from Garcia-Rossi *et al.* (2004).

biomass was statistically nil in the Virginia population. Herbivory reduced shoot length 1.2-fold, shoot number 1.6 fold and leaf number 1.7-fold in this native population of cordgrass (Figure 2). The reductions caused by the herbivore in all traits were much greater for plants that have evolved in the absence of the planthopper, ranging from reduction of 2.2-fold for shoot number in Willapa Bay *S. alterniflora* to a 7.7-fold reduction in shoot number and leaf number for *S. anglica* from Puget Sound.

Plant mortality paralleled the results for the other measures of tolerance to the planthopper. None died during growth without the planthopper. With planthoppers, plant mortality was zero for Virginia *S. alterniflora*; 25% for Willapa Bay *S. alterniflora*; 43% for Australia *S. anglica*, 50% for Puget Sound *S. anglica*, and 50% for Tasmania *S. anglica* (test of *a priori* hypothesis of lower mortality in the long standing cordgrass–planthopper associations, Kruskal-Wallis Test, $X^2 = 7.4$, df = 1, $P = 0.006$).

Planthopper population growth measures the combined effects of resistance and tolerance. In the experiments, just as in nature, planthoppers became dense and caused chlorosis, curled and dead leaves, and hopper burn. Planthopper populations grew faster on plants from cordgrasses long-separated from the planthopper than on plants with continuous contact with the insect. Densities grew from 0.5 to 1.9/cm of stem on the native Virginia *S. alterniflora* and to an average of 4.7 planthoppers/cm shoot on plants of the long-separated cordgrasses over one generation (10 weeks) of the planthopper ($t = 2.25$, df = 38, $P < 0.03$). For the long-separated plants, final density was 3.1 on *S. alterniflora* from Willapa Bay, and on the *S. anglica*, 4.7 from Australia, 9.3 from Puget Sound, and 2.6 from Tasmania.

Intrapopulation variation

S. alterniflora from Willapa Bay has been separated from the planthopper for *ca.* 100 years. This cordgrass had much higher variation among genotypes in population growth rate of the planthopper, and in harm caused by it, than cordgrass from San Francisco Bay (never separated). The plant genotypes from Willapa Bay supported a wide range of densities, 1.3 to 12.8 planthoppers/cm stem (mean 11.5), *ca.* 10-fold the range for genotypes from San Francisco Bay (0.7 to 1.9 planthoppers/cm of stem, Figure 3). The poorest plant genotype for planthoppers from Willapa Bay was close to the average from San Francisco Bay. Most genotypes from Willapa Bay supported planthopper densities two to seven fold greater than any from San Francisco Bay. Seven of eight Willapa Bay genotypes had mean densities greater than the highest planthopper density of San Francisco Bay genotype.

The range of relative shoot survival (survival with the planthopper/survival without it) was higher among Willapa Bay genotypes (range = 0.72, from 0.3 to 1.1; overall mean survival = 0.6, se = 0.09) than among genotypes of cordgrass from San Francisco Bay (range = 0.6, from 0.8 to 1.5, overall mean survival = 1.0) of *S. alterniflora* (ordinate, Figure 3). Planthoppers killed more than half of the shoots of three of eight Willapa Bay genotypes, while they killed no shoots in one other from the same population. In contrast, they killed no

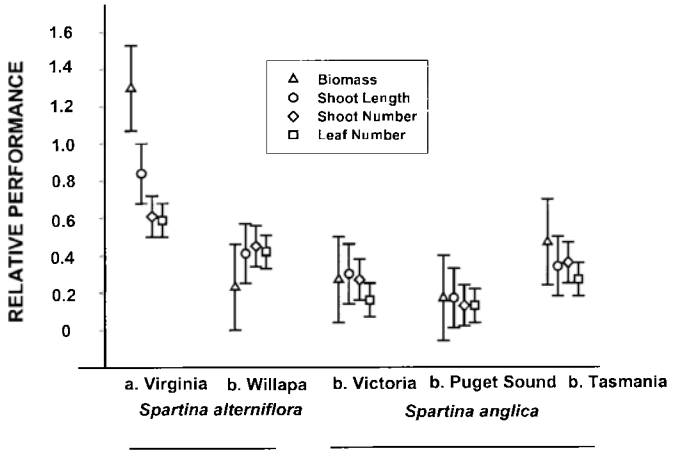

Figure 2. Relative performance of cordgrass measured as a ratio of the value of the trait for plants grown with *Prokelisia marginata* over that for plants growing without the planthopper, as a function of contact history. By *a priori* contrast, native cordgrass (a) from Virginia suffered less from herbivory in all four plant traits than did cordgrasses that had been separated from the planthopper for many generations (b). Redrawn from Garcia-Rossi *et al.* (2004).

more than a fifth of shoots of any San Francisco genotype. The coefficient of variation among genotypes was greater for Willapa Bay (44.9%) than for San Francisco Bay for relative shoot survival (20.8%, $P < 0.05$ by F test, Zar 1984). Plant mortality paralleled this pattern. The planthoppers killed *ca.* 35% of plants of a few Willapa Bay genotypes and none of the others ($X^2 = 14.6$, df = 1, $P < 0.0001$), while they killed very few plants of any genotype from San Francisco Bay ($X^2 = 4.1$, df = 1, $P < 0.5$).

Shoot survival decreased with increasing planthopper density (Figure 3). Genotypes from San Francisco Bay had highest survival and lowest planthopper density, with relatively little variation. Genotypes from Willapa Bay account for most of the relationship in Figure 3. The most interesting genotype in the study is the uppermost point in Figure 3. This genotype departs conspicuously from the rough negative correlation between tolerance and the densities of planthopper colonies that developed during this long experiment. While other genotypes from Willapa Bay were harmed by even quite low densities of the planthopper, this unusual genotype was virtually unaffected by the third-highest density (8.5 planthoppers/cm of stem) of the 17 genotypes in the experiment. Thus, this Willapa Bay genotype lacked resistance to the planthopper, while being quite tolerant of it. This genotype would promote biological control of other genotypes while resisting biological control itself.

Discussion

Cordgrasses *Spartina alterniflora* and *S. anglica* that have been separated from the specialist planthopper *Prokelisia marginata* for many generations are much more vulnerable to this herbivore than cordgrasses populations never separated from it. All comparisons of all traits investigated (three insect traits, five plant traits) showed the six invasive cordgrass populations, all estranged from *P. marginata*, were more vulnerable than the six populations that have never been separated from this insect.

Pertinent to enduring biological control, within-population variation in traits related to both tolerance and resistance was much greater among genotypes of *Spartina alterniflora* in a population that had long been separated from the planthopper than in a population that had never been separated from it. The relationship between cordgrass shoot survival and planthopper population growth epitomizes these results (Figure 3). Consistent with previous findings (Daehler and Strong 1995), San Francisco Bay genotypes varied little in this relationship, and none suffered greatly from the low densities of planthopper that built up over the 20-week experiment. In contrast, Willapa Bay genotypes varied greatly in both shoot survival and planthopper population growth. While the harm done to most genotypes of the estranged cordgrass population was substantial, a subset was different.

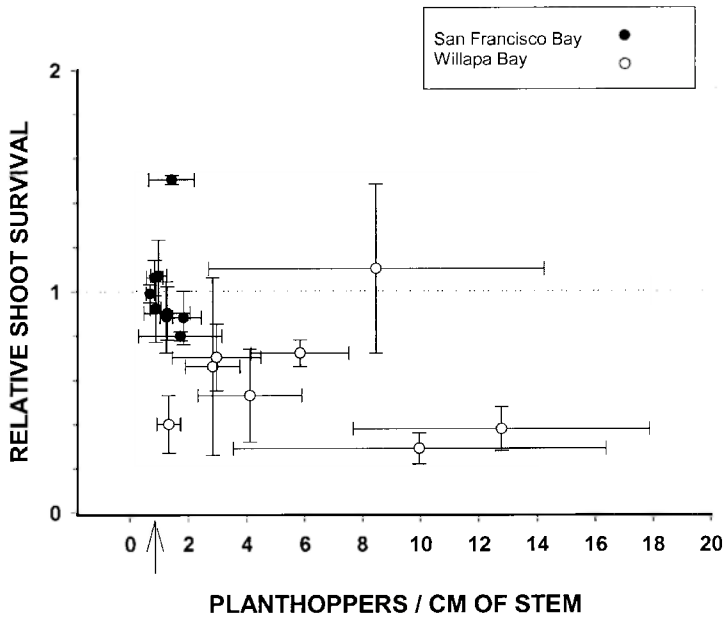

Figure 3. Variation among genotypes in population growth rate of *Prokelisia marginata* and in shoot death caused by this herbivore to *Spartina alterniflora* from Willapa Bay, which has been separated from the planthopper for *ca.* 100 years, and that from San Francisco Bay, which has never been separated from it. The arrow indicates the density of 0.66 planthoppers/cm of shoot at the beginning of this 20-week experiment.

One Willapa Bay genotype was virtually unaffected by the moderately high densities of the planthopper developing during the experiment. This genotype lacked resistance, but was tolerant of the planthoppers that grew upon it. Genotypes with this combination of traits could be self-defeating to biological control. Initially, such genotypes could accelerate control by producing many herbivores to harm the other, more vulnerable plant genotypes. In the longer-term, however, the effectiveness of the agent would decrease as the tolerant genotypes increased due to the selection pressure imposed by the biological control agent.

Genetic variation in vulnerability to natural enemies is important to biological control. Agents impose substantial natural selection (Gould *et al.* 1997), and the simplicity of foodwebs in biological control (Hawkins *et al.* 1999) can magnify selection differential due to enemies (Holt and Hochberg 1997). It is interesting that many plants have evolved resistance to chemical control (Georghiou 1990), while we have very few examples of evolved resistance to biological control; most concern insect pests (Muldrew 1953, Fenner 1983, Young 1986).

Our results provide an example of the potential for evolution of resistance to biological control, with an interesting twist of extra evolutionary dynamics caused by high variance in vulnerability of the weed. The enemy-free environment and relaxed selection in which invasive plants find themselves before biological control could lead to evolution of this increased variance (Colosi and Schaal 1992, Thompson 1998).

S. alterniflora has spread over approximately 6000 ha of previously open intertidal habitat during the 20th century in Willapa Bay, WA, amounting to about 30% of the 19,000 ha of intertidal lands suitable for this plant in the Bay. The invasion degrades habitat of shorebirds, waterfowl, fish, benthic invertebrates, and valuable clams and oysters (Anon. 1993, 1997). In summer of 2000, *P. marginata* from San Francisco Bay was introduced to Willapa Bay under permit of the Washington Department of Agriculture with unanimous approval of The Technical Advisory Group on Biocontrol of Weeds, of the US Department of Agriculture, APHIS. The introduction of this insect was made only after extensive host-specificity testing and disease screening (Davis *et al.* 2002, Grevstad *et al.* 2003).

In the event that planthopper densities grow sufficiently high (Grevstad *et al.* 2004), the result could be decreased spread rate or abundances of some *S. alterniflora* genotypes (those resembling the open points on the lower half of Figure 3). Initial success of biological control of this sort could drive natural selection favouring genotypes tolerant of the planthopper, which in the longer run could erode the effectiveness of biological control. In this scenario, other measures such as mechanical or chemical control (Patten 2002) would be necessary to prevent spread of cordgrass genotypes that are impervious to the planthopper. One could advocate the choice of agents with impact so severe that no host genotypes survive (extremely high virulence), but such agents are unknown in the specific case under discussion and not very frequent in cases of weed control (Kennedy *et al.* 1987, Julien 1992). An understanding of the spatial distribution of tolerant/vulnerable genotypes could lead to strategies to minimize the evolution of tolerance. For instance, if there was clear spatial segregation between these categories of genotypes, one could release only in susceptible-dominated areas.

Cases at least reminiscent of ours include biological control of rush skeletonweed, *Chondrilla juncea* in Australia (Burdon *et al.* 1981). A fungus, *Puccinia chondrillina*, and an eriophyid mite, *Aceria chondrillae*, were introduced and attacked one of the three forms of the weed preferentially to the other forms. In less than a decade after introduction of the agents, the geographical distribution of the attacked form of rush skeletonweed had decreased greatly, while that of the other two forms, which compete with the attacked form, increased concomitantly. A second case is the contemplated biological control of *Lantana camara* by the leaf spot pathogen, *Mycovellosiella lantanae* var. *lantanae*, in South Africa. Some biotypes of *L. camara* are resistant to all of the fungal isolates (Den Breeyen 2004). This situation at least raises the possibility of replacement of *L. camara* biotypes that are vulnerable to this fungus with biotypes that are not.

Evolution of weeds is a pertinent topic for biological control. Invasive species can experience strong selection in their new environments. The processes of founder effect during colonization, spread, and possible hybridization with native or subsequent introduction of other strains, interaction with native species and with introduced agents can result in a mix of genotypes very different from those of the propagules or the population of origin.

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Predicting climate compatibility of biological control agents in their region of introduction

M.J. Byrne,[1] J. Coetzee,[1] A.J. McConnachie,[1] W. Parasram[1] and M.P. Hill[2]

Summary

Despite the presence of their host plants, many biological control agents of weeds fail to establish, apparently because of climatic incompatibility in the country of introduction. We examined the thermal physiology, in particular the lower development threshold (t), rate of development K, and CTMin and lower LT_{50}, of four biological control agents. These parameters were used in degree–day, CLIMEX™, and minimum temperature models to compare the predicted distribution of the insects with their actual establishment. None of the models precisely accounted for all establishments or failures. However, incorporation of CTMin and LT_{50} thermal limits, in conjunction with the "Match Climates" module in CLIMEX may improve pre-release selection of agents or populations of agents, and thereby improve the probability of successful establishment.

Keywords: prediction, climate compatibility, biological control agents, introduction.

Introduction

Forty-four per-cent of weed biological control agents fail to establish because of climatic incompatibility of the agent, usually an insect, to its new area of introduction (McEvoy & Coombs 2001). This represents an enormous waste of time and money invested in foreign exploration and quarantine testing, which could be saved if some indication was available in advance of release of the physiological capabilities of the biocontrol agents. Here we examine methods that could contribute to improved forecasting of an agent's likelihood of establishment.

Empirical field-testing of thermal physiology in the country of origin has been instructive (Papadopoulos *et al.* 1996), and successfully incorporated into models of both the potential and realized distribution of the Mediterranean fruit fly (Vera *et al.* 2002). Modelling the potential distribution of an organism in its country of introduction is also relatively successful, but is generally achieved by inferring the new geographical range based on locality records from the native range, or known range of establishment (Kriticos & Randall 2001). This works particularly well for weed species whose native or new range of establishment is well known (e.g. Robertson *et al.* 2001). One of the most widely used tools for this task is CLIMEX (Sutherst & Maywald 1985) which requires the user to create a template of physiological parameters for the species, which the program then uses in conjunction with meteorological data to infer the potential range of the species being introduced. The parameters in CLIMEX models are inferred from the distribution records. Confidence in CLIMEX models is gained through comparison of projected potential distribution with locality records that were not used in the model-fitting process. Locality records for the species from a fairly broad range of climates are required to test the model before it can be used with any assurance (Sutherst 2003). Nevertheless, meaningful models have been generated for many organisms (Sutherst *et al.* 1999).

Insect development (degree–day) models, using temperature and time to predict the number of generations that an insect can complete at a given locality, use only empirical data and are sometimes successful at predicting whether an insect can establish at a particular locality (e.g.

[1] Ecophysiological Studies Research Programme, School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, WITS 2050, South Africa.
[2] Department of Zoology and Entomology, Rhodes University, PO Box 94, Grahamstown 6140, South Africa.
Corresponding author: M.J. Byrne <marcus@gecko.biol.wits.ac.za>.

McClay & Hughes 1995). Used on their own, these models appear to work best with extreme climates, involving univoltine insects that have defined overwintering strategies. If the introduced species can only complete one or less generations in a year, it is predicted to be unable to establish at that locality (McClay 1996). In more temperate climates with multivoltine insects, the results may not be so conclusive and additional data on egg production and overwintering may be required (Stewart *et al.* 1996). The major shortcoming of this method is the time and effort required to rear insects at fixed temperatures over long periods. These drawbacks led McClay (1996) to suggest that because of the labour involved, degree–day models should be reserved as a post-release research tool for agents that are difficult to establish. A simple pre-release test, which could predict the likelihood of establishment of an introduced species, would be extremely valuable to biocontrol workers.

Insects have two straightforward thermal responses to extremes of temperature, and these values are easily measured. The first is the critical temperature (CTMax or CTMin), being the temperature extremes at which the insect immediately loses locomotory function. Beyond these temperatures the insect cannot respond to any further change in temperature in the same direction, and therefore becomes vulnerable to predation, catastrophe or further temperature excess. Lethal temperatures (upper and lower LT_{50}) define extreme temperature limits from which organisms cannot recover after a prolonged exposure (in this case two hours). These thermal limits can be determined in a few days of experimentation, by exposing small numbers of the insects to extreme temperatures in a controlled water bath. The data are analysed by probit analysis, and an LT_{50} is produced for the upper and lower limits of the lethal temperature, while the CTMin and CTMax are calculated from the mean values across the temperature range at which a response was recorded.

The aim of this paper is to compare methods that can be used to predict the probability of establishment of classical biological control agents prior to their release.

Methods

Data on the thermal limits of 16 insect species were collected from the literature and unpublished data, and were compared to detect any correlation between the insect's habitat and its thermal physiology.

Different aspects of the thermal biology of four different insects species (marked * in Table 1) were used to assess the usefulness of these parameters in predicting the establishment of these insects in South Africa. CTMin and CTMax, and LT_{50} were determined using the methods of Mitchell *et al.* (1997). To generate an LT_{50} for the bud-galling wasp *Trichilogaster acaciaelongifoliae*, uneclosed pupae and adults that had not emerged from galls were exposed to the experimental temperature for two hours, then dissected out of the galls and examined for survival. Adults were scored as alive if they were able to self-right 24 hours after removal from the experimental temperature. Treated pupae were placed in separate wells of a 96-well enzyme-linked immunosorbent assay (ELISA) plate and kept at 25°C, 95% relative humidity (RH) until they emerged. A control sample received the same treatment but was never exposed to temperatures above 25°C. For all other species adult insects were used.

Degree–day models were calculated for three insects using a variety of fixed rearing temperatures, depending on the species. The values of K and t for each species were derived from the reduced major axis regression method of Ikemoto & Takai (2000). These were then used to calculate accumulated degree–days according to the methods of Campbell *et al.* (1974) at each location in the CLIMEX meteorological database, which has monthly mean maximum and mean minimum temperatures for 128 South African localities. The number of generations per annum each species could theoretically complete was calculated and projected onto contour maps of South Africa created with ARCVIEW. These data were compared with the number of generations in the native range of the insect. Because the mirid *Eccritotarsus catarinensis*, a natural enemy of water hyacinth, failed to overwinter at a high altitude site in Johannesburg, the number of generations able to survive the highveld winter months from April to August was also calculated and presented as above.

Results and discussion

Insects generally have thermal limits that reflect the environments in which they have evolved (Table 1). However, this relationship does not yield any sensible correlation between estimates of environmental temperature and lower thermal limits (CTMin and LT_{50}), primarily because we know so little about the microclimate in which the insects live (McConnachie 2004), and not least because of the multitude of methods and exposure times used by different workers to measure these limits.

Explicable correlations of thermal limits with environmental temperature

Trichilogaster acaciaelongifoliae is a bud-galling wasp of *Acacia longifolia*. Adult wasps were found to have an upper LT_{50} of 41.1°C ($Y = 109.067 - 2.651x$, $r^2 = 0.855$), while the pupae to have an upper LT_{50} of 41.3°C ($Y = 31.782 - 0.767x$, $r^2 = 0.396$) (Fig. 1), which is well above the January mean maximum of 26°C for Sydney, in its Australian native range, and satisfactorily explains why the wasp has been able to thrive on the South African highveld, and the KwaZulu Natal lowveld, despite the predictions of Dennill (1990), who used Walter and Leith's (1960) climate diagrams to

Table 1. Thermal limits of insects, in relation to their native range.

Species[a]	Family	Region of origin	Stage tested	Upper LT_{50}	CTMax	Lower LT_{50}	CTMin	Environmental low temp[b]	Exposure time	Source
Acyrthosiphon svalbardicum	Homoptera	High Arctic, Europe	Eggs			–33 SCP[c]		–16.7	1 min	Strathdee *et al.* (1995)
Rhopalosiphum padi	Homoptera	N. temperate, Europe	Eggs			–36 SCP		1.7	1 min	Strathdee *et al.* (1995)
Dendroides canadensis	Coleoptera Pyrochroidea	N. temperate, Canada	Larvae			–20 SCP		–32.8	?	Olsen *et al.* (1998)
Pringleophaga marioni	Lepidoptera	Subantarctic islands	Caterpillars		38.7	–9	–0.6	0	1 hour	Klok & Chown (1997)
Celatoblatta quinquemaculata	Dictyoptera, Blattidae	Alpine >1300m New Zealand	Nymphs			–9.5		–7	5 mins	Block *et al.* (1998)
Hodotermes mossambicus	Isoptera	Subtropical South Africa	Major workers	42.9	47.3	2.84	7.1	–1.8	2 hours	Mitchell *et al.* (1993)
Spodoptera exempta	Lepidoptera Noctuidae	Subtropical South Africa	Caterpillars		50		10	2.8	1 hour	Klok & Chown (1997)
Imbrasia belina	Lepidoptera Saturnidae	Subtropical South Africa	Caterpillars		48		10	2.8	1 hour	Klok & Chown (1997)
Cirina forda	Lepidoptera Saturnidae	Subtropical South Africa	Caterpillars		47		11	2.8	1 hour	Klok & Chown (1997)
Desert tenebrionids	Coleoptera Tenebrionidae	Subtropical Namibia	Adults		50		10		?	Roberts *et al.* (1991)
Philonthus sanamus	Coleoptera Staphylinidae	Subtropical South Africa	Adults	42.82					2 hours	Byrne (1998)
Philonthus labdanus	Coleoptera Staphylinidae	Subtropical South Africa	Adults	40.48					2 hours	Byrne (1998)
**Trichilogaster acaciaelongifoliae*	Hynemoptera Pteromalidae	Mediterranean Australia	Adults Pupae	41.1 41.3					2 hours	Byrne (unpublished data)
**Gratiana spadicea*	Coleoptera Chrysomelidae	Subtropical Argentina	Adults			–7.1	4.9	5.0	2 hours	Byrne *et al.* (2002)
**Stenopelmus rufinasus*	Coleoptera Curculionidae	Tropical Florida	Adults			–12.1	1.3	16.1	2 hours	McConnachie (unpublished data)
**Eccritotarsus catarinensis*	Hemiptera Miridae	Tropical Brazil	Adults	37	49.6	–3.5	1.2	13.9	2 hours	Coetzee (unpublished data)

Notes: CTMax and CTMin, upper and lower are defined in the text. [a] Insects marked with an asterisk are mentioned in the text. [b]Environmental low temp. is the lowest mean minimum temperature in the region of origin. [c]SCP = super cooling point.

suggest that the wasp would fail to establish because of high summer temperatures in these areas.

The lower CTMin of *Gratiana spadicea* (4.9°C; Table 1) is close to the lower average winter temperatures recorded at Buenos Aires, its most southerly collection site and the lower LT_{50} of –7.1°C is close to the lower extreme temperatures at South African release sites, where the beetle has had a patchy establishment and overwinters in very low numbers (Byrne *et al.* 2002).

Eccritotarsus catarinensis has a CTMin of 1.2°C. Those locations in South Africa that experience mean minimum temperatures below this level include Johannesburg, where there has been an establishment failure, and are to the south of successful establishment sites (Fig. 2). However, this parameter fails to explain the lack of establishment at some Western Cape sites.

Inexplicable correlations of thermal limits with environmental temperature

Stenopelmus rufinasus is thought to originate from the Florida region of the United States of America and has successfully established throughout South Africa wherever *Azolla filiculoides*, the target weed, occurs (McConnachie *et al.* 2003). The CTMin of 1.3°C and lower LT_{50} of –12.1°C bear little relationship to the climate of the country of origin (Table 1). However, these extreme values did give us the confidence to predict that the weevil would survive the cold winters of the high altitude interior of South Africa. *Stenopelmus rufinasus* has also established in Britain (Richard Shaw, CABI, pers. comm.), which is not that surprising given its lower LT_{50}.

Degree–day model successes

The degree–day model predicted a minimum of 4, to a maximum of 20 generations of *S. rufinasus* per year at various localities around South Africa (Fig. 3). This has been confirmed by the widespread establishment of the beetle, and field sampling suggests that these figures may be slightly low (A.J. McConnachie, unpublished data).

Degree–day model failures

The moth *Parachaetes insulata* released against *Chromoalaena odorata* in South Africa is predicted to complete four to six generations per year at subtropical release sites in Kwazulu Natal (Fig. 4). The moth has so far failed to establish a viable permanent population, but this might be because of severe larval predation (Kluge 1994) or low humidity levels (W. Parasram, unpublished data).

Eccritotarsus catarinensis was predicted to complete from 3 to 14 generations per year at different

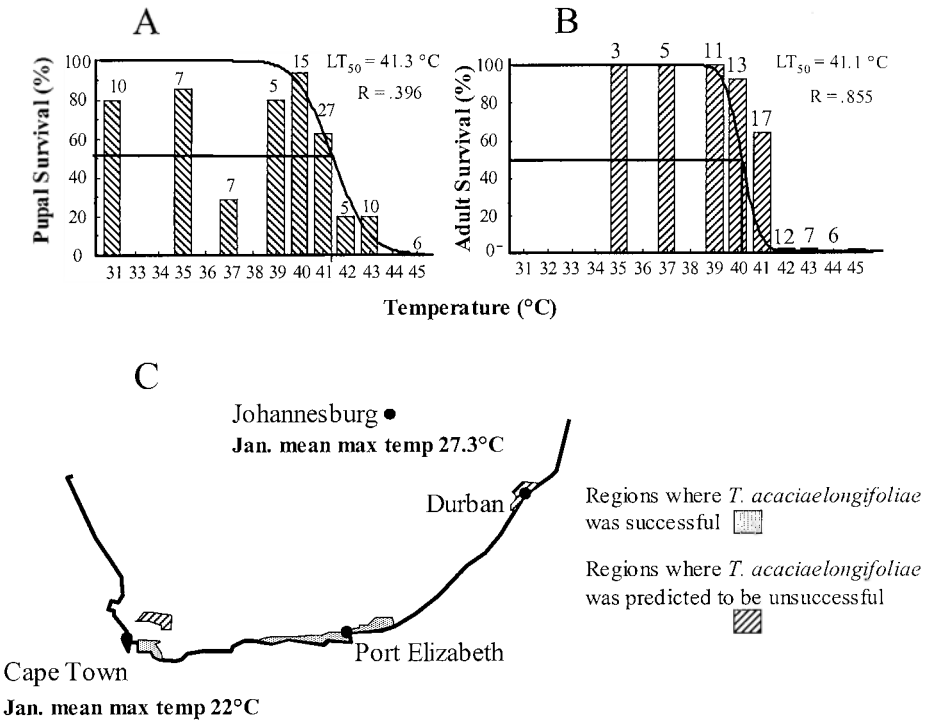

Figure 1. Upper LT_{50} of pupal (A) and adult (B) *Trichilogaster acaciaelongifoliae*, in relation to mean maximum January temperatures at sites where the wasp has been introduced and was predicted to establish or fail (C). Numbers at the tops of histogram bars refer to the sample size tested at that temperature.

localities in South Africa, and five generations at the Johannesburg site where it failed to overwinter. Extremes of temperature at this site exceed the lower thermal limits measured for the mirid. However, small populations have established on the Vaal River, which experiences similar low winter temperatures. The inability to develop sufficiently during the winter months may hinder overwintering of this insect, which survives as an adult for only 50 days (Fig. 5). The mirid can only develop through one generation during the winter months of April to August at the Johannesburg site, but can complete 1.3 generations at a site 80 km away near Pretoria where the insect has established (Fig. 5). Such bottlenecks probably force the population into non-overlapping generations, which makes them even more vulnerable to extremes of weather. However, the lack of establishment in the Western Cape where 1.7 generations are predicted does not yield to this explanation where winter rainfall and exposure to wind may also play a role.

Conclusion

Unfortunately none of the methods reviewed above has been revealed as an ideal technique for identifying an agent's thermal shortcomings at an early stage of laboratory testing. Upper thermal limits are generally well above average environmental temperatures, but may be below microhabitat extremes which active insects would be expected to avoid. However, the lower thermal limits, and in particular the LT_{50}, show some utility for estimating an insect's chances of surviving extreme winter conditions. The present weakness of the measure, which prevents cross species comparisons

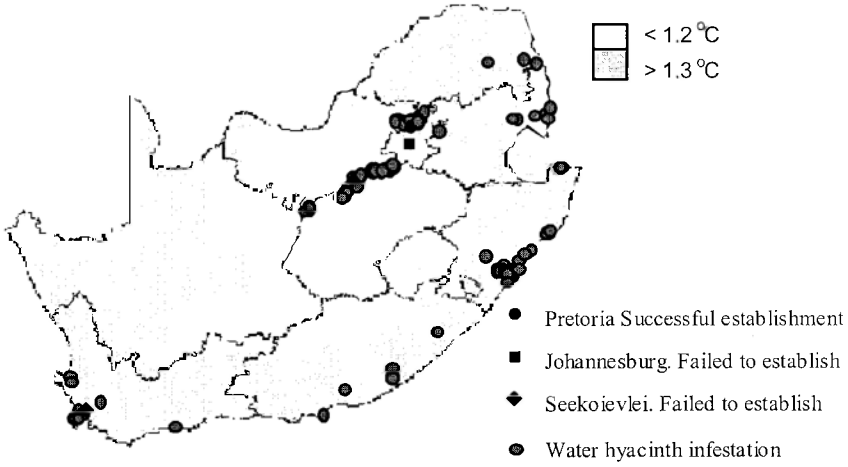

Figure 2. Areas in South Africa that experience temperatures above or below 1.2°C, the CTMin of *Eccritotarsus catarinensis*. Note the boundary between the Pretoria establishment and the Johannesburg failure sites.

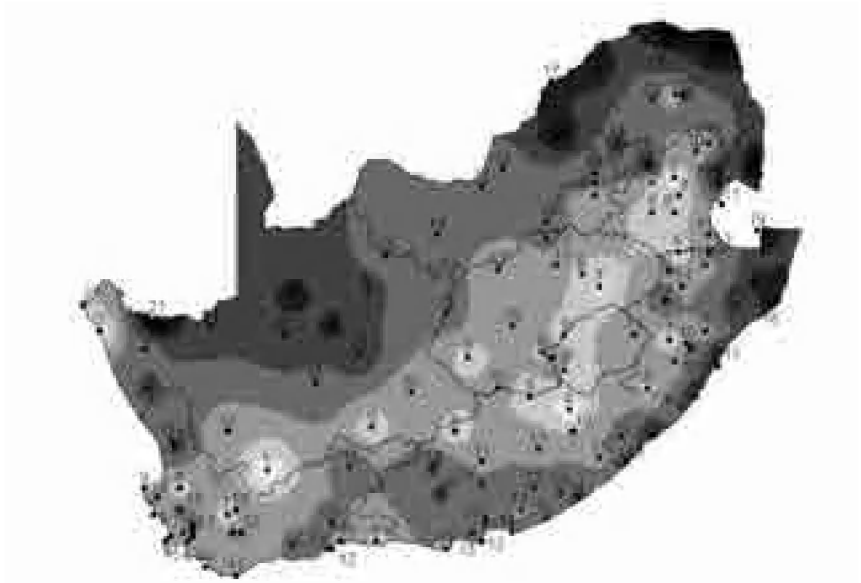

Figure 3. Map of South Africa indicating the number of generations that *Stenopelmus rufinasus* is expected to complete within a year.

and detection of large-scale patterns, is that low temperatures may have a cumulative effect, causing insects to die of cold stress, before the LT_{50} is reached. Lack of standardization with regard to exposure and recovery times over which the LT_{50} is measured has also contributed to its vague value. We propose that an exposure time of two hours, with 24 hours for recovery, measured by the ability to self-right, should be used for measurements of LT_{50}. Two hours at an extreme temperature represents a reasonable approximation of an overnight "cold snap" that could decimate a local insect population.

At this stage, the CTMin appears to be a weak measure of cold tolerance because most insects, whatever their geographical origin, go into torpor close to 2°C. A series of days where the temperature drops below the CTMin will presumably produce physiological stress. It may be instructive to compare patterns of sequential days below the CTMin at establishment and failure sites to estimate the effects of accumulated cold stress on the insects (Vera *et al.* 2000).

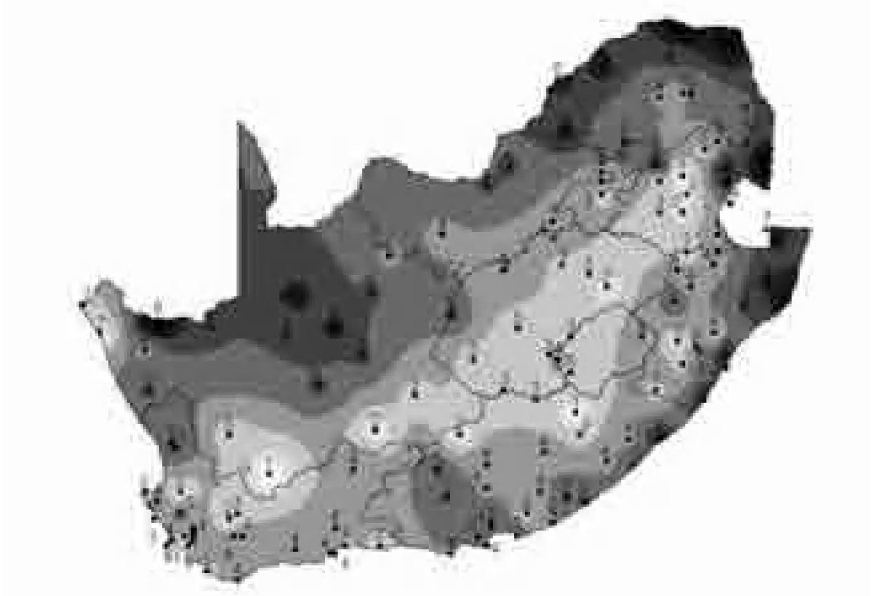

Figure 4. Map of South Africa indicating the number of generations that *Parachaetes insulata* is expected to complete within a year.

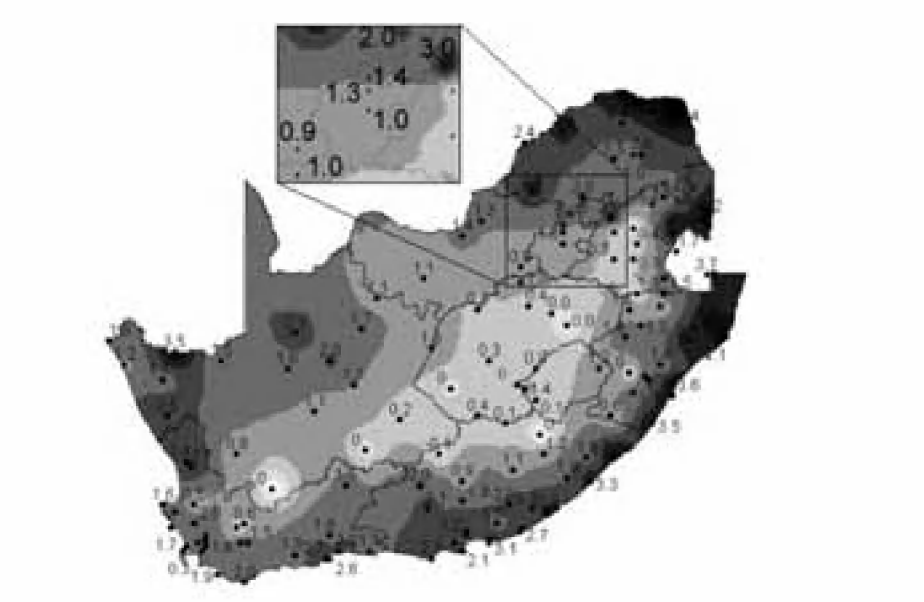

Figure 5. Map of South Africa indicating the number of generations that *Eccritotarsus catarinensis* is expected to complete within five winter months of April to August.

The degree–day model is most satisfying because the results appear sensible, and are useful for different geographical areas. However, in this review it has only worked well for one of the three examples given, largely because *S. rufinasus* is extremely cold tolerant. Comparing species reveals a pattern that reflects the underlying isotherms of the local climate, expressed as the number of potential generations. Our modification of this model to account for longevity and replacement of the parental generation adds a new dimension to prediction of the number of favourable months available to a species at a particular locality. This could be improved by including a pre-oviposition period, combined with an oviposition threshold and population structure. Nevertheless, the number of insects required and the time involved in gathering data for a degree–day model remains daunting.

Two recommendations emerge for steps to reduce climate-incompatibility failures-to-establish in classical biological control. Firstly, before any foreign exploration is undertaken, a climatic characterization of the native and introduced geographical range of the weed is prepared, followed by a comparison using CLIMEX "match climates", to identify areas in which suitable control agents should be sought. Secondly, a prompt experimental determination of the CTMin and LT_{50} values of the candidate agents should be carried out while they are still in quarantine, followed by a general comparison of these data with the extremes of climate in the proposed area of introduction, to estimate the chances of survival of the potential agents.

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The need to build biological control capacity in the Pacific

Liz Dovey,[1] Warea Orapa[2] and Suzy Randall[1]

Summary

Whilst clearing of native vegetation and unsustainable harvesting pose serious threats to the Pacific Islands, invasive species are considered to pose the biggest threat to the remaining biodiversity. The Pacific weed problem is huge – whole forests are smothered by vines, suppressing the birds and bats that rely on forest resources and which in turn disperse the forest species, as well as causing problems for agriculture and in subsistence gardens. All 22 Pacific island countries and territories face major weed problems, ranging from their impacts on simple island ecosystems and on Islanders' ways of life, to the logistics of tackling the problems, including access and capacity issues, and resource, information and technique limitations. The capacity of individual Pacific countries and territories to tackle weeds is very limited, in terms of people with skills, and technology, policy and infrastructure. Pacific countries and territories therefore work collectively through intergovernmental agencies such as the South Pacific Regional Environment Programme (SPREP) and the Secretariat of the Pacific Community (SPC) to address common issues with the help of key donors and partners. The Pacific has developed a Regional Invasive Species Strategy and many countries are developing national strategies and cross-sectoral committees. Pacific weed efforts are focusing on identifying what weeds are present in each country, noting other species that may be invasive if introduced, strengthening country capacity to prevent their establishment, and building capacity of each country and their people to better address the problems. There are a few successful examples of control – including biocontrol – and eradication that lend us heart. The Pacific Island countries and territories need effective collaboration with partners who have developed or could develop weed control techniques that work safely in the tropical conditions of the Pacific, such as biological control. This needs to be supported by gathering necessary information and developing or modifying appropriate techniques, plus the expertise to safely apply them.

Keywords: biocontrol, biodiversity, biological control, capacity, invasive species, Pacific, partners, weeds.

Introduction

The Pacific Islands region consists of thousands of mostly tiny islands and atolls – only seven have land areas of over 700 km^2 – in an ocean of 33 million km^2 (Power 2003) – less than 2% land.

Pacific biodiversity is globally significant. Species on islands are predisposed to genetic drift and natural selection towards endemism because of their relative isolation and reduced opportunities for mixing with continental populations. The number of species groups present declines eastwards as distance from Asia and Australia increases. Opportunities for new arrivals to radiate into unfilled niches can lead to unusual habitat selections, often in the absence of the larger predators of the continents. For example, lizards living in tidal zones, ground-nesting birds and land-dwelling crabs all occur in island situations. Populations of species restricted to one or a few islands are therefore often very small and thus especially vulnerable to any catastrophic event, whether natural, such as cyclones or volcanic eruptions, or otherwise, such as the wide range of impacts that people can inadvertently cause. The rate of extinction of native species has been higher on islands than anywhere else in the world.

[1] South Pacific Regional Environment Programme, PO Box 240, Apia, Samoa.
[2] Plant Protection Service, Secretariat of the Pacific Community, Private Mail Bag, Suva, Fiji.
Corresponding author: Liz Dovey <lizd@sprep.org.ws>.

In global analyses of conservation importance, the Pacific ranks highly, despite its minimal land area. The island of New Guinea is considered one of the biologically most diverse parts of the world (Mittermeier and Mittermeier 1997) and the Micronesian–Polynesian region is considered one of the most biologically rich and threatened regions of the world (Mittermeier *et al.* 2000). Many more species are endangered in the region than would be expected on the basis of its land area. The Pacific harbours a quarter of the world's globally threatened birds (Hilton-Taylor 2000) of which more than half are restricted to their islands or the region (Stuttersfield *et al.* 2000). Many plant and animal groups remain incompletely studied so it is likely that figures for biodiversity and endemism for this region will continue to rise. The living connection between biodiversity and people in the Pacific provides an additional social and cultural layer to be considered in addressing conservation needs in the region. The Pacific Islands are home to a great number of indigenous cultural groups, who have retained their robust cultural traditions, over a thousand distinct languages, and strong traditional attachments to the land, sea and natural resources.

Invasives: the biggest threat to biodiversity

Pacific island countries and territories are particularly vulnerable to the effects of invasive species such as weeds – island species are far more prone to extinction than continental species. A regional invasive species review (Sherley 2000) concluded that invasive species pose the greatest threat to remaining biodiversity of the Pacific. On a global scale, after clearing and habitat loss, invasive species are responsible for more species extinctions than any other cause. The *Convention on Biological Diversity* (CBD) under Article 8(h) recognizes the importance of this global problem and has called on contracting Parties to "prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats and species". Many of the Pacific Island countries are signatories to the Convention[1] but lack the capacity to implement the required measures to protect their countries.

Island ecosystems have been totally changed by the introduction of a wide variety of species – of pigs, cattle, and goats for food, of cats and dogs for company, of mongooses and mynas for control of other pests such as rats or cattle ticks, and by accidentally introduced pests, such as various rats, ants and snails. Many of these species have become part of more complex ecological interactions, and have led to interspecies "chain reactions" of problems (Sherley 2000).

Invasive plants – or weeds – also have dramatic impacts, causing a wide range of ecological changes that significantly degrade native ecosystems. Whole forests are smothered by vines, suppressing in turn the native flora and the fauna that depend on the forest products and that in turn disperse the tree seeds to replenish the forest. In addition to the permanent extermination of endemic species, impacts on the native vegetation can include: reduction in diversity and abundance of native species, including other native species that in turn depend on them; less complex vegetation structure (fewer vertical layers of plants available, so fewer niches available for other species); competition for light; and displacement of native species by more vigorous cosmopolitan species. In addition to the direct threats on biodiversity, invasive weeds cause changes to essential ecosystem processes such as soil and water quantity and quality, water retention and nutrient cycling that also affect them (Sherley 2000).

However, the most obvious impacts of invasive weeds are those to the economic, agricultural, health, social and cultural sectors. The impacts on ecosystems listed above affect people as well as native species, as does the loss or reduction in the ability of native species to continue to provide other benefits such as providing traditional medicines, firewood, building materials and food sources. In addition, some invasive weeds impact on human and domestic-animal health.

Prevention is the most cost-effective response, followed by rapid response to incursions and eradication where feasible, but several weed species are already well established, widespread and causing harm to a country.

Pacific weed challenge and response

The existing Pacific weed problem is huge – all 22 Pacific island countries and territories face major weed problems, ranging from the various impacts they have on native island ecosystems and on islanders' ways of life, to the scale of the logistics necessary to tackle the problems. Hundreds of invasive and agricultural weed species have been recorded from Pacific Island countries and territories (Swarbrick 1997, Pacific Island Ecosystems at Risk Project, see <http://www.hear.org/pier>).Weed species known to occur in each country have been recorded, as well as highlighting species likely to become invasive should they be imported to the country either deliberately or accidentally.

Pacific Islanders live so close to the land that their very livelihoods and lifestyles are impacted when an invasive species causes problems. As an example, the introduction of taro-leaf blight disease, caused by

1. Pacific island country signatories to the CBD and date of accession: Cook Islands (20/4/1993); Fiji (25/2/1993); Kiribati (16/8/1994); Marshall Islands (8/10/1992); Micronesia (20/6/1994); Nauru (11/11/1993); Niue (28/2/1996); Palau (6/1/1999), Papua New Guinea (16/3/1993); Samoa (9 /2/1994); Solomon Islands (3/10/1995); Tonga (19/5/1998); Tuvalu (08/06/1992); Vanuatu (25/03/1993). Tokelau is in the process of acceding. Several Pacific territories of France are also included as France is a signatory to the CBD.

Phytophthora colocasiae, to Samoa in 1993 not only decimated the country's biggest export crop, but also affected the traditional diet, way of life and livelihoods of thousands of Samoans. The economic cost to Samoa of this unwanted, introduced species is estimated to have been in the order of $US40 million – more than the impact of two major cyclones (Peter Sinclair, Secretariat of the Pacific Community, pers. comm.).

Addressing weed problems is a major challenge from many points of view: the geographical size of the Pacific; the large number of mostly tiny islands; and the large number of autonomous Pacific island countries and territories, with populations as small as 49 on Pitcairn Island, 1500 on Niue and 1300 on Tokelau, ranging to 5.13 million in Papua New Guinea. With many depending on foreign aid for survival, the Pacific's capacity to tackle weeds is very limited in terms of people with skills, or technology, policy, infrastructure and other resources. Many government departments comprise only a handful of people, some of whom wear many hats, and there are few specialized scientists or research institutions. Baseline ecological information is lacking or difficult to access for most countries and most lack effective invasive species prevention or management strategies and so continue to face the many associated problems.

Regional collaboration

Fortunately, however, the Pacific island countries and territories work collectively through various intergovernmental regional agencies to address the issues they hold in common and to ensure their voice is heard in world forums. The South Pacific Regional Environment Programme (SPREP), the agency responsible for supporting the members to tackle their environmental issues, and the Secretariat of the Pacific Community (SPC), responsible for assisting with their agriculture and health issues, are the key intergovernmental agencies that work with the countries and territories to address invasive species and weed issues with the help of key donors and partners through the Invasive Species Programme of SPREP and the Plant Protection Service of SPC. There are encouraging signs of efforts to improve collaboration between regional organizations to address the threats of invasive weeds. The focus is on both managing existing weeds as well as preventing the spread of risk species between the islands, thus helping countries fulfil their obligations under the Convention of Biodiversity.

A result of this collective work include a Regional Invasive Species Strategy, collectively developed and endorsed by the SPREP member countries (Sherley 2000), focusing on means to address invasive species affecting terrestrial biodiversity. A wider sectoral representation (including regional and national agricultural officers) at a regional meeting hosted by the Global Invasive Species Program in 2002 agreed that the strategy was equally applicable to marine, freshwater and agricultural sectors as well and recommended that the strategy be revisited to reflect these wider sectoral interests and to encourage inter-sectoral collaboration.

At the country level, many members are developing national invasive species strategies and cross-sectoral approaches to the issue. Current Pacific invasive weed efforts are focusing on identifying weeds present in each country, noting other species that may be invasive if introduced, strengthening country capacity to prevent their establishment and building the capacity of each country and their people to better address the problems.

Most existing weed management efforts are associated with, and limited to, agricultural production areas only and usually rely heavily on the use of herbicides, rather than addressing a specific weed problem from a multi-sectoral and ecological perspective. Since 1951, SPC's Plant Protection Service has been involved in addressing most of the past and present regional pest and disease problems, in collaboration with national partners, focusing on species of agricultural and health concern, but including many species known to be invasive. Emphasis by SPC is placed on prevention of weed introductions (by supporting national quarantine capabilities), preparation (improving capacity to address new weed, pests and disease cases) and management of well-established problem species. An Invasive Species Programme was established at SPREP in 1998 to focus more on plant, animal or microbe species of particular biodiversity concern, and works closely with SPC on species of mutual concern.

There is an increasing number of examples of weed eradication and management in the Pacific, with emphasis on biological control of specific environmental weeds, such as eradication of *Sphagneticola trilobata* and *Mimosa diplotricha* in Niue, eradication of some populations of *Mikania micrantha* in Palau (Joel Miles, pers. comm.), an eradication project on *Falcataria moluccana* in American Samoa's National Park (Tavita Togia, pers. comm.), and biological control of *Chromolaena odorata* in Guam, Palau, Federated States of Micronesia (Bamba 2002) and Papua New Guinea (PNG) (Orapa et al. 2002). Successful past cases of biological control in the Pacific include the efforts against water hyacinth (*Eichhornia crassipes*) in PNG (Julien and Orapa 1999, 2001), the successful control of *Salvinia molesta* in the Sepik River in PNG which resulted in immense socio-economic benefits for thousands of villagers and the restoration of natural ecology to its original stage (Thomas and Room 1986) and to some extent the partial suppression of *Lantana camara* in the Pacific Islands (W.O., personal observation).

Priority Pacific weeds and biocontrol

Several key weed species are too big and widespread to tackle by hand or by chemicals. Many weeds are alien species introduced intentionally or unintentionally since humans first arrived in the Pacific, most arriving without their guild of natural enemies or diseases that would otherwise keep their numbers under control. Many of these weeds are therefore good targets for biological control programs.

There has been longstanding interest in the use of biocontrol in the Pacific. Seminal work for the region was undertaken by Waterhouse (1993, 1997, Waterhouse *et al.* 1998) and, in 1995, a Pacific Biocontrol workshop was held and one of the outcomes was the development of guidelines for conduct of biological control in the Pacific.

Successful biological control of several weeds has already occurred in some Pacific island countries where safe and effective agents have been released (Waterhouse & Norris 1987, Room 1993, Julien & Griffiths 1998, Julien & Orapa 1999, 2001). However, much past biological control work against weeds in the region has been done on an ad-hoc basis – future regional biological control programs need to be developed, structured and implemented following set guidelines that minimize the chance of releasing species that can in turn become pests on non-target species. It is only when inappropriately tested agents are considered that problems can arise.

No cases of a weed biocontrol agent adversely affecting non-target species are known from the Pacific and the suspected extinction of the coconut moth *Levuana iridescens* of Fiji by the tachinid fly *Bessa remota,* introduced from Malaya in 1925, remains the only documented case of a biological control agent exterminating its (pest) host anywhere in the world (Kuris 2003). However, there have been unfortunate cases of biocontrol agents leading to the extinction of non-target native partulid land-snail species in the Pacific (Cowie 1992), so great care to ensure specificity of biological control agents is extremely important. Regardless, there are no known cases of weed biocontrol agents producing unexpected deleterious impacts and, unless inadequately screened for host preferences, biological control will remain the principal and preferred tool for managing major invasive weeds in the Pacific islands.

Attempts have been made to develop prioritized lists of agricultural weeds for the region, starting with Waterhouse (1997). During the 2002 Regional Technical Meeting on Plant Protection (RTMPP) in Nadi, Fiji, the region's 45 most important weeds were identified and ranked according to importance (Anon. 2000). Most weeds of significance for agriculture are also key ecological threats. Some of the most serious weeds for the region, as identified by the countries, include nutsedge (*Cyperus rotundus*), the vines mile-a-minute (*Mikania micrantha*) and *Merremia peltata*, the two sensitive weeds *Mimosa diplotricha* and *M. pudica*, lantana (*Lantana camara*), wedelia (*Sphagneticola triloba*), water hyacinth (*Eichhornia crassipes*) and African tulip (*Spathodea campanulata*). Some of these weeds are already a major problem in many or most countries, impacting upon both agriculture and the environment, but little has been done to control them. The top 24 of these species are listed in Table 1, together with an indication of the level of significance of their impact and suggested potential for biocontrol. It can be noted that this list does not include some tree species that may only be of considerable concern to the environment rather than to agriculture, such as the albizias *Albizia chinensis* and *Falcataria moluccana.*

Of the prioritized weed list (Anon. 2000), the majority (69%) have no known biological control agents available. For the rest, 18% have had at least one natural enemy released in or outside the Pacific region with no follow-up work or evaluation in the region, while 13% of weeds listed by the RTMPP have good biological control agents already available in some Pacific island countries or outside the region which could be assessed for use in the affected countries. There is an urgent need to conduct new research into new possible biological control agents and to re-visit previously released but forgotten biological control agents for the management of some of the region's most serious weeds.

Few or no original biological control research and development programs against weeds have been attempted in the Pacific region because of the large initial costs and length of time that may be involved. The only attempt at initiating biological control in the region was the preliminary exploration for natural enemies of Honolulu rose (*Clerodendron chinensis*) in Vietnam and southern China, but this did not proceed to the next step. Not all SPC and SPREP member countries and territories have the capacity to run separate biocontrol projects. Only a few (Fiji, PNG, Guam, and New Caledonia) have undertaken biological control programs and have some capacity to undertake biological control against weeds and pest problems.

Development and customization of Pacific-appropriate control measures, such as new biocontrol agents, is an important task for the Pacific. The use of biocontrol is highly suited to countries with limited technical capacity to maintain sustained control programs using other techniques, although it must be undertaken using best practice standards to ensure that the chosen agent will not become a new pest in its own right.

The Pacific island countries and territories need strong technical and resourcing partners who have developed or could develop techniques that work safely in the tropical conditions of the Pacific. We need help to learn from other successful control projects. We need partners who can help by gathering the needed information and developing or modifying appropriate techniques. We also need help to successfully and safely apply them.

Table 1. The top 24 potential candidate weeds for biological control in Pacific island countries and territories (PICTs).

Weed name	Number of PICTs identifying the species as a key invasive species/weed in 2002[a]			Regional agricultural weed ranking in 1997[b]	Possible biological control response (BCA = biological control agent)
	Key pest	Important pest	No. of countries ranking in their top 10		
Mikania micrantha Mile-a-minute weed	11	1	12	3	Investigate use of biological control using the leaf-feeding butterfly *Actinotes anteas* and possibly reinvestigate *Liothrips mikaniae*. Investigate current CABI trials in India.
Cyperus rotundus Nutgrass	10	5	10	1	Re-look at using the BCA *Bactra* spp., esp *B. minima*
Merremia peltata Merremia	10	1	10	23=	Explore for potential BCAs
Mimosa diplotricha (= *M. invisa*) Giant sensitive weed	8	2	9	2	Rear and distribute the psyllid *Heteropsylla spinulosa*
Mimosa pudica Sensitive plant	7	6	7	5	Explore for potential BCAs
Lantana camara Lantana	5	9	3	4	Redistribute all available BCAs and introduce additional host-specific BCAs released in Australia and elsewhere
Sphagneticola trilobata Wedelia	5	4	5		Explore for potential BCAs
Bidens pilosa Cobbler's pegs	4	9	4	6	Explore for potential BCAs
Eichhornia crassipes Water hyacinth	4	3	3	10	Consider biological control. Introduce the weevils *Neochetina* spp. and the moths *Xubida infusellus, Niphographta albigutalis* and the bug *Ecritotarsus catrinensis*
Spathodea campanulata African tulip-tree	4	2	5		Explore for potential BCAs; check work in northern Australia
Antigonon leptopus Chain of hearts	4	2	4		Explore for potential BCAs; eradicate or undertake integrated weed management, quarantine exclusion
Chromolaena odorata Siam weed	4	0	4	21=	Rear and distribute BCAs already released in region; introduce additional agents *Calycomyza eupatorivora, Lixus aemulus,* and *Actinote thalia-pyrrha*
Cassia tora Foetid cassia	2	8	2		Explore for potential BCAs
Stachytarpheta urticifolia Blue rat's tail	2	12	3	20	Explore for potential BCAs
Sida acuta Spinyhead sida	2	7	1	17	Introduce and release *Calligrapha pantherina* and *Eutinobothris* sp.
Kyllinga polyphylla Navua sedge	2	5	2	26=	Explore for potential BCAs
Clidemia hirta Koster's curse	2	4	1		Introduce and release *Liothrips urichi* (already present in Fiji)
Clerodendrum chinense Honolulu rose	2	0	1	9	Introduce *Phyllocharis undulata* already released in Thailand; screen other BCAs identified in Vietnam or South China surveys by Julien (see 1995 report in Proceedings Pacific Biocontrol workshop, Fiji)
Sida rhombifolia Paddy's lucerne	1	11	1		Introduce and release the BCAs *Calligrapha pantherina and Eutinobotris*
Solanum torvum	1	7	1	8	Explore for potential BCAs
Clerodendrum paniculatum Pagoda flower	1	1	1		Explore for potential BCAs
Costus speciosus Crape ginger	1	1	1		Explore for potential BCAs
Miconia calvescens Velvet leaf	1	0	1	28=	Environmental weed; pending Hawaii Dept Agriculture (HDOA) research results; HDOA exploring and testing potential BCAs in Hawaii and ?Tahiti; improve detection mechanism for other PICTs
Merremia tuberosa Wood rose	1	0	1		Explore for potential BCAs

[a] Derived from assessments made at the 2002 Regional Technical Meeting on Plant Protection, Fiji.
[b] Derived from Waterhouse (1977, p. 78, Table 15).

SPREP and SPC and the Pacific countries and territories are actively seeking to build new partnerships for projects to address invasive species, one specifically on biocontrol development. Objectives of the latter proposal are to reduce the impact of major weeds on agriculture, communities and the environment in member countries and territories by suppressing weed populations to levels below the ecological/economic thresholds, both by using known classical biological control agents as well as seeking to develop new or little known biological control agents of major regional weeds.

The Pacific needs help with this task, which is expensive and technically complex. Components of the project for which assistance would be welcomed include:

- collection and redistribution of suitable biological control agents (BCAs) already released on the target species in some Pacific island countries or territories (or nearby countries) to those countries and territories needing control of a target weed, ensuring that appropriate specificity requirements are met. A regional or subregional rearing facility may be needed to carry out this important activity and the next
- revisit and conduct research into the possibility of using potential weed BCAs that have been released only once in the past and forgotten
- exploration for new potential biological control agents for very important weeds requiring urgent suppression;
- testing of weed-management strategies suitable for Pacific island farming systems and natural area management systems.

The potential impact of this work, if developed and implemented properly, would be seen across all sectors in the Pacific island countries and territories. Production loss due to weeds should decline, there should be a contribution to improvement of the livelihoods of Pacific Islanders and the impact of invasive weeds on the ecosystems on which all Islanders depend would be reduced. The level of threat facing the region's globally significant and threatened biodiversity would also be reduced, especially if species that cause widespread habitat degradation were better controlled.

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The new encounter concept: centres of origin, host specificity and plant pathogens

Harry C. Evans and Carol A. Ellison[1]

Summary

The new encounter concept is analysed, initially drawing on plant pathology examples from agriculture. The two selected neotropical tree crops, rubber and cocoa, appear to show evidence for and against the hypothesis. There are no unique or new encounter diseases in the palaeotropical exotic range of rubber, whilst new major diseases, as well as pests, have moved rapidly from indigenous forest hosts and adapted to cocoa wherever it has been grown in the Old World tropics. On closer examination, however, it is concluded that the best (most damaging and host specific) hypothetical classical biocontrol agents, for both cocoa and rubber, are still the coevolved pathogens from their Amazonian centres of origin. A similar analysis of the fungal pathogens associated with three important invasive alien weeds of neotropical origin—*Chromolaena odorata*, *Lantana camara* and *Mikania micrantha*—in both their native and exotic ranges, shows that, in general, more fungi have been recorded from the Palaeotropics. Nevertheless, these comprise heterogeneous assemblages of opportunistic pathogens with wide host ranges, which have had no long-term or constraining impact on the invasive weed populations. In contrast, however, all these plant species are generally non-weedy in the Neotropics and coevolved pathogens, typically obligate or biotrophic fungi, are considered to be major natural control factors and which, consequently, have potential as classical biocontrol agents. Both these sets of examples provide evidence for classical biological control, or the enemy release theory, and against the new encounter hypothesis. However, some perplexing cases of new encounters, involving host range extensions of rust fungi on *Lantana* and *Senecio* species, are also presented and discussed.

Keywords: *Chromolaena,* cocoa, host specificity, *Lantana, Mikania,* new encounter pathogens, rubber.

Introduction

Coevolved natural enemy associations have traditionally been favoured for the biological control of alien or exotic pest organisms (DeBach 1964). However, based on an analysis of biocontrol programs involving insect agents, almost exclusively parasitoids, Hokkanen & Pimentel (1984) concluded that there is a greater chance of success (*ca.* 75%) when non-coevolved or new encounter natural enemies have been selected; although these results could not be analysed statistically because of the relatively few instances where new associations have deliberately been exploited. This concept or hypothesis, therefore, remains theoretical rather than practical. Certainly for invasive alien weeds, current biocontrol programs continue to follow the central tenet that the best—defined usually as the most host specific and highly damaging—natural enemies or potential biocontrol agents are to be found in the native range or centre of origin of the target invasive alien species (Greathead 1995).

Nevertheless, Hokkanen & Pimentel (1984) argued that, in terms of evolutionary ecology, such coevolved associations must lead to a mutual balance, or to an interspecific homeostasis, and that logically, therefore, the most damaging natural enemies should be new encounter associations. Subsequently, Hokkanen (1985) followed this up by analysing the diseases of several pantropical crops, and concluded that many of the important diseases are caused by new encounter pathogens that do not even occur in the native continental ranges of these crop plants. He recommended that surveys for potential biological control agents of alien weeds should not be restricted to their centres of

[1] CABI Bioscience, UK Centre (Ascot), Silwood Park, Ascot, Berks. SL5 7TA, UK.
* Corresponding author: Harry C. Evans <h.evans@cabi.org>.

origin and that screening should be widened to include non-coevolved natural enemies.

These findings are now re-examined to determine if the new encounter concept has validity, and, if so, whether or not there are lessons to be learned for the biological control of invasive alien weeds.

Lessons from agriculture?

An analysis of the history and the exploitation of two important tree crops of neotropical origin, rubber (*Hevea brasiliensis*) and cocoa (*Theobroma cacao*), shows evidence both for and against the new encounter hypothesis. Both these commodity crops, which have been introduced into all the humid tropical regions of the world over the past century, evolved in the forests of the Amazonian basin (Schultes 1984) and proved to be difficult if not impossible to grow on a commercial scale in their region of origin due to disease pressure from coevolved pathogens (Purseglove 1968; Davis 1997; Evans 2002a). Indeed, as the crops were moved around Latin America, these pathogens eventually caught up with their hosts with devastating results. A stark example is that of witches' broom disease of cocoa, *Crinipellis perniciosa* (Stahel) Singer, which since its arrival in the Brazilian State of Bahia a little over a decade ago, has reduced crop yields from about 400,000 tons per annum to somewhere in the region of 100,000 tons (Evans 2002a). The fact that cocoa had escaped its coevolved pathogen for so long is due to the geographic isolation of Bahia from Amazonia (Evans 1981).

The importance of natural barriers in separating natural enemies is graphically illustrated in cocoa both within and between continents, and a pod disease, caused by *Crinipellis* (*Moniliophthora*) *roreri* (Cif.) H.C. Evans, which evolved in the isolated forests of north-west Ecuador and Colombia on a locally endemic *Theobroma* species (*T. gileri* Cuatr.), has been on an invasive front for the past 20–30 years with potentially even greater impacts on crop yield than witches' broom disease (Evans *et al.* 1998, Evans 2002a). Thus, this appears to offer support for the new encounter concept and it seems that the forest pathogen moved to cocoa after it was brought across the Andes in Pre-Colombian times (Schultes 1984). This host extension has involved both morphological as well as physiological changes in the pathogen, with that from cocoa differing from the forest progenitor in producing a significantly greater proportion of round, thick-walled spores, probably as an adaptation to the more variable and drier conditions in cocoa plantations compared to the buffered forest ecosystem (Evans *et al.* 2003a,b). Nevertheless, this example also begs the question as to what constitutes a new encounter. Clearly, from the morphological and molecular evidence (Evans *et al.* 2003a), this adaptation has involved an evolutionary event.

New encounter natural enemies of cocoa, throughout its exotic palaeotropic range, are many and varied and, in contrast to this South American experience, these host extensions have not involved any inherent morphological or physiological changes. Thus, within a relatively short period of time after the introduction of cocoa to West Africa, the new crop was beset by new insect pests and diseases: the cocoa capsids (Miridae) appear to have moved extremely rapidly from indigenous sterculiaceous hosts (Dudgeon 1910, China 1944); whilst the mealybug-transmitted cocoa swollen shoot virus (CSSV) made an equally rapid transition from indigenous trees (Legg 1972). A new invasive *Phytophthora* species, *P. megakarya* Brasier & Griffin, has been identified more recently but evidence suggest that it had been around on cocoa in this area of origin for some time but had been confused with the more cosmopolitan *P. palmivora* (Butl.) Butl. (Brasier & Griffin 1979). A non-sterculiaceous, indigenous host of *P. megakarya*, has now been found in the ancient forest along the Cameroon–Nigerian border (Holmes *et al.* 2003), perhaps justifying Hokkanen's statement that: "...many pathogens will always be 'hiding in the jungle' waiting for new host species to be introduced into the area" (Hokkanen 1985). Remarkably similar incidences of new and damaging pest and disease associations have also occurred in Asia, with the cocoa pod borer (*Conopomorpha cramerella* (Shellen), Lepidoptera, Gracillariidae) and vascular streak die-back disease (*Oncobasidium theobromae* Talbot & Keane) moving from as yet unidentified forest trees (Talbot & Keane 1971, Prior 1980).

In contrast, however, rubber has remained free of any comparable new and highly damaging pest–pathogen associations in the Palaeotropics and the threat, which "continues to hang like a Damoclean sword over the neck of the industrial world" (Davis 1997), comes from its coevolved natural enemy *Microcyclus ulei* (Henn.) Arx, the causal agent of a devastating leaf blight which is still restricted to the New World.

There are, therefore, mixed messages from these two crop examples, as there are from those selected by Hokkanen (1985), which are very much dependent on correctly interpreting the information they contain. Thus, in the hypothetical but not entirely unjustified scenario that these tree crops were to become invasive, given the problems of cinnamon and quinine tree invasions in small island systems (Cronk & Fuller 1995), there is no doubt that, in the case of rubber at least, the control agent selected would be the coevolved pathogen *Microcyclus ulei*. Furthermore, it is our opinion that the neotropical pathogens of cocoa would be much more efficient, potentially reducing fecundity to zero, and sufficiently specific to be used as classical agents compared to any of the new encounter, palaeotropic pathogens. The case of *Crinipellis roreri*, is perhaps unique in that, although it has moved to an exotic host (i.e. cocoa), it has modified both its morphology and physiology: an example of a recent evolutionary event rather than a new encounter (Evans *et al.* 2003a).

Pathogens and invasive alien weeds

The fungal pathogens associated with the pantropical weeds *Chromolaena odorata* (L.) King & Robinson, *Lantana camara* L., and *Mikania micrantha* Kunth, have been documented in both their native and exotic ranges (Barreto & Evans 1994, 1995, Barreto *et al.* 1995). In all cases there were significant qualitative and quantitative differences in the mycobiotas between the exotic palaeotropic and the native neotropical ranges and, for both *C. odorata* and *L. camara*, more pathogens were recorded from the Old World, whilst only a few species were common to both situations (Table 1). In contrast to the fungi recorded from the exotic range, which comprise a heterogeneous assemblage of generalist opportunistic pathogens, those from the Neotropics are more specialized, often obligate biotrophs, which led Evans (1995) to conclude that: "This is a clear indication of the continuing isolation of the mycobiotas and provides evidence for the role of fungal pathogens in the natural control of weed populations because many of these plants are non-weedy or of minor importance in their centres of origin, and further strengthens the case for adopting classical biological control as a weed management strategy".

Table 1. Comparison of fungal pathogens recorded from native and exotic ranges of some major invasive weeds.

Weeds	No. of fungal species[a]		No. of species in common
	Neotropics (Native)	Palaeotropics (Exotic)	
Chromolaena odorata	17	21	4
Lantana camara	26	32[b]	6
Mikania micrantha	32	25	6

[a] Source: Barreto & Evans 1994, 1995, Barreto *et al.* 1995, Evans 1995, Sreenivasan & Sankaran 2001, Herb. IMI records.

[b] Although Mukerji & Juneja (1975) listed 30 new fungal records from this host in India, these were associated with dead or moribund tissues and such opportunists are not included here.

Recently, a similar but much larger analysis of the fungal pathogens recorded on invasive alien weeds has been carried out in the USA, comparing those found in both the exotic and native ranges (Mitchell & Power 2003). Significantly more specialized biotrophs were reported in the areas of origin, prompting the authors to conclude that the enemy release theory, or classical biological control as it is more usually known, offers a viable management strategy for invasive alien weeds.

Interpreting pathogenicity

Hokkanen (1985) argued that many pathogens are capable of infecting new hosts because specific host defence mechanisms have not developed in such new encounters. Whilst this may be true for the opportunistic facultative pathogens listed by Hokkanen (1985) on exotic tropical crops, and those recorded from many invasive weeds (Evans 1987, 1995), the biotrophic plant pathogens traditionally exploited as classical biocontrol agents have a much more complex gene-for-gene relationship with their coevolved hosts. Pathogenicity initially involves a parasitic phase which bypasses host defence mechanisms and is maintained by the presence of specific stimulators in the susceptible host. Such obligate pathogens are thus intimately linked in with their hosts and, as a consequence, these generally have a limited and stable host range. However, some perplexing new associations are now presented which raise questions about host specificity and host range extension.

Lantana rusts

Two rust species, *Prospodium tuberculatum* (Speg.) Arthur and *Puccinia lantanae* Farl., occur on *Lantana camara* throughout its native neotropical range (Barreto *et al.* 1995). *Prospodium tuberculatum* has been tested extensively against a range of *Lantana* species and biotypes and a strain from Minas Gerais (Brazil) which attacks several major invasive biotypes of *L. camara* in Australia has now been released in Queensland (Evans 2002b). A strain of *Puccinia lantanae,* which is especially common in the humid Neotropics, from the Amazonian region of Peru proved to be more damaging to a greater range of biotypes within the *L. camara* complex than *P. tuberculatum.* However, when these rust species were screened against an invasive *L. camara* biotype from the Galápagos Islands, as well as an endemic species (*L. pedicellaris*), the results were not as predicted. Thus, whilst the broader host range *P. lantanae* strain fully infected *L. camara*, there were no symptoms on the native species. Not surprisingly, the narrower host range *P. tuberculatum* strain failed to infect the particular *L. camara* biotype, but in an unexpected development, rust pustules formed in abundance on the leaves of *L. pedicellaris,* although spore density was significantly lower than on susceptible *L. camara* biotypes. This could, therefore, be interpreted to be an example of a new encounter, with the island species having evolved in isolation and with limited defence mechanisms against the *Prospodium* rust. Nevertheless, such an interpretation, in accordance with that proposed by Hokkanen & Pimentel (1984) and Hokkanen (1985), is simplistic since *L. pedicellaris* should also be susceptible to the broader host range *P. lantanae.* We can only conclude that *L. pedicellaris* evolved from a Central American *Lantana* species which was vectored to the islands by birds, since *P. tuberculatum* has been recorded on a range of *Lantana* species from that region (Cummins 1940, Léon-Gallegos & Cummins 1981).

Puccinia lagenophorae Cooke

This rust appears to be the only recognised example of a "successful" new encounter of a biotrophic pathogen in weed biocontrol, although, ironically, this was not the result of a deliberate classical release (Evans & Ellison 1990). The first record of *Puccinia lagenophorae* in the UK was in 1961 on groundsel (*Senecio vulgaris* L.) from a locality in southern England. However, by the end of 1964, the rust had spread to most counties in the UK, as well as in Eire (Wilson & Walshaw 1965). The latter authors concluded that it was morphologically indistinguishable from Australian rusts belonging to the *P. lagenophorae* "group", which has been recorded on a number of genera of Asteraceae in that country. This rust has now become an important regulator of groundsel populations in both the UK and Europe (Paul & Ayres 1986, Muller-Scharer & Rieger 1998). However, although alien *Senecio* species, such as *S. madagascariensis* Poir. (fireweed), are hosts of *P. lagenophorae* in Australia, the impact of the rust has not been sufficient to prevent them becoming weedy invasives (Parsons & Cuthbertson 1992). *Puccinia lagenophorae* has also been found on *S. madagascariensis* in its somewhat restricted native range in southern Madagascar (H.C. Evans, pers. obs.), where it appeared to be impacting severely on the fireweed populations. It appears, therefore, that the *P. lagenophorae* story presents evidence both for and against the new encounter hypothesis. The situation in Europe suggests that the exotic rust is a potent biocontrol agent of its new groundsel host, whilst the purportedly same rust in Australia has proven to be ineffective against invasive *Senecio* species. Clearly, an in-depth investigation, including molecular, morphological and cross-infectivity studies, is required to determine if distinct species and/or pathotypes occur within this rust complex and which should also provide evidence for or against the new encounter hypothesis.

Discussion

The evidence to support the new encounter concept is still fragmentary, and most examples tend to confirm that the classical approach using coevolved natural enemies should remain the priority strategy for the management of invasive alien weeds. Indeed, the evolutionary ecological reasoning put forward by Hokkanen & Pimentel (1984) and Hokkanen (1985) to support their hypothesis, specifically that relating to interspecific homeostasis, is, at least for fungal pathogens, somewhat naïve and misleading. In the case of cocoa and rubber for example, the hosts and pathogens do achieve a natural balance within the forest ecosystem (H.C. Evans, pers. obs.). This is due to a combination of low host density, high pressure from natural enemies of the natural enemies ("...little fleas have lesser fleas..."), such as hyperparasites, and complex physiological inter-relationships when these host plants are moved from buffered forest ecosystems. In agricultural situations, with high host density, increased host vigour and low hyperparasitism, coevolved fungi literally undergo a population explosion with catastrophic impacts on the host crop. This explains why biotrophic fungi such as rusts and smuts have proven to be successful biocontrol agents of invasive alien weeds, and why the hemibiotrophic pathogens of both cocoa and rubber have decimated plantations wherever they have caught-up with their coevolved hosts (Evans 2002a).

A closer examination of the fungal pathogens listed by Hokkanen (1985) on tropical crop plants demonstrates a similar simplistic interpretation of the data. Although many "new encounter" pathogens are documented on these crops in their exotic ranges, as for example in coffee where more than 60% of the total are pathogens from the Neotropics. The most damaging ones are those biotrophs or hemibiotrophs from the native range in the Old World. Thus, coffee leaf rust (*Hemileia vastatrix* Berk. & Broome) and coffee leaf disease (*Colletotrichum kahawae* Waller & Bridge, formerly *C. coffeanum*) from Africa are the most feared diseases; whilst coffee berry borer, *Hypothenemus hampei* (Ferrari), also from Africa, is the most serious insect pest (Flood *et al.* 2001, Cadena & Baker 2001). Similarly, although cassava has a number of new host–pathogen associations in Africa, these are not serious constraints to production, unlike the very real and potential threats from neotropical pathogens (Cassava Mosaic Virus), as well as from coevolved arthropods (*Phenacoccus manihoti* Matile-Ferrero, cassava mealybug and *Mononychellus tanajoa* [Bondar], cassava mite), from South America (Lyon 1973, Greathead 1995).

Undoubtedly, there are examples of destructive new encounter pathogens and several invasive *Phytophthora* species with eclectic host ranges and high virulence have the capacity to alter whole ecosystems, notably *P. cinnamomi* Rands in Australia and *P. ramorum* Werres, De Cock Man Veld in the USA (Weste & Marks 1987, Rizzo *et al.* 2002). However, there is no situation in which such generalist pathogens would ever be considered as classical biocontrol agents. There are several rust species that also have become accidentally invasive and have extended their host ranges. Nevertheless, in the case of *Puccinia lagenophorae,* the species was known to have a wide host range within the Australian Asteraceae so perhaps it was not surprising that new host–pathogen associations would have arisen. However, in such unpredictable associations there are anomalies: *Senecio vulgaris* proving to be highly susceptible and the closely-related *S. jacobaea* L. remaining immune.

In summary, therefore, we concur with the statement by Mitchell & Power (2003), who analysed the *biotrophic* pathogens of over 470 invasive alien weeds in the USA, and concluded that: "Noxiousness increased

with greater pathogen escape, implying that pathogens limit plant populations in their native range and supporting the idea that classical biocontrol can mitigate the costs of noxious weeds". However, there does appear to be a case for exploiting new encounter associations in genus-specific natural enemies. This is particularly suitable for arthropods, which tend to have less stringent specificity requirements than pathogens; although it is essential, of course, that members of that genus do not occur in the exotic range of the target weed.

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Phytomyza vitalbae, *Phoma clematidina*, and insect–plant pathogen interactions in the biological control of weeds

R.L. Hill,[1] S.V. Fowler,[2] R. Wittenberg,[3] J. Barton,[2,5] S. Casonato,[2] A.H. Gourlay[4] and C. Winks[2]

Summary

Field observations suggested that the introduced agromyzid fly *Phytomyza vitalbae* facilitated the performance of the coelomycete fungal pathogen *Phoma clematidina* introduced to control *Clematis vitalba* in New Zealand. However, when this was tested in a manipulative experiment, the observed effects could not be reproduced. Conidia did not survive well when sprayed onto flies, flies did not easily transmit the fungus to *C. vitalba* leaves, and the incidence of infection spots was not related to the density of feeding punctures in leaves. Although no synergistic effects were demonstrated in this case, insect–pathogen interactions, especially those mediated through the host plant, are important to many facets of biological control practice. This is discussed with reference to recent literature.

Keywords: *Clematis vitalba*, insect–plant pathogen interactions, *Phoma clematidina*, *Phytomyza vitalbae*, tripartite interactions.

Introduction

Biological control of weeds is based on the sure knowledge that both pathogens and herbivores can influence the fitness of plants and depress plant populations (McFadyen 1998). We seek suites of control agents that have combined effects that are greater than those of the agents acting alone (Harris 1984). However, recent research suggests that predicting which combinations of agents are likely to generate that effect is difficult, and may be misleading. This is particularly true for interactions between insects and plant pathogens, because entomologists and pathologists tend to work exclusively in their own discipline (Agrios 1980, Connor 1995, Caesar 2000).

Hatcher & Paul (2001) have succinctly reviewed the field of plant pathogen–herbivore interactions. Simple, direct interactions between plant pathogens and insects (such as mycophagy and disease transmission) are well understood (Agrios 1980), as are the direct effects of insects and plant pathogens on plant performance. Very few fungi are dependent on insects for the transmission of their spores, but spores transmitted by insects have a greater chance of reaching a suitable site compared with spores dispersed by water and wind (de Nooij 1988). The reciprocal effects of plants on pathogens and insects through such mechanisms as wound responses, induced resistance, systemic acquired resistance, and hypersensitive reactions are acknowledged, if imperfectly understood (Zidack 1999). However, the potential indirect effects of plant pathogens (especially biotrophs) on insects (and vice versa) mediated through the host plant are often cryptic, poorly understood, but common. Hatcher (1995) identified a range of possible outcomes for such tripartite relationships, and these have considerable relevance for future biological control practice.

This paper describes a manipulative experiment designed to examine some of the interactions between

[1] Richard Hill & Associates, Private Bag 4704, Christchurch, New Zealand.
[2] Landcare Research, Private Bag 92170, Auckland, New Zealand.
[3] CABI Bioscience Switzerland Centre, 1 Rue des Grillons, CH-2800 Delémont, Switzerland.
[4] Landcare Research, PO Box 69, Christchurch, New Zealand.
[5] Present address: 353 Pungarehu Rd, RD5, Te Kuiti, New Zealand.
Corresponding author: R. Hill, Richard Hill & Associates Ltd, Private Bag 4704, Christchurch, New Zealand <hillr@crop.cri.nz>.

two biological control agents introduced to New Zealand to attack the invasive weed *Clematis vitalba* L. (old man's beard). It also explores the importance of plant pathogen–herbivore relationships to the future practice of biological control of weeds.

Material and methods

The hypothesis

Clematis vitalba (Ranunculaceae) grows throughout central and southern Europe, and extends as far as the Caucasus. It was introduced to New Zealand as an ornamental before 1920, and is now naturalized throughout. Vines can climb tall forest trees, forming a dense light-absorbing canopy that suppresses vegetation beneath it. These can become large enough to pull down trees, and also scramble over the ground, suppressing regeneration. Infestations threaten the existence of small forest remnants, and create a nuisance in many other habitats (Hill *et al.* 2001).

The old man's beard leaf-mining fly *Phytomyza vitalbae* Kaltenbach (Diptera: Agromyzidae) and the fungus *Phoma clematidina* (Thümen) Boerema were introduced in 1996 (Gourlay *et al.* 2000). Both agents established and spread quickly (Hill *et al.* 2001). The speed with which *P. clematidina* dispersed within New Zealand and the co-occurrence of the two agents at new sites suggested that the fungus was carried from place to place by the fly. Before *P. clematidina* was introduced, *P. vitalbae* leaf-mines were usually brown. Following establishment of the fungus, leaf mines were usually black. Although the cause of the discoloration was never formally identified, it was suspected that the new fungus was invading mines. These were commonly surrounded by a yellow halo, a common symptom of infection by plant pathogens (Agrios 1988), suggesting that fungal invasion of leaves could occur from within mines. These observations raised the possibility that the two agents were synergistic in their effects on old man's beard leaves.

This hypothesis was reinforced when it was shown that newly emerged flies were capable of transferring *P. clematidina* by walking on a culture on an agar plate and transmitting it to a fresh plate. While short-range transport of the spores was therefore feasible, adult flies appeared to actively avoid the black *Phoma*-infected parts of leaves. The frequency with which flies transmitted the fungus between plants remained unclear (R. Wittenberg, unpublished data). The larvae of *P. vitalbae* produce characteristic mines, but adults can also damage leaflets. Female flies pierce the leaf surface using the ovipositor, and then feed on leaf exudates. Hundreds of feeding punctures can be made in a leaflet, and eggs are laid in just a few of these (R.L. Hill, J. Fröhlich, A.H. Gourlay & C. Winks, unpublished data). Our hypothesis was that feeding punctures formed by adult flies provided a point of entry for the necrotrophic fungus to infect the leaf and/or the mines, either directly via the ovipositor, or indirectly by the fungus invading the wounds. If so, then the damage to the leaf was likely to be greater than if either agent was working alone – an additive or a synergistic effect. To examine the relationship more closely, we designed an experiment to investigate whether the fly was facilitating the performance of the fungus. The aims were to determine:

1. how long *P. clematidina* conidia survived on the bodies of adult flies
2. whether adult flies introduced the fungus into the leaf through penetration by the ovipositor
3. whether feeding punctures on leaves facilitated invasion by water-borne inoculum of *P. clematidina*.

Methods

Clematis vitalba plants were obtained from two sources. Several hundred seedlings, 5–10 cm tall, were dug from beneath a single *C. vitalba* plant at Kaituna Valley, mid-Canterbury, in mid-December 1999, and were replanted in planter bags (PB3.5). Plants were placed in a shade house, and were ready for use 6 weeks later (batch 1). At this stage, plants bore one pair of fully formed leaves (each with five leaflets), and a second pair of leaves was developing. At the same time, seeds collected from a plant at Lincoln Golf Course, mid-Canterbury, in the previous spring were sown in a seed tray. In late January 2000, seedlings were potted as described above, and were ready for use 6 weeks later (batch 2).

Preliminary experiments established that treatment with 0.5% sodium hypochlorite (NaOCl) successfully stopped infection of *C. vitalba* leaves by *P. clematidina*, but did not prevent infection when conidia were later applied to surface-sterilized leaves. The culture of *P. clematidina* used in these experiments was a subculture of an isolate originally collected from *Clematis ligusticifolius* Nutt. in 1991 in the USA and subsequently released in New Zealand as a biological control agent for *C. vitalba* (A. Spiers, unpublished HortResearch client report 1995). Inoculum was prepared from 15-day-old cultures, grown on 15% V8 agar (made with V8 juice clarified with calcium carbonate) in 9 cm Petri dishes, and incubated at 20°C under white lights with a 12 h photoperiod. A spore suspension was prepared by initially adding 3 mL of sterile distilled water (SDW) to one plate, dislodging spores with a sterile glass rod, filtering through a sterile cell strainer (Falcon, 70 µm nylon, Becton Dickinson, USA), and adding the filtrate to 97 mL of SDW. Conidial density was estimated using a haemocytometer, and the suspension was used to harvest conidia from additional plates until an adequate conidial density was obtained. Suspensions were prepared on three separate occasions.

Survival of conidia on flies

Fly pupae of even age were collected from the general culture, soaked in 0.5% NaOCl for 15 min to kill any

Phoma spores, washed and dried. Flies were allowed to emerge for 48 h in two boxes (200 × 200 × 200 mm) containing a small surface-sterilized sprig of *C. vitalba* as food. One box was then misted with 25 mL of a suspension of *P. clematidina* (1×10^6 conidia mL^{-1}), using a de Vilbiss atomizer attached to an air line, and the other was misted with sterile water. Boxes were placed in ambient laboratory conditions for the following 72 h.

Within 60 min of spraying with the conidial suspension, or with water, 10 flies from each treatment were individually captured in clean glass tubes and killed by narcotizing with CO_2. In a laminar flow cabinet, and using sterile techniques, flies were wiped onto potato dextrose agar (Difco Labs, USA) amended with 0.02% streptomycin (Sigma, USA), contained in 9 cm Petri dishes, one fly in each of four quadrants. Plates were incubated at 20°C and 12 h photoperiod. After 10 days, promising cultures were transferred to 15% V8 agar to allow identification of *P. clematidina* colonies. A further 10 flies were assessed 24 h after conidia were applied, and the remaining five flies were assessed 72 h after application (four sprayed with SDW, and one with the conidial suspension).

Plant-to-plant transmission of Phoma clematidina *by* Phytomyza vitalbae

Eleven *C. vitalba* plants (batch 2) were selected for medium size, and convenient leaf size. Four days prior to experimentation, plants were surface-sterilized with 0.5% NaOCl. On each plant, two leaves (each bearing five leaflets) were selected. Three basal leaflets were enclosed in clip cages. The clip cages were made from 5 cm Petri dishes, and had a 3 cm diameter panel of fine steel gauze inserted in one face and a cotton-wool-stoppered hole in the other face. A hole in the edge of the closed cage accommodated the petiole of the leaflet, and gaps around the petiole were plugged with cotton wool.

Fly pupae of even age were collected from the general culture, soaked in 0.5% NaOCL for 15 min to kill any *Phoma* spores on the puparium, washed and dried. Flies were allowed to emerge for 48 h in two boxes (200 × 200 × 200 mm) containing a small surface-sterilized sprig of *C. vitalba* as food. One box was misted with a suspension of *P. clematidina* spores (1×10^6 conidia mL^{-1}) using a de Vilbiss atomizer attached to an air line, and the other box was misted with sterile water.

Flies were transferred to plants 60 min after application of conidial suspension. Large flies (presumed to be females) were captured individually in clean glass tubes (5 × 1 cm). Flies were briefly narcotised with CO_2, and transferred to clip cages through the stoppered hole. Clip cages had been randomly assigned to three treatments (no flies, flies sprayed with *Phoma* spores, and flies sprayed with water), with one set of clip cages per leaf, two leaves per plant and a total of 11 plants. One leaflet on each plant was painted with the same spore suspension to monitor the susceptibility of each plant to *P. clematidina*. After application of spores, leaflets were immediately enclosed in a zip-lock plastic bag (5 × 8 cm) to maximize the likelihood of infection. After 40 h, flies, clip cages and plastic bags were removed from leaflets. Plants were misted with sterile water, and placed haphazardly in two closed acrylic plastic boxes for 24 h at 20–23°C. The box was removed, and plants were maintained at approximately 70% relative humidity and 20–23°C under lights for 48 h. Leaflets were harvested, and the number of feeding punctures present on each leaflet was counted. Leaflets were placed singly in Petri dishes on moist filter paper. These were incubated at 20°C under lights at 12 h photoperiod for 2 weeks to induce sporulation (A. Spiers, pers. comm.). Leaflets turned black and developed many fungal colonies. These were examined microscopically to confirm the presence or absence of *P. clematidina* on each leaflet.

The effect of Phytomyza vitalbae *feeding punctures on the infection rate of* Phoma clematidina

Flies less than 2 days old can pierce the epidermis of leaves to feed, but cannot oviposit. All flies were therefore extracted from the bulk culture at 2-day intervals to ensure that no flies exceeded this age. For the 1-day-old damage treatment, insufficient young flies were available, and mixed-age flies from another general culture were used instead. The experiment was evaluated before eggs laid by these flies could hatch and produce mines.

Eighteen plants (batch 1) were selected haphazardly. Twelve were placed in individual acrylic plastic boxes (500 × 300 × 300 mm or 600 × 300 × 300 mm) in a temperature-controlled room set at 19–21°C with a 16 h photoperiod. Five assumed pairs of *P. vitalbae* (five large flies and five small flies) were added to each box. The remaining six plants were placed in a single box and no flies were added. Flies were removed after 24 h (day 1). On each of the 12 plants exposed to flies, five damaged leaflets were selected and marked (where possible one leaflet per leaf), and the number of feeding punctures was recorded. In some cases, fewer than five leaflets on the plant were damaged. In this case, all damaged leaflets were labelled. We also labelled five leaflets on each of the six plants that were not exposed to flies. Plants were returned to the shade-house and positioned haphazardly. Three further sets of 18 plants were treated for 24 h using the same technique beginning on days 2, 4 and 6. Thus, after 8 days, plants bearing 7-, 5-, 3- and 1-day-old fly damage had been produced. On day 8, six damaged plants from each treatment were sprayed to run-off with sterile water (10–15 mL per plant). The remaining six damaged and the six undamaged plants from each treatment were sprayed to run-off with a suspension of *P. clematidina* conidia that was adjusted to 1.5×10^4 conidia mL^{-1} (10–15 mL per plant). Three further untreated plants were taken from the shade-house, leaflets were marked,

and the plants were sprayed with sterile water. Each plant was covered with a tall plastic cylinder (200 mm diameter × 300 mm) to ensure free moisture remained on the leaves, and plants were haphazardly placed on a bench in a temperature-controlled room set at 19–21°C.

After 18 h, covers were removed from all 75 plants, and the temperature was reduced to a constant 15°C. After 7 days, marked leaflets were removed from the plants, examined at 10× magnification using transmitted light, and the number of infection sites present (each identified as a dark lesion with a yellow halo) was recorded. The leaflet opposite the marked leaflet (or if this was damaged, the nearest undamaged leaflet on the same leaf) was also removed and assessed.

The data were analyzed by fitting linear mixed-effects models in S-Plus 2000 using function l me and maximum likelihood estimation. The number of spots was taken as the dependent variable, with models including fixed effects for age of feeding damage (1, 3, 5 or 7 days), treatment (flies+water, flies+phoma, no flies+phoma) and damage (damaged leaflet or undamaged leaflet), plus all possible interactions. Plant (1 to 11) and leaf (1 or 2) were included as random effects. Fixed effects were tested by comparing nested models using likelihood ratio tests. The dependent variable was square-root transformed prior to analysis to help satisfy model assumptions.

Results

Survival of conidia on flies

No *P. clematidina* colonies were isolated from flies sprayed with water alone. Of the 10 flies plated immediately after conidia were applied, only three yielded *P. clematidina* colonies. No *P. clematidina* colonies were obtained from the 10 flies treated with conidia and plated after 24 h, or the one plated after 72 h.

Plant-to-plant transmission of *Phoma clematidina* by *Phytomyza vitalbae*

Of the 11 control leaflets painted with the conidial suspension, 9 survived to be assessed. Seven of these developed abundant *P. clematidina* infection spots, indicating that the plants used were susceptible to this fungal isolate. These 7 plants bore 14 sets of leaflets that could be assessed reliably. None of the leaflets to which no flies were added, or flies sprayed with water were added, developed *P. clematidina* infection. Of the 14 leaflets to which flies sprayed with the conidial suspension were added, 4 had no feeding damage, possibly because the flies added were males, or because flies died prematurely. Omitting these, and omitting those plants in which susceptibility to the isolate could not be proven, 10 replicates remained. Only one of these (10%) developed *P. clematidina* infection. This leaflet carried 510 *P. vitalbae* feeding punctures, the second-most damaged of all of the leaflets.

Effect of *Phytomyza vitalbae* feeding punctures on the infection rate of *Phoma clematidina*

Few infection spots appeared on leaves not sprayed with *P. clematidina* (Table 1), and these were probably attributable to other micro-organisms. There was no feeding damage on leaves not exposed to *P. vitalbae*. Leaf infection spots typical of *P. clematidina* were observed on leaves sprayed with the conidial suspension, whether damaged by adult flies or not (Table 1), indicating that the conidial suspension was capable of inducing disease symptoms.

Microscopic examination of the leaves revealed that infection spots occurred apparently randomly across the leaf surface. Fungal invasion of the leaf lamina appeared to be independent of the position of feeding punctures, and there was no evidence of invasion of feeding puncture margins by *P. clematidina*.

Table 1. Mean number (±SE) of *Phoma clematidina* infection spots observed per leaflet when *Clematis vitalba* seedlings were exposed to (1) both *Phytomyza vitalbae* adult feeding damage of different ages and *P. clematidina*, (2) *P. vitalbae* alone, (3) *P. clematidina* alone. Control leaflets were not exposed to feeding damage by *P. vitalbae* adults.

	Phytomyza and *Phoma*			*Phytomyza* and water			*Phoma* alone		
	Feeding punctures	Spots on damaged leaflets	Spots on control leaflets	Feeding punctures	Spots on damaged leaflets	Spots on control leaflets	Feeding punctures	Spots on undamaged leaflets	Spots on control leaflets
7-day-old damage	81.2 ± 12.2 n = 25	1.5 ± 0.4 n = 25	2.0 ± 0.9 n = 25	79.5 ± 20.6 n = 17	0.06 ± 0.06 n = 17	0.06 ± 0.06 n = 17	0 n = 30	3.2 ± 1.0 n = 30	2.9 ± 1.1 n = 30
5-day-old damage	86.2 ± 12.2 n = 25	7.1 ± 1.7 n = 25	6.2 ± 2.3 n = 25	87.4 ± 10.6 n = 25	0.08 ± 0.08 n = 25	0 n = 25	0 n = 30	2.5 ± 0.8 n = 30	2.9 ± 0.9 n = 30
3-day-old damage	54.1 ± 10.1 n = 28	5.5 ± 1.5 n = 28	5.0 ± 2.1 n = 28	48.0 ± 7.0 n = 24	0.7 ± 0.4 n = 24	0.8 ± 0.7 n = 24	0 n = 30	2.6 ± 0.8 n = 30	4.4 ± 1.3 n = 30
1-day-old damage	121.8 ± 13.1 n = 25	7.8 ± 2.1 n = 25	15.0 ± 3.5 n = 25	315.4 ± 26.0 n = 12	0 n = 12	0.6 ± 0.3 n = 12	0 n = 30	12.9 ± 3.8 n = 30	13.3 ± 3.4 n = 30

As no damage was possible on plants not exposed to flies, observations from plants treated with 'no flies+phoma' were omitted from models that included damage as an effect. Comparing the model with damage (and its interactions with other factors) with the model with day and treatment effects only shows no evidence that numbers of spots differed between damaged and undamaged leaflets (χ^2_8 = 9.92, P = 0.271). This suggests that the presence of punctures does not improve the chance of infection. Replacing the factor damage with the number of punctures gave similar results, with no evidence that greater numbers of punctures lead to more spots (χ^2_8 = 13.0, P = 0.111)

To test for treatment and age of damage effects we included all observations. There was very strong evidence that number of spots differed between treatments, and that the size of these differences depended on the number of days since flies were put in the boxes (χ^2_6 = 38.3, P < 0.0001). Numbers of spots were consistently higher on the plants treated with *P. clematidina* than on the water-treated plants, on which few spots were found. For plants treated with *P. clematidina*, mean spot numbers were not significantly different between the with- and without-fly treatments for day = 1, 3 or 7 (P > 0.2 for all three days). However, spot counts were significantly lower for the "no flies + *Phoma*" treatment than the "flies + *Phoma*" treatment on day = 5 (P = 0.004).

Discussion

Even though flies were treated with a dense suspension of *P. clematidina* conidia, and were rolled onto a substrate conducive to spore germination, the fungus could only be isolated from flies for 60 min after treatment. Even then, only three of the 10 flies tested yielded colonies. It is not known if this apparently low infectivity is a result of preening by adult flies, death of conidia on flies, or a methodological difficulty in recovering the fungus, but the results suggest that transport of conidia between plants by flies does not have a high probability of success. If conidia are as short-lived as this experiment suggests, then long-distance transport is particularly unlikely.

Similarly, adult flies sprayed with a dense suspension of *P. clematidina* conidia showed limited ability to transmit the disease directly to a leaf through adult feeding or oviposition. Many of the treated leaves could not be scored due to lack of leaf damage or lack of infection in positive controls, but only one of the 11 remaining replicates developed disease symptoms. Again, the probability of flies contributing significantly to the incidence of disease appears low. However, this frequency of facilitation may be sufficient to explain the field observations of *P. clematidina* invading leaves from mines.

The third experiment sought to determine whether feeding punctures created by *P. vitalbae* predisposed leaflets to infection by waterborne spores of *P. clematidina*. Haloes were sometimes observed around feeding punctures but these did not develop disease symptoms. Portions of leaves that were heavily punctured occasionally shrivelled and turned black, but this was never associated with typical disease symptoms or with a halo around the necrosis. In all of the leaflets examined, there were no cases where black infection sites were associated with feeding punctures. Invasion seemed to occur successfully in the absence of flies, directly through the leaf surface, often at depressions in the leaf or petiole where free water might accumulate. Given these observations, it is not surprising that statistical analysis was unable to detect any significant relationship between the number of feeding punctures per leaflet and the number of infection spots present, irrespective of age. There was a slight indication in the 1-day-old damaged plants that heavy adult feeding might reduce infection by *P. clematidina.* If this is true, the mechanism may be mechanical, as feeding of this intensity reduced the amount of leaf lamina available for fungal invasion, or it may be a resistance response. These three experiments provide complementary evidence that if there is any behavioural synergy between these agents, it is minor. Infection by pathogens does not necessarily cause disease every time, in all plant parts, or in all plant ages (Barbosa 1991). The amount of disease could also be dependent on the age of the plant and/or the leaf age (Barbosa 1991). This may have been a possible cause for the lack of visible infection noted around the wounding sites in *C. vitalba.*

The concept of three-way complex interactions between plants, plant pathogens and insects is well established, and is depicted simplistically in Figure 1. Interactions between insects and fungi can be direct (mycophagy, spore dispersal), as can interactions between plants and either insects or fungi (e.g. infection, phytophagy, defoliation, plant resistance to insects, plant resistance to fungi). However, the presence of insect damage can influence the performance of fungi (and vice versa) indirectly through host-plant responses. Of particular importance for biological control is the concept of cross-resistance, where resistance to pathogen infection induced in the host plant by a pathogen can confer incidental resistance to a herbivore (and vice versa). The presence of insects or pathogens can also alter nutrient fluxes within the plant, and these can influence the performance of other organisms either positively or negatively. The array of possible outcomes from indirect fungus–insect interactions range from synergistic effects, where the impact on a plant variable is significantly greater than that obtained from either species alone, to inhibitory, where a plant variable is affected less than by the weaker of the two agents alone (Hatcher 1995). Hatcher & Paul (2001) provide a range of examples relevant to biological control of weeds that demonstrate these effects. Small-scale experiments to assess the interactions

between *Rumex* spp., the rust *Uromyces rumicis,* and the leaf-feeding chrysomelid beetle *Gastrophysa viridula* indicated reciprocal negative effects between the insect and the pathogen. It was predicted that the combined effects over the life of the plant could be inhibitory. However, in longer trials, effects varied, ranging from inhibitory in one case, to additive in another. Explanations for this variation included behavioural changes by the beetle to select plant material that was not infected, and increased consumption by the beetle in response to reduced foliage quality (see Hatcher & Paul 2001). Hatcher & Paul (2000) have also shown that the impact of *G. viridula* on infection of *Rumex obtusifolia* can be systemic, conferring protection from fungal attack on leaves not attacked by the beetle.

Hatcher & Paul (2001) provide other examples relevant to biological control of weeds that demonstrate the real but complex nature of these interactions. For example, the effect of the weevil *Perapion antiquum* and the fungus *Phomopsis emicis* on the accumulated dry weight of *Emex australis* was equivalent to one of the agents working alone, but the effect on stem length and fruit weight was inhibitory. In another case, the combined effect of three beetles with the rust *Puccinia carduorum* on the performance of *Carduus thoermeri* was considered to be universally positive.

There are many other examples available in the literature. For example, de Nooij (1988) showed that the weevil *Ceutorhynchidius troglodytes* provided an entry wound for the pathogenic fungus *Phomopsis subordinaria* in the plant tissue of *Plantago lanceolata*. The weevils were indispensable for the infection process to occur, with no infection occurring in the absence of the weevil. However, wounding of the stalk did not always result in penetration of the pathogen. In another example, Connor *et al.* (2000) found that there were no significant combined effects of *Platyrepia virginalis* and the fungus *Phoma pomorum* on *Cynoglossum officinale* (houndstongue) in laboratory studies, and that larvae appeared to avoid damaged leaves. On the other hand, Teshler *et al.* (1996) proposed a synergistic interaction between an insect and pathogen feeding on *Ambrosia artemisiifolia.*

Effects are not restricted to root (Caesar 2000) or foliage organisms. In the field, the gall wasp *Dryocosmus dubiosus* experiences significant mortality due to a fungus. Galls with heavy fungal infection generally did not contain living larvae compared with galls without the fungus (Taper *et al.* 1986). Similar studies have begun to examine the tripartite interaction between the white smut *Entyloma ageratinae*, the gall fly *Procecidochares alani*, and the weed *Ageratina riparia* (mist flower) (S. Casonato, unpublished data, Fröhlich *et al.* 2000).

One system that Hatcher & Paul (2001) did not review is the recent research into the relationships between the thistle *Cirsium arvense*, the biotrophic rust *Puccinia punctiformis*, and the insect fauna that attacks the thistle in Europe. Friedli & Bacher (2001a,b) claimed a mutualistic interaction between *Apion onopordi* (Curculionidae) and *P. punctiformis* on *C. arvense*. The weevil benefited the rust fungus by transmitting urediniospores in the process of oviposition, increasing the incidence of rust-infected stems in the following year. The rust benefited the weevil because adults emerging from rust-infected stems were significantly larger than those developing in healthy stems. Bacher *et al.* (2002) have expanded this research. However, this mutually positive relationship does not hold with all insects that feed on *C. arvense*. Kluth *et al.* (2001) found that while larvae of *A. onopordi* were more abundant in infected stems, several other endophages preferred uninfected stems. The incidence of

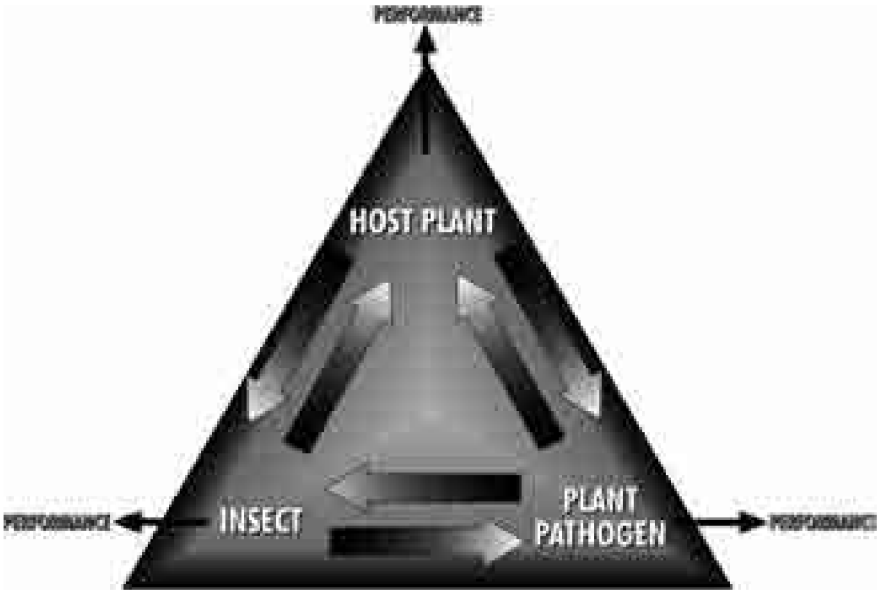

Figure 1. The tripartite relationship between insects, plant pathogens and their host plants.

ectophages on stems appeared unaffected by rust infection. However, on the same host plant, Kruess (2002) showed that the chrysomelid beetle *Cassida rubiginosa* consumed more, developed faster, survived better and was larger when fed on healthy leaves rather than leaves from plants systemically infected by the necrotrophic fungus *Phoma destructiva*. In thistle populations where both pathogens are present, the potential interactions are likely to be complex and variable (see also Kok & Abad 1994).

The complexity of interactions presented here, and by Hatcher & Paul (2001), shows that insects can change plant conditions to the advantage or detriment of fungi, and vice versa (Carruthers *et al.* 1986). As a result, the impact on host-plant performance can range from synergistic to inhibitory. In fact, real-world situations would include plant–plant interactions such as competition and environmental variables that can induce plant stress (such as drought and soil type), and pathogen–pathogen and insect–insect interactions. Even with pathogen–insect interactions that are synergistic, the effect of the interaction is dependent on various circumstances and may be reliant on the biocontrol agents "attacking" the plant at critical times. It is clear that the host plant should be taken into account when considering insect–fungal interactions. However, Hatcher & Paul (2001) observed that while experimental studies of plant pathogen–insect interactions exist, field studies that might shed light on such complex interactions are rare.

What does this mean for day-to-day practice of biological control of weeds? There have been repeated calls for pathologists and entomologists to work together to gain a better understanding of the nature of relationships and how they can be used to improve levels of control (e.g. Cullen 1996, Caesar 2000, Kremer 2000). However, plant pathogen–insect interactions have relevance in almost every stage of the biocontrol process, not just efficacy.

The legislation under which biological control agents are introduced into New Zealand requires the importer to identify and assess all reasonable and foreseeable risks associated with the proposed control agent (A. Sheppard, unpublished data). As tripartite relationships become more widely known, it is likely that risk assessment of such potential interactions will be required, however difficult or impractical that might be.

Wilding conifers are becoming a major threat to environmental and ecological values in southern hemisphere countries. Biological control of cones and seeds seems to be a logical approach to reducing the rate of spread, but cone-feeding insects may spread the devastating pine pitch canker, *Fusarium subglutinans* f.sp. *pini* (Hoover *et al.* 1996). Assessment of the risk posed by the introduction of new cone-feeding insects is under way in South Africa (Moran *et al.* 2000) and is beginning in New Zealand (even though the disease does not exist here).

It is conceivable that host-range testing of control agents in the country of origin could be compromised by plant pathogen–insect interactions. If tests are conducted on test-plant material in which changes have been initiated by fungal infection or insect attack, there is the possibility of overpredicting or underpredicting host range if a strong interaction occurs. Researchers could minimize this risk by ensuring that test material is obtained from plants that are free of fungal or insect damage.

The successful control of *Eichhornia crassipes* in several countries has been enhanced by the infection of insect-damaged plants by indigenous micro-organisms acting as secondary invaders (Charudattan 1986). While the likelihood of such an interaction might have been predicted before release of the introduced herbivores (Hatcher & Paul 2001), the resident organism likely to cause the rots could not. The contribution of indigenous pathogens to the successful control of *Opuntia inermis* in Australia is a similar case (Martin & Dale 2001).

The establishment success of a pathogen or insect could be affected either by unpredicted post-release inhibitory interactions with resident organisms, or by the omission of a necessary relationship from the country of origin. At present, agents are selected for complementary modes of action, minimizing competition for resources, and separating agents spatially and temporally (e.g. Morin *et al.* 1997, Fröhlich *et al.* 2000, Hill & Gourlay 2002). Increased knowledge about the tripartite interactions and the risks of cross-resistance could allow selection of agents that will not interfere with each other on release (Zidack 1999), and there is the prospect of "designing" synergistic combinations of control agents. It may be important to introduce agents in the correct order to maximize the likelihood of establishment. Hence tripartite interaction studies could potentially increase the success of biological control programs by introducing synergistic agents, rather than those that are inhibitory or equivalent (Hatcher 1995). There is also potential to reduce costs by introducing agents that appear to complement each other in their effect on the target weed.

Sheppard (2003), McEvoy & Coombs (1999) and others advocate selection of only those control agents that have demonstrated efficacy in pre-release evaluation in their home range. The predicted efficacy of an agent may be underestimated or overestimated if strong, but cryptic interactions are acting in the country of origin. The corollary is that an agent may behave differently when introduced to a new range without the tripartite relationship that influenced its performance in the home range. If a target weed accumulates pathogens and phytophagous insects in its new range, new tripartite relationships could change the expected performance of the control agent following release.

A long-time tenet of biological control of weeds has been that increasing "cumulative stress" on weeds by

serial introduction of control agents will increase the level of control (Harris 1984). As Hatcher & Paul (2001) observed, "one clear message from studies of pathogen–herbivore interactions is that 'more species' does not necessarily equal 'greater stress' due to potential negative interactions between organisms". On the other hand, studies to date suggest that few tripartite relationships have proven strictly inhibitory. Infection or infestation rates vary between sites, between plants, and even within plants (Hatcher & Paul 2001). Even if an inhibitory interaction between potential control agents did exist, it is likely that the inhibition would only be expressed in part of the potential range of each agent. Hatcher & Paul (2001) have pointed out that there are too few studies available upon which to generalize the importance of tripartite interactions for biological control success. As shown here, this applies to other facets of biological control practice as well. All we know is that a wide range of potential interactions exist. This leaves biological control researchers with a familiar conundrum – whether to invest in detailed research to reveal those tripartite relationships before introduction, or to "suck it and see". There appear to be both future opportunities and risks for biological control of weeds in this under-researched field.

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Can population modelling predict potential impacts of biocontrol? A case study using *Cleopus japonicus* on *Buddleja davidii*

Toni Withers, Brian Richardson, Mark Kimberley, John Moore, Malcolm Kay and Diane Jones[1]

Summary

As weed biological control comes under much closer scrutiny from legislators and risk managers, increasingly we are asked to provide evidence on the potential impacts a biological control agent will exert on the weed we want to control. Invariably this evidence is required well before the potential biocontrol agent can be released from quarantine. When quantitative data cannot be readily accessed from the country of origin, tools such as population modelling become invaluable. From laboratory studies, the biology of *Cleopus japonicus* (Curculionidae) had already been ascertained in relation to temperature and day length. Additional studies were undertaken on the leaf area consumption by larvae and adults. Using these data, we simulated the population dynamics of the weed biocontrol agent as if it was being released in a non-limiting monoculture of its host plant, the weed buddleia (*Buddleja davidii*; Buddlejaceae), in the central North Island of New Zealand. The results are useful for predicting the potential impacts on the weed, the rate of population build-up, and how many generations can be expected per annum in the likely distribution of the agent. The model predicts that only two generations of *Cleopus japonicus* can be expected per year and that overwintering survival is critical to population build-up. Experiments that ascertained the consumption of leaf area by larvae and adults showed that the leaf area index (LAI) for buddleia will be significantly reduced only from mid-summer until mid-winter, leaving the spring flush undamaged. The extent to which population modelling such as this will be utilized and accepted as a predictive tool before the release of weed biological control agents will depend upon the verification of predictions such as these.

Keywords: *Cleopus japonicus, Buddleja davidii*, functional relationships, leaf consumption, modelling impact on plant, population dynamics.

Introduction

There are unique challenges faced when undertaking weed management in plantation forests. Managed forests tend to require intensive weed control during the establishment phase. Often in the first few years following harvest, with its attendant disturbance, and during replanting, rapid weed growth is most problematic. At this time, weeds will compete with young plantation trees for nutrients, water and, in the central North Island forests, especially light (Richardson *et al.* 1996). Biological control provides one good option for sustainable weed suppression.

Numerous examples exist where biological control using insects has resulted in excellent suppression of the target weed. Unfortunately, there are also many examples where introductions have failed to affect the weed status or management requirements of the target plant (Cullen 1990, McFadyen 1998). With increased costs and stricter legislation concerning the introduction of exotic agents, we can no longer afford the luxury of a trial-and-error approach. Kriticos *et al.* (1999) recommend that studies on the population dynamics of the target weed be carried out before the implementation of biological control programs. In this way, critical

[1] Forest Research, PB 3020, Rotorua, New Zealand. Corresponding author: Toni Withers <toni.withers@forestresearch.co.nz>.

life stages could be targeted for attack by specific biological control agents in order to maximize the likelihood of success. Additionally, population models of the proposed biological control agent, incorporating the effects of feeding damage on the plant, could also be a useful tool to predict the impact they will have on suppression of weed populations. This in turn has the potential to predict the beneficial impact of biological control upon the growth of pine plantations where weed competition is known to reduce growth (Zabkiewicz *et al.* 1998).

In this paper, we examine, before implementation of a biological control program, whether a population dynamics model of a proposed agent (*Cleopus japonicus* Wingelmüller, Curculionidae) could be used to predict its effectiveness against populations of buddleia (*Buddleja davidii*; Buddlejaceae) in New Zealand. There are two stages to the project. The first requires development of a model that describes the development of insect and weed populations and their interactions (i.e. the effect of the insect on weed population development). The second stage is model validation, which can only be accomplished if *C. japonicus* is eventually released in New Zealand.

Previously published data were utilized on the development and survival of different life stages of *C. japonicus* at the range of temperatures representative of New Zealand's central North Island region, where buddleia is prevalent (Zhang *et al.* 1993). We also know that the higher the number of larvae per plant, the more leaf area they consume (Brockerhoff *et al.* 1999). However, in order to model leaf area consumption, we needed to calculate how daily leaf consumption of both larvae and adults was influenced by temperature and larvae or adult age. This paper describes how leaf area consumption was measured and incorporated into a model to predict the impact *C. japonicus* would have on buddleia. Specific details of all the parameters based on that data and used to run a population dynamics model of *C. japonicus* are not included.

Materials and methods

Laboratory experiments

Offspring of a New Zealand laboratory colony of *Cleopus japonicus*, imported into Forest Research Invertebrate Quarantine in 1992 from Hunan province in China, were used for all experiments. *C. japonicus* is a multivoltine external leaf-feeding weevil (Zhang *et al.* 1993) with two damaging life stages, the larva and the adult. The adult female also causes minute damage when depositing the eggs singly within the leaf, but this was not taken into account. The amount of leaf area consumed per larva from when it exits the leaf as a neonate to when it ceases feeding at pupation, and the daily rate of leaf area consumed per adult from eclosion and for 30 days of the pre-oviposition period, were gathered at 10°, 15°, 20°, and 25°C in Contherm environmental chambers set with a 14:10 light:dark cycle. Photoperiod is reported to have no significant impact on *C. japonicus* growth and development (Zhang *et al.* 1993).

Individual newly emerged larvae or adults were caged on a sprig of *B. davidii* foliage whose base was resting in water. Twice a week the sprig of foliage was replaced and the area of leaf area consumed during the previous few days was calculated by tracing the outline of the feeding track onto square millimetre grid paper under a 20× microscope. At the conclusion of the experiment (30 days), all weevils (replicated 20 times for both adults and larvae) were sexed by dissection.

Model description

The *Cleopus japonicus* model was implemented using SAS macros (SAS Institute, Raleigh, NC) and used climatic information to predict the survival and development of cohorts on a daily time-step.

The model identifies five discrete life stages of *C. japonicus*: egg, larva, pupa, pre-ovipositional adult and adult (Fig. 1). Movement between life stages is based on cumulative development of physiological age, which is calculated by the average daily temperature cycle. In this way, development is cumulative and all individuals move to the next life stage when their physiological development reaches one. The other function for which there were some data available was the rate of mortality in the adult stage. The one exception to this is post-oviposition adult mortality, which, due to a lack of data, was set as a gradual linear daily rate of adult mortality with a maximum adult lifespan of 500 days.

Three functions in the current model are not based entirely on the data in Zhang *et al.* (1993). The first is low temperature-induced egg mortality. The linear relationship described by 24% survival at 16°C and 91% survival at 20°C produced excessively high egg mortality at temperatures less than 20°C. Therefore the linear relationship was attenuated by also assuming 24% survival at 12°C. Mortality is calculated similarly,

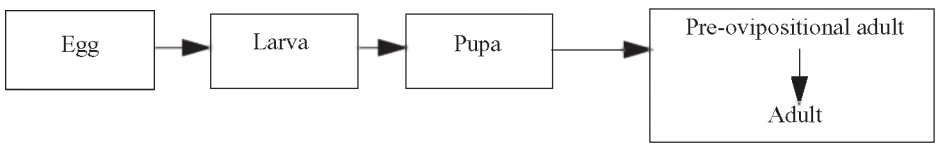

Figure 1. Schematic representation of the lifecycle module of *Cleopus japonicus* weevil used in the population dynamics model.

based on equations of increasing rates of mortality at temperatures significantly above or below the optimum. Consequently, we ran the model using one of two constants to describe daily mortality rates (proportion of the population dying each day). These were 0.007 (derived from 75% survival of pre-ovipositing adults over 36 days at 16°C (Zhang *et al.* 1993) and 0.012 (derived from 75% survival of pre-ovipositing adults over 22 days at 20°C (Zhang *et al.* 1993). These mortalities were applied to both pre-ovipositional and ovipositing adults. Thirdly, fecundity was based on an assumed sex ratio of 50:50 and daily rates were obtained from total fecundity of 8–12 pairs monitored for one month, calculated from four different temperatures (T. Withers & D. Jones, unpublished data). These were compared to total lifetime fecundity figures in Zhang *et al.* (1993) to obtain a best approximation for a temperature-driven daily rate of egg laying that ranged from 0.4 eggs per day (at 10°C) to 2.6 eggs per day (at 20°C).

The model is driven by a meteorological data set based on daily minimum and maximum temperatures which is calculated from the daily minimum and the daily maximum using a 12 segment sine curve and then used to drive the growth and mortality processes. In this case, an eight-year sequence of maximum and minimum temperatures was obtained from the national climate database for the Rotorua Airport climate station. Rotorua is considered close to the centre to the major New Zealand buddleia infestation, where a release of *C. japonicus* is most likely to occur in the future.

Results

Leaf area

Data on the rate of leaf area consumption by *C. japonicus* larvae under four different temperature regimes were analyzed by normalizing them with respect to both daily leaf consumption and age for each temperature. This relationship was found to be independent of temperature and was modelled using a modified version of the equation describing a beta probability density function. Parameter estimates were obtained using nonlinear least squares regression. Leaf area consumption by larvae increases with age, rapidly tailing off to zero when approximately 0.75 of the total larval period is reached (Fig. 2a), while that of newly emerged adults increases rapidly over the first week and then tails off to a steady rate per day (Fig. 2b). The relationship between temperature and maximum daily consumption rate was nonlinear, increasing with increasing temperature up to a maximum at approximately 21°C then decreasing with any further increases in temperature above this point (Fig. 2). There was also no significant difference in mean leaf area consumption according to the sex of the larval *C. japonicus* (two-way ANOVA; $F = 0.17$; df = 1; $P = 0.7$), but there was a highly significant impact of temperature (two-way ANOVA; $F = 3.9$; df = 3; $P < 0.014$).

In the simulation model, by combining the models described above, consumption was calculated on a two-hourly step based on temperature and the predicted age and size of each cohort for larvae and adults (only these life stages consume buddleia leaves). This was then summed to give total daily consumption.

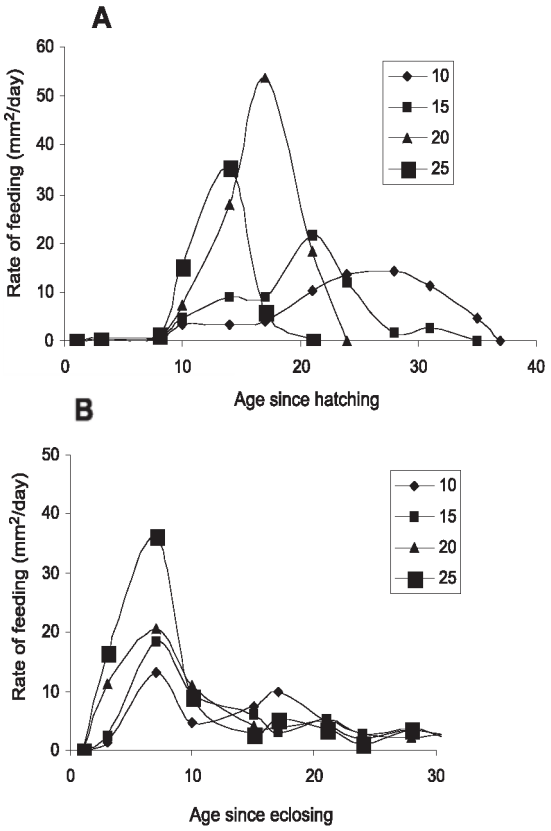

Figure 2. Daily rate of buddleia leaf consumption by (A) larval and (B) adult *Cleopus japonicus* at a range of temperatures (in °C).

Model of population dynamics

We initiated the simulations of the population dynamics of *C. japonicus* with 100 eggs "released" per day during January 1990. The simulation was then run for eight years using the actual daily temperatures recorded at Rotorua Airport for those years. Due to a lack of data on the expected rates of mortality of adult *C. japonicus*, we ran the model using estimates of adult daily mortality rates (proportion of the population dying each day) of both 0.007 and 0.012. These mortality rates were independent of temperature.

Significantly different results were obtained for each mortality rate. With the higher adult mortality rate (1.2% mortality per day) the population did not expand, but instead died out within three seasons. Most adults produced from the second, late-summer generation die by the following spring, meaning there are less adults

surviving to contribute to egg-laying. In this case, the model predicts that the agent will fail to establish (Fig. 3).

Assuming the lower adult mortality rate of 0.7% mortality per day, the model predicted the population gradually increases every year (Fig. 4). Sufficient adults from the second generation over-winter to initiate significant egg-laying in the spring.

Under the lower adult mortality scenario we were able to calculate the leaf consumption. As expected, leaf consumption is related to larval and adult numbers. Predicted daily leaf area of buddleia eaten by *C. japonicus* larvae and adults during the third year of the simulation (1993) peaked at a mere 0.04 m^2 at the end of February (late summer). Total leaf area consumed by the population over this calendar year was a modest 4.4 m^2. However, the leaf area eaten over the entire simulation is shown in Figure 5 and, by the eighth season, is peaking in late summer at 2.3 m^2 of buddleia leaf area eaten per day (Fig. 5). The total leaf area removed by both larvae and adults in the final year was predicted to be 25,000 m^2 (2.5 ha).

Discussion

Simulation models of the population dynamics of insects are only as good as the data that have been used to construct them. We were fortunate that the insect under study already had many experiments undertaken on its biology under controlled conditions (Zhang *et al.* 1993; Brockerhoff *et al.* 1999). Therefore, we believe that *C. japonicus*, the potential biocontrol agent for buddleia, has been modelled using more reliable data than have many other insects. Despite this, we must acknowledge that there are at least three functions within the model

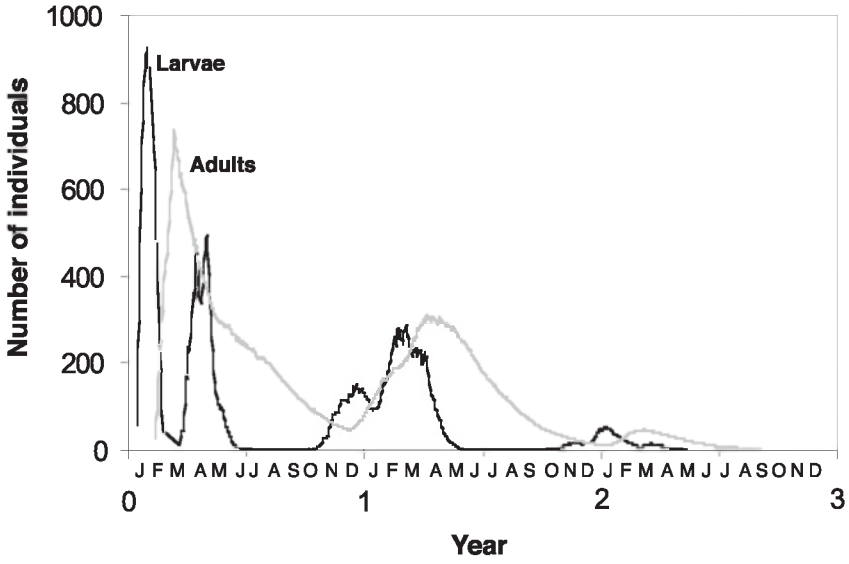

Figure 3. The predicted *Cleopus japonicus* population dynamics (only larvae and adults are shown) under a 1% daily adult mortality regime.

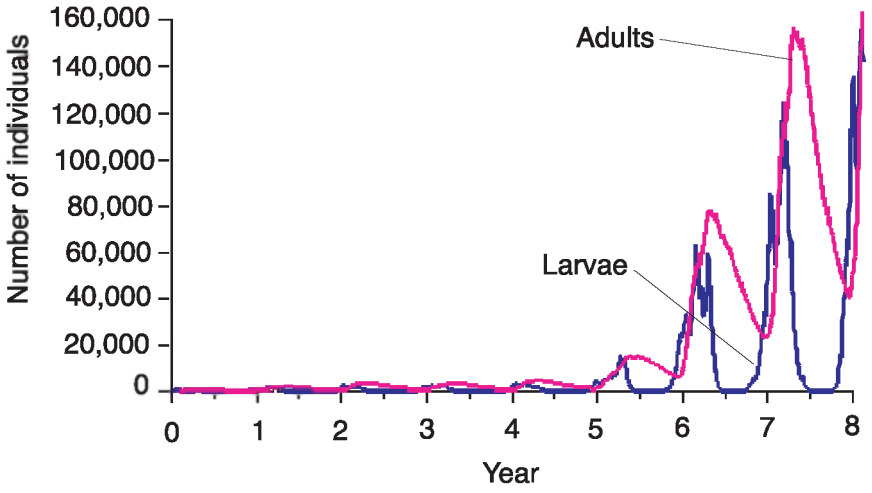

Figure 4. The predicted *Cleopus japonicus* population dynamics (only larvae and adults are shown) under a low 0.7% daily adult mortality regime.

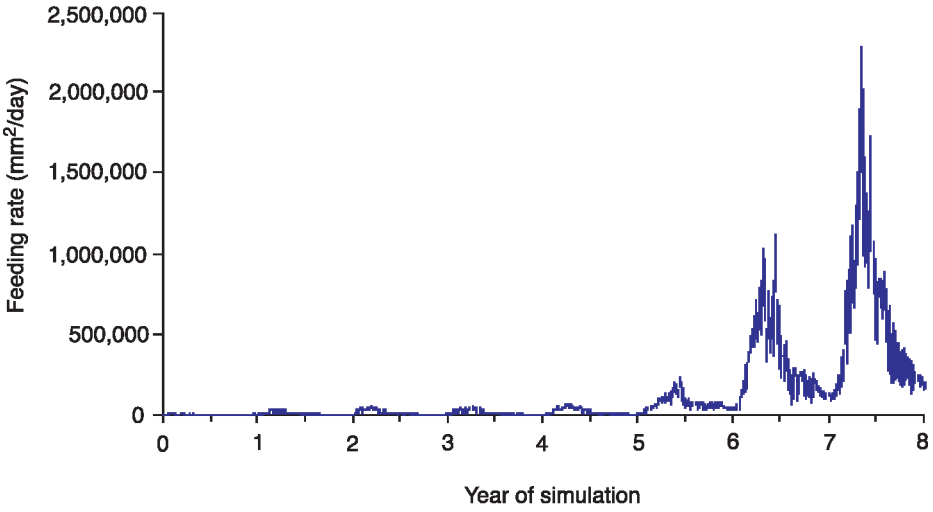

Figure 5. The predicted area of *Buddleja davidii* leaf consumed daily by larval and adult *Cleopus japonicus* under a low 0.7% daily adult mortality regime.

that have a considerable degree of uncertainty associated with them. These include egg mortality and adult mortality. When we ran multiple simulations, it was immediately obvious that adult mortality, and the associated measure of maximum longevity of adults, is crucial to the success of this insect as a biocontrol agent in New Zealand. Indeed the data suggest that only two generations will occur per annum, which is at least one less than that predicted by Zhang *et al.* (1993). The discrepancy here may be as simple as the choice of meteorological data file used to run this set of simulations. However, the sensitivity of the predicted outcome to the two assumed mortality rates emphasizes the need to collect more data to improve our confidence around the mortality functions. Additionally, there is considerable room for improvements to our model, e.g. by including a stochastic component.

Adult longevity is particularly crucial, as adults comprise the life stage that leads to spring egg laying as soon as temperatures allow. However, the possibility that the overwintering survival of pupae may have been underestimated should not be ignored. If the main life stage to successfully overwinter without mortality is pupae, then adults arising from these may assume the role of ovipositing the next generation of eggs in spring. Longer-term laboratory experiments at a range of temperature regimes are required to improve our understanding of mortality factors and to improve the model.

The aim of this research is to evaluate whether we can predict the effectiveness of a biological control agent before its release. In this case, we have been able to predict that a population arising from 3100 eggs could lead to the equivalent of 2.5 ha of green buddleia leaf area being removed each season, eight years later. While this is encouraging, we are not yet at a stage where figures such as this can be related to individual *B. davidii* leaf-area indexes. This is because no population density functions have been built into the model. We do not know how many individual weevils remain feeding on a plant before density dependent factors come into play, prompting adults to move to fresh plants. Adults are likely to move between plants as they are capable fliers, though larvae are not quite as mobile and are likely to only move when all fresh leaf tissue has been removed from the plant on which they emerged. These kinds of data are always going to be difficult or nearly impossible to collect within the confines of quarantine laboratories.

Other important considerations in being able to predict the impact of populations of an agent on populations of a weed are the type of damage, its timing, and the plant's response to that herbivory. For instance, this model predicts that *C. japonicus* leaf feeding will peak in late summer, while being minimal throughout springtime. This has important implications for whether or not this particular agent will be effective on seedlings. To the best of our current knowledge, seedlings germinate throughout the year, so it is possible that those present in springtime will temporarily escape feeding damage (Miller 1984). We will not know whether the peak periods of *C. japonicus* leaf consumption equate to peak periods of plant growth until the insect is established in New Zealand. In the meantime, we have field research under way to model buddleia growth in response to different levels and timings of defoliation.

Ultimately, many of the predictions made in this paper can only be tested when permission is given for the weevil *C. japonicus* to be released from quarantine in New Zealand. At the time of writing, official approval had not yet been sought, but it was likely to be under way by the end of 2003.

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Tobacco mild green mosaic virus: a virus-based bioherbicide

R. Charudattan, M. Elliott, J.T. DeValerio, E. Hiebert and M.E. Pettersen

Plant Pathology Department, University of Florida, PO Box 110680, Gainesville, Florida 32611-0680, USA

Tropical soda apple (*Solanum viarum*; TSA) is a serious noxious weed in pastures, sod fields, and natural areas in Florida and other states in the south-eastern United States. During a search for a biocontrol agent for this weed, we discovered that tobacco mild green mosaic *tobamovirus* (referred to herein as tobacco mild green mosaic virus or TMGMV; ICTV decimal code 71.0.1.0.011; = tobacco mosaic virus U2 strain) causes a systemic, hypersensitive response and kills seedling and mature TSA plants. Younger plants are killed faster than older plants. Inoculated plants develop necrotic foliar lesions, systemic necrosis of petioles and stem tips, and systemic wilting in rapid succession, beginning 12–14 days after inoculation. TSA is also susceptible to Tomato mosaic *tobamovirus* and Tobacco mosaic *tobamovirus* (strain U1), but these viruses induce only nonlethal mosaic and/or mottle symptoms. Among 31 solanaceous plants screened against TMGMV in a greenhouse, only *Capsicum annuum* (most of the 23 cultivars tested), a previously known host to this virus, developed hypersensitive reaction comparable to that seen on TSA. Other hosts were symptomless or exhibited systemic mosaic symptoms or local lesions. In repeated field trials, TMGMV caused 83 to 97% mortality of TSA plants of different sizes and ages. Typically, hypersensitive reaction is expressed as necrotic foliar spots; lethal systemic hypersensitive reaction to virus infection is uncommon and usually occurs in seedlings. Thus, TMGMV has the unique capacity to kill TSA plants of all ages and therefore can be used as an highly effective biological control for TSA. Attempts are under way to develop and register TMGMV as the first virus-based bioherbicide.

Molecular ecology of broom twig miner: implications for selection and release of biological control agents

H.M. Harman,[1] K. Armstrong,[2] S.P. Worner[2] and P. Syrett[3]

[1]Landcare Research, PB 92170, Auckland, New Zealand

[2]Lincoln University, PO Box 84, Lincoln University, Canterbury, New Zealand

[3]Landcare Research, PO Box 69, Lincoln, Canterbury, New Zealand

There has been ongoing debate as to how to increase the chances of establishing effective biological control agents in a new environment. Factors that have been considered include the level of genetic variability gathered in collections from source populations, climate matching, release size, and number of releases in space and time. The importance of these factors can be addressed through theoretical studies, specifically designed field tests, and retrospective analyses of introductions. As an example of the last mentioned, we present results from a study of a successful colonizer and biological control agent for broom, the broom twig miner (*Leucoptera spartifoliella*), which was accidentally introduced to New Zealand. Molecular techniques (AFLPS) were used to investigate population structure and the genetic consequences of long-distance colonization. Populations from the insect's native range in Europe showed little differentiation indicating high gene flow. Within New Zealand, there was stronger differentiation which was most marked with the most recently established of the populations studied. New Zealand populations showed some loss of genetic variability in comparison with the European populations but this may largely be accounted for by the loss of less frequent alleles. Overall, there is little loss of genetic variability in New Zealand populations of broom twig miner despite establishment from a presumed small number of founders. For a purposeful introduction, the lack of population differentiation in this species' native range indicates that the determination of the part of that region to collect individuals to enable successful colonization in New Zealand would not have been critical.

The significance and variability of predation of weed seeds in an agricultural landscape in Western Australia

David Minkey[1] and Helen Spafford Jacob[2]

[1]Faculty of Natural and Agricultural Sciences, Western Australian Herbicide Resistance Initiative, University of Western Australia, 6009, Australia

[2]Cooperative Research Centre for Australian Weed Management, Faculty of Natural and Agricultural Sciences, School of Animal Biology, University of Western Australia, Western Australia 6009, Australia

Understanding the fate of weed seeds is critical to successful integrated weed management particularly with the increase in herbicide-resistant weeds and reduced-tillage practice. However, the fate of weed seeds over summer in agricultural fields is not well understood. It is widely accepted that granivory plays a role in regulating plant populations and is a major contributor to seed loss in natural ecosystems. But this relationship has not been thoroughly researched or proven to significantly reduce seed banks of weed species in an agricultural environment. Three years of study in the Western Australian Wheatbelt has shown that the predation levels of wild radish, wild oat, and annual ryegrass seeds are highly significant but can be extremely variable. The major findings thus far are as follows; (1) predation levels can vary from 0 to 100% within the same field, (2) predation levels tend to be higher on the edges of field rather than in the centre, (3) ants play the dominant role in seed harvesting, (4) ants exhibit preferences for particular weed seed species, and (5) the presence of particular ant species can possibly be used as a predictor of weed seed predation levels. These findings may lead to recommendations for conservation of granivorous ants that are known to consume surface weed seeds during the summer. These results also contribute to our understanding of the weeds' life cycles and fate of their seeds.

The value of using taxonomists to survey for potential biological control agents of weeds

Riaan Stals,[1] E. Grobbelaar,[1] S. Neser[2] and W.A. Palmer[3]

[1]South African National Collection of Insects, Biosystematics Division

[2]Weeds Research Division, ARC–PPRI, Private Bag X134, Pretoria, 0001 South Africa.

[3]Alan Fletcher Research Station, Queensland Department of Natural Resources and Mines, PO Box 36, Sherwood, Queensland 4075, Australia

A critical step in biocontrol of an alien invasive plant is surveying for phytophagous organisms in the plant's native range. These surveys are usually conducted by a visiting biocontrol scientist or a contracted local scientist. A novel approach is to employ a team of taxonomists residing in the weed's country of origin. Such taxonomists know local conditions (biologically, geographically, politically), and are acquainted with the local biota. Specialist taxonomists are likely to be most efficient in collecting their focus taxa, and possess in-depth knowledge valuable to biocontrol surveying. Two South African case studies, where taxonomic teams were contracted for biocontrol surveying, are presented. The Biosystematics Division, ARC–Plant Protection Research Institute (ARC–PPRI), South Africa, was contracted to perform comprehensive surveys on two plant species, both indigenous to South Africa, but serious environmental weeds elsewhere. In 1996, the Queensland Department of Natural Resources, Australia, commissioned a survey on *Acacia nilotica* (prickly acacia, Fabaceae); in 1998 the Agricultural Research Service, United States Department of Agriculture, commissioned a survey on *Delairea odorata* (Cape ivy, Asteraceae). Taxonomists involved in the surveys included specialists of several phytophagous insect orders, Acari, and Fungi. An ARC–PPRI weeds scientist monitored the taxonomists' activities throughout. Most identifications were performed by ARC–PPRI taxonomists, who could also comment on the biologies and biocontrol potential of species collected. Both surveys were highly successful, and discovered several potential biocontrol agents. Compared to previous surveys on *A. nilotica* in Pakistan and Kenya, both performed by biocontrol specialists, the taxonomist approach in South Africa yielded two to three

times more narrowly associated phytophages. Species accumulation curves indicate near complete sampling. No previous survey had been done on *D. odorata*. Apart from the actual discovery of agents, using a taxonomic team offers other advantages, such as linking taxonomists with longer term biocontrol projects, and being cost effective.

Theme 2:

Target and Agent Selection

Pathogens for the biological control of weedy stipoid grasses in Australia: completion of investigations in Argentina

Freda Anderson,[1] William Pettit,[1,2] Louise Morin,[3] David Briese[3] and David McLaren[4]

Summary

Nassella trichotoma (serrated tussock) and *Nassella neesiana* (Chilean needle-grass) are the two most widespread and damaging species of stipoid grasses that have been introduced into Australia. A project was set up in 1999 in Argentina to investigate the potential of pathogens as biological control agents for these species. A Corticiaceae fungus found at a few sites, growing in association with *N. trichotoma* plants severely affected by root and crown necrosis, could not be studied in detail because all attempts to isolate it in pure culture failed. Infection of inflorescences of *N. trichotoma*, *Nassella tenuis* and *Nassella tenuissima* was achieved in the glasshouse with the smut *Ustilago* sp. (within *U. hypodytes sensu lato*), seen causing drastic reduction in seed production on both target plant species in the field. However, technical difficulties regularly encountered during experimental work compromise the prospect of further studies on this pathogen. The bulk of the investigations concentrated on the rust *Puccinia nassellae* which infects both target plants and, on the basis of field data, showed the greatest potential for biological control. Rust isolates from *N. trichotoma* were previously found to infect a wide range of *N. trichotoma* accessions and a non-target native Australian species. Host-specificity tests conducted in this study showed that rust isolates from *N. neesiana* were able to develop mature uredinia on *N. neesiana* plants grown from seed collected in Australia, but none of the tested isolates infected the Australian native species *A. aristiglumis* and *A. scabra*. Further testing is still required to clarify the nature of this rust's life cycle and to investigate differences in specificity between isolates from both host species.

Keywords: *Austrostipa* species, biological control, *Nassella neesiana*, *Nassella trichotoma*, pathogens.

Introduction

Nassella trichotoma (Nees) Arech. (serrated tussock) and *Nassella neesiana* Trin. & Rupr. (Trin. & Rupr.) Barkworth (Chilean needle-grass) are the two most widespread and damaging species of stipoid grasses that have been introduced into Australia (McLaren *et al.* 1998). *Nassella trichotoma* has been estimated to infest over 1 million ha through New South Wales and Victoria (McLaren *et al.* 1998) and costs the Australian grazing industry in New South Wales alone, around $40 million per year (Jones & Vere 1998). *Nassella neesiana* is considered a very serious environmental weed that is spreading rapidly and threatens to infest extensive areas of native grassland in south-eastern Australia (McLaren *et al.* 1998). A project was set up in 1999 in Argentina to investigate the potential of pathogens as biological control agents for these species

[1] CSIRO Entomology, c/o CERZOS, Universidad Nacional del Sur, CC 738 (8000), Bahía Blanca, Argentina.
[2] Present address: CSIRO Entomology, Tropical Ecosystems Research Centre, PMB 44, Winnellie, Northern Territory 0821, Australia.
[3] CSIRO Entomology, CRC for Australian Weed Management, GPO Box 1700, Canberra, ACT 2601, Australia.
[4] Victorian Department of natural Resources and Environment, Keith Turnbull Research Institute, CRC for Australian Weed Management, Frankston, Victoria 3199, Australia.
Corresponding author: Freda Anderson, CERZOS, Universidad Nacional del Sur, CC 738 (8000), Bahía Blanca, Argentina <anderson@criba.edu.ar>.

(Briese & Evans 1998, Briese *et al.* 2000). On the basis of preliminary field observations it was decided to prioritize for evaluation as potential biological control agents the smut *Ustilago* sp. (within *Ustilago hypodytes* (Schlecht.) Fr *sensu lato*) and the rust fungus *Puccinia nassellae* Arth. & Holw. A third species, a soil fungus believed to belong to the Corticiaceae, was included, with reservations, as a third prospective candidate.

The Corticiaceae fungus has been found at three of the 73 sites surveyed from 1999 to 2002 and was always associated with dying patches of *N. trichotoma* plants showing root and crown necrosis (Briese & Evans 1998, Anderson *et al.* 2002). All attempts to isolate the pathogen on artificial media have failed, as have artificial inoculations of plants. In glasshouse and field host-specificity tests reported in Anderson et al. (2002), which included target and non-target plant species, the control *N. trichotoma* plants were not infected by the Corticiaceae fungus under the given conditions, precluding any conclusions to be drawn on the specificity of this pathogen.

The smut *Ustilago* sp. was seen in the field preventing seed formation on severely attacked plants of *N. trichotoma* and *N. neesiana* (Anderson *et al.* 2002). However, the incidence of the disease in the field was usually low, with only a few exceptions. A severe outbreak of the disease on *N. trichotoma* was recorded at two of the surveyed sites, whilst such a high level of disease on *N. neesiana* was found at only one. Interestingly, at the latter site where *N. neesiana* plants were severely diseased, a large population of neighbouring *N. trichotoma* plants showed no signs of infection, suggesting that cross-infection between smut isolates from these plant species does not occur in the field (Anderson *et al.* 2002). In a preliminary host-specificity test, Anderson *et al.* (2002) demonstrated that the South American native *Nassella tenuissima* (Trin.) Barkworth was susceptible to a smut isolate from *N. trichotoma*. However, no infection could be recorded on the control *N. trichotoma* plants because they failed to flower, despite the application of gibberellic acid, which is known to trigger flowering. Therefore, this test could not be considered conclusive for the other plant species tested (*Austrostipa scabra* (Lindley) S.W.L. Jacobs & J. Everett, *Nassella tenuis* (Phil.) Barkworth and *N. neesiana*) that did not become infected.

The bulk of the investigations concentrated on the rust fungus *P. nassellae*, which infects both target weed species and has been partially reported on previously (Anderson *et al.* 2002). Only uredinia of the rust have been found on *N. trichotoma*, whilst both uredinia and telia have been recorded on *N. neesiana.* Levels of infection in the field depend highly on environmental conditions, ranging from hardly detectable after prolonged dry periods to severe outbreaks that kill plants under favourable wet conditions. Cross-inoculations of *P. nassellae* isolates between the two target stipoid species have not resulted in any infection, indicating the presence of different strains of the rust adapted to specific hosts (Anderson *et al.* 2002). Anderson *et al.* (2002) found that rust isolates from *N. trichotoma* infected all tested accessions of this plant species, including representatives from Australian populations. Two of the tested isolates infected and developed mature uredinia on the Australian native species *Austrostipa aristiglumis* (F. Mueller) S.W.L. Jacobs & J. Everett, but none of the isolates infected either the three tested South American stipoid grasses from different genera or the Australian native *A. scabra*. In contrast, preliminary tests showed that rust isolates from *N. neesiana* were capable of infecting only the plant accessions from which they originated (Anderson *et al.* 2002).

In this paper, we report on the most recent findings obtained during the last phase of the project in Argentina. These include results from further host-specificity tests with *Ustilago* sp. and isolates of *P. nassellae* from *N. neesiana*. We also report on further attempts to elucidate the life cycle of *P. nassellae*, as well as results from screening additional rust isolates from *N. neesiana,* in order to identify one that is pathogenic on an Australian accession of *N. neesiana*. Future possible courses of action for this project are discussed in the light of these findings.

Materials and methods

Ustilago sp.

Cross-inoculation test: Pre-germinated seeds of *N. neesiana* and *N. trichotoma* collected at site 64 (Table 1) were dusted with large quantities of dry freshly harvested smut spores and then sown at a 2-cm depth in potting mix contained in plastic trays with 3-cm diameter cavities. Undusted pre-germinated seeds of these two species were planted in a different tray as a control. Spores collected from smut-infected inflorescences of *N. trichotoma* at site 07 were used to inoculate *N. neesiana*, whilst spores collected from smut-infected inflorescences of *N. neesiana* at site 64 were used to inoculate *N. trichotoma*. A total of 42 *N. neesiana* and 16 *N. trichotoma* plants emerged from inoculated seeds in the glasshouse (temperature range 16–26ºC) while 22 and 19 plants, respectively, emerged from the untreated control seeds. After three months, all plants were transferred to 10-cm pots containing potting mix and kept in the glasshouse until the onset of flowering. Plants were monitored weekly to detect the first appearance of smut symptoms on the inflorescences.

Host-specificity test: Pre-germinated seeds of different accessions of *N. neesiana* and *N. trichotoma* from Argentina and Australia, *A. scabra* from Australia, and *N. tenuis*, *N. tenuissima*, *Piptochaetium napostaense* (Speg.) Hack , *Stipa clarazii* Ball and *Stipa gynerioides* Phil. from Argentina were inoculated, using the same method as above, with dry smut

spores collected from smut-infected inflorescences of *N. trichotoma* at site 07. Undusted pre-germinated seeds of each of the species were planted as control. Inoculated and untreated seeds were sown as above in potting mix contained in different plastic trays in the glasshouse and plants that emerged were transferred to pots after 12 weeks. Plants were monitored weekly to detect the first appearance of smut symptoms on the inflorescences.

Table 1. Details of sites mentioned in the text.

Site ID	Site location (nearest town)	Coordinates	
		°E	°S
07	Villa La Gruta	38.15033	62.08607
16	Alcira	32.73529	64.34049
27	Bahía Blanca	38.66602	62.23448
30	Villa Ventana	38.03206	61.98911
45	Tornquist	38.36551	62.28152
52	Coronel Suarez	38.02574	61.38724
64	El Crucero	31.90762	64.52332
94	Napaleofú	37.40969	58.97501
99	Tandil	37.41076	59.15270

Puccinia nassellae

Life cycle: *Nassella neesiana* plants were grown from seed collected at site 16 in potting mix contained in 3-cm diameter pots. Two different methods were used to inoculate plants (*ca.* 2-months-old) with telia (*ex.* 16) that had previously been treated to break dormancy (Anderson *et al.* 2002). In one method, treated but ungerminated teliospores were transferred with a needle onto the upper surface of leaves under a stereomicroscope. The second method involved an adaptation of the "leaf-disc method" used by Morin *et al.* (1992), which consists of inverting a Petri dish containing telia with germinating teliospores stuck to the surface of water agar over plants, thus allowing basidiospores to fall freely onto leaves. Basidiospores recovered from the surface of the water agar were inspected under the microscope to check germination. Inoculated plants were transferred to a controlled environment cabinet at 18ºC, approximately 100 % relative humidity and a 12 h photoperiod (fluorescent 18W). Plants were visually assessed for any type of symptoms or development of aecia after 2–3 weeks. Ten inoculations involving six to eight plants each were performed over time using each of the methods.

Host-specificity test: A series of trials was performed to test the susceptibility of different accessions of *N. neesiana* from Argentina and Australia, *A. aristiglumis* and *A. scabra* from Australia, and *S. clarazii* from Argentina to isolates of *P. nassellae* recovered from *N. neesiana* at sites 27, 52, 94 and 99. Leaves of healthy plants (*ca.* 2 months old), grown in potting mix contained in 3-cm diameter pots, were inoculated by dusting dry urediniospores using a small paint brush under the stereomicroscope (27, 94 and 99 isolates) or by spraying to run-off a suspension of urediniospores in distilled water (52 isolate) onto plants. Urediniospores that had been dried and kept in the fridge at 4°C for approximately 3 months were used for the 94 and 99 isolates, whilst freshly harvested urediniospores were used for the 27 and 52 isolates. Inoculated plants were misted with water and placed in a controlled environment cabinet (conditions as above) for 2–3 weeks. Plants were then visually assessed for presence of fully developed uredinia.

Results

Ustilago sp.

Cross-inoculation test: Slightly more than 80% of the plants of each species grown from inoculated seeds produced inflorescences. For plants grown from the control untreated seeds, 100 and 63% of the *N. neesiana* and *N. trichotoma* plants, respectively, flowered. None of the inflorescences of the control or inoculated plants developed symptoms of the smut fungus.

Host-specificity test: For seven of the accessions of the various species, more plants grown from untreated seeds flowered than those grown from smut-inoculated seeds (Table 2). However, *N. tenuissima* plants grown from inoculated seeds flowered as well as plants grown from untreated seeds, whilst more of the inoculated *A. scabra, P. napostaense* and *S. gynerioides* plants produced inflorescences. Only a small percentage of flowering plants of *N. tenuis* (12%), *N. tenuissima* (3%) and the Australian accession of *N. trichotoma* (5%) were found to be susceptible to the *Ustilago* sp. isolate from *N. trichotoma* collected at site 07 (Table 2).

Puccinia nassellae

Life cycle: Telia incubated under the described conditions germinated profusely producing hundreds of basidiospores which were also observed to germinate readily on water agar. However, none of the *N. neesiana* plants inoculated with either method developed any sign of infection.

Host-specificity test: Rust isolates collected from *N. neesiana* at various sites infected accessions of *N. neesiana* from which they originated, but also infected at least one other *N. neesiana* accession (Table 3). Two of the four isolates tested on an Australian accession of *N. neesiana* developed mature uredinia on some of the inoculated plants. Neither of the rust isolates *ex.* 94 and 99 tested against the *Austrostipa* species infected plants.

Discussion

Anderson *et al.* (2002) found inflorescences of both *N. trichotoma* and *N. neesiana* infected by *Ustilago* sp. at a number of field sites, but observed that cross-infection between the two stipoid species does not seem to occur. Although results from the cross-inoculation experiment presented here seem to be in agreement

Table 2. Susceptibility of various accessions of *Nassella neesiana*, *Nassella trichotoma* and other non-target plant species to an isolate of *Ustilago* sp. collected from infected inflorescences of *N. trichotoma* at site 07.

Plant species	Origin of plant accessions (Argentina site ID or Australian location)	Control seeds (untreated)			Smut-inoculated seeds		
		Total no. of plants emerged	Flowering plants (% of total emerged plants)	Infected plants (% of total flowering plants)	Total no. of plants emerged	Flowering plants (% of total emerged plants)	Infected plants (% of total flowering plants)
Austrostipa scabra	La Trobe, Vic., Australia	15	27	0	36	36	0
	Mont Park, ACT, Australia	19	47	0	23	22	0
Nassella neesiana	27	14	93	0	35	77	0
	ACT, Australia	23	91	0	48	85	0
N. tenuis	30	22	50	0	22	36	12
N. tenuissima	27	23	97	0	31	97	3
N. trichotoma	30	20	30	0	30	13	0
	Dalgety, NSW, Australia	23	70	0	37	54	5
Piptochaetium napostaense	27	20	40	0	45	42	0
Stipa clarazii	27	24	67	0	43	42	0
S. gynerioides	Caldenal, Argentina	28	93	0	17	100	0

Table 3. Susceptibility of various accessions of *Nassella neesiana* and other non-target plant species to isolates of *Puccinia nassella* collected from *N. neesiana* at different sites.

Plant species	Origin of plant accessions (site ID or country)	Disease incidence (% of infected plants)[a]			
		Origin of rust isolates (site ID)			
		99	94	52	27
N. neesiana	16	–[b]	–	70	–
	52	100[c]	80	100[d]	–
	94	–	10	–	–
	99	80	–	–	–
	ACT, Australia	0	0	10	62[e]
Austrostipa aristiglumis	Australia	0	0	–	–
A. scabra	Australia	0	0	–	–
Stipa clarazii	Argentina	–	0[f]	–	–

[a] Based on a total of 10 plants unless otherwise indicated.
[b] Not tested.
[c] Based on a total of four plants.
[d] Based on a total of 12 plants.
[e] Based on a total of 13 plants.
[f] Based on a total of five plants.

with these field observations they are by no means conclusive. The same comment is applicable for the results obtained in the host-specificity test. The very low rates of infection obtained in the tests reported herein and all previous inoculation trials involving the *Ustilago* sp. (Anderson *et al.* 2002) undermine the validity of the negative results obtained. Ideal conditions for infection to occur may not have been provided in these experiments. Nevertheless, it would appear that such conditions are not easily met in nature either since low disease incidence is the most common situation is the field. There may only be a very narrow window of opportunity during seed germination for infection to take place.

Results from the host-specificity test performed in this study concurred with previous findings (Anderson *et al.* 2002) demonstrating that *Ustilago* sp. collected from *N. trichotoma* can also infect other congeneric species such as *N. tenuis* and *N. tenuissima*. However, it is possible that additional species are also susceptible to this smut, but failed to develop symptoms in these host-specificity tests because of the very low rate of infection obtained. Apart from the fact that the low levels of infection obtained during experiments with *Ustilago* sp. hindered glasshouse studies on this pathogen, the low rates of disease spread within host populations observed in the field suggest that the potential of this pathogen as a classical biological control agent is most likely limited.

On the basis of field observations, the rust *P. nassellae* showed the greatest potential for biological control of *N. trichotoma* and *N. neesiana* (Anderson *et al.* 2002). However, the nature of the rust's life cycle on either host species has still not been fully elucidated. The rust's life cycle could only be studied experimentally using isolates from *N. neesiana* because teliospores have never been found on *N. trichotoma* in the field. Although *N. neesiana* plants were subjected to a strong inoculum pressure of germinating basidiospores (*ex. N. neesiana*) in this study, no infection was obtained on plants originating from the same location as the rust isolate used. This finding strongly suggests that *P. nassellae* is not autoecious. It is noteworthy though that Holway, who made the collection in Bolivia of the type specimen of *P. nassellae* on *Nassella caespitosa* Griseb., reported that aecia were repeatedly found on surrounding *Desmodium* sp. plants associated with the rusted grass (Greene & Cummins 1958). During field surveys conducted in Argentina over the years for this project, aecia-bearing plants belonging to this genus or other genera in the Fabaceae have never been found associated with rust-infected *N. neesiana* plants (unpublished data). However, several other aecia-bearing species have been observed growing close to rust-infected *N. neesiana* plants, but no single species was consistently found to be seriously considered as an alternative host for *P. nassellae*. Nevertheless, further investigations of these aecia-bearing species are required before completely disregarding these as possible alternative hosts.

In contrast to results from previous experiments reported by Anderson *et al.* (2002), it was demonstrated in this study that isolates of *P. nassellae* collected from *N. neesiana* at different sites successfully infected *N. neesiana* plants that did not share the same origin as the isolates. Moreover, two of the isolates tested (*ex.* 27 and 52) were able to develop mature uredinia on *N. neesiana* plants grown from seed collected in Australia, suggesting that isolates from *N. neesiana* are not as specific as previously believed (Anderson *et al.* 2002). Notwithstanding, neither of the two additional tested isolates (*ex.* 94 and 99) infected the Australian native species *A. aristiglumis* and *A. scabra*. This suggests that rust isolates from *N. neesiana* may behave differently from those from *N. trichotoma*, which were found to develop mature uredinia on *A. aristiglumis* in previous work (Anderson *et al.* 2002).

In conclusion, technical difficulties regularly encountered during the investigations of two of the candidates, *U. hypodites sensu lato* and a member of the Corticiaceae, have not allowed a complete body of information to be built on them, thus not permitting a thorough evaluation of their potential to be made at this stage. Studies on the third prospective candidate, *P. nassellae*, have been more successful and proceeded further, but have provided conflicting evidence which needs to be resolved. Anderson *et al.* (2002) reported that isolates from *N. trichotoma* and *N. neesiana* did not infect congeneric *Nassella* species, but showed that *N. trichotoma* isolates were able to develop sporulating uredinia on *A. aristiglumis*. In contrast, the study presented here found that isolates from *N. neesiana* did not infect any of the three *Austrostipa* and *Stipa* species tested. These preliminary findings suggest that rust isolates from *N. neesiana* may pose a lesser risk to non-target plants than those from *N. trichotoma*, but may be adequate for the control of *N. neesiana* only, because of their higher specificity. Additional host-specificity testing and cross-inoculation trials between both target weeds using a wider range of isolates are required to fully clarify these issues.

No other severely damaging pathogens were encountered on *N. trichotoma* during the extensive field surveys conducted in Argentina, with the exception of a *Septoria* leaf spot, and this under exceptionally wet weather conditions (Briese & Evans 1998, unpublished data). The limited number of damaging pathogenic fungi on *N. trichotoma* in Argentina does not therefore offer other alternatives for the biological control of this weed in Australia. In contrast, the prospects for possible biological control of *N. neesiana* with *P. nassellae* or another rust species recently found are more encouraging. Trap plants of *N. neesiana* grown from seed from ACT (Australia) and planted in a field plot at Bahía Blanca recently became heavily infected with another rust fungus tentatively identified as

Uromyces pencanus (Diet. & Neger) Arth. & Holw. (unpublished data). This rust species had been reported previously on *N. neesiana* in Argentina by Lindquist (1982) and has been found only once on an Argentinean accession of this same host during this project (unpublished data). The fact that it had not been recorded during previous surveys may indicate that infection is dependent on uncommon environmental conditions, but since this rust fungus is one of the two autoecious species known to infect grasses of the genera *Stipa* and *Nassella* (Greene & Cummins 1958) and its host range appears to be confined to the genus *Nassella* (Greene & Cummins 1958, Lindquist 1982), it may prove profitable to explore its potential as a biological control agent for *N. neesiana.*

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Psylliodes chalcomerus (Coleoptera: Chrysomelidae: Alticinae), a flea beetle candidate for biological control of yellow starthistle *Centaurea solstitialis*

Massimo Cristofaro,[1] Margarita Yu. Dolgovskaya,[2] Alexander S. Konstantinov,[3] Francesca Lecce,[4] Sergey Ya. Reznik,[2] Lincoln Smith,[5] Carlo Tronci[4] and Mark G. Volkovitsh[2]

Summary

Yellow starthistle, *Centaurea solstitialis* (YST), is an invasive noxious weed in USA, Chile, Australia, and South Africa. Several insect species have been introduced against this weed, but with limited success. Thus, other biological agents are being sought. Among them, a flea beetle, *Psylliodes chalcomerus* Illiger, with stem-boring larvae and leaf-feeding adults, seems one of the most promising. Several "biotypes" of this species have been collected on different host plants (YST, *Onopordum acanthium*, and *Carduus nutans*). Biological and morphological features of these biotypes were studied in the field and laboratory. The results suggested that each biotype is closely associated with its respective host plant. Field studies in natural conditions revealed negative correlation between plant biomass and insect infestation, suggesting high impact on the target plant, which is encouraging for biocontrol.

Keywords: *Carduus nutans, Centaurea solstitialis, Onopordum acanthium, Psylliodes chalcomerus*, *Psylliodes chalcomera*.

Introduction

Yellow starthistle, *Centaurea solstitialis* L. (YST), is an invasive noxious weed in the western USA, Chile, Australia, and South Africa. It originated in the Palaearctic (Maddox 1981, Sheley *et al.* 1999). YST is highly invasive in mediterranean and grassland habitats where it can dominate local plant communities, displacing forage and native plants (Carlson *et al.* 1990, DiTomasso 1998). It also causes the lethal disease, nigropallidal encephalomalacia in horses (Cordy 1978). Conventional chemical control strategies have been inadequate and thus research on biological control of yellow starthistle was initiated (Rosenthal *et al.* 1994). Since 1984, a number of insect species were released, all of which attack flowerheads. Lack of effective control indicates the need to broaden the search to find agents that attack roots, stems and leaves (Turner & Fornasari 1995). Thus, other potential biological agents are being evaluated. Among them, flea beetles of the genus *Psylliodes*, which have stem-boring larvae and leaf-feeding adults, appeared to be very interesting. During field explorations in the northern Caucasus (Russia) and central Italy, larvae and adults of *Psylliodes chalcomerus* Illiger were repeatedly collected from YST, from Scotch thistle, *Onopordum acanthium* L., and from musk thistle, *Carduus nutans* L., which are also considered invasive weeds. Although this nominal species has previously been evaluated (Dunn & Rizza 1976, 1977) and released in USA for biological control of *C. nutans* (Dunn & Campobasso 1993), it appeared to us that host-specific cryptic species or biotypes may exist.

[1] ENEA, C.R. Casaccia, Rome, Italy.
[2] Zoological Institute, 199034, St. Petersburg, Russia.
[3] Systematic Entomology Laboratory, USDA, National Museum of Natural History, Washington, DC 20013-7012, USA.
[4] BBCA-onlus, Rome, Italy.
[5] USDA/ARS Western Regional Research Center; Albany, CA, 94710, USA Corresponding author: M.Yu. Dolgovskaya, Zoological Institute, 199034, St. Petersburg, Russia <rita@MD12306.spb.edu>.

Material and methods

Field collections, experiments and observations were conducted in Krasnodar territory (Russia) and in the Latium region (central Italy). Laboratory studies were conducted at Biotechnology and Biological Control Agency facilities within ENEA (Italian Institute for the Environment, the Energy and the New Technologies, Rome, Italy), and in the Zoological Institute, Russian Academy of Sciences (St Petersburg, Russia). Plants for the laboratory experiments were grown in greenhouses. Host plants were mostly grown from seeds collected in natural conditions in Russia and Italy and also obtained from USDA–ARS Exotic Invasive Weeds Research Unit, Western Regional Research Centre, Albany, California. Safflower, *Carthamus tinctorius* L., plants were grown from seeds of two varieties obtained from CDFA, Sacramento, California.

For insect rearing, biological observations and experiments, individual plants were covered with transparent cages. In certain experiments, separate leaves (leafstalks wrapped with wet cotton and placed in a small plastic tube filled with water) were used to feed individual adults in Petri dishes. In this case, host plant leaves were changed every second day and eggs laid were collected, counted and transferred onto wet filter paper in small Petri dishes. Newly eclosed larvae were collected daily.

Adult feeding specificity was tested with two main methods. First, survival and oviposition of individual females in choice/no-choice conditions were recorded in Petri dishes with host and/or non-host plant leaves (as described above). Second, adult feeding, oviposition and progeny survival were checked in choice/no-choice conditions on potted plants.

Most biological observations were made in artificial climate chambers with 15 h light : 9 h dark and constant temperatures of 15, 20, and 25°C. Host-specificity tests with potted plants were conducted in greenhouse conditions (temperature ranging from 22 to 27°C). Other details of the methods are given with the results. Data were analyzed by conventional statistics (in the text and tables, means and SD are given).

Results

Russian populations

Adults of two biotypes of *P. chalcomerus* were collected in Krasnodar territory in 2001–2002:

1. the "YST-biotype": "Volna location", Temryuk region, *ca.* 10 km S Taman (45°07'36"N, 36°41'35"E), feeding on *Centaurea solstitialis*, and
2. the "*Onopordum*-biotype": "Krasnyj Oktyabr location", Temryuk region, near Krasnyj Oktyabr village (45°10'59"N, 37°39'55"E), feeding on *Onopordum acanthium*.

Adult fecundity and longevity

To estimate adult fecundity and longevity, beetles of both biotypes were placed individually in Petri dishes and fed with leaves of their respective host plant. Ten adults of each biotype were monitored at constant temperatures of 20 and 25°C from 11 April 2002 until death. Life duration, daily and lifetime fecundity showed significant differences between the YST and *Onopordum* biotypes ($p<0.01$, two-way ANOVA test) at both studied temperatures (Table 1).

Duration of development

In the YST-biotype the duration of egg development at 15, 20, and 25°C was, respectively, 17 ± 3.2, 9.9 ± 1.0, and 7.4 ± 1.0 days. In the *Onopordum*-biotype, it was 15 ± 4.5, 8.9 ± 0.9, and 7.2 ± 0.9 days at the same temperatures. Thus, the rate of egg development in both biotypes was linearly dependent on temperature, while in the *Onopordum*-biotype embryos developed slightly (insignificantly) faster. In both biotypes, the total duration of development of one generation (from egg to adult) was 30–40 days at constant temperature of 25°C.

Host specificity

No-choice tests with individual females were conducted in Petri dishes simultaneously and with the same methods that were used for the estimation of life duration and fecundity. All specificity tests were conducted at 20°C. Three plant species were used: YST, *O. acanthium*, and *Carthamus tinctorius* (10 females per treatment).

Females of the YST-biotype, when fed with *O. acanthium* or safflower, demonstrated much lower life duration and lifetime fecundity, compared to the controls, i.e. females of the YST-biotype fed with YST at the same conditions (Table 2). As for the *Onopordum*-biotype, YST and *O. acanthium* seem to be more or less equally suitable for adult feeding. When fed with safflower, however, both survival and fecundity were much lower. As for the

Table 1. *P. chalcomerus* adult fecundity and longevity in laboratory conditions.

	Longevity (days)		Fecundity (eggs laid)		Daily fecundity (eggs/female/day)	
Temperature	20°C	25°C	20°C	25°C	20°C	25°C
Psylliodes:						
YST-biotype	79±13	20±9	231±19	156±36	8.4±1.5	9.2±3.5
Onopordum-biotype	23±4	37±11	55±15	38±21	6.5±3.0	4.1±1.9

embryo's survival (percentage of hatching larvae in relation to the total number of eggs), in both biotypes it was significantly higher when fed on their "native" host plant.

No-choice tests with adults in potted plants in greenhouse conditions gave generally the same results (Table 3). In all cases, where *Psylliodes* adults were placed in cages with their "native" host plants, adults intensively fed, survived longer and obviously laid eggs (larvae, pupae and adults were found). In most of these cases, the plants were almost dead or heavily damaged. Neither of two biotypes survived for a long time or reproduced on safflower. The YST-biotype was able to feed on *O. acanthium*, although with lower survival, and to reproduce on this plant. The *Onopordum*-biotype also fed and reproduced when adults and larvae were forced to feed on YST. However, in both cases feeding and reproduction was much less intensive than on the "native" host, as indicated from the lower rate of damage.

Finally, the host specificity of both biotypes was tested with the possibility of choice. To do this, eight adults were placed in a cage with six plants (two plants of each test plant species). Under choice conditions, beetles demonstrated approximately the same range of host specificity: successful development on the "native" host plant, oviposition and some larval development on the closely related host, and no damage nor reproduction on safflower (Table 4). In combination, these data suggest that both biotypes are fairly host-specific, although YST seems to be rather acceptable for the *Onopordum*-biotype.

Impact of YST-biotype on the host plant

On 26–27 May 2001, field sampling was conducted at "Volna location". Height, weight, diameter of the stem, and number of *Psylliodes* larvae were recorded for each YST plant separately. Mean values ($n = 94$) were: height, 69 ± 20 cm; stem diameter, 5.3 ± 1.7 mm; weight, 20.5 ± 12.3 g; *Psylliodes* infestation, 2.6 ± 2.1 larvae per plant. When estimating impact on the host, only plants with stem diameter ≥ 3 mm were selected, to exclude small plants from overcrowded patches. Statistical treatment revealed a significant negative correlation between plant weight and *Psylliodes* infestation ($r = –0.30$, $n = 79$, $p < 0.001$). During 16–17 July 2001 field sampling was conducted at "Primorskij location" (Krasnodar territory, Russia). Means ($n = 150$) were: height, 39 ± 16 cm; stem diameter, 2.7 ± 1.4 mm; weight, 4.9 ± 7.9 g; *Psylliodes* infestation, 1.7 ± 1.9 larvae per plant. Statistical treatment also revealed a

Table 2. Survival and lifetime fecundity of the *Centaurea solstitialis*(yellow starthistle,YST) and the *Onopordum*-biotype of *Psylliodes chalcomerus* when fed with YST, *O. acanthium* and safflower (no-choice tests in laboratory conditions). Data for each biotype indicated by different letters in the same column are significantly ($p < 0.05$) different by ANOVA test (means) or by χ^2 test (percentages).

Psylliodes	Host plant	Survival (days)	Lifetime fecundity (eggs/female)	Embryo survival (%)
YST-biotype	YST	79±13 [a]	231±19 [a]	72.8 [a]
	O. acanthium	20±9 [b]	15±11 [b]	27.5 [b]
	Safflower	25±9 [b]	7±3 [b]	4.5 [c]
Onopordum-biotype	YST	32±9 [a]	33±16 [a]	21.4 [b]
	O. acanthium	23±4 [a]	55±15 [a]	51.8 [a]
	Safflower	9±2 [b]	8±2 [b]	52.0 [a]

Table 3. No-choice test with adults of *Psylliodes chalcomerus* in potted plants in greenhouse conditions (5 beetles per plant, 2–3 plants per each biotype/host combination).

Psylliodes	Test plant	Adult survival during one month	Plant state in one month	New generation recorded
YST[a]-biotype	YST	100%, $n = 15$	Dead plants	Larvae, pupae, adults
	O. acanthium	40%, $n = 15$	Medium damage	Larvae, pupae, adults
	Safflower	0%, $n = 15$	Light to medium damage	Absent
	O. acanthium	100%, $n = 9$	Dead plants	Larvae, pupae, adults
Onopordum-biotype	YST	67%, $n = 9$	Medium damage	Larvae, pupae, adults
	Safflower	0%, $n = 9$	Light damage	Absent

[a] Yellow starthistle, *Centaurea solstitialis*

Table 4. Host-specificity tests for biotypes of *Psylliodes chalcomerus* with possibility of choice.

Psylliodes	Plant state in one month			New generation development		
	YST[a]	*Onopordum*	Safflower	YST	*Onopordum*	Safflower
YST-biotype	Dead plant	Medium damage	Light damage	Larvae, pupae, adults	Larvae	Absent
Onopordum-biotype	Small damage	Heavy damage	Light damage	Larvae	Larvae, pupae, adults	Absent

[a] Yellow starthistle, *Centaurea solstitialis*

negative correlation between plant weight and *Psylliodes* infestation ($r = -0.32$), but because of the relatively small number of plants with stem diameter ≥ 3 mm ($n = 35$) the significance of the correlation was lower ($p = 0.06$).

Italian populations

Psylliodes chalcomerus (*Carduus*-biotype) was collected at two locations near Rome in 2002;

1. Martignano, near Martignano lake, 35 km N of Rome (42°4'60"N, 12°16'0"E), feeding on *Carduus nutans*, and
2. Vivaro, 45 km SE of Rome (41°40'60" N, 12°46'60" E), feeding on *C. nutans*.

Host specificity was evaluated in greenhouse conditions. In no-choice tests, single potted plants of *C. nutans*, YST, safflower, and *O. acanthium* were enclosed in 23 × 23 × 100 cm cages (five replicates per plant species/variety). Six adults were put in each cage and allowed to oviposit. In 20 days, insects were removed and plants were carefully inspected for feeding damage. High leaf damage was recorded only on *C. nutans*, YST was moderately damaged, while safflower and *O. acanthium* were only slightly damaged.

In 50 days from the beginning of the experiment, all plants were harvested and cages inspected. New generation adults were recorded only on *C. nutans* ($n = 10$), YST ($n = 1$), and *O. acanthium* ($n = 2$), while no progeny were recorded on both safflower varieties.

In choice conditions, three different situations were presented to the insects (Table 5). Each set of plants was potted together and placed in a 30 × 30 × 120 cm cage. Three replicates were made of each set. Ten adults were put in each cage and allowed to feed and oviposit during 20 days. Then the insects were removed from the cages. Inspection of the plants showed extensive feeding and oviposition on *C. nutans* plants in all treatments, while only one *O. acanthium* plant showed feeding attack on the stem. In 34 days after the beginning of the experiment, all plants were harvested and dissected under a binocular microscope to find larvae (Table 5).

Taxonomic and morphological notes

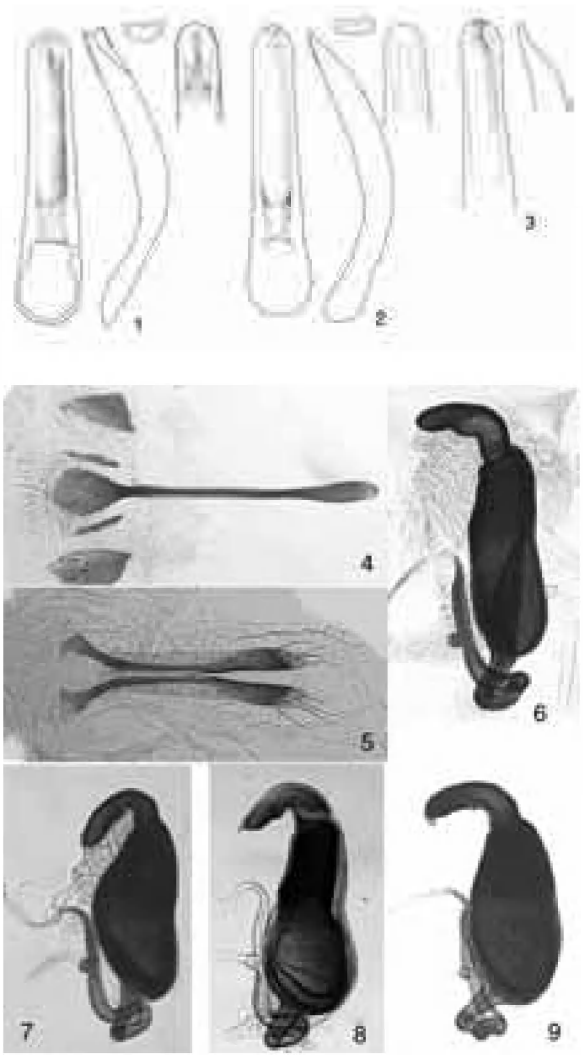
Psylliodes chalcomerus Illiger was described from Portugal (Illiger 1807). It was subsequently recorded in most of Europe, except for the extreme north (Gruev & Döberl 1997). In eastern Europe, and Russia in particular, it is known to occur from taiga (Yakovlev 1902) to steppe (Ogloblin 1925) and in all mountain regions including the Carpathians (Zybenko 1958), Crimea (Shapiro 1961) and Caucasus (Yablokoff-Khnzorian 1961, Yaroshenko 1982, 1986). Further east it is reliably recorded in western Kazakhstan. Other eastern records, for example the Russian far east and China (Gruev & Döberl 1997) need to be verified. The polymorphic nature of this species has been known for some time. Several varieties and aberrations have been described (Gruev & Döberl 1997), but their taxonomic status also needs further study.

We compared males and females from different "biotypes" in an attempt to find reliable diagnostic characters. Study of external morphology did not reveal such characters, despite the fact that specimens collected from *Onopordum* seem slightly larger than those from YST. This result is not unusual, since many, especially closely related, species of flea beetles are nearly indistinguishable externally. Most flea beetle species have unique genitalia, which have been used extensively for diagnostic purposes (Konstantinov 1998). The distribution of several characters of the male genitalia in specimens under consideration has been studied in detail. These include the shape of the median lobe, particularly the apex from ventral, lateral and dorsal views; a relative depth, width and general shape of ventral groove (Figs 1–3). For well-established species these characters would have enough diagnostic states, but in our case, significant intrapopulational variability effectively leaves no characters to separate the YST-biotype from those on *Onopordum* and *Carduus*. The same is true for the female genitalia. The tigna and vaginal palpi (Figs 4, 5) are similar in all the specimens, while the spermatheca are of two distinct shapes which differ in the width of the receptacle: one with the receptacle wide near the middle, the other with the receptacle much thinner (Figs 6–9). However, both shapes occur in the YST-biotype and the *Onopordum*-biotype.

Table 5. Results of indoor choice tests with *Psylliodes chalcomerus*.

Treatment	Plants used	Leaf damage	Stem damage by larvae	Total larvae on three plants
A	YST[a]	low	low	7
	C. nutans	high	high	278
	Safflower	absent	absent	0
B	YST	absent	low	1
	C. nutans	high	high	121
	Safflower	absent	absent	0
C	YST	absent	low	1
	C. nutans	high	high	145
	O. acanthium	low	absent	7

[a] Yellow starthistle, *Centaurea solstitialis*



Figures 1–9. Genitalia of *Psylliodes chalcomerus*. **Figures. 1–3.** Male genitalia (ventral, lateral, dorsal, and proximal views). 1. *Centaurea solstitialis*, yellow starthistle (YST), Krasnodar territory, Anapa, 2001. 2. YST, Krasnodar territory, Volna, 2002. 3. YST, Krasnodar territory, Anapa, 2002. **Figures 4–9.** Female genitalia: 4. tignum. 5. vaginal palpi. 6–9. spermathecae. 6. YST, Krasnodar territory, Anapa, 2002. 7. YST, Turkey. 8. *Carduus*, Italy. 9. *Onopordum*, Krasnodar.

Discussion

Laboratory investigations on the biology of the three biotypes of *Psylliodes chalcomerus* (i.e. two Russian populations associated with YST and *O. acanthium*, and two Italian populations both associated with *C. nutans*), in combination with the results of field observations, suggested that there is rather strict specificity of larval and adult feeding by all considered biotypes, while those that were found on YST and *C. nutans* seem to be more specific than those found on *O. acanthium*. Such intraspecific differentiation has been observed in other phytophagous insects (Bush 1969, Phillips & Barnes 1975, Fox & Morrow 1981, Via 1990). For example, the leaf beetle *Lochmaea caprea* L., which has been extensively investigated, includes up to five races differing in their host specificity and other biological and morphological characteristics. The authors (Mikheev & Kreslavsky 1986, Kreslavsky *et al.* 1987) suggest that more strictly specialized biotypes may originate from less specialized by a single or a few mutations. A similar case of "race" formation based not on host, but on habitat specificity shift, was recently reported for another leaf beetle, *Galerucella nymphaeae* L. (Nokkala & Nokkala 1998).

As for its biocontrol potential, estimation of the impact on the host in field conditions and measurement of host-plant specificity in laboratory experiments suggest that the YST-biotype of *P. chalcomerus* could be promising for YST biocontrol because of its relatively strict host specificity and the fact that it attacks both leaves and stems of the target plant. We also conclude that the other biotypes of *P. chalcomerus* undoubtedly deserve further, broader investigations to determine if they could be suitable agents to control *O. acanthium* and *C. nutans*. The hope is that such investigations, including genetic and taxonomic aspects, will clarify the biological and taxonomic status of *Psylliodes chalcomerus* biotypes.

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Ecological basis for selecting biocontrol agents for lantana

Michael D. Day[1] and Alan J. Urban[2]

Summary

Over the last century, more than 40 natural enemies have been released against the noxious weed lantana (*Lantana camara* L.) in over 40 countries or regions. Biocontrol of lantana remains inadequate, however, except on a few islands. Three of the main factors preventing adequate biocontrol of lantana are its unresolved parentage, resilience to established agents and climatic adaptability. These factors form the ecological basis for the current Australian–South African lantana biocontrol research project, which is tackling three main topics: (1) *Host plants*: the aim is to counteract the effects of the genetic heterogeneity of the weed by selecting agents from (a) the most probable parent species, (b) several species closely related to the probable parents or (c) ornamental cultivars growing in the native range of the probable parents. (2) *Agent guilds*: we aim to reduce the growth and reproductive vigour and resilience of lantana, by selecting agents that multiply quickly, or feed on the stems or roots. (3) *Climatic adaptations*: we seek to counteract lantana's ability to grow in an extensive range of climatic conditions by selecting agents that can bridge periods of plant dormancy and/or leaflessness caused by cold and/or dry conditions. Candidate biocontrol agents, including pathogens and mites, selected on the basis of these ecological considerations, are currently being investigated and are showing considerable promise.

Keywords: climatic adaptation, ecology, genetic heterogeneity, guilds, *Lantana.*

Introduction

Numerous ornamental forms of lantana were bred in glasshouses in Europe by selection and hybridization of unknown, imported, parental species, probably obtained from the New World (Stirton 1977). Of the over 600 named varieties of "*Lantana camara* L." that now exist, different aggregations became environmental and agricultural weeds in different countries. At least 40 recognizable weedy lantana varieties are present in South Africa, and 29 in Australia, threatening ecosystem biodiversity and reducing pasture productivity (Howard 1969, Smith & Smith 1982, Graaff 1986, Swarbrick *et al.* 1998).

Biocontrol of lantana began in 1902, with the introduction of 23 insect species into Hawaii. Over the last 100 years, 41 biocontrol agents were introduced into over 40 countries or islands worldwide and 27 species established (Julien & Griffiths 1998, Baars & Neser 1999, Day *et al.*, unpublished data) (Table 1). Despite some agents causing sporadic, localized damage, lantana is still not under adequate control in most regions. Day & Neser (2000) and Broughton (2000) suggested six factors that thwart adequate biocontrol of lantana:

1. the plant species from which potential agents were collected
2. the phenotype of the target plant
3. plant biology
4. the climate where agents were released
5. parasitism of agents
6. release techniques.

To improve biocontrol of lantana, efforts have recently been made to select candidate agents that address three of these key factors: host plants, agent guilds and climatic adaptations. This paper discusses each these factors in relation to agent selection, and provides information on some of the candidate agents currently being investigated that show considerable promise.

[1] Alan Fletcher Research Station, PO Box 36, Sherwood, Qld 4075, Australia.

[2] Plant Protection Research Institute, Private Bag X134, Pretoria 0001, Republic of South Africa.

Corresponding author: Michael D. Day <Michael.Day@dnr.qld.gov.au>.

Table 1. A list of agents introduced deliberately or accidentally for the biocontrol of *Lantana camara*, the *Lantana* species from which they were collected, their feeding guild and climatic requirements, and the number of countries in which they have been introduced and established.

Order	Family	Species	Origin	Host plant	Guild	Climatic requirements	No. of countries introduced into	No. of countries established in
Coleoptera	Apionidae	*Apion* sp. A	Mexico	*L. urticifolia*	flower feeder	Not known	1	0
		Apion sp. B	Mexico	*L. urticifolia*	seed feeder	Not known	1	0
	Cerambycidae	*Aerenicopsis championi*	Mexico	*L. urticifolia*	stem borer	Tropical, coastal	2	0
		Parevander xanthomelas	Mexico	*L. urticifolia*	root feeder	Not known	1	0
		Plagiohammus spinipennis	Mexico	*L. hirsuta*	stem borer	Tropical, dry	5	1
	Chrysomelidae	*Alagoasa parana*	Brazil	*L. tiliifolia*	leaf feeder	Subtropical, coastal	2	0
		Charidotis pygmaea	Brazil	*L. fucata*	leaf feeder	Subtropical, coastal	2	0
		Octotoma championi	Costa Rica	*L. urticifolia*	leaf miner	Tropical, tablelands	4	1
		O. scabripennis	Mexico	*L. urticifolia*	leaf miner	All except temperate	12	6
		Uroplata fulvopustulata	Costa Rica	*L. urticifolia*	leaf miner	Tropical, tablelands	3	1
		U. girardi	Argentina, Brazil	*L. tiliifolia*	leaf miner	All except temperate	26	24
		U. lantanae	Brazil	*L. tiliifolia*	leaf miner	Not known	2	0
Diptera	Agromyzidae	*Calycomyza lantanae*	Trinidad	unknown	leaf miner	All except temperate	15	15
		Ophiomyia camarae	Florida	*L. urticifolia*	leaf miner	Tropical, coastal	1	1
		O. lantanae	Mexico	*L. urticifolia*	seed feeder	All except temperate	28	24
	Tephritidae	*Eutreta xanthochaeta*	Mexico	*L. urticifolia*	stem galler	Temperate, dry	3	1
Hemiptera	Membracidae	*Aconophora compressa*	Mexico	*L. urticifolia*	stem sucker	Temperate, dry	1	1
	Miridae	*Falconia intermedia*	Jamaica	*L. urticifolia*	sap sucker	All except temperate	2	1
	Ortheziidae	*Orthezia insignis*	Mexico	*L. urticifolia*	sap sucker	Not known	7	6
	Pseudococcidae	*Phenacoccus parvus*	unknown	*L. urticifolia*	sap sucker	Subtropical, inland	2	2
	Tingidae	*Leptobyrsa decora*	Colombia, Peru	unknown	sap sucker	Tropical, tablelands	10	2
		Teleonemia bifasciata	Trinidad	unknown	flower feeder	Not known	1	0
		T. elata	Brazil	unknown	leaf & flower feeder	Not known	5	0
		T. harleyi	Trinidad	unknown	flower feeder	Not known	1	0
		T. prolixa	Brazil	unknown	flower feeder	Not known	1	0
		T. scrupulosa	Mexico	*L. urticifolia*	sap sucker	Subtropical, dry	31	29
Lepidoptera	Depressariidae	*Ectaga garcia*	Brazil	*L. fucata*	leaf feeder	Subtropical, coastal	1	0
	Gracillariidae	*Cremastobombycia lantanella*	Mexico	*L. urticifolia*	leaf miner	Not known	2	2
	Lycaenidae	*Strymon bazochii*	Mexico	*L. urticifolia*	flower feeder	Tropical	3	2
		Tmolus echion	Mexico	*L. urticifolia*	flower feeder	Not known	2	1
	Noctuidae	*Autoplusia illustrata*	Colombia	unknown	leaf feeder	Not known	2	0
		Diastema tigris	Panama	*L. urticifolia*	leaf feeder	Not known	10	1
		Hypena laceratalis	Kenya	*L. urticifolia*	leaf feeder	All except temperate	15	15
		Neogalea sunia	USA	unknown	leaf feeder	Subtropical, coastal	5	3
	Pterophoridae	*Lantanophaga pusillidactyla*	Mexico	*L. urticifolia*	flower feeder	All except temperate	12	12
	Pyralidae	*Pseudopyrausta santatalis*	Mexico	*L. urticifolia*	leaf feeder	Not known	3	0
		Salbia haemorrhoidalis	Cuba, USA	unknown	leaf feeder	Tropical, coastal	13	7
	Tortricidae	*Epinotia lantana*	Mexico	L. urticifolia	flower feeder	All except temperate	9	9
Mycosphaerellales	Mycosphaerellaceae	*Mycovellosiella lantanae*	Brazil	N/A	pathogen	Subtropical, coastal	1	1
Uredinales	Pucciniaceae	*Prospodium tuberculatum*	Brazil	N/A	pathogen	All except temperate	1	1
Coelomycetes	Sphaeropsidaceae	*Septoria* sp.	Ecuador	N/A	pathogen	Not known	1	1

Host plants

The first step in any biocontrol program is to know the identity of the target weed (Schroeder & Goeden 1986). For lantana, this has not been achieved. The name "*Lantana camara*" has been loosely applied by collectors and authors to many species and hybrids, with the result that much of the recorded host-plant information in botanical, horticultural and ecological literature is unreliable. The introduction of cultivated forms back into neotropical regions has allowed interactions with the native gene pool, producing further complex morphological variation (Mendez Santos 2002). Even after decades of considerable effort by morphological taxonomists and molecular biologists, lantana remains a taxonomic enigma. However, there have been some significant achievements: DNA studies showed that the common pink-flowering lantana variety from Australia, Fiji and Vanuatu has a greater affinity with *L. urticifolia* from Mexico than with any other species of lantana tested (Scott *et al.* 2002).

The DNA studies also suggested that the progenitors of lantana in other regions of the world may be different from those of lantana in Australia, Fiji and Vanuatu. For example, lantana plants from Hawaii and the Solomon Islands are similar to one another, but different from those from Australia (Scott *et al.* 2002). As yet, the DNA studies have not determined the progenitors of lantana from Hawaii, the Solomons or many other countries.

In addition, some lantana specimens from Australia and South Africa have been identified (using morphological characters) as *L. urticifolia* × *L. camara* hybrids (R. Sanders, Botanical Research Institute of Texas, pers. comm.). These findings, along with the DNA studies, suggest that many of the weedy lantana varieties that were assumed to be *L. camara* could be derivatives of *L. urticifolia*.

Retrospective studies have revealed the following:

1. most insects and mites collected as potential biocontrol agents in surveys in Mexico and Brazil have discrete hosts (less than 10% were found on more than one *Lantana* species) (Winder & Harley 1983, Palmer & Pullen 1995)
2. a greater proportion (73%) of agents that were collected from *L. urticifolia* or *L. camara* established on weedy lantana than agents collected from other species of *Lantana* (25%) (Day *et al.* 2002, Day *et al.*, unpublished data)
3. agents that were found on three or more *Lantana* species in their native range had a greater establishment rate (82%) on weedy lantana than those that were found on only one or two *Lantana* species (36%) (Day *et al.*, unpublished data).

Entomologists are now selecting agents that are either (a) specific to *L. urticifolia* or *L. camara*, as they should be better adapted to the weedy hybrids, or (b) stenophagous to several *Lantana* species, as they may have a wide enough host range to be able to utilize the hybrid forms of lantana in the target countries.

Agent guilds

Leaf- and/or flower- and fruit-feeding insects have been utilized successfully against many weeds (Julien & Griffiths 1998). However, they have not been able to achieve similar results against lantana. Lantana has the ability to withstand a wide range of climatic conditions and can survive in hot, dry regions as well as those areas susceptible to frost or prolonged droughts (Swarbrick *et al.* 1998). During periods of drought, plants can lose all their leaves, and populations of biocontrol agents, especially those of leaf- and flower-feeding insects, can decrease dramatically. Insect populations can increase again once conditions improve and the plant becomes healthy (Day *et al.*2004). As a result, populations are not maintained at levels sufficient to control the plant. Rather than leaf- and flower-feeding insects suppressing plant growth and reproduction, it appears that they may respond to the health of the plant.

Historically, most agents selected for the biocontrol of lantana were insect members of leaf- or flower-feeding guilds (possibly as these were the most conspicuous and easiest to test). Other guilds such as stem borers and root feeders and groups such as pathogens require more effort and were often overlooked. Worldwide, the proportion of the 41 agents introduced (and the 27 established) in the different guilds were: leaf feeders 56% (60%); flower or fruit feeders 24% (19%); stem feeders 10% (11%); and root feeders 2.5% (a single species which did not establish). Three fungal pathogens have also been introduced and all have established where released.

When considering individual countries or regions, the effort put into biocontrol varies considerably. Australia (30), Hawaii (25) and South Africa (24) have imported the most agents, with most other countries importing 3–5 agents (Julien & Griffiths 1998, Day *et al.*, unpublished data). However, some agents have been introduced into only one country or region and no country has introduced all 41 agents. In most countries or regions, only leaf- or flower-feeding insects have established. In South Africa, 71% of the 14 insect agents that have established feed on leaves, 21% feed on flowers/fruits, while no insects that feed on the stems or roots have established. One pathogen has established. In Australia, 69% of the 16 insect agents established feed on leaves and 19% feed on flowers/fruits. One stem-feeding insect and one pathogen have also established. Both countries can claim only partial control of lantana. Other countries, such as Fiji (seven agents established), India (seven) and Federated States of Micronesia (six) have only leaf- or flower/fruit-feeding agents and adequate control has also not been achieved.

In contrast, in Hawaii, where lantana is reported to be under control in some areas, a smaller proportion of

the established agents feed on leaves (53%), 29% attack flowers/fruits and a greater proportion attack stems (12%). One pathogen has also established. The stem-boring cerambycid *Plagiohammus spinipennis*, and the stem-galling tephritid *Eutreta xanthochaeta*, have established only in Hawaii. Both agents cause substantial damage to lantana, especially in the dry areas of the big island and aid control, such that lantana is not considered a major weed there (Davis *et al.* 1992). These insects were introduced into Australia and South Africa, but failed to establish.

For most countries where only a few agents have been introduced and established, introducing the main damaging agents such as *Octotoma scabripennis*, *Uroplata girardi* and *Teleonemia scrupulosa* would be a priority. However, most perennial weeds can tolerate a single defoliation (Harris 1973). Lantana is adapted to repeatedly losing its leaves due to drought and/or frost. This defoliation tolerance is the basis of the resilience of lantana to most of its biocontrol agents (Broughton 2000, Day & Neser 2000). For this reason, added pressure is needed on the plant, to further deplete carbohydrate reserves accumulated for regrowth. For countries such as South Africa, Australia and India, priority should be given to the utilization of pathogens and obtaining agents that attack stems or roots. Accordingly, in the past few years, a number of promising new candidate agents has been imported and released into South Africa or Australia.

One major advance in the biocontrol of lantana has been the recent introduction of two pathogens, *Mycovellosiella lantanae* in South Africa and *Prospodium tuberculatum* in Australia. Only one other pathogen, *Septoria* sp. in Hawaii, has been utilized against lantana (Trujillo & Norman 1995). All pathogens have established in their respective countries or regions of introduction but it is too early to determine their impact on the weed (Tomley & Riding 2002, A. den Breeÿen, pers. comm.). Pathogens induce toxic effects that disrupt physiological processes and may complement the actions of insects. Surveys by Barreto *et al.* (1995) found several other pathogens, such as *Puccinia lantanae* and *Ceratobasidium lantanae-camarae,* that are also worthy of further study.

The stem-boring beetle *Aerenicopsis championi* has been released in Australia and Hawaii. It has failed to establish in both regions, probably due to the small numbers released. It is particularly damaging, tunnelling down and killing branches of lantana. In the field, plants attacked by *A. championi* are stunted and less fruitful. So far, the beetle has proved difficult to rear and establish. If a successful rearing method can be developed to produce large enough numbers of insects for successful establishment, *A. championi* may markedly improve biocontrol of lantana. At present, South Africa and Australia are working on new methods to rear the insect.

The root-feeding beetle *Longitarsus* sp. AcSN2440, undergoing quarantine testing in South Africa, is host-specific (Simelane 2001) and very damaging to plants. Adults feed on leaves, but the significant damage is due to larvae feeding on secondary roots (Baars & Neser 1999), disrupting the uptake of water and nutrients by the plant, thereby increasing plant stress and reducing plant growth (Simelane 2001). The adults have a diapause stage enabling over-wintering and the insect is relatively easy to rear.

The eriophyid budmite *Aceria lantanae* has been tested in South Africa and is deemed host-specific. *A. lantanae* causes undifferentiated inflorescence buds to form microphyllous galls instead of flowers, fruits and seeds. The galls probably also act as a metabolic sink, debilitating the plant and reducing its competitive ability (Baars & Neser 1999, Urban *et al.* 2001).

Climatic adaptations

Despite the introduction of several new, potentially damaging agents, the climatic adaptability of lantana may still prove an immense problem to overcome. Lantana is found in a wide range of climatic and geographical areas (Henderson 2001, Day *et al.* 2004) and agents are unlikely to be suitable for all regions. As an example, at the limits of the distribution of lantana in Australia (33°S), only two agents, the leaf-mining hispine *Octotoma championi* and the fruit-mining agromyzid *Ophiomyia lantanae* are found. Both are found in only low numbers, such that damage to the weed is negligible, even in late summer (Day *et al.* 2004). In addition, few agents are found on lantana under canopy, or at altitudes greater than 200 m in temperate areas. Agents such as *O. scabripennis* cease oviposition when temperatures drop below 15°C and may complete only two generations a year (C. Clech & M. Day, unpublished data). This is insufficient for populations to build up and be maintained at damaging levels.

The herring-bone leaf miner, *Ophiomyia camarae*, was released in South Africa in 2001 and has established widely (Simelane 2002). Larvae tunnel along the leaf veins, disrupting translocation of water to, and photosynthates from, the lamina, and causing premature abscission of leaves. Field trials suggest that it indirectly reduces plant growth and reproduction (Simelane 2002). *O. camarae* appears to perform well in sheltered areas where lantana grows as an understorey. Even though this agent is a leaf feeder, it may improve biocontrol in cooler and sheltered areas where few agents are present.

Apart from the diverse climates in which lantana can grow, many areas where lantana is a problem have distinct wet and dry seasons. During the dry season, lantana may lose its leaves, causing populations of leaf-feeding agents to crash. It is therefore desirable to select agents that can bridge periods of leaflessness. The petiole-galling apionine *Coelocephalapion camarae* is a small, fast-breeding, host-specific beetle that indirectly stunts root growth (Baars & Heystek 2001).

Adults feed on the leaf laminae, while the larvae form galls in the petioles, causing early abscission of leaves, or occasionally in the peduncles of inflorescences, reducing flowering and seed set (Baars & Neser 1999). The petiole galls disrupt the transport of water and nutrients, causing a reduction in root growth (Baars & Heystek 2001). The long-lived adults have been recorded at altitudes of 1600 m (Kissinger 2000), are clearly adapted to areas where lantana is leafless in winter, and should contribute to biocontrol in such areas.

Discussion

Lantana has been the subject of biocontrol programs for 100 years and adequate control has not been achieved in most regions. Recent developments have suggested that better biocontrol could be obtained with the introduction of better-adapted and more effective agents. While it is generally hard to predict which agents or even guilds of agents are likely to be the most damaging to a weed, there is sound reason to suggest that targeting agents that have certain characteristics would be advantageous.

Sheppard (1992) suggests that genetically variable weeds are more difficult to control through biological means than weeds that are genetically homogeneous. Therefore, potential agents should be collected from the most closely related plant species, as those agents should be better adapted to the target weed. However, the most closely related species to the weedy forms of lantana were, for many years, just not known. The results of recent DNA studies suggest that potential agents should be collected from *L. urticifolia* and some of these agents are showing considerable promise in the laboratory.

A method for rating the effectiveness of agents, using a number of criteria such as host specificity, type of damage inflicted, phenology of attack and number of generations per year, was developed by Harris (1973) and later modified by Goeden (1983). The benefits of defoliating insects have been well documented, especially in annual weed species. They can reduce plant growth, flowering, seeding and the accumulation of carbohydrate reserves. However, the prime concern in controlling perennial weeds is the destruction of existing plants. Defoliating insects do not necessarily achieve this and their effectiveness is restricted to the summer months during which the weed is vulnerable to insect attack (Harris 1971).

Selecting agents that feed on the stems or roots may be more effective for suppressing the weed, as they do not rely on plants to be in leaf all year round (Harris 1971). Stem-boring or root-feeding agents remove biomass and/or disrupt the transport of water and nutrients and can severely weaken the plant. Stem-boring larvae, being internal feeders, can survive independent of the condition of leaves, while root-feeding agents can have dramatic impact on plant health and populations (Harris 1973, Blossey & Hunt-Joshi 2003). Furthermore, root feeders have contributed more to control of weeds than other agents. Over 50% of established root feeders contribute to control of weeds, compared to only 30% of aboveground feeders (Blossey & Hunt-Joshi 2003). There are several *Longitarsus* species that have been utilized against weeds and they appear to make a valuable contribution to the control of annual weeds (Julien & Griffiths 1998). The group's impact on perennials, however, is unknown.

The effect of gall-forming agents is often underestimated. Galls can act as metabolic sinks and their effect is often greater than their size or physical damage to the plant would indicate. Galls can disrupt the translocation of water and nutrients to growing shoots and nutrients to roots, increasing water stress and reducing plant growth. There are a number of stem- and flower-galling agents causing substantial damage to perennial weeds, e.g. *Cecidochares connexa* on *Chromolaena odorata* in Guam and Indonesia, and *Trichilogaster acaciaelongifoliae* on *Acacia longifolia* and *Uromyces tepperianum* on *A. saligna* in South Africa (Julien & Griffiths 1998). In addition, other *Aceria* spp. have already been used successfully against weeds (Julien & Griffiths 1998). Therefore, the potential of gall-forming agents, such as *A. lantanae* and *E. xanthochaeta,* to contribute to the control of lantana would seem to merit further research.

Pathogens are still grossly under-utilized. In fact, they make up only a small proportion of all agents released against weeds. Until recently, there was some concern about the use of pathogens, but pathogens have now successfully controlled weeds, such as rubber vine and noogoora burr (Julien & Griffiths 1998). Pathogens may be highly specific and may actually be limited in their impact, especially on weeds that are genetically diverse. However, it is possible that not all varieties of lantana would be susceptible to any one pathogen. Pathogens have a number of attributes that are superior to those of insects. They have different modes of attack to insects and are able to build up into large populations faster and disperse more quickly than insects. Pathogens can usually be mass-cultured more cheaply than insects while some pathogens can also be prepared and applied as a target-specific mycoherbicide when and where required.

While targeting specific guilds may appear beneficial in theory, the diverse range of climates in which lantana can grow may limit the effectiveness of many potential agents, especially in the cooler regions. CLIMEX has been particularly useful in identifying possible search areas, given that lantana has a wide distribution in neotropical regions.

Biocontrol of lantana has been conducted for over a century, yet suppression of the weed remains inadequate. Today, practitioners have the advantage of being able to review, analyze and learn from past attempts, and to utilize new technology. Applying this knowl-

edge in selecting agents that are ecologically better adapted to the target plant and its environment, and are able to markedly suppress plant growth and reproduction, should offer some improvement in the overall biocontrol of lantana.

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Eriophyid mites for the biological control of knapweeds: morphological and biological observations

E. De Lillo,[1] R. Baldari,[1] M. Cristofaro,[2] J. Kashefi,[3] J. Littlefield,[4] R. Sobhian[5] and C. Tronci[6]

Summary

During 2001 and 2002, Turkish populations of eriophyid mites infesting *Centaurea solstitialis* L. and *C. squarrosa* Willd. (Asteraceae) have been examined. The analyses of the morphometric data, induced symptoms, and the morphological comparison with the descriptions of known species, allowed us to identify three new *Aceria* species, here described and illustrated.

Aceria solcentaureae and *A. solstitialis* were collected on *C. solstitialis*, and *A. squarrosae* was associated with *C. squarrosa* in Cappadocia, Turkey. The infested plants were stunted, showing a reduced growth, a heavy broom-like appearance, being bushy, with the apical parts of the stems and flowerheads still green and fresh during the hot and dry season, less spiny than usual, and producing smaller seedheads.

Additional information is given about the ecology of these associations and on the potential role of these eriophyids as control agents.

Key words: biological control, Eriophyiidae, knapweeds, mites, weeds.

Introduction

Plants of the genus *Centaurea* (Asteraceae) are collectively referred to knapweeds and starthistles. The genus comprises over 1000 species of predominantly Eurasian origin (Wagenitz 1975, Roché & Roché 1991). The interest in herbivores of these *Centaurea* spp. is relevant to the "weed" status acquired by some of these host plants accidentally introduced into North America during the mid-1800s (Rosenthal 1996, Piper 2001).

Ten species of eriophyid mites have been found and described on plants belonging to the genus *Centaurea*: *Aculops centaureae* (Farkas) and *Epitrimerus jaceae* Liro are considered vagrant species; *Aceria acroptiloni* Kovalev & Shevtchenko, *A. calathidis* (Gerber), *A. grandis* (Nalepa) and *A. paniculatae* (Cotte) cause severe deformations of flower- and seedheads; similar damage is reported for *A. prima* (Cotte); *Aceria brevisetosa* (Cotte) and *A. centaureae* (Nalepa) cause blistering on leaves and stems; *Aceria thessalonicae* Castagnoli causes abnormalities in growth, with a broom like appearance (J.W. Amrine Jr. & E. de Lillo, unpublished electronic database, 2002). None of these species have been found so far on *Centaurea solstitialis* in the field, and only *A. centaureae* was able to develop stable populations on this target weed during laboratory host-specificity tests (Sobhian *et al.* 1989). Unfortunately, most of the eriophyid occurrences on different *Centaurea* species have been recorded only on the basis of the symptoms observed on the hosts, without any morphological specific identification of the associated mite populations. *Aceria centaurea* therefore seems to have a large geographical and host distribution (Amrine & de Lillo, unpublished electronic database, 2002) that requires confirmation.

[1] Istituto di Entomologia Agraria, University of Bari, Italy.
[2] ENEA, C.R. Casaccia, Rome, Italy.
[3] USDA ARS European Biological Control Laboratory, Thessaloniki, Greece.
[4] USDA –APHIS PPQ, Bozeman, MT, USA.
[5] USDA ARS European Biological Control Laboratory, Montpellier, France.
[6] Biotechnology and Biological Control Agency onlus, Largo S. Stefano 3, 00061 Anguillara Sabazia, Rome Italy.
Corresponding author: E. De Lillo, Istituto di Entomologia Agraria, University of Bari, Italy <delillo@agr.uniba.it>.

During 2001–2002, surveys were conducted in Turkey, attempting to find additional biological control agents mainly against yellow starthistle. The purpose of the present paper is to describe the new eriophyid mites found on *C. solstitialis* and *C. squarrosa*, and to report ecological observations.

Materials and methods

Specimens were recovered from dried and ethanol (70%) preserved plant material and were prepared and slide mounted according Keifer's method (Jeppson *et al.* 1975). Lindquist's (1996) terminology and setal notation of the morphological details has been adopted in the descriptions. All measurements of mites were made according to Amrine and Manson (1996), given in micrometers, with measurements and means are rounded off to the nearest integer; range values being given in brackets. The classification of the genus according to Amrine (1996) and Hong and Zhang (1997) was followed.

Type materials are deposited at the Dipartimento di Biologia e Chimica Agro-forestale e Ambientale (Di.B.C.A.), Entomological and Zoological Section, University of Bari, Italy.

Drawing abbreviations

AP1, internal female genitalia; CS, lateral view of a caudal region; DA, dorsal view of the prodorsal shield; E, empodium; ES, lateral view of annuli; GF, coxal and genital region of a female; L, foreleg; SA, lateral view of anterior region.

Aceria solcentaureae de Lillo, Cristofaro et Kashefi

Female (Fig. 1) – Body wormlike, colour whitish, 278 (243–310, n = 10) long, 72 (63–78) wide and 64 (50–75) thick. Gnathosoma 27 (22–30) projecting obliquely downwards, chelicerae 24 (22–28) long, seta *d* 9 (7–10) long. Prodorsal shield 40 (36–43) long, 39 (35–42) wide, semicircular in anterior shape with anteriormedian lobe over gnathosoma base 6 (5–7) long; shield pattern composed of median line, admedian, and submedian lines; The submedian lines end about 1/4 before the rear prodorsal shield margin. Some short dashes are included between the lines, many dashes are on the median fields. Tubercles *sc* are on the rear shield margin 32 (28–35) apart, *sc* setae 50 (45–55) long.

Foreleg 40 (36–44) long, tibia 10 (9–11) long, tarsus 9 (8–10) long, *w* 10 (9–10) long distally rounded, empodium simple, 7 (7–8) long, 5-rayed. Hindleg 33 (26–38) long, tibia 8 (7–8) long, tarsus 8 (7–9) long, *w* 10 (9–11) long distally rounded, empodium simple, 7 (7–8) long, 5-rayed.

Coxae ornamented by short striae and coarse granules; *1b* setae 13 (11–14) long, *1b* tubercles 16 (15–18) apart, *1a* setae 28 (24–30) long, *1a* tubercles 14 (11–14) apart, *2a* setae 56 (50–62) long, *2a* tubercles 31 (29–34) apart. Prosternal apodeme 8 (6–9) long.

Opisthosoma with 74–87 annuli. Pointed microtubercles on the rear margins of the annuli. Setae c_2 30 (24–35) long on annuli 9–10, *d* setae 63 (50–72) long on annuli 25–28; *e* setae 24 (20–31) long on annuli 42–48; *f* setae 23 (21–24) long on annuli 68–79. Last 6–7 annuli with elongated and linear tubercles. Setae h_2 59 (50–65) long very thin at the apex, h_1 setae 5 (4–6) long.

Genitalia 22 (18–25) long, 33 (30–35) wide. Female genital coverflap with 15–17 striae; *3a* setae 25 (23–27) apart, 27 (24–31) μm long.

Male – Similar to the female, 247 (220–260 n = 3 specimens) long, prodorsal shield 40 long; *sc* setae 39 (38–41) long; opisthosoma with 72–73 annuli.

Host plant – *Centaurea solstitialis* L. (Fam. Asteraceae), yellow starthistle.

Type locality – Goreme (38°39.87' N, 35°49.73' E), near Kayseri, Cappadocia, Turkey.

Type material – Holotype: 5 females and 1 male on a slide, dated 2, August 2001; Paratypes: many slides prepared from material collected in the same locality on the same date.

Collected by – Kashefi J.

Other material – Ethanol preserved stems, flowerheads and leaves from which the above slides were made.

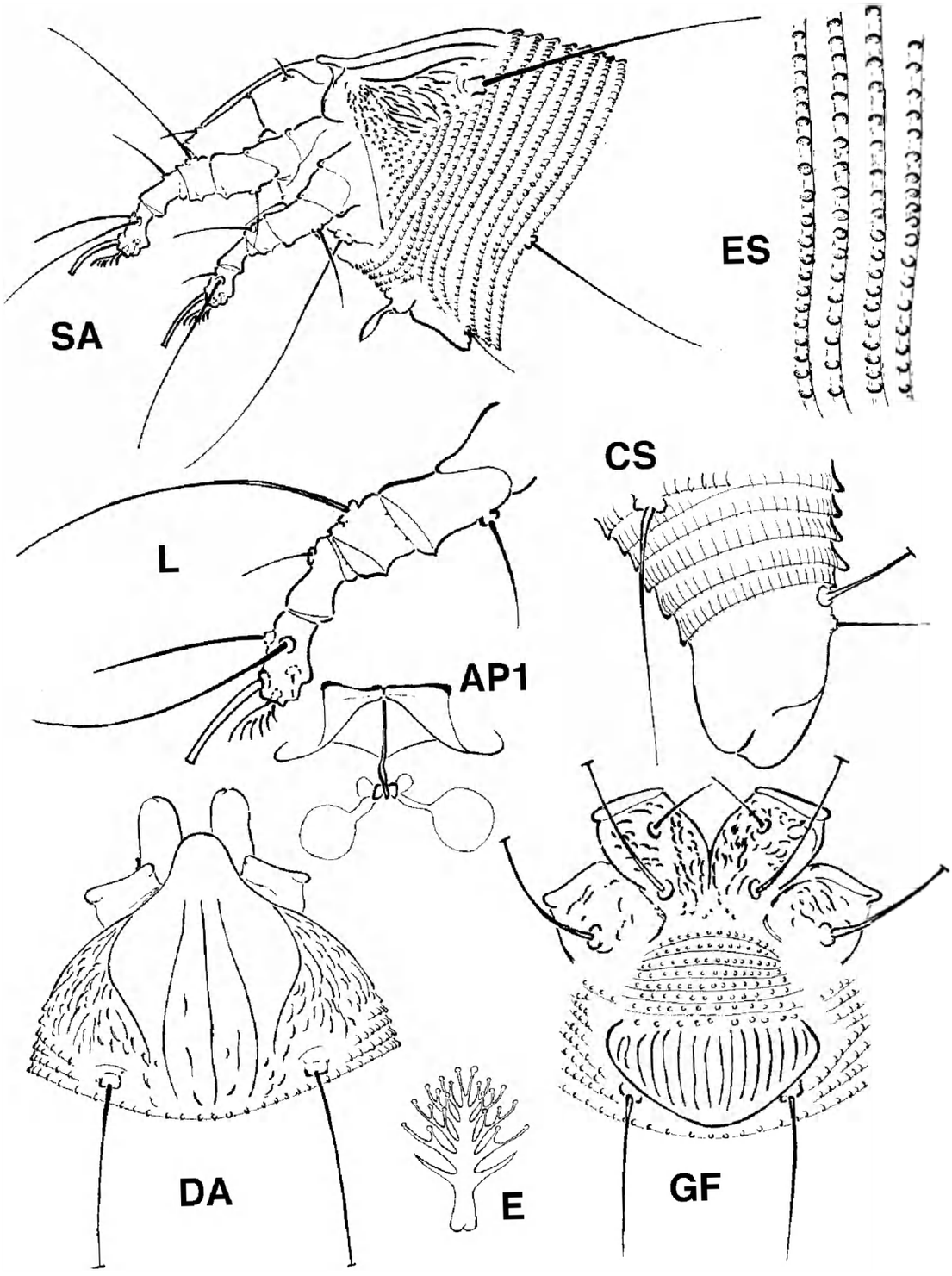

Figure 1. *Aceria solcentaureae* de Lillo, Cristofaro et Kashefi: semischematic drawings.

Aceria solstitialis de Lillo, Cristofaro et Kashefi

Female (Fig. 2) – Body wormlike, colour whitish, 235 (215–280, *n* = 10) long, 47 (43–50) wide and 46 (40–56) thick. Gnathosoma 28 (27–30) projecting obliquely downwards, chelicerae 23 (20–26) long, seta *d* 8 (6–10) long. Prodorsal shield 35 (31–38) long, 35 (33–38) wide, semi-elliptical in anterior shape with anteriormedian lobe over gnathosoma base 6 (5–8) long; shield pattern composed of a median, admedian, and submedian lines; the submedian lines do not reach the rear prodorsal shield margin. A few dashes are included between the admedian field close to the rear prodorsal shield margin, many dashes are on the median fields. Tubercles *sc* are on the rear shield margin 27 (20–30) apart, *sc* setae 42 (38–45) long.

Foreleg 35 (30–40) long, tibia 8 (7–8) long, tarsus 7 (6–7) long, *w* 10 (8–11) long distally rounded, empodium simple, 7 (6–8) long, 6-rayed. Hindleg 31 (28–34) long, tibia 6 (5–9) long, tarsus 7 (5–8) long, *w* 12 (10–13) long distally rounded, empodium simple, 8 (7–8) long, 6-rayed.

Coxae ornamented by short striae and coarse granules; *1b* setae 12 (10–14) long, *1b* tubercles 15 (13–15) apart, *1a* setae 23 (20–25) long, *1a* tubercles 10 (9–13) apart, *2a* setae 47 (40–50) long, *2a* tubercles 27 (25–30) apart. Prosternal apodeme 6 (5–8) long.

Opisthosoma with 76–90 annuli. Rounded microtubercles on the rear margins of the annuli. Setae c_2 21 (19–26) long on annuli 11–15, *d* setae 52 (45–60) long on annuli 29–33; *e* setae 15 (12–19) long on annuli 48–52; *f* setae 16 (15–20) long on annuli 70–79. Last 5–6 annuli with elongated and linear tubercles. Setae h_2 51 (42–65) long very thin at the apex, h_1 setae 5 (5–6) long.

Genitalia 18 (15–20) long, 27 (22–30) wide. Female genital coverflap with 17–20 striae; *3a* setae 21 (18–24) apart, 16 (11–20) µm long.

Male – Similar to the female, 176 (160–185 *n* = 6 specimens) long, prodorsal shield 29 (25–32) long; *sc* setae 32 (28–36) long; opisthosoma with 64–77 annuli.

Host plant – *Centaurea solstitialis* L. (Fam. Asteraceae), yellow starthistle.

Type locality – on the road from Nevsehir to Aksaray, about 1200 m asl, Cappadocia, Turkey.

Type material – Holotype: 2 females and 1 male on a slide, dated 25 September 2001; Paratypes: many slides prepared from material collected in the same locality on the same date. Other population collected in Goreme, Central Cappadocia, Turkey on 21 June 2002.

Collected by – Cristofaro M., Tronci C.

Other material – Ethanol and dried preserved stems, flowerhead and leaves from which the above slides were made.

Aceria squarrosae de Lillo, Cristofaro et Kashefi

Female (Fig. 3) – Body wormlike, colour whitish, 227 (195–240, *n* = 10) long, 41 (35–48) wide and 45 (40–50) thick. Gnathosoma 27 (24–28) projecting obliquely downwards, chelicerae 23 (20–26) long, seta *d* 7 (6–7) long. Prodorsal shield 27 (24–30) long, 27 (24–33) wide, semi-elliptical in anterior shape with anteriormedian lobe over gnathosoma base 6 (5–7) long; shield pattern composed of median line, admedian, and submedian lines; the submedian lines are curved and posteriorly end in a space included between the admedian line posterior end and the *sc* tubercle. A few dashes are included between the admedian field; many dashes are on the median fields. Tubercles *sc* are on the rear shield margin 21 (19–24) apart, *sc* setae 47 (42–50) long.

Foreleg 31 (27–34) long, tibia 7 (5–8) long, tarsus 7 (6–8) long, *w* 8 (8–9) long distally rounded, empodium simple, 6 (5–6) long, 6-rayed. Hindleg 26 (25–28) long, tibia 5 (4–5) long, tarsus 6 (5–7) long, *w* 10 (9–10) long distally rounded, empodium simple, 6 (5–6) long, 6-rayed.

Coxae ornamented by short striae; *1b* setae 13 (12–15) long, *1b* tubercles 10 (8–12) apart, *1a* setae 23 (21–25) long, *1a* tubercles 8 (7–9) apart, *2a* setae 48 (45–52) long, *2a* tubercles 20 (19–23) apart. Prosternal apodeme 4 (4–5) long.

Opisthosoma with 61–75 annuli. Rounded microtubercles on the rear margins of the annuli. Setae c_2 29 (23–33) long on annuli 8–10, *d* setae 60 (55–65) long on annuli 22–27; *e* setae 22 (20–27) long on annuli 38–44; *f* setae 22 (20–25) long on annuli 58–69. Last 5–6 annuli with elongated and linear tubercles. Setae h_2 76 (66–80) long very thin at the apex, h_1 setae 7 (6–8) long.

Genitalia 12 (10–14) long, 21 (20–22) wide. Female genital coverflap with 12–18 striae; *3a* setae 16 (14–18) apart, 20 (18–22) µm long.

Male – Similar to the female, 176 (165–185 *n* = 4 specimens) long, prodorsal shield 27 (27–28) long; *sc* setae 38 (36–42) long; opisthosoma with 61–62 annuli.

Host plant – *Centaurea squarrosa* Willd. (Fam. Asteraceae), squarrose knapweed.

Type locality – 30 km to Askary, on the road from Nevsehir to Aksery (38°29.39' N, 34°16.20' E), Cappadocia, Turkey.

Type material – Holotype: 2 females on a slide, dated 30 July 2001; Paratypes: many slides prepared from material collected in the same locality on the same date.

Collected by – Kashefi J.

Other material – Ethanol-preserved stems and leaves from which the above slides were made.

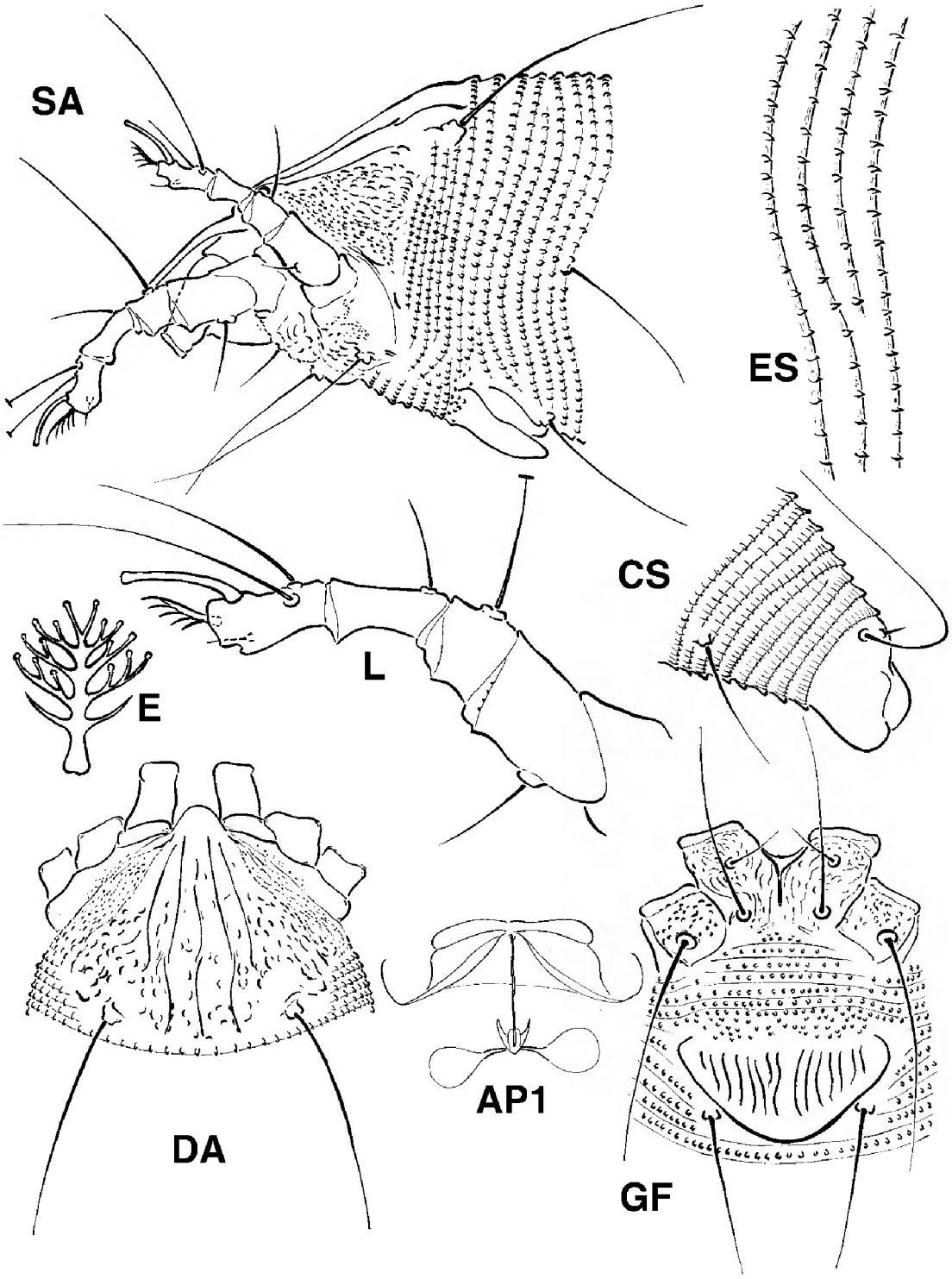

Figure 2. *Aceria solstitialis* de Lillo, Cristofaro et Kashefi: semischematic drawings.

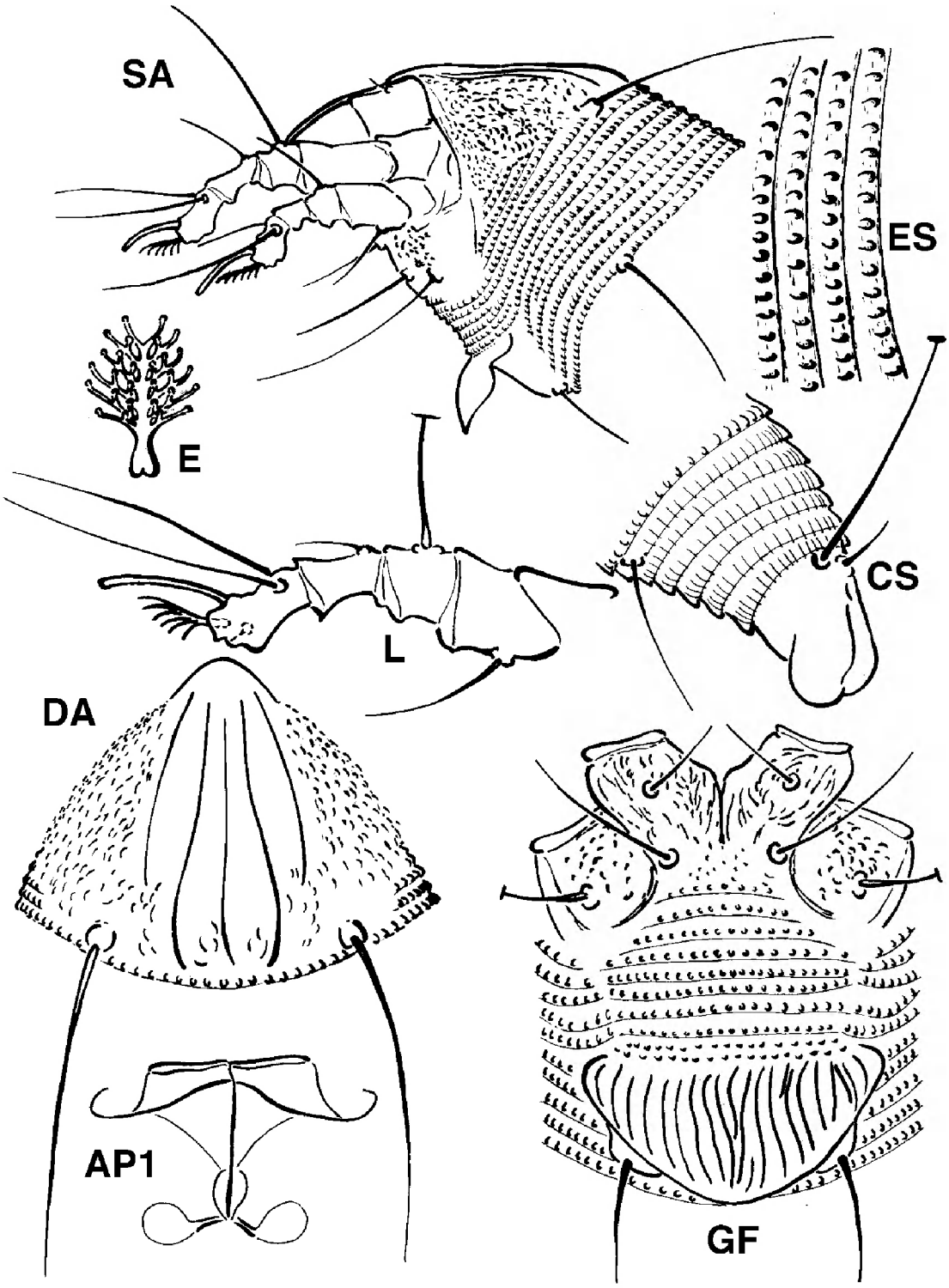

Figure 3. *Aceria squarrosae* de Lillo, Cristofaro et Kashefi: semischematic drawings.

Ecological aspects

The mite populations of these new *Aceria* spp. were found in Cappadocia, a particular highland region of central Turkey, characterized by a dry continental climate. They occur in dry open habitats, with sandy or rocky soil, on stunted plants showing a reduced growth (15–20 cm tall instead of 70 cm tall), with a heavy broom-like and bushy appearance. The apical stems and the inflorescences are fresh and green during the hot and dry season, and the smaller seedheads have flexible, soft spines (Fig. 4). First symptoms were observed on rosettes in early June, while the distortion of the flowerhead spines appeared in early July. Galled plants remained green in the field for a longer period than did the healthy plants, until the end of September. The damage apparently causes a reduction of biomass, especially for young plants. The most typical damage is the distortion and failure of flowerheads to develop, consequently reducing seed production. Unfortunately, these symptoms are similar for all the *Aceria* found on *Centaurea* in Turkey, so it is not possible, at least at present, to clearly distinguish these species based on the morphology of infested plants. Moreover, no plants have been found containing populations of both *A. solstitialis* and *A. solcentaureae*. Nor do we have reason to presume the presence of deutogynes. More field observations and laboratory tests could provide a better understanding.

These symptoms cannot be confused with those produced by *A. centaureae* and *A. brevisetosa*, which induce blister galls, discoloration etc. on the leaves of many *Centaurea* spp. (Cotte 1924, Castagnoli & Sobhian 1991).

Discussion and conclusions

Three new eriophyid mite species have been described on *C. solstitialis* and *C. squarrosa*. They induce similar effects on the developmental growth of stems and flowerheads. Similar symptoms and morphology have been previously observed for other *Aceria* found on other knapweeds. The very small morphological differences between the different eriophyids infesting closely related host plants might be explained by the large number of *Centaurea* species and by a co-evolution process that has been inducing a pool of sibling *Aceria* species, specifically adapted to each plant species.

Eriophyid mites are considered extremely important for biological control of weeds (Briese & Cullen 2001). In the current case, their narrow host range, combined with a strong impact on the target plant, multivoltine life cycle and great fertility, give these agents the possibility of playing a key role in controlling both annual and perennial knapweeds. Their attack often produces an apparent decrease of the biomass and seed production of the target weeds. Further evaluation of the eriophyoid species associated with weeds belonging to the genus *Centaurea* could result in the discovery of effective agents for the biological control of these target weeds in North America, especially considering the apparent high degree of host plant specificity.

Figure 4. *Centaurea solstitialis* L. showing symptoms produced by *Aceria solstitialis* (above), and *Aceria solcentaureae* (below).

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Flea beetles (Coleoptera: Chrysomelidae) associated with purple loosestrife, *Lythrum salicaria*, in Russia

Margarita Yu. Dolgovskaya,[1] Alexander S. Konstantinov,[2] Sergey Ya. Reznik,[1] Neal R. Spencer[3] and Mark G. Volkovitsh[1]

Summary

Purple loosestrife, *Lythrum salicaria* L., has become one of the more troublesome wetland exotic invasive weeds in Canada and the United States from initial introductions some 200 years ago. In the US, purple loosestrife has spread to most of the contiguous 48 states (no records from Florida) with the highest density in the north-east. *L. salicaria* is now recorded in all Canadian provinces with the exception of Yukon and the North-West Territories. A biological control effort begun in the 1970s resulted in the introduction in the 1990s of four insect species: a root-boring and a flower-feeding weevil, and two leaf beetle species (both adults and larvae are leaf feeders). As long-term impact assessments of these introductions are conducted, additional research is looking at other potential biological control agents, particularly insect species attacking both leaves and roots of the target plant. Thus, flea beetles with root-feeding larvae and leaf-feeding adults may be of value. Purple loosestrife is widespread in Russia in wet meadows, riverbanks and other moist habitats from the Baltic region to eastern Russia. Literature searches, studies of museum collections and ecological observations in the field and the laboratory suggest that a number of flea beetle species feed on *L. salicaria*, of which the oligophagous *Aphthona lutescens* with a flexible life cycle and two-fold impact on the host (larvae are root-borers and adults are leaf feeders) appears to be a particularly promising biocontrol agent.

Keywords: *Aphthona lutescens*, flea beetle, biological control, *Lythrum salicaria,* purple loosestrife.

Introduction

Purple loosestrife, *Lythrum salicaria*, is a deep-rooted perennial plant of Eurasian origin infesting wetlands and semi-aquatic habitats. It has become a particularly troublesome species in both the US and Canada, spreading over 48 states from Maine to California and in all but the two most northern provinces of Canada (Stuckey 1980, Thompson *et al.* 1987, Mal *et al.* 1992, Mullin 1998). Since the 1970s, a biological control research program targeting this weed has resulted in the introduction of four phytophagous insect species. Two weevil species, the root-borer, *Hylobius transversovittatus* Goeze, and a flower and seed-feeding weevil, *Nanophyes marmoratus* Goeze (Coleoptera: Curculionidae), are now established in the US and Canada. Two leaf beetles, *Galerucella calmariensis* L. and *G. pusilla* Duft. (Coleoptera: Chrysomelidae) whose adults and larvae feed on the above ground portions of *L. salicaria* have also become widely distributed (Batra *et al.* 1986, Blossey & Schroeder 1995, Hight *et al.* 1995).

The four introduced insect biocontrol agents are well established and local impact on purple loosestrife has occurred (Hight *et al.* 1995, Katovich *et al.* 1999, Katovich *et al.* 2001). However, The Invaders database (www.invader.dbs.umt.edu) lists purple loosestrife as noxious in 18 states (Anderson 1995, Hager & McCoy 1998, Mullin 1998, Blossey *et al.* 2001), although it has been argued that the environmental impact of purple loos-

[1] Zoological Institute, 199034, St Petersburg, Russia.
[2] Systematic Entomology Laboratory, USDA, National Museum of Natural History, Washington, DC 20013, USA.
[3] USDA/ARS/PPRU, Federal Plant Soil & Nutrition Lab, Tower Road, Cornell University, Ithaca, New York 14853, USA.
Corresponding author: M. Yu. Dolgovskaya, Zoological Institute, 199034 St Petersburg, Russia <rita@MD12306.spb.edu>.

estrife in North America has been overrated (Hager & McCoy 1998). Based on future potential need, research on new biocontrol agents was conducted.

Previously, the search for loosestrife biocontrol agents was concentrated in western and central Europe (Batra *et al.* 1986, Blossey 1995), although the natural range of *L. salicaria* is spread over Eurasia. In Russia, purple loosestrife is rather common in wet meadows, riverbanks and other flooded locations. For this reason, exploration for new biocontrol agents has been conducted in Russia. Among other phytophagous insects associated with *L. salicaria*, four species of flea beetles (Coleoptera: Chrysomelidae) were studied in the field and two species were screened for host specificity for purple loosestrife.

Materials and methods

Most of the field collections and field observations were conducted in Krasnodar territory (Russia). In addition, the search for potential purple loosestrife biocontrol agents was performed in natural stands of *L. salicaria* from north-western Russia (Karelia and Leningrad province) to the Caspian Sea (Kalmykia and Astrakhan province).

Laboratory studies were conducted in the Zoological Institute of the Russian Academy of Sciences, St Petersburg, Russia. Plants for the laboratory experiments were grown in a greenhouse and biological observations made as noted below. Standard moderately wet soil in 500 mL pots was used and artificial light was provided by special fluorescent lamps (Osram Fluora®) adapted to photosynthesis. *L. salicaria* plants were started from roots and stem parts collected in natural environments in Krasnodar territory. Raspberry (*Rubus idaeus*) and strawberry (*Fragaria magna*) plants used for host-specificity tests were grown under the same conditions from commercially supplied transplants of local varieties. These two plant species were selected for preliminary host-specificity tests because earlier studies with *Galerucella calmariensis* L., another leaf beetle that fed on *L. salicaria,* have shown that several Rosaceae, and particularly *Fragaria* × *ananassa,* were rather suitable for adult feeding and survival (Kaufman & Landis 2000).

For insect rearing, biological observations and experiments, certain plants were covered with transparent individual cages of 20 cm diameter and 35 cm height. Separate leaves (leafstalks wrapped with wet cotton and placed in a small plastic tube filled with water) were used to feed individual adults in Petri dishes. Every second day, host plant leaves were changed, and laid eggs collected and counted. Collected eggs were placed on damp filter paper in small Petri dishes. Eclosed larvae were collected daily and transferred with a small brush onto stem bases of potted plants under the same greenhouse conditions.

Adult host specificity was tested using several methods. Feeding, survival and oviposition of individual females under choice/no choice conditions was recorded in Petri dishes with host and/or non-host plant leaves (as described above). In addition, adult feeding, oviposition and progeny survival were checked under choice/no choice conditions on potted plants. Larval feeding specificity was tested in no-choice conditions by transferring neonate larvae to the stem bases of host and non-host plants.

Most of the biological observations were made in bioclimatic chambers with a 15h photoperiod and constant temperatures of 15, 20, and 25°C. Biological observations and host specificity-tests with potted plants were conducted under greenhouse conditions (11h photoperiod, temperature ranging from 22 to 27°C). Other details of the methods are given with the results. Data obtained were treated by standard descriptive statistics (in text and tables, means and SD are given). When necessary, means were compared by Student's t-test.

Results

Following is the list of flea beetle species collected from *L. salicaria* with short notes on their biology and biocontrol potential.

Altica lythri Aube.

This species was quite common on *L. salicaria* in Krasnodar territory. Preliminary observations in field and laboratory conditions suggest that *A. lythri* exhibited a rather wide host range. Under natural conditions, adult feeding was recorded on various plants from different families (Medvedev & Roginskaya 1988, Dubeshko & Medvedev 1989). Thus, this species was not used in the further studies.

Longitarsus callidus Warch.

As far as we know, species of this genus have never been recorded on *Lythrum*, at least in most of the Palaearctic (Medvedev & Roginskaya 1988, Doguet 1994). *L. callidus* is known to occur from France to Kazakhstan (Gruev & Döberl 1997). Its host associations are very poorly known. It is tentatively recorded on *Lysimachia vulgaris* L, *Teucrium* sp. and *Stachys* sp. (Doguet 1994), but these records may be the results of misidentifications. In nature, *L. callidus* was collected at the beginning of May, only on *L. salicaria*. Only 12 adults were collected and 5 of them were ovipositing females. Under laboratory conditions, adults markedly preferred purple loosestrife to strawberry or raspberry leaves (practically no damage to these two species was recorded). The mean lifetime duration was 21 ± 15 days from the beginning of observation, daily fecundity was 2.8 ± 2.4 eggs/female/day, total lifetime fecundity, 54 ± 30 eggs.

Lythraria salicariae Pk.

This species was occasionally collected on *L. salicaria* in Krasnodar territory, together with *Aphthona lutescens*. The data from the literature are rather contradictory. Medevdev & Roginskaya (1988) listed *Lysimachia* spp. as the most common host plants, although they also noted feeding on *L. salicaria*. Dubeshko & Medvedev (1989), however, stated that feeding on *Lythrum* is doubtful. Our observations suggest at least adult feeding on *Lythrum*, but because of the small number of individuals collected, laboratory tests were not performed.

Aphthona lutescens Gyll.

This species was the main object of our investigations. It is fairly widespread in European Russia, Caucasus, middle Asia, northern Africa, southern Siberia and Mongolia (Konstantinov 1998). According to the literature, adults and larvae fed mostly on *L. salicaria* (Medvedev & Roginskaya 1988, Dubeshko & Medvedev 1989). It was also recorded on *Filipendula ulmaria* (Putele 1970) and *Mentha aquatica* (Konstantinov 1996), but these records need verification.

At the end of May 2002, ca. 20 adults of *Aphthona lutescens* were collected from *L. salicaria* in Krasnodar territory. Laboratory biological observations were started on May 29. At this time, only two females laid eggs. Soon, one after another, females started to oviposit. At temperatures of 20 and 25°C, the mean fecundity of ovipositing females was 3.1 ± 2.9 and 5.0 ± 4.1 eggs/female/day, egg survival was 73 and 51%, and the mean time of embryo development was 11.9 ± 1.2 and 7.7 ± 1.2 days, respectively. At the end of August, under both temperature regimes, a few females collected in May continued to oviposit, but by the end of October, all had died. Maximal lifetime fecundity in these females was 418 eggs/female (see Table 1).

Eggs laid by females collected in May were used to establish a laboratory colony and, in the middle of July, the first adults of the new laboratory generation emerged. The larvae developed on roots of potted *L. salicaria* plants under greenhouse conditions. Under these conditions, development from egg to adult stage took about 30 days. Some of the females of the F_1 laboratory generation soon started to oviposit. The F_1 generation, reared again in the greenhouse, produced adults in 30–40 days (F_2).

In July 2002, more than 100 *A. lutescens* adults were collected from the same location. Beginning July 15, oviposition was recorded in the laboratory at constant temperatures of 15, 20, and 25°C. The mean fecundity of young ovipositing females at these temperatures was 2.1 ± 1.6, 5.7 ± 4.9 and 6.7 ± 4.8 eggs/female/day, respectively, i.e. significantly (t-test, $p<0.05$) higher than in overwintered beetles under the same conditions. The mean time of embryo development at 15, 20 and 25°C was 27 ± 3, 11.5 ± 1.3 and 7.7 ± 0.8 days, respectively. Thus, the rate of embryo development depends linearly on temperature with an approximate threshold of ca. 10°C and the sum of effective temperatures estimated at ca. 120 degree-days.

Most of the females collected at the end of summer did not lay eggs. Dissections revealed well developed fat body, suggesting diapause. At the end of October, diapausing adults were placed in low temperature conditions to imitate wintering (food was still provided). After four months (end of February), beetles were transferred to high temperatures of 15, 20 and 25°C. At all temperatures, oviposition was observed in certain of the females suggesting successful reactivation.

Host specificity of adult feeding was estimated in no-choice tests conducted with the females of the first natural generation simultaneously collected at the same location. The results (Table 2) suggest that both strawberry and raspberry leaves are suitable for adult feeding and survival (at least, at the studied sample size, the decrease in adult survival was insignificant). However, the percentage of ovipositing females also decreased when fed with non-host plants, and the sharp decrease in mean total fecundity was significant.

In choice feeding tests, adults markedly prefer purple loosestrife to strawberry or raspberry. When the choice between purple loosestrife and strawberry was offered to six adults kept in Petri dishes and the feeding of each beetle separately recorded every second day during 60 days, feeding on purple loosestrife was observed on 87% of the occasions while that on strawberry on only 7% of occasions. Observations conducted in the tests with the choice between purple loosestrife and raspberry gave similar results (81 and 14%, respectively).

Table 1. Results of laboratory biological observations on *Aphthona lutescens* collected in natural conditions (Krasnodar territory, Russia).

Data	Collection dates		
	May 23	July 6	July 28
Total number of adults observed	11	41	10
Ovipositing females (number and % of adults)	6 (55%)	17 (42%)	1 (10%)
Preoviposition period (days)[a]	4.7 ± 7.7	9.6 ± 2.6	0
Mean fecundity during the observation period (eggs/female/day)	3.4 ± 1.0	5.7 ± 3.6	2.6
Total fecundity during the observation period (eggs/female)	208 ± 136	157 ± 130	129
Total adults survival till November 1 (%)	0	54	60

[a] The period from the beginning of observation to first oviposition.

Table 2. Biological characteristics of *A. lutescens* when fed with purple loosestrife, strawberry, or raspberry in no-choice adult feeding test.

Data	Food plant		
	Purple loosestrife	Strawberry	Raspberry
Number of females studied	41	10	10
Ovipositing females (%)	42	30	30
Total fecundity, eggs/female	157 ± 130	34 ± 39 [a]	11 ± 9 [a]
Adults survival during 60 days, %	70	60	40

[a] Significantly ($p<0.05$) different from the control fed with purple loosestrife.

Table 3. No-choice test with adults in potted plants in greenhouse conditions (5 beetles per plant, 3 plants per each plant species for 30 days).

Test plant	Adult survival	Plant damage		New generation recorded
		Leaves	Roots	
Purple loosestrife	53%, *n*=15	Medium damage	Heavy damage	Larvae, pupae, adults
Strawberry	33%, *n*=15	Medium damage	No damage	Absent
Raspberry	40%, *n*=15	Medium damage	No damage	Absent

In the middle of August, feeding and oviposition tests were conducted on potted plants with the first generation adults. The results (Table 3) agree well with our earlier data. Larval transfer on potted plants in no-choice conditions revealed that successful larval development occurred only on *L. salicaria*.

Impact on the host was evaluated under laboratory conditions. Larvae fed on small secondary roots and at high density (more than 15 mature larvae per plant) caused significant damage and wilting of the potted host plant.

Discussion

Field observations, data obtained from literature (Medvedev & Roginskaya 1988, Dubeshko & Medvedev 1989), and unpublished data from collections of the Zoological Institute (St Petersburg, Russia) suggested that *Aphthona lutescens* is fairly widespread and common from European Russia to eastern Siberia, although the flea beetle can be collected only in large dense patches of purple loosestrife. *A. lutescens* is the only species of the genus *Aphthona* known to feed on purple loosestrife. It can sometimes be confused with *Lythraria salicariae* since both are of similar body shape, size and yellowish in colour with a darker strip along the suture. *A. lutescens* can be easily separated from *L. salicariae* based on the confused punctation of elytra. Other characters are given by Konstantinov & Vandenburg (1996).

The combination of our field survey and laboratory observations suggests the following life cycle of *A. lutescens* in Krasnodar territory. Adults overwinter in leaf mulch or in the soil and emerge at the beginning of May, soon after purple loosestrife produces vegetative growth. This time of emergence is suggested by the relatively short preoviposition period in adults collected on May 23 (Table 1). Specifically, most of the females collected were already ovipositing and only one female started oviposition 20 days after the beginning of observation (which indicates that it was still in diapause when collected). On the other hand, no beetles were collected in the same location in the middle of April. Thus, adult emergence can be approximately positioned at the end of April to early in May.

Under laboratory conditions, the mean duration of oviposition in overwintered females was 70 days (i.e. till the middle of August, although the last female of this cohort continued to lay eggs until the end of September). However, none of the adults collected in May survived until November, which suggested the absence of the second winter diapause, at least among the adults studied. Thus, we suppose that, under field conditions, some of the overwintering adults survive to the end of summer, although the sharp decrease in the adult population recorded in June, suggest that many of them died relatively soon after emergence.

Under greenhouse conditions, at a mean temperature of ca. 25°C, the duration of development from egg to adult stage was ca. 30 days. Considering the temperature in May–June (daily means, *ca.* 20°C) we suppose that, in natural conditions, emergence of the first generation adults begins at the end of June, two months after the reactivation of the maternal females. In the beginning of July, a sharp increase in population density was recorded during our field survey.

At least some of the newly emerged adults of the F_1 laboratory generation soon started to lay eggs. Almost half the adults collected in the field in the Krasnodar territory on July 6 started to oviposit when observed in the laboratory, although the mean pre-oviposition period in this cohort of females was almost twice as long as in the overwintering generation (Table 1), suggesting that many of the females were not yet ready to oviposit and the development of their ovaries continued in the laboratory.

Under natural conditions, the second generation may not emerge until the middle of August. It is not clear if some of them laid eggs before diapause, but obviously most of the second-generation adults enter diapause controlled by some environmental cues, which have not been investigated in this species. Even at the end of July, only one of 10 females collected was ovipositing, yet more than half survived until November (i.e. diapaused).

Our results made it possible to develop a preliminary description of the life cycle that contributes to the survival of *A. lutescens* under a wide range of climatic conditions and natural zones from dry steppes (north Caucasus) to cold wet meadows close to the Arctic Circle (Karelia) and also in sharply continental areas of eastern Siberia. All the overwintered females laid eggs. Then, in each following generation, some of the adults diapaused just after emergence while some of females started oviposition but retained the ability to diapause. This flexible life cycle is rather common among insects, particularly in leaf beetles. The Colorado potato beetle represents one of the best-studied examples (Hare 1990).

Rather strict host specificity is suggested both by field and laboratory observations. Under natural conditions, *A. lutescens* adults were collected only from *L. salicaria*. In the laboratory, while adult beetles fed on purple loosestrife and non-host plants, larval development was recorded only on purple loosestrife.

We conclude that the oligophagous *A. lutescens*, with a flexible life cycle, and two-fold impact on the host (larvae are root-borers, while adults are leaf feeders) may be an effective agent for the biological control of purple loosestrife. The earlier published conclusion by Blossey (1995) giving *A. lutescens* a relatively low score as a potential biocontrol agent for purple loosestrife control was partly based on the hypothesis that it was a univoltine species, which is true only for the northern part of its geographic range.

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The significance of intraspecies pathogenicity in the selection of a rust pathotype for the classical biological control of *Mikania micrantha* (mile-a-minute weed) in Southeast Asia

Carol A. Ellison,[1] Harry C. Evans[1] and Judith Ineson[2]

Summary

Mikania micrantha, commonly known as mile-a-minute weed (Asteraceae), is a vine of Neotropical origin, which has become an important, invasive weed within the moist tropical zones of Southeast Asia. A classical biological control program focusing on the potential of fungal agents, evaluated three rust pathogens, *Puccinia spegazzinii, Dietelia portoricensis* and *Dietelia* sp. nov., which occur within the native range of the plant. These rusts were found to have distinct and disparate geographical distributions. *Puccinia spegazzinii* is the widest-ranging species and 16 pathotypes were collected from eight countries, together with one isolate each of the other two species. Using molecular techniques, the genetic variability of *M. micrantha* throughout its native range was analyzed (21 accessions from nine countries) and compared to that in the exotic range (29 accessions from nine countries). The results show that great genetic variation occurs within the Neotropics, whilst in the exotic or palaeotropic range the genetic base is narrow, indicating that those populations originated from only a few introductions. The molecular data were compared with an extensive cross-inoculation program undertaken between selected accessions of *M. micrantha* (25) and rust pathotypes (9). These studies have been instrumental in the selection of the rust strain most suitable for the first target area of release (southeast India). An isolate of *P. spegazzinii* from Trinidad has been recommended for introduction. This will be the first fungal agent to be released against any weed in Southeast Asia and permission to import this rust into quarantine has been granted by the Indian Authorities. The anticipated success of this rust in relation to the results of the intraspecies specificity testing is discussed.

Keywords: intraspecies variation, invasive alien weed, *Mikania micrantha*, molecular techniques, rusts.

Introduction

Mikania micrantha Kunth. ex H.B.K. (Asteraceae) is a Neotropical invasive weed that can smother both agroforestry and natural forest ecosystems, as well as many crops within home garden and plantation production systems in the tropical moist forest zones of Southeast Asia; tea and plantain are particularly severely affected (Holm *et al.* 1977, Waterhouse 1994). It was deliberately introduced into Asia, particularly for use as a cover crop in rubber (Wirjahardja 1976), from as early as 1918 (Cock *et al.* 2000). It is regarded as a major weed in many countries, and is still in its invasive phase. Current control focuses on cultural (slashing) and chemical (herbicides) methods, but this is expensive, often ineffective, not sustainable and can be environmentally damaging (Palit 1981, Muniappan & Viraktamath 1993).

[1] CABI Bioscience UK Centre (Ascot), Silwood Park, Ascot, Berks., SL5 7TA, UK.
[2] CABI Bioscience UK Centre (Egham), Bakeham Lane, Egham, Surrey, TW20 9TY, UK.
Corresponding author: C. Ellison, CABI Bioscience UK Centre (Ascot), Silwood Park, Ascot, Berks., SL5 7TA, UK <c.ellison@cabi.org>.

A collaborative project that ran between 1996 and 2000, to investigate an IPM approach for the control of the weed in the Western Ghats, India, was funded by the UK Department for International Development (DFID), through the Natural Resources Institute's Crop Protection Program. The project involved three Indian organisations; Kerala Forest Research Institute (KFRI), Project Directorate of Biological Control (PDBC) and Assam Agricultural University (AAU), as well as CABI Bioscience (UK). It was concluded that classical biological control (CBC) was the most appropriate long-term solution for the control of this weed (Ellison 2001, Sankaran *et al.* 2001).

A broad range of fungal pathogens has been recorded on *M. micrantha* from its neotropical native range (Evans 1987, Barreto & Evans 1995). From this evaluation, three coevolved, autoecious, microcyclic rust species were selected for further assessment as potential CBC agents against the weed in southern India. These rusts, *Dietelia portoricensis* (Whetzel & Olive) Buriticá & JF Hennen, *Dietelia mexicana* sp. nov. and *Puccinia spegazzinii* de Toni, are all highly damaging to their host in the field, causing leaf, petiole and stem infections leading to cankering and whole plant death. None were found in the exotic range of the weed.

The nine pathotypes (seven of *P. spegazzinii* and one each of the two *Dietelia* spp.) were evaluated in the CABI Bioscience (UK) quarantine glasshouse, and an isolate of *P. spegazzinii* from Trinidad (W1761) was considered to be the prime candidate (Ellison 2001). This pathotype proved to be virulent against accessions collected from a wide range of Indian populations of the weed, and infected all of the accessions from the 10 populations sampled from the DFID target region of the Western Ghats.

A dossier was produced by CABI Bioscience for the Indian collaborators, containing detailed data on *P. spegazzinii*, following the FAO Code of Conduct (FAO 1996, Ellison & Murphy 2001). This was submitted to the India Directorate of Plant Quarantine & Storage by PDBC, together with a letter detailing that permission had been given by the Ministry of Agriculture Land and Marine Resources of Trinidad and Tobago for the use of their genetic resources, following the Convention on Biodiversity (http://www.biodiv.org/). Permission to import the rust into quarantine in India was granted in September 2002 and hand-carriage of the rust to quarantine facilities in Delhi was scheduled for mid-2003. Release in the Western Ghats and Assam was planned for the following year.

From this work it was apparent that these three, coevolved rusts demonstrate intraspecies specificity; each isolate only infecting a selected number of genotypes of its host. From field observations, considerable morphological variation, and hence, potential biotypic differentiation, is apparent within the *M. micrantha* species. This has ramifications for the potential success of CBC of this weed with the selected rust isolate. It is important to know how much genetic variation exists within the exotic range of the weed, and whether the Trinidad isolate has the inherent ability to be successful throughout the range of the weed. Consequently, a detailed cross-inoculation study was undertaken, whereby accessions of *M. micrantha* taken from populations in its native and exotic ranges, were challenged by a range of rust pathotypes. This was paralleled by a molecular analysis of these plant accessions. The preliminary conclusions are presented here.

Materials and methods

Field collections

Over the last decade, samples of living *M. micrantha* plants have been collected by CABI Bioscience personnel throughout both the Neotropics, and the paleotropical invasive range of the weed. These plant samples consisted of one or a few plant accessions, collected from within a population of the plant. Samples of rusts were also collected and brought back to the CABI Quarantine Unit (UK). Since these rusts do not survive drying, it was necessary to transport them on living plants. Each rust isolate was established in quarantine from a single pustule, assumed to have originated from a single basidiospore.

Rust inoculation procedure

Plants used for rust-inoculation studies were propagated from cuttings and grown in a 1:1 mixture of general purpose, peat-based potting compost and John Innes No. 2 soil-based compost. Pre- and post-inoculated plants were maintained in an air-conditioned, quarantine greenhouse chamber set at 22 ± 5ºC and with a humidity of between 50 and 80%. The chamber had a 12-hour light/dark cycle and was fitted with metal halide, full spectrum lamps, providing a light intensity ranging from 8000 to 13,000 Lux, depending on the ambient light. Vigorous test plants, with developing shoots or meristems, were mist sprayed with distilled water and then inoculated by suspending mature rust-infected material *ca.* 5 cm above the shoot apices, using plant ties attached to a wire frame. The plants were transferred to a dew chamber (Mercia Scientific, Birmingham, UK) set at 20ºC, for 24 hours. Under conditions of high humidity, basidiospores were shot-off from the teliospores (*P. spegazzinii*) or aecioid teliospores (*Dietelia* spp.) embedded in the plant tissue, and landed on the fresh host shoots, where they germinated and potentially infected. After removal from the dew chamber, inoculated plants were returned to the quarantine chamber for daily observation.

Molecular characterisation

More than 70 accessions of *M. micrantha* were collected throughout its native and introduced ranges

during the course of the study. A wide, representative selection of 51 accessions was included in the molecular characterization. Full site details are given in Ellison & Murphy (2001). *Mikania micrantha* can be an out-crossing species and thus the purity of each line is maintained by clonal propagation.

The genetic variability of weed samples was assessed by amplified fragment length polymorphism (AFLP). DNA was extracted from fresh leaf material using a Nucleon Phytopure DNA extraction kit (Tepnel Life Sciences, Manchester, UK). The AFLP protocol used was adapted from Mueller *et al.* (1996). The only variation from the published protocol was the introduction of a pre-amplification step to help increase the yield and uniformity of the selective AFLP profiles. A total of five selective primers was used with the following selective nucleotides; AC, AG, CG, CT, and GT. The AFLP profiles were separated by electrophoresis through 1.5% (w/v) agarose gels (SeaKem LE, BMA, Wokingham, UK), which were run at 100V for 6 hours, stained with ethidium bromide and photographed. Gel photos were imported into GelCompar (Applied Maths, Kortrijk, Belgium) and a composite dendrogram of all five primers was produced using the unweighted pair group method using arithmetic averages (UPGMA) and derived with the Dice coefficient.

Cross-inoculation studies

A representative range of nine rust pathotypes was selected for this study, from the 16 that had been collected during the CABI surveys. Their site details are given in Ellison & Murphy (2001). All plants used were clonally propagated from original stock plants. Three plants were inoculated per individual cross-inoculation, and this was repeated at least twice following a fully susceptible response, and four times when no symptoms or a semi-resistant response was observed.

The following pathogenicity scores were used for the evaluation of the rusts:

0 No macroscopic symptoms.
1 Necrotic spots on inoculated vegetative parts — no sporulation.
2 Abnormal infection site: chlorotic patches on vegetative parts with very low teliospore or aecioid teliospore production around edges of chlorosis.
3 Abnormal infection site: pustules reduced in size with low teliospore or aecioid teliospore production in relation to compatible-host pathogen interaction.
4 Normal pustule formation, in relation to compatible-host pathogen interaction.

Results and discussion

Distribution of rust pathogens

The current records of the three rust species within the Neotropics are shown in Figure 1. *Puccinia spegazzinii* is the most widespread and was collected at altitudes ranging from near sea level to ~1200 m, whereas *D. portoricensis* appears to be restricted to Central America and *D. mexicana* sp. nov. has been recorded from Mexico only. All three species are highly damaging and appear to be restricted to their host. Due to the wide distribution of *P. spegazzinii*, suggesting a broad environmental adaptability, which is supported by the glasshouse data, this species was selected as the primary classical biological control agent for *M. micrantha* in its exotic range.

Figure 1. Distribution of rust pathogens infecting *Mikania micrantha* in its native range (★ = *Puccinia spegazzinii*; ▲ = *Dietelia portoricensis*; ● = *Dietelia* sp. nov.).

Molecular characterisation

The dendogram constructed from the *M. micrantha* accessions is given in Figure 2, and the following generalizations can be drawn from these data:

- The genetic diversity in the native range is greater than that in the exotic range.
- The results do not provide information on the origins of the exotic range weed populations; with the exception of one accession from Indonesia that is appears to be similar to a genotype from Jamaica.
- With the exception of Jamaica, the accessions from the native range and the exotic range show a maximum of 67% similarity (between Australia and Brazil/Peru).
- There are numbers of genetic types which appear in more than one region in the exotic range, suggesting possible roots of distribution of the weed. Examples include the following: Sri Lanka and India; Nepal and India (Assam); PNG and Indonesia (West Java); Malaysia, Philippines and India.

- Populations from Indonesia appear to be more genetically diverse than those from India, although both regions appear to have a similar number of separate introductions of the weed (5).

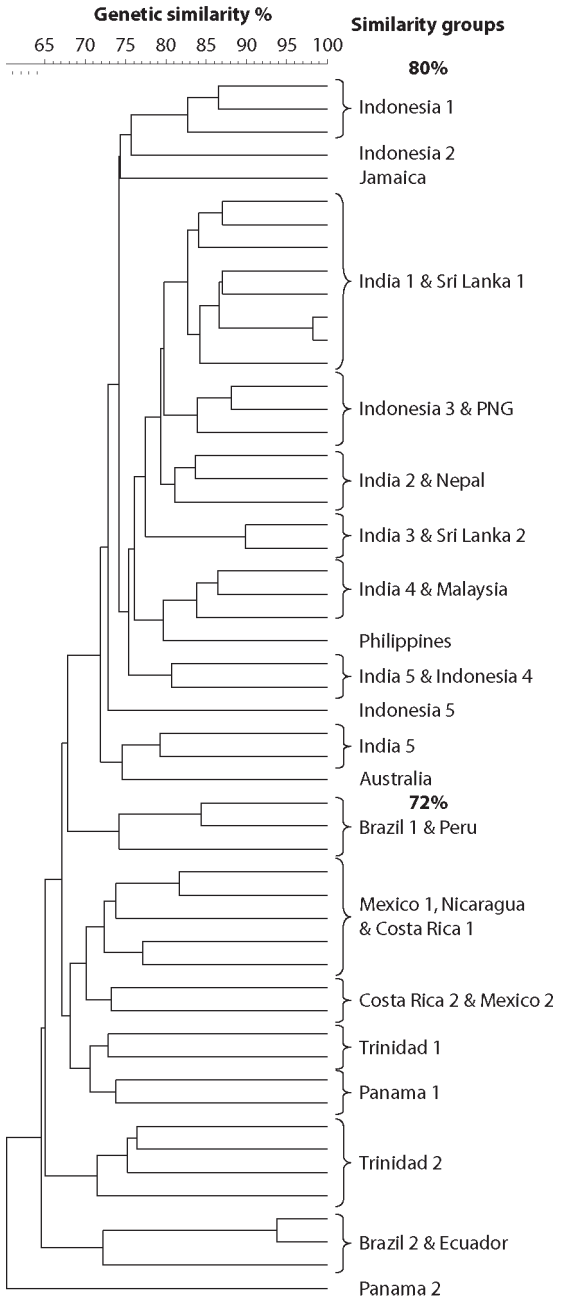

Figure 2. Dendogram of *Mikania micrantha* populations.

Cross-inoculation studies

The results are shown in Table 1. The following two overall generalisations can be made of these summarized data:

- The biotypes of *M. micrantha* within its native range are resistant to most of the rust pathotypes not present within their area of distribution.
- Most accessions of *M. micrantha* from its exotic range are susceptible or at least partially susceptible to all rust pathotypes.

The differential infection type responses occurring in particular host accession – pathogen isolate combinations indicate that qualitative resistance appears to be widespread in this interaction (Thrall & Burdon 2002). Most of the rust isolates that were studied in detail came from highly disparate populations of *M. micrantha.* Hence, it is not possible to draw conclusions about the size of each pathotype-susceptible population and whether more than one pathotype exists within a population; or indeed about the extent of variation within individual plant populations.

The absence of resistance to the rust pathotypes within the exotic range weed populations provides superficial support for the idea that resistance of this type has a metabolic cost that is selected against in the absence of the pathogen – as would occur when the plant is carried to a new environment (Thompson 1990). However, this possibility cannot be definitively concluded from these data, since the resistance status of the plants originally introduced into the Neotropics is unknown. It is conceivable that all introduced lines were from rust-susceptible Neotropical populations.

Exotic range populations of the weed were observed to have a vigorous growth form when compared with plants growing in their native range. All genotypes retained their field characteristics when grown under the same conditions in the glasshouse. This may suggest that gene-for-gene resistance, or perhaps a linked factor, carries a significant metabolic load. In addition, all three rusts, when inoculated onto fully susceptible populations of the plants in the exotic range, are highly aggressive (large pustules, plant death common). Conversely, these rust pathotypes are significantly less aggressive on their susceptible, native range biotypes (smaller pustules, less severe plant damage). However, again, without detailed knowledge of the plants that were originally introduced, this remains only an interesting, but unsubstantiated observation. Indeed, since the plants were originally introduced as a cover crop, it is likely that the most vigorously growing plants were selected.

The intermediate, semi-resistant rust pathogenicity could be governed by either gene-for-gene resistance, or pathotype-non-specific, multi-gene, horizontal resistance, though it would be expected that all the genotypes present in the exotic range would show a similar response to all the pathotypes if horizontal resistance was responsible (J.J. Burdon, pers. comm.). The intermediate pathogenicity reaction was equally expressed in both the native and exotic ranges of the plant, which may suggest that this type of resistance is not so readily lost from the exotic range populations as the gene(s) governing an immune response. However, again, lack of information on the resistance status of the plants originally introduced allows only speculation. If

Table 1. Summary of intraspecies pathogenicity of *Puccinia spegazzinii* and *Dietelia portoricensis* isolates against world-wide populations of *Mikania micrantha*.

Mikania collections[a,b]/ selected population	Host reactions to rust isolates[c]								
	Puccinia spegazzinii[a]							*Dietelia portoricensis*[a]	
	Argentina (1)	Peru (1)	Brazil (4)	Ecuador East [Na] (1)	Ecuador West [Im] (1)	Trinidad (4)	Costa Rica [telia] (1)	Costa Rica [aecia] (1)	Mexico [aecia] (1)
Argentina (1)	✔	–	–	–	–	✗	–	–	✗
Peru (1)	✗	✔	✗	✗	✗	✗	✗	–	✗
Brazil (6)	–	–	✔	–	–	✗	✗	✗	✗
Ecuador Napo (eastern)	✗	±	✗	✔	✗	✗	✗	–	✗(?)
Ecuador Imbabura (western)	–	–	–	–	✔	±	–	–	–
Trinidad (4)	✗	✔(–)	±	✗	±	✔	✗	✗	±
Jamaica (1)	✔	±	✗	✔(–)	✔	✔	✔	–	±
Mexico W1904	–	–	✔	–	–	✗	✔(?)	✔	✔
Costa Rica 17–1	✗	±	±	✗	✗	✗	✔	✔	✔
Nicaragua	±	✔	✗	✗	✔(–)	✗	✔	–	–
Panama (2)	±	–	±✗	✔	–	±✔	✔	✔±	±
India south-west (10)	✔	✔	±✔,✗	✔	✔	✔	✔	✔,✗±	✔,✗
India north-east (7)	✔±	✔(+)	±✔(–)	±✔(–)	✔(–)	±✔	±	✔	✔(+)
Nepal (1)	✔	✔	✔	✔	✔	✔	✔	✔	✔
Sri Lanka (1)	–	–	–	–	–	✔	–	–	–
Malaysia (2)	✔	–	✔±	✔	✔	✔	✔	–	✔±
Philippines (1)	–	–	✔	–	–	✔	–	–	–
Indonesia (6)	✔	✔	✔(–)	✔(–)	✔	±4	✔	–	✔
Papua New Guinea (1)	✔		✔	✔		✔			
Australia (1)	✔	✔(–)	✔±	✔(–)	✔(+)	✔(–)	✔	✗	±

[a] Number of collections or isolates assessed.
[b] Not all combinations have been assessed. ? = Unclear result, confirmation still required.
[c] Host reactions: ✔ = fully compatible (pathogenicity score ✔); ✔(+) = first choice if Trinidad pathotype not fully compatible; ✔(–) = Fully compatible, but number and size of pustules reduced in comparison to controls; ± = semi-resistance response (pathogenicity score 2/3); ✗ = not compatible (pathogenicity score 0/1); – = not tested;

the semi-resistance response is governed by a number of genes, it could be argued that the horizontal resistance is gradually being eroded within the exotic range, but requires a longer period of evolution than the vertical resistance based on single genes. The metabolic cost of keeping these genes (or those linked to them) may not be as significant. Indeed, some populations of the weed are fully susceptible to all pathotypes of the rusts, and perhaps these populations originate from the earliest introductions of the weed. Alternatively, the multi-gene resistance may still be useful to the plant in reducing susceptibility to generalist pathogens in the exotic range.

Conclusions

Although the centres of origin of most of the exotic range population of the plant were not elucidated by the molecular characterisation, the results of the cross-inoculations did not suggest that it is necessary to obtain rust isolates from the specific area of origin of the weed genotype. The cross-inoculation studies indicated that most or possibly all the populations present in the exotic range of the weed are fully susceptible to one or more rust pathotypes. This may be because the original populations that were introduced into Asia were taken from populations of *M. micrantha* that were susceptible to the rust. Conversely, resistance may have been lost in the exotic range populations, isolated from their coevolved rusts. Nevertheless, the presence of a semi-resistance interaction necessitates the need for using the most virulent rust pathotype(s) for the genetic types of the weed present in a particular invaded region.

The relatively narrow genetic base of the weed in its palaeotropical range, confirms the evidence in the literature of a small number of deliberate introductions of the plant (Wirjahardja 1976, Cock *et al.* 2000). This factor makes the concept of selecting different pathotypes for different target regions a feasible approach for CBC of the weed. It is proposed that a relatively quick and inexpensive DNA screen may facilitate this selection. For example, it is clear that the isolate of *P. spegazzinii* selected for use in the Western Ghats region of India is not the optimal pathotype match for the

genetic weed types present in Assam. The isolate from Peru, or *D. portoricensis*, would be the optimal choice available. A full host-specificity screening program would be required before other rust species could be considered for introduction, but it is not clear whether this would be required for the introduction of an additional pathotype of the same species. It is important that careful monitoring of the weed is undertaken after the release of a rust pathotype, since there is a risk that less susceptible genotypes of the weed will fill the vacant niche (Burdon 1991). Fortunately, this work suggests that there is a wealth of pathotypes to select from within the native range of the weed.

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Two shoot miners as potential biological control agents for garlic mustard: should both be released?

E. Gerber,[1] H.L. Hinz,[1] B. Blossey[2] and S. Bacher[3]

Summary

Two shoot-mining weevils, *Ceutorhynchus alliariae* and *C. roberti,* both potential biological control agents for *Alliaria petiolata* in North America, show high temporal and spatial niche overlap. To select an appropriate future release strategy, we investigated the capacity of different weevil combinations to attack the target plant. We tested *C. alliariae* alone and *C. alliariae* in combination with *C. roberti*, both in field sites and under experimental conditions. The comparison of attack levels as an indirect estimate of their potential to damage garlic mustard revealed that in both cases, *C. alliariae* is at least equally as effective in attacking garlic mustard alone as in combination with *C. roberti*. Under experimental conditions, *C. alliariae* alone reached even higher infestation levels than the mixed species treatments. However, the higher attack levels did not result in a higher impact on garlic mustard. Provided *C. alliariae* and *C. roberti* prove to be equally specific once host-range tests are completed, two release strategies can be envisioned: a) only one of the two species will be released to minimize potential non-target effects. Its establishment and impact will be closely monitored, and the second species will only be released if the first species fails to establish in all habitats or does not provide the expected impact. b) Both species will be released together. Replicated releases of different combinations of the two species, i.e. *C. alliariae* alone, *C. roberti* alone, and both together, would provide us with a unique opportunity to test the conclusions from our pre-release investigations and thereby to test the predictive power of pre-release studies.

Keywords: biological weed control, *Alliaria petiolata*, *Ceutorhynchus alliariae*, *Ceutorhynchus roberti*, pre-release studies.

Introduction

It is a matter of controversy whether successful biological control results from the impact of a single agent (Myers 1985) or the combined effect of multiple agents (Harris 1981, Schröder & Goeden 1986). A recent tendency in biological control is to reduce the number of insects released, since each additional introduction adds an increment of environmental and economic risk (McEvoy & Coombs 1999). We agree with McEvoy & Coombs (1999) that only a subset of the most promising organisms in terms of safety and effectiveness should be released. Presuming that for each weed biocontrol system, there is a "certain" number of agents required to achieve control, each species released in addition to that number is redundant. In the best-case scenario, releasing an extra species has little or no influence, in the worst case, the additional introduction may reduce the overall impact on the target plant (Myers 1985). The risk of redundancy among biological control agents is particularly high if several insects occupying similar feeding niches on the target plant are co-introduced.

Two shoot-mining weevils, *Ceutorhynchus alliariae* Brisout and *C. roberti* Gyllenhal, are currently being investigated as potential biological control agents for *Alliaria petiolata* (Bieb) Cavara & Grande in North America. Studies on their biology and ecology revealed high temporal and spatial niche overlap (Gerber &

[1] CABI Bioscience Centre Switzerland, Rue des Grillons 1, CH-2800 Delémont, Switzerland.
[2] Department of Natural Resources, 122E Fernow Hall, Cornell University, Ithaca, New York 14853, USA.
[3] Zoologisches Institut, Synökologie, Baltzerstr. 6, 3012 Bern, Switzerland.
Corresponding author: Esther Gerber <e.gerber@cabi-bioscience.ch>.

Hinz, unpublished data). In addition, host-range tests have so far not shown any major differences in their specificity (Gerber *et al.* 2002).

We will therefore face the selection of the appropriate release strategy. Should both species be released? Or is one species sufficient to successfully control garlic mustard – and which one of the two species should we choose for release? One important criterion for this decision is the potential impact of the two weevils on garlic mustard. In general, only agents that reach high population levels have the potential to successfully control a target weed (Gassmann 1996). We therefore considered weevil attack levels as an indirect estimate of their potential to damage garlic mustard and therefore their effectiveness.

In this paper, we compare the results of two different approaches to investigate the effectiveness of *C. alliariae* alone and together with *C. roberti*. Attack levels on *Alliaria petiolata* were measured: 1) at field sites where both species occur and sites where only *C. alliariae* occurs, and 2) in a manipulative experiment, where both species or only *C. alliariae* were released onto potted plants in a common garden. The latter approach was also used to collect quantitative data on the impact of, and potential competitive interactions between, the two species.

Materials and methods

Study organisms

Ceutorhynchus alliariae and *C. roberti* are sibling species in the family Curculionidae. They have almost identical life histories. They overwinter as adults and start to lay eggs in spring. We found no differences in average fecundity or oviposition period (Gerber *et al.*, unpublished data). Larvae of both species mine during April and May in shoots of bolting plants, but also in petioles of rosettes of garlic mustard. No spatial or temporal niche segregation for larvae was found, and they cannot be distinguished morphologically (Gerber & Hinz, unpublished data). Mature larvae leave the plant to pupate in the soil, and adults of the F1-generation emerge about four weeks later. The two species do differ, however, in their geographical distribution. *C. alliariae* and *C. roberti* occur both in geographically isolated (allopatric) and associated (sympatric) populations. Both are considered as monophagous on garlic mustard, a plant of Eurasian origin that was introduced into North America in the 19th century (Cavers *et al.* 1979). The plant has since become one of the most serious invaders in forested areas of the north-eastern and mid-western United States (Blossey *et al.* 2002). Garlic mustard is a strict biennial in the family Brassicaceae that reproduces entirely by seed. Seedlings emerge in early spring and form rosettes over summer. These start to bolt in March/April of the following year and siliques form by June.

Field data

We collected and compared attack data between field sites where only *C. alliariae* occurs (allopatric, $n = 6$) and where both occur (sympatric, $n = 10$). At each site, we randomly collected 12–332 bolting garlic mustard plants along a transect and brought the plants back to the laboratory for dissection under a stereo microscope. The number of larvae and exit holes were recorded separately for each shoot. To calculate attack levels (i.e. average number of larvae per shoot), one exit hole was counted as one larva that had left the shoot.

Experimental data

A manipulative experiment was conducted in a common garden at the CABI Bioscience Centre Switzerland, in Delémont, Switzerland, (47°21'N, 7°22'E) in 2000. Potted, bolting plants of garlic mustard were dug into the ground about 50 cm apart on 12 April 2000. We covered each plant individually with gauze bags (55 cm diameter, 150 cm high), and applied the following treatments: 1, 2, 4 and 8 pairs of *C. alliariae*, 1, 2, 4 and 8 pairs of *C. roberti* and 2, 4 and 8 pairs of the combination of both species in a frequency of 1:1. Plants without weevils were established as controls. Each treatment was replicated 10 times. Adults of both species were collected at garlic mustard sites in Switzerland and southern Germany. The fertility of females was tested and fertile weevils placed on the plants according to treatments on 14 and 24 April. Between 14 and 19 June, we cut the shoots of all plants and stored them at 2°C. Between 15 June and 20 July, we dissected all shoots and recorded the number of larvae still present in shoots and the number of exit holes. We also recorded the impact of the different weevil combinations and densities on the growth and reproductive output of garlic mustard as well as the effect on competitive interactions between the two species. These data will be presented in forthcoming papers. In this paper, we will only present and compare data of attack levels from treatments with 2, 4 and 8 pairs of *C. alliariae* and 2, 4 and 8 pairs of the combination of both species.

Results

Data collected at field sites revealed that garlic mustard shoots are extensively utilized resources. We recorded equally high proportions of attacked shoots and plants at sites where only *C. alliariae* occurred and at sites where both species were present. In the allopatric area, on average 86.9% ± 4.5 (mean ± SE) of plants (range: 70 –100%) and 74.7% ± 8.6 of shoots (range: 40.8 –100%) were attacked, while 87.1% ± 5.4 of plants (range: 40.0–100%) and 78.0% ± 5.9 of shoots (range: 37.7–100%) were infested in the sympatric area (independent samples t-test: plants: $t = -0.27$, df = 14, $P = 0.979$; shoots: $t = -0.331$, df = 14, $P = 0.746$). About

four larvae were found mining in each attacked shoot, irrespective of whether only *C. alliariae* or both species were present (Fig. 1, independent samples t-test: t = –0.552, df = 14, P = 0.589).

Under experimental conditions, *C. alliariae* reached higher attack levels than the combination of *C. alliariae* and *C. roberti* (Fig. 2, Mann-Whitney test: U = 300.00, P = 0.027). Because increasing weevil densities did not increase attack levels (Kruskal-Wallis: *C. alliariae*: χ^2 = 0.919, df = 2, P = 0.632; both species: χ^2 = 0.000, df = 2, P = 1.000), data were pooled over the different densities.

Discussion

Considering the results from our field and experimental studies, we reach the same conclusion. In both cases, *C. alliariae* alone is at least equally as effective in attacking garlic mustard as in combination with *C. roberti*. Under experimental conditions, *C. alliariae* alone reached even higher attack levels compared to the mixed species treatments. However, the higher attack levels did not result in a higher impact on garlic mustard (Gerber & Hinz, unpublished data). Adults as well as larvae of *C. alliariae* tend to be smaller than *C. roberti*, which might explain this result.

The manipulative experiment also allowed us to investigate potential interactions between the two species. Competitive interactions between agents have been documented in several biological weed control programs (Briese 1991, Woodburn 1996, Story *et al.* 2000). In the worst-case scenario, the more effective agent is displaced by a more competitive, but less effective, agent, thereby reducing the overall impact on the

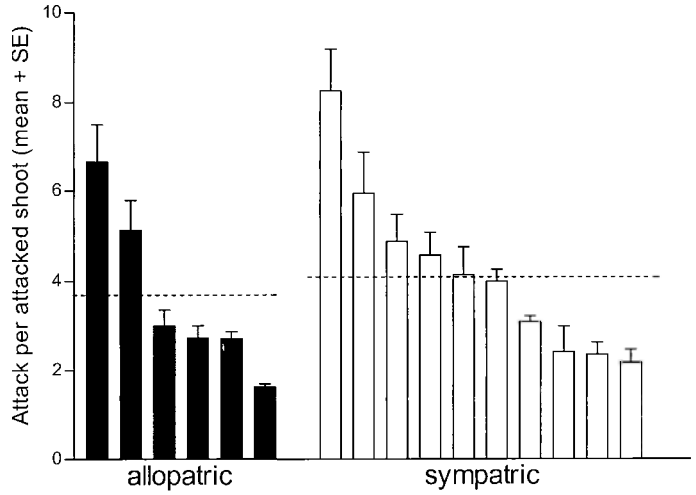

Figure 1. Attack of *Alliaria petiolata* at different field sites – allopatric = field sites in the range where only *C. alliariae* occurs; sympatric = field sites in range where both species occur; black bars = attack by *C. alliariae* at six field sites, white bars = attack by both species at 10 field sites.

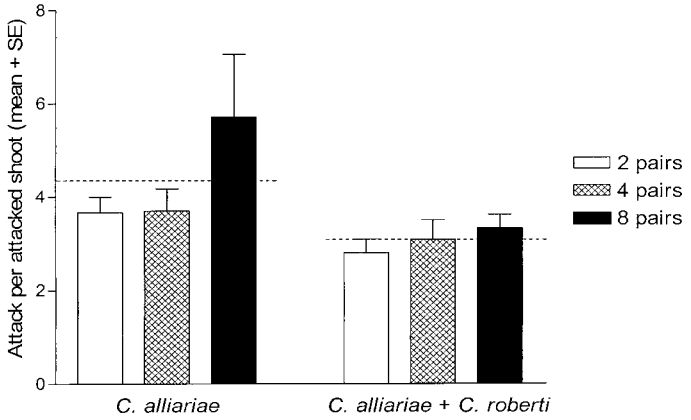

Figure 2. Attack of *Alliaria petiolata* under experimental conditions. Bars are means of 10 replicates (plants) each – dotted lines = overall mean for each weevil composition. Attack corresponds to the sum of all larvae and exit holes found upon dissection.

target weed (Woodburn 1996). In the case of *C. alliariae* and *C. roberti*, the release of increasing weevil densities did reduce the number of offspring produced per female, however the reduction was the same, whether females of the same or females of both species were released, i.e. intra- and interspecific competition were equally strong (Gerber & Hinz, unpublished data). We therefore do not expect that the two species would negatively affect each other's establishment or impact if co-released. In a similar way to *C. alliariae* and *C. roberti* on garlic mustard, the two leaf beetles *Galerucella calmariensis* L. and *G. pusilla* Duftschmid occupy the same fundamental niche on purple loosestrife (*Lythrum salicaria* L.) and have identical competitive abilities (Blossey 1995a). They were jointly released in 1992 for the control of this invasive weed in North America. Up to now, no signs of competitive exclusion or negative effects on their establishment or impact have been observed (B. Blossey, pers. observ.).

For the biological control of leafy spurge, *Euphorbia* spp., five flea beetle species, *Aphthona* spp., were released in North America (Gassmann *et al.* 1996). Larvae and adults of the different species have the same feeding niche on their host plant, but the species differ in their habitat preferences (i.e. three species predominate in open dry habitats, two prefer moister sites), which led to differential establishment according to habitat (Nowierski *et al.* 2002). In the case of the two weevils investigated, *C. alliariae* is reported to prefer shaded habitats, while *C. roberti* is supposed to occur more frequently in open habitats (Pencke 1928, Strejcek 1969). Our investigations confirmed the latter, but we did not find evidence for habitat preferences of *C. alliariae* (Gerber *et al.*, unpublished data). Nevertheless, such subtle differences might contribute to ultimate differences in the impact of these two weevils in different microhabitats.

The advantage of comparing attack levels of two potential agents at field sites is that the species can freely move between plants and choose which shoots to infest, leading to realistic results obtained under natural environmental conditions. In the case of our investigation, the comparison of attack levels is however confounded by the fact that the two ranges, i.e. allopatric and sympatric, lie in different geographical regions that differ in climatic conditions. We cannot exclude the possibility that the conditions in the allopatric area are intrinsically more suitable for the development of *C. alliariae*. In addition, the population of *C. alliariae* in the allopatric area might be higher due to the absence of a key predator or parasitoid. These potential differences are excluded in our experimental approach, conducted under controlled and standardized conditions. Data on the attack levels of *C. alliariae* alone and in combination with *C. roberti* are therefore directly comparable. The disadvantage is, however, that females were confined to individually potted plants, which in itself might influence the outcome of the experiment if, for instance one of the species is more sensitive to these conditions. Hence, results of such experiments cannot necessarily be extrapolated to natural conditions. In addition, if one of the two species is, for example, more vulnerable to parasitoids or predators, its population size could be limited, and in turn its effectiveness as a biological control agent. Such differences would not have been detected under the experimental conditions used.

Provided *C. alliariae* and *C. roberti* prove to be equally specific once host-range tests are completed, two release strategies can be envisioned:

1. Only one of the two species is released to minimize the danger of potential non-target effects. At the moment, we would suggest that *C. alliariae* should be released first, because it is found equally often in all habitat types (Gerber & Hinz, unpublished data). Its establishment and impact would be closely monitored, and *C. roberti* would be only released if *C. alliariae* fails to establish in all habitats or does not provide the expected impact.
2. Both species are released together. In this case, we would make replicated releases of different combinations of the two species, i.e. *C. alliariae* alone, *C. roberti* alone, and both together, thereby testing the conclusions from our pre-release investigations with the following predictions: firstly, both species will establish and co-exist, and secondly, each species alone will have a similar impact as both species together. Such carefully planned release experiments provide a unique opportunity to test the predictive power of pre-release studies conducted in the area of origin of the target weed and are essential if we want to improve the success rate and credibility of biological weed control programs (Malecki *et al.* 1993, Blossey 1995b).

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We would like to thank E. Gault, N. Guazzone, A. Hoffmann, K. Jackson, J. McKenney, S. Michler, C. Thalman and M. Zuefle for their assistance in the field and laboratory and to B. Klander who provided additional data on attack levels of *C. alliariae* in northern Germany. Dr U. Schaffner is gratefully acknowledged for fruitful discussions and suggestions concerning the experimental design and Dr A. Barker for revisions of the text.

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Pre-release evaluation and host-range testing of *Floracarus perrepae* (Eriophyidae) genotypes for biological control of Old World climbing fern

J.A. Goolsby,[1] J.R. Makinson,[2] D.M. Hartley,[3] R. Zonneveld[2] and A.D. Wright[2]

Summary

A biological control program for *Lygodium microphyllum*, an invasive climbing fern in Florida, USA was initiated in 1997. Surveys for natural enemies were conducted in the fern's native range which includes Australia, Asia and Oceania. Twenty-two herbivores were documented, including an eriophyid mite, *Floracarus perrepae* Knihinicki & Boczek. Molecular diagnostics were used to match the origin of the invasive Florida population with the native range. The population from Cape York, Queensland was found to be an exact match with the invasive populations in Florida for the two chloroplast DNA sequences analyzed. Field studies of *F. perrepae* were conducted, which found that the mite was active year-round, with populations peaking during periods of ample soil moisture. Predator mites and a pathogen had significant impacts on *F. perrepae* populations, but heavy plant damage was still observed. Pre-release field impact studies revealed that *F. perrepae* caused more than 50% impact on *L. microphyllum* biomass production over a two-year period. Several genotypes of *F. perrepae* were identified from south-eastern Queensland, New Caledonia, China, Thailand, India/Sri Lanka, and Cape York. Each of these populations was screened for their acceptance of the invasive Florida genotype of the climbing fern. The populations from Cape York and Thailand performed best and came from fern genotypes that were most closely related to the Florida genotype.

Keywords: agent selection, matching plant origin, screening mite genotypes.

Introduction

Old World climbing fern, *Lygodium microphyllum*, is an invasive weed in southern Florida, USA, including the Everglades (Pemberton & Ferriter 1998). It is indigenous to the wet tropical and subtropical regions of the Old World (Pemberton 1998). Although the fern was introduced into Florida in the 1890s, it did not become a serious invasive weed until the 1990s. A biological control program was initiated for this weed in 1998, which is a part of the National Everglades Restoration Program.

As part of the surveys for natural enemies of *L. microphyllum,* plant samples were collected to be used for molecular analysis with the aim of matching the invasive Florida population with populations in the native range. We initially used RAPDs to distinguish populations, but then switched to gene sequencing, which proved to be more informative. Several genes were initially sequenced including: CO1, ITS1, and D2, but they failed to show significant differences. The chloroplast genes TrnF-TrnL and rps4-TrnS showed the greatest variation among populations. We used the technique developed by Thomson (2000) for the chloroplast genes and identified unique *L. microphyllum* genotypes from Ghana, Australia (Queensland), New Caledonia, China, Thailand, India/Sri

[1] United States Dept. of Agriculture, Agricultural Research Service, Australian Biological Control Laboratory, 120 Meiers Rd, Indooroopilly, Queensland 4068, Australia.
[2] CSIRO Entomology, Australian Biological Control Laboratory, Long Pocket Laboratories, 120 Meiers Rd. Indooroopilly, Queensland 4068, Australia.
[3] CSIRO Entomology, Canberra, ACT 2601, Australia.
Corresponding author: J.A. Goolsby, <john.goolsby@csiro.au>.

Lanka and Australia (Cape York). The population at the tip of Cape York at the Iron Range National Park was found to be an exact match with the invasive populations in Florida for the chloroplast DNA sequences.

Exploration for natural enemies of this weed was conducted between 1997 and 2002 in Australia, China, India, Indonesia, Japan, Malaysia, New Caledonia, Singapore, Taiwan, Thailand, and Vietnam. Two species of mites and 20 insect species were collected (Goolsby *et al.* 2003). Over 500 collections were made across the range of the plant over several years and during all seasons. We did not find the plant to be dominant or weedy at any location and it was always found in a mosaic of other plant species. The eriophyid mite *Floracarus perrepae* was the most widely distributed of the herbivores and appeared from field observations to gradually debilitate the plant over time. Feeding by the adults and immatures causes formation of leaf roll galls, which leads to necrosis and premature defoliation of *L. microphyllum* pinnules, impacting on plant growth. Based on its narrow field host range and apparent impact on *L. microphyllum*, *F. perrepae* was prioritised for further evaluation (Goolsby *et al.* 2003).

Mite phenology and impact

Field and laboratory studies of *F. perrepae* were initiated in south-eastern Queensland to learn about its phenology and quantify its impact on *L. microphyllum*. Four field sites were located to the north and south of Brisbane at Bribie Island and near Logan, respectively. The sites are typical habitats within the native range of both *L. microphyllum* and *F. perrepae* in subtropical, eastern Australia. All the sites are seasonally inundated, with standing water common during the summer months. Monthly surveys of *F. perrepae* on *L. microphyllum* were conducted at each site from November 2000 to March 2003. At each site, 30 newly expanded sterile pinnules were selected at random and returned to the laboratory for counting. The numbers of infested and uninfested subpinnules were counted for each pinnule. This count provided a measure of the proportion of infested subpinnules, or mite damage, at each location. From this sample of infested subpinnules, a subsample of 30 was removed to count the numbers and stages of *F. perrepae* within each curl (Fig. 1). We also identified and counted the predator mites within each subpinnule and assessed the presence or absence of the mite pathogen *Hirsutella thompsoni*.

The field studies found that populations of the mite were positively correlated with minimum temperatures and soil moisture levels. Populations of *F. perrepae* were lowest during hot, dry summer conditions. The impact of the predators and the pathogen were also significant, though even with high levels of natural enemies, the mite still caused obvious visual damage to the fern.

Although the use of eriophyid mites in biological control of weeds shows great promise, several authors, including Briese & Cullen (2001) have stated that there are not yet any dramatic successes that can be attributed to the singular impact of an eriophyid. Bearing this in mind, we sought to measure the impact of *F. perrepae* on *L. microphyllum* in an experimental field setting in the native range. We used a field plot design with 32 pairs of *L. microphyllum* plants to measure the mite's impact on biomass production. One plant in each pair was sprayed monthly with Agrimec® miticide to exclude the mite (Fig. 2). Each quarter, over a two-year period, four pairs of the plants were harvested and the dry weights of the roots, stems and leaves were measured. We found that the mite caused a greater than 50% reduction in biomass over the two-year period. The other significant aspect of this experiment was that the local south-eastern Queensland population of *F. perrepae* did not feed and develop on the Florida genotype of *L. microphyllum*. We concluded that the locally collected *F. perrepae* had a significant impact on the south-eastern Queensland genotype of *L. microphyllum*, but that we needed to search more widely for a biotype of the mite that accepted the invasive Florida genotype of the fern.

Performance of mite genotypes

To characterize *F. perrepae*, populations from throughout its native range were collected and analyzed using sequence data from nuclear rRNA D2 and mitochondrial CO1 genes using the methods of DeBarro *et al.* (2000). This technique identified genotypes from south-eastern Queensland, New Caledonia, China, Thailand, India/Sri Lanka, and Cape York. Each of these unique mite genotypes corresponded with a unique fern genotype. To screen these genotypes of *F. perrepae* for acceptance of the Florida *L. microphyllum*, portable screening methods were developed to allow for in-country testing (Fig. 3). Mites were field-collected from each location and hand-transferred to Florida and Queensland genotype sporeling ferns. Mites were held on the sporeling ferns for 3 to 4 weeks until completion of leaf curling, oviposition and development of progeny. The development of leaf curls and the numbers of progeny produced on the Florida and Queensland ferns were recorded for each mite population tested. *F. perrepae* populations from Cape York and Thailand performed best on the Florida genotype of the fern. The south-eastern Queensland mite genotype performed best on its own co-evolved south-eastern Queensland genotype of the fern, but did not develop on the Florida fern. The mite genotype from New Caledonia was intermediate in its performance on the Florida fern genotype. Genotypes from China and India/Sri Lanka performed poorly on the Florida fern genotype. In summary, the mites collected from the fern genotypes that matched or were very similar to the Florida genotype performed best. The population of the mite from Cape York was selected for release in Florida pending the results of full host-range testing and approval by the US regulatory authorities.

Figure 1. Subpinnule of *Lygodium microphyllum* showing marginal leaf curl induced by *Floracarus perrepae*.

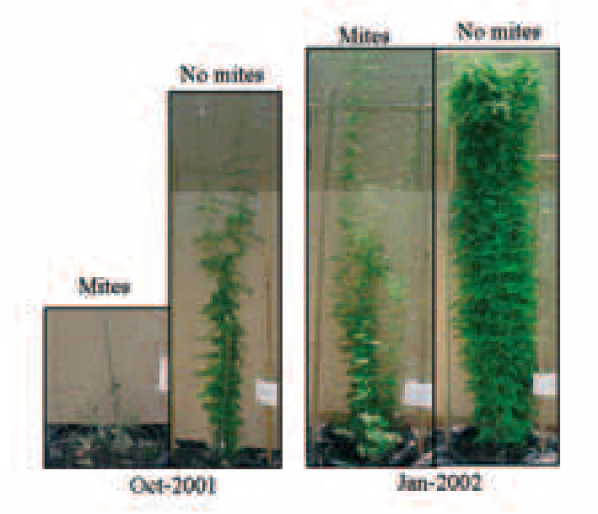

Figure 2. Impact of *Floracarus perrepae* on *Lygodium microphyllum* growth and biomass production. Plants shown from the field plot form a paired replicate with one plant treated with Agrimec® miticide.

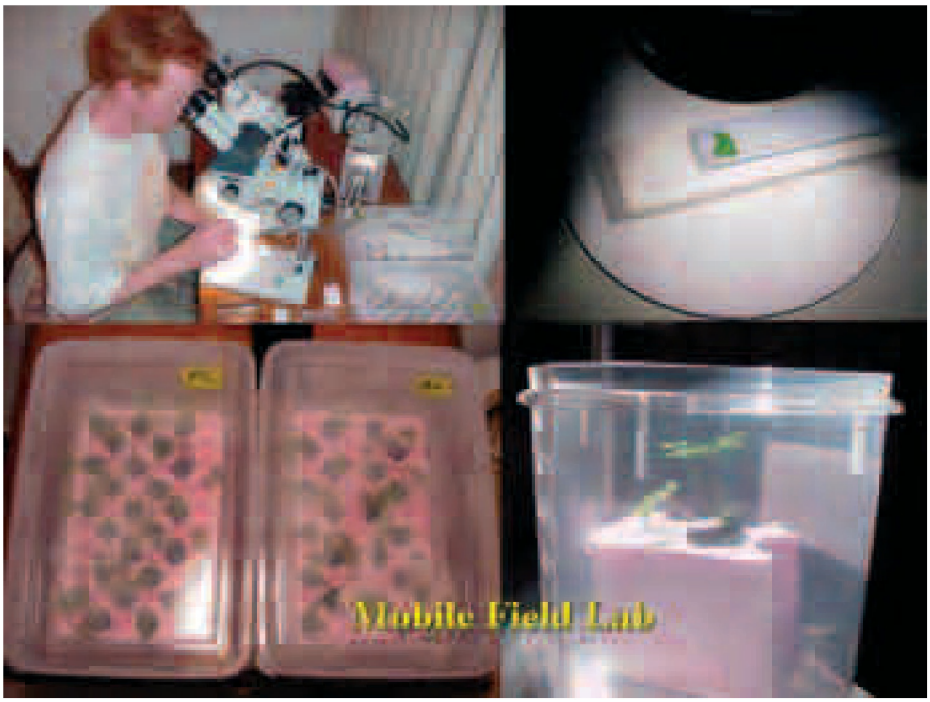

Figure 3. Mobile field laboratory for in-country screening of mites: top left to bottom right: dual microscope set-up used to select and transfer mites; field-collected infested leaf curls; containers of Florida and Queensland sporeling ferns used in screening tests; close-up of sporeling, *L. microphyllum* held in container to maintain high levels of humidity during transfer process.

Conclusions

We concluded that *F. perrepae* was the best candidate agent based on its widespread distribution, its extremely narrow field host range and obvious damage caused to *L. microphyllum* across its native range in Asia, Australia and Oceania. Field studies were conducted which confirmed and quantified the impact of the mite on the fern. Field studies also elucidated the effect of climatic factors and natural enemies on population dynamics of the mite. These studies indicated that mite populations were active year-round and highest during periods of ample soil moisture and moderate temperatures. The impact of predators and pathogens was significant but did not negate the impact of *F. perrepae* on *L. microphyllum*. Finally, the molecular diagnostics used in the biological control program were critical to discovery of the origin of the invasive fern and selection of the best adapted mite genotype. This result may have implications for other biological control programs, in that knowledge of the origin of the invasive species may lead to discovery of the most efficacious natural enemies.

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Studies in Argentina on two new species of *Thrypticus* (Diptera: Dolichopodidae) as agents for the biological control of water hyacinth, *Eichhornia crassipes*

M. Cristina Hernández,[1] Hugo A. Cordo[1] and Martin P. Hill[2]

Summary

For about thirty years *Thrypticus* spp. (Diptera, Dolichopodidae) were considered as possible candidates for biocontrol of water hyacinth (*Eichhornia crassipes*) in its adventive range. Initially it was thought that there was only one species attacking plants within the Pontederiaceae. However, five new species of *Thrypticus* have been identified from water hyacinth. Due to their abundance and wide geographical distribution, *T. truncatus* and *T. sagittatus* (provisional names) were prioritized for further investigation. Both species have similar behaviour and appear to share the same similar ecological niche. The larvae bore a horizontal mine in the petiole, making a small incision in the vascular bundles. The larvae then feed mainly on the exuded sap. In the Southern Hemisphere, the flies reproduce from spring through to the end of summer. During autumn and winter, no oviposition was recorded, suggesting that both *Thrypticus* species spend the winter months as larvae in the petioles. One generation in summer requires about 7 weeks. Preliminary host-range testing, conducted in the laboratory and in the field by interspersing test plants among infested water-hyacinth plants, showed that none of the following plants were attacked: *Eichhornia azurea, Pontederia cordata* var. *cordata* and var. *lancifolia*, *P. rotundifolia, Echinodorus grandiflorus, Canna glauca, Myriophyllum aquaticum, Heteranthera reniformis, H. callifolia* and *Monochoria africana*. During field surveys, both species of flies were only reared from *E. crassipes*. These results indicate that both species warrant further studies on their biology and specificity.

Keywords: biological control, *Eichhornia crassipes, Thrypticus* spp., water hyacinth.

Introduction

Water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laub.) is a very damaging aquatic weed occurring on water bodies in more than 50 countries with warm climates (Mitchell & Thomas 1972). This weed has different strategies for growing in a broad range of environments, including sexual and vegetative reproduction, a very fast growth and dispersal rate in tropical regions, an ability to survive attack by a complex of natural enemies, and seeds which remain viable for long periods of time.

The control strategies include biological, chemical and integrated methods. Seven biological agents have been released in 33 countries (Julien & Griffiths 1998). While success has been achieved in some areas, results in other areas, including some areas of South Africa (Hill & Olckers 2001) have been less successful. Therefore, new agents are being considered for release around the world, including the petiole-boring flies in the genus *Thrypticus*. For about 30 years, *Thrypticus* spp. (Diptera, Dolichopodidae) have been considered as possible candidates for biocontrol of water hyacinth in its adventive range (Bennett 1968). These flies were suspected to have a wide host acceptance, but the studies being carried out at the South American Biological Control Lab (SABCL) suggested that they might be suitable (Cordo *et al.* 2000). This paper presents a summary of the advances in the knowledge of these

[1] South American Biological Control Lab, USDA–ARS, Hurlingham, Buenos Aires Province, Argentina.
[2] Department of Zoology and Entomology, Rhodes University, PO Box 94, Grahamstown, 6140, South Africa.
Corresponding author: M. Cristina Hernández <crisher@speedy.com.ar>.

promising biological-control agents, including studies on the taxonomy, biology and host specificity.

Materials and methods

The surveyed area for *Thrypticus* on Pontederiaceae included the Parana–Paraguay river catchments, south-east of Brazil, and the upper Amazon River near Iquitos in Perú. Adult flies were obtained by placing petioles of Pontederiaceae from the field into emergence boxes. The adult flies were kept in cold dishes to separate species and sexes. Microscope slides were prepared for descriptions of genitalia for taxonomic examination. After identification of the species, the two most abundant species on water hyacinth in Argentina were selected for further studies. We assigned them provisional names of *Thrypticus* sp1 and *Thrypticus* sp2. Observations on the behaviour of the larvae and adults were made in both the laboratory and the field. In the laboratory, observations were made with pure colonies of each species. In host-specificity tests, the appearance of mines was taken as evidence of oviposition due to the small size of the adults and their eggs. This implies that the female found the substrate suitable for egg laying, and that the larvae emerged and accepted the petiole for feeding. Consequently, the results of the specificity tests recorded both oviposition and larval development.

All plants used were obtained from seeds or collected as small plants from the field. The trials were conducted with non-clonal plants. Water hyacinth was cultured in pools (2 × 1.4 × 0.6 m) with water and 15 cm of soil in the bottom as source of nutrients. The identifications of the species of plant used were based on Castellanos (1959), Cabrera (1969), Eckenwalder *et al.* (1986) and Horn (1987).

Host-range testing

Two type of tests were conducted to determine the host range of selected species of *Thrypticus.*

Field-based host-specificity trials

Field trials were conducted in a canal (200 × 10 m) connected to Carabelas Grande River (34°4.98'S; 58°48.6'W), Buenos Aires Province. This river belongs to the delta of Paraná River and is representative of the temperate environment of this catchment basin. The water hyacinth mat covered the whole canal and supported a natural population of *Thrypticus* sp1 and *Thrypticus* sp2. Five sites were marked 15 m apart along the canal. At each site, one plant of each of the test-plant species and one non-infested water-hyacinth plant (control) were interspersed.

The test species used were: Pontederiaceae – *Eichhornia azurea* (Swartz) Kunth, *Pontederia cordata* L. var. *lancifolia* (Muhl.) Torrey, *P. rotundifolia* (L.f.); Alismataceae – *Echinodorus grandiflorus* (Chamisso et Schlechtendahl) Micelli.; Cannaceae – *Canna glauca* L.; and Haloragaceae – *Myriophyllum aquaticum* (Velloso) Verdcourt.

These species were selected because they possess aerenchyma. This tissue is important in the development of these *Thrypticus* species. The plants were in position for a mean duration of 14 days. After the exposure, the test-plant species and the water-hyacinth control were removed and returned to the laboratory to record the development of larval mines. This experiment was repeated five times during the summers of 2001 and 2002.

Laboratory-based host-specificity trials in garden pools

The oviposition tests, to establish the host range of two selected species of *Thrypticus,* were carried out in two walk-in cages each containing a plastic garden pool (2 × 1.4 × 0.6 m). The water-hyacinth culture, started 5 months before the experiment, had 90 plants per pool during the period of the trials. Each pool was divided into 30 quadrats. Each quadrat was assigned at random to the test plants or water-hyacinth control plants. Five plants of each of five test-plant species plus five water hyacinth controls were exposed, one plant per quadrat, simultaneously to the flies. It was necessary to maintain the pools filled with water hyacinth as the canopy they formed was necessary to prevent the *Thrypticus* flying toward the mesh of the cage when released. The test plants (all Pontederiaceae) were from the species: *Eichhornia azurea; Pontederia cordata, P. cordata* var. *lancifolia; Heteranthera reniformis* Ruiz & Pavon; *H. callifolia* Rchb. ex. Kunth; *Monochoria africana* (Solms-Laub.)N.E. Brown. Test plants were kept in pots with soil in the pools.

Tests with *Thrypticus* sp1 and *Thrypticus* sp2 were performed separately. From 23 to 30 January 2002, 99 females + 76 males of *Thrypticus* sp1 were released in one cage. From 14 to 30 January 2002, 444 females + 366 males of *Thrypticus* sp2 were released in the other cage. The mean temperature inside the cages for the period of the trials was 22.9°C, with a maximum of 41.1°C and a minimum of 12.5°C.

Results

Taxonomy

The taxonomy of the group of species that utilize water hyacinth and other Pontederiaceae as host plants was studied by Dr Daniel Bickel (Australian Museum, Sydney) and M.C. Hernández (SABCL, Argentina). They described five new species from water hyacinth: *Thrypticus truncatus* Bickel & Hernández, *T. sagittatus* Bickel & Hernández, *T. yanayacu* Bickel & Hernández, *T. chanophallus* Bickel & Hernández and *T. circularis* Bickel & Hernández.

Thrypticus truncatus (provisional name: *Thrypticus* sp1) and *T. sagittatus* (*Thrypticus* sp2) were selected for further studies because they are the most abundant species on water hyacinth in Argentina. Both species are mostly metallic green with silvery dust, but each with a particular distribution of this colour. Additionally, the shape of the abdomen differs in dorsal view; *Thrypticus* sp1 is oval shape, while *Thrypticus* sp2 is more conical.

Biology

Adults

Both species, *Thrypticus* sp1 and *Thrypticus* sp2, reproduce in the same habitat. They coexist in the protected microenvironment under the water-hyacinth canopy, where they are very elusive insects. The individuals remain in the basal part of the petioles where they make short flights up and down or between the petioles. They walk backwards, descending the petiole toward the water surface. The adults emerge around noon and mating takes place in the warmer part of the day. Before copulation, the male moves near the female and jumps repeatedly over her, up and down. In some of these jumps, he alights on the female for an instant. If the female remains in the same place for a following jump, mating occurs. Mating lasts from 1 to 2 min. *Thrypticus truncatus* adults live for about 5–9 days and their complete development (egg to adult) takes 7 weeks in summer.

Larvae

The larvae of *Thrypticus* sp1 and *Thrypticus* sp2 have no evident morphological differences and show similar behaviour. The first instar larva mines across the septa of the aerenchyma joining the vascular bundles spread in the tissue. The larva scrapes a small portion in each bundle. Although the larva eats the tissues to dig the mine, it feeds mainly on the sap that exudes from the damaged bundles. There is some doubt whether it is true phytophagy or if the larvae are feeding on bacteria, yeasts or fungi in the plant wound (D. Bickel, pers. comm.). Inside the mine, the larva moves back and forth re-visiting the damaged bundles and enlarging the mine to accommodate its increasing diameter. The larva does not leave the mine although there are openings at each end. Moreover, the larvae do not survive out of the mine nor do they have the ability to form another new mine if transferred to a new petiole.

Pupa

The late-instar larva cuts an epidermal operculum near one of the orifices in the petiole and digs a chamber. After sealing the chamber, pupation occurs.

Reproductive period

In the southern part of its distribution, near Buenos Aires, both species of *Thrypticus* reproduce from spring to the end of summer. New mines were not recorded in autumn or winter. They spend the cold season as larvae in the basal part of the petioles. These parts remain alive during regular winters even when freezing temperatures kill the laminae and distal part of the petioles.

Host-range tests

Field-based host-specificity trials with Thrypticus *sp1 and Thrypticus sp2.*

Mines of *Thrypticus* were produced only on water-hyacinth control plants. Mines were not recorded in any of the test plants. The larvae did not complete their development in the water-hyacinth controls because the petioles decayed rapidly when the plants were transported back to laboratory conditions.

Laboratory-based host-specificity trials in garden pools enclosed in walk-in cages

Both species of *Thrypticus* produced mines on water hyacinth, but not on any of the test plant species. Forty-five days after the first release, all the test plants and water-hyacinth controls were examined for mines. The mean number of mines per plant in controls of *Thrypticus* sp1 was 1.4 (SD 0.89). For *Thrypticus* sp2, the mean number of mines per plant in controls was 2.2 (SD 3.27). With the methodology used, the development time of the larvae is prolonged and the petioles deteriorate before they can complete their development.

Discussion

This study has achieved several objectives. The taxonomy of this group has now been revised and will be published shortly. Most aspects of the biology have been quantified. However, further studies are required to quantify the impact of the flies on water hyacinth. The most promising aspect of this study is that both the laboratory and the field-based host-specificity trials concur with the field surveys in Argentina that both *Thrypticus* sp1 and *Thrypticus* sp2 are monophagous on water hyacinth. According to current evidence, both species are safe for use as biocontrol agents of water hyacinth around the world.

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Population structure, ploidy levels and allelopathy of *Centaurea maculosa* (spotted knapweed) and *C. diffusa* (diffuse knapweed) in North America and Eurasia

Ruth A. Hufbauer,[1] Robin A. Marrs,[1] Aaron K. Jackson,[1] René Sforza,[2] Harsh Pal Bais,[3] Jorge M. Vivanco[3] and Shanna E. Carney[4]

Summary

Understanding the origins and basic biology of non-indigenous invasive plants can help lay a strong foundation for successful control of such invaders. *Centaurea maculosa* (spotted knapweed) and its congener *C. diffusa* (diffuse knapweed) were introduced into North America from Eurasia. These species have diploid and tetraploid forms, and they are thought to hybridize. We are investigating: 1) the Eurasian origins of these plants and their population structure in North America using cpDNA sequence data; 2) ploidy levels of introduced populations; and 3) production of a potentially allelopathic root exudate ([–]-catechin) by *C. maculosa* and putative hybrids. We sequenced four noncoding regions of the chloroplast genome (4,050 bp) of 14 individuals. For two of the regions (2,161 bp) we sequenced an additional 12 individuals. The sequence data show complex patterns. Haplotypes do not segregate neatly between *C. maculosa* and *C. diffusa*. The data suggest that at least two distinct introductions of *C. maculosa* into North America have occurred – one of individuals related to those in southern France and one of individuals with haplotypes found in western Europe and Ukraine. The cytology shows that *C. maculosa* populations in North America comprise both diploid and tetraploid individuals, while *C. diffusa* populations are predominantly diploid. We examined root exudates from individuals collected from what appeared to be a hybrid swarm. Offspring from *C. maculosa* phenotypes produced the most (–)-catechin, while offspring from putative hybrids produced almost no (–)-catechin. This suggests that the ability to produce (–)-catechin is lost through hybridization. This research will aid in focusing the search for new biological control agents. In addition, it lays the foundation for testing two important hypotheses: that introduced populations have evolved to be more aggressive than their native counterparts, and that herbivores and pathogens from the area of origins of introduced plants make more effective biological control agents.

Keywords: allelopathy, area of origin, knapweed, population genetics, tetraploids.

Introduction

The ecological and evolutionary potential of a population is a function of both levels of genetic variation and the specific traits present in the population. For invasive organisms, amounts of genetic variation and the traits present in the new range depend in large part on how many propagules of a species were introduced, and from what location(s) they originated. This makes discerning the area(s) from which introductions were

[1] Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80523, USA.
[2] European Biological Control Laboratory, Campus International de Baillarguet, CS 90013 Montferrier-sur-Lez, 34988 St. Gély du Fesc Cedex, France.
[3] Department of Horticulture and Landscape Architecture, Colorado State University, Fort Collins, CO 80523, USA.
[4] Department of Biology, Colorado State University, Fort Collins, CO 80523, USA.
Corresponding author: R. Hufbauer <hufbauer@lamar.colostate.edu>.

made and the effects of the introduction and invasion processes on levels of genetic variation critical to understanding the ecological characteristics and evolutionary potential of invaders. Here we present preliminary results on (1) the geographic origins of the introductions of *Centaurea maculosa* Lam. (syn. *C. stoebe stoebe* L. and *C. stoebe micranthos* Gugler Hayek; spotted knapweed; Asteraceae)) and *C. diffusa* Lam. (diffuse knapweed) into North America inferred from chloroplast DNA sequence data, (2) the ploidy levels in the introduced and native ranges of the plants, and (3) the allelopathic root exudates of hybrids.

Two long-term goals of this research are to facilitate effective biological-control efforts, and more fundamentally to understand the factors that contribute to effective biological control. Biological control of weeds is phenomenally successful at times, yet only 10–18% of all introductions have provided good to complete control (Crawley 1989, Lawton 1990). It is clear that factors such as making introductions into compatible climates and avoiding Allee effects (e.g. Grevstad 1999) are important in successful classical biological control, but other issues must contribute to the variation in success. Knowing the sources of an invasion may facilitate finding specialized and efficacious biological control agents. To our knowledge, this idea has not been explicitly tested. A long-term goal of this project is to compare the levels of specialization and of inflicted damage between phytophagous insects from the source of introductions with other areas in the native range. Knowing the sources of introductions will also make possible a more rigorous test of the hypothesis that invaders evolve increased competitive ability (Blossey & Notzold 1995).

Materials and methods

Study system

Spotted knapweed comprises diploid western European populations (*C. stoebe stoebe*), and tetraploid eastern European or Asia Minor populations (*C. stoebe micranthos*) (Ochsmann 2000). For simplicity, we use *C. maculosa* here, and specify ploidy level when known. *Centaurea diffusa* also has diploid and tetraploid forms thought to originate in western Europe, and eastern Europe or Asia, respectively. It is uncertain which cytotypes of both taxa are found in North America, but it is the tetraploid form of *C. maculosa* that is thought to be present (Ochsmann 2000). Both species likely were introduced in the late 1800s as a contaminant in alfalfa, either from Asia Minor (Watson & Renney 1974, Ochsmann 2000) or Germany (Watson & Renney 1974). The source of their introductions remains unclear. The uncertain identity of the cytotypes of the introduced North American populations impedes formulating effective strategies for their control.

Gáyer (1909) described hybrids between *C. maculosa* and *C. diffusa* as *Centaurea* × *psammogena*. Hybridization between these species could have profound implications for their control, whether chemical or biological. New hybrid genotypes may contribute to the invasion (Ellstrand & Schierenbeck 2000), and hybrids can be either more or less susceptible to specialized herbivores than the parent plants (Whitham et al. 1999). It is currently unknown how frequently hybridization occurs between these species in North America.

Centaurea species exude chemicals from their roots that can have allelochemical activity (Callaway *et al.* 1999, Bais *et al.* 2002, 2003). Bais *et al.* (2002) isolated (±)-catechin from the root exudates of *C. maculosa* and showed that (–)-catechin can have strong allelopathic effects in sterile culture. Interestingly, although the chemical shows no autotoxicity to *C. maculosa*, it is toxic to *C. diffusa*. It is unknown whether hybrids produce (–)-catechin and, if they do, whether they are autotoxic or immune.

cpDNA phylogeography

The initial cpDNA analysis presented here includes individuals of both species from the native and introduced range collected as fresh plant tissue stored in desiccant (Drierite® ($CaSO_4$)) or as seeds (Table 1). We extracted genomic DNA from fresh (grown from seed) or dried leaf or bud tissue using the Qiagen DNeasy® Plant Mini Kit (Qiagen). Three universal chloroplast primer pairs were used to amplify regions of interest (trnSb–trnfMa from Demesure *et al.* (1995), trnK2–trnQr from Dumolin-Lapègue *et al.* (1997), and B48557–A50272 from Taberlet *et al.* (1991)). We amplified these regions in 50 µL polymerase chain reactions containing 5 µL genomic DNA, 1X PCR Buffer (20 mM Tris-HCL, pH 8.4, 50 mM KCl), 2 mM $MgCl_2$, 0.2 mM each dNTP, 2 pmol of each primer, 2.5 units *Taq* polymerase (Life Technologies), and 0.5 µL *Taq*Start antibody (Clonetech). Amplification conditions were one cycle of 2 min 30 s at 94°C, 30 cycles of 40 s at 94°C, 40 s at the annealing temperature (62, 47, and 51°C, respectively), 2 min at 72°C, and a final extension step of 10 min at 72°C (Hybaid PCR Express and Hybaid PCR Sprint thermocyclers). PCR reactions were purified using the Qiagen QIAquick® Gel Extraction Kit (for regions SbFma and K2Qr) or the QIAquick® PCR Purification Kit (BA region).

Davis Sequencing <www.davissequencing.com> ran the sequencing reactions using the amplification primers on the PCR products with BigDye Terminator® Cycle Sequencing. Reaction products were separated on an ABI 3730 automated sequencer (PE Applied Biosystems). We visually inspected all trace files for accuracy of base calls. Overlapping regions between and within samples were compared where possible. The trnSb–trnfMa and B48557–A50272 regions both contained overlapping areas that were sequenced in both the forward and reverse direction, but the trnK2–trnQr region did not. Differences in sequences of overlapping areas were conservatively recorded following individual visual inspection of trace

files for all samples in the region. Data were aligned in SeqMan (DNASTAR, Inc.) and by eye. Sequences are available from GenBank (trnK accession numbers): AY316594–AY316607; trnQr accession numbers: AY316608–AY316633; trnSb–trnfMa accession numbers: AY316634–AY316647, B28557–A50272 accession numbers: AY316648–AY316673). We constructed two haplotype networks using TCS 1.1.3 (Clement *et al.* 2000). TCS uses parsimony to construct unrooted networks of relationship between non-recombining sequences. The first was based on a total of 4,050 base pairs (1,126 from trnSb–trnfMa, 1,434 from the AB region, 835 from trnK2 and 727 from trnQr) for 14 individuals (Table 1). The second was based on 2,161 base pairs (the AB region and trnQr) for the same 14 individuals plus an additional 12 individuals (Table 1). We treated insertion-deletion sites (indels) as a fifth state. If the indel was more than one nucleotide long, we coded it as only a single base pair in the analysis, to prevent longer indels from overwhelming other signal in the data set.

Ploidy levels

We assayed ploidy levels of individuals from eight *C. diffusa* populations and 10 *C. maculosa* populations. Seeds from between 6 and 35 individual parental plants from each sample location were sown in plug trays. To determine ploidy levels, we cut off the root meristems, soaked them for 1–3 h in a 0.001% solution of colchicine to halt microtubule formation in the mitotic cells, then fixed them in 1:3 glacial acetic acid:ethanol for 2–24 h. Each root tip was transferred to a microscope slide and cleared for 1 min in a drop of 45% acetic acid. We dissected the meristems into small pieces under a light microscope, stained them with 2% aceto-orcein over a flame and squashed them with the slide cover slip. We counted the stained mitotic chromosomes using a compound microscope.

Allelopathy

We quantified root exudates of plants grown from seeds collected at a single sample location in Hood River, Oregon, USA. This population appeared to be a hybrid swarm containing *C. maculosa*, *C. diffusa* and intermediate phenotypes spanning the spectrum between them that match descriptions of hybrids (Ochsmann 1998) and backcrosses between both parent species and the hybrids. We collected samples from five phenotypic categories: spotted, diffuse, and three intermediate categories ranging from more like spotted to more like diffuse. To measure (–)-catechin produced by individuals from the hybrid swarm, we extracted root exudates following the protocols of Bais *et al.* (2002). Briefly, seeds were surface sterilized in 50% bleach and germinated on static Murashige and Skoog

Table 1. Individuals of three species from the native and introduced ranges sequenced for the regions specified. Abbr. shows the abbreviations and formatting used in Figure 1. Individuals in bold are *Centaurea maculosa*, except for *Cv* (*C. vallasiaca*), others are *C. diffusa*. Underlined individuals are from the introduced range.

Species		Site	Code	Abbr.	SbfMa	AB	K2	Qr
C. diffusa	Native range	Turkey Site 6	CD8	TRa		*	*	*
		Turkey Site 6	CD17	TRb		*		*
		Ukraine A–B	UK DK 7	UA	*	*	*	*
	North America	California	Low Lem DK 6	CA	*	*	*	*
		Colorado, Ft. Collins	Ft. CO DK 6	COa	*	*	*	*
		Colorado, Ft. Collins	Ft. CO DK 5	COb		*		*
		Wyoming, Afton	Afton DK 9	WYa	*	*	*	*
		Wyoming, Afton	Afton DK 2	WYb	*	*	*	*
C. maculosa	Native range	Basel plant 4	CM 46	**CHa**		*		*
		Basel plant 5	CM 47	**CHb**		*		*
		France 20	France 20	**F1a**	*	*	*	*
		France 28	France 28	**F1b**	*	*	*	*
		Kembs plant 2	CM 26	**F2a**		*		*
		Kembs plant 3	CM 27	**F2b**		*		*
		Ukraine Site 31	CM 23	**UA1**		*		*
		Ukraine site 7	CM 4	**UA2a**		*	*	*
		Ukraine site 7	CM 3	**UA2b**		*		*
	North America	BayfieldWI#8	SK 6	**WIa**	*	*	*	*
		BayfieldWI#8	SK 7	**WIb**	*	*	*	*
		California	LJ 13A	**CAa**	*	*	*	*
		California	LJ 18	**CAb**	*	*	*	*
		Montana, Hamilton	SK48	**Mta**	*	*	*	*
		Montana, Hamilton	SK52	**MTb**		*		*
	Australia	Canberra1	Can1	**AUa**		*		*
		Canberra2	Can2	**AUb**	*	*	*	*
C. vallasiaca	Native range	Brigerbad, CH	Br2	***Cv***	*	*	*	*

culture medium (Murashige & Skoog 1962) under constant light. After 9 days, we transferred seedlings to liquid Murashige and Skoog medium and grew them on an orbital platform shaker at 90 rpm under constant light for 30 days. Five-hundred µL of the growing medium containing root exudates were extracted with 500 µL of hexane. The hexane fraction was dried down and remaining solids were re-suspended in 500 µL 100% methanol and stored at –20°C. Samples were run on an HPLC–mass spectrometer (Summit Dionex, Sunnyvale, CA).

Results

cpDNA Phylogeography

The non-coding cpDNA regions showed extensive sequence variation as single base pair substitutions and insertion–deletion mutations. Clear, consistent differences between *C. diffusa* and *C. maculosa* were not apparent. However one group of *C. maculosa* was quite distinct (Fig. 1). This group includes two samples from southern France and two from California, USA. The differences between this group and the others suggest at least two introductions to North America of *C. maculosa*.

Ploidy levels

The European populations of both *C. diffusa* and *C. maculosa* were diploid (Fig. 2). Most *C. diffusa* in the North American samples were diploid, but two individuals appeared to be tetraploid (Fig. 2a). A very different pattern was seen among the North American *C. maculosa* populations. The two sample locations from California had only diploid individuals, while 40–90% of the individuals from the Montana, Idaho and Wisconsin populations were tetraploid (Fig. 2b). Offspring from two *C. maculosa* individuals from Canberra, Australia proved to be diploid and all of the individuals in the allelopathy work discussed below were also diploid.

Allelopathy

Seeds grown from the two phenotypic categories morphologically closest to pure *C. maculosa* produced the most (–)-catechin, and seeds from phenotypically hybrid parents produced almost none (Fig. 3). Parental phenotype explained 62% of the variation in (–)-catechin production ($F_{4,66} = 27.2$, $P < 0.0001$). The HPLC runs of individuals intermediate between hybrid and diffuse phenotypes did detect some (–)-catechin (Fig. 3).

Discussion

cpDNA Phylogeography

Two main conclusions can be drawn from the cpDNA sequence data. First, *C. diffusa* and *C. maculosa* from both the native and introduced range share many of the haplotypes. This suggests either hybridization or recent common ancestry. To sort out these complex patterns more fully and to pinpoint the sources of North American populations, regions from the nuclear genome will be needed, and other species in the *C. stoebe* group should be included in the analysis. Second, despite the complexity of the data, one group is quite distinct. This group includes individuals from an isolated population of diploid *C. maculosa* from Southern France and diploid *C. maculosa* from California. This consistent grouping across the four sequence regions suggests that the California populations of *C. maculosa* may represent a separate introduction event.

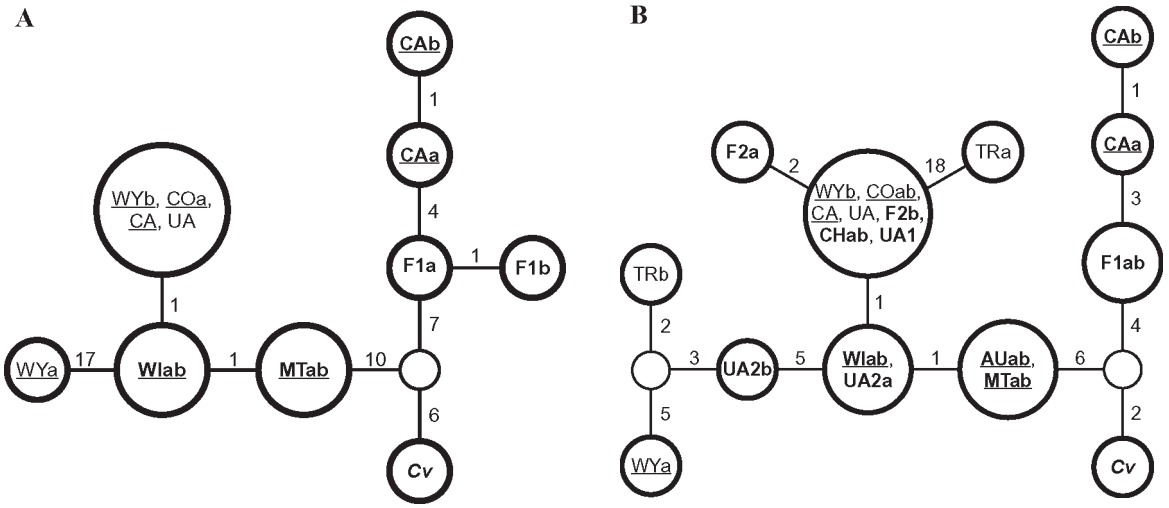

Figure 1. Haplotype networks constructed from parsimony analysis of cpDNA sequence data in TCS. **A**. Based on 14 individuals and four sequence regions (4,050 bp). **B**. Based on 26 individuals and two regions (2,161 bp). Abbreviations follow Table 1.

Ploidy levels

The discovery of both diploid and tetraploid *C. maculosa* individuals also suggests a minimum of two distinct introductions of that species. Before now, it was thought that only tetraploids were present (Ochsmann 2000). More surprising than finding both cytotypes is that they occur commonly in mixed stands, which are unlikely to represent interbreeding populations due to barriers to successful crossing between ploidy levels. The mixture of cytotypes suggests that at least two introductions occurred, one of diploids and one of tetraploids. If both cytotypes established in the same area, subsequent spread of propagules could very likely have been of mixed ploidy. It is also possible that *de novo* polyploidization in North America contributes to the pattern. The ecological consequences of polyploids for the invasiveness of these plants is yet unknown. As with other systems, the tetraploid plants may be more aggressive invaders than the diploids (e.g. Galatowitsch *et al.* 1999).

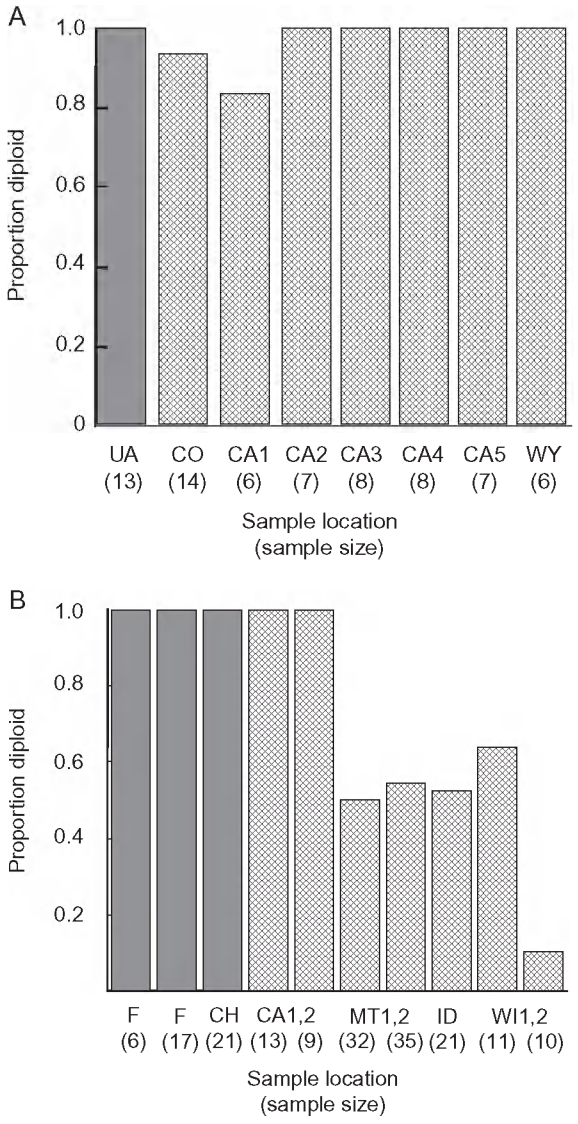

Figure 2. Proportion of diploid individuals from native European and introduced North American samples **A**. *Centaurea diffusa*, **B**. *C. maculosa*. Grey bars represent samples from the native European range and cross-hatched bars represent samples from introduced populations in North America. Samples are as follows: UA (Ukraine), CO (Colorado), CA (California), WY (Wyoming), F (France), CH (Switzerland), MT (Montana), ID (Idaho), and WI (Wisconsin). Sample size is given in parentheses.

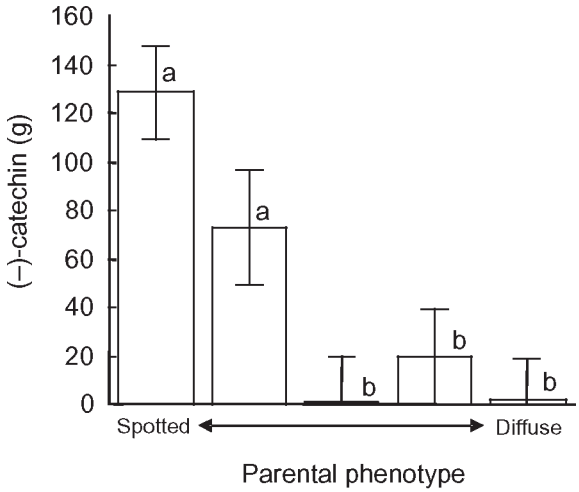

Figure 3. Mass in µg of the root exudate (–)-catechin extracted from 500 mL growth medium. Means ± SE of offspring from five phenotypic classes in an apparent hybrid swarm in Hood River, Oregon, USA. Bars with different letters are significantly different using Tukey-Kramer HSD.

Allelopathy

In some cases it has been shown that hybrids are more invasive than their parental species (Ellstrand & Schierenbeck 2000). Plants that are morphologically intermediate between *C. maculosa* and *C. diffusa* have been documented in many different locations. Do these putative hybrids make the invasion a greater threat? Our data do not support this hypothesis. When assayed for production of (–)-catechin, offspring from plants along a phenotypic gradient from spotted to diffuse showed high production on the spotted end of the continuum and low or no production on the diffuse end. Callaway *et al.* (1999) demonstrated that allelopathy can give *C. maculosa* an advantage over its competitors in a greenhouse. If allelopathy is indeed a key component of the invasion of *Centaurea* species, the putative hybrids' lack of (–)-catechin could put them at a disadvantage. However, some individuals on the diffuse end of the spectrum did produce some (–)-catechin, suggesting the possibility of introgression of the trait into the *C. diffusa* genome.

Conclusions

For the field of classical biological control to provide safer and more effective suppression of invasive pests, our underlying assumptions must be analysed critically and we need to know more about the basic biology and characteristics of our invasive plants. To test the long-standing idea that insects and pathogens from the source of an introduction make more effective and specific biological control agents, we must first know the provenance of invasive species. This research lays the groundwork for comparing specialized insects and pathogens from the appropriate native populations of *C. diffusa* and *C. maculosa* to test this idea. Our characterization of the allelopathy of hybrids suggests that they may not pose an additional invasive threat, butr the response of biological control agents to hybrids and the different ploidy levels of the parent species is unknown.

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Thirty years of exploration for and selection of a succession of *Melanterius* weevil species for biological control of invasive Australian acacias in South Africa: should we have done anything differently?

F.A.C. Impson[1,2] and V.C. Moran[1]

Summary

The question of how we can be simpler, faster and better in exploring for and selecting successful agents for weed biological control has been on the agenda since these Symposia began. We give a brief account of the development of some of these ideas on how to "pick a winner". For about 30 years, South African scientists have made exploratory trips to Australia to select agents for release against various alien acacias, and the related *Paraserianthes lophantha.* Besides two species of gall-forming wasps, a rust fungus, and far more recently, a cecidomyiid pod-galler, five seed-feeding *Melanterius* weevil species were chosen, and have proved to be highly successful. *Melanterius ventralis* was released against *Acacia longifolia* (in 1985); *M. acaciae* on *A. melanoxylon* (1986); *M. servulus* on *P. lophantha* (1989); *M. servulus* on *A. cyclops* (1991); *M. maculatus* on *A. mearnsii* (1994), and on *A. dealbata* and *A. decurrens* (2001); and *M. compactus* against *A. saligna* (also in 2001). With reference to this singular group of weevils, the question is, in retrospect, whether we should or could have done anything differently? The basic ingredients for success in exploration and selection still require that the agents are available, amenable and appropriate (politically, climatically, and in their niche selection and ability to inflict critical damage), *and* that the agents must be acceptably host-specific, and sufficiently prolific and peripatetic. We conclude, as many others have before us, that successful agent selection is a serendipitous blend of biological and ecological knowledge, and pragmatic circumstances.

Keywords: biological control, invasive acacias, *Melanterius* weevils, seed-feeders, South Africa.

Introduction

Implicit in the title of this Symposium session ("Ecology in exploration and agent selection") is the notion that a better understanding of the biology and population dynamics of prospective agents, and their target weeds, may allow the formulation of generalities and principles that would expedite the practice of biological control. That is, we would be able to choose the best agent(s) that would inflict maximal damage, and reduce population densities of the target weed in the shortest time. Biological control practitioners have surely been gnawing on this old bone since the practice began: the concern is that weed biological control is not quantitative enough, not sufficiently predictable, and thus not "scientific" enough (Huffaker 1976), and may be more of an art than a science (e.g. Harris 1976).

Having said that, however, it is also true that a number of ideas on how to optimize the selection of agents and their targets have been well entrenched in the literature for decades. Many of these concepts have

[1] Zoology Department, University of Cape Town, Rondebosch 7701, South Africa.
[2] Plant Protection Research Institute, Private Bag X5017, Stellenbosch 7599, South Africa.
Corresponding author: F. Impson, Plant Protection Research Institute, Private Bag X5017, Stellenbosch 7599, South Africa <Vredfi@plant3.agric.za>.

been widely applied consciously or otherwise by weed biocontrol practitioners. Table 1 provides a summary of these main concepts, together with a list of key references in which these ideas are variously discussed as they apply to the selection of *insect* agents. The latter information is gleaned from a review of the full-length articles on insect-agent and target selection that have been published, since 1980, in the six *Proceedings of the V–X International Symposia on Biological Control of Weeds*. The fifth Symposium was taken as the starting point for this review because it was the first of the major Symposia at which 100 or more delegates attended.

By the mid-1980s, most of the concepts listed in Table 1 had already been formulated. The only apparently novel idea that sparked some debate was the hypothesis that evolutionary "new-associations" between potential agents and their host plants could profitably be exploited in weed biological control (see Table 1 and Hokkanen & Pimentel 1984, Dennill & Moran 1988). However, even that idea was not really recent or novel – Room had already mooted it formally in 1981. Indeed, the "new-associations" concept must have been accepted even during the earliest days of weed biological control when *Cactoblatis cactorum* and cochineal insects were deployed as agents against *Opuntia* weed species (see Moran & Zimmermann 1984, and the critique by Goeden & Kok 1986).

We discuss achievements by entomologists over the years in their exploration for and selection of agents that have been used for the biological control of invasive Australian acacias in South Africa. One species of rust fungus, *Uromycladium tepperanium* has been spectacularly successful against *Acacia saligna* in South Africa (Morris 1999). But in this paper, the emphasis is on *insect* agents, and particularly on a group of seed-destroying weevils in the genus *Melanterius*. We review the history of these introductions and ask whether we could have or should have done anything differently to improve the levels of seed destruction achieved.

The history of exploration for and selection of insect species for the biological control of Australian acacias in South Africa

The exploration for natural enemies of Australian *Acacia* species that had become invasive in South Africa began some 30 years ago (Neser & Annecke 1973, Van den Berg 1973, 1977, 1980a,b,c,d, 1982a,b,c). Thorough surveys were carried out, in Australia, (mainly by Drs S. Neser and M. Van den Berg) to discover as many natural enemies as possible. From the outset, the economic importance to South Africa of several of the Australian *Acacia* species was a crucial consideration in the selection of agents. The focus was on agents that would reduce the reproductive capabilities of the plants, but which would not otherwise damage the commercially important (albeit invasive) Australian species in South Africa (Dennill & Donnelly 1991). In this context, seed-attacking agents were recommended and given preference because of their tendency to be host-specific (Janzen 1971, 1975, Annecke 1978).

From long lists of natural enemies (Van den Berg 1980a,b,c, 1982a,b,c), a number of potential agents were proposed, amongst which were *Trichilogaster* species (Hymenoptera: Pteromalidae), *Bruchophagus* species (Hymenoptera: Eurytomidae) and *Melanterius* species (Coleoptera: Curculionidae) (Van den Berg 1977, 1980d), as well as the mirid bug, *Rayieria* sp., (which was rejected because it was not host-specific), cecidomyiid flies and eriophyiid mites. Further consideration of the cecidomyiids and eriophyiids was shelved very early on due to a lack of knowledge regarding their taxonomy, biology and host ranges and also because of the lack of finances and suitably qualified people to work on additional agents (S. Neser, pers. comm. 2003). Cecidomyiids have recently been studied in earnest for use against several of the Australian *Acacia* species (Adair 2000, 2002), and *Dasineura dielsii* is now widely established on *A. cyclops* in South Africa.

Bruchophagus species were imported into quarantine in South Africa on several occasions during the 1980s (Kluge 1989) and more recently, but were never released for biological control of any of the Australian acacias. They are far less readily available on acacias in Australia than the *Melanterius* species; there are still questions about the taxonomy of the group (New 1983); and there are almost insurmountable technical difficulties in rearing the insects and in proving their host specificity. Thus, the focus of attention in the early years of the biocontrol program against Australian acacias was on the *Trichilogaster* and *Melanterius* species.

It was initially believed that seed-destroying agents would be acceptable to all stakeholders (including the owners of black wattle plantations – *A. mearnsii* – in South Africa) because they would be able to slow the reproduction of the invasive target plants while not destroying their useful attributes. However, serious concerns were raised by the wattle industry (Stubbings 1977), at a stage when exploratory surveys were well under way in Australia. These concerns hampered the progress and implementation of biological control for some years. The issue was apparently resolved and, in 1982, the biological control of Australian acacias in South Africa began with the release of the bud-galling wasp *T. acaciaelongifoliae* on long-leaved wattle, *A. longifolia*, and with concerted efforts to collect and import *M. ventralis* (Dennill & Donnelly 1991). The bud-galling wasp established throughout the range of *A. longifolia* and drastically reduced the reproductive potential of its host plant (Dennill 1985, 1988, 1990, Neser 1985).

Table 1. Optimizing target and agent selection in weed biological control using insects: how to "pick a winner". A summary of the main concepts is given. Authors that have written on one or more of these aspects in the *Proceedings of the International Symposia on Biological Control of Weeds,* since 1980, are listed in chronological order. General key references, in which many of these concepts are reviewed, are given at the bottom of the table.

Main concepts	Authors who comment on these concepts
1. Ensure accurate identification (including molecular techniques); establish genetic (biotypes/strains), phenotypic and geographical variability of target weed and potential agents; optimize genetic variability of agent	Burdon *et al.* 1981, Forno 1981, Marshall *et al.* 1981, Harris 1985, Johnson 1985, Lawton 1985, Room 1985, Chaboudez & Sheppard 1995, Palmer 1995, O'Hanlon *et al.* 2000, Ruiz *et al.* 2000
2. Establish exact provenance and native range of target weed and potential agents (central versus peripheral populations)	Forno 1981, Myers & Sabath 1981, Room 1981, Palmer 1995
3. Conduct pre-release studies and experiments on the ecology of the target weed and potential agents in native land (and in land of introduction)	Lawton 1985, Müller 1990, Pecora & Dunn 1990, Ehler 1995, Scott & Adair 1995, DeClerck-Floate 1996, Louda 2000
4. Predict possible impacts on beneficial plants, other non-target plants and native plants	Andres 1981, Harris 1985, Johnson 1985, Room 1985, Cullen 1990, DeClerck-Floate & Bourchier 2000, Louda 2000, Louda & Arnett 2000
5. Predict most suitable, damaging and virulent agent, and most vulnerable stage of target plant	Andres 1981, De Loach 1981, Harris 1985, Müller 1990, Chaboudez & Sheppard 1995, Ehler 1995, DeClerck-Floate 1996, Gassmann 1996, Cappuccino 2000, DeClerck-Floate & Bourchier 2000, Kluge 2000
6. Estimate possible impact on target weed, estimate risk and conduct cost–benefit analyses	Lawton 1985, Palmer & Miller 1996, Kluge 2000, Louda 2000
7. Ensure climatic matching	Harris 1985, Room 1985
8. Identify "vacant niches" on the target plant, or the most vulnerable stage, or time for attack	De Loach 1981, Lawton 1985, Room 1985, Müller 1990, Pecora & Dunn 1990
9. Determine the number and sequence of agent species to be released	Pecora & Dunn 1990
10. Choose seed-destroying agents for early phases of program, or to minimize conflicts	De Loach 1981, Cloutier & Watson 1990
11. Determine most vulnerable weeds and potentially successful agents from their "track record", i.e. the evolutionary or historical record	Crawley 1990, Kovalev & Zaitzev 1996
12. Consider agents that have "new associations" (in evolutionary terms) with the target weed	Room 1981, Ehler 1995
13. Acknowledge that pragmatic aspects may outweigh theoretical considerations in selecting the most suitable biocontrol agents	Myers & Sabath 1981, Cullen 1995, Ehler 1995, Scott & Adair 1995, Palmer & Miller 1996, Kluge 2000
14. Accept that the biological complexities, and other requirements, preclude the formulation of useful generalities and that "picking a winner" in weed biological control should be done on a case-by-case basis, relying on accumulated wisdom and on the intuition of the biologists concerned	Chaboudez & Sheppard 1995, Cullen 1995, Ehler 1995
General key references	Harris 1973, 1991, Wapshere 1974, 1981, 1985, Andres *et al.* 1976, Sands & Harley 1981, Goeden 1983, Schroeder & Goeden 1986, Crawley 1989, Peschken & McClay 1995, and Schroeder *et al.* 1996

Before the success of *T. acaciaelongifoliae* had been properly evaluated, however, *M. ventralis* was released in 1985 (Dennill & Donnelly 1991). The weevils readily established at all release sites. Although populations were slow to increase, levels of seed destruction ranged from 14.9% to 79.5% after only three years (Dennill & Donnelly 1991). The weevils were particularly useful in destroying the seeds on *A. longifolia* plants growing close to rivers, where, despite the dramatic effects of *T. acaciaelongifoliae*, trees were able to produce many more pods per branch than in the drier areas (Dennill *et al.* 1999).

Even though *T. acaciaelongifoliae* (in combination with *M. ventralis*) was clearly successful, other *Trichilogaster* species were not considered for acacia biocontrol for several years. There may be at least two reasons for this. Firstly, in 1985 and 1987, cohorts of *Trichilogaster* species had been introduced into quarantine from Australia to see whether establishment would occur on *Acacia pycnantha*, and the results did not look at all promising (Dennill & Gordon 1991). Secondly, it had always been very evident that *T. acaciaelongifoliae* galls were acting as a nutrient sink and were thus very damaging to *A. longifolia*. Galling by the wasps greatly

inhibits reproductive and vegetative growth, and causes branches and whole trees to collapse under the weight of galls, features that would have alarmed commercial wattle growers who perceived their industry to be under threat. Irrespective of these fears, a second *Trichilogaster* species was later established on the invasive, non-beneficial *A. pycnantha* in 1995 (Hoffmann *et al.* 2002).

Buoyed by the ready establishment of *M. ventralis* in 1985, the following years were dominated by research on the *Melanterius* group of weevils. In 1986, a second weevil species, *M. acaciae,* was released on *A. melanoxylon* (Dennill & Donnelly 1991). Conflicts with commercial wattle growers arose yet again in 1987 with the pending release of *M. servulus* for the biological control of the Australian *Paraserianthes lophantha* (a close relative of the acacias). Quarantine testing had demonstrated that *M. servulus* could also oviposit and develop within seeds of *A. mearnsii* (Donnelly 1992). This was an obvious concern, but despite earlier agreements with the wattle industry regarding acceptable levels of seed damage, the release was opposed. The program was suspended temporarily, but a compromise was later reached, in 1989, when it could be proven that *A. mearnsii* seeds in orchards could be chemically protected from the weevils (Donnelly *et al.* 1992). *Melanterius servulus* was then cleared for release. Shortly after this, in 1991, *M. servulus* was also released onto *A. cyclops*. Eventually, in 1993, the first releases of *M. maculatus* were made on *A. mearnsii*, which was the mainstay of the wattle industry and the subject of most of the conflict over the years. Although initial releases of *M. maculatus* were restricted to the Western Cape Province, release of this species has now been extended to cover much of the country. More recently (in 2001), *M. maculatus* was also introduced onto two closely related wattles, *A. dealbata* and *A. decurrens*. Lastly, but also in 2001, a fifth weevil species, *M. compactus*, was introduced and established on *A. saligna* (to supplement the action of the rust fungus *U. tepperanium*).

The *Melanterius* seed-feeding weevils used for biological control in South Africa

To date, some 88 species of *Melanterius* have been described (R. Oberprieler, pers. comm. 2002). This large group of curculionid weevils is, for the most part, native to Australia, and appears to be associated exclusively with Australian *Acacia* species (Auld 1983, New 1983, Donnelly 1992). The *Melanterius* species used in South Africa are small (3–5 mm), univoltine weevils, that breed in spring, coinciding with the peak period of pod production of their acacia hosts. Adult weevils feed mainly on green, developing seeds during this time, and to a lesser extent, at other times of the year, on buds, flowers, new vegetative growth and young pods. Mating and oviposition follow the spring feeding. The female weevils chew small holes through the walls of the swollen green pods, through which they insert a single egg. The eggs are placed onto, or near, the developing seeds. The newly hatched larvae burrow into the seeds, where they feed and complete their larval development. Generally only one larva develops per seed, during which time the entire contents of the seed are consumed, leaving the hard outer coat. (In some *Melanterius* species, a single larva may devour more than one seed.) Fully developed larvae then chew their way out of the pods, and drop to the ground and pupate in the soil. Some larvae remain in the soil until the following breeding season, but most of the adult weevils emerge from the soil 6–8 weeks later. These adults remain mostly inactive for the cooler months, sheltering under the bark of their host or other plants in the vicinity, and only become evident in large numbers again during the next spring.

Although *Melanterius* species each seem to be specific to a very narrow range of *Acacia* species, the host-plant and phylogenetic relationships of Australian acacias and their *Melanterius* weevils are poorly understood. Certainly some patterns of host association are evident. For example, *M. ventralis*, which is morphologically and phylogenetically distant from other *Melanterius* species used for biocontrol in South Africa (Clarke 2002), is specific to *A. longifolia*, the only target species belonging to the section Juliflorae in the genus *Acacia*. In the less specific *M. maculatus*, the main hosts (*A. mearnsii*, *A. dealbata*, *A. decurrens* and *A. baileyana*) all belong to the section Botrycephalae (Oberprieler & Zimmerman 2001). Such associations in *Melanterius* can be accurately determined only from a comprehensive and detailed study of specimens reared through from seeds or pods of the actual host plant. Adult *Melanterius* weevils that are found on various *Acacia* species in Australia (where they sheltering under bark or in the canopy) can create incorrect assumptions about host-plant relationships.

Much of the recent evaluation on the impact of *Melanterius* species on acacias has been on *M. servulus* on *A. cyclops* (Impson *et al.* 2000). Evaluations have also been done on *P. lophantha* (Schmidt *et al.* 1999), *A. mearnsii* (F. Impson, unpublished data), *A. longifolia* (Dennill & Donnelly 1991) and *A. melanoxylon* (Donnelly 1995). Seed destruction is the combined consequence of *Melanterius* feeding, ovipositional activities, and larval development. From the data accumulated thus far, it seems that the various *Melanterius* species have similar impacts on their different acacia hosts in South Africa, so it is possible to generalize about what can be expected and achieved from biological control efforts using these agents.

Early records of *M. ventralis* on *A. longifolia*, and of *M. acaciae* on *A. melanoxylon*, indicate slowly increasing levels of seed damage over several years,

followed by gradual dispersal of the weevils away from the release sites (Dennill & Donnelly 1991, Donnelly 1995). *Melanterius servulus* on *A. cyclops* shows the same pattern. Although the weevils cause negligible damage to the buds and immature pods, the greatest damage to the host plant is that inflicted on the almost-mature green seeds. Following release of the weevils, gradual increases in seed damage were recorded, from only 7% to 95% at some sites, after approximately five years (Impson *et al.* 2000). (Seed destruction by the weevils is unlikely to result in a reduction in the density of the target plants because this requires consistently high levels of seed mortality, i.e. >99%. However, seed destruction is a substantial aid to management of these invasive trees, a matter that is more fully discussed by Moran, Hoffmann & Olckers in a separate contribution in these Symposium Proceedings).

Several factors play a role in the levels of seed destruction achieved (e.g. fires and manual clearing), and the rate of build-up is also affected by the initial numbers of weevils released at a site, the relative seed abundance, and the rate of weevil dispersal. Dispersal rates of *Melanterius* species are relatively slow (approximately 2 km per year; F. Impson, unpublished data). However, *Melanterius* species are easy to redistribute manually, which substantially increases their effectiveness.

Discussion and conclusions

It is instructive to review 30 years of effort in the biological control of Australian acacias that have become invasive in South Africa, and to question whether these efforts could have been more effective. It would be trite to note that more time and money could have been expended in collecting more *Melanterius* weevils, and other agents, more widely and more often. Bearing in mind that much of the information from the literature (e.g. Table 1) was not available in the 1970s, the question is whether there are some aspects from these "guidelines" for exploration and selection of biocontrol agents that were omitted or ignored and which, if now implemented, could improve the levels of seed destruction that have been achieved. The answer is probably not.

In retrospect, the past emphasis on *Melanterius* weevils seems obvious and appropriate. They are sufficiently, but not always completely, host-specific (they do not feed on or oviposit in any native acacias in South Africa or on any other plants). They were readily available, in that South African scientists were allowed access to Australia, and the weevils were relatively easy to collect. In addition, earlier biological control program in South Africa using seed-feeding weevils (*Erytenna consputa* on *Hakea sericea*; see Kluge & Neser 1991) had set a favourable precedent for the use of these types of agents. As seed-destroying agents, *Melanterius* weevils were grudgingly eventually accepted by commercial growers of Australian acacias (mainly *A. mearnsii*) in South Africa, as suitable for importation. The weevils had no difficulty adapting to the climate in their country of introduction and they have built up hugely in numbers over the years. The successes that have been achieved are largely a tribute to the skills, knowledge and intuition of the naturalists who were given the initial task of exploring for and selecting agents for acacia biocontrol in South Africa, more than 30 years ago (in particular, Drs S. Neser and M. Van den Berg).

This review of the literature on insect-agent and target selection in weed biological control suggests two realities. (i) There is acceptance that non-biological, extraneous factors, such as political pressures, permits, transport difficulties, funding etc. may dominate in the exploration for and selection of agents. (ii) There is also widespread acknowledgement that, while the checklist of concepts listed in Table 1 represents an essential starting point, the reality of selecting insect agents in the field has to be determined on a case-by-case basis, and will seemingly always rely on a serendipitous blend of biological and ecological knowledge, and pragmatic circumstances.

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Insects for the biocontrol of weeds: predicting parasitism levels in the new country

Rachel McFadyen[1*] and Helen Spafford Jacob[2]

Summary

Parasitism in the new country can be a major problem with insects used as weed biocontrol agents, with some otherwise successful agents parasitised so heavily that their impact is negligible. This increases the difficulties involved in choosing the best agent. Can we predict parasitism levels? Are certain taxonomic or habitat groups more liable to parasitism in the new country? The crofton weed gall fly, *Procecidochares utilis,* is heavily parasitised in some countries, such as India, South Africa, New Zealand, and Australia, but not in others, such as Hawaii and China. Why is the similar gall fly, *Cecidochares connexa,* not parasitised in Indonesia? Why do no parasitoids attack the pseudococcid, *Hypogeococcus festerianus,* in Australia despite a large number of native and introduced parasitoids attacking pseudococcids? The various theories are discussed in an attempt to discover some guidelines for predicting parasitism levels in different countries.

Keywords: agent impact, agent selection, predicting parasitism.

Introduction

The issue of parasitism of weed biocontrol agents in the new country, and whether this can be predicted in advance, was first reviewed 25 years ago (Goeden & Louda 1976). Since then, there have been many publications on parasitism levels, size of parasitoid guilds, and theories of parasitism levels, with Hawkins' 1994 monograph the main source of information and references. There is general agreement among most biocontrol scientists (both weed and arthropod biocontrol) (Frankie & Morgan 1984, Hawkins 1990) that, in insect populations, overall mortality due to parasitism is correlated with the total number of parasitoid species, although this is not a straightforward linear relationship and is probably not causative (Hawkins 1994). The impact of parasitism on the host population is also affected by the ability of the parasitoid populations to "keep up" with the host population. This may be critically affected by periods when the host population is very low, or is unavailable to the parasitoid, e.g. through spatial isolation (Frankie *et al.* 1984) or in diapause.

The following issues have been seen as important influences on the number of parasitoid species moving onto a herbivorous insect in a new country. In support, we collated records of parasitism of weed biological control agents from as many sources as possible including Julien & Griffiths (1998), other published references (Appendix 1), and personal communication with colleagues. From these records we have compiled a data set (which is not exhaustive) which we use to discuss some of the hypotheses presented. Fifty-four records of parasitism from 343 weed biological control agents were included.

Factors affecting parasitism

Taxonomic position of the insect

There is a perception that certain insect orders (e.g. Lepidoptera or Diptera) have more parasitoids than

CRC for Australian Weed Management
[1] Queensland Department of Natural Resources and Mines, Alan Fletcher Research Station, PO Box 36, Sherwood, Queensland 4075, Australia.
[2] School of Animal Biology, University of Western Australia, 35 Stirling Highway, Crawley, Western Australia 6009.
* Present address: 80 Meiers Road, Indooroopilly Queensland 4068, Australia.
Corresponding author: Rachel McFadyen <rachel.mcfadyen@nrm.qld.gov.au>.

others (e.g. Heteroptera or Orthoptera: Hawkins 1994). For example, among leaf miners in the Neotropics, Lewis *et al.* (2002) reported higher parasitism on dipterous species compared with Lepidoptera or Coleoptera. A galling nematode has no known parasitoids (Harris & Shorthouse 1996) although most other galling species are heavily parasitised (Frankie & Morgan 1984).

In our data set, the overall rate of parasitism is 15.7%. Dipterans experience the highest rates of parasitism of weed biological control agents in their introduced range (Table 1). Lepidoptera have a marginally higher rate of parasitism than the mean, while the other major insect groups have a lower rate of parasitism. Only 9% of curculionids and chrysomelids were parasitised, compared with 17% of tephritids and 62% of cecidomyiids. However, those Coleoptera that were parasitised in one country had a much higher chance of being parasitised in another country than either Diptera or Lepidoptera. Therefore, the taxonomic grouping of the insect does appear to have an effect on whether it is attacked in its introduced range and our results support the hypotheses.

The phylogeny of the insect also relates to the country of introduction, in that the presence of closely related native insects will affect the likelihood of parasitism. For example, *Hydrellia pakistanae* and *H. balciunasi* (Diptera: Ephydridae), released against *Hydrilla verticillata*, have both been attacked by a parasitoid of native *Hydrellia* species (Doyle *et al.* 2002, Wheeler & Center 2001).

Habit of the insect

Whether the insect is an open or concealed feeder (gall former, leaf miner, leaf roller, root feeder or above ground; feeding inside seeds or shoots) is agreed to be "the single most important correlate of how many parasitoid species a herbivore is known to support" (Hawkins 1994, p. 24: see also refs. therein). Parasitism rates are highest on concealed feeders such as leaf rollers, leaf miners and case bearers, and low in borers and root feeders, with gallers intermediate. Most species have part of the lifecyle (eggs, early larval instars, later instars, pupae) inside plant structures or otherwise concealed, and part exposed, and this will affect the parasitoid guilds at each stage. Furthermore, there is consensus that, for hymenopterous parasitoids, those attacking unprotected external and active stages are generally koinobionts (feeding inside the still-living host) and tend to be specialists, less likely to move onto new host insects, while those attacking protected or immobile stages are more usually idiobionts (feeding on a dead or paralysed host) and therefore generalists that more readily move onto new hosts (Hawkins 1994: Cornell & Hawkins 1993). However, the important dipterous group Tachinidae is usually polyphagous although they are koinobionts usually attacking active external feeders (Hawkins 1994).

In our analysis we did not determine percentage parasitism for the feeding guilds of weed biocontrol agents, but report instead the numbers of species parasitised in each guild (Fig. 1). Although we are unable to directly test the prediction that some guilds experience higher rates of parasitism than others, we do note that we have found no reported cases where a root feeder has been attacked in its introduced range.

For galling insects (both Hymenoptera and Diptera), several authors have reported high mortality due to parasitoids in their native range (Frankie *et al.* 1984, Ehler *et al.* 1984, Frankie & Morgan 1984); however, as introduced biocontrol agents, only the cecidomyiid gall inducers are heavily parasitised (Harris & Shorthouse 1996). The stem-boring moth *Coleophora parthenica* is heavily parasitised in its native country (Baloch & Mustaque 1973), while in the introduced range in California, it is attacked by eight parasitoids, all generalists, with a low overall parasitism rate (Muller & Goeden 1990). For leaf miners in the Neotropics, Lewis *et al.* (2002) reported that parasitism was a major mortality source and most parasitoid species were generalists; similar results were reported by Hawkins (1994). Several authors report only or mainly specialist parasitoids on surface-feeding lepidopterous larvae (Janzen & Gauld 1997, Lei *et al.* 1997), though their pupae (possibly concealed in leaf litter or among bark) may be attacked by generalist parasitoids (Lei *et al.* 1997). An exception is surface-feeding hairy arctiid caterpillars, where the main parasitoids are tachinids, usually generalists, but overall mortality due to parasitoids may also be low (Stireman & Singer 2002, Bennett & Cruttwell 1973). The

Table 1. Percentage of parasitism of weed biological control agents.

Taxonomic order	% of agents parasitised	% parasitised in more than one country	Families parasitised
Heteroptera	11.5	0	Cicadellidae; Tingidae; Aphididae
Hymenoptera	40[a]	0	Eurytomidae; Pteromalidae
Diptera	36	30	Agromyzidae; Tephritidae; Cecidomyiidae; Ephydridae
Coleoptera	10	42	Bruchidae; Chrysomelidae; Curculionidae
Lepidoptera	17	21	Tortricidae, Pyralidae, Arctiidae, Coleophoridae, Geometridae, Lyonetiidae, Oecophoridae Noctuidae, Phytcitidae, Carposinidae

[a] The small sample size (n=5) of Hymenopterans released for weed biocontrol suggests that this is not an accurate assessment.

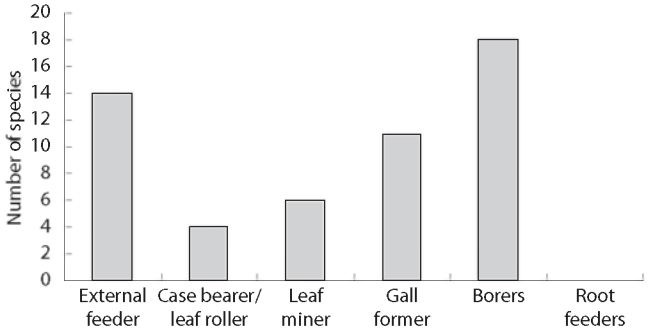

Figure 1. Number of species of weed biocontrol agents parasitised in the introduced range, against the feeding habit of the attacked stage.

surface-feeding arctiid *Pareuchaetes pseudoinsulata* in its introduced range has few parasitoids; pupal parasitism was not studied (McFadyen 1997). On the other hand, *Cactoblastis cactorum* in South Africa with protected larvae has few larval parasitoids, but many species on the exposed pupae (Pettey 1948).

Factors relating to the plant host

There is a view that increased plant "apparency" increases the size of the parasitoid guild on the insects feeding on that plant. That is, perennials support a larger parasitoid guild than do annual plants, shrubs more than herbs, and trees more than shrubs (Hawkins 1994). On the other hand, Muller & Goeden (1990) reported the relatively high number of eight parasitoid species attacking the introduced stem-boring moth *Coleophora parthenica* on the annual plant *Salsola australis* in California. Plant apparency is related to the insect population cycle: if the insect population is low or unavailable to the parasitoid for long or unpredictable periods (due to diapause or seasonally or locally very low numbers in an extreme or unpredictable climate), then the parasitoid population may never be able to "catch up" sufficiently to control the host (Frankie *et al.* 1984). If the host insect has a diapause, the life-cycle of generalist parasitoids may not be synchronized to this. Conversely, if the host insect has multiple generations on a perennial plant in an equable climate and is therefore always present, the parasitoid populations can maintain damaging levels (Stone *et al.* 1995, Stireman & Singer 2002, Lewis *et al.* 2002).

Factors relating to the country of introduction

Climate has already been mentioned above. In general, more extreme climates (with severe winters or hot dry summers, or unpredictable long dry periods) will make it more difficult for generalist parasitoids to maintain synchrony with the host populations, and will tend to result in lag effects, whereby parasitism levels are seldom sufficient to reduce the impact on the host plant.

Phylogeny interacts with country, in that if there are closely related insects in the new country (same or similar genera), then it is likely that specialist parasitoids from native species can move onto the introduced species. Edwards (1998) identified the possibility of Australian native *Eurytoma* wasps attacking *Mesoclanis polana* (Diptera: Tephritidae) after its introduction into Australia for the control of bitou bush. *M. polana* is heavily parasitised by *Eurytoma* sp. in South Africa and is now parasitised in Australia. Conversely, if the new country has a very impoverished fauna in that group (e.g. hispine beetles in Australia), there may be few or no specialist parasitoids available.

Size and isolation of the new country may be a factor. Oceanic islands such as Hawaii or New Zealand may have a reduced parasitoid fauna with few species available to move onto introduced insects (Duan & Messing 2000). Large, but isolated, continents such as Australia may also have reduced faunas in certain groups, compared with the Americas or the Eurasian land mass.

Predictability

For all of these factors, however, the magnitude and reliability of the effect is very debatable. That is, will a given endophagous insect in a new country have 50% more parasitoid species than an equivalent ectophagous species, or 100% more, or 200%? And what is the probability that these effects will occur? If the introduced insect has a congener in the new country (e.g. the rubbervine moth *Euclasta whalleyi* in northern Australia (McFadyen & Marohasy 1990), is it certain or only probable that all parasitoid species will transfer across? And can we predict their impact on the agent population?

Discussion

The impact of parasitism on weed biological control agents is not an issue of establishment according to Lawton (1986), but of overall effectiveness of the agent and hence subsequent control of the target weed.

Reduced effectiveness of an agent is increasingly an issue with increased regulatory and financial restrictions on the number of agents that can be released. Every agent that is heavily parasitised after release becomes more costly – economically, ecologically, and perhaps socially.

Our analysis has demonstrated the need for more published records of parasitism of weed biological control agents and for these to be included in an active data base from which we can start examining patterns of parasitism. Hill & Hulley (1995) report 40% parasitism of weed biocontrol agents in South Africa. If we take this as a benchmark for the worldwide rate of parasitism, then there is every indication that parasitism is underreported (our data show an overall rate of 15.7%). Furthermore, reports of parasitism are dispersed within an immense literature on efficacy of agents. Some papers explicitly address the parasitism of biological control agents (Wilson & Andres 1986, Wehling & Piper 1988, Hoffman *et al.* 1993, Hill & Hulley 1995, Hoebeke & Wheeler 1996, Lang & Richard 1998, Manongi & Hoffmann 1995, Newton & Sharkey 2000). Most papers make reference to the parasitism of agents as a note or in vague terms. Part of the difficulty is obtaining clear identification of the parasitoid(s) involved. In some countries the native insect fauna is not well known and it is difficult to identify parasitoids, some of which may be unknown until the agent's introduction (e.g. *Stethynium* sp. nov. a parasitoid of *Zygina* sp. (Joder *et al.* 2002)). It is difficult to gauge how frequently the occurrence of parasitism goes unrecorded, but our opinion is that parasitism of biological control agents occurs at a much higher frequency than is reported or published. This may be because parasitism is determined to be at such a low level as to not warrant reporting or further attention, or there are insufficient resources for follow-up studies. In any case, under-reporting reduces our ability to predict parasitism.

We have not assessed the impact of parasitism or the number of parasitoids in relation to time since release of the agent. These analyses would also be beneficial in helping us to predict parasitism.

Hill & Hulley (1995) argue that no potential agent should be rejected for release on the basis of predicted parasitism. We support this approach in principle. However, when choosing between two or more potential agents, knowledge of potential parasitism is an important factor to consider. Not only can parasitism significantly reduce the effectiveness of an agent, but it also reduces confidence in the ecological safety of biological control. There is little information about the trophic web effects resulting from parasitism of weed biological control agents. Yet increased host availability can be expected to lead to an increase in the parasitoid population. Consequently, there may be significant shifts in the parasitoid pressure on the native hosts. Therefore, being able to predict parasitism effectively becomes more critical as the ecological effects of biological control are coming under increasing scrutiny.

In summary, we urge all biological control practitioners to consistently observe and report, preferably in published papers, all parasitism of weed biological control agents.

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Appendix 1. List of weed biological control agents parasitised in their introduced range.

Weed species	Biological control agent	Reference
Acacia longifolia	*Melanterius ventralis*	Hill and Hulley 1995
Acacia longifolia	*Trichilogaster acaciaelongifoliae*	Manongi and Hoffmann 1995
Ageratina adenophora	*Procecidochares utilis*	Julien and Griffiths 1998, Hill and Hulley 1995
Asparagus asparagoides	*Zygina* sp.	Joder *et al.* 2002
Baccharis halimifolia	*Rhopalomyia californica*	Julien and Griffiths 1998
Carduus nutans	*Rhinocyllus conicus*	Lawton 1986, T. Woodburn pers. comm.
Centaurea nigra	*Urophora jacaeana*	Hoebeke and Wheeler 1996
Centaurea spp.	*Urophora affinis*	Lang and Richard 1998
Chondrilla juncea	*Cystiphora schmidti*	Julien and Griffiths 1998
Chromolaena odorata	*Pareuchaetes pseudoinsulata*	Julien and Griffiths 1998
Chrysanthemoides monilifera	*Comostolopsis germana*	R. Holtkamp pers. comm.
Chrysanthemoides monilifera	*Mesoclanis polana*	R. Holtkamp and T. Willis pers. comm.
Cirsium arvense	*Cassida rubiginosa*	Tipping 1993
Cirsium arvense	*Larinus planus*	McClay *et al.* 2001a
Clematis vitalba	*Phytomyza vitalbae*	Hill *et al.* 2001, M. Grodowitz pers. comm.
Clidemia hirta	*Ategumia ebulealis*	Julien and Griffiths 1998
Cordia curassavica	*Eurytoma attiva*	Julien and Griffiths 1998
Cyperus rotundus	*Bactra venosana*	Julien and Griffiths 1998
Cytisus scoparius	*Leucoptera spartifoliella*	Julien and Griffiths 1998
Euphorbia esula	*Spurgia esulae*	Julien and Griffiths 1998
Hakea gibbosa	*Erytenna consputa*	Hill and Hulley 1995
Hakea sericea	*Carpinosina autologa*	Hill and Hulley 1995
Hakea sericea	*Erytenna consputa*	Hill and Hulley 1995
Hydrilla verticillata	*Hydrellia balciunasi*	Grodowitz *et al.* 1997
Hydrilla verticillata	*Hydrellia pakistanae*	Wheeler and Center 2001, Doyle *et al.* 2002, M. Grodowitz, pers. comm..
Hypericum perforatum	*Aphis chloris*	Hill and Hulley 1995
Hypericum perforatum	*Aplocera efformata*	Julien and Griffiths 1998
Hypericum perforatum	*Zeuxidiplosis giardi*	Hill and Hulley 1995
Lantana camara	*Calycomyza lantanae*	Hill and Hulley 1995
Lantana camara	*Hypena strigata*	Hill and Hulley 1995
Lantana camara	*Neogalea sunia*	Julien and Griffiths 1998
Lantana camara	*Neogalea sunia*	Julien and Griffiths 1998
Lantana camara	*Octotoma scabripennis*	Julien and Griffiths 1998, Hill and Hurley 1995
Lantana camara	*Ophiomyia lantanae*	Hill and Hurley 1995
Lantana camara	*Salbia haemorrhoidalis*	Julien and Griffiths 1998
Lantana camara	*Uroplata girardi*	Hill and Hulley 1995
Matricaria perforata	*Omphalapion hookeri*	McClay *et al.* 2001b
Matricaria perforata	*Rhopalomyia tripleurospermi*	McClay *et al.* 2001b
Opuntia aurantiaca	*Mimorista pulchellalis*	Hill and Hurley 1995
Opuntia ficus-indica	*Cactoblastis cactorum*	Hill and Hurley 1995
Orobanche cumana	*Phytomyza orobanchiae*	Julien and Griffiths 1998
Prosopis spp	*Algarobius bottimeri*	Hill and Hurley 1995
Prosopis spp	*Algarobius prosopis*	Hoffmann *et al.* 1993
Prosopis spp	*Neltumius arizonensis*	Coetzer and Hoffmann 1997
Salsola australis	*Coleophora klimeschiella*	Julien and Griffiths 1998
Salsola australis	*Coleophora parthenica*	Julien and Griffiths 1998
Salvinia minima	*Samea multiplicalis*	Newton and Sharkey 2000
Schinus terebinthifolius	*Episiumus utilis*	J. Cuda pers. comm.
Senecio jacobaeae	*Tyria jacobaeae*	Julien and Griffiths 1998
Sesbania punicea	*Neodiplogrammus quadrivittatus*	Hill and Hurley 1995
Solanum elaeagnifolium	*Leptinotarsa texana*	T. Olckers and J. Hoffmann pers. comm.
Solanum mauritianum	*Gargaphia decoris*	T. Olckers pers. comm..
Solanum sisymbrifolium	*Gratiana spadicea*	Byrne *et al.* 2002, M. Byrne and M. Hill pers. comm.
Sonchus arvensis	*Cystiphora sonchi*	McClay and Peschken 2001
Ulex europaeus	*Agonopterix ulicetella*	Julien and Griffiths 1998

Biological control of *Rubus fruticosus* agg. (blackberry): is the leaf rust the only option for Australia?

Jean Louis Sagliocco[1] and Eligio Bruzzese[1,2]

Summary

Rubus fruticosus aggregate (European blackberry) is a complex weed that is listed as a Weed of National Significance because of its economic and environmental impacts in temperate Australia. A biological-control program for *R. fruticosus* agg. commenced in the late 1970s and resulted in the release of the rust fungus *Phragmidium violaceum* (Uredinales), because of its specificity and potential to suppress the weed through defoliation. The impact of the rust varies between *R. fruticosus* taxa and between locations and years. While the introduction of additional isolates of *P. violaceum* is being investigated by the Cooperative Research Centre for Australian Weed Management, a literature review was carried out to identify additional natural enemies that may have potential for the biological control of *R. fruticosus* agg. Results reveal that a fungus, an eriophyid mite and a number of insects deserve further consideration.

Keywords: arthropods, biological control, fungi, *Rubus fruticosus*.

Introduction

Rubus fruticosus L. aggregate (European blackberry) is a complex weed that is listed as a Weed of National Significance in Australia, because of its economic and environmental impacts in the temperate climatic zones of the south-east and south-west of the continent. The Weeds of National Significance Blackberry Strategic Plan (ARMCANZ *et al.* 2001) states the following research and extension opportunity: "Concentrate on further biological control options for blackberry growing in situations where current biological control is not effective".

Phragmidium violaceum (Schultz) Winter (blackberry leaf rust) is a useful and effective biological-control agent for *R. fruticosus* agg. in temperate southern Australia (Marks *et al.* 1984, Bruzzese & Field 1985, Bruzzese & Hasan 1986, Mahr & Bruzzese 1998, Evans & Bruzzese 2003). The Cooperative Research Centre for Australian Weed Management has a current project aimed at the introduction of additional isolates of *P. violaceum* with improved pathogenicity and targeted at a wider range of taxa of *R. fruticosus* agg. in Australia (Evans *et al.* 2003). There are, however, limitations to its effectiveness in suppressing this complex weed comprising of several taxa and these are well documented (Evans *et al.* 1998, Pigott *et al.* 2001). The current study is a review of the natural enemies recorded on *R. fruticosus* agg. in the literature with the view of identifying additional natural enemies that may complement the impact of the *P. violaceum* and increase the suppression of *R. fruticosus* agg. throughout its range in temperate Australia.

Materials and methods

An extensive literature survey was undertaken in abstracts referring to *R. fruticosus* agg. and possible associated organisms using the following resources: reference works held in Australian libraries, Review of Applied Entomology (1913–1972) and the electronic databases CAB Abstracts (1972–2003), Agricola (1992–2002) and Current Contents. The Victorian Plant Pest and Disease Collections Database, Agriculture Victoria (2001) was also consulted to identify

[1] Department of Primary Industries, Keith Turnbull Research Institute, PO Box 48, Frankston, Victoria 3199, Australia.
[2] Cooperative Research Centre for Australian Weed Management, PO Box 48, Frankston, Victoria 3199, Australia.
Corresponding author: J.L. Sagliocco <JeanLouis.Sagliocco@nre.vic.gov.au>.

diseases of *R. fruticosus* agg. already recorded in Australia. All organisms found associated with *R. fruticosus* agg. were checked for other associations and organisms not restricted to *R. fruticosus* agg. were rejected.

Results

A review of Australian and international databases and literature has identified additional fungal pathogens, insects and mites attacking *R. fruticosus* agg. in the Northern Hemisphere. Any fungal pathogen, insect or mite recorded on *Rubus idaeus* (raspberry) and any *Rubus* spp. not included in the *R. fruticosus* agg. (Tutin *et al.* 1968) was excluded from the list of potential biocontrol agents (Table 1). Additional exclusions were fungal pathogens and insects already recorded attacking *Rubus* spp. in Australia (Bruzzese 1980a,b) or recorded in the Victorian Plant Pest and Disease Collections Database. This list may not be exhaustive as a number of rare taxonomic texts on insects, mites and fungi could not be accessed at the time of this study.

Septocyta ruborum (lib.) Petrack (Coelomycetes) (blackberry purple blotch)

This fungus is widespread in Europe on wild and commercial cultivars of *R. fruticosus* agg. (Koellreuter 1952, Oort 1952, Punithalingam 1980, Kövics 1997). It is also recorded as a disease of blackberry cultivars "Marion", "Evergreen", "Kotata" and "Waldo" in Oregon, United States of America (USA) (Strik 1996) and on thornless blackberry cultivars "Thornfree" and "Hull Thornless" in Hungary (Kövics 1997). There is only one record of this disease on a Rosaceae other than *Rubus*, *Potentilla sterilis* (Feige *et al.* 2001), but this needs to be confirmed. This disease was commonly recorded causing dieback of first-year canes of *R. fruticosus* agg. in southern Europe by Bruzzese (1982b). *Septocyta ruborum* infects only first-year canes and symptoms only develop after a chilling requirement is met. Severely affected canes die the following spring prior to flowering in the USA (Pscheidt 2002).

Eriophyes rubicolens (Canestrini) Acarina: Eriophyidae

This eriophyid mite was described from specimens collected from *R. fruticosus* agg. at Veneto, Italy (Canestrini 1891), and was included in the fauna of Italy (Canestrini 1892). The mite (also known as *Phyllerium rubi* Fr.= *Erineum rubi* Pers.) is described to produce erineum on the lower side of leaves of several wild species of *Rubus* spp. (Nalepa 1892) in eastern France (Nalepa 1929). The mite has no alternative host (Amrine & Stasny 1994).

Ectoedemia erythrogenella (de Joannis) Lepidoptera: Nepticulidae

Originally described from France (Joannis 1907), this univoltine Lepidoptera species has also been

Table 1. Organisms with potential for the biological control of *Rubus fruticosus* agg.

Organism	Distribution	Host plants	Plant association	References
Fungi Coelomycetes				
Septocyta ruborum	Northern Hemisphere	*Rubus fruticosus*	Stems	Koellreuter (1952), Oort (1952), Punithaligam (1980)
Acarina Eriophyidae				
Eriophyes rubicolens	Italy, France	*Rubus fruticosus*	Leaf erineum	Canestrini (1891, 1892), Nalepa (1892, 1929), Amrine & Stasny (1994)
Lepidoptera Nepticulidae				
Ectoedemia erythrogenella	Western Europe	*Rubus fruticosus*	Leaf miner	Joannis (1907), Heath (1976)
Hymenoptera Cephidae				
Hartigia albomaculata	Southern Europe	*Rubus fruticosus*	Stem borer	Scheibelreiter (1979), Bruzzese (1982a, 1982b)
Hymenoptera Tenthredinidae				
Claremontia alternipes	Europe, Mongolia, Siberia	*Rubus* spp.	Leaf feeder	Lacourt (1999)
Empria excisa	Europe, northern Africa, Mongolia	*Rubus ulmifolius*	Leaf feeder	Lacourt (1999)
Macrophya militaris	Central and southern Europe	*Rubus* spp.	Leaf feeder	Lacourt (1999)
Macrophya montana montana	Central and southern Europe, Turkey, Iran	*Rubus* spp.	Leaf feeder	Lacourt (1999)
Monophadnoides ruficrucis	Central and southern Europe, Turkey	*Rubus ulmifolius*, *Rubus* spp.	Leaf feeder	Lacourt (1999)

recorded from the United Kingdom (UK), Switzerland, Austria, Italy and Majorca (Heath 1976). Females lay eggs on leaves in July and larvae develop in leaf mines throughout summer until early winter. The literature gives *R. fruticosus* agg. as the only recorded host plant.

Claremontia alternipes (Klug.) Benson, Hymenoptera: Tenthredinidae

This sawfly is reported to feed on *R. fruticosus* (Lacourt 1999) and is one of the few sawfly species reported to feed on *Rubus* spp. Other species are *Empria excisa* (Thomson) Enslin, *Macrophya militaris* (Klug.) Taschenberg, *Macrophya montana montana* (Scopoli) Kirby and *Monophadnoides ruficrucis* (Brullé) Benson. None of these species are mentioned in the recent literature and a further literature search through old references is required to evaluate their host range. They are thought to have leaf-feeding larvae, but the number of generations each year is unknown.

Hartigia albomaculata (Stein) Hymenoptera: Cephidae

This sawfly was identified in the literature and from field surveys in Europe for organisms attacking *R. fruticosus* agg. (Scheibelreiter 1979). *Hartigia albomaculata* is univoltine and parthenogenetic and attack is restricted to first-year canes of *R. fruticosus*. Females were observed to lay eggs in May with larvae developing in canes for 6 weeks before overwintering in a cocoon, with pupation occurring during the following spring (Bruzzese 1982a). Field surveys in southern France showed that *H. albomaculata* was common on *Rubus ulmifolius*. Plants of *Rubus caesius*, *Rosa canina* and *Rosa rubiginosa* present at the same survey sites were never found to be attacked.

Discussion

More than 50 oligophagous arthropod species are reported associated with *R. fruticosus* agg., not to mention a greater number of polyphagous species. Among the organisms identified, some were not previously considered for the biological control of *R. fruticosus* agg., while one insect was the subject of preliminary investigations and testing.

The only fungal pathogen of additional interest as a potential candidate for the biological control of *R. fruticosus* agg. is the blackberry purple blotch fungus, *S. ruborum.* Although this fungus has been recorded on some commercial cultivars containing principally American genetic material in the USA and on some thornless blackberry cultivars of mixed American and European parentage in Europe, the extent of cultivation and importance of these cultivars in Australia needs to be established. Although Punithalingham (1980) reports that no physiologic specialization of this pathogen has been reported, the significant dieback of first-year canes caused by this disease warrants further studies to determine whether specialization and levels of pathogenicity to different taxa of *R. fruticosus* agg. and commercial cultivars exist. This fungus would complement the impact of *P. violaceum* by causing dieback of first-year canes in early spring, prior to defoliation of *R. fruticosus* agg. by the rust.

The absence of reported association of the lepidopteran *E. erythrogenella* with blackberry species other than *R. fruticosus* agg. strongly suggests that this insect is specific to the weed. However, because the insect has only one generation per annum and larvae develop in a mine in a single leaf, it is unlikely that this damage would significantly contribute to the biocontrol of *R. fruticosus* agg.

The cephid *H. albomaculata* was identified in the late 1970s as a potential biocontrol agent for *R. fruticosus* agg. and field surveys have clarified its biology and distribution. Host-specificity tests done in cages have shown oviposition on some *Rubus* spp. and *Rosa* spp. (Bruzzese 1982a). However, it is believed that these results were due to the testing procedure in the laboratory because this insect was never recorded to attack plants other than *R. fruticosus* in the field. Further host-specificity field tests under natural conditions in the region of origin should confirm the host range of this insect. If specificity is confirmed, *H. albomaculata* could be introduced to complement the effect of *P. violaceum* because of its mechanical destruction of first-year canes.

The eriophyid *E. rubicolens* is the only mite species which appears to be restricted to *R. fruticosus* agg. Apart from information given by Nalepa (1892) on the production of erineum induced by the mite's feeding, no information is available on its biology, abundance or host range within taxa of *R. fruticosus* agg. This can only be confirmed through surveys in the region of origin and host testing.

This study has identified that the choice of additional natural enemies for biological control of *R. fruticosus* agg. in Australia is restricted. The organisms listed in Table 1 have some potential and require further investigation. The reason for the restricted list of candidates is because there are a number of native *Rubus* spp. in Australia and a small but significant brambleberry industry that uses blackberry cultivars of mixed European and American parentage. Any candidate biocontrol agent needs to have the potential to inflict significant damage to the weed and has to be shown to be host-specific before permission for its release into the Australian environment is given.

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The CSIRO Mexican Field Station: history and current activities

Ricardo Segura P.L.[1] and Tim A. Heard[2]

Summary

Many weeds in Australia have their origins in the neotropical America, including Mexico, Central America, the Caribbean Islands and South America. The CSIRO Mexican Field Station, located at Veracruz on the gulf coast of Mexico, conducts research on plants native to tropical America, which are weeds in the tropical northern parts of Australia and other countries. The major focus is to find biological control agents and to investigate various aspects of plant and insect ecology. The station has been operating continuously since 1984. Currently, the major target weeds are *Mimosa pigra, Sida* spp., *Jatropha gossypiifolia, Hyptis suaveolens, Parkinsonia aculeata* and *Argemone* spp. Through the work of the Mexican Field Station, 14 agents have been released in Australia against four weed targets.

Keywords: *Argemone* spp., field surveys, *Hyptis suaveolens*, *Jatropha gossypiifolia*, *Mimosa pigra*, neotropical America, *Parkinsonia aculeata*, *Sida* spp.

History

The Entomology Division of Australia's Commonwealth Scientific and Industrial Research Organisation (CSIRO Entomology) has long been involved in weed biocontrol. From the late 1960s, CSIRO Entomology has conducted surveys for potential biological control agents of weeds from the Americas. A field station led by Ken Harley with John Winder as officer-in-charge (OIC), was set up in Curitiba, Brazil, to provide a base for this work. Early targets included lantana (*Lantana camara* L.), salvinia (*Salvinia molesta* D.S. Mitchell), water hyacinth (*Eichhornia crassipes* (Martius) Solms-Laubach, water lettuce (*Pistia stratioides* L.), alligator weed (*Alternanthera phylloxeroides* (Martius) Grisebach), mimosa (*Mimosa pigra* L.) and hyptis (*Hyptis suaveolens* (L.) Poit.). The station was closed in February 1982. In March 1984, CSIRO Entomology established the Mexican Field Station on the western coast of Mexico at Acapulco, Guerrero State. The leader was Ken Harley and John Gillett was OIC. This site closely matched the climate of northern Australia where the target weeds occur. Surveys on mimosa and hyptis that had commenced in Brazil were continued and new projects were initiated on *Sida* spp. In January 1987, the Station was transferred to Boca del Rio, Veracruz State, Mexico (lat. 19°08.6'N, long. 96°07.0'W, elevation 33 m). Since then, Ricardo Segura has been the Officer-in-Charge. Wendy Forno led the station from 1990 to 1999, followed by Tim Heard until the present. Additional target species surveyed from the Veracruz station include bellyache bush (*Jatropha gossypiifolia* L.), Mexican poppy (*Argemone mexicana* L. and *A. ochroleuca* Sweet) and parkinsonia (*Parkinsonia aculeata* L.). Collection of biocontrol agents for other target weeds were made for other institutions.

Scope of activities

The station undertakes the activities required in the early stages of biological control research. First, target-plant populations are located, often using the assistance of local botanists and herbaria records. Then fieldwork is conducted to collect the organisms associated with the plant, with their ecological and geographical data. Contact is made with local research institutions for advice and with government departments to obtain permits to study and export insects. Collected specimens are preserved, labelled, and sent to experts for

[1] CSIRO Mexican Field Station, A. Carlon No. 5, Ejido 1 de Mayo, Boca del Rio, A.P.14 Veracruz, CP 94297, Mexico.
[2] CSIRO Entomology, 120 Meiers Road, Indooroopilly, Queensland 4068, Australia.
Corresponding author: Ricardo Segura <Ricardo.Segura@csiro.au>.

identification, usually in North America where most interest and knowledge about American invertebrates resides. This results in lists of natural enemies (e.g. Gillett *et al.* 1991, Harley *et al.* 1995). Preliminary host-specificity testing is conducted on species that appear promising (e.g. Heard *et al.* 1997) or to validate the results of laboratory testing (e.g. Heard *et al.* 2004). Experiments or observations on biology, phenology and ecology of the target weed are also conducted (e.g. Lonsdale & Segura 1987). At all stages, the vast amount of information is captured in relational databases.

Up to 40 field trips to find biological control agents have been carried out in the Americas, namely to Mexico, United States of America, Belize, Guatemala, Honduras, Nicaragua, Costa Rica, Venezuela, Brazil, Cuba, Dominican Republic, Puerto Rico, Curaçao and Trinidad.

A field plot, required for open field experiments and growing plants, is maintained at La Aguada (lat. 19°03.0'N, long. 96°01.8'W, elevation 50 m). A web-maintained insect collection is central to achieving our goals. Our insect collection contains approximately 9400 pinned specimens with 800 duplicate specimens in alcohol. Approximately 5000 specimens have been sent for identification, including some new species. The herbarium collection contains approximately 900 specimens.

Target weeds

The Mexican Field Station has been the involved in a number of biological control research projects initiated by CSIRO (Table 1), as well as collaborating with other agencies working on other target weeds (Table 2).

The following list shows organizations that have collaborated with the station on specific weed projects, or were local research institutions or government departments that provided advice and permits to study and export insects. The organizations that have collaborated with the station on specific weed projects, or have provided advice and permits to study and export insects include:

- United States Department of Agriculture (USDA), United States of America
- Alan Fletcher Research Station, Queensland Government, Australia
- Northern Territory Government, Australia
- University of Queensland, Australia
- International Institute of Biological Control (now CABI BioScience), United Kingdom
- Plant Protection Research Institute, Pretoria, Republic of South Africa
- Secretaria de Agricultura, Ganaderia, Desarrollo Rural, Pesca y Alimentación (SAGARPA), Mexico
- Instituto de Ecología (IE) Xalapa, Veracruz, Mexico

Table 1. Target weeds comprising core CSIRO biological control research projects.

Common name	Scientific name	Family
Giant sensitive plant, mimosa	*Mimosa pigra* L.	Mimosaceae
Sida retusa	*Sida rhombifolia* L.	Malvaceae
Spinyhead sida	*Sida acuta* Burm. F.	Malvaceae
Flannel weed	*Sida cordifolia* L.	Malvaceae
Hyptis	*Hyptis suaveolens* Poit.	Lamiaceae
Bellyache bush	*Jatropha gossypiifolia* L.	Euphorbiaceae
Physic nut	*Jatropha curcas* L.	Euphorbiaceae
Mexican poppy	*Argemone mexicana* L.	Papaveraceae
Mexican poppy	*Argemone ochroleuca* Sweet	Papaveraceae
Parkinsonia	*Parkinsonia aculeata* L.	Caesalpinaceae

Table 2. Target weeds for which CSIRO has collaborated with other institutions to undertake biological control research.

Common name	Scientific name	Family
Leucaena	*Leucaena leucocephala* Lam. De Wit.	Mimosaceae
Silverleaf nightshade	*Solanum elaeagnifolium* Cav.	Solanaceae
Lantana	*Lantana camara* L.	Verbenaceae
Siam weed	*Chromoloena odorata* (L.) R.K.&H. Rob.	Asteraceae
Sicklepod	*Senna obtusifolia* L.	Caesalpinaceae
Parthenium weed	*Parthenium hysterophorus* L.	Asteraceae
Mimosa	*Mimosa asperata* L. (=*Mimosa pigra* var. *berlandieri*)	Mimosaceae
Crofton weed	*Ageratina riparia* (Reg.) R.K.&H.Rob.	Asteraceae
Mile-a-minute weed	*Mikania micrantha* Kunth	Asteraceae

- Universidad Autónoma del Estado de Morelos (UAEM), Mexico
- Colegio Profesional de Biólogos del Estado de Veracruz A.C. Delegación Veracruz, Boca del Río, Mexico
- Ministerio da Agricultura, Abastecimiento e Reforma Agraria da Brasil
- Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA)
- Centro Nacional de Pesquisa de Recursos Genéticos e Biotecnología (CENARGEN), Brasil
- Instituto de Investigaciones Forestales de Cuba (IIF)
- Universidad Central de Maracay, Instituto de Zoología Agrícola, Venezuela
- Consejo Nacional de Ciencia y Tecnología (CONICIT), Venezuela
- Ministerio de Agricultura y Cría (MAC), Venezuela
- Ministerio del Ambiente y de los Recursos Naturales, Venezuela
- Universidad Nacional Agraria (UNA), Managua, Nicaragua.

Agents released in Australia

Through the work of the Mexican Field Station, 14 agents have been released in Australia against four weed targets (Table 3) (Heard & Segura 2004). Most of these have established and several have made an impact on their target, especially *Neurostrota gunniella* (Lonsdale & Farrell 1998), *Carmenta mimosa* (Steinbauer 1998) and *Calligrapha pantherina* (Flanagan *et al.* 2000). Many other agents have been assessed for their potential and been rejected (e.g. Heard *et al.* 1998). In total, about 40 insects have been sent to Australian quarantine for further study. Additionally potential agents have been selected for *Sida cordifolia*, *Hyptis suaveolens, Argemone mexicana,* and *Parkinsonia aculeata.* Although only employing between two to three staff members, the Mexican Field Station has made a crucial contribution to weed management in Australia.

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Table 3. Agents released against target weeds in Australia as a result of work carried out by the CSIRO Mexican Field Station.

Weed and agent	Plant part attacked	Year of release
Mimosa pigra (Released: eight insect species and two fungal pathogens)		
Chrysomelidae		
Malacorhinus irregularis	Leaves, roots	2000
Curculionidae		
Coelocephalapion aculeatum	Flower-buds	1992
Coelocephalapion pigrae	Leaves and flower-buds	1994
Chalcodermus serripes	Mature green seed	1996
Sibinia fastigiata	Young green seed	1997
Gracillariidae		
Neurostrota gunniella	Tunnels in pinnae and small stems	1989
Sesiidae		
Carmenta mimosa	Tunnels in large stems	1989
Geometridae		
Macaria pallidata	Leaves	2002
Fungi		
Phloeospora mimosae-pigrae	Leaves, stems and pods	1995
Diabole cubensis	Leaves	1996
Sida acuta* and *Sida rhombifolia (Released: three insect species)		
Chrysomelidae		
Calligrapha pantherina	Leaf-feeding	1989
Curculionidae		
Eutinobothrus sp.	Stem-boring	1994
Eutinobothrus pilosellus	Stem-boring	1994
Jatropha gossypiifolia (Released: one insect species)		
Scutelleridae		
Agonosoma trilineatum	Feeds on fruits	2003

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Surveys for natural enemies of giant hogweed (*Heracleum mantegazzianum*) in the Caucasus region and assessment for their classical biological control potential in Europe

Marion K. Seier,[1] Rüdiger Wittenberg,[2] Carol A. Ellison,[1] Djamila H. Djeddour[1] and Harry C. Evans[1]

Summary

Heracleum mantegazzianum (Apiaceae) (giant hogweed), a biennial or perennial herb indigenous to the Caucasus mountains, has become an important invasive alien weed throughout Europe as well as in parts of North America. First introduced into botanical gardens in central and northern Europe in the late 1800s, *H. mantegazzianum* was subsequently widely planted as an ornamental and has now become invasive in most of its introduced range in western Europe. Giant hogweed not only replaces the native flora and alters ecosystems along waterways, but also poses a risk to human health by causing phytophotodermatitis after contact with its sap.

In January 2002, a collaborative European Union-funded program was initiated aiming to develop an integrated strategy for management of *H. mantegazzianum* in its exotic range. Biological control forms a central theme in this program and a series of surveys for both arthropod and pathogen natural enemies are being undertaken in the Caucasus region. The initial surveys have revealed an extensive mycobiota associated with *H. mantegazzianum*, most species of which are new records for this host. At least four, purportedly co-evolved pathogens belonging to the genera *Ramulariopsis, Septoria, Phloeospora* and *Phoma* were collected, of which the first three are under evaluation regarding their potential as biological control agents. The most prominent phytophagous insect present on *H. mantegazzinanum* was found to be the stem-boring curculionid *Lixus iridis*. Other insects collected attacked different parts of the host plant: Diptera species in the roots, Lepidoptera feeding on the leaves and flowers, and thrips species sucking on leaves, stems and flower heads. Likewise, the potential of these insect agents is currently being assessed.

Keywords: biological control, Caucasus, fungal pathogens, *Heracleum mantegazzianum*, phytophagous insects.

Introduction

Heracleum mantegazzianum Somm. & Lev., commonly known as giant hogweed, is a biennial or perennial herb belonging to the Apiaceae. Native to the western part of the Caucasus, the mountain range stretching from the Black Sea to the Caspian Sea, the plant was first introduced into botanical gardens in central and northern Europe in the late 19th century (Briggs 1979, Lundström 1984). With its impressive height of up to 5 metres and its large showy leaves and flowerheads, *H. mantegazzianum* was subsequently promoted by nurseries and actively planted as an ornamental curiosity in large private gardens and parks. Since then, the plant has escaped, spreading naturally by seed propagation, with each individual potentially producing up to 120,000 seeds per year (Dodd *et al.* 1994). The initial spread occurred mainly along rivers

[1] CABI Bioscience UK Centre, Silwood Park, Ascot, Berkshire, SL5 7TA, UK.
[2] CABI Bioscience Switzerland Centre, 1 Rue des Grillons, CH-2800 Delémont, Switzerland.
Corresponding author: Marion Seier, CABI Bioscience UK Centre, Silwood Park, Ascot, Berkshire, SL5 7TA, UK. m.seier@cabi.org

and streams, affecting riparian habitats (Caffrey 1994; Dodd *et al*. 1994). However, due to human activities, *H. mantegazzianum* has colonized a variety of habitats such as agricultural land, abandoned fields, and road and railway embankments, as well as urban sites (Pyšek 1994, Tiley *et al.* 1996). It is now a widespread weed in most of its introduced European range as well as in parts of North America (Morton 1978).

Rapid growth and a large leaf area forming dense canopies makes giant hogweed highly competitive and capable of shading out native herbaceous species, thus posing a significant threat to the local biodiversity and altering whole ecosystems (Vogt Andersen 1994, Pyšek & Pyšek 1995, Otte & Franke 1998). Following its annual winter die-back the weed exposes large areas of bare ground leaving the soil vulnerable to erosion, especially along riverbanks (Williamson & Forbes 1982). The sap, which is present in all parts of *H. mantegazzianum*, contains a number of furanocumarins giving it photosensitizing properties (Hipkin 1991). Skin contact with the plant causes severe blistering and dermatitis in affected humans, making the plant a serious public health hazard, especially when growing in amenity areas (Dodd *et. al*. 1994).

Current methods of control are based on herbicide application, particularly as glyphosate, animal grazing and mechanical control, such as cutting and ploughing (Dodd *et al*., 1994, Lundström & Darby 1994). However, overall, these methods have failed to give lasting control of the weed (Sampson 1994). While biological control has been considered as an alternative or additional strategy for the management of giant hogweed, little research has been undertaken in this field to date (Sampson 1990, 1994, Fowler *et al*. 1991, Caffrey 1994).

The need to develop an integrated management strategy that comprises effective, practicable and sustainable means to control the spread of *H. mantegazzianum* in its introduced European range, led to the initiation of a collaborative multidisciplinary project funded by the European Union in January 2002. Biological control, particularly the evaluation of co-evolved natural enemies from the native range of the plant as classical biocontrol agents, forms a central theme of this project.

Materials and methods

Field surveys

Two field trips were undertaken to the Russian part of the Caucasus mountains to establish the mycobiota and herbivore fauna associated with *H. mantegazzianum* in its native range and to assess the damage caused by individual fungal and arthropod species to this host, as well as to related plant species. The surveys were undertaken at the beginning of June and July 2002, respectively, covering 11 field sites between Pyatigorsk in the east and the Krasnodar territory in the west. The altitude of these sites ranges from 510 to 1670 m.a.s.l. A map showing the location of individual field sites is given in Figure 1.

Figure 1. Map showing the sites surveyed (☆) in the Caucasus region in 2002. (ref. http://caspian.hypermart.net/caucasus.gif)

Fungal pathogens

At each field site, a representative number of *H. mantegazzianum* plants and related species was assessed, comprising all ages of plants present. Level and type of pathogen damage was recorded and samples were taken. These were brought back in a plant press into the quarantine facilities at CABI Bioscience UK Centre where all subsequent work was carried out. Suspected biotrophic pathogens were isolated immediately onto *H. mantegazzianum* plants grown from seeds ex Kew Botantical Gardens, purportedly obtained from original plant collections in the Caucasus. Facultative pathogens were isolated onto potato carrot agar. A representative range of fungal specimens was taxonomically identified and deposited either in the culture collection or the dried reference collection of the CABI Bioscience fungal herbarium (Herb. IMI).

Plant inoculations with fungal pathogens were undertaken using spores obtained either from infected plant material or from agar cultures. Spore suspensions in sterile distilled water containing 0.01% Tween 80 were applied to leaf surfaces of *H. mantegazzianum* at a concentration of 10^6 spores ml^{-1} using a fine paintbrush. Either both upper and lower leaf surfaces were treated, or exclusively one or the other. Inoculated plants were placed in a dew chamber (Mercia Scientific, Birmingham, UK) for two days at 16°C and were subsequently maintained in a controlled environment room at 20°C, with a light regime of 12h light/12h dark. Treated plants were regularly assessed for the development of macroscopic symptoms of infection, and disease development was closely monitored and recorded.

Initial host-specificity studies were conducted using the following test plant species belonging to the same subfamily (Apioideae) within the Apiaceae as *H. mantegazzianum*: *Angelica archangelica* L. (angelica), *Coriandrum sativum* L. (coriander), *Daucus carota* L. (carrot), *Ferula communis* L. (giant fennel) and *Pastinaca sativa* L. (parsnip). Three plants were tested per species and a range of different leaf stages was inoculated. Inoculations and the subsequent maintenance of treated plants were carried out as outlined above. A test run was regarded as positive once the pathogen sporulated on the three inoculated plants of *H. mantegazzianum* included as positive controls. Individual test plant species were closely monitored for the development of any disease symptoms related to the respective fungal agent for at least double the period of time required by the pathogen to sporulate on its host.

Herbivores

Plants were taken at random and dissected according to a protocol developed beforehand. Besides general data about the field site, selected parameters of the plants were recorded in datasheets: plant height, diameter of the stem, diameter of the root, number of leaves and the length of the longest leaf, number of flower heads and the diameter of the central flower head. Observations of phytophagous insects on and in these parts of the plant were also noted. Each plant was dug out completely and all stems, petioles, and roots were dissected. In addition to these randomly chosen plants, as many plants as possible were assessed for signs of insect attack.

All stages of insects found were collected together with pieces of the plant parts, where they were feeding, and kept in plastic boxes. When necessary, plant material was replaced with fresh material. The samples were transported into quarantine at the CABI Switzerland Centre, where they were transferred onto potted plants. Adults emerging out of the rearing cages are being identified.

As with fungal pathogens, initial host-specificity tests were carried out using the following closely related native and economically important plants: coriander, carrot, *Foeniculum vulgare* Miller (fennel) and *Heracleum sphondylium* L. The adult feeding and oviposition tests were carried out under single-choice as well as multiple-choice conditions, using potted plants in cages. All plants were examined for feeding traces and dissected for eggs.

Results

Fungal pathogens

An extensive mycobiota was found to be associated with *H. mantegazzianum* in its native Caucasus region, with a number of species newly recorded from this host. At least four potentially co-evolved pathogens were found during the early season survey (June), though their overall abundance was very low. These pathogens were subsequently identified and deposited as: *Septoria heracleicola* Kabát & Bubák (Herb. IMI no. 389651); *Ramulariopsis* sp. nov. (Herb. IMI nos. 389652, 389653, 389656); *Phoma* sp. (possibly *Phoma longissima* (Pers.)Westend. (Herb. IMI no. 389654)); *Phloeospora heraclei* (Lib.) Petr (Herb. IMI nos. 389658, 389659). By mid season (July 2002) some additional fungal species, identified as *Ramularia heraclei* (Oudem.) Sacc. (Herb. IMI no. 389655) and *Erysiphe heraclei* DC. (not deposited), were recorded. It was noted that the abundance of all pathogens had markedly increased both on first year and mature plants. The genera *Phloeospora* and *Ramulariopsis* were also found on related *Heracleum* species and the inter-relationships are being investigated. Further evaluations of *P. heraclei* (Herb. IMI 389658), *S. heracleicola* and *Ramulariopsis* sp. nov. (Herb. IMI 389652) have commenced under quarantine conditions in the UK.

Field observations indicated that the coelomycete fungus *P. heraclei* might have a high potential as a biocontrol agent since it occurred at all sites, causing significant damage in the form of leafspot and die-back to *H. mantegazzianum* plants of all ages, and particularly to plants at the seedling stage. Laboratory studies

revealed *P. heraclei* to be a true biotroph or obligate parasite since it could be cultured only on its living host. Infection of *H. mantegazzianum* occurs through the lower leaf surface leading to chlorotic spots *ca.* 7 days after inoculation and subsequent sporulation *ca.* 2–3 days later. The pathogen forms conspicuous large pale brown acervuli bearing hyaline, curved conidia on the upper leaf surface of its host. These are associated with black crusts, considered to represent either feeding or survival structures, forming on the lower leaf surface. Under controlled environment conditions, infection of *H. mantegazzianum* with *P. heraclei* was consistently high. Initial host specificity studies showed that *P. heraclei* can sporadically infect parsnip and coriander, showing restricted sporulation with smaller pustules than those formed on *H. mantegazzianum*. To date, no symptoms have been recorded on the test plant species angelica, carrot and giant fennel.

Septoria heracleicola closely resembles *P. heraclei*, both in the disease symptoms caused as well as in macromorphology. In the field, mixed infections of both pathogens were frequently encountered on *H. mantegazzianum,* with *P. heraclei* being the dominant agent. Therefore, an accurate assessment of the impact of *S. heracleicola* on its host alone is still lacking. *Septoria heracleicola* can easily be cultured *in vitro* and produces infective conidia. Inoculation using conidia from agar culture leads to the formation of necrotic leaf spots on *H. mantegazzianum* after *ca.* 15 days. Sporulation on these necrotic lesions could be induced by incubation in a humid chamber for two days. Initial studies showed that *H. mantegazzianum* is susceptible to *S. heracleicola* under controlled environment conditions. A preliminary assessment of the host specificity of this pathogen has commenced and the results are pending.

The cercosporoid fungus *Ramulariopsis* sp. nov. is a hitherto undescribed species and is the first record of the genus *Ramulariopsis* for the family Apiaceae (J.C. David, pers. comm.). It remains to be established whether the different specimens deposited in the culture collection of the CABI Bioscience herbarium represent isolates of the same species. The pathogen causes angular lesions with a distinct cottony appearance once sporulation occurs. Its impact on *H. mantegazzianum* in the field can be variable. *Ramulariopsis* sp. nov. produces infective conidia *in vitro* and initial pathogenicity studies have shown that the first symptoms of disease, seen as necrotic lesions on infected leaves, appear *ca.* 10 days after inoculation. The pathogen sporulates predominantly on the lower leaf surface of its host.

Herbivores

While there were no feeding traces on any of the roots during the first survey in June 2002, Diptera larvae were found to mine the roots later in July. The species have not yet been identified and some of them feeding on the outer parts of the root may be saprophytic. The most obvious phytophagous insect present on the plants was *Lixus iridis* Ol. (Curculionidae), which was found on most sites, with a high abundance at some sites. It was not uncommon to find 10 larvae in one stem. The adults mate on the highest parts of the plants and, after copulation, eggs are laid inside the hollow stem. Dissections and field observation of the adults show that eggs and larvae occur mainly in the stem, but also in the larger petioles of the leaves. Adults and eggs of this weevil were also recorded on another *Heracleum* sp. (most probably *Heracleum asperum* (Hoffm.) M. Bieb.) during the survey. Some 74 adults were brought back into quarantine at the Centre. Besides these root and stem-feeding insects, some unidentified leaf-feeding larvae were found, especially in the western part of the surveyed area. Two thrips species were found in very high numbers on *H. mantegazzianum*, one feeding on the leaves and the other on the stems and flower/seed heads. The latter species had a dramatic impact on the development of flowers on single plants. Unfortunately, it was subsequently determined as the rather polyphagous *Thrips vulgatissimus* (Haliday). *Depressaria* spp. (Oecophoridae) larvae were found feeding within the flower heads.

Preliminary host-range testing with *L. iridis* showed that they did not feed or oviposit on three plants of economic importance, i.e. coriander, carrot and fennel, under single as well as multiple-choice conditions. However, during the tests only nine eggs were found on *H. mantegazzianum*, but feeding was recorded on almost all host plant replicates.

A previously undetermined noctuid larvae feeding gregariously on the leaves of *H. mantegazzianum* in the Caucasus, was also used in preliminary host-range testing. After adult emergence, the species was identified as *Mamestra brassicae* (L.) Barathra, a well known pest on *Brassica* spp. In single-choice tests the larvae fed on all the test plants offered.

Discussion

In order to develop an integrated strategy for management of *H. mantegazzianum* in Europe, biological control needs to be considered as one approach, potentially contributing to long-term control of the weed. Areas with restricted infestations of giant hogweed can be effectively managed employing mechanical and chemical control, but the only sustainable solution for large populations of the weed would be classical biological control. While a range of herbivores and fungal pathogens has been recorded from giant hogweed in its exotic distribution, these comprise mainly polyphagous insect species, which generally have little impact on the plant; and generalist pathogens, usually exhibiting a relatively wide host range (Sampson 1990, Bürki & Nentwig 1998). Hence, such insect and fungal species are considered to have little or

no potential as biological control agents. In contrast, knowledge about the natural enemy complex that co-evolved with *H. mantegazzianum* in its native Caucasus range is still scarce. The recent survey work documented here has established that a diverse mycobiota and herbivore complex is associated with giant hogweed in its centre of origin, with a number of species constituting first records for this host.

Phloeospora heraclei appears to have high potential as a biological control agent due to its impact on *H. mantegazzianum*. Being particularly damaging to *H. mantegazzianum* at the seedling stage, the pathogen would affect the weed at a critical phase of its life cycle given the inability of giant hogweed to spread vegetatively (Ochsmann 1996). However, the host specificity of *P. heraclei* will need to be critically evaluated given its apparent ability to infect non-target species, such as parsnip and coriander, under controlled environment conditions. Artificial host range extension of plant pathogens in greenhouse screening is a well-documented phenomenon (Cother 1975, Evans 1995) and restricted sporulation, as seen for *P. heraclei* on these test species, is generally viewed as an expression of plant resistance (Heath 1982). However, records in the CABI Bioscience fungal herbarium revealed that *P. heraclei* has been reported from parsnip as well as from the indigenous *H. sphondylium* in the UK and other European countries. The incidence of *P. heraclei* on these hosts reported from Europe, as well as a potential presence of the pathogen on *H. mantegazzianum* in its exotic range, has to be established and pathogen strains from different hosts and regions need to be characterized and compared.

Regarding the other two fungal pathogens currently under evaluation, *S. heracleicola* and the cercosporiod fungus *Ramulariopsis* sp. nov., their impact on *H. mantegazzianum* needs to be determined and their host specificity assessed. Fungal pathogens belonging to the genus *Septoria* as well as other cercosporoid fungi have already been used, apparently with success, in biocontrol programs against invasive weeds, as for example *Septoria passiflorae* Syd. against Banana Poka (*Passiflora tripartita* (Juss.) Poir var. *tripartita* Holm-Nie. Jörg & Law) in Hawaii (Trujillo *et al.* 2001) and *Cercospora rodmanii* Conway against waterhyacinth (*Eichhornia crassipes* (Martius) Solms-Laubach) in South Africa (Morris & Cilliers 1992).

The field surveys conducted in 2002 for arthropods attacking *H. mantegazzianum* indicate that seed feeders might be the most promising insects as biocontrol agents, e.g. *Depressaria* spp. The field observations found three insects to be most damaging to the host plant, but subsequent identification of these insects revealed that they are polyphagous, and thus cannot be considered as potential biocontrol agents. Some of the insects collected as larvae have yet to emerge, whilst others have not yet been identified.

In 2003, additional surveys will be undertaken both in the invasive and the native range of *H. mantegazzianum* in order to complete the inventory of fungal pathogens and herbivores associated with this host. It is hoped that further visits in the Caucasus at different times of the year and other field sites will reveal additional agents for further studies.

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Candidates for the biological control of teasel, *Dipsacus* spp.

René Sforza[1]

Summary

Dipsacus fullonum L., wild teasel, and *D. laciniatus* L., cut-leaf teasel (Dipsacaceae), native to Eurasia, were introduced into North America in the 1700s. Primarily cultivated for its seedheads, *D. fullonum* escaped from cultivation and colonized waterways, waste ground, fallow fields and pastures, outcompeting native plants. This study reports on foreign exploration for biological control agents against teasel in its native range. Countries from France to Russia were surveyed, with a particular emphasis on insects feeding either on rosettes or seedheads. Two potential candidates were collected, namely the checkerspot butterfly, *Euphydryas aurinia* (Lepidoptera: Nymphalidae), and the leaf beetle, *Galeruca pomonae* (Coleoptera: Chrysomelidae). This is the first report of these two insect species feeding on teasel. Both were collected in the same locations in northern Turkey, and may feed concurrently on the same plants. *Galeruca pomonae* was also collected from south-eastern Russia. Preliminary host-choice and no-choice test experiments showed that *G. pomonae* can complete its entire development on teasel but does not feed on carrot, radish, cabbage, or lettuce. *Euphydryas aurinia* populations from Turkey were parasitized by a tachinid fly, *Erycia furibunda*. Ecological considerations and host specificity are discussed for potential biological control programs.

Keywords: Biocontrol, Chrysomelidae, Dipsacaceae, Endothenia, *Euphydryas, Galeruca,* invasion, Nymphalidae, teasel, Tortricidae, weeds.

Introduction

Teasel is an invasive species in North America. Research on herbivores of *Dipsacus fullonum* and closely related species has been conducted since 2000. This paper gives an overview of the current research on selection of natural enemies against teasel and understanding its ecology in its native range. The term "teasel" will be used in this paper and refers to *Dipsacus* species in general.

Taxonomy and distribution in its native range

Dipsacus fullonum L. (syn. *D. sylvestris*, *D. sativus* for the cultivated teasel), the wild teasel, is native in Eurasia, from north-western Africa to the northern middle east. Botanists are not clear about the binomial terminology of wild and cultivated teasel (Werner 1975b). This plant belongs to the Dipsacaceae family (300 species worldwide) divided into three tribes, which comprise only 12 North American species among the genera *Dipsacus*, *Knautia*, *Succisella*, *Cephalaria* and *Scabiosa*, all aliens. Closely related families are Caprifoliaceae (500 spp., mainly in Asia), Valerianaceae (400 spp. worldwide), Morinaceae (15 spp. in Asia) and Adoxaceae (1 spp. in North America) (Verlaque 1985b, Wahlberg 2001). With 75% of the total species, the Mediterranean basin and Middle East are considered the likely centre of origin for Dipsacaceae. The genus *Dipsacus* comprises 19 species worldwide; nine distributed in Eurasia, including *D. fullonum, D. laciniatus, D comosus, D. ferox, D. bulgaricus, D. gmelini*, eight in Asia, and two in Africa (Verlaque 1985a).

Biology and ecology of teasel

This robust monocarpic biennial to perennial herb reproduces by seed, producing over 3,000 seeds per plant. No vegetative reproduction has been observed. Depending on conditions, up to 30–80% of the seeds

[1] USDA-ARS, European Biological Control Laboratory, Campus International de Baillarguet, CS 90013 Montferrier sur Lez, 34988 St Gely du Fesc, France <email: rsforza@ars-ebcl.org>.

will germinate, so each plant can produce many offspring. Seeds also can remain viable for at least two years. Seeds typically do not disperse far; most seedlings will be located around the parent plant (Werner 1975b). Parent plants often provide an optimal nursery site for new teasel plants after the adult dies. Dead adult plants leave a relatively large area of bare ground, formerly occupied by their own basal leaves, which new plants readily occupy. Teasel grows in open sunny habitats producing rosettes with puckered leaves having scalloped edges during its first year of growth, after which a 2 m tall prickly flower stem emerges. It flowers from July to September. Found on a variety of soils, it prefers abundant moisture in poorly drained areas, tolerating spring flooding very well. When established in a new area, a teasel population may remain for decades in the same field or roadsides.

In North America, numerous ecological studies on teasel were undertaken to evaluate rosette development, population growth rates, and the effect of the invasion on plant communities (Caswell & Werner 1978, Werner 1977). Bolting, flowering and the size of the remaining rosette vegetation are highly correlated with the size of the vegetative rosette in the first year. Rosette size gives a better prediction of plant fate than does age (Werner 1975a).

Why is it a problem?

Common teasel and *D. laciniatus*, the cutleaf teasel, were primarily introduced from Europe into North America possibly as early as the 1700s. No hybrids have been described so far. Until the 1950s, cultivated teasel was considered a valuable commercial product in North America for its head, used to "tease" or raise the nap on wool cloth, and as a horticultural plant. It has been cultivated in Europe since Roman times for the same purpose. An interesting historical fact is that the Popes of Avignon (France) awarded prizes to the grower cultivating teasels which were then used in the manufacture of the cloth for their vestments. In North America, in the last 20–30 years, it escaped from cultivation and colonized natural areas extending from central Maine south to south-eastern Virginia and west to Utah in the US, and from Ontario to British Columbia in Canada. Lack of natural enemies allowed teasel to proliferate, and it rapidly became common and abundant, probably aided by construction of the American interstate highway system. Whereas the plant is not a problem on Eurasian agricultural land, it is considered a noxious weed locally in Colorado, New Mexico, Missouri and Iowa. Teasels are invading plains, waste grounds, old fields, and pastures and along ditches and edges of forests. If left unchecked, teasel can quickly form large monocultures excluding all native vegetation. Cut-leaved teasel is considered more aggressive than common teasel and has severely threatened several northern and central Illinois natural areas. Teasel may be resistant to control measures (Glass 1991) and spread over high-quality natural communities, as shown by its threatened displacement of a native plant of sensitive conservation status, *Cirsium vinaceum* (Woot. & Standl) in central New Mexico (Huenneke & Thomson 1995). Huenneke & Thomson report that, in the greenhouse, growth of *C. vinaceum* rosettes was significantly reduced by the presence of *Dipsacus*, but the invader was unaffected by the thistle.

Literature records of pathogens and invertebrates on teasel

There is currently a very short list of natural pathogens and invertebrates reported worldwide attacking teasel.

Pathogens

In the US, an aphid-borne potyvirus is reported from California inducing leaf mottling and malformation on teasel and *Scabiosa atropurpurea* (Stoner 1951). The species *Macrosiphum rosae* (L.) and *Myzus persicae* (Sulz) were reported as vectors. Several ubiquitous American fungi hosted by teasel were listed by the USDA (cited by Werner (1975b)), e.g. *Cercospora elongata* Pk, *Mycosphaerella asterinoides* (Ell & Ev.) Fairm., *Peronospora dipsaci* Sacc. *Phyllactinia corylea* Pers. and *Phymatotrichum omnivorum* (Shear). None of these fungi have been studied in detail for teasel infection, but they cause diseases in crops (fruits, potatoes) and have low potential for biocontrol.

Invertebrates

A non-specific nematode, *Ditylenchus dipsaci*, is reported from Idaho (USA), first collected on *Dipsacus* in 1888 but also proved to infect potatoes (Thorne 1945). In the Old World, several rosette invertebrates are reported to attack teasel but without causing any damage. A fly, *Phytomyza ramosa* Hd. (Agromyzidae), is known to attack teasel in the UK (Topham 1968). In addition, teasel flowerheads support larvae of certain tortricid species, namely *Endothenia gentianaeana* (Hübner) and *Cochylis roseana* (Haworth) (Cheesman 1996). A butterfly, *Euphydryas desfontainii* (Godart), is sometimes reported as feeding on *Dipsacus fullonum* (Wahlberg 2001). The list of insects from flowers is great, including many insect orders, mostly Hymenoptera and Diptera acting as pollinators (Judd 1983). A potential candidate for biocontrol is a lepidopteran larva, identified as *Epiblema* near *pflugiana* (Tortricidae), reared out from immature buds of teasel collected in the northern Caucasus (G. Campobasso, pers. comm.).

Survey and insect collection

Field surveys in 2001 and 2002 were conducted in Turkey, south-east Russia (Kransnodar region),

France, Spain and Ukraine to look for insects on different species of teasel. Ukraine and Turkey were intensively surveyed. We focused on the rosette stage for collecting phytophagous insects attacking plants before flowering. The flowerhead stage was also of interest for insects that may reduce the seedbank. In spring, rosettes of different sizes were inspected and dug up. Roots were inspected for the presence of pathogens or root-feeding insects. All insects found on leaves as larval stages were caged into cardboard tubes with fresh teasel leaves until they reached the quarantine greenhouse in Montpellier. From each collection site at different time of the year 10 dried flower heads in which a tortricid larva was present were cut off at 10 cm below the top, then transferred into cardboard tubes for transportation.

Preliminary experiments

Rearing

Insects (*Galeruca* (Chrysomelidae) and *Euphydryas* (Nymphalidae)) of foreign origin were reared out on French teasel rosettes grown from seeds in quarantine in pots of 12 cm diameter with perlite and compost (50/50). From April to August, insects were maintained in Plexiglass cages at 22°C, under natural light, and under an average RH of 60%. Plants were removed when defoliated and replaced with fresh material one to two times per week depending on feeding activity. Fifty 2nd instar larvae of *Galeruca* were isolated in Petri dishes each with a teasel leaf disc, and were measured at each instar. All Petri dishes were sealed with parafilm. Duration between stages was evaluated.

Host specificity

A preliminary choice and no-choice test was undertaken with *Galeruca*. Radish, carrot, lettuce, turnip and cabbage were selected for economic value, and for their similar leaf structure with teasel. A leaf disc of 4-cm in diameter was used for each plant and stored at 22°C, 16/8 (L:D) using artificial fluorescent illumination, and under an average RH of 80%. In the no-choice test, a single disc was placed in a Petri dish with three 2nd instar larvae, which were surveyed for feeding activity every day until they died. In the choice test, several combinations with five plants plus teasel were used. Three leaf discs were put in each Petri dish with three larvae of 2nd instar, and were surveyed under the same conditions as before. There were two to three replicates in each treatment. Three positive controls consisted of teasel leaf discs with three larvae each, kept under the same conditions as test Petri dishes.

Results

Teasel distribution and ecological data

Teasel was common from southern Spain to south-eastern Russia. Teasel was rare in west and central Ukraine; the main populations were near the Carpathian mountains, and in south-eastern Crimea. Teasel is very widely distributed in northern Turkey, from Ankara to the Black Sea, but rare or absent in the rest of the country. In general, the largest populations were found when moisture is maintained at relatively high levels throughout the growing season, along rivers, and in abandoned, moist fields. Elevation of teasel sites ranged from sea level to 1875 m (in northern Turkey).

List and collection sites of insect species on teasel

Table 1 shows all the insects collected from Palaearctic sites. Large numbers of chrysomelid beetles, *Galeruca pomonae*, were collected on *Dipsacus*. The largest populations of *Galeruca* species were found in Turkey and Russia. Tortricid larvae were collected from each country surveyed and from most sites within a country.

Table 1. Insects collected from *Dipsacus* plants in Eurasia in 2001–2002.

Insect family	Species	Collecting area	Part of the plant attacked
Coleoptera			
Chrysomelidae	*Longitarsus luridus* (Scopoli)	Krasnodar region (Russia)	rosette
	L. brisouti Heikertinger	Krasnodar region (Russia)	rosette
	Phyllotreta nigripes (F.)	Krasnodar region (Russia)	rosette
	Chaetocnema tibialis (Illiger)	Krasnodar region (Russia)	rosette
	Galeruca (Circassica) pomonae Scop.	Kastamonu (Turkey)	rosette
	G. pomonae Scop.	Krasnodar region (Russia)	rosette
Lepidoptera			
Nymphalidae	*Euphydryas aurinia* Rottemburg	Kastamonu (Turkey)	rosette
Tortricidae	*Endothenia gentianaena* (Hübner)	France, Ukraine, Russia, Turkey	flowerhead
	Diceratura ostrinana (Guenée)	Southern France	flowerhead
	Not identified	Krasnodar region (Russia)	rosette tip

Damage observed

Galeruca

In the field, larvae of all stages of *Galeruca* fed on the leaf blades and on the tip of the rosettes. In April, feeding activity observed was high, with punctures and holes on all leaves and with development of necrosis around holes. Damage due to feeding activity on a rosette depended on the number of larvae per plant, their stage, and the size of the rosette. In Russia, larvae per rosette ranged from 4 to 33 (average number = 20 for 5 rosettes). This beetle can be very damaging, causing whole mats of rosettes to be defoliated. Both larvae and adults were teasel feeders.

Euphydryas

In the field, late larvae are solitary and damage the rosette by intense feeding on margins of the leaves. In the quarantine laboratory, larvae were gregarious at all stages, and colonized rosette leaves, enclosed in wax webs. In July, they were feeding heavily before entering diapause.

Tortricidae

Endothenia gentianaena was confined to one larva per seedhead, feeding on the pith in the central cavity. We did not observe significant damage to seedheads. Several unidentified species inducing severe damage to the plant were collected (data not shown) in the rosette tips.

Biology and ethology of two selected species

General data on *Euphydryas aurinia*

Euphydryas aurinia was found in northern Turkey, near Kastamonu city (1100 m elevation), in semi-alpine areas. Teasel was the only observed food plant. In April, mature larvae were moving from plant to plant, from rosettes to old teasel stems, probably looking for a secure place to pupate. Large larvae were feeding actively on rosette leaves. In Europe, *E. aurinia* is known for its gregarious larval behaviour in the spring (Porter 1982), but my observations in Turkey indicated that they were more solitary in nature. Insect larvae collected were then reared on French teasel in quarantine. Pupation occurred on the top of cages in mid June. Most of the pupae were parasitized by a single tachinid fly, *Erycia furibunda* Zetterstedt. From pupae, we obtained 40 adults that were kept on *Knautia* flowers. The rearing of *E. aurinia* was continued past the adult stage. The females were given *Dipsacus* plants for oviposition. White eggs, rapidly turning yellow, were laid in clusters on the leaves. The subsequent egg batches were collected and the hatching larvae were reared on teasel but larvae did not survive diapause. Both American and French teasel plants served as food plants and were accepted by insects.

General data on *Galeruca pomonae*

Insects were found from 300 to 1100 m elevation in the field in Turkey and at sea level in Russia. We observed high resistance of larvae to moisture inside the rosettes. In August, a *G. pomonae* female was found buried 10-cm deep into the soil along a *Dipsacus fullonum* root. This behaviour was observed in quarantine; after mating, females were found in the pots, sometimes dead. Egg clusters were collected along the inner edge of the pots. Brown in colour and covered by ornamentation, eggs were stuck to soil particles and in between them. We were not able to obtain a second generation. After collecting 1st instar larvae in April, the first adults emerged in June and survived until August in the quarantine on teasel plants. Females may mate several times before egg laying. Table 2 shows partial life tables for *Galeruca pomonae* originating from Turkey and maintained on teasel leaves.

Host specificity

Figure 1 shows survival of *G. pomonae* on different cultivated plants in no-choice and choice conditions. In all treatments, teasel was the only plant attacked. In no-choice tests, survival never exceeded 16 days (radish), and was less than 10 days for lettuce, cabbage and turnip. For radish, 8 of 9 larvae died before 12 days. In choice tests, larvae seemed to live longer than in no-choice as they were associated with teasel leaf discs. Only teasel discs were eaten. A few tentative feedings were observed on cabbage in the treatment carrot+cabbage+*Dipsacus*, but only on the day before the larvae died. In many treatments, even in no-choice, larvae moulted. Controls were the only treatment in which larvae continued their development until adult emergence. For positive controls, we stopped the experiment at 40 days, when 5 (of 9 in the three replicates) individuals were still alive.

Table 2. Measurements of larvae and pupae and duration of 3rd to pupal instar for *Galeruca pomonae*.

Larval stage	3	4	5	Pupa
Mean length in cm ± SE	4.39 ± 0.53 (N = 49)	6.09 ± 0.59 (N=42)	7.63 ± 0.89 (N=41)	7.52 ± 0.80 (N=40)
Mean duration in days ± SE		7.26 ± 1.16 (N=38)	15.79 ± 1.66 (N=39)	3.50 ± 1.08 (N=39)

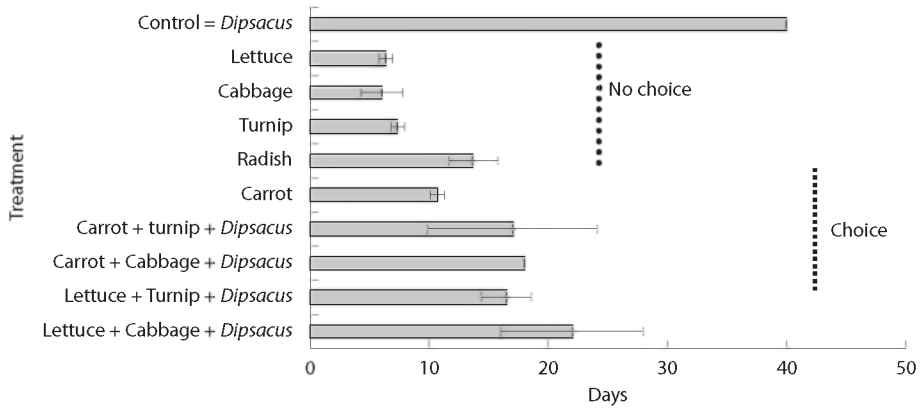

Figure 1. Preliminary choice and no-choice tests with *Galeruca pomonae*.

Conclusions

Evaluation of potential biocontrol agents for further studies

Chrysomelidae

This group is an important source of introduced insects for biological control of weeds in North America (White 1996). Based on the literature, just a few chrysomelid beetles collected in this study were previously reported on *Dipsacus*. Of these, *P. nigripes* and *C. tibialis* were probably accidental feeders (A. Konstantinov, pers. comm.), as they were known from other host plant families. *Longitarsus luridus* is known from other Dipsacaceae, and might be a candidate for further studies. *Galeruca* species might be promising agents for biocontrol of teasel. Our specificity test was only an indicator and showed real association with teasel plant. The short list of test plants does not reflect monophagy of this population of *G. pomonae*, but opens to discussion the role of this species for controlling teasel. It is clear that more Dipsacaceae plants should be included in subsequent host-specificity tests, as well as genera from closely related families, like *Gentiana* sp., *Lonicera* sp., *Plantago* and *Centaurea* sp.

Nymphalidae

Euphydryas aurinia is reported feeding on plant species from three different families: Caprifoliacae, Gentianaceae, and Dipsacaceae (Wahlberg 2001). Within the latter, the genera *Succisa, Scabiosa, Cephalaria and Knautia* are reported as host plants. Our findings show a particular adaptation of *E. aurinia* for Dipsacaceae. It is surprising that *Dipsacus* sp. is not reported as a host plant, as *E. aurinia* is commonly distributed in northern Turkey and is a species widely studied in the Palaearctic region. Nevertheless, this result is confirmed by recent molecular studies reporting that *E. aurinia* is closely related to *E. desfontainii* (both within the named *E. aurinia* clade) feeding on *Dipsacus fullonum*, and on two other plant species belonging to Dipsacaceae (Wahlberg 2001). The importance of certain secondary chemicals known as iridoid glycosides and seco-iridoids to host use by butterflies has been shown (Jensen *et al.* 1975). The *E. aurinia* clade from the Palaearctic mainly utilizes plants in Dipsacaceae containing only seco-iridoids that are also present in Caprifoliaiceae or Gentianaceae on which a few populations of *E. aurinia* feed (Wahlberg 2001).These results have to be compared with Nearctic species in the *Euphydryas* group that are able to exploit a wide range of plants containing both iridoid metabolites, suggesting that *E. aurinia* originating from Eurasia became specialized on Dipsacaceae, which contain only seco-iridoids. In fact, the evolutionary history of host-plant use in the *Euphydryas* group is somewhat linked to the biogeography of the group and suggests promising expectations for a biocontrol program using these species. Special attention should now be devoted to the plant families having only (or mainly) seco-iridoids, such as Dipsacaceae, Gentianaceae, and Oleaceae (Zimmermann *et al.* 2000). On this basis, the choice of host plants for testing specificity will be facilitated by using chemical signatures of selected plants. The main concern will be on the potential split of *E. aurinia* clade onto North American native plants, but it is known that *Euphydryas* species are monophagous or at most oligophagous at the population level (Mazel 1986, Singer 1983), which must be ascertained by host-plant experiments. Potential for biocontrol is also supported by observed mortality occurring when insects are experimentally put on plant species other than the natural one (Mazel 1982). For further collections, we need to consider larval parasitism, as we collected a tachinid fly, previously reported from *E. aurinia* and *E. desfontainii* (Ford and Shaw 1991), and the status of endangered species for the *E. aurinia* clade, which is protected in France.

Tortricidae

As yet, no precise evaluation of seed reduction by these tortricid larvae in *Dipsacus* flowerheads has been made. It is reported for *E. gentianaena* that the mean

number of 10 seeds per head is damaged, which is very low compared to teasel seed production (Cheesman 1996). For another tortricid species, *Cochylis roseana,* not found in this study, larvae are held together by solidified, compacted frass, and tend to remain in the teasel heads. The impact should not be negligible as up to 30 individuals may be contained in one single head (Cheesman 1996). Comparing the two microlepidoptera, it seems that *C. roseana*, if specific to teasel, would have more potential than *E. gentianaena* in terms of damage, as many larvae may feed in one flower head instead of a single for *E. Gentianaena.*

Perspectives for further studies

For the Turkish populations of *E. aurinia* and *G. pomonae*, open field tests could take place in the same location, as the insects are sympatric. At the same time, investigations should now focus on French populations of these two groups. Insects of French origin feeding on teasel will be reared and studied outside quarantine, making host-specificity tests easier to undertake. The *Euphydryas aurinia* clade is distributed is southern France, and we will evaluate their specificity for teasel. Concerning chrysomelid beetles, the well-known polyphagy of *Galeruca pomonae* has to be clarified for these Russian and Turkish populations. A recent study reported differentiation between biological and genetic data for characterization of the species status of a weevil, for which morphology was not sufficiently discriminating (Fumanal *et al.* 2002). Other *Galeruca* species might be of interest, as larvae are active leaf feeders. The Lepidoptera is an interesting group to focus on for rosette feeders because, in addition to Nymphalidae, several other species feeding in the rosette tip might have a severe impact on teasel growth as the main shoot is destroyed.

To conclude, surveys on other plant species among the Dipsacaceae, such as *Cephalaria leucantha*, *Knautia arvensis* and *Succisa pratensis*, might provide other potential candidates, as all Dipsacaceae in North America are introduced species.

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Quantitative field surveys for the selection of biological control agents for *Genista monspessulana*, based on host range and efficacy assessment

Andy Sheppard and Thierry Thomann[1]

Summary

Surveys for potential biological control agents of weeds provide opportunities to collect detailed quantitative data on the community structure of phytophagous species associated with particular plant species and their close relatives. Such studies are still few and far between, but offer increased understanding of assemblage rules of species with different degrees of host-plant specialization and the numbers and abundances of species in different feeding guilds. Including a range of closely related host plants also allows comparison of natural enemy community structure across similar host-plant species with different local abundances and regional distributions. When such surveys also measure agent impact, they allow agent selection to be based on efficacy as well as specificity. The preliminary results of quantitative surveys of natural enemy communities on species in the tribe Genisteae, particularly *Genista monspessulana* (French, Montpellier or Cape broom), around the Mediterranean, are presented. Sampling consisted of fixed beating-tray samples on up to ten individual flowering plants per site. Seed pods were also collected from the plants when they matured, and then dissected to quantify attack and abundance of seed feeders. Insects collected were sorted to species, and counted and analyzed for species diversity by site and region. Sample sites were selected based on the co-occurrence of two to several host-plant species to allow comparison of host use and abundance. Analysis of the preliminary results is discussed together with the value of quantitative field surveys in biological weed control.

Keywords: agent selection, insect–plant interactions, natural enemy communities, seed predation, species abundance.

Introduction

Surveys of potential biological control agents for weeds are most frequently made by qualitatively listing the natural enemy species found on the target, and perhaps co-occurring species in the same genus, during trips throughout its native range, together with simple descriptions of known feeding habits, likely specificity from literature records and geographical distributions (e.g. Zwölfer 1963), or by listing sites where each insect was sampled (O'Donnell 1986). Syrett & Emberson (1997) extended this approach to quantitative sampling of insects on plants in full flower on all co-occurring species in the same tribe as the target, and analyzing these data to look at likely specificity of the main insects found. For more precision, sampling can then be focused on one or a few sites where agent damage (Hosking 1995) and abundance (Mazay 1993) can be measured more precisely. These approaches allow information on the abundances, specificity and damage levels of the different natural enemies in the community to be relatively quickly obtained without investing years on the detailed ecology of the system (e.g. Waloff 1968).

Quantitative sampling of the invertebrate communities on several closely related hosts also provides explanations of the effects of host-plant phylogeny, architecture, spatial pattern and abundance on natural

[1] CSIRO European Laboratory, Campus International de Baillarguet, 34980 Montferrier-sur-Lez, France. Corresponding author: Andy Sheppard <andy.sheppard@csiro-europe.org>.

enemy species richness (Lewinsohn 1991, Lawton *et al.* 1993), species packing (Zwölfer 1987, Lawton 1990), guild structure and levels of specificity (Frenzel & Brandl 1998, Prado *et al.* 2002). Such data sets can also be used to explore community assemblage rules (Gaston & Lawton 1990, Hanski & Gyllenberg 1993) and to compare the structure of native communities with those newly developed in the exotic range (Lawton 1982, Moran & Southwood 1982, Memmott *et al.* 2000). Despite this, very few quantitative data sets exist of natural enemy communities across a group of closely related hosts with associated records of abundance, guild structure and specificity that can help understand whether these communities evolved through sequential adaptation or competition (Frenzel & Brandl 1998). Biological-control surveys provide a great opportunity to collect such data to help explain how and why natural enemy communities differ between closely related host plants (Zwölfer 1987)

In this paper, we present the preliminary results of a quantitative survey approach adopted for natural enemies on plants in the tribe Genisteae in the Mediterranean region focused around the target *Genista monspessulana* (L.) L.A. Johnson. This approach goes one step further in complexity than previous studies (e.g. Syrett & Emberson 1997), by attempting to combine field assessment of host range, abundance and damage across the whole native range of the target, thereby incorporating host range and efficacy as equally important in the agent selection process (McFadyen 2003, Sheppard 2003). At each site, quantitative data were separately collected per plant of the natural enemy community present from all plant species in the tribe during peak flowering and within mature seed pods. The specific aim was to develop a prioritized list of potential biological control agents for use against *G. monspessulana* and other widespread Mediterranean weeds in the Genisteae. This approach also provides an opportunity to explain how the phytophagous community attacking species in the Genisteae is organised in relation to local differences in host frequency, abundance and geographical distribution.

Materials and methods

Literature search

To complement the published species lists from previous biological control survey trips against target weeds in the Genisteae (e.g. Zwölfer 1963, O'Donnell 1986, Syrett & Emberson 1997, Syrett *et al.* 1999), a standard online literature search was made of CABI Abstracts and Zoological Record for all references containing the key words either *Genista, Cytisus* or *Ulex*, as well as searching the standard taxonomic reference books on the phytophagous arthropods and plant pathogens of Europe. The results of the literature search was used, in combination with the comparative quantitative survey data, to generate a list of stenophagous to monospecific arthropods found on *G. monspessulana* during field surveys. It was also used to list those genera where separate species are known to occur on *G. monspessulana*, *Cytisus scoparius* (L.) Link and *Ulex europaeus* L.

Surveys

Conventional survey trips were carried out throughout most of the native distribution of *G. monspessulana* around the Mediterranean, on the north coast from Greece to Portugal, and to Tunisia on the south coast. Areas intensively searched were typical native habitat, i.e. rainfall 600+ mm per annum, less than 1000 m altitude on acid soils that support oak or pine-overtopped maqui vegetation. A separate trip was made to Tenerife and Gomera in the Canary Islands, because, while *G. monpessulana* does not occur there, these islands are a centre for diversification of very closely related *Genista* spp. (= *Teline*), including the exotic weed *Genista stenopetala* Webb & Berth (Percy 2003), and the only native range of *Chamaecytisus proliferus* (L.f.) Link (tagasaste), a key test plant for Australia where it is also grown as a forage species for livestock. The surveyed areas support many co-occurring species in the Genisteae, so sites were selected to include several species in the tribe where possible, and where not, samples were taken in large monospecific stands of the common species present. Particular effort was made to find sites where *G. monspessulana* co-occurred with *C. scoparius* or *U. europaeus* for comparison, as focused survey trips have been made for these species in the past and the natural enemy community found on them is relatively well understood (Zwölfer 1963, Syrett *et al.* 1999).

Quantitative sampling

Two trips were made to each site. On the first "mid-flowering" visit (between March and May) sampling consisted of three sharp taps (with a shortened broom handle) to 10 plants per Genisteae species per site (where possible) with a 1.5 m × 1.5 m beating sheet held under each plant. All arthropods were collected with an aspirator except for very numerous species where a subsample was collected from a random section of the beating sheet and the numbers of individuals calibrated up for the whole sheet. Immature stages of herbivorous species where adults clearly were not present (e.g. Lepidoptera larvae) were placed in separate rearing boxes with the food plant. Attempts were made to rear out adults for identification. Plants were also searched visually to collect any obvious endophagous species not sampled by beating, including leaf miners, gall formers, stem and root borers and obviously pathogenic fungi. Such species were recorded as present or absent. Samples from individual plants by host species by site were kept separately. Herbarium samples were taken to confirm plant identifications.

All arthropods were sorted, counted and identified as far as possible (to family or genus) in the laboratory in Montpellier on return, and voucher specimens were sent for identification from all species clearly on *G. monspessulana* alone and all species in the following orders/families: Lepidoptera, Diptera, Curculionidae, Apionidae, Chrysomelidae, Cerambycidae, Bruchidae, Buprestidae, Aphididae, Cicadellidae, Psyllidae and Miridae.

Another visit was made to each site (except sites in Greece) just before seed-pod maturation (in June to July) in the previous, same or subsequent year and all the pods from 10 randomly selected plants per species were collected and dry-stored separately per plant in ventilated plastic boxes. If arthropod species exited the green pods as larvae to pupate in the soil prior to collection then they were noticed from their emergence holes in the pods. Those larvae that did emerge from the pods soon after collection were placed in rearing dishes of moist vermiculite until adult emergence. After a minimum of three-months storage, the samples were sorted for emerged adult phytophagous arthropod species from the whole sample and then 30 pods per plant were dissected to quantitatively assess the attack rate and impact of the different arthropod species on total plant seed production, by relating damage characteristics to phytophagous species.

Analysis

The quantitative natural enemy species data from the beating trays were combined for each site sampled and the number of each species found per plant that were a) specific to the *G. monspessulana*, b) specific to the tribe Genisteae, c) specific to the family Fabaceae and d) other generalist species (including flower visitors) was calculated for each site and region. These data were then used to calculate Shannon diversity indices, *H*, per plant for each site and region. The pod dissection data were used to calculate the percentage seed loss per plant for each pre-dispersal insect seed predator identified a) for the seven most common Genisteae species sampled across all sites and b) between regions where *G. monspessulana* was sampled.

The data from the first site sampled with high abundance of *G. monspessulana* and several other Genisteae, Romanya de la Selva in north-eastern Spain, were used to assess the efficiency of the sampling regime at locating the total number of species present at a site. This site was also selected because the number of natural enemy species was relatively high (>25) compared with other sites sampled during the early surveys. At this site, two extra beat samples were taken, providing a total of 12 samples. The average number of species sampled from 1 through to 12 samples was calculated for all combinations of sample order. By plotting this against the number of samples, a rarefaction curve was generated, the asymptote of which estimates the number of samples necessary to have captured all the species present at the site (Müller-Schärer *et al.* 1995).

Results

Literature search

The literature search generated a list of 183 insects recorded from hosts in the genus *Genista*, of which 28 had already been recorded from *G. monspessulana*, and 134 insects recorded from hosts in the genus *Ulex,* of which 87 had already been recorded from *U. europaeus*. The literature search found no significant additions to the known list of 243 insect species recorded from *C. scoparius* (Syrett *et al.* 1999). This search supported the argument that historical sampling effort on *U. europaeus* and *C. scoparius* had led to much higher known natural enemy communities on these weeds, but that a similar sampling effort on *G. monspessulana* and other species in the Genisteae would improve understanding of the natural enemy community within the tribe.

Sites and sampling

The coastal surveys have so far included 10 sites in Spain (in the north-east and south-west), four sites in Portugal, four sites in coastal France, 10 sites in Corsica, three sites in Sardinia, five sites in western Italy and Sicily and four sites in Greece. The density of sampling reflected the frequency and abundance of *G. monspessulana.* Sampling was also carried out at 16 sites in the Canaries and six sites in Tunisia on other species in the Genisteae. Species in the Genisteae sampled throughout these surveys are included in Table 1. Sites surveyed and analysed in this paper are given in Figure 1. Beat samples were taken at 30 sites containing *G. monspessulana* and pod samples were taken at 25 of these sites. The remaining unsurveyed regions within the native range of *G. monspessulana* include the eastern coast of Italy and the Balkan coast, Turkey and Morocco.

The assessment of the efficacy of the beating tray sampling is presented in Figure 2 from the site in north-eastern Spain. According to this relationship, the sample size of 10 plants per site used throughout the surveys would be expected to find 93% of the total number of species estimated to be present at that site. It appears that the sample size chosen was sufficient to collect the vast majority of species during this survey at the time of sampling.

Natural enemies of *G. monspessulana*

The quantitative beating-tray and pod-sample surveys in the northern Mediterranean region have so far found 85 species of phytophagous arthropod on *G. monspessulana*. Of these, 26 are considered to be specific to the level of the tribe Genisteae and 8 are specific to the genus *Genista* (Table 2). The rust *Uromyces genistae* Fuckel was also observed attacking old leaves in late spring and summer (Guynot & Massenot 1958).

Table 1. Species of Genisteae sampled since January 1999 and whether or not arthropods were found. Surveys included Greece, France, Italy, Spain Portugal and Tunisia. Nomenclature follows <http://www.ildis.org/LegumeWeb/>.

Species	Number of sites sampled alone	Number of sites sampled together with other Genisteae	Total number of sites sampled	Number of sites where arthropods were found on the plant
Genista monspessulana [a]	9	34	43	42
Genista stenopetala [b]	0	3	3	2
Genista canariensis [b]	0	1	1	1
Genista corsica	0	2	2	1
Genista ferox	2	0	2	2
Genista linifolia [a]	0	1	1	0
Genista microcephala	3	0	3	3
Genista tricuspidate	2	0	2	2
Cytisus villosus	5	16	21	21
Cytisus scoparius [a, b]	1	8	9	7
Cytisus arboreus	0	7	7	7
Chamaecytisus proliferus [a]	4	7	11	10
Spartium junceum [a, b]	2	6	8	6
Calicotome spinosa [a]	0	3	3	3
Calicotome villosa	4	10	14	14
Adenocarpus foliolosus [b]	0	4	4	2
Adenocarpus telonensis	0	4	4	4
Spartocytisus filipes [b]	0	1	1	1
Stauracanthus boivinii	0	1	1	1
Retama raetam [a, b]	1	1	2	0
Ulex europaeus [a, b]	1	7	8	8

[a] Species that are also exotics.
[b] Species only (or also) sampled in the Canary Islands.

Figure 1. Map of the sample sites (O) of agent prospecting surveys for the biological control of *Genista monspessulana* around the Mediterranean. Quantitatively sampled sites are shaded.

Most foliar damage observed was caused by the psyllid *Arytinnis hakani* (Loginova). The psilid fly *Chyliza (Chyliza) leptogaster* (Panzer) and the buprestid *Agrilus antiquus* Mulsat et Rey (Schaefer 1949) were the only species observed killing mature plants, although only in a restricted part of the native range in south-eastern France. Amongst the seed feeders, the bruchid beetle, *Bruchidius lividimanus* (Gyllenhal) was the commonest species, followed by the apionid *Lepidapion (Lepidapion) argentatum*

(Gerstäcker) and weevil *Pachytychius sparsutus* (Olivier). A population of *Bruchidius villosus* (F.) in north-eastern Spain was found restricted to *G. monspessulana* despite the presence of *Cytisus villosus* Pourret. *Cytisus scoparius* is the commonest host of this species in northern Europe (Haines *et al.* 2004), but our surveys also found a second population restricted to *Spartium junceum* despite the presence of *G. monspessulana* in southern France. This suggests *B. villosus* may also attack these other species in the Genisteae in Australia, New Zealand and North America, where it has been introduced as a biological control agent for *C. scoparius*.

A comparison of Table 2 with a similar list for *C. scoparius* (Syrett *et al.* 1999), suggests it contains very few species in all orders except the Coleoptera and that there remain many species not yet detected in our surveys from *G. monspessulana*. Several species were also found during the literature search (e.g. Emmet & Heath 1992), which have not yet been seen

Table 2. The abundance and frequency of the 32 phytophagous arthropod species that the literature suggests are at least specific to the tribe Genisteae, and that were sampled during the beating-tray survey of 30 *Genista monspessulana* sites in Greece, Italy, France, Spain and Portugal. Information includes their likely specificity, their phytophagous feeding guild and other genera of the Genisteae from which these species were also collected during these surveys.

Species	Specificity[a]	Guild[b]	Insects plant^{-1}	Frequency (%)[c]	Other Genisteae genera
Hemiptera					
Arytaina genistae (Latreille)	2	1	4.00	3	*Cytisus*
Arytinnis hakani (Loginova)	1	1	11.98	70	*Cytisus, Calicotome*
Acyrthospihon pisum ssp. *spartii* (Koch)	2	1	0.15	7	*Genisteae*
Gargaria genistae (F.)	1	1	0.43	37	*Cytisus, Spartium*
Heterocordylus ? leptocerus (Kb)	2	1	8.05	7	*Cytisus*
Orthotylus ? adenocarpi (Perris)	2	1	11.82	40	*Cytisus*
Diptera					
Chyliza leptogaster (Panzer)	1?	7	1.5	16	
Asphondylia sp. (galls)[d]	2	3	0.22	53	
Lepidoptera					
Agonopterix nervosa (Haworth)	2	2	0.71	17	*Calicotome*
Agonopterix scopariella (Heinemann)	1	2	0.09	25	*Cytisus*
Callophrys rubi (L.)	2	2	0.04	3	
Pseudoterpna pruinata (Hufnagel)	2	2	0.10	3	*Cytisus, Calicotome*
Oecophoridae sp.[d]	2	2	0.08	13	
Pyralidae sp.[d]	2	2	0.12	13	*Cytisus*
Tortricidae sp.[d]	2	2	0.19	13	*Genista, Cytisus, Calicotome*
Coleoptera					
Chrysomelidae					
Gonioctena (*Spartoxena*) sp.[d]	2	2	0.20	7	
Bruchidae					
Bruchidius villosus (F)	2	5	0.67	33	*Cytisus, Calicotome, Spartium*
Bruchidius lividimanus (Gyll.)	2	5	3.61	57	*Genista, Cytisus, Calicotome*
Buprestidae					
Anthaxia sp., *Agrilus antiquus* & *Agrilus cinctus*	2	7	0.09	7	
Apionidae					
Exapion fuscirostre (F)	1	5	0.20	3	*Cytisus, Calicotome*
Exapion nr. *putoni* (Ch. Brisout)	1	5	0.60	7	*Genista, Calicotome*
Lepiapion argentatum (Gerstäcker)	1	5	1.39	37	
Oryxolaemus ? scabiosus (Weise)	1	3	0.20	3	*Cytisus, Calicotome*
Pirapion ? immune Kirby	2	3	0.13	3	*Cytisus*
Protopirapion attratulum (Gemar)	2	4	0.08	7	
Curculionidae					
Pachytychius sparsutus (Ol)	2	5	0.38	7	*Cytisus, Calicotome*
Peritelus senex (Boheman)	2	6	0.93	3	
Pleurodrusus carinula (Olivier)	2	6	0.09	7	*Cytisus, Spartium*
Sitona gressorius (F.)	2	6	1.00	3	
Sitona regensteinensis (Herbst)	2	6	0.84	20	*Cytisus, Calicotome, Spartium*

[a] Specificity: 1 = specific to genus, 2 = specific to tribe.
[b] Guild: 1 = sap sucker, 2 = defoliator, 3 = leaf miner/galler, 4 = flower feeder, 5 = seed feeder, 6 = root feeder, 7 = stem feeder.
[c] Percentage of *G. monspessulana* sites where species sampled.
[d] Detailed rearing and identification required.

in the field. Table 3 summarises the currently known specialist arthropod community on *G. monspessulana, C. scoparius* and *U. europaeus* developed from both the literature search and field collections from *G. monspessulana.* This table focuses on arthropod genera where the literature suggests there are different species using these three closely related hosts.

Table 3. A comparison of the specialist arthropod community on *Genista monspessulana,* and the previously documented community on *Cytisus scoparius* (Syrett *et al.* 1999) and *Ulex europaeus* (Zwölfer 1963) generated from the literature search and field collections. Species in bold type are the extreme specialists that appear to be restricted to one host or the other.

Family	Genus	Species on *G. monspessulana*	Species on *C. scoparius*	Species on *U. europaeus*
Eriophyidae	***Aceria***		***genistae***[a]	***genistae***
	Tetranychus			***lintearius***[a]
Psyllidae	***Arytaina***		***genistae***	
	Arytinnis/Arytainilla	***Arytinnis hakani***[a]	***Arytainilla spartiophila***[a]	
Aphididae	*Acyrthosiphon*	*?spartii*	*spartii*	
	Aphis	***genistae***	***sarothamni***	***ulicis***
Membracidae	*Gargaria*	*genistae*	*genistae*	*genistae*
Pentatomidae	*Piezodorus*	*lituratus*	*lituratus*	*lituratus*
Miridae	***Heterocordylis***	***genistae,*** *leptocerus*	***tibialis***, *leptocerus*	*parvulus*
	Globiceps	*fulvicollis,* ***genistae***	*fulvicollis*	
	Orthotylus	*adenocarpi, beieri, virescens*	*adenocarpi, beieri, virescens*, ***concolor***	
	Platycranis	***boreae***	*bicolor*	*bicolor*
Geometridae	***Chesias***		***legatella***	
	Isturgia		***limbaria***	
	Pseudoterpna		*pruinata*	*pruinata*
Oecophoridae	***Agonopteryx***	*scopariella, nervosa*	***assimilella,*** *scopariella, nervosa*	***ulicetella,*** *nervosa*
Lyonetiidae	***Leucoptera***	***laburnella***	***spartifoliella***[a]	
Nepticuliidae	***Trifurcula***	***serotinella***	***immundella***	
Gelechiidae	***Mirificarma***	***cytisella***	***mulinella***	***ulicinella***
Gracillariidae	***Phyllonorycter***	***stainoniella***	***scopariella***	***uilicicolella***
Tortricidae	***Cydia***	*succedana*	*succedana,* ***scopariana***	*succedana*[a], ***ulicetana, internana***
Psilidae	***Chyliza***	***leptogaster***		
Cecidomyiidae	***Asphondylia***		***sarothamni, pilosa***	***ulicis***
Tenthredinidae	*Rhogogaster*		*genistae*	
Cerambycidae	*Deilus*	*fugax*	*fugax*	*fugax*
Buprestidae	*Agrilus*	*antiquus, cinctus*	*antiquus, cinctus*	
	Anthaxia	*funerula*	*funerula*	*funerula*
Bruchidae	*Bruchidius*	*lividimanus, villosus*	*lividimanus, villosus*[a]	*lividimanus*
Chrysomelidae	*Gonioctena*	***sexnolatus, gobanzi****, variabilis*	***olivacea,*** *variabilis*	
Apionidae	***Lepidapion***	***argentatum***,[a] *squamigerum*	*squamigerum*	***pseudogallaecianum***, *squamigerum*
	Exapion	*?plutoni*	***fuscirostre***,[a] *plutoni*	***ulicis***[a]
	Pirapion	*immune*	*immune*	*immune*
	Protopirapion	*attratulum*	*attratulum*	*attratulum*
Curculionidae (roots)	***Sitona***	*regensteinensis,* ***gressorius***	*regensteinensis,* ***puberulus***	*regensteinensis,* ***striatellus***
	Polydrusus	***?cervinus***, *prasinus*	***confluens***, *prasinus*	
	Peritelus	*senex*	*?*	*?*
Curculionidae (seeds)	***Tychius***		***parallellus***	
	Pachytychius	*sparsutus*[a]	*sparsutus*	*sparsutus*

[a] Released or studied as a biocontrol agent.

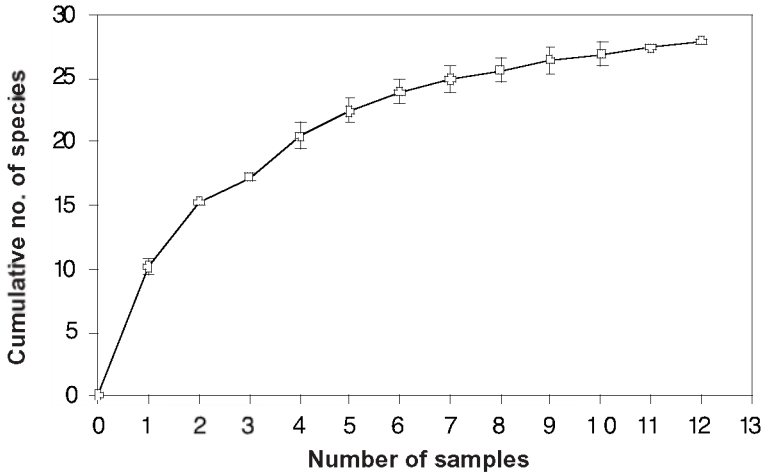

Figure 2. The rarefaction curve for the number of herbivorous arthropod species detected on *Genista monspessulana* against the number of plants sampled using the described beating method at Romanya de la Selva, Sierra de Gavarres, south-eastern Girona, south-western Spain. This analysis includes all arthropod species collected; both specialists and generalists.

Quantitative analysis

The percentage of beat samples from *G. monspessulana* that included each natural enemy specific at least to the tribe Genisteae and the number of individuals of that species per sampled plant are given in Table 2. The average number of species found per site in each region and the total number of species sampled per region are presented in Figure 3 for species sampled that were a) specific to the *Genista monspessulana*, b) specific to the tribe Genisteae, c) specific to the family Fabaceae and d) all other insects found including generalist flower visitors. Shannon diversity index *H* mean values calculated for each region surveyed are presented in Figure 4 for a) species found at least specific to tribe Genisteae, b) species found at least specific to the family Fabaceae and c) all insects found including generalist flower visitors. The diversity of the largely specialist insect species collected suggests that the centre of origin of *G. monspessulana* is in the western Mediterranean.

Data from the pod dissections from each of the 25 *G. monspessulana* sites were used to estimate pre-dispersal seed losses to insects for the different Genisteae species sampled across sites (Table 4) and variation in seed loss per site to the different pod feeding insects between regions (Figure 5). The overall average seed predation level in the pods of *G. monspessulana* was 22%. This was higher than for any other co-occurring species in the Genisteae except *Calicotome spinosa* (L.) Link, although only two populations of this were sampled. *Lepidapion argentatum* damage was the highest, but bruchids also caused comparable losses (Table 4). There was large variation is seed losses to the different seed predators across plants and sites and in overall seed losses per seed predator species between native range region where *G. monspessulana* occurs (Figure 5), ranging from 6 to 39% across regions and 1 to 63% across sites.

Discussion

Quantifying the natural enemy community

A comparative approach is starting to show how communities of natural enemies differ between closely related host plants (e.g. Table 3). We have also started to turn a qualitative picture of the natural enemy community into a quantitative description of the patterns of abundance and diversity of all species in this community in relation to their specificity and host use. With such a description, community assemblage rules can be explored that may explain what determines the abundance and number of highly specific and damaging species using individual hosts (Gaston & Lawton 1990, Hanski & Gyllenberg 1993). Understanding community assemblage rules would also assist biological control in its attempts to create stable natural enemy communities on weeds in their exotic range that have the capacity to suppress host populations.

Quantitative biological control surveys also provide valuable information on the potential damage species may inflict on their hosts if released. Here we have started to show the variation in damage levels observed for seed feeders as well as the mean. Natural enemies that show wide variation in the damage they inflict across many sites are more likely to be suppressed by extrinsic bottom up (plant density) or top down (predation) ecological processes, which they might escape from following release. This assists agent efficacy evaluation prior to release (Sheppard 2003).

Table 4. Percentage seed loss per plant overall and for the five seed predators across 7 species of Genisteae at 25 sites containing natural populations of *Genista monspessulana* in the native range in Spain, France and Italy.

Genisteae	*n*	Mean total % seed loss per plant	*Bruchidius lividimanus*	*Bruchidius villosus*	Apionid spp.[a]	*Pachytychius sparsutus*	Lepidoptera
Genista monspessulana	25	22.03 ± 3.40	5.33 ± 1.18	2.92 ± 1.12	8.38 ± 3.49	2.00 ± 0.55	3.40 ± 0.96
Cytisus villosus	8	9.73 ± 4.54	4.76 ± 1.24	0.06 ± 0.05	4.74 ± 4.49	0.02 ± 0.02	0.15 ± 0.12
Cytisus scoparius[b]	2	1.19 ± 0.26	1.01 ± 0.43	0.00	0.00	0.00	0.17 ± 0.17
Cytisus arboreus	2	12.78 ± 4.56	8.60 ± 1.90	0.00	2.05 ± 1.81	0.88 ± 0.77	1.25 ± 0.08
Calicotome villosa	6	16.35 ± 5.14	10.94 ± 5.65	0.00	4.78 ± 3.58	0.36 ± 0.40	0.03 ± 0.03
Calicotome spinosa	2	34.27 ± 2.02	2.94 ± 1.87	0.00	0.00	31.02 ± 0.16	0.31 ± 0.31
Adenocarpus telonensis	2	4.36 ± 3.52	0.00	0.00	0.00	0.00	4.36 ± 3.52

[a] *Lepidapion argentatum* on *G. monspessulana*, *Exapion ? subparallelum* (Dbr.) on *C. villosa*, *Exapion ? fuscirostre* on *C. villosus* and *C. arboreus*.

[b] Data only for sites where *C. scoparius* co-occurs with *G. monspessulana* (Corsica); other studies in the native range have estimated seed loss of *C. scoparius* to be 15–23% (Hosking 1995), 0.4–24% (Mazay 1993), 26% (Hinz 1992).

Selecting effective agents also requires clear understanding of the population dynamics of the target weed in the exotic environment. There is good understanding of the population dynamics and ecology of *G. monspessulana* (Pareja 1999, Lloyd 2000), *C. scoparius* (Rees & Paynter 1997, Sheppard *et al.* 2002) and *U. europaeus* (Rees & Hill 2001). All these studies suggest that the best agent for these woody weeds with seed-based reproduction is an agent that can reduce lifetime seed production. A stem or root borer that prematurely kills adults would therefore receive a high priority, but these studies also show that agents that directly reduce seed production can also be very useful, particularly in habitats of low fertility or where seedling mortality is naturally high (Sheppard *et al.* 2002). They would also be useful for weeds that are still spreading significantly, by both reducing rate of population spread and reducing the control efforts required for other management strategies.

Selecting agents for *G. monspessulana*

The preliminary results of these surveys suggest that the most damaging agents are the psyllid *A. hakani* attacking the foliage, the fly *C. leptogaster* and buprestid *A. antiquus* attacking the stems and roots, and the beetles *B. lividimanus*, *L. argentatum*, *B. villosus* and *P. sparsutus* attacking the seeds. The only pathogen found so far was only present in significant amounts on old leaves.

That a psyllid appears on this list is highly desirable from a specificity perspective. The genera *Lupinus* and *Ulex* in the Genisteae have no recorded psyllid species and the four genera of arytainine psyllids known to feed

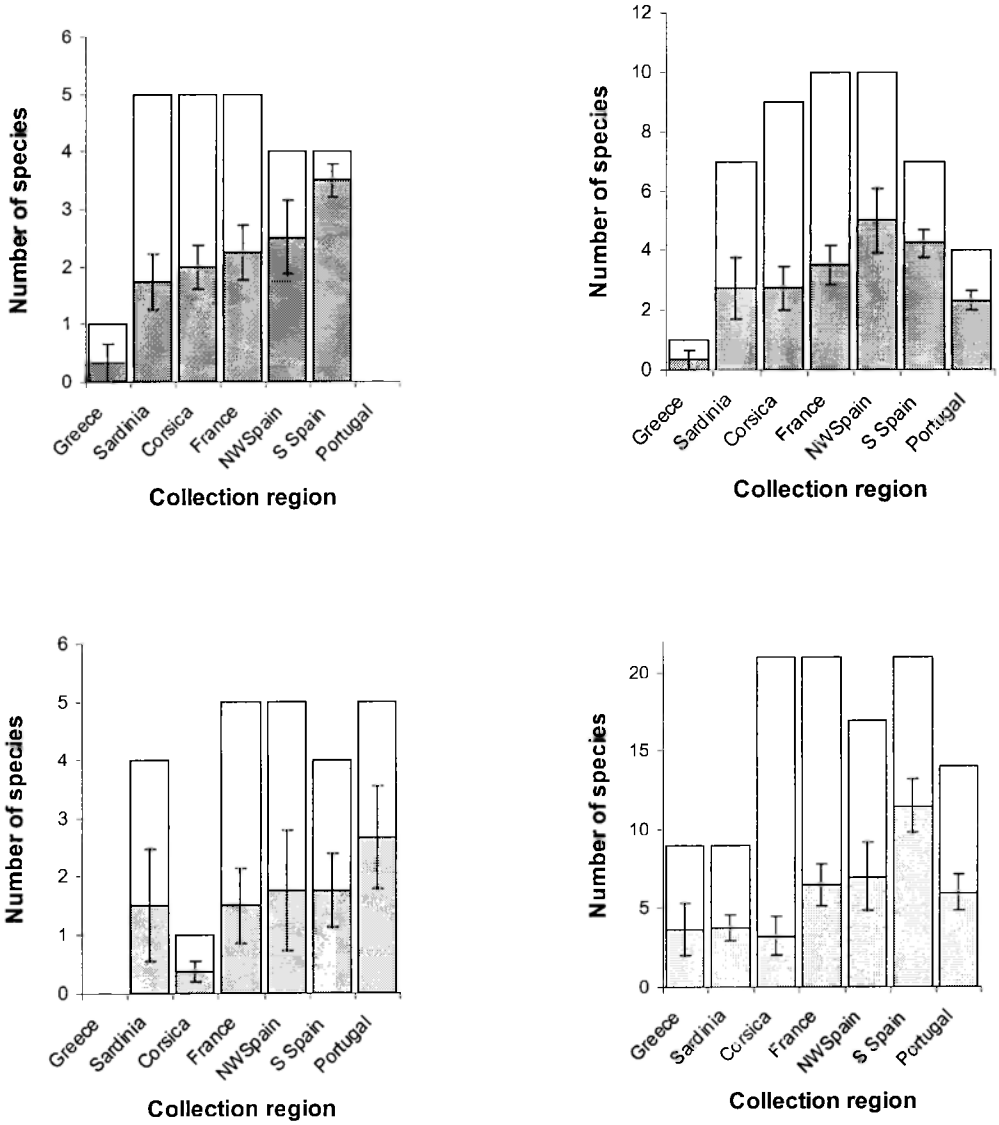

Figure 3. Mean number of species sampled on *Genista monspessulana* per site (filled section ±SE) and total number (bar height) for each region sampled for species a) specific to *Genista*, b) specific to the Genisteae, c) species specific to the Fabaceae and d) generalist flower visitors etc.

on host plants in the Genisteae are restricted within the tribe (Hodkinson & Hollis 1987, Burckhardt 1989) with a high percentage of monospecific species (Percy 2003). Only one species in the genus *Arytinnis*, *Arytinnis modica* (Loginova) comb. n., has hosts in two genera (*G. stenopetala* and *C. proliferus*) and an analysis using the molecular phylogeny of arytainine psyllids to date the separation of these two host races suggests divergence occurred 70,000–121,000 years ago (D. Percy unpublished data). *Arytinnis hakani* has only ever been recorded from *G. monspessulana* and has a relatively wide geographical distribution (northern and southern coasts of the western Mediterranean from Portugal to Italy and Morocco to Algeria). As molecular and morphological evidence suggests the genus *Arytinnis* probably originates from the Canaries (Percy 2001), where *G. monspessulana* does not occur, the association between this psyllid and *G. monspessulana* may be recent. We found *A. hakani* only on *G. monspessulana* and only in the western Mediterranean. We found no evidence of other species of psyllids using *G. monspessulana* as a host. *Arytaina genistae* (Latreille) was found on *C. scoparius* at sites where this co-occurred with *G. monspessulana*, but *A. genistae* was clearly not using *G. monspessulana* (though this species will develop on *C. proliferus*; S. Fowler, pers. comm.). Evidence from California, where *A. genistae* has been accidentally introduced, but is only found on *C. scoparius* and not *G. monspessulana*, supports this.

Of the stem borers, the psilid fly *C. leptogaster* was only observed through the Massif des Maures in France, but appears to be a significant cause of early plant population decline at this sample site. The larvae tunnel under the bark, either ring-barking whole branches or causing widespread necrosis of cambium tissue. This genus of 57 species worldwide (Iwasa 1989) from a small family are considered to be bulb and stem miners, however, very few of these have known host plants. *Chyliza leptogaster* has been recorded from nut-like wood galls on *Physocarpus* and *Spiraea* sp. (Rosaceae) in northern Europe, however Collin (1944) talks about slight morphological differences between his *C. leptogaster* and a "southern form" described by Rondani in Italy in 1876 which the latter called *Chyliza premixta* Rondani. Rondani records no host plant for his species. Chandler (1975) comments that this genus had fairly "chaotic taxonomy". However, a slight concern is the tendency of some species in the genus to appear to only oviposit into existing wounds (e.g. *Chyliza annulipes* Macquart on *Pinus*, Lyneborg 1987).

The buprestid *A. antiquus,* found in the same region as *C. leptogaster*, was also observed to be associated with plants that had died prematurely in low density populations of *G. monspessulana.* Like *C. leptogaster*, it was not found in nearby *C. villosus* and *Calicotome villosa* (Poiret) Link stands, although the literature suggests it will attack many species in the Genisteae.

Of the seed feeders, *B. lividimanus* appears to have too broad a host range to be useful in countries where native or commercially important species in the Genisteae occur. The seed-feeding apionid *L. argentatum* is also likely to be highly specific to *G. monspessulana*. The genus *Lepidapion* has *ca.* 16 Mediterranean and Canary Island species and two subgenera and shows a high degree of monospecificity. Hosts in the genus include members of *Genista, Ulex, Retama, Spartocytisus* and *Cytisus* (Alonzo-Zarazaga 1985, Ehret 1990). A major revision of the genus is required. Currently, *Genista umbellata* (L'H & eacute; r.) Poiret and *Adenocarpus* sp. have been included in the host range of *L. argentatum*, and *Lepidapion acuminatum* (Schilsky) has also been recorded attacking *G. monspessulana* near Cadiz in southern Spain (Alonzo-Zarazaga 1985), but there is probably only one highly

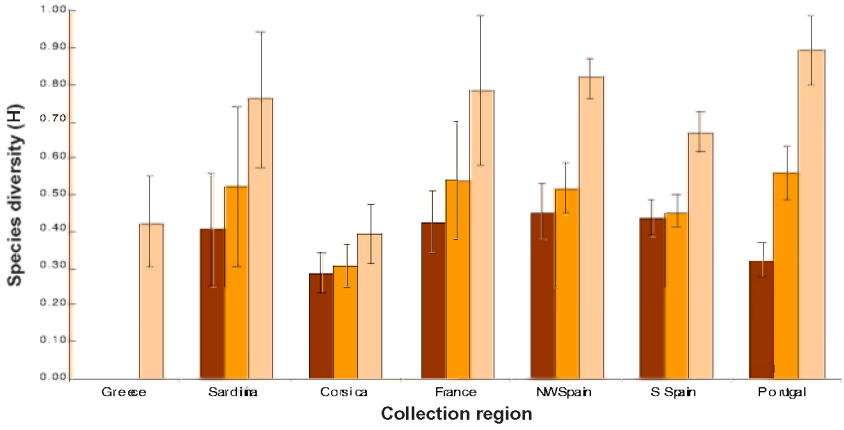

Figure 4 Mean Shannon species diversity indices per *G. monspessulana* plant for each region sampled at flowering for natural enemies a) at least restricted to the tribe Genisteae (dark shading), b) at least restricted to the family Fabaceae (mid-shading) and c) all natural enemies found (light shading) (±SE).

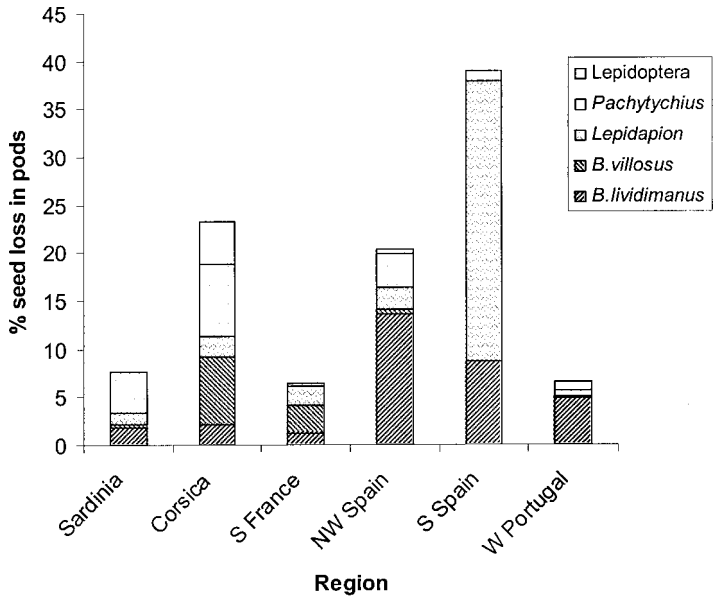

Figure 5. Percentage seed loss per plant in *Genista monspessulana* to pre-dispersal seed predators (Lepidoptera, the weevil, *Pachtychius sparsutus*, the apionid *Lepidapion argentatum* and the two bruchids, *Bruchidius villosus* and *B. lividimanus*) at 25 sites in 6 distinct geographical regions throughout south west Europe.

specific species on *G. monspessulana* and *L. argentatum* may not be its correct name (M. Alonzo-Zarazaga, pers. comm.). Molecular and morphological comparisons will need to be made of *Lepidapion* species on Genisteae throughout the Mediterranean to clearly understand both the taxonomy and host range of species in this genus. *Pachytychius sparsutus* is less specific, but also has potential as a biological control agent. The currently known hosts do not include either *Lupinus* or *Ulex* (Hoffmann 1958, Freude *et al.* 1981), although it would probably feed on *C. proliferus*.

There remain several groups and species, notably the Lepidoptera, that are still too poorly understood, but may have potential for the biological control of *G. monspessulana*.

Conclusion

In this paper, we have tried to emphasize how quantitative agent surveys can be a valuable way of understanding both the host range and damage capacity of natural enemies on target weeds in their native range. This can provide benefits for agent selection, which we are applying in the case of *G. monspessulana*, but we have also outlined the benefits the resulting databases may offer to our general ecological understanding of the structure of natural enemy communities on plants.

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Avoiding and exploiting trophic cascading: its role in the selection of weed biological control agents

Lincoln Smith[1]

Summary

Ecologists have long argued that the "world is green" because natural enemies, rather than host plants, limit the size of herbivore populations. Theoretically, in the absence of these higher trophic natural enemies, herbivore populations would increase until they overexploit their host plants, causing the populations to crash. Such drastic reductions of target weed populations are exactly what biological control practitioners are trying to accomplish. Historically, the pre-release evaluation of candidate biological control agents has focused primarily on finding agents that are host-specific, and secondarily on those that impact the target plant. Nevertheless, regardless of how much "impact" an individual natural enemy has on the target plant, successful classical biological control also depends on the production of large numbers of natural enemies. A biological control agent is likely to fail if its reproduction and survival are limited by factors such as incompatible climate, poor host-plant suitability, or attack by higher trophic natural enemies. The first two factors are usually considered in biological control projects, but the last is often overlooked. As a consequence, for example, two species of coleophorid moths introduced to control *Salsola tragus* (Russian thistle) in the western United States, became widely established, but they are heavily attacked by predators and parasitoids and have not reduced the weed population. A tetranychid mite introduced to control *Ulex europaeus* (gorse) in the north-western United States began heavily damaging the weed until predators responded and reduced the mite populations.

Pre-release evaluation of biological control agents can be improved by looking for natural enemies that are: 1) gregarious on the target plant, 2) primarily attacked by specialist predators and parasitoids (which do not occur in land of release), or 3) well defended from generalist predators and parasitoids.

Keywords: foreign exploration, parasitoid, predator, selection, trophic cascade.

Biological control theory

Biological control of weeds is an applied science which is gradually evolving from being an empirical "art" towards becoming more scientifically based (e.g. McEvoy 1996, Withers *et al.* 1999, Van Drieiche *et al.* 2000). But, we are severely challenged by the complexity of ecological interactions and the difficulty of testing hypotheses by repeated experiments that must occur on large landscapes over long time periods.

[1] United States Department of Agriculture–Agricultural Research Service, Western Regional Research Center, 800 Buchanan Street, Albany, CA 94710 USA <lsmith@pw.usda.gov>.

Although the discipline has been successful in predicting the host range of agents before release, it has been less successful in predicting which agents will successfully control the target plant (Cruttwell-McFadyen 2000, Pemberton 2000). Thus, practitioners operate as best as possible using a general theory (Harris 1991, Bellows & Headrich 1999, Goeden & Andres 1999 and references therein) and basic pre-release evaluations, but still depend largely on trial and error: not knowing whether an agent will be effective until after it is released (Harley & Forno 1992, Marohassy 1997). In what ways can we further refine our theory to help guide the selection and application of classical biological control agents?

Criteria for selecting an effective agent

There is a substantial literature proposing what criteria should be used to select agents that are most likely to be safe and effective (e.g. Wapshere 1985). Scoring systems have been proposed and revised (Harris 1973, Goeden 1983), but this approach has not been widely adopted (Cullen 1995, Marohasy 1997, Anon. 1998). Nevertheless, it is generally recognized that effective agents should be host-specific, should be adapted to the target climate, and should attack "vulnerable" parts or stages of the plant. It may be implicitly recognized that agents must be able to achieve large populations in order to affect the population of the target plant, although this factor usually receives little or no attention in pre-release evaluations.

In the continental United States, because of increasing requirements to not harm non-target species, practitioners have focused most pre-release evaluation efforts on determining that prospective agents are safe. A possible consequence of this emphasis may be that many recently released agents have either failed to establish or have not achieved sufficient densities to significantly impact the target weed population. Some examples are presented in Table 1. Current practitioners are increasingly aware of the need to find agents that are adapted to the climate of the release region and to the biotypes of the target weeds. The potential negative impacts of higher trophic predators, parasitoids and pathogens is also recognized (Goeden & Louda 1976). However, the latter factor may not be receiving as much attention during foreign exploration and pre-release evaluation as it should. It appears that the most successful agents in North America are not significantly limited by third trophic natural enemies (e.g. *Longitarsus jacobaeae* and *Tyria jacobaeae* [Turner & McEvoy 1995], *Chrysolina quadrigemina* [McCaffrey *et al.* 1995], *Rhinocyllus conicus* [Brinkman *et al.* 2001]), although some effective agents have been so affected (e.g. *Microlarinus lypriformis* [Goeden & Kirkland 1981]).

Tritrophic interactions

The theory of trophic cascading developed from the study of food chains and is now a mainstream theory in ecology (e.g. Pace *et al.* 1999, Polis *et al.* 2000 and references therein). The theory poses that in the absence of herbivores, a plant population will increase. Adding a herbivore to the food chain will directly reduce the plant population. However, adding a third trophic level (e.g. predator, parasitoid or disease of the herbivore) will reduce the herbivore population and consequently allow the plant population to increase. Thus, the success of a biological control of weed program depends on avoiding third trophic interactions. The theory also argues that adding a fourth trophic level should permit the herbivore population to increase and thus reduce the plant population, but pursuing such a strategy is likely to be too complex to permit reliable pre-release assessment of efficacy and safety. Interference of biological control agents by the third trophic level is most typically caused by pre-existing generalist predators or parasitoids in the region of release that accept the introduced biological control agent as a new prey or host. Accidentally introduced pathogens or parasitoids are another obvious source, although standard quarantine procedures are designed to prevent this. Purposeful introduction of biological control agents of arthropods can also interfere with biological control of weeds when the agents are not specific enough.

Evolution of life-history characteristics

Understanding what factors limit the population of a prospective natural enemy in the land of origin may help us to determine whether it will be able to achieve sufficiently large populations after release. Life-history characteristics (e.g. fecundity, survivorship, sex ratio, dispersal, gregariousness, and diapause) evolve in response to natural selection (Stearns 1992). Environmental factors that select for high fecundity, high survivorship and gregariousness rather than dispersal should

Table 1. Biological control agents of weeds in North America that may have failed because of interference by predators or parasitoids.

Agent	Target weed	Notes	Reference
Bangasternus orientalis (Coleoptera: Curculionidae)	*Centaurea solstitialis*	Egg predation	M. Pitcairn (pers. comm.)
Coleophora klimeschiella (Lepidoptera: Coleophoridae)	*Salsola tragus* (= *australis*)	Native parasitoids	Halstead (1989)
Coleophora parthenica (Lepidoptera: Coleophoridae)	*Salsola tragus* (= *australis*)	Parasitoids, spiders & rodent predators	Muller & Goeden (1990), Müller *et al.* (1990), Nuessly & Goeden (1983, 1984)
Tetranychus lintearius (Acari: Tetranychidae)	*Ulex europaeus*	Reduced by predaceous mites	Pratt *et al.* (2003)
Tyta luctuosa (Lepidoptera: Noctuidae)	*Convolvulus arvensis*	Cryptic external larvae; generalist predators	Ciomperlik *et al.* (1992) Tipping & Campobasso (1997)

be favourable for producing an effective biological control agent.

Let us consider some factors that may regulate a herbivore population and their potential consequences on life-history characteristics:

Plant defences

If the plant defences (e.g. chemical, structural, phenological) in the region of release are the same as those in the region of exploration (i.e. same phenotype), then the biological control candidate may or may not be likely to be an effective agent. However, if the plants in the region of release are more acceptable to the agent than in the region of exploration, then the agent would have a better chance of being effective. Because host-specific agents must have already evolved ways to overcome the plant's defences, this situation is most likely to apply to generalist herbivores, which are usually not of interest for biological control. Thus, the role of plant defences is more important to the natural selection of herbivores that are host-specific (i.e. safe) than it is to producing herbivores that are likely to be effective in controlling the plant in the adventive region.

Plant scarcity

This fits the classical model of metapopulation local extinction, in which the natural enemy so overwhelms a patch of the plant population that it produces local extinctions (e.g. McEvoy *et al.* 1993). The plant species (metapopulation) persists by establishing new populations that are isolated enough in space and time to permit multiplication before the herbivore finds and destroys them. This should be an ideal agent, because it presumably possesses the ability to find isolated patches, aggregate and dramatically reduce the plant population. However, in evaluating such an agent, it is important to determine whether the plant is rare because of the prospective agent or because of other environmental factors. Observation of high densities of the agent on small plant patches that decrease over time is one clue. Conducting insect-exclusion field experiments at natural field sites and in garden experiments should also help resolve this question.

Control by higher trophic level

The plant may be common or abundant in the region of exploration and the prospective agent may also be common, but is often heavily attacked by predators, parasitoids or pathogens. This situation may select for herbivores with characters such as:

- increased fecundity (to compensate for mortality)
- defence (gall thickness, behaviour, webbing, toxins, aposematic colouration)
- avoidance (crypsis, dispersal, rarity).

If the third trophic level consists primarily of specialist parasitoids or predators, which are not present in the region of release, then the high fecundity rate would result in abnormally high population densities that may overwhelm the plant in an adventive region lacking such enemies, which is auspicious. Existence of defences against third trophic attack may also be interesting, but if the agent is never observed at high densities in the region of exploration, then further analysis should be done to determine what conditions would permit such agents to be "released" from control in the region of release. If the enemies are specialist species that do not exist in the region of release, this would be favourable. The last category, avoidance of attack, appears to be an undesirable characteristic for biological control because it presumably is more effective for a species that exists at typically low population densities, which is not likely to be sufficient to affect the plant's population.

Application to biological control

The application of classical biological control is based on the theory that alien plants become invasive weeds because they are no longer controlled by higher trophic natural enemies. Practitioners currently focus on finding natural enemies that are specific enough to pass regulatory requirements. However, it is increasingly important to go further and discover natural enemies that have the added likelihood of creating high population densities. Prospective biological control agents that appear to be well defended from specialist predators or parasitoids should be more likely to continue to display the same mechanisms in the region of release. Such defences could be recognized by characteristics such as the feeding habit of the insect on the plant (internal versus external feeding larvae), presence of aposematic colouration, behavioural defences, and low level of gregariousness. For example, it is doubtful that an insect that has cryptically coloured, solitary, external feeding larvae could ever significantly affect the population of its host plant (at least, in systems that are not severely disrupted by insecticides), because such a survival strategy depends on being uncommon to escape the attack of its natural enemies.

In order to increase the likelihood of producing high population densities of biological control agents after release, we should focus on discovering prospective agents that are either 1) gregarious on the target plant, 2) heavily attacked by specialist parasitoids, predators or pathogens that are not known to occur in the region of release, or 3) well defended from generalist predators and parasitoids.

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New research on *Alternanthera philoxeroides* (alligator weed) in its South American native range

Alejandro J. Sosa,[1] Mic H. Julien[2] and Hugo A. Cordo[1]

Summary

Alternanthera philoxeroides (alligator weed) is a herbaceous amphibious weed of the Amaranthaceae, native to southern South America. Several agents from Argentina, e.g. *Agasicles hygrophila* and *Arcola malloi*, have been used to control aquatic *A. philoxeroides* in Australia and the USA. However, in Australia, the weed continues to pose a serious problem, particularly in terrestrial situations. In Argentina, *A. philoxeroides* is distributed along the catchments of the Paraná and Uruguay rivers in the north, and in the catchments of the San Borombón and Salado rivers in the centre of Buenos Aires province. Two forms are recognized: *A. philoxeroides* f. *philoxeroides* in the southern range and *A. philoxeroides* f. *angustifolia* in the northern range. There appears to be preferential attack by flea beetles on *A. philoxeroides* f. *angustifolia*. In 2000, the CSIRO initiated a collaborative research project with the USDA South American Biological Control Laboratory in Argentina to search for new biological agents. After the initial year of surveys, the natural enemies that may have biological control potential included: two species of leaf-feeding beetles, *Systena* spp.; a tip-galling Cecidomyiidae fly; and two agromyzid flies, one that causes node galls and another that mines leaves. Two fungi were also found: one probably *Nimbya alternantherae*, known to have a wide host range, and another, thought to be a new *Sphaceloma* species, that causes a characteristic "corky" deformation on the stem and leaf surfaces. Surveys will be extended and the interactions between these herbivores and pathogens with *A. philoxeroides* will be studied.

Keywords: alligator weed, *Alternanthera philoxeroides*, native range, natural enemies.

Introduction

In its introduced range, *Alternanthera philoxeroides* (Martius) Grisebach (alligator weed; Amaranthaceae) is often a serious aquatic and terrestrial weed. Surveys for natural enemies were carried out in the 1960s in parts of its native range in South America (Vogt 1961), and the flea beetle *Agasicles hygrophila* Selman and Vogt, the moth *Arcola malloi* (Pastrana) and the thrips *Amyinothrips andersoni* O'Neil were released in the USA. Good control was obtained in aquatic habitats, largely attributed to the flea beetle and the moth (Spencer & Coulson 1976). The flea beetle and the moth were subsequently released in Australia and controlled the weed in warm temperate aquatic habitats (Julien 1981). However, terrestrial growth of *A. philoxeroides* and aquatic growth in cooler regions of Australia continue to cause serious concern (Julien & Bourne 1988, Julien & Stanley 1999).

Alternanthera philoxeroides consists of several taxa in both its native and adventive ranges. It was first described by Martius in 1826, and named *Bucholzia philoxeroides*. Covas (1939, 1941) considered two varieties of alligator weed: *A. philoxeroides* var. *obstusifolia* (Moquin) Hicken and *A. philoxeroides* var. *acutifolia* (Moq.) Hicken. The former was characterized by the presence of ovate lanceolate leaves with obtuse or sub obtuse apex; whereas the latter variety, *acutifolia*, has lanceolate leaves with acute apex. Pedersen (1967) indicated that Martius did not consider two varieties of alligator weed and he considered that the specimen deposited in Brussels, which had ovate or elliptic and

[1] South American Biological Control Laboratory, USDA-ARS, Bolivar 1559 (B1686EFA), Hurlingham, Buenos Aires, Argentina.
[2] CSIRO Entomology, Long Pocket Laboratories, 120 Meiers Road, Indooroopilly, Queensland 4068, Australia.
Corresponding author: A.J. Sosa <alejsosa@speedy.com.ar>.

obtuse leaves, as a good lectotype. However, Pedersen (1999) suggested that *A. philoxeroides* be divided into two and he referred to these as forms: *A. philoxeroides* f. *philoxeroides* (Mart.) Griseb. and *A. philoxeroides* f. *angustifolia* Süssenguth.

Records from the United States suggest that both forms were introduced there. Ganstad & Solymosy (1973) and Weldon *et al.* (1973) reported that some plants of *A. philoxeroides* had small stems, and in some cases the internodes were solid, and apparently this tended to produce a deficient plant that is not preferred by the flea beetle *A. hygrophila*. Kay & Haller (1982) found two different forms in the USA, which they differentiated as the narrow-stemmed alligator weed (NSA) biotype and the broader-stemmed alligator weed (BSA) biotype. The NSA biotype was characterized by the presence of slender stems with short internodes and obtuse and rounded leaves. The BSA biotype has broader and longer stems and longer and acute leaves. They pointed out a different pattern in the damage caused by *A. hygrophila*. Populations of NSA seem to be attacked less than the BSA populations in the USA and both biotypes responded differently to herbicides (Kay 1992). Wain *et al.* (1984) demonstrated genetic differences between the two biotypes in the USA using isozyme pattern analyses. In contrast, no genetic variation was detected within or between populations of *A. philoxeroides* in China using RAPD analysis (Xu *et al.* 2003).

In 2001, the CSIRO (Australia) initiated a cooperative research project with the USDA South American Biological Control Laboratory in Argentina to update the list of natural enemies known from *A. philoxeroides* and to identify potential new biological control agents.

Materials and methods

Surveys were conducted, mostly in Argentina, but also in Uruguay, Paraguay and south-eastern Brazil, between October 2001 and November 2002. Natural enemies were sampled at 93 *A. philoxeroides* sites. Sites where *Alternanthera aquatica* (Parodi) Chodat (ex *A. hassleriana*) occurred were also sampled. At each *A. philoxeroides* site, adult insects were collected either by direct aspiration from plants or after sweeping with a net, placed in 70% ethanol and sent to taxonomists for identification. Other immature insects were collected alive and reared in the laboratory to adult stage. These were also sent for identification.

The extent of the native ranges of both forms of *A. philoxeroides* were approximated using the local distribution of the weed, its morphology, and assessments of where it grew, i.e. natural areas or highly disturbed locations such as town drains. Herbarium specimens from representative sites, six stems per site, were collected. For each of the six stems, two younger leaves were removed and stored in 96% alcohol for genetic analysis. RAPD analyses were conducted on material from seven sites located from Posadas, on the northern Argentina border with Paraguay, to Tandil, well south of Buenos Aires.

In the field, the presence of fruits and seedlings of *A. philoxeroides* was recorded, and collected for cultivation in the laboratory. Seedlings and stem cuttings were collected from four localities and also grown in the laboratory. Two localities represented the southern form, Tandil and Mar del Plata in Buenos Aires province, and two represented the northern form, Santa Fé, Santa Fe province, and Hurlingham, Buenos Aires province. After 6 months of growth in identical conditions, 27 plant parameters were measured and compared using principal components analysis. Three factors were extracted that explained 71% of the total variance. They were: diameter of internode of leaf one, length/width ratio of leaf one, and leaf apical angle of leaf one. ANOVA was carried out to evaluate mean differences and Tukey test was used for multiple comparisons among pairs of means based on unequal sample of sizes. All statistical analysis was carried out using Statistica 5.5.

Results

Alligator weed: native ranges, morphological variation and biology

Alternanthera philoxeroides f. *philoxeroides*, the southern form, was distributed along the catchments of San Borombón and Salado rivers in Buenos Aires province (Fig. 1). It was not found in western Buenos Aires province. It was also found in some sites in the north west of Argentina, in township drains, possible outside the native range. This form has small, ovate leaves, short internodes and slender stems. It occurred in semi-aquatic conditions, in ditches next to roads or along the shores of lakes. Flowering was abundant in summer, with short, small inflorescences.

The second, northern form, *A. philoxeroides* f. *angustifolia*, was distributed along the Paraná River from Posadas and along the Paraguay River from Pantanal region (Brazil). It also occurred along the Uruguay River downstream from Santo Tomé (Corrientes province). Thus, it extended from the northern parts of Buenos Aires province through the north-east wetlands of Argentina (including Entre Rios and Corrientes province, western Misiones and eastern parts of the provinces that have their eastern borders along the Paraná and Paraguay rivers), and along the south and east coast of Uruguay and possibly into south-east coastal areas of Brazil. This form was also found in the north-west of Argentina and at one site in the north of Patagonia in Río Negro province (Fig. 1). In these cases they were only associated with human activities and were considered to be outside the native range. Vogt *et al.* (1979) suggested that north-western populations were isolated relicts or introduced populations. We suggest the latter.

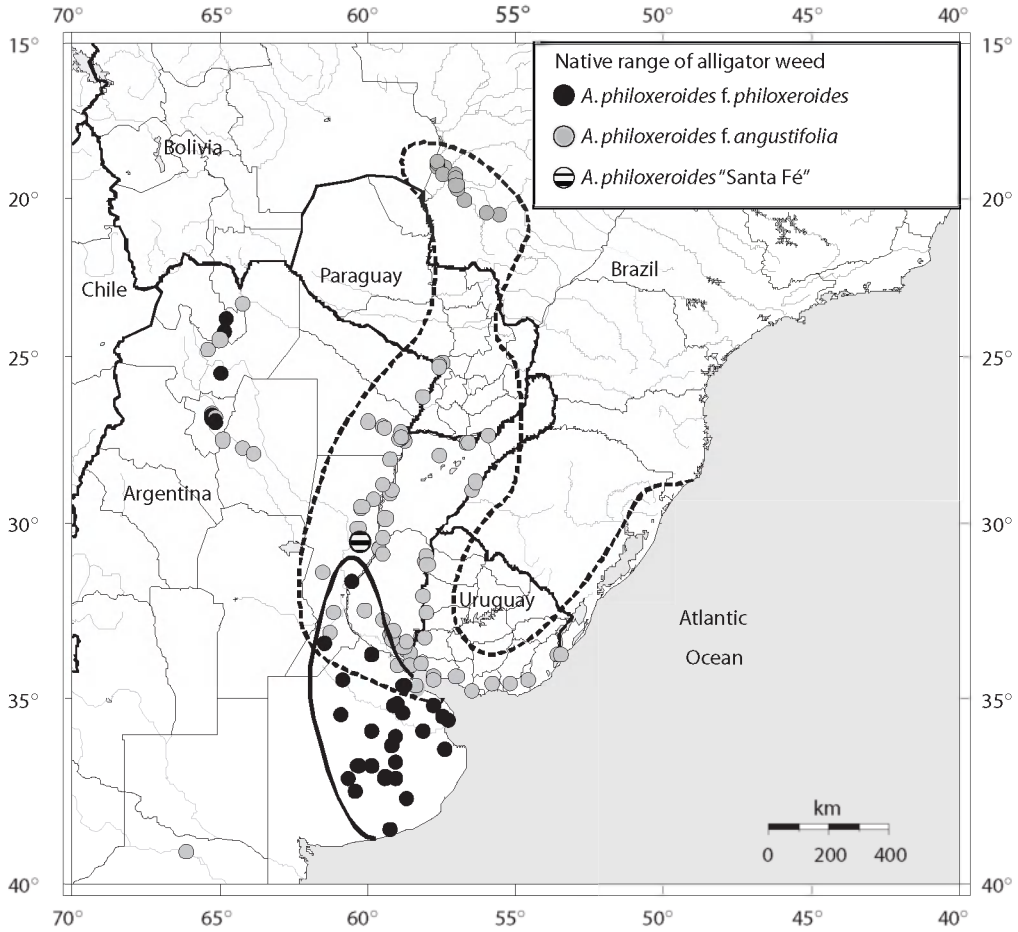

Figure 1. Distribution of *Alternanthera philoxeroides* in Argentina, Paraguay, Uruguay and the south-eastern coast of Brazil. Closed lines indicate the native range for *A. philoxeroides* f. *philoxeroides* in the south and broken lines indicate the native range of *A. philoxeroides* f. *angustifolia* in the north and east.

The growth comparison experiments distinguished the two forms, but also indicated the possibility of a third form. *Alternanthera philoxeroides* f. *philoxeroides*, grown from material from Tandil and Mar del Plata, had significantly more slender stems (smaller diameter internodes) and ovate (higher values in length/width ratio and leaf apical angle) and smaller leaves compared with the northern form, *A. philoxeroides* f. *angustifolia*. The latter grown from Hurlingham material, was significantly differentiated by its acute and long leaves (less value in leaf apical angle and length/ width ratio) and broader stems. This form was more frequently attacked by the flea beetle *A. hygrophila*.

Plants grown from Santa Fé material resembled the *angustifolia* form by having broader stems; and the *philoxeroides* form by having ovate and smaller leaves. The Santa Fé site is located just outside the overlap regions of the sympatric distributions for the two forms (Fig. 1). It is therefore uncertain if plants from this locality represent a third form or a hybrid. The RAPD analyses of samples from seven sites that included material representing both recognized forms of *A. philoxeroides* gave ambiguous results. Further analyses using AFPL technique are planned.

Sexual reproduction in alligator weed

Seeds and, for the first time, seedlings were observed in the field. Initially these were found at several sites near the Salado River at Dolores and along Route 30 between Azul and Tandil, Buenos Aires province, from the *philoxeroides* form. Later, seeds were collected from the *angustifolia* form from Chaco province and were germinated in the laboratory. Laboratory garden plants of both forms also set viable seeds. Vogt (1961) collected seeds from San Miguel del Monte, Buenos Aires province, probably from the *philoxeroides* form, and germinated these in small tins while travelling.

Natural enemies – insects

A range of natural enemies was found (Table 1) and some could be considered promising because of their abundance, damage to the plant and their probable narrow host range. The chrysomelid beetles, *Agasicles* spp., that feed on leaves and stems, are among those promising candidates. They were collected at 40 sites, mostly on *A. philoxeroides* f. *angustifolia*, and most specimens were identified as *A. hygrophila*, the known successful biological control

agent. Some of the specimens were identified as *A. vittata* Jacoby and *A. conexa* (Boheman) based on Vogt *et al.* (1979) and Selman & Vogt (1971). The three species of *Agasicles* are very similar, difficult to distinguish and inconsistencies are apparent. A revision of this genus would assist in determining the number of species involved and how to differentiate them.

Disonycha argentinensis Jacoby was found at 24 sites, mostly in conjunction with *Agasicles*, except in San Miguel del Monte (Buenos Aires province) where this flea beetle seemed to be dominant. This beetle was studied and released as a possible biological agent for the terrestrial *A. philoxeroides* in Australia and New Zealand but failed to establish, apparently due to environmental reasons (Julien & Chan 1992, Julien & Griffiths 1999).

Large populations of one species of *Systena* were found at 20 sites, mainly in Santa Fé and Chaco provinces and its biology is being studied in the laboratory. Vogt (1961) reported three species of *Systena*, considered them as "minor biotic suppressants", and pointed out that one of them (found in Posadas, Misiones province and in Barranqueras, Chaco province, Argentina), was the most abundant. These areas were searched in this study and specimens from this genus were found at both. In Barranqueras and near Corumbá (Pantanal, Brazil) another species of *Systena* was found on *A. aquatica*.

The thrips, *Amynothrips andersoni* O'Neill was found at every site at every visit. This is the most ubiquitous insect on *A. philoxeroides* regardless of plant form. It has also been observed attacking *A. aquatica*. The abundance of this thrips, its presence throughout the plant's growth cycle, in terrestrial and aquatic habitats, and its known host specificity strongly suggest it as a promising candidate for Australia. It has been introduced to the USA where it became established, but apparently has not contributed to the control of *A. philoxeroides* (Julien & Griffiths 1999). Details of its biology and rearing methods are recorded in Vogt 1961, Maddox *et al.* 1971, Maddox & Mayfield 1972 and Maddox 1973.

Table 1. Natural enemies of *Alternanthera philoxeroides* found in Argentina.

	Species	Form of *A. philoxeroides*	Observations
Chrysomelidae:			
Alticinae	*Agasicles hygrophila*[a,b]	*angustifolia*	Specific
	Agasicles conexa	*angustifolia*	A revision of this genus is
	Agasicles vittata	*angustifolia*	necessary
	Systena spp.	*angustifolia*	Being studied
	Disonycha argentinensis	*angustifolia*	Specific
Galerucinae	*Paranapiacaba significata*	*angustifolia* *philoxeroides*	Polyphagous species, considered a pest
Thysanoptera:			
Phlaeothripidae	*Amynothrips andersoni*[b]	*angustifolia* *philoxeroides*	Specific
Lepidoptera:			
Phyticidae	*Arcola malloi*[a,b]	*angustifolia* *philoxeroides*	Specific
	"leaf-tying moth"	*angustifolia* *philoxeroides*	Host-range unknown
Diptera:			
Cecidomyiidae	*Clinodiplosis alternantherae*	*angustifolia* *philoxeroides*	Probably specific Being studied
Agromyzidae	*Ophiomyia alternantherae*	*angustifolia* *philoxeroides*	Probably specific Being studied
	Ophiomyia marellii	*angustifolia*	Biology unknown
	Ophiomyia possibly *buscki*	*philoxeroides*	Host-range unknown
Cicadellidae:			
Typhlocibinae	*Empoasca curveola*	*angustifolia*	Not specific
	Empoasca aculeata	*philoxeroides*	Not specific
Membracidae	*Membracidae* sp. 1	*angustifolia*	Host range unknown
Fungi			
	Nymbia alternantherae ?	*angustifolia* *philoxeroides*	Not specific
	Sphaceloma ?	*angustifolia* *philoxeroides*	Host range unknown

[a] Species already released and established in Australia and USA.
[b] Species already released and established in the USA.

The fly *Clinodiplosis alternantherae* n. sp. Gagné was abundant in Buenos Aires province and at several sites in Uruguay, and was also present through most of the native range of *A. philoxeroides*. The mesophyll and main vein of leaves are enlarged due to larval activity. In many cases the gall causes severe stunting of the inflorescence peduncle. We do not know if the gall interferes with the production of seeds or with the size and numbers of flowers. The fly is abundant, has a short life cycle, is multivoltine and appears to be restricted to *A. philoxeroides* and the closely related *A. aquatica*. Vogt (1961) reported a species that formed terminal galls, but provided no other information. Gagné (1994) reported a species of *Clinodiplosis* that damages the terminals of *A. philoxeroides* in Argentina, Brazil and Uruguay. Specimens have been recently sent to Gagné and a paper with descriptions of this fly, as *C. alternantherae*, is in preparation.

Three species of *Ophiomyia* were found and probably correspond to those agromyzids mentioned by Vogt (1961). They are: *O. alternantherae* (Spencer), *O. marellii* (Brethes) and *Ophiomyia* possibly *buscki* (Frost). *Ophiomyia alternantherae*, the smallest one, is a leaf miner that was found in many (37) of the localities visited, and was frequently parasitized by wasps. There is very little information about its biology and the damage it causes to plants. Its probable short life cycle and its abundance in the field make it a potential candidate for biological control of *A. philoxeroides*. *Ophiomyia marellii* forms node galls and appears to be restricted to *A. philoxeroides*. It probably has a long life cycle. Its galls were mostly found on underground stems but on a few occasions they were found just above ground. This species was found at only seven sites, mostly on the *angustifolia* form and exclusively in terrestrial situations. The larvae of *Ophiomyia* possibly *buscki* are stem-miners. A few specimens only were collected in one location near Tandil, Buenos Aires province. A taxonomic revision of this species is necessary because discrepancies appeared between specimens collected and those deposited in museums.

Natural enemies – fungi

The pathogen, *Nymbia alternantherae* (Hyphomycetes) was found at most locations (82). This species has been studied in Brazil and the USA and has potential as a mycoherbicide. It has recently been found in Australia (B. Auld, R. Gilbert & B. Hennecke, pers. comm.) where further studies will be conducted. A species of *Sphaceloma* (Coelomycete) was observed at 27 sites on both forms of *A. philoxeroides*. Damaged parts of stems and leaves take on a cork-like texture and greyish colouration. Very little is known about this pathogen.

Discussion

Two species of *Alternanthera* were found in Argentina: *A. philoxeroides* and *A. aquatica*. Both grow in terrestrial and aquatic habitats. When growing in terrestrial conditions they are morphologically similar and it is difficult to differentiate them (Bona & Lange de Morretes 1997). Some insects attack both species; for example, the tip galler *C. alternantherae* and a flea beetle *Systena* sp. were observed on both species in Pantanal, Brazil, and Chaco province, Argentina.

Alternanthera philoxeroides exhibits morphological variation. The differences observed between *A. philoxeroides* growing in aquatic or terrestrial habitats may be due to phenotypic expressions. However, the existence of at least two genetic entities under the name *A. philoxeroides* appears to be possible, but requires confirmation. This, along with the existence of genetically different form(s) in Australia and other countries, is currently being assessed. Such information may be important for management of the weed, as genetically different forms may respond differently to management strategies. Preferential attack by *A. hygrophila* on different morphotypes of *A. philoxeroides* in Argentina and the USA is already known.

The two currently recognized forms of *A. philoxeroides* in Argentina produce fertile seeds. In the USA, Ganstad & Solymosy (1973) collected seeds, but they failed to germinate unless the utricle was extracted. They were not able to identify pollination processes. *Alternanthera philoxeroides* has not been observed producing seeds in other exotic ranges (Julien 1995). The factors, if any, preventing development of seeds in the exotic range must be studied. *Alternanthera philoxeroides* grows along much of the east coast of Brazil more or less contiguously with the Argentina/Uruguay populations. However, it is generally considered that the native range centres around river systems in Argentina (Vogt *et al.* 1979), and that the Brazil populations are part of the extended range.

Several host-specific agents are known from *A. philoxeroides*: *A. andersoni*, *D. argentinensis* and *Agasicles* spp. The biology and host ranges of other potential agents are being studied in Argentina. They include *C. alternantherae*, *Systena* spp. and *Ophiomyia* spp. There is no doubt that *A. andersoni* is host specific on *A. philoxeroides*, causing intensive damage. The lack of contribution by this insect to the control of *A. philoxeroides* in the USA should not discourage its release in Australia. Many agents work well in one habitat, region or country, but not in another. *Disonycha argentinensis* has been studied previously and its host range and rearing techniques are known. This insect was found at most sites in the northern distribution of *A. philoxeroides*. It is considered a potential candidate for the control of terrestrial *A. philoxeroides* and a renewed effort to import and establish it in Australia is suggested.

Once the *Agasicles* taxonomy is clarified, biology and preliminary host-range studies can be carried out for species other than *A. hygrophila*, which has already been studied. Additionally, more information is

required about the pathogens of *A. philoxeroides*, including more detailed field surveys to be conducted by a plant pathologist, and biology and host-range studies on the *Sphaceloma* sp. Native range studies will be continued and surveys for new natural enemies will be extended.

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Survey of potential biological agents to control yellow bells, *Tecoma stans* (L.) Kunth. (Bignoniaceae), in southern Brazil

M.D. Vitorino,[1,2] J.H. Pedrosa-Macedo,[3,4] A.O. Menezes Jr.,[5,6] C.J. Andreazza,[2] E.A. Bredow[4] and H.C. Simões[6]

Summary

Tecoma stans (yellow bells), commonly referred to as *amarelinho* in Brazil, was introduced as an ornamental in the late 1800s, mainly in the southern Brazilian regions. Nowadays, this plant has invaded more than 50,000 ha in Paraná State, with 10,000 ha totally unproductive. The most infested areas are located in the northern regions of Paraná State and between the coastline and the city of Santa Maria in the Rio Grande do Sul State. Field surveys were conducted monthly in the southern Brazil states to look for potential natural enemies of *T. stans*. The most infested areas were determined in each state to select areas for surveying. Several insects and pathogens were found on *T. stans* during the surveys. The biology, specificity and damage to plants are being studied for selected insects (mainly the leaf roller *Eulepte* spp. and the mite *Tetranychus ludeni*) and the rust fungus *Prospodium appendiculatum*. The project is continuing until March 2004 and is financially supported by the Brazilian Environmental Ministry (MMA), the Brazilian National Research Council (CNPQ) and the World Bank.

Keywords: *Eulepte* spp., *Prospodium appendiculatum*, rust fungus, *Tecoma stans*, *Tetranychus ludeni.*

Introduction

The plant *Tecoma stans* (L.) Kunth (yellow bells; Bignoniaceae), native to Mexico and the southern United States, is commonly referred to as *amarelinho*, *ipê de jardim* or *caroba amarela* in Brazil. It was introduced in Brazil as an ornamental in 1871 (Mello 1952). *Tecoma stans* is considered a weed in the United States, Nicaragua, Argentina (Morton 1981) and Brazil, where it has invaded more than 50,000 ha of pasture land in Paraná State, with 10,000 ha totally infested and non-productive (Kranz 1997, Lorenzi 2000, Vitorino & Pedrosa-Macedo 2001). This weed is also found associated with native vegetation alongside roads through 121 cities in Paraná State. Most infested areas are located in southern Brazil, principally in the north and north-east of Paraná, on the south-western border with Santa Catarina and in the central and mountains areas of the state of Rio Grande do Sul.

In Santa Catarina, *T. stans* is common as an ornamental plant along the sides of streets and in backyards. Infested areas are only found in the field near the city of Concórdia. In the Rio Grande do Sul State, *T. stans* is abundant in the region of the Serra do Rio das Antas, mainly between the cities of Veranópolis and Bento Gonçalves, and also in the mountains near the city of Santa Maria. In this state, high density infestations of the weed are common. The presence of *T. stans* along roadsides indicates that seeds are effectively disseminated via wind and rain. The fruits are dehiscent siliquas, which, when mature, release windborne seeds similar in shape to those of the genus *Tabebuia*. Plants are generally not cut or controlled because of the beautiful flowers they produce.

[1] Blumenau Regional University – FURB, Forest Engineer Department, Brazil.
[2] Monitoring and Forest Protection Lab. – LAMPF, Brazil.
[3] Federal Paraná University – UFPR, Forest Sciences Department, Brazil.
[4] Neotropical Biological Control Laboratory – LNCB, Brazil.
[5] Londrina State University – UEL, Agronomy Department, Brazil.
[6] Entomology Laboratory, Brazil.
Corresponding author: M. Vitorino.

Tecoma stans is known as an alternative host for pathogens and insect pests such as *Alternaria tenuis* (Kranz 1997), an important pathogen causing damping-off of different agricultural and forest crops, and the rust mite *Polyphagotarsonemus latus*, an important pest of citrus fruit. The parasitic plant *Cuscuta* spp. is also found associated with *T. stans* in the south of Brazil. Other pests such as *Aphis spiraecola and A. gossypii* (Homoptera: Aphididae), *Acrogonia citrina, Parathona gratiosa* and *Sonesimia grossa* (Homoptera: Cicadellidae) (these last three species known as vectors of diseases in *Citrus* orchards), are also associated with this weed.

The project presented here was developed through collaboration between the Blumenau Regional University, the Federal Paraná University and the Londrina State University. Its establishment was motivated by the real possibility that *T. stans* could invade and establish in the natural ecosystems of southern Brazil, as this plant is used throughout Brazil as an ornamental, is dispersed as seed by rain and wind and is highly aggressive and plastic.

The main objective of the project was to select and study potential agents (either insects or pathogens) for the biological control of *T. stans* in southern Brazil. Potential agents were identified during monthly field expeditions in areas infested with *T. stans* and their biology and ethology subsequently studied in more detail. Multiple and non-choice specificity tests were also conducted as well as experiments to determine their impact on the weed under laboratory and field conditions. Another important objective of the project was to determine the phenology of *T. stans* in three southern Brazilian states, in order to provide data to support studies with the selected biocontrol agents. A management program for this weed, that will include the introduction of economically important tree species to infested areas, will be developed based on results from these preliminary biological control studies.

Materials and methods

The field surveys are being conducted by staff from the three universities involved, in the central and northern regions of Paraná State and in the Santa Maria city region to the mountain range of the Rio Grande do Sul State. Every month, the selected areas infested with *T. stans* have been visited to identify potential biological control agents, collect insect and pathogen specimens and check field experiments. Biology studies, specificity tests and impact experiments have then been conducted on the collected natural enemies back at the universities. Most studies so far have concentrated on the rust fungus *Prospodium appendiculatum* (Teliomycetes: Pucciniaceae), the leaf roller *Eulepte* spp. (Lepidoptera: Pyralidae) and the leaf mite *Tetranychus ludeni* (Acari: Tetranichychidae), and have been carried out in the field and laboratory.

Results and discussion

The project is currently investigating the possibility of using the rust pathogen *P. appendiculatum,* apparently only associated with *T. stans* in southern Brazil, as a biological control agent for *T. stans*. *Prospodium appendiculatum* was recorded causing severe damage to flowers, fruits, shoots and leaves, mainly in the Londrina city region, north of the Paraná State. The severity of damage caused by the rust, particularly deformation of infected plant tissues, would likely inflict major stress on plants growing in a dense infestation. The specificity of the rust is currently under investigation. Other pathogens were also found associated with *T. stans*: *Alternaria* sp. (Kranz 1997), *Aspergillus* sp., *Fusarium* sp., *Glomerella* sp., *Pestalotia* sp., *Phialophora* sp. and *Sporothrix* sp.

The leaf roller *Eulepte* spp. found during surveys also shows potential for use in the biological control program. This insect was recorded in all areas infested with *T. stans*, but populations were higher in the northern Paraná region. The biology and host range of this leaf roller is currently under study. The mite *T. ludeni*, which damages leaves of *T. stans*, was also identified as a potential biological control agent. It causes impressive damage and can defoliate and kill *T. stans* seedlings.

Native pathogens and insects that have *T. stans* as an alternative host and pose limited risks to non-target desirable plants should be considered in the biological control program against this weed. They could be mass-reared and released in the field in areas densely infested with *T. stans* in order to reduce the weed population. However, the possibility, even remote, of finding specific agents associated with this plant in Brazil during this project should not be discarded.

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The patterns of harvester ant removal of wild radish seeds in the native range: the importance of generalist seed predators to weed management

Janine Vitou,[1] John K. Scott[2] and Andy W. Sheppard[1]

Summary

Raphanus raphanistrum L., wild radish, is a major weed of cropping systems in Australia, and is being targeted for biological control. Wild radish is a winter annual; consequently, biological control agents that significantly reduce the quantity or quality of seed production are likely to be effective. Surveys and ecological studies in the region of origin, the Mediterranean, included an assessment of the impact of phytophagous organisms on this weed and, in particular, on seed production. Seed production of wild radish in sown plots was also monitored in southern France. In previous surveys, we found that seed-harvesting ants caused the greatest seed losses of maturing seeds in southern France (11–91% seed loss per unit area). The ant species involved were *Messor* species, in particular *Messor sancta* Forel, a species native to the Mediterranean region, and *Messor rufitarsis*, common in Central Europe and throughout the Mediterranean south-east. In spring, the ants cut segments from green, nearly mature siliqua. The cut segments fall to the ground and some of them are then opened by the ants that carry the seeds to the nest. Remaining siliqua mature naturally, fall to the ground during summer and break into segments. Ants also harvested these mature segments on the ground prior to germination in autumn. We measured the impact of this seed predation by counting all the siliquae on each plant soon after ant harvesting started in late April and when wild radish plants were fully mature in late May. The results suggest ants are likely to have a significant impact on native wild radish populations. While these ants can not be considered as biological control agents, their overriding effect relative to other seed predators in the native range suggests associations between ants and other generalist seed predators on wild radish in Australia may also be providing some form of natural control.

Keywords: ant harvesters, *Messor* sp., *Raphanus raphanistrum*, seed predation, wild radish.

Introduction

Wild radish (*Raphanus raphanistrum* L.) (Brassicaceae) is distributed throughout the world and is a common weed of cultivation and disturbed areas (Piggin *et al.* 1978, Parsons & Cuthbertson 1992). *R. raphanistrum* occurs naturally in the Mediterranean region and occasionally forms dense populations. It is one of the most important weeds of grain crops in southern Australia and has developed herbicide resistance (Walsh *et al.* 2001). Biological control of wild radish is being investigated for its potential to provide a supplementary management option for this weed (Scott *et al.* 2002).

Surveys for potential biological control agents were undertaken in the Mediterranean region, considered as the native range of wild radish (J. Scott & J. Vitou, in preparation). Southern parts of Portugal, France, Greece and northern parts of Tunisia were surveyed. About 50 species of phytophagous insects were found associated with wild radish, but most of these have recorded host ranges that include other Brassicaceae,

[1] CSIRO European Laboratory, Campus International de Baillarguet, 34980 Montferrier-sur-Lez, 34980 France.

[2] CRC for Australian Weed Management and CSIRO Entomology, Private Bag 5, PO Wembley. WA 6913, Australia.

Corresponding author: Janine Vitou <janine.vitou@csiro-europe.org>.

including economic species such as *Brassica napus* (canola), *Raphanus sativus* (garden radish), cabbage etc. (Scott *et al.* 2002). Genetic studies have shown wild radish is closely related to these important crops and may share a close ancestry with edible radish (J.F. Martin *et al.*, unpublished). This makes this weed a very difficult target and essentially restricts the study of potential agents to those reducing seed production (Scott *et al.* 2002). *R. raphanistrum* produces seeds in siliquae that average between four and seven single-seeded segments per siliqua (Vitou & Scott 2002).

Messor species are the very large-headed ants of the seventeenth century fable "La Cigale et La Fourmi" by Jean de La Fontaine, where the ants build up a stock of seeds in their nest while the cicadas are singing! *Messor* species are typical harvester ants, feeding on seeds collected in the foraging area and storing seeds in nest chambers (Hahn & Maschwitz 1985). In arid countries such as Algeria, the ant-nest granaries can contain more than 100 L of seeds (Bernard 1968). Ant activity decreases, but does not stop, throughout the summer and early autumn, and ants also harvest pod segments on the ground before the seeds germinate in autumn. Probably originating in North Africa, the *Messor* genus consists of about 40 species living exclusively in the Old World, with eight species present in France. *Messor rufitarsis* is common in Central Europe and throughout the Mediterranean south-east, while *Messor sancta* is common in sunny and rocky Mediterranean fields (Bernard 1968, Hahn & Maschwitz 1985).

An insecticide and fungicide exclusion experiment was set up in southern France to study the impacts of natural enemies on wild radish throughout its growing season. This study identified a 7.5-fold increase in wild radish seed production per unit area in April 2002 across twenty 0.5 m^2 insecticide and fungicide treated quadrats (J. Vitou *et al.*, in preparation). The current study describes and tests the patterns of harvester-ant activity within this variable arena of resource availability.

Materials and method

An experiment was set up at Vendres, France (43°16'14"N, 03°13'30"E), in fallow land that had previously been a vineyard. The site was at 16 m altitude on a sandy-clay soil that had not been ploughed for at least 10 years. The plant community at this site was dominated by the evergreen shrub *Dittrichia viscosa* (L.) Gaertner (Asteraceae). Abundant wild radish plants were present in the neighbouring field.

A 30 m × 10 m plot was fenced to prevent disturbance from livestock or other large animals. Soil was professionally cultivated. Five blocks of six 0.5 m^2 quadrats were set up 3 m apart and 2.5 m from the fence. Quadrats were arranged in two rows of three with a separation of 1.5 m between quadrats (Fig. 1).

On 11 September 2001, 240 single-seeded segments of wild radish siliqua were sprinkled over four quadrats selected at random within each block and covered with soil to a depth of 2–3 cm. Each quadrat was treated with insecticide and/or fungicide or water, sprayed every three weeks, following a factorial experimental design. Every three weeks, weeds were removed by hand from the quadrats so that only *R. raphanistrum* plants remained. Twice a year, the plot was mown around the quadrats. By April, these treatments led to large spatial variation in seed production per quadrat that represented a 7.5-fold overall variation (Fig. 1). This study focuses on the responses of harvester ants to this variation. The results of the treatments are the subject of another paper (J. Vitou *et al.*, in preparation).

Abundant ant activity was observed in early spring, when the siliquae were well developed but not yet lignified. Collections of ants were sent to specialists and identified as *Messor* sp., *Messor sancta* Forel, and *Messor rufitarsis* Fabricius. The position of ant nest exit holes were recorded within the design (Fig. 1). All of the siliquae on each plant in each of the four quadrats per block were counted soon after ant harvesting started in late April. Siliquae, already harvested by the ants, were included in these counts through the persistence of the pedicels so this gave a total number of siliquae and the number left after an initial period of ant harvesting. In late May, when wild radish plants were fully mature, all of the remaining siliquae were collected in each quadrat of blocks 1, 3 and 5. The plants were uprooted and placed in a separate paper bag for each quadrat. In the laboratory, the number of siliquae in each quadrat was counted. Siliqua number was converted into estimated seed number based on an average number of 4.92 (± 0.04) seeds per siliqua obtained from a sample of 2185 siliquae collected and analyzed on this site the previous year (J. Vitou, unpublished data).

A simple experiment was set up on 11 September 2001 to measure segment collection from the ground by ants in late summer and early autumn, before the autumn rains. Twenty 10 × 10 × 5 cm high pots were prepared and filled with site-soil free of wild radish seeds. The pots were buried so that the pot rims were at ground level to allow the ants to enter the pots. In each block, four pots were laid down between the two rows of quadrats (Fig. 1). Ten pots selected at random received five segments and ten pots received 50 segments of *R. raphanistrum* collected at Vendres in May 2001. In each block, two pots of each density selected at random had the segments covered with soil from the site (0.5 cm depth), and in the other two pots the segments were placed on the surface. In November, when the new season seedlings were established, the pots were brought back to the laboratory where the soil of each pot was sieved, and the seedlings and remaining segments were counted.

Statistical analysis

Generalised linear models were used for analysis of seed numbers using the GLIM statistical package (McCullagh & Nelder 1983). The factors used were plant, quadrat and block. Dependent variables were total seeds per plant and per quadrat, seeds left per plant in late April and seeds left per quadrat in late May (Log(n+1) transformed). Seed loss per plant and per quadrat and per pot was analyzed using survival analysis with binomial errors, i.e. the initial total seed number as the binomial denominator and scaled for over-dispersion in the data (Crawley 1993).

Results

Ants were omnipresent during all our field surveys, and the impact of the harvester ants in the genus *Messor* was obvious. Ants harvested between 11% and 91% of available seeds per quadrat over a two-month period. Harvester ant activity on wild radish in the quadrats started in March 2002 and increased as the fruits started to mature. In early April, as fruits matured, the ants climbed the plant and removed only one or two segments of three to five siliquae per plant. Every week more were removed as they became suitable. When whole siliquae were suitable, the ants cut the pedicel causing the siliqua to fall to the ground. All seeds were removed on some plants. Ant activity was visible on the ground at the base of each plant where abundant pod fragments and empty siliqua segments were distributed. Ants cut large siliquae into segments and tended to open some segments to take only the seed or a fragment to their nest.

Seed production per quadrat available to the harvester ants ranged from 7410 to 56,974 seeds m^{-2}. The slope of the log–log plot of total number of seeds per individual plant and seeds left after the ant harvesting in April was one (Fig. 2). This slope indicates that the proportion of seeds harvested per plant was independent of the size of the plant. In contrast, the log–log plot of the number of seeds per quadrat over the same time period before and after ant harvesting (Fig. 3) had a slope significantly less than one, indicating that quadrats with greater numbers of seeds lost a higher proportion of seeds to the ants. By the end of May, this was even more noticeable, when the remaining numbers of seeds per quadrat was no longer a function of the initial seed production per quadrat (Fig. 4).

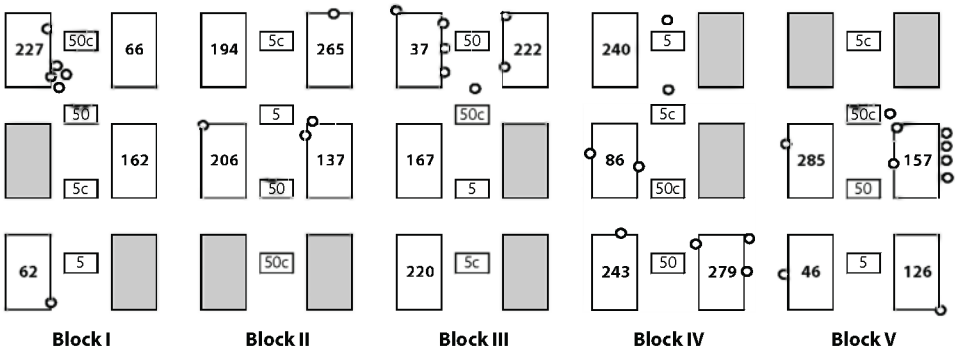

Figure 1. Field experiment design with five blocks of six quadrats at Vendres, France. Number (multiples of 100) in each quadrat represents the seed production, grey quadrats represent the trap gardens (*R. sativus* and canola). Between the two rows of quadrats, four pots per block were buried at random. Two pots received five seeds (5), one was covered with soil (5c), two pots received 50 seeds (50), and one was covered with soil (50c). Ant nest holes are represented by the black rings.

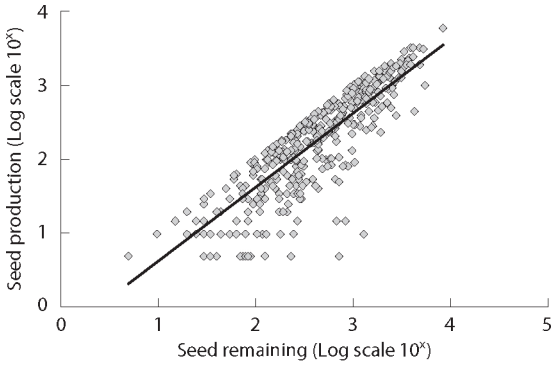

Figure 2. Seed survival by late April (Y) versus seed production (X) per plant. Y = 1.00X – 0.37. $R^2 = 0.74$.

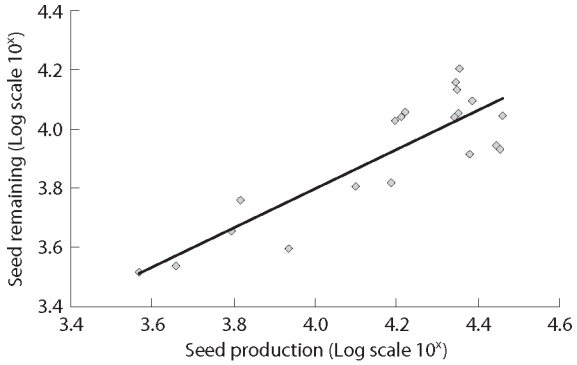

Figure 3. Seed survival by late April (Y) versus seed production (X) per quadrat. Y = 0.66X + 1.16. $R^2 = 0.75$.

With the tray experiment set up before germination, the percentage of the seed survival was significantly lower ($F_{1,19}$ = 34.11: $P < 0.000$) than in soil covered seeds. Initial number of seeds added to the trays had no significant effect ($F_{1,19}$ = 3.98: $P = 0.06$) on the seed survival (Fig. 5).

Discussion

Seed harvesting was density-dependent at the scale of the quadrat but plants of all sizes lost the same proportion of seeds to ants. The efficacy of the ants at harvesting seeds in relation to their density per quadrat levelled out the initial 7.5-fold difference in seed density between quadrats within 2 months of harvesting.

As a winter annual weed, wild radish produces abundant siliquae and seeds. Seed production from wild radish infestations ranged from 292 seeds m^{-2} from 1 plant m^{-2} to 17,275 seeds m^{-2} from 52 plants m^{-2} (Reeves *et al.* 1981). Total seed production prior to ant harvesting activity in our field plot where 960 seeds m^{-2} were planted and kept weed-free was between 698 to 26,508 seeds m^{-2} in 2001 (J. Vitou & J. Scott, unpublished), and between 7410 to 57,878 seeds m^{-2} and between 5 to 8226 seeds per plant in this study on the same site in 2002. When these 2002 seed densities were available to the harvester ants, the residual seed density dropped to between 4126 and 6892 seeds m^{-2}. Ant activity therefore eliminated any differences in seed production resulting from the initial treatments. The number of viable seeds that the ants left behind may be a harvesting threshold below which the ants turn their attention to other resources. Nonetheless, this appeared to be more than enough for sufficient wild radish recruitment in the next germination period to ensure population replacement, particularly if the seeds became incorporated into a buried seed bank. The seed density and burial experiment showed that the ants harvested seeds in proportion to their density when on the surface or buried in the soil. For seeds on the surface, seed survival of 50 seeds per pot was lower than for 5 seeds per pot. Though this difference was not significant, it suggested a similar tendency to the density-dependent harvesting observed from the quadrats. Ants appear to be five times less successful at harvesting buried seeds (Fig. 5).

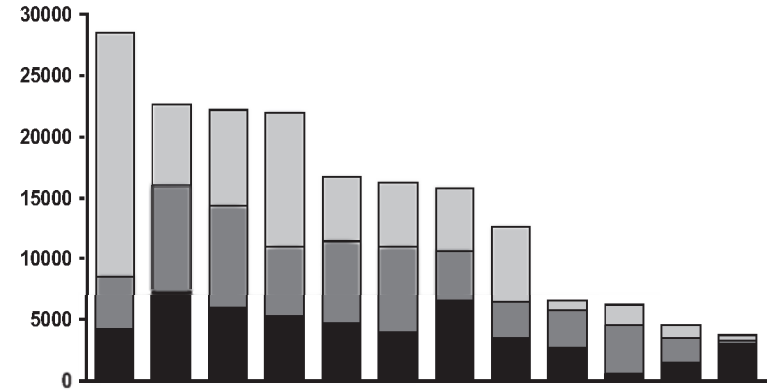

Figure 4. Initial and final (black) seed numbers per quadrat, and numbers harvested by ants in April (light grey) and May (dark grey). Quadrats arranged in order of decreasing initial seed density.

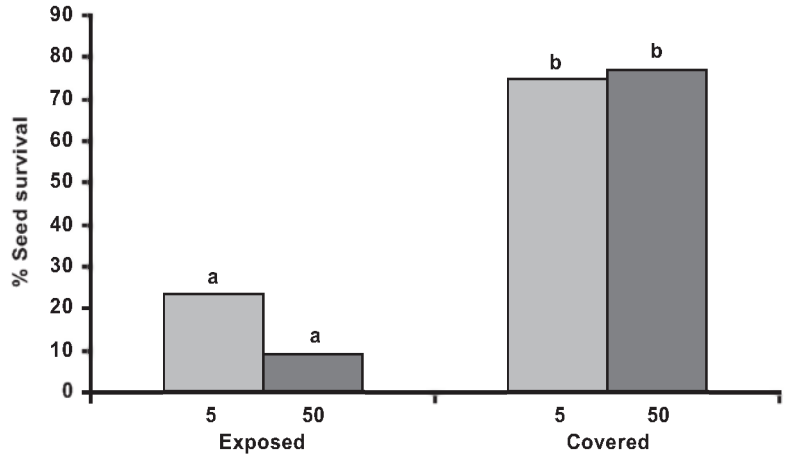

Figure 5. Seed survival per exposed and covered seeds and two densities [5 seeds (light grey) and 50 seeds (dark grey)]. Bars with different letters are significantly different within seed exposition treatments.

Harvester ants should only be able to maintain high abundance in plant communities that exhibit high seed production. Mediterranean herbaceous communities dominated by annuals exhibit this trait, and are a common habitat for *Messor* sp. (Wolff & Debussche 1999). Reyes-López & Fernández-Haeger (2002) observed that harvester ants tend to gather the most abundant and/or larger seeds within such communities, and that the superabundance of a given seed type in the environment prompts increased activity. Hahn & Maschwitz (1985) also found that ants were attracted to rich seed sources when they were available. The abundance of the large-seeded wild radish siliquae generated by this experimental design may have attracted higher than average harvester ant activity at this particular site. With reduced numbers of seeds available, ant activity decreased.

In Australia, McGeown (1999) suggested that ants (a total of 18 morphospecies) trapped within the study site, were the primary remover of the seeds of wild radish in north-eastern Victoria and southern New South Wales. Borger *et al.* (2002) point to the importance of ants for removal of wild radish seed in Western Australia, with 24 of 30 species known to consume such seeds (Minkey & Spafford Jacob 2002). Australia has a rich seed-harvester ant fauna. Eight harvester species were recorded from some Australia tropical study plots (Andersen *et al.* 2000), and harvester ants are capable of inflecting severe seed losses (Briese 1982). Recent studies in Australia suggest up to 90% of weed seeds are removed by ants in cropping systems and attracting ants for this purpose is now being considered (D. Minkey, pers. comm.).

This suggests wild radish seed harvesting by ants is important in both the native and exotic range of this weed. Any proposal to use a seed-removing biological control agent needs to be considered carefully. Unless the proposed agent could reduce seed density to levels below the harvesting threshold of the local ants, then such ants are likely to nullify any impacts of such agents. Further work on harvesting ants on wild radish seeds in Australia is clarifying this.

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Exploration for plant pathogens against *Taeniatherum caput-medusae* (medusahead ryegrass)

T.L. Widmer and R. Sforza[1]

Summary

Taeniatherum caput-medusae (medusahead ryegrass) is an invasive weed in the United States of America with origins in the Mediterranean region extending to central Asia. It is a member of the grass family, with seed germinating in the fall and continuing to grow all winter. Currently, it infests millions of hectares of land, primarily in California, Colorado, Nevada, Oregon, and Utah. *Taeniatherum caput-medusae* crowds out native plant species and is almost worthless as forage. Current management strategies have been ineffective. This study examines biocontrol, through the use of plant pathogens, as a possible management strategy. In the literature, a few plant pathogens, including *Ustilago* spp., *Tilletia bornmuelleri, Puccinia* spp. and *Fusarium culmorum*, have been reported to occur naturally or to infect *T. caput-medusae* through artificial inoculation. Several *Ustilago* spp., a *Tilletia* sp., two *Puccinia* spp. and *Fusarium arthrosporioides* have been found during surveys in the native habitat of *T. caput-medusae*. Preliminary studies have begun to identify the species and determine their host range and impact. A completed study involving *F. arthrosporioides* showed that it was not host-specific and, therefore, it is not being pursued as a biocontrol agent. Further explorations will continue to search for new pathogens and assess their host specificity.

Keywords: invasive species, *Puccinia*, *Tilletia*, Triticeae, *Ustilago*.

Introduction

Taeniatherum caput-medusae (L.) Nevski (medusahead ryegrass) is considered a noxious weed in many western states of the United States of America (USA). Its rapid spread has presented a serious problem to wildlife and rangeland managers. As attempts to control this annual weed have generally resulted in failure (Horton 1991), this study reports for the first time foreign exploration for pathogens to be used in a biological control strategy.

T. caput-medusae, previously misidentified as *Elymus caput-medusae* L., is a member of the Triticeae tribe of the grass family. The name is derived from the Greek taenia (ribbon) and ather (awn), alluding to the flat-based lemma awns. Apparently, the genus *Taeniatherum* has a genome that is distinct, but faintly related to those of *Dasypyrum*, *Eremopyrum* and *Hordeum* (Frederiksen & Bothmer 1989). Three subspecies, *caput-medusae, asperum,* and *crinitum*, are known to occur in the native range. The subspecies *asperum* is the one introduced into the USA and differs from the others by having pronounced barbs, coated with silica on the awns (Young 1992). This winter annual has its origins in areas bordering the Mediterranean Sea extending eastward to central Asia (Frederiksen 1986).

T. caput-medusae is a slender annual grass, 5–60 cm high, which is predominantly self-pollinated. It grows in areas where extended periods of intense cold are lacking. Soils with high clay content, well-developed profiles and those receiving run-off from infested areas are most susceptible to invasion (Dahl & Tisdale 1975). The species matures later than other annual grasses and may require clay soils for their high water-holding capacity (Young & Evans 1970). Well-drained soils and coarse-textured sands with poorly developed profiles are less likely to be utilized by *T. caput-medusae*. The species overlaps in range and local

[1] USDA–ARS European Biological Control Laboratory, CS 90013 Montferrier sur Lez, 34988 St-Gély du Fesc, France.
Corresponding author: T. Widmer <tlwidmer@ars-ebcl.org>.

habitat with *Bromus mollis* and *B. tectorum* (cheatgrass) in California and Oregon, also introduced plant species. It is reported that *T. caput-medusae* is displacing *B. tectorum* on more mesic sites (Harris 1977). *T. caput-medusae* germinates in the fall with the roots beginning to grow immediately and continuing to grow all winter. Seed dormancy is due to inhibitory substances in the awns of fresh seed, which are lost by early fall (Nelson & Wilson 1969). In terms of spread and growth, *T. caput-medusae* has probably not reached its ecological limit. If the requirements completely overlap those of *B. tectorum*, it could spread widely in the Great Basin and beyond.

T. caput-medusae was first collected in the USA in Oregon in 1887, and spread rapidly in the 1930s (Young 1992). It is now invasive across millions of hectares of large semi-arid areas of intermountain rangelands in western US states, in which it is considered a noxious weed (e.g. Colorado, California, Oregon, Nevada and Utah). In Oregon, 1.0 million ha are included within the boundary of known infestations; more than 300,000 ha in Idaho (Hironaka 1961); at least 50,000 ha in eastern Washington; 40,000 ha in northern California; as well as portions of north-eastern California, northern Nevada and western Utah. Its primary range includes areas with 25–50 cm of annual precipitation, although it has been noted in areas with up to 1 m of precipitation. This weed is a major problem on Nature Conservancy preserves in the interior valleys of Oregon and California where it crowds out native species by producing a thick thatch of highly siliceous plant matter, outcompeting perennial grass seedlings.

T. caput-medusae has substantially impacted ecosystem functioning in a way that ensures its persistence. An important life-history trait that enables persistence is its ability to germinate in the fall. A tolerance for cool soil temperatures allows root development and resource capture earlier in the spring than other plant species. *T. caput-medusae* threatens rangelands with sparse native plant communities, as well as more complex communities degraded by overgrazing, fire, or cultivation, particularly *Artemisia–Agropyron–Poa*-dominated communities (Dahl & Tisdale 1975). It has been reported to have a slightly higher seed production per unit area than *B. tectorum*. The greater seed production and inhibition of *B. tectorum* germination by mat formation are thought to be two reasons allowing *T. caput-medusae* to invade *B. tectorum*-infested areas. It is worthless as forage for cattle and sheep, although animals will graze it for a short time in the early spring during the pre-seedhead stage (Miller *et al.* 1999). It has been estimated that grazing capacity of an area can be reduced by 50 to 80% after a few years of infestation (Hironaka 1961). Different strategies that include burning, grazing, plant competition, restoration of natives, and chemicals are being used for control. Before our study, no biocontrol strategies had been studied.

Fusarium arthrosporioides, isolated from the crown of *T. caput-medusae* in Greece, is the only report of a soil pathogen attacking the grass in its native range (Siegwart *et al.* 2003). The authors reported the effect of the pathogen on *T. caput-medusae* under experimental conditions, but also on cultivated cereals, including wheat, barley, and oats. In North America, the effects of five soil fungi, endemic to the western USA, were evaluated on five grass species including *T. caput-medusae* (Grey *et al.* 1995). In this study, *T. caput-medusae* was susceptible to crown rot caused by *Fusarium culmorum*. It appeared that *Fusarium* spp. may have a potential for reducing growth of *T. caput-medusae*.

Rust fungi are widespread on Triticeae, with some species being more host-specific than others (Cummins 1971). It is reported that the multiple-host rust fungus *Puccinia graminis* successfully infected *T. caput-medusae* when inoculated under artificial conditions (Holubec *et al.* 1997). *Puccinia striiformis* and *Puccinia hordei* are also reported from *T. caput-medusae* (Watson & Dallwitz 1992).

Smuts are a general term for fungi that attack the reproductive structures of the plant. Smuts have been studied specifically on *Aegilops* and *Hordeum* species, which are closely related to *Taeniatherum* spp. (Nielsen 1985, 1987). These studies reported successful experimental inoculation of *T. caput-medusae* subspecies *crinitum* with *Ustilago tritici*, but not with *U. nuda*. According to the literature, the only natural smut infection reported on *T. caput-medusae* was by *U. phrygica* found in Iran (Vanky & Ershad 1993). In Iran, 54 species of Ustilaginaceae are recorded on plants. *Ustilago phrygica* is also known from south-eastern Europe, North Africa and Asia, and is widely distributed in Turkey (K. Vanky, pers. comm.). One species in the genus *Tilletia* (Tilleciaceae), *T. bornmuelleri* (syn. *T. serbica*), has been recorded on *T. caput-medusae* in Iran (Hdjaroude & Abbasi 2000). However, no evaluation of disease severity and impact of these naturally occurring smut fungi has been undertaken.

Microorganisms other than fungi, may also have an impact on the growth and spread of *T. caput-medusae*. For example, rhizobacteria that actively colonize roots may have deleterious effects on weedy species with the potential to control weeds without the undesirable effects associated with the application of herbicides (Kennedy & Kremer 1996).

Materials and methods

Survey

Areas of the natural habitat of *T. caput-medusae* were targeted for surveys. Since 2000, foreign exploration has been specifically carried out in Spain, France, Ukraine, Cyprus, Turkey and Greece. All growth stages

of *T. caput-medusae* were observed from April to September.

Pathogens collected

Whole plants with disease symptoms were collected and maintained individually in plastic bags for transportation, until they could be transferred to a quarantine facility for observation, identification, and cultivation of the causal organisms. Pieces of diseased tissue were surface-sterilized and plated on water agar amended with 100 mg/L streptomycin contained in Petri dishes. Any fungi growing from the diseased tissue was subcultured and maintained on half-strength potato dextrose agar until its pathogenicity could be evaluated.

Methods of evaluation

T. caput-medusae plants were germinated from seed on moistened filter paper in a Petri plate and then planted into autoclaved soil. The plants were maintained in a growth chamber at 25°C. Isolated fungi that could be cultured on artificial media were grown on autoclaved wheat seed contained in Erlenmeyer flasks for 1 week. The air-dried, infested wheat seed was ground to a coarse powder and added to the soil at a rate of 10%. *Taeniatherum caput-medusae* seedlings were transferred to the infested soil and the subsequent mortality rate was assessed. Obligate parasites were evaluated based upon methods described in the literature (Royer & Rytter 1985, Sampson & Watson 1985, Jones & Dhitaphichit 1991).

Results

Distribution of pathogens and symptoms

Pathogens listed in Table 1 were collected during surveys. They belong to the classes Urediniomycetes (rusts), Ustomycetes, Septomycetes (smuts) and Hyphomycetes (*Fusarium* sp.). To date, none of the rusts or smuts have been identified to species.

The *Tilletia* sp. was collected in central Turkey in several patches of a few square metres of *T. caput-medusae*. However, not all the individual plants in the patches were diseased. Sori present in the ovaries of infected plants were swollen, ovoid to elongate, brown, partly hidden by the glumes. For some individual, infected plants, all seed were affected by the systemic infection.

In the case of the *Ustilago* spp., infected plants were collected from May to September in Turkey and late March in Cyprus. Infection was sparse, with two to three infected plants together and then no infection occurring for a few metres. Typical symptoms of *Ustilago* spp. infection included sori, usually comprising the whole spike, leaving intact stunted and deformed awns, slightly bullate, subepidermal. A blackish-brown, powdery spore mass appeared upon rupture of the epidermis. During flowering, the seeds were replaced by the sori, thus effectively breaking the life cycle of this annual plant.

Rusts, identified as *Puccinia* spp., were collected in locations where an infection was localized within a radius of 100 m^2 on the sunny slope of rocky hills. Rust infections were found at only one location each in Turkey and Cyprus. The rust collected in Turkey forms leaf and stem pustules that are red in colour. Histological sections of prepared tissue infected by this rust showed paraphyses in the pustules. The rust collected in Cyprus forms bright orange pustules on the adaxial side of leaves only, which turn to brown upon maturity and teliospore formation. Histological sections of prepared tissue revealed no paraphyses in the pustules of this rust.

The plants from which *F. arthrosporioides* was isolated showed no distinct symptoms in the field except that they generally appeared weaker. Infection and seedling death were demonstrated under controlled inoculation studies in the laboratory (Siegwart *et al.* 2003).

Discussion

Several pathogens collected during our surveys show promise as potential biocontrol agents. The symptoms of smut infection detailed in Nielsen's studies (1985, 1987) were similar to those we observed in Turkey. However, this does not confirm the identification of the

Table 1. Pathogens collected on *Taeniatherum caput-medusae* in 2001–2003.

Pathogen	Stage of the plant attacked	Location
Smut fungi = Ustilaginales		
Ustilago spp. (Ustilaginaceae)	seedhead	All Anatolia (Turkey)
Tilletia spp. (Tilletiaceae)	seedhead	Nicosia (Cyprus) Erzurum province (Turkey)
Fusarium		
Fusarium arthrosporioides	collar	Thessaloniki (Greece)
Rust fungi = Uredinales		
Puccinia spp. (Pucciniaceae)	Leaves and stems	Erzurum province (Turkey) Nicosia (Cyprus)

smuts. According to the literature, the only natural infection of *T. caput-medusae* by a smut was found in Iran and caused by *U. phrygica* (Vanky & Ershad 1993). Although we do not have yet the identification of the *Tilletia* sp. that we collected, we believe that it is most likely *T. bornmuellerii*, a potentially valuable candidate that should be studied in more detail. This smut is reported from the former Yugoslavia (Vanky 1994) to Iran, and therefore it is probably well distributed into Turkey where we collected our specimen.

Considering the high diversity of smut and rust fungi in the Middle East, which is also considered as the centre of diversity of the genus *Taeniatherum*, it appears that cultivated cereals are highly susceptible to smuts. Further studies with these pathogens as potential biocontrol agents will need to pay particular attention to host specificity. The narrower the host range of an *Ustilago* species suggests a greater specialization of the species. Research has recently been initiated on the use of pathogens as possible biological control agents for *B. tectorum* (Meyer *et al.* 2001). The authors explored the potential of using a naturally occurring pathogen (*Ustilago bullata*) that causes head smut for *B. tectorum* control. Fungal infection of the seed head by the smut predominated amongst spring cohorts resulting in up to 30% mortality (Mack & Pyke 1984). It was suggested that low seed production by fall cohorts may be offset through increased seed production by later cohorts. This implies that control measures should be applied in the spring, after most cohorts with a high probability of seed set success have germinated, but before their inflorescences have had a chance to mature.

Until we find candidates for biocontrol on *T. caput-medusae* in France, where our research laboratory is located, we will have to undertake all our experiments in the confined structure of a quarantine facility. Artificial conditions in a quarantine always lead to questions about repeatability under natural conditions. To address this concern, an open garden will be set up in Eastern Turkey to evaluate the impact and specificity of both the *Tilletia* and *Ustilago* species under natural conditions. Particular attention will be paid to the closely related grasses, including wheat, rye, oats etc., that will be tested alongside *T. caput-medusae*. In addition, field surveys will be continued in order to collect pathogens in southern France where *T. caput-medusae* also occurred.

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The potential for classical biological control of invasive grass species with special reference to invasive *Sporobolus* spp. (Poaceae) in Australia

A.B.R. Witt and A.J. McConnachie[1]

Summary

Sporobolus africanus, *S. natalensis* and *S. pyramidalis* were accidentally introduced to Australia from Africa and have the potential to invade approximately 223 million hectares. Mechanical and chemical controls are largely ineffective and expensive, hence the search for potential biological control agents in southern Africa. Mycoherbicides are being used more widely today for the control of some invasive grass species in agricultural situations although no pathogen has been released as a classical biocontrol agent. Arthropods have been largely ignored as potential agents until very recently because it was assumed that the simple architecture of grasses and the lack of secondary compounds would militate against the evolution of monophagy. However, in recent surveys of *Phragmites australis* and *Calamagrostis epigejos* in Europe, some monophagous insect species have been found, and *Prokelisia marginata* (Delphacidae) has been released for the control of *Spartina alternifolia* on the west coast of the United States. Many *Tetramesa* spp. (Eurytomidae) are apparently monophagous and a species that has been reared from *S. pyramidalis* in South Africa is extremely damaging. A number of other damaging insects have been collected on these *Sporobolus* spp. but can only be considered as potential agents once they have undergone further trials. Many pathogens have also been collected, including a leaf rust (*Uromyces tenuicutis*), but a smut (*Ustilago sporoboli-indici*) appears to have the most potential. The biggest obstacle to the biological control of invasive *Sporobolus* spp. in Australia is the fact that there are 13 native *Sporobolus* spp., which will largely govern which agents can be selected for biocontrol. This paper considers the various factors which make grasses amenable to biological control and criteria used in the selection of agents, with particular reference to invasive *Sporobolus* species in Australia.

Keywords: grasses, pathogens, rust, smut, *Sporobolus*.

Introduction

Grasses cover more of the world's land surface than any other vegetation type. Grasses are the most important food crops in the world and are also utilized extensively for building materials, essential oils, ornamental plants, lawns and pastures. As a result, grass species have been introduced, either accidentally or intentionally, to many regions worldwide.

Species in the *Sporobolus indicus* complex, like *S. africanus* (Poir) Robyns & Tournay, *S. pyramidalis* P. Beauv. and *S. natalensis* (Steud.) Dur. & Schinz., were accidentally introduced to Australia from Africa and have subsequently become invasive, posing a major threat to the environment and livestock production. All of the introduced species are unpalatable to livestock and the carrying capacity of invaded pastures can be reduced by 10–80%, resulting in a potential loss of A$60 million per annum to the livestock industry in northern Australia (Department of Natural Resources and Mines 2001). It has been estimated that this complex of invasive species could invade approximately 223 million hectares (Department of Natural Resources and Mines 2001). Chemical and mechanical

[1] Queensland Department of Natural Resources and Mines, South African Field Station, c/o ARC–Plant Protection Research Institute (ARC–PPRI), P/Bag X134, Queenswood, 0121, South Africa. Corresponding author: A.B.R. Witt <rietaw@plant2.agric.za>.

control measures have proved to be either ineffective, impractical or expensive, hence the search for potential biological control agents in southern Africa.

A number of potential agents have been found in surveys of *S. africanus*, *S. pyramidalis* and *S. natalensis* in South Africa, Swaziland and Botswana. In this paper, we report on progress towards the selection of control agents for this complex of *Sporobolus* spp. and comment more broadly on the selection of grasses as targets for biological control.

Sporobolus spp. taxonomy and biology

There are approximately 160 *Sporobolus* spp. in tropical and subtropical areas (Clayton & Renvoize 1986). Of the 21 *Sporobolus* species in Australasia, 13 are endemic (Simon & Jacobs 1999). However, the recognition of many of these species, especially those in the *S. indicus* complex, is difficult because of the morphological intergradation in the genus (Simon & Jacobs 1999). *Sporobolus pyramidalis*, *S. africanus* and *S. natalensis* are all known to hybridize, making field identification very difficult (Van Wyk & Van Oudtshoorn 1999).

Species in the *S. indicus* complex occur on all soil types and generally in areas with high rainfall (Van Wyk & van Oudtshoorn 1999). *Sporobolus pyramidalis* occurs throughout tropical Africa as well as Madagascar, Mauritius and Yemen while *S. africanus* and *S. natalensis* are found from southern Africa to East Africa as far north as Ethiopia (Van Wyk & van Oudtshoorn 1999). Weedy *Sporobolus* spp. can mature in as little as three months under favourable conditions (Department of Natural Resources & Mines 2001). Seed viability is 90–100%, with as many as 150,000 seeds/m^2 in infested pastures and a seed bank which may remain viable for as long as 10 years (Department of Natural Resources & Mines 2001).

Grasses as targets for biological control

According to Randall (2002), 18,146 plants species have become invasive worldwide. However, Randall (2002) has included 20,081 names which includes synonyms for various species. Of these 15,605 are dicotyledons, and 4476 are monocotyledons, of which 2176 are species in the family Poaceae. The exact figures are therefore smaller than those indicated but the ratio between monocotyledons and dicotyledons should remain fairly constant. The family with the greatest number of invasive species is the Asteraceae followed by the Poaceae and Fabaceae (Table 1) (Randall 2002). The top five species of weed worldwide, based primarily on the impact they have in agriculture in control costs and yield reduction (Holms *et al.* 1977), are in the Cyperaceae or Poaceae, with *Cyperus rotundus* L. being the worst weed worldwide (Holm *et al.* 1977).

To date, species in 40 plant families have been selected as targets for biological control (Julien & Griffiths 1998). Most are in the families Asteraceae (31 spp.), Cactaceae (23 spp.), Fabaceae (Mimosoideae, Caesalpinioideae, Papilionoideae) (19 spp.) and Rosaceae (4 spp.) (Julien & Griffiths 1998). Control programs have never been initiated against any species in the Poaceae until very recently and only two species in the Cyperaceae have had agents released for their control, despite the abundance of weedy species in these two families. This is possibly because grasses are perceived as lacking specific herbivores, and as being too similar in morphology, physiology and ecology to crop species (Gill & Blacklow 1984, Evans 1991). The apparent absence of host-specific arthropods has been ascribed to their simple structure and lack of secondary compounds, which reduces the evolution of monophagy (Evans 1991). This view was entrenched by surveys on *Imperata cylindrica* and *Cyperus rotundus* in the early 1970s (Simmonds 1972) and *Sorghum halepense* in northern Italy in the 1980s (Domenichini *et al.* 1989) which found that arthropods on these species were not sufficiently host specific and/or damaging. As a result, arthropods were widely discounted as potential control agents for grasses, with most attention focusing on the use of mycoherbicides (Evans 1991).

Table 1. The number of genera and species in each family classified as weeds by Randall (2002) together with the total number of species in each family (Mabberley 1997) and the percentage of weed species in each family.

Family	Genera	Species	Total species	% weeds
Asteraceae	1528	2373	22,750	10.4
Poaceae	668	2176	9,500	22.9
Fabaceae	643	2147	18,000	11.9
Cyperaceae	98	627	4,350	14.4
Rosaceae	95	550	2,825	19.5
Lamiaceae	251	497	6700	7.4

However, recent evidence would appear to suggest that even simple plants like grasses support large numbers of arthropods. A recent literature survey by Tewksbury *et al.* (2002) found more than 160 arthropod species associated with *Phragmites australis* (Cav.) Trin ex Steud. *Spartina alternifolia* Lois. has more than 24 arthropod species which have potential as biological control agents (F.S. Grevstad, University of Washington, pers. comm.) while *Calamagrostis epigejos* (L.) has 10 endophagous arthropod species (Dubbert *et al.* 1998). In any case, the number of species associated with a plant should not necessarily deter from its selection as a target species. Many simple plants like

Opuntia spp. and water weeds have been successfully controlled despite the fact that they have few arthropod species associated with them in their native ranges (Moran 1980, Julien & Griffiths 1998).

The fact that alkaloids are only present in less than 0.2% of grasses while other noxious terpenoids and chemical compounds are completely absent (McNaughton *et al.* 1985) should also not deter from their selection as target species. Recent evidence suggests that the role of plant toxicity in fostering monophagy has been overemphasized and that other explanations may be preferable (Futuyma & Keese 1992). Structural defences like trichomes, silica bodies and others may also play a role in driving monophagy in insects (Djamin & Pathak 1967).

Weed species with no closely related native species or crops are seen as better targets than weeds with native congeners (Pemberton 2000). Oligophagous species like *Cactoblastis cactorum* (Bergroth) and *Dactylopius opuntiae* (Cockerell) could be released against *Opuntia* spp. in South Africa because there are no native species in the Cactaceae and no closely related major crop species (Moran 1980). The family with the most species targeted for biological control, the Asteraceae (Julien & Griffiths 1998), contains no major crop species other than sunflower (Simmonds 1976). In contrast, the Poaceae which has no species targeted for biocontrol, has the highest percentage of weedy species and has more than 20 species of major crops, more than any other family (Simmonds 1976). Nevertheless, weed species have been selected as targets despite being closely related to major crops (Julien & Griffiths 1998). *Solanum elaeagnifolium* was selected as a target weed in South Africa despite there being many major crops in the same genus (Olckers *et al.* 1999). However, agents released for the control of invasive *Sporobolus* spp. in Australia will need to be extremely host specific to appease environmentalists because there are 13 (62%) endemic *Sporobolus* spp. in Australasia and two of these species are listed as rare and one as vulnerable in Queensland (Simon & Jacobs 1999).

Introduced invasive grass species may also be overlooked as biocontrol targets because they are not noticed in native grasslands, especially if they have many native congeners, and their impact is therefore seen as being negligible. Until the public can distinguish between native and introduced grasses and is made aware of the impact they have on native ecosystems, grasses will continue to be ignored unless a problem in agricultural situations.

Selection of biological control agents for grasses

According to Moran (1980), the arthropod complex on simple plants should be dominated by endophagous species, e.g. *Opuntia* spp. where 79% of the phytophagous species are borers (Lepidoptera and Coleoptera) (Moran 1980). Grasses, being simple plants, should therefore also be dominated by endophages. However, according to Tscharntke & Greiler (1995) grasses are dominated by ectophages, which is what we found on *Sporobolus* spp. in our surveys. However, in *P. australis*, there are virtually equal numbers of ectophages and endophages (Tewksbury *et al.* 2002), probably because the large culms provide niches for a large number of arthropods. Endophagous species are also abundant in other large semi-aquatic grasses like *S. alternifolia* and *C. epigejos*.

Unlike the situation in many dicotyledons, where the arthropod fauna is often dominated by species in the Coleoptera (Curculionidae and Chrysomelidae) (Syrett *et al.* 1996), grasses have a relatively poor beetle fauna (Tewksbury *et al.* 2002). Only eight beetle species have been collected on *P. australis* worldwide (Tewksbury *et al.* 2002). However, in smaller grasses, like *Sporobolus* spp. and *Nasella trichotoma*, beetles are relatively abundant, but the majority of these are generalist pollen feeders. Diptera (Agromyzidae, Chloropidae) are generally more common in grasses than in dicotyledons, with 32 species in the Chloropidae, most of them endophagous, collected on *P. australis* (Tewksbury *et al.* 2002). Herbivores with apparent specialization on *S. alternifolia* are mainly hemipterans with only 2 of the 24 arthropod species being coleopterans (Mordellidae, Curculionidae) (F.S. Grevstad, University of Washington, pers. comm.).

Host specificity of agents on grasses

Chewing insects on grasses are generally oligophagous (Bernays & Berbehenn 1987), but many other taxa are monophagous. There is a close association between many species in the Cecidomyidae and particular grass hosts (Barnes 1946) and many grass-feeding homopterans also have a small host range (Southwood & Leston 1959, Gibson 1976). Many stem-boring and stem-galling dipterans found in grasses have a limited host range (Nye 1959, Mowat 1974), with more than 20 monophagous chloropid species attacking *P. australis* (Tewksbury *et al.* 2002). Other families with a large number of monophagous species on *P. australis* are the Agromyzidae and Delphacidae, while species in the Pseudococcidae, Coccidae and Noctuidae are generally polyphagous (Tewksbury *et al.* 2002). Of the nine endophagous insects collected on *C. epigejos*, two are considered to be monophagous (Eurytomidae, Chloropidae) (Dubbert et al. 1998).

Many species in the Eurytomidae are known to be host specific. Martinez *et al.* (1999) found 18 different species of eurytomids in 10 sympatric species of grasses, with no species occurring in more than one species of grass. The position in which the larvae develop on the culm is also specific for many species (Boucek 1988) as demonstrated by the endophages on *C. epigejos* (Dubbert *et al.* 1998).

Many pathogens on grasses also only have a single host with head smuts and many rusts being extremely host specific (Valverde *et al.* 1999). The host specificity of biotrophic pathogens in general can be extremely narrow, sometimes being restricted to a particular biotype as demonstrated with the rust *Puccinia chondrillina* Bubak & Syd. released for the control of skeleton weed in Australia (Burdon *et al.* 1981). A pathogen that exhibits biotype selectivity within a single species should not infect plants from closely related species.

Level of damage caused by agents on grasses

Arthropods on grasses can be extremely damaging and result in the death of the attacked plant. A sap-sucker, *Prokelesia marginata* (Van Duzee) (Homoptera: Delphacidae), recently released for the control of *S. alternifolia* on the west coast of the United States, was placed in cages with *S. alterniflora* plants from Willapa Bay (Daehler & Strong 1997) and *S. anglica* plants from Puget Sound (Wu *et al.* 1999). Attacked plants from both species were severely stunted or died.

Although eurytomids are not known to kill plants they can reduce crop yields substantially. *Eragrostis teff* (Zucc.) Trotter was introduced to the United States where it was attacked by the stem-boring eurytomid *Eurytomocharis eragrostidis* (Howard),,, causing a reduction in forage yields of over 70% in one year (McDaniel & Boe 1990). Spears & Barr (1985) also found that *Tetramesa* spp. reduced seed weight in *Aristida longiseta* Steud., *Sitanion hystrix* (Nutt.) J.G. Smith, *Sporobolus cryptandrus* (Torr.) A. Gray and *Stipa comata* Trin. & Rupr. by 47, 33, 46 and 60%, respectively. This resulted in a reduction in seed germination for all four species with as many as 99% of seeds of *A. longiseta* not germinating (Spears & Barr 1985).

A stem-borer, *Tetramesa* sp. (Hymenoptera: Eurytomidae), collected on *S. pyramidalis*, *S. africanus* and *S. natalensis* in southern Africa, was also found to be damaging. Of 144 *S. pyramidalis* culms randomly collected at a particular site, 33% were infested with *Tetramesa* sp. larvae. The inflorescences of 60% of these infested culms were malformed. The culms of infested plants were also significantly shorter: 470 mm ($n = 48$) versus 656 mm ($n = 96$) df = 79, $t = -6.385$, $P < 0.001$).

Numerous pathogens damage cereal crops throughout the world, with smuts and rusts being particularly abundant. A smut, *Sporisorium ophiuri*, which is being considered for the control of *Rottboellia cochinchinensis* in Costa Rica, is very damaging and as a sole agent could reduce the density of itchgrass by 90%, with an annual infection rate of about 88% (Smith *et al.* 1997). This level of infection is unlikely to be achieved consistently, but indicates how damaging a smut can be. Infected plants have significantly fewer tillers and leaves and flower earlier than healthy individuals.

Of the five primary pathogens collected on the three *Sporobolus* spp., the smut *Ustilago sporoboli-indici* L. Ling appears to be the most promising agent. The other pathogens, a leaf rust (*Uromyces tenuicutis* McAlp.), tar spot (*Phyllachora sylvatica* Sacc. & Speg.), choke disease (*Parepichloë cinerea* Berk. & Br.) and ear blight (*Bipolaris crustacea* (Henn.) Alcorn) are already present in Australia (R. Shivas, Curator: Plant Pathology Herbarium, Queensland, Australia, pers. comm.) while the smut has only ever been recorded in parts of Africa, Asia and the Philippines (K. Vánky, pers. comm.). Research into the use of *B. crustacea* as a mycoherbicide found that it was not suitable anyway because of its low rates of infection and the timing of infection in relation to seed production (Hetherington & Irwin 1999).

Ustilago sporoboli-indici produces sori on the leaves and stems and usually prevents the production of an inflorescence. The disease appears to be systemic and usually all shoots of an infected plant are affected and sterile. In preliminary surveys, 10 randomly collected *S. pyramidalis* plants at each of five localities were separated into individual tillers, and only 6% (15/250) of infested tillers had inflorescences compared to 50% (547/1085) of uninfested tillers. The culms of infested tillers were also significantly shorter than uninfested tillers: 74.6 cm ($n = 15$) versus 101.8 cm ($n = 547$); df = 14, $t = 3.46$, $P < 0.002$. In transect surveys at five localities, an average of 54% (range = 15–70%) of grass clumps had at least one infested tiller.

Conclusions

There does not appear to be any valid reason why grasses should not be considered as targets for classical biological control programs. Recent surveys on a number of grass species clearly demonstrate that there are large number of arthropods, especially on large species, and that many of them are monophagous. We are optimistic that some of the agents we have selected as potential biocontrol agents for *Sporobolus* spp. will be both damaging and host specific.

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Prospects for the search for weed biocontrol agents in Russia

Vadim F. Zaitzev,[1] Sergey Ya. Reznik,[1] Mark G. Volkovitsh,[1] Margarita Yu. Dolgovskaya,[1] Alexander S. Konstantinov[2] and Neal R. Spencer[3]

Summary

Invasive weeds of Palaearctic origin constitute a major portion of weed problems in the Nearctic region and elsewhere. Specifically, 33 of 37 noxious invasive North American weed species recorded in 10 or more US states originated from the Palaearctic. In the Palaearctic region, these species are almost evenly distributed between three regions: (1) western and central Europe, (2) eastern Europe and the Middle East, and (3) the European part of Russia, including the northern Caucasus. A little less often, south-western Siberia, and rarely, south-eastern Siberia are included as a "cradle land" of North American weeds. In contrast, the overwhelming majority of field explorations aimed at the classical biocontrol of weeds have been conducted in western and central Europe (*ca.* 75%) and in eastern Europe and the Middle East (*ca.* 20%). Only 2% of past explorations were conducted in Russia and other republics of the former Soviet Union. Overcoming this imbalance, will provide new opportunities for both old and new weed targets of the Palaearctic origin. To support this conclusion, the results from exploration and research in Russia, which has targeted several invasive weed species, are described. The potential for new weed research programs in Russia is extraordinary.

Key words: biological control, explorations, Palaearctic, Russia, weeds.

Introduction

Exotic invasive weeds are a major problem in agriculture, forestry and natural areas, posing a threat to biodiversity conservation (Pimentel *et al.* 2000; Mirkin & Naumova 2002). Introduction of natural enemies from the native area of a target weed is considered one of the most efficient and biologically safe methods to control these plants (Strong & Pemberton 2000). However, biological control of weeds has a rather low "success rate". The percentage of research programs resulting in successful introductions and efficient control of invasive weeds is about 10% by the estimation of different authors (Harris 1991, 1993, Gassmann 1995, Williamson & Fitter 1996), although it is rather difficult to define a biocontrol program's success or failure. Finding a potential biocontrol agent with the required host specificity and efficacy is difficult, time consuming, and increasingly expensive. However, when a suitable biocontrol agent is collected, investigated and successfully introduced, it will pay for the research and the benefit:cost ratio could be quite high (McFadyen 1998). Thus, the advantage of a weed biocontrol project markedly decreases with increase in the number of investigated, but rejected or ineffective agents studied. Various selection methods and "scoring systems" have been proposed to predict efficiency and to avoid the time, effort and expense involved with the study of inappropriate candidates (Harris & Zwölfer 1968, Harris 1973, Goeden 1983, Lawton 1985). Most of the authors agree that the probability of quickly finding effective control agents clearly increases when searching on the target weed in its native range (Harris & Zwölfer 1968, Harris 1973, Goeden 1983, Lawton 1985, Schroeder & Goeden 1986, Gassmann 1995, McFadyen 1998), although Hokkanen & Pimentel (1984) stated that the success rate could be higher for agents collected from plants other than target species

[1] Zoological Institute, 199034, St Petersburg, Russia.
[2] Systematic Entomology Laboratory, USDA, National Museum of Natural History, Washington, DC 20013-7012, USA.
[3] USDA/ARS/PPRU, Federal Nutrition Lab., Cornell University, Tower Road, Ithaca, New York 14853, USA.
Corresponding author: M.Yu. Dolgovskaya, Zoological Institute, 199034, St. Petersburg, Russia <rita@MD12306.spb.edu>.

(but see also Goeden & Kok 1986). Hence, it could be expected that native areas of the greatest number of invasive weeds would be explored more frequently. In the present paper, we attempted to check this hypothesis with invasive weeds originating from the Palaearctic, one of the largest biogeographic regions including Europe, northern and central Asia.

Materials and methods

The list of invasive weeds of USA and Canada was taken from the INVADERS database supported by the USDA Agricultural Research Service and available on the Internet at http://invader.dbs.umt.edu (Skinner *et al.* 2000). Data on introductions of weed biocontrol agents were taken from *Biological control of weeds: a world catalogue of agents and their target weeds* fourth edition (Julien & Griffiths 1999). In the case where a single species was repeatedly introduced, each introduction was included in this analysis. The native distribution range of Palaearctic plants was taken from the comprehensive *Flora of the USSR* (1934–1964).

Results and discussion

To limit the number of weeds under consideration, we selected from the database only the "top" invasive weeds recorded in 10 or more states of the USA. Forty records met this requirement, but three of them, *Cuscuta* spp., *Cardaria* spp., and *Brassica* spp. were excluded from consideration, as these weeds comprise a complex of species. Notwithstanding, we did include the species, *Cardaria draba* in our analysis. Of the remaining 37 invasive weed species, only 4 (*Solanum carolinense, S. elaeagnifolium, Nassella trichotoma*, and *Sorghum almum*) originated from outside the Palaearctic region. Thus, 33 from the 37 most widespread invasive American weeds are native to the Palaearctic and are the subject of our consideration. This distribution is not surprising as it is well known that plants of the Palaearctic origin constitute a major portion of exotic invasive weed species in the Nearctic region and elsewhere (Gassmann 1995; Pimentel *et al.* 2000).

For further analysis, we divided the wide Palaearctic region into four zones divided not only by their biogeographical characteristics, but also by political boundaries: western and central Europe; eastern Europe (including the Balkans and Asia Minor); Russia and other former Soviet Union republics; and other Palaearctic.

Analysis of the native distribution of the selected 33 plant species in these four main regions of the Palaearctic showed (Fig. 1) that western and central Europe, eastern Europe, and Russia are almost equal in the number of native plant species that have become weeds (note that the native area of a species may be spread over several parts of the Palaearctic region). Less often, other parts of the Palaearctic are included as a "cradle land" of North American weeds. Inside Russia, the European part of Russia (including the northern Caucasus and lower Volga) is the richest in native plants which have been introduced and subsequently become invasive in North America (32 species), while western Siberia (including the Urals) has 25 species, and Eastern Siberia (including the Russian Far East) has 12 species.

Further, we estimated the intensity of field explorations for weed biocontrol agents conducted in the above listed parts of the Palaearctic. The number of introductions (Julien & Griffiths 1999) was used to measure this intensity. At this stage of analysis we considered not only introductions into the Nearctic region, but also releases of biocontrol agents in Australia, New Zealand, Africa, and South America, where invasive weeds originating from Eurasia, have become a part of the local flora.

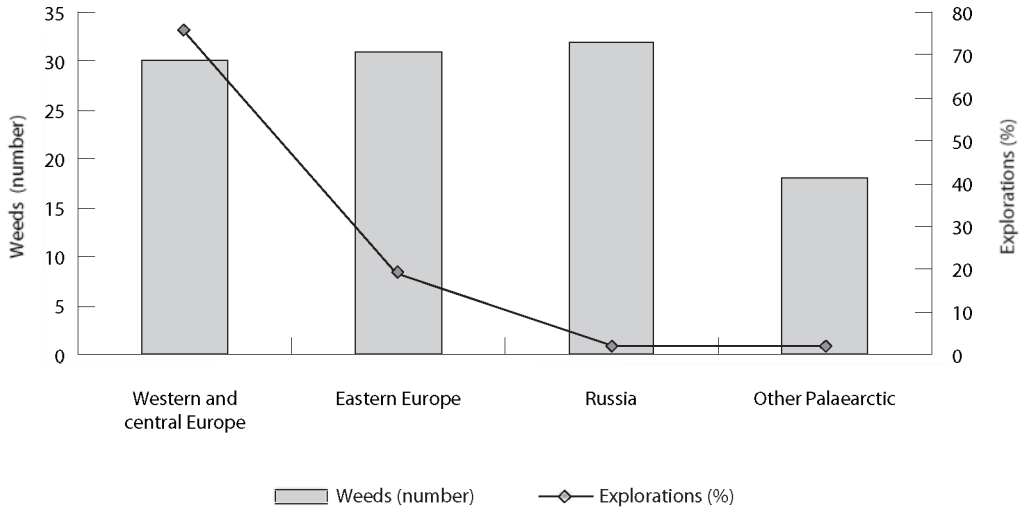

Figure 1. Origins of Palaearctic species that are important weeds in the Nearctic region: number of weed species from four native areas (bars) and percentage of biocontrol explorations conducted in each area (line).

A total of 398 introductions of 181 biocontrol agents against 45 invasive weeds originating from the Palaearctic were considered. Almost half of biocontrol agents fall into two coleopteran families; both popular and successful in weed biocontrol: Chrysomelidae (21%) and Curculionidae (22%). The remaining 57% are other insect species, mites, nematodes, and fungi. A majority of the introductions (65%) occurred in North America, 28% in Australia and New Zealand, and the remaining 7% on other continents.

The areas where exploration was conducted and biocontrol agents collected were unevenly distributed over the Palaearctic region. Overwhelming, the majority of field collections (76%) occurred in western and central Europe: primarily in France, Italy, Switzerland, Germany, and Austria. Only 19% of the collecting was done in eastern Europe, the Balkans and Asia Minor (mainly Hungary, Greece, and Turkey). The remaining 5% of the collections was equally shared by the vast territories of the former Soviet Union and other Palaearctic countries (Pakistan, China etc.).

Thus, the current distribution of native areas of invasive plant species and the areas of search for biocontrol agents for the same plants that have become noxious weeds in naturalized areas are markedly different (Fig. 1). The western Palaearctic was studied much more frequently, while the eastern Palaearctic remains much less explored than would be expected given the above bio-geographical data. This imbalance is obviously a result of the former political divisions that no longer exist. The necessity to increase the intensity of field explorations in this sparsely investigated part of the world was noted some time ago (e.g. Schroeder & Goeden 1986, Pemberton 1990). The country of Russia comprises a considerable part of the Palaearctic region and includes a number of biomes from tundra to desert and various climates from mild sea regions to sharp continental areas in eastern Siberia. These many different biomes have given rise to a multitude of plant and animal species; providing substantial opportunities for biocontrol exploration and research. In the past 10 years, numerous projects aimed at biological control of various weeds have expanded their field research to include Russia.

Leafy spurge (*Euphorbia esula*), an aggressive deep-rooted perennial weed of Eurasian origin, is one example. Fourteen or more insect species have been released in North America for leafy spurge biological control (Julien & Griffiths 1999). Economic losses caused by this noxious weed are still extremely high (Gassmann & Schroeder 1995, Gassmann 1996, Gassmann *et al.* 1996). Hence, a search for additional biocontrol agents was conducted in 1998–1999 in Krasnodar territory (south-east Russian lowlands and the Caucasus), Novosibirsk province (south-west Siberia), and Irkutsk province (south-east Siberia). As a result of these field explorations, numerous new natural enemies of leafy spurge were discovered. Among these potential new biocontrol agents, one genus, *Aphthona* (Coleoptera: Chrysomelidae), yielded six species feeding on *E. esula* from climatically similar areas to the major leafy spurge infestation in North America (Konstantinov *et al.* 2000). *Aphthona russica* Konst., found in the Taman Peninsula and described as a new species, is considered a promising candidate for the biological control of leafy spurge (Konstantinov *et al.* 2001). The biology of this flea beetle was investigated both in the field and under laboratory conditions (Volkovitsh *et al.* 2000). Strict host specificity and a detrimental impact on the host in its native range, suggest a high potential for the control of leafy spurge in areas where it has become naturalized and weedy.

Among other insects worth mentioning is the spurge sawfly, *Arge beckeri* Tournier (Hymenoptera: Argidae), which was also collected in Krasnodar territory and investigated under laboratory conditions in the Zoological Institute (St Petersburg, Russia). The preliminary data suggest that *A. beckeri* deserves further intensive studies as a potential agent for the biological control of leafy spurge. In addition to the insect species, several pathogenic fungi were isolated from diseased *Euphorbia* plants collected under natural conditions and tested in the same laboratory (Dolgovskaya *et al.* 2000). However, recent explorations aimed at the search for new biocontrol agents in Russia are not limited to leafy spurge.

Since 1999, an international USDA-funded team has conducted field and laboratory studies aimed at biological control of Yellow starthistle, *Centaurea solstitialis* (YST), a noxious invasive weed in the USA, Chile, Australia, and South Africa. Among the insects found feeding on YST, is a flea beetle *Psylliodes chalcomera* with stem-boring larvae and leaf-feeding adults. This insect has been repeatedly collected from YST and Scotch thistle, *Onopordum acanthium,* another invasive thistle. Field observations suggested each of the plants is being attacked by different "ecological forms" of this flea beetle. Laboratory tests have shown these insects to be very host specific. Taxonomic research is being conducted at the Systematic Entomology Laboratory (USDA, ARS, Washington, DC) to investigate whether these "forms" may represent sibling species. Quantitative field sampling demonstrated significant impact on the host, suggesting that *P. chalcomera* may be an important biocontrol agent for YST in areas where it have become invasive (see also paper by Cristofaro *et al.* in this volume).

More recently, purple loosestrife, *Lythrum salicaria,* another exotic invasive weed in North America has become the object of studies conducted by the same Russian team working in cooperation with the USDA/ARS in Ithaca, New York, USA. In Russia, purple loosestrife is widespread in wet meadows, riverbanks and other moist habitats from the Baltic region to the Black sea and Eastern Siberia. Field explorations and studies of museum collections revealed a number of flea beetle

species feeding on *L. salicaria*. *Aphthona lutescens* collected in Krasnodar territory and studied under laboratory conditions seems to be particularly promising as a potential biocontrol agent due to narrow host specificity and (in contrast to biocontrol agents earlier introduced against purple loosestrife) two-fold impact on the host with root-feeding larvae and leaf-feeding adults (see also paper by Dolgovskaya *et al.* in this volume).

Hoary cress, *Cardaria draba*, is another invasive weed of western North America, listed among the 37 most invasive weed species of the United States. In cooperation with the Northern Plains Agricultural Research Laboratory in Sidney, Montana, numerous phytophagous insects were collected from *C. draba* during field trips in southern Russia. A flea beetle *Psylliodes wrasei* Leonardi and Arnold, not considered as a potential *C. draba* biocontrol agent earlier because its host range was unknown, looks promising. *Psylliodes wrasei* was collected in Krasnodar territory at the end of May and adults were frequently observed feeding on *C. draba* under natural conditions. Host-specificity tests conducted in the laboratory with field-collected beetles demonstrated that adults strongly prefer *C. draba*. Field observations support this conclusion: no adults of this species were found on other neighbouring cruciferous plants. In combination, these preliminary data suggest that *P. wrasei* definitely needs further investigation.

Last year, black and pale swallow-worts (*Vincetoxicum nigrum* and *V. rossicum*) were also included among our targets. Both species originated in the east Palaearctic region. *Vincetoxicum rossicum* is reportedly endemic to southern Russia. Black and pale swallow-worts are serious, highly aggressive, exotic weed species, rapidly increasing in area infested in both the US and Canada (Christensen 1998). A literature search and data from insect collections of the Zoological Institute suggest that some leaf beetles collected feeding on *Vincetoxicum* spp. in the northern Caucasus and southern Siberia are potential biocontrol agents (see also Spencer *et al.* 2003).

Gaint hogweed (*Heracleum mantegazzianum*) is the target for another new weed biocontrol project supported by the European Community. This project began with literature and museum research and then moved into intensive field exploration in the Russian northern Caucasus, reported to be the centre of origin of this highly aggressive and dangerous invasive plant (visit the project home page at http://www.flec.kvl.dk/giant-alien/).

The opportunities for significant weed population reduction using environmentally benign biological methods against both old and new weed targets of Palaearctic origin have not been exhausted by this short review. The large expanse of the country of Russia across Europe and Asia and the many endemic plants and animals to be found there, provides excellent opportunities for reducing the impact of many naturalized weeds, native to the Palaearctic region, through the introduction of host-specific biocontrol agents. Today, Russia is a country opening itself to the world, with a strong background in science and an interest in partnerships.

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Natural impact of the flea-beetle, *Longitarsus* sp., on *Heliotropium amplexicaule* in Argentina and its potential for use as a biological control agent in Australia

M. Zapater,[1] N. Bartoloni,[1] G. Perez-Camargo[1] and D.T. Briese[2]

Summary

Heliotropium amplexicaule (Boraginaceae) was introduced into Australia from South America over 100 years ago and has since become a serious weed in summer pastures. In 1998, CSIRO started a classical biological control project against *H. amplexicaule* to search for and evaluate natural enemies in its native range in Argentina. This study quantifies the impact of one such natural enemy, the flea-beetle *Longitarsus* sp. (Coleoptera: Chrysomelidae), on *H. amplexicaule*, to determine its potential for control of the weed in Australia. *Longitarsus* sp. adults chewed small feeding holes in the leaves of *H. amplexicaule* plants, leading to necrosis and death of the remaining leaf tissue. Feeding started from the basal leaves, with new leaves attacked as they developed on the growing plants. Unattacked control plants achieved maximum size by early March. The mean growth of attacked plants at the two attacked sites was only 54 and 48%, respectively, of that shown by these control plants. Moreover, these plants showed greatly reduced vigour and half of them had died by mid-March at one site and mid-May at the other. There was no plant mortality at the control site. Finally, control plants produced significantly larger quantities of flowers and seed over a longer period than did attacked plants. The overall impact of *Longitarsus* sp. is due to a combination of adult feeding on leaf tissue and larval feeding on the root system. The study also demonstrates a synergism between the flea-beetle and pathogenic micro-organisms causing subsequent leaf necrosis. Such impact on plant survival and reproductive potential suggests that *Longitarsus* sp. could be a good biological control agent for *H. amplexicaule* in Australia, provided it demonstrates sufficient host specificity.

Keywords: biological control, *Heliotropium amplexicaule*, impact assessment, *Longitarsus* sp.

Introduction

Blue heliotrope, *Heliotropium amplexicaule* (Boraginaceae) is a deep-rooted, semi-prostrate, perennial herb, native to South America in northern and central Argentina, southern Bolivia, Uruguay and the extreme south of Brazil (Johnston 1928). It was introduced into Australia as an ornamental plant over one hundred years ago, and has expanded its range considerably during the past four decades. There are now widespread infestations in south-eastern Queensland and northern New South Wales, with isolated populations in South Australia (Parsons & Cuthberton 1992). Blue heliotrope can compete successfully in agricultural systems with desirable crops and pasture species and cause a decline in livestock performance as a result of toxicity from the pyrrolizidine alkaloids it contains (Glover & Ketterer 1987). Its increasing rate of spread and difficulties in controlling it by conventional herbicides, make *H. amplexicaule* a serious threat to Australian agriculture.

In Argentina, blue heliotrope is a coloniser of recently disturbed areas, but populations either do not persist or remain at low densities. Survey work suggests that natural enemy attack reduces the plant's capacity to

[1] Facultad de Agronomía, Universidad de Buenos Aires, 1417 Buenos Aires, Argentina.
[2] CSIRO Entomology, GPO Box 1700, Canberra 2601, Australia
Corresponding author: M. Zapater <mmzapater@arnet.com.ar>.

compete successfully with later successional vegetation during the same season, resulting in *H. amplexicaule* being much shorter-lived in its native range than in Australia (Briese & Zapater 2001).

H. amplexicaule has a perennial life-cycle. Plants produce several new semi-prostrate branching shoots from a central root-crown in spring. These continue to grow throughout spring and summer, if rainfall is sufficient, and may produce several flowering flushes until autumn. The roots grow and thicken during this growth period, storing up nutrient reserves. As temperatures fall in late autumn, the shoots die back and the plants pass winter as an extensive root system, before using nutrient reserves to reshoot in the following spring and repeat the cycle. *H. amplexicaule* may reproduce either from seed or vegetatively from roots just below the surface. Based primarily on the life-cycle of *H. amplexicaule*, Briese & Zapater (2002) described a two-pronged strategy for the biological control of this weed in Australia, in which they recommended the use of complementary agents to attack the above-ground biomass of the plant (photosynthetic tissue) and the root system (nutrient reserves), in order to reduce plant longevity, regrowth and reproduction, and thus render it less competitive with more desirable pasture species.

Surveys in the native range of *H. amplexicaule* in Argentina had identified four insect species with potential for biological control: the leaf-feeding beetle, *Deuterocampta quadrijuga* (Coleoptera: Chrysomelidae); the flea beetle, *Longitarsus* sp. (Coleoptera: Chrysomelidae), which feeds on leaves as an adult and roots as a larva; the bug, *Dictyla* sp. (Hemiptera: Tingidae), which sucks saps from the cells of leaves, killing them; and the thrips, *Haplothrips heliotropica* (Thysanoptera: Phlaeothripidae), whose feeding causes deformation of leaves and buds (Briese & Zapater 2001, 2002). Additionally, an open-field experiment conducted in Argentina showed that the four insects had very restricted host ranges and might satisfy more extensive host-range testing in quarantine in Australia (Briese *et al.* 2002). The leaf-beetle, *D. quadrijuga*, was selected as the best agent to target above-ground biomass and, after quarantine testing had determined that it posed minimal risk to non-target plant species (Briese & Walker 2002), it was released in 2002 following approval by Australian regulatory authorities. *Longitarsus* sp., a 2 mm long, dark-brown halticine flea-beetle, was the only agent surveyed that attacked the root system. Laboratory studies showed that female *Longitarsus* sp. enter gaps in the soil to lay eggs directly onto the roots and emerging larvae then feed on the fine feeder roots that extend from the larger storage roots (A. Walker, pers. comm.). However, adult feeding also produces small shot-holes in the leaves, which subsequently become necrotic, causing more extensive leaf damage and death. This ability to damage both leaves and roots makes this species an interesting possibility for the control of *H. amplexicaule*, particularly as other *Longitarsus* species already have a good track record and interest as weed biological control agents (see Wapshere 1982, Ireson *et al.* 1991, McEvoy *et al.* 1991, Jordan 1997).

Before investing in the costs of introduction and quarantine clearance of a candidate agent, it is essential to determine whether it has the capacity to cause damage that could affect the population dynamics of the target weed in its introduced range. This paper therefore describes studies on the impact of naturally occurring populations of *Longitarsus* sp. on *H. amplexicaule* plants in its native range in Argentina, designed to determine whether it had the potential to fulfil a complementary role to *D. quadrijuga* in the biological control of the weed.

Materials and methods

The experimental area

Experimental plots were set up on abandoned roadside strips of land containing localised populations of *H. amplexicaule* at km 49 (hereafter named as site 49), km 50 (site 50) and km 51 (site 51) of Route 188, near Pergamino in Buenos Aires province, Argentina. Latitudes and longitudes of these three sites were 33°43'56"S by 60°26'15"W, 33°44'16"S by 60°26'23"W and 33°44'29"S by 60°26'38"W, respectively. Most plants at site 51 and some at site 50 had germinated the previous year in areas overgrazed by horses, while those at site 49 had germinated during the current year after a winter fire. The dominant vegetation in sites 50 and 51 was the invasive grass, *Cynodon dactylon,* while site 49 had a dense mixed grass sward. Plants from site 51 had been observed to be in good condition during the season before this study.

The studies

At each site, 20 plants of *H. amplexicaule* were individually identified with numbered plastic tags and visited fortnightly on 11 occasions from December 1999 to June 2000; on December 17, January 3 and 15, February 1 and 22, March 7 and 23, April 6 and 25, May 18 and June 15. At two sites (49 and 51) all plants were maintained under natural conditions to evaluate *Longitarsus* sp. damage while, at the third site (50), control plants were treated at each visit with a systemic insecticide to prevent insect attack and provide a baseline for quantifying the impact of the flea-beetle. Surveys ceased once the aerial parts of *H. amplexicaule* plants had senesced with the approach of winter.

Longitarsus sp. populations were estimated by counting adults on all marked plants and calculating the mean density per plant for each site and date. It should be noted that, since the *Longitarsus* sp. females lay their eggs directly onto the roots in the soil and larvae feed underground on the hair roots of *H. amplexicaule*, it was impossible to sample either eggs or larvae non-

destructively *in vivo*. The feeding damage produced by adults, which chew small feeding holes in the leaves, was estimated by randomly selecting three stems on each plant and dividing them into 10 nodes, ranging from 1 (the oldest) to 10 (the youngest) leaves. A leaf from each node was then selected and the number of flea-beetle feeding holes in it counted. The amount of leaf tissue lost due to necrosis around the feeding holes was rated on a scale of 0–4; where 0 = no leaf necrosis, 1 = < 10% necrosis, 2 = 10–50% necrosis, 3 = 50–90% necrosis and 4 = > 90% necrosis. Leaf necrosis was a direct consequence of the *Longitarsus* sp. feeding holes, which facilitated infection of surrounding plant tissue by saprophagous plant pathogens.

Overall plant size was estimated by measuring the widest cross-section (D_1) of an individual *H. amplexicaule* plants and the cross-section at 90° to this (D_1) and calculating plant area using the formula: Area = $\pi((D_1 + D_2)/4)^2$. Plant vigour was rated visually for each tagged plant as either 0 (in poor condition), 1 (intermediate) or 2 (vigorous). Dead plants were counted separately. These plant data were collected from all sites at each of the 11 sample dates to determine plant growth and changes in condition. Plant reproduction was estimated by counting the numbers of cymes and classing them either as flowering, if 10–100% of the buds were in flower, green (if more than 90% of flowers had progressed to the green fruit capsule stage, and mature (if cymes contained all ripe fruit capsules). Counts were made on February 22 and April 25 at the three sites. The earlier date corresponded to the maturity of earlier flowering flushes, while the latter date corresponded to the maturity of flowers produced just prior to plant senescence.

Results

Longitarsus *sp. population density*

Longitarsus sp. adult population densities at the three sites during the season are shown in Figure 1. The population at site 49 fluctuated in December and January, peaked rapidly in late February–March and declined to very low levels from April on. The population at site 51 was lower than at site 49 and showed a slightly earlier, less pronounced and less persistent peak in population in early February before declining to low levels from March on. These population peaks corresponded with the periods of maximum plant size (see below) and hence greatest foliage quantity at the respective sites, and there was a significant correlation between plant size and *Longitarsus* density; Spearman coefficient of rank correlations (r_s) being 0.557 ($P<0.01$) for site 49 and 0.524 ($P<0.01$) for site 51. Towards the end of the survey period, *Longitarsus* numbers at site 51 became concentrated on the few surviving plants, and then adults were often observed on fruits, instead of leaves, as these were the only parts of the plants remaining green.

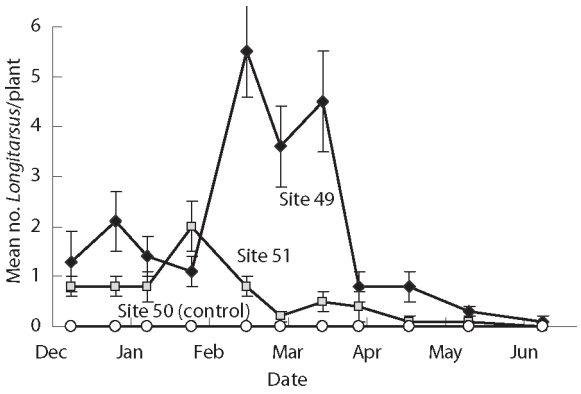

Figure 1. Changes in adult *Longitarsus* sp. population density on *Heliotropium amplexicaule* at three sites near Pergamino, Argentina.

Longitarsus sp. adults are highly mobile and frequently jump to neighbouring plants when disturbed (Briese *et al.* 2002). At the study sites, adults were observed on other plant species within the neighbourhood of a *H. amplexicaule* plant. Some feeding holes were found on only one other species, *Convolvulus* sp. (Convolvulaceae), between February 22 and March 23, but only on plants within a radius of 0.80 m. Further away, *Convolvulus* sp., although present in large numbers was never seen to be fed on or even visited by *Longitarsus* sp. Interestingly, no *Longitarsus* sp. adults were observed on plants of the more closely related *Echium plantagineum* (in the same family Boraginaceae as *H. amplexicaule*) either close to or far from *H. amplexicaule*.

Feeding damage and leaf necrosis

In spite of the apparently low average numbers of adults observed per plant, the resulting feeding damage was important. As indicated earlier, although *Longitarsus* sp. adults produced only small shot-holes in the *H. amplexicaule* leaves when feeding, necrotic lesions rapidly spread out from feeding holes causing more extensive leaf damage and death (Fig. 2). In contrast, unattacked leaves exhibited low levels of necrosis as they aged. As expected, the larger population of *Longitarsus* adults at site 51 resulted in heavier feeding damage to the *H. amplexicaule* leaves, reaching a mean of 18 holes per leaf, compared with six holes per leaf at site 51 (Fig. 3). Feeding started from the basal leaves (lower nodes) with newer leaves attacked along the growing shoot as they developed and the more basal ones died (Fig. 3). As the season progressed, leaves on the higher nodes successively became necrotic following the pattern of *Longitarsus* feeding (Fig. 4). There was a low, though significant correlation between the number of feeding holes per node and the extent of subsequent leaf tissue necrosis per node; Spearman coefficient of rank correlations (r_s) being 0.138 ($P<0.01$) in site 49 and 0.170 ($P<0.01$) in site 51.

Figure 2. Progressive development of necrotic lesions from *Longitarsus* sp. feeding holes in leaves of *Heliotropium amplexicaule*.

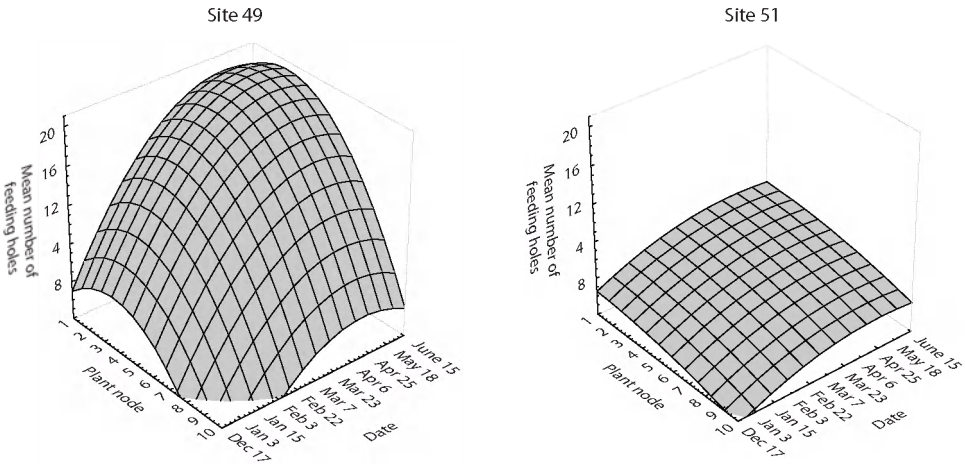

Figure 3. *Longitarsus* sp. feeding damage on the leaves of *Heliotropium amplexicaule* plants at sites 49 and 51, near Pergamino, Argentina.

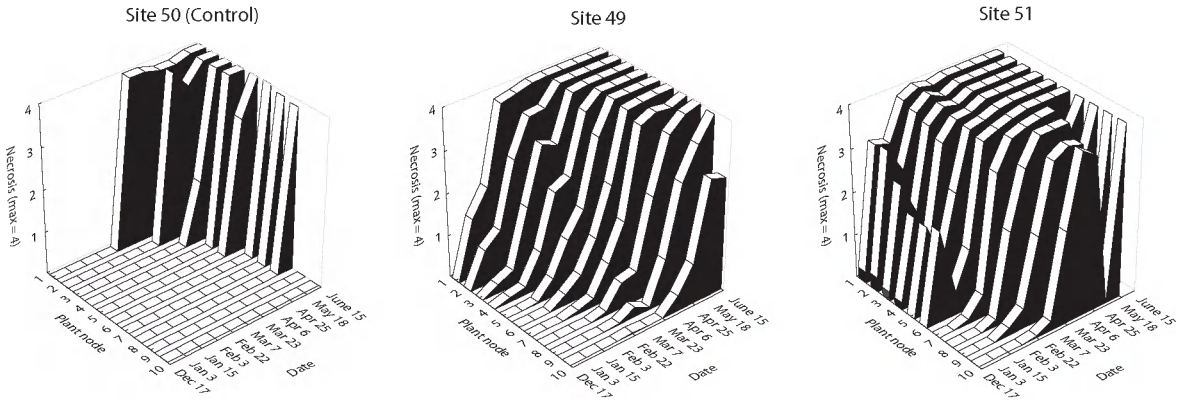

Figure 4. Progressive development of necrotic lesions in the leaves of *Heliotropium amplexicaule* following feeding damage by *Longitarsus* sp. Site 50 shows natural senescence of leaves from unattacked plants (node 1 leaves are closest to the base and node 10 at the growing tips of shoots).

At the unattacked control site 50, the oldest basal leaves began to senesce in late February with a progression of senescence of leaves along the shoot. At the last sample date on June 15, the youngest tip leaves still remained healthy (Fig. 4). The condition of senescing leaves declined much more rapidly than those affected by necrotic lesions (cf. slopes of rates of necrosis between sites 50 and 49 in Figure 4). The pattern of development of necrotic lesions differed between the two sites. Interestingly, leaf necrosis was more rapid at site 51 than at site 49, despite there being less *Longitarsus* feeding damage. Most leaves were destroyed in the lower half of the shoot by mid January at site 51, whereas only the basal leaves showed this level of necrosis at site 49. Most leaves were very heavily necrosed by the end of April at site 51 and, apart from a dip in May due to new leaf production after heavy rains, were all dead by mid-June. Necrosis progressed more slowly at site 49 with some terminal leaves still remaining when sampling ended on June 15. *Longitarsus* feeding and subsequent leaf necrosis therefore led to considerable loss of photosynthetic potential and earlier senescence of *H. amplexicaule* plants.

Plant growth

Plants at sites 50 and 51 (870 ± 264 cm^2 and 816 ± 140 cm^2, respectively) were larger than those at site 49 (415 ± 68 cm^2) at the start of sampling, confirming the observation that they were already present the previous year, whereas the population at site 49 had recently germinated. The unattacked control plants at site 50 continued to grow and increase in size until March–April, when plants gradually deteriorated as the above ground vegetation senesced. Figure 5 shows the growth and decline of *H. amplexicaule* plants during the sampling period. Sites 50 (control) and 49 followed a similar pattern, peaking in size during March and then declining, though control plants attained a greater maximum area (1467 ± 217 cm^2) than did the attacked plants at site 49 (2846 ± 868 cm^2). Attacked plants at Site 51, which exhibited earlier and more extensive necrosis than site 49, peaked in size in February (1825 ± 418 cm^2), followed by a more gradual decline (Fig. 5). The mean overall plant sizes of the two attacked *H. amplexicaule* populations were 48% (site 51) and 54% (site 49), respectively, of that shown by the control population (Fig. 5). While plants from site 49 could have been expected to remain smaller than the unattacked control plants, due to their smaller initial size, those at site 51 would have been expected to grow similarly, suggesting that the reduced growth was due to insect attack and subsequent necrosis. Moreover, the leaves of plants at site 51 were smaller (3–5 cm) than those at sites 49 and 50 (5–6 cm), indicating reduced plant vigour.

Plant vigour and mortality

Plants attacked by *Longitarsus* sp. at both sites declined in overall vigour rating between February and April, whereas those at the control site remained in good condition until natural senescence of the above-ground vegetation commenced in May (Fig. 6). This decline in vigour of plants at site 51, which showed a higher rate of necrosis, preceded that at site 49 by two weeks (Fig. 6). Moreover, none of the control plants died during the sampling period, whereas 70% of plants at site 49 and 95% of plants at site 51 were dead by the end of sampling on June 15. This mortality occurred over the second half of the sampling period from March to June (Fig. 6). *Longitarsus* larval feeding on the roots would have contributed to this mortality, but it was not possible to partition the effects of adults and larvae. A year after the experiment all plants from these two sites were confirmed dead.

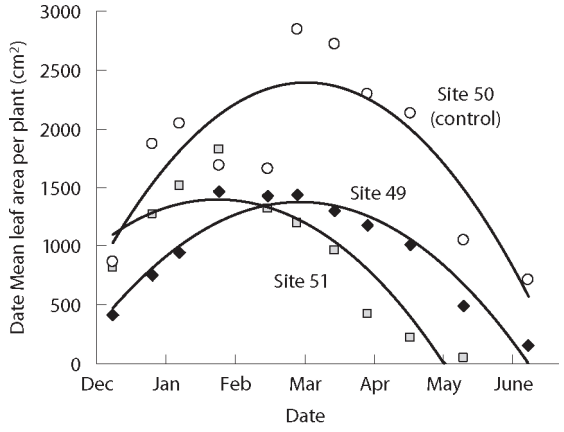

Figure 5. Changes in the mean size of *Heliotropium amplexicaule* plants over the growing season at sites unattacked (site 50) and attacked (sites 49 and 51) by *Longitarsus* sp.

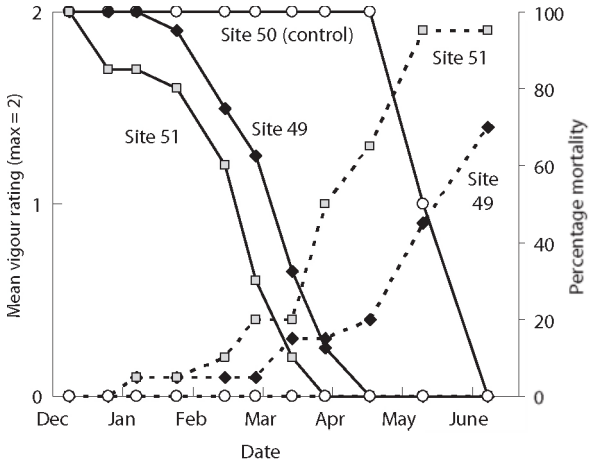

Figure 6. Effect of *Longitarsus* sp. on *Heliotropium amplexicaule* plant vigour (solid line) and mortality (dashed line) at the three sites near Pergamino, Argentina.

Plant reproduction

H. amplexicaule can produce several flushes of flowering in a season, commencing in late spring. At the first sampling date in February, there was no significant difference (t-test comparisons with unequal variance) between the number of flower cymes produced at the three sites (45 ± 4 at control site 50, 52 ± 6 at site 49 and 39 ± 5 at site 51). However, flowering phenology was different, as 53% of the cymes had inflorescences at the control site, compared to 16% and 11% at sites 49 and 51, where plants were subject to *Longitarsus* attack, respectively (Fig. 7). This could indicate a slowdown in new flower production at the attacked sites. When sampled in April, the number of flower cymes per plant at the control site had increased to 89 ± 19, with 9% still flowering and 38% fully mature (Fig. 7). In contrast, there was no increase in the mean number of cymes at site 49 (52 ± 8), and almost 90% of cymes were mature with no new flowers. At site 51, there had been no further production of flowering cymes since February (Fig. 7). Hence, fruit production in the second part of the growing season was greatly reduced in the two sites that had been attacked by *Longitarsus* sp.

Discussion

Longitarsus sp. showed a close synchrony with the life-cycle of *H. amplexicaule* in its native range. Adults emerged in early spring, following the onset of new shoot growth by the plant, which they used as a food source. From laboratory studies on the time for development, the flea-beetle could undergo at least two generations in the field. The sharp population peaks in February at Site 51 and during March at site 52 most likely indicated the emergence of new generation adults from larvae that had been feeding on the roots of *H. amplexicaule*. These larvae would have hatched in late spring from eggs laid by the first generation adults. Such emergence coincided with the period of maximum plant size, and the subsequent decline in flea-beetle numbers was partly due to mortality and partly to dispersal from plants that were deteriorating due to feeding damage and necrosis. *Longitarsus* sp. are the most mobile of the insects found on *H. amplexicaule* and similar dispersal away from cut host-plants was observed during an open-field host choice experiment (Briese *et al.* 2002). Although not actually observed, the absence of adults over the winter period suggests that the beetle spends the winter, when *H. amplexicaule* foliage has senesced, in the soil as either larvae or pupae laid by the second generation.

It is instructive to compare the response of *H. amplexicaule* plants to different types of damage. *H. amplexicaule* has an extensive root system that acts as a nutrient reserve. When subject to instantaneous mechanical defoliation by cutting shoots, the reserves enable these to be rapidly replaced by vigorously growing new shoots from the central meristem (M.Z., pers. obs.). Insect defoliation alters this response. The leaf-feeding beetle, *D. quadrijuga*, already released in Australia for control of the weed, is much larger than *Longitarsus* sp. and feeds on foliage as both adult and larva. It can completely defoliate plants over a period of several days to a few weeks, but tends to leave much of the stem tissue intact. Plants respond similarly to mechanical defoliation, but more slowly, taking several weeks to produce new shoots, which tend to be shorter than those reshooting following rapid mechanical defoliation (Briese & Zapater 2001). This is probably because nutrients are still being directed towards the shoots as they are defoliated, which leads to depletion of root reserves and a consequent reduced regrowth once defoliation ceases. Plants are weakened, but do not die.

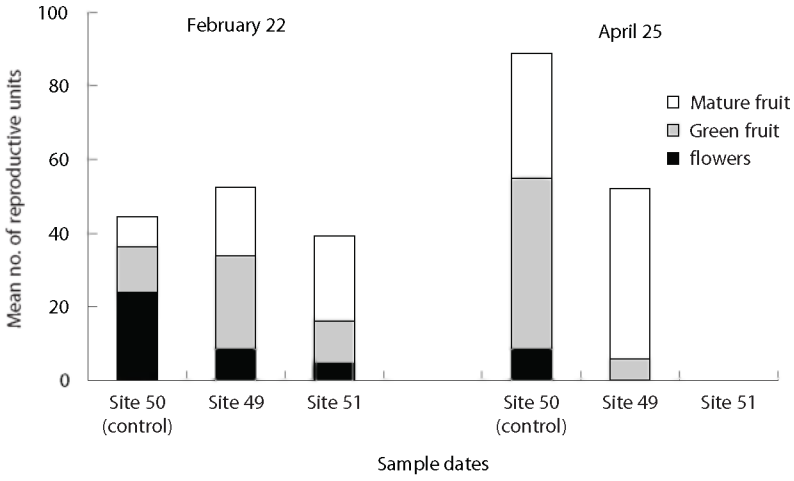

Figure 7. Average number of cymes per plant bearing flowers, green fruits and mature fruit capsules on February 22 and April 25 at sites unattacked (site 50) and attacked (sites 49 and 51) by *Longitarsus* sp.

In the case of *Longitarsus* sp., the degree of defoliation is much less than for *D. quadrijuga*, but extends over a longer period of several months and, coupled with the synergistic damage due to leaf necrosis, can also eventually lead to total defoliation of the plant. Depletion of root reserves is therefore likely to be greater as nutrients are directed towards the attacked shoots for a longer period. Critically though in the case of *Longitarsus* sp., defoliation is coupled with the destruction of feeder roots by larvae in the soil and a subsequent reduction in nutrient and water uptake. This leads to even greater exhaustion of root reserves during the growing season, the observed loss of plant vigour and even plant death. Another indicator of this larval root damage was the observation that attacked *H. amplexicaule* plants wilted in mid-summer, whereas unattacked control plants and neighbouring plants of other species did not, suggesting that they were suffering greater water stress under the same conditions.

Damage by *Longitarsus* sp. appears to be cumulative in its effect. With a season, this could be seen by the measurements of plant reproductive effort, where flea-beetle damage had little impact on the early production of flowers and fruit, but greatly reduced subsequent reproductive success. There is also a suggestion that damage can be cumulative across seasons, as there was a greater reduction in plant vigour and higher mortality of plants at site 51, which were known to have been present and subject to *Longitarsus* sp. attack during the previous year. Such cumulative impact would be beneficial in Australia, where *H. amplexicaule* is considered an aggressive competitor in summer pastures and where there are presently no natural enemies of significance (D.B., pers. obs.). In its native range, *H. amplexicaule* is a coloniser of disturbed habitats (e.g. earth movement, burning, overgrazing) but populations do not persist for many years and tend to be replaced by other vegetation until the next disturbance (M.Z., pers. obs.). From the results of this study, *Longitarsus* sp. can play an important role in driving this process.

This study also demonstrates a synergism between two types of organism leading to more effective biocontrol. Interestingly, the degree of necrosis can be similar, even when there is a substantial difference in the original feeding damage (cf. sites 49 and 51). The extent and rate of development of necrotic lesions may therefore depend on other factors such as humidity, temperature and other plant stressors. While the pathogenic organism causing the necrosis was not identified, it was noted that *H. amplexicaule* plants reared in quarantine conditions did not exhibit necrosis around *Longitarsus* feeding holes (A. Walker, pers. obs.), suggesting the absence of a causal agent. The presence or not of effective saprophytes in the field in the country of introduction could therefore have an important impact on biological control success.

Overall, the results obtained on the impact of *Longitarsus* sp. on plant vigour, survival and reproductive potential suggests that it could be a good biological control agent for *H. amplexicaule* in Australia. The observation that it fed on one non-target species gives cause for some concern, though this appears to be due to short-term "overflow" feeding in the neighbourhood of the main host, *H. amplexicaule*, at a time when flea-beetle populations were at there peak, rather than colonisation. Detailed host-specificity tests are currently being carried out at the Black Mountain Quarantine Facility, Canberra, Australia, to confirm whether it is safe to release *Longitarsus* sp. in that country.

Acknowledgements

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A Canadian strain of *Pseudomonas syringae* causes white-colour disease of *Cirsium arvense* (Canada thistle)

Wenming Zhang,[1] Michelle Sulz,[1] Tania Mykitiek,[1] Xiujie Li,[1] L. Jay Yanke,[2] Hyesuk Kong,[3] Jeffrey S. Buyer[3] and John Lydon[3]

Summary

Patches of white-coloured *Cirsium arvense* (Canada thistle) plants were recently found on roadsides, pastures and market gardens in Devon, Mulhurst, Stony Plain and Edmonton, Alberta, Canada. The diseased plants showed apical chlorosis, sometimes with dark and necrotic leaf spots. These symptoms were also associated with stunted growth, fewer shoots, inhibition of flowering and/or sterility. A total of 101 bacterial strains were isolated from the leaves, stems and flowers of white-coloured *C. arvense* plants. A bacterial species (one strain designated CT99B016C) was consistently isolated from diseased plants and was found to produce similar symptoms on *C. arvense* under both greenhouse and field conditions. The organism was reisolated from inoculated, diseased plants, thereby fulfilling Koch's postulates. The optimal bacterial cell concentration to achieve maximum disease was within the range of 10^8–10^9 colony forming units (cfu)/mL, while the optimal surfactant concentration was 0.15–0.3% Silwet L-77®. The CT99B016C strain also caused severe disease of *Sonchus oleraceus* and *S. asper* (annual and spiny sowthistle) and *Taraxacum officinale* (dandelion). The disease severity on these weed species was even greater than that on *C. arvense*. Results of phenotypic tests and fatty-acid analysis clearly placed the CT99B016C strain within the *Pseudomonas syringae* group. Fatty-acid analysis also indicated that isolate CT99B016C is more closely related to *P. syringae* pv. *tabaci* and *P. syringae* pv. *syringae* than *P. syringae* pv. *tagetis*. Results from polymerase chain reactions (PCRs) with primer sets TAGTOX-9 and TAGTOX-10 also indicated that CT99B016C is different from *P. syringae* pv. *tagetis*. The exact pathovar identification of CT99B016C remains to be determined.

Keywords: apical chlorosis, bacterial identification, Canada thistle, *Pseudomonas* sp., white-colour disease.

Introduction

Cirsium arvense (L.) Scop. (Canada thistle) is a serious perennial weed in almost all cereal, oilseed and pulse crops grown in Western Canada. It is also a problematic, competitive weed in many forage crops, pastures and conservation sites. *Cirsium arvense* is a weed contributing to a proportion of the $600 million in annual weed-related yield losses in Western Canada (Swanton *et al.* 1993). In wheat on the Canadian prairies, Peschken *et al.* (1980) estimated that *C. arvense* caused average annual losses of $3.6 million despite herbicide use. *Cirsium arvense* is not utilized by livestock because of its spiny leaves and in pastures it tends to dominate fertile, moist sites. Schreiber (1967) demonstrated that two plants per 0.09 m^2 caused losses in alfalfa of 16.5 t/ha over 4 years. Unfortunately, the *C. arvense* population of the prairie provinces has been increasing over the last 10 years (Thomas *et al.* 1998). From 1986–1989 to 1995–1997, its frequency increased from 23% to 50% in wheat and from 42% to 54% in canola, while its relative abundance over these years rose by five rankings in wheat (from ranking No.

[1] Alberta Research Council, Vegreville, Alberta, Canada T9C 1T4.
[2] Agriculture and Agri-Food Canada, Lethbridge Research Centre, 5403 1st Ave South, Lethbridge, Alberta, Canada T1J 4B1.
[3] USDA/ARS/Sustainable Agricultural Systems Laboratory, Beltsville, Maryland, USA.
Corresponding author: W. Zhang <zhangw@arc.ab.ca>.

9 to No. 4) and four rankings in canola (from ranking No. 10 to No. 6). It is difficult to control *C. arvense*. Various strategies are used and/or under study for *C. arvense* control, however this weed still remains problematic.

Recently, severe bacterially infected *C. arvense* plants were discovered and collected from pastures and market gardens in Devon, Mulhurst, Stony Plain and Edmonton, Alberta, Canada. Typical symptoms of the infected plants were very similar to previously reported *C. arvense* disease caused by *Pseudomonas syringae* pv. *targetis* (PST) (Johnson *et al.* 1996), including apical chlorosis, sometimes with dark and necrotic leaf spots. The infected plants show reduced vigour, stunted growth, fewer shoots and inhibition of flowering. In some cases, severe infections cause plant death. One hundred and one bacterial strains were isolated from those samples. In order to evaluate the possibility of utilizing these bacteria for control of *C. arvense* and to compare the Canadian bacterial strain to previously reported PST, the objectives of this study were: (1) to determine of the pathogenicity of the isolated bacteria using Koch's postulates and selection of the most efficacious isolate; (2) to evaluate the infectivity of the selected pathogenic bacterial isolate to *C. arvense* as well as *Taraxacum officinale* (dandelion), *Sonchus oleraceus* and *S. asper* (annual sowthistle); and (3) to characterize and identify the selected bacterial isolate.

Materials and methods

Inoculum production

Cryovials, each containing 2 mL of an individual bacterial strain in 15% glycerol, which had been stored at –80°C, were thawed to room temperature in a 36°C water bath. A 50 µL suspension of each strain was added individually to 2 mL of nutrient glucose broth (NGB; 8 g nutrient broth (Difco), 2.5 g glucose in 1000 mL distilled water) in test tubes (150 mm × 18 mm diameter). Tubes were then set on an orbital shaker at 200 rpm for 24 h at room temperature, or until a high cell density was apparent. A 1 mL aliquot from each tube was used as seed culture to aseptically inoculate 75 mL of sterile NGB in a 250 mL Erlenmeyer flask. Flasks were incubated on an orbit shaker at 200 rpm for 24 h at room temperature or until a high cell density was apparent. Unless otherwise specified, a 30 mL aliquot of each culture was then centrifuged for 10 minutes at 5400 rpm (3749 g) and 23°C. Supernatant was discarded and the cell pellet was resuspended in 15 mL 0.01 M phosphate buffer (pH 7). The bacterial concentration of the inoculum varied from 10^8 to 10^{10} colony forming units (cfu) per mL unless otherwise specified.

Plant preparation

Seeds of *C. arvense* were planted in 25 × 25 cm trays containing pasteurized soil mix and incubated in a greenhouse at 24/20°C day/night temperature with a 12-h photoperiod and 20–50% relative humidity (RH) and were watered daily. Seedlings at the cotyledon stage of growth were transplanted to 10-cm diameter peat pots of soil mix, one plant per pot, and returned to the greenhouse chamber.

Inoculation procedure

Seedlings at the 2–3 leaf stage were sprayed with 5 mL of inoculum per pot using an airbrush at 100 kPa. After spraying, pots were placed in a randomized complete block design in the greenhouse with conditions mentioned as above, unless otherwise specified.

Pathogenicity of bacterial isolates

Koch's postulates were applied to all 101 bacterial isolates. Inoculum applied to *C. arvense* plants was prepared as described above and amended with Silwet L-77® (0.2% v/v) prior to application. Control treatments were inoculated with Silwet L-77® (0.2% v/v) and 0.01 M phosphate buffer (pH 7). Three replicate pots of each treatment were included. Plants were assessed for any symptoms of disease daily for 2 weeks after spraying using a modified disease severity scale of Johnson *et al.* (1996), where 0 = healthy, 1 = detectable chlorosis, 2 = moderate chlorosis, 3 = severe chlorosis, 4 = severe chlorosis and necrosis, and 5 = dead plant.

Effect of Silwet L-77® and bacterial cell concentrations on disease severity of Canada thistle caused by isolate CT99B016C

The experiment was a 5 × 6 factorial experiment arranged in a randomized complete block design with three replicate pots per treatment. Silwet L-77® concentrations of 0.1%, 0.15%, 0.2%, 0.25% and 0.3% v/v and bacterial cell concentrations of approximately 0, 10^8, 5×10^8, 10^9, 5×10^9 and 10^{10} cfu/mL were included. Bacterial cell concentrations were adjusted to the highest concentration of about 10^{10} cfu/mL by concentrating cultures from flasks 10 times through centrifugation and resuspension in 0.01 M phosphate buffer. Serial dilutions of this concentrate were then performed to achieve lower concentrations. The actual number of viable cells in the bacterial suspensions was determined using the dilution plate count method with spread plating of cells on nutrient glucose agar. Disease severity was recorded 1, 2 and 3 weeks after inoculation using the 0–5 disease severity assessment scale previously described. The data were analysed by SAS analysis of variance (ANOVA).

Pathogenicity of isolate CT99B016C to *Sonchus* spp. and *T. officinale*

Sonchus spp. and *T. officinale* are problematic weeds taxonomically related to *C. arvense*. The infec-

tivity of isolate CT99B016C was therefore tested on these hosts. Seeds of two different populations of *Sonchus* (*S. oleraceus* and *S. asper*) and of *T. officinale* were planted, incubated and transplanted to 10-cm diameter peat pots of soil mix as described for *C. arvense*. *Cirsium arvense* plants were at the 2–3 leaf stage, while the *Sonchus* spp. and *T. officinale* were at the 3–5 leaf stage at the time of inoculation. Bacterial inoculum was produced as described, amended with Silwet L-77® (0.2% v/v) and applied to plants as described. Control treatments were inoculated with Silwet L-77® and buffer. After inoculation, plants were placed in the greenhouse with conditions as previously described. Disease severity was assessed 1 and 2 weeks after inoculation using the 0–5 disease severity assessment scale previously described. Three replicate pots of each treatment were included.

Characterization and identification of isolate CT99B016C

Bacterium isolate CT99B016C was characterized and identified based on phenotypic and genotypic analysis. The phenotypic analysis included physiological and biochemical characterization and fatty acid composition. The physiological and biochemical characterization included gram staining, motility, carbon substrate assimilation, oxidase and other physiological activities using previously described methods (Hu *et al.* 1991). Carbon substrate assimilation tests were performed using auxanographic API 50CH strips (bioMerieux) as recommended by the manufacturer. The fatty-acid composition (MIDI-FAME) was analyzed using the method described by MIDI (2002a,b). Polymerase chain reaction (PCR) analysis of bacterial strains was conducted using the primer sets TAGTOX-9 and TAGTOX-10 as described by Kong *et al.* (2004).

Results

Pathogenicity of bacterial isolates

Of the 101 isolates tested, only isolate CT99B016C was pathogenic to *C. arvense*. All other bacterial isolates produced no symptoms. Plants inoculated with the pathogenic isolate developed moderate to severe apical chlorosis (severity rating of 2–3) within 1 week of inoculation. Only tissue that developed after treatment was yellow or white, sometimes with necrotic lesions. Tissue formed before treatment did not develop chlorosis, but sometimes showed slight necrosis.

Effect of Silwet L-77® and bacterial cell concentrations on disease severity of *C. arvense* caused by isolate CT99B016C

Silwet L-77® concentration, bacterial cell concentration and interaction between these two factors significantly affected disease severity of *C. arvense* caused by isolate CT99B016C ($p > 0.0003$) (Fig. 1). When a Silwet L-77® concentration of 0.1% was used, no disease was observed at any of the bacterial cell concentrations tested. The greatest disease severity was observed at 0.3% Silwet L-77® and a bacterial cell concentration of 10^8 and 5×10^8 cfu/mL. A high level of disease was also observed at 0.15% Silwet L-77® and a bacterial cell concentration of 10^8 cfu/mL and 0.2% Silwet L-77® and a bacterial cell concentration of 10^9 cfu/mL. When the bacterial cell concentration was too high (generally above 10^9 cfu/mL), less disease severity was observed at all Silwet L-77® concentrations tested. More than 0.2% Silwet L-77® also caused some phytotoxicity to control plants.

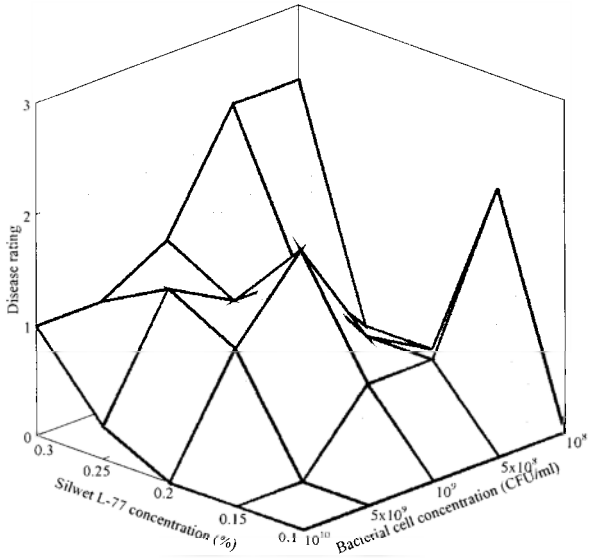

Figure 1. Effect of Silwet L-77® and bacterial cell concentrations on disease severity of *Cirsium arvense* caused by isolate CT99B016C 1 week after inoculation. A modified disease severity scale of Johnson *et al.* (1996) was used where: 0 = healthy, 1 = detectable chlorosis, 2 = moderate chlorosis, 3 = severe chlorosis, 4 = severe chlorosis and necrosis, and 5 = dead plant.

Pathogenicity of isolate CT99b016C to *Sonchus* spp. and *T. officinale*

Sonchus plants of the Bruce population sprayed with the bacterium showed symptoms similar to those seen on *C. arvense* with moderate chlorosis (severity rating of 2) of new growth 1 week after inoculation. *Sonchus* plants of the Olds population and *T. officinale* were more diseased, with moderate to severe chlorosis with necrotic lesions and necrotic lower leaves (severity rating of 3–4) within 1 week of inoculation.

Characterization and identification of isolate CT99B016C

Isolate CT99B016C was found to be a gram negative rod, 0.5–0.75 × 1–2 µm, motile, and strictly aerobic. No soluble pigments were produced, but fluorescent pigment was weakly produced on Kings B medium. This isolate is oxidase, indole, arginine and lysine dihydrolase, urease, lecithinase and ß-galactosidase negative. Gelatin liquification, citrate utilization and ß-glucouronidase were positive. The isolate grew well at 37°C. Tests based on the assimilation of 53 carbon sources indicated that isolate CT99B016C utilized 20 carbons as sole carbon source (data not shown). All of these characteristics matched very well with that of *Pseudomonas syringae*, making it highly probably that this isolate belongs to this species. Based on these results and similar studies conducted by others with PST (Trimboli *et al.* 1978, Styer *et al.* 1980, Bowden & Percich 1983, Shane & Baumer 1984, Young & Triggs 1994, Gardan *et al.* 1999), the only characteristic of CT99B016C that was consistently different from PST was the ability of CT99B016C to utilize trehalose as a carbon source. Thus, the physiological and biochemical properties of CT99B016C were not significant enough to tell whether it was PST or a closely related pathovar.

Based on the fatty-acid methyl-ester analysis, isolate CT99B016C clustered with all 14 of the *P. syringae* pathovars tested at an Euclidian distance of less than 8. It is generally accepted that an Euclidian distance of 10 or less indicates that isolates are the same species, providing strong evidence that CT99B016C is a *P. syringae* species (MIDI 2002b). Twelve of the *P. syringae* pathovars, including PST, fell within a cluster with an Euclidian distance value of 6 (the accepted cut-off value for subspecies), demonstrating that the fatty-acid methyl esters in these pathovars are very similar. Fatty-acid analysis indicated that CT99B016C is more closely related to the pathovars *tabaci* and *syringae* (Fig. 2) than to *tagetis*.

PCRs with the TAGTOX-9 primers and DNA from isolate CT99B016C produced an amplicon of approximately 750 base pairs (bp) in size, significantly larger than the amplicon produced in PCRs with DNA of PST strains (507 bp) (Fig. 3A). Similarly, PCRs with the TAGTOX-10 primers and DNA from isolate CT99B016C produced an amplicon of about 400 bp, which was significantly smaller than the 733 bp amplicon produced by PST strains (Fig. 3B).

Based on the four methods of analysis described here, we conclude that isolate CT99B016C is a *P. syringae* species that is different from PST. The exact pathovar identification of CT99B016C remains to be determined.

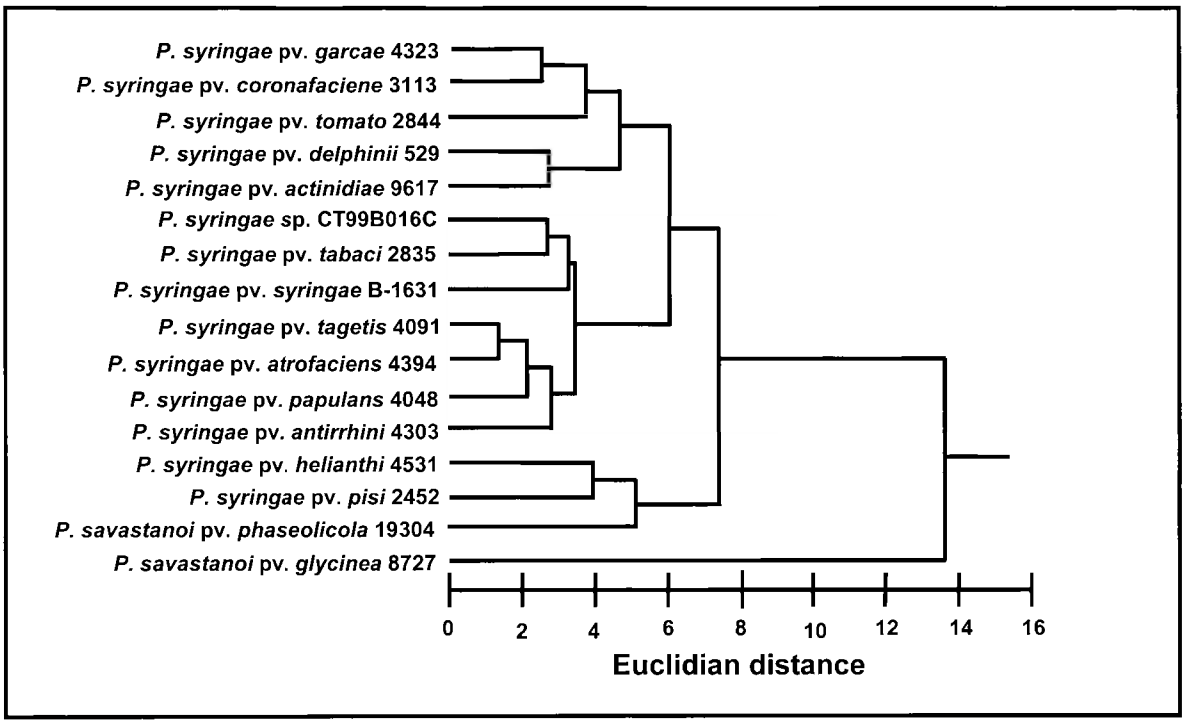

Figure 2. Relationship of *Pseudomonas syringae* and *Pseudomonas savastanoi* pathotypes with isolate CT99B016C based on fatty-acid analysis. A Euclidian distance of 10 or less indicates that isolates are the same species, 6 or less indicates that isolates are the same subspecies or biotype, and 2.5 or less indicates that isolates are the same strain.

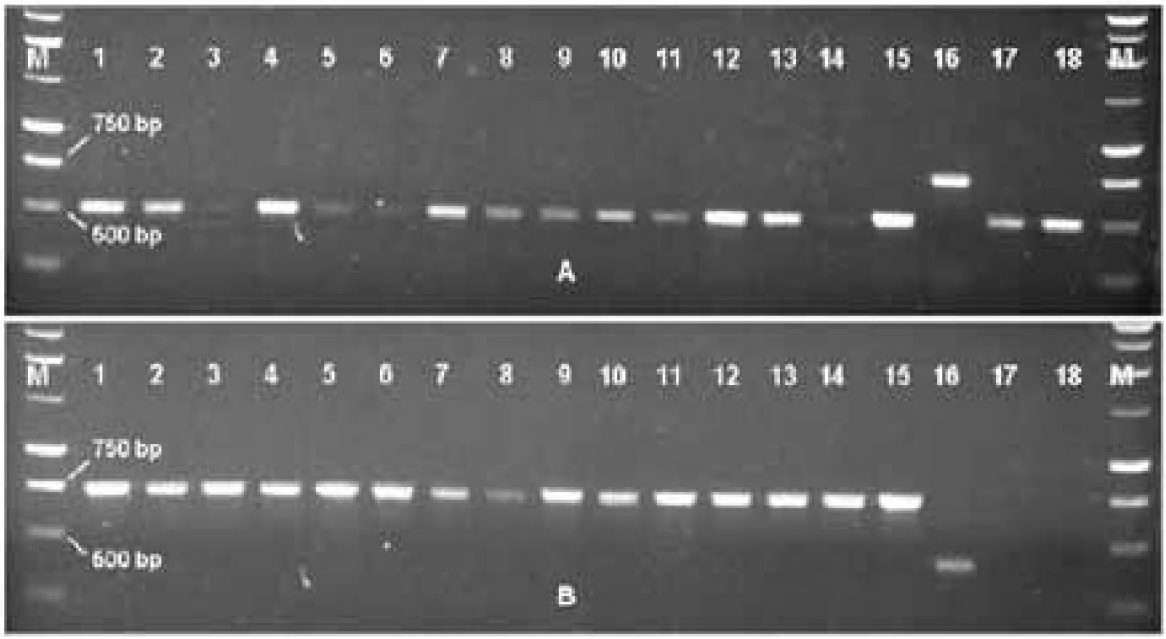

Figure 3. Electrophoresis gels of amplicons produced from polymerase chain reaction (PCR) amplifications with the (A) TAGTOX-9 and (B) TAGTOX-10 primer sets and genomic DNA from *Pseudomonas syringae* strains. Lanes: M, DNA marker; 1, *P. syringae* pv. *tagetis* 1-0392; 2, *P. syringae* pv. *tagetis* 1-502a M2; 3, *P. syringae* pv. *tagetis* 1-1065x; 4, *P. syringae* pv. *tagetis* 1-1332a; 5, *P. syringae* pv. *tagetis* 1-1394; 6, *P. syringae* pv. *tagetis* 1-2386; 7, *P. syringae* pv. *tagetis* 4091; 8, *P. syringae* pv. *tagetis* 5866; 9, *P. syringae* pv. *tagetis* 6371; 10, *P. syringae* pv. *tagetis* 6564; 11, *P. syringae* pv. *tagetis* 26808; 12, *P. syringae* pv. *tagetis* 26816; 13, *P. syringae* pv. *tagetis* 43127; 14, *P. syringae* pv. *tagetis* 43128; 15, *P. syringae* pv. *tagetis* EB037; 16, *P. syringae* sp. CT99B016C; 17, *P. syringae* pv. *tagetis* 349392; and 18, *P. syringae.* pv. *tagetis* 349393.

Discussion

This is the first report on white-colour disease of *C. arvense* in Canada. Johnson *et al.* (1996) reported that PST caused severe disease on *C. arvense* in the United States. Our bacterial isolate CT99B016C caused disease symptoms similar to PST. However, further characterization and identification revealed that CT99B016C was not PST. Therefore, further development of CT99B016C for *C. arvense* control possesses its own merits as, being indigenous to Canada, there would be less regulatory hurdles and it would possibly be better suited to the Canadian climate than strains imported from more southern latitudes. Moreover, our bacterial strain also infects other Asteraceae weed species such as *Sonchus* spp. and *T. officinale*. Therefore, there is potential to develop this bacterium as a biocontrol agent against Asteraceae weed species in Canada.

Acknowledgements

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Silybum marianum: another host for *Puccinia punctiformis*

D.K. Berner, L.K. Paxson, W.L. Bruckart, D.G. Luster, M. McMahon and J.L. Michael

United States Department of Agriculture, Agricultural Research Service, Foreign Disease–Weed Science Research Unit, 1301 Ditto Avenue, Fort Detrick, MD 21702, USA

The rust fungus, *Puccinia punctiformis*, is well known as a pathogen of *Cirsium arvense* throughout the world. This association is so common that the fungus has been thought to have *C. arvense* as its only host. Recently, we found the fungus parasitizing *Silybum marianum* in our quarantine greenhouse. Apparently, fungus spores from *C. arvense* moved to adjacent *S. marianum* plants and caused infection without any environmental manipulation. Analysis of ribosomal internal transcribed spacer sequences from fungal spore DNA isolated from the two hosts showed the organism to be the same. Initial symptoms on *S. marianum* were abundant fragrant spermagonia on large leaves. These symptoms occur on secondary shoots of *C. arvense* and are indicative of systemic fungus infection. It is unknown whether this is also the case for *S. marianum*. As the fungus infection developed on *S. marianum*, uredinia and urediniospores were produced. Urediniospores from infected leaves were harvested and sprayed onto young *S. marianum* plants grown in isolation from *P. punctiformis*. These plants also became infected and produced urediniospores. Older infected leaves also produced teliospores. In nature, *C. arvense* and *S. marianum* occupy different ecological areas: *C. arvense* is found predominantly in temperate habitats while *S. marianum* is found in habitats with a Mediterranean climate near coasts. Life cycles of each host are also different: *C. arvense* is a perennial that emerges in spring and dies back in winter, while *S. marianum* is a winter annual that emerges in fall and dies in late spring. Thus, *P. punctiformis* from *C. arvense* may rarely encounter susceptible *S. marianum* plants in the field. However, since fungal spores can be produced routinely on artificially inoculated *S. marianum*, there might be potential to use *P. punctiformis* for biological control of *S. marianum*. This would depend on understanding *P. punctiformis–S. marianum* interactions.

Evaluation of variable temperature regimes on bioherbicidal activity of non-indigenous fungal pathogens for biological control of green foxtail

S.M. Boyetchko,[1] G. Peng,[1] K. Sawchyn,[1] K. Byer,[1] S. Chandramohan[2] and R. Charudattan[2]

[1] Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada S7N 0X2
[2] Department of Plant Pathology, University of Florida, Gainesville, FL 32611

Three fungal pathogens (*Drechsclera gigantea* and two *Exserohilum* species) with bioherbicidal activity against seven grass weed species from Florida, are being explored for potential to control green foxtail (*Setaria viridis*), one of the most abundant annual grass weeds in the Canadian prairies. A thermogradient apparatus, consisting of temperature-controlled cells with day/night temperature combinations between 0 and 45°C, at 5°C intervals, was used to determine the potential of these non-indigenous pathogens as biocontrol candidates in the Canadian prairies. Optimal radial growth rate of *D. gigantea* occurred at temperatures ranging from 20–30°C, while it was 25–35°C for the two *Exserohilum* spp. Green foxtail plants inoculated with these fungal pathogens at the 2 to 3-leaf stage were also exposed to various temperatures and their efficacy compared with that of an indigenous Canadian fungal isolate, *Pyricularia setariae*. Generally, greater amounts of disease and plant biomass reduction increased as temperatures increased. *Drechslera gigantea* provided significant weed control across all temperature regimes tested, with highest disease of 74% and 69% occurring at 30/25°C and 30/15°C, respectively. *Pyricularia setariae* was slightly less effective than *D. gigantea*, especially at lower temperatures, causing a maximum of 57% disease at 25/20°C. *Exserohilum rostratum* was not a highly effective pathogen, causing only 2 to 5% disease at all temperature regimes. The results indicate that *D. gigantea* is

comparable to *P. setariae* and shows promise as a bioherbicide for green foxtail in the Canadian prairies, while *E. rostratum* is not a suitable bioherbicide candidate for this weed under the environmental conditions tested.

Biological control of the southern African *Chromolaena odorata* biotype using pathogens – the search continues

Alana den Breeyen

ARC–PPRI, Weeds Research Division, P/Bag X5017, Stellenbosch, 7599 South Africa

Chromolaena odorata, originating from tropical America, is one of the most troublesome invasive plant species in the warm, moist subtropical and temperate areas of southern Africa. There is strong evidence to suggest that the biotype of *C. odorata* invading southern Africa originates from the islands of the Northern Antilles, particularly Cuba, Jamaica or Puerto Rico. Several pathogens have been reported on *C. odorata* in recent years and, as a result, several exploratory survey trips to South, North and Central America were undertaken from 1988 until 1997, to record and collect pathogens on *C. odorata*. A number of isolates of several pathogens were collected and screened against South African *C. odorata* plants. These pathogens, including *Pseudocercospora eupatorii-formosani*, *Mycovellosiella perfoliata* and *Septoria ekmaniana*, were of necessity collected on other biotypes of *C. odorata* from other parts of its native range. To date, only *P. eupatoriiformosani* and *M. perfoliata* isolated from diseased leaf material collected in Jamaica have been found to be pathogenic on the South African biotype of chromolaena. In order to ensure compatibility between potential agents and host plant, it has become important to find the origin of the southern African biotype so that biological control agents can be collected from neotropical *C. odorata* populations which match the southern African biotype. Results of a field survey to Cuba and Jamaica in October 2002 focused on the collection of pathogens from *C. odorata* populations matching the southern African biotype.

Competition experiments for pre-release evaluation of the potential efficacy of new biological control agents

Ted D. Center,[1] Thai K. Van,[1] Teresa Rebelo,[2] F. Allen Dray,[1] Paul Pratt[1] and Min B. Rayamahji[3]

[1] US Dept. Agriculture, Agricultural Research Service, Invasive Plant Research Laboratory, 3205 College Avenue, Fort Lauderdale, FL 33314, USA

[2] Faculdade de Ciencias de Lisboa, Departamento de Zoologia e Antropologia, Centro de Biologia Ambiental, Lisbon, Portugal

[3] University of Florida, Institute of Food & Agricultural Sciences, 3205 College Avenue, Fort Lauderdale, FL 33314, USA

Two factors are of concern when considering a new biological control agent: biosafety and ability to control the weed. Methods for evaluating safety are well known, but scant attention has been given to assessment of the candidate's potential value. This is understandable inasmuch as the agent's performance depends on the role of regulating factors that differ between donor and recipient regions. Also, important subtle effects of seemingly benign biological control agents are not easily discerned. These, however, can become apparent when the targeted plant is subjected to other stresses, like interspecific competition. Additive series analysis (inverse linear models) of competition between the weed and a competitor as mediated by the prospective agent has been proposed for judging the value of new agents. We examined this possibility by comparing the abilities of two congeneric waterhyacinth weevils, *Neochetina eichhorniae* and *N. bruchi*, to modify competition between waterhyacinth and waterlettuce. The competition analysis revealed that, without weevils, 41 waterlettuce plants were required to produce an effect equivalent to a single waterhyacinth plant on waterhyacinth yield, i.e. intraspecific

competition was 41 times stronger than interspecific competition. Exposure to weevils reduced the intraspecific to interspecific competition ratio to near unity, indicating parity between the competing species. Nonetheless, *N. bruchi* was more effective than *N. eichhorniae*, and the two combined were only slightly better than *N. bruchi* alone. Similar results were obtained with ramets or flowers as yield components. Nutrient limitation did not alter relative results, although all yield components were reduced in lower nutrient environments. We conclude that important effects of these weevils act through modification of water hyacinth competitive ability. This approach could allow assessment of the value of proposed introductions by pre-empting the release of risky agents with little control value, while increasing the valuation of those that cause seemingly trivial damage.

Foreign explorations and preliminary host-range and field impact bioassays of two promising candidates for the biological control of yellow starthistle in eastern Europe

M. Cristofaro,[1] L. Smith,[2] M. Pitcairn,[3] R. Hayat,[4] S. Uygur,[5] M. Yu. Dolgovskaya,[6] S. Ya. Reznik,[6] M. Volkovitsh,[6] B. A.Korotyaev,[6] A.Konstantinov,[7] E. Colonnelli,[8] C. Tronci[9] and F. Lecce[9]

[1] ENEA, C.R. Casaccia, Rome, Italy
[2] USDA–ARS, Albany, CA, USA
[3] CDFA, Sacramento, CA, USA
[4] Ataturk University, Erzurum, Turkey
[5] Cukurova University, Adana, Turkey
[6] Zoological Institute, 199034, St Petersburg, Russia
[7] Systematic Entomology Laboratory, USDA, Washington, DC 20013-7012, USA
[8] University of Rome "La Sapienza", Rome, Italy
[9] BBCA-onlus, Rome, Italy

In the search for biocontrol agents of yellow star thistle (*Centaurea solstitialis*; Asteraceae), surveys have been carried out regularly in Turkey and southern Russia since 1990s. Yellow starthistle is common in dry habitats, although some subspecies (sub. *carneola*) can be found in moist areas, e.g. the Adana region. The largest populations and their associated natural enemies have been recorded in central and eastern Turkey, especially in highland areas with a range of altitude from 1000 to 1900 m above sea level. On the contrary, the weed is common in southern Russia (Krasnodar territory) just a few metres above sea level. Although the weed has a large distribution in Europe and western Asia (all of the Mediterranean Basin), eastern European countries like southern Russia and Turkey are real "goldmines": in addition to all the selected biocontrol agents, five new natural enemies have been found in large numbers (flea beetles, weevils, tingids and eriophyid mites). In particular, two among them are very promising: a root-borer weevil (*Ceratapion basicorne*) and a stem-borer flea beetle (*Psylliodes* sp.), due to their restricted host range (according to our field and laboratory evaluations), and their strong impact focused on early phenological stages of the target weed.

Prospects for classical biological control of torpedograss, *Panicum repens* (Poaceae), in the USA

J.P. Cuda,[1] G.E. MacDonald[2] and C.G. Hanlon[3]

[1] Department of Entomology and Nematology, Institute of Food and Agricultural Sciences, University of Florida, PO Box 110620, Gainesville, FL 32611-0620, USA
[2] Department of Agronomy, Institute of Food and Agricultural Sciences, University of Florida, PO Box 110500, Gainesville, FL 32611-0500, USA
[3] South Florida Water Management District, PO Box 24680, West Palm Beach, FL 33416-4680, USA

Torpedograss (*Panicum repens*) is a non-native, perennial grass species that is found throughout much of the southeastern United States. This aggressive rhizomatous grass thrives in a variety of agricultural and natural settings, and is considered an invasive weed of terrestrial, wetland and aquatic environments in tropical and subtropical regions worldwide. Current control strategies in the USA have focused exclusively on mechanical and chemical methods, either alone or in combination. However, these conventional weed-management practices are non-selective, expensive, and rarely provide long-term control of torpedograss in most situations. In order to achieve effective long-term suppression of torpedograss in the USA, all available management options should be considered including classical biological control. Torpedograss is not presently a candidate for classical biological control, but its biology, distribution, damage, and other control methods are under investigation. In addition, domestic surveys of the fauna using torpedograss as a host plant have been initiated in Florida. As is the case with all weedy members of the Poaceae, the botanical position of torpedograss makes it a high-risk target for biological control because the grasses are the most important group of plants in terms of their value to human society. They not only provide food for humans and forage for livestock, but also form extensive grassland ecosystems that support countless grazing animals and complex food webs. Consequently, the feasibility of initiating a classical biological control program for torpedograss was critically examined using the Peschken–McClay scoring system. By using this approach, the suitability of torpedograss as a legitimate target for classical biological control was objectively assessed. Land managers charged with controlling torpedograss infestations can use this information to decide whether public agencies should allocate resources for implementing a classical biological control program against torpedograss in the USA.

Sub-specific differentiation in the selection of a suitable biotype of *Dactylopius tomentosus* for biocontrol of *Opuntia fulgida* var. *fulgida* in South Africa

C.W. Mathenge,[1,3] J.H. Hoffmann[2] and H.G. Zimmermann[1]

[1] Plant Protection Research Institute, Agricultural Research Council, P. Bag X134, Pretoria 0001, South Africa
[2] Zoology Department, University of Cape Town, Rondebosch 7700, South Africa
[3] Postal address: GPO Box 2486, Sydney, NSW 2001, Australia

Opuntia fulgida var. *fulgida* (Cactaceae), commonly known as rosea cactus, was until recently referred to as *Opuntia rosea* in South Africa, where it was introduced, supposedly from Mexico, for ornamental purposes and subsequently became a noxious weed with infestations developing in the Free State, Northern Cape, and Limpopo provinces. Mechanical and chemical control measures have proved ineffective, and biological control has become the preferred method to curtail the spread of this weed. Cochineal insects (*Dactylopius* spp.) feed exclusively on cactus plants and some species consist of different strains or biotypes which are extremely host-specific. This may explain why *D. tomentosus*, a supposedly generalist species on "chollatype" cacti, has successfully controlled *Opuntia imbricata* in

South Africa since 1970, but has had very little impact on rosea cactus. Examination of several provenances of *D. tomentosus* from different hosts and localities in Mexico revealed the existence of several distinct strains or biotypes within this species. The host-plant relationships of the cochineal biotypes were used to verify the identity of rosea cactus and its taxonomic relatedness to other similar cactus species. The *D. tomentosus* biotype obtained from *O. rosea* in Mexico failed to survive on rosea cactus during host-specificity tests, confirming that *O. rosea* in South Africa had been misidentified. Subsequent botanical examination showed that rosea cactus conformed to *O. fulgida* var. *fulgida* and not *O. rosea*. This discovery enabled the search for biocontrol agents to be narrowed to *O. fulgida* var. *fulgida* and closely related species. Host-specificity tests showed that a biotype of *D. tomentosus* collected from *O. cholla* had a significantly higher rate of development, survival and fecundity on *O. fulgida* var. *fulgida* than other biotypes from *O. imbricata*, *O. rosea*, *O. fulgida* var. *fulgida* and *O. fulgida* var. *mamillata*. This particular biotype of *D. tomentosus* has the potential to cause more damage than the other biotypes and is consequently considered the most suitable entity for biological control of *O. fulgida* var. *fulgida* in South Africa.

The role of ecology in selecting target species and agents for biological control

P.B. McEvoy

Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, 97331-2902, USA

Weed biological control has been compared to a lottery in which many control organisms are released to find the few that are effective. Two symptoms of biocontrol under the "lottery model" are "runaway importation rates" and the "monitoring and evaluation gap". Alternatives to the lottery model include designing biological control systems to minimize the number of control-organism species introduced based on (1) attributes of enemies, weeds, and environment critical to success, (2) targeted disruption of weed life cycles, and (3) combinatorial ecology involving coordinated manipulation of herbivore, competition, and disturbance regimes. Models are becoming indispensable for linking phenomena that occur on very different scales of space, time, and ecological organization in biological control systems. Recent models applied to biological control reflect the general trend for ecological theory to become more useful as it more faithfully attends to the details of life histories and the mechanisms governing encounters between enemies and their hosts. Ecology's contribution to biological control may have been modest in the past, but the prospects for the future are brighter owing to better monitoring at each stage in the development of a program; use of appropriate mathematical and experimental models (e.g. incorporating spatial information and movement); and a closer alliance between basic biocontrol research and implementation.

(This presentation was a keynote address for Theme 2)

Aspects of the biology and host range of *Alcidodes sedi* (Curculionidae: Mecysolobini), a potential biological control agent for the introduced plant *Bryophyllum delagoense* (Crassulaceae) in South Africa and Australia

A.J. McConnachie and A.B.R. Witt

Queensland Department of Natural Resources and Mines, South African Field Station, c/o PPRI, Private Bag X134, Pretoria 0001, South Africa

Despite the fact that *Bryophyllum delagoense* (mother-of-millions) was introduced into southern Africa from Madagascar more than 130 years ago, it has not become as invasive as in Queensland, Australia. Several factors may be contributing to the current situation, one of which is the possibility that native insects in southern Africa have extended their host ranges to include *B. delagoense*. One indigenous species, *Alcidodes sedi*, has been collected on *B. delagoense* and is particularly damaging. Larvae of this weevil are stemborers, and high larval densities often result in plant death in laboratory situations. These observations resulted in the selection of *A. sedi* as a potential biological control agent for *B. delagoense* in Australia, and possibly southern Africa through augmentative releases. Aspects of the biology and host range of the weevil were therefore investigated. Preliminary data on the biology of the weevil suggests that it has a developmental time of 62.1 ± 16.3 days (n = 19) (range:15–30°C). Preliminary host-range trials indicate that the weevil is able to feed and develop on several species of *Bryophyllum*. There is no significant difference in the morphometrics and developmental times of the adults emerging from any of the species tested so far, suggesting that *A. sedi* has a wider host range than initially anticipated. Further host-range trials will be undertaken to determine if this weevil can complete its development on indigenous *Crassula* and *Kalanchoe* spp. and on species native to southern Africa in other closely related families. Future work will also explore the impact of the weevil on plant vigour in the laboratory and field, as well as its distribution and natural host range.

Compatible interactions between the pathogen, weed and environment make the bridal creeper rust a successful biological control agent

Louise Morin,[1] Joel Armstrong,[1] Wolfgang Wanjura,[2] Felice Driver[2,3] and Darren Kriticos[1]

[1] Cooperative Research Centre for Australian Weed Management, CSIRO Entomology, GPO Box 1700, Canberra ACT 2601, Australia

[2] CSIRO Entomology, GPO Box 1700, Canberra ACT 2601, Australia

[3] Present address: C-Qentec Diagnostics Pty Ltd, 51 Rawson Street, Epping NSW 2121, Australia

Bridal creeper, *Asparagus asparagoides*, is listed as a Weed of National Significance in Australia. It invades native vegetation through bird dispersal of seeds and establishes an underground mat of rhizomes and tubers that support its persistence in seasonally dry environments. Following extensive pathogenicity screening and host-specificity testing, an isolate of the rust fungus *Puccinia myrsiphylli*, originating from South Africa, was released in Australia in 2000. The rust established readily at release sites across the country and has already demonstrated its ability to cause destructive localized epidemics. The released isolate of *P. myrsiphylli* was chosen because of its pathogenicity and aggressiveness towards representative Australian accessions of bridal creeper. In contrast, some of the other isolates tested did not develop profusely on plants. DNA sequence data confirmed that Australian bridal creeper populations originate from the winter rainfall region of the Western Cape Province of South Africa, where the selected rust isolate was collected. *Puccinia myrsiphylli* can infect leaves and

stems of bridal creeper at any growth stage. In Australia, the optimum conditions for rust infection (at least 8 hours of leaf wetness; 16–20°C) are common during the cool months in winter-dominant rainfall areas where bridal creeper occurs. The rust produces a large number of dormant teliospores throughout the growing season of bridal creeper which allow its survival during the dry summer months, when the host foliage is dead, and the initiation of a new disease cycle the following season. The abundance of bridal creeper growing in dense patches in Australia favours the rate and extent of disease epidemics. However, the rust has been relatively slow at dispersing between bridal creeper patches. An extensive redistribution program has consequently been established to manually disseminate the rust to most bridal creeper infestations across southern Australia.

Biological control of weeds program, Parana, Brazil; problems and progress in current research on Brazilian weeds in Parana State

J.H. Pedrosa-Macedo

Centro de Ciencias Florestais, DECIF–SCA, Universidade Federal do Parana, Av. Lothario Meissner, 3400, 80.210-170 Curitiba, Paraná, Brazil, Laboratorio Neotropical de Controle Biologico de Plantas

Investigations on the arthropod natural enemies attacking various native Brazilian species of *Senecio* and *Tecoma stans* are being conducted. Insects are being studied to determine their potential for biological control of *Senecio*, *Tecoma stans* and other toxic plants in pasture situations. Cattle deaths have been attributed to various toxic plants in these genera. For example, in the state of Rio Grande do Sul, the loss of cattle due to consumption of *Senecio* spp. is estimated to cost US$7.5 million annually. In 2002, faunal surveys of toxic plants in the genus *Senecio* and on *Tecoma stans* were initiated. The remaining projects are all cooperative research projects with foreign universities studying the natural enemies of Brazilian plants species that have become pests elsewhere in the world, including Brazilian pepper (BP) – *Schinus terebinthifolius*. The impact caused by *Pseudophilothrips ichini* on BP has been quantified, but the tests must be repeated. Other BP natural enemies are being studied. Five potential biological control agents have been selected for strawberry guava and preliminary studies on their biology and host range are being done. Four agents against BP have been identified, and currently two of them are under study for their biology, host range and impact on the plant. The toxicity of the BP sawfly has been tested in a preliminary test with cattle. Exploratory studies on *Tibouchina herbacea* natural enemies have been continued with special efforts on *Anthonomus partiarius* biology and host-range tests. The identification of the main candidate agent for *Solanum mauritianum* has been confirmed and nine Solanaceae species are being used in field tests.

The use of molecular taxonomy in the exploration for a cold-hardy strain of the tansy ragwort flea beetle *Longitarsus jacobaeae* (Coleoptera: Chrysomelidae)

Kenneth P. Puliafico,[1] Jeffrey L. Littlefield,[1] George P. Markin[2] and Urs Schaffner[3]

[1] Entomology Department, 333 Leon Johnson Hall, Montana State University, Bozeman, MT 59717, USA

[2] USDA Forest Service, Forestry Science Laboratory, Bozeman, MT 59717, USA

[3] CABI Bioscience, Delémont, Switzerland CH-2800

An extensive infestation of tansy ragwort, *Senecio jacobaea* L. (Asteraceae), in north-western Montana has renewed the search for a cold-hardy strain of the tansy ragwort flea beetle *Longitarsus jacobaeae* (Waterhouse) (Coleoptera: Chrysomelidae). Early reports suggested that Swiss flea-beetle populations

were pre-adapted to colder winters than those collected from the Mediterranean regions of Europe. Our comparison of the two strains' phenotypic characteristics in the field also indicated that the Swiss populations are better suited for the biological control of *S. jacobaea* in continental climates. Using mitochondrial DNA sequencing techniques, we identified five Swiss populations as con-specifics of the Italian strain of *L. jacobaeae* collected from three populations in Oregon. Species identification utilized diversity in the cytochrome oxidase I and II and tRNA leucine genes. Variability in the cytochrome oxidase subunits was particularly informative for investigations of variability within and among *L. jacobaeae* populations. The *L. jacobaeae* populations were genetically distinct from the cryptic sister species *L. flavicornis* (Stephens), with 16 to 25 nucleotide substitutions between species. Parsimony analysis using two distantly related *Longitarsus* species helped elucidate the differences between *L. jacobaeae* and *L. flavicornis*. In rooted phylogenetic trees, the distant out-groups clearly illustrated the recent genetic divergence of the sister species that was predicted by their morphological and behavioural similarities. The use of mtDNA sequencing provided an accurate and quick method for the verification of our *Longitarsus* species, especially in cases where traditional identifications based on morphological characters may be uncertain. With the positive verification of Swiss flea-beetle populations as *L. jacobaeae*, releases were made in autumn 2002 for tansy ragwort control in Montana.

Will further exploration find effective biological control agents for *Hydrilla verticillata*?

M.F. Purcell and J.A. Goolsby

USDA–ARS Australian Biological Control Laboratory (ABCL), c/- CSIRO Entomology, Meiers Road, Indooroopilly, Queensland 4067, Australia

The submersed aquatic plant *Hydrilla verticillata* (Hydrocharitaceae) is native to Australia, Asia and central Africa, and was introduced into the United States in the early 1950s. It has now greatly expanded its range from Florida to Delaware on the east coast and westward to Texas and California. Hydrilla forms dense mats at the water surface, impeding water flow. It causes extensive environmental, economic and recreational problems. Herbicidal and mechanical controls have been ineffective and very expensive. Biological control is considered to be the long-term solution. Following worldwide surveys for biological-control agents, many phytophagous insects were found, though few were selected as agents due to their low specificity, availability or impact. The four insects released in the US, two leaf-mining *Hydrellia* flies (Ephydridae) and two *Bagous* weevils (Curculionidae), are yet to provide adequate control, and new agents will be needed if biological control is to be successful. In Florida, hydrilla has now invaded over 40% of water bodies, and recently it has become a serious problem in the Rio Grande Valley, Texas. For this reason there has been a renewed interest in finding new agents. Previous surveys for agents in Southeast Asia were limited. Within that region, the plant is rarely problematic, with excessive growth occurring only in disturbed or artificial water bodies. It usually grows as part of a balanced aquatic ecosystem, often improving water quality. Natural enemies appear to keep hydrilla under control, and new surveys are being undertaken in this region as well as unsurveyed areas of Australia. After initially assessing the impact and biology of insects already released in the US, research is focusing on determining the efficacy of specific agents that have fully aquatic lifecycles. Genetic characterization techniques, which were not available when the original surveys were conducted, are also being employed to identify new herbivores.

Impact of two invasive plants, purple loosestrife (*Lythrum salicaria*) and reed canary grass (*Phalaris arundinacea*), on wetland plant and moth communities in the Pacific north-west, USA

S.S. Schooler,[1] P.B. McEvoy[1] and E.M. Coombs[2]

[1] Oregon State University, Department of Entomology, Corvallis, OR, USA
[2] Oregon Department of Agriculture, Plant Division, Salem, OR, USA

Introduced plant species may affect local plant and animal community diversity and species richness. We studied the association between varying densities of two introduced wetland plants, purple loosestrife (*Lythrum salicaria*) and reed canary grass (*Phalaris arundinacea*), on plant and moth species richness at sites within 24 palustrine emergent wetlands in Oregon, Washington, and Idaho. Seven wetlands were dominated by canary grass, seven were dominated by loosestrife, and ten were reference wetlands dominated by neither canary grass nor loosestrife. We measured plant community composition as percent cover and sampled the moth community using blacklight traps. One hundred and sixty-nine plant and 178 moth species were identified. As the mean percent cover of canary grass and purple loosestrife increased from 0 to 91%, plant species richness declined from 45 to 4. We found a strong positive correlation between moth and plant species richness in rural wetlands. However, urban wetlands did not show this relationship. A strong negative association between reed canary grass and purple loosestrife abundance and plant and moth species diversity suggests that these two invasive species reduce local biodiversity. In addition, our data suggest that the influence of urban landscapes reduces moth species richness and abundance.

The use of trap gardens in biological control: the case of blackberry, *Rubus fruticosus* and its agent, the rust *Phragmidium violaceum*

John K. Scott,[1] Mireille Jourdan[2] and Katherine J. Evans[3]

[1] Cooperative Research Centre for Australian Weed Management and CSIRO Entomology, Private Bag 5, PO Wembley, Western Australia 6913, Australia
[2] CSIRO European Laboratory, Campus International de Baillarguet, 34980 Montferrier-sur-Lez, France
[3] Cooperative Research Centre for Australian Weed Management, and Tasmanian Institute of Agricultural Research, New Town Research Laboratories, 13 St Johns Avenue, New Town, Tasmania 7008, Australia

Trap gardens, where a garden of the target weed is planted in a region of origin and monitored for potential biological control agents, have been used extensively for the selection of agents. We review the approach used in past projects and examine the practicalities of this method using, for example, the garden of blackberry clones (*Rubus fruticosus* L. agg.) that was established to help selection of effective strains of the biological control agent, the rust *Phragmidium violaceum* (Schultz) Winter. Nineteen blackberry clones from Australia with known genotypes were imported into the CSIRO European Laboratory in France and established in a garden of four replicated blocks. Rust disease soon appeared on all plants, but not all weed clones showed the same susceptibility in the timing and degree of infection. Strains of the rust fungus were cultured from single pustules and these are undergoing genetic analyses to determine whether or not they are different from the rust fungus found in Australia. The host range of the purified strains is also being analysed. The garden was managed so as to prevent any gene flow to other European blackberries growing wild nearby. Eventually, the aggressive nature of some of the planted weed clones has required that we start a progressive destruction of the garden. The elimination of the trap garden started in 2002 and it will be important to monitor the garden site so as to confirm that no plants remain. We have shown that a trap garden of identified clones on blackberry

is feasible, safe and enables the rapid isolation of potential biological control agents for a plant where both the identity and exact origins are uncertain. Further gardens, perhaps with fewer clones, should be planted in other regions of interest, such as regions of high diversity of *Rubus* species, for example southern England, or in regions of climatic similarity to southern Australia, for example southern Portugal.

Progress with the biological control program for Japanese knotweed

Richard H. Shaw and Djamila H. Djeddour

CABI Bioscience, UK Centre (Ascot) Silwood Park, Ascot, Berks, UK, SL5 7TA, UK

Japanese knotweed is a rhizomatic perennial weed introduced into the UK, mainland Europe and the USA as a desirable ornamental plant during the 19th century. It soon lost its charm and was recognized as the potential weed that it has since become. It is now considered the most pernicious weed in the UK with an awesome reputation for displacing native vegetation and even concrete during its exponential spread. Previous studies suggested that *Fallopia japonica* is a very good potential target for biological control given its lack of natural enemies and apparent clonal nature. Phase 1 of a biocontrol program for the UK and USA was initiated in 2000. It involved a literature review and set-up mission to Japan. This visit/survey revealed a plant under severe natural enemy pressure with representatives from the more promising groups of arthropod and fungal potential agents, including a ubiquitous and damaging rust species. The promising results from this phase, along with observations in quarantine on some of the natural enemies encountered, are presented. This project has a good chance of being the first successful biological control program against a weed in Europe, as long as the political obstacles can be successfully negotiated.

Biological control of privet in La Réunion: the story so far

Richard H. Shaw and Harry C. Evans

CABI Bioscience, UK Centre (Ascot) Silwood Park, Ascot, Berks, UK, SL5 7TA, UK.

Privet, *Ligustrum robustum* ssp. *walkeri,* is a major invasive weed in the Mascarenes, threatening what is left of the native forest. Its impact on the now depauperate island of Mauritius led to the initiation of a classical biocontrol program for the neighbouring French island of La Réunion where the plant had recently arrived and was spreading rapidly. Molecular techniques revealed that the area of origin was Sri Lanka, but the apparent lack of suitable co-evolved agents, and fungi in particular, led the team back along the path of speciation eventually arriving at the centre of diversity of the genus in China. Although a different suite of natural enemies was found in each region surveyed, the most promising fungal agents belong to the little-studied and notoriously challenging Dothideales. One member of this order, *Thedgonia ligustrina*, does attack the target and remains of interest. Fortunately, this was one of the first classical programs to combine from the outset both entomology and pathology and, although the two survey disciplines can seem incompatible at times, it does result in a more efficient and cost-effective approach. Numerous arthropods were rejected on the grounds of specificity, but a moth from Sri Lanka, *Epiplema albida,* proved to be suitably specific for consideration as a biocontrol agent. Cut foliage starvation tests were carried out on 89 plant species, followed by live plant tests on indigenous non-target Oleaceae. They revealed a high level of physiological specificity. Further lab testing confirmed that this insect is capable of completing development on only one species of non-target plant found in La Réunion and is highly unlikely to lay eggs on any species other than the target in the field. The decision whether to release the moth has yet to be made.

Biological control of invasive alien weeds in the UK: new initiatives

Richard H. Shaw and Robert H. Reeder

CABI Bioscience, UK Centre (Ascot) Silwood Park, Ascot, Berks, UK, SL5 7TA, UK

European Union member states have been the source countries for over 380 biocontrol releases against weeds around the world, yet have never benefited from such a program. Despite the considerable inertia hindering its use in the UK, this alternative approach is gaining credence as governments come to terms with their commitments to the Convention on Biological Diversity. It is likely that the UK Government's recent review of its non-native species policy will open the door to the expansion of biocontrol through commitment to funding as well as improved legislation and education. Initiatives against Japanese knotweed (*Fallopia japonica*), giant hogweed (*Heracleum mantegazzianum*), bracken (*Pteridium aquilinum*), rhododendron (*Rhododendron ponticum*), buddleia (*Buddleja davidii*) and water fern (*Azolla filliculoides*) demonstrate the flexibility of biocontrol, as well as its many and varied challenges. The conclusion is drawn that the popularity of this tried and tested method of weed control will increase in Europe, but that its novelty will remain a hindrance until success overcomes prejudice.

Bionomy, seasonal incidence and influence of parasitoids of the field bindweed stem borer fly *Melanagromyza albocilia* (Diptera:Agromyzidae) in Slovakia

Peter Toth,[1] Massimo Cristofaro[2] and Ludovit Cagao[1]

[1] Slovak Agricultural University, Department of Plant Protection, A. Hlinku 2, 949 76 Nitra, Slovak Republic

[2] ENEA C.R. Casaccia, INN BIOAG–ECO, via Anguillarese 301, 0060, Rome, Italy

Studies on population density, seasonal incidence, impact on host plant and percentage parasitism of *Melanagromyza albocilia* were carried out in maize and sunflower fields infested by field bindweed (*Convolvulus arvensis*) in south-western Slovakia during 1998–2001. *Melanagromyza albocilia* infests field bindweed in Slovakia from May to October and completes two generations per year. The larvae of *M. albocilia* mine field bindweed shoots, causing them to dry up. The infestation, initially low during the first generation, reaches its peak from August till the end of the season (second generation). In natural conditions, the infestation rate of attacked plants ranges from 30 to 100%. The host range of *M. albocilia* is restricted to the target weed *C. arvensis*. Although feeding punctures (caused by adults) were observed on species in the closely related genera *Calystegia* and *Ipomoea* in no-choice laboratory tests, no larval feeding was recorded. A complex of seven hymenopterous parasitoids was shown to have a high impact on the populations of the stem borer fly. *Chorebus cyparissa* and *Bracon picticornis* (Braconidae) and the chalcid *Sphegigaster truncata* (Pteromalidae) were the most numerous, causing together up to 96.3% parasitism. *Sphegigaster aculeata*, *Cyrtogaster vulgaris* (Pteromalidae), *Macroneura (Eupelmus) vesicularis* (Eupelmidae) and *Aneuropria foersteri* (Diapriidae) were less abundant (about 3.7%). Parasitoids reduced the agromyzid population by about 80.0% in field conditions. Despite parasitization, *M. albocilia* was shownto be suitable for biological control because of its specificity and its high level of effectiveness.

Varietal resistance in lantana: fact or fiction?

A.J. Urban,[1] D.O. Simelane,[1] P.F. Mpedi,[1] M.D. Day,[2] S. Neser[1] and C. Craemer[1]

[1] ARC–Plant Protection Research Institute, Private Bag X134, Pretoria 0001, South Africa

[2] NRM–Alan Fletcher Research Station, PO Box 36, Sherwood, Queensland 4075, Australia

It is firmly established that lantana (*Lantana camara*) comprises a large complex of polyploid hybrids of widely diverse genetic composition. Variable performance of biocontrol agents at different sites is often ascribed to different levels of insect-resistance in the lantana varieties, but usually without experimental evidence. Here we report evidence of the high degree to which varietal resistance within lantana affects the performance of two biocontrol agents. In the first study, standard numbers of sexed, newly emerged, adult lantana mirids, *Falconia intermedia*, were isolated on three replicates of six Australian varieties of lantana. Reproductive performance, measured as the mean number of adult progeny per parent per unit time, varied sigificantly, from 1.4 to 20.6 i.e. by 15-fold. In the second study, two replicates of ten South African and six Australian lantana varieties were exposed to the Florida, USA, biotype of the lantana flower gall mite, *Aceria lantanae.* Suppression of lantana reproduction varied significantly between varieties, from 10 to 95% in the South African varieties, and 0 to 30% in the Australian varieties. To make the impact of biocontrol on the lantana complex more uniformly intense, it is therefore necessary to select candidate agents of many species and biotypes, from several Camara Group *Lantana* species, varieties and hybrids.

Biocontrol initiative against cat's claw creeper, *Macfadyena unguis-cati* (Bignoniaceae), in South Africa

Hester Williams and Terry Olckers

ARC–Plant Protection Research Institute, Private Bag X134, Pretoria 0001, South Africa

A biocontrol program was initiated against cat's claw creeper in 1996, and the first biocontrol agent, the golden-spotted tortoise-beetle *Charidotis auroguttata*, was released in 1999. Several releases were made at sites in different climatic regions of South Africa, including the warmer subtropical parts of Mpumalanga and Limpopo, the colder inland areas of KwaZulu-Natal, and the frosty highveld areas of Gauteng and the North West Province. Establishment was confirmed in some of these regions. It was clear from the start that the impact of the tortoise beetle alone would not be severe enough to curb the aggressiveness and spread of cat's claw creeper. Therefore, several more insect species were collected on cat's claw creeper during a survey in Argentina and Brazil in 2002. Among these are a leaf-tying moth, a leaf-mining buprestid and a leaf-sucking tingid, all causing severe damage to cat's claw creeper plants under glasshouse conditions. Funding is being requested to enable biological and host-specificity studies to commence during 2003.

Theme 3:

Risk Analysis

Oviposition preference: its definition, measurement and correlates, and its use in assessing risk of host shifts

Michael C. Singer[1]

Summary

To predict evolution in plant–insect systems we can begin by defining potentially heritable traits of plants that describe how they interact with insects and potentially heritable traits of insects that describe how they interact with plants. Examples are "acceptability" as a plant trait and "preference" as an insect trait. Some practical applications of this approach and of dissecting preference into its components are discussed. The question: Given one population of insects and 2 categories of plant, which category of plant do the insects prefer, and is this preference adaptive? seems like a simple question, but testing it can be confounded by two problems. First, plants vary both within and among species, and we don't know how to classify them from the insects' perspective. Second, insects vary along axes of preference that we hadn't imagined. An example is given from butterflies in which variation among insects in how they rank plant individuals (within species) can masquerade as variation in which species they prefer. An apparent solution to this problem would be to offer each insect a different, randomly chosen pair of plants in the two plant categories being compared. But insects don't interact with plants at random in nature, and we show that forcing them to do so in an experiment generates misleading results.

From a practical perspective I argue that risk assessment would benefit from incorporation of the concept of "motivation" alongside "preference" and that candidate species should be tested at maximum levels of motivation. I also describe how taking advantage of detailed behavioural traits of a study insect allows the development of a preference-testing technique. The technique itself may or may not transfer to other systems; what should transfer is the approach to exploiting natural traits of the insect, whatever they may be. This approach also includes a rationale for identifying and testing the assumptions underlying the design of a preference test.

Introduction

Critiques of biocontrol procedures have been based on observed use of non-target plants as hosts by introduced insect control agents (Louda *et al.* 1997). Such critiques underline the need to estimate as precisely as possible the risks that such events will occur (Zwölfer & Harris 1971, McEvoy 1996, Simberloff & Stiling 1996, McFadyen 1998, Withers 1999, van Klinken & Edwards 2002). These efforts could involve estimating the likelihoods of acceptance of particular specified non-targets, and/or the general propensity of a candidate agent to expand its host range in a general sense. An important aspect of risk assessment is to understand the relationship between the results of preference tests performed on captive insects and the likelihood that these insects would attack low-ranked (less-preferred) hosts in nature. Here, I discuss the definition and testing of preference and summarize prior work on the conceptual and practical separation between insect preference and plant acceptability. I also consider the ways in which the internal state of the insect may affect its motivation, or readiness to feed or oviposit, and the manner in which the concept of "motivation" might be useful in risk assessment. Finally, I suggest that we do not yet know whether a monophagous population of an oligophagous species poses a greater risk as a candidate agent than an equally monophagous population of an entirely monophagous species.

For many herbivorous insects, especially flying insects, oviposition preference is the principal mechanism by which the insect–host relationship is established. It is this trait of the insect that interacts with spatial distributions, abundances and acceptabilities of plants to generate patterns of insect–host association across the landscape. The arguments presented here,

[1] Integrative Biology, University of Texas, Austin, TX 78712, USA. sing@mail.utexas.edu

developed specifically from studies of Melitaeine butterflies (genera *Melitaea* and *Euphydryas*), provide a worked example of how oviposition preference might be defined, measured and related to events in the field. Readers are left to deduce the extent to which these conceptual approaches and techniques may be useful for understanding the insects with which they work.

Definitions of terms

I use the following definitions, modified slightly from those suggested earlier (Singer 1982, 1986, 1994, 2000, Singer *et al.* 1992b):

- An "encounter" occurs between plant and insect when the insect arrives at a distance from which it could perceive stimuli emanating from the plant. For example, an insect may encounter a plant visually, perceive it, approach or alight upon it and then encounter it chemically and physically.
- "Acceptance" is a positive response made by an insect to a plant that has been encountered. For example, a flying insect may accept visual stimuli by turning towards a plant and alighting on it. The insect may then accept (or reject) contact chemical stimuli by feeding or ovipositing (or not).

Insect traits:

- "Motivation" is a general tendency to feed or oviposit, without reference to any particular host. A motivated insect is sensitive and responsive to stimuli that may lead it to feed or oviposit (Singer *et al.* 1992b).
- "Perceptual ability" is the set of likelihoods of perceiving a particular specified set of plants that are encountered.
- "Preference" is the set of likelihoods of accepting a particular specified set of resources that are perceived (Singer 1986, 2000). In practice, it would normally be measured as the set of likelihoods of accepting resources that are encountered. One aspect of preference is "host range", the set of plants that would be accepted under specified conditions. In a conservative assessment of a candidate agent we should be principally interested in the host range at maximum motivation.
- "Specificity" has been defined (Singer 1982, 1986) and used by some biologists (e.g. Courtney *et al.* 1989, Thompson 1998) to mean the strength of preference, regardless of its direction. However, this usage has not become well-established and there are several current uses of "specificity". It is sometimes synonymous simply with "host affiliation" or with "insect diet" and sometimes has a wider meaning incorporating both preference and performance (van Klinken & Edwards 2002).

Plant traits:

- "Apparency" is the set of likelihoods that a plant will be perceived by a specified set of insects (Feeny 1976, Singer 1986).
- "Acceptability" is the set of likelihoods that a plant will be accepted after being encountered by a specified insect or set of insects (Singer 1986, 2000).

The definition given here renders "preference" a useful trait in thinking about the potential for evolutionary change because it is a trait of the insect whose variation can be measured among individuals and populations. In contrast, "preference" is often defined by ecologists as the proportion of a particular resource in the diet as a function of the availability of that resource in the habitat (Hassell & Southwood 1978, Crawley 1984). This ecologically important parameter is an emergent trait of the plant–insect interaction rather than a trait of insect or plant (Singer & Parmesan 1993, Singer 2000). A partial solution to the difficulty posed by the diversity of meanings of "preference" is to use the term *"electivity"* (Ivlev 1961, Singer 2000) for the ecological parameter and "preference" for the behavioural parameter.

With the definition of "preference" used here, experiments in which an insect is offered a single plant (no-choice tests) should not strictly be called tests of "preference," although a series of such tests might be so called. An insect cannot have a "preference" for a single resource. We might better describe it as having an *"affinity"* for such a resource.

Sequence of events in host search by Melitaeine butterflies: responses to visual, chemical and physical stimuli

I'll begin with a description of the oviposition behaviour of our study insects, from which our conceptual approaches have been derived. While the results of this study may not be directly applicable to different types of insect, the approach and manner of analysis could be more widely relevant. In *Euphydryas editha* at Rabbit Meadow, Sequoia National Forest, California, alighting was primarily or entirely in response to visual stimuli, as evidenced by strong relationships between fixed (non-learned) alighting bias and plant visual traits (Parmesan *et al.* 1995). After tasting a plant and finding it chemically acceptable, the Melitaeine curls its abdomen under a leaf, extrudes its ovipositor, and probes the lower surface of the leaf. This probing is clearly a response to chemistry because it can be stimulated by placing the insect on a dampened filter paper on which an ethanol wash of host leaf surface has been evaporated. There is apparently no chemical sense on the ovipositor: eggs are readily laid on non-host or even non-plant material, all that is necessary is that the tarsi of the insect contact the host; the ovipositor does not need to do so. Once the plant has been chemically

accepted, oviposition depends principally on physical features of the site such as the size, shape and orientation of the leaf and the extent to which it yields when pressed by the ovipositor.

Pre-alighting butterfly preference and host apparency

Apparency was first visualized by Feeny (1976) as a property of a plant that influenced its susceptibility to being found by herbivores. Singer (1986) defined it as the set of likelihoods that a plant will be perceived by some specified insect or set of insects. The property of the insect that interacts with plant apparency is the insect's perceptual ability, defined here as the set of likelihoods of perceiving a particular specified set of plants that are encountered. The concept of apparency has fallen into disuse. This may be because apparency is, in practice, hard to measure and/or to separate from perceptual ability. We usually cannot tell whether an insect that passes over a plant without stopping fails to perceive the plant or perceives it and decides against alighting on it. Whether or not the plant is perceived depends on an interaction between insect perceptual ability and plant apparency. Whether or not the insect alights on a plant that it has perceived depends on an interaction between the insects' pre-alighting host preference and plant acceptability.

With present knowledge and techniques these factors may be difficult or impossible to tease apart in practice. However, defining them in principle is, I think, useful to help generate the incentive to understand the mechanisms at work. In one case, our group has made progress in identifying pre-alighting preference rather than the apparency/perceptual ability relationship as a cause of observed patterns of alighting. *E. editha* at Rabbit Meadow had added to their diet a novel host, *Collinsia torreyi,* just a few (<20) generations prior to our study. The butterflies found this novel host very inefficiently: in the habitat patches where it was the principal host, the proportion of alights upon it was lower that the proportion the butterflies would have achieved by alighting on vegetation at random (Mackay 1985, Parmesan *et al.* 1995). This inefficiency of finding *Collinsia* could have resulted from two causes:

1. evolutionary lag in the insects' pre-alighting preference, such that *Collinsia* was perceived but not preferred for alighting (even though many insects accepted it readily on contact).
2. failure of the insects to perceive *Collinsia* by virtue of the interaction between its apparency and the insects' perceptual abilities.

The first explanation was suggested by Mackay (1985). At that time *Collinsia* had been used by the Rabbit Meadow butterflies for less than 20 generations. Parmesan (1991), by comparing populations that had undergone host-shifts in different directions, showed that *Collinsia* was found efficiently when it was the traditional, rather than the novel host. She therefore attributed the inefficiency of finding *Collinsia* at Rabbit Meadow to an evolutionary lag in the response to natural selection on pre-alighting preference, rather than to an evolutionary constraint associated with the failure of the insects to perceive *Collinsia* at all.

Post-alighting oviposition preference and host acceptability

In the same manner that perceptual ability can be viewed as an insect property that interacts with plant apparency, preference can be viewed as an insect property that interacts with plant acceptability (Singer 1986, 2000). The simultaneous variation of both preference and acceptability creates a series of difficulties for experimental design and interpretation (Singer 2000, Singer & Lee 2000, Singer *et al.* 2002). Despite these difficulties, it has been possible in one case to illustrate how variation of both preference and acceptability made independent contributions to patterns of insect–plant association in the field (Singer & Parmesan 1993). Two populations of *Euphydryas editha* chose different host species, partly because of a genetic difference between the sites in acceptability of one of the two host species and partly because of a genetic difference in insect oviposition preference.

Preference-testing technique: development of the sequential choice test for Melitaeines

The most common form of preference-testing used with butterflies is to place the insect in a cage with several test plants and allow oviposition to occur for a day. At the end of the day the eggs on each plant are counted. The positions of the plants are then rotated to control for "position effects", and the experiment is repeated on the following day (Thompson 1993, Bossart & Scriber 1995, Wehling & Thompson 1997). This technique doesn't work well with Melitaeines, for several reasons. First, the insects don't duplicate natural flight behaviour in small cages. During the time-period when a caged butterfly would naturally be searching for hosts if it were at liberty, it is likely instead to sit on the walls of its cage. Therefore, by the time the butterfly does move sufficiently to encounter plants, it is highly motivated and likely to accept the first host that it finds (see below). Second, these insects each lay few, large egg clusters. This experimental design therefore produces few data from each individual butterfly when the data are numbers of egg clusters. There is insufficient statistical power to compare preferences of individuals. In response to this difficulty, our group has developed a testing technique for post-alighting preference that generates more data from each individual than the number of egg clusters that it lays. This technique is the sequential choice test (Singer 1982, 1986, Singer *et al.*

1992b), a technique that overcomes the problem of low egg cluster number by staging a series of encounters between a butterfly and test plants and using as data the results of each encounter, while preventing the insects from actually ovipositing. An insect that is not allowed to oviposit will continue to show acceptances and rejections of plants that it encounters, thereby providing more information than could have been obtained from a single oviposition.

This test takes advantage of the manipulability of Melitaeines. A female placed gently on a host, either in the field or in the greenhouse, appears to behave as though she had naturally alighted on that host. But does she? Indeed she does! Rausher *et al.* (1981) found that manipulated butterflies duplicated the choices they had made before they had been captured.

A second test of the relevance of manipulated trials to actual host use was made by testing the preferences of insects captured naturally ovipositing on different species at the same site (Singer 1983, Singer *et al.* 1993). There was a strong association between the tested preferences and the observed ovipositions. Again, this shows that a test using manipulated butterflies measures *something* that is connected with the observed variation of host use.

How to perform a sequential choice test

Manipulated tests are clearly pertinent to events in the field. What are the actual procedures involved in the testing? Each insect is offered a series of staged encounters at, say, 15-minute intervals. Each encounter lasts a maximum of three minutes. Acceptance is judged from pressing of the extruded ovipositor against the plant for a count of three. Rejection is the absence of this behaviour during the entire three-minute period. An insect that accepts is not allowed to oviposit, but is manually removed from the plant before the first egg has been laid.

The test is based on the observation that, as time passes, the probability that a particular plant would be accepted, if it were encountered, jumps from 0 to almost 1 very rapidly, in the space of just a few minutes (Singer 1982). That probability then remains close to 1, at least during the principal hours when oviposition is likely (noon to 4pm) until oviposition occurs. Suppose that an insect is offered the same plant over and over and over and over and over again, in repeated staged encounters, and is prevented from actual oviposition as described above. There is a rejection phase when the plant is consistently rejected, followed by an acceptance phase when the plant is accepted about 95% of the time (Singer 1982). Now suppose that the same insect is offered staged encounters with two plants, X and Y, in alternation. If we indicate a rejection by R and an acceptance by **A**, we may observe one of three types of sequence, shown below. Each acceptance or rejection in these sequences is the result of an *entire* three-minute trial, with no account taken of the time to acceptance *within* any such trial:

1. RX; RY; RX; RY; RX; **AY**; RX; **AY**; RX; **AY**; **AX; AY; AX; AY; AX**
2. RX; RY; **AX**; RY; **AX**; RY; **AX**; RY; **AX**; RY; **AX**; RY; **AX**; RY; **AX; AY**
3. RX; RY; RX; RY; RX; RY; RX; RY; **AX; AY; AX; AY; AX; AY; AX; AY**

This procedure would constitute a sequential choice test. In case (1) we would say that Y is preferred because X is rejected in encounters that follow acceptance of Y. In case (2) X is preferred, and in case (3) no preference is detected.

To the extent that the behaviour of manipulated butterflies really represents what they would do if they were at liberty, then the result shown in (1) estimates the length of time that a butterfly would search in the motivational state where encounter with Y but not X would result in oviposition, before reaching the motivation at which either X or Y would be accepted, whichever were the next plant to be encountered. This length of time is called the "discrimination phase" (Figure 1). It is a measure of the strength of preference for Y over X. The discrimination phase in (1) is shorter than that in (2), so the preference for Y over X shown in (1) is weaker than the preference for X over Y in (2).

Because the insect cannot be offered continuous exposure to both plants, the length of the discrimination phase cannot be measured precisely. Its minimum length is the time difference between the first acceptance of the preferred host and the last rejection of the second-ranked host. In practice, this minimum length is the value that has been used, partly because the maximum cannot be estimated for insects that never accept the second-ranked host. Use of the minimum value gives us the freedom, if we so choose, to utilize data from butterflies that escape or die under interrogation, before they have accepted all the hosts in the test series.

It is impossible to estimate the length of a discrimination phase unless one begins the test sequence before *any* of the test plants are acceptable. If the first staged encounter with just one of the test plants results in acceptance, then the discrimination phase has already begun. If this happens, it may be possible to obtain a rank order of preference, but estimation of discrimination phase length requires allowing the insect to oviposit and recommencing the test. The test, once begun, should ideally not be interrupted. An insect that rejects both test plants before such an interruption may switch directly to accepting them both when testing recommences. In such a case the opportunity to discover which plant would have been accepted first has been lost. The insect should be allowed to oviposit and its test re-started.

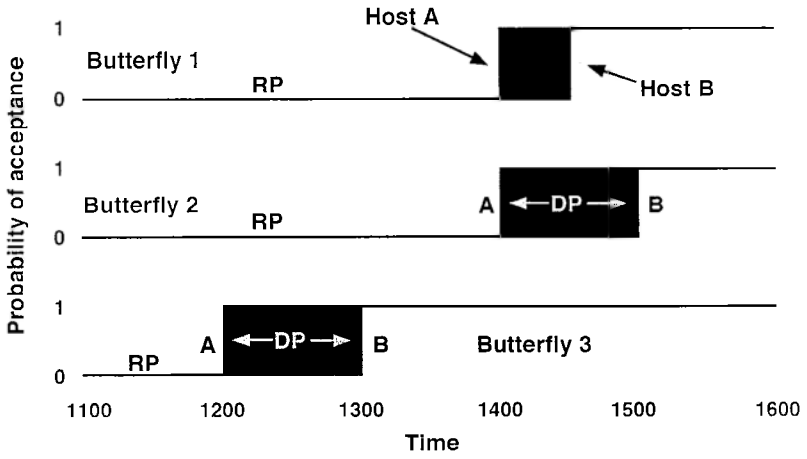

Figure 1. Stylized depiction of changes in responses of insects to two plants, when repeated encounters are staged and oviposition is NOT allowed. Records are shown for three butterflies, each of which prefers plant A over plant B. Discrimination phases are indicated by "DP". (Modified from Singer *et al.* 1992b.)

Expressing the results of the sequential choice test

The original description of the sequential choice test (Singer 1982) suggested that the test allowed preference to be described in two ways. The "rank order" of preference is the order in which the different plants are first accepted, while the strength of preference or "specificity" is estimated from the length of the discrimination phase. This distinction has been adopted by some authors who have found it useful in order to argue that rank order is more highly conserved in evolution than strength of preference (Courtney *et al.* 1989, Thompson 1993). In Melitaeines both aspects of preference can vary simultaneously, giving rise to a bell-curve of preferences (Figure 2). In this figure, specificity is depicted as the distance along the abscissa from the "no preference" point, and "rank order" is opposite on either side of this point. The minimum length of discrimination phase, again on the abscissa, is determined using only time differences during the period (11:30am to 4:30pm) when oviposition is likely. Therefore 5 hours in the figure is equivalent to 1 day, 10 hours to 2 days, etc.

Figure 2 shows that the range of plants that would be accepted, if they were encountered, expanded at different rates in different individual butterflies sampled from the same population. In *E. editha* this type of variation is heritable (Singer *et al* 1988), and responds rapidly to natural selection (Singer *et al.* 1993).

Assumptions of the sequential choice test

Any preference test carries baggage in the form of assumptions of varying testability, and ours, alas, is no exception! The following section identifies some of our assumptions and discusses the extent to which they have been tested.

Assumption 1. There is a precise time at which an insect switches from a "rejection phase", during which a particular plant would be consistently rejected if encountered, to an "acceptance phase", during which that plant would be consistently accepted. The timing of the switch from rejection to acceptance differs in responses to different plant categories. If this assumption were not true, the "discrimination phase" would not be real. How true is it? This can be tested by asking what is the frequency of rejection of plants that have been previously accepted, when no oviposition has intervened. For plants that were moderately or highly acceptable to the insects, the frequency of such rejections was typically 5% or less, but in one case a plant that was first accepted several days after the highest-ranked host was never consistently accepted (Singer 1982). For most hosts, a plant that had been accepted was accepted again with about 95% probability, provided that no oviposition had occurred, that there was no adverse change in the weather and that the end of the day was not at hand.

Assumption 2. When a test covers more than one day, we assume that the motivational state of the butterfly at the beginning of the second day's test is the same as its motivational level at the end of the first day's test. This is not easy to test, and we have not explicitly tested it, but the behaviour of *E. editha* is consistent with the assumption in the following manner. When we commence testing at some time between 11:30 and noon, we usually observe that the range of plants that are accepted resembles the range that had been accepted at 4pm the previous afternoon. It is unusual for additional plants to be accepted at this

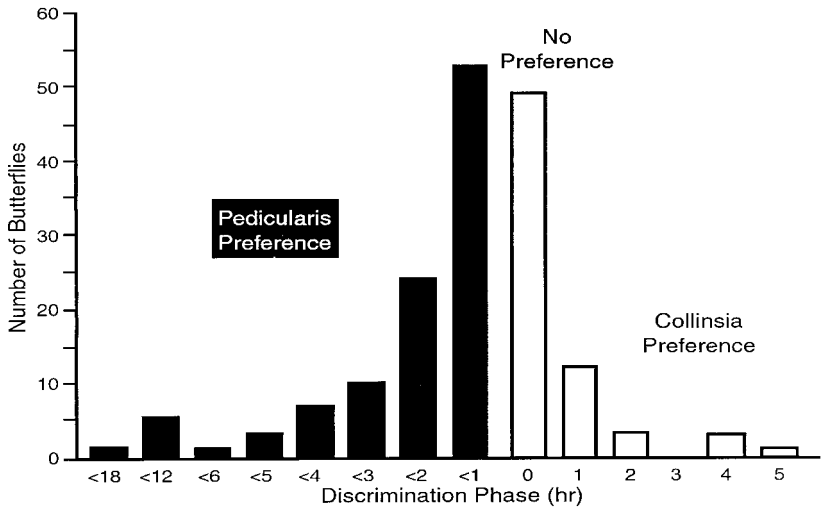

Figure 2. Distribution of measured discrimination phases at Rabbit Meadow in 1981. Lumped data from two adjacent habitat patches, one where *Collinsia* was used and one where *Pedicularis* was used. (Modified from Singer 1983.)

time, or for plants to be rejected that had been accepted the day before. We tentatively conclude that the passage of time between 4:30pm and 11:30am has little effect, and time during this period is not included in the calculation of the discrimination phases.

Assumption 3. We assume that an encounter with plant A at time 1 has no effect on the insect's responses to either plant A or plant B at some subsequent time. This assumption is obviously violated when a butterfly has just accepted a host and is transferred quickly to another one. There is a clear "carry-over" effect making the second host much more likely to be accepted than if the butterfly were made to fly or allowed to rest in a cage with no hosts for a few minutes. Therefore, when we observe an acceptance, we allow the insect at least five minutes' rest before testing another plant.

Apart from this effect, experiments consistently fail to show any effect of manipulated experience on host acceptance. Two such experiments are described below:

a) We collected wild females each morning at Rabbit Meadow and split them into two groups. One group was offered 9–10 repeated encounters with C (*Collinsia*), the other with P (*Pedicularis*). In the afternoon of the same day all butterflies were offered the same test plant species. This test plant was sometimes C and sometimes P, on alternate days. So, each day's experiment asked whether butterflies with different recent experience of host encounter differed in their responses to a single test plant. No such effects were found (Thomas & Singer 1987)

b) We collected teneral females (as mating pairs with no prior host encounter) at Rabbit Meadow and offered some of them alternating encounters with P and C, while others were offered only C or only P. We then offered each insect a single test with either C or P on the afternoon of the second day of its adult life. Again, no effects were detected (Singer 1986).

Assumption 4. Handling the butterflies does not affect their responses. In fact, handling does have a clear effect: it increases the likelihood of oviposition. A butterfly can be "encouraged" to oviposit by being picked up and quickly replaced on the test plant. Perhaps picking the insects up and replacing them makes them respond as though they were encountering plants more frequently, and they may be sensitive to plant density. We don't know. Our method of dealing with this violation of assumption 4 is that, whenever an insect appears to be rejecting a plant, she is picked up and replaced at least three times during each three-minute staged "encounter", before the result of the test is recorded as rejection. By this means we attempt to "encourage" oviposition equally in all of our test subjects.

It is clear from this account that the assumptions of our technique are to some extent violated, and that subjectivity cannot be totally eliminated from these sequential choice trials. We cannot be sure of the exact relationship between the test results and the behaviour of the butterflies in the field. However, several tests have shown that variation among individuals or populations in natural behavior in the field is parallelled by variation in the results of preference tests administered subsequently (Rausher *et al.* 1981, Singer *et al.* 1993).

Distinguishing in practice between "preference" and "motivation"

Starting with Dethier's (1959) paper on "mistakes" made by ovipositing butterflies and continuing to the

present day, there has been continued discussion in the literature about the frequency with which insects oviposit on hosts that are suboptimal, hosts that are not preferred, or even on non-hosts that are toxic (Chew & Robbins 1984, Feldman & Haber 1998). These discussions have often involved questions about the roles played by unusual oviposition events in evolution of diet (Thomas *et al.* 1987). Perhaps such events are preludes to host-shifts? Whether or not this is true depends on the behavioural mechanisms that cause unusual ovipositions and on the likelihood that insects performing such unusual acts do so because of heritable preferences (Karowe 1990, van Klinken 2000).

In this context our ability to test preferences of freshly-captured Melitaeines in the field has enabled us to investigate the behavioural mechanisms that underlie observations of natural oviposition on low-ranked hosts. Why might a Melitaeine be found ovipositing on a plant other than its preferred host? There are two possibilities. First, the insect has been searching for a long time without finding its preferred host. Second, its discrimination phases are short and it does not search for long before it would accept a second or third-ranked host. In the first case, we could describe the butterfly as highly motivated to oviposit. In the second, we could say that its preference is weak or its specificity is low. Why should we bother to make this distinction? The evolutionary consequences are different in the two cases. Differences among individuals in motivation caused by differences in length of search are not likely to be heritable, while differences in length of discrimination phase could be heritable, and indeed, are likely to be so (Singer *et al.* 1988, 1992b). This argument is pertinent to questions about the consequences of a single event in which an introduced agent feeds on a non-target plant.

To clarify the distinction between preference and motivation, I have depicted in Figure 1 (taken from Singer *et al.* 1992b) stylized records for three butterflies, two of which (#2 and # 3) differ in motivation but not in preference and two of which (#1 and #2) differ in strength of preference but not in motivation. The figure indicates that, at 13.50h, butterfly 3 would accept plant A if that plant were encountered, but butterfly 2 would reject it. This would be ascribed to the difference in motivation. At 14.50h, butterfly 2 would reject plant B while butterfly 1 would accept it. This would be ascribed to their difference in strength of preference. We have shown experimentally that variation of motivation and of preference occur simultaneously in the field and that these variables can be teased apart (Singer et al. 1992b).

Correlates of preference

1. Relationship of preference to fecundity

Preference is often thought to be driven by "eggload". An insect that feels increasing "egg pressure" might be increasingly motivated to oviposit. Differences among individuals in fecundity or rate of egg maturation would then generate differences in strength of oviposition preference (Courtney & Hard 1990). However, we (Agnew & Singer 2000) suspected that several of these conclusions had been derived from incorrect attributions of cause to observed correlations in the field. In our own study insects, the individuals that matured eggs fastest were *not* the individuals with the fastest increase in their range of accepted hosts.

2. Relationship of maternal preference to offspring performance

Discussion of relationships between preference and performance typically confounds several different questions. Three of the most important ones are:

1. Is preference correlated with performance among populations? In other words, is preference variation among populations associated with performance variation in the same set of populations?
2. Is preference correlated with performance within populations? In other words, do individual mothers with particular preferences produce offspring with particular performances?
3. Is host choice adaptive at the population level? To what extent is the rank order of plants in the insects' preference hierarchy concordant with the rank order of the same plants in their ability to support larval growth and survival (cf Wiklund 1975, Jaenike 1990, Mayhew 1997)?

All three of these types of correlation occur in *E. editha* (Rausher *et al.* 1981, Ng 1988, Singer *et al.* 1988, 1994). The second type even occurs with respect to variation among individual host plants (Ng 1988).

3. Correlations among preferences

Preference for A versus B may not be independent of preference for C versus D (Courtney *et al.* 1989). Then again, it may be! (Singer *et al.* 1992a).

Novel axes of variation revealed by preference-testing of Melitaeines

Melitaeines make substantial discriminations within as well as among host species. This process generates complexity because discrimination within species is not nested within discrimination among species, as one might reasonably expect. Preference-testing of insects on conspecific and heterospecific plants has revealed novel axes of variation, the existence of which threatens

many standard and apparently sensible experimental designs. Three examples are discussed below.

1. Individual *Melitaea cinxia* butterflies varied in the relative importance they assigned to variation within and among host species (Singer & Lee 2000). Singer & Lee showed how variation in discrimination within plant species might falsely appear as variation in discrimination among species. This could be an important, general and overlooked problem in experimental design.
2. When *Euphydryas aurinia* butterflies and their hosts were sampled randomly, we obtained the odd result that insects from populations feeding on *Gentiana, Lonicera and Cephalaria* all preferred over their own hosts a plant species, *Succisa pratensis*, that they never encountered in the field (Singer *et al.* 2002). This appearance of maladaptation was an artefact of sampling host populations at random. It disappeared when the populations were sampled differently, using naturally-accepted plants (Singer *et al.* 2002). This result casts doubt on experiments that ask whether host choice is adaptive by manipulating insects to feed on randomly-chosen members of different host species. Alas, this category includes many of our own experiments (e.g. Singer *et al.* 1994).
3. *E. editha* at Rabbit Meadow were offered *Pedicularis* plants in sequential choice trials. Newly hatched larvae were then placed on the plants to ask whether plants that were generally preferred supported higher offspring survival. They did not (Ng 1988). However, this apparently simple result, that discrimination is NOT adaptive, disguised an unexpected complexity. Individuals that discriminated among *Pedicularis* plants produced offspring that survived better on plants preferred by discriminating individuals. Offspring of insects that did not discriminate survived equally well on plants accepted or rejected by discriminators (Ng 1988). If we put the question in the form: "are the plants that are most acceptable in a general sense also the most suitable in some general sense?" we get a misleading result!

These three effects create considerable difficulties for the design of experiments that manipulate plants and insects into specific interactions and then examine the consequences of those interactions for either or both partners.

Conclusions

Changes in oviposition preference are intimately involved in observed diet shifts in nature (Singer *et al.* 1993, Singer & Thomas 1996). These natural observations validate the approach of incorporating detailed studies of preference in risk-assessment (e.g. Heard & van Klinken 1998, Barton-Browne & Withers 2002). Here, I have described the approach to defining and measuring preference that our group has developed in working with populations of Melitaeine butterflies. This approach first introduced the role of time since last oviposition (TSLO) as an important cause of changes in host acceptance (Singer 1982, 1986). For reasons detailed earlier in the report, we have chosen to describe these time-dependent changes in observed host range as driven by changes in motivation, and to define "preference" independently of motivation. So, when an insect that is deprived of opportunity to feed undergoes physiological changes that cause it to accept a wider range of hosts, this is a change in motivation. Its preference is measured by the manner in which these changes occur. Learning (Cunningham *et al.* 1999) influences preference and thereby affects the relationship between motivation and acceptance.

While the detail of this study may not be broadly applicable, what are the more general messages of this approach for risk assessment? First, that candidate insects should be tested at the maximum levels of motivation that they are likely to attain in the field. In many cases we don't know what conditions maximize motivation, so this requires study. Time of day, insect age and body temperature may be important. In insects whose motivation varies with TSLO, as do our study insects, maximizing motivation entails prolonged testing with deprivation of opportunity to feed or oviposit, as suggested by Barton-Browne & Withers (2002). No-choice tests are useful in this context since allowing feeding/oviposition on a preferred resource may hold motivation to low levels at which low-ranked resources are not accepted. In any case, insects normally encounter plants sequentially rather than simultaneously. What may appear to the experimenter to be a choice situation may from the insects' perspective be a series of no-choice situations. Even when choice tests in which test plants are juxtaposed resemble natural situations that occur frequently, they don't represent all the natural conditions. Some individual insects are sure to wander away from the hosts on which they developed, encounter habitats that don't contain those hosts, and experiment with hosts that they would not have attacked if they had stayed at home (Thomas *et al.* 1987; Singer *et al.* 1992b). Situations are bound to occur where the control agent is exposed to the non-target plants and not to the target. The important question then becomes, not which plant is preferred, but whether the non-target is acceptable to the insect in a no-choice situation. For this purpose no-choice tests are the tests of choice (cf Hill 1999).

A second question that arises from our work is this: do we have any use for species such as *E. editha,* in which populations may be either oligophagous or monophagous? In other words, is a monophagous population of an oligophagous species useless as a candidate agent, despite its population-level monophagy? Our preference testing shows that a sample taken from a monophagous population may comprise entirely insects that would search for several

days for their preferred host species before accepting a second choice. These are specialized insects that might indeed be suitable candidate agents. But would such a population be more likely to indulge in a host shift because it is sampled from an oligophagous species rather than from a monophagous one? The answer to this question isn't known, but is susceptible to analysis by molecular phylogenetic techniques. Pending such analysis, all we can say is that when these insects have undertaken host shifts in the past they haven't necessarily speciated. We can't say that host shifts have occurred with higher frequency over time than in other groups of insects that have speciated with each host shift. If I continue this line of reasoning further, I'll get into discussion of the definition of "species", which lies far outside the purview of this paper. However, as several recent works (e.g. Hoffman et al. 2002) indicate, we should not worry too much about how to define species, host races and biotypes, but we SHOULD worry that the candidate agents that are introduced belong to EXACTLY the same entity, be it a species, a biotype or a host race, that has been subjected to specificity testing. Insects vary among populations in their host adaptations, and we should never assume that a sample originating from one population will behave in the same way as the same "species" sampled elsewhere.

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Biological control safety within temporal and cultural contexts

Robert W. Pemberton[1]

Summary

The safety of biological control of weeds in the United States of America (USA) and elsewhere is being debated because of the adoption of native plants by introduced biological control agents such as *Rhinocyllus conicus*. To attempt to understand the patterns related to the adoption of non-target native plants by introduced agents, I conducted an analysis of non-target plant use resulting from natural enemy introductions in continental USA, Hawaii and the Caribbean between 1902 and 1994. Fourteen of the 117 agents introduced have adopted 45 native plants as developmental hosts. All but one of these plants are closely related to the target weeds. The non-target use was predictable, based on known host ranges of the insects in their native areas and host-specificity testing. No evolution of host range was needed for the adoption of these plants. The single case in which a plant unrelated to the target weed was adopted involves the lantana lacebug (*Teleonemia scrupulosa* introduced to Hawaii in 1902), which was thought to be a lantana specialist but apparently is not. Almost all (13/14) of the insects adopting native plants were introduced between 1902 and 1972. During this period, 20% (13/63) of the agents introduced have adopted native plants. This compares to only 1.8% (1/54) of the agents introduced between 1973 and 2002, after native plants were given more legal protection. In contrast to this native-plant use, none of the 117 introduced natural enemies have adopted agricultural plants. These results suggest that biocontrol science and decision-making regarding appropriate risk functioned well to prevent harm to agricultural plants, but allowed harm to native plants before 1980, when native plants began to be considered more fully in risk assessment. Greater consideration of potential risk to native plants in biological control was stimulated by, and concurrent with, heightened interest in native plants in the USA during the 1970s and 1980s, as indicated by both federal legislation and the growth of native plant societies. From 1973–83, the federal government passed four important laws and regulations protecting rare native plants. Before 1970, there were only four native plant societies, whose members are primarily amateur plant enthusiasts and conservationists.

Keywords: biocontrol safety, non-targets, risk.

Introduction

The safety of biological control in the United States of America (USA) and elsewhere is being debated because of the adoption of native species as hosts for introduced biological control agents (Hawkins & Marino 1997, Louda *et al.* 2003). Recent reviews of non-target effects in biological control indicate that the use of native species by introduced biological control agents is due to pre-adaptation, not evolution (or expansion of host range of the agents) (Pemberton 2000, Louda *et al.* 2003).

Non-target native-plant use

In my review of these effects in biological control of weeds, an analysis was made of non-target native-plant use (complete development in the field) resulting from natural enemy introductions in continental USA, Hawaii, and the Caribbean between 1902 and 1994 (Pemberton 2000). Fourteen of the 117 agents introduced have adopted 45 native plants as developmental hosts. All but one of these plants is closely related to the target weeds. The non-target use was predictable, based on known host ranges of the insects in their native areas and/or host-specificity testing. The single case in which

[1] Invasive Plant Research Laboratory, United States Department of Agriculture–Agricultural Research Service, 3205 College Avenue, Fort Lauderdale, Florida 33314, USA <bobpem@saa.ars.usda.gov>.

a plant unrelated to the target weed was adopted involves the lantana lace bug *Teleonemia scrupulosa* Stal, introduced into Hawaii in 1902. This lace bug has been thought to be a lantana specialist but apparently is not. These results indicate that projects against weeds with close native-plant relatives in the flora where biological control agents are introduced are riskier than are projects against weeds which lack close relatives in that area. Target weed selection for biological control should recognize the greater risk associated with weeds with close relatives, and consider the resulting need for more specialized agents. Finding enemies with the suitable specificity can be expected to entail more field survey and more host-specificity research, with the resulting increased costs, and the possibility that natural enemies with the needed specificity may not be found (Pemberton 2002a,b). For these reasons, targeting weeds with fewer native relatives may be a better use of limited resources, although the severity of the weed problems must also be considered.

Changing considerations

All 14 of the insects adopting native plants were introduced between 1902 and 1974. In contrast to native plants adopted as non-target hosts, none of the 117 introduced natural enemies have adopted agricultural plants. These results suggest that biological control science and decision making regarding appropriate risk functioned well to prevent harm to agricultural plants, but it allowed the introduction of agents that could adopt native plants before 1980. Greater consideration of potential risk to native plants in biological control was stimulated by, and concurrent with, the heightened interest in native plants in the United States during the 1970s and 1980s, as indicated by both federal legislation for the protection of endangered plants and the great growth of native-plant societies (clubs). It is interesting to note that biological control practice was modified to better consider risks to native plants before most non-target effects to native plants were generally recognized. Unfortunately, the use of native plants by insects introduced long ago gives the impression that contemporary biological control of weeds carries more ecological risk that it actually does.

Conclusions

Although the biological control of weeds safety with regard to native plants has greatly improved in the United States during the past 20 years, several problems remain. Differences in what constitutes acceptable risk can lead to demands for unwise biocontrol introductions by the sectors most affected by the weeds, and to introductions of agents in one state or country deemed too risky for use in adjacent states or countries. This involves both the movement of established agents, such as *Rhinocyllus conicus* (Frolich), and new introductions to North America. In 2000, the USDA–Animal and Plant Inspection Service, in the first action of this sort, revoked permits for interstate movement of this weevil (Louda *et al.* 2003). Risk to native plants from biological control introductions needs to be considered more strongly from a bioregional perspective.

Improved environmental safety in biological control of weeds, and importantly, the recognition of safer practice will allow this urgently needed tool to be available and well supported for use against invasive plants of both agriculture and natural areas.

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A fuller version of this manuscript is in preparation and will be published elsewhere.

Non-target impacts of *Aphthona nigriscutis*, a biological control agent for *Euphorbia esula* (leafy spurge), on a native plant *Euphorbia robusta*

John L. Baker, Nancy A.P. Webber and Kim K. Johnson[1]

Summary

Aphthona nigriscutis Foudras, a biological control agent for *Euphorbia esula* L. (leafy spurge), has been established in Fremont County, Wyoming since 1992. Near one *A. nigriscutis* release site, a mixed stand of *E. esula* and a native plant, *Euphorbia robusta* Engelm., was discovered in 1998. During July of 1999, *A. nigriscutis* was observed feeding on both *E. esula* and *E. robusta*. A total of 31 *E. robusta* plants were located and marked on about 1.5 ha of land that had an *E. esula* ground-cover of over 50%. Eighty-seven percent of the *E. robusta* plants showed adult feeding damage. There was 36% mortality for plants with heavy feeding, 12% mortality for plants with light feeding, and no mortality for plants with no feeding. By August of 2002, the *E. esula* ground-cover had declined to less than 6% and the *E. robusta* had increased to 542 plants of which only 14 plants (2.6%) showed any feeding damage. For the four-year period, the *E. esula* ground-cover was inversely correlated to *E. robusta* density and positively correlated to *A. nigriscutis* feeding damage, showing that as *E. esula* density declines so does *Aphthona nigriscutis* feeding on *E. robusta*.

Keywords: *Aphthona*, density, *Euphorbia*, mortality, non-target impacts.

Introduction

A parcel of land 4.8 km (3 miles) south-west of Lander, Fremont County, Wyoming has been infested with *Euphorbia esula* (leafy spurge) for over 30 years. Owned for many years by the Majdic family, it was used mainly for livestock grazing during the summer. In 1995, the land was subdivided and today is only grazed occasionally by antelope and deer. In the late 1970s, the land was treated with herbicides on a regular basis, but a groundwater contamination in the area stopped the use of herbicides and the *E. esula* reestablished at the site and spread into new areas. *Aphthona nigriscutis* was released on the Christiansen property, just west of the site, in 1990 and the insects established well. Many redistribution releases were made between 1993 and 1996 on the Majdic land from those earlier-established populations. These sites were monitored annually to assess the establishment of the bioagents. There was a strong contrast between the Majdic land and the Christiansen properties where the insects were prospering and impacting the spurge in a dramatic way. *Euphorbia esula* ground-cover fell from the 50–70% range to 5–10% by 1998 at Christiansen's, but at Majdic's the spurge continued to spread. At Christiansen's, it was possible to sweep over 100 beetles in one swing of the net in hot spots, while just 0.8 km (0.5 miles) away, we seldom averaged one beetle per sweep. It was while monitoring *A. nigriscutis* on the Majdic property that we observed a small colony of a native spurge, *Euphorbia robusta* Engelm.

Early in the *E. esula* biocontrol effort, *E. robusta* had been identified as a species of interest because it is closely related to *E. esula*, both belonging to the subgenus *Esula*, is a perennial which could support the long life cycle of *Aphthona* beetles and is sympatric with *E. esula* (Pemberton 1985). Since the late 1980s, Fremont County Weed and Pest had supplied the

[1] Fremont County Weed and Pest, 450 N 2nd Street, Lander, WY 82520, USA.
Corresponding author: John L. Baker <larbaker@wyoming.com>.

United States Department of Agriculture–Agricultural Research Service with *E. robusta* plant material. Typically, *E. robusta* is found sparsely growing on rocky, wind-swept ridges where the tools of extraction were pry bars rather than shovels. We had to force the rock layers apart to follow the roots and it took hours to find and collect just a few plants. The Majdic site was refreshingly different with the *E. robusta* growing in deep soil and the digging was easy. We returned often to this site to get more plant material.

In 1997 and 1998, we observed *E. robusta* plants with feeding scars on the leaves and occasionally saw *A. nigriscutis* feeding on the plants. A few of these *E. robusta* plants were photographed and marked with pin flags for future reference. The next yea,r the marked plants were gone. Actually, this feeding activity by *A. nigriscutis* could be anticipated. An examination of the petitions to introduce *Aphthona flava* (Pemberton & Rees 1990), *A. cyparissiae* (Pemberton 1986) and *A. czwalinae* (Pemberton 1987) into the United States showed that acceptance of *E. robusta* was almost as large as for *E. esula*. *Euphorbia robusta* was not used for host-testing *A. nigriscutis* (Pemberton 1989).

Early host-plant testing was designed to demonstrate that new biocontrol of weeds agents would not attack economically valuable crop species. In recent years, concern has shifted toward the impacts, both direct and indirect, that biological agents might cause to native species. *Rhinocyllus conicus*, a biological control agent for *Carduus nutans* L. (musk thistle) has been found to impact a wide variety of native thistles, some endangered (Gassmann & Louda 2001). Increased concern has stimulated a call for greater scrutiny of new biological control agents, more thorough study of the target species before release, and post-release tracking of host range under field conditions (Waage 2001). It is in the spirit of post release evaluation that these data are offered.

Dr Peter Harris (Ag Canada, Lethbridge, Alberta) suggested that the feeding we were seeing on *E. robusta* was incidental and would probably be inversely related to distance from the host. We set up an experiment to evaluate the impact of *Aphthona nigriscutis* feeding on *E. robusta* at a location where there was a gradient of *E. esula* density and distribution associated with *E. robusta*.

Materials and methods

Between May and August of 1999, the Majdic site was visited several times and *E. robusta* plants were located, marked and photographed. The soils are red loam, 50 to 150 cm deep. The site slopes 10 to 20 degrees to the north-east. Average annual precipitation is 33 cm, although over the last five years the rainfall has been 50 to 75 percent of normal.

Each plant was marked with a numbered wooden stake driven into the ground 60 cm north of the plant to avoid shading the plant or injuring the root. The latitude and longitude of each plant was determined with a Garmin IIIa global positioning system (GPS) device. This device is not capable of differential correction for atmospheric errors, but is generally accurate to within 3 m most of the time since selective availability was switched off in the United States.

A study site boundary was established using three *Aphthona nigriscutis* release locations marked with steel posts on the west and a road on the east with parallel north and south lines to enclose a rectangle of about 2 ha where 36 *E. robusta* plants had been marked. *Euphorbia esula* was heaviest along the western boundary. The *E. robusta* was roughly distributed in two groups toward either side of the site, plants 1–20 on the west where the *E. esula* was heaviest, and 26 to 36 on the east where the *E. esula* was lighter.

The degree of adult feeding was determined by visual examination of the *Euphorbia robusta* plants. If more than half of the leaves on more than half of the stems showed feeding activity, it was categorized as "heavy". Feeding on less than half of the stems and leaves down to 25% was "medium" damage. If the feeding was on less than 25% of the stems and leaves, the damage was "light". If there was no discernable feeding injury zero damage was recorded.

The distance from each *E. robusta* plant to the nearest *E. esula* plant was measured up to 3 m in 1 m increments. Experience suggested that the beetles, although capable of flight, simply did not move very far if host plants were abundant. A few years after release, there would often be distinct "craters" in the *E. esula*, centered on the point of release. The craters were seldom more than 3 m across until the *E. esula* really began to die out at the point of release. Additionally, there were few *E. robusta* plants that were not within 10 m of at least one *E. esula* plant. At other *E. robusta* sites in the area, where the closest *E. esula* was hundreds of metres away, there was no feeding damage to the plants from *A. nigriscutis*. Preliminary observations indicated that the *E. robusta* plants with feeding damage were growing within jumping distance of the *E. esula*.

In 2000, the *E. esula* population was in dramatic decline and there were many new *E. robusta* plants which were marked and evaluated. In addition to assessing feeding damage on *E. robusta* and measuring the distance to the nearest *E. esula*, we counted the number of *E. esula* plants inside a 1 m square frame centred on each *E. robusta* plant. *Euphorbia esula* density for the whole site was taken by walking in a roughly grid pattern back and forth across the site guided by the GPS unit. The grid consisted of a series of transects roughly 15 m apart with waypoints along them every 15 m. A 1 m square frame was dropped at each waypoint and *E. esula* and *E. robusta* stems were counted. The locations for all *E. robusta* were plotted using geographical information system (GIS) software and compared to the population density map developed from the grid data.

In 2001, we re-sampled the marked *E. robusta* locations and identified and marked new plants. We also established a permanent grid across the site on 15 m intervals. At each intersection, we measured *E. esula* and *E. robusta* density with a 1 m square frame and took ground-cover readings inside the frame with a point frame (Levy & Madden 1933) recording the first contact only for each wire in the frame. As a result of mapping the density of *E. esula*, the boundaries of the study site were altered to eliminate most of the north-western quadrats where no *E. esula* was present. This reduced the site to 1.5 ha and ensured that *E. esula* was present within 15 m of each grid point. Five *Euphorbia robusta* plants were now outside the study area, reducing the number to 31 plants marked during 1999. On 25 May 2001, 12 *E. robusta* plants, 8 from within the study area, were dug up to examine the roots for presence of *A. nigriscutis* larvae and feeding damage. In 2002, the same data were collected as in 2001.

Results and discussion

Upon returning to the site on 7 June 2000, we observed a dramatic change. The *E. esula* that was so dominant the year before was nearly gone. Visually, the Majdic site resembled other sites where the ground-cover had been reduced to less than 10% by *A. nigriscutis* feeding. This was unexpected as the *A. nigriscutis* numbers were always low across the site. There had never been craters at the points of release, and the *E. esula* had been expanding and becoming denser every year. Yet, the *E. esula* was no longer a major component of the site. Four of the 31 marked *E. robusta* plants were gone, but many new plants were observed. Several trips were made to the site to locate new plants, resulting in a total of 163 new *E. robusta* plants marked during 2000. A real increase of *E. robusta* had taken place at the same time the *E. esula* had declined.

It is not possible to explain adult feeding damage to *E. robusta* as a function of distance to *E. esula* plants or *A. nigriscutis* population centres (Table 1): depending on the year, the correlation coefficient was positive or negative.

In 2000, the correlation coefficient between *E. esula* and *E. robusta* densities was 0.01, –0.25 in 2001, and –0.51 in 2002, perhaps suggesting that, over time, feeding damage on *E. robusta* became more common where *E. esula* densities were lower. Moreover, the density data do not reflect the decrease in size of individual *E. esula* stems. In 1999, they were large, 25 to 50 cm tall and heavily branched, while in later years they were mostly less than 20 cm tall, unbranched and non-flowering. The decline in *E. esula* ground-cover was a result of reduced stem size and vigour rather than a reduction in stem density.

Aphthona nigriscutis adult feeding does appear to have a have a relationship to *E. robusta* mortality. Mortality in 2002 for each level of feeding is listed by year in Table 2. The data reflect a higher mortality rate for plants with heavy and medium feeding compared to plants with light or no feeding.

Table 1. *Euphorbia robusta* adult feeding damage correlation.

Variable	1999	2000	2001	2002
Distance to 1994 *Aphthona nigriscutis* release from *E. robusta*	–0.56	–0.18	–0.11	+0.06
Distance to nearest *Euphorbia esula* plant from *E. robusta*	–0.43	–0.01	+0.21	+0.20
Density of *E. esula* at each *E. robusta*	No data	0.01	–0.25	–0.51

Table 2. *Euphorbia robusta* 2002 mortality as a function of feeding damage by adult *Aphthona nigriscutis*.

Year	Feeding	No. of plants	Mortality	Percentage
1999	Heavy	11	4	36
	Light	16	2	13
	None	4	0	0
2000	Medium	8	3	38
	Light	85	3	4
	None	59	8	14
2001	Light	19	1	5
	None	221	20	9

In retrospect, it is questionable that adult feeding could actually kill these perennial plants. Heavier adult feeding might have been an indicator of oviposition and larvae attacking the roots. Since our focus in 1999 was on tracking the long-term impacts of *A. nigriscutis* on individual *E. robusta* plants, no attempt was made at the time to dig up the attacked plants to determine if *A. nigriscutis* larvae were present on the roots. Twelve plants each of *E. robusta* and *E. esula* were dug up on 25 May 2001 and the roots were examined for the presence of *Aphthona nigriscutis* larvae. None was found and the roots looked healthy and intact. Larvae were found on *E. esula* roots along the western edge of the research site in the fall of 2000, but none was found on *E. esula* inside the site boundaries at that time either.

In the host-specificity testing, the host range of *A. nigriscutis* was found to be the subgenus *Esula* in the genus *Euphorbia*. *Euphorbia robusta* is in the subgenus *Esula* (Pemberton 1985) and was thought to be an acceptable host even though, as indicated above, it was not tested. The *E. robusta* plants in culture were consumed during tests of the three *Aphthona* (*A. flava*, *A. cyparissiae* and *A. czwalinae*), all of which accepted

E. robusta as a laboratory host. Pemberton's conclusion in the petition to release *A. nigriscutis* was that its level of specificity was similar to and perhaps somewhat narrower than the other tested *Aphthona* species. The decision to petition for release of *A. nigriscutis* and other *Aphthona* species in the western United States was made because research indicated that they could use only two native *Euphorbia* species (*E. robusta* and *E. incisa*) that were perennial and partly sympatric with leafy spurge. This level of risk was considered to be modest when one considers that there are 112 native *Euphorbia* (*sensu lato*) in the US that could be potential hosts of *Euphorbia* feeding insects (Pemberton 1985), and the leafy spurge problem was severe in the United States. Because *A. nigriscutis* larvae were not sampled when the population of the beetle was high, it is not clear whether the observed impact of *A. nigriscutis* on *E. robusta* was due to adult feeding alone. Adult feeding might be correlated with oviposition and thus larval damage to the plant's root system (R.W. Pemberton 2003, pers. comm.).

No ground-cover measurements were made in 1999 or 2000, but the appearance of the Majdic site was very similar to other sites in the area where ground-cover and density data have been collected for many years. *Euphorbia esula* at the Majdic site was estimated to contribute between 10 and 90% to the ground-cover east to west across the site, and conservatively would have averaged 50% in 1999, dropping to less than 10% in 2000. Ground-cover of *E. esula* was 6.3% in 2001 and 5.7% 2002.

As the *E. robusta* population increased, the number of plants with feeding damage first increased, then decreased in real numbers. Percentage of plants fed upon declined annually over the period (Fig. 1). When compared to the change in *E. esula* ground-cover over the same period, it appears that there is a competitive impact from the *E. esula* at the site and as the *E. esula* declines the *E. robusta* population takes advantage of the open space. The feeding damage lags a year behind the ground-cover decline (Fig. 1) suggesting that the adult feeding by *Aphthona nigriscutis* in 2000 is more closely related to the *E. esula* ground-cover in 1999 than in 2000.

Even though host-specificity testing predicts that *E. robusta* should be a good host for the *Aphthona* beetles (Pemberton 1986, 1987, 1989), observations at the Majdic site indicate that *Aphthona nigriscutis* only fed heavily on *E. robusta* when its primary host *E. esula* was plentiful and able to support the biological control agent in large numbers. When the *E. esula* ground-cover declined, feeding by *A. nigriscutis* on *E. robusta* declined as well while *E. robusta* plant numbers increased (Fig. 1). Even with a 17-fold increase, *E. robusta* still did not show up in the ground-cover measurements and did not replace the habitat and food source that the *Aphthona nigriscutis* had enjoyed when the *E. esula* ground-cover component averaged 50%.

While it is not known if *A. nigriscutis* can complete its life cycle on *E. robusta* in the field, the strong correlation between the decline in *E. esula* with the decline in beetle damage to *E. robusta* suggests that we observed an adult feeding effect. If *E. robusta* was a good developmental host for the beetle, then it would have been unlikely for the adult feeding to decline and the density of the *E. robusta* plants to increase with *E. esula* decline.

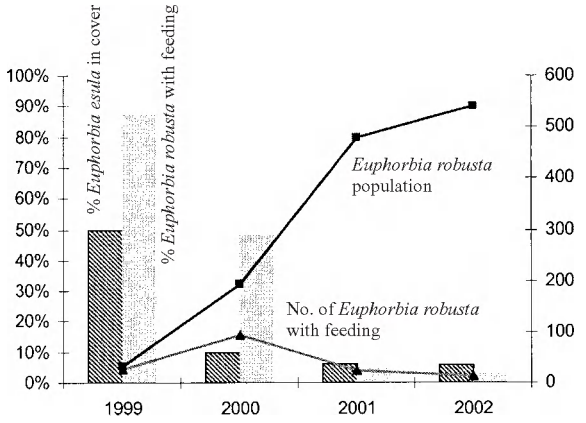

Figure 1. Time course for *Euphorbia esula* cover (%), and *E. robusta* population size, feeding incidence (%), and number of *E. robusta* with feeding.

This is in keeping with observations made in 1998 and 2001 at Camel's Hump, west of Medora, North Dakota. In 1998, this site was heavily infested with *E. esula* which was supporting an epidemic population of *Aphthona nigriscutis* and *A. lacertosa*. The insects were super-abundant and millions were collected for redistribution in just a few hours. Every blade of grass had notches in the leaves and the insects could be observed feeding on every plant species present. Upon returning to the site in 2001, *E. esula* was nearly gone. Although a number of people attempted to collect *Aphthona* for redistribution, the populations were too low. At that time, we observed no *Aphthona* sp. feeding activity on any species other than *E. esula*. Waage (2001) reports two parallel occurrences where weed biocontrol agents attacked non-target species during the epidemic period of agent development when the host plants were abundant. A lace bug, *Teleonemia scrupulosa*, released against *Lantana camara* in sesame crops in Uganda attacked the crop at peak populations (Davies & Greathead 1967), and a leaf beetle, *Zygogramma bicolorata*, released against *Parthenium hysterophorum*, attacked sunflowers in India during population explosions (Jayanth *et al.* 1993). In both cases, a decline in host-plant numbers resulted in a decline in the biological control agent and the non-target feeding stopped (Davies & Greathead 1967, Jayanth *et al.* 1993).

The *Aphthona* beetles are proving to be excellent biological control agents that severely impact their

target weed, *Euphorbia esula*, in the United States (Nowierski & Pemberton 2002). Their reputation can only be enhanced by these recently observed modest transient effects on their most likely non-target host, *Euphorbia robusta* (R.W. Pemberton 2003, pers. comm.).

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Are mono-specific agents necessarily safe? The need for pre-release assessment of probable impact of candidate biocontrol agents, with some examples

J.K. Balciunas[1]

Summary

Historically, weed biocontrol practitioners searched for highly effective agents that were also safe. Protecting agronomic crops was the original focus for risk evaluation, but to this has been added protecting native plants, especially those related to the target weed. Host range tests are now weed biocontrol's hallmark tool, with concern about the efficacy of the candidate agent sometimes being secondary. However, even a highly specific agent can disrupt ecosystem pathways in unpredictable ways, especially if it becomes abundant on its target, but fails to reduce the target weed's populations. I review some of the current concerns about non-target impacts, both direct and indirect, as well as criticisms about the inefficient "lottery" approach that wastes scarce resources in introducing many agents, some of which never contribute to controlling the target weed. Effective agents can help alleviate some of these concerns, and there is increasing demand that we should strive to release agents that are not only narrowly host specific, but also have demonstrated their ability to damage the target weed. While still not yet routine, pre-release consideration of the proposed agent's probable efficacy is receiving increased attention. This is usually done overseas, in the native range of both the target weed and candidate agent. I review some of the different approaches used in these overseas evaluations. However, pre-release impact assessments can also be performed under containment conditions in quarantine. I discuss the results of two "dosage" trials I conducted with a gall-making fly that is being considered as a biological control agent for Cape ivy (*Delairea odorata*). Plants exposed to both low and high densities of gall flies, were smaller, and had fewer leaves than the ungalled controls. Pre-release evaluations of a candidate agent's potential impact should lead to fewer ineffective agents being released, thereby making weed biocontrol more efficient, and reducing [but not eliminating] the possibility of negative indirect impacts on non-targets.

Keywords: efficacy, indirect impacts, ineffective agents, non-target impacts, risk reduction.

Introduction

Within the subdiscipline of biological control of weeds, those involved in selecting potential agents have always been concerned that the agent would contribute to the eventual control of the target weed. Early numerical scoring systems for prioritizing potential weed biocontrol agents were heavily weighted towards an agent with demonstrated impact (Harris 1973, Goeden 1983). In the early decades of weed biocontrol, concern for non-target effects concentrated on possible impacts to crops and other agronomic plants. However, by the early 1970s this concern began to shift to native plants (Pemberton 2003). The host specificity of potential weed biocontrol agents became of paramount importance, and test plant lists became quite lengthy, with concern about the efficacy of the candidate agent sometimes being secondary. Despite this emphasis on host specificity, biological control of weeds continues to draw criticism, both from practitioners and outside observers. The most troubling criticisms can be lumped into two categories: 1) non-target impacts and 2) inefficiency.

[1] USDA-ARS Exotic & Invasive Weed Research Unit, 800 Buchanan St, Albany, California 94710 USA.

Direct non-target impacts

Non-target impacts caused by biological control agents appears to be a current "hot topic", with two recent books (Follett & Duan 2000, Wajnberg *et al.* 2001) and a review article (Louda *et al.* 2003) being devoted primarily to this subject. The impacts resulting after the release of an agent can be classified as either *direct* or *indirect* (usage follows Fowler *et al.* (2001) and Lonsdale *et al.* (2001)). The former results from direct action (feeding, galling, oviposition) by the agent on a target or non-target. Indirect impacts include those resulting from the decrease of the target weed or the agent becoming a food source or disease vector, and can affect species that do not even directly encounter the biological control agent. As pointed out by Lonsdale *et al.* (2001), both forms of impacts are desired end products for a successful weed biocontrol project. For example, the agent should directly reduce the abundance of the target weed, and this reduction should indirectly allow for replacement by native plants, crops or forage plants. But an agent can also cause undesirable, negative impacts on non-targets, both directly and indirectly. These are among the outcomes of biological control to which McEvoy & Coombs (2000) apply Tenner's (1996) term "revenge effects".

Due primarily to the long-standing concern by weed biocontrol scientists about the host specificity of the agents that they release, our subdiscipline has an enviable record of safety with very few instances of direct impacts on non-targets (McFadyen 1998). For example, although more than 350 organisms have been released to control 130 weed species world-wide, only eight examples of impacts on non-target plants are known and most of these were predictable from the pre-release tests (Julien & Griffiths 1998). In a more recent review by Pemberton (2000) of the data on field host use by the 117 biological control agents released against weeds in the continental USA, Hawaii, and the Caribbean since 1902, he found that 15 insect agents also utilize 41 native plant species. All but one of these plants are closely related to the target weed, and the potential for attack by the agents could have been predicted from host-specificity testing. Though Stiling & Simberloff (2000) might argue otherwise, unpredicted non-target impacts from weed biological control agents appear to be relatively uncommon.

It should be remembered that, although direct impacts to a native plant are undesirable, the mere possibility of this occurring does not necessarily disqualify a potential agent from being released, even under the current more stringent regulatory environment. Adequate host range testing, coupled with good information on the distribution and ecology of both the weed and the potential non-target host can allow for reasonable predictions of severity of non-target attack as well as a risk–benefit analysis. For example, Willis *et al.* (2003) review nine weed biocontrol projects in Australia that included a proposed agent whose pre-release host range evaluations indicated a risk to a native Australian plant. In all nine cases, the agents were ultimately approved and released.

Indirect non-target impacts

Direct impacts to non-targets are usually predictable from host-specificity evaluations. Host range data can also provide guidance about the most plausible kind of indirect impacts – those arising from the "knock-on" (Fowler *et al.* 2001) or "downstream" effects from the [hoped for] decrease in the population of the target weed [or other host]. If, for example, the weed now serves as food or shelter for a native species, a conflict of interest might exist. The biological control of *Tamarix* spp. (salt cedar) in the western United States was delayed for many years because of concerns for the western willow flycatcher, a threatened bird subspecies that had started to nest in this invasive shrub (Stenquist 2000). Stiling & Simberloff (2000) also cite as an example Hayes *et al.* (1995) concerns that *Cactoblastis* moths are destroying the prickly pear on a Bahamian islet, where it is the major food source for the San Salvador rock iguana.

Similar "downstream" indirect impacts might be expected if the agent also has other hosts. This is the case for the now notorious *Rhinocyllus conicus* weevil whose attack on native North American thistles is also displacing some of the native insects that feed on them (Louda *et al.* 1997, Louda 2000). There does, however, now seem to be a consensus that the damage to native thistles by this weevil was predictable from the pre-release and early post-release evaluations (Gassmann & Louda 2001).

Host range evaluations are of little use, however, in predicting other indirect impacts that might arise from any of the myriad possible disruptions that a weed biocontrol agent might cause to complex food webs. While such food-web disruptions have been theorized as possible for weed biocontrol agents (Simberloff 1991, Simberloff & Stiling 1996), there have been few documented cases of negative indirect impacts to food webs. We do, however, know that new tritrophic relationships arise after release of biocontrol organisms, including those used for weeds. Many weed biocontrol insects soon acquire native parasitoids that find them acceptable hosts (McFadyen 2003). An example is the native wasp, which now parasitizes up to 30% of the pupa of the *Hydrellia* spp. flies that were introduced in the USA to control the aquatic weed *Hydrilla verticillata*, and which is suspected of reducing the effectiveness of these agents (Balciunas *et al.* 2002, Grodowitz 2003). The populations of this wasp are now undoubtedly higher, but the impact of these higher parasitoid populations on their original native *Hydrellia* hosts is unknown.

Although not accepted by everyone, perhaps the most convincing evidence of food-web disruption by a weed biocontrol agent is the recent research into predation by native deer mice on the larvae of the *Urophora* spp. gall flies released to control spotted knapweed. Released in Montana nearly 30 years ago, these gall flies have not been found attacking any native plants, and they are now ubiquitous and very abundant, but they have failed to arrest the spread of the knapweed, and their larvae are now the preferred prey for native deer mice, whose populations appear to have increased as a result (Pearson 1999, Pearson *et al.* 2000). Since these mice are the primary vectors of hantavirus, the incidence of this serious disease in humans may increase (Stiling & Simberloff 2000). While the more dire of the possible ecosystem perturbations flowing on from the abundance of these gall flies may not be confirmed, I, for one, accept that weed biocontrol agents can and do modify food webs.

Inefficiency of the lottery approach

One result of the current emphasis on host-specificity of weed biocontrol agents is that many of the most damaging potential agents are rejected for consideration for release because they also damage other plant species. Frequently, agents causing less damage are selected for release, and often many different agents are released against the same weed target in the hope that one or several, acting cumulatively, might provide adequate control. This approach, termed the "lottery model" by Myers (1985) does not appear to have clear-cut superiority in providing control, and it does have drawbacks. McEvoy & Coombs (1999, 2000) criticize the lottery model as being inefficient, lacking enough post-release monitoring, and being prone to "revenge effects". They urge a more "parsimonious" approach, and this recommendation is echoed by other reviewers (e.g. Strong & Pemberton 2001, Sheppard 2003).

Testing candidate agents for potential impact

I agree with those who believe indirect impacts, such as those that *Urophora* flies are having on deer mice, cannot readily be predicted before release. However, I believe that, with additional testing beyond the traditional host-specificity tests, the probability of such indirect impacts can be reduced. Intuition indicates that non-target impacts from a biological control agent are most likely to occur if the agent becomes very abundant. While biocontrol specialists hope that, after release, the agent will establish and become abundant, these high populations of the agent should be followed by the collapse of the populations of the target pest, and subsequent decline of the agent. Likewise, Holt & Hochberg (2001, p.31) conclude from their theoretical studies of a "shared predation" model that "a control agent which is only moderately effective at limiting target species numbers may be much more abundant than an effective agent, and thus pose a greater risk of incidental attack". Ecological models (Lynch *et al.* 2001, Kriticos 2003) and construction of quantitative food webs (Memmott 2000) can assist in delineating some of these non-target risks. But the prudent approach will be to demonstrate, before release, that a candidate agent has the potential to reduce populations of the target weed. Agents causing lethal damage to the target weed may not need to undergo this additional testing for impact. Pre-release demonstration of potential efficacy of agents should allow for selection of more effective agents. This should not only reduce the likelihood of indirect non-target impacts and other "revenge" effects, but will counter other objections to the "lottery" approach, as well as making weed biocontrol more "parsimonious".

Table 1. The "International Code of Best Practices for Classical Biological of Weeds" as approved at the X International Symposium on Biological Control of Weeds in Bozeman, Montana, July 1999. Guideline #3 specifies the use of effective agents, while Guideline #7 mandates monitoring for impact. Guideline #8 specifies how to react to the results of monitoring.

1.	Ensure target weed's potential impact justifies release of non-endemic agents
2.	Obtain multi-agency approval for target
3.	Select agents with potential to control target
4.	Release safe and approved agents
5.	Ensure only the intended agent is released
6.	Use appropriate protocols for release and documentation
7.	Monitor impact on target
8.	Stop releases on ineffective agents, or when control is achieved
9.	Monitor impacts on potential non-targets
10.	Encourage assessment of changes in plant and animal communities
12.	Monitor interaction among agents
13.	Communicate results to the public

Within the subdiscipline of classical biological control of weeds, the need for selecting effective agents is receiving new emphasis, and pre-release assessment of a candidate's potential impact is often urged (Harris 1991, Cullen 1995, McEvoy & Coombs 2000, Hopper 2001, Strong & Pemberton 2001, Sheppard 2003). Likewise, the "International Code of Best Practices for Classical Biological Control of Weeds" (Table 1) urges practitioners to select effective agents and, after release, to monitor them for both beneficial and non-target impacts (Balciunas 2000). As a result, pre-release evaluations of impact are becoming more common. These pre-release assessments of a potential agent's efficacy are usually performed in the native range of both the target and agent, under non-containment conditions. They can take several forms. Overseas surveys of natural enemies

can be quantified, thereby providing relative abundances and field attack rates that can greatly aid in agent prioritization (Balciunas *et al.* 1995a, 1995b, Sheppard *et al.* 1994, 1995, Briese 2000). One type of pre-release assessment that can be performed only in the native range is "Exclusion Studies" where the target weed is protected from attack by potential agents. For example, an 11-year insecticidal exclusion study in Britain demonstrated that broom bushes protected from insect herbivores outgrew those that did not (Waloff & Richards 1977). In a more recent study in Australia, the home of *Melaleuca quinquenervia* (Cav.) Blake, Balciunas & Burrows (1993) used insecticides to exclude insects from attacking the "control" saplings of melaleuca, and demonstrated that sprayed saplings quickly outgrew those that were unprotected (Fig. 1), and were able to infer that two insect species were likely responsible for this suppression of sapling growth. The impact of potential agents can also be assessed in the native range through experimental manipulations of their density under field conditions (Hasan & Aracil 1991 Brun *et al.* 1995) or in cages (Briese 1996).

While assessments of potential agents' probable efficacy are increasingly being performed in their native range, they are seldom done under the more constrained conditions a quarantine facility. Recently, I demonstrated that assessment of probable impact of a potential agent is possible under the strict containment conditions of an approved quarantine facility. I conducted two trials exposing test *Delairea odorata* (Cape ivy) plants to two different densities of *Parafreutreta regalis* gall-forming flies, and, after approximately two months, comparing the growth of the galled vines to similar vines that had not been exposed to flies. Under both the high density (10 pairs of flies/ plant) (Fig. 2) and low density (2 pairs/plant) treatments, the galled vines exhibited visible stunting, and the non-galled plants were statistically longer, and had more nodes and larger leaves. These trials confirmed that relatively subtle, sublethal impacts on the target can be quantified, even under strict containment conditions, and this should encourage others to more routinely, prior to release, to assess the potential impact of prospective agents on their proposed target.

In conclusion, the renewed interest in a candidate agent's efficacy is leading to more pre-release evaluations of their potential impact. This should lead to the release of fewer ineffective agents, making weed biocontrol more effective and less susceptible to "revenge effects", including indirect impacts on non-targets.

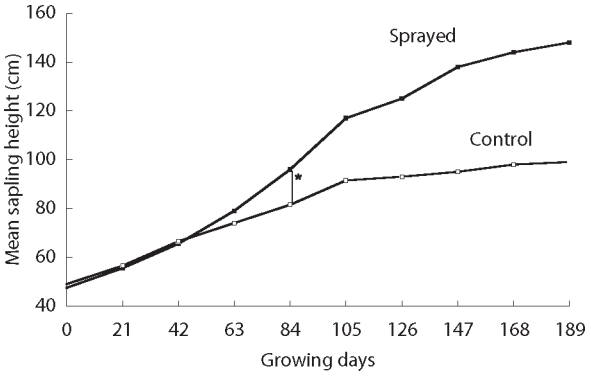

Figure 1. Comparison of the mean height of 20 pairs of potted *Melaleuca quinquenervia* saplings growing inn Townsville, Australia. One sapling from each pair was sprayed with a systemic insecticide every two weeks, while the other (the control) was left untreated. After just 84 days, the difference in height had become statistically different (Student's T-test, $P < 0.05$) (from Balciunas & Burrows 1993).

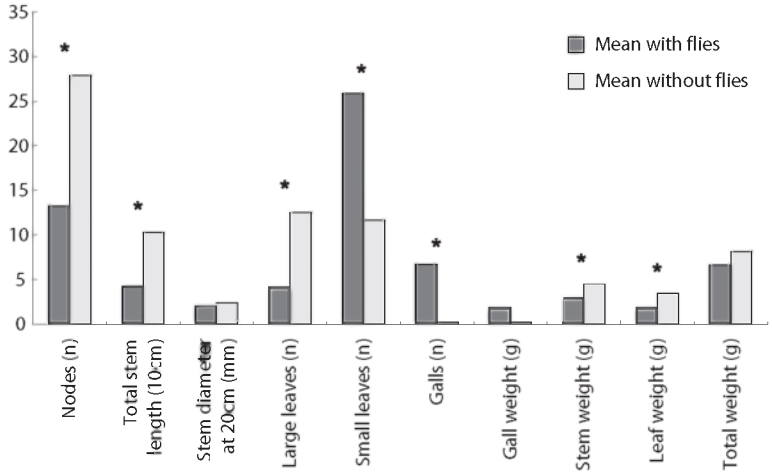

Figure 2. Comparison of the mean measurements, after two months, for the four control *Delairea odorata* (Cape ivy) plants, with the means for the six Cape ivy plants that were continuously exposed to 10 pairs of *Parafreutreta regalis* gall-forming flies. The asterisk * above the columns indicates that the means were significantly different (Student's T-test, $P < 0.05$) (Balciunas, unpublished data).

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Four years of "Code of Best Practices": has it had an impact?

Joe Balciunas[1]

Summary

In 1999, during the Xth International Symposium on Biological Control of Weeds in Bozeman, Montana, the delegates overwhelmingly voted to endorse the "Code of Best Practices for Classical Biological Control of Weeds". I review why the Code was proposed, and subsequent attention [or lack thereof] that this Code has received from those within the biological control of weeds community, and from other observers of our craft. I also present the results of a short questionnaire on experiences with the Code that was circulated during this symposium in Canberra. It is clear that although a few individuals have found the Code important and useful, many individuals involved in biological control of weeds are still unaware of the Code. A small number of individuals cite the Code, and continue to popularize its existence and utility. With time, the number of practitioners who recognize the value of adhering to the Code should increase, thereby making our subdiscipline safer and more effective – and possibly forestalling further legislative restrictions on our craft.

Keywords: international standards, risk management, survey results.

Introduction

Initially, classical biological control of weeds was practised by a small group of scientists who knew each other, and who could provide oversight and informal consent over each other's activities. When the First International Symposium on Biological Control of Weeds was held in Rome in 1969, there were 21 attendees (Simmonds 1970). The current symposium, like several that preceded it, had over 200 participants. This illustrates the rapid expansion and acceptance of our subdiscipline over the past few decades.

Because there are probably several thousand practitioners now involved in some aspect of biological control of weeds, informal oversight by peers is no longer possible. With such a diversity of personnel now involved in biocontrol of weeds, their levels of training, experience, and tolerance for risk, vary greatly. Therefore, some international standards are desirable. Accordingly, at the Xth International Symposium on Biological Control of Weeds, held in Bozeman, Montana, in 1999, I presented a draft of 10 guidelines that I felt could form a framework for a "Code of Best Practices" for our subdiscipline. During that symposium, an evening workshop on "A Proposed Code of Best Practices" was held, with over 30 delegates attending. The result was an "International Code of Best Practices for Classical Biological Control of Weeds" comprised of 12 guidelines (Table 1) (Balciunas 2000). During the final business meeting at the Bozeman Symposium, a resolution (Table 2) urging all practitioners of biological control of weeds to adhere to the principles presented in the newly revised Code was overwhelmingly ratified by the attendees – there were only two dissenting votes.

Unlike previous guidelines and regulations (e.g. FAO 1996, USDA 1998, 2003) that only apply to the importation and first releases of weed biocontrol agents, the Code is meant to serve as a guide for everyone involved in weed biocontrol, including those making redistribution releases, as well as stakeholders and administrators. While reemphasizing the use of safe and approved agents, the Code directs that only appropriate targets be selected, and stresses that only effective agents be used. Four of the guidelines emphasize post-release monitoring. The purposes of the Code are to: reduce the idea that biological control of weeds is conducted in a "willy nilly" manner, increase safe use

[1] USDA-ARS, Exotic & Invasive Weed Research Unit, 800 Buchanan St., Albany, California USA 94710 <joe@pw.usda.gov>.

of biocontrol agents, help improve efficacy in both environmental and economic terms, and improve the public's confidence in biocontrol by increasing professionalism within our ranks.

Table 1. International Code of Best Practices for Classical Biological Control of Weeds.

1. Ensure target weed's potential impact justifies release of non-endemic agents
2. Obtain multi-agency approval for target
3. Select agents with potential to control target
4. Release safe and approved agents
5. Ensure only the intended agent is released
6. Use appropriate protocols for release and documentation
7. Monitor impact on target
8. Stop releases of ineffective agents, or when control is achieved
9. Monitor impacts on potential non-targets
10. Encourage assessment of changes in plant and animal communities
11. Monitor interaction among agents
12. Communicate results to the public

Table 2. Resolution (ratified 9 July 1999, by the delegates to the X International Symposium on Biological Control of Weeds, Bozeman, Montana).

Delegates and participants to the X International Symposium for Biological Control of Weeds, recognizing the need for professional standards in the subdiscipline of classical biological control of weeds, urge practitioners of the subdiscipline to voluntarily adopt the CODE OF BEST PRACTICES FOR BIOLOGICAL CONTROL OF WEEDS, as published in the proceedings of the Symposium, and adhere to the principles outlined in the code.

Code available at <http://wric.ucdavis.edu/exotic/techtran/Code_of_Best_Practices.htm>.

Impact of the Code

The Code was noteworthy enough to be mentioned by the scientific writer covering the Bozeman Symposium (Malakoff 1999), and several of the ecologists that attended the symposium praised it. Several of the workshop participants have incorporated the Code into talks at other meetings, and in other proceedings (e.g. McEvoy 2001, Balciunas 2002). A discussion of the Code is one of the introductory chapters in the forthcoming textbook "Biological Control of Invasive Plants in the United States" (Balciunas, in press).

For this Symposium, I prepared a brief questionnaire in order to elicit responses from attendees about their experiences with the Code. Unfortunately, very few completed questionnaires (n = 21) were returned, so making strong inferences is not warranted. The replies of the respondents are tabulated in Figure 1. A third of the respondents had not heard of the Code, and for the majority of the rest, it appears not to have had any impact. Although at the Bozeman Symposium several attendees voiced concerns that the Code might make their jobs more difficult, there is little evidence that this has occurred. Only one respondent cited difficulty arising from the Code, but interestingly, this same person noted that he had not previously heard of the Code!

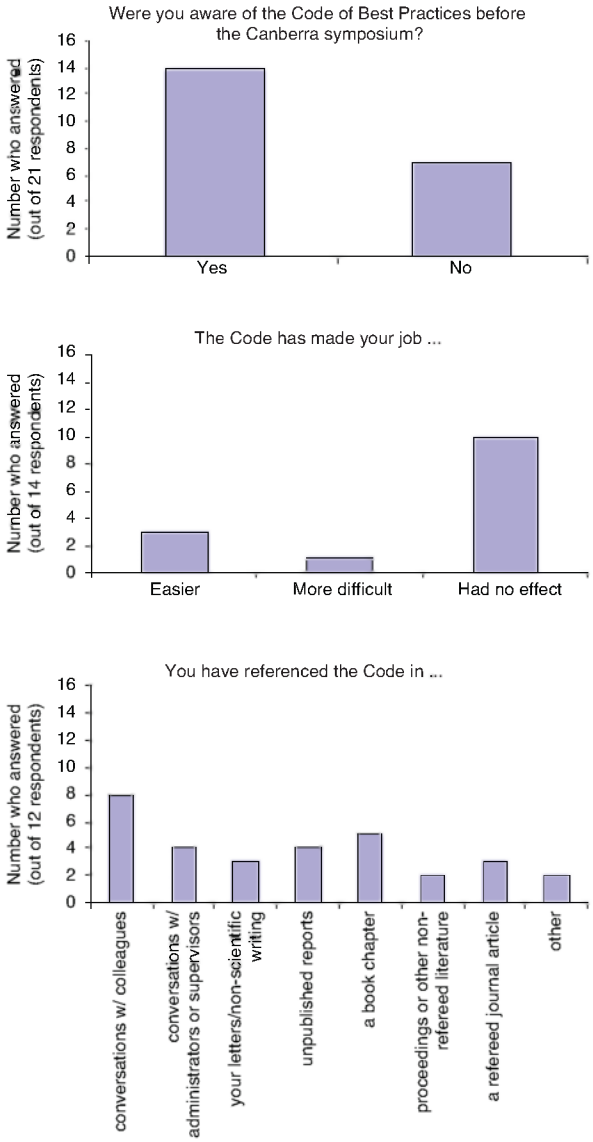

Figure 1. Responses to the three questions in the questionnaire on the Code of Best Practices by participants at the XI International Symposium on Biological Control of Weeds.

It is evident that the Code's importance and relevance is appreciated and being publicized by some participants at this symposium in Canberra. At least five have cited the Code in book chapters, and three have cited the Code in refereed journals, indicating that interest and acceptance is increasing and that the Code is beginning to have a positive impact. Several of the speakers during this Canberra Symposium have favourably mentioned the Code, and one (Del Fosse) even urged that all those involved in biological control of weeds keep a copy of the Code posted on their office (laboratory) doors.

Conclusions

After four years, many practitioners in our subdiscipline are still unaware of the existence of the Code, and most do not feel that the "International Code of Best Practices for Classical Biological Control of Weeds" has had an impact on their research and projects. However, a small number of individuals (including academics and state practitioners) continue to cite the Code, and to popularize its existence and utility, indicating a small but positive impact. It is hoped that, with additional time, the number of practitioners who recognize the value of having a Code will increase. Adherence to the Code should help in discriminating "good" biocontrol projects from those that are risky, poorly conceived, or lacking appropriate thoroughness and safeguards. A widely observed "Code of Best Practices" may help forestall the international trend towards increasing legal and regulatory restraints on classical biological control of weeds.

Acknowledgements

The inappropriate attacks by some colleagues on the meticulous research of Svata Louda galvanized me to search for some form of self-regulation that would allow outside observers to distinguish "good" biocontrol from the bad and ill-conceived. A subsequent conversation with Jennifer Marohasy about her experiences with Queensland Sugar Grower's Code of Best Practices inspired this approach. The sustained assistance of Eric Coombs during the Bozeman Symposium allowed the presentation and ratification of the "Code of Best Practices for Classical Biological Control" there, and his persistence, along with that of Peter McEvoy, have helped keep the Code viable since then.

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Setting safety zones for a biological herbicide: a New Zealand case study

Graeme W. Bourdôt,[1] Geoff A. Hurrell,[1] David J. Saville,[1] Meindert D. de Jong,[2] Hans J. Erbrink[3] and Jan C. Zadoks[4]

Summary

The use of wide-host-range aerially dispersed plant pathogens as biological herbicides carries with it a risk of additional disease in neighbouring susceptible crops. This additional disease risk may be quantified as the ratio of inoculum added to the susceptible neighbouring crop's environment from the upwind biological herbicide source, to the density of inoculum created by natural infections of the pathogen in the crop's environment. The spatial pattern in this ratio beyond a biocontrol site provides an objective basis for assessing the risk posed by the biological herbicide and enables a safety zone to be set. This approach for assessing and managing risk is illustrated by a case study conducted in New Zealand in which safety zones were estimated for average *Cirsium arvense*-infested sheep and dairy pastures treated with the plant pathogen *Sclerotinia sclerotiorum* using models of the escape and aerial dispersion of its spores. Assuming a 1:1 ratio of added to naturally present spores was acceptable, no safety zone was necessary for either of the pastures modelled. A ten-fold ratio (1:10 added to natural) necessitated safety zones of 300 and 150 m for the sheep and dairy pasture, respectively. Uncertainties associated with extrapolation of this conclusion from average to individual pasture management scenarios, and to other years and climatically different regions are discussed.

Keywords: Biocontrol, *Cirsium arvense*, mycoherbicide, risk analysis, *Sclerotinia sclerotiorum*.

Introduction

Plurivorous plant pathogens that are air-dispersed may often have qualities (e.g. high pathogenicity and ease of culture, scale-up and storage) that make them good candidates for development as bioherbicides. However, such pathogens may be needlessly rejected by bioherbicide researchers because of the perceived risk of added disease occurring in susceptible crops downwind of biocontrol sites. This additional crop disease risk may be defined as the ratio of "added" to "natural" inoculum in the crop environment (de Jong *et al.* 1999) and its evaluation in space enables estimation of a safety zone around a biocontrol site (de Jong *et al.* 1999). Here we discuss the simulation modelling approach taken in a New Zealand case study in which safety zones for market-garden cropping land were estimated for *Cirsium arvense* (L.) Scop.-infested sheep and dairy pastures treated with the plant pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary (de Jong *et al.* 2002a).

[1] New Zealand Pastoral Agriculture Research Institute Ltd., P.O. Box 60, Lincoln, New Zealand
[2] Biological Farming Systems, Wageningen University and Research Centre, Marijkeweg 22, 6709 PG Wageningen, The Netherlands
[3] KEMA Power Generation and Sustainable, P.O. Box 9035, 6800 ET Arnhem, The Netherlands
[4] Herengracht 96 C, 1015 BS Amsterdam, The Netherlands
Corresponding author: G. Bourdôt, New Zealand Pastoral Agriculture Research Institute Ltd., P.O. Box 60, Lincoln, New Zealand <graeme.bourdot@agresearch.co.nz>.

Materials and methods

In essence the approach taken was to (1) simulate the concentration of "naturally-occurring" *S. sclerotiorum* ascospores in the air above an area of market garden crops, (2) simulate the concentration of mycoherbicide-derived ascospores beyond a biocontrol source in both sheep and dairy pasture, and (3) locate in two-dimensional space around each biocontrol source, the concentration contour of added spores that equates to the median concentration in the market garden area (1:1 ratio of added to natural spores) and to one tenth of the

median market garden concentration (1:10 ratio). These contours are the safety zones for their respective ratios of added to natural ascopores for mycoherbicide-treated sheep and dairy pasture. The salient features of the method are presented below while full details are given by de Jong *et al.* (2002a).

The Gaussian plume model is a valuable tool in predicting the atmospheric transport of fungal spores (Spijkerboer *et al.* 2002). It was used, as implemented in the air quality management computer programme PC STACKS (Erbrink 1995), to estimate the atmospheric concentration, C, of *S. sclerotiorum* ascospores at distances x (m) downwind of a virtual 100 m × 100 m biocontrol pasture source (added inoculum) and within a virtual market garden area represented (in PC STACKS) by a 7 × 7 matrix of 49 identically parameterised adjacent 100 m × 100 m sources (natural inoculum). In PC STACKS, the Gaussian plume model is given as

$$C_{(x,y,z,H)} = \frac{PQ}{2\pi \bar{u} \sigma_y \sigma_z} \times \left[e^{\frac{-(z-H)^2}{2\sigma_z^2}} + e^{\frac{-(z+H)^2}{2\sigma_z^2}} \right] \times e^{\frac{-y^2}{2\sigma_y^2}} \times C_{ls} \quad (1)$$

where y is horizontal distance (m) from the plume axis, z is height (m) above ground, H is source height (m), P is the inversion layer penetration fraction, Q is the emission rate of the source (spores s^{-1}), $\bar{u}$ is mean wind speed (ms^{-1}), C_{ls} is a reflection term and σ_y and σ_z are respectively horizontal (cross-wind) and vertical dispersion terms and are functions of atmospheric stability and x. A plume was modeled for every hour from 1 Sept 1996 to 30 Nov 1996, the time of year when sporulation occurs in pasture (Bourdôt *et al.* 2001), using 1996 Canterbury weather records to estimate P, $\bar{u}$, σ_y and σ_z. Using all plumes (2184 for each of the sheep and dairy pasture sources and 107,016 for the market garden area), contour plots of 91-day average spore concentrations within and beyond the sheep and dairy pasture biocontrol sources, and within the market garden area, were calculated. Safety zones corresponding to two levels of risk averseness are defined by the concentration contour of added spores that equates to (a) the median concentration in the market garden area (1:1 ratio of added to natural spores) and (b) one tenth of the median market garden concentration (1:10 ratio).

The source term in the Gaussian plume model (1) was calculated as

$$Q = R_{spor} \times a \times E_v \quad (2)$$

where R_{spor} is the release rate of ascospores from apothecia at the source (spores m^{-2} ground s^{-1}), a is the area of the source (10,000 m^2), and E_v is the proportion of the released spores vertically escaping the pasture or market garden crop canopy. R_{spor} was calculated as

$$R_{spor} = S \times A \times f \quad (3)$$

where S is the density of sclerotia (number m^{-2}) in the soil in the autumn, A is the size of the sporulating apothecial disc surface (mm^2 sclerotium^{-1}) and f is the flux of ascospores from the apothecia (spores mm^{-2} disc surface s^{-1}). The values for parameters S, A and f were taken from data collected in a Canterbury pasture. S was set to 125 for both the sheep and dairy pasture biocontrol sources and to 8.8 for each of the 49 sources making up the market garden area (Bourdôt *et al.* 2000) and A was varied with time as in Figure 5d of Bourdôt *et al.* (2001). Parameter f followed a diurnal pattern differing between frosty and frostless days according to the data in Figure 9 of Bourdôt *et al.* (2001). Parameters A and f were assumed to be the same in both the biocontrol and market garden sources.

The escape fraction, E_v, was a mathematically derived function of mean wind speed, u (ms^{-1}) and pasture leaf area index, LAI (leaf area/ground area),

$$E_v = \exp\left[-b\frac{LAI}{\sqrt{u}}\right] \quad (4)$$

with the shape parameter $b = 0.934$. The derivation of this simple model of spore escape from a vegetation canopy has been given previously (de Jong *et al.* 2002b). LAI was set to a constant 1.0 for the market garden area; a mean value that allowed for the fact that sporulation could occur in a variety of situations on market garden land such as on bare soil ($LAI = 0$), between widely spaced rows of vegetable crops ($LAI = 0$), and under crop canopies ($LAI > 1$). For the sheep and dairy pasture sources, LAI varied with time of year and was obtained by linear interpolation between LAI values measured in sheep and dairy pasture in Canterbury during the 1996 spring and summer period (Figure 5 in de Jong *et al.* (2002a)).

Results

The daily means of ascopore emission, Q (ascospores ha^{-1} s^{-1}) varied throughout the 91-day simulation period. This variation was a result of the underlying temporal variation in apothecial surface area A, ascopore flux f, and the escape fraction E_v, the latter driven by the imposed LAI profiles and hourly variation in wind speed, u. Of the two biocontrol pasture sources, Q was lower with dairy cattle grazing than with sheep, due to a lower escape fraction in dairy pasture, which in turn was due to the higher LAI in the dairy pasture. In the market garden source, Q was much lower than in either of the biocontrol pasture sources notwithstanding the assumed low LAI of 1.0 and therefore a relatively high escape fraction. The market garden Q was lowest because of the relatively low density of soilborne sclerotia in market garden soils ($S = 8.8$ cf. 125 in biocontrol pastures) giving rise to low values of R_{spor}.

The simulated aerial density of *S. sclerotiorum* ascospores generated by naturally diseased crops within the virtual market garden area (not illustrated) varied

asymmetrically across the 49 ha area, partly as a result of the prevailing NE wind in Canterbury during the spring period. The median of the 49 average densities generated by PC-STACKS, one value per 1 ha cell in the 7 × 7 matrix representing the market garden area, was 2,880 × 10^6 ascospores/(m^3 of air at a height of z=1.0 m).

The contour line for ascospores dispersing from the virtual sheep pasture biocontrol source at the density equal to the median average density in the virtual market garden area (1:1 added to natural), was located within the boundaries of the biocontrol source. By contrast, the contour representing the 1:10 ratio of added to natural ascospores was located beyond the biocontrol pasture source, at a maximum distance of 300 m from the edge of the biocontrol source (Fig. 1). The asymmetry in the contour is, as was the case for the market garden area, partly the result of the prevailing NE wind allowing more spores to disperse in the SE that in the NE direction over the 91-day period.

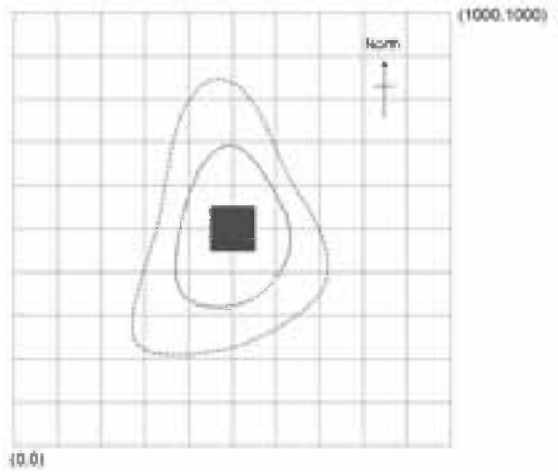

Figure 1. Safety zones for a 1:10 level of risk for sheep (- - - - - -) and dairy (———) pastures in Canterbury, New Zealand, treated with *Sclerotinia sclerotiorum* to control *Cirsium arvense*. The central square represents the 100 m × 100 m (1 ha) biocontrol source of ascospores. The contour lines represent the position where the ascospores dispersing from the biocontrol site, at a height above ground of z = 1.0 m, are at an average density equal to 10% of the median density for the market garden area.

The contour line for ascospores dispersing from the virtual dairy pasture biocontrol source representing the density equal to the median density in the virtual market garden area (1:1 ratio of added to natural spores), was also located within the biocontrol source. Here again the contour representing the 1:10 ratio was located beyond the biocontrol pasture source, but closer than for the sheep pasture, at a maximum distance of 150 m from the edge of the biocontrol source (Fig. 1). Again, the asymmetry is partly the result of the prevailing NE wind.

Discussion

The question posed was "how near to land growing susceptible market garden crops can biological weed control using *S. sclerotiorum* be practised in pasture without 'unacceptably' increasing the disease risk to these crops?" We might expect that (1) the probability and/or severity of Sclerotinia disease in these crops would be increased above the natural level if they are subjected to large numbers of additional airborne ascospores emitted by the pasture undergoing biocontrol, and (2) that a separation distance between such crops and a biocontrol site may therefore be necessary.

It has been suggested (de Jong *et al.* 1990a,b) that it may be acceptable to define a safety zone around a biocontrol site by calculating the distance at which the density of added ascospores has declined to the natural level in the air above a susceptible crop; this corresponds to a doubling of the ascospore density above the crop. On this basis a safety zone would have been unnecessary for the sheep and dairy pastures modelled here. In the case of a sheep pasture biocontrol source, a more risk-averse safety zone could be defined by calculating the distance at which the density of added ascospores is 10% of the market garden level (1:10 ratio of added to natural ascospores). In this case, 300 m in any direction would have been adequate in 1996 (Fig. 1). In the case of dairy pasture, this 1:10 ratio distance was halved (Fig. 1), a result of the greater spore-trapping ability of the dairy pasture due to its higher *LAI* (de Jong *et al.* 2002b). This dramatic impact of pasture *LAI* on downwind spore density suggests that withholding grazing, or reducing the frequency and/or duration of grazing during the sporulation period (September–November) in the year following an application of *S. sclerotiorum*, when ascospore emission is maximal (Bourdôt *et al.* 2001), would be options for shrinking the safety zone.

The safety zones calculated here are likely to be conservatively wide because in the model, the apothecial surface area per sclerotium, A, in the biocontrol pasture sources (and in the market garden sources) was based on data measured in a non-grazed sheep pasture. Under normal grazing it is highly probable that a proportion of the apothecia produced at the biocontrol pasture sources will be destroyed by treading. Although we have no supporting data for such an effect, any such damage would reduce the apothecial surface area per sclerotium. If, for example, apothecial surface area A (in Equation 3) is halved at the biocontrol sites in the presence of grazing animals, then Q and thus also ascospore density C (Equation 1), would be halved. This would result in narrower safety zones than are indicated in Figure 1 since the aerial density of ascospores dispersing from the biocontrol sources would be 50% lower at all distances from the sources.

The safety zones estimated here are based on the average ascospore emission over the 91-day period in

spring in Canterbury when *S. sclerotiorum* sporulates in pasture (Bourdôt *et al.* 2001) and can therefore be expected to be reasonably robust. Nevertheless, they are also based on (1) the average sheep and dairy pasture and so do not account for extreme management scenarios that may result in either very low or very high pasture *LAI*s, and consequently very high or very low escape fractions (Equation 4), and (2) just one year of meteorological data. Since *LAI* and meteorological conditions are driving forces in both the escape (Equation 4) and dispersal (Equation 1) of the ascospores, safety zones can be expected to vary substantially between alternative pasture management strategies and between different years and climatic regions. In addition to these sources of variation, there may be between-year variation in the length of emission period and the seasonal pattern of the release rate of ascospores, R_{spor}, within this period, which may both contribute to between-year variation in the safety zone.

Because of this expected variation, the safety zones calculated here for pasture treated with an *S. sclerotiorum*-based mycoherbicide may not be generally applicable to all pasture management scenarios, regions and years. Quantification of the effects of these sources of variation would enable regulatory authorities to make a judgment as to the general applicability of these estimates, and/or the need to modify them to enable the safe (risk averse) use of *S. sclerotiorum* as a mycoherbicide in pastures.

The dispersion and escape models discussed here may be applied to other aerially dispersed pathogens, intended as bioherbicides, to set safety zones where there are perceived risks of additional disease in adjacent crops. Full methodological details are provided by de Jong *et al.* (2002a).

Acknowledgements

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Safety in New Zealand weed biocontrol: a retrospective analysis of host-specificity testing and the predictability of impacts on non-target plants

S.V. Fowler,[1] A.H. Gourlay,[1] R.H. Hill[2] and T. Withers[3]

Summary

A retrospective analysis revealed that all weed biocontrol agents released in New Zealand were subjected to generally appropriate host-range tests, although there were several examples where, by modern standards, significant plant species were not tested. Surprisingly, the first three agents released in the 1920s and 1930s were tested against several key native plant species, but then it was not until 1990 that native species became consistent components of all test plant lists. The results of this analysis have been used to focus field surveys on the most likely non-target plant species to be attacked by biocontrol agents in New Zealand. For example, *Tyria jacobaeae* (cinnabar moth) did feed on some *Senecio* species in the original host-range tests, so the occasional field attack on native New Zealand fireweeds such as *S. minimus* was predictable. To date, this is the only weed biocontrol agent in New Zealand (of the total of 32 established in the field since 1929) that has been recorded attacking a native non-target plant species in the field. Test results, and field data from Australia, predict that two native *Hypericum* species may also be attacked occasionally by agents released against *H. perforatum* (St John's wort), but the necessary manipulation experiments in New Zealand have yet to be conducted. There are just two cases where test results did not predict potentially substantial non-target impacts: *Bruchidius villosus* (broom seed beetle) and *Cydia succedana* (gorse pod moth), attacking seed of non-target, exotic Fabaceae. For *B. villosus*, the omission of no-choice tests and limited replication, rather than a host-range expansion, appear to be the explanation. For *C. succedana*, research is ongoing into whether the no-choice tests were too short in duration, whether there are unusual issues of seasonal timing of the moth and its host plants in New Zealand, or whether there are issues of source provenance of the moths imported from Europe.

Keywords: host-range testing, New Zealand, predictability of non-target effects, safety in weed biocontrol.

Introduction

The safety record of weed biocontrol has been questioned recently, and several cases of damage to non-target plants have been reported, although few have been quantified (Louda *et al.* 1997, Pemberton 2000). The two best documented examples were predictable from the host-range testing results: *Rhinocyllus conicus* Fröhlich attacking native *Cirsium* species in the United States of America (USA), and *Cactoblastis cactorum* (Bergroth) attacking native *Opuntia* species, also in the USA (Pemberton 1995, Louda *et al.* 2003). There are also examples of damage to non-target plants that were not predicted from safety testing, but most of these appeared to be transitory, "spill-over" effects when agents are extremely abundant (MacFadyen 1998, Fowler *et al.* 2000). Encouragingly, there is no evidence of evolutionary changes in the fundamental host range of weed biocontrol agents after release (van Klinken & Edwards 2002, Louda *et al.* 2003).

[1] Landcare Research, PO Box 69, Lincoln 8152, New Zealand.
[2] Richard Hill & Associates, Private Bag 4704, Christchurch, New Zealand.
[3] Forest Research, Private Bag 3020, Rotorua, New Zealand.
Corresponding author: S.V. Fowler <fowlers@landcareresearch.co.nz>.

However, the lack of monitoring of non-target impacts in most weed biocontrol programs has led to the suggestion that the few examples of non-target impacts must be "a minuscule fraction of those that have occurred" (Simberloff & Stiling 1996). This has prompted research into the safety record in weed biocontrol in New Zealand. Here we present a retrospective analysis of host-range testing for all biocontrol agents released and established against alien weeds in New Zealand, asking whether testing methods used were adequate, and whether the range of plant species tested was reasonable by modern standards. Using results of field surveys, we then ask whether the host-range testing predicted observed non-target impacts.

Methods

Records of the host-range tests carried out before weed biocontrol agents were released in New Zealand were "interrogated" using a standard set of questions, producing a summary of testing procedures and results for each agent. Where the same tests had been conducted more than once, the data with the highest level of attack on non-target species were analyzed. Where additional host-range testing was available after the agent had been first released in New Zealand, this was also summarized, but used only to clarify issues where necessary (e.g. whether test results predicted non-target effects). The following were recorded for each data set:

1. whether feeding tests and/or oviposition tests were used
2. whether tests were choice (normal host present) and/or no-choice (normal host absent)
3. whether tests were of short duration, or longer, e.g. allowing complete development
4. the scale of the test, e.g. were tests small-scale laboratory containment, field cage or open field tests, or were data based on field host records?
5. the extent of replication of test plants and agents
6. the source, age and quality of test plants and agents
7. whether plants or agents were used more than once in tests.

Testing methods were assessed relative to currently accepted practice (Wapshere 1989, Withers *et al.* 1999). Plant species tested were listed in the summary data for each agent, and this list was assessed to determine whether species related to the target weed, native plants, or other important plants, had been omitted from the testing.

The 11 agents in New Zealand that were either released too recently, or remain too rare, to allow reliable field detection of any non-target effects were excluded. The two fungal agents released were also excluded as we have yet to survey for non-target effects of these in New Zealand. The summary data for each remaining agent species were assessed to determine whether tests predicted any attack on non-target plants in the field. Agents were placed in three categories: (1) agents for which there was no evidence of anything other than extremely minor or "trace" levels of feeding, even in no-choice "starvation" tests, so no non-target attack was predicted; (2) agents for which there was attack, even occasionally to moderate or high levels, in no-choice tests in confinement, but for which the level of attack declined to low levels in tests in larger arenas or in the field, so minor and/or sporadic non-target effects were predicted; (3) agents for which testing predicted major potential non-target effects because non-target plants were extensively attacked in realistic trials in large arenas or in the field.

Since 1999, systematic surveys have been conducted, mainly on the non-target plants that the analyses suggested might be attacked by particular control agents. Various methods were used to detect non-target attack, and will be reported in detail elsewhere. Detection methods mirrored those used successfully to detect establishment of the agents on their target weeds (Landcare Research 1996). Where possible, sites were selected where the agent, the target weed and the potential non-target species were all present. Non-target species were sometimes sampled at distances greater than 100–500 m from the nearest known target plant, to establish whether an infestation was due to larval mobility, or clearly had to result from oviposition on the non-target plant. Where our records indicated that the agent dispersed well, we assumed that any substantial stand of the non-target plant was effectively exposed to the agent, even if the target weed occurred several kilometres away. Other methods used included questionnaire surveys of the biosecurity staff of regional councils for visually obvious, but sporadically occurring agents such as *Tyria jacobaeae* (L.) (cinnabar moth). For more cryptic agents, material was collected and/or dissected to see what emerged. Where the identity of dissected larvae could be confused, they were reared through to adults to confirm identifications. For the agents of thistles in the tribe Cardueae, the only valued plants in the tribe in New Zealand are minor crops such as *Cynara scolymus* L. (globe artichoke). To date, no quantitative surveys have been conducted, but several commercial growers of globe artichoke have been contacted and asked about levels and types of insect attack that they experience on their crops.

Results and discussion

Past investigations of non-target effects of weed biocontrol agents in New Zealand have been sporadic, and systematic surveys were only carried out on a local scale if a report of suspected non-target damage was received. The search for non-target effects is now an integral part of biological control practice in New Zealand, and this is the first report on this initiative. Biological-control introductions began in New Zealand in 1929, with the numbers of agents introduced per decade reaching 13 in

the 1990s (Fig. 1). All species of biocontrol agents released in New Zealand against alien weeds were subjected to host-range tests to determine their safety prior to release. In general, testing methods were acceptable by modern standards, typically involving feeding, development and oviposition tests on appropriate life-history stages. Tests in the presence and absence of the normal host plant were carried out in most cases, but there were a few exceptions, which are discussed on a case-by-case basis below. In some cases, details of testing procedures were poorly reported, e.g. number of replicates, source of insects/plants, whether they were reused in sequential tests, or the state or age of plants.

The generally satisfactory nature of methods for host-range testing of potential weed biocontrol agents in New Zealand was an encouraging finding from this retrospective analysis, given the relatively recent development of standard protocols in host-range testing (Wapshere 1989, Withers *et al.* 1999). More surprising was the very early inclusion of native New Zealand plants of no economic importance in the tests of the first two agents to be released (Fig. 1): *Tyria jacobaeae* (cinnabar moth), released in 1929 against *Senecio jacobaea* L. (ragwort), was tested using eight native *Senecio* species (Miller 1970); *Exapion ulicis* (Forster) (gorse seed weevil), released in 1931 against *Ulex europaeus* L. (gorse), was tested using three native Fabaceae (Miller 1970). None of these native species were of economic significance, and so this was an unusually early example of concern for avoiding damage to native plant species in weed biocontrol programs. However, from 1943 to 1982, 13 introductions relied on testing carried out by programs for other countries, so native New Zealand test plants were invariably not tested (Fig. 1). Since 1990, native plant species have always been included in the host-range testing of weed biocontrol agents for New Zealand.

Testing was considered adequate or good for 72% of the 32 weed biocontrol agents established in New Zealand (Table 1). Of the nine agents where some inadequacies were identified in the host-range tests, seven were older examples where modern programs would have included additional plant species related to the target weed. In one case, *Bruchidius villosus* Fabricius, there appeared to be some omissions in the testing methods. In the last, very recent example, *Cydia succedana* (Dennis & Schiffermüller), research is ongoing. These nine cases are discussed further below. With 59% of agent species, the host-range testing was considered to have predicted a complete lack of attack on any non-target plant species. There were no examples where potentially serious attacks on non-target plant species were predicted to occur from agents that had been adequately tested. This was probably because such agents were rejected (e.g. Syrett *et al.* 1995).

For the 10 agent species where testing was considered adequate, and the agents are sufficiently common in the field, no effects on any non-target plant species have been detected in the field in New Zealand (Table 2). All of these species have been surveyed or tested quantitatively in the field, with the exception of the four thistle biocontrol agents; *Rhinocyllus conicus, Trichosirocalus mortadelo* Alonzo-Zarazaga & Sánchez-Ruiz, *Urophora solstitialis* (L.), *and U. stylata* (Fabricius)). The most closely related valued plants to thistles in New Zealand are minor crop species in the tribe Cardueae such as *Cynara scolymus* (globe artichoke). Growers of *C. scolymus* report that they experience no insect damage on their crops (despite being in areas where thistle biocontrol agents are present), and as a result do not need to use insecticides. We take this as good preliminary evidence that the widespread thistle agents in New Zealand are not having any non-target effects on *C. scolymus*. To summarize, where testing

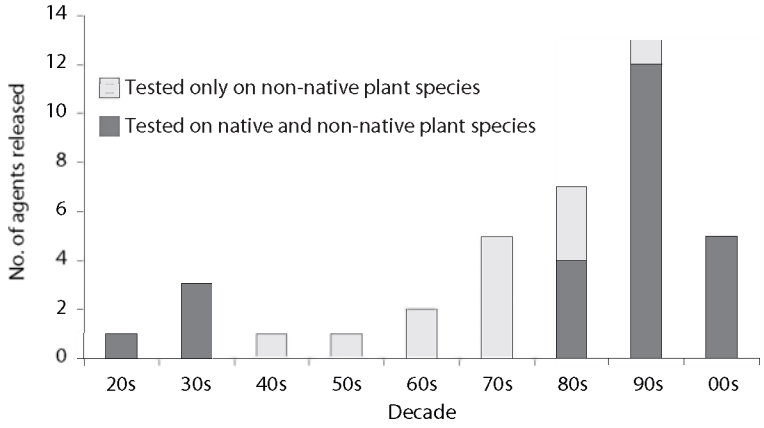

Figure 1. The number of weed biocontrol agents released against alien weeds in New Zealand per decade, distinguishing those species where native plant species were included in the host-range tests prior to release of the agent, from those where only economically important, non-native plant species were tested.

was adequate (methods used and plant species tested) the predictions of non-target effects in the field appear to be conservative; even where testing suggests that some minor effects might be expected, our field surveys have failed to find any such effects.

In the following section, we examine the safety record of the nine agent species established (and sufficiently common) in New Zealand where we consider that the host-range testing was, by modern standards, inadequate in some way (Table 2). These include the one example in New Zealand, noted in several previous publications (e.g. Fowler *et al.* 2000), where a deliberately released weed biocontrol agent, *Tyria jacobaeae* (cinnabar moth), is attacking native New Zealand plant species. *Tyria jacobaeae* was tested against eight native and one exotic *Senecio* species. However, only one of these native species, *S. lautus* Willd., a coastal specialist, is still classified in the genus *Senecio* (Webb *et al.* 1988). The effects that occur on the two native non-target plants that were not tested, *S. minimus* Poiret and *S. biserratus* Belcher, are sporadic, and appear to occur only when *T. jacobaeae* larvae defoliate the target weed, *S. jacobaea* (ragwort). According to modern standards, these native *Senecio* species should have been tested. However, they were previously classified in the genus *Erechtites* (Allan 1961), probably explaining why they were overlooked for testing back in the 1920s. The other biocontrol agent for *S. jacobaea* in Table 2, *Botanophila jacobaeae* (Hardy) (ragwort seed fly), was not tested against any other *Senecio* species prior to its release in New Zealand. It has a limited distribution in New Zealand, and has not been surveyed yet. We anticipate that this agent will be shown to be specific to *S. jacobaea* because tight host specificity is a typical characteristic in gall-forming insects.

Another example where testing omitted a plant species involves *Agasicles hygrophila* Selman & Vogt and *Arcola malloi* (Pastrana), released in New Zealand to target *Alternanthera philoxeroides* (Martius) Griseb. (Alligator weed). There are no native plant species in the same family as the target weed, but in recent years an exotic congener, *Alternanthera sessilis* (L.) Roemer & Schultes, has started to be used as a minor vegetable, producing a possible conflict of interest. Manipulation experiments will be needed to test for potential non-target effects, as *A. sessilis* is not common in the wild in New Zealand. The host-range testing suggests that *Arcola malloi* will attack other *Alternanthera* species, but that *Agasicles hygrophila* is more tightly host-specific (Maddox *et al.* 1971).

The only potentially serious, predicted impact of established weed biocontrol agents on non-target native plant species in New Zealand comes from the two *Chrysolina* species and possibly *Zeuxidiplosis giardi* (Kieffer), released against *Hypericum perforatum* L.

Table 1. Adequacy of host-range testing methods according to modern standards, and degree of impact on non-target plant species predicted from the testing, for biological-control agents established against weeds in New Zealand.

Predicted non-target effects	Testing standard good or adequate	Some inadequacies in testing	Totals
None	15	4	19
Minor ± sporadic	8	2	10
Potentially major	0	3	3
Totals	23	9	32

Table 2. Agent species from Table 1, after excluding agents that are rare or were released too recently to allow assessment of non-target effects in the field.

Predicted non-target effects	Testing standard good or adequate	Some inadequacies in testing
None	*Exapion ulicis* *Procecidochares utilis* Stone *Rhinocyllus conicus* *Trichosirocalus mortadelo* *Urophora solstitialis* *Urophora stylata*	*Botanophila jacobaeae* *Agasicles hygrophila* *Bruchidius villosus* *Cydia succedana*
Minor ± sporadic	*Tetranychus lintearius* Dufour *Phytomyza vitalbae* Kaltenbach *Lochmaea suturalis* Thompson *Longitarsus jacobaeae* (Waterhouse)	*Arcola malloi* *Tyria jacobaeae*
Potentially major		*Chrysolina hyperici* (Forster) *Chrysolina quadrigemina* (Suffrian) *Zeuxidiplosis giardi*

(St John's wort) from 1943 to 1963. Host-range testing from overseas was used to assess the safety of these insects for release in New Zealand. This testing showed that other, mostly unspecified *Hypericum* species might be attacked by all three agents, although the two New Zealand natives, *H. japonicum* Murray and *H. gramineum* Forster, were probably not tested. More recent observations from Australia show *H. gramineum,* also a native species there, does suffer a high level of attack when it is growing close to *H. perforatum* hosting high numbers of *C. quadrigemina* (A.J. Willis, pers. comm.). In contrast, *Z. giardi* was not found attacking *H. gramineum* in similar circumstances (A.J. Willis, pers. comm.), so this gall former may be more tightly host-specific. The two native *Hypericum* species are not common in New Zealand, and the agents and the target weed now occur sporadically, so manipulation experiments are planned to check for non-target impacts. It is ironic that this, the only complete success of weed biocontrol claimed in New Zealand (Fowler *et al.* 2000), would probably not be sanctioned under current regulations.

An example where host-range testing appears in hindsight to have been inadequate involves *Bruchidius villosus* (broom seed beetle) unexpectedly attacking *Chamaecytisus palmensis* (Christ) Bisby & Nicholls (tagasaste), an exotic relative of *Cytisus scoparius* (L.) Link (broom) (Fowler *et al.* 2000). The cause appears to be the omission of no-choice oviposition tests, combined with low replication, rather than an increase in the fundamental host-range of *B. villosus* (Haines *et al.* 2004).

The final example of unpredicted effects on non-target plant species in New Zealand involves *Cydia succedana* (gorse pod moth). With *C. succedana*, a complex array of host-range tests was conducted including no-choice tests. Field records indicated the moth was narrowly oligophagous in Europe, and the host plants recorded there were tested. Minor attack on non-target flowers and pods was recorded in laboratory tests, but the apparent host range narrowed in more natural tests. Hosts recorded in Europe appeared no more susceptible than other plants tested, and *U. europaeus* was strongly preferred. Hill & Gourlay (2002) concluded that the agent was safe to release. Approval was granted, and *C. succedana* was released in New Zealand in 1992 (Hill & Gourlay 2002). *Cydia succedana* is now abundant in New Zealand, and has recently been reared from the pods of several exotic species in the family Fabaceae other than *U. europaeus*. Surveys have not revealed any attack on native plant species. The phenomenon appears highly variable both temporally and spatially, and could be a "spill-over" effect caused by high moth population density. Research is under way to clarify the extent of the unpredicted non-target impact. We hypothesize that this unpredictability could be due to insufficient duration of the no-choice oviposition tests, variable sources of the introduced populations, or differences in seasonal phenology of the host plants and the moth in its native versus the introduced range. Interestingly, both *B. villosus* and *C. succedana* utilize seasonally ephemeral resources, whose phenology is different in Europe and New Zealand, potentially producing novel no-choice situations to agents in the field in New Zealand.

We need to continue to improve safety-testing methods (Withers *et al.* 1999), but in practice, we probably need to avoid relying on one set of tests, or one set of evidence, for assessing safety. It may also be that agents using discrete, seasonal resources need particular care in host-range testing, and in interpreting data from the field in the native range. However, the overall reliability of methods for host-range testing in past weed biocontrol programs for New Zealand has been high. With the more rigorous regulatory legislation in place in New Zealand, it is important that we can demonstrate a good past safety record for weed biocontrol agents, and that the methods we use for host-specificity testing allow a reliable assessment of risk.

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Ruling out a host-range expansion as the cause of the unpredicted non-target attack on tagasaste (*Chamaecytisus proliferus*) by *Bruchidius villosus*

Melanie L. Haines,[1] Pauline Syrett,[2] Rowan M. Emberson,[1] Toni M. Withers,[3] Simon V. Fowler[2] and Sue P. Worner[1]

Summary

Scotch broom (*Cytisus scoparius*) is a woody shrub of European origin that is an invasive weed in New Zealand. *Bruchidius villosus* was released in New Zealand in 1986 as a biological control agent of Scotch broom, after tests indicated that it was specific to this species. However, in 1999, *B. villosus* was discovered developing in the seeds of an unpredicted host, tagasaste or tree lucerne (*Chamaecytisus proliferus*). Although the original choice tests carried out in quarantine failed to predict acceptance of *C. proliferus* by ovipositing females, the current population in New Zealand clearly finds this species an acceptable host. An investigation of the original host-testing procedures revealed a number of possible limitations in the tests conducted in the 1980s. Concerns that a host-range expansion might have occurred in a weed biological control agent led to this study in which beetles from the original population (Silwood Park, United Kingdom) were reimported and the original handling and host choice tests were replicated. Despite showing a strong preference for Scotch broom, the beetles tested in this study accepted *C. proliferus* for oviposition. These results allow us to rule out the possibility that a host-range expansion has occurred.

Keywords: *Bruchidius villosus*, *Chamaecytisus proliferus*, *Cytisus scoparius*, host-range expansion, host-specificity testing.

Introduction

Scotch broom, *Cytisus scoparius* (L.), Link is a woody shrub of European origin that is an invasive weed in many countries, including New Zealand, Australia and North America. The broom seed beetle *Bruchidius villosus* (F.) (previously referred to as *B. ater* (Marsham)) was identified as a potential biological control agent for New Zealand's Scotch broom weed problem because it was thought to attack only *Cytisus* species. Host-specificity testing began in the Inted Kingdom (UK) in 1985 and consisted of no-choice oviposition tests with adults being confined to either whole potted plants or to single branches of larger plants inside cotton mesh sleeve cages (Syrett & O'Donnell 1987). All hosts were required to be bearing young green pods (the stage of pod on which the broom seed beetle oviposits) at the time of testing. Thirteen species of non-target plants were tested, also seven species of potted non-target plants were tested together with *C. scoparius* in a choice test within a field cage in the UK (Syrett & O'Donnell 1987). In all these assays, eggs were only laid on *Cytisus* species (*C. scoparius* and *C. praecox* cv. Allgold). The insect was released as a biological control agent in New Zealand in 1986.

In 1985, *B. villosus* was imported into quarantine in New Zealand as newly emerged beetles from the UK *C. scoparius* pods. Normally in the UK, such beetles would overwinter for about six months, feeding on flowers in the following spring, pbefore becoming

[1] Ecology and Entomology Group, Soil, Plant and Ecological Sciences Division, PO Box 84, Lincoln University, Canterbury, New Zealand.
[2] Landcare Research, PO Box 69, Lincoln, New Zealand.
[3] Forest Research, PB 3020, Rotorua, New Zealand.
Corresponding author: M. Haines <hainesm2@lincoln.ac.nz>.

reproductively mature. However, because New Zealand is in the Southern Hemisphere, the beetles were fed on arrival with bee pollen, honey water and/or fresh flowers for approximately eight weeks (without overwintering), bringing them into reproductive maturity for the oviposition tests. The results of the choice tests with nine species of pod-bearing plants (six New Zealand natives and three exotics), indicated that *B. villosus* would be host-specific to *C. scoparius* in New Zealand (Syrett & O'Donnell 1987). One of the exotic species was tagasaste, *Chamaecytisus proliferus* (L. f.) Link (known as *C. palmensis* (Christ) Bisby & K. Nicholls in New Zealand).

In spring 1994, adults of *B. villosus* reared from *C. scoparius* from New Zealand were imported into quarantine in Australia. The results of choice tests on 18 native Australian species and 10 exotic species supported the New Zealand results, also indicating that *B. villosus* was host-specific to *Cytisus* species (A. Sheppard, unpublished data).

In 1999, however, *B. villosus* was found emerging from *C. proliferus* seeds in New Zealand, and further studies showed that this plant was a suitable and commonly utilized alternative host (Syrett 1999). At the time *C. proliferus* had only been tested in choice tests with *C. scoparius* as a control, in quarantine in both New Zealand and Australia. It was not included in the UK no-choice and choice tests because it does not produce pods in the colder climate of the UK. *C. proliferus* is native to the Canary Islands, which have a significantly warmer climate than the UK, and is grown abundantly in New Zealand where it has naturalized extensively. It is regarded as weedy in some places in New Zealand (Williams & Timmins 1990), but also has benefits including use as fodder in high country farms when there is drought (Douglas *et al.* 1996), as a pollen source for beekeepers (Dann & Trimmer 1986), and as a supplementary food source for the threatened native pigeon in New Zealand (McEwan 1978).

Although it has now been shown that choice tests including the target species are not the most robust method for observing acceptance of lower ranked alternative host plants (Marohasy 1998, Edwards 1999, Hill 1999, Heard 2000, Purcell *et al.* 2000, Barton Browne & Withers 2002), we are nevertheless surprised that the original choice tests in New Zealand did not reveal the relative acceptability of *C. proliferus*. It seemed plausible that a host-range expansion (Dennill *et al.* 1993), otherwise referred to as a host shift (Howarth 1991), had occurred in the population of established beetles in New Zealand some time in the 14 years since its introduction (Syrett 1999). Many purported host-range expansions, defined by Marohasy 1996 as "feeding by biological control agents on plant species other than those on which they were known to feed prior to their release", have been reported in weed biological control. Marohasy (1996) argued that these were caused by other phenomena, such as preadaptation (established behavioural concepts), threshold change as a result of host deprivation, or effects of experience (learning). This study investigates the possibility that a host-range expansion may have occurred in *B. villosus*. Oviposition acceptance behaviour of the current New Zealand population of *B. villosus* was compared with beetles collected from Silwood Park, the same field site where the original beetles had been collected for shipment to New Zealand in the 1980s. Our hypothesis was that, if British beetles still refused to accept *C. proliferus* for oviposition, while their New Zealand progeny now accepted it, then a host-range expansion would indeed be the most likely explanation.

Materials and methods

In June 2002, adult *B. villosus* were beaten from *C. scoparius* at Silwood Park, UK. These beetles were placed into 1 m diameter by 2 m long, 1 mm mesh sleeve cages on branches of *C. scoparius* bearing young pods. In July, infested pods were picked from the sleeves and held in a glasshouse in mesh bags until emergence. The emerged adult beetles were reimported into quarantine in New Zealand in August 2002. Repeating the same procedure as carried out in 1985, 150 adults were maintained in Perspex cages with ample bee pollen and honey water, followed by *C. scoparius* flowers, under a 22:16°C (day:night) temperature regime with a day length of 14:10 L:D. Relative humidity was approximately 70%.

Host-specificity tests undertaken in the original study in 1985 were replicated as far as possible in 2002 using UK beetles, and in 2001 using New Zealand beetles (field collected from *C. scoparius*). The procedures recorded in the original quarantine laboratory books were followed as closely as possible, however minor differences were required with regard to timing and experimental design.

Perspex boxes (220 × 130 × 100 mm), with flexible push-on lids and four, 25-mm diameter gauze-covered holes for ventilation, were used as test cages. Moistened blotting paper was placed at the bottom of the cage, and several pieces of tissue paper were included to absorb excreta. *Cytisus scoparius* twigs, approximately 200 mm long, bearing young green pods, were placed in vials of water in each test cage. A disc of plastizote, 6 mm thick, with the twigs pushed through its centre, acted as a stopper for the vial, which was supported at an angle to ensure the shoot remained in the water. Twigs of each test plant were selected such that they had approximately equal amounts of pod material and pods judged to be at an equivalent developmental stage to the *C. scoparius* pods. Test material of the different plant species, prepared in the same way as the *C. scoparius*, was placed in each cage with an equivalent amount of *C. scoparius*, to constitute paired choice tests comprising *C. scoparius* and a test plant (Syrett & O'Donnell 1987).

Test-plant material was collected from at least three different plants for each species. Beetles were held in each test cage for 6 days during the tests. Beetles were fed pollen and provided with cotton dental rolls soaked in a honey–water solution. After the beetles were removed, all plant material and cages were carefully examined for eggs. The numbers of eggs found on the pods of *C. scoparius* and each of the test species were recorded. Each phase of the experiment was conducted when each of the test-plant species had pods available at the appropriate stage of development (Table 1). Not all plant species were tested at the same time, therefore. Every attempt was made to ensure laboratory conditions, cage type used, number and sex ratio of beetles, bee pollen source, twig size, approximate number of pods presented, the presentation of pod material, duration of assays, and approximate timing of presentation of various host plants, were the same as in the 1985 experiments (Table 1).

In the original choice tests conducted in 1985, one or two replicates were used for each test plant species, whereas 4 replicates for each test plant were used in 2001 and 10 in 2002. Each replicate contained five male and five female beetles.

Results

In 1985 female *B. villosus* laid a mean of between 4.2 and 18.4 eggs each on *C. scoparius* and 0 eggs on the test plants (Table 2). In 2002 tests, *B. villosus* laid a mean of between 3.0 and 12.3 eggs each on *C. scoparius*, and 0.7 eggs on the test plant *C. proliferus*. The range of eggs laid on *C. proliferus* in 2002 was between 0 and 2.6 eggs per female and only 4 out of 10 replicates had eggs laid on them at all. In the 2001 tests using beetles field caught from *C. scoparius* in New Zealand, female beetles laid a mean of between 18.1 and 25.5 eggs on *C. scoparius* and a mean of 1.0 egg each on the test plant *C. proliferus.* The range on *C. proliferus* was between 0.2 and 2.6 eggs per female and in each of the 4 replicates at least 1 egg had been laid.

In the four replicates of the 2001 tests with New Zealand field-collected beetles, a total of 20 eggs was laid on *C. proliferus* by a maximum of 12 females. In

Table 1. Timing of two-choice tests with material presented to *Bruchidius villosus* from various origins. The tests included the target weed *Cytisus scoparius* and the following plant species: *Carmichaelia australis* G. Simpson, *Carmichaelia petriei* T. Kirk, *Carmichaelia stevensonii* (Cheeseman) Heenan, *Carmichaelia williamsii* T. Kirk, *Chamaecytisus proliferus*, *Clianthus puniceus* (G. Don.) Sol., *Cytisus multiflorus* (L'Her) Sweet., *Genista monspessulana* (L.) L.A.S. Johnson, *Laburnum anagyroides* Medikus., *Sophora microphylla* Aiton, and *Sophora prostrata* J. Buchanan.

Weeks	1985 UK import (1 or 2 reps)	2001 NZ origin (4 reps)	2002 UK import (10 reps)
1–5. (2nd week Sept – 3rd week Oct)	*C. scoparius* flowers and bee pollen		*C. scoparius* + *C. proliferus* flowers and bee pollen
6. (4th week Oct)	*C. scoparius* flowers, green pods and bee pollen	Beetles collected continuously off *C. scoparius*	C. scoparius vs *C. proliferus* No eggs laid
7. (1st week Nov)	*C. scoparius* flowers, green pods and bee pollen First eggs laid	*C. scoparius* vs *C. proliferus* First eggs laid	C. scoparius vs *C. proliferus* No eggs laid
8. (2nd week Nov)	*C. scoparius* vs *C. proliferus*	*C. scoparius* vs *S. microphylla*	*C. scoparius* vs *C. proliferus* First eggs laid
9. (3rd week Nov)	*C. scoparius* vs *S. microphylla*	*C. scoparius* vs *S. prostrata*	*C. scoparius* vs *S. microphylla*
10. (4th week Nov)	–	*C. scoparius* vs *C. multiflorus*	*C. scoparius* vs *C. australis*
11. (1st week Dec)	*C. scoparius* vs *C. australis*	*C. scoparius* vs *G. monspessulana*	*C. scoparius* vs *C. petriei*
12. (2nd week Dec)	*C. scoparius* vs *C. petriei*, *C. williamsii, G. monspessulana*, *C. puniceus* & *C. multiflorus*	*C. scoparius* vs *L. anagyroides*	*C. scoparius* vs *C. williamsii*
13. (3rd week Dec)	Repeated *C. scoparius* vs *C. multiflorus*		*C. scoparius* vs *C. puniceus*
14. (4th week Dec)	–		*C. scoparius* vs *C. multiflorus*
15. (1st week Jan)	–		*C. scoparius* vs *G. monspessulana*
16. (2nd week Jan)	–		*C. scoparius* vs *C. stevensonii*
17. (3rd week Jan)	*C. scoparius* vs *C. stevensonii*		*C. scoparius* vs *L. anagyroides*

Note: in Syrett and O'Donnell (1987), *C. proliferus* was referred to as *C. palmensis* (Christ) Bisby & Nicholls, *C. australis* as *C. ovata* G.Simpson, and *C. stevensonii* as *Chordospartium stevensonii*.

the 10 replicates in 2002 with UK imported beetles, a total of 33 eggs was laid by a maximum of 19 females in only 4 of the replicates. There was no significant difference in the overall mean number of eggs laid per female per replicate on *C. scoparius* between the sequential choice tests conducted in 1985 and 2002 with beetles imported from the UK (*t*-test, $P = 0.5$, df = 12). The overall mean number of eggs per female per replicate was 9.1 and 7.9, for beetles in 1985 and 2002, respectively (excluding *Laburnum anagyroides* which was an extra plant in the 2002 sequence). The overall mean number of eggs laid per female per replicate in the 2001 tests on New Zealand field-collected beetles was 22.3, which is more than double the mean in the other tests.

Discussion

For an expansion in fundamental host range to occur in phytophagous insects, so that an insect can move from one host plant to another, a "host race" must first develop. To be classified as a host race (defined in Marohasy 1996) populations must first fulfil the following criteria: (1) be non-interbreeding and sympatric; (2) differ in biological characteristics, but not (or only marginally) in morphology; and finally (3) be prevented from interbreeding as a result either of preference for different host-plant species, or as a consequence of physiological adaptation to different host-plant species.

So which of the above criteria have either been fulfilled or have the potential to be fulfilled in New Zealand with *B. villosus*? Firstly, it seems that *B. villosus* adults emerging from both *C. scoparius* and *C. proliferus* are interbreeding. Beetles emerging from each species of pods at similar times have been observed mating (M. Haines, personal observation). Furthermore, both plant species frequently grow in the same area, and within the same habitats in New Zealand (no geographical isolation). *Bruchidius villosus* shows high mobility, and therefore it appears the insects continue to interbreed after emerging from different host pods. Seasonal asynchrony is, however, a possible mechanism that could also lead to sympatric speciation. Certainly *C. proliferus* flowers earlier than *C. scoparius* in spring and is the first available pollen source to *B. villosus* when it emerges from its overwintering period (Fowler *et al.* 2000). However, *C. proliferus* flowers for a longer period and simultaneously with *C. scoparius* over summer, suggesting seasonal asynchrony in New Zealand may be insufficient to lead to sympatric speciation or to prevent interbreeding.

Secondly, the possibility that *B. villosus* has begun to develop different biological characteristics on the two host plants has also started to be investigated. Field observations and initial data gathering in 1999 (M. Haines, unpublished results) and in 2000 (Wittenberg & Thomann 2001) have suggested there is phenotypic plasticity in body size and colour of *B. villosus* depending on the host-plant seed in which they have developed. Adults emerging from seeds of *C. proliferus* are generally larger and sometimes browner in colour than those emerging from the usual host *C. scoparius,* which are smaller and blacker in colour. Whether this phenotypic plasticity is suggestive of different performance or suitability of genotypes according to host plant has yet to be ascertained, but the development of different biological characteristics cannot be ruled out. Laboratory studies will be used to investigate whether or not lines of *B. villosus* reared from different host-plant pods retain oviposition preferences for the species of pod in which they spent their larval development.

Thirdly, we need to establish that *B. villosus* is in the process of being prevented from interbreeding as a result of a preference developing for the new host-plant species. *Cytisus scoparius* remains the preferred host over *C. proliferus* in all choice tests to date (M. Haines, unpublished results), suggesting that no preference has yet developed for *C. proliferus.* The 2001 test results confirm this (Table 2), as beetles randomly collected from the field laid on average 25 times as many eggs on *C. scoparius* as on *C. proliferus.*

So it appears that the criteria that would indicate that a host race has developed, or is in the early stages of developing in *B. villosus,* are not met. The fact that reimported UK beetles accepted *C. proliferus* suggests that a host-range expansion has not occurred in New Zealand, but that for some reason the 1985 tests failed to elicit oviposition on *C. proliferus.*

There are at least two possible explanations for the discrepancy in laboratory testing results between 1985 (no eggs were laid on *C. proliferus*) and 2002 (some eggs were laid on *C. proliferus*). There were differences in the number of *B. villosus* tested (smaller sample sizes in 1985), and beetles may have been treated subtly differently between tests despite best attempts to replicate conditions (Table 1). For example, the 1985 beetles were held for two weeks before testing with very small pods and flowers of *C. scoparius*, which may have caused an unusual degree of excitation towards *C. scoparius* in 1985. In 2002, beetles were held before testing with pods of both *C. scoparius* and *C. proliferus* at the same time, to check for onset of oviposition. In both cases, all beetles imported from the UK had never experienced *C. proliferus* pods before being imported into New Zealand quarantine. All testing was conducted sequentially, but in both 1985 and 2002, the same groups of beetles were reused for each test plant, whereas in 2001, independent groups of beetles were used for each test plant in the sequence.

Having ruled out a host-range expansion, why did the original choice tests not indicate some acceptability of *C. proliferus* pods? The hierarchy-threshold model of host selection (Courtney *et al.* 1989) hypothesizes that insects rank hosts in a hierarchical fashion and that selection of diet by individual insects is determined by

Table 2. Mean number of eggs laid per *Bruchidius villosus* female per replicate in paired cut-shoot choice tests run sequentially over time. The tests included the host plant *Cytisus scoparius* and the following test-plant species: *Carmichaelia australis* G.Simpson, *Carmichaelia petriei* T. Kirk, *Carmichaelia stevensonii* (Cheeseman) Heenan, *Carmichaelia williamsii* T. Kirk, *Chamaecytisus proliferus*, *Clianthus puniceus* (G. Don.) Sol., *Cytisus multiflorus* (L'Her) Sweet., *Genista monspessulana* (L.) L.A.S. Johnson, *Laburnum anagyroides* Medikus., *Sophora microphylla* Aiton, and *Sophora prostrata* J. Buchanan.

Plant material	1985 UK import		2001 New Zealand field		2002 UK import	
	test plant	*C. scoparius*	test plant	*C. scoparius*	test plant	*C. scoparius*
Chamaecytisus proliferus	0	18.4	1	25.5	0.7	12.3
Sophora microphylla	0	6.2	0	19.3	0	4.8
Carmichaelia australis	0	12.2	–	–	0	7.4
Carmichaelia petriei	0	7	–	–	0	9.2
Carmichaelia williamsii	0	4.2	–	–	0	8.5
Genista monspessulana	0	10	0	18.1	0	9.5
Clianthus puniceus	0	7.6	–	–	0	4.3
Cytisus multiflorus	0	12.2	0	21.7	0	9.0
Carmichaelia stevensonii	0	4.2	–	–	0	5.3
Sophora prostrata	–	–	0	25.2	–	–
Laburnum anagyroides	–	–	0	24.8	0	3.0

the host's "acceptability". One prediction of the model is that female oviposition behaviour is influenced by female egg load, such that when egg load is high, so is the tendency for a wider range of hosts to become acceptable (Courtney *et al.* 1989). The overall mean number of eggs laid per female was significantly higher in the New Zealand field-collected beetles tested in 2001 (more than twice that of both 1985 and 2002 UK imported beetles), suggesting these beetles had a higher egg-load than their imported counterparts. So could egg-load explain why the original host tests were not indicative of field host range? The number of eggs laid per female in the 2002 imported beetles was almost three-fold less than that of the 2001 New Zealand field-collected population. Yet, the lower-ranking host *C. proliferus* was still accepted for oviposition at an equivalent rate despite the reduced egg-laying. In both experiments, the minimum number of eggs laid per female on *C. proliferus* was 1.7, and more eggs were laid on *C. proliferus* by beetles with a comparatively low egg-load in an equal number of replicates. So, it appears unlikely that egg-load is responsible for the discrepancy in test results.

We conclude that a host-range expansion has not occurred, but that the 1985 host testing failed to detect the non-target impact of *B. villosus* on *C. proliferus*. From the 2001 and 2002 test results indicating that *B. villosus* laid 18–26 times as many eggs on *C. scoparius* as on *C. proliferus* (Table 2), we might have predicted that its non-target impact in the field would be minor, but the level of seed attack by the beetle in New Zealand is in fact substantial (M. Haines, unpublished data). The implication for biological control releases is that we cannot assume non-target impacts will be insignificant on the grounds that results of choice tests indicate a strong preference for the target plant. On a more positive note, despite the non-target attack on the exotic plant *C. proliferus*, *B. villosus* remains a useful agent against *C. scoparius* in New Zealand as all the test results consistently predict that no native Fabaceae are under any risk of attack (M. Haines, unpublished data).

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Limited success of open field tests to clarify the host range of three species of Lepidoptera of *Mimosa pigra*

Tim A. Heard,[1] Ryan Zonneveld,[1] Ricardo Segura[2] and Moises Martinez[2]

Summary

The search for biological control agents for *Mimosa pigra*, a serious weed in northern Australia, has continued since 1979. Despite heavy damage from released and established agents that feed on the stems, flowers and seeds, more control is needed. Three species of moths that feed on leaves and tie leaves together to form a protective shelter, cause heavy damage in the native range. The species are: *Apotoforma rotundipennis* (Tortricidae), *Aristotelia* sp. (Gelechiidae) and *Pococera gelidalis* (Pyralidae). All species were imported into Australian quarantine for evaluation of their safety for release. Aspects of their biology were studied and reported here. Steps in the insect's natural host selection behaviour are not expressed in cages, resulting in indiscriminate oviposition in the quarantine laboratory. As oviposition preferences could not be tested in these circumstances, we tested the innate developmental host range of larvae. Although *Mimosa pigra* was the superior host for all three moth species, development to adult occurred on other plant species. These tests may have generated false positive results thereby over-estimating the field host range. We then conducted open field tests in semi-natural conditions in Mexico. We grew four individuals of 26 test plant species of Australian and American origin in a field plot. We made successive releases of cohorts of laboratory-reared adults of the three species. All resulting leaf ties were bagged to capture emerging adults. The numbers of adults reared on the 48 *Mimosa pigra* plants was low particularly for the first two species, being 11, 17 and 103 for *Apotoforma rotundipennis*, *Aristotelia* sp. and *Pococera gelidalis*, respectively. The numbers of adults originally released were 295, 437 and 150, respectively. No adults of *Aristotelia* sp. were reared from the test plants but two of *Apotoforma rotundipennis* were reared from the closely related *Mimosa asperata*. However, not much value was placed on the results for *Apotoforma rotundipennis* and *Aristotelia* sp., as the poor return of reared to released adults suggests that the oviposition pressure on plant species was too low. These tests may produce false negative results, thereby under-estimating the field host range. Hence, the realized host range of these species remains unknown. One useful result was that adults of *Pococera gelidalis* were reared from a test plant species, *Desmanthus virgatus,* indicating that this species is probably not sufficiently specific to release in Australia.

Keywords: *Apotoforma rotundipennis*, *Aristotelia* sp., host-specificity tests, *Pococera gelidalis*.

Introduction

The insects used in this study were potential biocontrol agents of the weed, *Mimosa pigra* (Mimosaceae), an introduced, thorny, perennial shrub that forms impenetrable monocultures along watercourses and in floodplains in northern Australia and Southeast Asia (Lonsdale 1992). *Mimosa pigra* (hereafter called mimosa) has been the focus of a biological control program since 1979 (Harley *et al.* 1995, Heard & Segura 2003). Thirteen agents have been released to target various plant parts (foliage, stems, roots, flowers, green pods and mature pods). Damage to the leaves in Australia is still minimal while in the native range it is very apparent. Much of this damage is due to several

[1] CSIRO Entomology, 120 Meiers Rd, Indooroopilly, Queensland 4068, Australia.
[2] CSIRO Mexican Field Station, Carlón No. 5, Ejido 1° de Mayo, Boca del Río C.P. 94297, Veracruz, Mexico.
Corresponding author: Tim Heard, CSIRO Entomology, 120 Meiers Rd, Indooroopilly 4068, Brisbane, Australia <tim.heard@csiro.au>.

species of Lepidoptera that eat leaves and tie them together forming distinctive shelters, in which they remain concealed and protected. The primary aim of this paper is to examine the potential for biological control of the three species of leaf-tying Lepidoptera that could be collected in sufficient numbers for host-specificity testing. A summary of the three insect species under investigation follows.

Apotoforma rotundipennis (Walsingham) (Tortricidae: Tortricinae: Tortricini) was identified by D.J.L. Agassiz (CABI, IIE) and J. Razowski (Polish Academy of Sciences) by examination of pinned specimens. *A. rotundipennis* is reported by Razowski (1993) from the USA (Florida and Texas), the Caribbean Islands and as far south as Brazil. The only recorded hosts for *Apotoforma rotundipennis* are *Acacia macrantha, Acacia arabica* (=*Acacia nilotica)* and marabú (*Dichrostachys cinerea*) in Cuba (Razowski 1966, Razowski 1993). Such a broad host range is unsuitable for a biocontrol agent. However, Razowski stated that the species is variable. Hence, we continued to assess this Mexican taxon in the chance that it was a race or sibling species with a narrower host range.

Aristotelia sp. (Gelechiidae: Aristoteliinae) was identified by D.J.L. Agassiz (CABI, IIE) as probably undescribed and closest to *Aristotelia dasypoda* Walsingham. This was re-assessed by K. Sattler (CABI, IIE), who confirmed it was probably undescribed but stated that the female genitalia most closely matched *Aristotelia hieroglyphica* Walsingham and *Aristotelia pyrodercia* Walsingham. In summary, it is clear that the species belongs to *Aristotelia sensu stricto*, but is probably undescribed.

Pococera gelidalis (Walker) (Pyralidae: Epipaschiinae) was identified by A. Solis (USDA, ARS, SEL). This species ranges from southern Texas to Central America (Solis *in litt.*). Species of *Pococera* have been thought to be highly host specific but this is not true for at least one species (Solis 1993).

Materials and methods

The species were introduced into Australian quarantine from Veracruz state, Mexico, for laboratory determination of host specificity. On arrival, the larvae were placed on mimosa plants and allowed to develop and emerge as adults. The adults were separated into species and pure colonies were maintained.

To keep laboratory colonies variable and viable, several shipments per year of larvae were made over four years from 1996 to 1999. The total number of individuals introduced into quarantine to contribute to colonies for each species was 88 *Apotoforma rotundipennis*, 60 *Aristotelia* sp. and 131 *Pococera gelidalis*.

All species were reared in gauze-covered cages, dimensions 0.5 × 0.5 × 1 m tall. Pairs of adults were placed into cages and allowed to mate and lay eggs. The larvae hatched and developed on the plant. More plants were added if required to feed the larvae. Pupation occurred on the plants and in concealed places around the pot and cage.

The laboratory was artificially lit 14 hours/day plus limited oblique natural lighting; temperature was approximately 27 ± 1°C during the day and 23 ± 1°C at night; RH was 60% ± 10%.

Host-specificity tests

Three methods were used to assess host specificity: 1) laboratory based, larval development tests, 2) field tests of combined adult host searching, oviposition and larval development, and 3) surveys of leaf-tying insects on legumes species.

Laboratory larval development tests

The laboratory larval development tests provide a measure of the suitability and acceptability of plant species for the feeding, growth and development of the immature insect stage. Newly emerged adults were placed in cages with a living potted plant to allow oviposition and larval development. The design was no-choice. The adults were left to die in the cage. The plant was held for emergence of adults of the next generation. One trial consisted of the target weed (*Mimosa pigra*, the control plant) with about five test plant species each in their own cage. After the final emergence of adults from the control cage, the test plant species were examined for damage and evidence of larval development. The trial finished either because there was no larval feeding damage, or if so, after all adults had emerged. The number of adults placed in cages depended on our experience of the number necessary to reliably infest the plant with a number of eggs approximately equal to the ability of the plant to support the resulting larvae. The number of adults was eight pairs for *Apotoforma rotundipennis*, four pairs for *Aristotelia* sp., and six pairs for *Pococera gelidalis*. Sexing of all species was easily done with the naked eye, based on external abdominal morphology. When adults emerged in sufficient numbers from a test plant, these adults were placed back onto the same plant species to determine their viability.

Between 24 and 31 species of plants, most with four replicates, were included in this trial. The species were tested to heavily represent two subfamilies within the Mimosaceae: Mimosae and Acaciae. Mimosae is the subfamily to which *Mimosa* belongs. Acaciae is the subfamily which contains the large and important *Acacia* genus.

Field tests

To confirm the accuracy of the laboratory tests, field tests of combined adult oviposition and larval development were performed. These were conducted in a field plot of the CSIRO Mexican Field Station at La Aguada,

Veracruz State (lat. 19°03.0'N, long. 96°01.8'W, elevation 50 m) in the wet season from June 2000 until March 2001. Adults were reared at the laboratory and, after parasites were excluded, were taken to the field plot for release in the afternoon. Releases were not made every day so some adults were held in the laboratory before release. This was perceived to be advantageous as it increased the chances of females being mated. The numbers released were 295, 437 and 150 for *Apotoforma rotundipennis*, *Aristotelia* sp. and *Pococera gelidalis,* respectively. The numbers of release events were 14, 23 and 10, respectively, between October 2000 and January 2001. Time of day of releases was varied to increase chances of successful infestation on plants within the plot.

Over the following months, all plants in the plot were checked at least weekly. Any leaf ties were enclosed in a bag made of fine gauze. The bags were checked regularly and any emerging adults were collected. These adults were pinned, labelled and identified.

The test plants grew in a series of 4 × 4 Latin square plots. Each plot consisted of a control plant and three test plant species, each with four replicates. A total of 12 plots gave 48 individuals of mimosa and 4 individuals each of 34 test plant species. However, not all the plant species grew well. Plants were visually rated from 1 to 5 on general condition. If the mean condition was below 2.5, then that species was excluded. After excluding eight unhealthy plant species, a total of 26 test plant species and the control species remained in the analysis. The plant species included in the open field trials did not exactly match those in the laboratory trials as many species from the laboratory trials did not grow well in Mexican field conditions.

Field surveys

Leaf-tying insects from other legumes growing in the field were collected opportunistically and reared to adult, pinned and labelled for identification. These collections did not result from the growing of test plants or the release of insects but simply from collections made during general field work.

Results

Biology

Apotoforma rotundipennis. Eggs were laid singly on stems, primary and secondary rachises and the underside of leaves. Young but fully expanded leaves are preferred for oviposition. Eggs are ovoid in shape and 0.9 × 0.3 mm in size. The average development time from adult emergence to adult emergence is 33 days under laboratory conditions. Adults live for a maximum of 10 days. Oviposition starts on the first night and peaks at around the third night.

***Aristotelia* sp.** Eggs were laid singly in concealed places in old bark, between buds and stipules of leaf axils and between pinnae of young unfolded leaves. They are ovoid in shape, 0.5 × 0.3 mm, and cream in colour with orange spots. They hatch in 6–7 days. Larvae build leaf ties from silk and feed within the ties. Larvae do not appear to move from the ties. Larvae are not gregarious and each builds its own tie. However, when conditions are crowded, the ties overlap. Leaves of all ages are used. The average development time from adult emergence to adult emergence is 36 days under laboratory conditions. Adults live for at least 7 days. Oviposition starts on the second night and peaks at around the fourth night.

Pococera gelidalis. Eggs were laid singly on all parts of leaves (upper side) and stems. Eggs are flattened 0.5 × 0.3 mm, and light green or pink in colour. They hatch in 5–6 days. The average development time from adult emergence to adult emergence is 45 days under laboratory conditions. The first sign of larval feeding is frass and silk webbing. Larvae then weave a few pinnules together to form a small leaf tie. Gradually more pinnules are tied together. At about 2 weeks, larvae are tying adjacent pinnae together. Larvae continue to feed, often gregariously, within the leaf tie, until pupation within the ties. Adults live for a maximum of 13 days. Oviposition starts on the first night and peaks at around the third night. After the fifth night, oviposition almost ceases.

Laboratory larval development tests

Mean numbers of adults emerging from mimosa in laboratory larval development tests were consistently high at 157, 128 and 57 for *Apotoforma rotundipennis*, *Aristotelia* sp. and *Pococera gelidalis*, respectively (Table 1). No test plant species produced such high levels of emerging adults. For all insect species, the best performing test species produced about half the number of adults as did the control.

Eight of the 27 test plant species produced adults of *Apotoforma rotundipennis*. For *Aristotelia* sp., this number was 12 of 32, and for *Pococera gelidalis* 16 of 32.

There appeared to be no relationship between insect species in their ability to develop on particular plant species. For example, *Acacia farnesiana* and *Neptunia major* were good hosts for *Apotoforma rotundipennis* but not the other insects. *Acacia deanei* was a good host for *Aristotelia* sp. but not the other insects. Similarly, *Mimosa pudica* was a good host for *Pococera gelidalis* but not the other insects. No plant was a good host for all insect species.

The results of some continuation trials, in which adults emerging from a test plant were placed back onto the same plant species to determine their viability, are presented here. For *Apotoforma rotundipennis* only the adults reared from *Neptunia monosperma* were tested. These were viable, being able to support second

Table 1. Combined results of the laboratory and field host-specificity tests for *Apotoforma rotundipennis*, *Aristotelia* sp. and *Pococera gelidalis*. The data for the laboratory tests are the mean numbers of adults emerging from a cage containing that plant species; those for the field test are the total numbers of insects reared of the plants of that species in the plot.

	Apotoforma rotundipennis		*Aristotelia* sp.		*Pococera gelidalis*	
	Lab test	Field test	Lab test	Field test	Lab test	Field test
Mimosa pigra	157.1	11	127.7	17	56.8	103
Mimosa asperata		2		0		0
Mimosa invisa	0		0.3		0.5	
Mimosa pudica	0	0	5.3	0	33.5	0
Neptunia dimorphantha		0	5.5	0	5.0	0
Neptunia gracilis		0		0	2.0	0
Neptunia major	55.5	0	0.3	0	4.3	0
Neptunia monosperma	75.3	0	3.0	0	21.0	0
Neptunia plena		0		0		0
Adenanthera pavonina	0		0			
Calliandra callocephala		0		0		0
Desmanthus virgatus	14.8	0	0	0	0	1
Dichrostachys cinerea	0		0		0	
Dichrostachys spicata	0					
Entada phaseoloides			0			
Leucaena leucocephala	0	0	0	0	0	0
Prosopis hybrid			0			
Prosopis juliflora	0				0	
Acacia baileyana			5.0			
Acacia botrycephala			0			
Acacia bruinoides					0.5	
Acacia cardiophylla	2.4		0			
Acacia conferta	0					
Acacia deanei	4.3	0	63.5	0	5.0	0
Acacia farnesiana	71.8	0	1.0	0	0	0
Acacia filicifolia	0	0	0	0	1.0	0
Acacia fimbriata	0		0		2.0	
Acacia glaucocarpa	0	0	6.5	0	0.5	0
Acacia holosericea	0		0			
Acacia irrorata	0		0		2.3	
Acacia mangium			0			
Acacia mollifolia			18.5		6.3	
Acacia nilotica	0	0		0	0	0
Acacia oshanesii	0		3.6		1.8	
Acacia parramattensis	4.0	0	12.5	0		0
Acacia saligna			0			
Acacia simsii	1.0	0		0	1.0	0
Acacia spectabilis			41.0		1.0	
Parachidendron muellerianum	0					
Parachidendron pruinosum	0		0			
Paraserianthes lophantha			0		0	
Tamarindus indica	0	0	0	0		0
Caesalpinia pulcherrima		0		0		0
Parkinsonia aculeata		0		0		0
Peltophorum pterocarpum					0	
Senna emarginata		0		0		0
Senna obtusifolia		0		0		0
Senna occidentalis		0		0		0
Aeschynomene americana			0			
Aeschynomene indica	0					
Arachis hypogea		0		0		0
Eriosema violaceum		0		0		0
Sesbania macrocarpa		0		0		0

generation larval development to adult. For *Aristotelia* sp., these tests showed that adults from *Mimosa pudica*, *Neptunia monosperma*, *Acacia baileyana*, and *Acacia deanei* were viable, producing adults in the next generation, albeit in low numbers. Tests for *Aristotelia* sp. on *Neptunia dimorphantha* and *Acacia oshanesii* failed to produce next generation adults. For *Pococera gelidalis*, two species, *Neptunia monosperma* and *Acacia deanii*, were tested. Adults reared off *Neptunia monosperma* proved to be viable by successfully producing next generation adults.

Field tests

A total of 309 adults emerged from the plants in the plot, of which 175 were species not released or relevant to this study. Of the remaining 134 individual insects, only three were reared from the test plant species with 131 from the *Mimosa pigra* control plants.

Despite large numbers of adults being released successively into the field plot over a period of months, only limited attack occurred on the control plant for *Apotoforma rotundipennis* (11 adults) and *Aristotelia* sp. (17 adults). Reasonable numbers of *Pococera gelidalis* were reared from the control plant (103 adults).

In addition to the control plant, two individuals of *Apotoforma rotundipennis* were reared from the closely related *Mimosa asperata*. No adults of *Aristotelia* sp. were reared off test plants. One adult of *Pococera gelidalis* was reared off the test plant *Desmanthus virgatus* (Table 1).

Field surveys

Leaf-tying insects were reared from non-target legume species in the field. The plant species searched were *Mimosa pudica, Mimosa dormiens, Acacia cornigera*, *Acacia farnesiana, Neptunia plena* and *Senna* spp. Only one relevant species was reared: a specimen of *Pococera gelidalis* from *Neptunia plena*.

Discussion

The combination of laboratory tests, open field tests and field surveys was of limited use for predicting the field host range of the three species of Lepidoptera. Sufficient evidence was gained to indicate that one of the three species, *Pococera gelidalis*, was not sufficiently specific for safe release in Australia. However, the other two species may not be testable.

In the laboratory trials, all insect species showed development on non-target plant species. Samples of the resulting adults proved to be viable by producing next generation adults. No test plant species produced such high levels of emerging adults as the control plant. This may be due to the plant being less attractive for oviposition or being less suitable for development. These two possibilities were not separated in this study. To separate them, eggs counts would be necessary.

We believe that the laboratory larval development tests may have generated false positive results. Hence, the host range was possibly over-estimated because the larvae may have developed on some plants species which they would not select for oviposition in the field. These plants are within the developmental host range of the species but perhaps not within the ovipositional host range. Adult oviposition tests could not be performed in laboratory conditions, as adults lay eggs indiscriminately on cages walls and well as plant surfaces (Withers & Barton Browne 1998). For this reason, we did the field tests.

Unfortunately, the field test may have produced false negative results, especially for *Apotoforma rotundipennis* and *Aristotelia* sp. and hence possibly underestimated the host range. That is, they indicated some plants to be outside the host range when they are hosts in nature. This is because the ovipositional pressure on the plants, under the conditions of this study, was low. The low number of emerging adults from the control plants indicates low levels of egg laying. It is possible that the habitat was not acceptable for the field-released adults, and they may have moved rapidly away. Reasonably high numbers of *Pococera gelidalis* emerged from the control plants in the open field plot, indicating high ovipositional pressure and lower probability of false negative results. Indeed, for this species an adult was reared from a test plant.

There were disturbing disparities between the results of laboratory tests compared with field tests and field surveys. The only test plant attacked in the open field trial by *Pococera gelidalis* was *Desmanthus virgatus.* But this plant was not attacked in the laboratory trials. Also, there are disparities between laboratory results and literature records for *Apotoforma rotundipennis*. According to the literature, marabú (*Dichrostachys cinerea*) is a host plant (Razowski 1966; Razowski 1993). But *Dichrostachys cinerea* (and *Dichrostachys spicata*) were tested in the laboratory with negative results. We explain these disparities by high levels of intraspecific variation in discrimination within plant species by this insect. Singer & Lee (2000) show how discrimination within host species can mask or confound discrimination among species. Variation in host use can also result from host plant distribution and abundance (Singer *et al.* 1989).

Field tests are increasingly being used to test the host specificity of potential biocontrol agents (Heard & van Klinken 1998). They have an important role in clarifying ambiguous laboratory results (Briese 1999). They are considered by many to offer the most realistic method for assessing host range, as laboratory testing can produce false positive results leading to the rejection of potentially safe agents (Briese 1999). Field tests are still not common because they are often not required and because of political and logistical constraints (Clement & Cristofaro 1995). Also, they have been criticised as they can produce false negative results when

the agent is unresponsive to lower-ranked hosts (Marohasy 1998), due to low agent densities and high target abundance in the native range (McFadyen 1998). Hence, open field tests are more likely to produce accurate results when the ratio of agents to target is high. For two of the three species included in this trial, agent densities could not be kept sufficiently high to lend confidence to the result of no attack on the test plant species.

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Biotypes, hybrids and biological control: lessons from cochineal insects on *Opuntia* weeds

J.H. Hoffmann[1]

Summary

The quest for genetic diversity in newly released biological control agents and matching of agent genotypes with particular strains of the target weed are widely assumed to ensure enhanced effectiveness of weed biological control. However, there has seldom been concern that these practices may be detrimental. The use of cochineal insects for biological control of cactus weeds in South Africa has disclosed circumstances where adverse effects may arise. Two host-specific biotypes of the cochineal species, *Dactylopius opuntiae* (Homoptera: Dactylopiidae), have been identified and used for biological control of *Opuntia ficus-indica* and *Opuntia stricta* in South Africa. Host specificity breaks down when the two biotypes crossbreed. F_1 progeny of such crosses are able to survive equally well on both opuntia species. The production of hybrid populations should benefit biological control in areas where the two weed species are sympatric, because the chances of a passively dispersed nymph landing on a suitable host plant and surviving will increase proportionately with the combined abundance of the plants. However, the unusual lecanoid chromosome system (i.e. in males, only maternally inherited genes are passed to progeny) found in cochineal insects complicates the issue, because F_2 progeny from crosses between hybrid parents and from backcrosses between hybrids and true-bred parents produce both generalist and specialist progeny in combinations that depend on parental phenotypes. The situation is further complicated because hybridisation in the field is asymmetrical. Males move predominantly from *O. ficus-indica* to mate with females on *O. stricta* but seldom do so in the other direction. Under these circumstances, females may produce nymphs that are genetically maladjusted to the natal host and are thus disadvantaged, to the detriment of biological control. This example shows that the use of sub specific entities to increase genetic diversity of introduced agents may not produce the desired results, and may even have harmful consequences for biological control in some situations. For the control of cactus weeds in South Africa, stringent measures are being employed to ensure that only pure strains of cochineal are released on the target weed species, with positive results for biological control. More generally, a precautionary approach is advised when releases of sub specific mixtures of agent types are planned for other weed species, at least until all possible outcomes have been investigated in full.

Keywords: Cactaceae, cross breeding, *Dactylopius opuntiae*, host specificity.

Introduction

Biological control of *Opuntia* species (Cactaceae) in South Africa has been enhanced recently with verification that *Dactylopius opuntiae* (Cockerell) (Homoptera; Dactylopiidae) comprises at least two distinct host-specific biotypes, each with a restricted host range (Githure *et al.* 1999, Volchansky *et al.* 1999). The two biotypes have been called the "ficus" biotype, which is normally associated with *Opuntia ficus-indica* (L.) Millar and related tree-like species, and the "stricta" biotype, from *Opuntia stricta* (Haw.) Haw. and related shrub-like species. During 1997, the "stricta" biotype, which has contributed to the control of *O. stricta* elsewhere in the world (Dodd 1940, Moran & Zimmermann 1984, Hosking *et al.* 1994, Julien & Griffiths 1998), was introduced into South Africa from Australia, resulting in the spectacular collapse of many infestations of *O. stricta* (Hoffmann & Zimmermann 1999).

The introduction of the "stricta" biotype of *D. opuntiae* into South Africa has brought the two

[1] Zoology Department, University of Cape Town, Rondebosch 7700, South Africa <hoff@botzoo.uct.ac.za>.

known biotypes ("stricta" and "ficus") of *D. opuntiae* into close proximity and has opened opportunities for them to interbreed, which they do readily (Hoffmann *et al.* 2002). Laboratory studies of the results of interbreeding on the host specificity of hybrid progeny have revealed some distinct patterns due to the extraordinary (lecanoid) chromosome system found in cochineal insects (Hughes-Schrader 1948, Brown 1959, Bull 1979, Nur 1990). The consequences of the lecanoid chromosome system on inheritance of host-specificity traits in cochineal insects are addressed by Hoffmann *et al.* (2002). In essence, crosses of the true-bred "stricta" and "ficus" biotypes produce F_1 hybrid progeny which, unlike their parents, are not host specific, developing equally well on either *O. stricta* or *O. ficus-indica*. Some F_2 crosses produce batches of progeny in which all the siblings are either hybrids or all are true-bred genotypes, while other crosses produce batches of siblings in which half of the individuals are hybrids and half are true-bred, depending on the combination of parent genotypes (Table 1) (Hoffmann *et al.* 2002). Variation in the proportions of genotypes produced by cross breeding has potential consequences for biological control of both *O. stricta* and *O. ficus-indica* in South Africa because, in common with other scale insects, survival of passively dispersed, first-instar nymphs ("crawlers") of *D. opuntiae* is dependent on chance encounters with a suitable host plant.

Table 1. Phenotypes of progeny produced by possible crosses between true-bred and hybrid cochineal insects.

Parents		Progeny
♀♀	♂♂	
true-bred	hybrid	true-bred (100%) OR hybrid (100%)
hybrid	true-bred	true-bred (50%) AND hybrid (50%)
hybrid	hybrid	true-bred (50%) AND hybrid (50%)

The probability that a passively wind-borne nymph will land within range of a suitable host plant increases proportionately with the density of available hosts in the vicinity of the host plant (Moran *et al.* 1982). For each of the true-bred "ficus" and "stricta" biotypes, the effective target area for dispersing nymphs will be determined by the respective abundances of *O. ficus-indica* and *O. stricta* plants. In the case of hybrid phenotypes of *D. opuntiae*, there is a relatively greater chance of individual crawlers reaching a suitable host because either *O. ficus-indica* or *O. stricta* will support the nymphs and the combined abundance of the two plant species is the effective target area for the insects. Production of hybrid individuals should benefit biological control because losses of nymphs during passive dispersal will be less frequent and this will enable the insects to more readily reach higher population levels.

The situation is not that simple because back crosses in the F_2 and subsequent generations produce mixtures of both hybrid phenotypes and true-bred phenotypes (Table 1). The advantage of having hybrid individuals in the population will be negated if substantial numbers of the true-bred crawlers are born on host plants that are incompatible with their genotypes. For example, a hybrid female that has matured on *O. ficus indica* and has mated with a "stricta" male will produce a mixture of progeny, half of which are "hybrids" and half of which are "stricta" phenotypes. In such a situation, the "stricta" crawlers will not reach maturity unless they vacate the natal *O. ficus-indica* plant and disperse to an *O. stricta* plant. Under most circumstances many will perish without encountering a suitable host.

The mating patterns of the two biotypes of *D. opuntiae* were observed in the field to measure the extent and uniformity of crossbreeding in an attempt to determine whether or not the insects are benefiting from crossbreeding under natural conditions. The findings are reported and discussed.

Materials and methods

Batches of mature females of *D. opuntiae* were collected from *O. ficus-indica* and *O. stricta* growing in a mixed infestation of the two cactus species near Salem (33°29'S 26°27'E) in the Eastern Cape Province of South Africa. Samples were gathered on five occasions over a five-year period between 15 April 1998 and 14 January 2002. On each occasion, cladodes with cochineal were brought to the laboratory where up to 35 females that were producing crawlers were removed from both *O. ficus-indica* and *O. stricta*. The females were kept isolated in vials. Sixty of the crawlers produced by each female were removed from the vials within 30 hours of being born and divided into two batches. Half of the crawlers in each batch were placed on an isolated cladode of *O. stricta* while the other half was placed on an isolated cladode of *O. ficus-indica*. The cladodes with crawlers were retained in an insectary at 27°C on a 14-hour daylight cycle while the insects developed to maturity and mated. As soon as one of the females on a cladode started to produce crawlers (after about 35 days), all the females were removed from the cladode, weighed and kept separately until they produced crawlers or died without doing so. This quantified the numbers of females reaching maturity, their body masses at maturity and the percentage that had mated.

These three measurements were used to calculate a comparative rate of increase for the batches of siblings on each of the two host plants (see Hoffmann *et al.* (2002), for details of calculations and the interpretation of assays). The measurements revealed the proportion of females that were producing exclusively true-bred progeny on each host plant species, as opposed to females with all or some hybrids among their offspring. The ratios revealed the patterns and extent of crossbreeding between the two biotypes.

Results and discussion

Breeding for more than one generation between the two biotypes of *D. opuntiae* results in the production of cohorts of progeny which include both hybrids and true-bred genotypes (Hoffmann *et al.* 2002). If pairing of males and females is random within the population, then the proportions of "stricta", hybrids and "ficus" phenotypes among the progeny will be 1:2:1, respectively. In mixed stands of *O. ficus-indica* and *O. stricta* the high proportion (50%) of hybrids will ensure that survival of crawlers is enhanced because either plant species will be a suitable host for half (i.e. the hybrids) of the individuals in the population.

Assays revealed that, under field conditions, crossbreeding between the newly-introduced "stricta" biotypes and the long-established "ficus" biotypes of cochineal had commenced within 12 months of the introduction of the "stricta" biotype into the survey site (Fig. 1). Levels of crossbreeding were consistently higher among females collected from *O. stricta* than among females from *O. ficus-indica* (Fig. 1). This discrepancy widened until very few (2.5%) of the females collected from *O. stricta* in the most-recent sample produced exclusively true-bred offspring. In comparison, approximately 90% of the females on *O. ficus-indica* were still producing exclusively true-bred progeny after five years.

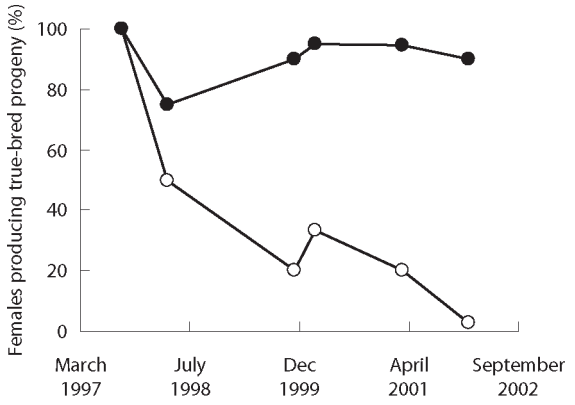

Figure 1. Frequency (%) of *Dactylopius opuntiae* females producing true-bred progeny on *Opuntia stricta* (open circles) and *Opuntia ficus-indica* (closed circles) over a five-year period in a mixed stand of the two cactus species. The "stricta biotype of *D. opuntiae* was released in the area during September 1997.

The results show that crossbreeding was not random but asymmetrical; with crosses between "ficus" males and "stricta" females much more common than those between "stricta" males and "ficus" females. The reasons for this asymmetry are not clear, but four possible hypotheses could explain the phenomenon: (i) "ficus" males are more mobile than "stricta" males; (ii) "stricta" females are more attractive" to "ficus" males than "ficus" females are to "stricta" males; (iii) progeny from crosses between "stricta" females and "ficus" males are less viable than those from other crosses; and (iv) "ficus" males physically outnumber "stricta" males in the region. While the validity of each of these hypotheses needs to be tested, the consequences for biological control are of more immediate relevance.

In South Africa, "stricta" genes have persisted in the cochineal insect populations in hybrid individuals. However, the trend shown in Figure 1 indicates that these genes could be lost completely, especially during unfavourable periods for *D. opuntiae*, when populations of the insects decline to low levels (producing genetic "bottlenecks") e.g. during high rainfall periods (Moran & Hoffmann 1987, Moran *et al.* 1987). Even if the "stricta" genes are not swamped out entirely, the decline in their frequency is almost certain to reduce the effectiveness of the insects as biological control agents of *O. stricta*, and the situation may worsen with time.

An additional indirect consequence of the imbalanced pairing (i.e. asymmetrical crossbreeding) between the two genotypes will be a disproportionately high number of crosses between hybrid females and "ficus" males and between hybrid females and hybrid males whose maternal, and therefore functional, genes are of "ficus" origin. In regions where *O. stricta* is relatively more abundant than *O. ficus-indica*, these crosses will result in many crawlers being born on plants that are not suitable hosts (i.e. "ficus" biotype individuals being born on *O. stricta*). Many of these progeny will fail to reach maturity, either because they settle on an incompatible host or because they failed to disperse onto a suitable host before settling. This attrition of crawlers will negate the benefits of hybridisation. While the potential losses should not affect the biological control performance of *D. opuntiae* on *O. ficus-indica*, the populations of cochineal on *O. stricta* will be suppressed, with a corresponding drop in levels of damage inflicted on these plants.

The possible detrimental effects resulting from the asymmetrical patterns of crossbreeding in cochineal insects have not been quantified as yet. When this is done, it may be found that other factors serve to alleviate potential problems due to the attrition of "stricta" genes. Until the situation is clarified, every effort is being made not to mix the two biotypes of *D. opuntiae* in areas where *O. ficus-indica* and *O. stricta* do not yet occur together. This is a precautionary measure based on the current extent of our knowledge. The outstanding success of the "stricta" biotype in monocultures of *O. stricta* (Hoffmann & Zimmermann 1999) indicates that this approach is paying dividends.

There remains the intriguing possibility that the relatively recent releases of the "stricta" biotype in South Africa could weaken biological control of one or both of the target weed species. The potentially undesirable, and irreversible, consequences for biological control of mixing biotypes from different provenances should be

a cautionary lesson for biological control workers in general, and provide sufficient motivation for including these considerations in pre-release tests aimed at determining the biology and host specificity of new agents.

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Putting the phylogeny into the centrifugal phylogenetic method

Dean G. Kelch[1] and Alec McClay[2]

Summary

Phylogeny has long been recognised as an important concept in constructing test plant lists for use in host specificity testing. In the absence of detailed, explicit phylogenies, taxonomic classifications have often been used as a framework for test plant selection. Unfortunately, traditional taxonomies often do not reflect phylogenies accurately. These inaccuracies can take several forms; some taxonomic classifications represent unnatural (polyphyletic) groups. However, the most common instances reflect paraphyletic classifications, in which some, but not all, descendents of a most recent common ancestor are grouped taxonomically. Explicit phylogenies, many based on comparison of DNA sequences, are becoming available for many plant groups. The Internet is an excellent guide to these phylogenies, as one can find references to peer-reviewed articles, abstracts, and unpublished research information. Explicit phylogenies should provide a basis for test plant choice, but the process will also be informed by such guideline criteria as economic importance, regulatory interest, geographic proximity, and ecological similarity. Common phylogenetic patterns include those in which the target taxon is equally distantly/closely related to plant species of concern. This scenario indicates a broad, equally distributed choice of exemplar taxa for host specificity testing. If the target taxon is more closely related to some taxa of concern than it is to others, then a graduated sampling strategy is indicated. Some specific examples are discussed that illustrate these common outcomes.

Keywords: biocontrol, phylogenetic method, phylogeny, weeds.

Introduction

In assessing potential agents for the biological control of weeds, food plant specificity studies play a vital role. Because these studies entail assessing the potential agent's response to a significant number of plant species or cultivars, they utilize a considerable portion of the time and money allotted for agent development. Therefore, one must use criteria for choosing test plants that are both efficient and effective in evaluating potential agents.

There are several major criteria that are used in choosing test plants for food plant specificity studies including propinquity, relationship, and importance. Propinquity refers to the occurrence of the test plant within the release region. Generally speaking, a broad interpretation of release area is preferred, as the vagility of the potential agent is rarely known. In addition, once an agent is approved for release, there are rarely significant regulations preventing the release of an agent throughout the country (see Nechols 2000, Louda & O'Brien 2001). In the case of large countries such as the US and Australia, this can lead to spreading the agent throughout an entire continent. Degree of relationship is an important criterion for choosing test plants, as there are strong correlations between agent host range and taxonomy. Importance of the plant chosen for testing has traditionally applied to agricultural and important range species, but can equally apply to ecologically important plants, as well as rare or endangered species.

The ideal test plant would fulfil all three criteria mentioned above. Nevertheless, the most important criterion is degree of relationship. It is the close relatives of the target weed that are most likely to share the critical features that allow an agent to feed and breed successfully (e.g. chemistry, morphology, and phenology). Ideal biocontrol agents should be monophagous (restricted to the target plant) or, if

[1] University and Jepson Herbaria, University of California, Berkeley, CA 94525, USA.

[2] Alberta Research Council, Bag 4000, Vegreville Alberta T9C 1T4, Canada.

Corresponding author: Dean G. Kelch <dkelch@sscl.berkeley.edu>.

oligophagous (restricted to the target plant and a few related plants), then the non-target plants should not occur in the release area and/or they should be troublesome weeds themselves. It is the close relatives to the target weed that will provide the acid test of monophagy.

Wapshere (1974) emphasized phylogenetic (evolutionary) relationships as the most important criterion for choosing plants to test (although he also acknowledged non-phylogenetic criteria). He called this the centrifugal phylogenetic method. At the time, explicit phylogenies, that is to say detailed hypotheses of evolutionary relationships among organisms, were rare. However, as taxonomy was viewed as being based primarily on phylogeny, it was recommended that the hierarchical taxonomic groupings be used as a guide to test plant choice.

Phylogeny versus taxonomy

The field of systematics has undergone major changes in the last 30 years, the most profound of which is the development of phylogenetic systematics. This field attempts to reconstruct the evolutionary tree of life. A huge body of literature has been generated on the theoretical bases and practical methods for inferring evolutionary relationships and constructing phylogenies. Putative relationships are illustrated by using branching diagrams or trees. Although interpreting these trees quickly entails some preparation, they are powerful cognitive tools. In addition, the development of comparatively inexpensive and rapid methods for sequencing genes and other molecular markers has resulted in a source of new data for use in phylogenetic analysis. By comparing the sequences of particular genes across taxa, systematists have been achieving great insights into the structure of the tree of life. What study after study has found is that traditional taxonomy is not necessarily a pure reflection of phylogeny. Although artificial (polyphyletic) groups are rare in land plants, many taxonomic groups are paraphyletic. This can create problems for planning food plant specificity studies.

Polyphyletic groups are composed of organisms not closely related to each other. They generally are associated on the basis of convergent evolution. Traditional taxonomists were, by and large, able to avoid polyphyletic taxa by comparing whole suites of morphological characters. Polyphyletic groups are more likely to result from single character taxonomy, which was occasionally practised, but more often criticized, by systematists. Nevertheless, when few characters were available to distinguish taxa, polyphyletic groups sometimes resulted. The evidence from DNA sequence data often allows us to identify these unnatural groupings. At the family level, Cornaceae (dogwood family) *sensu lato* (s.l.) and Saxifragaceae (saxifrage family) s.l. are two examples of polyphyletic groupings. In Cornaceae, the small starry flowers with inferior ovaries resulted in dogwoods and the related *Davidia* (dove tree) being classified with the unrelated (to Cornaceae and to each other) *Aucuba* and *Corokia* (see Bremer *et al.* 2002, Xiang *et al.* 2002) (Fig. 1). The herbaceous Saxifragaceae *sensu stricto*, mainly based on the possession of a bicarpellate ovary, traditionally has been associated with several woody taxa such as *Ribes* (gooseberries), *Hydrangea*, *Philadelphus* (mock orange), *Argophyllum* and *Brexia* (Fig. 1). Current evidence indicates that, of these, only *Ribes* is closely related to Saxifragaceae (Savolainen *et al.* 2000, Soltis *et al.* 2000, Soltis *et al.* 2001). The traditional family Scrophulariaceae, which contains a number of weedy genera such as *Linaria*, *Verbascum*, *Veronica*, *Plantago* and *Striga*, has been shown to be composed of at least five distinct monophyletic groups (Olmstead *et al.* 2001).

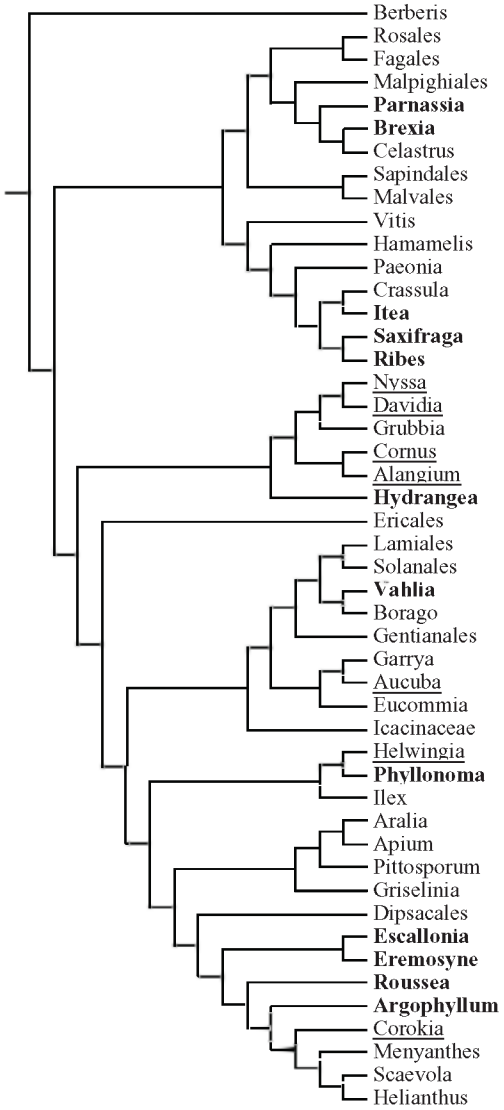

Figure 1. Phylogeny of eudicots showing polyphyly of Cornaceae and Saxifragaceae. Taxa marked in bold represent taxa traditionally placed in Cornaceae. Taxa underlined represent taxa traditionally placed in Saxifragaceae. Adapted from references in text.

At the generic level, recent evidence indicates that the large, woody, legume genus *Acacia* is polyphyletic. *Acacia* subgenus *Acacia*, consisting of about 160 species of pantropical trees and shrubs, is not closely related to subgenera *Phyllodineae* and. *Aculeiferum* (Miller & Bayer 2001, 2003, Robinson & Harrison 2000). *Acacia* subgenus *Phyllodineae* comprises about 960 species from Australasia. In this group are found several economically important species, including some that are noxious weeds in warm temperate and subtropical areas. Unless there is a successful petition to change the type of the genus *Acacia*, the large subgenus *Phyllodinaeae* will become the genus *Racosperma* C. Martius (Maslin *et al.* 2003). As more evidence becomes available, other polyphyletic genera are likely to be identified, especially in large families with poorly differentiated genera (e.g. Compositae, Umbelliferae, and Cruciferae).

Paraphyletic taxonomic groups include some, but not all, of the descendents of a most recent common ancestor. Usually, allied taxa not included within the paraphyletic group display one or more characteristics that make them seem significantly different from their close relatives. Paraphyly is very common in modern taxonomic systems, because, traditionally, paraphyly was ignored or implicitly accepted by systematists.

A classic example of a paraphyletic group is the Pongidae (great ape family), that are paraphyletic to the Hominidae (human family). Based on both morphological and molecular evidence, chimpanzees are more closely related to humans than they are to other great apes (Goodman *et al.* 1998). Therefore, the Pongidae include some, but not all, descendents of the most recent common ancestor of chimps and orangutans. In a purely hypothetical example, if chimpanzees were to become an agricultural pest, one would be in error if one tested potential biological control agents only against members of the Pongidae. Using phylogeny as a guide, such agents, even if narrowly oligophagous, would be much more likely to attack humans than other great apes.

In a plant group that includes weedy taxa, a good example of paraphyly is found in the genus *Brassica* (mustards), which is paraphyletic to *Raphanus* (radishes) (Fig. 2). These genera are distinguished by their fruit morphology; *Brassica* has the typical siliques of the Cruciferae, while *Raphanus* has indehiscent fruits that break into one-seeded sections. Evidence from DNA sequence data shows that *Raphanus* evolved from within the genus *Brassica* (Yang *et al.* 1998, 1999). Both of these groups include weedy strains as well as important food cultivars. Any food plant preference studies carried out within this group should include test plants chosen based on phylogenetic relationships rather than taxonomic grouping. Other paraphyletic plant groups include *Arabis* and Chenopodiaceae. *Lepidium* (Cruciferae) is paraphyletic to the weedy genus *Cardaria* (Mummenhoff 2001). Chenopodiaceae is paraphyletic to the family Amaranthaceae (Downie 1998); this has led to the proposal to unite the two families under the older name Chenopodiaceae.

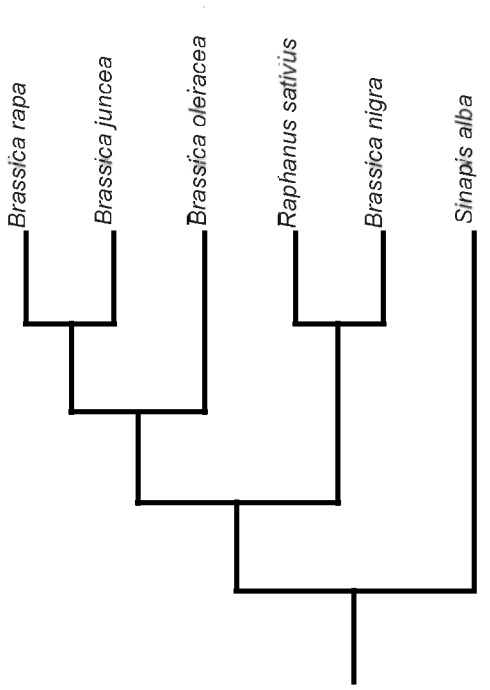

Figure 2. Paraphyly of *Brassica* in regard to *Raphanus*. Phylogenetic tree based on Yang *et al.* (1999).

Phylogeny and biocontrol

Briese *et al.* (2002), in their study of potential biocontrol agents of *Onopordum* (scotch thistle), illustrated how a phylogeny, even if it is not fully resolved, can be used in guiding test plant choice. Briese *et al.* (2002) numbered the clades (groups) or nodes on a simplified phylogeny of the Compositae based on degree of relationship. Theoretically, those taxa in clade 1 would be more heavily sampled than those in clade 2, but not in clade 1. At the distant level of 4 and 5, no sampling was deemed necessary. The authors point out that using this information allows one to save time and money by excluding distantly related plants, even if they are classified in the same family.

Hypericum perforatum (St John's wort), native to Europe, is a noxious range weed in large areas of western North America. *Hypericum* is a large genus of 350–400 species; in North America alone, there are about 60 taxa of *Hypericum*. In the literature on the biocontrol of *H. perforatum*, there are accounts of differential feeding by potential agents on species within the genus. As there are too many taxa to test them all, a phylogenetic framework would be invaluable in choosing the most critical *Hypericum* species for inclusion in food plant specificity studies. Preliminary results of a molecular systematic study (Park & Kim 2001) indicate that *H. perforatum* is more closely

related to some native North American species of *Hypericum* than it is to others. *Hypericum* section *Hypericum*, which contains *H. perforatum* as well as some North American species (e.g. *H. concinnum*) is paraphyletic. Thus, there is evidence from the phylogenetic structure within *Hypericum* that differential sampling within the genus would be indicated in studies of potential biocontrol agents of *H. perforatum*.

The genus *Cirsium* (true thistles) represents an interesting problem in North America. There are introduced species that are noxious weeds (e.g. *C. arvense* and *C. vulgare*), as well as over 90 native taxa, some of which are critically endangered. The traditional taxonomy implies that infrageneric groups (sections) are distributed in both the Old and New Worlds (Petrak 1917). However, a preliminary phylogeny of the genus (Kelch & Baldwin 2003) indicates that the North American native taxa form a clade separate from all Old World *Cirsium* (Fig. 3). Therefore, all North American *Cirsium* are equally distantly related to any Old World species. This result calls for an even sampling of North American taxa in food plant specificity studies.

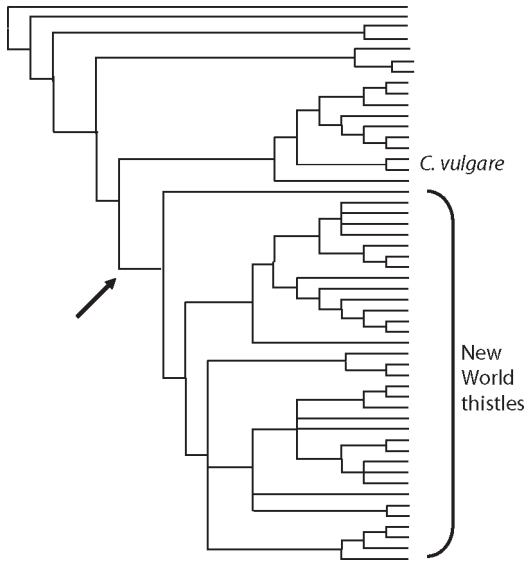

Figure 3. Phylogeny of *Cirsium* (true thistles) indicating New World Clade (arrow) and placement of weedy taxa *C. arvense* and *C. vulgare*. Based on Kelch and Baldwin (2003).

Conclusions

If a well-resolved phylogeny is available, we can use phylogenetic inference to avoid wasting resources on superfluous sampling. In a hypothetical example, two sampled taxa are attacked by the potential biocontrol agent. It is quite likely that all members of the clade representing all descendents of the most recent common ancestor of the two attacked taxa are potential food plants for the agent being studied. Based on the evidence indicated, one cannot rule out that there will be feeding beyond the clade of interest. Nevertheless, this information allows one to concentrate sampling on those untested taxa most likely to be attacked by the potential agent. Note that phylogenetic inference allows information from studies from other geographic regions to inform test plant choice in another region. In addition, inferring the potential pool of vulnerable plant taxa based on limited sampling allows early rejection of candidate agents that show feeding patterns that are too broad for desirable biocontrol agents.

Criteria other than degree of relationship are important in test plant choice as well. These include economic importance, rare or endangered species, ecologically important species, and species of particular concern to government agencies (e.g. wetland species in the US). Nevertheless, all of these secondary criteria should be considered within a phylogenetic framework. Sampling distantly related taxa is a waste of time and resources (Pemberton 2000, Briese 2003).

Much information on plant phylogeny is available on the Internet, but as yet there is no central repository of information. A search should start on the Treebase website <http://www.treebase.org/treebase/>, which is meant as a source for information on the phylogeny of all life. However, as submission of information is voluntary, the results of many studies do not appear in this database. Many journals demand that authors of manuscripts including nucleotide sequences submit all such sequences to Genbank <http://www.ncbi.nlm.nih.gov/>. Multiple sequences for the particular gene in a specific plant group generally indicates a phylogenetic study published or in press. Most entries also include information regarding the purpose of the research that generated the sequence, as well as any pertinent publications. Primary literature database services such as Biosis <http://www.biosis.org>, Web of Science <http://wos.mimas.ac.uk/>, and Agricola <http://www.nal. usda.gov/ag98/> are excellent sources of citations of phylogenetic studies. General Internet search engines such as Google <www.google.com> often can provide information on plant phylogeny. Many professional scientific societies post abstracts of their meetings and many scientists have professional webpages that cite their research interests. These can be a useful source of information on publications and/or addresses of potentially informative personnel.

Acknowledgements

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Risk assessment of *Gratiana boliviana* (Chrysomelidae), a potential biocontrol agent of tropical soda apple, *Solanum viarum* (Solanaceae) in the USA

J. Medal,[1,2] D. Gandolfo,[3] F. McKay[3] and J. Cuda[1]

Summary

Solanum viarum (Solanaceae), known by the common name tropical soda apple, is a perennial prickly weed native to north-eastern Argentina, south-eastern Brazil, Paraguay, and Uruguay, that has been spreading at an alarming rate in the USA during the 1990s. First detected in the USA in 1988, it has already invaded more than 1 million acres (*ca*. 400,000 ha) of improved pastures and woody areas in nine states. Initial field explorations in South America for potential biocontrol agents were initiated in June 1994 by University of Florida researchers in collaboration with Brazilian and Argentinean scientists. The leaf beetle *Gratiana boliviana* (Chrysomelidae) was evaluated as a potential biocontrol agent of tropical soda apple. The only known hosts of this insect are *S. viarum* and *Solanum palinacanthum*. Open field experiments and field surveys were conducted to assess the risk of *G. boliviana* using *Solanum melongena* (eggplant) as an alternative host. In an open field (choice-test) planted with tropical soda apple and eggplant there was no feeding or oviposition by *G. boliviana* adults on eggplant. Surveys conducted (1997–2002) of 34 unsprayed fields of eggplant confirmed that this crop is not a host of *G. boliviana.* Based on these results, the Florida quarantine host-specificity tests, the open field tests in Argentina, and the lack of unfavourable host records in the scientific literature, we concluded that *G. boliviana* is safe to release for biocontrol of tropical soda apple. A petition submitted for field release to the Technical Advisory Group (TAG) for Biological Control Agents of Weeds was unanimously approved on April 2002, and an APHIS permit for field release was issued in May 2003. Field releases in Florida were initiated on 14 May 2003.

Keywords: *Gratiana boliviana*, risk assessment, Solanaceae, weed biocontrol.

Introduction

Tropical soda apple is a perennial weed, native to South America, that has been spreading throughout Florida at an alarming rate during the 1990s. The pasture land infested in 1992 was estimated as approximately 150,000 acres (1 acre = *ca*. 0.4 ha)(Medal *et al*. 1996, Mullahey *et al*. 1993), and this infested area increased to more than 750,000 acres in 1995–96 (Mullahey *et al*. 1997). Currently, the infested area is estimated at more than one million acres (Medal *et al*. 2002a). Tropical soda apple was first reported in the USA in Glades County, Florida in 1988 (Coile 1993, Mullahey & Colving 1993). This weed is also present in Alabama, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee, Texas, and Puerto Rico (Bryson & Byrd Jr. 1996, Dowler 1996, Mullahey *et al*. 1997), although infestations in these states have not reached high levels. The potential range of tropical soda apple in the United States can extend even further based on studies of the effects of temperature and photoperiod conducted by Patterson (1996) in controlled environmental chambers. This invasive exotic weed was placed on the Florida and Federal Noxious Weed Lists in 1995, and is listed as one of the most invasive species in Florida by the Florida Exotic Pest Plant Council (1999).

[1] University of Florida – Department of Entomology & Nematology. POB 110620. Gainesville, Florida 32611. USA.
[2] INTA-Cerro Azul, Misiones Province, Argentina.
[3] USDA-ARS South American Biological Control Laboratory. Hurlingham, Argentina.
Corresponding author: J. Medal, University of Florida – Department of Entomology & Nematology. POB 110620. Gainesville, Florida 32611, USA <medal@mail.ifas.ufl.edu>.

In addition to invading pasture lands and reducing cattle carrying capacity (Mullahey *et al.* 1993), tropical soda apple is a host of at least six viruses that affect vegetable crops including tomato, tobacco, and pepper (McGovern *et al.* 1994a, McGovern *et al.* 1994b, McGovern *et al.* 1996). Tropical soda apple is also an alternative host of several major insect pests such as the silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring (Homoptera: Aleyrodidae), a worldwide pest of many field, horticultural, and ornamental crops; the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), a major foliage feeder of potato in North America; the tomato hornworm *Manduca quinquemaculata* (Haworth) (Lepidoptera: Sphingidae), and the tobacco hornworm, *Manduca sexta* (L.), major pests of tomato and tobacco; and several other polyphagous insects such as the southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae); the tobacco budworm, *Helicoverpa virescens* (Fabr.) (Lepidoptera: Noctuidae); and the suckfly, *Tupiocoris notatus* (Distant) (Heteroptera: Miridae) (Medal *et al.* 1999, Sudbrink *et al.* 1999, Habeck *et al.* 1996). Although it is very difficult to estimate the real (direct and indirect) economic losses due to this invasive weed, Mullahey (unpublished data) estimated the annual production loss to Florida ranchers in 1993 at US$11 million.

Native to Brazil, Paraguay, north-east Argentina, and Uruguay (Nee 1991), tropical soda apple has spread into other parts of South and Central America (confirmed growing in Nicaragua, but its presence in Honduras and Costa Rica is unconfirmed). This weed has also spread into other regions including the Caribbean (confirmed in Puerto Rico), Africa, India, Nepal, and China (Chandra & Srivastava 1978, Coile 1993). The rapid spread in south Florida can be partially attributed to the plant's high reproductive potential (Akanda *et al.* 1996, Pereira *et al.* 1997), and effective seed dispersal by cattle and wildlife, such as deer, feral hogs, raccoons, and birds that feed on fruits (Mullahey *et al.* 1993, Bryson *et al.* 1995, Brown *et al.* 1996). One tropical soda apple plant can produce an average of 41,000 to 50,000 seeds with a germination rate of at least 75% (Mullahey *et al.* 1993, Pereira *et al.* 1997). Infested areas are increasing rapidly, making this a national problem rather than just a Florida problem.

Management practices for tropical soda apple in Florida pastures are based on herbicide applications combined with mechanical (mowing) practices (Mislevy *et al.* 1996, Sturgis & Colvin 1996, Akanda *et al.* 1997, Mislevy *et al.* 1997). These control tactics provide temporary weed suppression, and costs are estimated at US $75.00 per acre to control dense infestations of tropical soda apple (Mullahey *et al.* 1996). In addition to being expensive, application of chemicals is not always feasible in rough terrain or inaccessible areas.

A biological control project on this highly invasive non-native weed was initiated in 1997 by J. Medal (University of Florida) in collaboration with R. Pitelli (Universidade Estadual Paulista, Jaboticabal campus, Brazil), and D. Gandolfo (USDA-ARS South American Biological Control Laboratory, Hurlingham, Buenos Aires province, Argentina). Host-specificity tests and field surveys were conducted from 1997 to 2002 to determine the suitability of the leaf beetle *Gratiana boliviana* Spaeth (Chrysomelidae) for biological control of tropical soda apple. In this article we report the results of an open-field experiment conducted with *G. boliviana* exposed to tropical soda apple and eggplant (choice test) in Misiones, Argentina, and a survey of eggplant fields in South America to assess the specificity and safety of *G. boliviana* as a biocontrol agent of tropical soda apple in the USA.

Material and methods

Field experiment in Argentina

An open-field experiment (choice) was conducted at the Instituto Nacional de Tecnología Agropecuaria (INTA)-Agriculture Experimental Station in Cerro Azul, Misiones province, Argentina. A natural population of *G. boliviana* was monitored in a field planted with tropical soda apple and eggplant (cultivars: Black Beauty and Long Purple). The tropical soda apple plants tested were transplanted from fields close to the area, and the eggplants were grown from seeds obtained from a commercial supplier of local varieties. They were planted in pots held in a greenhouse and then transplanted to the field on December 6, 1999 when approximately 10–15 cm in height. Fifty plants of each of the two species tested (total:100 plants) were randomly assigned in five replicates of 20 (10 of each species). All plants were thoroughly examined once a week (from January 5 to April 3, 2000) and feeding, number of beetles, and oviposition on the plants were recorded.

Field surveys in Argentina, Brazil, Paraguay, and Uruguay

Thirty-four eggplant fields inside the area of distribution of *G. boliviana* were surveyed from January 1997 to March 2002 in Argentina (16), Brazil (16), Paraguay (1), and Uruguay (1). These fields were not treated with pesticides during the growing season, or were fields where fungicides and/or insecticides were applied to eggplants only at the beginning of the growing cycle. The number of eggplants in the fields surveyed varied from 6 to approximately 1200. All plants were thoroughly examined above ground for insects when the field had fewer than 100 plants. When there were more than 100 plants, a sample of 50 to100 plants was randomly selected to have representatives from most of the areas in each field. Insect specimens found on plants were collected, identified, or sent to specialists for identification or confirmation.

Results

Field experiment in Argentina

In the open-field planted with tropical soda apple and eggplants in an area with a natural population of tropical soda apple and *G. boliviana* growing in the proximity, there was no feeding or oviposition by *G. boliviana* adults on either of the two eggplant cultivars (Black Beauty and Long Purple) during their vegetative and reproductive stages of growth. Almost all tropical soda apple plants that were thoroughly examined once a week (from January 5 to April 3, 2000), showed some leaf-feeding damage (5–20% of the leaf area) by *G. boliviana*. A total of 16 *G. boliviana* adults, 26 larvae and 21 eggs were found on tropical soda apple plants, but no *G. boliviana* stages were found on the eggplants.

Field surveys in Argentina, Brazil, Paraguay, and Uruguay

In the field surveys of insects attacking eggplant in 34 fields, no *G. boliviana* were found feeding on this crop (Table 1). Insects found feeding on eggplant included mainly the leaf-feeding beetle *Diabrotica speciosa* Germar (Coleoptera: Chrysomelidae), the green peach aphid *Myzus persicae* (Sulz.) (Heteroptera: Aphididae), the tobacco hornworm *Manduca sexta* Paphus (Lepidoptera: Sphingidae), and an unidentified species of spider-mite (Acari: Tetranichidae). Four of the eggplant fields in Argentina and Brazil were examined at least once a month for insects during their vegetative and reproductive stages of development. Larvae, pupae and adults of *G. boliviana* were found on tropical soda apple plants that were growing intermixed, or close to (sometimes a few metres away) the eggplant fields. The South American growers that have been growing eggplant for many years have also never found *G. boliviana* attacking their crops.

Discussion

The field release of a non-indigenous leaf feeding insect to control tropical soda apple in the continental USA should have little negative effect on non-target organisms. No adverse impacts are expected on the six solanaceous species listed as threatened or endangered in Hawaii and Puerto Rico (US Fish and Wildlife Service 1997). Indirect beneficial effects on wildlife populations may be expected due to recolonization by native plants that have been displaced by the rapidly growing and highly competitive tropical soda apple plants.

The eggplant fields surveyed in South America, and the host range tests conducted in quarantine in the USA and in South America (Gandolfo et al., 1999, Medal et al., 2002b) indicated that *G. boliviana* is safe to release. Occasional temporary feeding might occur on some very closely related *Solanum* species such as *Solanum torvum* Sw. (on the Federal Noxious Weed list, introduced from West Africa) and on *S. elaeagnifolium* Cav. (an important weed in agricultural areas in North America) (Medal *et al.* 2000, Medal *et al.* 2001). Based on the surveys of 34 unsprayed eggplant fields reported here, noticeable damage to eggplant seems unlikely. Further field and laboratory experiments conducted in Argentina (Gandolfo et al., unpublished data) and the lack of unfavourable host records in the scientific literature also corroborate the specificity and safety of *G. boliviana*. Therefore, control of tropical soda apple by this leaf beetle is not expected to have any significant, long-term negative impacts on non-target organisms.

The petition to release the South-American tortoise beetle *G. boliviana* for the control of tropical soda apple in the USA was approved and a permit was issued by the USDA-APHIS in May 2003. Field releases in Florida were initiated on 14 May 2003.

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Table 1. Eggplant fields surveyed in Argentina, Brazil, Paraguay and Uruguay.

Location	Plants checked/total plants	Date	*G. gratiana*
Argentina			
Misiones (13 fields)	6–100/6–800	Feb 98/Jan 00	0
Buenos Aires (3 fields)	20/20	Dec 98/March 00	0
Brazil			
São Paulo (7 fields)	40–100/40–1200	Jan 97/June 98	0
(9 fields)	7–100/7–640	Nov 98/March 01	0
Paraguay			
Itapua (1 field)	40/40	January 2000	0
Uruguay			
Canelone (1 field)	100/600	April 1999	0

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Host-specificity testing of the boneseed (*Chrysanthemoides monilifera* ssp. *monilifera*) leaf buckle mite (*Aceria neseri*)

Thomas B. Morley[1]

Summary

The eriophyid mite *Aceria neseri* is a candidate agent for biological control of *Chrysanthemoides monilifera* ssp. *monilifera* (boneseed), one of two weedy *Chrysanthemoides* taxa in Australia. Based on testing carried out in a shadehouse at the Agricultural Research Council's Plant Protection Research Institute premises in Stellenbosch, South Africa, the host range of *A. neseri* was found to be restricted to *Chrysanthemoides*. Sixty-two plant species were tested including a few *Chrysanthemoides* taxa other than boneseed, and three species (*Osteospermum fruticosum, Calendula officinalis* and *Dimorphotheca sinuata*) in the same tribe as boneseed (Calenduleae). No signs of feeding or damage attributable to *A. neseri* were observed on species other than *Chrysanthemoides*. *A. neseri* would be safe to release in Australia since the only representatives of *Chrysanthemoides* in Australia are pests.

Keywords: *Aceria neseri*, *Chrysanthemoides monilifera*, erineum, Eriophyiidae, host specificity.

Introduction

After two decades of research and development, it has so far proven difficult to develop effective biological control for *Chrysanthemoides monilifera* (L.) Norl., two of whose taxa, *C. m.* ssp. *monilifera* (boneseed) and *C. m.* ssp. *rotundata* (DC.) Norl. (bitou bush), are serious weeds in Australia (Weiss *et al.* 1998). The first eight agents released in Australia have either failed or achieved only limited success to date. They include two foliage-feeding moths, four foliage-feeding chrysomelid beetles and two seed-feeding flies. Two further organisms are currently under development as biological control agents: an eriophyid mite *Aceria neseri* Meyer and a rust fungus, *Endophyllum osteospermi* (Doidge) comb. nov. This paper reports on the host-specificity testing of *A. neseri*.

A. neseri is a small whitish, worm-like mite up to 175 microns long and about 50 microns wide. Feeding by *A. neseri* on developing boneseed leaves induces the formation of *erinea* (patches of densely packed hair-like outgrowths) that are initially white but turn brown with age and are associated with distorted leaf growth. Erinea are composed of non-photosynthetic tissue, reduce photosynthetic efficiency and provide shelter and substrate in which *A. neseri* colonies grow. Heavily infested *C. monilifera* plants in the native range, South Africa, are unthrifty and appear to have lower reproductive outputs and less vigorous growth than uninfested plants.

Following several unsuccessful attempts to establish a culture of *A. neseri* in quarantine at the Keith Turnbull Research Institute (KTRI) in Australia operations were transferred to the Agricultural Research Council's Plant Protection Research Institute (PPRI) premises in Stellenbosch , South Africa, where tests could be conducted in an outdoor shadehouse. This removed the constraints of quarantine on test plant propagation and *A. neseri* colony maintenance, and allowed easy access to local populations of *A. neseri* for inoculum.

Materials and methods

A host-specificity test list was compiled by Adair (1999) along the lines of the method described by Wapshere (1974) (i.e. centrifugal phylogenetic method plus safeguard criteria) and approved in accordance

[1] Keith Turnbull Research Institute, Department of Primary Industries, PO Box 48, Frankston, Victoria 3199, Australia

with the protocol described on the web site of the Department of Agriculture, Fisheries and Forestry – Australia (Anon.). In addition, a selection of opportunistically available *C. monilifera* and *C. incana* accessions, whose subspecific identity was uncertain, were also tested as hosts for *A. neseri*. Taxa tested are listed in Table 1. Test plants were acquired from commercial nurseries or propagated from seed and grown in 20 cm diameter plastic pots. *C. m.* ssp. *monilifera* plants were collected as seedlings from roadsides and other waste places and transplanted into 20 cm pots for use as controls.

Most of the host-specificity tests were conducted between December 2001 and May 2002 in a shadehouse at PPRI in batches of one to several test taxa. The remainder were done in a PPRI quarantine glasshouse (two batches, March to May 2002) or in a KTRI quarantine controlled environment room (one batch, June/July 2002) depending on quarantine status and availability of test plants. (Sufficient experience with *A. neseri* had been gained by June 2002 to enable its successful use in host-specificity tests in quarantine at KTRI.) Test batches were inoculated with *A. neseri* collected from populations residing in natural stands of *C. m.* ssp. *monilifera* in Cape Town and environs, South Africa. A vegetative, actively growing shoot tip on each of three to eight (usually five or six) replicate test taxon plants was inoculated with two to three healthy erinea-bearing live *A. neseri*. Erinea were nestled in the shoot tip by gravity or by anchoring them in such a way that when the mites exited the drying inoculum they were likely to encounter the growing tip of the test plant.

Tests were controlled in three ways: 1) An uninoculated plant of each test taxon was used as a control for test plant inoculation effects. 2) In order to check that inoculum was infective, for each test plant batch, five replicate *C. m.* ssp. *monilifera* plants (positive controls) were inoculated with the same batch of inoculum as was used for that test plant batch. 3) In order to check that erineum development on positive controls was due to inoculation and not to *A. neseri* from ambient sources (e.g. wind-borne), for each test plant batch, five replicate uninoculated *C. m.* ssp. *monilifera* plants (negative controls) were incubated under the same conditions as test and positive control plants. Tests were considered valid only if the inoculated shoot tip continued to grow throughout the test, erinea developed normally on four out of the five positive control plants for that batch and no erinea developed on the negative control plants.

Test and control plants were inspected daily for development of erinea and other abnormalities that might be attributable to *A. neseri*. Erinea were counted and their surface area was estimated three weeks after they appeared on a majority on positive controls. Test plants were examined microscopically for *A. neseri* four to five weeks after inoculation.

Results

Results are presented in Table 1. The only genus affected by *A. neseri* was *Chrysanthemoides*. Normal erinea and *A. neseri* colonies were routinely induced on the inoculated shoot tip of positive control plants. Erinea usually appeared on these plants six to ten days after inoculation. Erinea did not develop on uninoculated shoot tips. No taxa in any other genera developed erinea or any other galls or sustained damage that could be attributed to or showed signs of infestation with *A. neseri*, nor were any *A. neseri* found on any of those taxa at the conclusion of tests.

Erinea developed on one of the unidentified *C. monilifera* and three of the unidentified *C. incana* accessions, although these responses were generally weaker than those that occurred on the positive controls.

Discussion

The tests described here indicate that *A. neseri* is restricted to the genus *Chrysanthemoides*, a favourable result in terms of its potential as a biological control agent, and that it would be safe to release in Australia, since the only representatives of *Chrysanthemoides* in Australia are pests and are accepted as biological control targets.

The results also give some indication that the laboratory host range of *A. neseri* accessions from *C. m.* ssp. *monilifera* includes taxa from *C. incana* as well as *C. monilifera*. Whether these laboratory hosts would be suitable as hosts in the field was not determined. However, given the generally weaker response of *A. neseri* in these tests to *Chrysanthemoides* taxa other than *C. m.* ssp. *monilifera* it would appear that *A. neseri* accessions from *C. m.* ssp. *monilifera* prefer that taxon.

A. neseri has also been observed on *C. m.* ssp. *rotundata* and *C. m.* ssp. *pisifera* (L.) Norl. (Adair 1999) and these mite populations are probably distinct biological races. As an adjunct to the tests described above, an *A. neseri* accession from *C. m.* ssp. *pisifera* was tested for its ability to induce erineum formation on *C. m.* ssp. *monilifera*. The response of *C. m.* ssp. *monilifera* to this accession was much weaker than that of *C. m.* ssp. *monilifera* to *A. neseri* accessions from *C. m.* ssp. *monilifera*. I also observed an erineum-forming eriophyid (probably another race of *A. neseri*) infesting an unidentified *C. incana* taxon in Cape Town and was able to induce erineum formation with it on *C. m.* ssp. *monilifera*.

Providing accessions of *A. neseri* that cause severe leaf distortion and abundant erineum formation on Australian forms of *Chrysanthemoides* can be located, the potential for suppression of Australian infestations is good.

Table 1. Taxa tested as hosts for *A. neseri* and erineum development on inoculated shoot tips three weeks after erinea appeared on the majority of test batch *C. m.* ssp. *monilifera* positive controls.

Taxon	Proportion of replicates that developed erinea	Mean number of erinea (*s*) per replicate	Mean total area of erinea (mm^2) (*s*) per replicate
C. monilifera ssp. *monilifera*	80/90[a]	10.0 (10.5)	57.6 (81.6)
C. monilifera unidentified taxon 1[b]	0/3	0	0
C. monilifera unidentified taxon 2[c]	2/4	1.8 (2.1)	2.3 (2.6)
C. monilifera unidentified taxon 3[d]	0/3	0	0
C. monilifera unidentified taxon 4[e]	0/5	0	0
C. incana unidentified taxon 1[f]	4/8	0.5 (0.5)	1.3 (1.4)
C. incana unidentified taxon 2[f]	3/5	5.8 (7.4)	12.8 (14.7)
C. incana unidentified taxon 3[g]	2/4	1.3 (1.5)	5.0 (5.8)
C. incana unidentified taxon 4[h]	0/6	0	0
Calendula officinalis	0/6	0	0
Dimorphotheca sinuata	0/5	0	0
Osteospermum fruticosum	0/5	0	0
Actites megalocarpa	0/6	0	0
Lactuca sativa	0/5	0	0
Cichorium intybus	0/6	0	0
Cichorium endivia	0/3	0	0
Tragopogon porrifolius	0/6	0	0
Arctotheca calendula	0/6	0	0
Cymbonotus preissianus	0/5	0	0
Gazania rigens	0/5	0	0
Artemisia dracunculus	0/6	0	0
Chamaemelum nobile	0/5	0	0
Cotula turbinata	0/6	0	0
Cotula coronopifolia	0/6	0	0
Chrysanthemum morifolium	0/5	0	0
Tanacetum cinerariifolium	0/5	0	0
Callistephus chinensis	0/6	0	0
Olearia axillaris	0/6	0	0
Cynara scolymus	0/5	0	0
Carthamus tinctorius	0/6	0	0
Stemmacantha australis	0/6	0	0
Ageratum houstonianum	0/6	0	0
Dahlia pinnata	0/5	0	0
Helianthus annuus	0/5	0	0
Helianthus tuberosus	0/6	0	0
Melanthera biflora	0/6	0	0
Tagetes patula	0/6	0	0
Bracteantha bracteata	0/6	0	0
Cassinia aculeata	0/6	0	0
Leucophyta brownii	0/6	0	0
Ozothamnus turbinatus	0/4	0	0
Gerbera jamesonii	0/5	0	0
Senecio odoratus	0/6	0	0
Senecio hybridus	0/6	0	0
Tussilago farfara	0/6	0	0
Allocasuarina verticilliata	0/3	0	0
Acacia sophorae	0/6	0	0
Eucalyptus grandis	0/6	0	0
Banksia integrifolia	0/6	0	0
Actinidia chinensis	0/6	0	0
Mangifera indica	0/5	0	0
Annona reticulata	0/6	0	0
Campanula medium	0/6	0	0
Humulus lupulus	0/6	0	0
Carica papaya	0/6	0	0
Ipomoea batatas	0/5	0	0
Beta vulgaris	0/6	0	0
Brassica napus	0/6	0	0
Vaccinium corymbosum	0/6	0	0
Persea americana	0/4	0	0
Pisum sativum	0/5	0	0
Trifolium repens	0/6	0	0
Allium cepa	0/6	0	0
Asparagus officinalis	0/6	0	0
Linum usitatissimum	0/6	0	0
Musa sapientum	0/4	0	0
Lolium perenne	0/6	0	0
Oryza sativa	0/6	0	0
Protea burchellii	0/3	0	0
Capsicum annuum	0/5	0	0
Camellia sinensis	0/6	0	0
Apium graveolens	0/6	0	0
Daucus carota	0/6	0	0
Zingiber officinale	0/6	0	0

[a] Includes all *C. m.* ssp. *monilifera* positive control plants from all test batches.

[b] Suspected of being ssp. *pisifera*. Purchased from Sonderpry's Nursery, Somerset West, Cape Town.

[c] Suspected of being ssp. *pisifera*. Propagated by Mitchell's Nursery

[d] Suspected of being an intermediate between ssp. *rotundata* & ssp. *pisifera*. Purchased from Helderberg Nature Reserve Nursery, Somerset West, Cape Town.

[e] Suspected of being an intermediate between ssp. *rotundata* & ssp. *pisifera*. Source unknown.

[f] Purchased from Good Hope Nursery, Cape Peninsula.

[g] Purchased from Helderberg Nature Reserve Nursery, Somerset West, Cape Town.

[h] Purchased from Nursery on the West Coast, Melkboschplaas, Cape Town.

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Evaluating off-target movement of *Xanthomonas campestris* pv. *poannua* following application as a biocontrol agent for *Poa annua* on golf turf

Joseph C. Neal,[1] Nancy D. Williams[2] and Eric B. Nelson[3]

Summary

Poa annua var. *reptans* is a prostrate perennial grass and one of the most difficult to control weeds of golf course turf. *Xanthomonas campestris* pv. *poannua* (*Xcp*) has shown promise as a highly selective biological control agent for this species. Because *Xcp* must be applied at high concentrations and requires a wound for entry, limited off-target impacts have been assumed. However, since *Xcp* may infect some closely related species, an understanding of its movement and persistence is essential. A rifampicin-resistant strain of *Xcp*, was applied to a simulated golf green at a rate of 150 mL of 1×10^9 cfu/mL to 26 cm^2 areas (5.8×10^9 cfu/cm^2) in the centre of 4 m^2 plots of *P. annua* var. *reptans* mowed thrice per week at 0.6 cm. Turf and thatch samples were extracted every other day from the point of inoculation, and at 35, 70, 105 and 140 cm in four directions for 49 days. *Xanthomonas campestris* pv. *poannua* was quantified by plating on selective medium. The experiment was conducted twice, in randomised complete block designs, with four replications. By nine days after inoculation (DAI), fewer than 1×10^4 cfu/cm^2 *Xcp* were recovered from the inoculated areas. By 49 DAI no *Xcp* was recovered from the area of inoculation. Off-target movement was minimal but detectable up to 140 cm from the point of inoculation. Maximum off-target *Xcp* recovery of between 75 and 400 cfu/cm^2 was observed 35 cm from the point of inoculation between 3 and 11 DAI. By 49 DAI no *Xcp* was recovered. No differences between quadrants were detected; therefore, mowing direction does not appear to influence off-target movement. These data suggest that the application of *Xcp* to golf turf does not present a significant risk of off-target movement and that *Xcp* populations at the site of inoculation will dissipate rapidly.

Keywords: dissipation, off-target movement, *Poa annua*, turf, *Xanthomonas campestris* pv. *poannua*.

Introduction

Poa annua L. (annual bluegrass or annual meadow grass) is one of the most widespread and difficult to control weeds of sports turf, especially golf greens (Bogart & Beard 1973). *Poa annua* is well adapted to frequent and close mowing, high nitrogen fertility, compaction, disturbance and frequent irrigation common to these sites. There are two distinct biotypes of *P. annua*: *P. annua* var. *annua*, a winter annual with an ascending growth habit and *P. annua* var. *reptans*, a perennial with a more prostrate growth habit (Warwick 1979). *Poa annua* var. *reptans* is the predominant form on golf greens in northern USA. Due to the species' perennial life cycle, pre-emergence herbicides labeled for *P. annua* control in turf have not provided adequate control. Current control procedures rely upon selective plant-growth regulators and cultural practices that reduce the competitiveness of *P. annua* and encourage more desirable turfgrasses (Cooper *et al.* 1987, Gaussion & Branham 1989). However, these practices have not provided adequate control and must be carefully managed to avoid unacceptable injury to the desirable turfgrass.

[1] Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609, USA.
[2] City Sprouts, Inc., 3040 Lake Street, Omaha, NE 68111, USA.
[3] Department of Plant Pathology, Cornell University, Ithaca, NY 14853, USA.
Corresponding author: J.C. Neal, Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609, USA

Xanthomonas campestris pv. *poannua* (*Xcp*) is a facultative parasite that causes bacterial wilt in *P. annua* (Roberts *et al.* 1985). It is highly selective for *P. annua* and has shown promise as a selective biocontrol agent of *P. annua* in the USA and Japan (Savage 1991, Zhou & Neal 1995, Imaizumi *et al.* 1997). However, *P. annua* is often considered to be a desirable component of turfgrass swards. Consequently, an understanding of the fate of applied *X. campestris* pv. *poannua* and potential for off-target movement are imperative before widespread use. Therefore, the objectives of this study were to monitor *Xcp* populations in turfgrass following application as a biocontrol agent for annual bluegrass and to assay adjacent *P. annua*-infested turfgrass for off-target movement.

Materials and methods

The experiment was conducted on an established stand of *Agrostis stolonifera* (creeping bentgrass) 'Seaside' heavily infested with *P. annua* var. *reptans*. The experiment was conducted from July through September 1992, and was repeated from August through October 1992 on a separate turf sward. The experiment location was Ithaca, New York, USA. Each trial of the experiment was replicated four times in a randomised complete block design. Turfgrass was mowed three times a week at 6.5 mm and irrigated as needed.

A rifampicin-resistant strain of *Xcp* (from strain MSU-450) was selected on Kings B medium amended with 100 ppm rifampicin. All studies were conducted with this rifampicin-resistant strain. Inoculum was applied at a rate of 150 mL of approximately 1×10^9 cfu/mL to 103 cm^2 in the centre of each plot (about 5.8×10^9 cfu/cm^2). Grass was mowed immediately following application (previously mowed borders separated each plot). Based on results from a preliminary experiment (Webber & Neal 1992), turf and thatch samples were extracted every other day for 2 weeks and weekly thereafter to 49 days after treatment. Samples were extracted using a 1-cm diameter cork borer from the inoculated centre area, and at 35, 70, 105 and 140 cm from the centre in four directions (Fig. 1). Turf and thatch samples were blended with sterile water then aliquots were quantified by plating on selective medium containing 100 ppm rifampicin.

Results and discussion

Xanthomonas campestris pv. *poannua* populations in the inoculated area declined rapidly following application. By 9 days after inoculation (DAI), fewer than 1×10^4 cfu/cm^2 of the bacterium were recovered from the inoculated areas (Fig. 2). Previous greenhouse and field trials have suggested that inoculum populations below 1×10^4 cfu/cm^2 produce no significant control of *P. annua* (Webber *et al.* 1992). By 49 DAI no *Xcp* was recovered. Similarly, Nishino *et al.* (1997) have

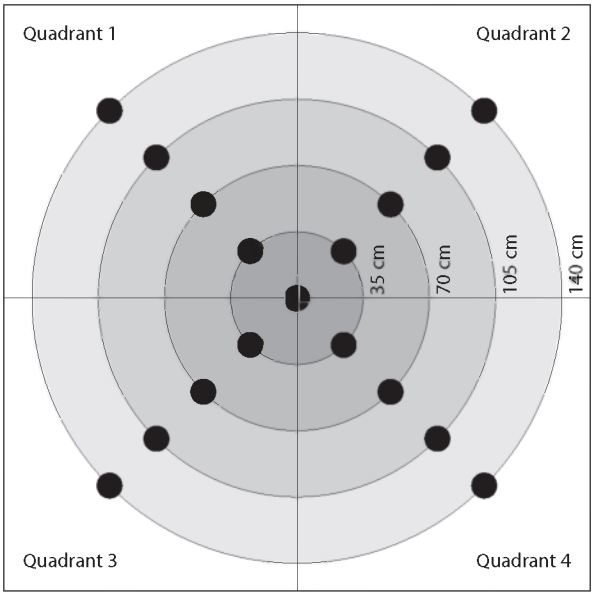

Figure 1. Turf and thatch sampling pattern for recovery of rifampicin-resistant *Xanthomonas campestris* pv. *poannua*. Circles represent sample points – inoculated area in the centre, and at 35, 70, 105 and 140 cm from the centre, in four quadrants of each plot. The centre 103 cm^2 was inoculated with approximately 5.8×10^9 cfu/cm^2.

reported rapid dissipation of a Japanese isolate of this bacterium in turfgrass. In their studies, populations were below their detection limit of 1×10^3 cfu/g dry soil after 3 days in moist soil, and after 3 weeks in dry soil.

No differences in bacterium recovery were observed between quadrants; therefore data were pooled for analysis and presentation. Off-target movement was minimal but detectable up to 140 cm from the point of inoculation in both trials of the experiment (Fig. 3). Maximum off-target *Xcp* recovery in the July through September experiment was 75 cfu/cm^2, 35 cm from the area of inoculation at 3 DAI. In the August through October trial, maximum recovery was 400 cfu/cm^2 at the 35 cm sample points 11 DAI. When averaged over all data, the only recoveries statistically greater than zero were at 35 cm from the area of inoculation between 7 and 21 DAI (Fig. 3). By 49 DAI no *Xcp* was recovered in either trial of the experiment. This was consistent with results from preliminary tests on the same site in 1991. In contrast, Imaizumi & Fujimori (1999) have reported movement up to 16 m from the area of inoculation and the potential for secondary infections through movement on mowing equipment. Greater movement and persistence in the Japanese tests could be due to many factors including differences in virulence of the bacterial isolate used, the predominant biotype of annual bluegrass present or local environmental conditions.

These data demonstrate a rapid decline in populations of *Xcp* following application as a biological control agent for *Poa annua* var. *reptans*. This rapid decline in

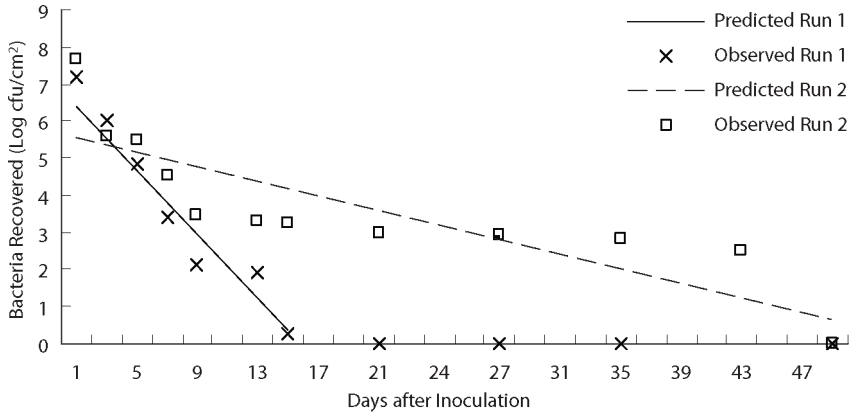

Figure 2. Dissipation of applied rifampicin-resistant *Xanthomonas campestris* pv. *poannua* from the area of inoculation. Data for the two 1992 studies were fitted to a linear regression analysis. Lines are the predicted values based on the following equations. For the July through September trial (run 1): $LogY_1 = 6.81 - 0.43 \times DAI$; $R^2 = 0.92$. Equation for the August through October trial (run 2): $LogY_2 = 5.66 - 0.102 \times DAI$; $R^2 = 0.74$. Xs and squares are the observed means for the two trials of the experiment.

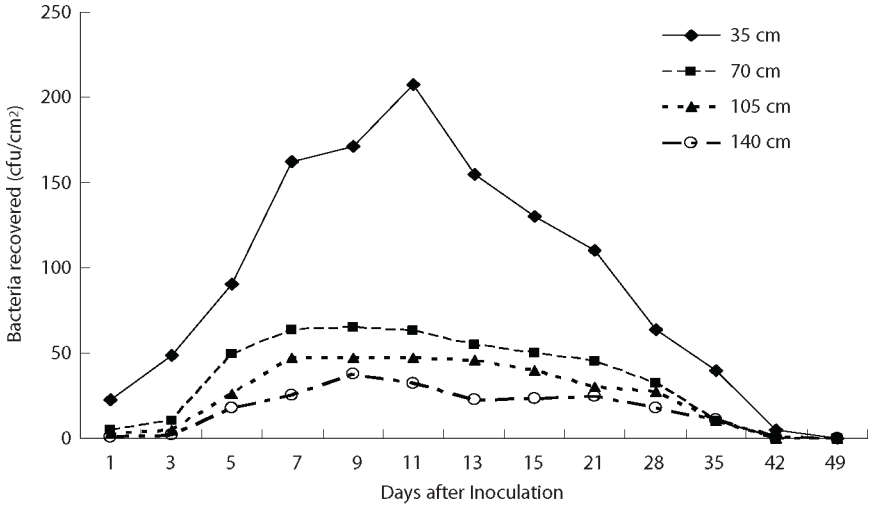

Figure 3. Recovery of rifampicide-resistant *Xanthomonas campestris* pv. *poannua* at 35, 70, 105 and 140 cm from the area of inoculation over time. Data are pooled for four quadrants and for two trials of the experiment. The only statistically significant recoveries were from the 35 cm samples between 3 and 21 days after inoculation. Note: values are presented as actual cfu/cm^2 and not log values (as presented for recoveries from the area of inoculation in Fig. 2).

bacterial populations may, in part, explain why repeated applications at high doses have been required to achieve suppression of the perennial type of *P. annua* (Zhou & Neal 1995). Furthermore, although research conducted in Japan suggests secondary infections and movement on mowing equipment is possible, we observed no evidence of this in our trials. These results suggest that the application of *Xcp* to golf turf does not present a significant risk of off-target movement in the northeastern United States, and that bacterial populations at the site of inoculation will dissipate rapidly.

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Risk analyses of recent cases of non-target attack by potential biocontrol agents in Queensland

William A. Palmer[1]

Summary

Three insects considered for biocontrol of weeds in Queensland have potential or realized non-target risks associated with them. The membracid *Aconophora compressa* was released for control of *Lantana camara* in 1995. By 1999, the insect was found also attacking the verbenaceous, exotic, ornamental tree, *Citharexylum spinosum*, around Brisbane. The circumstances of this attack in relation to the host-testing program, and community attitudes to it, are described. A geometrid moth, *Isturgia disputaria,* was considered for the biological control of *Acacia nilotica* ssp. *indica*. Although highly host-specific, the insect was able to feed and develop on some Australian acacias under laboratory conditions, at much reduced development and survival rates. CLIMEX and DYMEX models were used to demonstrate the low likelihood of significant attack on Australian acacias. Despite this, it was decided not to release the insect. The weevil *Osphilia tenuipes* was the first agent introduced for a new biocontrol target, *Bryophyllum delagoense*. It was very destructive in laboratory trials and appeared a very promising agent because its host range was confined to some exotic genera of the Crassulaceae. However, it attacked the exotic, closely related *Kalanchoe blossfeldiana,* which is a popular flowering plant sold through nurseries. The argument being developed to support its release through the Biological Control Act is described. These examples are discussed within the general framework of risk analysis for introduced biocontrol agents.

Keywords: *Aconophora compressa*, biological control, *Isturgia disputaria*, *Osphilia tenuipes*, risk analysis.

Introduction

In recent years, there has been increasing emphasis on ensuring that detrimental effects of introduced biological control agents are minimized. Although there is not yet an example where an introduced agent has caused serious and persistent damage to an agricultural or otherwise commercial crop, there have been examples where attack on native plants has been noted (Pemberton 2000, Louda & O'Brien 2002, Louda *et al.* 2003). More general authors (Howarth 1991, Low 1999) have also been cautious or critical of biocontrol practices because of risk to non-target organisms.

This paper presents information about one introduction and two potential introductions in Queensland where non-target effects have had to be considered.

Aconophora compressa attack on *Citharexylem*

The membracid bug *Aconophora compressa* Walker was introduced from Mexico into Australia as an agent for *Lantana camara* L. (Verbenaceae). Host testing was conducted in both Mexico and Australia. The insect was tested against 62 plant species, including seven species in five genera of the Verbenaceae *sensu stricto* (Cantino *et al.* 1992). Approximately 30 species in 10 genera, all exotic and most weedy, represent the Verbenaceae in Australia. Host tests and field information indicated that the insect had a narrow host range confined to *Lantana, Lippia, Duranta* and possibly

[1] Alan Fletcher Research Station, Queensland Department of Natural Resources and Mines, PO Box 36, Sherwood, Queensland 4075, Australia.

other closely related verbenaceous genera and it was approved for release (Palmer *et al.* 1996) under the standard procedures of review by 21 agencies and by the Australian Quarantine and Inspection Service (AQIS) and Environment Australia (McFadyen 1997).

Some years after the initial releases in 1995, *A. compressa* was found on an exotic ornamental tree *Citharexylum spinosum* L. (Verbenaceae), known as fiddlewood. The insect was found in high numbers, suggesting that this tree was a very acceptable host. On occasions, large insect populations were damaging to these sometimes large trees. Although *C. spinosum* is native to the West Indies, there are at least 11 congeners native to Mexico and some sympatric with *A. compressa.* There is no previous record of this insect attacking *Citharexylum* spp. in its native range.

Citharexylum spinosum was not included in the approved host-plant test list for this insect and further investigation has not revealed a case among the 28 agents introduced to Australia over a period of some 80 years when it had been tested. The reason for not testing this plant was presumably that it was neither a native nor an agricultural crop. Fiddlewoods are very fast growing and were popular some decades ago as "screen" trees planted in rows to give privacy and protection from wind, especially in new housing developments. They have now been largely replaced by native species, are often considered "messy", and often appear on council "recommended not to plant" lists because their shallow root systems interfere with drainage lines. They only appear occasionally in retail nurseries in Queensland. Although fiddlewoods are now included in the approved host test list for insects of lantana, attack on them might not preclude an insect's release.

Perhaps fortuitously, summer heatwaves with maximums of over 37°C for just 2–3 days duration are capable of reducing *A. compressa* to the point of local extinction. For this reason, most of Queensland and New South Wales is unsuitable for this insect. However, it has firmly established in a number of the coastal suburbs of Brisbane and will undoubtedly spread to other suburbs and localities following milder summers.

In the first few months of 2003, the insect again become abundant and there was considerable enquiry from the public to local nurseries, "tree doctors", and departmental extension services. Homeowners with these trees had to decide whether to put up with the damage, remove the tree or treat the tree with insecticide recommended by their local nursery. Some people complained more stridently. The concerns were the condition of grown trees and, if they were killed, the cost of removing them from the properties. Concerns were also expressed that the bugs deposit honeydew on laundry and that populations might overflow onto surrounding plants.

All necessary approvals were obtained for the release of this insect. The Department of Natural Resources and Mines is providing information on the bug and the undesirable aspects of fiddlewood trees to improve the understanding of concerned people, and also advice on how to treat the trees. In that respect, the use of systemic insecticides by stem and soil injection is being investigated. These compounds may give 12–18 months protection. This, together with hot summers in some years, should be sufficient to protect valued trees.

The development of *Isturgia disputaria* on some native acacia species

The geometrid moth *Isturgia disputaria* (Guenée) was imported as a prospective agent for the biocontrol of prickly acacia, *Acacia nilotica* ssp. *indica* (Benth.) Brenan. This insect is known from various subspecies of *A. nilotica* in both Africa and the Indian subcontinent. There are also a few specimens extant that were purportedly collected in Africa from the Australian species *A. mearnsii* De Wild. and *A. decurrens* Willd., but details of these collections are unknown.

Host testing of any agent against prickly acacia needs to be particularly discriminating because Australia has more than 950 native *Acacia* spp. (Maslin 2001). *Isturgia disputaria* was therefore tested against an approved host list of 73 plant species including 45 native *Acacia* spp. The insect was narrowly stenophagous, but larvae were able to develop through from neonate to adult on some test plants. The group of *Acacia* showing most susceptibility to *I. disputaria* was the section Botrycephalae. This section of 42 bipinnate species (Maslin 2001) is a temperate group with species found from southern Queensland to Tasmania. There is little overlap between the group and the prickly acacia infestations, which occur in the tropics. Further, a CLIMEX model (Skarratt *et al.* 1995) indicated that *I. disputaria* was also essentially a tropical/subtropical species and would not survive over most of the geographical range occupied by the *Botrycephalae.*

Although larvae could develop through from neonate to pupa on some Botrycephalae, such as *A. mearnsii* De Wild., *A. decurrens* Willd. and *A. deanei* (Baker) Welch, Coombs & McGlynn, these species were clearly less suitable as hosts. Larval development on these species was characterized by higher mortality, longer development times and sometimes lighter pupal weights. A simple population model, constructed using DYMEX (Maywald *et al.* 1999), indicated that there would be one less generation a year on any of these species and that population increases would be at least 100-fold less than on prickly acacia over the course of a summer.

The results were then discussed in a seminar with a group of biocontrol and plant ecology scientists. Despite the findings, there was still considerable unease

expressed by various people about the proposal to release this insect. The general consensus was that *Acacia* is a particularly important genus in terms of numbers of species, public recognition (Australia's official floral emblem is *A. pyncnantha* Benth.), and ecological significance. There was also a strong feeling that any attack detected on native acacias might generate strong community discussion and perhaps bring a degree of opprobrium to biocontrol in general. It was therefore thought unlikely that approval would be obtained for its release.

Although there is only an extremely low probability that this insect would damage any native acacia and even less likelihood of detriment at the population or ecosystem level, it was decided the consequences of this low probability becoming a reality were unacceptable and thus permission to release was not requested.

Osphilia tenuipes attack on *Kalanchoe blossfeldiana*

A recent project undertaken by my organization has been the biocontrol of *Bryophyllum delagoense* (Eckl. & Zeyh.) Schinz (mother-of-millions) and *B. delagoense* × *B. daigremontianum* (Ramet-Hamet and H. Perrier), hybrid mother-of-millions. The genus *Bryophyllum* is endemic to Madagascar, though the hybrid is thought to be a horticultural cultivar developed in the United States (Hannan-Jones & Playford 2002). These weeds are poisonous to livestock (McKenzie & Dunster 1986) and have quite dramatically increased in abundance in Australia in the last decade.

A stem-boring weevil, *Osphilia tenuipes* (Fairmaire), was found in mother-of-millions in Madagascar and its host range evaluated in the laboratory in both South Africa and Australia. The insect had a narrow host range confined to most *Bryophyllum* spp. and some *Kalanchoe* spp. The genera *Bryophyllum* and *Kalanchoe* are very closely related and are synonymized by some authorities (Boiteau & Allorge-Boiteau 1995). *O. tenuipes* did not attack any Crassulaceae endemic to Australia.

Unfortunately, the ornamental *Kalanchoe blossfeldiana* Poelln. was a good host for *O. tenuipes*. This species was first discovered in Madagascar in 1924. A few plants were collected at the time, and from the cultivation and breeding of these few plants a significant garden ornamental which is now sold worldwide has been developed (Van Voorst & Arends 1982). In Australia, the kalanchoe industry is valued at A$5 million annually (R. Edwards, unpublished). While Queensland is a major grower, the largest retail market is in Victoria, situated well south of the present distribution of the weedy *Bryophyllum* spp.

A key question is whether the insect could survive in the southern Australian states and particularly Victoria. Unfortunately, this could not be predicted initially using CLIMEX because the climatic limits for the insect are not naturally tested. The insect is endemic to the island of Madagascar so that its range is limited by the seas rather than by climate. Supplementary cold tolerance tests were therefore conducted within the quarantine facility. Adults and late-instar immatures survived 3 days of 16 hours at 27°C/8 hours at –4°C. Immatures survived 7 days of 6 hours at 12°C/18 hours at 6°C (simulating some of Melbourne's worst winter weather). The information was incorporated into the CLIMEX model, which then predicted that the insect would survive in Melbourne.

The next issue addressed was whether there were insecticides available to control this insect should a nursery become infested. It was felt that if the insect could be controlled by insecticides then the nursery industry might not be overly concerned about the release of the insect. Early results indicated that adults can be controlled by most insecticides already being used by commercial nurseries and early instars in the stems can be controlled by some systemics.

The major concern of the kalanchoe industry is the possibility of attack in the commercial wholesale nurseries. The flowering plants are normally sold in winter as "pots of colour". Most are purchased for apartments and patios and are discarded when the flowers fall. Once plants are purchased they are unlikely to be exposed to *O. tenuipes*, which in any case would cause little damage before the plant was discarded. On the other hand, the wholesale nurseries are concerned about their "crops" being infested, particularly because there are quality control issues for those supplying large quantities to supermarket chains.

Because there are only a few wholesale nurseries in Australia, it may be possible to work with them to demonstrate that if a few hygiene procedures are implemented (elimination of any mother-of-millions from surrounds, ensuring starter stock is free of *Osphilia,* routine surveillance for the insect etc.) there is unlikely to be a problem.

However, because the release of this insect has the potential to affect a non-target plant and potentially cause significant loss to a substantial nursery industry, it was felt that the insect should be released in Australia under the authority of the Commonwealth Biological Control Act and the mirror state legislation. Application was therefore made for mother-of-millions and *O. tenuipes* to be included with other proposed targets and agents being nominated under the Act.

Discussion

The three examples indicate three different approaches to non-target attack.

The absence of fiddlewood from traditional host-test lists for lantana insects indicates, albeit implicitly, that an exotic plant with little commercial value and equivocal aesthetic value was not considered sufficiently important to impede the release of a potential biocontrol agent. This assumption may well be tested in the

coming years. Although it is most unlikely there will be significant economic damage, there may well be some community angst and a tarnishing of the biocontrol image held by the community. The experience may well provide useful guidelines for future risk studies involving exotic ornamentals.

As it now appears likely that *A. compressa* has permanently established on the non-target fiddlewood and is likely to induce significant damage on this tree (albeit in limited areas), this example may be one of the first of sustainable damage to a non-native plant of value to some in the community. The insect was thoroughly tested before release, perhaps more comprehensively than any other lantana insect before it. One recommendation that might be drawn from the experience is that it is highly desirable to include species of perceived worth in the tests where possible and particularly when dealing with agents with host ranges broader than monophagy.

The decision to withdraw *I. disputaria* from consideration represents a low public risk approach that recognized the significant or public profile of acacias generally. Although it would be quite probable that the insect would be recorded from native acacias at some point in the future, it is most improbable that it would have built up to populations damaging to individual trees, let alone plant communities or ecosystems. This example demonstrates that prickly acacia is indeed a difficult target weed for biological control in Australia because the numbers and importance of congeners appreciably reduce the number of prospective agents. Indeed, Louda *et al.* (2003) have recommended that targeting weeds with close native relatives sharply increases ecological risk.

A risk analysis such as that for *I. disputaria* should incorporate more information than simply the results of host-range testing. It is very appropriate to attempt to evaluate host specificity in an ecological context (Louda *et al.* 2003). In this case, the modelling applications CLIMEX and DYMEX were employed and made useful contribution to the analysis.

The decision to invoke the Biological Control Act to cover the release of *Osphilia* is a logical decision as this Act was designed to handle conflicts of this nature. However, it now appears that the process of placing a target or agent under this Act is not at all straightforward. The Biological Control Act was promulgated in 1984 but has, in fact, only been used since for three issues (Paterson's curse, blackberries and rabbit calicivirus) and there is now very little administrative experience available within governments on the implementation or implications of this Act. One possible difficulty in using the Act for cases other than those of significant national importance is that the application and later approval processes need the unanimous support of state and Australian government ministers with agricultural and natural resource portfolios. On the other hand, there has been increased pressure from some government agencies for the Biological Control Act to be utilized more often in Australia, and at least one agency has suggested that all biocontrol introductions be put through the Act. How and in what circumstances the Act may be utilized is now under consideration at a national level through relevant state and Australian government departments.

Two of these three case studies involve non-target attack on garden ornamentals. The attitude to attack on ornamentals is perhaps not clearly defined as they often fall between the two major groups of concern, the native flora and the agricultural crops and pastures. There is also a perception that attack on ornamentals can be easily addressed with insecticides, or consumers and supplying nursery industries can easily switch to alternative plants. There are also issues of changing, and perhaps cyclical, popularity of particular plants to consider. In the case of fiddlewood, it was no longer popular and its planting was being discouraged long before *Aconophora* was considered for release. Although these issues will have to be evaluated on a case-by-case basis, it is recommended that the nursery and ornamental sector (past, present and future) be properly assessed before release of stenophagous agents.

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Progress on weed biocontrol projects in Paraná State, Brazil: targeting plants that are invasive in Brazil and elsewhere in the world

J.H. Pedrosa-Macedo[1]

Summary

Biological control projects against weeds in Paraná State, Brazil are currently focused on six species or species groups. Two projects, including native *Senecio* species (Asteraceae) and the exotic *Tecoma stans* (L.) Kunth (Bignoniaceae), involve studies on the ecology and natural enemies of plants that are problematic in Brazil. Native *Senecio* species are particularly problematic in Brazilian pastures, where losses of livestock, notably cattle, due to toxicity have been estimated to cost US$7.5 million annually in the State of Rio Grande do Sul alone. The Central American *T. stans*, which has become an increasing problem in pastures in Paraná State, is currently the subject of botanical studies and studies on associated insects and fungal pathogens. The remaining four projects represent cooperative research programs with international organisations and involve studies on the ecology and natural enemies of native Brazilian plants that are invasive elsewhere in the world. These species include *Schinus terebinthifolius* Raddi (Anacardiaceae) and *Tibouchina herbacea* (Melastomataceae), both of which are problematic in Hawaii, *Psidium cattleianum* Sabine (Myrtaceae), which is invasive in Florida (USA), and *Solanum mauritianum* Scopoli (Solanaceae), which invades the high rainfall regions of South Africa. This paper updates the progress achieved with these six projects.

Keywords: *Psidium cattleianum, Schinus terebinthifolius, Senecio* spp., *Solanum mauritianum, Tecoma stans, Tibouchina herbacea.*

Introduction

The Federal University, Paraná, Brazil (UFPR), has encouraged studies on agricultural weeds as well as the training of weed specialists in the departments of Forest Sciences, Veterinary Studies and Entomology since 1990. These programs led to the establishment of international programs aimed principally at the development of biocontrol agents against Brazilian species that have become pests elsewhere.

Several species of *Senecio* kill cattle in Rio Grande do Sul State (Riet-Correa *et al.* 1991). *S. brasiliensis* is the most problematic species, as it is a significant weed in pastures in the southern states of Brazil. It is a large, perennial plant that can form significant stands within 2–3 years. The economic losses due to intoxication induced by *Senecio* species are estimated at US$7.5 million (Mendez 1997, Riet-Correa & Medeiros 2000, Karam *et al.* 2002). Our intention is to augment populations of the most damaging insects in the hope that they will reduce stands significantly.

The "amarelinho" (yellow bells), *Tecoma stans*, is another priority species. This plant, which has spread throughout several parts of the world, presents conflicts of interest in that it is considered either as a weed, an ornamental or a medicinal plant (Kranz & Passini 1997). It is considered an invasive alien plant in the Brazilian savanna (Mendonça *et al.* 1998).

International cooperative studies are being conducted against strawberry guava (*Psidium cattleianum* – Myrtaceae), Brazilian peppertree (*Schinus terebinthifolius* – Anacardiaceae), glory bush

[1] Centro de Ciências Florestais – DECIF-SCA – Universidade Federal do Paraná, Av. Lothário Meissner, 3400, 80.210-170 Curitiba, Paraná, Brazil. Laboratório Neotropical de Controle Biológico de Plantas <johpema@netpar.com.br>.

(*Tibouchina herbacea* – Melastomataceae) and bugweed (*Solanum mauritianum* – Solanaceae). There is a wide range of insects that cause damage on leaves, growth buds, flower buds, flowers and fruits of these plants. Over 18 species are undergoing intensive field and laboratory research aimed at elucidating their biology, host specificity and impact. To facilitate field and laboratory studies, an arboretum has been developed containing a variety of species in the following families: Asteraceae, Anacardiaceae, Bignoniaceae, Convolvulaceae, Lauraceae, Melastomataceae, Poaceae, Solanaceae, Myrtaceae and Rutaceae. Research is carried out in collaboration with various UFPR units. FUPEF (Fundação de Pesquisas Florestais do Paraná) is a university foundation that administers most foreign cooperative agreements (currently University of Hawaii, University of Florida, and Plant Protection Institute of South Africa). Besides these agreements, there are cooperative arrangements with local universities and government agencies, principally UNICENTRO (Universidade Estadual do Centro-Oeste, Irati, PR), FURB (Fundação Universidade de Blumenau, SC), UEL (Universidade Estadual de Londrina, PR), and UFPel (Universidade Federal de Pelotas, RS).

The study region

Universidade Federal do Paraná has an ideal location in Curitiba due to its proximity to several major habitats, viz. Atlantic Forest (tropical, from sea level to 650 m), *restinga* (sea level to 20 m) and Araucaria Forest (subtropical – 650 to 1100 m). All are within 90–100 km of the university. This closeness enables studies of insects and diseases associated with the target plants to be undertaken throughout the year under natural conditions, as well as in the greenhouses and arboreta. The conditions at Curitiba were advantageous enough that CSIRO (Australia) maintained a biological control laboratory there during the 1970s and 1980s. Coastal *restinga* vegetation consists of a wide variety of plants growing in sandy substrate with a high water table. The forest today is all secondary and of short stature, not more than 5 m. There are a few protected areas, but the region has marginal agricultural potential so disturbance is minimal. The climate is hot and humid with year-round average temperature between 18–22°C (min. 0.9°C, max. 38°C) and annual rainfall between 1430 and 2450 mm (Maack 1968). Droughts are possible in June and July (Carpanezzi *et al.* 1986). The Atlantic Forest ranges from 50–700 m. It is mostly secondary forest with some pockets of primary vegetation. The climate is humid, tropical, with temperatures from 15 to 19°C (min. 5°C, max. 38°C) and annual rainfall between 1250 and 2500 mm. In Paraná, much of this forest is protected. However, some limited subsistence agriculture is practised on a small scale in some areas (Carpanezzi *et al.* 1986). The subtropical first plateau ranges from 650 to 1100 m. The climate is hot and humid with temperatures between 15 and 19°C (min. –10°C, max. 35°C), 0–40 frosts per year and 900–1200 mm annual rainfall. Droughts are extremely rare (Carpanezzi *et. al.* 1986). Within the region there are several protected forest areas, including *Araucaria angustifolia* (Araucariaceae) cloud forest, as well as extensive secondary forest and submontane fields available for studies.

Biological control research projects

The current research program at Curitiba includes efforts to control *Tecoma stans* in Paraná State and *Senecio brasiliensis* (Asteraceae) in southern Brazil (Paraná, Santa Catarina and Rio Grande do Sul). The cooperative program with the University of Florida (Brazilian peppertree control project) and with the University of Hawaii (strawberry guava and Tibouchina biological control projects) are continuing at an advanced phase of agent selection, host range and impact tests. The cooperative program with the Plant Protection Research Institute, South Africa is also active, testing the host range of one only agent on six Solanaceae species.

Tecoma stans (Bignoniaceae) – amarelinho – yellow bells

The plant is found throughout much of Brazil, from Amazonia (Manaus) south to Rio Grande do Sul (Butiá). In the north and northeastern regions of the Paraná state, it occupies over 50,000 ha of pastureland. The infestation is centered around Londrina, where over 10,000 ha of pasture has been lost to weed infestations. Yet, in Curitiba and vicinities, it is used as in urban forestry from where it has dispersed to abandoned grounds.

We have initiated phenological studies. A number of insects have been collected, including Lepidoptera (2 spp.), Coleoptera (9 spp.), Homoptera (2 spp.), Hymenoptera (6 spp.), Hemiptera (5 spp.) and Thysanoptera (1 sp.). Two unidentified Lepidoptera species attack the plant: a leaf-roller (Crambidae) and a fruit borer (Olethreutidae). The other associated insects are being collected for future taxonomic and biology studies. At Bogotá savanna (Colombia), there are 41 associated insect species: Coleoptera (1 sp.), Diptera (3 spp.), Hemiptera (6 spp.), Homoptera (9 spp.), Hymenoptera (14 spp.), Lepidoptera (3 spp.), Neuroptera (2 spp.) and Thysanoptera (3 spp.). In addition, there are also three Acari species (Lee *et al.* 2000). In Nicaragua (Masaya National Park), the species observed to date include: Lepidoptera (1 sp.), Coleoptera (3 spp.), Homoptera (2 spp.) and Hymenoptera (3 spp) (Pedrosa-Macedo, personal notes, 2002). At Blumenau, SC (FURB) studies on associated insects and fungal diseases are being conducted (*Prospodium*

appendiculatum), while at Londrina (UEL), the studies include botanical and entomological aspects.

Senecio (Asteraceae) in Brazil

There are about 150 species of *Senecio* in Brazil, but the biennial *S. heterotrichius, S. selloi, S. brasiliensis* and the annual *S. oxyphyllus* are the most common in pastures (Karam *et al.* 2002). The most abundant potential control agents are: *Pericopis sacrifica* (Lepidoptera), *Phaedon confinis*, *Agathomerus subfasciatus* and *Systena s-littera tenus* (Coleoptera) (Pedrosa-Macedo *et al.* 2000, Karam *et al.* 2002). All these species are native to the regions of infestation. Thus, their use in an augmentation biological control program is limited because their natural enemies are also present. Potential control agents from other areas, including *Longitarsus jacobaeae* Waterhouse and *L. flavicornis* Stephens (Coleoptera: Chrysomelidae), could be used as a "short cut" project, as both are already used as biological control agents for *Senecio jacobaea* L. (Asteraceae) (tansy ragwort). Further evaluation depends on international cooperation.

Psidium cattleianum (Myrtaceae) – strawberry guava

Strawberry guava is established in at least 31 countries (mostly islands) in the subtropical region (Wikler 1999). In the Hawaiian archipelago it is a significant weed of native forest, where it forms monotypic stands. Ecosystem disturbance, particularly by non-indigenous feral pigs, is the principal mechanism of establishment and intensification (Diong 1982). Manual control, though expensive, is feasible, but the ecological and often archaeological damage is unacceptable. Chemical control is increasingly difficult as more and more suitable herbicides become banned due to long-term undesirable ecological effects. Biological control is the last resort. Potential biological control agents have been discovered in the Atlantic Forest and associated areas in Brazil. They include, in order of suitability: *Tectococcus ovatus* (Homoptera: Eriococcidae); a leaf galler, *Dasineura gigantea* (Hymenoptera: Cecidomyiidae); a bud galler, *Eurytoma psidii*; and other gall-formers, either *Eurytoma cattleianii* or *Eurytoma desantisi* (Hymenoptera, Eurytomidae) (Angelo 1997, Vitorino 2001). *Neotrioza tavaresi* (Hemiptera: Psyllidae), another leaf galler, appears to have insufficient impact on the plants to be useful (Butignol & Pedrosa-Macedo 2001). None of the above species attack the congeneric *P. guajava*, an important agricultural fruit crop.

The taxonomy of the *P. cattleianum* group needs further research. Field tests demonstrated that the yellow-fruited form of *Psidium cattleianum* and *P. spathulatum* are heavily attacked by *T. ovatus*, whereas the red-fruited form of *P. cattleianum* and *P. longipetiolatum* appear to be resistant. Insects are frequently extremely capable discriminators between species. It is somewhat incongruous that one form of a currently accepted species is not attacked by an insect, whereas another species is.

Solanum mauritianum (Solanaceae) – bugweed (fumo-bravo)

This plant was taken to South Africa by Portuguese navigators in the 16th century (Roe 1972, Olckers 1999). It is a significant weed in reafforestation, agriculture and conservation areas, urban space, river margins and road margins. It is listed as a Category I weed in the South African biological control program (Henderson 2001). The weevil *Anthonomus morticinus* Clark (Coleoptera: Curculionidae) from south-eastern Brazil is being studied on nine congeners: *S. capsicoides, S. diflorum, S. fastigiatum, S. gilo, S. granuloso-leprosum, Solanum melongena, S. palinacantum, S. tuberosum,* and *S. viarum.* Field studies are conducted at various sites around Curitiba and laboratory and controlled environment studies at the Arboretum Juvevê. None of the above *Solanum* species is attacked by *A. morticinus*, except *S. mauritianum* and *S. granuloso-leprosum*. In "no-choice" tests in the laboratory, however, this weevil can feed on some of these species.

Schinus terebinthifolius (Anacardiaceae) – Brazilian peppertree (aroeira)

Brazilian peppertree is an aggressive plant in Florida, USA, especially in the Everglades National Park, and also in the Hawaiian archipelago. It was brought to the USA as an ornamental plant in 1840 (Bennet & Habeck 1991) and again in 1891 (Workman 1978). It is native to southern Brazil, Paraguay and northern Argentina. It has been spread subsequently to several parts of the world, including American Samoa, Australia, Fiji, New Caledonia, Mauritius, Micronesia, Puerto Rico, and Tahiti. Four potential biological control agents are being studied: *Heteroperreyia hubrichi* (Hymenoptera: Pergidae), *Calophya terebinthifolii* (Homoptera: Psyllidae), *Epsimus utilis* (Lepidoptera: Tortricidae) and *Pseudophilothrips ichini* (Thysanoptera: Phlaeothripidae). *H. hubrichi* was approved after host range tests in Florida, but not in Hawaii where it also attacks the endemic *Rhus hawaiiensis*. Its toxicity to wild animals and cattle is being evaluated because there are some suggestive reports from Australia. A preliminary test with a calf, where 100 final-instar larvae were mixed with the food, resulted in no signs of poisoning after 24 and 72 hours. The droughts associated with the "El Niño" phenomenon have resulted in a significant reduction in *H. hubrichi* populations, curtailing the program temporarily. We have not developed a reliable mass-rearing technology to date.

The leaflet galler, *C. terebinthifolii*, has a disjunct distribution on the First Plateau of Paraná and the littoral area. There are different impacts in the two

regions. Both of these results have caused some concern as to the suitability of this potential agent. Further studies on its biology, as well as captive rearing studies, are in progress.

Epsimus utilis provides a reasonable degree of specificity to the Brazilian peppertree. Preliminary tests with *E. utilis* larvae on young Brazilian peppertree plants resulted in a mean biomass loss of 42% per leaflet, but the general impact of the larvae on the plant was not established; we have not developed a sufficiently robust means of evaluation.

Pseudophilothrips ichini attacks Brazilian peppertree throughout its range, except in the littoral areas of Paraná, where it is rare. "No-choice" tests registered attacks on *Mangifera indica*, *Anacardium ocidentale* and *Rhus sandwicensis* (Anacardiaceae), but in "multiple choice" tests these species were not attacked. Two types of impact have been established: 11% reduction of plant growth and 45% increase in branching, causing excessive branching of the plant. *P. ichini* tests on the ornamental *Schinus molle* are being prepared.

Tibouchina herbacea

T. herbacea was introduced into Hawaii as an ornamental plant. It is originally from Brazil, and the main population occurs at Serra Gaucha, Rio Grande do Sul state, although it is known from other Brazilian states such as Santa Catarina and in the cerrado (Brazilian savanna) region (Mendonça *et al.* 1998). It belongs to a species complex whose systematics is unclear. Two closely related species, *Tibouchina cerastifolia* and *T. gracilis*, are common. Two other related species, *Acisanthera variabilis* and *Rhyncanthera* sp., are also frequent. All four species are attacked by at least three different species of as yet unidentified weevils. Among 35 insect species associated with ruderal herbaceous Melastomataceae, six are potential agents: *Schrenkensteinia* sp. (Lepidoptera: Schrenkensteinidae), whose presence was verified only in Rio Grande do Sul state, though Barreto (pers. comm.) recorded it in Minas Gerais; *Syphrea uberabensis* Bechyné (Coleoptera: Chrysomelidae); *Margaridisa* sp. (Coleoptera: Chrysomelidae); *Lius* sp. (Coleoptera: Buprestidae); an unidentified Geometridae caterpillar (Lepidoptera); and *Anthonomus partiarius* Boheman (Coleoptera: Curculionidae). The last insect was found on *T. herbacea, T. cerastifolia* and *Acisanthera variabilis*, feeding on flower buds, flowers, pollen and seeds. Studies on its biology, ecological behaviour and host range are being conducted. *A. partiarius* was not found on *T. gracilis* and *Rhyncanthera* sp. (Pedrosa-Macedo *et al.* 2000)

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Determining the suitability of a European cone weevil, *Pissodes validirostris*, for biological control of invasive pines in South Africa

Alain Roques,[1] Géraldine Roux-Morabito,[2] John H. Hoffmann,[3] Marc Kleinhentz[1] and Andrei Gourov[1]

Summary

Several Mediterranean pine species introduced to South Africa have become invasive plants which displace native flora and deplete limited water resources. A proposal to release host-specific, seed-destroying insects to arrest these pine invasions has created a potential conflict with the lucrative forest industry which is predominantly based on pine species from North America. A survey of European cone insects revealed that pine cones are heavily damaged by larvae of a cone weevil, *Pissodes validirostris* (Coleoptera: Curculionidae). To determine the host specificity, weevils were collected on 10 pine species throughout Europe. Adult responses to European and North American *Pinus* species were recorded using both natural choice tests and no-choice tests. Cone use was significantly dependent on the larval host of the weevils with adults originating from northern and alpine pines (*P. sylvestris* group) being incapable of developing on Mediterranean pines (*P. pinaster* and *P. pinea*) and *vice versa*. Neither group of beetles utilized cones of five-needle pines or *P. patula*. Observations of adult maturation-feeding on seedlings produced similar patterns of host specificity. Morphometric and genetic (mitochondrial DNA) analyses on the different populations confirmed that *P. validirostris* probably consists of a complex of sibling species specialized on different host pines rather than a single generalist species. Therefore, cone weevils originating from *P. pinaster* appear to be suitable for release in South Africa.

Keywords: biological control, cones, host specificity, insect damage, invasive *Pinus*, *Pissodes validirostris*, South Africa.

Introduction

With one exception, there are no native conifers in the genus *Pinus* in the Southern Hemisphere, but pines mostly originating from Europe and North America are extensively planted in many countries throughout the region. During the late 17th century, Mediterranean pine species, notably *P. pinaster* Aiton and *P. pinea* L., were introduced into South Africa to develop commercial plantations. Within a short time span, *P. pinaster* in particular started to invade natural vegetation around plantations (Richardson and Higgins 1998). Today, several pine species, including *P. pinaster* and another Mediterranean pine, *P. halepensis* Mill., as well as several North American species, are extremely problematic in conservation areas, where native plant species are displaced, and in water catchments, where the large trees diminish water-flow in rivers (Richardson *et al.* 1996). There is therefore a pressing need to remove pines from areas that have been invaded and to prevent the reinvasion of these areas, or at least slow the rate at which this happens.

The best option for combating problems caused by invasive pines is classical biological control. However,

[1] INRA, Zoologie Forestière, Ardon, BP 20619, F-45166 Olivet Cedex, France.
[2] Biologie des Ligneux, Orléans University, BP 6759, F-45067 Orléans, France.
[3] Zoology Department, University of Cape Town, Rondebosch 7700, South Africa.
Corresponding author: Alain Roques <Alain.Roques@orleans.inra.fr>.

South Africa has a lucrative timber industry based mainly on pine species from North America, including *P. elliotti* Engel., *P. patula* Sch. et Deppe and *P. taeda* L. and to a lesser extent *P. radiata* D. Don. The prospects for the biological control of alien invasive *Pinus* species must therefore take into account this potential intersectoral conflict. Similar conflicts have been successfully managed in South Africa. An example is mesquite (*Prosopis* spp.), a North American leguminous tree, which invades dry areas in South Africa, but which has also many uses as an agro-forestry plant (Zimmermann 1991, Moran *et al.* 1993). The release of two seed-feeding bruchids, which have become widely established and abundant, led to the destruction of copious quantities of seeds without affecting the useful attributes of mesquite plants (Hoffmann *et al.* 1993, Coetzer & Hoffmann 1997). Similarly, seed-feeding insects have been used against Australian acacias that are both invasive and exploited in South Africa (e.g. Dennill & Donnelly 1991, Dennill *et al.* 1999) and there are precedents in which the invasiveness of alien perennial tree species has been reduced by suitable, host-specific, seed-feeding insects (Hoffmann & Moran 1991, 1998).

The situation requires one or more biological control agents that: (i) are associated entirely with cones and seeds; (ii) are monospecific or attack only a limited number of pine species; (iii) do not affect either productivity or growth of the economically important pine species; and (iv) do not transport pathogens between plants. The arthropod fauna exploiting the cones and seeds of *Pinus pinaster, P. pinea* and *P. halepensis* in the native Mmediterranean habitats consists of 16 species (15 insect species and 1 mite species) of which 13 (i.e. 81%) are cone-specific (Roques & El Alaoui 2004). Among these species, a cone weevil, *Pissodes validirostris* Gyll. (Coleoptera: Curculionidae), was considered as the most promising candidate for biological control of invasive pines because it frequently destroys >80% of the annual cone crop in parts of Europe, and 2–3 weevil larvae are enough to destroy a cone and all its seeds (Roques 1976).

Pissodes validirostris is widely distributed throughout the Palaearctic region, from Portugal and Scandinavia to north-eastern China, and has been recorded as attacking both the target Mediterranean pines as well as species in the section *silvestris* (*Pinus sylvestris* L., *P. mugo* Turra, *P. uncinata* Mill., *P. nigra* Arnold and *P. leucodermis* Antoine), and some North American pines that have been introduced to Europe (*P. contorta* Doug. ex. Loud.) (Roques 1983). This apparent lack of host specificity, and the fact that adult weevils feed on pine shoots for maturation before females lay eggs on two-year-old cones (Roques 1976), cast doubts on the potential usefulness of *P. validorostris*. To clarify the situation, a combination of behavioural experiments, morphometric analyses and genetic analyses were used to determine how adult weevils from different larval hosts and geographical origins responded to cones and shoots of target and non-target pine species.

Material and methods

Surveys of cone weevils

From 1998 to 2002, a total of 116 cone samples were collected from 90 different sites in 10 countries throughout Europe and North Africa (Finland, 2; France, 28; Greece, 8; Italy, 2; Morocco, 3; Portugal, 44; Romania, 1; Spain, 23; Switzerland, 3; Turkey, 2). The samples were selected to cover the known range of *P. validirostris* (Fig. 1) but they were also extended to areas where the host plants were growing without records of cone weevils. Collections primarily focused on the three targeted Mediterranean pines (*Pinus halepensis*: 18; *P. pinaster*: 44; *P. pinea*: 7), but they were extended to four other native pine species (*Pinus brutia* Tén. 2; *P. nigra*: 11; *P. sylvestris*: 21; *P. uncinata*: 5) and 3 exotic, introduced species (*P. contorta*: 2; *P. radiata*: 5; *P. taeda*: 1). At ten sites, cones were sampled on pine species growing sympatrically to examine differences in natural levels of damage. Cones were collected during late summer (from 5 August to 5 September, depending on location), just before adult emergence commenced. Wherever possible, up to 100 cones were collected from 10 different trees.

Cone preferences of adult weevils

Two different experiments were performed to determine the range of species that the weevils would use for oviposition and larval development. In one set of experiments, adult weevils were released in an arboretum (Bormes) in southern France, where 34 native and exotic pine species had been planted for trials to compare growth performances. Before the experimental releases, *P. validirostris* did not occur in the arboretum. More than 3000 adult weevils were released during October 1998 (2800) and 1999 (320). Only the pine species known to produce cones regularly in the arboretum were used. Batches of 100 weevils were put near the trunk base of five trees of *Pinus patula*, *P. pinaster*, *P. pinea*, *P. radiata* and *P. taeda*, and batches of 50–60 weevils were put near *P. brutia, P. coulteri* D. Don, *P. eldarica* Medw., *P. flexilis* James, *P. nigra, P. ponderosa* Laws., *P. rudis* Endl., *P. stanckewiezii* Sukacz., *P. rigida* Mill., and *P. pseudostrobus* Lindl. About half (1450) of the weevils originated from a stand of Scots pine in the Alps (Briançon area) and the remainder came from a stand of *Pinus pinea* near Valladolid in Spain. Trees in the arboretum were surveyed for signs of cone damage during the summer from 1999 to 2002. As far as the annual cone crop permitted, up to 100 cones (10 per tree on 10 different trees) were randomly collected on each of the 15 pine species used for the weevil's release. The following variables were then measured: percentage of cones with feeding punctures; percentage of cones with egg-laying punctures; percentage of cones with successful larval development; and percentage of dead cones. Cone length, cone width, and total number and

quality (percentage of filled seeds) of surviving seeds was also measured using X-ray inspection of the cones.

The second experiment consisted of no-choice tests in which weevils were confined in gauze sleeves enclosing one or several branches bearing second-year cones. An adult couple (1 male and 1 female) was placed in each sleeve during early May; when the beetles were normally mating and ovipositing. The adult weevils originated from four different pine species (*P. pinaster* from Buçaco, Portugal; *P. pinea* from Valladolid, Spain; *P. sylvestris* from Briançon, France; and *P. nigra* from Orléans, France) and each couple was offered cones of one of the following pine species: *P. pinaster*, *P. pinea*, *P. halepensis*, *P. taeda*, *P. radiata* or *P. patula*. Sleeves without insects were used for controls in each pine species. Originally, 20 replicates per test were planned, but large fluctuations in cone production on the different pine species prevented a balanced design. The tests were carried out at the Bormes arboretum in 1999, 2000 and 2001. The same variables as measured in the free choice surveys described above were measured to assess insect responses.

Response of adult weevils during maturation feeding

Tests were carried out at INRA Orléans within large (2 × 2 × 2 m) outdoor cages. Each cage enclosed 25, 80–100 cm-tall potted pine seedlings and was supplied with beetles from a single origin, either *Pinus pinaster* from northern Portugal, *P. pinea* from central Spain, or *P. sylvestris* from the southern French Alps. To test weevil preferences during maturation feeding on leaders, five species (*P. pinaster*, *P. pinea*, *P. elliotii*, *P. patula* and *P. halepensis* – the latter replaced by *P. sylvestris* in cages with weevils originating from that species) were randomly arranged in each cage and 200 to 295 newly emerged adult weevils were released in the centre of the cage, during October in 2000 and 2001. In May of the following year, the number of feeding punctures per trunk and branch, and the number of dead shoots were recorded on each plant.

Variation in adult morphology

Length of snout and total body length along the midline were measured in individuals from 12 populations originating from seven pine species (*P. pinaster*, *P. pinea*, *P. nigra*, *P. contorta*, *P. halepensis*, *P. sylvestris*, and *P. uncinata*) and different geographical areas (France, Portugal, Spain, Finland).

Genetic variability of weevil populations

The same populations that were used in the morphometric study, plus 14 additional populations from the same host trees, were subjected to molecular examination. This set covered the European range of *P. validirostris*. Genomic DNA was extracted using the phenol–chloroform method. Only the wing muscles were used in order to prevent any contamination with parasites, fungi and nematodes. This DNA was used as a template for amplification of mitochondrial DNA (mtDNA) fragments by polymerase chain reaction (PCR) using the primers designed by Simon et al. (1994) and Langor and Sperling (1995). A segment *ca.* 900 base pairs long of the cytochrome oxidase I (COI) gene (mtDNA) was amplified. PCR-amplified fragments were digested with 13 endonucleases in restriction fragment length polymorphism (RFLP) analysis.

Results

Survey and samples of cone weevils

More than 22,000 weevils were obtained from the cone collections, including some from areas where they had not previously been observed, e.g. southern Portugal and northern Greece (Fig. 1). *Pissodes validirostris* was not found in North Africa, Corsica, far-southern Spain, and southern Greece. No weevils were found to be associated with the native species, *P. brutia*. On exotic, introduced pines, damage was observed on *P. contorta* but not on *P. taeda*. Weevil-like damage was observed on one occasion on *P. radiata* in Spain but no weevils emerged from the cones.

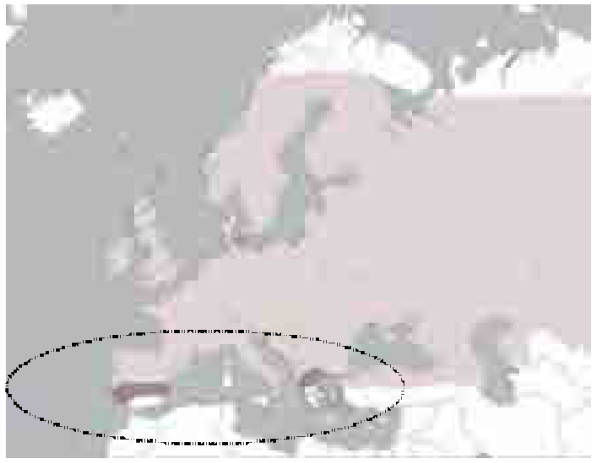

Figure 1. Known range of *Pissodes validirostris* (pale grey) compared to that of Mediterranean pines (dashed line), and new weevil records (dark grey).

Cone preferences of adult weevils

In the arboretum, the percentage of cones attacked (i.e. those with egg-laying punctures) by weevils was higher than 35% in both years in the Mediterranean pines but no damage was observed at all on *P. patula* and *P. radiata* as well as on five-needle pines (*P. cembra*, *P. strobus*). *Pinus taeda* was attacked but the cones were never killed, in contrast to the situation on the Mediterranean pines where 15–35% of the cones were dead. Dissections revealed that the weevil larvae were apparently not capable of penetrating into the cones of *P. taeda* after hatching.

In the no-choice tests, all the pine species were used by weevils but damage patterns differed with both the weevil origin and the pine species (Fig. 2). There was no significant difference in the number of feeding punctures per pine species whatever the larval host (Fig. 2A). However, weevils originating from *P. pinaster and P. pinea* laid significantly more eggs than those from *P. sylvestris* except on *P. sylvestris* (Fig. 2B). Weevils from *P. sylvestris* laid very few eggs on Mediterranean pine cones and there was no larvae development (Fig. 2C).

Larvae of weevils from *P. pinaster* and *P. pinea* developed equally well 0n the Mediterranean pines but usually failed to develop on *P. sylvestris*. On *P. radiata*, larval survival was higher for weevils from *P. pinea* than from *P. pinaster*. The decrease in percentage of filled seeds per attacked cone did not differ between weevils from *P. pinea* and *P. pinaster*, but the impact of these two provenances was significantly higher than that caused by beetles from *P. sylvestris,* except on *P. sylvestris* (Fig. 2D).

Response of adult weevils during maturation feeding

Regardless of number of feeding punctures, no seedlings or leader shoots were killed in any pine species during both years. Weevil damage expressed as the mean number of feeding punctures per cm of branch did not differ significantly between years but damage was significantly different between pine species and weevil origin. Figure 3 presents the average results for 2000 and 2001.

The weevils that originated from the Mediterranean pines fed significantly more on *P. pinaster* than on *P. elliotti* and *P. patula* (ANOVA followed by Tukey's test: $F_{4,21} = 24.41$, $P = 0.0000$ for weevils from *P. pinaster*; $F_{4,21} = 14.1$, $P = 0.0000$ for weevils from

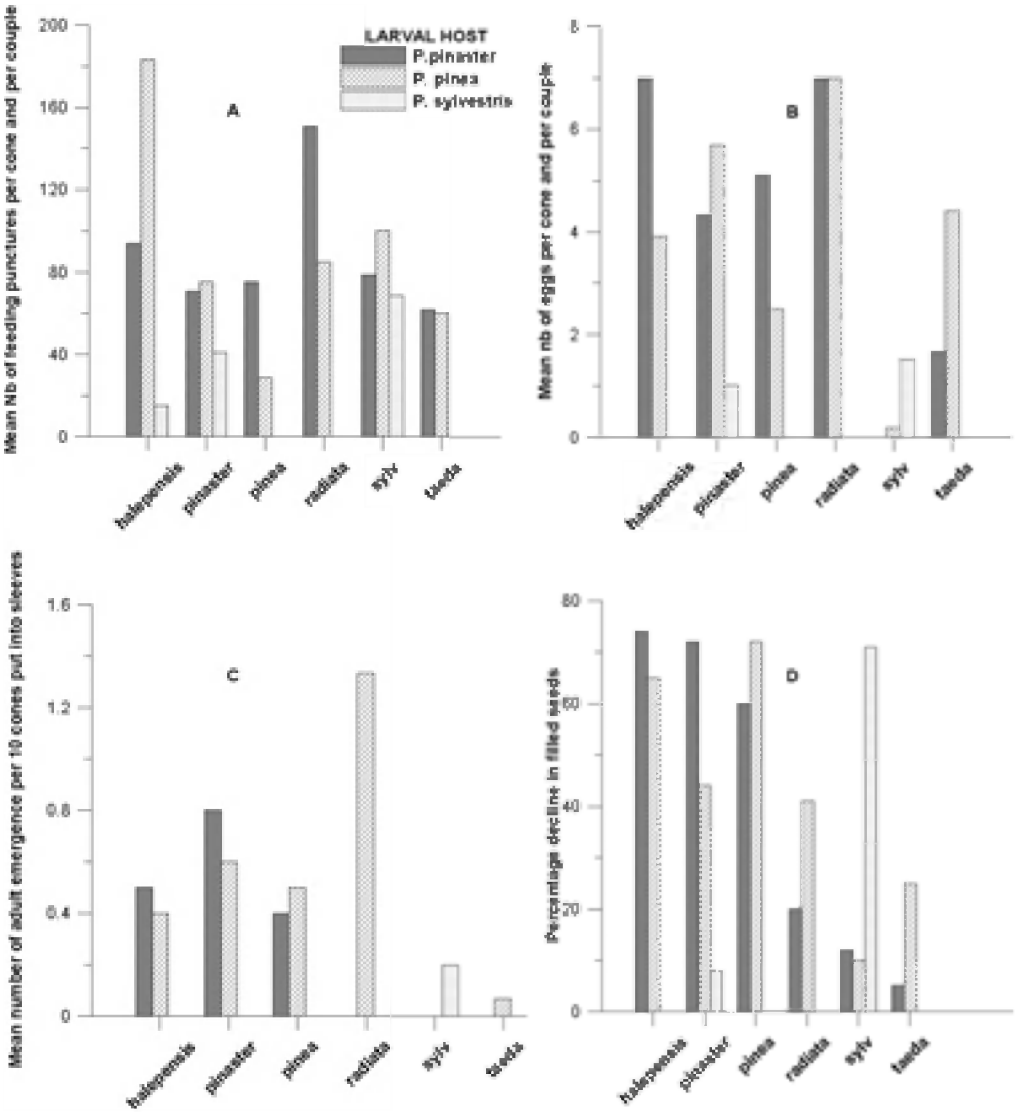

Figure 2. Response of adult cone weevils to pine cone species in no-choice tests according to larval host. A – feeding activity on cones; B – egg-laying; C – success in larval development; D – damage to seeds.

P. pinea). In contrast, weevils from *P. sylvestris* did not show preferences in feeding except on *P. patula*, which in all cases had few feeding punctures.

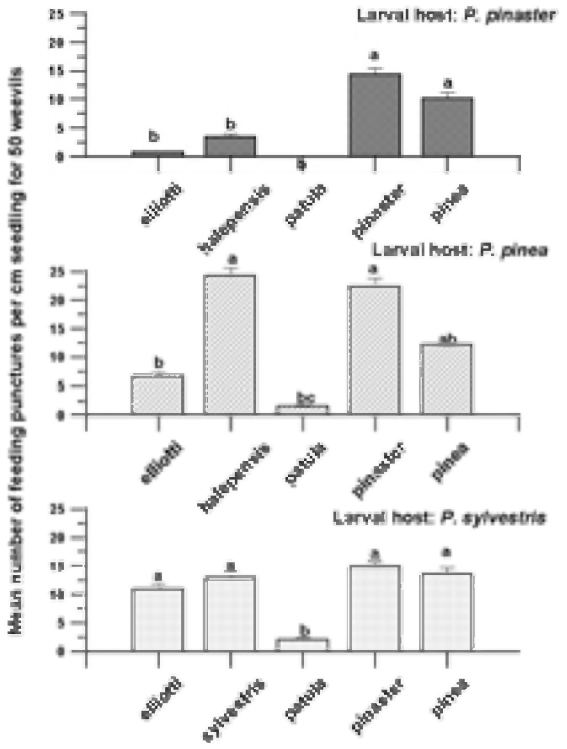

Figure 3. Response of adult cone weevils to seedlings of different pine species according to larval host. Results for 2000 and 2001 combined. Columns with the same letter are not significantly different by Tukey's test at $P = 0.05$).

Variation in adult morphology according to host and geographic origin

Table 1 presents the results for 12 populations sampled in 1999. The insects emerging from cones of Mediterranean pines (*P. pinaster, P. pinea, P. halepensis*) were significantly larger for the two measured traits than those emerging from cones of pines of the *sylvestris* section (*P. sylvestris, P. nigra, P. uncinata*). Insects from *P. contorta* were smaller than these of the latter group. No difference was observed among the Mediterranean pines as well as among the pines of the *sylvestris* group. Frequently, females have a longer snout (except in *sylvestris*) but a smaller body.

Genetic variability of weevil populations

Four of the 13 tested enzymes revealed similar restriction sites for all populations, and thus nine appeared to be polymorphic. Two enzymes (Mspl and Bsp1431) separated the populations into two main groups, corresponding to those developing on northern and alpine pines, plus *P. halepensis* and those developing on Mediterranean pines (*P. pinaster, P. pinea*), respectively. An enzyme (ACCI) discriminated between *P. pinaster* (Portugal) and *P. pinea* (Spain) whereas all the sampled Portuguese populations developing on *P. pinaster* did not differ from the south to the north of the country. We also observed a large within-population variability, probably due to heteroplasmy (DNA composition differing between mitochondria within the same specimen) as has been shown in some other *Pissodes* species.

Discussion

Species in the genus *Pissodes* are all associated only with conifers on which, with one exception, they inhabit either boles or terminals. *Pissodes validirostris* is the only species whose larvae develop within cones – an association that requires a high degree of specialization (Turgeon *et al.* 1994). Convergent results from studies of adult behaviour, morphometry and genetics suggest that *P. validirostris* probably

Table 1. Variation in adult morphology of cone weevils according to host and location.

Pinus host	Country	Site	No.	Rostrum length (1/10 mm)	Body length (1/10 mm)
P. contorta	France	Orléans	12	10.7a[1]	60.7a
P. halepensis	France	Montpellier	12	16.2bc	86.3cd
P. pinaster	Portugal	Alto Espinho	12	17.3c	90.4d
P. pinaster	Portugal	Ansaies	12	17.9c	92.0d
P. pinaster	Portugal	Buçaco	12	18.4c	93.5d
P. pinaster	Portugal	Pardelhas	12	16.6bc	88.9d
P. nigra	France	Orléans	22	15.3b	78.5bc
P. pinea	Spain	Valladolid	20	17.8c	93.1d
P. sylvestris	France	Briançon	25	14.8b	76.7b
P. sylvestris	France	Fontainebleau	25	15.1b	78.6bc
P. sylvestris	France	Orléans	20	14.8b	78.6bc
P. uncinata	France	Montgenèvre	10	15.7bc	76.4bc

[1] Numbers in the same column followed by the same letter are not significantly different following by Tukey's test at $P = 0.05$).

incorporates discrete taxa (species, subspecies, strains or biotypes) that may be monophagous. In a related species, the white pine weevil, *P. strobi* (Peck), Phillips and Lanier (2000) showed similar differences in the host specificity of the insects from different geographical regions and genetic divergence with host associations of weevil populations. Unacceptability of eastern white pine for western populations of weevils from Sitka spruce was shown to be under genetic control, rather than influenced by prior host experience. These authors suggested that *P. strobi* can exist as small breeding populations which facilitate host specialization.

Differences in adult size of *P. validirostris* are possibly dependent on the host resource, the cones of Mediterranean pines being much larger than these of the *sylvestris* group, but preliminary measurements on progeny produced from cross-rearings indicate that these differences are at least partly genetically based. In *Pissodes* species from North America, Williams and Langor (2002) also showed that bole-inhabiting species tend to be larger, with proportionally longer and more slender snouts than the terminal-inhabiting ones. A strong preference of adults of *P. validirostris* for the larval host has been shown for oviposition as well as for sexual maturation feeding. Successful larval development followed the same patterns of host specificity. As a result, the weevil populations obtained from *P. pinaster* and *P. pinea* demonstrated the greatest potential to decrease the seed yield of Mediterranean pines. In addition, apart from restricted amounts of egg-laying, complete larval development was never observed, even under no-choice conditions, in cones of three of the pine species that are of economic importance in South Africa (*P. patula*, *P. taeda*, and *P. radiata*; no cones of *P. elliottii* were available in Europe while this study was under way). Adult maturation feeding on pine leaders and seedlings was insignificant in *P. patula* and *P. elliottii* at least. It would be informative to compare the volatile profiles of these pine species with those of the native hosts. Dormont and Roques (2002) suggested that weevil host choice is mediated by olfactory cues (cone volatile monoterpene profile) which explained why *Pinus cembra*, a European five-needle pine, did not suffer any damage by the weevils.

Further genetic analysis is needed to confirm that populations from *P. pinea* are really separate from *P. pinaster* or whether the observed differences only correspond to a geographical pattern because the populations off *P. pinea* that have been analyzed so far came from Spain, while those off *P. pinaster* came from Portugal. Regardless, the cone weevil taxa originating from the Iberian Peninsula show traits that make them suitable and safe candidates for biological control of Mediterranean pines. Preliminary analyses on several hundred adults did not reveal any pathogenic fungi attached to the body.

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Conflicts of interest associated with the biological control of weeds

Margaret C. Stanley and Simon V. Fowler[1]

Summary

The introduction of weed biological control agents may be delayed or prohibited where the plant targeted for control also has beneficial attributes. There is usually opposition from at least one interest group in most current biological programs. Conflicts fall into one of several categories: 1) those conflicts where one or more groups value the target plant for economic and/or cultural use; 2) those associated with the non-target effects of biological control; 3) those related to biocontrol programs against native plants; and 4) those related to the ecological effects of successful biocontrol as a result of weed use by native biota. In the past, the majority of conflicts and delays to biocontrol have had an economic basis. While industry-based conflicts still dominate, there has been a shift towards conflicts associated with the ecological effects of weed biocontrol. The benefits of weeds to ecosystems, particularly where weeds provide resources for native fauna, are becoming an important part of cost–benefit analyses for weed biocontrol programs. We review examples where weed biocontrol programs have been delayed because of economic and ecological conflicts. We also discuss conflict resolution and the high costs of risk assessment currently faced by biocontrol programs. At present, weed biocontrol programs are usually initiated only when the risk of conflict is low. Where conflict does occur, communication and cost–benefit analyses are key to ensuring resolution is found. However, cost–benefit analyses, particularly those encompassing ecological interactions, are expensive and time-consuming, causing substantial delays to weed biocontrol programs and ongoing environmental damage as a consequence of weed invasion.

Keywords: beneficial, biological control, conflicts, ecological, economic, non-target, native, risk.

Introduction

Serious conflicts of interest can arise from consideration of a plant as a weed by one interest group, but as a valued plant by another. The use of biological control agents may be prevented or impeded where a plant also has beneficial attributes (Cullen & Delfosse 1985). Biological control feasibility studies often reveal conflicts associated with the benefits of the weed, usually economic or ecological benefits. At this point, consultation should begin with various interested groups to determine how serious the conflict of interest is before the biological control program can be initiated (Pieterse & Boucher 1997). According to Julien & Griffiths (1998), biocontrol agents have been released for 133 weed species worldwide, but conflicts of interest associated with weed biocontrol are rarely reported in the literature. However, only recently have interest groups been able to lodge their formal opposition to biocontrol through appropriate authorities and processes. It is therefore likely there have been many unreported conflicts of interest associated with the initiation of weed biocontrol.

Much of the current biocontrol research is opposed by at least one interest group (Table 1). For example, about half the biocontrol research in South Africa is conflict driven, that is, the type of agent used for biocontrol is determined by the nature of the conflict (the part of the weed that is perceived as useful) and the stakeholders (H. Zimmermann, pers. comm.). Internationally, almost all biocontrol conflicts result in some delays in the release of biocontrol agents, and several conflicts have resulted in delays lasting up to two decades, seriously compromising biocontrol programs (Table 2).

[1] Landcare Research, Private Bag 92170, Auckland, New Zealand. Corresponding author: Margaret Stanley <stanleym@landcareresearch.co.nz>.

Table 1. Economic conflicts of interest associated with consideration of biological control of a target weed species.

Weed	Conflict location	Adversely affected by biocontrol	Biological control status	References
Acacia cyclops (Rooikrans)	South Africa	*Wood industry* – timber, bark extracts, firewood *Stabilization* – of dunes	Biocontrol delayed – but seed-eating biocontrol agents provide an acceptable alternative because these agents make the plant less invasive but the vegetation will remain useful. A seed-eating weevil first released 1991.	Neser & Kluge (1986) Olckers *et al.* (1998) Dennill *et al.* (1999) Impson *et al.* (2000)
Acacia mearnsii (black wattle)	South Africa	*Wood industry* – timber, bark extracts (tannins), firewood *Horticulture* – South African Wattle Growers Union provide seed and seedlings for forestry	Biocontrol delayed (about 20 years) – seed-eating weevil eventually released in 1994. Seed-eating agents only acceptable alternative, but release delayed – onus on researchers to prove that chemical protection of wattle seed orchards from the seed-eating biocontrol agent was possible.	Neser & Kluge (1986) De Selincourt (1992) Donnelly *et al.* (1992) Pieterse & Boucher (1997) Dennill *et al.* (1999)
Acacia melanoxylon (blackwood)	South Africa	*Wood industry* – timber, bark extracts, firewood	Biocontrol agent release delayed but eventually released after discussion. Seed-attacking biocontrol agents provided an acceptable alternative.	Neser & Kluge (1986) Geldenhuys (1986) Pieterse & Boucher (1997) Olckers *et al.* (1998)
Acacia saligna (Port Jackson willow)	South Africa	*Wood industry* – both formal industry as well as impoverished locals (townships and squatter camps surrounding Cape Town) who derive income from selling wood and use it domestically (but see Morris & Zimmerman 1993 for argument against this) *Farmers* – use it for shade, animal fodder and as a sandbinder	Delayed but gall-forming rust released in 1987 (Julien & Griffiths 1998). Poor minority farmers not consulted, in hindsight probably should have released seed-eating agent rather than devastating fungus (but see Morris & Zimmerman 1993 for discussion). Higgins *et al.* (1997) showed that the economic benefits of the biological control program far outweighed the potential loss of benefits.	De Selincourt (1992) Morris & Zimmermann (1993) Higgins *et al.* (1997) Pieterse & Boucher (1997) Morris (1999)
Cannabis sativa (marijuana)	United Nations	*Drug producers* – immense monetary worth	Agent (fusarium wilt) found and host specificity tested but not released because of international politics and because violent retributions were feared.	Bennett (1985) McCain & Noviello (1985)
Carduus nutans (nodding thistle)	New Zealand	*Apiarists* – valuable nectar source	Biocontrol put on hold for a brief time, but agents then released. Nectar production more likely to be reduced by herbicide applications than by biological control.	Syrett *et al.* (1985) Julien & Griffiths (1998)
Centaurea solstitialis (yellow star thistle)	USA	*Apiarists* – flowers valuable source of nectar	Biocontrol agent released but committee recommended investigation of plants useful to beekeepers to replace weed.	Huffaker (1957) Turner (1985) Campobasso *et al.* (1998) Julien & Griffiths (1998)

Table 1. (cont'd) Economic conflicts of interest associated with consideration of biological control of a target weed species.

Weed	Conflict location	Adversely affected by biocontrol	Biological control status	References
Chondrilla juncea (skeleton weed)	Australia	*Farmers* – used by graziers to fatten lambs andfeed stock in dry periods	Biocontrol agent released. Small losses compared to economic benefits of controlling the weed.	Cullen (1985) Cullen & Delfosse (1985) Julien & Griffiths (1998)
Chromolaena odorata (triffid weed/ Siam weed)	Africa	*Farmers* – considered to be a valuable fallow species for farmers who use shifting agriculture techniques	Release of biocontrol agents still blocked in most of West Africa (R. McFayden, pers. comm.)	Kluge (1990) Wallich (1995) McFadyen (1996) Strathie-Korrubel & Zachariades (2001)
Cytisus scoparius (Scotch broom/ English broom)	USA	*Horticulture* – used as an ornamental	Biological control agents released.	Turner (1985)
	New Zealand	*Horticulture* – used as an ornamental *Apiarists* – pollen important (high protein) during periods of critical shortage *Farmers* – potential source of fodder *Non-targets* – tagasaste (*Chaemaecytisus palmensis*) also affected by control agent in NZ – tagasaste also used in all above situations	Release of new agents indefinitely suspended. More detailed investigation of costs/benefits required.	Williams (1983) Porteous (1993) Clout *et al.* (1995) Fowler *et al.* (2000b)
	Australia	*Horticulture* – used as an ornamental	Biocontrol agents released, but call for impact assessments before new agents are released.	Atkinson & Sheppard (2000)
Echium plantagineum (Paterson's curse)	Australia	*Apiarists* – flowers produce large quantities of nectar and pollen, particularly at critical times (salvation jane)	Court proceedings led to an injunction on release (1980). Release finally lifted in 1988 and control agent released.	Cullen & Delfosse (1985) Delfosse (1985) Julien & Griffiths (1998)
Eichhornia crassipes (water-hyacinth)	India	*Production* – paper, mulch, alternative source of fuel (biogas), sewage purification *Medicine* – used by locals for various remedies	Release delayed while potential benefits investigated. Delay lasted several years, feasibility of utilization found to be low and eventually permission to release biocontrol agents was granted.	Bennett (1985) Freeman & Charudattan (1985) Julien & Griffiths (1998) Oudhia (1999)
Lantana camara (lantana)	USA	*Horticulture* – commercial growers of ornamental lantana in Florida opposed to biocontrol	Request to release agents for lantana declined because of opposition from commercial growers, dependent on conventional control. No agents for lantana released on mainland USA.	Morton (1994) Julien & Griffiths (1998) J. Coulson (pers. comm.)
Leucaena leucocephala (leucaena)	South Africa	*Wood industry* – timber, fuel *Farmers* – animal fodder	Seed-eating bruchid beetle released after considerable delay. Seed-eating beetles were found to be compatible with utilization of plant's beneficial features and valuable seed plants could be protected from beetle relatively easily (S. Neser, pers. comm.)	Neser (1994)

Table 1. (cont'd) Economic conflicts of interest associated with consideration of biological control of a target weed species.

Weed	Conflict location	Adversely affected by biocontrol	Biological control status	References
Opuntia ficus-indica (prickly pear cactus)	Hawaii	*Farmers* – cactus used by ranchers as forage andsource of water during drought conditions	Biocontrol delayed for several years, poorer ranches were able to develop better water systems and eventually a biological control agent was released.	Fullaway (1954) Julien & Griffiths (1998)
Papaver sommiferum (opium poppy)	United Nations	*Drug producers* – immense monetary worth	Agents found and host specificity tested but not released because of international politics and because violent retributions were feared.	Bennett (1985)
Passiflora mollissima (banana poka/banana passionfruit)	Hawaii	*Hunters* – feral pigs and the introduced Kalij pheasant (*Lophura leucmelana*) use fruit as a food source	Biocontrol agents released. Destructive impact of the weed (and the feral pigs) far outweighed any benefits of the weed.	Warshauer *et al.* (1983) LaRosa (1992) Ref? Markin *et al.* (1992) Markin & Yoshioka (1992) Julien & Griffiths (1998) Trujillo *et al.* (2001)
Pennisetum clandestinum (kikuyu grass)	Hawaii	*Farmers* – agricultural value as forage in some regions	Investigation into biocontrol of kikuyu abandoned because of agricultural value.	Gardener *et al.* (1995)
	New Zealand	*Farmers* – agricultural value in some regions	Now in early stages of investigation into biocontrol. Likely to be opposition.	Pervical (1978) Piggot & Morgan (1987) Fowler *et al.* (2002)
Pinus spp. (pine – wilding pines)	New Zealand	*Wood industry* – *P. radiata* the most important commercial timber species, *P. contorta* the most serious invasive species Also commonly used as shelter belts and ornamentals	In early stages of investigation into biocontrol. Investigation of potential biocontrol agents is restricted to seed or cone eaters only. However, likely to be strong opposition to biocontrol from large *Pinus* forestry industry.	Harding (1990) Brockerhoff & Kay (1998) McGregor (2001)
	South Africa	*Wood industry* – timber	Biocontrol research for *P. radiata* and *P. patula* abandoned for reasons including importance to forestry and possibility of agents acting as vectors for diseases (pitch canker). Research and host range testing of seed/cone-eaters continues for *P. ptnaster* and *P. halepensis*.	Moran *et al.* (2000)

Table 1. (cont'd) Economic conflicts of interest associated with consideration of biological control of a target weed species.

Weed	Conflict location	Adversely affected by biocontrol	Biological control status	References
Prosopis spp. (mesquite)	South Africa	*Farmers* – shade, source of fuel, seed pods used for livestock fodder in arid areas *Apiarists* – source of nectar	Biocontrol delayed for several years. Three seed-eating biocontrol agents eventually released. No biocontrol agents that eat flowers or vegetative parts can be released, but further control is necessary to have effective control of *Prosopis*. Use of agents other than seed-eaters may become more acceptable in the future as landowners realise the extent of *Prosopis* impact.	Neser & Kluge (1986) Harding (1987) Moran *et al.* (1993) Impson *et al.* (1999)
Rubus fruticosus (blackberry)	Australia, New Zealand	*Horticulture* – opposition from berryfuit producers because control agents not specific enough to blackberries (e.g. raspberries, loganberries, strawberries at risk) *Apiarists* – nectar useful	New Zealand initially abandoned biological control investigations because an agent specific to blackberry could not be found. When blackberry rust fungus was selected in Australia as a promising biocontrol agent, its release was blocked for several years by the Tasmanian government on behalf of apiarists and berryfruit producers. The fungus was illegally released in Victoria (1984) and eventually self-introduced to NZ (1990) from Australia. Ongoing biocontrol programs for blackberry in both countries to obtain the best rust strains for the *Rubus fruticosus* aggregate.	Field & Bruzzese (1985) Syrett *et al.* (1985) Stahle (1997)
Rubus strigosus *Rubus parvtflorus* *Rubus spectabilis*	Canada	*Horticulture* – berryfruit producers value genetic contributions to raspberry breeding programs, fruit valued by berryfuit pickers *Rubus* spp. also have ornamental value *Stabilization* – bank stabilization, especially in avalanche areas, dune stabilization	Biological control research ongoing.	Oleskevich *et al.* (1996) (references therein)
Salix spp. (willow)	New Zealand, Australia	*Stabilization* – used for erosion and river (flood) control, shelter belts *Recreation* – promoted as preferred habitat for trout (exotic but New Zealand's most important freshwater fishing resource) *Aesthetic* – public preference for willowed landscape, shade, shelter	Conventional control occurs at present on a site-by-site basis following assessment of benefits and costs of control. Biological control is being considered, but opposition is likely to be great. Crack willow (*Salix fragilis*) and grey willow (*Salix cinerea*) are the most invasive and detrimental species. Several species (including crack willow) are still being planted.	Van Kraayenoord & Hathaway (1986) Warren (1994) West (1994) Cremer *et al.* (1995) Wilkinson (1999) Agriculture *et al.* (2000) Sagliocco & Bruzzese (2001) Stanley (2002)

Table 1. (cont'd) Economic conflicts of interest associated with consideration of biological control of a target weed species.

Weed	Conflict location	Adversely affected by biocontrol	Biological control status	References
Schinus terebinthifolius (Brazilian peppertree/Christmas berry)	Hawaii,	*Apiarists* – nectar important, bee maintenance during the winter months	Hawaii – After a considerable delay, a compromise was reached with beekeepers and biological control agents were released in Hawaii during the 1950s. More biocontrol is currently required by ranchers, but the weed is not spreading and the beekeeping industry opposition is very strong; so no further releases have been made.	Yoshioka & Markin (1991)
	Mainland USA	*Apiarists* – one of Florida's best nectar-producing plants, particularly during the winter months *Horticulture* – used as an ornamental, minor opposition because gardeners now realize adverse impact	Release of biocontrol agent is imminent. Benefits of biocontrol outweigh any associated costs. Apiarist conflict likely to resolved through discussion – the beekeeping community may be willing to tolerate biocontrol because it is less radical and immediate than herbicides or mechanical removal.	Morton (1978) Bennett and Habeck (1991) Sanford (1997) Medal *et al.* (1999) J. Cuda (pers. comm.)
Solidago gigantea and *Solidago canadensis* (goldenrods)	Europe	*Apiarists* – good source of nectar *Horticulture* – still sold in nurseries, widely used as ornamentals in gardens	Biological control considered but abandoned because of opposition. Currently, weed still spreading but biocontrol not considered.	Capek (1971) Greathead (1976) Weber (2000) M. Cock (pers. comm.)
Tamarix ramosissima (salt cedar)	USA	*Horticulture* – ornamental *Apiarists* – food plant for honeybees *Hunters* – provides nesting sites for game birds, white-winged dove (*Zenaida asiatica*) and mourning doves (*Z. macroura*)	Release of biocontrol agents delayed for four years primarily because of flycatcher (and Endangered Species Act) (see Table 2). Benefits of control far outweigh costs.	Andres (1981a) Andres (1981b) DeLoach *et al.* (1996) Stenquist (2000) Zavelata (2000) DeLoach (2001)
Ulex europaeus (gorse)	New Zealand	*Apiarist* – valuable pollen source at a critical time *Farmers* – hedge, shelter plant, forage plant (particularly for the growing goat industry)	Considerable delay (1982–1989), mostly due to apiarists. The benefits of gorse control were found to outweigh any loss due to biological control. Six agents have been released since 1989.	Krause *et al.* (1984) Syrett *et al.* (1985) Hill (1987) Hill *et al.* (2000)

Conflicts usually fall into one of several categories: 1) those conflicts where one or more groups value the target plant for economic and/or cultural use; 2) those associated with the non-target effects of biological control; 3) those related to biocontrol programs against native plants; and 4) those related to the ecological effects of successful biocontrol as a result of weed use by native biota. In this paper, we review examples where biological control programs have been delayed because of conflicts of interest that result from economic use of the plant, non-target effects, and integration of the plant species into the ecosystem. We also discuss conflict resolution and the high costs of risk assessment currently faced by biocontrol programs.

Economic conflicts – plants targeted for biocontrol

There is a general trend for conflicts associated with the biological control of weeds to be industry-based. Of the 27 weeds associated with conflicts in Table 1, apiarists benefit from 37% of these weeds, the horticultural industry (nurseries, berryfruit producers) from 30%, farmers from 30% and the forestry or wood-fuel industry from 22%. Economic conflicts associated with a target weed usually consist of 1) opposition from one economic group, such as apiarists, to biocontrol of a target weed advocated by another economic group, such as farmers or ranchers, or 2) opposition to the biocontrol of an environmental weed by an economic group that uses the target weed. The same type of economic conflict can arise in more than one country where a plant is invasive and control is being attempted (Table 1). For example, berryfruit producers and apiarists in both New Zealand and Australia objected vigorously to the biocontrol of blackberry (*Rubus fruticosus*) (Syrett *et al.* 1985, Stahle 1997). The forestry industry in New Zealand and South Africa have both expressed strong opposition to biocontrol for *Pinus* spp., particularly for *Pinus radiata*, for which biocontrol research has now been abandoned in South Africa (Moran *et al.* 2000).

Conflicts between economic groups

Where values differ, the definition of a weed becomes blurred and specific to the economic group and their use of the weed. The classic illustration of this is *Echium plantagineum* (Paterson's curse to most farmers and landowners, Salvation Jane to graziers in areas prone to drought) in Australia, where the biocontrol of *E. plantagineum* was proposed by farmers and landowners, but opposed by graziers in drier regions in collaboration with apiarists. This conflict resulted in a delay of nearly ten years in the release of biocontrol agents for *E. plantagineum* (Cullen & Delfosse 1985). The proposed biological control of several weeds in New Zealand, such as gorse (*Ulex europaeus*) and nodding thistle (*Carduus nutans*), has caused similar conflicts between apiarists and farmers, and considerable delays (Table 1). Plants can also be valued differently within an economic group. For example, pasture and crop weeds such as skeleton weed (*Chondrilla juncea* – Australia), prickly pear cactus (*Opuntia ficus-indica* – Hawaii) and mesquite (*Prosopis* spp. – South Africa) have been seen as valuable sources of animal fodder by some farmers, especially those in drought-prone regions (Table 1). The biocontrol research programs involving these weeds were subject to long delays while the conflicts were resolved.

Economic conflicts can also arise *post-hoc* with the development of new industries that use exotic plants. Perceptions and values change over time. During the 1930s and 1940s, biocontrol agents were released to control prickly pear cactus (*Opuntia ficus-indica*), which at that stage infested 1 million ha of land in South Africa. However, the 1990s has seen the development of a fast-growing cactus pear fruit industry (spineless fruit variety of *Opuntia ficus-indica*), which now accuses the South African Department of Agriculture of negligence in having allowed the release of control agents (Zimmermann 1992). St John's wort (*Hypericum perforatum*), a noxious pasture weed, is now gaining popularity in the natural pharmaceutical industry as an anti-depressant and is currently being grown as a crop in some regions (Rey & Walter 1998, Reichard & White 2001).

Economic plants as environmental weeds

Most environmental weeds are those that have been introduced for use by industry or to create a new industry. For example, several *Acacia* species introduced to South Africa are highly invasive and have a

Table 2. Outcome of economic biocontrol conflicts driven by weed benefits to apiarists, horticulture industries, farmers, or wood industries.

Biocontrol outcome	Interest group			
	Apiarists ($n = 6$)	Horticulture ($n = 4$)	Farmers ($n = 6$)	Wood Industries ($n = 6$)
Agents released	1	0	1	0
Delayed (but eventually released)	4	1	3	5
Abandoned (or still delayed)	1	3	2	1

severe impact on natural areas such as the Fynbos (Dennill & Donnelly 1991, Pieterse & Boucher 1997). However, *Acacia* species are also highly valued by the forestry industry and this has generated intensive discussion, interaction and conflict resolution, which have almost inevitably resulted in compromise (Dennill & Donnelly 1991, Pieterse & Boucher 1997, Zimmermann & Klein 2000). Five of Australia's worst environmental weeds are introduced tropical pasture plants and two are shrubs introduced for shade and fodder (Low 1997). Of the 463 grasses and legumes introduced to northern Australia to improve pasture productivity, only 5% improved productivity, but over 60% of the remaining species naturalized and became weeds (Lonsdale 1994).

Industry often limits which plant species can be targeted for biocontrol. For example, ornamentals, such as ginger (*Hedychium* spp.), cannot be targeted for biocontrol in Hawaii because they are seen as being of high value to the Hawaiian tourist industry, despite being serious invaders of Hawaiian forest (Gardner *et al.* 1995). Several alien pasture species (e.g. kikuyu grass, *Pennisetum clandestinum*) in Hawaii that aggressively invade natural systems cannot be targeted for biocontrol because they are valued by the agricultural industry (Gardner *et al.* 1995). There are similar issues with pasture grasses in New Zealand and Australia (Lonsdale 1994, Fowler *et al.* 2002). Biocontrol of lantana (*Lantana camara*) has been blocked in Florida because it is a popular ornamental plant (Table 1; Morton 1994). Olives (*Olea* spp.) have become a major environmental weed of bushland remnants in Australia (Dellow *et al.* 1987, Spennemann & Allen 2000). However, because of conflicts associated with an expanding olive industry in Australia, it is highly unlikely that olive trees will ever be considered a target weed for biological control (Low 1999, Spennemann & Allen 2000). There is, however, increasing pressure on the olive industry to control feral olive trees and minimize the risk of avian dispersal of olive seeds (Jupp *et al.* 1999). Another important horticultural plant, kiwifruit (*Actinidia* spp.), is becoming a serious environmental weed in New Zealand. Although it is improbable that biocontrol would ever be considered for this weed, the kiwifruit industry has recently committed to contributing to the costs of wild kiwifruit control (Mather 2000).

It is not surprising that many horticultural plants become environmental weeds because they are selected on the basis of fast growth rate, and a wide range of adaptations (Richardson 1998 and references therein). The traits that make them suitable for forestry or horticulture are often characteristics of early successional species and therefore make them potential invaders. Information about the use and management of native plants is often lacking, and thus their value is not so readily appreciated by economic groups (Turner 1985, Richardson 1998). Successful management techniques are available for well-studied and used alien species, and it is with these species that industry groups prefer to work (Richardson 1998). Although recent attention has been given to selecting species that pose less risk to the environment, factors such as the fast growth rate, low cost and adaptability of alien species often override such considerations (Richardson 1998, Stanley 2002).

Non-target conflicts

Several conflicts have emerged as a result of the potential effects of biological control agents on one or more non-target plant species that have beneficial or valuable attributes. The proposed biological control agents for noogoora burr (*Xanthium pungens*), an important agricultural weed in Australia, attacked sunflower plants (*Helianthus annuus*) in host-specificity tests (Cullen & Delfosse 1985). However, the substantial economic importance of controlling noogoora burr was considered to far outweigh the risk to the small sunflower industry in Australia, and subsequently the biocontrol agents were released. Conversely, the biological control program for sweet briar (*Rosa rubiginosa*) in New Zealand was abandoned because of strong opposition from the rose-growing industry because of the perceived risk to cultivated roses (Syrett *et al.* 1985). The root weevil (*Rhabdorhynchus varius*), a proposed biocontrol agent of houndstongue (*Cynoglossum officinale*) in Canada was rejected after host-specificity testing revealed that it fed on *Echium vulgare*, a valuable nectar-producing plant for honey bees in southern Ontario (De Clerck-Floate & Schwarzländer 2002).

The release of an agent (*Melanterius servulus*) to control stinkbean (*Paraserianthes lophantha*) in South Africa was delayed substantially because the agent was found to attack seeds of commercially important Australian *Acacia* species (Donnelly 1990). The South African Wattle Growers Union strongly objected to the release of this agent, and release was delayed several years. Subsequent negotiations put the onus on researchers to prove to the Growers Union that chemical protection of the wattle seed orchards was possible. Once this had been achieved, the weevil was finally released (Donnelly 1990, Donnelly *et al.* 1992, Dennill *et al.* 1999).

Non-target conflict is causing serious delays in the biological control of broom (*Cytisus scoparius*) in New Zealand (Fowler *et al.* 2000b). Broom has been declared a noxious weed and is a problem for forestry, agriculture, and areas of high conservation value (Syrett *et al.* 1999, Fowler *et al.* 2001). Two biological control agents have been released since 1981. However, an application to release broom leaf beetle (*Gonioctena olivacea*) was declined in 1997 because the broom leaf beetle attacked tagasaste (*Chamaecytisus palmensis*) seeds, and there was insufficient information on the value of tagasaste relative to the broom problem and on the risk the agent posed to tagasaste. Tagasaste is currently being

promoted as an important source of fodder for farmers, is a food source for endemic pigeons (*Hemiphaga novaeseelandiae*), and is used as a nurse plant for revegetation of native forest (Fowler *et al.* 2000b, 2001). This conflict has not been resolved, and data on these issues are being gathered to strengthen a revised application to release the agent.

The conflicts involving stinkbean and broom are interesting because in both cases the non-target plants are seen as weeds by some interest groups. Several *Acacia* species are serious weeds of conservation areas such as the Fynbos in South Africa (Pieterse & Boucher 1997), and tagasaste is seen as a weed of roadsides in New Zealand (Roy *et al.* 1998) and Australia (Swarbrick & Skarratt 1992, Muyt 2001). However, the commercial value of non-target plants may prevent or substantially delay the release of agents to combat important weeds. This is likely to result in more expensive biological control programs with longer delays associated with finding agents acceptable to commercial groups.

In recent years, where extensive host-specificity tests reveal a native species at risk of being a non-target food source for the agent, the proposed agent is rejected before any application for release is made (Syrett *et al.* 1995). However, some agents have been released that have attacked native plants on release (Turner 1985, Diehl & McEvoy 1989, McFadyen 1998, Fowler *et al.* 2000a). For example, *Rhinocyllus conicus* released in North America to control musk thistle (*Carduus nutans*) in the 1960s, now attacks several native *Cirisium* thistle species (Louda *et al.* 1997). The possibility of some damage to native thistles was anticipated through host-testing, but the seriousness of the weed problem (economically and ecologically) resulted in a decision to release the agent (Zwoelfer & Harris 1984, Boldt 1997, Louda 2000). Recent research demonstrates that the reduction in seed production in native thistle species has been significant, this phenomenon has been geographically widespread, and use of native thistles by this agent is increasing (Louda *et al.* 1997, Louda 2000). However, in some cases, rare, non-target native plant species may already be negatively affected by the broad-spectrum chemical herbicides used in the absence of biocontrol agents (Harris 1988). For example, Sukopp & Trautmann (1981) estimated that 89 of 589 rare plants in Germany are declining as the result of herbicide application.

Native plant species as weeds

In several regions, the densities of some native plant species have increased substantially due to changes in land use and overgrazing (Buffington & Herbel 1965). This has resulted in a number of plant species being considered weeds within their native range (DeLoach 1978, 1995). Many native "weeds" in the south-western United States and in Canada have been proposed as targets for biological control (DeLoach 1980, Teshler *et al.* 2002). However, Pemberton (1985) argues that site-specific control methods that are less persistent may be more appropriate solutions to native plant problems. Although native "weeds" cause serious problems for agriculture and conservation where they displace other species, they often form an integral part of the ecosystem and provide an important resource for other native biota because they have evolved alongside them. They may also provide many materials and uses for human cultures, since people have coexisted with native plants for substantial periods of time. Where a native plant species is targeted for biological control, the conflict is more intense and more difficult to resolve since benefits are more numerous, the ecological effects of control are complex, and the general public may find it difficult to view a native species as a weed. Before biological control can proceed, the possible impacts on beneficial uses of the plant must be considered, as must the effects of reducing the abundance of the native species, which is usually a dominant species in the ecosystem. We illustrate such conflicts with three case studies.

Case study 1: bracken (*Pteridium aquilinum*)

Bracken is native to Great Britain, but also causes problems for agriculture, conservation, forestry and recreation (Pakeman & Marrs 1991, 1993). Links have also been suggested between bracken in the landscape and the incidence of certain types of human cancers and livestock losses (Pakeman & Marrs 1993, Pakeman *et al.* 1993). The spread of bracken is historically linked to rural depopulation, the replacement of hill cattle with sheep (which do not trample fronds) and a decline in the use of bracken as a resource in rural areas (Pakeman *et al.* 1993). Bracken invades areas of high conservation value, including heather moorlands, heaths and grasslands, often threatening rare species as a result. However, because bracken is a native species, it also provides habitat for many bird and invertebrate species and several native plant species flourish under a dense canopy of bracken (Pakeman *et al.* 1993). Although continued invasion by bracken appears to result in reduced species richness and loss of valuable plant communities, the high conservation value of bracken in some areas has raised concerns about using an indiscriminate control technique such as biological control (Lawton 1988, Pakeman & Marrs 1993, Pakeman *et al.* 1993). As a result, biological control agents have so far not been released for use against bracken (R. Pakeman, pers. comm.; S. Fowler, pers. comm.).

Case study 2: manuka (*Leptospermum scoparium*)

Manuka is a successional species native to New Zealand that is seen by New Zealand farmers as a problematic woody weed of marginal land, encroaching on

pasture. However, manuka is important in preventing erosion on steep hill country and plays a significant role in the regeneration of native forest as well as providing habitat for native fauna (Wardle 1991). During the 1940s, large areas of manuka began dying following the self-introduction of a scale insect (*Eriococcus orariensis*) from Australia. Conflict arose when farmers widely distributed infected plant material from localized infected areas to other parts of the country until the scale insect was widespread in New Zealand (Syrett *et al.* 1985). Control of manuka by the scale insect was extremely effective for some years until an insect pathogenic fungus (also self-introduced) reduced the efficacy of the scale insect. Another Australian *Eriococcus* species was discovered that could result in even greater damage to manuka, but no introductions were made due to concerns associated with the beneficial aspects of manuka as a native species (Hoy 1959, Syrett *et al.* 1985). Manuka is currently a highly valued source of nectar, and manuka honey has antiseptic properties (Cooper & Cambie 1991).

Case study 3: mesquite (*Prosopis* spp.)

The density of mesquite in the rangelands of the south-western USA has increased greatly since grazing livestock were introduced. Mesquite now competes with grasses for limited soil water, which results in livestock losses (DeLoach 1985). The increase in mesquite density has also caused a shift in natural plant communities in the region from grasslands to shrublands. However, mesquite also provides food and habitat for native fauna, is grown as a shade tree, is a valued source of nectar for honeybees, and has various other minor uses (DeLoach 1980). Although rangeland ranchers are still pushing for biological control of mesquite, no agents have been released yet due to misgivings about the use of biological control on a native species and the potential loss of useful attributes of the plant (DeLoach 1985, J. Coulson, pers. comm.).

Agents are being investigated for several other native rangeland weeds, and agents have already been released for snakeweed (*Gutierrezia* spp.) after assessment revealed this species had few beneficial values (DeLoach 1995). Conflicts of interest must be resolved for several other native rangeland species such as creosotebush (*Larrea tridentate*) before biocontrol can be initiated (DeLoach 1995). The deliberate release of introduced agents to control prickly pear cacti on Santa Cruz Island, California, and snakeweed in New Mexico and Texas, are the only examples of native species being targeted for biological control in USA (Goeden *et al.* 1967, Pemberton 1985, Turner 1985, DeLoach 1995). The cactus moth (*Cactoblastis cactorum*) was introduced to the Caribbean islands, Nevis, Montserrat and St Kitts, in 1957 to control native invading cacti. This was successful; however, *Cactoblastis* has now spread (naturally and deliberately) to almost all other Caribbean islands and has now reached Florida where it poses an enormous threat to the unique *Opuntia* spp. diversity of Mexico and the USA (Zimmermann *et al.* 2001).

Investigation of the costs and benefits of biological control in the bracken and mesquite case studies suggests the detrimental aspects of reducing the density of a native species can be outweighed by the benefits gained (DeLoach 1985, Pakeman & Marrs 1991). The substantial density increases and continual spread of these native species brought on by grazing and changes in management practices have more potential to result in the loss of threatened species and natural communities through displacement than the reduction of that plant species. Biological control is likely to reduce native "weeds" to natural densities rather than allow continued spread, which may result in the homogenization of several ecosystems in the region. However, biological control of large numbers of native species in the western rangelands of USA has generally been an attempt to improve agricultural use of the land, and the ecological value and function of these plants (usually ecologically dominant) has been inadequately investigated (Pemberton 1985). Pemberton (1985) suggests changes in land use and grazing may be necessary to restore the original vegetation and would be more appropriate than the use of biological control for native plants. However, long-term experiments excluding livestock have shown no decrease in the density of native weeds once they are established (DeLoach 1995).

Ecological conflicts

The beneficial attributes of weeds, particularly those associated with the use of weeds by native fauna, are becoming an important part of cost–benefit analyses for biological control. There have been public concerns in New Zealand and elsewhere, that successful biocontrol of a weed could leave native fauna without essential resources (Scott *et al.* 2000, DeLoach 2001, Fowler *et al.* 2001). The use of weeds as sources of food and habitat by native wildlife is becoming common in many countries, due to clearance of native vegetation and/or its replacement by invasive weeds. The risk of releasing biocontrol agents is more critical when the weed seems essential to the survival of an endangered or "iconic" species.

The best known example of biocontrol being impeded because of an ecological conflict involves the use of saltcedar (*Tamarix* spp.) by an endangered flycatcher in the USA. The biocontrol program for the highly invasive saltcedar was delayed for several years because the endangered willow flycatcher (*Empidonax traillii extimus*) began to nest in saltcedar following the displacement of native willow trees by saltcedar (DeLoach *et al.* 1996, Stenquist 2000). Ecological studies found that nesting success in saltcedar is considerably lower than in the native willows, and flycatcher decline has subsequently been attributed to a range of

factors associated with nesting in saltcedar, such as increased brood parasitism and lethal high temperatures (Stenquist 2000, DeLoach 2001). Concern was expressed that saltcedar increases soil salinity to such a degree that native vegetation would not return rapidly enough following biocontrol to prevent adverse effects on flycatcher (DeLoach 2001). However, biocontrol researchers anticipate that dispersal of biocontrol agents, and the control exerted, will be slow, and the agents will take 10–20 years to reach the flycatcher nesting sites (up to 1000 km from the release sites), by which time preferred native vegetation will have regenerated at other sites (DeLoach 2001). Experimental removal of saltcedar by mechanical means, followed by controlled flooding, has been shown to favour the establishment of native tree species (Taylor *et al.* 1999). Flycatchers, which have been absent from saltcedar-dominated sites, or have bred in monotypic stands of saltcedar (with low reproductive success), are now nesting in regenerating willows (DeLoach 2001). Biocontrol agents were finally released from field cages in 2001 (DeLoach 2001).

One issue currently delaying the release of biocontrol agents for broom in New Zealand is whether kereru, or native pigeons (*Hemiphaga novaeseelandiae*), rely on broom as a food source, particularly where there has been extensive clearing of native vegetation (Fowler *et al.* 2000b, 2001). Although kereru do consume broom leaves and buds, they also consume a wide variety of vegetation (McEwen 1978). In addition, many landowners currently use herbicides to control broom, therefore reducing the amount of broom currently available for kereru. Feeding on broom also provides some problems for kereru. For example, Dunn (1981) suggested that feeding in broom low to the ground is more energetically expensive for kereru than feeding on native trees because of increased vigilance and flights made to tall trees between foraging bouts on broom. Radio-tracking studies have revealed a number of kereru deaths associated with stoat predation while feeding in broom (Clout *et al.* 1995), and kereru deaths have also been attributed to collisions with motor vehicles while feeding on roadside weeds such as broom (Manders *et al.* 1998, N. Egerton, pers. comm., Alistair Hutt, pers. comm.). The death of even a few breeding adults may have detrimental effects on kereru population size and structure (Clout *et al.* 1995). A reduction in broom density as a result of biocontrol is therefore unlikely to have serious adverse consequences for kereru.

The almost exclusive dependence of threatened red-tailed black cockatoos (*Calyptorhynchus banksii samueli*) on doublegee (*Emex australis*) seed as a food source in some regions of Western Australia caused substantial delays in the release of biological control agents for this weed (Scott *et al.* 2000). However, it was determined that any reduction in seed production would only be a problem for bird abundance in a small geographical region that is a recent (150 years) range extension associated with the spread of agricultural development. It was also anticipated that seed production would not be significantly reduced by biocontrol compared to the extent of current herbicide use. Conservation efforts are being directed towards the provision of native foods for diet diversification and provision of nesting sites (Scott *et al.* 2000).

Management of the effects of weed control

It is easy to observe the positive effects that weeds have on native fauna, but the negative effects (often more important) are far more difficult to document (Loyn & French 1991). Exotic weed species may provide suboptimal food and habitat for native fauna relative to native plant species (Williams & Karl 2001), and there are substantial problems in extrapolating current use of plants and habitat to recommendations of food and habitat requirements (Gray & Craig 1991). It is more likely that availability of food and habitat translates into use of weeds that now often dominate natural systems. Most vertebrates tend to be dietary generalists (which is why they are seldom used as biocontrol agents) and, as such, do not often rely exclusively on a particular weed species as a food source. The species benefiting from weed invasions are usually common generalists that would not be driven to extinction by reduction or eradication of the weed (Schiffman 1997). Although some species are found to benefit or even depend on a weed species, the overall biodiversity of systems usually decreases rapidly as a result of the weed invasion (Braithewaite *et al.* 1989, Griffin *et al.* 1989, Samways *et al.* 1996, Ekert & Bucher 1999). For example, the delay in the biocontrol of *Tamarix* spp. in the USA because of use by the threatened flycatcher occurred despite at least five other endangered or threatened animals being adversely affected by the *Tamarix* invasion (DeLoach *et al.* 1996).

Where rare or threatened species do depend on a weed targeted for control, there are management techniques that can be used to ensure survival and population growth of the dependent species. Most weed control researchers suggest gradual, staggered removal of weeds and concurrent revegetation with native plant species (Whelan & Dilger 1992, Ekert & Bucher 1999). Biocontrol of weeds is not usually instantaneous, and revegetation, or even regeneration, could occur during the control period (DeLoach 2001). If natural replacement is too slow, supplementary feeding can be initiated as well as the provision of artificial or natural nests (Pereira *et al.* 1998, Scott *et al.* 2000), depending on what factors are limiting the rare/threatened species.

Weeds as nurse plants

In some situations, exotic plants can function as facilitators of native forest restoration, particularly on highly

degraded sites (De Pertri 1992, Williams 1997). Exotic species may facilitate rapid forest restoration, despite the ability to grow in adverse conditions that has allowed them to become invasive. Some weeds thought to facilitate forest regeneration have been targeted for biocontrol, and this has resulted in conflicts of interest and delays (Hill 1987, Pieterse & Boucher 1997, Fowler *et al.* 2001). Gorse (*Ulex europaeus*) and broom (*Cytisus scoparius*) have been advocated as facilitators of forest regeneration in New Zealand, although there has been considerable disagreement in the literature as to their effectiveness in all situations (Williams 1983, Lee *et al.* 1986, Hill 1987, Partridge 1992, Wilson 1994, Smale *et al.* 2001). More recently, research has shown that although these weeds may facilitate forest restoration, successional pathways are altered. Smith (1994) found that broom altered successional processes in Australian sclerophyll forests to produce mesic conditions that favoured cool temperate rainforest species and inhibited eucalypt regeneration. Williams (unpubl. data) found gorse succession in New Zealand excludes important podocarp species. More research is required to quantify the benefits of using weeds as nurse plants, but their role in ecological processes should be explored in cost–benefit analyses before biocontrol is initiated.

Conflict resolution

Often biocontrol programs only get under way once conflicts associated with the target plant have been investigated and it has been determined that the program is likely to go ahead unhindered and without substantial delays (Gardner *et al.* 1995). Weeds are thus selected as targets for biocontrol programs not only because agents specific to the target weed are likely to be available, but also because the risk of conflicts of interest are low for this weed (Pieterse & Boucher 1997). At present, initiation of biocontrol programs where the risk of conflicts is high usually only goes ahead when the plant is highly invasive and causing serious problems for several sectors of the community.

Communication and stakeholder involvement

For those biocontrol programs with an element of conflict, communication is the key to ensuring resolution is found. It is vital to have full stakeholder participation from the beginning and to maintain contact and information flow. In South Africa, where conflicts of interest are common, this is usually achieved through a steering committee (H. Zimmermann, pers. comm.). Conflict resolution in New Zealand for the biocontrol of broom involved apiarists, who were reassured after discussions that successful biocontrol would not reduce the weed to a level that would deprive their bees of an important pollen source (Syrett *et al.* 2000). It is important that all parties meet in discussions, so that the gravity of the conflict is understood by all, and comparisons of gains and losses can be made among stakeholders. If this does not occur, and if legal biocontrol using appropriate channels is made too difficult, expensive, or slow, individuals or groups who are suffering economic losses from the weeds may act outside the law, with enormously increased risk of undesirable side effects, such as the illegal release of a blackberry rust by interest groups in Australia (Stahle 1997, McFadyen 1998). Stakeholder representatives, including apiarists who opposed biocontrol, met in California in 1959 to discuss the costs and benefits of yellow starthistle (*Centaurea solstitialis*) biocontrol and to resolve the biocontrol conflict (Turner 1985). The outcome of this meeting was the unanimous recommendation to go ahead with the biocontrol of yellow starthistle. However, a vital consequence of this meeting was the recommendation of an investigation of plants useful both to livestock and to the beekeeping industry to replace a valuable source of nectar (Turner 1985).

Cost–benefit analyses

Cost–benefit analyses are an important part of resolving conflicts of interests, particularly between two economic groups where monetary value can be estimated for the gains and losses to each party as result of biocontrol (Higgins *et al.* 1997). Decision-makers often find arguments couched in monetary terms to be more convincing. The distribution of costs and benefits, that is, the number of individuals that stand to gain or lose from the biocontrol program, can also influence decision-makers. However, the value of the plant to any one interest group may change over time (Zimmermann 1992), complicating long-term cost–benefit analyses.

It is substantially more difficult to quantify the environmental and/or social benefits of biocontrol on natural communities or the impact of weeds in monetary terms, and few attempts have been made to do this (but see Greer & Sheppard 1990, Zavaleta 2000, de Wit *et al.* 2001). As well as assessing the economic losses predicted to result from the biocontrol of melaleuca (*Melaleuca quinquenervia*) in Florida, such as losses to apiarists, Diamond & Davies (1991) assessed the economic losses attributed to the spread of melaleuca in Florida. This assessment included the current costs of conventional control, loss of and restricted use of parks and recreation areas by residents, hunters and tourists, costs and losses to due to the extreme fire hazard posed by melaleuca, costs associated with a lowered water table, and costs of allergy treatment associated with melaleuca pollen (Diamond & Davis 1991, Turner *et al.* 1998). Zavaleta (2000) assessed the value of ecosystem services, such as water provision, flood control and wildlife habitat, lost to *Tamarix* invasion in the USA, to be an estimated $7–16 billion over 55 years.

Tangible social benefits, as well as economic benefits, can be gained from weed biocontrol. South

Africa's Working for Water Program creates job opportunities for the poorer sections of the community, as well as securing precious water resources (Zimmermann & Neser 1999). Removal of alien woody plants throughout riparian zones and wetlands in South Africa replenishes available stocks of water, since 7% of South Africa's mean annual water run-off is lost through transpiration by these alien plants (Zimmermann & Neser 1999). Large weed clearing operations also create employment for unskilled labour, while biocontrol prevents reinvasion of cleared areas (Zimmermann & Neser 1999).

Use of exotic plants

The most important point to convey to all stakeholders in any biocontrol program is that biocontrol agents are very unlikely to eradicate the weed; instead they make it less invasive. Exotic plants can still be used even where biocontrol has been overwhelmingly successful, particularly where trees are controlled by seed-eaters (Zimmermann & Neser 1999). In some cases, where seed growers may be affected by biocontrol, insecticide can be used to protect useful plants from highly effective seed-eating agents (Pieterse & Boucher 1997, Zimmermann & Neser 1999). Most biocontrol conflicts in South Africa are now resolved by introducing seed-feeding agents (Klein 2001). Seed-feeding agents have been introduced to South Africa in an attempt to reduce the invasiveness of plants such as mesquite (*Prosopis* spp.), so that they can be cultivated in manageable sections without forming dense, unusable thickets and without spreading into surrounding areas (Olckers *et al.* 1998). An additional advantage of seed-eaters is the gradual shift they cause towards low weed population densities, during which time less aggressive species can be cultivated to replace the weed (Olckers *et al.* 1998). Biocontrol of *Acacia* spp. using seed-eating agents has been accepted by the timber industry in South Africa following much interaction and discussion among interested parties (Dennill *et al.* 1999). Although a biocontrol program has been initiated investigating seed-eating agents for *Pinus* spp. in South Africa (Moran *et al.* 2000, J. Hoffmann, pers. comm.), biocontrol for *Pinus* spp. in New Zealand even with the use of seed-eaters is still a long way off, due to the importance of *Pinus* species to both the forestry industry and the national economy (Kay 1994, McGregor 2001). There is also a perceived risk that insect biological control agents may act as vectors for the disease, pine pitch canker (*Fusarium* sp.) (Moran *et al.* 2000, McGregor 2001).

Exploitation of an exotic plant species for timber, such as *Acacia* species in South Africa and *Pinus radiata* in New Zealand, often relieves pressure on harvesting of indigenous trees (Wardle 1991, Pieterse & Boucher 1997). However, it is frequently assumed that introducing exotic trees is the best way to create production systems that give priority to use for human needs. The presumed advantages of exotics over native species are their apparently greater economic value, better tolerance of unfavourable conditions, and the absence of specialized natural enemies (Ewel *et al.* 1999). Experts tend to depend on exotic species with proven management techniques, regardless of their appropriateness for site conditions (Butterfield & Fisher 1994). However, indigenous trees often do as well as exotics, are better adapted to local conditions (Butterfield & Fisher 1994, Haggar *et al.* 1998, Leaky & Simons 1998), and enemies of exotic plants eventually arrive or new indigenous enemies are acquired (Madden & Bashford 1977, de Groot & Turgeon 1998). Although native plants have been used extensively by local indigenous people, they have often been overlooked by scientists, managers and governments for use in production systems (Ewel *et al.* 1999). There is a need for evaluation of potentially useful indigenous plants, rather than the routine recommendation of exotic plants (Butterfield & Fisher 1994, Leaky & Simons 1998).

Due to a revision of weed legislation, use of invasive exotic plants in South Africa now comes with additional responsibilities and requirements. Provision has been made in the legislation for the continued utilization of invasive plants with beneficial properties, provided growers take responsibility for the control of these plants and contain them within the boundaries of their property (Klein 2001). Before the revision of this weed legislation, conflict between the beneficial and harmful aspects of invasive plants in South Africa prevented the inclusion of many harmful plant species in weeds legislation because they were being used by industry.

Assessing indirect effects of biological control

Host-specificity testing and risk assessment requirements for most countries involved in biological control are now very time consuming and result in the delayed release of agents (Fowler *et al.* 2000a). In some countries, host-specificity testing is mandatory for rare and endangered species, and investigation is required even for those species thought to be extinct (Pemberton 1985, Scott *et al.* 2000, De Clerck-Floate & Schwarzlaender 2002). Although this may ensure non-target plants are not affected by the agents, host-specificity testing now presents huge and costly problems in acquiring and also growing the material (R. De Clerck-Floate, pers. comm.). Louda (2000) argues that ecological criteria and investigation of possible direct and indirect ecological interactions should be included in pre-release criteria to prevent detrimental impacts on native species. Such effects on non-target organisms and ecosystems, such as possible shared predators, must now be investigated in addition to host specificity before permission can be obtained to release biological

control agents into the New Zealand environment (Fowler *et al.* 2000a). However, Fowler *et al.* (2000a) argue that if it becomes necessary to assess the more subtle "ripple" ecosystem impacts of the organism, biocontrol programs will in effect cease to exist because the research required would be prohibited by time and expense.

Faced with major threats to food production and ecosystem destruction, biological control researchers in Africa take a pragmatic approach to minimizing the risks of undesirable environmental effects of biological control (Neuenschwander & Markham 2001). Threats to crops from weeds and insect pests result in additional clearing of forest by landowners, with devastating impacts on biodiversity. There are also serious social risks associated with the current broad-scale spraying of weeds and insect pests due to poor handling of pesticides. Many farmers can neither read the hazard warning labels, nor afford protective clothing. Neuenschwander and Markham (2001) suggest a social dimension be added to any cost–benefit analysis. People living in extreme poverty are not in any position to make long-term decisions about protecting their environment, and pragmatic decisions concerning the environmental effects of biocontrol should take social factors into account (Neuenschwander & Markham 2001).

Conclusion

There are numerous conflicts of interest and risks in releasing biocontrol agents. However, classical biocontrol often remains the only safe, practical and economically feasible method of weed control that is sustainable in the long term (McFadyen 1998). We are reminded by McFadyen (1998) that lengthy delays or even prohibition of biocontrol because of risks to the environment is not "benign neglect". Uncontrolled invasive weeds cause increasing and ongoing environmental and economic damage (Fowler *et al.* 2000a). Therefore, it is important to resolve conflicts of interest promptly and minimize possible negative effects from biocontrol agents. Clearly, any cost–benefit analysis must be inclusive of economic, environmental and social factors, but the cost of such analysis must also be weighed against the consequences of delays or impediments that could result in escalating weed impacts far worse than the risk the biocontrol agent poses to the environment.

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Microbial toxins in weed biocontrol: a risk or an aid?

Maurizio Vurro[1]

Summary

Weed pathogens are able to produce a wide array of toxins, bioactive metabolites with different biological activities, chemical structures, mechanisms of action, specificity with respect to plants, environmental impact and stability. Usually bioactive compounds produced by plant pathogenic fungi, including those attacking weeds, are considered to be a risk. They have been intensively studied, mainly in relation to the risks posed to human and animal health when these toxins accumulate in agricultural commodities and are absorbed through nourishment. Often very promising mycoherbicides have been discarded during the final evaluation processes just because they produce powerful and dangerous toxins *in vitro*. The evaluation of the "real" risk should be ascertained by considering the global environmental impact, i.e. determining the exact production of those metabolites when fungi are formulated, or when they are applied against, and grown on, target weeds; the toxicity to non-target organisms; the stability of toxins in plants or the absorption by soil particles; and the risk of water drift. On the other hand, toxins could be used to directly or indirectly enhance the efficacy of weed biocontrol agents, depending on their biological and chemical characteristics, through: 1) the selection of organisms overproducing toxins; 2) their synergistic use with biocontrol agents; 3) their use as biomarkers; 4) their use as sources of natural herbicides; and 5) their synthesis.

Keywords: efficacy enhancement, natural herbicides, risk assessment, toxigenic fungi, toxins.

Introduction

Fungi represent an immense and still almost unexplored source of metabolites. Toxic metabolites produced by fungal pathogens can: 1) have a wide array of chemical structures including glycosides, peptides, phenolics, terpenoids; 2) act in different ecological and environmental roles, such as to be important factors for pathogenicity or virulence; and 3) have different mechanisms of action. Fungal species belonging to the same genus are able to produce a wide variety of metabolites. *Alternaria* or *Claviceps* species, for instance, are known to be producers of more than 100 toxic metabolites, and *Fusarium* biosynthesizes more than 130 bioactive metabolites. A further source of variability is that toxins belonging to the same structural group can be produced by different microorganisms belonging to many different genera. This is the case, for example, for cytochalasins, produced by more than 30 different fungal species (Vurro *et al.* 1997); trichothecenes, a group including more than 50 different compounds, produced by different genera, such as *Fusarium* (more than 25 different trichothecenes), *Myrothecium* (producing roridins and verrucarins), *Stachybotrys* (satratoxins) and *Trichoderma* (trichodermins) (Lacey 1985); and destruxins, metabolites known for their herbicidal properties, in addition to their insecticidal activities, that are produced in at least 35 different forms (Pedras *et al.* 2002).

There are hundreds of species of fungi that have not yet been evaluated for toxin production just within the known toxigenic genera. There are also huge differences between strains within the same species. While phytotoxins from fungal pathogens have received considerable attention mainly in the understanding of disease development and in setting up strategies for disease control, much less attention has been given to the secondary metabolites produced by weed pathogens. Usually bioactive compounds produced by plant pathogenic fungi, including those attacking weeds, are

[1] Institute of Sciences of Food Production, C.N.R., Viale Einaudi 51, 70125, Bari, Italy.
Corresponding author: M. Vurro <maurizio.vurro@ispa.cnr.it>.

considered to be a risk. They have been intensively studied, mainly in relation to the risks posed to human and animal health when these toxins accumulate in agricultural commodities and are absorbed through nourishment. In addition to quite a few families of metabolites, such as aflatoxins, ochratoxins, trichothecenes, fumonisins, zearalenols and alkaloids of *Claviceps*, which was proved to be responsible for severe human and animal poisonings, there are many other metabolites that are not so dangerous and, even if they were, could nevertheless represent interesting tools for improving the efficacy of weed biological control agents. Some of the possible risks and applications are discussed here.

Risk of toxin production

Often very promising mycoherbicides have been discarded during final evaluation processes just because they produce powerful and dangerous toxins *in vitro*. The evaluation of the "real" risk should be ascertained by considering the global environmental impact, i.e. determining the production of those metabolites when fungi are formulated, or when they are applied to, and grown on, target weeds; the toxicity to non-target organisms; the stability of toxins in planta or the absorption by soil particles; and the risk of water drift.

With regard to the production of toxic metabolites by mycoherbicides, one of the main problems is to ascertain their biosynthesis and eventual release into the environment. In fact, many fungi are able to produce very high amounts of secondary metabolites when grown for some weeks on solid media. In such conditions, where the fungi have at their disposal large amounts of nutrients, they produce conspicuous amounts of biomass, as happens during storage of kernels, and in food contamination.

The accumulation of metabolites can be different when a fungus is formulated as dried spores or chlamydospores, as mycoherbicides usually are. When distributed in the field, the biocontrol agent is usually applied to young plants or seedlings. The available nutrients are not so plentiful, and usually the fungus is able to cause a high level of disease within a few days, and then disappear together with the diseased plants. So, the potential to produce and accumulate high amounts of toxins appears very limited. This is also confirmed by the scarcity of information about the detection of phytotoxins in plants. For example, *Myrothecium verrucaria*, proposed as an agent for the control of *Pueraria montana* var. *lobata* (Kudzu), produced a wide range of macrocyclic trichothecenes, such as epiroridin E, verrucarin A and J, and others, when grown *in vitro* in both liquid and solid culture (Abbas *et al.* 2001). Concentrations of these trichothecenes ranged from more than a hundred to fractions of milligrams per gram of culture. On the other hand, none of those metabolites were detected by high-performance liquid chromatography (HPLC) analysis in diseased tissues of kudzu treated with a spore suspension of the fungus.

Another aspect that should be considered is the transformation of fungal metabolites by microbial or plant metabolism, their immobilization by soil particles and the physical and chemical changes that can occur, leading to the possible inactivation of the compounds.

Enhancement of virulence through toxin overexpression

Toxins can be either pathogenic factors responsible for the ability of the pathogen to cause disease, and/or virulence factors, involved in the severity of expression. The development of pathogens with enhanced biocontrol activity by selection or by the introduction of genes responsible for toxin biosynthesis seems a reasonable possibility. In fact, several genes in the biosynthetic pathways of fungal toxins have already been identified and cloned, such as many of those encoding the biosynthetic pathway of trichothecenes, toxins produced by several species of different fungal genera such as *Fusarium* and *Trichoderma*. Among those genes, Tox5, a trichodiene synthase gene responsible for cyclization of a trichothecene precursor, was first isolated from *Fusarium sporotrichioides* and subsequently cloned into *Escherichia coli*. The *E. coli* transformant overexpresses soluble trichodiene synthase (Cane *et al.* 1993).

The protein NEP1 is a potent phytotoxin isolated for the first time from culture filtrates of a strain of *Fusarium oxysporum* pathogenic on *Erythroxylum coca*, and later found as a product of many other strains of the same species (Bailey *et al.* 1997). The toxin induces necrosis in leaves and is largely responsible for the natural virulence of the species producing it, and the gene, nep1, encoding that proteinaceous toxin, has been identified. Recently, Amsellem *et al.* (2002) have transferred the nep1 gene to a weak pathogenic strain of *Colletotrichum coccodes*, a potential mycoherbicide for the biological control of *Abutilon theophrasti*. The resulting transgenic strain proved quickly able to kill abutilon plants at the three-true-leaf stage, whereas the wild type was only sporadically able to kill those plants, and only if applied to the young seedlings. Furthermore, the virulence of the pathogen was strongly increased. The transgenic strain applied at a concentration nine-fold lower than that used for the wild type, was able to cause the same level of disease. In addition, a shorter dew period was required by the transgenic strain to infect plants (usually one of the limiting factors of mycoherbicide application) and symptoms appeared much faster than for the wild strain.

Synergistic use with agents

The development of a plant disease is the result of several biochemical and physical interactions between the plant and the potential pathogen. Induced accumu-

lation of antimicrobial phytoalexins, synthesis of ethylene, deposition of lignin and other wall-bound phenolic compounds, and synthesis of proteins such as chitinases, wall-associated hydroxyproline-rich glycoproteins and pathogenesis-related proteins are among the responses observed in plants attacked by pathogens. The use of compounds that could weaken the physical and/or biochemical defences of the target plant, or increase the aggressiveness of the pathogen, as tactics to increase pathogen efficacy in weed control have been widely considered (Hoagland 1996). Toxins could be used to indirectly enhance the efficacy of the pathogen, applied together with it, if they proved able to weaken the reaction of plants. For example, this was observed in *Helminthosporium oryzae*, the causal agent of rice brown spot pathogen, whose infection and consequent symptom appearance were delayed if phenol metabolism was stimulated in rice plants. When its fungal toxin was applied to chemically treated and stimulated leaves, their phenolic content decreased rapidly, providing good evidence of the toxin's role in the suppression of rice plant defence mechanisms (Vidhyasekaran *et al.* 1992).

Ascochyta caulina, an interesting fungus proposed for biological control of *Chenopodium album*, was able to cause severe disease symptoms in young plants within 7–10 days after the application of a spore suspension. If the fungus was sprayed together with a mixture of purified toxins, produced and purified from the culture filtrate of the same pathogen, its efficacy was strongly enhanced, both in terms of the speed of disease appearance, with effects already appearing 2–3 days after treatment, as well as in the entity of symptoms (Vurro *et al.* 2001).

Similarly, when Nep1 protein, produced by *F. oxysporum*, was applied as a foliar spray with an appropriate wetting agent and together with *Pleospora papaveracea* for biological control of *Papaver somniferum* (opium poppy), the treatment caused higher necrosis ratings than either component alone. In greenhouse experiments, the necrosis ratings for plants treated with the combination of Nep 1 protein and spores ranged between 60 and 95%, against a rate between 7 and 50% for the pathogen alone and almost none for the protein alone. Similar results were also obtained in field experiments (Bailey *et al.* 2000).

A restriction to the practical application of pathogens in biocontrol is the host range of the fungus. If a broader host range could improve the use of the pathogen, allowing its application to a larger number of weeds, it would pose a higher risk due to the uncontrollability of the pathogen once it has been released in the field. Since some pathogens are able to produce host-specific toxins, having the same host range as the pathogen producing them, their use could increase the efficacy of the pathogen only against the target weeds, or change the spectrum of action of the pathogen. For example, the spores of *Helminthosporium victoriae*, a pathogen of oats, could not penetrate into maize tissues unless applied with BZR-toxin, produced by *Bipolaris zeicola* Shoemaker race 3, a pathogen of rice and maize (Xiao *et al.* 1992).

A further approach could be the use of non-specific toxins with non-specific pathogens. This could transform a weak pathogen into a good biocontrol agent. This would also permit a single pathogen to be used against a larger number of targets, without the risk of uncontrolled diffusion in the environment. In this case, disease could be obtained only where the pathogen was introduced in presence of the toxins. In the absence of the toxin, the pathogen would not be virulent. *Alternaria* toxins have shown these effects. Application of AK-toxins, produced by a virulent strain of *A. alternata* f. sp. *kikuchiana* caused treated plants to leak electrolytes and enabled a non-virulent strain to establish infections at a level equal to that of toxin-producing spores (Otani *et al.* 1985).

Use as biomarkers

If toxins produced by pathogens were used as biomarkers this could indirectly improve biocontrol agents. In fact, one of the main difficulties in weed biological control is the assessment of virulence and its comparison among different fungal strains. If toxins proved to be virulence factors, meaning that there was a positive correlation between toxin production and aggressiveness of the candidate agent, analytical methods could be developed (when the chemical structure of toxic metabolites was determined) to measure the absolute concentrations of the toxins in culture filtrates or partially purified extracts. This would allow the selection of more virulent strains of the pathogen simply by testing the *in vitro* production of toxic metabolites and choosing the highest toxin producers. For example, the fungal metabolite botrydial was recently detected for the first time in plants, in a wound inoculated with conidial suspensions of *Botrytis cinerea* in ripe fruits of *Capsicum annuum* (sweet pepper) (Deighton *et al.* 2001). In this system, the most aggressive isolate of *B. cinerea* was also the highest producer of botrydial in the soft rot regions of the infections on *C. annuum.*

In the case of *A. caulina*, the biocontrol agent of *C. album*, a method of high-performance anion exchange chromatography and pulsed amperometric detection was developed, allowing a quick and simple quantification of the three main metabolites produced by the pathogen in liquid culture. Preliminary observations carried out on some pathogenic strains seemed to support a positive correlation between toxin production and virulence of the strains, and thus the idea of using toxins as biomarkers for biocontrol agent selection (Evidente *et al.* 2001). In contrast, the same approach was not successful in the selection of phytopathogenic strains of *F. oxysporum* for biological control of parasitic weeds. Fusaric acid, a well known toxin produced by *Fusarium*

species, has also been considered a virulence factor (Kern 1970). In a wide survey with the aim of finding potential agents for biological control of *Orobanche ramosa*, a parasitic weed infesting among others, tomato, tobacco and cabbage, several phytopathogenic *F. oxysporum* strains were isolated, and their virulence and fusaric acid production were determined, but no correlations were observed (Abouzeid *et al.* 2004).

Use as sources of natural herbicides

New bioactive metabolites have often been obtained by screening extracts from different microbes. This approach can be useful if applied to a general, and not a focused, screening for novel bioactive metabolites. This technique has a low percentage of success due to the different biological activities such compounds can possess and the constraints to evaluate them. It can also be an inefficient screening process because of the almost infinite number of organic compounds with low molecular weights that can be produced and the difficulties in explaining why a microorganism should produce secondary metabolites having biological properties far from the ecological needs of the microorganism that produced them. In the case of bioherbicides, a simpler approach would be to limit the study to phytopathogenic microbes that have demonstrated potential as weed biocontrol agents and could provide a rich source of metabolites active against weeds. Many toxins produced by fungal and bacterial weed pathogens have been already isolated, purified, chemically and biologically characterized, and proposed as potential herbicides (Table 1).

Modification of metabolic pathways

Fungal extracts can be obtained by fermentation on a sufficient scale to allow follow-up testing and to provide material for studies of structure–activity relationships. In certain cases, the activity of a produced metabolite and its potency in the field could make commercial production by fermentation a realistic possibility. Production of metabolites can be further manipulated by the use of specific growth media to modify the biosynthetic pathways for the production of the compounds. This offers the possibility of obtaining "non-natural" natural products, and this could be accomplished by simply adding chemical analogues of key biosynthetic intermediates to the growth medium. These chemicals are recognized by biosynthetic enzymes and enter into the pathway. The end products are analogues of the normal product or intermediates that are not substrates for subsequent transformations.

A further approach could be the use of strains having altered biosynthetic abilities. For example, mutant strains of *Fusarium graminearum* obtained by disruption of Tri8, a gene probably encoding an esterase, were able to accumulate 3-acetyl T-2 toxin, 3-acetyl neosolaniol and 3,4,15-triacetoxyscirpenol, rather than T-2 toxin (McCormick & Alexander 2002). This approach would allow, as final products, metabolites that are only intermediates in the "natural" biosynthetic pathways, and that could have different biological properties with respect to the end products. This was also observed in the case of *Fusarium sporotrichioides* where, by disruption of Tri11, a gene encoding a cytochrome P-450 monooxygenase, was able to produce four

Table 1. Examples of phytotoxins produced by fungal pathogens of weeds.

Toxin	Producer	Host weed
Alteichin	*Alternaria eichhorniae*	*Eichornia crassipes*
Ascaulitoxin	*Ascochyta caulina*	*Chenopodium album*
Ascochytine	*Ascochyta hyalospora*	*C. album*
Bipolaroxin	*Bipolaris cynodontis*	*Cynodon dactilon*
Bostricin	*Alternaria eichhorniae*	*E. crassipes*
Brefeldin A	*Alternaria zinniae*	*Xanthium occidentale*
Cytochalasins	*Pyrenophora semeniperda*	*Bromus* spp.
De-O-methyldiaporthin	*Drechslera siccans*	*Lolium* spp.
Dihydropirenophorin	*Pyrenophora avenae*	*Sorghum halepense*
Epoxidon	*Phoma sorghina*	*Phytolacca americana*
Exserohilone	*Exserohilum holmii*	*Dactyloctenium aegyptium*
Maculosin	*Alternaria alternata*	*Centaurea maculosa*
Monocerin	*Exserohilum turcicum*	*S. halepense*
ß-nitropropionic acid	*Septoria cirsii*	*Cirsium arvense*
Ophiobolins	*Drechslera sorghicola*	*S. halepense*
Putaminoxin	*Phoma putaminum*	*Erigeron annuus*
Tentoxin	*Alternaria tenuis*	*S. halepense*
Tryptophol	*Drechslera nodulosum*	*Eleusine indica*

trichothecenes not observed in the culture of the parent strain (McCormick & Hohn 1997).

New derivatives

Knowledge of toxin structure can allow the preparation of appropriate derivatives and/or analogues that are essential to study structure–activity relationships, to understand their mechanisms of action and to determine the active sites of the toxins that could be used as the backbone of new compounds. Many studies have shown that changes in the active sites of microbial metabolites cause modification of their biological activities. Putaminoxin and pinolidoxin, two structurally related nonenolides, isolated respectively from the organic extracts of *Phoma putaminum* and *Ascochyta pinodes* cultures, together with some of their natural analogues and synthetic derivatives, were used in structure–activity relationship studies using phytotoxic, antifungal and zootoxic assays (Evidente *et al.* 1998). The strongest phytotoxic compounds proved to be putaminoxin and pinolidoxin, and their toxic activity seemed related to the integrity and presence of both hydroxyl groups and an unmodified propyl side chain.

The biological activity of cytochalasins, produced by many fungal species, such as *Phoma exigua* var. *heteromorpha*, and that of several derivatives, proved to be related mainly to the size of the macrocyclic ring, and to its conformational freedom. Furthermore, modifications of the hydroxy group on C-7 were shown to affect the toxic properties of the toxins (Bottalico *et al.* 1990).

Abbas *et al.* (1995), in a search for analogues of fumonisin B and AAL-toxin retaining high phytotoxicity but with lower mammalian toxicity, tested many analogues for toxicity to duckweed, tomato, black nightshade and mammalian cell lines, and found only one compound having all those properties, thus indicating some potential for the development of safe and effective natural herbicides.

Possible syntheses

The inability of microorganisms to produce large amounts of a toxin or the high costs of purification represent potential constraints to their practical use as natural herbicides. However, these could be overcome by the chemical syntheses of the fungal metabolites. Several fungal pathogens, especially belonging to the genera *Alternaria* and *Cochliobolus*, produce host-selective toxins that are virulence and/or pathogenicity factors. These compounds are active against the same plant species as the fungal pathogens and low (physiological) concentrations of the toxin are able to reproduce symptoms of the natural infections. These plant-specific metabolites have received attention as models for new herbicides. For example, the synthesis of host-specific toxins has been extensively investigated by Crombie *et al.* (1999), particularly AK-toxin I and AK-toxin II produced by *A. alternata* (Japanese pear pathotype), which causes disease in pears. These toxins, affecting the Nijisseiki varieties of pear, but not other cultivars, are active at a concentration of 5×10^{-9} M. AF-toxins *A. alternata* (strawberry pathotype) have also been considered for synthesis. Recently, another host-specific toxin, the cyclic dehydrodepsipeptide AM-toxin II, produced by *A. alternata,* the fungal agent of apple tree leaf spot disease, was efficiently synthesized using a solid-phase method (Horikawa *et al.* 2001). The synthesis included C-terminal peptide elongation and cyclization followed by oxidative cleavage and formation of the double bond. It has also been shown that this methodology could be very useful in synthesising unsaturated compounds using solid-phase chemistry.

Seiridin and its structural isomer isoseiridin are two phytotoxic butenolides produced by three species of *Seiridium*, fungi associated with the canker of *Cupressus sempervirens* (cypress trees) in the Mediterranean area. Since those compounds are available only at very low levels, and after a long process of purification of the fungal culture filtrates, the synthesis of these toxins was considered. This has led to the first enantioselective synthesis of seiridin, which provided large quantities of the toxin, a possible tool for genetic selection of resistant cypress plants (Bonini *et al.* 1995).

Dehydrocurvularins are produced by a number of phytopathogenic fungal species, such as *Curvularia, Penicillium, Cochliobolus* and *Alternaria*. These metabolites possess interesting biological properties, and are related to octaketide and nonaketide analogues such as lasiodiplodins, resorcyclide, zearalenones and monocillin. The interest in investigating the mechanism of action and production in larger quantities has led to the study of biosynthetic incorporation of precursors into dehydrocurvularin, allowing an understanding of the fundamental steps of its biosynthesis (Liu *et al.* 1998).

Conclusions

Toxins represent important tools for improving, directly or indirectly, the efficacy of weed biocontrol agents. First of all, toxicity against non-target organisms should be evaluated, as well as the toxicological risk due to the introduction of pathogens, potential producers of toxic metabolites. The evaluation should also be carefully carried out at the field level, to avoid discarding potential and promising mycoherbicides only because they produce toxic metabolites *in vitro*. The availability of new methods of toxin purification, structure elucidation, fermentation processing, synthetic production, formulation, knowledge of biosynthetic pathways and molecular tools for their transformation may provide further incentives to investigate the use of natural metabolites in weed control strategies.

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Regulatory approval processes for release of *Puccinia* spp. for biological control of *Carduus* and *Centaurea* spp. in the United States

W.L. Bruckart and D.G. Luster

United States Department of Agriculture, Agricultural Research Service, Foreign Disease–Weed Science Research Unit, 1301 Ditto Avenue, Fort Detrick, MD 21702, USA

Puccinia carduorum and *P. jaceae* var. *solstitialis* have been evaluated and proposed for introduction into the United States (US) for biological control of musk thistle (*Carduus nutans*) and yellow starthistle (*Centaurea solstitialis*), respectively. In each case, limited non-target infections were noted under containment greenhouse conditions. Also in each case, a related *Puccinia* species from the US was used in greenhouse comparisons with the candidate agent to resolve questions about potential non-target effects in nature. A strain of *P. carduorum* already present on slenderflower thistle (*Carduus tenuiflorus*) in California, USA, was used in comparison with the candidate isolate from musk thistle. The yellow starflower thistle rust infected safflower (*Carthamus tinctorius*) under greenhouse conditions, and a US isolate of safflower rust, *Puccinia carthami*, was used for comparison. During each risk assessment, interest groups were informed about conclusions that non-target species would not likely be damaged by the use of either organism. Artichoke and safflower growers in California, and representatives of the US Fish & Wildlife Service (F&WS) working with listed (Endangered or Threatened) plant species, were included as contacts. All requests for additional tests were honoured. The state departments of agriculture in Virginia and California, where releases were proposed, also provided approval to federal regulators. Proposals for release of each candidate also were reviewed by the Technical Advisory Group (TAG) and the Animal and Plant Health Inspection Service (APHIS), based on the recommendation of the TAG. A field study for *P. carduorum* was approved for one location in Virginia, and the rust has subsequently spread across the US to California. Notice of the proposal for *P. jaceae* has been published in the Federal Register for comment. A Finding of No Significant Impact (FONSI), thus concluding the approval process, is expected from APHIS. Release of *P. jaceae* is planned in CA, if approved.

Biology and host range of the Brazilian thrips *Pseudophilothrips ichini*, a candidate for biological control of *Schinus terebinthifolius*: US quarantine tests

J.P. Cuda,[1] J.C. Medal,[1] J.L. Gillmore[1] and J.H. Pedrosa-Macedo[2]

[1]Department of Entomology & Nematology, Institute of Food and Agricultural Sciences, University of Florida, PO Box 110620, Gainesville, FL, USA 32611-0620

[2]Neotropical Biological Control of Weeds Laboratory, Department of Forestry Sciences, Federal University of Parana, Curitiba, PR, Brazil 80210-170

Brazilian peppertree, *Schinus terebinthifolius* Raddi (Anacardiaceae), is an evergreen shrub or small tree native to Argentina, Paraguay and Brazil. This invasive plant, known as aroeira, aroeira-vermelha or aroeirada-praia in Brazil, was introduced into the United States as a landscape ornamental in the 19th century. Brazilian peppertree readily invades disturbed sites as well as natural communities where it forms dense thickets of tangled woody stems that completely shade out and displace native vegetation. It is a serious problem for natural resource managers in Florida and Hawaii, USA, because it reduces the biodiversity of the native plant and animal communities. In addition, direct contact with a toxic resin present in the leaves, flowers, and fruits can irritate the skin and respiratory passages of sensitive humans. Exploratory surveys conducted in Brazil produced several promising insect natural enemies. One of the most damaging is the thrips *Pseudophilothrips* (= *Liothrips*) *ichini* (Hood) (Thysanoptera: Phlaeothripidae). Feeding by the nymphs and adults kills the meristems and causes flower abortion. This type of feeding damage suppresses the growth rate of young plants and curtails seed production

in mature trees. Host-specificity studies (no-choice development, no-choice and multiple choice oviposition tests) were conducted in the Florida, USA, quarantine laboratory using 30 plant species in 11 families. Laboratory tests indicated that *P. ichini* is capable of continuous reproduction only on Brazilian peppertree and its congener *S. molle* L., a prized ornamental tree in California native to Peru that is becoming invasive in some areas. If approved for release in the USA, *P. ichini* is unlikely to survive in the arid environment where *S. molle* thrives in California. In addition, field surveys in Brazil confirmed that under natural conditions where both *Schinus* species coexist, *S. molle* is not attacked by *P. ichini*.

The nature of risk from biological control

Ernest S. Delfosse

USDA–ARS, 5601 Sunnyside Avenue, Room 4-2238, Beltsville, MD 20705-5139, USA

Risk is a relative concept commonly used when *uncertainty* can be quantified. The probabilities of possible outcomes are estimated, such as risk of damage to a non-target species from a biological control agent. "Acceptable risk" is used when uncertainty is quantified to the subjective satisfaction of a viewer. Uncertainty is measured by the deviation from "expected values", which may also be difficult to quantify. Thus, when probabilities of different outcomes are unknown, *uncertainty is transformed into risk*, where probabilities of outcomes are weighted according to their likelihood of occurrence. Each potential outcome is weighted by its probability of occurrence (by past trends, subjective judgments, experimentation etc.), and the weighted outcomes are summed to arrive at a mean, or expected, value. Incomplete information complicates objective estimates of risk, so the subjective valuation of risk is biased, and usually overstated. Herein lies the problem for biological control. Most risk (and most fears) in biological control is measured by the *assumption of potential* damage to non-target species. However, there is an equal risk to non-target species from *not* using biological control to manage invasive pests. Also, it is difficult to isolate the exclusive impact of potential risk by biological control agents on non-target species, mainly because environmental factors other than natural enemies influence risk, and if omitted, bias (overestimate) estimates of risk. It is also difficult to compare across different types of risk. Clearly, in biological control, the risk to non-target species from a macrocyclic, autoecious rust fungus such as *Puccinia chondrillina* or *Uromyces heliotropii*, is far less than the potential risk from an oligophagous or polyphagous biological control agent. It is argued that the term "the risk from biological control" is meaningless and a risk analysis model is proposed for use with biological control of weeds.

(This presentation was a keynote address for Theme 3)

Host-specificity investigations of a gall midge for the biological control of alien invasive hawkweeds in North America

Gitta Grosskopf,[1] Linda Wilson,[2] Lindsay Smith[3] and Pauline Syrett[3]

[1]CABI Bioscience Switzerland Centre

[2]University of Idaho, Moscow, Idaho, USA

[3]Landcare Research, Lincoln, Canterbury, New Zealand

Several hawkweed species of Eurasian origin have been deliberately or accidentally introduced into other parts of the world and some have become invasive weeds. Thus, in New Zealand, where there are no indigenous *Hieracium* spp., *Hieracium pilosella* is a severe weed in pastures, reserves and national parks. *H. caespitosum*, *H. glomeratum*, *H. praealtum* and *H. aurantiacum* are weeds in rangelands, national parks and clear-cut areas in North America. One of several insect species studied for the biological control of hawkweeds in New Zealand is the multivoltine gall midge *Macrolabis pilosellae*. Gall midge attack leads to shorter stolons and reduced numbers of leaves and flower heads. Host-specificity investigations carried out for New Zealand showed that the gall midge is at least genus-specific, developing on *H. pilosella*, *H. caespitosum* and *H. praealtum*. Therefore, *M. pilosellae* was selected as a potential biological control agent of alien invasive hawkweeds in North America. In

contrast to the situation in New Zealand, there are native hawkweed species in North America, and so a narrower host range is necessary. To assess its potential field host range, the gall midge is being tested on a range of North American test plant species including native and invasive *Hieracium* spp. using different test designs. North American invasive alien hawkweed species are in the subgenus *Pilosella,* whereas the native ones are in the subgenera *Hieracium* and *Stenotheca*. All those hawkweed species from the subgenus *Pilosella* on which normal gall development occurred in no-choice tests and which were tested under less restricted conditions were also accepted as hosts in these test designs. *Hieracium* spp. from the subgenera *Hieracium* and *Stenotheca* were accepted to a varying extent in no-choice gall formation tests, but not or only to a very limited extent under more natural conditions.

Our changing perception of *Cactoblastis cactorum* in North America

Stephen D. Hight,[1] Robert W. Pemberton,[2] Kenneth A. Bloem,[3] Stephanie Bloem[4] and James E. Carpenter[5]

[1]USDA–ARS, Tallahassee, FL, USA
[2]USDA–ARS, Ft. Lauderdale, FL, USA
[3]USDA–APHIS, Tallahassee, FL, USA
[4]Florida A&M University, Tallahassee, F., USA
[5]USDA–ARS, Tifton, GA, USA

Control of prickly pear cacti, *Opuntia* spp. (Cactaceae), by the South American cactus moth, *Cactoblastis cactorum* (Pyralidae), is a classic example of successful weed biological control. Unfortunately, in 1989 *C. cactorum* was found in the Florida Keys feeding on endangered *O. corallicola*. The insect attacks all six native Florida opuntias. The insect was not introduced into Florida as a biological control agent, but most likely as a Caribbean immigrant on ornamental cacti. Of major concern is the potential spread of *C. cactorum* to the opuntia-rich areas of the western US and Mexico. This could have devastating effects on the landscape and biodiversity of this region. In addition, the forage and vegetable opuntia industries in Mexico will likely be severely impacted by this "pest". This study is addressing three objectives: 1) determine the current distribution and spread of *C. cactorum* in North America; 2) determine the potential impact of native natural enemies on the spread (and possible control) of *C. cactorum*; and 3) explore the potential of the inherited sterile insect technique (SIT) to control *C. cactorum*. The moth's range continues to expand and now reaches as far north as Charleston, SC along the Atlantic and the Florida Panhandle along the Gulf of Mexico. The moth is spreading most quickly on cacti along the coast. However, infestations noted in the interior are becoming more common. Parasitoids (Tachinidae, Ichneumonidae) found attacking the native cactus moth, *Melitara prodenialis* (Pyralidae), were also found attacking *C. cactorum,* but at lower rates. Irradiation studies have determined the dose at which *C. cactorum* males are 100% sterile and the deleterious effects inherited by the F1 generation minimized. A SIT program may be useful in controlling *C. cactorum* along its leading edge to limit geographical range, to eradicate isolated populations far in front of the leading edge, or as an abatement program to protect rare and endangered *Opuntia* spp.

Attack on and use of a native Hawaiian plant by the biological control agent *Teleonemia scrupulosa* introduced against *Lantana camara*

Stephen D. Hight,[1] Robert W. Pemberton,[2] Patrick Conant[3] and Tracy Johnson[4]

[1]USDA–ARS, Tallahassee, FL, USA
[2]USDA–ARS, Ft. Lauderdale, FL, USA
[3]Hawai'i Department of Agriculture, Hilo, HI, USA
[4]USDA–Forest Service, Volcano, HI, USA

The lantana lace bug, *Teleonemia scrupulosa,* was introduced into Hawaii to control the invasive weed *Lantana camara* around 1900. The insect is common on lantana throughout the Hawaiian Islands and is an important factor reducing lantana in wetter parts of the islands. A brief note of this insect feeding and reproducing on a native Hawaiian plant (naio, *Myoporum sandwicensis*) not closely related to lantana was suggested in the mid-1960s. The objective of this study was to follow-up on this claim and to quantitatively evaluate this potential host-range expansion with surveys and controlled rearing studies. Naio along the coast of Oahu may be a different species of *Myoporum* from naio on Hawaii, at least a different variety. Target plants (lantana) and non-target plants (Hawaiian naio) were surveyed on two islands; Oahu and Hawaii. Field surveys verified the use of naio on Oahu, but not on Hawaii. Reproducing populations of lace bugs (adults and nymphs) were found on Oahu naio, Oahu lantana, and Hawaii lantana. No lace bugs were found on Hawaii naio even though insects were present on adjacent lantana plants. This represents the broadest host shift recorded for a classical weed biological control agent, i.e. onto a family in a different order. Preliminary greenhouse rearing experiments showed that lantana collected lace bugs survived poorly and failed to reproduce on the naio from Hawaii, although they did well on lantana. Oahu naio-feeding lace bugs survived on lantana, but performed poorly on Hawaii naio. Rearing studies are being repeated and will include insect populations from Oahu naio, Oahu lantana, and Hawaii lantana on four different host plants (Oahu naio, Oahu lantana, Hawaii naio, and Hawaii lantana).

Genotyping of pathogens with potential for biological control of invasive weeds

Douglas G. Luster, Leonard F. Yourman, William L. Bruckart and Michael B. McMahon

United States Department of Agriculture, Agricultural Research Service, Foreign Disease–Weed Science Research Unit, 1301 Ditto Avenue, Fort Detrick, MD 21702, USA

Genetic characterization of microbial biological control agents is an essential part in the process of evaluation and release, allowing researchers to identify and discriminate between beneficial plant pathogen strains for purposes of release monitoring, risk assessment and liability. We have applied molecular techniques with proven utility in plant pathology to the analysis of fungal pathogens considered for release as biological controls for invasive weeds. We have utilized both amplified fragment length polymorphism (AFLP) and random amplified microsatellites (RAMS) to characterize *Puccinia jaceae*, a rust fungal pathogen of *Centaurea solstitialis* (yellow starthistle; YST). Unique AFLP patterns were identified for a *P. jaceae* strain targeted for release on YST in California, USA. PCR primers were engineered from the DNA sequence of a RAMS amplicon generated by PCR from the *P. jaceae* strain. The primers were found to be specific for the *P. jaceae* strain and thus will prove useful in monitoring the spread and establishment of the pathogen once released. Ribosomal RNA gene internal transcribed spacer (ITS) regions were sequenced from *Puccinia carduorum,* a rust fungal pathogen of *Carduus thoermeri (*musk thistle). Discrete differences in DNA sequence were identified between strains of *P. carduorum*, allowing us to discriminate between those specific to individual *Carduus* host species. ITS sequencing was subsequently applied to the identification and monitoring of a *P. carduorum* strain released in Virginia USA, which has since spread as far west as California, USA. The application of such "genotyping" techniques to the study of beneficial weed pathogens illustrates the potential of the techniques and their utility in practical post-release applications.

Argentinian fungi for Bathurst burr fail preliminary host-specificity tests

Louise Morin,[1] Bruce Auld,[2] Harry Evans,[3] Carol Ellison,[3] Robert Reeder,[3] Freda Anderson[4] and Bill Pettit[4]

[1]Cooperative Research Centre for Australian Weed Management, CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia
[2]Orange Agricultural Institute, NSW Agriculture, Forest Road, Orange, NSW 2800, Australia
[3]CABI-Bioscience, Silwood Park, Buckhurst Road, Ascot, SL5 7TA, UK
[4]CSIRO Entomology, c/o CERZOS, Universidad Nacional del Sur, CC 738 (8000), Bahia Blanca, Argentina

Xanthium spinosum (Bathurst burr) is a widespread summer annual weed in rangeland, pasture and crops in eastern Australia. Prospects for classical biological control of this weed were investigated in the 1990s by carrying out a series of surveys for fungal pathogens attacking *X. spinosum* in Argentina, the putative country of origin of this plant. The powdery mildew *Erysiphe cichoracearum* and the facultative parasite *Cercospora xanthicola* were the most frequently recovered pathogens and were widely distributed within the regions of Argentina surveyed. Significant damage was associated with the presence of *E. cichoracearum*, which sporulated profusely on both leaf surfaces, stems and shoots. Infection by *C. xanthicola* was spectacular at several sites in northern Argentina in March 1995, but it appeared that the pathogen was favoured by humid environmental conditions. In following surveys, infection was scattered, restricted to lower leaves and rarely damaging. The pathogenicity of isolates of *E. cichoracearum* was tested on Bathurst burr, other *Xanthium* spp. and a selection of species from related genera of Asteraceae. Severe infection and heavy sporulation developed on Bathurst burr plants while the other *Xanthium* spp. developed only mild symptoms. All other Asteraceae tested proved to be immune or resistant, but three of the eight sunflower cultivars tested became heavily infected. The most aggressive isolate of *C. xanthicola* tested in the laboratory produced necrotic lesions on Bathurst burr that expanded to cover most of inoculated leaves within 3 weeks. However, no stem lesion ever developed and plants recovered rapidly. The fungus required a minimum of 2 days under humid conditions to infect plants. All sunflower cultivars tested were susceptible to the pathogen and developed necrotic lesions. The lack of specificity of *E. cichoracearum* and *C. xanthicola* militates against their possible use as biological control agents for Bathurst burr in Australia.

Biological control of saffron thistle with fungi: limited prospects

Louise Morin[1] and Mireille Jourdan[2]

[1]Cooperative Research Centre for Australian Weed Management, CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia
[2] CSIRO European Laboratory, Campus International de Baillarguet, 34980 Montferrier-sur-Lez, France

Carthamus lanatus (saffron thistle), a native of the Mediterranean region, affects livestock, pasture and grain production throughout temperate and subtropical Australia. Herbicides are effective in controlling this weed, but the cost of this control method prohibits its use over the vast areas infested. Biological control, if successful, is likely to be the only solution to effectively manage saffron thistle populations in Australia. Following surveys carried out in Greece, two pathogens, *Puccinia sommieriana* (microcyclic rust) and *Septoria centrophylli* (facultative parasite), were identified as potential candidates for classical biological control of saffron thistle. A preliminary study was conducted to determine the susceptibility to these pathogens of Australian accessions of saffron thistle and cultivars of the closely related crop, safflower (*Carthamus tinctorius*). All isolates of *Puccinia sommieriana* tested produced, within 5–6 days after inoculation, small chlorotic flecks on leaves of all Australian accessions of saffron thistle tested. Flecks had developed into mature telia by 14 days after inoculation. However, the rust also infected leaves, bracts and stems of safflower cultivars and developed mature telia within the same time frame.

Septoria centrophylli infected Australian accessions of saffron thistle tested. Small necrotic lesions were first observed at 8 days after inoculation and developed into necrotic lesions by 13 days after inoculation. All safflower cultivars inoculated with *S. centrophylli* also developed large necrotic lesions. The finding that safflower is also a host for the two pathogens isolated from *C. lanatus* in Greece raises concerns about the suitability of these pathogens for biological control of saffron thistle in Australia. Although the safflower industry is shrinking in Australia, farmers are still contracted to grow this crop for the Japanese market because of the high quality oil produced. It is likely that a conflict of interest would emerge with this industry should this biological control program be pursued.

Assessing the risks associated with the release of a flowerbud weevil, *Anthonomus santacruzi*, against the invasive tree *Solanum mauritianum* in South Africa

T. Olckers

ARC–Plant Protection Research Institute, Private Bag X6006, Hilton 3245, South Africa

Biological control of *Solanum mauritianum* Scopoli, a major environmental weed in the high-rainfall regions of South Africa, is dependent on the establishment of agents that can reduce fruiting and limit seed dispersal. The flowerbud weevil, *Anthonomus santacruzi* Hustache, is a very promising fruit-reducing agent, despite ambiguous results obtained during host-specificity evaluation in quarantine. Adult no-choice tests showed that although feeding is confined to *Solanum* species, normal feeding and survival occurred on the foliage (devoid of floral material) of cultivated eggplant (aubergine), potato and several native South African *Solanum* species. During paired choice tests involving floral bouquets in 10-litre containers, *A. santacruzi* oviposited in the flowerbuds of 12 of the 17 test species, including potato and eggplant, although significantly more larvae were recovered on *S. mauritianum* than on eight species. Larvae survived to adulthood on all 12 species, with survival significantly lower on only four species than on *S. mauritianum*. However, during multichoice tests, involving potted plants in a large walk-in cage, *A. santacruzi* consistently displayed significant feeding and oviposition preferences for *S. mauritianum* over all of the 14 *Solanum* species tested. Analyses of the risk of attack on non-target *Solanum* plants suggested that, with the possible exception of two native species, none are likely to be extensively utilized as hosts in the field. Also, host records and field surveys in South America have suggested that *A.santacruzi* has a very narrow host range and that the ambiguous laboratory results are further examples of artificially expanded host ranges. These and other considerations suggest that *A. santacruzi* should be considered for release against *S. mauritianum* in South Africa and an application for permission to release the weevil was submitted in 2002.

A global review of risk–cost–benefit assessments for introductions of biological control agents against weeds: a crisis in the making?

A.W. Sheppard,[1] R. Hill,[2] R.A. DeClerck-Floate,[3] A. McClay,[4] T. Olckers,[5] P.C. Quimby Jr.[6] and H. Zimmermann[5]

[1]Cooperative Research Centre for Australian Weed Management/CSIRO Entomology European Laboratory, Campus International de Baillarguet, 34980 Montferrier-sur-Lez, France

[2]Research & Consultancy Services, Private Bag 4704, Christchurch, New Zealand

[3]Agriculture & Agri-Food Canada, PO Box 3000, Lethbridge, AB T1J 4B1, Canada

[4]Alberta Research Council, Bag 4000, Vegreville, Alberta T9C 1T4, Canada

[5]Plant Protection Research Institute, Agricultural Research Council, Private Bag X6006, Hilton 3245, South Africa

[6]USDA–ARS European Biological Control Laboratory, Campus International de Baillarguet, 34980 Montferrier-sur-Lez, France

Risks of non-target effects resulting from introductions of exotic organisms for the biological control of exotic pests are a growing major concern because: a) a small number of previous introductions are having significant negative impacts on rare native species, b) exotic organisms are an increasing global threat to sustainable agriculture and biodiversity, c) risk assessment, as applied to environmental threats of species invasions and harmful effects of releases of genetically modified organisms, is a burgeoning new field, and d) biological control is increasingly being used in complex natural ecosystems where indirect impacts are harder to predict. As a result, governments are adopting a more risk-averse attitude to biological control as they assess such releases from an environmental as well as agricultural stand point. In this paper we review the risk assessment processes used by regulatory bodies around the world to pre-judge biological control introductions against weeds in light of risk–benefit–cost (RBC) assessment theory. The aim is to publicize both strengths and weaknesses and to help encourage existing risk-assessment processes to be fair to all without blunting the value of biological control as a recognized effective tool against increasingly damaging exotic weeds. The six key components of formal RBC assessment are: 1) a comparative assessment of the RBC of biological introductions relative to other types of introductions, 2) a full identification of hazards, benefits and costs, 3) exposure analysis of identified hazards and benefits, 4) clearly defined procedure, responsibility and democracy in the decision process, 5) procedures to manage risks where appropriate, and 6) procedures to evaluate outcome in relation to the risk assessment, 7) adequate communication/consultation on RBC at all levels. Currently, only New Zealand addresses the concepts of a formal ecological RBC assessment of biological-control introductions with a precautionary approach, open consultation, broad definition of risk taken in the release application, and a judicial basis to the decision. What is also clear is that the benefits of biological control remain poorly understood by the public, such that the risks are given disproportionate attention. We make recommendations to address this and discuss the outcomes of the review with respect to the inherent social risks of making assessment of biological control releases an overly protracted process.

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The first genuine root-attacker (*Longitarsus* sp., Coleoptera:Chrysomelidae: Alticinae) for *Lantana camara*

D.O. Simelane

ARC–Plant Protection Research Institute, Private Bag X134, Pretoria, 0001 South Africa

Despite the establishment of 11 leaf-, flower-, fruit- and shoot-attacking insect agents, biological control of *Lantana camara* L. (lantana) in South Africa is not sufficiently effective. Not only does this shrub compensate for insect damage, the populations of lantana biocontrol agents also decline during autumn or winter, particularly in areas prone to frost and drought. In the present initiative against lantana, the South African biological control program is targeting niches not affected directly by natural enemy attack. Accordingly, a flea beetle, *Longitarsus* sp., was collected from *L. camara* in Mexico and introduced into quarantine in South Africa because of its ability to damage the root system, a niche not exploited by any of the previously introduced lantana biocontrol agents. The *Longitarsus* sp. larva is highly damaging to the roots, leading to reduction in plant growth rate, which could in turn hamper flower and seed production. Host-range tests were carried out on 52 plant species in 11 families. Only 11 plant species, all in the family Verbenaceae, supported complete development of the root beetle during no-choice tests. The root beetle showed a very strong preference for *L. camara* during paired-choice and multi-choice tests. The narrowing of the host range, particularly during the multi-choice tests, demonstrated that the 10 marginally suitable plant species attacked during the no-choice tests were only attacked due to the inability of the insect to exercise its real host-selection ability under laboratory conditions. Under natural conditions, its host range is expected to be confined to the target weed. It was therefore concluded that this root feeder is sufficiently host-specific to *L. camara* and poses no threat to non-target plant species. Application for permission to release this agent was submitted to the relevant authorities.

Biological control of ragwort (*Senecio jacobaea*): monitoring nontarget impacts of *Cochylis atricapitana* and *Platyptilia isodactyla* on native Australian *Senecio* species

K.A. Snell[1] and D.A. McLaren[2]

[1]Department of Primary Industries, Keith Turnbull Research Institute, PO Box 48, Frankston, Victoria 3199, Australia

[2]Cooperative Research Centre for Australian Weed Management

Despite rigorous host-range and specificity testing before the approved release of a biological control agent, there is still a great need to monitor the post-release safety of non-target species. This is to ensure that biological-control programs are delivering their intended outcomes without causing any detrimental impacts upon non-target species. Laboratory host-specificity testing before release of the ragwort (*Senecio jacobaea*) crown boring moths, *Cochylis atricapitana* and *Platyptilia isodactyla,* had shown some low-level feeding damage to several native *Senecio* species. To monitor any potential non-target impacts, six sites across Victoria where *C. atricapitana* and *P. isodactyla* have established were sampled to determine whether any non-target native *Senecio* species were being attacked by these biological control agents. Native *Senecio* plants were collected at each of the six sites, whilst bolting ragwort plants and rosettes were collected from three and four sites, respectively. Thirty native *Senecio* and bolting ragwort specimens and 15 ragwort rosettes were randomly collected from each of the available sites. In total, 180 native *Senecio* plants, 90 bolting ragwort plants and 60 ragwort rosettes were assessed in detail for attack by *C. atricapitana* and *P. isodactyla*. The visible signs of damage to individual plants were recorded, whilst the pupae and larvae found in individual plants were identified and recorded to determine agent attack rates. The presence of pupae and larvae from a common native moth *Patagoniodes farinaria* were also recorded. The direct attack upon ragwort by *C. atricapitana* and *P. isodactyla* was found to be quite high, but there was no evidence of any direct attack upon non-target

native *Senecio* plants. Damage was notable for 54.5% of the native *Senecio* plants sampled with 15.3% of this damage caused by the native moth *Patagoniodes farinaria* and the rest by other unidentified insect agents. Of the ragwort plants damaged, definite attack (presence of an agent) by *C. atricapitana* or *P. isodactyla* occurred upon 100% of the rosettes and 51% of the damaged bolting and flowering ragwort plants. The results from this study support the results obtained during detailed host-specificity studies, which indicated that *C. atricapitana* and *P. isodactyla* are host-specific to *S. jacobaea* and therefore pose a very low risk to the native flora.

Host specificity of *Megamelus scutellaris* (Hemiptera, Fulgoromorpha, Delphacidae), a potential agent for the biological control of waterhyacinth

A.J. Sosa,[1] H.A. Cordo,[1] M.A. Szudruk,[1] M.C. Hernandez[1] and M. Hill[2]

[1]South American Biological Control Laboratory, USDA–ARS, Bolivar 1559 (B1686EFA) Hurlingham, Buenos Aires, Argentina

[2]Department of Zoology and Entomology, Rhodes University, PO Box 94, Grahamstown, 6140, South Africa

The host range of *Megamelus scutellaris* was studied in the laboratory using two types of multiple-choice test (including and excluding water hyacinth), and one type of non-choice test. In the first multiple-choice test, plants other than Pontederiaceae were used, whereas in the second one only Pontederiaceae were used. For the non-choice test, five species and two varieties in the Pontederiaceae were included, and this test included maize and rice. These last two plants were included because they are host of many species of Delphacidae. Feeding damage was difficult to quantify, so the preference for each plant was indirectly measured using an index that related the number of insects on a given plant and the number of insects alive in the cage used. Mortality was also measured. When given a choice, *M. scutellaris* significantly preferred waterhyacinth to other plants and it did not show preference to a particular plant when waterhyacinth was absent. The mortality after 48 hrs in the tests where waterhyacinth was present was significantly lower than those where waterhyacinth was absent. In the non-choice trial, *M. scutellaris* reached the adult stage on only three plants: waterhyacinth, *Pontederia cordata lancifolia* and *P. rotundifolia*. However, nymphal mortality was lower, and the duration of the whole immature stage was significatively shorter in waterhyacinth than on the other two plants. These results, along with the fact that, despite extensive surveys, *M. scutellaris* has been recorded from waterhyacinth in only Argentina and Brazil, indicate that the insect is monophagous and a safe agent to be introduced into other countries for the biological control of this weed.

Realized host-specificity testing of *Bruchidius villosus* (Coleoptera: Chrysomelidae) in Europe

Thierry Thomann and Andy Sheppard

CSIRO European Laboratory, Campus International de Baillarguet, 34980 Montferrier-sur-Lez, France

Bruchidius villosus, a broom seed feeder, was introduced into New Zealand in 1987 from the UK and from New Zealand into Australia in 1995 as a biological control agent against Scotch broom (*Cytisus scoparius*) a leguminous shrub native to Europe. Introduction followed extensive testing in the UK, New Zealand and Australia that showed it to be host specific. Contrary to test results, *Bruchidius villosus* was found emerging from pods of tagasaste (*Chamaecytisus palmensis*), an exotic fodder species closely related to broom, in New Zealand in 1999. The same year, a field trial of the host range

of several agents (including *Bruchidius villosus*) was set up in a garden at CSIRO European Laboratory at Montferrier (FR 34). Two blocks of three rows, each row containing three plots of ten plants of *Cytisus scoparius, Chamaecytisus palmensis,* and *Genista monspessulana*, were used. A naturally occurring population of *Bruchidius villosus* on the surrounding Spanish broom plants (*Spartium junceum)* colonized the plots. The pods of test plant species were collected when mature in June 2000 and allowed to dehisce in boxes. Several individuals of *Bruchidius villosus* emerged from the tagasaste seeds. The next summer, another garden trial was set up in the same field to grow 40 *Lupinus arboreus* that produced flowers and pods collected when mature. Seed dissection revealed also an attack of *Bruchidius villosus* with adult emergence. Those two trials showed for the first time in Europe that using big healthy plants, a natural population of *Bruchidius villosus* could attack and develop on tagasaste seeds under field conditions. We also detected for the first time a small attack level of this insect within the genus *Lupinus* outside the subtribe Genistineae. This result has wide significance for the use of *B. villosus* in North America.

Specificity tests with *Heteroperreyia hubrichi* (Hymenoptera: Pergidae) and *Calophya terebinthifolii* (Homoptera: Psyllidae) potential control agents against Brazilian peppertree *Schinus terebinthifolius* (Anacardiaceae) in the United States

M.D. Vitorino,[1] G. Barbieri,[2] J.H. Pedrosa-Macedo[3] and J.P. Cuda[4]

[1]Blumenau Regional University, Brazil, Forest Engineer Department, Forest Protection and Monitoring Laboratory

[2]Blumenau Regional University, Brazil, Forest Protection and Monitoring Laboratory

[3]Federal University of Parana, Brazil, Neotropical Biological Control Laboratory

[4]Entomology and Nematology Department, University of Florida, USA

The Brazilian peppertree *Schinus terebinthifolius* (Anacardiaceae) was introduced into the southern United States as an ornamental, where it established has become a weed. This plant is native to South America, mainly Brazil. In the beginning of the 1990s, field surveys were initiated in southern Brazil looking for potential natural enemies. Some insects were selected showing potential for biological control of this weed; in particular a sawfly *Heteroperreyia hubrichi* (Hymenoptera: Pergidae), the Brazilian peppertree leaf feeder, and *Calophya terebinthifolii* (Homoptera: Psyllidae) a leaf gall maker. Multiple and non-choice tests were carried out using the methodology proposed by Wapshere. Twenty plant species belonging to nine different botanical families were tested with both agents. A total of 80 no-choice tests was carried out for the sawfly, with 1,497 first-instar larvae. Oviposition tests with adult females of *H. hubrichi* were conducted on the plant species where development had occurred to the pupae stage. The sawfly is oligophagous and was shown to be specific to the *Schinus* genus, Anacardiaceae family. The insect was assessed against Goeden's evaluation of entomological agents considered for biological control of weeds. *H. hubrichi* obtained a sufficient score on Goeden's criteria, therefore being considered safe for introduction as a biological control agent against *Schinus terebinthifolius* in Florida. The leaf galler *C. terebinthifolii* is under testing (specificity and damage effects), but also shows potential for future introduction.

Specificity tests with *Tectococcus ovatus* (Heteroptera: Eriococcidae) a potential control agent against strawberry guava *Psidium cattleianum* (Myrtaceae) in the United States

M.D. Vitorino,[1] A.L. Buss,[2] J.H. Pedrosa-Macedo[3] and J.P. Cuda[4]

[1] Blumenau Regional University, Brazil, Forest Engineer Department, Forest Protection and Monitoring Laboratory
[2] Blumenau Regional University, Brazil, Forest Protection and Monitoring Laboratory
[3] Federal University of Parana, Brazil, Neotropical Biological Control Laboratory
[4] Entomology and Nematology Department, University of Florida, USA

The strawberry guava *Psidium cattleianum* Myrtaceae, a plant native to southern Brazil, was introduced into Hawaii in the 1880s as an ornamental and as a fruit crop, where it has since disseminated and established. Nowadays, this plant is considered a weed in many countries and is also noted as an alternative host for fruit flies. A search for natural enemies in its native distribution was initiated in the 1990s with surveys in south Brazil, mainly in Parana state. Many enemies were recognized, in particular *Tectococcus ovatus* Hempel (Heteroptera: Eriococcidae) a leaf gall maker that shows real potential to control this plant. Biological studies showed high infestations from *Tectococcus ovatus* cause severe stress at all ages of the plant, directly affecting flowers and fruit production and also the growth rate. This insect attacks also young tissues like buds. Multiple-choice and non-choice specificity tests were conducted to show the gall maker's host specificity. About 20 species in different genera from the Myrtaceae family were tested and also plants of commercial interest in Brazil and USA. The results show the specificity of this agent to the *Psidium* genus and confirm its potential for introduction.

The trimorphic lantana flea-beetle *Alagoasa extrema* not suitable for release in Africa, is suitable for biocontrol in Australia

Hester Williams[1] and Martin Hill[2]

[1] ARC–Plant Protection Research Institute, P.Bag X134, Pretoria, 0001, South Africa
[2] Department of Zoology and Entomology, Rhodes University, Grahamstown, 6140

Biological and host-specificity studies on *Alagoasa extrema* Jacoby (Coleoptera: Chrysomelidae: Alticinae) showed that this flea-beetle is able to oviposit and develop on indigenous South African *Lantana* and *Lippia* species. The relative suitability of these test plant species to support the development of a population of *A. extrema*, compared to that of *L. camara*, was measured by means of a risk-analysis. Insect performance factors that were taken into consideration for the analysis were adult plant preference, oviposition preference, oviposition performance and larval survival. The results showed that *Lippia* sp. B was 72%, *L. rehmannii* 62% and *Lantana trifolia* 16% as suitable a host as *L. camara*. Continuation trials, determining the ability of test plant species to support a population over three generations, showed that *Lippia* sp. B and *L. rehmannii* are able to support viable populations. Due to the risk to indigenous *Lippia* species, it is recommended that *A. extrema* not be released in Africa. As no indigenous *Lantana* or *Lippia* species are present in Australia, the highly damaging and possibly predator-resistant *A. extrema* is suitable for biocontrol in Australia.

Pre-release studies on *Lixus aemulus*, a new biocontrol agent on *Chromolaena odorata*: biology, host range and impact

Costas Zachariades[1], Milly Gareeb[1] and Robert L. Kluge[2]

[1] ARC–Plant Protection Research Institute, Private Bag X6006, Hilton 3245, South Africa
[2] 8 Brindy Mews, Brindy Road, Hilton 3245, South Africa

Stem-attacking insects are an important element in attempts to suppress *Chromolaena odorata* (Asteraceae) using biological control. In the quarantine laboratory, adults of the stem-borer *Lixus aemulus* (Coleoptera: Curculionidae) feed on the young leaves of *C. odorata*. Between one and about eight eggs are laid per stem, just above the nodes. The larvae hatch after a week and tunnel inside the stem, feeding mainly on the pith. Pupation takes place in a chamber inside the stem, and in summer the adult progeny emerge three to four months after oviposition. Average adult lifespan is between three and six months with a maximum of one year. Results from no-choice tests indicate that although *L. aemulus* adults can feed on a wide range of asteraceous species, oviposition and larval development is largely confined to members of the tribe Eupatorieae, with preference for plants with young, non-woody, upright stems containing pith and having a minimum diameter of about 3 mm. Plant species from which adult progeny were obtained in the no-choice tests (five South African weeds of American origin and one indigenous weed) were used in single-choice tests with *C. odorata*. In these tests, *Ageratum conyzoides* was the only species that approached *C. odorata* in terms of adult feeding and oviposition. Because *L. aemulus* was collected from a different biotype of *C. odorata* to that invasive in southern Africa, multi-choice tests were set up to determine adult preference for different *C. odorata* biotypes, but no strong patterns emerged. Results from damage trials conducted in the laboratory indicate that *L. aemulus* larval development markedly decreases stem growth rate, often causes die-back and dramatically suppresses achene production. Pre-release studies therefore suggest that *L. aemulus* is host specific and a potentially damaging agent. Permission for its release in South Africa is pending.

Theme 4:

Integration and Management

Integrated weed management – could we be doing better? Lessons from controlling the invasive wetland shrub, *Mimosa pigra*

Quentin Paynter[1] and Grant J. Flanagan[2,3]

Summary

1. Partial control; i.e. where biological control has an impact, but other control methods are still required, is the most common result of biological control programs against weeds. For this reason, optimizing integration of biological control with other methods should be a research priority. However, there are relatively few examples where biological control and other control methods have been integrated and have led to enhanced control.
2. Potential reasons why biological control may fail to integrate with other methods are discussed and a large-scale integrated control trial to investigate the impacts of herbicide application, crushing by bulldozer and burning, either alone or in combination, on both the introduced wetland weed *Mimosa pigra* and its biological control agents are described.
3. In isolation, herbicide, bulldozing and fire were not effective control measures, but several combinations of techniques cleared mimosa thickets and promoted establishment of competing vegetation which inhibited seedling establishment.
4. Depending on the species, biological control agent abundance relative to mimosa was either unchanged or increased following herbicide and/or bulldozing treatments.
5. All agents re-colonised regenerating mimosa within one year of the fire treatment. Abundance of *Carmenta mimosa* declined, whereas *Neurostrota gunniella* increased dramatically following the fire.
6. We attribute increased abundance of *N. gunniella* in response to all treatments, to attack by this species being aggregated along stand edges. By reducing mimosa populations from monocultures to smaller patches or individual plants, control treatments will have increased the ratio of 'edge' to 'thicket' plants and, therefore, the proportion of plants susceptible to *N. gunniella* attack.
7. We conclude that integrating control techniques can successfully control dense mimosa thickets; and that biological control integrates well with other control options and should, therefore, lead to significant cost reductions in the management of mimosa. To maximise this benefit, integrated weed management plans should be designed to fully integrate biological control with other methods, rather than separate them spatially or temporarily.

Keywords: integrated management, *Mimosa pigra*, *Neurostrota gunniella*, *Carmentia mimosae*, fire, herbicides, bulldozing.

Introduction

Recent analyses (Hoffmann 1995, McFadyen 1998, Fowler *et al.* 2000, Briese 2000) indicate that biological control of weeds has been more successful than previously supposed. However, complete successes, where biological control is so dramatic that no other control methods are required, only account for approximately one-third of all completed programs (McFadyen 1998) and programs that deliver 'substantial' or 'partial' control, where biological control contributes to management, but other control methods are still required, are more typical. For example, Hoffmann (1995) considered biological control programs resulted in complete control of six weeds (26%) and contributed

[1] CSIRO Entomology, Tropical Ecosystems Research Centre, PMB 44, Winnellie, 0822 NT, Australia.
[2] Northern Territory Department of Business, Industry and Resource Development, PO Box 79, Berrimah, NT 0828, Australia.
[3] Present address: Kangaroo Island Natural Resources Board, PO Box 520, Kingscote, SA 5223, Australia.
Corresponding author: Dr Q. Paynter <Quentin.Paynter@csiro.au>.

to control of 13 out of 23 weeds (57%) in South Africa. In New Zealand, six completed biological control programs have resulted in complete control of one weed (17%), partial control of four weeds (67%) and one failure (Fowler *et al.* 2000). In Australia, 19 out of 45 projects (42%) resulted in a "substantial contribution to control" (although not necessarily complete control) and the remaining 26 (58%) resulted in "some contribution to control" (Briese 2000). These figures indicate that some form of integration with other management options is required for well over half the weeds targeted by biological control programs. In this context, it seems remarkable how few documented examples exist where such integration has been demonstrated to result in improved weed management (e.g. Trumble & Kok 1980ab, Charudattan 1986, Briese 1996, Hoffmann *et al.* 1998). In this paper, we investigate why this may be the case and suggest how the value of biological control in integrated management programs can be increased.

What is integrated weed management?

Integrated weed management (IWM) is defined here as a sustainable approach to managing weeds that combines biological, cultural, physical and chemical methods in a way that maximizes their effectiveness while minimizing economic, health and environmental risks. It is a form of ecosystem management, and requires sufficient knowledge of the ecology of the weed and the invaded system to allow prediction of the outcome of control efforts.

Biological control can be integrated with other methods of weed control in a number of ways (Watson & Wymore 1990). In this paper, we refer to the integration of "classical" or "inoculative" biological control, rather than "augmentative" or "inundative" control (e.g. the use of native pathogens as a mycoherbicide described by Daniel *et al.* 1973).

Why is including a biological control component desirable in an IWM program?

At the request of the members of the Convention on Biological Diversity, experts in the Global Invasive Species Program (GISP) reviewed the methods for controlling invaders and concluded that because biological approaches and land-management practices are environmentally benign they should "form the cornerstone of IPM programs" (Baskin 2002). Numerous papers have noted the potential benefits of integrating biological control with other methods (e.g. Briese 1990, Watson & Wymore 1990, Miller *et al.* 1992, King *et al.* 1996, Scott & Yeoh 1996, Rees & Paynter 1997, Smith *et al.* 1997, Shea & Kelly 1998, Rees & Hill 2001, Huwer *et al.* 2002). However, whether potential for improved control can be realized is often debatable. For example, Rees & Paynter (1997) and Rees & Hill (2001) predicted that, where biological control does not provide complete regulation, integrating agents that reduce plant fecundity with control methods such as mechanical control or fire, to reduce the proportion of reproductive plants, should enhance suppression of woody legume populations. However, a criticism of these models is that they assumed additional control methods do not affect biological control agent abundance, which may not be the case. Indeed, scientists have often presumed classical biological control and techniques such as chemical weed control were incompatible (Harris 1991). However, herbicides (e.g. Andres 1982, Harris 1991, Messersmith & Adkins 1995, Lindgren *et al.* 1999, Paynter 2003) or fire (Briese 1996) are not necessarily unfavourable to biological control agent populations.

Recent work on the integrated management of *Mimosa pigra* L. (Paynter *et al.* 2000; Paynter & Flanagan 2002) has enabled the impact of a number of control techniques on a suite of biological control agents to be investigated.

Mimosa

Mimosa pigra, henceforth mimosa, is now a pantropical weed, native to tropical America, which poses the most serious of all invasive threats to tropical wetlands (Cronk & Fuller 1995).

In 1979, a biological control program against mimosa was established in Australia (Forno 1992). Four insect biological control agents are now widespread throughout much of the introduced range of mimosa, namely: the stem-mining moths *Neurostrota gunniella* Busck and *Carmenta mimosa* Eichlin & Passoa (both first released in 1989), the flower-feeding weevil *Coelocephalapion pigrae* Kissinger, and the seed-feeding bruchid *Acanthoscelides puniceus* Johnson, which were first released in 1994 and 1983, respectively. Two agents are established locally: *Chlamisus mimosae* Karren, a leaf-feeding chrysomelid that was first released in 1985, only established on the Finniss River catchment, despite widespread releases, and its impact is trivial. *Malcorhinus irregularis* Jacoby is also a chrysomelid with leaf-feeding adults, but the larvae of this species live in the soil and feed on germinating seeds, small seedlings and roots and root nodules. It was first released in 2000 and was recently confirmed to have established and become seasonally abundant at one release site.

This program could currently be described as a partial success: *A. puniceus* destroyed only 0.8% of seed and was considered a failure (Wilson & Flanagan 1991). *N. gunniella*, however, was associated with a decline in fecundity of 58–78%, compared to pre-biological control levels, and stunted growth of both mature plants (Lonsdale & Farrell 1998) and seedlings (Paynter & Hennecke

2001), but damage was not considered sufficient to bring about complete control of mimosa (Lonsdale & Farrell 1998). Ongoing evaluation work indicates *Carmenta mimosa* is beginning to have a major impact (Q. Paynter, unpublished data), however, alternative control methods are currently required (Paynter & Flanagan 2002).

Physical and chemical control

A number of control techniques have been tried, but are rarely effective in isolation: aerial herbicide application is most effective in the wet season, though spraying may not achieve 100% kill and plants often regenerate from the seed bank, so follow-up control is required (Miller & Siriworakul 1992). Re-growth and regeneration from the seed bank generally occurs after mechanical control such as chaining and bulldozing (Siriworakul & Schultz 1992). Green mimosa is difficult to burn and, even if a fire does carry through a stand, burnt plants often regrow from buds at the base of stems, while fire can enhance mimosa germination (Miller & Lonsdale 1992). Miller *et al.* (1992) suggested an integrated approach should, therefore, provide the most effective management strategy for mimosa.

The mimosa integrated control experiment

A split-plot experiment was performed at Wagait Aboriginal Reserve, on the Finniss River catchment, Northern Territory (NT) (12°56'S, 130°33'E altitude *ca.* 20 m asl) to measure the impact of herbicide, crushing by bulldozer, and burning on both mimosa and its biocontrol agents (Fig. 1a). The design, originally described by Paynter *et al.* (2000), was modified to include additional herbicide treatments (Fig. 1b). We intend to publish this work elsewhere (Paynter & Flanagan 2004) so detailed descriptions of the site, methods and analyses are not presented here. Four replicates of the following herbicide treatments were performed, with or without a bulldozing treatment:

- control (no herbicide)
- single herbicide applications (fluroxypyr: Starane 300®; DowElanco Co, Frenchs Forest, Australia) diluted to 0.5% v/v at 1.5–2 L ha^{-1}) April 1998, January 1999, December 1999)
- double herbicide applications (April 1998 + January 1999, April 1998 + December 1999, January 1999 + December 1999)
- a triple herbicide application (April 1998 + January 1999 + December 1999).

The April 1998, January 1999 and December 1999 herbicide treatments, corresponding to the 1997/8, 1998/9 and 1999/2000 wet seasons, are henceforth referred to as the 1997, 1998 and 1999 wet season treatments, respectively.

Following construction of a firebreak around the study site perimeter, the burn treatment was conducted on 3 November 2000. Fire passed through all plots, burning for at least two weeks, until it was extinguished by heavy thunderstorms.

Quantitative data on the impacts of the control treatments on mimosa, competing vegetation and on the relative abundance of four biological control agents (*N. gunniella*, *C. mimosa*, *C. pigrae*, and *Chl. mimosae*) was collected. *A. puniceus* could not be quantified (by rearing beetles from seed: Wilson & Flanagan 1991) because mimosa seeds most prolifically shortly after the wet season (Lonsdale 1988), when the field site could not be accessed due to seasonal flooding. *M. irregularis* was not present at the field site during the course of this study.

Results and discussion

Impact of control treatments on mimosa

Mimosa was initially present as a virtual monoculture (mean percentage cover, aboveground biomass per hectare and number of stems per hectare was estimated at 96.3%, 39,279 kg (dry-weight) and 15,137, respectively). Nevertheless, impenetrable thickets were turned into productive, biologically diverse, grassland within just a few years. Single treatments, however, did not provide substantial control. For example, by November 2000, mimosa cover in plots left untreated following single herbicide applications in the 1998 wet season was just *ca.* 25% less than levels in the control plots (Table 1). Similarly, mimosa cover in bulldozed only plots was *ca.* 50% of control levels and fire reduced mimosa cover by only *ca.* 30% in unsprayed, non-bulldozed plots (Table 1).

Repeat herbicide applications were generally more effective than single applications, as were combined herbicide and bulldozing treatments, compared to either treatment in isolation (Table 1). Fire was generally most effective in bulldozed plots where compaction of dead mimosa branches should have enabled a hotter, more destructive, fire to occur (Lonsdale & Miller 1993).

Impact of control treatments on biological control agents

Biological control agent populations were remarkably resilient, indicating that they either survived the control treatments or their dispersal abilities were sufficient to rapidly re-colonise plots. *N. guniella* and *C. pigrae* are known to disperse extremely rapidly over many kilometres. For example, *N. guniella* spread at least 160 km within two years of release (Wilson & Forno 1995). Abundance of all agents was unaffected by, or even increased following, herbicide and bulldozing treatments (e.g. *N. gunniella*; Fig. 2a) and fire was only detrimental to *C. mimosa* (Fig. 2c). The decline of *C. mimosa* following the fire was probably because few regenerating plants were large enough to support larvae, rather than a poor ability to recolonise plots. However, *C.*

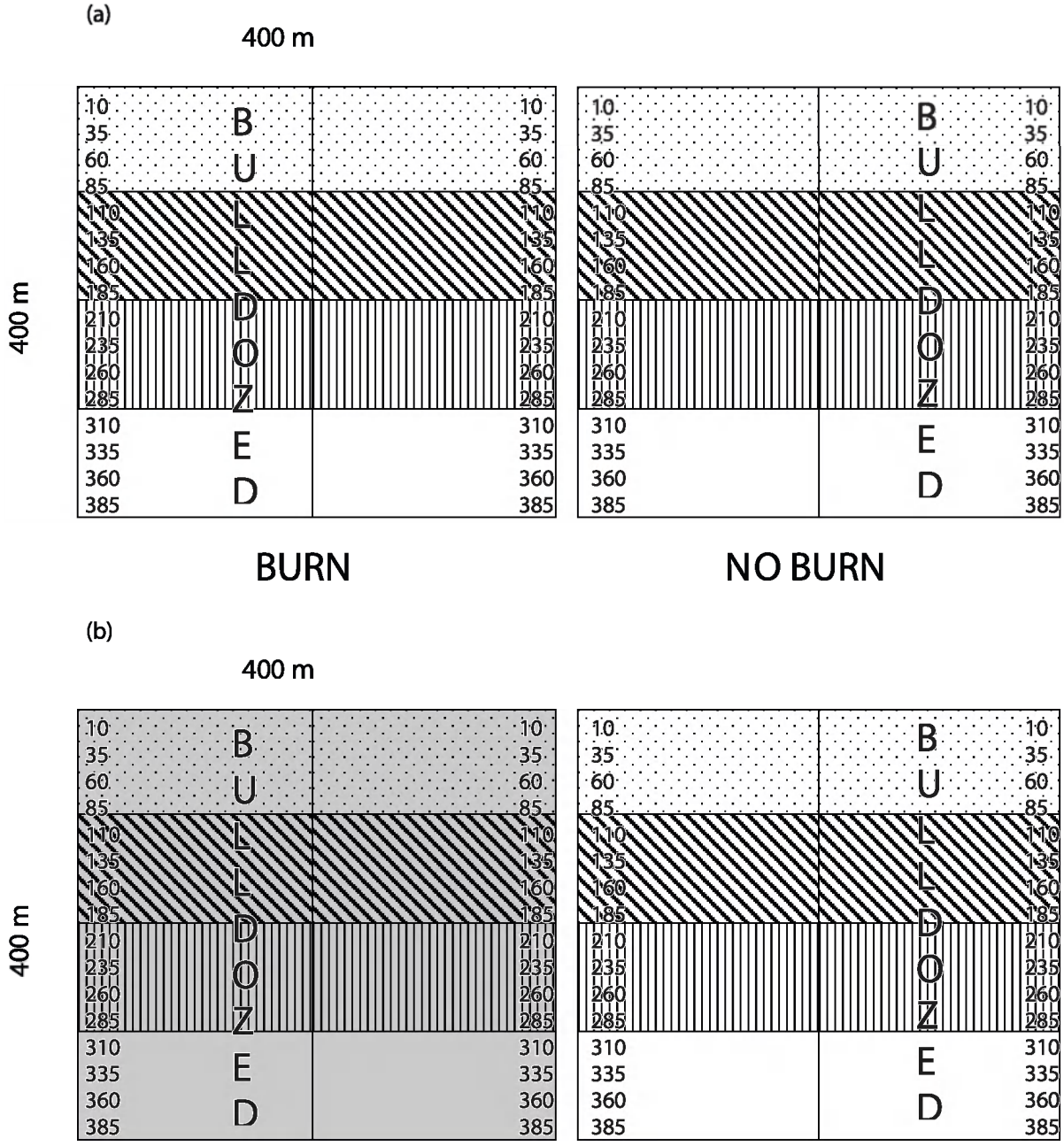

Figure 1. Summary of treatments for one replicate of: (a) the original experimental design. Bulldozed = plots crushed by bulldozer. Burn = sub-block to be burnt in the 1999 dry season. Herbicide applications: no shading = unsprayed; vertical shading = 1997 wet season; diagonal shading = 1998 wet season; stippled = 1997 + 1998 wet seasons. (b) The final design: herbicide and bulldozing treatments as above, but grey shading = plots also sprayed in 1999 wet season. Both sub-blocks were burnt. Numbers within each plot refer to positions of permanent quadrats (m).

mimosa is less mobile than other agents — invading at a rate of *ca.* 2 km yr^{-1} (Ostermeyer 2000), so this agent may be prone to local extinction when very large or isolated mimosa stands are treated.

The best combination of treatments to clear thickets

Fire is essential to clear thickets (including deadwood) and enable vehicular access if mimosa-infested floodplains are to be cleared for cattle grazing. This allows cattle to be herded and other property maintenance to be conducted and facilitates ground control of regenerating seedlings to prevent reinvasion, especially in heavily grazed or trampled areas. Although this study indicates bulldozing or a single herbicide application should enable a successful fire treatment (Table 1), in practice, these treatments left sharp stumps that could stake car tyres. A single herbicide application, followed by a bulldozing treatment and fire, cleared stands more effectively.

Table 1. The effect of herbicide and bulldozing treatments on percentage cover of mimosa recorded in November 2000 (before the fire treatment) and in December 2000 (after the fire treatment).

Treatment	Approximate reduction in percentage cover of mimosa			
	Nov 2000 (pre-fire)		Dec 2000 (after fire)	
	– bulldozed	+ bulldozed	– bulldozed	+ bulldozed
Control	0%	50%	30%	95%
Herbicide (1997)	60%	95%	65%	100%
Herbicide (1998)	25%	90%	70%	100%
Herbicide (1999)	99%	99%	100%	100%
Herbicide (97+98)	95%	100%	99%	100%
Herbicide (97+98+99)	95%	99%	99%	100%

Table 2. Approximate costs of the control treatments (M. Ashley & C. Deveraux, personal communication). The cost for the fire treatment is the estimated cost of creating and supervising a firebreak around the perimeter of a 100 ha mimosa stand.

Treatment	Approximate cost ha^{-1} (A$)			
	– bulldozing		+ bulldozing	
	– fire	+ fire	– fire	+ fire
Control	0	30	60	90
Single herbicide application	20	50	80	110
Two herbicide applications	40	70	100	130
Three herbicide applications	60	90	120	150

If vehicular access is unnecessary, so that fire is not required to clear deadwood (e.g. when controlling patches of mimosa in a national park), control costs are considerably lower, and the potentially adverse effect fire has on competing vegetation that can suppress mimosa seedling establishment (Lonsdale & Farrell 1998) can be avoided. This study indicates two aerial herbicide applications in consecutive years (costing *ca.* \$40 ha^{-1}; Table 2) would greatly reduce the mimosa infestation (Table 1) and enhance *N. gunniella* abundance whilst having no detrimental impacts on *C. mimosa* (Fig. 2).

Conclusions

An unexpected feature of this study was the low degree of mimosa reinfestation following control. For example, mimosa cover remained at very low levels in bulldozed plots treated with repeat herbicide applications in 1997 and 1998 (at a cost of *ca.* \$130 ha^{-1}), almost two years after any control measures had been applied to those plots (Table 2). This is remarkable, considering the highly invasive nature of this weed during the 1970s and 1980s, when populations doubled in size every 1.2 years and thickets advanced at a rate of 76 m yr^{-1} (Lonsdale 1993). This also compares very favourably to a cost of *ca.* \$1000 ha^{-1} spent on a five-year management program from 1991–1996 (Anon. 1997), during which an infestation was treated with herbicide the equivalent of three times, yet only reduced mimosa cover by *ca.* 80–90%, so that continued control efforts were still required (Cook 1996). We believe there are two explanations for this:

1. Eradication of feral water buffalo *Bubalis bubalis* Lydekker during the late 1980s and early to mid-1990s will have reduced overgrazing of competing vegetation, allowing competitive perennial species to recover (Braithwaite & Roberts 1995) and, therefore, reduced the ability of mimosa to reinvade (Lonsdale 1993).
2. Spatial models of invasive legume shrubs (Rees & Paynter 1997, Rees & Hill 2001) suggest that by reducing fecundity (Lonsdale & Farrell 1998) biological control may have enhanced the impact of control treatments by reducing seedling regeneration, due to smaller seed banks and reduced reinvasion from dispersing seed. High levels of *N. gunniella* herbivory in treated plots (Fig. 2a) should have enhanced this effect by stunting seedlings, thus reducing their probability of survival during wet season floods (Paynter & Hennecke 2001), when entire cohorts of seedlings can drown (Lonsdale & Abrecht 1989). It is also likely that *N. gunniella* herbivory delays sexual maturity of regenerating mimosa, so that plants can be treated by ground control operations before they set seed (Colin Deveraux, personal communication).

There are two potential explanations why *N. gunniella* abundance increased relative to mimosa in treated plots. Firstly, *N. gunniella* larvae can complete their development before plants treated with herbicide die, so a treatment that kills most plants should increase

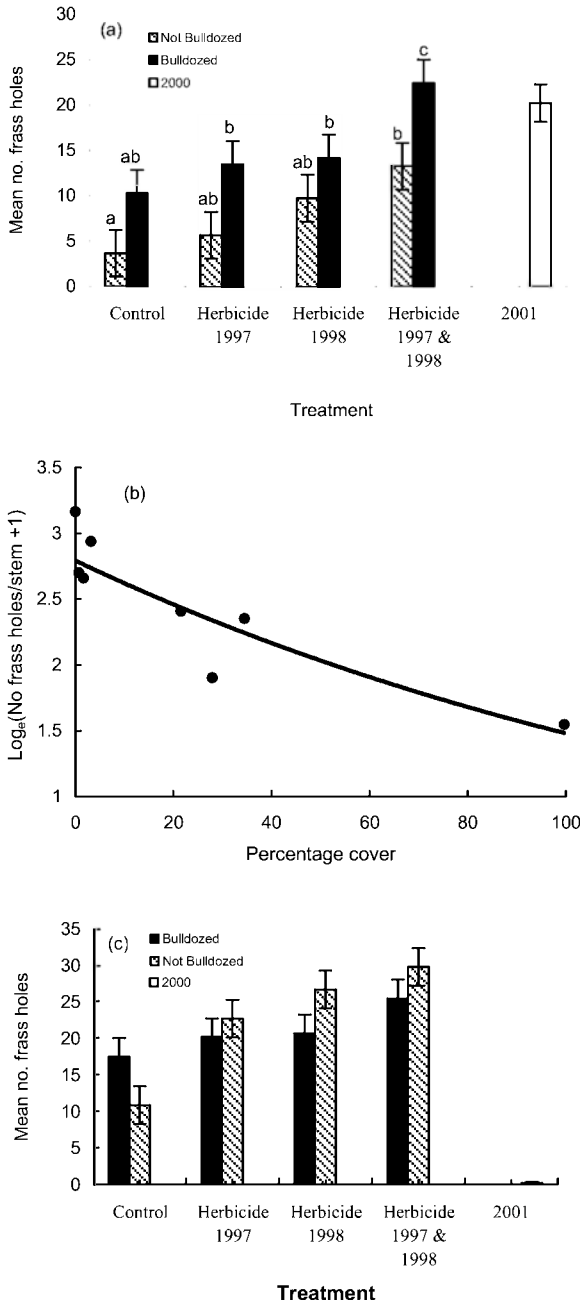

Figure 2. (a) Effect of herbicide and bulldozing treatments on *Neurostrota gunniella* abundance [mean no. frass holes per 50 cm stem (±SE)] in 1999 and overall abundance in 2001, following the fire. Columns with the same letter are not significantly different (LSD).
(b) *Neurostrota gunniella* abundance versus percentage cover of mimosa recorded in 1999. Log_e (no. frass holes +1) = $2.793e^{-0.0063x}$, R = 0.91, P<0.001.
(c) Effect of herbicide and bulldozing treatments on *Carmenta mimosa* abundance [mean no. frass holes per 5 min count (±SE)] in 1999 and overall abundance in 2001, following the fire.

N. gunniella abundance, relative to surviving mimosa plants (Paynter 2003). However, this could not explain increased *N. gunniella* abundance following the fire (Fig. 2a), which would have also killed *N. gunniella*. We believe a second explanation, based on the finding that *N. gunniella* attack is aggregated at stand edges (Smith & Wilson 1995), is more likely. By reducing mimosa populations from dense thickets to smaller patches or individual plants, control treatments will have increased the ratio of "edge" plants to "interior" plants and, therefore, the proportion of plants susceptible to *N. gunniella* attack. Indeed, *N. gunniella* abundance increased exponentially as the percentage cover of mimosa declined (Fig. 2b).

Failure of *N. gunniella* to control untreated mimosa populations, despite high levels of recorded damage (Lonsdale & Farrell 1998) may, therefore, be due to the aggregative attack of this species creating "partial refuges" for plants in the centre of thickets, which escape high levels of herbivory. By greatly increasing the proportion of plants susceptible to herbivory by *N. gunniella*, herbicide application, bulldozing and fire will have enhanced the impact of biological control. This study, therefore, demonstrates the value of integrating biological control with other options to significantly reduce the cost of managing weeds and supports the suggestion by Lindgren *et al.* (2000), who noted that the use of herbicides to clear infestations of weeds may accelerate the classical control of weeds.

As for many weed control programs (e.g. Cullen 1996), previous "integration" of techniques for mimosa was limited to using herbicides in areas where complete control was required and relying on biological control in areas where eradication was no longer an option. For example, mimosa growing in riverine habitats in the upper river catchments has been considered a priority for herbicidal control, to prevent more seed being dispersed downstream. Biological control is considered to be the most suitable option for large, intractable infestations on floodplains. We believe this viewpoint may no longer hold true. Although most agents are widespread, *Carmenta mimosa* remains absent, for example, from the upper Adelaide and Finniss Rivers (Ostermeyer 2000). Plants overlooked by control operations in these habitats are highly fecund compared to plants growing where *C. mimosa* is common (Q. Paynter, unpublished data). We believe releases of *C. mimosa* should be made in these riverine areas, even if there is a risk that release sites will be treated with herbicide. Furthermore, the control work at Wagait has shown that large infestations can be cleared, using herbicides, and the floodplain then used for profitable cattle grazing enterprises, despite the presence of large stands of untreated mimosa on neighbouring properties acting as sources of seed (C. Deveraux, pers. comm.).

Implications for IWM

To summarise, the major findings of the above work are as follows.

1. Herbicide, bulldozing or fire treatments do not necessarily reduce biological control agent attack and may even result in an increase in the level of damage they inflict on the target plant.
2. Theoretically, the impacts of control treatments on attack rates could have been predicted from observations of insect behaviour (e.g. length of life cycle, dispersal ability, pattern of attack) and plant response to herbicides (e.g. time to morbidity and death of plant tissues utilized by biological control agents).
3. To maximise the benefits of biological control for integrated weed management of mimosa, a priority should be to investigate how to fully integrate biological control with other methods, rather than to separate them spatially.

To develop further recommendations based on these observations, it is useful to consider why integrated control may fail or not be adopted or not even be appreciated. Potential reasons for the failure of integration fall into three main categories:

Insufficient data exist

The extreme case will be where there are no impact data at all. There will clearly be a low priority for incorporating biological control in an IWM strategy if the benefits of biological control have not been demonstrated. Quantitative evaluation of biological control agent performance is therefore essential, if cynics are to accept the recently quoted *ca.* 80% success rate for biological control of weeds, which includes partial successes (e.g. McFadyen 1998). This can be a challenge, as the full benefits of control may not be confined to the sites where biological control agents are deployed. For example, a successful biological control program against ragwort in western Oregon (Coombs *et al.* 1996) caused a reduction in plant fecundity, which was correlated with a decline in the rate of new ragwort infestations many miles away in eastern Oregon (Isaacson *et al.* 1996). Indeed, an unsuccessful program may, theoretically, provide benefits that outweigh costs, even though a weed might continue to invade and its impact increase. For example, Paynter *et al.* (1996) predicted that seed-feeders were unlikely to have a major impact on existing Scotch broom stands, but should reduce the rate of invasion, making infestations easier to contain with herbicides. Therefore, even if the ongoing Australian Scotch broom program fails to control existing stands, the introduction of *Bruchidius villosus* L. may have provided benefits that outweigh the costs of the program, by slowing the invasion — even if broom continues to invade.

Treatments cannot be integrated

In this instance, control options are incompatible – for example, where mortality of the target weed may result in high mortality and even local extinction of biological control agents, reducing their effectiveness (e.g. Zimmermann & Neser 1999). Mimosa agents integrated well with control treatments (Fig. 2). This ability was not only related to their ability to survive certain treatments, but also their capacity to rapidly recolonise regrowth following control treatments that would have cause local extinction (i.e. the fire treatment). More work is required if we are to be able to understand why agents can survive or even thrive following control treatments. For example, are multiple-brooded species, with overlapping generations, less vulnerable to herbicide treatments because a proportion of individuals will always be at a stage in their life cycle that can survive the death of a host plant (e.g. Paynter 2003)? In contrast, a sub-optimally timed herbicide application might kill an entire generation of a single-brooded species. Are agents that aggregate on isolated or "edge" plants more likely to thrive in an IWM program because their good dispersal abilities enable them to locate isolated plants regenerating after control treatments have been applied? Should this influence how we select potential biological control agents?

As well as investigating optimal timing of control applications, it has also been postulated that, where treatments are harmful to biological control agents, maintaining a mosaic of treated and untreated areas as sources for insect reinvasion might benefit a control program (Briese 1996). However, such a policy may also allow a weed to reinvade. Clearly, the importance of weed reinvasion from remnant patches will depend on the relative importance of regeneration from the seed bank versus regeneration from dispersing seed. This is unlikely to be an issue on floodplains infested with mimosa: the scale of the mimosa problem and the good dispersal abilities of the agents make it likely that some patches of mimosa, "within agent range" of mimosa regenerating from control, will always be overlooked by control operations or left untreated in areas where it is uneconomic to attempt control.

All other control options are uneconomic

In this scenario, the land is of such low economic value and the weed is so widespread that biological control is the only control option available — other options are simply too costly.

To summarise, we suggest the following are essential to improve the uptake and appreciation of the benefits of biological control within IWM programs:

- evaluation of agent impact on plant performance
- sufficient understanding of the ecology of a control agent and the weed to understand the impacts of control treatments on agent and weed populations,

and the consequences, to both agent and weed populations, of modifying treatments (e.g. optimal timing of treatment applications or the provision of untreated patches as 'refuges' for reinvasion) to enhance the effectiveness of control

- modelling or good quantitative data, in conjunction with collection of economic data, to determine the impacts and financial benefits of partial successes on weed populations in IWM programs.

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The transfer of appropriate technology; key to the successful biological control of five aquatic weeds in Africa

Martin P. Hill[1] and Mic H. Julien[2]

Summary

The rivers and lakes of Africa have been subjected to invasion by alien aquatic vegetation since the early 1900s. Water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), salvinia (*Salivinia molesta*), parrot's feather (*Myriophyllum aquaticum*) and red water fern (*Azolla filiculoides*), all native to South America, have been recorded in many countries in Africa. Vast mats of these weeds impact on all aspects of water utilisation and severely degrade aquatic biodiversity. Biological control programs have been initiated against all of these weeds from the early 1980s onwards and they have been successfully brought under control in many areas, although in some areas water hyacinth remains problematic. The successful biological control of aquatic weeds in Africa has been ascribed to several factors, including: the reliance on fundamental research performed on the target biocontrol agents in developed countries obviating the need to screen agents for host specificity in resource-poor countries; the development of simple mass-rearing techniques, ensuring the release of high numbers of healthy insects; and a standard post-release monitoring technique allowing comparison between different control sites. However, the single most important factor contributing to the success of these programs was the involvement of dedicated individuals who understood the potential of biological control and who ensured that the projects progressed. These people developed community involvement, another important factor in the success of projects, in mass rearing and agent distribution.

Keywords: biological control, integrated control, technology transfer, water hyacinth.

Introduction

There are five important aquatic plant species in Africa, which warrant control: *Azolla filiculoides* Lamarck (Azollaceae) (red water fern); *Myriophyllum aquaticum* (Vellozo Conceição) Verdcourt (parrot's feather); *Salvinia molesta* D.S. Mitchell (Salviniaceae) (salvinia); *Pistia stratiotes* Linnaeus (Araceae) (water lettuce) and *Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae) (water hyacinth). The native ranges for all five of these species is South America, but they have all been introduced to many parts of the world, where they have become problematic, especially in tropical and subtropical regions (Holm *et al.* 1977). Two issues contribute to their invasiveness – the lack of co-evolved natural enemies in their adventive range (Buckingham 1994) and the presence of nitrate and phosphate enriched waters associated with urban, agricultural and industrial pollution (Heard & Winterton 2000).

These five species form dense mats on rivers and dams throughout Africa and degrade aquatic ecosystems and limit their utilisation. The economic and environmental losses due to these weeds are huge, to the extent that the problem threatened the development of Africa. One of the key issues that separates aquatic weed from terrestrial weed programs is that the impact of water weeds on riverine communities is easy to quantify which makes it easier to generate funding, unlike many of the terrestrial weeds whose impacts, notably on biodiversity, are more difficult to quantify. Here we report on the successful biological control of these five weeds in Africa.

[1] Department of Zoology and Entomology, Rhodes University, PO Box 94, Grahamstown 6140, South Africa.

[2] CSIRO Entomology, 120 Meiers Road, Indooroopilly, Queensland 4068, Australia.

Corresponding author: M. Hill, Department of Zoology and Entomology, Rhodes University, PO Box 94, Grahamstown, 6140, South Africa <m.p.hill@ru.ac.za>.

Azolla filiculoides (red water fern)

Azolla filiculoides has become a weed of small dams and slow-moving rivers in a number of countries around the world. The first record of this plant in Africa was from South Africa in 1948, where it was introduced as a pond ornamental (Oosthuizen & Walters 1961). Phosphate-enriched waters, the lack of natural enemies (Hill 1998a) and dispersal between water bodies by man and waterfowl facilitated an increase in its distribution and abundance.

Azolla filiculoides is able to undergo rapid vegetative reproduction throughout the year by elongation and fragmentation of small fronds, and under ideal conditions, the daily rate of increase can exceed 15%. This translates to a doubling time for the weed of 5–7 days (Lumpkin & Plucknett 1982). In addition, the fern can reproduce sexually via the production of spores, especially when the plant is stressed, which can overwinter and are resistant to extreme desiccation, allowing re-establishment of the fern after drought (Ashton 1992).

Among the major consequences of the dense mats (5–30 cm thick) of the weed on still and slow-moving water bodies are: reduced quality of drinking water caused by bad odour, colour and turbidity; an increase in waterborne, water-based and water-related diseases; increased siltation of rivers and dams; reduced water surface area for recreation (fishing, swimming and water-skiing) and water transport; deterioration of aquatic biological diversity (Gratwicke & Marshall 2001); clogging of irrigation pumps; drowning of livestock; and reduced water flow in irrigation canals.

Control

Mechanical and herbicide control options have been suggested for *A. filiculoides*. However, the impractical nature of mechanical control and undesirability of herbicide control in the aquatic environment suggested that *A. filiculoides* was a suitable candidate for biological control. The weevil *Stenopelmus rufinasus* Gyllenhal (Curculionidae) was prioritised as a biological control agent for *A. filiculoides*. This frond-feeding weevil was imported to South Africa from Florida (USA) in late 1995, underwent host-specificity testing in quarantine (Hill 1998b) and was cleared for release in late 1997 (Hill 1999a).

The weevil has been released at some 110 sites throughout South Africa. The information available on these sites is that the weevil had been responsible for clearing 72 of them completely, all within a year (McConnachie, unpublished data). For the remaining 38 sites, either the weed has been washed away, or they have not been evaluated. The weevil has dispersed up to 300 km from the release sites The weed returned at 7% of the original sites within two years after control, but the weevil has been able to locate and control these infestations (McConnachie, unpublished data). This weevil has also dispersed to Zimbabwe and Mozambique unaided. Five years after the first release of *S. rufinasus* in South Africa, the weed no longer poses a threat to aquatic ecosystems in southern Africa.

Myriophyllum aquaticum (parrot's feather)

Myriophyllum aquaticum was introduced to Africa in the early 1900s (Jacot Guillarmod 1979). Unlike the other four aquatic weeds, *M. aquaticum* is rooted to the substrate and can grow in water up to 1.5 m deep, with emergent shoots which protrude some 200–500 mm above the water surface. Outside of its native range, propagation is entirely vegetative due to the absence of male plants (Henderson 2001). The plants grow throughout the year in tropical and subtropical regions of the world. In the more temperate areas, the emergent shoots die back due to frost damage, but sprout from nodes during the spring (Cilliers 1999). Problems caused by *M. aquaticum* are similar to those caused by *A. filiculoides*, which include a reduction in stream flow, blocking of pumps and interference with recreational activities (Chickwenhere 2001).

Control

Herbicides are not translocated well down the stems of the plants and its rate of growth makes mechanical control impractical. Therefore a biological control program was initiated in South Africa in 1991, which resulted in the release of the leaf-feeding beetle, *Lysathia* sp. (Chrysomelidae) in late 1994 (Cilliers 1999).

This insect has established at more than 25 localities throughout South Africa. The damage caused by the beetle resembles that of frost with a die-back of the emergent vegetation. However, regrowth occurs from the submerged stems. After several years of defoliation, the mat of the weed collapses and no regrowth occurs (Cilliers 1999). However, an additional agent, *Listronotus marginicollis* (Hustach) (Coleoptera: Curculionidae), the larvae of which bore into the stems of *M. aquaticum*, is being considered for release. It is hoped that this species will reduce the time taken to achieve biological control.

Salvinia molesta (salvinia)

Salvinia molesta is a free-floating fern that inhabits still and slow-moving freshwater bodies across the world. This fern is sterile and reproduces by vegetative growth of the rhizomes (Forno & Julien 2000). The negative impacts caused by this weed are the same as those for other floating aquatic weeds, where dense mats constrain the utilisation of impoundments and rivers for agricultural, recreational and conservation purposes (Cilliers 1991). *S. molesta* became a major problem on Lake Kariba as it was filling in the late 1950s. By 1962,

some 1000 km^2, or 22% of the lake was covered by a thick mat of the weed (Mitchell & Rose 1979).

Control

Salvinia molesta can fairly easily be controlled with the use of herbicides. However, concerns over the use of chemicals in the aquatic environment prompted the search for a more sustainable control option. Several agents have been released against *S. molesta* in Africa. The weevil *Cyrtobagous salviniae* Calder and Sands has been the most successful and has now been released in many countries in Africa (Julien & Griffiths 1998).

The adults feed on the growth tips of *S. molesta*, stunting its vegetative growth. The larvae feed on the buds and the roots and then burrow into the rhizome of the plant, causing the plants to rot and sink (Julien *et al.* 1987). Although this insect is not a particularly good disperser (Forno & Julien 2000), it has been a successful biological control agent wherever it has been introduced in the world. *Salvinia molesta* is under complete biological control in Africa and no longer requires any manual removal or herbicide application (Cilliers 1991). In addition, a congeneric species, *Cyrtobagous singularis* Hustache, was released in Botswana and Zambia in the 1970s with little success (Julien & Griffiths 1998). The moth *Samea multiplicalis* (Guenée) (Pyralidae) was released on Lake Kariba in 1970, but did not establish (Mitchell & Rose 1979). The grasshopper *Paulinia acuminata* (DeGeer) (Paulinidae) was released in Zimbabwe in 1969 and Zambia in 1970 (Julien & Griffiths 1998). This agent was damaging on *S. molesta* on Lake Kariba and by 1973 the area covered by the weed had been reduced to 5% and by 1980 *S. molesta* covered less than 1% of the dam, a situation which has been maintained since then (Mitchell & Rose 1979). Although there has been some dispute as to the efficacy of the grasshopper in controlling *S. molesta*, it certainly did play a role in the control of this weed on Lake Kariba, although this was most certainly in conjunction with a reduction in the nutrient status of the water body as the dam settled (Mitchell & Rose 1979).

Pistia stratiotes (water lettuce)

Pistia stratiotes is a rosette-type plant that floats freely on still or slow-moving water bodies. The undersides of the leaves contain spongy parenchyma and the leaves are covered with dense hairs on both surfaces. This plant reproduces through the formation of stolons and daughter plants (Forno & Julien 2000). The role of sexual reproduction is considered less important than the vegetative reproduction, although seed germination is an important factor in the dynamics of water lettuce populations (Dray & Center 1993). The negative impacts caused by this weed are the same as those for the other aquatic weed species, where dense mats limit all aspects of utilisation of rivers and dams.

Control

Once again, this weed was targeted for biological control as it was deemed the most suitable control option and the Brazilian weevil, *Neohydronomus affinis* (Hustache), which was previously incorrectly referred to as *Neohydronomus pulchellus* Hustache was introduced via Australia to a number of countries in Africa (Julien & Griffiths 1998). The larvae form extensive mines in the leaves, sometimes severing the leaf from the plant, while the adults chew characteristic round feeding holes in the leaves. Since the mid-1980s, this agent has been released in seven countries in Africa, where is has been very effective at controlling the weed.

Eichhornia crassipes (water hyacinth)

Eichhornia crassipes has been the most damaging weed in waterways in Africa. It has been present on this continent since it was first recorded in Egypt in the late 1800s (Gopal 1987) and South Africa since the early 1900s (Cilliers & Neser 1991). The impact of this weed on aquatic ecosystems has been staggering, and none more so than on Lake Victoria, East Africa. In the mid-1990s, the lake was infested by up to 20,000 ha of *E. crassipes*, which floated around in huge mats and infested inlets and fishing beaches along the shoreline (Moorhouse & Albright 2002). It was estimated that some 80% of the Ugandan shoreline was infested with a permanent fringe of the weed extending out to around 10 m (Ogwang & Molo 1999). The proliferation of water hyacinth on Lake Victoria has been linked to an increase in the eutrophication of the water due to changes in land-use practices in its catchment (Bright 1998). The dominant socio-economic activities around Lake Victoria and its catchment, which include agriculture, hydroelectric power generation, fisheries, lake transport, recreation and water supply for both domestic and industrial use, were heavily impacted by *E. crassipes*. Trade in and out of the ports and in turn the productivity of the three countries surrounding the lake (Kenya, Uganda and Tanzania) were seriously affected (Hill 1999b).

Control

Since the first introductions in Zambia in 1971, five arthropod species and one pathogen species have been released against *E. crassipes* in Africa, with varying levels of success (Julien & Griffiths 1998). However, of these, it has been two weevil species, *Neochetina eichhorniae* Warner and *N. bruchi* Hustache, that have been credited with most of the success in the biological control of this weed (Julien *et al.* 1999). The first introductions of these weevils were made to Lake Victoria in 1995. By 1998, there was a significant reduction in the extent of the weed on the Ugandan shoreline and by

December 1999, some 75% of the mats on the Kenyan side of the lake had sunk (Anon. 2000). Between February 1998 and February 2001, the percentage coverage of *E. crassipes* on Lake Victoria had been reduced from about 20,000 ha to below 2000 ha (Moorhouse & Albright 2002). There has been much speculation regarding the causes for this dramatic crash on Lake Victoria and some authors (e.g. Moorhouse & Albright 2002) have cited physical factors such as the *El Niño* phenomenon during which the water level in the lake rose some 3 m. While there is little doubt that the increase in wind and wave action would have promoted mortality of the *E. crassipes* plants, it was the action of the weevils, which broke up the tight structure of the mats, that allowed the increased turbulence on the lake to further destroy the plants.

The biological control of *E. crassipes* has also been highly successful in a number of other countries in Africa, notably Benin, where the biological control of this weed is estimated to have resulted in an increased in income of some US$ 30.5 million per year. This translates to a benefit to cost ratio of some 124:1 (De Groot *et al.* 2003). However, this study did not attempt to quantify the benefit to biodiversity due to reduction of the mats of water hyacinth.

South Africa has had an active biological control program on *E. crassipes* since the mid-1980s and has released the highest number of species against this weed (Hill & Cilliers 1999). However, the results from this country have been variable and ascribed to a temperate climate, characterised by cold winters, eutrophic water bodies and interference from other control actions such as herbicide application and manual removal of the mats (Hill & Olckers 2001). This has prompted several courses of action, including surveys for additional agents which might be better cold adapted, inundative releases of agents already released at the start of spring, implementing stricter water quality guidelines, and an attempt to better integrate alternative control options with biological control (Hill & Olckers 2001).

Discussion

The biological control of the five aquatic weeds, with few exceptions, has been highly successful in Africa, to the point where, provided the water body is correctly managed and biological control is implemented, none of these weeds should present problems. The success of these programs on this continent can be ascribed to several factors:

- the reliance on fundamental research performed on the target biocontrol agents in developed countries obviating the need to screen agents for host specificity in resource-poor countries, thereby increasing the speed with which new agents can be introduced
- the development of simple mass-rearing techniques, ensuring the release of high numbers of healthy insects
- standard post-release monitoring techniques allowing comparison between different control sites in different countries
- the involvement of dedicated individuals who understood the potential of biological control and who ensured that the projects progressed. This is probably the single most important factor, as without these individuals stationed in research institutes throughout Africa, the concept of biological control would not have been communicated to affected riverine communities and politicians would not have been convinced as to the potential of this technique. Further to the involvement of biocontrol researchers *in situ* in these countries, biocontrol scientists from research institutes in the developed world, with the aid of various funding agencies were pivotal in the transfer of appropriate technology
- community involvement was an important factor in the success of these projects, in particular for mass rearing and agent distribution.

The key to the success of any biocontrol program in Africa is that the technology that is transferred must be appropriate to the situation and that all programs must be flexible. No biological control program will succeed unless it has political support. This support has been engendered through the publicizing of success, where impacts can be observed at the landscape level and real benefits accrue to affected communities.

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Toxicity of herbicides and surfactants to three insect biological control agents for *Cytisus scoparius* (Scotch broom)

Kathrin Affeld, Katherine Hill, Lindsay A. Smith and Pauline Syrett[1]

Summary

Integrated weed management is disrupted if chemicals applied to suppress weeds harm insect biological control agents. Three herbicides and two surfactants for control of *Cytisus scoparius* (Scotch broom) were topically applied to *Arytainilla spartiophila* (broom psyllid), *Bruchidius villosus* (broom seed beetle), and *Leucoptera spartifoliella* (broom twig miner) at a range of concentrations. *Arytainilla spartiophila* was the most susceptible to all substances tested. Glyphosate was less toxic to *A. spartiophila* than triclopyr and picloram + triclopyr, but at a concentration equivalent to recommended field rate, caused significant mortality. *Leucoptera spartifoliella* adults were significantly more tolerant of herbicides, except triclopyr, than of the two surfactants. Combined with the surfactant polydimethylsiloxane, glyphosate caused significantly increased mortality to *A. spartiophila* and *L. spartifoliella*. All substances caused mortality in the test insects at concentrations below recommended field rates, except triclopyr applied to *B. villosus*, and surfactants were generally more toxic at lower concentrations. However, there was no significant difference between adult emergence from *L. spartifoliella* pupae topically sprayed with herbicides or glyphosate + surfactant and the controls. We conclude that surfactants harm all three insects, *A. spartiophila* is susceptible to all forms of chemical control, but *L. spartifoliella* and *B. villosus* are likely to tolerate glyphosate applied at field rate, without surfactant.

Keywords: *Cytisus scoparius*, herbicide toxicity, insect biological control agents, surfactant toxicity.

Introduction

Toxicity of herbicides and surfactants to insects used for weed biological control can disrupt integrated weed management programs. In New Zealand there is concern that newly-established biological control agents of *Cytisus scoparius* (L.) Link (Fabaceae) (Scotch broom) might be harmed by conventional chemical controls. *Cytisus scoparius* is a leguminous shrub native to western and central Europe that has become a serious invasive weed of forest, agricultural, and conservation land in New Zealand, Australia, and the USA, where it has been introduced (Hosking *et al.* 1998). The economic damage and threat to indigenous species have forced these countries to implement extensive control measures to manage *C. scoparius*, and herbicide application is the most commonly used method. However, the associated high costs, varying results, and potential risks that chemicals pose to the environment and non-target species have led to a change in management strategies with the focus on long-term control of *C. scoparius* using classical biological control (Syrett *et al.* 1999).

Three biological control agents are established for control of *C. scoparius* in New Zealand. The twig mining moth *Leucoptera spartifoliella* Hübner (Lyonetiidae) was accidentally introduced before 1950, while the broom seed beetle *Bruchidius villosus* F. (Chrysomelidae) and the psyllid *Arytainilla spartiophila* Förster (Psyllidae) are recent intentional introductions, first released in 1987 and 1993, respectively (Harman *et al.* 1996). The moth is now established throughout most of the country and causes severe damage to *C. scoparius* (Memmott *et al.* 1997). *Bruchidius villosus* has been released throughout New Zealand, and is confirmed as established at a number of

[1] Landcare Research, P.O. Box 69, Lincoln 8152, New Zealand
Corresponding author: P. Syrett, Landcare Research, P.O. Box 69, Lincoln 8152, New Zealand. rockview@actrix.co.nz

sites (Syrett *et al.* 2000) where it destroys a substantial number of *C. scoparius* seeds. *Arytainilla spartiophila* is also widely established, but more time is needed to assess its impact (Syrett *et al.* 1999). Because biological control is unlikely to completely suppress *C. scoparius* below required thresholds, especially in the short term, herbicides will continue to be used for its control. So, for a successful, integrated management program for the weed, it is desirable that a spray regime cause minimal disruption to biological control agents.

In 1999 a survey was conducted to identify the herbicides and surfactants most commonly used for *C. scoparius* control by farmers and spraying contractors in North Canterbury, New Zealand (M. Kilvington unpublished data). Active ingredients of the most commonly used herbicides are picloram + triclopyr (Tordon® brushkiller), triclopyr (Grazon®), glyphosate 360 (Roundup®), and glyphosate in granular form (Trounce™). The active ingredients of two of the most commonly used surfactants (Pulse® and Boost®) are polydimethylsiloxane and dimethicone copolyol, respectively.

Previous studies examining the effects of these herbicides on insects have shown that some herbicides at low concentrations are compatible with biological control programs. Glyphosate was relatively safe to *Eccritotarsus catarinensis* (Carvahlo) (water hyacinth mirid) (C. Ueckermann & M.P. Hill unpublished data) and was considered harmless to two aphelinid parasitoids (Teran *et al.* 1993) and the water hyacinth weevil *Neochetina eichhorniae* (Ding *et al.* 1998, C. Ueckermann & M.P. Hill unpublished data). Triclopyr amine was safe to the chrysomelid beetle *Galerucella calmariensis* (Lindgren *et al.* 1997). Picloram + triclopyr was found to have no effects on butterfly populations (Bramble *et al.* 1997), whereas R. Adams (unpublished data) reported low mortality to the chrysomelid beetle *Longitarus jacobaeae* (Waterhouse) under indirect application outdoors. This indicates that some insect groups might be more susceptible to herbicides than others. However, there has been concern over the possible toxicity of surfactants when they are used with herbicides. Oil-based surfactants were found to cause high mortality in silverleaf whitefly (Sieburth *et al.* 1998) and to act on the tracheal system of mosquito larvae (Corbet *et al.* 2000). Goodwin & McBrydie (2000) examined the effect of polydimethylsiloxane and dimethicone copolyol on honeybees in the laboratory and observed high mortality at concentrations as low as 10% of the recommended application rate.

We aimed to test the effects of different concentrations of herbicides and surfactants commonly used for control of *C. scoparius* in New Zealand on adult *A. spartiophila*, *B. villosus*, and *L. spartifoliella* moths and pupae. We sought to determine which active ingredients were responsible for any observed effects.

Materials and methods

Renovate® (triclopyr), Roundup® (glyphosate), Tordon® Brushkiller (picloram+triclopyr), Pulse® Penetrant (polydimethylsiloxane) and Boost® (dimethicone copolyol), commonly used products for controlling *C. scoparius* (M. Kilvington unpublished data), were chosen for this study. *Arytainilla spartiophila* and *B. villosus* adults were beaten from *C. scoparius* growing at the Landcare Research facilities at Lincoln, Canterbury immediately prior to each trial. *Leucoptera spartifoliella* adults were collected as pupae. *Cytisus scoparius* branches infested with *L. spartifoliella* cocoons were cut from plants at a site near Burnham, Canterbury and transferred to the laboratory for eclosion. Pupae attached to pieces of twig were placed in clear, ventilated, acrylic boxes with close-fitting lids until adults emerged. Newly emerged adults were harvested prior to each trial.

Insects were exposed to herbicides and surfactants at concentrations below, at, and above their recommended rates for application with a spray gun or knapsack (Table 1). In some instances, substances were not tested at concentrations below the recommended field rate because of no, or very low, mortality at field rate. Fifteen insects were individually sprayed, each in a single dish, with several concentrations of the test substance and with water. The lid and base (lined with a sheet of filter paper) of 85-mm-diameter Petri dishes were each sprayed with 3 ml of the test substance using a Burkart® "Potter Tower". Water was applied first, followed by the lowest, and then increasingly higher concentrations of the solutions. Lids were sprayed first, to allow the herbicide/surfactant to dry before they were placed over the base containing the sprayed test insect, thereby reducing the risk of insects sticking to droplets on the plastic surface. After each treatment, the dish containing an individual insect was replaced with a clean one. Each group of insects sprayed with the same test substance was placed in a separate plastic bag to avoid contamination and kept in a controlled environment rearing room under 16:8 h light/dark, 21.6:12.8°C day/night temperature and 64% relative humidity.

Adult trials

At the start of each trial, unsexed insects were placed in a cool room for 10–20 min to reduce their activity before transferring to individual Petri dishes. Immediately before spraying, moths and psyllids were cooled by placing in a freezer for 2–5 min (6–7 dishes at a time) to ensure they did not escape from the Petri dish while being sprayed. This was not necessary for the beetles. All individuals were checked to see that they were alive immediately before and after spraying. All dishes were misted with water the morning following the application to prevent insects drying out. Insects were monitored 12, 24 and 48 h after spraying. Dead individuals were removed and numbers recorded. The Petri dishes containing *B. villosus* were covered with a clear acrylic sheet to prevent the beetles from escaping.

Table 1. 48-h mortality from herbicides and surfactants applied topically to adults of *Arytainilla spartiophila*, *Bruchidius villosus*, and *Leucoptera spartifoliella*, and *L. spartifoliella* cocoons at a range of concentrations. "Field rate" shaded.

Chemical	Concentration (ml/litre)	48-h mortality (%)			
		Arytainilla spartiophila	*Bruchidius villosus*	*Leucoptera spartifoliella*	*L. spartifoliella* cocoons
Glyphosate	Control	6.7	0	0	27
	1.925	33			
	3.75	40			
	7.5	87			
	10	60	6.7	0	13
	15	93			
	100	100	20	0	27
	300	93	53	33	27
Polydimethylsiloxane (S1)	Control	13	0	0	
	0.01	20			
	0.1	100		27	
	0.2	93			
	0.3	100			
	0.4	100			
	0.5	100		40	
	1	100		60	
	2	100	13	80	
	20			93	
Glyphosate + S1	Control	6.7	0	0	6.7
	S1 + water	100	13	87	60
	5	100		60	40
	10	100	27	87	0
	100		6.7	67	27
	300		0	60	
Triclopyr	Control	6.7	0	0	
	0.6	6.7	0	0	
	1.5	73	0	6.7	
	3	100	0	40	
	30	100	13	100	
Picloram + triclopyr	Control	6.7	0	0	27
	0.75	20			
	1.5	47			
	2.5				20
	3	67			
	6	93			33
	12	100	6.7	13	13
	25				47
	120	100	13	100	
	300		73		
Dimethicone copolyol	Control	13	0	0	
	0.01	13	0		
	0.1	93	0	33	
	0.5	93	13	73	
	1	100	27	60	
	10		93	100	

Cocoon trials

Cut pieces of *C. scoparius* stem, 20–30 mm long, with one live *L. spartifoliella* cocoon attached, were placed into individual Petri dishes, with the cocoons on the upper surface, before being positioned into the "Potter Tower". All dishes were misted with water the morning following the application, and once or twice weekly to prevent the cocoons from drying out. Cocoons were monitored twice daily for 14 days and then once or twice weekly for a further 21 days before being dissected. Hatched adults were removed and numbers recorded.

Statistical analysis

Adult mortality was compared using the Pearson chi-square test between insect species and herbicides. For 2×2 tables where the expected values were < 5, Fishers exact test (FET) (two-tail) was used. Test results with a *P*-value <0.05 were considered statistically significant. The effects of increasing concentrations on mortality were tested by comparing mortality at field rate with that at 10 times the concentration or, where this was not available, with half the field rate. Probit analysis using the POLO package (Russell *et al.* 1977) was used to determine the concentrations that killed 50% of insects (LD_{50}s) and their 95% confidence intervals.

Results

The three insects responded differently to the various formulations when tested at the recommended field rates (Fig. 1). *Arytainilla spartiophila* was most susceptible, and *B. villosus* least susceptible, to all of the formulations tested. The mean mortality for the control groups of *A. spartiophila* was 13.3% and for *L. spartifoliella* adults and *B. villosus* was 0%. Glyphosate caused lower mortality in *A. spartiophila* than did any of the other substances, while the other herbicides and the surfactants were highly lethal to them. The addition of the surfactant polydimethylsiloxane to glyphosate caused higher mortality than glyphosate alone: the difference was significant for *L. spartifoliella*. Glyphosate and picloram + triclopyr was less harmful to *L. spartifoliella* than were the other formulations including the two surfactants. However, these were equally harmful (FET $P = 0.31$). There was no significant difference in mortality caused by polydimethylsiloxane alone or in combination with glyphosate (FET $P = 0.62$). All formulations tested had similar effects on *B. villosus*, with greater than 60% survival.

Increasing the concentration of chemicals increased *A. spartiophila* mortality, except for glyphosate + polydimethylsiloxane, which caused 100% mortality at the lowest concentration tested (half the field rate) (Table 1). All chemicals were harmful to *A. spartiophila* at concentrations below the recommended field rate, but the two surfactants caused higher mortality and at lower concentrations than any of the three herbicides. *Arytainilla spartiophila* mortality from herbicides and surfactants applied at field rate was not significantly different from that at concentrations above field rate ($P = 0.99$) because a 100% kill rate resulted from applications at and below field rate.

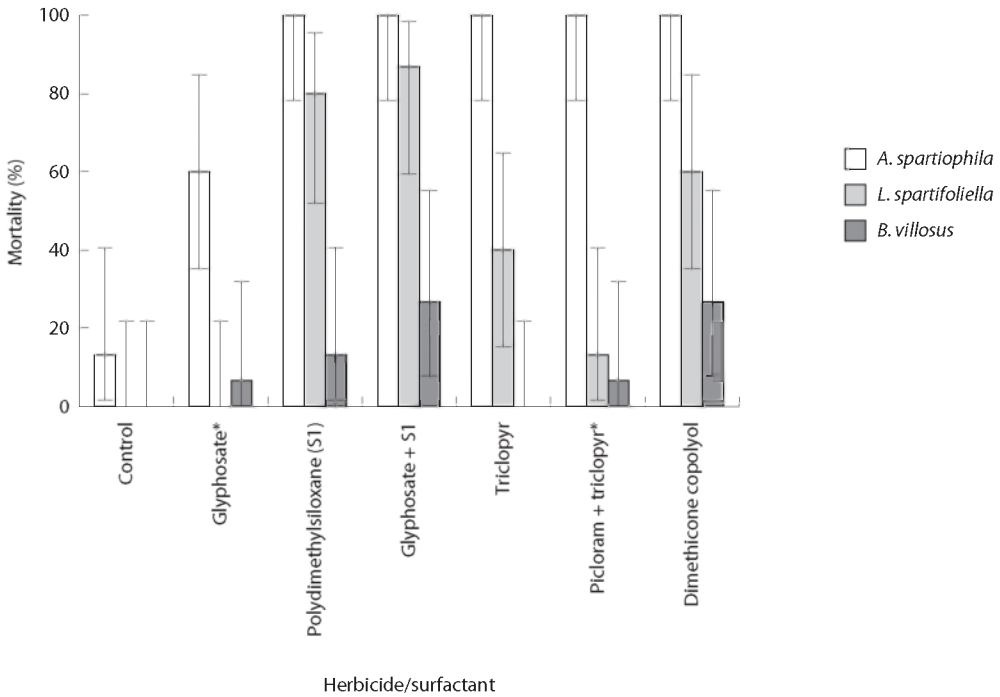

Figure 1. Mortality (%) (95% C.I.) of adult Arytainilla spartiophila, Leucoptera spartifoliella and Bruchidius villosus 48 h after exposure to a range of herbicides and surfactants at "field rate".

At concentrations below the field rate that were tested with *B. villosus*, dimethicone copolyol caused mortality, but triclopyr had no effect (Table 1). Mortality increased with increase in concentration for most formulations, but decreased for glyphosate + polydimethylsiloxane application. Dimethicone copolyol was most toxic to the beetles, while triclopyr caused the least harm of the three herbicides.

Leucoptera spartifoliella mortality increased with increased concentration except for glyphosate + polydimethylsiloxane (Table 1). The two surfactants were the most harmful substances and equally lethal, while triclopyr caused least harm below field rate. Increased mortality, related to an increase in concentration, caused by dimethicone copolyol and triclopyr above the field rate was significant (FET $P = 0.017$ and FET $P = 0.001$, respectively). Results of tests with *L. spartifoliella* pupae were variable, and often not significantly different from controls, which had a high mean mortality of 20%. The proportion of *L. spartifoliella* pupae that died after application of picloram + triclopyr at field rate was lower (FET $P = 0.047$) than at twice this concentration.

The lowest LD_{50}s concentrations were estimated for glyphosate, triclopyr and picloram + triclopyr for *A. spartiophila*, polydimethylsiloxane for *L. spartifoliella*, and dimethicone copolyol for *B. villosus*. They were below the recommended field rates for both *A. spartiophila* and *L. spartifoliella*, but above for *B. villosus* (Table 2).

Discussion

Most herbicides are thought to have low direct toxicity to insects because their active ingredient has been specifically selected to act on systems found only in plants, e.g. photosynthesis inhibitors. Because surfactants act by reducing the surface tension of herbicides, and in some cases dissolving the waxy cuticle of plants, they may be more harmful to insects than herbicides. They may reduce the protection provided by the insects' waxy cuticle making them more prone to dehydration and chilling, and block their spiracles, thus interfering with gas exchange. Results reported here indicate that surfactants, and to a lesser extent some herbicides, that are commonly used for control of *C. scoparius* in New Zealand, do harm insects (Fig. 1). However, the three insect species tested (representing three different insect orders: Hemiptera, Coleoptera and Lepidoptera) showed markedly different levels of susceptibility. *Arytainilla spartiophila* (Hemiptera) was the most susceptible, while *B. villosus* (Coleoptera) was least affected. This result is in agreement with the finding of C. Ueckermann & M.P. Hill (unpublished data) who reported higher susceptibility to herbicides of *E. catarinensis* (Hemiptera) compared with *N. eichhorniae* (Coleoptera). *Leucoptera spartifoliella* (Lepidoptera), showed an intermediate level of susceptibility in results reported here.

The results also indicate that surfactants are more toxic than herbicides, and that the combination of glyphosate and polydimethylsiloxane is more toxic than the herbicide alone (Fig. 1). This is consistent with findings of Goodwin & McBrydie (2000) who showed that the surfactants polydimethylsiloxane and dimethicone copolyol at concentrations of 0.02% and 0.01%, respectively, and higher, were highly toxic to honeybees, and Sieburth *et al.* (1998) who showed that oil-based surfactants caused high mortality in silverleaf whitefly. Studies by Wright & Skilling (1987), who tested the herbicides dichlorprop and 2,4-D with and without the surfactants AF302® and Chem 100®, respectively, found that the surfactants increased the toxicity of the herbicide to *N. eichhorniae*.

For all three insects, there was a general increase in mortality with an increase in concentration of the herbicides and surfactants tested except that mortality in *L. spartifoliella* and *B. villosus* decreased when sprayed with increasing concentrations of a combination of glyphosate and polydimethylsiloxane. A possible explanation for this apparently anomalous finding is that the surfactant (at the recommended field rate) may becomes less effective in reducing the surface tension as the herbicide concentration increases. Tests with *L. spartifoliella* pupae showed that glyphosate, glyphosate + polydimethylsiloxane and picloram + triclopyr had no significant impact on the emergence of adults from cocoons when applied at field rate. The cocoons presumably provide effective protection against the herbicides and the observed mortality is likely to be the result of natural causes rather than the treatment as it was not significantly different from the control.

Where LD_{50}s could be estimated, they were considerably lower than the recommended field rate, with the exception of dimethicone copolyol for *B. villosus* (Table 2), confirming that all substances tested with *A.*

Table 2. LD_{50}s for *Arytainilla spartiophila, Leucoptera spartifoliella* and *Bruchidius villosus* for a range of herbicides and surfactants tested.

Insect	Herbicide/surfactant	Field rate (ml/l)	LD_{50}s (ml/l)	95% confidence interval
A. spartiophila	Glyphosate	10	3.4	2.1 – 4.9
	Triclopyr	3	1.1	0.87 – 1.4
	Picloram+triclopyr	12	1.7	1.1 – 2.4
L. spartifoliella	Polydimethylsiloxane	2	0.52	0.19 – 1.1
B. villosus	Dimethicone copolyol	1	1.9	1.1 – 3.8

spartiophila, and polydimethylsiloxane with *L. spartifoliella*, would be unsafe for these biological control agents if applied at field rate.

The herbicide glyphosate was the least toxic substance tested (Fig. 1), and would therefore be the most compatible with biological control of *C. scoparius* if used without surfactant. Even so, *A. spartiophila* is likely to be adversely affected. As the psyllids spend a large proportion of their life cycle in immobile egg and larval stages in *C. scoparius* stems, they are likely to be seriously affected if their host is killed through spraying during this period. Survival of adult *A. spartiophila* from glyphosate application was 40%. Although control mortality was high for *L. spartifoliella* cocoons tested, indications are that they are relatively resistant to treatment compared to adult moths, and that adult moths might successfully emerge from herbicide-treated *C. scoparius*. *Bruchidius villosus* is more resistant to all the substances tested, and provided their host plant is sprayed during the period when the beetles are in the mobile, adult stage, it is likely that most spray regimes would be compatible with their survival. However, sub-lethal effects of chemicals have not been considered.

From these preliminary results, we conclude that it is possible to devise a herbicide regime that would be compatible with preservation of insect biological control agents. Of course, this is relevant only if some reservoir of *C. scoparius* remains in the area, as otherwise the insects will die through lack of available food plants. Insect survival will be enhanced if herbicides are used without surfactants. Future work should measure LD_{50}s of commonly applied herbicides and surfactants for all insect biological control agents for *C. scoparius*, and identify at what times of year each insect species is in a mobile stage. Thence an optimal time for herbicide application could be determined that would effectively suppress *C. scoparius* with minimal disruption to insect activity.

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Community involvement in the distribution of the biological control agents for bridal creeper, *Asparagus asparagoides*

Kathryn L. Batchelor,[1] Louise Morin,[2] Anthony Swirepik[2] and Tim L. Woodburn[1]

Summary

Asparagus asparagoides (bridal creeper) is a widespread weed of bushland and remnant vegetation across southern Australia. It is recognized as a major threat to biodiversity in those habitats and is one of Australia's Weeds of National Significance. The bridal creeper leafhopper *Zygina* sp. and rust fungus *Puccinia myrsiphylli* were released in Australia in 1999 and 2000, respectively. Damage caused by both agents is obvious; the leafhopper's silver zigzag spotting and the rust's orange pustules on foliage are readily recognizable. These features of the agents' biology made them ideal candidates for rearing and distribution by non-specialists. The leafhopper can be reared by school and community groups as it requires little more than a cage and healthy bridal creeper plants. The rust fungus is easy to distribute from infected foliage, following a basic protocol. The mechanisms and infrastructure required to involve community members in distribution of each agent are outlined.

Keywords: *Asparagus asparagoides*, bridal creeper, community involvement, *Puccinia myrsiphylli, Zygina* sp.

Introduction

Asparagus asparagoides (L.) Druce (bridal creeper), is an exotic weed that poses a major threat to biodiversity and conservation in Australia's temperate natural ecosystems. Originally introduced as a garden plant in the 1850s, it became naturalized in the early 1900s and is now listed as a Weed of National Significance. In 1991, surveys for biological control agents in the weed's native range, South Africa, identified several potential agents. Three agents have since been approved for release in Australia following extensive studies on their host range: the leafhopper *Zygina* sp. (Batchelor & Woodburn 2002a), rust fungus *Puccinia myrsiphylli* (Thuem.) Wint. (Morin *et al.* 2002) and leaf beetle, *Crioceris* sp. (Batchelor & Woodburn 2002b), in 1999, 2000 and 2002 respectively.

Until biological control was implemented, bridal creeper was managed through hand weeding and herbicide application. As bridal creeper fruits are dispersed by birds (Raymond 1996; Stansbury 2001), it has the potential to infest pristine ecosystems as well as reinvade weeded areas, increasing the frustration and apathy amongst land managers trying to control this weed. When the leafhopper and rust fungus were approved for release, suitable release sites were sought using Landcare/Bushcare information networks. The response was immediate and overwhelmingly positive. Rearing facilities at the time were not prepared or funded for a nationwide redistribution program, hence it was decided to involve community groups in the rearing and redistribution of the agents. The simple biology of the leafhopper and its ability to readily establish indicated that it could be reared and released by non-specialists (Batchelor & Woodburn 2002c). Whilst the rust has a complicated lifecycle and requires specific environmental conditions to establish, community members could release the rust by following a set of instructions.

[1] CSIRO Entomology, CRC for Australian Weed Management, Private Bag 5, PO Wembly, Western Australia 6913, Australia.

[2] CSIRO Entomology, CRC for Australian Weed Management, GPO Box 1700, Canberra, Australian Capital Territory 2601, Australia.

Corresponding author: Kathryn Batchelor, CSIRO Entomology, CRC for Australian Weed Management, Private Bag 5, PO Wembly, Western Australia 6913, Australia. e-mail kathryn.batchelor@csiro.au

Both agents damage bridal creeper by attacking the cladodes. The leafhoppers feed on mesophyll cells and their damage is seen as white spotting on the leaf surface (Witt & Edwards 2000). The rust fungus infects stems and cladodes of bridal creeper and is easily recognizable as yellow lesions on the upper side of the cladode and by corresponding orange pustules on the underside. Severe infestations of both agents result in reduced photosynthesis, premature defoliation and reduced tuber production (Batchelor & Woodburn 2002a; Morin *et al.* 2002).

Participation by community groups in the redistribution of biological control agents is recognized as playing an important part in technology transfer (Briese & McLaren 1997). This paper provides a summary of the techniques especially developed for community and school groups for rearing and releasing the leafhoppers and the rust fungus. It outlines the approaches taken to teach, engage and disseminate knowledge to interested groups and the development of monitoring protocols for such groups to measure the spread of agents and impact of their control techniques. The benefits of community involvement in the biological control of bridal creeper are discussed.

Material and methods

Rearing and release techniques

Rearing of leafhoppers

Batchelor and Woodburn (2002c) outline in detail the technique developed for community/school groups to rear leafhoppers. In summary, rearing leafhoppers requires a stock of healthy bridal creeper plants, a colony of leafhoppers and a rearing cage to prevent adults from escaping. Participating groups established a nursery of 100–200 potted bridal creeper plants to ensure a steady stream of plants for rearing. They then prepared a rearing cage, usually comprised of an old aquarium with a fine mesh cover pegged to the top, and filled it with bridal creeper plants. When plants had sufficient foliage, a colony of leafhoppers, consisting of adults caged on bridal creeper plants plus three-four plants hosting eggs and nymphs, was posted or delivered by the project staff and introduced to the cage. The leafhoppers fed and oviposited into bridal creeper leaves within the cage. Every few weeks or when the plants were 50% white from leafhopper feeding damage, the foliage was rustled to shake off adults and the plants removed and replaced with fresh stock plants.

Release of leafhoppers

Plants infested with leafhopper eggs and nymphs were taken to a bridal creeper field infestation and placed amongst the foliage for a release. The eggs on the damaged plants eventually hatched and nymphs moved on to cladodes at the local infestation. After six weeks the potted plants were usually collected from the field site and returned to the nursery.

Release of rust fungus

The release process involves transferring spores from infected foliage to the underside of the plant foliage in the field and maintaining a high humidity for up to a day to facilitate spore germination and hence fungal infection of plants (Morin *et al.* 2002). To enable community members to infect bridal creeper with the rust fungus, a practical and simple field protocol comprising of five steps was devised:

Materials: Plastic sheet, pegs, spray bottle containing water, bridal creeper with erupting rust pustules, surveyors flagging tape.

Protocol:

1. Find a bridal creeper clump, preferably a "column", about 0.5m from the ground out of direct sunlight.
2. Rustle the rust-infected foliage vigorously in an upward motion through the field foliage. This will dislodge rust spores ensuring most land on the underside of the cladodes of field plants.
3. Mist the foliage with water using a spray bottle. Rust requires moisture for spore germination and infection.
4. Wrap the "column" of bridal creeper with a plastic sheet (secured with pegs or tape) for 24 hours to ensure high leaf humidity and ensure a good level of infection.
5. Remove plastic sheet and mark the release site with fluorescent flagging tape. First signs of rust symptoms will be visible after 2–3 weeks.

The rust fungus was supplied to community members on either dry-rooted bridal creeper plants or as field harvested foliage. Once the rust fungus had established at a field site, infected foliage could then be harvested and used to infect another site using the method described above. The rust fungus can usually be found in the field from April to October, depending on autumn temperatures and rainfall.

Monitoring protocols

Two types of monitoring protocols were developed and implemented by the project, the first focusing on agent establishment and spread, the second on medium to long term vegetation change at bridal creeper control sites. To gain a broad indication of agent performance, community groups involved in releasing the agents were asked to monitor their establishment and spread from release sites. An accurate indication of the establishment of the leafhopper proved difficult to gain through community groups as small populations of the insect are difficult for the untrained eye to detect. Conversely, community groups could reliably identify the rust fungus due to its greater rate of increase and more obvious symptoms. The second form of monitoring was focused at community groups who had received funding from the Natural Heritage Trust for

bridal creeper management. A performance indicator of their projects was to measure the impact of their control activities (whether biological control or herbicide). A suitable protocol was thus developed in collaboration with a national steering committee for bridal creeper management, which includes representatives from all states of temperate Australia. The protocol involves recording vegetation types and their percentage cover along a series of permanent transects. Repeated measurements over time, should demonstrate whether or not bridal creeper is declining as a result of the management technique implemented, and if native plants, or other weeds, are subsequently increasing.

Dissemination and collection of information

Workshops/field days

Between 1999 and 2002, a series of "train the trainer" workshops was conducted in Western Australia, South Australia and New South Wales to demonstrate how to rear and release the leafhopper and release the rust fungus (Woodburn *et al.* 2002). Workshops were advertised through landcare networks, natural resource and agricultural officers in state and local government, teachers and community Landcare representatives. The workshop program provided information on: bridal creeper biology; principles of biological control; the biology of each agent; rearing and release techniques; record keeping; finding and monitoring agents after release; and redistribution of the agents. Workshops were generally conducted adjacent to a bridal creeper infestation to enable practical demonstrations of the release techniques.

Brochure, website and promotion

A brochure and website, www.ento.csiro.au/bridalcreeper, was created to enable community and school representatives unable to attend the workshops to participate in the project. The website outlines: the biology of each agent; a step by step guide to rearing and releasing the leafhoppers; a technique for releasing the rust; agent release site locations; and the monitoring protocols. As many collaborators do not have internet access, some of this information was published in a fold-out brochure outlining, in different sections, the lifecycle of the plant, leafhopper and rust, release techniques and who to contact for more information and/or an initial supply of agents. An additional brochure outlining the processes involved in implementing a biological control program was produced to address community concerns over host-specificity of agents. The project was widely publicised in the media, especially in regional/community print and radio. Community and regional media often feature stories on public-good activities, especially those that involve school children. The project was featured on national television as well as in several national radio and print media.

Record collection

When a release of either agent was made, school and community groups were requested to provide information about where and when releases had taken place (Batchelor & Woodburn, 2002c). A "release details" form was supplied with agents and was also available as a download from the bridal creeper website. Returned forms were incorporated into a central database and published online on the bridal creeper website. In July 2002, a letter was sent to all participants encouraging the return of release details forms.

Results and discussion

Involving community groups and schools in a biological control program is an effective method to increase the number of release sites. By the end of 2002, within three years of the first release, the leafhopper and rust fungus had each been released at over 700 locations across southern Australia. The number is likely to be far greater as release site details were supplied by only approximately one third of the people involved in a release, and communities are likely to have redistributed the agents from established sites without providing details to the central database. Over 100 primary schools and community groups have been involved in rearing leafhoppers, contributing to at least 450 release site locations.

Rearing leafhoppers was relatively easy for most schools and community groups, but occasionally the leafhopper colonies took either a long time or failed to establish (Batchelor & Woodburn, 2002c). This was mostly a problem for groups rearing leafhoppers on plants suffering from transplant shock. On the whole, community members had no difficulty releasing the rust fungus in the field following the 5-step protocol. Project staff strongly emphasized the importance of misting the foliage and wrapping with plastic as missing these steps generally resulted in no infection. It is possible to infect foliage with the rust fungus without following the entire protocol, but only on cool rainy days when high humidity persists for at least 8 hours (Morin, unpublished). It was generally advised that if the weather was not predictable over this time period, misting and plastic wrapping for 24 hours was essential.

Involving the community in this project was also extremely effective as a vehicle to communicate the impact of bridal creeper on bushland and raise the profile of other environmental weeds (Batchelor & Woodburn 2002c). The leafhopper and rust fungus became a valuable educational tool, especially in schools looking for practical assignments to complement weed education lessons. Communities benefited by being able to apply a sustainable weed control technique that had no negative impact on surrounding vegetation. However, community involvement is not appropriate for all biological control programs, especially if rearing is involved. Communities working with agents that are difficult to rear and establish are likely to become disappointed with the process

and have less enthusiasm to continue (Briese & McLaren 1997). Ideal agents for community rearing should be those with a proven ability to establish readily, have a simple lifecycle with multiple generations/year, high fecundity, an exposed juvenile or spore stage and show visible signs of damage throughout development. Both the leafhopper and rust fungus meet these criteria. The weed itself should be easy to propagate and handle. An example of an agent unsuitable for community rearing is the bridal creeper leaf beetle, *Crioceris* sp., as it has one to two generations/year, and consumes only young, expanding cladodes and shoots. Adults lay eggs only on shooting tips and both the adults and larvae are difficult to handle.

Project staff found that most community groups and school teachers wanting to be involved had limited knowledge of biology and needed considerable help initially to understand the biology and release techniques for the leafhopper and rust fungus. It was therefore found just as essential to first teach the processes of biological control in order for community groups to understand that it is a long-term weed control strategy and that the agents are host-specific. Biological control practitioners considering involving the community in their programs are advised to prepare extensive supporting materials to help community groups understand these concepts.

Obtaining feedback on new releases or spread from release sites was a weakness in the project. All collaborators were encouraged to redistribute from established sites, but many failed to return release details forms, despite being reminded. Although redistribution increases the speed at which the agents reach weed infestations, it reduces the ability for project staff to keep a complete record of releases and monitor the natural spread of each agent. However, those that returned release details forms proved reliable to help study the spread and disease intensity of the rust fungus from the release sites. In 2002, establishment and spread data on the rust fungus for 56 sites across southern Australia were forwarded to the researchers. However, it is unrealistic to expect community groups to participate in the long-term monitoring of agent activities. Community groups are fluid entities and as members and priorities change it will be difficult to maintain consistency in data collection over time.

However, some community groups, such as those that received funding for bridal creeper management, are interested in determining the effectiveness of their management technique and are therefore likely to participate in longer term monitoring activities.

To date, the biological control project for bridal creeper has greatly benefited from the involvement of community groups and schools, and vice versa. The project has introduced these groups to the damage invasive plant species can cause to bushlands, particularly why some introduced plants become weeds. The project's media exposure in the wider community raised the profile of bridal creeper and biological control as an environmentally-friendly approach for weed management. As a whole, this project will be a useful case study for others who may be interested in involving the community in biological control programs for other weeds.

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Release strategies for the establishment of the leaf spot pathogen, *Mycovellosiella lantanae* var. *lantanae*, on *Lantana camara* in South Africa

Alana Den Breeÿen[1]

Summary

Lantana camara is a poisonous shrub from South and Central America that has invaded much of the moist, warm subtropical areas of South Africa. In the past decade evidence of a conspicuous and damaging mycobiota on *L. camara* in the Neotropics has persuaded researchers to consider fungi as potential biocontrol agents for this plant. Preliminary pathogenicity testing of several fungi isolated from diseased *L. camara* leaves collected during field surveys in South, North and Central America from 1987 to 1997 showed the leaf spot fungus *Mycovellosiella lantanae* var. *lantanae* to be a promising biocontrol agent against *L. camara* biotypes in South Africa. Results from host-specificity tests indicated a very restricted host range, making this pathogen a suitable candidate for use as a classical biological control agent. Permission to release *M. lantanae* var. *lantanae* in South Africa was granted in September 2001. Release strategies include the use of a combination of isolates to target a wide range of *L. camara* biotypes in the field, and releases under different environmental conditions ranging from tropical and subtropical to mediterranean in the KwaZulu-Natal and the Eastern Cape provinces. The impact of the agent on the growth rates and fecundity of individual plants, and on populations over time, will be monitored.

Keywords: *Lantana camara*, *Mycovellosiella lantanae* var. *lantanae*, release strategies.

Introduction

Lantana camara L. (lantana; Verbenaceae), originating from South and Central America (Holm *et al.* 1977), is a cosmopolitan weed in the tropical and subtropical regions of the world. In South Africa it is presently naturalised in the subtropical and temperate regions of the Northern, Gauteng, Mpumalanga and KwaZulu-Natal provinces, as well as the southern coastal regions of the Eastern and Western Cape provinces (Fig. 1) (Stirton 1977). *Lantana camara* is a poisonous, but highly decorative garden plant, ranging in size from a compact shrub (< 1m high) to an untidy scrambler (≥ 3 or more metres high). However, it reduces the biodiversity of natural ecosystems, interrupts the regeneration processes through allelopathic suppression of indigenous plant species (Gentle & Duggin 1997) and rapidly invades disturbed areas, including areas cleared of other invasive weeds.

Declared a weed in South Africa in the early 1940s, *L. camara* has been targeted for classical biological control in South Africa since the 1960s. Cilliers & Neser (1991) and Baars & Neser (1999) reviewed the biological control program initiatives undertaken on *L. camara* in South Africa covering the period 1960 to 1999. Despite these efforts, biological control of the weed has had limited success. One of the main reasons for this is the genetic diversity of *L. camara*, which presents the natural enemies with several morphological and physiological barriers to utilisation (Cilliers 1983, Cilliers & Neser 1991, Baars & Neser 1999).

Mycovellosiella lantanae (Chupp) Deighton var. *lantanae* was selected as a potential biocontrol agent against *L. camara* in South Africa based on the research and field evidence of Evans (1987), Barretto *et al.*

[1] ARC-PPRI, Weeds Research Division – Weeds Pathology, P/Bag X5017, Stellenbosch, 7599 South Africa <vredadb@plant3.agric.za>.

(1995) and M.J. Morris (pers. comm.). These authors undertook several field surveys to South, North and Central America from 1987 to 1997 to collect pathogens to test for potential as biocontrol agents on *L. camara* biotypes from South Africa (Fig. 2). Results indicated that *M. lantanae* var. *lantanae* was pathogenic on several South African *L. camara* biotypes and had a very restricted host range, making this pathogen a suitable candidate for use as a classical biological control agent (Den Breeÿen & Morris 2003). It is intended for release as a classical biocontrol agent because, according to Barretto *et al.* (1995) and the author's observations, all the isolates grow very slowly and sporulate irregularly, making it unsuitable for mycoherbicidal development.

Permission to release *M. lantanae* var. *lantanae* in South Africa was granted in September 2001. Releases were carried out in the KwaZulu-Natal (KZN) and Eastern Cape provinces. This paper reports on the release strategies for the establishment of *M. lantanae* var. *lantanae*. These include: i) the use of a combination of isolates in order to target a wide range of *L. camara* biotypes in the field; ii) two different inoculation methods, namely an oil-based spore suspension and an aqueous spore suspension; and iii) the initial monitoring of the first release sites in KZN.

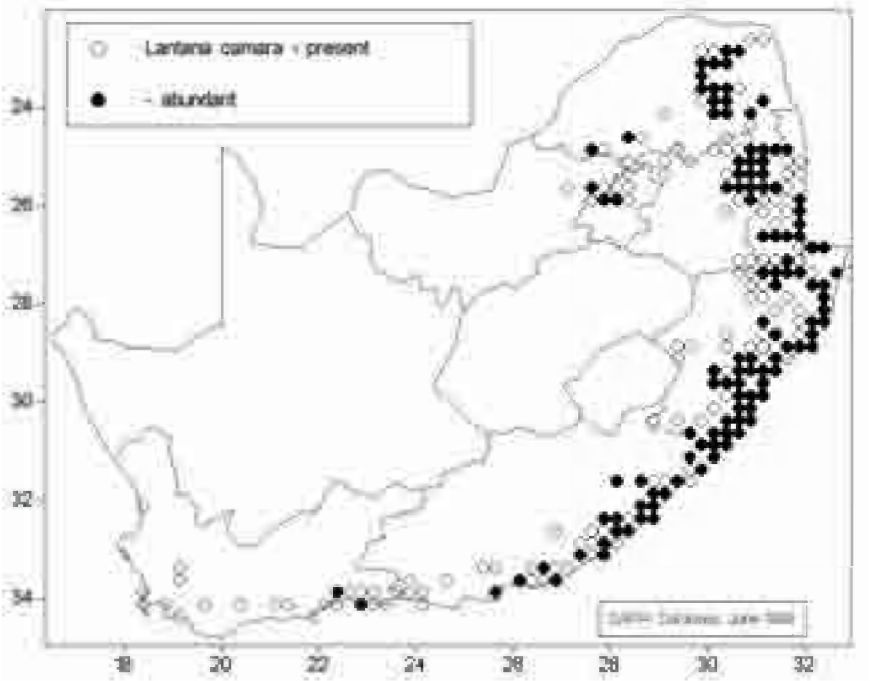

Figure 1. Distribution of *Lantana camara* throughout South Africa (photograph courtesy of Lesley Henderson, SAPIA database)

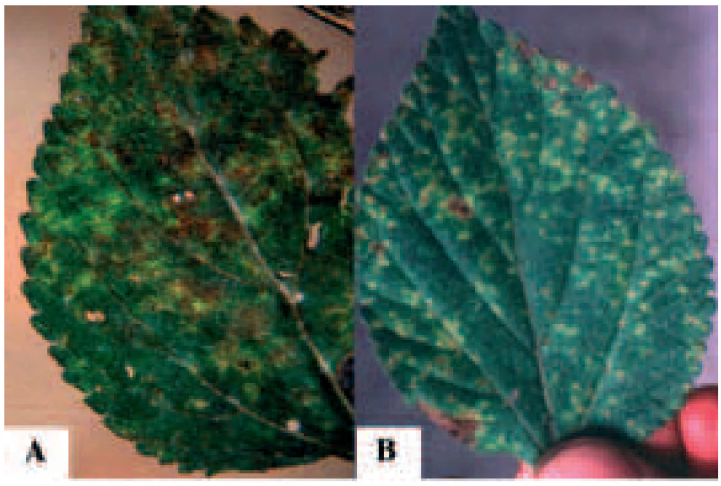

Figure 2. Symptoms of *Mycovellosiella lantanae* var. *lantanae* on naturally infected *Lantana camara* in (A) South America and (B) Florida, USA.

Materials and methods

Due to the variation in virulence of *M. lantanae* var. *lantanae* when tested for pathogenicity on South African *L. camara* biotypes (Den Breeÿen & Morris 2003), a combination of three isolates, C442, C470 and C493, was applied to target a wider range of biotypes in the field. Production of *M. lantanae* var. *lantanae* on a large scale was undertaken at the ARC–PPRI Vredenburg Production Laboratory in Stellenbosch. The three isolates were induced to sporulate on *L. camara* leaf decoction glucose agar (LDGA) plates by streaking the surface of these plates with mycelia from PDA slant cultures and incubating these at 19°C for 10 days under near UV and white light for 24 hours (Den Breeÿen & Morris 2003).

Six sites infested by *L. camara* were selected. These included sites in both coastal and inland areas. The fungus was released at these sites in December 2002. For the field releases, an aqueous spore suspension (2×10^5 spores/mL) and an oil-based spore suspension (1×10^5 spores/mL), was sprayed on 10 branches per treatment on successive plants at each of the sites. The release sites were monitored 12 weeks after release to assess establishment and local spread of the fungus. Inoculated *L. camara* branches, uninoculated branches within the same plant and *L. camara* plants within a 5–10 m radius were examined for symptoms of establishment. Where symptoms were found, samples of the diseased leaf material were collected. Leaves with typical *M. lantanae* var. *lantanae* lesions were incubated in dew chambers at 25°C for 24 hours and single-spore isolations were made.

Results

Twelve weeks after its release, typical lesions were found on inoculated branches and neighbouring plants at three of the six release sites in KZN for both the oil-based and aqueous spore suspensions. At one site, infected plants were found up to 10 m away from the inoculated plants. Sites will be monitored again every three months for the first year post-release and then annually. *Mycovellosiella lantanae* var. *lantanae* was reisolated from symptomatic leaves and grew into characteristic colonies on PDA.

Discussion

At three of the six release sites in KZN the fungus had established and caused secondary infections within 12 weeks. The three sites where no establishment was recorded were sites further inland and at the time of release were undergoing a drought (i.e. no rain for up to 12 months). The best site was situated along the south coast of KZN. The impressive rate of spread at this site was probably due to the windy and humid conditions during the three months following the release. The fungus was released at a further seven sites in the Eastern Cape province and these will be monitored at the end of June 2003. While is too soon to determine the likely impact of *M. lantanae* var. *lantanae* on weedy *L. camara* biotypes in South Africa, the results of monitoring of the first releases in KZN are promising.

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Integration of *Aphthona* spp. flea beetles and herbicides for leafy spurge (*Euphorbia esula*) control in the habitat of the western prairie fringed orchid (*Platanthera praeclara*), a threatened species

Ann M. Erickson and Rodney G. Lym[1]

Summary

Leafy spurge is a serious threat to maintaining biodiversity in rangelands and pastures of the northern Great Plains in the United States and Canada. Leafy spurge is threatening the habitat of the western prairie fringed orchid (*Platanthera praeclara* Sheviak & Bowles), a federally listed threatened species in the US and endangered species in Canada. *Aphthona* spp. flea beetles, biological control agents for leafy spurge, have established and controlled leafy spurge in the region, but not in habitat of the orchid. Also, current law prohibits use of herbicides in areas where the orchid grows. Previous research had shown that leafy spurge could be controlled with imazapic or quinclorac with minimal or no injury to the orchid. The purpose of this research was to evaluate leafy spurge control and *Aphthona* spp. establishment in habitat of the orchid, using imazapic or quinclorac in combination with the flea beetles. This combination method in other habitats has resulted in better leafy spurge control than either method used alone and has increased biological control agent establishment. Leafy spurge was treated with *Aphthona* flea beetles, herbicides, and *Aphthona* flea beetles plus herbicides. Leafy spurge stem density decreased from about 114 to 4 stems/m^2 the season following treatment with herbicides alone and from an average of 126 to 1 stem/m^2 the following season, when the treatment included both *Aphthona* flea beetles and herbicides. Stem density of leafy spurge treated with only flea beetles decreased from 150 to 41 stems/m^2. The population of flea beetles the season following release was estimated. Fewer than 1/m^2 *Aphthona* flea beetles were collected with sweep nets in plots where the flea beetles were not released, 6/m^2 in plots treated only with flea beetles, and 2/m^2 in plots treated with both flea beetles and herbicides. This is the first establishment of a leafy spurge biological control agent in the habitat of this orchid.

Keywords: *Aphthona*, imazapic, IPM, leafy spurge, quinclorac, threatened species.

Introduction

Platanthera praeclara Sheviak & Bowles, the western prairie fringed orchid, is a native plant of the tallgrass prairie that was placed on the federal threatened species list in 1989 (US Fish and Wildlife Service 1989). Various threats to the survival of *P. praeclara* exist and include habitat invasion by *Euphorbia esula* L., leafy spurge (Sieg & Bjugstad 1994, US Fish & Wildlife Service 1996, Wolken *et al.* 2001).

E. esula is one of the most widespread and competitive noxious weeds in North America, where it invades mainly non-cultivated areas such as native prairie and rangeland (Hanson & Rudd 1933, Selleck *et al.* 1962). *E. esula* decreases forage production for range animals, suppresses native plant species, and decreases biodiversity (Westbrooks 1998).

E. esula is very difficult to control with methods other than herbicides, but herbicides cannot be used in areas where the orchid is located due to its status as a

[1] Department of Plant Sciences, North Dakota State University, Fargo ND 58105

federally listed threatened species. Biological control of *E. esula* in the habitat of the threatened species would seem to be the least harmful approach. Biological control of *E. esula* with the use of *Aphthona* spp. flea beetles in North Dakota began in the mid-1980s (Carlson & Mundal 1990), but establishment of the flea beetles in the habitat of *P. praeclara* has not yet been successful (Lym 1998; Mundal *et al.* 2000).

An experiment to evaluate herbicides for *E. esula* control in North Dakota in the early 1990s had to be discontinued two years after establishment due to the appearance of *P. praeclara* in areas treated with fall-applied herbicides (Kirby *et al.* 2003). Subsequent research found imazapic and quinclorac provided good *E. esula* control with little or no injury to the orchid (Kirby *et al.* 2003, Sterling *et al.* 2000a, b). Therefore, this study was initiated to evaluate the interaction of imazapic and quinclorac with *Aphthona* spp. flea beetles for *E. esula* control in the habitat of *P. praeclara*.

Aphthona spp. flea beetles (Coleoptera: Chrysomelidae) are some of the most promising biological control agents of *E. esula*. These insects are natural enemies of *E. esula* in eastern and central Europe. Years of research with *Aphthona* spp. flea beetles were conducted on several plant species to determine the host range of the insects (Harris *et al.* 1985). Six species have been released in the US, but the three most effective species of these flea beetles are *A. czwalinae* Weise., *A. lacertosa* Ross. and *A. nigriscutis* Foudr. (Hansen *et al.* 1997, Mundal *et al.* 2000).

Aphthona spp. flea beetles oviposit eggs at the base of an *E. esula* plant and the eggs hatch in 14 to 19 days (Mundal *et al.* 2000). The larvae go through three instars, and with each instar, the larvae feed on progressively larger roots (Gassmann *et al.* 1996, Hansen *et al.* 1997, Mundal *et al.* 2000). *Aphthona* larvae prefer to feed on previously attacked root sections, so aggregate feeding occurs, which destroys the root sections (Gassmann *et al.* 1996). *A. abdominalis* is the only multivoltine *Aphthona* species released in the US, with four generations per year, and overwintering in the adult stage (Fornasari 1993). All five other *Aphthona* species released in the US are univoltine (Gassmann *et al.* 1996). Third instar larvae of univoltine species overwinter and pupate the following spring (Gassmann *et al.* 1996, Mundal *et al.* 2000). Adult univoltine flea beetles emerge from late May to early July and live for 2 to 3 months, during which time they feed on the leaves and stems of *E. esula*, but do not contribute greatly to its control (Gassmann *et al.* 1996, Hansen *et al.* 1997). Larval feeding may kill the plant directly by disrupting water and nutrient transport or indirectly by creating pathways for plant pathogens to enter the plant (Hansen *et al.* 1997).

E. esula densities may be reduced when *Aphthona* spp. flea beetles become established (Kirby *et al.* 2000), but the control of *E. esula* with the flea beetles has not occurred in all habitats (Lym 1998). Establishment of *Aphthona* spp. flea beetles has been variable because they usually do not survive well in habitats that are moist, shady, contain sandy soil, or have high *E. esula* densities (Lym 1998, Mundal *et al.* 2000), which are characteristics of the habitat of *P. praeclara*.

The use of *Aphthona* spp. flea beetles is an ecologically favourable control method for *E. esula*, but, as noted, the flea beetles generally do not survive well in the sandy, mesic habitat of *P. praeclara*. In fact, no releases made in the habitat of the orchid have effectively established (Lym 1998). Establishment of *Aphthona* spp. flea beetles in the habitat of *P. praeclara* may be improved using herbicides (Lym & Nelson 2002, Nelson 1999). An apparently unsuccessful *Aphthona* spp. population increased rapidly from an average of 14 flea beetles swept/m^2 to an average of 76 flea beetles swept/m^2 1 year after herbicide application in a study by Lym & Nelson (2002). *E. esula* stem density decreased from 114 stems/m^2 to 8 stems/m^2 one year after fall application of imazapic, and no *E. esula* stems remained in the study area two years after herbicide application. *E. esula* control generally occurred more rapidly and was maintained for longer periods when herbicides were used in conjunction with *Aphthona* spp. flea beetles than when either method was used alone in a number of experiments by Lym & Nelson (2002). *Aphthona* flea beetles also are compatible with other methods of *E. esula* control, such as sheep grazing and prescribed burning, and the integration of the flea beetles with either method controls *E. esula* better than any of the control methods used alone (Beck & Rittenhouse 2000, Fellows & Newton 1999).

Materials and method

An experiment to evaluate the interaction of imazapic and quinclorac with *Aphthona* spp. flea beetles for *E. esula* control in the habitat of *P. praeclara* was established in June 2001. The experiment was located near a large population of orchids.

The experiment was arranged as a randomized complete block-design with a split-plot arrangement and four replicates. Whole plots were 3.05 m wide and 9.15 m long. Whole plots consisted of herbicides alone, and subplots consisted of flea beetles plus herbicides, flea beetles alone, and an untreated control (neither flea beetles nor herbicides) (Nelson 1999). Measures were compared to an untreated check using an ANOVA, and individual treatment means were separated using Fisher's-protected LSDs calculated at the 95% levels of confidence.

The soil at the site was classified as a Hecla–Hamar–Arveson association, which is sandy, mixed, frigid Oxyaquic Hapludolls; sandy, mixed, frigid Typic Endoaquolls; and coarse-loamy, mixed, superactive, frigid Typic Calciaquolls; respectively (US Soil Conservation Service 1975). The soil was 75:20:5 sand:silt:clay and was analyzed for nutrients (Table 1).

Table 1. Western prairie fringed orchid research site soil characteristics at two depths.

Depth (cm)	N (kg/ha)	P (ppm)	K (ppm)	pH	EC	OM (%)
0 to 15	12	3	140	7.1	0.12	4.3
15 to 30	9	3	75	NA	NA	NA

A mixture of *A. czawalinae* and *A. lacertosa* was collected from an established population near Lisbon, North Dakota, approximately 29 km from the experiment's location. Approximately 350 adult *A. czawalinae* and *A. lacertosa* were released into insect cages on June 27, 2001, and 100 additional beetles were released on July 17, 2001 to ensure appropriate sex ratios (Olson & Mundal 1999). Cages were 1.8 by 1.8 by 1.8 m with a PVC frame covered by a plastic screen (32 × 32 Lumite) (Nelson 1999, Lym & Nelson 2002, Nelson & Lym 2003). Imazapic and quinclorac were applied on September 20, 2001 using a CO_2-pressurized backpack sprayer delivering 80 L/ha at 240 kPa with four flat-fan 8001 nozzles. Cages were removed from the plots for the winter prior to herbicide application (Nelson 1999, Lym & Nelson 2002, Nelson & Lym 2003).

The effects of the interaction of imazapic and quinclorac with *Aphthona* spp. were evaluated by counting the number of larvae that developed into adults from soil cores collected in October 2001 and May 2002, and by counting adults in the field in late June and early July 2002.

Four soil cores were collected per subplot using a golf cup-cutter. The golf cup-cutter was placed over an *E. esula* root crown, and cores 10.8 cm in diameter were cut to a depth of 15 cm. Soil cores collected in the fall were placed in plastic bags, transported, and stored in a refrigeration unit for 75 days at 3°C. Vernalization induced larvae to pupate and emerge as adults. After 75 days, the soil cores were placed into 0.9 L paper cups. Cups were covered with 2 L clear plastic cylinders. The covered cups containing soil cores were maintained in the laboratory at 21°C with a 16h photoperiod under artificial lighting. Soil cores collected in the spring were brought directly into the laboratory. Adults emerging from soil cores from both fall and spring collections were counted and removed from trap chambers daily. Soil cores were discarded 2 weeks after the last adult was collected (approximately 4 weeks) (Nelson 1999, Nelson and Lym 2003).

To estimate *Aphthona* flea beetle density in the field, vegetation in the subplots was swept for adults with a sweep net having a 38 cm diameter hoop. Quarters of the subplot and portions of the subplot border, each totalling 1 m^2, were swept in five sweeps in the spring, and adults captured were counted and returned. *E. esula* control was monitored by counting *E. esula* stems in four 0.25 m^2 areas in each subplot both before and after treatment (Lym and Nelson 2002, Nelson 1999).

Results

Imazapic or quinclorac applied alone or with *Aphthona* spp. flea beetles reduced *E. esula* density more than flea beetles alone (Table 2). *E. esula* stem density was reduced from an average of 150 to 41 stems/m^2 (53% control) with *A. czwalinae/lacertosa* alone compared to a reduction from an average of 114 to 4 stems/m^2 (96% control) and from an average of 126 to 1 stem/m^2 (99% control) with herbicides alone and herbicides with flea beetles, respectively, one season following treatment. Imazapic and quinclorac provided similar *E. esula* control one season following treatment regardless of application rate.

Table 2. Control of leafy spurge one season following release of *Aphthona* spp. flea beetles, herbicide application, or both.

Treatment	Rate (g/ha)	Leafy spurge density (stems/m^2)	
		June 4, 2001	June 5, 2002
Imazapic	140	104	7
Imazapic + *Aphthona*[a]	140	115	<1
Imazapic	210	105	1
Imazapic + *Aphthona*	210	150	0
Quinclorac	840	96	4
Quinclorac + *Aphthona*	840	132	0
Quinclorac	1120	149	3
Quinclorac + *Aphthona*	1120	107	2
Control	–	99	80
Control + *Aphthona*	–	150	41
LSD (0.05)		44	11

[a] Three hundred and fifty *Aphthona* flea beetles per subplot were added on June 26, 2001, and an additional 100 *Aphthona* flea beetles per subplot were added on July 17, 2001.

Imazapic and quinclorac did not affect *A. czwalinae/lacertosa* adult emergence from soil cores collected in the fall or spring (Table 3). However, approximately 15 flea beetles emerged per subplot from soil cores collected in the fall while only an average of 7 flea beetles emerged per subplot from soil cores collected in the spring. This 47% decrease in the number of flea beetles that emerged may have been due to low soil temperatures in the winter of 2001 to 2002 caused by little snow cover. A similar study reported that soil temperatures in the winter of 1995 to 1996 that were colder than normal for an extended period of time caused a 60% winter kill of *A. nigriscutis* adults taken from soil cores collected in the spring compared to soil cores collected in the fall (Nelson 1999, Nelson and Lym 2003). When that study was repeated in 1996, snow accumulated in record amounts in the winter of 1996 to 1997, which insulated the soil and prevented winter kill of larvae.

The numbers of *A. czwalinae/lacertosa* adults collected in the field were higher in subplots without herbicide treatment compared to subplots with herbicide treatment. An average of six *Aphthona* flea beetles

per m^2 was collected with sweep nets from subplots that received flea beetles alone compared with an average of 2 flea beetles per m^2 collected from subplots that received both imazapic or quinclorac and flea beetles (Table 4). Even though fewer *Aphthona* adults were collected where herbicides were applied, neither the choice of herbicide nor the application rate of the herbicide affected the numbers of flea beetles collected. Picloram plus 2,4-D did not affect the number of *A. nigriscutis* adults collected in the field in a similar study (Nelson 1999, Lym & Nelson 2002). However, *E. esula* density one season following the release of flea beetles and herbicide application was 55 stems/m^2 in the study by Lym & Nelson, whereas it was less than 1 stem/m^2 in this study (Table 2). Fewer *Aphthona* flea beetles collected from subplots that received imazapic or quinclorac plus flea beetles may have resulted because of the low densities of *E. esula* for adults to feed on the season following herbicide application.

Table 3. Effect of imazapic and quinclorac on *Aphthona* spp. flea beetle establishment according to emergence from soil cores.

Treatment	Rate (g/ha)	Emerged beetles (no./subplot)	
		Fall[b]	Spring[c]
Imazapic + *Aphthona*[a]	140	12	5
Imazapic + *Aphthona*	210	19	8
Quinclorac + *Aphthona*	840	13	7
Quinclorac + *Aphthona*	1120	16	4
Control + *Aphthona*	–	13	10
LSD (0.05)		NS	NS

[a] Three hundred and fifty *Aphthona* flea beetles per subplot were added on June 26, 2001, and an additional 100 *Aphthona* flea beetles per subplot were added on July 17, 2001.
[b] Soil cores were collected on October 18, 2001 and stored for 75 days at 3°C.
[c] Soil cores were collected on May 13, 2002.

Table 4. Population of *Aphthona* spp. flea beetles when estimated per five sweeps the season following flea beetle release and herbicide application.

Treatment	Rate (g/ha)	Collected *Aphthona* flea beetles (no./ m^2)	
		June 28, 2002	July 3, 2002
Imazapic	140	<1	0
Imazapic + *Aphthona*[a]	140	1	2
Imazapic	210	0	0
Imazapic + *Aphthona*	210	2	2
Quinclorac	840	<1	0
Quinclorac + *Aphthona*	840	1	2
Quinclorac	1120	<1	<1
Quinclorac + *Aphthona*	1120	1	2
Control	–	1	0
Control + *Aphthona*	–	5	6
LSD (0.05)		2	2

[a] Three hundred and fifty *Aphthona* flea beetles per subplot were added on June 26, 2001, and an additional 100 *Aphthona* flea beetles per subplot were added on July 17, 2001.

Discussion

This is the first reported establishment of *Aphthona* spp. flea beetles in the habitat of *P. praeclara*. However, an effective establishment may take up to five years. Using imazapic or quinclorac in conjunction with *Aphthona* spp. flea beetles may be useful to enhance establishment of flea beetles in the habitat of the orchid. Once the flea beetles become established, need for yearly herbicide applications decreases. An integrated approach to leafy spurge control may reduce costs for land managers (Lym & Nelson 2002).

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Insect performance and host-plant stress: a review from a biological control perspective

K.E. Galway,[1,4] R.P. Duncan,[1] P. Syrett,[2] R.M. Emberson[1] and A.W. Sheppard[3]

Summary

Three hypotheses predict how insect herbivores perform on stressed host plants. The plant stress hypothesis (PSH) predicts improved insect performance on stressed hosts. The plant vigour hypothesis (PVH) predicts that insects closely associated with their host, such as gall-formers, will perform better on vigorously growing non-stressed hosts. The third hypothesis, the Insect Performance Hypothesis (IPH) predicts that wood-feeders, sap-feeders and miners will perform better on stressed hosts, while leaf-feeders and gall-formers will perform better on non-stressed hosts. These hypotheses were developed, however, without separating different types of plant stress. In this review we tested these hypotheses across five insect feeding-guilds and twelve host-plant stress types, from more than 200 published studies on insect performance. When all host-plant stress types were pooled, the results suggested wood, sap and leaf-feeders performed better on stressed host plants, while miners and gall-formers performed better on non-stressed host plants, thus supporting the PVH. However, when all insect feeding-guilds were pooled, it was found that host-plant-stress type also influenced insect performance, which was generally higher when host plants were growing under reduced moisture, light or CO_2, increased soil nitrogen or on younger plants. When host-plant-stress type and insect feeding-guild were separated, it was found that insect performance across feeding guilds varied with the type of host-plant stress encountered suggesting that insects in different feeding guilds may respond to different physiological and morphological changes in the plant. This review highlights the fact that insect performance is often significantly affected by host-plant stress, but that the direction of the response is variable. Although this review did not fully support any of the three theoretical hypotheses tested, there were consistent relationships between some insect-feeding guilds and host-plant-stress types that would allow the prediction on whether a specific biological control agent might perform better under a specific host-plant stress.

Keywords: environmental stress, insect performance hypothesis, insect plant interactions, plant stress hypothesis, plant vigour hypothesis.

Introduction

Environmental stress is a factor that reduces plant performance below that achieved under optimal conditions (Price 1991). All plants encounter stress, because optimal conditions are rarely encountered in the field due to variations or fluctuations in environmental conditions. Several morphological and physiological changes may occur in plants under stress (Mattson & Haack 1987), depending on the plant species, and the severity, duration and type of stress encountered (Grime & Campbell 1991). Under moisture stress, for example, many plants show reduced leaf water, starch and carbohydrates,; and increased leaf nitrogen and soluble sugars (Miles *et al.* 1982, Mattson & Haack 1987, English-loeb *et al.* 1997). In contrast, low light levels can lead to reduced soluble sugars and increased leaf nitrogen and leaf water (Collinge & Louda 1988, Attridge 1990, Potter 1992).

[1] Ecology and Entomology, PO Box 84, Lincoln University, Canterbury, New Zealand.
[2] Landcare Research, PO Box 40, Lincoln, Canterbury, New Zealand.
[3] CSIRO Entomology, GPO Box 1700, Canberra ACT 2601, Australia.
[4] Present address: 18 Kuringal Drive, Ferny Hills, Queensland 4055, Australia.
Corresponding author: K. Galway, 18 Kuringal Drive, Ferny Hills, Queensland 4055, Australia <kepnz@optusnet.com.au>.

The morphological and physiological changes that occur in plants under stress may affect the performance of insect herbivores feeding on those plants (Mattson & Haack 1987). Several authors have suggested that changes in insect performance under host-plant stress may be predictable, although different authors have suggested different responses by insects to host-plant stress. White (1969, 1993) suggested that plants under stress become more susceptible to insect herbivores, with the increase in insect performance driven by increases in leaf soluble nitrogen (the plant stress hypothesis; PSH). In contrast, Price (1991) suggested that certain insects would perform better on more vigorously growing (non-stressed) host-plants, particularly herbivores closely associated with their host-plant (the plant vigour hypothesis; PVH). Price (1991) suggested that "herbivores closely associated" would include insects where the female selects the oviposition site within a few centimetres of where larvae will feed and that hatching occurs soon after oviposition. This hypothesis was based on observations of insect herbivores preferentially attacking young and vigorously growing plants and plant parts, over older plants or plant parts. Combining elements of both the PSH and the PVH, Larsson (1989) suggested that certain insect feeding-guilds (wood-feeding, sap-feeding and mining insects) perform better on stressed host-plants, whereas other insect feeding-guilds (leaf-feeding and gall-forming insects) perform better on vigorously growing host plants. This was termed the insect performance hypothesis (IPH). The rationale for this hypothesis was based on: a) woody plants under stress have reduced oleresin flow, making them less resistant to attack by wood-feeders; b) sap-feeding insects generally encounter low nitrogen levels so that when a plant is stressed, with resulting higher nitrogen levels, insect performance improves; c) miners are able to avoid consuming harmful defensive compounds produced by the plant while taking advantage of the higher nitrogen content of stressed plants; d) leaf-feeders do not separate out the chemical fractions in their food as efficiently as other feeding-guilds that discriminate against defensive compounds in stressed plants, so do better on vigorously growing plants; and e) galling insects prefer large-sized buds, which are found on vigorously growing plants.

If insect herbivores perform differently when host plants are under stress, then this has important implications for the effectiveness of insect herbivores released as biological control agents. Herbivores may be more effective in reducing plant performance over certain parts of a plant species' range, depending on whether they perform better on stressed or vigorously growing plants. For example, knowing that an insect herbivore performs better on vigorously growing non-stressed host plants and that the same insect performs poorly on stressed host plants may indicate that an additional biological control agent that performs well on stressed plants, or other forms of control, are required in those parts of a plant's range where it is subject to stress.

Two studies have reviewed the evidence for relationships between insect performance and host-plant stress. Waring & Cobb (1992) assessed insect performance in relation to host-plant moisture and nutrient stress, reporting that the type of stress was a stronger predictor of insect performance than insect feeding-guild. In contrast, Koricheva *et al.* (1998) argued that the insect feeding-guild (using the same guilds as Larsson (1989)) could predict insect performance for host plants under moisture, light and pollution stresses. They used a meta-analysis to detect a weak but overall significant relationship consistent with the IPH.

The aim of this paper is to assess both the strength and variability of the relationship between insect performance and host-plant stress by collating the results of published studies that have examined insect performance across insect feeding-guilds and host-plant-stress types. If this relationship is to be of predictive use in biological control then it requires strong, consistent relationships between the performance of insect feeding-guilds and host-plant stress.

Materials and methods

Selection of insect feeding-guilds and stress types

This review assesses insect performance across five insect feeding-guilds on plants subject to 12 stress types. The insect feeding-guilds selected were wood-feeders, sap-feeders, miners, leaf-feeders and gall-formers. These are the same guilds considered in the IPH by Larsson (1989) and reviewed by Koricheva *et al.* (1998). These feeding-guilds can directly affect plant growth, unlike flower and seed-feeding guilds.

Stresses from five of the seven abiotic categories listed by Heinrichs (1988) as affecting plant growth were included: moisture (water deficit and excess), electromagnetic energy (light and ultraviolet-B radiation), physical and chemical properties of the soil (soil-nitrogen, salinity and acidity), air pollution (ozone, carbon dioxide, sulfur dioxide and acid rain) and mechanical damage (fire). Stresses caused by temperature or pesticides/growth regulators were not included, because these have strong, direct effects on insect performance as well as on host-plant growth. Plant age was also included as a "stress" because several authors have shown that insect performance is affected by age (Price *et al.* 1987, Caouette & Price 1989, Craig *et al.* 1989, Roininen *et al.* 1993), and age was a factor considered by Price (1991) in developing the PVH.

Selection of studies

Key word searches in CAB Abstracts were used to source studies. Studies were found by entering a stress type and herbivory (e.g. moisture and herbiv*) and by entering the name of each of the three hypotheses. Additional studies were found by searching the reference lists

of the papers collected. Studies were selected if they assessed the performance of individual insect species, where those species belonged to one of the five listed feeding-guilds, and where the host plant was under one of the 12 listed stresses. Studies measuring insect performance as changes in fecundity, abundance, growth rate or generation time in relation to host-plant stress were selected. Studies using only feeding rates or preferences were not included. Only papers written in English that could be obtained in New Zealand, and only studies that used herbivorous arthropods in the orders Insecta and Acari, were included.

Definition of a 'stressed host-plant'

A stressed host plant is defined as one with reduced growth relative to that experienced under optimal conditions (Price 1991). Stressed host plants were therefore found in environments with reduced moisture, light, UV-B, soil nitrogen, and CO_2; increased salinity, acidity, ozone, SO_2 and acid rain. Plants were also stressed after burning and as they aged.

Analytical approach

Meta-analysis is frequently advocated as the best approach for combining the results from several studies to provide an overall test of a hypothesis, because it assesses the magnitude of the effect across studies (Gurevitch & Hedges 1993). In this study, however, the primary interest was not in testing for an overall effect, but in examining variability in the outcome of studies assessing the relationship between insect performance and host-plant stress. A weak, but significant overall relationship between insect performance and host-plant stress, such as that found by Koricheva *et al.* (1998), may be of little practical significance if the aim is to reliably predict the performance of insect biological control agents on host plants in different parts of their range.

Vote counting has been criticised because it relies on the statistical significance reported in individual studies, which varies as a function of the sample sizes employed in those studies (Gurevitch & Hedges 1993). Studies could show a non-significant result but nevertheless show a consistent tendency towards a particular outcome, which would not be detected using a vote-counting approach, but is more likely to be detected using meta-analysis, which considers the reported effect sizes (Gurevitch & Hedges 1993). Nevertheless, the use of vote-counting in this study is justified on two grounds. First, most of the studies examined did not report the outcome of experiments in sufficient detail to be included in a meta-analysis. A vote counting approach allowed a greater number of studies to be included so that the variability of outcomes across different insect feeding-guilds and host-plant-stress types could be better assessed. Second, over three quarters of the studies examined reported a statistically significant result one way or the other, allowing insect performance with regards to host-plant stress to be clearly categorised.

The number of studies that showed a significant positive relationship, a significant negative relationship, or no significant relationship (including studies that showed a non-linear response: that is, insect performance initially increased as stress intensity increased, but subsequently decreased) between insect performance and host-plant stress were tallied. The following additional information from each study was also collated: stress type, insect feeding-guild, arthropod family and species, and plant species.

Results

Data were collated from 201 studies on insect performance in relation to host-plant stress, from 105 papers published between 1955 and 2000. These 201 studies investigated the performance of 132 arthropod species (from 47 families and 7 orders) on 86 plant species.

When all stress types were pooled, the variability in the response of insect herbivores to host-plant stress was highlighted (Fig. 1). Of the 153 studies showing significant results, 77 showed that insect performance increased significantly on stressed host plants, whereas 76 showed that insect performance decreased significantly on stressed host plants, giving little support to the PSH. This data set supported the PVH ($\chi^2_1 = 6.8$, $p = 0.009$), as it was found that miners and gall-formers (this review considered those two guilds to be closely associated with the host plant) were represented in a greater proportion of studies showing a negative relationship between insect performance and host-plant stress, compared with other guilds that tended to show the opposite. The data set did not support the IPH, with only 38% of wood-feeders, sap-feeders and miners performing better on stressed hosts ($\chi^2_1 = 0.001$, $p = 0.971$).

When all feeding guilds were pooled, insect performance was higher on host plants growing under reduced moisture, light or CO_2, increased soil nitrogen or on younger plants (Fig. 2).

The data set also suggested that insect performance differs between insect feeding-guilds, depending on the host-plant-stress type encountered (Table 1). For example, the performance of leaf-feeders improved when host plants were growing under reduced moisture, light or CO_2, or increased soil nitrogen. The performance of miners improved when plants were growing under reduced CO_2, or increased moisture or soil nitrogen.

Several studies assessing the performance of leaf-feeders and miners also measured plant physiological responses to the stress being imposed. From these data, the performance of leaf-feeders tended to improve with increased plant nitrogen, while no clear and consistent physiological response was found for miners.

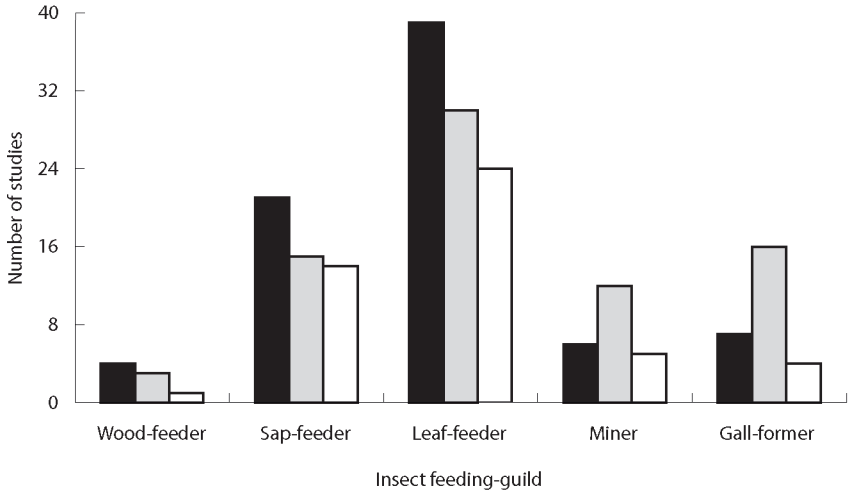

Figure 1. The number of studies where insect performance improved on stressed hosts (dark-shaded bars), improved on non-stressed hosts (light-shaded bars), or showed no relationship with host-plant stress (open bars), for five insect feeding-guilds.

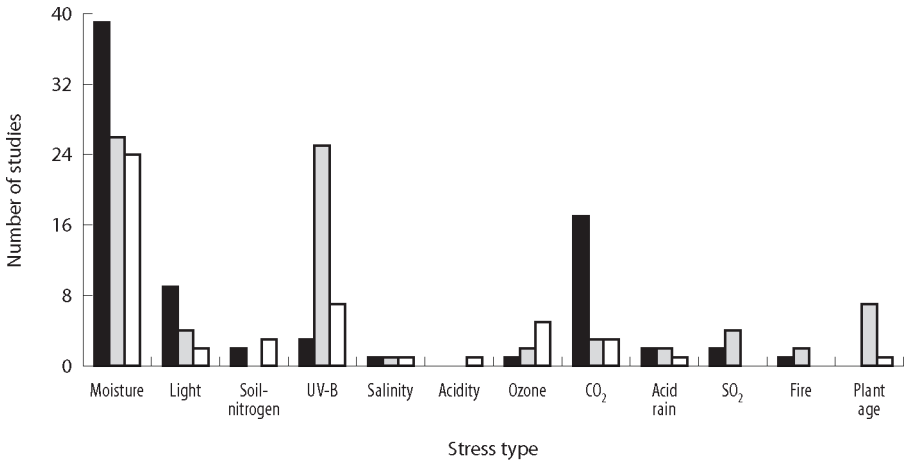

Figure 2. The number of studies where insect performance improved on stressed hosts (dark-shaded bars), improved on non-stressed hosts (light-shaded bars), or showed no relationship with host-plant stress (open bars), for 12 stress types.

Table 1. Predictions for improved insect performance on stressed host plants, on non-stressed host plants or when host-plant stress is not important across a range of stress types and insect feeding-guilds.

Insect feeding-guild	Improved insect performance		
	Stressed host plants	Non-stressed host plants	No relationship with stress
Wood-feeder			Moisture
Sap-feeder		CO_2	Moisture
Leaf-feeder	Reduced moisture Reduced light Reduced CO_2	Increased soil nitrogen	
Miner	Reduced CO_2	Increased moisture Increased soil nitrogen	Light
Gall-former		Increased soil nitrogen Reduced plant age	Moisture

Discussion

Hypotheses predicting relationships between insect performance and host-plant stress may allow the identification of parts of a host-plant's range where insect herbivores released for biological control are likely to be effective and where other control strategies will be required. For this to be of practical use requires consistent relationships between insect performance and host-plant stress. However, the results of this study suggest that insect response to host-plant stress can vary greatly according to the feeding-guild that the insect belongs to and on the form of stress encountered by the host plant.

Figure 1 highlights the variability in the response of insect herbivores to host-plant stress, where insects in the same feeding-guilds showed both positive and negative performance responses to host-plant stress. Though this data set supported the PVH, it does not provide a reliable basis for predicting the performance of insect feeding-guilds under host-plant stress, as only 56% of mining and gall-forming insects showed a positive response to non-stressed host plants.

Waring & Cobb (1992) reported that stress type, not insect feeding-guild, was the more important determinant of insect performance on stressed and non-stressed hosts, while Koricheva *et al.* (1998) found the opposite. These contrasting results may have arisen because each study reviewed a different range of stress types. Waring & Cobb (1992) focused on moisture and nutrient stress, while Koricheva *et al.* (1998) reviewed moisture, light and pollution stresses. Results presented here showed that insect performance generally improved when hosts were under moisture deficit, and decreased when experiencing nutrient deficit, as indicated by soil nitrogen and as predicted by Waring & Cobb (1992). Consistent with Koricheva *et al.* (1998), these results also showed improved insect performance when host plants were under moisture, light and pollution stresses. If Koricheva *et al.* (1998) had considered a wider range of stress types, and included nitrogen stress under which insect performance appears to decline, their results may well have showed that stress type is an important determinant of insect performance.

Plant nitrogen levels probably explain the general association found between stress type and insect performance. In this review, improved insect performance occurred under reduced host-plant moisture, light and CO_2, increased host-plant soil nitrogen, and on younger host plants (Fig. 2); all of which have been generally associated with increased plant nitrogen.

These results indicate that, to predict insect performance in relation to host-plant stress, both insect feeding-guild and stress type must be considered. Small sample sizes prevented detailed statistical analysis of insect performance by feeding-guilds with plant stress, though some patterns emerged (Table 1). For instance, under moisture stress, leaf-feeders performed better on stressed hosts. Waring & Cobb (1992) found that "chewers" (wood-borers, stem-borers, root-feeders, leaf-miners and leaf-chewers) all responded positively to moisture stress. As several feeding-guilds were included in this grouping, the results should be used cautiously, especially as our results indicated that miners performed better on non-stressed hosts. The results from this review support those of Waring & Cobb (1992) that leaf-feeders, miners and gall-formers performed better on fertilized (non-stressed) hosts and those of Koricheva *et al.* (1998) in that leaf-feeders performed better on light-stressed hosts. Bezemer & Jones (1998) found that, as CO_2 increased, sap-feeders' performance increased, and that the performance of leaf-feeders and miners decreased. This review supported those findings for leaf-feeders and miners, but insufficient data were collated on sap-feeders to compare findings. Wood-feeders showed little variation in performance in relation to stress type. This might be expected, given that the feeding site is somewhat removed from the actively growing host-plant tissue.

Larsson (1989) emphasized the need to first understand how plants respond to a stress, and then relate this to how it might affect insect performance. Different insect feeding-guilds may respond in different ways to different mechanisms. In this review, leaf-feeders appeared to respond positively to stresses (reduced moisture, light and CO_2 and elevated soil-nitrogen) associated with increased nitrogen, as did gall-formers. However, no clear plant-physiological mechanism was evident for miners, suggesting that they may be responding to another factor, such as changes in leaf morphology. Potter (1992) observed that changes in performance of a leaf-miner were related to changes in leaf structure rather than the nutritional quality of leaves. Details of plant physiological and morphological changes that occur under all the different stresses reviewed are beyond the scope of this study.

The results from this review indicate that the current plant stress – insect herbivory hypotheses do not adequately predict insect performance on stressed and non-stressed plants. However, based on the analyses, it is clear that the type of stress imposed on the plant, as well as the insect feeding-guild, is important in determining insect performance. There may be underlying associations between plant morphology and physiology and insect performance (e.g. available plant nitrogen) that are affected by the nature of the stress. The categories of feeding-guilds used in this analysis may also mask some trends in relationships. For instance, the category "miners" includes both stem and leaf miners, which may respond differently to applied stresses. Although current insect herbivory–plant stress hypotheses are too generalized at this stage to be helpful for improving weed biological control, our analysis does suggest possible benefits from the application of some of the results, particularly for leaf-feeders and miners.

For example, it is predicted that a leaf-feeding insect might perform better on host plants growing under drought-stressed conditions, in shade and with high soil nitrogen levels, while a miner might perform better in environments where host plants are receiving high moisture and soil nitrogen.

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Establishment of a weed biocontrol implementation program in South Africa

Penny Gillespie,[1] Hildegard Klein[2] and Martin Hill[3]

Summary

A program has been established in South Africa focusing on the distribution of biocontrol agents for invasive alien plants and the integration of biocontrol into alien plant clearing programs. One national and six regional biocontrol implementation officers liaise with biocontrol researchers, agricultural departments, the forestry industry and biodiversity managers, ensuring biocontrol agents are distributed to the extent of their ecological ranges. The program raises awareness of the effective and safe use of biocontrol. During the last 3 years, some 12.6 million individuals of 30 species of biocontrol agents were distributed in South Africa against 22 weed species.

Keywords: biocontrol implementation, invasive alien plants, Working for Water.

Introduction

Biocontrol of invasive alien plants commenced in South Africa in 1913 when *Dactylopius ceylonicus* (Green) successfully controlled *Opuntia vulgaris* Miller. Since then, 95 species of biocontrol agents have been released in South Africa, targeting 41 weed species. Of these, 10 weed species are considered to be under complete biocontrol and 13 species are substantially controlled (Olckers 1999, H. Klein, unpublished data).

During the past 6 years, the South African "Working for Water" (WfW) program, a national poverty relief initiative, has funded most research into the biocontrol of invasive alien weeds. A partnership has now been formed between the major research body and WfW, to ensure the optimal implementation of the products of this and further biocontrol research.

This paper outlines the roles and functions of the biocontrol implementation program, which is now a functioning arm of the WfW. It will also describe some of the issues that the program has dealt with and some lessons learnt.

[1] ARC – Plant Protection Research Institute, Private Bag X5017, Stellenbosch, 7599 South Africa.
[2] ARC – Plant Protection Research Institute, Private Bag X134, Pretoria, 0001 South Africa.
[3] Professor of Zoology, Rhodes University, Grahamstown, 6140, South Africa.
Corresponding author: Martin Hill <m.p.hill@ru.ac.za>.

The pre-WfW era of biological weed control in South Africa

Until the commencement of WfW, the biocontrol of weeds in South Africa was almost exclusively managed by the Plant Protection Research Institute (PPRI) of the Agricultural Research Council (ARC) or PPRI's predecessors, with funding mainly from the National Department of Agriculture (NDA).

The benefits of having a single research organization handling all aspects of biocontrol countrywide were offset by a lack of research capacity. The researcher who carried out the host-specificity testing was also responsible for the agent's mass-rearing, release and post-release monitoring. Financial constraints often forced scientists to limit the redistribution and post-release monitoring of one biocontrol agent in favour of processing the next candidate through quarantine. In some cases, universities took over the post-release monitoring phase and agricultural and nature conservation officers assisted with the distribution of agents.

Insufficient communication and extension often resulted in land managers hampering biocontrol efforts.

The WfW program

WfW was initiated in 1995 by the National Department of Water Affairs and Forestry, in response to a report commissioned by the Water Research Commission indicating that, without significant action, the reduction

in water runoff caused by invading alien plants would increase from 7% to 36% during the next 20 years (Versfeld *et al.* 1998).

By clearing weeds, the WfW program aims to enhance water-supply security and the ecological integrity of natural ecosystems, and to restore the productive potential of land. By employing mainly the marginalized sectors of the South African population, WfW provided them with jobs, training and the economic benefits of alien-plant-based industries, such as furniture-making (Anon. 2001). In 2001, WfW employed over 17,000 people and cleared over 608,000 ha of invading plants (Kasrils 2002).

The early WfW era in weed biocontrol

Initially, WfW used only physical and chemical weed-clearing methods, with an emphasis on job creation. With their strong financial support, WfW was able to clear weed infestations at an unprecedented rate.

WfW top management then recognized biocontrol as a crucial component of the WfW program to ensure the sustainability of weed suppression. During the past 6 years, WfW provided generous funding to biocontrol research, revitalizing this research field. Yet WfW did not reap the full benefits of the research they were funding. No formal communication channels existed between WfW clearing teams and biocontrol researchers. Within the ranks of WfW, biocontrol was relatively unknown, and often regarded with suspicion. It was disregarded as a potential control option, or even actively opposed. Researchers lacked the capacity to provide training and extension. It became evident that a special program was required within WfW to ensure that biocontrol was integrated into its clearing activities.

The weed biocontrol implementation program in WfW: initiation and structure

A national biocontrol implementation officer (the senior author), seconded from Australia in 1999 and employed by PPRI, planned and initiated the Biological Control Implementation (BCI) program.

The program operates in six of the nine South African provinces (see Fig. 1). WfW has appointed a BCI officer in each of these provinces to manage regional BCI programs. All but one of the officers have postgraduate qualifications in zoology or related fields. Their responsibilities are to ensure that all available biocontrol agents are distributed to their ecological range in South Africa and to facilitate the incorporation of biocontrol into WfW clearing programs (Gillespie 2003).

So far, the BCI program has focused mainly on insect agents and not pathogens, with the exception of "Hakatak", a commercially available mycoherbicide for the control of *Hakea sericea*.

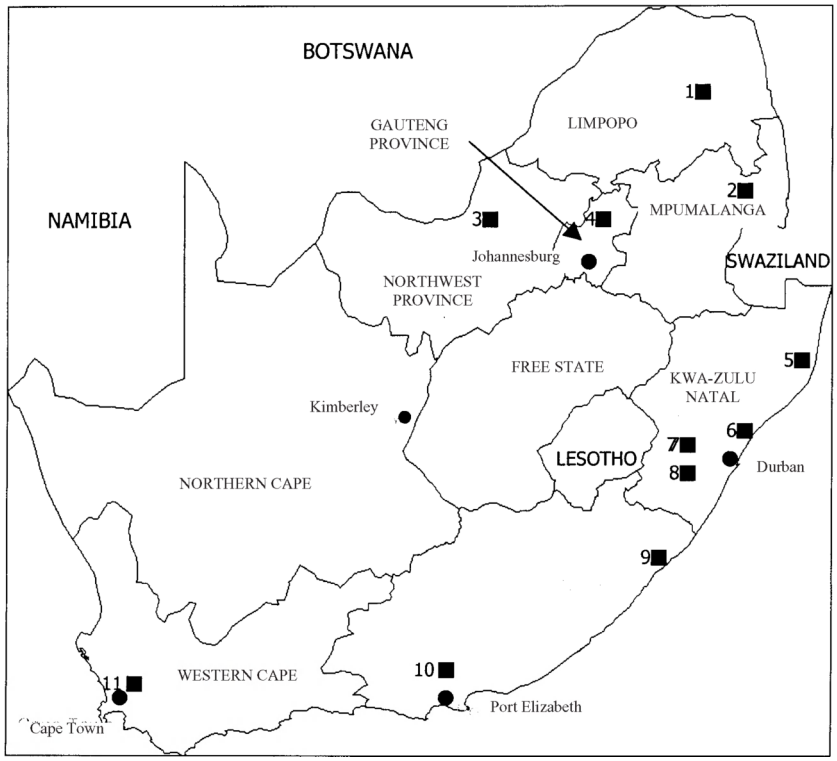

Figure 1. South African provinces in which the Biological Control Implementation (BCI) program operates.

Distribution of biocontrol agents

The BCI program relies on close cooperation and information exchange between biocontrol researchers and the BCI officers. Once permission is obtained to release a new biocontrol agent, the responsible researcher and regional BCI officers may release the first insects together using quarantine-reared stock. The researcher briefs the BCI officers on the insect's biology as well as rearing and release techniques, and provides them with a starter culture of the insect to mass-rear for future field releases. The BCI officers and researcher collaborate on troubleshooting any mass-rearing problems, monitoring the initial release sites and collecting data until establishment has been confirmed. Once established in the field, the BCI program manages the distribution and establishment of the agent across the country.

The national BCI officer uses information provided by the researcher to produce information sheets about the biocontrol agents, their mass-rearing and integration into alien plant clearing programs for use by all weed controllers and interested members of the public.

Source of insects for redistribution

After release from quarantine, insects are usually mass-reared in the regional BCI centres on potted plants in shade houses, or in insectaries using cut sprigs of plants as a food source. Once it is possible to collect a biocontrol agent species more easily from an established field site than it is to mass-rear it, mass-rearing is usually discontinued.

Certain biocontrol agents, particularly those insects that lay their eggs on the immature fruit or seeds of large, woody trees, cannot be mass-reared in a laboratory situation. As most of the agents used in the Western Cape region fall into this category, the Western Cape BCI program currently has no facilities for mass-rearing, but relies on field-collection as a source of insects for redistribution. Collecting times for seed or fruit-feeding agents are seasonal and provide temporary employment for small collection teams who might normally be employed by WfW for the chemical or manual control of weeds.

Highly mobile biocontrol insects are often so widely dispersed that suitable collecting sites can easily be found. Others, such as stem-boring beetles, reproduce slowly and often have a restricted distribution. Their breeding sites are valuable sources of insects for redistribution, and need to be protected. These sites are registered as "biocontrol reserves" (discussed later).

During the last 3 years, some 12.6 million individuals of 30 species of biocontrol agents were distributed in South Africa against 22 weed species (Table 1).

Release site selection

The BCI program aims to distribute available biocontrol agents throughout their ecological range, to as many target weed infestations as possible. Whether releases are made on private or public land, the BCI officer consults with the land manager to ensure that the chosen site does not clash with other land-use priorities. Land owners are informed of the need to protect the site for a number of years to ensure establishment and natural spread of the insects.

The BCI program aims to have biocontrol operating in all catchments, whether or not WfW is actively clearing weeds there. Biocontrol could suppress weeds in low priority areas that have no other long-term weed management plan, thereby giving WfW a "presence" in the catchment. In catchments where WfW manages weed-clearing operations, the BCI program aims to incorporate biocontrol into these operations, providing an ongoing legacy of weed suppression. BCI officers liaise with clearing managers to ensure that suitable pockets of weeds are left to ensure the continued presence of the biocontrol agents. Guidelines are prepared to aid land managers in this respect.

Protection of released biocontrol agents

The *Conservation of Agricultural Resources Act, 1983* (Act 43 of 1983) (CARA), administered by the National Department of Agriculture (NDA), recognizes effective biocontrol as a valid control method and protects such sites from disturbance. CARA allows important biocontrol agent nursery sites to be registered as biocontrol reserves, protecting them from clearing.

When biocontrol agents are released, an undertaking is signed between the land user and WfW. It requires the land user to protect the agents for a maximum of 5 years or until notification by WfW, and protects the land user from prosecution by NDA. By applying biocontrol in parts of a weed infestation, land users are not absolved of their weed-management obligations in surrounding infestations. NDA is notified of all biocontrol agent releases, and once established, the release site can be registered with NDA as a biocontrol reserve.

Data management

All biocontrol agent release sites are recorded on a standard release form which includes global positioning system (GPS) coordinates, site descriptions, land managers involved, infestation characteristics, weather data and numbers of insects released. During post-release monitoring sessions, information regarding insect numbers and damage to the infestation is recorded. This information is stored on the WfW information management system as well as being sent to relevant researchers and NDA.

Table 1. Regional biological control implementation (BCI) program centres in South Africa, and biocontrol agents distributed by them.

Region	Location	Staff at centre	Insect being distributed by WfW	Target weed	Number released 2001/2	Number released 2002/3
Western Cape	WfW, Belville	1 officer	*Melanterius* spp.	*Acacia* spp.	7000	6500
			Erytenna consputa	*Hakea sericea*	2000	1000
			Carposina autologa	*Hakea sericea*	150	Not known
			Dasineura sp.	*Leptospermum laevigatum*	–	Unknown number
	PPRI, Stellenbosch		*Aphanasium* sp.[a]	*Hakea sericea*	–	121 adults and 272 eggs
	Heidelberg	1 worker	"Hakatak"[b]	*Hakea sericea*	–	2 trials established
Eastern Cape	Uitenhage	1 officer	*Melanterius* spp.	*Acacia* spp.	3000	6200
		1 technician	*Falconia intermedia*	*Lantana camara*	200,000	700,000
		2 workers	*Teleonemia scrupulosa*[a]	*Lantana camara*	–	53,000
			Gargaphia decoris[a]	*Solanum mauritianum*	–	?
			Sulcobruchus subsuturalis	*Caesalpinia decapetala*	5,00	58,000?
			Dactylopius spp.[a]	*Opuntia* spp.	–	33,000 infested cladodes
			Cactoblastis cactorum[a]	*Opuntia* spp.	–	9000
			Leptinotarsa defecta[a]	*Solanum elaeagnifolium*	–	800
			Stenopelmus rufinasus	*Azolla filliculoides*		400
			Various agents	*Eichhornia crassipes*		
KwaZulu-Natal	ARC, Cedara	1 officer	*Sulcobruchus bakeri*[a]	*Caesalpinia decapetala*	12,000	30,000
	Richmond and	2 workers	*Gargaphia decoris*[a]	*Solanum mauritianum*	62,000	91,000
	Kwambonambi (with SAPPI)		*Teleonemia scrupulosa*[a]	*Lantana camara*	–	91,000
	Futululu	none presently	*Falconia intermedia*	*Lantana camara*	249,000	500,000
			Pareuchaetes insulata[a]	*Chromolaena odorata*	–	807,000
			Melanterius spp.	*Acacia* spp.	1800	1000
	Mt Edgecombe (contract with SASEX)	4 technicians	*Charidotis auroguttata*	*Macfadyena unguis-cati*	100	–
			Cyrtobagous salviniae	*Salvinia molesta*	–	300
			Dactylopius spp.	*Opuntia* spp.	–	3000 infested cladodes
			Neohydronomus affinis[a]	*Pistia stratiotes*		100
Mpumalanga	White River	1 officer	*Ophiomyia camarae*[a]	*Lantana camara*	–	3000
	(Uplands School)	1 worker	*Falconia intermedia*	*Lantana camara*	–	571,000
			Sulcobruchus subsuturalis[a]	*Caesalpinia decapetala*	–	5000
			Melanterius spp.	*Acacia* spp.	–	600
			Leptinotarsa defecta	*Solanum elaeagnifolium*	–	Not known
			Alcidion cereicola	*Cereus jamacaru*	–	To be initiated
			Phenrica guérini	*Pereskia aculeata*	–	To be initiated
			Various species	*Eichhornia crassipes*	–	Not known

Table continued on next page.

Table 1. (cont'd) Regional biological control implementation (BCI) program centres in South Africa, and biocontrol agents distributed by them.

Region	Location	Staff at centre	Insect being distributed by WfW	Target weed	Number released 2001/2	Number released 2002/3
Limpopo	Tzaneen dam and other locations	1 officer	*Falconia intermedia*[a]	*Lantana camara*	1,800,000	120,000
		1 foreman	*Gargaphia decoris*[a]	*Solanum mauritianum*	7,000,000	60,000
		4 workers	*Sulcobruchus subsuturalis*[a]	*Caesalpinia decapetala*	103,000	535,000
			Melanterius spp.	*Acacia* spp.	600	_
			Alcidion cereicola	*Cereus jamacaru*	_	To be initiated
			Dactylopius spp.	*Opuntia* spp.	_	To be initiated
			Various species	*Eichhornia crassipes*	_	To be initiated
			Charidotis auroguttata[a]	*Macfadyena unguis-cati*	not known	_
Northwest and Gauteng	Groot Marico	1 Officer	*Alcidion cereicola*	*Cereus jamacaru*	_	Not known
			Falconia intermedia	*Lantana camara*	not known	-
			Ophiomyia camarae	*Lantana camara*	_	To be initiated
			Melanterius spp.	*Acacia* spp.	1000	600
			Dactylopius spp.	*Opuntia* spp.	_	Not known
			Charidotis auroguttata	*Macfadyena unguis-cati*	300	-
			Various species	*Eichhornia crassipes*	_	Not known

Note: WfW = Working for Water, PPRI = Plant Protection Research Institute, ARC = Agricultural Research Council, SAPPI = South African Paper and Pulp Industries, SASEX = South African Sugar Experiment Station.
[a] Insects are mass-reared in that province. [b] "Hakatak" is not an insect, but a commercially available mycoherbicide.

The BCI program produces maps upon request showing established release sites.

The web-based database (www.waterweeds.co.za) was established to store the BCI data. Different types of information are available to registered users depending on their requirements. All users have access to regional maps showing release points, what has been released and when. Biocontrol researchers are given detailed site references and data about release conditions and site monitoring. This web site will provide a useful tool for evaluating the effectiveness of biocontrol of alien plants in South Africa.

Other partnerships

The BCI program has united all organizations involved with weed control in a single forum, coordinating their activities, and avoiding duplication or counterproductive actions. Apart from biocontrol researchers, the following organizations participate in these liaison committees:

- NDA – administers the CARA regulations dealing with weeds, recognizing and protecting biocontrol. It is responsible for law enforcement and advice on weed control methods and has taken part in the development of the BCI program, including the funding of a technical officer position in one region.
- The forestry industry – as a major weed manager, the industry has a financial interest in the success of the WfW program. SAPPI (South African Pulp and Paper Industries), SAFCOL (South African state forestry organization) and Mondi actively participated in the BCI program development in three regions, and manage a number of insectaries for the program.
- The South Africa National Parks or equivalent nature conservation organizations – contribute actively to planning the release programs.
- Private conservancy or "Landcare"-type groups, municipalities and private landowners.

Education and training

Ninety per cent of WfW staff has low levels of formal education and technical expertise. They initially feared that biocontrol agents would kill all the weeds, and that their jobs would then be terminated. Another misconception, especially among managers, concerned host specificity of biocontrol agents, because they were unaware of the strict protocols followed in releasing agents.

A training program was developed to provide a more rational understanding of biocontrol and how, by focusing clearing programs on weed species not under biocontrol, it may be used to better achieve weed management goals. Training is provided to WfW personnel at all levels, from contractors in charge of clearing teams to management. Other partnership organizations have also requested training for their employees on the role of the BCI program and how they could cooperate. BCI officers deliver the training, with support from biocontrol researchers adding valuable depth to the technical discussion.

Approximately 400 people have attended one of 14 half-day biocontrol information sessions. The theory of biocontrol was outlined, how it is applied in South Africa and how biocontrol can be integrated into the alien plant-clearing program without affecting jobs. Demonstrations using live insects or pathogens and the plant damage they cause generated much interest. Course notes were issued to each participant, containing examples of release site maps, data record sheets and illustrated colour brochures on biocontrol agents and their associated weed damage.

Informal surveys indicated that there was a much greater acceptance and willingness to cooperate with the BCI program after these training sessions.

Public awareness

An important component of the BCI program is providing the public with information relating to the use of biocontrol of invading alien plants. The interest in biocontrol generated by this extension activity provided the BCI program with excellent release sites across the country.

Regional BCI officers are often invited to speak about their programs at local farm days, schools and conservancy meetings. The BCI program is represented in all WfW public displays and publications. A set of 26 colour brochures on biocontrol agents has been well received by the public and WfW employees.

Lessons learnt from developing the BCI program

- Cooperation between researchers and implementation officers is extremely important. The time researchers spend in briefing of or consultation with the BCI officers is a small investment to make for large returns in productivity, data collection, and access to long-term establishment information and extension. BCI officers obtain valuable information and useful techniques from the researchers.
- Data transfer between BCI officers and researchers maximizes the productivity of both programs. Data collected by the BCI program must be accurate and researchers must ensure the BCI program knows the locations of important research sites.
- After much initial debate, the present model, where researchers and BCI officers cooperate on initial releases and data gathering, with BCI officers taking over the distribution of agents once establishment has been confirmed, seems to function well.
- The current system of cooperation does not require the BCI officers to be highly trained entomologists.

Their skills in the field and technical information transfer widen the skills of the biocontrol fraternity.
- Releasing far larger numbers of insects per site than previously may result in establishment successes not previously experienced in South Africa.

Benefits of the BCI program

The BCI program has brought a new dimension to the control of invasive alien plants in South Africa, benefiting researchers and practitioners alike.

Biocontrol-related functions, once solely managed by research, are now being shared by partners across the country. This has resulted in a dramatic increase in numbers of insects released, thereby increasing the rate of successful establishment and visual impact, and showing up any need for intervention earlier. Biocontrol agents are rapidly distributed throughout their ecological range. By ensuring that biocontrol is operating wherever possible, taxpayers' money is saved in terms of reduced alien plant-clearing programs, protection of agricultural land and biodiversity, and potentially by increased water supplies.

Data collected by the BCI program can be used for a variety of reasons to support both research and extension programs. WfW managers are kept informed of the presence of biocontrol sites; they are now including biocontrol in their management programs and minimizing conflict between treatment methods. Local agricultural officers also gain first-hand information on the whereabouts and effectiveness of biocontrol agents in their regions. The BCI program is facilitating the creation of biocontrol reserves by obtaining agreement from the land user and integrating biocontrol into larger alien plant-clearing programs.

The mass-rearing centres provide an opportunity for employment and training, and create a feeling of ownership of biocontrol in the local communities. The public at large is now far more aware of and better informed on weed biocontrol.

Future plans

The BCI program is intended to continue for the duration of WfW in South Africa (currently projected as 2020). In the next few years, the BCI aims at convincing all clearing program managers that biocontrol is a useful tool, to be incorporated wherever possible.

It is essential that both implementation and research staff continue their cooperation and joint ownership in the program. The continued production of technical extension material targeted at the general public must be a joint effort between both partners.

Currently, the BCI program operates at approximately 3% of the total WfW alien plant-clearing budget. It is not planned to significantly increase annual funding to the program, but rather to direct funding towards obtaining quantitative data to motivate the continued existence of the program. Being a special government initiative, WfW requires quantitative data to accurately determine the BCI program's impact on alien weeds. This could be achieved by contracting ecologists, biocontrol experts and economists, as well as consulting similar international teams.

Conclusion

The biocontrol implementation arm of the WfW alien plant-clearing program is something that the country can be proud of. It will provide an ongoing legacy of weed control to South Africa, long after WfW has ceased to exist.

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Integrating biological control and land management practices for control of *Ulex europaeus* in Hawai'i

R. L. Hill,[1] Y. Buckley,[2] N. Dudley,[3] D. Kriticos,[4] P. Conant,[5] E. Wilson,[5] B. Beaudet[6] and M. Fox[7]

Summary

Despite our best efforts, most biological control programs do not adequately control target weeds. The number of "partially successful" programs in place around the world is legion. Recent advances in modelling allow us to simulate how biological control interacts with more traditional control techniques and with land-use choices, and to test scenarios for integrating control strategies to enhance the value of partially successful biological control projects. A comprehensive, integrated weed-management program for approximately 4000 ha of *Ulex europaeus* L. (gorse) at Humu'ula, Hawai'i, is under development. An existing, spatially explicit, population dynamics model created using *U. europaeus* population parameters from New Zealand predicts that long-term suppression of *U. europaeus* infestations is feasible within a range of combinations of seed predation, inter-specific seedling competition, and disturbance from fire or herbicides. Results from a field experiment at Humu'ula will be used to re-parameterise the model for Hawaiian conditions, and test the predictions of the model. Several biological control agents have already been established there, and the seed-feeder *Cydia succedana* will be introduced. The insights gained from the field experiment and from modelling will be used to enhance biological control, by developing an array of integrated control tactics and incorporating these into long-term management plans for *U. europaeus* at a landscape level. Provenance trials are underway to test the feasibility of forestry in the area. Management plans will be prepared with local stakeholders, and will take into account the relative viability of alternative land uses such as forestry and grazing.

Keywords: gorse, Hawai'i, integrated control, models, *U. europaeus*.

Introduction

In various estimates 50–83% of mature, well-resourced biological control of weeds projects mounted in countries across the world have provided economic benefits, or have contributed to environmental or social well-being (Hoffman 1995, McFadyen 1998, Fowler *et al.* 2000). In only 17–30% of these cases has complete control been achieved by biological control alone. For the rest, biological control is seen as valuable, but partial, or sporadic. One can look at this large bulk of partial successes either as an indictment of the success rate of such projects, or as a plethora of "near-successful" projects waiting to be realised.

One way to improve the value of such projects is to integrate the biological control system with other control techniques in a synergistic fashion (Syrett *et al.* 2000). Integrated weed management is a concept that is much discussed, but rarely implemented. Huwer *et al.* (2002) and Paynter & Flanagan (2003) have examined the results of the simultaneous application of alternative control tactics, but studies of this type are rare. More often, development of integrated weed manage-

[1] Richard Hill & Associates, Private Bag 4704, Christchurch, New Zealand.
[2] Imperial College, Silwood Park, Ascot, SL5 7PY, UK.
[3] Hawai'i Agricultural Research Centre, Aeia, Hawai'i 96701-3911, USA.
[4] CSIRO Entomology, GPO Box 1700, Canberra, Australia.
[5] Hawai'i Department of Agriculture, Hilo, Hawai'i 96729-4302, USA.
[6] Parker Ranch, PO Box 458, Kamuela, Hawai'i 96743, USA.
[7] The Nature Conservancy of Hawai'i, 923 Nu'uanu Ave, Honolulu, Hawai'i 96817, USA.
Corresponding author: Richard Hill, Richard Hill & Associates, Private Bag 4704, Christchurch, New Zealand <hillrl@xtra.co.nz>.

ment has been brought up short by the complex interactions of weed and agent ecology, management strategies, and environmental influences.

Models can be used to describe weed population dynamics and inform decisions about biological control options (e.g. Hoffman 1990, Lonsdale *et al.* 1995, Shea and Kelly 1999). Models also provide the means to analyse relationships between weed populations and natural enemies, and how these are influenced by plant competition, environmental variables, and control tactics. They can therefore provide insights into the complexity of real weed management systems and identify promising avenues for improving weed control (McEvoy & Coombs 1999, Buckley *et al.* 2001, 2003).

Rees & Paynter (1997) developed a spatially explicit simulation model for population size and ground cover of *Cytisus scoparius* L. (Link) (broom). They found that insects that fed on seeds were most likely to have substantial impact on the equilibrium plant cover if the rate of disturbance was high and survival of seedlings was low. Simulated reduction in annual seed production of as little as 75% had a dramatic impact on broom abundance in their model. Rees & Hill (2001) applied this modelling approach to *U. europaeus,* and again found that disturbance and seedling survival were critical determinants of plant cover in simulations. As with *C. scoparius*, 75% reduction in annual seed production (originally set at 20,000 seeds m^{-2}) resulted in decline in *U. europaeus* cover under certain combinations of disturbance and seedling survival. They took the approach further and explored the effects of management tools such as herbicides, fire and over-sowing on the key population parameters, and simulated their effect on the equilibrium cover of *U. europaeus*. The model predicted that both treatments were capable of either further depressing equilibrium *U. europaeus* cover at a given level of seed predation or that a lower level of seed predation by biological control agents might still yield effective control.

This paper describes a new project that aims to develop an integrated control program for *U. europaeus* in Hawai'i. The core of the program will be two models. The first will extend the exploratory power of the model developed by Rees & Hill (2001) to predict changes in *U. europaeus* cover under various management regimes. The second will be a process-driven model developed to further explore the impact of weed management practices.

Ulex europaeus L. is a spiny leguminous shrub that can grow to over 3 m tall, and has a life span of 20–30 years. Its natural range is western Europe, where it occurs singly or in small clumps on sandy heathland soils; but where it has colonised new environments it forms dense impenetrable thickets (Richardson & Hill 1998). It is regarded as a weed in New Zealand, Australia, Chile, Iran, Italy, Poland (Holm *et al.* 1979) and elsewhere. It first became naturalised in Hawai'i before 1910 (Wegner *et al.* 1990). It can be found on approximately 4000 ha of rangeland on the island of Hawai'i, on the flanks of Mauna Kea in a parcel of land called Humu'ula, where it forms dense thickets on 60% of this land. Some has been colonised relatively recently, but *U. europaeus* may have been present for over 80 years. The infestation is barely contained, but could potentially occupy an area at least 20 times larger than its current distribution. The affected land could be grazed by cattle, and annual production losses are estimated to be $US1.8million. *U. europaeus* also threatens the landscape and conservation values of the Hakalau National Wildlife Refuge, which invests at least $50,000 annually in preventing *U. europaeus* invasion, and is also invading open land and watercourses in neighbouring forests. It is also present on the islands of Maui and Molokai.

Key principles for sustainable management of *U. europaeus*

U. europaeus is a large, long-lived shrub that is difficult to kill and has a persistent soil seed bank. Its populations are therefore resilient, and achieving sustained management of an infestation is difficult. As with other woody weeds, successful management requires a long-term, coherent, and painstaking approach. Effective management over large areas requires clear understanding of the extent of the problem, the economic and environmental capacity of the area, and the preferred land uses for the infested area. This understanding is important because the level of control required, and hence the tactics employed, may vary with preferred land use, and at a landscape level, there may be a mosaic of land uses. For example, in heavily infested areas where the economic or environmental future of the land is uncertain, no weed management may be a valid option. To manage this complexity requires effective GIS-based mapping of the infestation, definition of its distribution, and an estimate of weed density. This allows subdivision into areas for which practical management plans can be developed. Control of woody weeds is expensive, and setting priorities for resource allocation is important. Containment of the infestation should be the most important priority, followed by action that will limit future costs. In particular, control of outlying or low-density infestations will be more effective in the long term than control of dense thickets.

It is likely that effective management of such weeds over a range of land uses, topography, and weed densities will require the full range of appropriate and practical biological, chemical and mechanical control tactics available, integrated to provide the best and most cost-effective management strategy. Population models now allow us to explore the effects of control-tactic combinations on future infestation levels, without having to rely heavily on experimentation. Modelling suggests that biological control is likely to have an important role to play in reducing the maximum age of

plants (an important determinant of cover in both *U. europaeus* and *C. scoparius*), possibly increasing seedling mortality, and perhaps making weeds seed-limited (Rees & Paynter 1997, Rees & Hill 2001).

For a weed like *U. europaeus*, seeds in the soil are capable of reestablishing a population for at least 20 years after plants are removed (Hill *et al.* 2001). Long-term management plans based on land-use aspirations and on optimal control strategies must be developed for each management unit. Failure to plan on this scale risks wasting the resources expended in the early stages of weed management, and rapid reinvasion by the weed. Operational plans therefore need to transcend changes in personnel and land tenure, and to be resourced for the long term. For this reason, all potential stakeholders need to agree to the plans, and commit to maintaining operations. Finally, the long-term management plan needs to be continually reviewed and refocused as circumstances change.

Operational planning for *U. europaeus* management at Humu'ula

Long-term management plans were developed by August 2003. In preparation for this, aerial photographs of the infested area were taken at a scale of 1:24 000, scanned at 15 μ, and assembled as small .tif images. "Farmdata" (www.farmdata.com) has been selected as the mapping package. It is a simple GIS and GPS-capable package that will be used for defining and locating management areas, and for long-term record keeping.

Biological control of *U. europaeus* at Humu'ula

Biological control, particularly of seed production, is predicted to be a critical factor in suppressing *U. europaeus* at Humu'ula. The seed-feeding moth *Cydia succedana* may be introduced from New Zealand where it was released in 1992 (Hill & Gourlay 2002). Twenty-seven valued Hawaiian plant species bearing flowers and pods will be sent to New Zealand, where experiments will assess the susceptibility of these plants to *Cydia* attack before permission is sought to release the moth in Hawai'i. Other biological control agents have already been released at Humu'ula (Markin *et al.* 1996). It is thought that further control agents for *U. europaeus* exist in Spain and Portugal. Two surveys will be conducted in southern Europe to seek additional potential control agents (A. Sheppard, CSIRO Montpellier, pers. comm.).

Parameter determination, and modelling integrated control at Humu'ula

The recent model of *U. europaeus* population dynamics prepared by Rees & Hill (2001) showed that three key determinants of its cover are the rate of disturbance of the environment, the rate at which seedlings are successfully recruited from the seed bank, and seed production. The size of the seed bank is also important. These ecological characteristics can be manipulated using biological control, herbicides, fire, and over-sowing (Paynter & Flanagan 2003). The Rees–Hill model was compiled using parameter estimates from New Zealand. The following experiment will measure those parameters at Humu'ula to validate the model for Hawaiian conditions.

Four similar blocks (250 m × 30 m) were selected at four accessible sites at Humu'ula. Blocks are oriented across the prevailing wind to assist management operations. Firebreaks (10 m wide) were cut around each block and through the blocks to create eight identical treatment plots in each. Blocks were fenced to minimise unplanned disturbance. In each block, eight combinations (presence/absence) of herbicide, fire, and over-sowing (+H/+F/–O etc.) were randomly assigned to produce a standard randomised block design, replicated four times.

Prior to treatment, *U. europaeus* stem and seed bank density were estimated at each end of every plot. At the time of peak growth, herbicide was applied by air to the four assigned plots in each block. After 3 months, each assigned plot was individually fired. Measurement areas (5 m × 5 m) were established 5 m from each end of each plot and equidistant from the sides (128 areas in total). For unburnt plots (four per block) this involved cutting access into the *U. europaeus*. A mixture of *Holcus lanatus* (velvet grass, fog), *Dactylis glomeratus* (orchard grass, cocksfoot), *Lolium multiflorum* (annual ryegrass) and *Pennisetum clandestinum* (kikuyu) seed (at a rate of 2:6:10:1 kg/ha) was sown onto the measurement areas in plots assigned for over-sowing (as opposed to treating the whole plot).

Two permanent quadrats (40 cm × 40 cm) were selected within 1 m of a random point within each measurement area. Seedling emergence from the seed bank is measured by serial removal of seedlings from one quadrat, and in the other, the survival of cohorts of individual seedlings is monitored (128 measures). These measurements will be made monthly for 16 months. We have labelled 10 randomly selected plants or crowns in each treatment plot, and will monitor the survival of mature plants following each treatment combination (320 plants).

At the same random point in each measurement plot, two seed traps measure the amount of seed that falls under intact plants, and pod infestation rate is monitored (initially monthly, then twice annually). These

figures will be used to estimate the impact of control agents on the annual seed crop. We also measure the rate at which control agents recolonise different treatments by noting their presence or absence monthly in all plots. Seeds have been buried at four sites and will be recovered at 6-monthly intervals to estimate whether seedbank decline is likely to be fast or slow at Humu'ula (Hill *et al.* 2001).

These measurements will provide reliable estimates of the key population parameters for *U. europaeus* at Humu'ula and allow us to better calibrate the model (Rees & Hill 2001) for local conditions. We will check the validity of the model by comparing model predictions with real measures of seedling recruitment under the different disturbance (treatment) regimes. After 2 years, the plots will also provide a statistically sound assessment of the role that each of the control tactics plays in limiting recruitment of *U. europaeus* seedlings at Humu'ula. This will assist the development of long-term plans for sustainable management of the weed.

Treatments were completed in October 2002, and measurements were conducted in December, January and March. Unseasonal drought since October 2003 resulted in poor grass germination in over-sowing treatments and this will profoundly affect the experiment. It is too early to draw any other conclusions from this experiment.

Forestry and agro-forestry

Afforestation can enhance sustainable management of *U. europaeus* by shading out germinating seedlings until the seed bank is effectively exhausted, or by providing sufficient cash flow to justify intensive weed control. We are looking at both approaches.

Three uniform sites have been selected that are broadly representative of the land available for forestry operations at Humu'ula. Two-hectare plots have been selected, fenced, and protected by firebreaks. A range of trees are being planted in randomised complete block or eight-tree-row plot designs to assess which tree species and which seed sources within species will be most successful operationally at Humu'ula. Different assemblages of trees are being planted at each site. There will be 25–30 treatments (seed sources) in a 5 × 5 configuration, at 2-m spacing within the row and 3 m between rows. There will be 4–6 replications per treatment per trial. Tree performance will be assessed by analysing differences in tree survival, growth rate, and stem diameter increment at 6, 12, 18 and 24 months. The trees selected for evaluation are *Acacia koa*, *Cryptomeria japonica* (Sugi), *Eucalyptus* spp., *Pinus* spp. and *Pseudotsuga menziesii* (Douglas fir). Data will also be collected on the costs of establishment and maintenance of forestry operations at Humu'ula.

Over 250,000 Christmas trees are imported into Hawai'i annually. We will examine the feasibility of growing such trees at Humu'ula to substitute for imports, and to provide short-rotation revenue to improve the economics of long-term *U. europaeus* control. The tree species selected for assessment are *P. menziesii, Abies concolor* (Concolor fir), *A. fraseri* (Fraser fir), and *A. procera* (Noble fir). The design is the same as for the provenance trial, and will be replicated twice. Trees will require periodic shearing and fertilisation, and this site-intensive management regime will be combined with tight control of *U. europaeus* among the trees.

A 2-ha silvo-pastoral agro-forestry trial will demonstrate how trees can be integrated with livestock grazing and forage operations in this environment. Examples may include forest grazing amongst low-density plantings, or fence-line plantations. Such systems can provide greater income per acre than either forestry or grazing alone. Grazing can also provide weed control in such systems, further enhancing sustainability. Related research has demonstrated that, if properly managed, forage production can be maintained while producing high-value timber. There is significant potential for this agro-forestry system to become widely adopted in rangelands across Hawai'i. The trees will be established in single-, double- and triple-row sets planted along the contour. The stocking rate for trees will vary from 60 to 160 trees per hectare depending on the number of rows planted. The pasture alley will be over-sown with the same grass mixture that will be utilised in the agronomic trials and the pasture alley width will be 15 m. The forest tree species chosen will be a sub-set of those included in the forestry trials. *U. europaeus* density and growth rates will be assessed within and around all trials to measure the effectiveness of these treatments in suppressing infestations. The area around each trial will be monitored to check whether any of the tree species are spreading. Potentially invasive species will be removed from the trials.

Discussion

Knowledge of population dynamics is important for evaluating control, especially where control is costly or risky or where eradication is not possible. Timing of control strategies can be important due to density dependence or interactions with other control methods (Buckley *et al.* 2001). We model interacting control strategies in two ways. The first approach uses DYMEX™ and incorporates process-based sub-models (driven by environmental variables) to describe how control strategies affect population dynamics and weed management options. The second approach uses a simple, spatially explicit population model and incorporates the effects of integrated control options by the estimation of how those integrated strategies affect the population parameters in the model. The experiment described in this paper will provide new estimates of population parameters under different integrated

control regimes, which will then be used in the models. The use of this approach will enable us to compare our results with those from the original population model (Rees & Hill 2001).

Modelling the effects of combinations of control methods allows the evaluation of far more strategies than can be tested in the field, providing an objective method for narrowing down a wide range of options (Buckley *et al.* 2003).

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The role of biological control agents in an IWM program for *Chrysanthemoides monilifera* subsp. *rotundata* (bitou bush)

Royce H. Holtkamp[1]

Summary

Bitou bush, *Chrysanthemoides monilifera* subspecies *rotundata*, is a native of South Africa, which was used extensively in Australia as a sand-stabilising plant and for revegetation of coastal areas mined for mineral sands. It has now become a serious environmental weed in eastern Australia, primarily of conservation areas, where it significantly reduces biodiversity. Since 1989, six species of insects have been released on bitou bush, four of which have established. These are having varied impacts on bitou bush with bitou tip moth, *Comostolopsis germana*, and bitou seed fly, *Mesoclanis polana*, being the most successful. An integrated weed management approach appears to be the best option for long-term sustainable control of bitou bush. This paper discusses the use of biological control agents in combination with other control options such as strategic herbicide applications, fire, physical removal and revegetation techniques.

Keywords: biological control, bitou bush, *Chrysanthemoides monilifera* subspecies *rotundata*, integrated weed management.

The plant

Chrysanthemoides monilifera subspecies *rotundata* (DC.) T. Norl. (bitou bush), is a competitive environmental weed of South African origin. It is primarily restricted to areas of summer rainfall (Parsons and Cuthbertson 1992) and infests coastal areas of southern Queensland, New South Wales (NSW) and Lord Howe Island. There is also a localised inland infestation at Menindee Lakes, NSW. In NSW it is common north of Sydney and occurs south to the Victorian border.

C. monilifera subsp. *rotundata* was first recorded in Australia from Stockton near Newcastle in 1908 (Weiss *et al.* 1998) where it appears to have been an accidental introduction in ships ballast. From 1946 to 1968, *C. monilifera* subsp. *rotundata* was used as a sand-stabilising plant and to revegetate coastal areas mined for mineral sands. The capacity of *C. monilifera* subsp. *rotundata* to invade native vegetation was then recognised and its recommendation for coastal planting was withdrawn. However, this action came far too late and by 1976 *C. monilifera* subsp. *rotundata* was naturalised along much of the NSW coast.

A survey conducted in 2001 by the NSW National Parks and Wildlife Service (NPWS) has shown *C. monilifera* subsp. *rotundata* to be present on 900 km (80%) of the NSW coastline and the dominant plant on over 400 km. Over approximately two-thirds of this area, it could completely dominate and eventually displace most of the existing native vegetation. This current distribution represents a 36% increase in the area over which it was present in a 1982 survey that was also conducted by NPWS (Holtkamp *et al.* 1999).

The importance of *C. monilifera* subsp. *rotundata* was officially recognized in early 1999 by both the NSW National Parks and Wildlife Service which listed it as a "key threatening process" under the *Threatened Species Conservation Act 1995* and by the Commonwealth of Australia which listed it as a Weed of National Significance under the National Weeds Strategy 1997.

C. monilifera subsp. *rotundata* is largely an environmental weed as it is easily controlled by stock grazing and cultivation. It is primarily restricted to non-agricul-

[1] NSW Agriculture, Weed Biological Control Unit and Cooperative Research Centre for Australian Weed Management, RMB 944, Calala Lane, Tamworth, NSW 2340, Australia.

tural areas such as national parks, forests, coastal dune ecosystems and other recreational land. The impact and control of *C. monilifera* subsp. *rotundata* have been discussed in more detail by Holtkamp *et al.* (1999) and Vranjic (2000).

Biological control

A biological control program against *C. monilifera* (which includes *C. monilifera* subsp. *rotundata* and *C. monilifera* subsp. *monilifera* (boneseed)) was approved by Standing Committee on Agriculture in 1987. Surveys in South Africa have indicated that there are more than 100 species of phytophagous insects associated with the *Chrysanthemoides* species complex (Scott & Adair 1990). Eighteen of these species were identified as having potential for the biological control of *C. monilifera*.

Six of these species have now been released on *C. monilifera* subsp. *rotundata*, but only four have successfully established. These are: bitou tip moth, *Comostolopsis germana* Prout; bitou tortoise beetle, *Cassida* sp.; bitou seed fly, *Mesoclanis polana* Munro; and bitou leaf roller, *Tortrix* sp.

More than 200 releases of *C. germana* were made between 1990 and 1997 at 72 sites in NSW, ranging from the Queensland border to Tathra in southern NSW. It is now established along most of the NSW coast and it is believed that this insect has spread throughout all *C. monilifera* subsp. *rotundata* infestations, with population levels still increasing in some areas. Populations in excess of 400 larvae m^{-2} have occurred at some sites (Holtkamp unpublished data) despite the presence of two hymenopteran parasitoids, one of which parasitizes up to 50% of *C. germana* larvae (Holtkamp 1993). In many areas *C. germana* is having a significant impact on flowering and seed production of *C. monilifera* subsp. *rotundata*.

M. polana was first released in very low numbers in August 1996 at Iluka Bluff and Dunbogan. Since then, nine releases have been made on the NSW North Coast. By August 1998, *M. polana* had been found from near Fraser Island in Queensland to Tathra, a total of over 1200 km of coastline (Edwards *et al.* 1999). Over much of this area, population levels are extremely high and reductions in seed production in excess of 50% have been recorded.

Cassida sp. was first released at La Perouse (a suburb of Sydney) in 1995. A total of 12 releases was made, with locations spread over most of the NSW coast. Recent surveys have shown it to be present at all of these sites, but only close to the original release sites and only in low numbers. It would seem that the likely impact of *Cassida* sp. will be minimal.

Releases of *Tortrix* sp. commenced in 2001. There have now been more than 50 releases made at 20 sites along the NSW coast in a combined CSIRO Entomology/NSW Agriculture project. Due to prolonged drought this species proved extremely difficult to establish. Multiple releases were made at sites that had the best plant quality and there are signs of a *Tortrix* sp. population persisting at some of these sites. It now appears as if the drought is breaking and high hopes are held for this insect.

Integrated weed management

Despite the success of two biological control agents and expectations of success for another, it is apparent that the only viable answer for long-term control of *C. monilifera* subsp. *rotundata* is the integrated weed management (IWM) approach discussed by Vranjic (2000). This includes such strategies as biological control, physical removal, herbicides and fire. In the past, these traditional techniques have been used to reduce infestations and limit spread of *C. monilifera* subsp. *rotundata*. Unfortunately, these techniques are limited in their use for *C. monilifera* subsp. *rotundata* control for a number of reasons.

Physical control techniques are extremely labour intensive and are usually carried out by local volunteer groups targetting small areas. These groups mainly organise working parties to remove *C. monilifera* subsp. *rotundata* plants by hand pulling, although painting cut stumps with glyphosate is also practised. The cut-stump method is preferred by many workers because it results in minimal soil disturbance and subsequent erosion. Physical removal is particularly effective in small areas of high conservation significance. Larger scale control using these methods is not practical because it is too labour intensive. The possibility of removing *C. monilifera* subsp. *rotundata* in areas infested for many years is compounded by large soil seed banks. Weiss and Milton (1984) recorded a soil seed bank of 2030 seeds per m^2 near Moruya on the south coast of NSW, and Holtkamp (unpublished data) has recorded a soil seed bank of up to 1968 viable seeds per m^2 at Port Macquarie.

Herbicides aerially applied using helicopters have proven to be extremely effective for broad scale *C. monilifera* subsp. *rotundata* control (Toth et al. 1996). There is a "window of opportunity" during the winter period immediately following peak flowering. At this time *C. monilifera* subsp. *rotundata* plants are highly susceptible to the herbicides glyphosate and metsulfuron methyl, while over 180 native species tested were virtually unaffected. Further herbicide treatments are required approximately every two years until the soil seed bank is exhausted. It is important that none of the regenerating plants be allowed to flower and set seed. Unfortunately, herbicide application is not suitable in a number of situations such as in the presence of rare or threatened flora.

The interactions between biological control agents and herbicides were discussed by Ainsworth and Holtkamp (1999), who reached the conclusion that

herbicide application was unlikely to significantly affect populations of *M. polana*. However, any integrated program which incorporates herbicides and biological control will need to consider all species of biological control agents present at the time of treatment, to ensure that sufficient agents remain to allow reestablishment.

The use of fire, especially following herbicide application, stimulates germination of virtually the entire *C. monilifera* subsp. *rotundata* soil seed bank. This then leaves these seedlings vulnerable to attack by foliage feeding biological control agents or further herbicide applications. However, fire is not suitable for all areas because ecosystems such as coastal dunes and rainforests are not fire adapted.

Integrated control of *C. monilifera* subsp. *rotundata* has the potential to reduce this weed to a minor component of invaded vegetation, but will never eradicate it. Any integrated program will have to ensure that sufficient biological control agents remain following other forms of treatment to ensure reestablishment of biological control agent populations. Continuing physical and herbicidal control by volunteer groups in areas of high conservation significance is also important. The regeneration of coastal areas cleared of *C. monilifera* subsp. *rotundata* by local volunteer groups also forms an important component of this program. It is essential that revegetation of disturbed habitat occurs quickly, to prevent the niche previously occupied by *C. monilifera* subsp. *rotundata* from being occupied by *C. monilifera* subsp. *rotundata* seedlings or by another, perhaps more serious, weed species.

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Progress on the biological control of gorse (*Ulex europaeus*) in Australia

John E. Ireson,[1,4] Raelene M. Kwong,[2,4] Hugh Gourlay,[3] Jamie T. Davies,[1,4] Richard J. Holloway[1] and Wade S. Chatterton[1]

Summary

Gorse, *Ulex europaeus*, occurs in all Australian states, but principally in Tasmania and Victoria. An early attempt at biological control in Australia resulted in the establishment of the gorse seed weevil, *Exapion ulicis*, in 1939. *E. ulicis* is now widespread in Tasmania and Victoria, but its impact has been limited. In Tasmania, the number of pods attacked annually ranges from 15–44%. Gorse was declared a target for biological control in Australia in 1995. Since then, two foliage-feeding agents, the gorse spider mite, *Tetranychus lintearius* (of mixed European origin via New Zealand), and the gorse thrips, *Sericothrips staphylinus* (of English origin via New Zealand), have been released. *T. lintearius* was first released in Tasmania and Victoria in December 1998. By spring 2001, it had become widely established throughout most of the major gorse infestations in Tasmania and over large areas in Victoria. However, predation by the introduced Chilean predatory mite, *Phytoseiulus persimilis*, and the native coccinellid, *Stethorus histrio*, is already widespread. *P. persimilis* has been associated with the destruction of entire *T. lintearius* colonies in both Tasmania and Victoria, and it is expected that both predators will significantly restrict its impact. *S. staphylinus* was first released in Tasmania and Victoria in January and March 2001, respectively. Post-release surveys in Tasmania show that the agent has successfully established but dispersal is slow. Acceleration of its dispersal will need to rely on planned redistribution programs. However, *S. staphylinus* of Portuguese origin (via Hawaii via New Zealand) is now being reared for field release in Tasmania and Victoria to determine whether it spreads more rapidly than *S. staphylinus* of English origin. Planned releases of two additional European agents established in New Zealand, the gorse pod moth, *Cydia succedana* (a seed feeder), and the oecophorid moth, *Agonopterix ulicetella* (a foliage feeder), will be dependent on the outcome of investigations into their host specificity that are now being conducted. In the long term it is hoped that the combined effect of the biological control agents can reduce the spread, vigour and longevity of gorse and become useful components of area-based integrated management strategies.

Keywords: *Agonopterix ulicetella*, *Cydia succedana*, *Exapion ulicis*, gorse, *Tetranychus lintearius*, *Sericothrips staphylinus*.

Introduction

Ulex europaeus L. (Fabaceae), gorse, was introduced to Australia from Europe during the early 1800s, primarily as a hedge plant, though there was also interest in its potential use as a fodder crop (Parsons and Cuthbertson 2001). It occurs in all Australian states, but the main problem areas are principally in Tasmania and Victoria, where it has invaded pastoral land, significantly reducing pasture and animal productivity. It is also a significant problem along roadsides, forest plantations and bushland margins. In 1999, gorse was listed as a Weed of National Significance.

An early attempt at biological control resulted in the establishment of the gorse seed weevil, *Exapion ulicis*

[1] Tasmanian Institute of Agricultural Research, 13 St John's Avenue, New Town, Tasmania 7008, Australia.

[2] Keith Turnbull Research Institute, Department of Primary Industries, PO Box 48, Frankston, Victoria 3199, Australia.

3 Landcare Research New Zealand Ltd, Canterbury Agriculture and Science Centre, PO Box 69, Gerald St., Lincoln, New Zealand

[4] Cooperative Research Centre for Australian Weed Management
Corresponding author: John Ireson, Tasmanian Institute of Agricultural Research, 13 St John's Avenue, New Town, Tasmania 7008, Australia <john.ireson@dpiwe.tas.gov.au>.

(Forster) (Coleoptera: Brentidae). Originally from Europe, *E. ulicis* was introduced from New Zealand to Tasmania in 1939 (Evans 1942). Gorse was declared a target for biological control in 1995 by the Standing Committee of Agriculture and Resource Management following increasing concerns about the extent of the problem and the difficulty and expense of control. Since 1995, four additional European biological control agents established in New Zealand, have been investigated. These are: the gorse spider mite, *Tetranychus lintearius* Dufour (Acari: Tetranychidae), the gorse thrips, *Sericothrips staphylinus* Haliday (Thysanoptera: Thripidae), the gorse pod moth, *Cydia succedana* Denis & Schiffermüller (Lepidoptera: Tortricidae) and the oecophorid moth, *Agonopterix ulicetella* (Stainton) (Lepidoptera: Oecophoridae). This paper reviews the progress of work relevant to Australia carried out to autumn 2003, discusses future directions and the prospects for biological control.

Agents released

Exapion ulicis (gorse seed weevil)

Exapion ulicis is now widespread in Tasmania and Victoria, but its distribution in other states is unknown. In New Zealand, gorse produces seed in both spring and autumn, but *E. ulicis* was found to damage only *ca.* 36% of the annual seed pods (Cowley 1983), as only those produced in spring were attacked (Hill *et al.* 1991). In Australia, it was suspected that similar restrictions on the efficacy of *E. ulicis* applied (Ireson *et. al.* 1999) and a recent study by J.T. Davies (unpublished data) has now confirmed this. The study was conducted at two Tasmanian sites at altitudes of 30 m and 300 m. *E. ulicis* was univoltine at both sites. Larvae fed on seeds only during spring and early summer and were not present during the second period of seed production during autumn/winter. The number of pods produced annually that were attacked ranged from 15–44%.

Tetranychus lintearius (gorse spider mite)

Tetranychus lintearius forms large colonies that prefer to feed on mature gorse foliage and are capable of causing severe damage. The suitability of *T. lintearius* for release in Australia was based on host-specificity tests conducted on over 130 plant species (Hill and O'Donnell 1991, Ireson *et al.* 2003). *T. lintearius* was first released in Tasmania and Victoria in December 1998. Rearing and release techniques that resulted in the subsequent establishment in Tasmania and Victoria were detailed by Ireson *et al.* (1999).

By spring 2001, widespread releases (over 200 sites in Tasmania and *ca.* 100 in Victoria), community redistribution programs and windborne dispersal had resulted in *T. lintearius* becoming widely established throughout most of the major gorse infestations in Tasmania and over large areas in Victoria. *T. lintearius*, was also established at a site in southern NSW following releases there in 2000 (A.E. Swirepik pers. comm.).

Two predators, the introduced Chilean mite, *Phytoseiulus persimilis* and the native coccinellid, *Stethorus histrio*, are already widespread amongst *T. lintearius* populations in Tasmania and Victoria, and both have the potential to significantly restrict the usefulness of *T. lintearius* as a biological control agent (Ireson *et al.* 2003). *Phytoseiulus persimilis* has been associated with the destruction of entire colonies of *T. lintearius* in both Tasmania and Victoria (Ireson *et al.* 2003) as well as in Oregon, USA (Pratt *et al.* 2003).

In Tasmania, *T. lintearius* has dispersed more rapidly in the warmer drier regions of the state compared to cooler, wetter regions (Ireson *et al.* 2003). Preliminary results from a Tasmanian field study on the impact of *T. lintearius* have shown significant reductions in gorse biomass 12 months after infestation (J.T. Davies, unpublished data).

Sericothrips staphylinus (gorse thrips)

Sericothrips staphylinus can feed on all green gorse foliage (Hill *et al.* 2001), including newly germinated seedlings. Host testing on *ca.*120 plant species or cultivars enabled approval to be obtained for the release of *S. staphylinus* in Australia (Hill *et al.* 2001, J.E. Ireson and A.H. Gourlay unpublished data). *Sericothrips staphylinus* of English origin (via New Zealand) were first released in Tasmania and Victoria in January and March 2001, respectively.

By autumn 2003, *S. staphylinus* had been released at 103 sites in Tasmania and at 21 sites in Victoria. Establishment assessments at 23 Tasmanian release sites one and two years post-release resulted in recovery from 19 (83%) of the release sites. However, dispersal has been slow, with *S. staphylinus* still mostly confined to the bushes on which it was released. Poor dispersal of *S. staphylinus* of English origin has also been recorded in New Zealand and Hawaii, but *S. staphylinus* originating from Portugal was also released in Hawaii where it is reported to have dispersed rapidly (Hill *et al.* 2001). *Sericothrips staphylinus* of Portuguese origin (via Hawaii) are now established in New Zealand and were imported to Australia (via New Zealand) in October 2002. They are now being mass reared for field release in Victoria and Tasmania and will be monitored in both states to determine whether they spread more rapidly than *S. staphylinus* of English origin.

A glasshouse study was conducted in Tasmania during 2002 to assess the impact of *S. staphylinus* (English origin), ryegrass competition and grazing on the growth and survival of gorse seedlings. All three treatments combined reduced seedling survival to 7%. In addition, the study found that the presence of *S. staphylinus* alone reduced shoot dry weight of gorse by a mean of *ca.* 57% (J.T. Davies unpublished data).

Agents under investigation

Agonopterix ulicetella (oecophorid moth)

Agonopterix ulicetella is a potentially important control agent for gorse because the larvae damage new growth during late spring and summer (Hill *et al.* 1995). This agent has established well in Hawaii where it has caused extensive feeding damage (Markin *et al.* 1996). However, in New Zealand, where it was first released in 1990, it has performed poorly. Populations are still surviving in the field, but only small numbers of adults have been recovered and no larval populations have been observed (A.H. Gourlay unpublished data).

Planned importations of *A. ulicetella* to Australia will be dependent on the approval of host-specificity tests which have been conducted on over 100 plant species or cultivars (Hill *et al.* 1995, J.E. Ireson and A.H Gourlay unpublished data). These results are scheduled for review in Australia during 2003.

Cydia succedana (gorse pod moth)

In contrast to *E. ulicis*, *C. succedana* is a bivoltine species whose larvae are active during autumn as well as spring (Suckling *et al.* 1999, T.R. Partridge personal communication). Studies at a site in Canterbury, New Zealand (T.R. Partridge personal communication) showed that *E. ulicis* and *C. succedana* together reduced the annual seed crop of gorse at this site by *ca.* 56%. The proportion of the autumn seed crop reduced by *C. succedana* was *ca.* 10%.

Host-specificity tests conducted by Hill and Gourlay (2002) enabled the release of *C. succedana* in New Zealand in 1992. The release of *C. succedana* in Australia is still pending the outcome of ongoing host specificity tests.

Prospects for control

Long-term control of gorse will be reliant on the development of integrated management strategies (Richardson and Hill 1998), in which a suite of complementary biological control agents will be useful components.

In Australia, although predators may significantly reduce the impact of *T. lintearius* (Ireson *et al.* 2003), studies in Tasmania (J.T. Davies unpublished data) have indicated that populations can still reach high densities. These populations can cause severe damage to gorse in localised areas, after which numbers start to decline; probably as a result of predation or migration triggered by the presence of predators, colony size and the decline in food quality.

Although a combination of *S. staphylinus*, simulated grazing and ryegrass competition significantly reduced gorse biomass under glasshouse conditions (J.T. Davies unpublished data), confirmation of these results under field conditions will be required to provide a useful basis for the development of an integrated control strategy involving this species. It is possible that *S. staphylinus* of Portuguese origin may spread more rapidly than *S. staphylinus* of English origin. However, the role of *S. staphylinus* in any integrated control program in the short term will depend on acceleration of its dispersal, either by artificial means through direct releases from culture, or local redistribution programs.

In New Zealand, establishment of *A. ulicetella* has been sporadic, and then at low densities (Richardson and Hill 1998). In Hawaii, *A. ulicetella* has established and spread widely (Markin *et al.* 1996), and this suggests it could be a useful biological control agent in Australia. However, in south-eastern Australia, foliage feeding biological control agents that spend most of their life cycle exposed on their host plant seem vulnerable to natural enemies (Briese 1986, McLaren *et al.* 2000, Ireson *et al.* 2002).

Simulation and analytical modelling by Rees and Hill (2001) showed that seed feeders were important in gorse control providing they were used in conjunction with other management practices that reduce seed recruitment and seedling survival. Control of gorse using seed-feeding agents alone would be difficult because of the need to consistently destroy a high proportion of the annual seed crop to achieve significant results (Rees and Hill 2001).

In Australia, as in New Zealand (Hill *et al.* 1991), studies in Tasmania (J.T. Davies unpublished data) have shown considerable variation in seasonal seed production on bushes within and between sites. Some sites produce seed during both late spring/summer and late autumn/winter and, at other sites, seed is produced only during the spring/summer period. New Zealand impact studies (T.R. Partridge personal communication) and modelling simulations (Rees and Hill 2001) show that the proportion of autumn produced seed that is reduced by the feeding of *C. succedana* will be insufficient to control gorse. The suite of biological agents needed to reduce gorse vigour and seed output should therefore include additional European agents (either biotypes of known species or different species) that may significantly reduce autumn seed production.

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Dispelling the myths of biological control: extension activities of the CRC for Australian Weed Management

R.M. Kwong[1,2]

Summary

Biological control has been utilised in Australia against some 60 weed species for over 90 years. During this time we have enjoyed many successes including the spectacular control of *Opuntia* species and, through diligent host testing, have an excellent safety record. While we stand here and pat ourselves on the back, the general community has a quite different perception. The cane toad's introduction and devastating effects on biodiversity across north-eastern Australia is often quoted as an example of "biological control gone wrong", and serves as a constant source of mistrust amongst the general community. High hopes held by desperate farmers for the eradication of rabbits by the myxomatosis virus has contributed to a belief that biological control does not work. In our attempts to dispel such myths we have tended to over-sell the potential of biological control, using the prickly pear story as a classic example. As such, the Australian community's perception of biological control ranges from fear about its safety to complete faith that biological control is the answer to all pest problems. The community has an important role to play in biological control. Countless examples worldwide have proven that community participation in the release of biological control agents speeds up the process of their establishment. Moreover, community support is critical in providing political pressure for continuing government and industry funding. This can be achieved only through effective communication. This paper discusses the various community education, awareness and technology transfer programs undertaken by the Cooperative Research Centre (CRC) for Weed Management Systems since 1995, and outlines strategies for future activities of the current CRC for Australian Weed Management.

Keywords: Community groups, technology transfer, weed biological control, Weeds CRC.

Introduction

There is a growing mistrust of science within the general community (Cribb & Hartomo 2002). The community knows from experience that new things are not always good things, and even good things bring bad things with them. Popular fiction fulfils the public belief that, even though the intentions of the researcher may be good, the tinkering with the natural order produces calamity. Dr Frankenstein, Dr Jeckyll, the Nutty Professor are just some examples of the yardstick by which real-life researchers are frequently misjudged.

So after some 90 years of weed biological control in Australia, what does the community think of the biological control scientist? Eccentric? A nutty professor? Why should we care about what the community thinks about biological control?

Community support and participation in biological control is a key element to achieving a successful outcome (Briese & McLaren 1997, Swirepik & Briese 1999, Batchelor & Woodburn 2002). But how do we enlist this support and increase adoption?

It is here that we refer to the basics of extension theory. It starts with getting to know the target audience — understanding their beliefs, values and needs — then tailoring messages to suit the various segments of the audience to influence their perception and promote a

[1] Keith Turnbull Research Institute, Department of Primary Industries PO Box 48, Frankston, Victoria, Australia 3199.
[2] CRC for Australian Weed Management
Corresponding author: R.M. Kwong <rae.kwong@dpi.vic.gov.au>.

change in behaviour, ultimately resulting in adoption of the technology.

It sounds quite basic really, but there are few examples of biological control implementation programs in Australia at least, that have been based on a needs analysis, or an understanding of the barriers to adoption (Norton 1996).

This paper looks at some of these barriers and misconceptions about biological control within the Australian community and discusses how collaborative action through Cooperative Research Centres is providing a unique opportunity to improve community awareness and adoption of biological control on a national scale.

Biocontrol myths

The most commonly held myth associated with biocontrol is that it is not safe. In Australia we refer to this as the "cane toad syndrome", spawned from the fear that, like the cane toad, the agent might switch hosts and become a pest itself. The cane toad syndrome is well entrenched within the minds of most Australians. Despite our best efforts to dissociate modern-day classical biological control from the cane toad, the reality is that the community is informed enough to understand that there are risks associated with this practice. In the past we have attempted to dispel the cane-toad myth by overemphasising that "biocontrol is safe because years of stringent testing prove that the agent is host-specific". In reality, we know that there is a degree of risk involved, but we risk making the public more sceptical if we are not open and honest about it. Hence, in communicating with the public, we need to help them understand that the risks aren't really as great as they fear. Any delusion that it is possible to force on the public a belief that something is safe or wholesome, contrary to their suspicions is liable to have counterproductive effects (Cribb & Hartomo 2002).

The second myth takes us to the opposite end of the spectrum. "Biological control is a silver bullet and the answer to all our weed problems." This misconception results from over-optimistic promotion by biocontrol practitioners. The dramatic success of *Cactoblastis cactorum* in controlling prickly pear is often quoted as the biocontrol success story (Briese 2000).

Impressive "before" and "after" photos promote an unrealistic expectation that biocontrol will eliminate the weed. Rarely, do we show images of partial control, an 80% weed infested property reduced to 60%, for example. Failing to recognise partial successes has most likely promoted the idea that unless the weed is completely controlled, biocontrol has been unsuccessful.

Following on from the silver bullet mentality is the belief that biocontrol is all that is needed; "I have biocontrol on my property, therefore I don't need to do anything else". Unfortunately, some landholders see biocontrol as an easy option, an excuse to avoid more expensive methods of weed control, while, for the majority of others, it is a lack of understanding of how biocontrol fits into the overall management of the weed. This is caused simply by a lack of available information on integrated weed management.

Engaging the community

As previously outlined, the Australian community's perception of biological control ranges from fear about its safety to complete faith that biological control is the answer to all pest problems. This presents a challenge to biocontrol practitioners attempting to improve the biocontrol image and increase adoption. However, the key is to engage both facets of the human character, the innately adventurous and the innately cautious.

Australian biocontrol practitioners are not oblivious to the community's perceptions of biocontrol (Briese 2000, McFadyen 1996), though most institutions lack the resources to fully address the issues adequately and to overcome the barriers to adoption.

Weeds are a societal issue and individual land managers are not able to appropriate all the benefits of classical biological control (Auld 1998). Hence, government plays the major role in the funding of biological control programs. There is no one particular organisation responsible for biocontrol research and development in Australia. The vastness of the Australian continent, its diversity of climates and habitats, distribution of weeds and state government weed priorities all contribute to weeds being of both state and national concern. As such, biocontrol research and development is carried out by state agriculture and natural resource management (NRM) departments, by universities and, federally, by CSIRO.

The agenda or charter of each agency, combined with expertise of constituent staff, naturally results in agencies having particular strengths in certain aspects of the biocontrol discipline. Collectively, this provides Australia with a huge intellectual resource. However, it is only relatively recently that a framework has been established that allows for the effective collaboration of weed biological control research across Australia.

This framework takes the form of a cooperative research centre (CRC); an Australian Government initiative aimed at pooling the resources of agencies working in related areas to carry out collaborative research. Weed biological control researchers have been involved in three CRCs; the Centre for Tropical Pest Management (CTPM) (1991–1998), the Cooperative Research Centre for Weed Management Systems (CRCWMS) (1995–2002), and the current, nationally-focused CRC for Australian Weed Management (Weeds CRC) (2001–2008). The Weeds CRC consists of 132 researchers from 19 participating agencies.

Cooperative Research Centre for Australian Weed Management

The activities conducted and benefits of the two former CRCs (CTPM and CRCMWS) in enhancing the efficiency of weed biological control have been outlined by Briese (1999, 2000) and McFadyen (1996). These CRCs were predominantly climatically (temperate and tropical) and species-focused, with the activities of the CRC largely researcher driven. The current Weeds CRC has a much broader focus and has endeavoured to align its mission and desired outcomes to meet the current and future needs of the community and government (as reflected in policies such as the National Weeds Strategy 1997). The most significant change has been in the formation of targeted end user groups (TEGS) to guide the research direction more from the users' perspective. An effective extension program is critical to support this client-led research, to ensure research results are relevant, applicable and embraced by the community.

The Weeds CRC consists of three systems-based research programs (Program 1 – Weed Incursion and Rapid Response, Program 2 – Sustainable Cropping Systems and Program 3 – Landscape Management), a communication provider (Program 4 – Community Empowerment) and an education provider (Program 5 – Education). Development of suitable biological controls, including mycoherbicides, for weeds in cropping systems is conducted within Program 2, while the development of generic models for enhancement of agent selection, improved methodologies for host testing and strategies for establishment and distribution is conducted within Program 3. Research is also conducted in Program 5, through CRC-funded Postgraduate Scholarships.

The "extension" arm of the CRC lies within Program 4 – Community Empowerment, and serves two main functions; 1) to increase community awareness of weeds, and 2) to increase the adoption of research findings. Unlike the other stand-alone programs, Community Empowerment is, in reality, a "service provider" to both the CRC research programs and the general community.

For the Weeds CRC to position itself as the "source of the best available information on weed management around Australia", a utilisation strategy has been formulated to ensure effective communication of weed research to the broader community and to facilitate the transfer and adoption of weed management technologies.

A communication strategy for biological control

Biocontrol research should ultimately provide products and technology that require transfer mechanisms to ensure adoption by end users. However, the route-to-market, communication/marketing strategy is a complex one for biocontrol. Traditional products of agricultural research, such as herbicides and farm equipment, are all cited as examples of technology readily taken up by farmers and other land managers. These are commercialised products that carry with them the fruits of technological advances. They are the "silver bullets" of research, commercialised, promoted, sold from the shelf and rapidly adopted. Biological control, however, is much less tangible than the silver bullets. Its technology is contained within a sea of information (such as weed ecology, insect ecology, plant pathology and integrated weed management) that, if incorporated into a management system, can make major improvements in the long-term management of weeds.

The fundamental difference between this technology and that contained in a silver bullet is that its adoption requires complex changes to the way landholders think and go about their daily work. In this respect, the adoption of biological control bears more resemblance to the embrace of a religious belief system than to the purchase of a product from a supermarket shelf. Transferring technology contained in a commercial product via its sales across a counter is a relatively simple process. On the other hand, the "transfusion" of information necessary to create a belief system is a far more intimate and intrusive procedure (Boomsma 1997).

For these reasons, communicating the benefits, limitations and techniques of biological control in a way that promotes acceptance of the technology and leads to its adoption requires a more complex communication strategy.

The Weeds CRC's approach to biological control awareness and adoption consists of three main elements; 1) to improve the capacity of CRC researchers to individually and collectively promote and deliver research outcomes, 2) to improve the general community's awareness of biological control, and 3) to enhance the adoption of biological control by land managers through the evaluation and refinement of best-practice approaches to biocontrol technology transfer.

Improving the capacity of CRC researchers to communicate research outcomes

The current Weeds CRC has recognised that the Community Empowerment Program (P4) alone cannot meet the communication needs of such a large organisation. As such, the CRC has attempted to address this in two ways. Firstly, communication and extension activities are built into research project agreements (RPAs). This requires each research project team, through the assistance of communication specialists from P4, to go through the process of planning how they will deliver findings and achievements to various audiences. The second initiative addresses the fact that many scientists are reluctant to communicate with the

wider audience because they have no training or little experience in science communication. To address this, the CRC has provided opportunities for staff and post-graduate students to gain confidence and enhance their communication skills through the provision of media skills workshops, conducted by science communicators and media professionals.

Improving community awareness and adoption of biological control

In implementing a communication strategy, firstly we need to consider who is the audience we are trying to reach, as the messages that we deliver and the strategies that we use to deliver these will be dependent on the target audience. This audience can be grouped into three broad categories (Fig. 1);

1. End users: these include landowners and managers of public and private land. The so called "early adopters" of the community often form groups called Landcare groups, to tackle local land management problems, such as weeds, pests, salinity and soil erosion.
2. Intermediate users: these are the providers of extension services such as specialist consultants, government extension officers and community group facilitators.
3. General public: this group also includes politicians and policy-makers, who are influenced by public opinion.

Ultimately, CRC research results in information, products and technology that need to be disseminated to the various audiences. The mechanism by which the CRC does this can be described as a funnelling approach (Fig. 2). This is a fairly standard communication technique, which graduates from impersonal type extension activities towards more personal approaches as progress is made from community awareness through to landholder adoption (Chamala & Mortiss 1990).

Increasing community awareness

The CRC utilizes a full range of communication technologies to raise community awareness of weeds, including media (press releases and media stories), newsletters (*Weed It and Reap*, *Weed Watch*), a web site (http://www.weeds.crc.org.au) and displays for field days and expos. A significant achievement has been the CRC's involvement in National Weedbuster Week which was instigated in 1997, and continues today to emphasize the importance and recognise the achievements of community weed action (Vitelli *et al.*1999). Community groups and schools participating in biocontrol agent releases is a popular activity during Weedbuster Week.

Increasing community awareness of biological control might stimulate interest but it does not automatically lead to adoption. Often, further impetus is required using other communication strategies tailored to the needs of the intermediate and end users.

Increasing the skills of intermediate users

Intermediate users are the front-liners, who are skilled in influencing end users to adopt technology. Many of these people have some form of tertiary or vocational education, but surprisingly have received little training in weed management and even less in biological control. Unless they are knowledgable and credible, intermediate users will have little hope of influencing community adoption of biocontrol. The Education Program of the CRC is addressing this through the development of weed management courses and curricula resources for vocational and tertiary education, and through the running of "train the trainer" type workshops. Extension materials, in the form of Best Practice Management Guides, the Weed Management Text Book and brochures are also produced.

Figure 1. The target audience of a Weeds CRC Biocontrol Communication Strategy is divided into three main categories; end users, intermediate users and the general public.

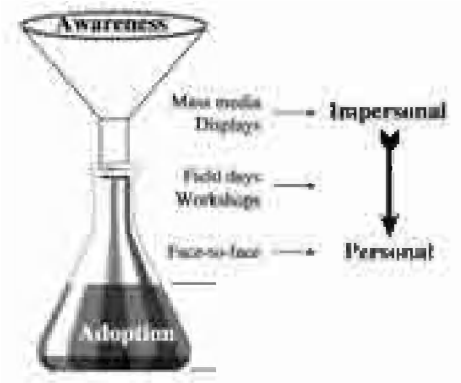

Figure 2. The funnelling approach is an effective communication technique that graduates from impersonal types of communication activities towards more personal approaches to influence a change from general awareness to adoption of the technology.

Increasing adoption of biological control

It seems not all that long ago that the involvement of community groups in biocontrol was considered an innovative way of getting more agents out into the field (Darby & McLaren 1993). This "top-down" approach to biocontrol delivery seems highly effective from the outside, with hundreds of releases being achieved in short periods of time, but they tend to disintegrate when there is no researcher continually providing a driving force.

The CRC has invested a lot of resources into improving the "community biocontrol network" model. Since 1995, a total of 33 biocontrol agents has been released against 13 weed species, and three biocontrol facilitator positions were funded to assist in the coordination of releases across state boundaries (Briese 1999).

The most significant advance of the CRC has been to change biocontrol delivery from a top-down approach to a bottom-up approach. Researchers are working directly with community groups and their facilitators to provide them with the skills necessary to be proactive in planning biocontrol integration into regional weed management plans. This results in greater ownership of the process and, therefore, increased adoption.

Outcomes of CRC research into integrated weed management, biocontrol agent release, establishment and monitoring strategies are being delivered directly to community groups and their facilitators through field days, workshops and site visits. These principles are also reinforced through extension material, such as specific biocontrol agent information kits.

The current and future activities of the CRC are focused in three main areas;

1. Expanding community biocontrol networks, particularly into northern Australia. This will be particularly challenging since there are few Landcare groups in this region and the majority of the land is owned by the Aboriginal community, whose capacity to adopt biocontrol may be hampered by cultural beliefs, poor English-language skills and low income.
2. Empowering weed extension officers to become local experts in biocontrol. This initiative, being conducted in association with the South Australian Government provides funding incentives to encourage regional weed officers to develop their skills in biocontrol delivery. Officers are firstly trained in the techniques of biocontrol implementation through workshops, with particular emphasis on integrating biological control into local weed management plans. They are then supported with funding to enable them to plan and conduct biocontrol projects on a range of weeds in association with community groups.
3. Educating the next generation. This project, called Weed Warriors, recognises that increasing the awareness of children about weeds is an effective way of nurturing a better-educated wider community (Kwong 2002). The CRC aims to develop a national Weed Warriors program, and is working in with the CRC Education Program and state education departments to develop curricula resources for teachers. The strengths of the program lie in the formation of partnerships linking students with members of their local community group to learn and participate in real-life biological control programs.

Discussion

Increasing public scrutiny and dwindling funds for research are tightening the noose around the weed biological control discipline. Biocontrol practitioners are largely to blame for this, for two main reasons. Firstly, the public is, and continues to be, largely disengaged from the biocontrol process. Lonsdale *et al.* (2003) indicated that New Zealand was the only country that currently had a public consultation phase enabling the public to consider the potential risks and benefits of biological control. The result of community disengagement is that the public is unable to weigh up the pros and cons of releasing biological control agents and, therefore, takes a precautionary view. Secondly, biocontrol practitioners either neglect to incorporate communication into research projects or are using out-of-date extension techniques such as the linear model of information transfer from research to extension. The result is that the community lacks the motivation, information and skills to adequately adopt biological control.

As biocontrol practitioners, we need to analyze the way we operate if we are to improve the implementation of biological control. In particular, we need to recognize that biological control needs to be communicated not as a reductionist approach, but as a systems approach. This latter approach takes on a holistic view of the situation and allows for interaction between the separate parts of the system to be analyzed and understood (Röling & Jiggins 1998). Once we know how to go about learning and understanding a complex situation, we have laid the foundations for the decision-making process that can lead to its improvement (Wilson 1998).

Finally, we need to recognize that biological control implementation is more about social science than applied research, and requires an understanding of the end-users values, needs and capacity to understand, desire and adopt biological control. The future challenge of the Weeds CRC is to encourage research organisations to engage in effective and focused dialogue with the general community, intermediate and end users that will result in sustainable improvements in weed management from the local to national level.

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Perspectives on biological control of invasive plants in Latin America

Julio C. Medal[1]

Summary

Invasive plants can cause a global average of up to 20–30% crop yield losses, or even higher in the Latin American region. Manual removal, mechanical tools, and herbicides are the major weed management practices currently used in the agricultural systems in Latin America. Biological control of invasive plants, using mainly host-specific insects, and to a lesser extent plant pathogens, has been traditionally practised in developed countries such as Australia, United States, South Africa, Canada, and New Zealand, primarily in rangeland situations, aquatic systems, and conservation areas. Biological control of invasive plants has not been utilized in most of the Latin American countries. This can be partially attributed to the lack of personnel trained in this discipline. Chile can be considered the pioneer country in the region. Research efforts were initiated there as early as 1952 to successfully control an invasive, non-native plant *Hypericum perforatum* L. (Clusiaceae). Others countries with some classical and/or non-classical weed biocontrol activities include Brazil, Argentina, and Mexico. Recent successes with biological control of invasive plants in non-crop and agricultural situations in developed countries could be implemented in the low-input farms and conservation areas of the Latin American region. Several of the most serious weeds in Latin America include *Rottboellia cochinchinensis* (Lour) Chyton, *Cyperus rotundus* L., and *Portulaca oleracea* L. These weeds are appropriate targets for classical biological control because they are not native to this region and they cause significant economic damage to justify the research costs. In addition to this, costs can be significantly reduced via the "short route". In conclusion, biological control with insects and/or pathogens can provide an effective, safe, and low-cost solution to the Latin American region's most important invasive plant problems.

Keywords: biological control, invasive plants, Latin America, training.

Introduction

Invasive plants can cause a global average of up to 20–30% crop losses, or even higher in the Latin American region. On the other hand, it is difficult to estimate the number of native species or biodiversity lost due to invasive plants. Manual removal, mechanical tools, and herbicides are the major weed management practices currently used in the agricultural systems in Latin America. Biological control of invasive plants, using mainly host-specific insects and, to a lesser extent, plant pathogens, has been traditionally practised in developed countries such as Australia, United States, South Africa, Canada, and New Zealand, primarily in rangeland situations, aquatic systems, and conservation areas (Julien & White 1997, Julien & Griffiths 1998). Biological control of invasive plants has not been utilized in most of the Latin American countries. Chile can be considered the pioneer country in the region where research efforts were initiated as early as 1952 to successfully control an invasive, non-native plant *Hypericum perforatum* L. (Clusiaceae) (Norambuena & Ormeño 1991). Other countries with some classical and/or non-classical weed biocontrol activities include Brazil, Argentina, and Mexico (Medal 2003).

Potential for biological control of invasive plants in Latin America

Recent successes with biological control of invasive plants in non-crop and agricultural situations in developed countries could be implemented in the low-input farms and conservation areas of the Latin American

[1] University of Florida, Department of Entomology & Nematology, POB 110620, Gainesville, Florida 32611, USA <medal@mail.ifas.ufl.edu>.

region. The important invasive plants in Central America (Table 1) include *Cyperus rotundus* L, *Rottboellia cochinchinensis*, (Lour) Chyton, *Portulaca oleracea* L., and *Sorghum halepense* L. (CATIE 1990, Muñoz & Pitty 1994, Pitty & Molina 1998, Medal 2003). A diverse number of arthropods (26 species) were found damaging *S. rhombifolia* in South America (Vogt and Cordo 1976). Several potential natural enemies were found feeding on *C. rotundus* and *S. halepense* in explorations conducted in South-East Asia (DeLoach 1990) A biological control project for *R. cochinchinensis* using the fungus *Sporisorium ophiuri* (P. Henn.) Vanky is under way by CABI (United Kingdom) and CATIE in Costa Rica. The smut fungus looks highly promising (Ellison 1993, Sanchez *et al.* 1997, H.C. Evans, pers. comm.). These weeds are appropriate targets for classical biological control because they are not native to this region and they cause significant economic damage to justify the research costs. In addition to this, costs can be significantly reduced via the "short route", which makes use of existing technology that has been successful in other regions of the world (Harley & Forno 1992). An example is the effective control of *Eichhornia crassipes* (Mart) Solms-Laubach by the two *Neochetina* weevils in Sinaloa, Mexico (Alejandro Pérez, pers. comm.). *Pistia stratiotes* L. is another aquatic plant that is causing significant economic damage in the Central America region sufficient to justify the biocontrol implementation costs.

Major constraints to the implementation of biological control of invasive plants in Latin America

Lack of personnel trained in this discipline in the region

The lack of personnel trained in this discipline in the Latin American region is a major limiting factor for the implementation of biological control projects against invasive plants using arthropods/or pathogens. Training efforts were initiated by the University of Florida in cooperation with the Universidad Nacional Agraria of Nicaragua (UNA), the Instituto Nacional de Tecnología Agropecuaria (INIA–Carillanca) of Chile, and the USDA–ARS South American Biological Control Laboratory in Argentina. A one-week intensive course was conducted in June 2002 in Nicaragua with 78 participants from 17 countries. This kind of training activity should continue to contribute to the development of the discipline in the region, and increase the possibility that Latin America could play a more important role in biological control of invasive plants in the near future.

Few quarantine buildings in the region

Most of the Latin American countries do not have quarantine facilities for the introduction of arthropods or pathogens for biocontrol of invasive plants. However, most of the countries in the region have quarantines for introduction (handling and screening) of parasites and/or predators for biocontrol of arthropod pests. These facilities could be adapted for the introduction of biocontrol agents for invasive plants (Norambuena 2003).

Limited funds

Funds for any type of agricultural research are scarce. The funds required to initiate a new project to control an invasive plant are relatively high. However, the ecological/or economical benefits obtained, if the biocontrol agent is successful, will recover the investment. Based on the limited experience in the region and on the limited resources available, it is recommended to initiate projects using the "short route" rather than initiating a completely new biocontrol program.

Conclusions

There is a great potential for the biological control of invasive plants with insects and pathogens in Latin America. These control tactics can provide a highly effective, environmentally friendly, low cost, and

Table 1. The most important invasive plants in Central America.

Scientific name	Common name	Family	Origin
Amaranthus spinosus	Spiny amaranths	Amaranthaceae	Tropical America
Cyperus rotundus	Purple nutsedge	Cyperaceae	India
Portulaca oleraceae	Purslane	Portulacaceae	India
Rottboellia cochinchinensis	Itchgrass	Poaceae	India
Sorghum halepense	Johnsongrass	Poaceae	Mediterranean
Bidens pilosa	Hairy beggarticks	Compositae	America
Sida rhombifolia	Sida	Malvaceae	Pantropica
Pistia stratiotes	Water lettuce	Araceae	South America
Eichhornia crassipes	Waterhyacinth	Pontederiaceae	South America

sustainable solution to the most important invasive plants in conservation areas and aquatic systems. Pathogens could play a major role in the most altered and complex combinations of multiple crops practised by farmers in the region. Continued training of local researchers in the principles and procedures for biological control of invasive plants is a key factor that can contribute to the greater utilization of this technique in the Latin American countries.

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Plant-mediated interactions between *Neochetina* spp. weevils and the fungal pathogen *Cercospora piaropi* on *Eichhornia crassipes* (water hyacinth)*

Patrick J. Moran[1]

Summary

Insect biological control agents of weeds may aid infection by plant pathogens by generating wounds or by vectoring. Pathogen infection may lead to plant biochemical changes that alter host suitability for insects. In *Eichhornia crassipes* (water hyacinth), *Neochetina bruchi* and *N. eichhorniae* adult weevils feed mostly on immature leaves, while symptoms of infection by *Cercospora piaropi*, a fungal pathogen, occur mostly on old leaves. This study examined associations between weevil scarring and fungal spotting (necrosis) on leaves, and determined if necrosis is associated with levels of biochemical factors that may influence weevil feeding. Scarring and necrosis scores were positively correlated across four field sites sampled at four times. At individual sampling times, two of the four sites tended to have higher scarring and necrosis scores, but scores were not correlated. Total available carbohydrate, potassium and phenolic contents did not vary across sites in the same manner as did necrosis scores. Peroxidase activities and potassium levels in furled leaves were positively correlated to necrosis scores in oldest non-senescent leaves. Phenolic content in late-season samples was also correlated to necrosis. Prior *C. piaropi* symptom production on old leaves of cultivated plants did not influence weevil feeding on young leaves. Leaf scarring and necrosis were related, but fungal infection did not alter the feeding of *E. crassipes* weevils by changing plant biochemical components.

Keywords: defence, induction, insect–pathogen synergism, nutrition, plant stress.

Introduction

Insects and plant pathogens have been employed as biocontrol agents against the same weed species. Examples include *Carduus* spp. (musk thistle) (Charudattan 2001) and *Chondrilla juncea* (skeletonweed) (Julien & Griffiths 1998). Many more weeds have been targeted with pathogens alone (Charudattan 2001). Little is known about the integrative effects of biocontrol by combinations of insects and pathogens (Zidack 1999; Caesar 2000). Many pathogens gain entry into plants via wounds made by insect feeding. Such 'direct' interactions (Hatcher 1995) could generate additive or synergistic biocontrol effects (Caesar 2000). Biological control by insects could be influenced by plant biochemical responses to pathogen infection (Zidack 1999). Fungal infection increases peroxidase activity and phenolic defences (Hammerschmidt & Kuć 1995) and alters the protein and carbohydrate nutritional composition of tissues in ways known to influence insect feeding and survival (Hatcher 1995).

Biological control of *Eichhornia crassipes* (Mart) (Solms.) (water hyacinth) in the south-eastern United States has involved, among other agents, two introduced weevil species, *Neochetina eichhorniae* (Warner) and *Neochetina bruchi* (Hustache), and a native fungal pathogen, *Cercospora piaropi* Tharp. Adult weevil feeding on mostly furled and young unfurled leaves generates scarring damage, and fungal infection accelerates leaf senescence, leading to the

[1] USDA, Agricultural Research Service, Beneficial Insects Research Unit, 2413 E. Hwy 83, Weslaco, TX 78596 USA. <pmoran@weslaco.ars.usda.gov>.

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development of necrotic lesions on mostly old leaves. Scarring and necrosis vary among field sites and environments (Freeman *et al.* 1981; Center *et al.* 1999). The objectives of this study were to determine if scarring and necrosis were associated in young and old leaves of field plants, and if necrosis influenced plant biochemical factors or altered weevil feeding.

Materials and methods

Field sampling

Four field sites in the Lower Rio Grande Valley of Texas were sampled four times: June–July (summer) 2001, November (fall) 2001, April (spring) 2002, and July (summer) 2002. Site 'Lake Canal' (LC) was a continuously flowing canal connecting two sides of a reservoir. Site 'Rio Grande' (RG) was located directly on the Rio Grande, at the mouth of a pumping station inlet. Site 'Inlet' (IN) was a closed, disused inlet, adjacent but not connected to the Rio Grande. Site 'Resaca Canal' (RC) was an irrigation canal adjacent to series of small reservoirs. Plastic pipe squares (0.5 × 0.5 m) were thrown into mats of plants to define sampling units (4–5 units per site per sampling time). The two youngest and two oldest unfurled leaves were collected from five plants in each unit. The percentage of the adaxial leaf laminar surface covered with feeding scars made by *Neochetina* spp. weevils was visually estimated in youngest and second-youngest unfurled leaves. Percentages were converted into scores based on the following scale: 0 = no scarring; 1 = < 5%; 2 = 5%–10%; 3 = 11%–20%; 4 = 21%–40%; 5 = > 41%–60%; 6 = > 60% coverage. Per cent adaxial leaf surface coverage with necrotic spots, indicative of *C. piaropi* infection, was visually estimated on the second-oldest and oldest leaves and converted to the following scores: 0 = no necrosis; 1 = < 5%; 2 = 6%–15%; 3 = 16%–25%; 4 = 26%–50%; 5 = > 50% coverage. Scores were summed across plants to yield one score for each unit.

Biochemical analyses

The furled leaf and the youngest unfurled leaf were collected from three plants per unit and were pooled and frozen at –80°C. All colorimetric analyses were performed on a Spectronic Genesys-2 spectrophotometer. Total soluble protein content and peroxidase activity were examined as in Showler & Moran (2003) using Bradford reagent (Sigma) at 595 nm for protein and guaiacol reagent at 470 nm for peroxidase. Total available carbohydrate (TAC) content was determined in 30 mg lyophilised samples as in Center & Van (1989) using anthrone reagent and glucose standard at 625 nm. To determine potassium content, 0.15 g lyophilized samples were ashed at 600°C for 6 hours, dissolved in 0.02N HCl and filtered through Whatman #4 paper. Samples were read with a Jenway PFP 7 flame photometer using potassium chloride solutions as standards. Water-soluble phenolic content was assayed using procedures modified from Center & Van (1989). Lyophilized samples (50 mg) were extracted once with 5 mL diethyl ether and three times with 5 mL 80% methanol: 1% HCl (99:1). Pooled methanolic supernatants were extracted with 6 mL hexane and concentrated under reduced pressure at 35°C. Phenolics were quantified with the Folin-Ciocalteu reagent (Sigma-Aldrich) at 760 nm. Chlorogenic acid, a common phenolic in *E. crassipes* leaves (Martyn & Cody 1983; Center & Wright 1991), was used as a standard.

Inoculations and bioassays in greenhouse

Eichhornia crassipes plants were grown in 1000 L outdoor tanks filled with irrigation water containing 5–7 ppm nitrogen (not augmented), 5 ppm phosphorus (P_2O_5) and 1ppm iron (chelated ferric form). Six five- to eight-leaf plants were placed into 20 L tanks containing water from the 1000 L tanks. *Cercospora piaropi* was cultured on potato dextrose agar. Conidia and mycelial fragments from two-week-old cultures were scraped off and suspended in water (1×10^6 fragments per mL) containing 0.1% Tween-20. The youngest unfurled leaves on *E. crassipes* plants in the 20 L tanks were gently abraded with sandpaper and inoculum applied with a pump aerosol sprayer. Mock-inoculated leaves received water containing 0.1% Tween 20. After two weeks of symptom development, tanks were caged with fine mesh, and 75 field-collected *N. bruchi* and *N. eichhorniae* beetles (species ratio approximately 2:1) were added to each cage. After one week, feeding scars on both surfaces of the laminae of inoculated leaves (four–five positions down from the shoot apex) and the two youngest unfurled leaves were counted. Leaf area was determined with a Li-Cor LI-3100 leaf area meter. Feeding was expressed as scars per cm^2 area.

Statistics

Poisson regression in PROC GENMOD (SAS Institute 1999) was used to determine the effects of field site and time on scarring and necrosis scores across all sampling times, and the effect of site on scores within each time. Pearson Chi-square adjustments to standard errors were used to correct for overdispersion when needed (Allison 2001). Pairwise contrasts tested for differences among sites, with P for significance adjusted to 0.0083 based on six independent comparisons. TAC and potassium content variation across sites and over time were examined with repeated measures analysis using PROC MIXED. Akaike's finite sample Information Criterion (AICC) (SAS Institute 1999) was minimized by specifying unstructured covariance with banding. Within sampling times, two- and one-factor analyses of variance in PROC GLM evaluated leaf age and site effects, and feeding in the greenhouse bioassay. Spearman rank correlations examined associations between scarring and necrosis scores and biochemical factors.

Results

Scarring and necrosis scores

Across all sampling times, summed scarring scores for youngest and second-youngest unfurled leaf laminae were strongly correlated ($r = 0.68$, $n = 64$, $P < 0.001$), as were summed necrosis scores on the second-oldest and oldest leaves ($r = 0.68$, $n = 64$, $P < 0.001$). Across all sampling times, scarring scores were positively, significantly correlated to necrosis scores, (e.g. scarring on the second-youngest leaf to necrosis on the second-oldest leaf, $r = 0.40$, $n = 64$, $P = 0.001$; to oldest leaf necrosis, $r = 0.52$, $n = 64$, $P < 0.001$). Scarring and necrosis scores were higher (1.4-fold and 1.8-fold, respectively) on plants from sites IN and RC than from sites LC and RG, although these two pairs of sites differed significantly only for necrosis on the oldest leaf (Fig. 1A). Scarring scores on second-youngest unfurled leaves varied across sites ($\chi^2 = 18.0$, df = 3, $P < 0.001$) and sampling times ($\chi^2 = 28.9$, df = 3, $P < 0.001$), as did necrosis scores on oldest leaves (site effect, $\chi^2 = 48.3$, df = 3, $P < 0.001$, time effect, $\chi^2 = 114.6$, df = 3, $P < 0.001$). However, site-to-site variation in scarring on second-youngest leaves did not usually occur at individual sampling times (Fig. 1B). Necrosis scores varied significantly by site at all four sampling times ($\chi^2 \geq 8.8$, df = 3, $P < 0.05$), but were never higher at both sites IN and RC than at sites LC and RG (Fig. 1C). The significant time effects and site-by-time interactions (necrosis only) ($\chi^2 \geq 55.4$, df = 9, $P < 0.001$) reflected increases in damage and necrosis between the summer and fall 2001 sampling times, and decreases by spring 2002 (Fig. 1B, 1C). Scarring and necrosis scores were not correlated at individual time points ($P > 0.05$).

Biochemical measures and necrosis

Total available carbohydrate content did not vary by site in furled leaves ($P > 0.05$) (data not shown). In youngest unfurled leaves, TAC content varied across sites (F = 11.4, df = 3, 16, $P < 0.001$) and sampling times (F = 34.1, df = 1, 40, $P < 0.001$) with linear (F = 3.6, df = 3, 40, $P = 0.02$) and quadratic (F = 53.7, df = 1, 40, $P < 0.001$) site-by-time interactions. However, patterns of variation among sites in TAC content were not consistent with the trend of higher scarring and necrosis at sites IN and RC (Fig. 2A). Potassium content varied by site in both furled leaves (F = 7.3, df = 3, 15, $P = 0.003$) and youngest unfurled leaves (F = 5.6, df = 3, 16, P = 0.008) but did not vary according to sampling time ($P > 0.05$). In youngest unfurled leaves, potassium never varied across sites in a manner consistent with trends in scarring or necrosis scores (Fig. 2B).

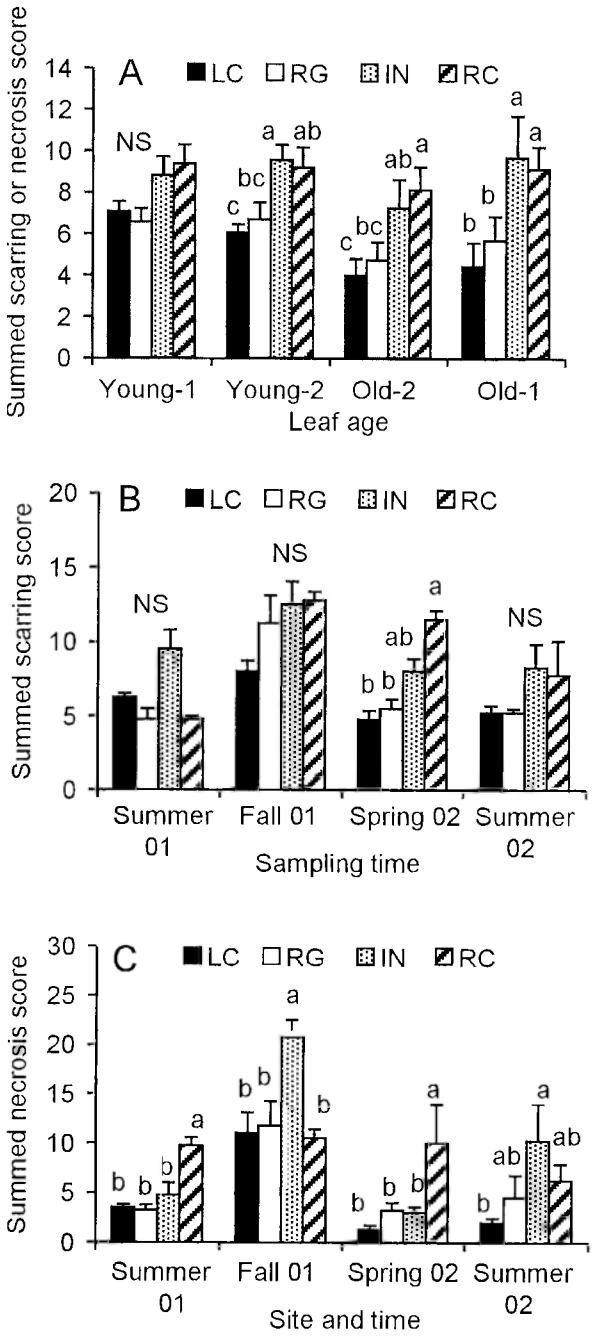

Figure 1. Leaf scarring and necrosis scores summed within samples of *Eichhornia crassipes* plants. **A**. Scarring scores on the youngest (Young-1) and second-youngest (Young-2) unfurled leaves and necrosis scores on the second-oldest (Old-2) and oldest (Old-1) leaves, averaged across all sampling times. **B**. Scarring scores on second-youngest unfurled leaves. **C**. Necrosis scores on oldest leaves. Values represent mean ± SE; $n = 4$–5 samples per site per time. Bars within leaf ages (A) or sampling times (B, C) with different letters are significantly different ($P < 0.05$). NS = no significant differences among means.

Phenolic content was examined in samples collected in fall 2001, the time at which necrosis scores were highest (Fig. 1C). Phenolic content varied significantly between sites LC and IN in youngest unfurled leaves ($F = 6.4$, $df = 7, 24$, $P < 0.001$; site effect, $F = 7.6$, $P = 0.001$) (Fig. 3). Phenolics were higher in furled leaves than in youngest unfurled leaves ($F = 22.0$, $P < 0.001$) in contrast to TAC and potassium contents, which did not consistently vary between leaf ages. Soluble protein, TAC and potassium contents, and soluble peroxidase activity were positively correlated between furled and youngest unfurled leaf ages ($r > 0.31$, $n = 55$–64, $P < 0.05$), as were phenolic contents in Fall 2001 samples ($r = 0.56$, $n = 16$, $P = 0.02$). Soluble peroxidase activity ($r = 0.35$, $n = 60$, $P = 0.005$) (Fig. 4A) and potassium content ($r = 0.44$, $n = 55$, $P = 0.009$) (Fig. 4B) in furled leaves were correlated to necrosis scores in oldest non-senescent leaves. Correlations between necrosis and soluble protein and TAC contents were not significant. In fall 2001 samples, necrosis scores in second-oldest leaves were positively correlated to phenolics in youngest unfurled leaves ($r = 0.56$, $n = 16$, $P = 0.02$).

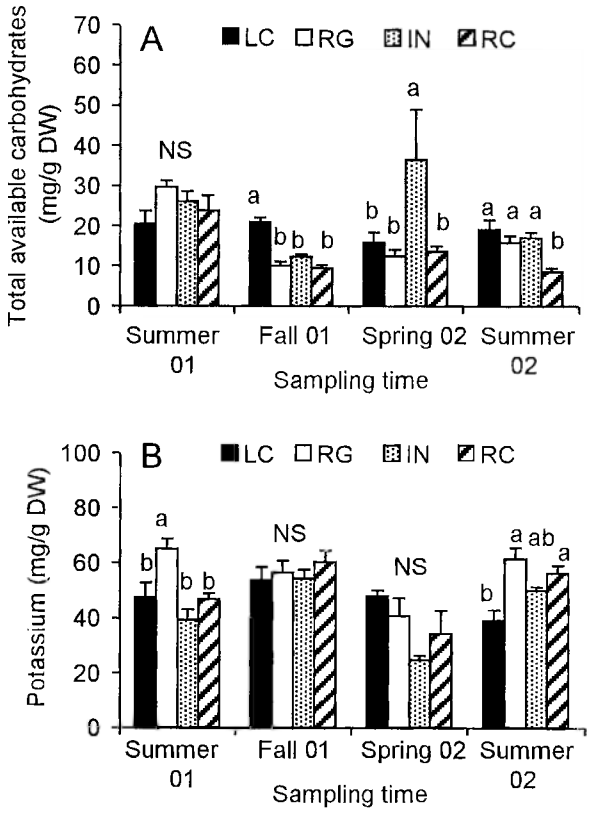

Figure 2. Biochemical components of youngest unfurled leaves of *Eichhornia crassipes* plants. **A.** Total available carbohydrate content. **B.** Potassium content. Values represent mean ± SE; n = 4–5 samples per site per time. Bars within sampling times with different letters are significantly different ($P < 0.05$). DW = dry weight; NS = no significant differences among means.

Bioassays with *Neochetina* spp. weevils

Inoculated leaf laminae had light symptom coverage (≤ 15%) 2 weeks after inoculation. Other leaves on inoculated plants were free of symptoms. Leaf scarring by *Neochetina* spp. weevils was not significantly different between infected and mock-inoculated plants on either inoculated leaves or on the two youngest unfurled leaves (data not shown).

Discussion

Leaf scarring on young *E. crassipes* leaves and necrosis on old leaves showed a positive association when examined across four field sites and four sampling times, even though scarring and necrosis were spatially separated. Most laminar scarring by adult *Neochetina* spp. weevils occurs on furled and newly unfurled, young leaves (Center & Wright 1991). Old leaves receive little new adult weevil damage (Center 1985) but show necrotic spotting resulting from earlier *C. piaropi* infection more commonly than do young leaves (Conway 1976). The bioassay and biochemical results suggest that *C. piaropi* symptom production did not alter weevil feeding, even though necrosis was associated with increased potassium and phenolics, potential determinants of feeding (Center & Van 1989). The field results may thus reflect chronic weevil scarring and the direct associations between scarring and infection observed previously (Charudattan 1986)

Variation in damage by *Neochetina* spp. and in stress related to pathogen infection or abiotic conditions are common among *E. crassipes* populations (Freeman *et al.* 1981; Center *et al.* 1999), including those in the Lower Rio Grande Valley of Texas (Moran unpublished data). Elevated feeding on furled and young unfurled leaves by *Neochetina* spp. at sites IN

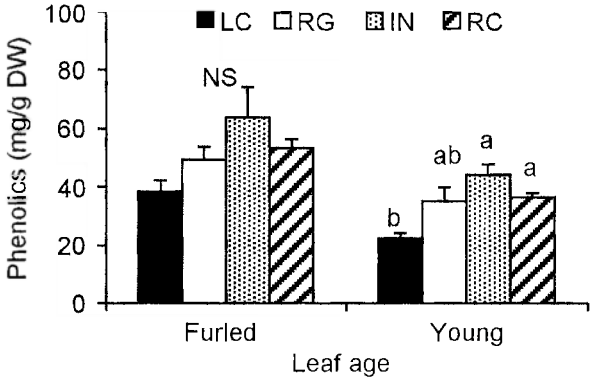

Figure 3. Phenolic content in furled and youngest unfurled ("Young") *Eichhornia crassipes* leaves sampled in fall 2001. Values represent means ± SE; $n = 4$ samples per site. Bars within leaf ages with different letters are significantly different ($P < 0.05$). DW = dry weight; NS = no significant differences among means.

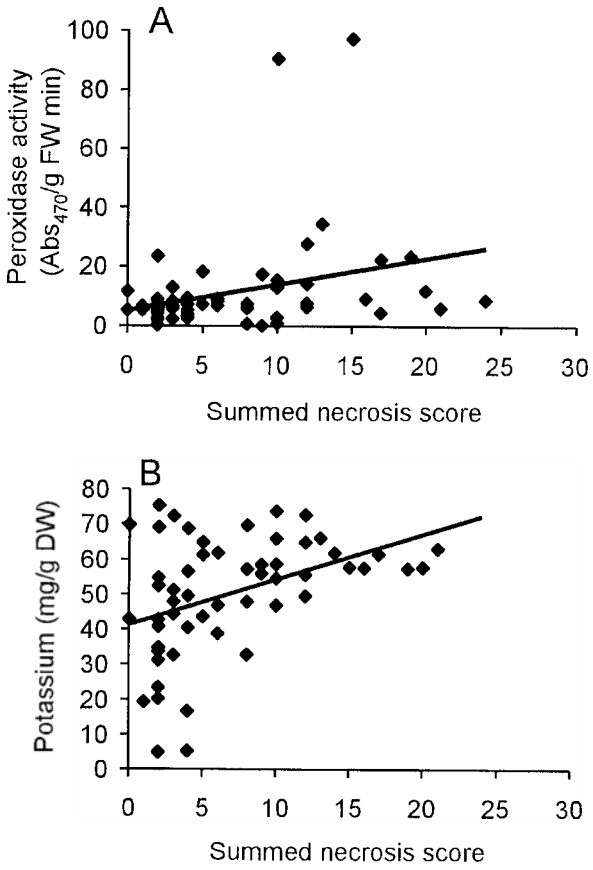

Figure 4. Correlations between necrosis scores in oldest leaves of waterhyacinth and biochemical factors in furled leaves. **A**. Peroxidase activity, n = 60. **B**. Potassium content, n = 56. Each point represents one sample of leaves collected at any of four sampling times. FW = fresh weight; DW = dry weight.

and RC may have increased fungal infection, leading to greater necrosis on these leaves when they were older (Charudattan 1986). A similar damage–pathogen association occurred between *E. crassipes* mites and the fungus *Acremonium zonatum* (Charudattan *et al.* 1978). Abiotic site characteristics common to sites IN and RC could have decreased plant growth and increased biological control (Charudattan 1986; Center *et al.* 1999). However, the two sites differed in disturbance related to water flow and mechanical control, which were present at site RC but absent at site IN. The scarring-necrosis correlation involving all time points was likely a function of variable weevil and fungal activity in individual sampling units, rather than site characteristics. The buildup of necrosis at most sites late in the field season in 2001 and the subsequent decline are consistent with previous studies of *C. piaropi* (Conway 1976; Cofrancesco *et al.* 1985).

Total available carbohydrate and potassium content in furled and young leaves, and water-soluble phenolic content in fall 2001 samples were not related on a site-by-site basis with necrosis scores. However, peroxidase activities and potassium content in furled leaves were positively correlated with necrosis in oldest leaves across all sites and times, and late-season phenolic content showed an association in one of four possible young-old leaf combinations. Infection by a foliar necrosis-inducing fungal or bacterial pathogen often leads to increases in plant proteins and sugars (Hatcher 1995), phenolics (Nicholson & Hammerschmidt 1992) and peroxidase activities (Hammerschmidt & Kuć 1995). These responses are dynamic over time. The timing of infection and symptom production relative to field sampling of *E. crassipes* is unknown. Peroxidases may increase the toxicity of phenolics via oxidation (Nicholson & Hammerschmidt 1992). Polyphenol-oxidases also contribute to oxidation in *E. crassipes* (Martyn & Cody 1983). The higher phenolic content in furled than in unfurled leaves agrees with past results (Center & Wright 1991). Nitrogen and potassium content in healthy plants is also highest in the furled leaves preferred by adult weevils (Center & Wright 1991).

Although necrosis may have increased potassium, peroxidase and phenolics in furled leaves, the bioassay results suggest that these effects did not lead to indirect, plant-mediated influences of prior infection on weevil feeding. Additive or synergistic biocontrol impacts of *Neochetina* spp. weevils and *C. piaropi* on *E. crassipes* (Charudattan 1986) can occur in the absence of fungus-induced changes in host plant suitability. Other biotic or abiotic sources of plant stress could influence the wounding-related weevil-fungus interaction if they alter host suitability.

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Politics and ecology in the management of alien invasive woody trees: the pivotal role of biological control agents that diminish seed production

V.C. Moran,[1] J.H. Hoffmann[1] and T. Olckers[2]

Summary

1. Biological control agents have been established on a total of 22 species of alien, invasive woody trees in Australia (and via Australia in Thailand, Vietnam and Malaysia), South Africa, the United States of America and Hawaii. Fifteen of these tree species (mostly Australian natives) are in South Africa.
2. Of the 39 agent species, 38 are insects (mostly weevils) and one is a pathogen. About 50% of these agents have been released in South Africa. Approximately two-thirds of these agents reduce seed production, directly or indirectly.
3. Usually, pre-dispersal seed mortality will not bring about a reduction in the density of invasive plants because weed populations can maintain near-saturation densities in spite of impressive deprivations of their seeds by biocontrol agents. However, when humans intervene to clear adult plants, and where recruited seedlings are systematically and repeatedly destroyed, even modest levels of pre-dispersal seed mortality can translate into substantial savings for weed-control managers. Fewer seeds mean easier management through lower recruitment rates, fewer seedlings, and slower dispersal.
4. In the context of the politics and ecology of alien invasive tree control, agents that reduce seed production can also prove to be decisive in resolving conflicts of interest, and can become key elements in rehabilitation and restoration processes. They have become the first-line of defence in the successful management of alien invasive tree species.
5. We illustrate these points with reference to three very different cases, namely biological control against *Sesbania punicea, Acacia cyclops* and *Acacia pycnantha* in South Africa. In each case, agents that reduce seeding have played a pivotal role in the biological control campaigns.

Keywords: biological control, invasive trees, management, seed-attacking agents.

Introduction

Only about 22 species of alien invasive woody trees have been targeted for biological control. Of these, 3 are in the United States of America, 4 in Australia (or via Australia) and 15 in South Africa (Table 1; though an exact species count is not possible because *Prosopis* has formed hybrid communities in Australia and in South Africa). Thirty-eight insect species (and one rust species) have become established as biological control agents on trees. About 50% of these agents are established in South Africa, mostly deployed against alien *Acacia* species from Australia (Table 1). About two-thirds of these agents reduce seeding of their host plants either directly through feeding or laying on, or developing in, the seeds, or indirectly by damaging the reproductive parts of the plant, or variously debilitate the plants, and thus inhibit seed formation.

Much has been published on seed dynamics (Harper 1977, Crawley 1997) and on the dynamics of plant–herbivore interactions (Crawley 1983). In the

[1] Zoology Department, University of Cape Town, Rondebosch, 7701, South Africa.
[2] Plant Protection Research Institute, Cedara, Hilton, 3245, South Africa. Corresponding author: V.C. Moran <vcmoran@iafrica.com>.

Table 1. *Alien* invasive tree species (and their countries of *origin*) that have been deliberately subjected to biological control. (Shrubs that less commonly become small trees, and large cactus species, are excluded.) The agents that have become *established* on each of the target tree species, and the countries of *introduction*, are listed. The data are mostly extracted from Julien and Griffiths (1998), but modified according to more recent information, indicated by superscript numerals in the table, as follows: (1) van Klinken, R.D. *et al.* (2002) and van Klinken, R.D. (2003, pers. comm.); (2) Adair, R. J. (3, 4, 5) Impson, F.A.C.; (6) Olckers, T. (2003, Report of the Plant Protection Research Institute, Pretoria); (7) Center, T.D. *et al.* (2000); (8) Wheeler, G.S. *et al.* (2001).

Tree species	Agents established	Countries of introduction
Acacia nilotica ssp. *indica* Prickly acacia (India)	*Bruchidius sahlbergi* (Coleoptera: Bruchidae)	Australia
Mimosa pigra Giant sensitive plant (tropical America)	*Acanthoscelides puniceus* (Coleoptera: Bruchidae)	Australia; to Thailand
	Acanthoscelides quadridentatus (Coleoptera: Bruchidae)	Via Australia (not established); to Thailand, Vietnam
	Carmenta mimosa (Lepidoptera: Sesiidae)	Australia; to Vietnam, Malaysia
	Chlamisus mimosae (Coleoptera: Chrysomelidae)	Australia
	Coelocephalapion aculeatum (Coleoptera: Apionidae)	Australia
	Coelocephalapion pigrae (Coleoptera: Apionidae)	Australia
	Malacorhinus irregularis (Coleoptera: Chrysomelidae)	Australia
	Neurostrota gunniella (Lepidoptera: Gracillariidae)	Australia
Parkinsonia aculeata Palo verde (tropical America)	*Mimosestes ulkei* (Coleoptera: Bruchidae)	Australia
	Penthobruchus germaini (Coleoptera: Bruchidae)	Australia
	Rhinacloa callicrates (Hemiptera: Miridae)	Australia
Prosopis spp. (various hybrids including *pallida* and *velutina*) Mesquite (North and Central America)	*Algarobius prosopis* (Coleoptera: Bruchidae)	Australia
	Evippe sp. #1[1] (Lepidoptera: Gelechiidae)	Australia
	Prosopidopsylla flava [1] (Hemiptera: Psyllidae)	Australia
Acacia cyclops Rooikrans (Australia)	*Dasineura dielsi* [2] (Diptera: Cecidomyiidae)	South Africa
	Melanterius servulus (Coleoptera: Curculionidae)	South Africa
Acacia dealbata Silver wattle (Australia)	*Melanterius maculatus* [3] (Coleoptera: Curculionidae)	South Africa
Acacia decurrens Green wattle (Australia)	*Melanterius maculatus* [4] (Coleoptera: Curculionidae)	South Africa
Acacia longifolia Long leaved wattle (Australia)	*Melanterius ventralis* (Coleoptera: Curculionidae)	South Africa
	Trichilogaster acaciaelongifoliae (Hymenoptera: Pteromalidae)	South Africa
Acacia mearnsii Black wattle (Australia)	*Melanterius maculatus* (Coleoptera: Curculionidae)	South Africa
Acacia melanoxylon Australian blackwood (Australia)	*Melanterius acaciae* (Coleoptera: Curculionidae)	South Africa
Acacia pycnantha Golden wattle (Australia)	*Trichilogaster* sp. (Hymenoptera: Pteromalidae)	South Africa
Acacia saligna Port Jackson willow (Australia)	*Melanterius compactus* [5] (Coleoptera: Curculionidae)	South Africa
	Uromycladium tepperianum (Fungus: Uredinales)	South Africa
Hakea gibbosa Rock hakea (Australia)	*Erytenna consputa* (Coleoptera: Curculionidae)	South Africa

Table 1. (Continued) *Alien* invasive tree species (and their countries of *origin*) that have been deliberately subjected to biological control. (Shrubs that less commonly become small trees, and large cactus species, are excluded.) The agents that have become *established* on each of the target tree species, and the countries of *introduction*, are listed. The data are mostly extracted from Julien and Griffiths (1998), but modified according to more recent information, indicated by superscript numerals in the table, as follows: (1) van Klinken, R.D. *et al.* (2002) and van Klinken, R.D. (2003, pers. comm.); (2) Adair, R. J. (3, 4, 5) Impson, F.A.C.; (6) Olckers, T. (2003, Report of the Plant Protection Research Institute, Pretoria); (7) Center, T.D. *et al.* (2000); (8) Wheeler, G.S. *et al.* (2001).

Tree species	Agents established	Countries of introduction
Hakea sericea Silky hakea (Australia)	*Carposina autologa* (Lepidoptera: Carposinidae)	South Africa
	Cydmaea binotata (Coleoptera: Curculionidae)	South Africa
	Erytenna consputa (Coleoptera: Curculionidae)	South Africa
Leptospermum laevigatum Australian myrtle (Australia)	*Dasineura* sp. (Diptera: Cecidomyiidae)	South Africa
	Parectopa thalassias (Lepidoptera: Gracillariidae)	South Africa
Paraserianthes lophantha Crested wattle (tropics and subtropics)	*Melanterius servulus* (Coleoptera: Curculionidae)	South Africa
Prosopis spp. (various hybrids including *glandulosa* and *velutina*) Mesquite (North America)	*Algarobius prosopis* (Coleoptera: Bruchidae)	South Africa
	Neltumius arizonensis (Coleoptera: Bruchidae)	South Africa
Sesbania punicea Red sesbania (Argentina, Brazil, Uruguay)	*Neodiplogrammus quadrivittatus* (Coleoptera: Curculionidae)	South Africa
	Rhyssomatus marginatus (Coleoptera: Curculionidae)	South Africa
	Trichapion lativentre (Coleoptera: Apionidae)	South Africa
Solanum mauritianum Bugweed (South America)	*Gargaphia decoris* [6] (Hemiptera: Tingidae)	South Africa
Melaleuca quinquenervia Paper-bark tree (Australia)	*Oxyops vitiosa* [7] (Coleoptera: Curculionidae)	Florida, U.S.A
Myrica faya Fire tree (Azores, Madeira, Canary Islands)	*Caloptilia* nr *schinella* (Lepidoptera: Gracillariidae)	Hawaii , U.S.A
Schinus terebinthifolius Brazilian pepper tree (South America)	*Episimus utilus* (Lepidoptera: Tortricidae)	Hawaii, U.S.A.
	Lithraeus atronotatus (Coleoptera: Bruchidae)	Hawaii, U.S.A
	Metastigmus transvaalensis [8] (Hymenoptera: Torymidae)	Florida, U.S.A

context of biological control of weeds, the majority of studies quantify the actual damage inflicted on the target plants by the biological control agents (e.g. the number of shoots destroyed, or the number of feeding holes in the leaves, and so on). Relatively few studies quantify the impact of the agents on the population dynamics of the target weeds (Crawley 1989) or their effects on the rate of spread of the weeds (Paynter *et al.* 1996). In particular, it has frequently been noted that biological control agents that reduce seed production will usually be ineffective in reducing host-plant densities (Myers *et al.* 1990, Cloutier and Watson 1990, Myers and Risley 2000). This is because the agents will have destroyed a surfeit of seeds that would have succumbed to numerous pre- and post-dispersal mortalities anyway, or failed because of the subsequent death of the seedlings they generated. Weed populations can often persist in spite of the destruction of high proportions of their seeds by biological control agents.

The situation is very different where humans intervene to harvest (see Crawley 1997) or to clear parent populations of the weed, and where recruited seedlings are systematically and repeatedly pulled out, chopped, poisoned or burned. Under these circumstances, even low levels of seed mortality brought about by biological control agents can reduce seedling densities and slow rates of spread, which in turn translates into substantial savings for weed-control managers in clearing and follow-up operations, and may determine whether or not control succeeds. In this paper, we discuss the pivotal role of seed-destroying agents in the management of invasive trees in South Africa.

Management of alien invasive trees in South Africa

South Africa has responded to the problems caused by alien invasive trees (Moran *et al.* 2000) through the concerted efforts of the Working for Water Programme. This ambitious program has been running successfully for several years, and costs about US$50 million per annum. It employs about 25,000 people who clear invasive trees and other alien plants, mostly from river courses and catchments. The task is daunting. For example, it is estimated that the Klein River in the South West Cape (which is about 40 km in length from its mountain sources to the sea), will take 20 years and US$4 million to clear (C. Maartens and L. Waller, Cape Nature Conservation, pers. comm.). Weed managers have reached the conclusion that success in clearing alien trees to acceptable densities in South Africa will be nearly impossible without biological control. Follow-up operations on regrowth, after parent populations have been cleared, are crucial and often need to continue for many years. It is in this context that seed-destroying biological control agents have such an important role to play in the management of alien invasive trees.

Management benefits of biological control agents that reduce seed-production

Recently, Hoffmann *et al.* (2002) described the effectiveness of a gall-forming agent *Trichilogaster* sp. (Pteromalidae) in reducing seeding in the Australian tree *Acacia pycnantha,* that has invaded the south-western parts of South Africa. Galled inflorescences of *A. pycnantha* produce no pods, and branches with more than 10 galls produce 95% fewer pods (and hence seeds). The gall-loads on *A. pycnantha* are enormous and thus, in aggregate, seed reduction is spectacular.

Over the last several years, Impson *et al.* (2000) and Impson (2003) have carefully monitored the effects of the weevil *Melanterius servulus* on seed destruction in *Acacia cyclops*, another highly invasive tree from Australia. The weevils were originally released at 16 sites around the southwestern Cape in 1994. Already, at eight of the sites, seed-destruction is greater than 65% and at three of the sites the beetles are destroying 90–95% of the seeds.

Hoffmann and Moran (1998) studied the effects of biological control agents on the population dynamics of the invasive South American tree, *Sesbania punicea*. They demonstrated that two agents, a bud-feeder, *Trichapion lativentre* (Apionidae), and a seed-feeder, *Rhyssomatus marginatus* (Curculionidae), in combination, resulted in a 99.7% reduction in seeding by the trees. Over a period of 10 years or more, this extremely high level of seed destruction resulted in only a marginal decline in plant density at only one of the study sites. *Sesbania punicea* is considered particularly vulnerable in this respect because it has no seed banks and the trees are relatively short-lived (12–15 years). Populations of other species of invasive trees are likely to be even less affected by high levels of seed destruction.

These studies raise the question, again, of whether biological control agents that reduce seed production, can ever be successful in controlling trees. If "success" in this context means reductions in the distribution or density of the target plants, then the answer is usually "no: the self-thinning argument applies", i.e. even with very high levels of seed destruction, there are still enough seeds left to replenish the adult plant populations.

The three cases discussed above are apparently disappointing from a biological control point of view. Massive reductions in seeding are predicted to have little impact on invasive plant population densities, even in the long term. What these bland conclusions hide, however, is the fact that management of the weeds is much easier after biological control agents have reduced the levels of seeding, and hence seedling recruitment. Without biological control, manual or other methods of clearing parent populations usually prove to be almost completely futile because of the resurgence of seedlings. After biological control using seed-destroying agents, the reduced levels of seedling recruitment greatly facilitate control, especially follow-up operations.

The Working for Water Programme has recorded the costs of clearing alien trees from river courses and catchments in South Africa. Each of the many species of invasive trees has attendant problems that affect the costs of clearing. However, on average, the relative costs per unit area can be estimated and these are shown in Table 2. These data illustrate the obvious point that it is much more expensive (up to 80 times) to clear high densities of mature plants than low densities of seedlings. This reinforces the imperative to follow-up the original clearing of parent populations as soon, and as frequently as possible. However, the most significant feature of Table 2 is the evidence that any reduction in the density of seedlings (that is brought about by biological control agents) will result in significant and disproportionately favourable reductions in the costs of follow-up control. For example, it is five times more costly to clear dense infestations of seedlings (where the area covered is between 51–75%) compared with lighter infestations (of only 6–25%).

Thus, if success in biological control means savings of time and money in clearing and follow-up operations, then agents that reduce seed production in their target hosts must be deemed to be highly successful. Seed destruction by biological control agents results in fewer seeds, fewer seedlings and lower costs of the original clearing operations, and these savings are

Table 2. The average relative costs per unit area of clearing alien invasive tree species (mainly *Acacia* species, *Eucalyptus* species, *Hakea* species from Australia and *Pinus* species from Europe), at different levels of maturity. The data are a summary of detailed species by species costs that are used by Working for Water to calculate rates of pay for the tree-clearing teams (C. Maartens and L. Waller, Cape Nature Conservation, pers. comm.)

	Area covered (%)				
	<5	6–25	26–50	51–75	76–100
Seedlings	1	3	7	15	20
Young Plants	3	5	19	43	57
Mature Plants	4	13	26	60	80

multiplied many-fold over the subsequent years of follow-up operations. The benefits of biological control agents that reduce the levels of seed production by their target plants may be summarised as follows:

1. there are fewer seeds (and seed banks) and seedlings
2. there are fewer and less expensive follow-ups
3. there is less pollution and habitat disturbance during clearing operations; there must be a slower spread of the invasive plants, although this has seldom been satisfactorily demonstrated (Paynter *et al.* 1996)
4. there will be quicker rehabilitation and easier restoration.

Besides these advantages, one of the most compelling "political" benefits of using seed-destroying agents against invasive trees is that they help to resolve conflicts of interest. For example, many invasive trees are highly problematic in conservation areas, but in other areas the same species may be cultivated and exploited commercially. *Acacia cyclops* in South Africa forms widespread, impenetrable infestations along the coastal areas in the southwest Cape excluding hundreds of species of native plants, yet it produces a valuable fuel wood. *Acacia mearnsii* is *the* major conservation problem in riparian and other habitats in South Africa, but this species is also the basis of a highly valuable tannin, wood-chip and paper industry in South Africa. The use of seed-destroying agents against these species does not detract from the beneficial attributes of the plants, but does reduce their aggressiveness and make them far more manageable. With seed-feeding agents we can have our cake and eat a piece of it!

Conclusion

There is overwhelming evidence from the studies of biological control of invasive trees in South Africa that any reduction in seeding levels aids management. Agents that reduce seed production should always be in the front line of the attack (De Loach 1981). As a general principle in weed biological control we advocate that agents that reduce seed production should take priority during the exploration phases and be amongst the first agents released. After seed-destroying agents are deployed, selected agent species that attack other parts of the plant should be considered, but only after seeding is controlled, as a first priority.

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Release strategies for the moth *Agonopterix ulicetella* in the biological control of *Ulex europaeus* in Chile

Hernán Norambuena, Sergio Escobar and Jorge Díaz[1]

Summary

The univoltine insect, *Agonopterix ulicetella*, was introduced into Chile from Hawaii and the UK in 1996 and 1997, respectively, for the biological control of gorse, *Ulex europaeus*. Release strategies to enable this agent to persist on gorse bushes were investigated. Five release sizes: 0, 2, 4, 8, 16 and 32 third-instar larvae, four times replicated, were made on gorse branches enclosed with a fine mesh sleeve. Over two seasons, agent population parameters and damage to the gorse branches were assessed. For *A. ulicetella*, the critical initial release size was eight larvae. The number of gorse shoots attacked by *A. ulicetella* was dependent on release size. The 8, 16 and 32 larval density levels resulted in a larger number of attacked shoots than did the lower larval densities.

Keywords: *Agonopterix ulicetella*, biological control, gorse, release strategies, *Ulex europaeus*.

Introduction

Gorse, *Ulex europaeus* L. (Fabaceae), is a perennial spiny shrub that originated in western Europe. It was introduced into Chile at the beginning of the 19th century and has since become a serious weed (Matthei 1995). The plant forms a dense, spiny, impenetrable scrub that gradually invades open ranges and competes with grass and forb species. Gorse is also a threat because it hinders the establishment and efficient management of exotic forest trees and constitutes a serious fire risk. In Chile, growth of gorse might reach a 30-fold increase per year (Norambuena 1995).

The gorse soft shoot moth, *Agonopterix ulicetella* (Stainton) (Lepidoptera: Oecophoridae), was first released in Chile in 1997, where it has one generation per year. The adult stage overwinters in the leaves, emerging during the spring to mate and lay eggs on the plant surface. Larvae feed and develop on leaves, particularly new growth, and then wander to pupate in the bushes. The new generation of adults emerge by early summer. This life cycle is similar to that reported by Hill *et al.* (1995).

One of the most critical problems following the introduction of weed biological control agents is the lack of experimental evidence relating to the optimal release strategy for successful colonization and establishment (Memmot *et al.* 1998). Currently, there are no theoretical grounds for making decisions about release size of biological control agents (Grevstad 1999a), and the optimal number of individuals to release at a site at any one time varies for different agent species. This number may depend on multiple factors (dispersal, ecoclimatic conditions, reproductive state, host phenology and quality etc.). Theoretical studies addressing the relationship between population size and persistence have indicated that, in general, persistence is predicted to be an increasing function of initial population size. Retrospective analyses of successful and unsuccessful deliberate introductions of biotic agents have also supported a positive correlation between initial colony size and establishment. The studies have compared establishment rates among species for which different numbers were released, rather than comparing the establishment rates of different sized releases within a species (Grevstad 1999b and references therein). As pointed out by Memmot *et al.* (1998) and Grevstad

[1] Instituto de Investigaciones Agropecuarias, Centro Regional de Investigación Carillanca, Casilla 58-D, Temuco, Chile.
Corresponding author: Hernán Norambuena <hnorambu@carillanca.inia.cl>.

(1999b), in order to improve our ability to make decisions about release sizes in biological control, a strategy for propagation and distribution based on manipulative experiments should be developed for each control agent.

Improved release strategies may be needed for *A. ulicitella*. Apart from Chile, the moth has also been introduced into New Zealand and Hawaii for the biological control of gorse, but establishment success has been variable. In Hawaii, the insect is well established above 1000 m altitude (Markin *et al.* 1996) and is still producing noticeable damage (G. Markin and R. Hill, 2003, pers. comm.). In New Zealand, where it was first released in 1990, researchers had to develop a sex attractant to enable its recovery in the field (Suckling *et al.* 2000).

In Chile, *A. ulicetella* successfully overwintered in six of nine localities after its first release during the 1997/1998 season (Norambuena *et al.* 2000). Although larvae and feeding damage were observed at two of the original release sites during the past four years, population increases at these surviving sites has been slow (Norambuena, unpublished results). Difficulties in detecting *A. ulicetella* life stages or larval damage have resulted in farmers and forestry biocontrol supporters becoming sceptical about the usefulness of this agent. Furthermore, the moth's univoltine life cycle, which includes an obligate adult diapause, has made its propagation a rather slow process, making it difficult to decide on an optimal release strategy (i.e. how many individuals to release and when and how to release them). There has therefore been a need to investigate release strategies in order to improve the possibility of field establishment .

This paper presents the results of a field experiment with *A. ulicetella* to calculate the number of larvae that can be released in sleeve cages, as well as determining survival thresholds and feeding damage.

Materials and methods

Experiments were conducted in a field site located 20 km northeast of Temuco, Chile (38°41'S) from December 2000 to January 2003. An invading three-year-old gorse infestation of approximately 0.1 ha in size on an abandoned cultivated field constituted the study area. New gorse branches with stems from 0.7 to 1 cm in diameter and with 12 to 20 shoots per branch were selected from the edges of the gorse front for use as experimental plots. The total mean gorse shoot length per branch ranged from 441 to 468 cm in the release size treatments (Table 1).

Six releases sizes consisting of 0, 2, 4, 8, 16 and 32 third-instar larvae four times replicated were randomly made on gorse branches, making a grand total of 248 larvae released on 20 branches. The control releases (0 larvae) were used for determining whether natural *A. ulicetella* infestations had occurred and for assessing any treatment effects on gorse.

Branches were enclosed with a fine gauze sleeve 45 × 145 cm (length × diameter) in size and open at both ends. A conical wire structure of 70 × 40 cm served to fasten the sleeve over the branches. The whole structure was further affixed by hanging it from a supporting wire line located about 40 cm above the sleeves. Before the release, one end of the sleeve was secured around the branch with a plastic twist-tie. The other end, including the distal part of the branch, was also secured with a plastic twist-tie immediately after the release of the insects. The gauze sleeves were replaced annually.

The larvae used in the experiment originated from a hybrid population (UK/Portugal) introduced from Hilo, Hawaii. Initially, larvae were reared in the field on gorse plants enclosed in walk-in cages made of mesh fabric (2 × 2 × 2 m), similar to the cages described by Briese et al. (1996). In the laboratory, selected third-instar larvae were randomly assigned to each release size treatment and then transported to the field in ventilated plastic vials containing pieces of gorse shoots. The larvae were kept in a cool box until their release on a single day in late spring (19 December 2000). The releases were made by opening the vials and encouraging the larvae onto the growing gorse shoots. Larvae were spread around the shoots using a fine camel hair brush.

A. ulicetella population censuses and measurements of host plant parameters (shoot length, branch diameter, healthy and damaged shoots) were made at about one month, and one and two years after the releases. Counts

Table 1. Shoot length and stem diameter of gorse.

Release size treatments	Shoot length (cm)[a]		Branch diameter (cm)		
	Dec 2000 Mean (SD)	Dec 2002 Mean (SD)	Dec 2000 Mean	Dec 2001 Mean	Dec 2002 Mean
32 larvae	468 (91)	Na	0.88	1.28	1.3
16 larvae	441 (48)	Na	0.83	1.15	1.23
8 larvae	445 (57)	Na	0.88	1.13	1.25
4 larvae	446 (56)	1809 (912)	0.80	1.30	1.48
2 larvae	456 (62)	2420 (1515)	0.85	1.25	1.55
0 larvae	443 (49)	1557 (616)	0.78	1.25	1.33

[a] = total shoot length per branch.
Na = not assessed due to the presence of *A. ulicetella*.

of *A. ulicetella* developmental stages were made by searching inside the gauze sleeves and carefully examining all gorse shoots present on each branch, as well as the faeces and remaining host-plant material accumulated at the bottom of the sleeve. Any pupae present were returned to the sleeves. Data were square-root-transformed. Percentages of damaged shoot were arcsin-transformed before being subjected to a nonlinear regression analysis to estimate detectable feeding damage thresholds.

Results

Sampling effectiveness

The consistent recovery rates for larvae and pupae one month after the initial releases showed that there was no difference in sampling efficiency between the five release sizes. The risk that any variation in numbers of *A. ulicitella* found in different release-size treatments might be due to a sampling effect was therefore considered low (Table 2). Throughout the sampling period, there was no evidence of *A. ulicetella* developmental stages in the control plots. This, and the similar length of gorse shoots and diameter of gorse branches, in both control and infested treatments at the onset of the experiment (Table 1), satisfied the requirements for evaluating the influence of the release size on any detectable feeding damage on gorse and on the survival threshold of the agent.

Table 2. Recovery rate of *A. ulicetella* per treatment after one month.

Treatment	Recovery rate (SD)
32 larvae	0.64 (0.07)
16 larvae	0.56 (0.15)
8 larvae	0.56 (0.36)
4 larvae	0.75 (0.35)
2 larvae	0.63 (0.47)
0 larvae	0

Estimation of net reproductive rate

To calculate the estimated net reproductive rate/individual ($R = p/\theta$), a constant value (p) and the probability of recovering one individual present on the branch (θ) were calculated (see Memmot *et al.* 1998). The θ value was estimated by dividing the total number of recovered *A. ulicetella* one month after the initial release, corresponding to the lowest and highest release sizes (87 larvae), by the released number of larvae of these treatments (136 larvae). This resulted in an insect recovery rate of 0.639 (Table 1).

To estimate the p value, the initial number of released larvae and the realized number of insects found 13 months later were transformed to square-root to stabilize the variance and the data were then fitted to a linear regression with the equation $y = px$. The function line was forced through the origin so that the slope of the line corresponded to square-root of p value = 1.09 ($r^2 = 0.62$). Back transformation yielded a value of $p = 1.04$. Therefore, the estimated net reproductive rate was $R = 1.63$ which means that the *A. ulicetella* released were able to replace themselves in the first year after the release. Regression analysis showed that the square root of the number of released larvae per branch explained 62% of the variation after 13 months in the field (Fig. 1.)

When the same model was applied to the data of *A. ulicetella* recovered after two years, (December 2002), but plotted against the realized number recovered the previous sampling date (January 2002), instead of using the original release sizes, the regression line explained only 53% of the variation in the dependent variable (Fig. 2). The net reproductive rate of the population during the second season resulting from p/θ ($p = 0.63$ and $\theta = 0.639$) was 0.99.

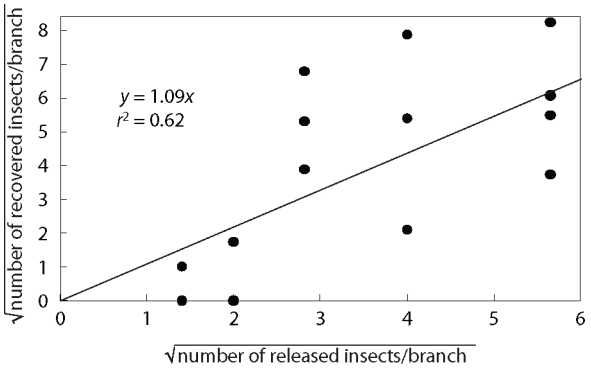

Figure 1. Relationship between the number of *A. ulicetella* recovered vs the number of larvae released one month earlier (both variables were square-root transformed).

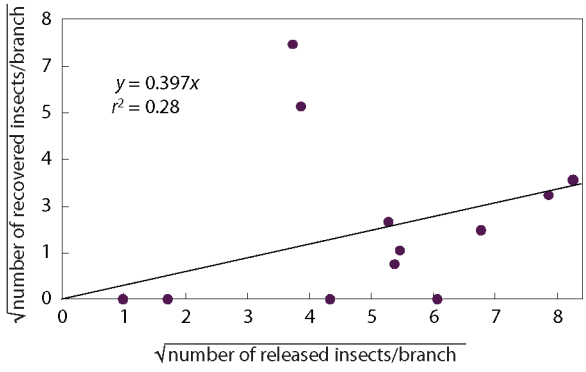

Figure 2. Relationship between the number of *A. ulicetella* recovered versus the number of larvae released one year earlier (both variables were square-root transformed).

The alternative method to estimate reproductive rates (Memmot *et al.* 1998) was used with each of the initial release sizes. The net reproductive rate one year after the releases indicated substantial differences between the two and four larvae releases size and the three highest initial larval release sizes. These increased

as the release sizes increased from 8 to 32 larvae (Fig. 3). This alternative method was also used to calculate the net reproductive rates of populations of 30, 37 and 37 *A. ulicetella*; populations that resulted from the initial release sizes of 8, 16 and 32 larvae, respectively. Recovered populations of the smaller release sizes were disregarded from the analysis as they did not produce any progeny after two years. The highest net reproductive rate ($R = 0.9$) resulted from the populations originating from initial release sizes of 32. The second highest rate ($R = 0.53$) resulted from the population originating from releases of eight larvae (Fig. 4).

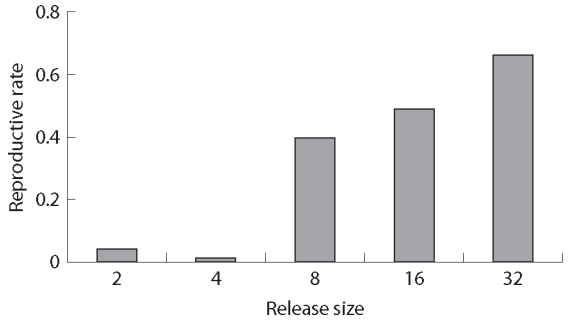

Figure 3. The relationship between reproductive rate of *A. ulicetella* and its release size after one gorse growth season.

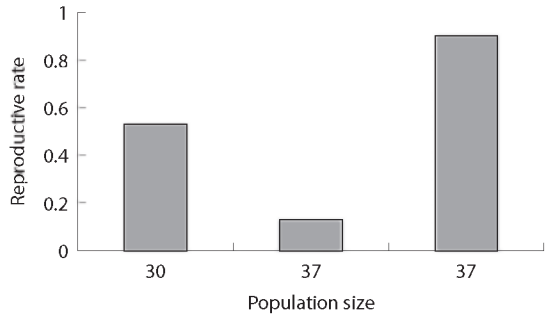

Figure 4. The relationship between reproductive rate of *A. ulicetella* and its release size after two years.

Colonisation patterns and release size

Colonization patterns of the 20 released populations are plotted in Figure 5. Population size varied markedly among the release sizes about one month after the initial release, ranging from 0 to 2, 1 to 4, 1 to 8, 7 to 12 and 18 to 22 for the 2, 4, 8, 16 and 32 larvae release sizes, respectively. All populations, except five belonging to the size releases of 2, 4 and 8 larvae, decreased in population size. One year after the initial releases, all populations of the 32 larvae release size treatments, and three populations out of four of the 16 and 8 release size treatments, increased in size. One population out of four of the four larvae release size treatment survived the first year before going extinct. Only one population of the release size of two larvae survived the first year. After two years, all populations decreased, excepting one of the 32 and one of the 8 larvae release sizes. Interestingly, one population belonging to the release size of two larvae, which was undetected after one month, reappeared the following season, but became extinct during the second year.

Feeding damage

Shoot damage data obtained for the varying release sizes of *A. ulicetella* one year after the initial releases were best-fitted to a logistic function (Fig. 6, $R^2 = 0.88$). Although the number of shoots damaged by *A. ulicetella* was noticeable in all the release sizes treatments, it was substantially higher in the release size treatments of 8, 16 and 32 larvae than in the smaller size releases. No additional benefits in terms of feeding damage were noticed by releasing 16 and 32 larvae, compared with the treatment where 8 larvae were released. When data of the percentages of damaged shoots were arcsin transformed before plotting, this relationship was even stronger ($R^2 = 0.98$) and indicated that eight larvae were able to attack about 86% of gorse shoots.

After two years, the number of damaged gorse shoots were also best fitted to a logistic function (Fig. 7, $R^2 = 0.49$). No damaged shoots were detected on branches exposed to the two and four larvae release size treatments. Similarly to the previous year, the eight larvae release size treatment was sufficient to demonstrate detectable feeding damage on the gorse branches. When data for the number of shoots damaged were transformed, this relation was $R^2 = 0.58$, indicating that about 52% of gorse shoots were damaged with releases of eight larvae.

Discussion

The decrease in most of the release sizes treatments about one month after the initial release (Fig. 5), particularly in the highest release size treatments, may have resulted from manipulation of the larvae during the infestation process. This pattern was less strongly expressed in the smaller release sizes, perhaps because the larvae were handled more carefully. However, it is also possible that the larvae in the lower release size treatments were easier to find. Population decreases following the initial release of weed biological control agents have also been reported by Grevstad (1999b) who attributed this result to stress due to the host change (i.e. when the insects used in field experiments are obtained from laboratory rearing). In our experiments, *A. ulicitella* was retained in the laboratory for only a short period before release, so manipulation might have been a more important factor on the pattern of colonisation after one month rather than at later sampling dates.

None of the populations of the smallest releases sizes treatments (two and four larvae) survived the second year. Demographic stochasticity was a likely reason for the extinction of these populations, although an Allee effect may have played a role because preda-

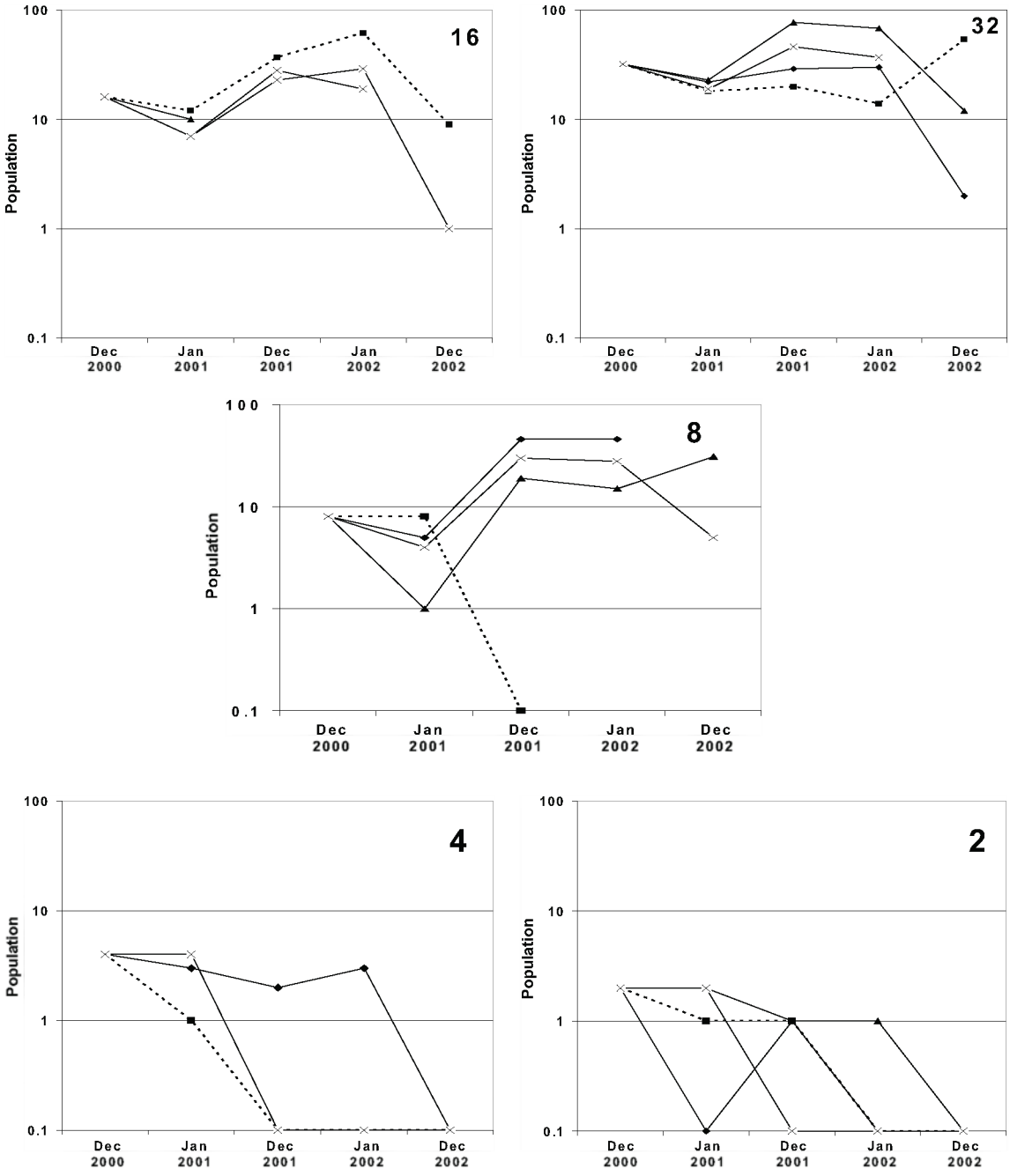

Figure 5. Numbers of *A. ulicetella* recovered at different sampling dates in each of the four replicates of the release size treatments.

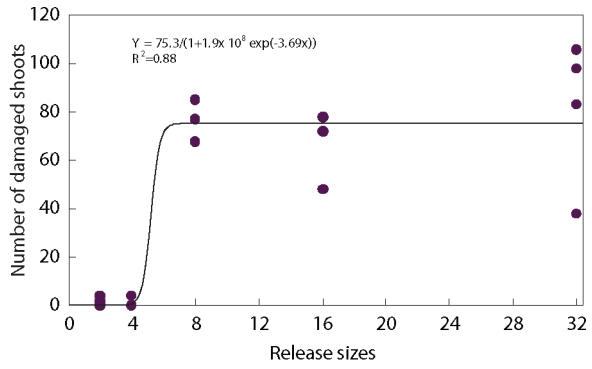

Figure 6. Numbers of shoots damaged by *A. ulicetella* in the release size treatments after one year.

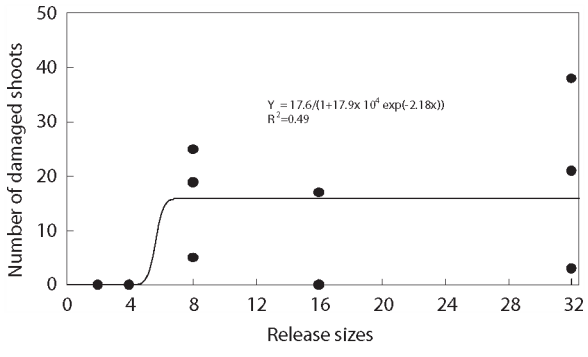

Figure 7. Numbers of shoots damaged by *A. ulicetella* in the release size treatments after two years.

tors (spiders and Carabidae larvae) were occasionally seen inside some of the sleeve cages. For instance, predation impact might have been less strong inside sleeve cages containing more *A. ulicetella* (larger release size treatments) than inside sleeve cages containing fewer larvae (smaller release size treatments) due to the strong defensive mechanisms of the fifth-instar larva, which, when disturbed, quickly moves backward inside a tunnel it builds with gorse spines. This behaviour may have favoured aggregation of *A. ulicetella* at more infested gorse branches and survival of sufficient individuals to ensure mating. Overall, populations of larger releases (8, 16 and 32 larvae) were clearly less likely to become extinct during the two-year study period than those originating from the smaller releases. The observed decline of some populations of the larger release sizes may have been due a differential shortage of the food resources provided by the host branch, as their shoots were highly damaged after one season (Fig. 6) and the sleeve cages prevented *A. ulicetella* from searching for a new food supply. This is coincident with the 0.99 net reproductive rate of the insect during the second season (which was calculated independently of the releases of two and four larvae) as compared with the 1.7 value obtained for the population originating from the initial release sizes during the first year.

Despite the decrease in larval numbers one month after the initial releases, a second generation of *A. ulicetella* was produced in all the release size treatments during the first year of the experiment, with survival rate increasing with release size (Figure 3). However, during the second year (Fig. 4) reproductive rates of populations originating from initial release sizes of 8 and 32 larvae were above 0.5 and 0.9, respectively, both of which were higher than populations originating from the 16 larvae release size. The lower survival rate of populations originated from the 16 larvae release size after two years might have occurred because a new generation was recorded in two of the three replicates that remained, and in one of these replicates only one larva was observed.

Thus, releases of eight third-instar larvae appear to be acceptable as a survival threshold of the control agent under sleeve cage conditions after one and two years. Furthermore, this release size was sufficient to demonstrate a detectable feeding damage of 88% of shoot damage after one year (Fig. 6), and of 49% (Fig. 8) after two years, from the onset of the experiment.

This experiment demonstrated that release size of *A ulicetella* did affect survival and had an impact on gorse shoot damage during two field seasons. Although releases of eight third-instar larvae appeared to be acceptable as the optimal release size for survival and detectable feeding damage, it cannot be assumed that this release size would lead to the field establishment of *A. ulicetella*, as the experiment was performed under confinement. Even so, sleeve cage releases could be a useful technique for initiating the colonization of *A. ulicetella* as well as being used to demonstrate the potential impact of *A. ulicetella* on gorse to farmers.

The experimental results might also become useful in the future establishment of *A. ulicetella* as part of the current gorse biocontrol project in Australia, where importation of *A. ulicetella* will be considered on the basis of the outcome of host-specificity testing (Ireson *et al.* 2004, this volume).

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Preliminary assessment of release and establishment of lantana herringbone leafminer, *Ophiomyia camarae* (Diptera: Agromyzidae), in South Africa

D.O. Simelane and M.S. Phenye[1]

Summary

The herringbone leafminer, *Ophiomyia camarae* Spencer (Diptera: Agromyzidae), was released in South Africa against *Lantana camara* L. (Verbenaceae), a noxious weed. About 14,500 flies were released between November 2001 and April 2002 at 20 sites in 5 provinces. Three techniques, namely: short-term caging in the glasshouse, short-term caging in the field, and release via the placement of infested leaves in the field were used to release *O. camarae*. The release via the placement of infested leaves directly into the field was the most successful and cost-effective method, as initial establishment occurred at all the sites where it was used, whilst it required considerably less time and resources than the other two techniques. Neither release size nor cultivars of lantana appeared to influence initial establishment of *O. camarae*. Although there was no indication that climate had any influence on initial establishment during the first summer, monitoring conducted 12 months after release showed that flies released at sites located at altitudes higher than 900 m failed to establish. Cold and dry winters, which often characterized these areas, might have directly hampered development and population build-up of *O. camarae*. Because of either frost or prolonged drought during the winter and spring seasons, host plants at high-altitude release sites underwent leaf abscission, thereby depleting the populations of *O. camarae*. All sites located at altitudes lower than 900 m were frost-free and most of the host plants retained their leaves during the winter season, enabling *O. camarae* to persist. Preliminary assessment of the release sites suggests that climate unsuitability will limit establishment of *O. camarae* in the high-altitude areas of South Africa characterized by frost and dry winter seasons.

Keywords: climate, establishment, *Lantana camara, Ophiomyia camarae*, release.

Introduction

Despite having been subjected to biological control for more than a century, *Lantana camara* L. (lantana) remains invasive in many parts of the world. It ranks among the world's worst weeds, competing with tree crops and infesting millions of hectares of natural grazing land.

In South Africa, only 8 of the 15 biocontrol agents that were deliberately introduced to control lantana have established. Factors inhibiting establishment include: low number of individuals released (Grevstad 1996, Memmott *et al.* 1996, Baars & Neser 1999, Broughton 2000, Day & Neser 2000, Swirepik & Briese 2000) and climate unsuitability, both directly and indirectly, by its effects on the host plant (Broughton 2000, Day & Neser 2000, F. Heystek, unpublished data). Certain biocontrol agents display preferences for certain cultivars of lantana (Radunz 1971, Harley & Kassulke 1974, Cilliers 1987a,b, Cilliers & Neser 1991, Urban & Simelane 1999), and some agents have reportedly failed to establish because of cultivar resistance (Cilliers & Neser 1991). Since a large proportion of biological control programs suffer from failure of agent establishment, it is imperative to ensure that a reliable and efficient release strategy is employed to improve establishment and redistribution.

[1] ARC – Plant Protection Research Institute, Private Bag X134, Pretoria, Queenswood 0121, South Africa.
Corresponding author: D.O. Simelane <rietdos@plant2.agric.za>.

This paper provides a preliminary assessment of different release strategies used during the release of *Ophiomyia camarae*, a natural enemy cleared for release against lantana in South Africa. Influence of altitude and cultivar preference on initial establishment of *O. camarae* are also discussed. This preliminary assessment will also serve as a guideline for future releases to be performed by *Working for Water*, a program involved with mass-rearing, release and distribution of biocontrol agents at a much larger scale in South Africa.

Materials and methods

Mass rearing

The agent was mass-reared under glasshouse conditions of 28 ±2°C, 60 ± 10% RH, and a photoperiod of 14:10 (L:D) h. Approximately 50 adults were confined with several potted host plants in a cage (1.4 × 1.0 × 0.6 m) for 10 days to allow mating and oviposition. Adults were offered a fine spray of water on a daily basis to increase their longevity (D.O. Simelane, unpublished. data). Infested leaves were harvested when typical fishbone-shaped mines, often centred along the midrib, with side-shoots along the lateral veins, had been formed (Simelane 2002). Prior to release, harvested leaves were kept in 5 L perforated containers at room temperature (25 ± 2°C) for 5 days to ensure completion of pupal development. To prevent rapid wilting of the harvested leaves while allowing pupation in some leaves to be completed, a moist paper towel was spread on the floor of the container. Based on biological studies conducted by Simelane (2002), the expected number of newly emerged adults from a set of harvested (infested) leaves was equivalent to 80% of the total number of infested leaves.

Release techniques

Short-term caging in the glasshouse

When adults were ready to emerge, that is eight days after infested leaves had been harvested, the leaves were enclosed with a potted host plant in a gauze-covered cage (0.55 × 0.55 × 1.00 m) in the glasshouse for 3 days. The cage was then taken to the field, and ovipositing adults, presumably mated during the 3-day period, were released under actively growing lantana plants.

Short-term caging in the field

A gauze-covered sleeve cage (0.55 × 1.00 × 1.4 m) was used to cover an actively growing lantana plant in the field. Infested leaves, with adults ready to emerge from pupae, were confined, together with the host plant in the sleeve cage for 5 days. In contrast to the short-term caging period in the glasshouse, field caging required a longer period due to relatively cooler nights that tended to delay adult emergence. The purpose of both short-term caging techniques discussed here was not to prevent dispersal and localize population build-up as reasoned by Briese *et al.* (1996), but to facilitate mating and completion of the pre-oviposition period prior to release.

Release via the placement of infested leaves

Leaves containing pupae were loosely packed in a green vegetable bag, with holes big enough for *O. camarae* adults to pass through. The bag was suspended under a host plant cleared of spiders and ants. On emergence, adults dispersed freely into the field.

Release sites

To optimize establishment, the ideal release sites had to have an abundant food resource, and be located in an area where plants were less likely to suffer frost during winter. Approximately 14,500 flies were released between November 2001 and April 2002 at 20 sites located in 5 South African provinces (Mpumalanga, Limpopo, North West, Gauteng and KwaZulu-Natal).

Results and discussion

Effect of release techniques on initial establishment of *O. camarae*

Initial establishment failed at all the three sites where short-term caging in the field was used (Table 1). Spiders and predatory flies that were observed during the caging period might have reduced the number of ovipositing adults. However, the effect of varietal resistance on initial establishment at these release sites could not be ruled out.

Initial establishment occurred at all the sites where flies were initially caged in the glasshouse prior to their release (Table 1). In contrast to caging in the field, predation in the glasshouse was negligible and therefore would not have reduced the release sizes. Use of this technique was only limited to nearby sites (20 km radius from the mass-rearing facility) as adults did not survive longer trips to more distant release sites. Considerably more time and resources were also required to accomplish each of the caging techniques.

Release via placement of infested leaves proved to be the most successful and cost-effective technique, as initial establishment occurred at all sites where it was employed (Table 1), with considerably less time and resources invested. This technique was not only simple and effective, but hands-on input by cooperators was also minimized (Briese *et al.* 1996), reducing the risk of human error during the release process. Given the cost of making cages, which could be as high as US$40 per cage (Heytek, pers. comm.) in South Africa, placement of leaves directly in the field was by far the cheapest technique. Use of cages to confine insect releases to prevent dispersal and localize population build-up (Briese *et al.* 1996) appeared to be irrelevant in as far as initial estab-

lishment of *O. camarae* was concerned. Populations of *O. camarae* increased exponentially during the two or more successive generations following releases at the sites where adults had not been caged (D.O. Simelane, unpublished data). The use of short-term caging as an attempt to facilitate mating prior to release into the field is also irrelevant for *O. camarae,* as initial establishment occurred at all the sites where release was made via the placement of infested leaves, and the emerging adults were unconfined.

Table 1. Success of three different techniques used in releasing *Ophiomyia camarae* in 2001–02.

Release technique	No. of release sites (*n*)	Percentage sites established
Short-term caging in the field	3	0
Short-term caging in the glasshouse	5	100
Release of infested leaves	12	100

Effect of release size on initial establishment of *O. camarae*

All the sites in which adults were released via the caging in the field technique were disregarded in this analysis. Release sizes ranging from 350 to 3500 individuals per site resulted in initial establishment (Table 2) of *O. camarae.* However, it is uncertain whether release sizes smaller than 350 individuals per site could have achieved initial establishment. Until more work has been done to determine the optimum release size required for initial establishment, release sizes less than 350 adults per site should be avoided as smaller populations are believed to establish less frequently in the field (Schaffer 1981).

Table 2. Effect of release size on initial establishment of *Ophiomyia camarae,* using only the two successful release techniques.

Number of adults released	Number of sites	Number of sites established
350	3	3
450–600	6	6
900–1000	6	6
3000–3500	2	2

Effect of altitude on establishment of *O. camarae*

All the sites in which adults were released via the caging in the field technique were disregarded in this analysis. Initial establishment occurred at all sites located at elevations ranging from 0 to over 1200 m (Table 3), and at least two generations were completed during summer/autumn of 2001/2. Monitoring conducted 12 months after first release showed that full establishment had only occurred at release sites located at lower altitudes, ranging between 0 and 900 m. Further assessment revealed that leaf abscission occurred on most host plants located at altitudes above 900m during winter and spring seasons. The higher-altitude areas were characterized by lower winter temperatures and prolonged drought during winter and spring. These factors could have induced leaf abscission, causing the populations of *O. camarae* to crash. Low winter temperatures could also retard the developmental rate of immature stages of *O. camarae*, making them more vulnerable to harsh environmental conditions. Further releases should therefore be centred around the lower altitude and coastal regions, which correspond well with the climates of the collection localities of *O. camarae* in Florida, USA.

Table 3. Relationship between altitude of release site and establishment of *Ophiomyia camarae,* when only the two successful release techniques were used.

Altitude range (m)	Number of sites	Number of sites initially established before first winter	Number of sites fully established after first winter
0–300	3	3	3
601–900	3	3	3
901–1200	4	4	0
>1201	7	7	0

Effect of lantana colour form on initial establishment of *O. camarae* at mixed and homogeneous sites

At a site where three different colour forms of lantana co-existed, *O. camarae* showed a significantly higher infestation intensity on white–pink and light-pink colour forms than on an orange–red one (Table 4). A significantly higher infestation intensity also developed on a white–pink (but not on a light-pink) than on an orange–red colour form at release sites where only one colour form occurred (Table 4). Cilliers (1987a) found that *Teleonemia scrupulosa* Stål (Hemiptera: Tingidae) was not only attracted to light-pink flowering forms, but also performed better on those cultivars. Although colour forms of lantana appeared not to prevent initial establishment of *O. camarae*, further work should be done to ascertain the relationship between long-term persistence, infestation intensity and impact of *O. camarae* on different lantana colour forms in the field.

Conclusions

Given the cost of biological control programs, it is imperative that the cheapest and most effective release technique is employed when releasing a biocontrol

Table 4. Infestation levels of *Ophiomyia camarae* on three different lantana colour forms at a mixed site compared to that at three sites where only one colour form was present.

Flower colour (cultivar)	% Leaves infested per branch in a mixed flower colour site		% Leaves infested per branch in single flower colour sites	
	Range	Mean ±SE[a]	Range	Mean ±SE
Orange–red	0–10	4.8 ± 1.2a	2–15	8.9 ± 1.4a
White–pink	14–60	35.4 ± 4.8b	70–100	86.8 ± 4.2b
Light-pink	6–21	33.7 ± 2.7b	1–18	5.3 ± 1.5a

[a] Means (in a single column) followed by the same letter are not significantly different by LSD ($P = 0.05$).

agent. For initial establishment, the best release technique is through the placement of pupa-infested leaves in the field. Until more work is done to determine the optimum release size required for initial establishment, release sizes less than 350 individuals per site should be avoided as smaller populations are less likely to establish. Future releases should be centred around the lower altitude and coastal regions, which are climatically better matched with that of the collection localities in Florida, USA. Since there is no indication that *O. camarae* totally avoids any colour form of lantana, future releases should not be limited to any particular flower colour form of lantana.

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Delivering pasture weed biological control through community networks in temperate Australia

A.E. Swirepik, M.J. Smyth and D.T. Briese[1]

Summary

Between 15 and 25 years worth of research effort has been invested in the development and delivery of a suite of agents for three broad-leafed pasture weeds (*Echium plantagineum*, *Onopordum* spp. and *Carduus nutans*) in temperate Australia. Seventeen agents have been released into Australia during this process, of which 11 have required redistribution beyond their initial releases. Seven of these species have a single generation per year, three have a partial second generation and one undergoes multiple generations. Due to the slow intrinsic rates of increase of the agents and the high demand for the agents, a delivery network involving three levels of government, the Australian Landcare movement and the community has been developed to speed up the delivery process. While the delivery model is built on a hierarchical structure, involving collaborators in the process of agent rearing, release and evaluation at a level commensurate with their training and/or experience, it has been designed to facilitate information flow to end users and provide feedback to researchers. Consequently, outcomes of the delivery model include a high level of community ownership of the agents and a more effective measurable impact of those agents on their target.

Keywords: Agent rearing, release network, nursery sites, evaluation.

Introduction

Biological control of temperate pasture weeds in Australia has proven to be a discipline requiring a long-term research commitment to the study of the target, the guild of agents and the delivery of the agents to the stakeholder. During the 1970s and 1980s, CSIRO Entomology initiated projects on three broad-leafed pasture weeds, *Echium plantagineum* L. (Paterson's curse), *Carduus nutans* L. (nodding thistle) and *Onopordum* spp. (Scotch and Illyrian thistle). In 2003, two of these projects (*Echium* and *Onopordum*) are still attracting industry funding, while the third (*Carduus*) has concluded, with control having been achieved (Swirepik & Smyth 2002). Each project began independently and has a unique history until 1997, when funding for the project through Australian Wool Innovation, Meat and Livestock Australia and the CRC for Weed Management Systems saw the three projects bought together to focus on speeding up the delivery of agents to stakeholders and evaluating the outcomes of agent releases.

The *Echium* project commenced in the early 1970s with initial surveys of *E. plantagineum* populations in southern France, Portugal and Morocco to select potential agents (Wapshere 1985). Host-range testing for seven species was completed during this period, but the project was placed on hold due to an injunction (sought by apiarists and a small number of graziers) placed on the project in 1980 by the High Court of Australia (Cullen & Delfosse 1985). Following the introduction of the *Biological Control Act 1984* and a subsequent inquiry into the biological control of *Echium*, the injunction was lifted in 1988 and the first agent *Dialectica scalariella* was released in the same year. Over the eight years that followed, six more species of agent were released into Australia (Table 1). Six of the seven agents have established (Table 1).

The *Carduus* project was initiated in the mid 1980s with studies of the interaction of potential agents and *C.*

[1] CSIRO Entomology, GPO Box 1700, Canberra 2601, Australia
Corresponding author: A.E. Swirepik, CSIRO Entomology, GPO Box 1700, Canberra 2601, Australia <anthony.swirepik@csiro.au>.

nutans in the home range (Sheppard *et al.* 1990, Sheppard *et al.* 1994) and the ecology of *C. nutans* in Australian pastures (Woodburn & Sheppard 1996). Three agents were released in Australia between 1988 and 1993 (Woodburn & Briese 1996), all of which have established (Table 1).

The *Onopordum* project commenced in 1987 with survey work in the home range (Mediterranean Europe) to select a potential suite of agents (Briese *et al.* 1994), while a study of *Onopordum* ecology in Australian pastures was being carried out concurrently (Pettit *et al.* 1996). Seven agents were released between 1992 and 2000 (Briese *et al.* 2002a); four have established (Table 1). Eleven of the 13 agents that established on the three target weeds have been subsequently redistributed (Table 1).

Evolution of the release process

Until the early 1990s, the three projects were primarily focused on the research components of biological control. As a result, CSIRO Entomology would generally make releases for research reasons rather than with the express purpose of providing agents to end users. The distribution of the weeds was important in the evolution of a release network, i.e. as *Echium* occurs across temperate Australia, it was recognised in the 1980s that individual states (New South Wales (NSW), Victoria, South Australia and Western Australia) would need to be involved in the rearing and release process. The two thistles have much more limited distributions (*Carduus* on NSW tablelands areas above 800 m and *Onopordum* primarily on the Southern Slopes and Monaro regions of NSW), whose accessibility from Canberra led to the evolution of a network that relied on CSIRO Entomology rearing the agents and collaborating with state, regional and local professionals for release purposes. A model strategy for the rapid redistribution of biocontrol agents with slow rates of increase (Briese *et al.* 1996) was used between 1993 and 1996.

The advent of the Cooperative Research Centre (CRC) for Weed Management Systems in 1995 coincided with negotiations for funding of these three projects with Meat and Livestock Australia and Australian Wool Innovation. These negotiations resulted in moves to formalise a rearing, release and evaluation framework with the primary focus of

Table 1. The number and status of agents releases against *Echium*, *Carduus* and *Onopordum* in Australia.

Year first released	Agent	Releases to date	Status
Echium			
1988	*Dialectica scalariella*[a]		Widespread, limited damage
1992	*Mogulones larvatus*[f]	889	Widespread, severe local damage
1994	*Mogulones geographicus*[f]	119	Established, measurable local damage
1995	Longitarsus aeneus	1	Failed[b]
1996	*Longitarsus echii*[f]	126	Established, measurable local damage
	Phytoecia coerulescens	5	Established locally[c]
	Meligethes planiusculus[g]	59	Established locally
Carduus			
1988	*Rhinocyllus conicus*[g]	15[d]	Widespread, target controlled
1991	*Urophora solstitialis*[g]	101[e]	Widespread, target controlled
1993	*Trichosirocalus mortadelo*[f]	102[e]	Widespread, target controlled
Onopordum			
1992	*Larinus latus*[f]	302[e]	Widespread, severe local damage
1993	*Lixus cardui*[f]	501[e]	Widespread, severe local damage
1995	*Tephritis postica*	5	Failed[e]
1997	*Trichosirocalus briesei*[f]	4	Established locally
1998	*Eublemma amoena*[h]	55	Established locally
1999	*Botanophila spinosa*	2	Failed
2000	*Urophora terebrans*[g]	2	Establishment not yet confirmed

[a] *D. scalariella* quickly dispersed across the range of *Echium*, release work ceased in 1990.
[b] It was not possible to resynchronise *L. aeneus* to southern hemisphere seasons under quarantine conditions. A direct release permit was obtained, but the sole release made failed to establish and work on the species stopped.
[c] Experimental work in Australia indicated that *P. coerulescens* would have no impact on *Echium*. A decision was taken to cease work on the species after making one release in each state.
[d] A decision was taken at the time of the initial releases of *R. conicus* to allow the species to disperse naturally rather than redistribute it. *R. conicus* can be found across the range of *C. nutans* in Australia.
[e] Agent has dispersed across the range of the target in Australia.
[f] Agent has been redistributed and completes a single generation per year.
[g] Agent has been redistributed and completes a partial second generation per year.
[h] Agent has been redistributed and completes multiple generations per year.

speeding up the delivery of biological control outcomes to stakeholders. To assist in the development of this framework, the CRC appointed a biocontrol facilitator who was based in Canberra with CSIRO Entomology. The CRC also organised and ran a workshop at Yanco, NSW in May 1997 that dealt with the key issues in the project at the time: rearing techniques, release strategy (release size, timing and method) and evaluating outcomes.

Rearing agents

Typically, when an agent is first approved for release after the completion of host-specificity testing there are relatively few individuals due to the constraints of rearing in a quarantine facility. Rapidly increasing this limited resource is the first factor that needs to be addressed, and CSIRO Entomology and the state departments provide officers and facilities to rear agents for release. However, a number of key technical issues need to be overcome; an understanding of agent biology, provision of suitable rearing facilities and training staff in rearing techniques.

Insight into agent biology begins with existing literature, which is generally incomplete. Key facets of agent biology/requirements that have been explored include fecundity and oviposition pattern, the carrying capacity of the host, suitable watering regimes for potted plants containing root-feeding larvae, and aestivation requirements.

Rearing facility requirements for this project are relatively simple. In order to synchronise agents with the season of particular regions, we have developed culturing protocols in which most of the agents' development takes place in pots or tubs (a standard pot size of 20 cm has been used for the rearing of *Echium* agents, while fibreglass tubs varying in size from 50 cm × 100 cm to 100 cm × 100 cm have been used to grow multiple plants and reduce labour). Host plants are maintained through winter in either unheated glasshouses or plastic tunnels to avoid the waterlogging effects of winter rainfall. Heated glasshouses or constant temperature rooms are used only for plant propagation and/or maintenance of stable oviposition rates by the insects. A protocol for rearing agents in an "in ground" setting has also been developed. These protocols require the construction of a mesh "shadehouse" that is planted out with a garden of the host plant. Agents are then introduced in a number commensurate to the carrying capacity of the garden. All states now have access to suitable facilities.

Culturing agents in several regional rearing facilities also reduces the impact of a single culture failure, whilst keeping individual cultures to a logistically manageable size. An unforeseen bonus of this structure is that a friendly rivalry between organisations over maximising rearing outcomes has been created. The wide distribution of *Echium* also dictates that agents should be cultured closer to where they will be released, e.g. if all *Echium* agents were to be cultured in cool climate Canberra, synchronising their development and subsequent emergence for release with the season in South Australia or Western Australia would be difficult.

Training collaborating staff has taken a number of forms. Before the 1997 Yanco workshop, CSIRO officers visited state rearing facilities on an *ad hoc* basis to work through culture management issues with collaborators, and financial constraints during this period meant that Western Australia and South Australia were not visited. However, a rearing/culture management package was provided to all collaborators and updated to include technique modifications every year from 1993–1996. The package was backed up with telephone support where necessary.

The Yanco workshop was a significant step forward for the project, as it was the first time all project staff had come together to discuss the project. The personal contact enhanced interstate collaboration to a level where state collaborators would contact each other to discuss issues rather than always directing questions to CSIRO Entomology. Another important outcome of the workshop was a decision to bring project staff together for a technical and management meeting annually. This happened every year until 2001 when a reduced budget saw the meeting dropped to conserve funds. During the period (1997–2001) resources were also available to allow all of the state rearing facilities to be visited by a CSIRO Entomology officer at least once.

The release network

The release network is formed around the three levels of government in Australia: federal (the Australian Government), state and local. The Australian Government, through CSIRO Entomology, leads the process, providing agents and the technical requirements for their rearing and release. State department officers that have reared the agents release them in collaboration with local government weeds officers or similar, who act as a first contact with end users in their community (Figure 1). The regulatory role that weeds officers fill (administering the Noxious Weeds Act), provides them with detailed knowledge of weed infestations in their local government area, and exposure to the biocontrol project allows them to work with landholders to ensure that biocontrol meets with legislative requirements.

The National Landcare Program is an Australian Government initiative that also plays a key role at the local level. Depending on the state, Landcare coordinators are either based within the state or local government offices and fill a similar local coordination role to that provided by weeds officers. A key difference is that Landcare is charged with providing collective action by communities to sustainably manage the environment and natural resources in partnership with government to

achieve sustainable rural industries. This gains weed biological control exposure as a component of natural resource management rather than as a stand alone solution to a weed infestation. The fact that Landcare coordinators do not have a regulatory role to play with regard to weed infestations has, on occasion, also been seen to enhance community collaboration.

The release process

The first objective of the release process is to establish agents on a regional basis across the range of the weed, to create a network of "nursery" sites. The project officer who has reared the agents, collaborates with the local coordinator to find the most suitable release site. Preferred sites are owned by landholders who readily understand the project's objectives and likely time frame for outcomes, whilst also showing a commitment to collaboration for the ongoing maintenance of the release. Once the site is selected, the agents are released according to current best practice and the details of the release recorded in a relational data base for future reference. At the time of release, those present (which may vary from local coordinator and landholder to a field day with 20–30 people) are given an oral overview of the project, along with specific information pertaining to the management of the release. The oral presentation is supported by an information kit that includes photographs of the agent, a description of agent biology, release site management information and links into integrated weed management (IWM).

Evaluating the outcome of an agent release

The release process goes hand in hand with evaluation (Figure 1), as monitoring the outcome of an agent release is essential to optimise release strategies and to hasten project outcomes, whilst using the results to communicate progress to end-users and funding agencies. Swirepik & Smyth (2003) described a three-tiered evaluation strategy that comprises agent establishment, spread and impact. The key outcome of tier-one monitoring, the most detailed, is to provide robust measures of the impact of each agent on the target species. To achieve this, it is often necessary to identify and quantify key transition stage relationships for the target (Woodburn & Cullen 1993, Sheppard *et al.* 1994, Woodburn & Cullen 1996, Smyth *et al.* 1997, Briese, 2000, Sheppard *et al.* 2001, Briese *et al.* 2002b). Tier two aims to provide an estimate of both target and agent population size, through simple one-off measurements, e.g. measuring *Echium* density and the rate of attack by *M. larvatus* in late winter successfully predicts weed mortality (Swirepik & Smyth 2003). Tier-three monitoring is the simplest form of evaluation in a biocontrol project. Its aim is to provide information on the establishment and spread of agents at all release sites and

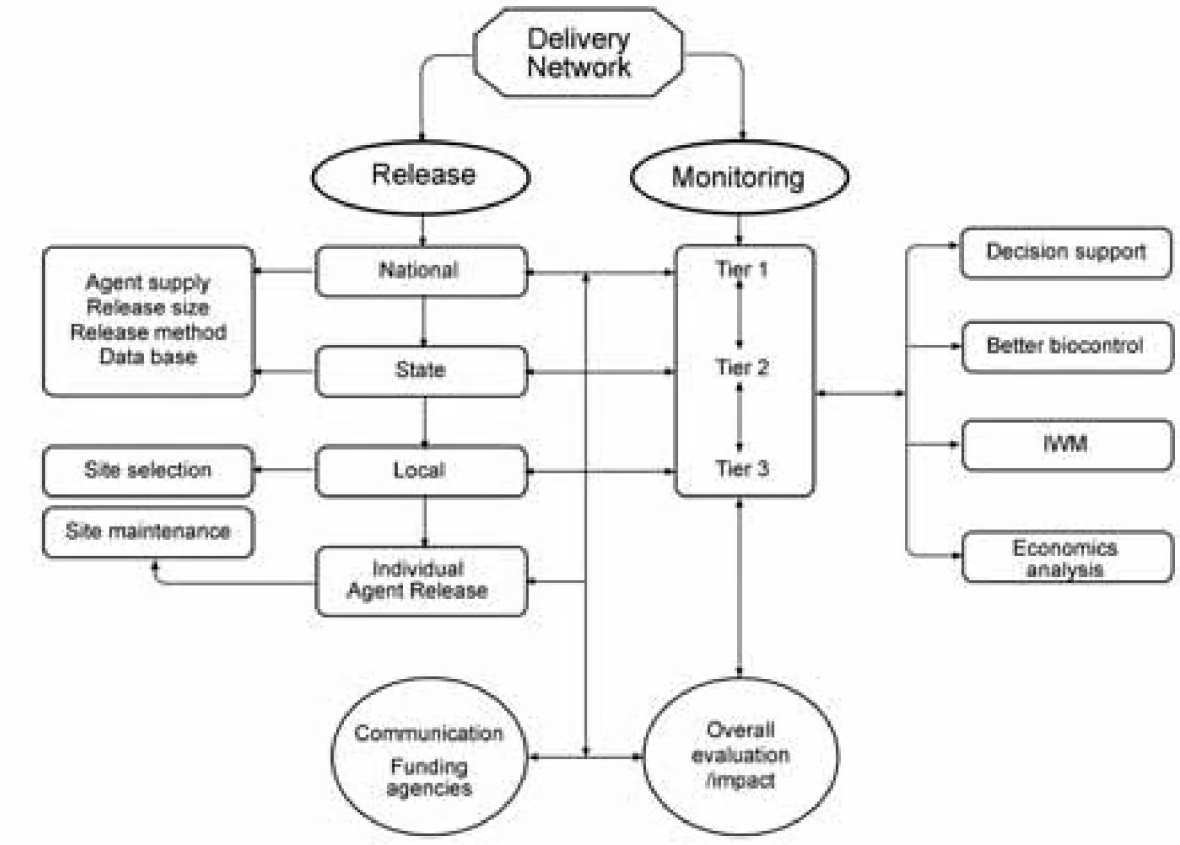

Figure 1. Schematic representation of the delivery and evaluation process and outcomes.

may involve landholders/stakeholders and/or members of the professional network collecting data.

Data gathered from tier three are then added to the release data base to be analysed and used as decision support for project management. By using an active adaptive management approach to steer the project, we have been able to quickly improve practices concerning the size, timing and method of release (e.g. whether to use a release cage or not). Tier three has also influenced the location of agent releases, e.g. the project no longer recommends the release of *M. larvatus* in drier/late autumn-break areas infested with *E. plantagineum*, but encourages the release of *M. geographicus* and especially *L. echii* due to their naturally longer aestivation period, which allows them to remain dormant through prolonged autumn drought and which has led to higher establishment rates (Swirepik & Smyth 2003).

Once tier-three evaluation indicates that agents are established and spreading at a release site, additional data may then be collected to estimate target and agent density at the site (tier two). This allows prediction of when a release site may be ready to be harvested by collaborators at a field day(s). The development of an agent population large enough to allow a field day to be held for collaborators to collect their own agents forms a transition of the site from "release" to "nursery" site. Running field days at nursery sites has been instrumental in accelerating the delivery of agents to stakeholders at national, state and local levels.

At the national level, the delivery of *M. larvatus* has benefited significantly from the use of a nursery site at Yanco, NSW for the annual collection of tens of thousands of weevils over the last six years. Officers from all states have travelled to the site to collect *M. larvatus* and have subsequently redistributed the agent in their states through their local networks. Alternatively, they have the agents shipped to them. The same nursery site has also been used to supply *M. larvatus* to all NSW Agriculture officers, and field days have been held for weeds officers from surrounding regions. Each state (apart from WA) now has at least one such nursery site for local supply. This process has resulted in 889 releases of *M. larvatus* across temperate Australia.

The delivery of *Larinus latus* provides a model for the use of field days to deliver agents at the local level. During the early 1990s, a regional network of release sites was set up across the southern slopes of NSW. Until 1997, populations of *L. latus* expanded slowly, providing limited opportunity to redistribute the species from field collection. However, in the spring of 1998 significant populations of *L. latus* were found at two sites near Harden, NSW. These sites were used to host field days (for up to 50 people/field day) over the next four years, for local landholders/stakeholders to be trained in the identification, collection and release of the weevil. Between 1992 and 1997, only 51 releases of *L. latus* had been made, whereas 244 releases were made from 1998 to 2001. This led to a high level of community ownership for the ongoing redistribution of this species and allowed the project to concentrate on the newer agents in the suite. A key aim of the project is to create such community ownership for each agent species. *L. latus* collected from these nursery sites have also been used to start a new biological control project against *Onopordum acaulon* in Western Australia and South Australia (Swirepik & Woodburn 2002).

Bringing it all together

Creating a widespread and high level of community ownership for an agent does not necessarily mark the conclusion of the project. Project officers have been able to build further on the knowledge obtained during the 15–25 year investment in the various biological control projects. The ecological understanding of temperate pasture dynamics and agent biology has fed directly into the design and implementation of integrated management trials for each of the target weeds (Huwer *et al.* 2002). Evaluation data have also been central to an economic analysis of investing in the redistribution of *Echium* agents (Nordblom *et al.* 2002), highlighting a need for investment in additional targeted releases of *M. larvatus* (in areas where release numbers are low and potential benefits are high) to speed up the control of *E. plantagineum*.

The discipline of biocontrol has benefited from the monitoring program, through the additional insights gained into how a suite of agents impacts on their host and how this will, in turn, influence field population dynamics. This knowledge will add decision support to agent selection for future projects. At the project level, the strategy implemented has provided evaluation data that have: 1) provided a decision support framework that has facilitated the targeting of agent releases to areas where they are best suited, and information as to how to best release those agents; 2) provided the ability to predict the time frame over which project outcomes may be realistically expected; 3) played an integral role in the extension of the project to community collaborators; and 4) provided a sound justification of project expenditure to our funding partners that in turn has realised a long-term partnership.

All of these benefits have not been derived without some difficulty; the main ones concerned with relationship management, which is not surprising in a collaborative project involving complex links between three levels of government and the community across four states and one territory. Within these jurisdictions, there are collaborators from many different backgrounds and organisational cultures, all of whom require a level of technical understanding about the project commensurate with their level of involvement. At times, the effective delivery of this knowledge has been time consuming and frustrating. However, the end result is a functional project worthy of both the long-term past investment, and the medium-term investment currently required to complete it.

The project is currently in the final phase of negotiations for three additional years of funding for delivery and evaluation activities. This funding period will allow us to develop a regional nursery site network for all of the newer species of agent indicated in Table1. The project beyond this point will then be able to focus on creating community ownership for each species whilst providing ongoing impact evaluation.

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Using GIS to integrate biological control into the integrated weed management program for *Spartina alterniflora* in Willapa Bay, Washington

Miranda S. Wecker, M.S., Teresa Alcock and Keven Bennett[1]

Summary

Willapa Bay is one of the most productive and highest quality estuaries remaining in the United States. A key fueling stop for migrating birds in the Pacific Flyway, it supports an abundance of marine species. Willapa Bay is also the site of the most extensive infestation of smooth cordgrass (*Spartina alterniflora*) in the region. Roughly 32% of the total 47,000 acres (*ca.* 19,000 ha) of intertidal habitat are infested. More than half of the Bay's mudflats are broken into approximately 500 private ownerships, while the rest is under federal or state jurisdiction. In 1994, government agencies launched an integrated weed management program. The toolkit of approved methods is limited to one herbicide, glyphosate, and various mechanical methods. University of Washington Olympic Natural Resources Center (UW-ONRC) and its partners added biological control in 2000 when *Prokelisia marginata* was released. Prior greenhouse trials had shown that certain plants would be resistant to *P. marginata* and therefore would have to be targeted for eradication with other tools. To target resistant plants, sophisticated and precise integration of control applications is necessary.

The challenges of integration in this case are legion. Four state and federal agencies have overlapping jurisdictions. Hundreds of private citizens own infested lands. The options for control vary in cost, sensitivity, and efficacy. The weed is spreading at different rates in different areas of the Bay. One of the greatest barriers to integrated planning has been the lack of analytical decision-support tools. In the past two years, UW-ONRC has developed a geographic information system (GIS) application specifically designed to aid in spartina management. An ARCVIEW™-based computer program, it integrates all available data sets with a dynamic model projecting the future spread of the weed. It included functionality that allows users to assess costs, equipment and staffing requirements of various scenarios. In this paper we describe this software and its utility in integrated planning.

Keywords: GIS, integrated weed management, invasive spartina.

Introduction

Willapa Bay is one of the most productive and highest quality estuaries remaining in the United States (US Fish and Wildlife Service 1997). A key fueling stop for migrating birds along the Pacific Flyway, the Bay is also the source of most of the private sector employment in this rural area of Washington State. Businesses harvesting, processing, and selling oysters, clams, crabs and finfish are the mainstay of the area's economy. The oyster industry in Willapa Bay leads the nation in terms of productivity, generating 60% of the oysters produced on the west coast of the US. The remarkable ecological and economic values of Willapa Bay are at great risk due to the continuing biological invasion of a cordgrass that is native to the eastern and southern US coastline, *Spartina alterniflora.* Spartina was unintentionally introduced in the early 1900s and spread slowly until the 1980s (Sayce 1988). Before the invasion, the estuary was characterized by 47,000 acres (1 acre = 0.405 ha) of open mudflats, with scattered eelgrass beds and oyster reefs. A ground survey conducted in 2002 using global posi-

[1] Olympic Natural Resources Center, University of Washington, PO Box 160, Naselle, WA, USA 98638.
Corresponding author: Miranda S. Wecker <mwecker@willapabay.org>.

tioning system (GPS) techniques showed that approximately 6000 acres of formerly open mudflats are now solid monotypic meadows and roughly 5300 acres are now infested with clone fields. Extreme outliers affect another 5000 acres. Studies have demonstrated that the changes taking place are far-reaching and deleterious to native biological diversity.

In 1993, several state agencies collaborated in the issuance of an environmental impact assessment which announced the state's official policy of "integrated weed management" (Washington State Co-Leads 1993). At the federal level, the US Fish and Wildlife Service conducted a separate but parallel environmental review and came to identical conclusions. The control program began in 1995 with a complex array of independent participants: five state agencies, two federal agencies, a local Native American tribe, two units of local government and a multitude of private interests, commercial and non-profit. The first two years produced little on the ground, as lawsuits blocked use of chemicals, numerous permit processes moved ahead at a slow pace, and the efficacy of control methods was tested on a relatively small scale. From the perspective of local residents, the government was reacting far too slowly, and the rate of spread was increasing alarmingly. In 1995, prodded by local political leaders, the Washington State Legislature unanimously declared the spartina invasion an environmental disaster, removed some of the key regulatory hurdles that delayed action, and called upon the agencies to attack the problem in a more aggressive and coordinated fashion.

A portion of the state environment impact assessment was devoted to the topic of integrated weed management (IWM) (Brueggeman 1993). In it, integrated management was defined as the "deliberate selection, integration, and directed utilization of plant population suppression measures on the basis of predicted economic, environmental and sociological consequences." [Klassen 1979]. "The approach is unique because it is predicated on ecological principles and incorporates multidisciplinary methodologies in developing ecosystem management strategies that are practical, effective, economical, and protective of public health and environmental health." The environmental impact study authors very helpfully provided cautionary words about the challenges or "shortcomings" of integrated weed management. They anticipated that IWM would be difficult due to the following features:

- failure among managers to recognize time-consumptive processes
- insufficient understanding of the concepts, philosophies, and goals of IWM
- shortage of knowledgeable individuals for development, implementation, and evaluation
- reluctance among agencies to administer integrated program complexity
- lack of information on plant biology and ecology, damage and action thresholds, and management method efficacies needed for program implementation
- inconsistent, inadequate financial support by responsible management agencies.

The prescience of this list of challenges is now obvious to those who have observed the history of the spartina control program to date. All of the difficulties listed above have in fact hindered the realization of a truly integrated program of spartina control. While not easy, integration is necessary for success of most projects. For our weed management project, there is no alternative. Many of the challenging complexities we face may be better met if we use the capabilities of geographic information system (GIS) tools.

Each weed problem presents its own context of particular and complex "economic, environmental and sociological" conditions. Introducing biological control approaches into the possible mix of conventional responses adds further complexity. It is difficult for managers to sort out the differing research requirements, the timing of costs and benefits, and the expectations associated with management. It is no wonder that after the panel on the subject of integration during the IX International Symposium on Biological Control of Weeds in South Africa, moderator J.M. Cullen described the concept of integrated management of weeds "still fairly loosely defined" (Cullen 1996). He suggested that there are various ways in which biological control can be integrated with other methods of management and proposed three categories: purpose-specific approaches, ecological integration and physiological integration. Purpose-specific approaches were those in which each tool has a specific job to do and operates separately in space or time. The second and most common form of integration, ecological integration, is one in which various tools are used, often at the same time on the same infestation, e.g. when herbicides are used to initially knockdown a widespread infestation and then biological control agents are used to maintain the weed at a lower and acceptable level. The third category, physiological integration, referred to cases in which synergistic interactions occurred, allowing one type of control tool to enhance the impact of another control tool. These categories are helpful in organizing our thoughts about the benefits of integration, as well as envisioning the many ways in which we may attempt to integrate biological control into an overall integrated weed management program. Any or all of these forms of integration would promote the overall success of the IWM program. Therefore it makes sense to intentionally broaden our strategy to attempt all of the aforementioned types of integration. It is useful to understand the different ways the biological control agents may be integrated so that we can be thorough in testing the practicality of each form of integration.

Our unique case: the unusual vulnerability of exotic spartina

In the late 1980s, Daehler & Strong (1997) discovered that moderate levels of the planthopper *Prokelisia marginata*, an insect common to native spartina marshes throughout the US coastline, had a devastating effect on exotic *Spartina alterniflora* plants from Willapa Bay. In greenhouse trials, 37% of the Willapa plants had been killed by the end of the second year of exposure. Most of the remaining plants were severely stunted. The same level of insect exposure had no effect on *S. alterniflora* from San Francisco Bay, or from its native range on the Atlantic coast. Additional trials found that exotic *Spartina anglica* from Puget Sound suffered the same fate when exposed to *P. marginata*. (Wu & Hacker 1999). One theory is that the vulnerability of Willapa spartina plants is an outgrowth of adaptation to a herbivore-free environment (D. Strong, pers. comm.). Greenhouse trials have demonstrated that not all Willapa spartina is vulnerable. Some plants managed to survive exposure to *P. marginata*. This variability of response in the greenhouse indicates that, even under the best of circumstances, we should not expect *P. marginata* to have a lethal effect on all the plants in the field.

The unusual nature of the opportunity for biological control of Willapa spartina was crucial to convey to the public and to our professional colleagues, so that their expectations for the biological control project were not excessive. Even if *P. marginata* repeated its dramatic performance on greenhouse plants in the field as we hoped, other tools would still be needed to remove resistant clones. We were also cognizant of a more subtle point to communicate. Once the insects were released, the "natural selection" clock would start ticking. As vulnerable plants were eliminated by *P. marginata*, only insect-tolerant survivors would be left to repopulate the Bay. Because this biological control project is likely to be self-defeating over time, we recognize the transient nature of the opportunity to use biological control. By releasing the insects, we assumed a share of responsibility to see that the overall control program was ready and capable of finishing the job.

Our initial expectation, in its most optimistic form, was that *P. marginata* might very rapidly knock back the infestation by killing the vulnerable 40% of the plants. More often, herbicides are used to "knock back" the infestation so that biological control agents have a better chance of offering long-term control. In our case, we hoped *P. marginata* would reduce the overall infestation in the short term, but envisioned less of a long-term contribution. If *P. marginata* were able to remove the bulk of the weed in the near term, then the limited funds currently available for other elements of the control program would become sufficient to eliminate the portion of spartina that remained. So far, these hopes have not been realized. Although we have established populations at several areas in the Bay, *P. marginata* has not reached the levels of abundance needed to achieve substantial impacts. The political context is such that there is little time to wait for *P. marginata* to deliver results: major progress must be made in the next two years or else the control effort will be abandoned. As a consequence, state and federal agencies have launched a very aggressive chemical control program. The biological control effort will be accommodated by avoiding chemical applications in areas reserved for *P. marginata*. While we are not waiting to see if *P. marginata* can knock back the infestation, we still hold out hope that its presence as a natural enemy will have a long-term beneficial effect in controlling spartina.

As discussed above, integration of our biological control effort into the overall spartina control program is particularly important in light of the unusual vulnerability of Willapa plants. The need to have an effective overall program led us to take on the much larger challenge of fashioning a decision-support tool to help develop and execute the overall integrated weed management strategy. GIS software provided the format for pulling together the full range of information necessary for efficient and comprehensive integrated planning.

Spartina GIS application: a desktop decision-support tool for managers

GIS applications have become standard tools for all manner of decision-making. Their capacity to visually present data make them ideal for communication of information and for group analysis and consensus-building. We therefore developed a GIS software application tailored for use in the spartina control program (SpartGIS). This software application is an extension of ARCVIEW™, and was specifically designed to be used by weed managers with little or no background or training in GIS. SpartGIS is suitable for routine performance of the key tasks associated with spartina management (Fig. 1).

SpartGIS contains a comprehensive range of digital data on natural features and biological resources in Willapa Bay. Some of the key datasets already installed as themes include the following: base layer bathymetry of the Willapa Bay; substrate types (mud, sand and mixed); spartina distribution over time; commercial and recreational shellfish resources, shellfish productivity classifications; migratory duck habitat; Brant habitat; shorebird habitat; eelgrass beds; tideland ownerships; bayfront ownerships; water quality monitoring sites; biological control release sites; and long-term monitoring sites. Additional themes can be brought into the system as they become available. Along with the datasets themselves, SpartGIS also makes available the "metadata" that explain how datasets were collected, when they were collected, by

whom they were collected etc. In sum, SpartGIS represents an archive of the widest range of relevant information configured to allow the viewing of physical, economic, and sociological features.

A dynamic model of spartina spread has been incorporated into SpartGIS to provide managers with the ability to test and compare control approaches over time. Functionalities have been installed that permit users to assess costs, equipment needs and staff requirements of various scenarios. Simple buttons and dialogue boxes allow users to adjust the values given to key factors such as the cost per acre, efficacy rate, and the treatment capacity per day. This allows managers to make take into account many of the complexities they encounter in implementing control in different settings in the field. For example, with one type of mechanical treatment device, no more than 5 acres could be treated in a single day. That treatment can be expected to produce about 90% efficacy at a cost of $US1000 per acre. Another treatment option, herbicide application, costs $US200 per acre and can be done on 30 acres in a day. However, a minimum of 12 hours of drying time is needed to reach 90% efficacy. With less drying time, efficacy is reduced to 40%. Default values have been assigned to characterize each tool option, but a dialogue box allows users to adjust them to reflect situations encountered in the field operations. The dynamics of the model also can be adjusted to reflect such influences as reduced seed production, slowed vegetative spread, and variability of seedling establishment. Drawing from studies done on Willapa spartina, the model is designed to replicate the plants' dynamics of vegetative spread, seed production and seedling establishment.

SpartGIS allows users to draw treatment blocks in any area of Willapa Bay, associate those blocks with treatment tools to be used, and then run the spread model to evaluate the results (Fig. 2). At the end of the model run, the GIS tool presents the user with information about the extent of the infestation during each quarter of the year. Treatments are scheduled for the appropriate season: mechanical control is done summer, autumn and winter. Chemical control is executed in the summer and autumn. After the control approach has been set and the spread model has run, the program is designed to ask a sequence of management questions. Users are asked whether they would like to purchase more tools to complete the control program and are offered an assessment of the costs of acquiring the tools. If the user is unable to afford the purchase of additional equipment, SpartGIS provides information on the infestation remaining to be addressed later. The output of the model is stored as tabular information in EXCEL spreadsheets. The system also outputs still images as the model runs that can be strung together to make an animated movie showing the control program in action.

Applying SpartGIS

In the remaining space, this paper will review the utility of this tool in addressing the array of complexities that can be described as physical, economic, or sociological.

Physical complexities

The sheer size and physical complexity of Willapa Bay present enormous logistical challenges for integrating the control program. SpartGIS offers an efficient tool for easily plotting the location and size of spartina meadows and clone fields. The extreme tidal ranges characteristic of Willapa Bay make it particularly difficult place to undertake large-scale herbicide applica-

Figure 1. SpartGIS's simple user interface.

tions. The one herbicide approved for use – glyphosate – is most effective when applied in conjunction with a very long period of drying. With at least 12 hours of drying time, efficacy rates exceeding 90% have been attained (Patten & Stenvall 2001). Such long periods between tidal inundations occur during a sequence of two low high tides. SpartGIS has been used to map the drying time periods for large blocks of spartina targeted for spraying in the upcoming season. We have used GPS devices to plot the relevant high tide lines. These data have been quickly and easily integrated into SpartGIS so that managers can now plan the spray program to take advantage of the best tides to treat the most difficult-to-reach areas. Another illustration of the value of SpartGIS is its capacity to account for key factors affecting the expansion rate of the weed. The substrate type is an important influence. In recent years, the impact of the increased fertility of certain clone fields has become more pronounced. Studies have shown that coalescing spartina clones in the two particular sub-areas of the Bay are producing much larger proportions of viable seed than other parts of the infestation (Davis 2002).

SpartGIS has helped us keep in sharp focus the ecological values we hope to protect. We have data on areas of importance to migratory birds, shellfish and finfish. Logistical problems of a physical nature can also be addressed with the GIS system. The eastern side of the Bay is almost entirely undeveloped forest lands with few points of access from which control operations can be launched. Through working with local landowners, we have plotted the private access roads and boat launches that have been offered for use.

Economic complexities

The economic complexities of the control program can be categorized as those associated with the costs of the control program and those arising from the varying economic damages caused by spartina. SpartGIS helps managers to understand and analyze both kinds of costs. As mentioned above, specific cost calculations for each control method are included in SpartGIS. After a treatment block has been drawn, the user may associate it with a control technique by simply pushing a button on the screen. The system will report the cost based on its calculation of the size of the infestation. SpartGIS has been used to compare the long-term costs and time requirements for strategies with emphases on different tools: herbicide-dominant versus mechanical tool-dominant approaches versus ones with a more even blend. SpartGIS also presents data visually on the economic values present in the Bay. One GIS layer shows the ranking of shellfish beds according to their commercial productivity. Another contains tax data indicating the assessed value of all privately owned lands. This kind of information is useful in considering the economic burdens landowners might be likely to bear in efforts to control spartina.

Sociological complexities

Willapa Bay's mudflats are divided into federal, state, tribal, local, commercial and residential ownerships. One federal agency, the US Fish and Wildlife Service, and three state agencies, the Washington Department of Natural Resources, the Washington

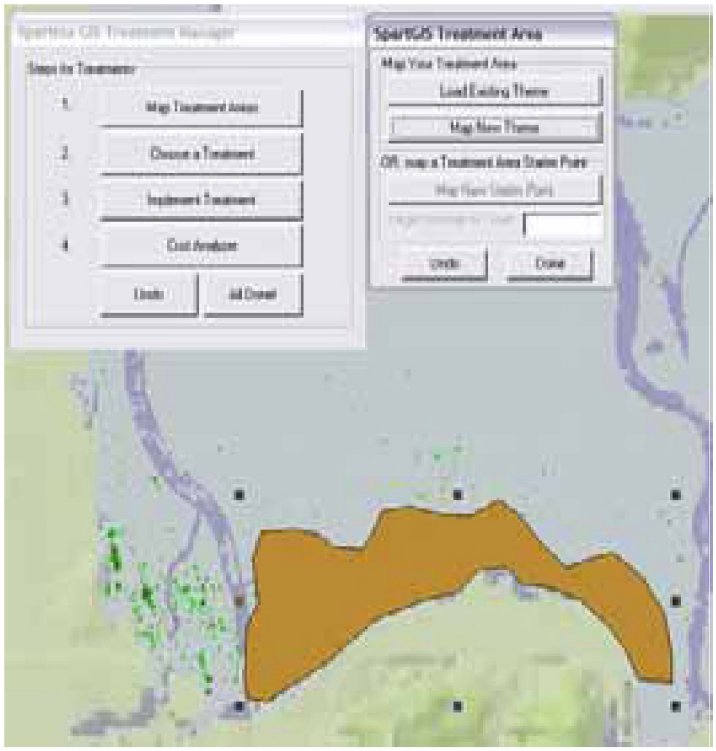

Figure 2. SpartGIS: drawing treatment blocks.

Department of Fish and Wildlife, and the Washington State Parks Department, own property in Willapa Bay. A fourth state agency, the Washington Department of Agriculture, has been assigned the responsibility for coordinating the spartina control program. Each agency operates under different mandates and must comply with an array of laws. The privately owned intertidal lands are broken into approximately 500 parcels of irregular shape and size. SpartGIS includes the best available information on this complex ownership pattern, allowing managers to accommodate the political sensitivities of individual landowners in the selection of the control tool used on their property. SpartGIS has also been helpful in developing and archiving information on landowners' willingness to cooperate. Landowners have provided legal permission allowing crews to enter their property to carry out control, to use private roads for access the Bay and to draw from water sources on their property. These donations have been recorded in SpartGIS and are represented on maps to help guide planning.

User-experience

We designed SpartGIS to be an easy-to-use desktop application. We envisioned that it would allow managers and citizens to call up a wealth of information "on the fly" during meetings. We hoped that it might become a standard desktop tool for agency staff. In 2000, we sponsored a two-day training course for agency staff involved in the spartina control program. The curriculum included coursework in the basic operation of ARCVIEW™ GIS software as well as the specialized capabilities of SpartGIS. We developed a step-by-step take-home manual and gave each participant a free copy of the SpartGIS application. We also offered to provide free technical support as needed. At the end of the course, we held a friendly competition to see who could design the most efficient long-term control program. As they worked with the tool, agency staff provided suggestions on how to adjust the model to better fit reality. We compiled these criticisms and later revised the application to address them.

In the years since our training workshop, some of the managers have continued to use of our system on their own. However, our GIS team has frequently had to provide technical support. In general, we have found that our GIS program is not as easy to use as most desktop software in wide use. Although it is very simple when compared to the most advanced GIS software, SpartGIS requires regular practice to maintain competence. We have concluded that until further advances are made in the underlying software, GIS applications will probably not become a standard desktop tool. For the foreseeable future, SpartGIS will be a very useful decision-support tool that requires the assistance of technicians to apply. Still, the benefits of our spartina system are evident in the expanded role our GIS technicians now play in providing mapping and analytical support. Dozens of specialized maps, images, and posters have been generated for official documents, public meetings, intergovernmental planning sessions, and briefing papers for decision-makers. The animated sequences we have generated with SpartGIS have had a particularly potent impact on audiences who say that these depictions make a far deeper and more immediate impression than hours of words. In the hands of competent technicians, SpartGIS has proven to be an enormously powerful tool to support the efficient integration of vast amounts of information. It has helped our managers overcome some of the challenges to integration outlined above.

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Novel techniques for increasing the survival of aestivating biological control insects

Paul B. Yeoh and Tim L. Woodburn[1]

Summary

Red apion, *Apion miniatum*, is a potential biological control agent for *Emex australis,* doublegee, a major annual weed in the Mediterranean climatic zones of southern Australia. Offspring from the univoltine weevil emerge from the plants in late spring/early summer. Over summer, the host plant is not available and the adults aestivate. It is desirable to release the red apion in autumn. For insects being held in the laboratory over the summer, we assessed the effects of food availability, temperature and lighting on the individual's subsequent reproductive output. Survival rates of red apion over the storage period were inversely related to temperature for 5 to 20°C. The most successful storage method was to store the normally leaf-eating insects at 8°C, whilst giving them access to simple carbohydrates. This temperature was just above their lower developmental threshold temperature. Infertility resulted at lower temperatures and fecundity and survival declined at higher temperatures. The techniques outlined have possible applications to other biological control programs.

Keywords: aestivation, *Apion miniatum,* biological control agent, doublegee, *Emex australis*, low temperature, reproductive output, summer storage.

Introduction

Typically in classical biological control programs, agents are collected overseas and imported in low numbers into quarantine facilities for host-specificity testing. If suitable, agents are removed from quarantine and a mass-rearing and redistribution program initiated. In this phase, like in any production plant, the aim is to maximize the output of the product without affecting quality, and the net output is inversely proportional to the efficiency of the system. Larger numbers of available agents maximize the chance of establishment with larger release numbers permitted at each site and they allow for releases to be made at more sites.

In Mediterranean climates, the summer is hot and dry with most annual plant species existing in the seedbank. Many adult insects are able to endure unfavourable environmental conditions by entering a reproductive diapause and even non-quiescent individuals in diapause have been shown to age far slower than non-diapause insects under identical conditions (Tatar & Yin 2001).

Our biological control project targets *Emex australis,* doublegee, a major weed in the Mediterranean climatic zones of southern Australia, using the red apion, *Apion miniatum* (Yeoh *et al.* 2002). This univoltine weevil is from Israel where its native host is *Emex spinosa,* the lesser jack. Red apion adults aestivate over the summer, becoming reproductive at the break of the season in autumn when their host plant, a winter annual, becomes available. Its host range is restricted to *Emex* spp. and some *Rumex* spp. During winter, it is only observed feeding on the leaves and petioles of the host plants and there are no published records of it feeding on nectar or flowers of any species at any time (Scott & Yeoh 1996). In both its native and intended new habitat, there is no suitable green foliage for it to feed upon during the summer.

Other summer aestivating, univoltine weevils, *Mogulones geographicus* and *M. larvatus*, are widely established in Australia and are causing significant damage to Paterson's curse, *Echium plantagineum* (Swirepik & Smyth 2001). Collecting and releasing teneral adults immediately onto field sites lessens husbandry requirements, but fully exposes them to the

[1] CSIRO Entomology, Private Bag 5, PO Wembley, Western Australia 6913, Australia.
Corresponding author: Paul B. Yeoh <paul.yeoh@csiro.au>.

hostile environmental conditions of summer. Despite entering diapause, many still die before reproducing. For the Paterson's curse project, autumn releases proved to be more likely to establish populations at sites and the insects are usually stored over summer in cages located under shade, with soil, leaf litter/mulch and dead host plants. Similarly, we opted for autumn releases for the red apion and this required us to develop a method of holding our aestivating insects over the summer.

The storage method developed for our red apion (our "host plant" method) gives good survival, but is resource intensive. It requires environmental chambers with light and temperature control, host plants grown out of their normal season and constant husbandry so as to control pests and prolong the life of the plants. We therefore sought an alternative, pest-free and more labour-efficient method. We also wanted a system that would expedite the process of collecting and releasing the apion in autumn as well as a system that could be used by others.

The aims of this paper are to 1) determine the value of supplementary feeding with host plants to the survival of red apion so as to develop a method of holding the mass-reared red apion over the summer and 2) investigate alternative low maintenance, low resource methods of storing the red apion over summer that do not require the use of host plants.

Methods

The value of host plants over summer

Laboratory observations confirmed that red apion enters a non-quiescent reproductive diapause over summer and will drink and feed if given the opportunity. During this period, *E. australis* is difficult to grow in pots due to the soil temperatures, so in quarantine, breeding colonies were maintained over summer by caging them at very low densities (10 per plant) on *Rumex crispus* plants. Once the mass-rearing phase of the biocontrol program began, it was necessary to increase the densities at which adult red apion were housed over summer.

The effects of supplementary watering and the provision of host plants over summer on red apion's survival were assessed using adults that had just entered aestivation. The experiment was set up as a three-level, one-way analysis of variance (ANOVA) with adults either having access to foliated host plants (*R. crispus*) and weekly applications of water to the soil, only weekly applications of water to the soil or neither host plants nor applications of water. Each cage was initially set up in December with 300 red apion. There were three replicates.

All cages consisted of a base (100 × 50 × 50 cm) filled with potting mixture and covered with a 100 cm tall, insect-proof, polypropylene netting ("Meteor anti-virus netting"). To provide potential refuge sites, all cages contained a 70 cm long × 10 cm diameter trunk section of stringy bark bottlebrush (*Callistemon* sp.), dead *E. australis* foliage/stems, *Acacia cyclops* stems/seeds/leaves, hessian material, crumpled paper towelling and mulch/leaf litter made from local trees (predominantly *Eucalyptus* spp.). All cages were located under a stand of *Eucalyptus citriodora* at CSIRO, Perth, Western Australia. Ants were recognized as potential predators and were prevented from entering the cages by trays of water, layers of oil and applications of tanglefoot. The over-summer survival was assessed at the commencement of the autumn rains (May) when individuals were recovered from the cages by attracting them to *E. australis* plants.

Alternatives free of host plants

The need to provide host plants to aestivating red apion over summer has associated high labour and resource investments. Storing the red apion at lower than ambient temperatures without host plants was therefore investigated. Two separate experiments were conducted: the first using a range of temperatures with no supplementary feeding and the second with a range of temperatures, but with supplementary feeding with simple carbohydrates.

Experiment 1 – no supplementary feeding

This commenced in summer 1999/2000 and was designed as a 4 × 3 factorial ANOVA with insects held at 5, 10, 15 or 20°C and given a 0:24, 14:10 or 24:0 (hours light: dark) photophase. The effect of light on survival and reproductive output was also incorporated into this experiment as fridges could substitute for environmental chambers if storage within the dark was acceptable. Storage with constant light has been shown to maintain aestivation in grasshoppers (Pener & Broza 1971). Each of 6 replicates consisted of 10 aestivating individuals. They had been previously housed using the host plant method as above for 1 month. Insects were housed within "vial" cages made from Cospak 40 Dram styrene vials (142 mL volume). A cotton wick was inserted into a water source through a 8 mm hole in the base: crumpled paper towel within the top half of the vial provided roosts for the insects and terylene voile in place of the lid gave ventilation. Heavy gauge nylon mesh (10 mm aperture) separated the water wick from the paper. The vial cages and their water source were then placed inside a 30 × 20 × 20 cm lightproof cardboard box. Relative humidity within the boxes was 75 ± 5%. A 20 cm × 20 cm metal conduction plate ensured the internal temperature matched the set temperature (within 0.5°C). A non-heat producing 12 volt, 6 cp white LED light source was attached to the metal plate and the lighting regime (0, 14 or 24 hours at 0.1 to 0.3 $\mu E/s/m^2$ within the box) controlled by timers on the power supply.

The number of live and dead insects within each vial cage was assessed monthly from the outside of the vial. Most insects came out of the paper to die and their

bodies could be easily seen on the base of vial. The experiment was terminated after 129 days as the winter rains had started and field releases begun. Surviving insects were weighed and measured. From then onwards they were maintained at ambient temperatures and with natural, but diffused daylight whilst their fecundity and fertility were assessed.

For assessing fecundity and fertility, we pooled individuals from different replicates within the same treatment. Each female was treated as a replicate and her egg production/hatch recorded until her death. Females were paired with males from the same treatment at 5°C but at 10°C there were no surviving males so males housed using the host plant over-summer storage method were substituted. The experimental design became unbalanced due to the absence of female survivors and the failure of some females to lay eggs. The fecundity and fertility of the individuals housed in the vial cages was therefore compared to those that were housed using the host plant over-summer storage method by treating the data as a one-way ANOVA (after pooling all homogenous factors identified within the initial two-way ANOVA).

Pairs of red apion were held on leaves of *E. australis* using 20 mm diameter clip-on cages. Each week, the pairs were transferred to a new leaf and the number of eggs laid was counted. At 2 and 4 months post-removal from the vial cages, the eggs were checked for viability. Eggs laid over a 3-day period were individually identified and then monitored daily (*in situ*) for up to 2 weeks. All surviving red apion were killed 6 months post removal. At this stage in the field, the breeding season had finished and all host plants were dead.

The potential reproductive output over the red apion's lifetime under each storage regime was estimated by multiplying the average over-summer survival rate by the average fecundity rate by the average egg fertility observed for that regime.

Experiment 2 – supplementary feeding with sugars

This commenced in the 2000–01 summer period and was designed as a 5 × 2 factorial experiment. The temperature range was similar (5, 8, 10, 15 and 25°C), but with the addition of an 8°C treatment because the lower developmental threshold temperature of red apion, during the egg to adult phase, had been determined to be 7.0°C (unpublished data). The second factor was the effect of supplementary feeding with simple carbohydrates (sugar and honey). Although adult red apion are only reported to feed upon foliage, this was included because escaped aestivating individuals were noticed feeding upon honey. Insects were either given both white sugar cubes and Australian honey (Wescobee) – or given neither. Three replicates each consisting of 20 random individuals were set up for all cells except at 25°C with carbohydrates, where only 10 males per replicate were set-up and at 25°C without carbohydrates, where no replicates were set. A fully balanced design was not utilized so as to conserve our breeding stocks. All individuals had been stored for 1 month using the host plant over-summer storage method prior to being set up in this experiment.

Experiment 2 utilized over-summering storage "capsules" specifically designed to hold moderately large numbers of insects and to facilitate the transport and release of the red apion at the start of the new breeding season. They were constructed from 1.25 L polyethylene terephthalate (PET) plastic drink containers (bases removed). An inverted plastic lid containing potting mixture was taped to the bottom of the container to act as a cage floor. This was to reduce the saprophytic fungal build-up observed in the smaller vial cages used in experiment 1. An open-celled foam plug blocked a 6 cm diameter access hole bored through the side of the bottle. This plug provided the only ventilation to the cage. Sugar cubes were placed on the potting mix at the base of the cage and the honey was smeared on the inner surface of the plug. Bundles of paper towelling were secured within the top half of the cage by wire. Water was provided *ad libidum* via a cotton wick protruding through the lid of a water filled 16-dram vial placed within the cage. The light regime, via florescent tubes, was 16 hours light: 8 hours dark. Cages were placed within brown paper bags to subdue the lighting (intensity inside bags 5–13 $\mu E/s/m^2$), elevate humidity (to 80 ± 10%) and prevent visual disturbances.

The red apion individuals were removed from their over-summer storage capsules when the *E. australis* plants became available in the field (180 days after set up). The egg production and hatch rates were then assessed, as for experiment 1, until the plants were no longer available in the field (December).

Statistical analysis

Statistica 99 was used for all statistical analysis. All averages are expressed as means ± SE. In tables, the sample size is shown in parentheses.

Results

The value of host plants over summer

There was almost complete mortality with an average survival of only 0.1 ± 0.1% when insects were housed for a 4-month period at summer ambient temperatures (average shade temperature = 21°C, range 6–40°C) in the tubs with assorted potential over-summering sites, but with neither host plants nor water. Supplying water gave slightly, but not significantly better results (5.1 ± 4.4%). Supplying host plants with green foliage did, however, significantly ($F_{2,6} = 21.1$, $P < 0.01$, Tukey HSD) improve survival to 67.1 ± 6.1%.

Based upon these results, our method of storing red apion over summer was further refined. To accommodate the numbers mass reared, more insects needed to

be housed within smaller cages using potted *Rumex* plants, but at 500 to 1000 red apion/cage, plants retained green foliage for only a fortnight. Floor space became limiting within the cages, but removing dead plants from the cages required us to disturb insects that were hiding in the dead leaves. The survival rate dropped to 48.3 ± 8.36% (n = 5).

When smaller cages (30 × 40 × 50 cm) containing 500 to 1000 apion were held within an environmental chamber at 15°C with 14 hours light/day, insect activity was slowed and plant life prolonged. Under these conditions, only one potted *R. crispus* plant per month needed to be introduced and old pots did not need to be removed. Paper towelling, when folded and hung inside the cages, was as good as or better than any other refuge substrate tried. Plastic covers loosely placed over the cages reduced desiccation by minimizing air movement. This has become our regular or proven method of holding red apion over summer, and gives 74.4 ± 2.48% (n = 26) survival. For future reference, this will be referred to as the "host plant" method.

Alternatives free of host plants – 1. no supplementary feeding

With only water supplied, the rate of decline in population size was inversely proportional to the temperature at which the red apion populations were stored with high mortality (41%) already being noticed at the highest temperature (20°C) after only 1 month (Fig. 1a). Comparison of survival curves using Cox proportional hazard models also found significant, but smaller influences due to the lighting regime (Wald statistic (WS) = 18.1, n = 720, $p < 0.001$ for light and WS = 460.6, n = 720, $p < 0.001$ for temperature). The survivorship rates between red apion housed under constant light or under a 14:10 (light:dark) diurnal light cycle did not differ significantly (n = 480, WS = 0.4, p = 0.54), but apion housed in the dark died earlier (WS = 12.4 and 17.6, respectively, both n = 480, $p < 0.001$). At the end of the storage period (Table 1), 15°C and 20°C were found to be completely unsuitable, with the resulting survival being 0.6% and 0%, respectively, hence they were not included in any subsequent analysis. An ANOVA performed on survival rates after 129 days failed to find the lighting regime to be a significant factor at this time (Table 1). Survival at 5°C (71.7%) was equivalent to that observed from red apion housed using the host plant method and both these were significantly better than the 11.1% observed at 10°C. Forecasts of when 50% mortality would occur in the vial cages can be estimated from regressions derived from the survival curve plots (Fig. 1b). To obtain a 50% survival rate over the observed 129 days before the rains began, using the vial cages, we would have needed to either store the insects in constant darkness at 6.0°C or have them with either fluctuating or constant lighting at 7.1°C.

Females stored under conditions of starvation within vial cages had longer pre-oviposition periods than those stored at 15°C with host plants (48.6 ± 2.40 days, n = 35 versus 30.8 ± 5.01 days, n = 13, $F_{1,46}$ =12.89, $p < 0.001$). For starved individuals, the storage temperature or lighting regime made no difference to the pre-oviposition period ($F_{5,29} = 1.15$, $p = 0.36$). Placing insects in complete darkness for the entire summer affected lifetime fecundity ($F_{2,43} = 3.29$, $p < 0.05$). Insects exposed to constant dark laid only 42 ± 8.0 eggs whereas those in constant light laid on average 106 ± 20.9 eggs (Tukey HSD, $p < 0.05$). A diurnal rhythm in the lighting was not essential (Table 1). Although red apion stored with a light source at 5°C laid approximately the same number of eggs as the females from the host plant method (all approx. 75 eggs/female), the average egg hatch rate was lower at only 43.7% compared with 93.8% for females from the host plant method. Females stored at 10°C had reasonable egg hatch (85.3%).

By combining the estimates of survival, fecundity and fertility (Table 1), it becomes obvious that red apion populations do not fare well when housed under laboratory conditions with only water. Although increasing survival to acceptable levels occurred with reduced temperatures, decreases occurred in fecundity and fertility so that even under the best conditions offered (5°C and constant light) offspring production was only 38.5% of that obtained under the host plant method.

Alternatives free of host plants – 2. supplementary feeding with sugars

The survival of red apion, when housed with only water over summer mirrored that of the previous experiment (Table 2). At 5°C, it was approximately the same as that observed using the host plant method (73%). Egg lay from colonies stored at 5°C with water was only half of that of females from the host plant method, but this was not significant due to high individual variation. Egg hatch was significantly lower and it was estimated that after combining the mortality, fecundity and fertility results, the net reproductive output per female would only be four offspring. A negative population growth would occur if the insects were stored at 10°C or higher (and this assumes that every egg survives to become a sexually mature adult).

The provision of simple carbohydrates virtually eliminated mortality regardless of the temperature, with over 93% survival occurring at even the highest tested temperature (25°C). Survival by itself is not, however, a good predictor of the success of the storage method for at 5°C with honey, eggs laid were not viable, with less than 2% hatching. As a result, less than 1 offspring could be produced from every initial female. An increase of only 3°C saw significant increases in both egg lay and hatch. Females stored at 8°C with honey each laid 73 more eggs than the 42 eggs/female

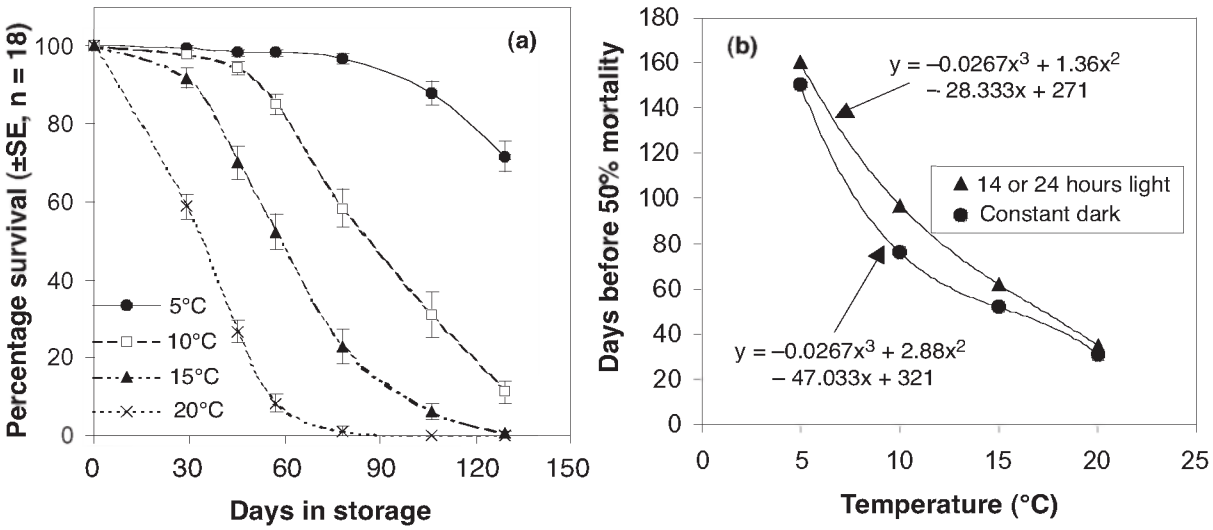

Figure 1. (a) Effect of temperature on the survival of red apion when stored with only water. For clarity, data across light regimes (the factor accounting for the least variation) have been pooled so $n = 18$ for each data set. (b) Storage time permitted over summer before red apion population size is decreased by 50% (individuals stored with only water at various temperatures with or without lighting). Note, response to 14 or 24 hours light per day was not statistically different.

Table 1. Lifetime reproductive output from red apion stored with only water and under various lighting regimes/temperatures over summer.

Temp. (°C)	Light (hours)	% survival	No. of eggs laid[1]	% egg hatch[2]	No. of offspring per initial female	As % of host plant method
5	0	63.3 ± 8.82 (6)	42.3 ± 8.69 (12) [a]	36.9 ± 15.34 (8)	9.9	10.5
	14	80.0 ± 5.16 (6)	92.9 ± 21.23 (14) [ab]	40.5 ± 12.08 (11)	30.1	31.9
	24	71.7 ± 6.01 (6)	92.7 ± 21.34 (12) [ab]	54.9 ± 12.40 (8)	36.4	38.5
All 5 pooled		71.7 ± 4.06 (18)[a]	76.8 ± 11.09 (38)	43.7 ± 7.48 (27) [a]	24.1	25.5
10	0	3.3 ± 2.11 (6)	32.0 (1) [a]	[88.3 (0)][3]	0.9	1.0
	14	8.3 ± 3.07 (6)	99.8 ± 20.63 (8) [ab]	88.3 ± 4.87 (7)	7.3	7.7
	24	21.7 ± 6.54 (6)	192.5 ± 35.06 (2) [ab]	74.9 ± 15.80 (2)	31.2	33.0
All 10 pooled		11.1 ± 3.01 (18) [b]	110.5 ± 20.63 (11)	85.3 ± 4.97 (9) [b]	10.5	11.1
All vial treatments		20.8 ± 3.52 (72)	84.4 ± 9.88 (49)	46.7 ± 6.60 (31)	9.8	10.4
Host plant method		75.7 ± 3.74 (12) [a]	133.2 ± 20.73 (19) [b]	93.8 ± 1.64 (13) [b]	94.5	

[1] Pooled by light regime for comparing to the host plant storage method. $(x + 0.5)^{0.5}$ transformed data.
[2] x^3 data transformation applied.
[3] No data as no eggs laid – given same value as 10°C 14 hours light.
Means with same letters are not significantly different at 5% (one-way ANOVA).

observed from those stored using the host plant method. The hatch rate from females stored at 8°C with honey was equal to that of those stored using the host plant method (both approx. 81%), but with a significantly better survival rate (98% versus 73%), the net result being an increase in lifetime reproductive output of 360%. For colonies stored at 8°C with honey over summer, 90 larvae can be expected from each initial female. Females housed during the summer using our host plant method had a reproductive output of only 25 larvae/female. At temperatures higher than 8°C, fecundity dropped, so that at 15°C with honey, the lifetime reproductive output was 71% of that observed from females stored using our host plant, high maintenance method. As no females were set up at 25°C, the results are unknown at this temperature.

Discussion

The duration of our storage experiments matched that of the local summer dry period. Individuals must survive the full period in order to breed, as the required host plants do not become available until after the rains begin. Our results indicate that without some supple-

mentary supply of energy, the red apion population would go to extinction if the mean temperature of their selected or available microclimate was 15°C or higher. Assuming red apion's selected microclimate is at ambient temperature, this means survival would not have been possible with water alone in either the area from which the red apion was originally collected (Israel) nor the main target area for our biocontrol program (the Western Australian wheat-growing region). In both these regions, mean summer temperatures exceed 22°C.

Supplying simple carbohydrates resulted in an incredible increase in survival rates for red apion stored over the summer period at what could be considered "normal summer temperatures". This would imply that these insects do in fact feed on some sort of nectar or sugar source in the wild. The absence of a suitable substitute may be the reason why, despite considerable effort, this insect appears not to have established in Australia (Yeoh *et al.* 2002). It should, however, be emphasized that nothing is known about the behaviour of the red apion in its native environment and the need to feed on sugar at warmer temperatures may in fact be an artefact of us providing the insects with totally unsuitable and unnatural microclimates during the summer period.

Host records for red apion have almost exclusively been restricted to plants within *Rumex* spp. and *Emex* spp. with the exception of an insect being "found" on blackcurrant. In this case, there was no mention of red apion feeding (Scott & Yeoh 1996). The adults typically produce "shot holes" in the leaves, but when plants are senescing, green foliage becomes rare. At this stage, they eat anything green, including petioles, stems, seed and flowers. Despite working on the red apion for almost a decade, whilst it is in its reproductive phase we have never seen it feeding on any nectar sources from any non-host plants. We have not even noticed it selectively feeding on the flowers or nectar of *Emex* although this may be because the flowers are small and insignificant.

The idea of supplementary feeding our phyllophagous insect with simple carbohydrates was initiated only because escapees within our over-summer holding room were found feeding upon honey used in a different experiment. If the red apion does feed upon nectar/pollen prior to aestivating, it is probably not unique, as *Mogulones larvatus* with its predominantly phyllophagous adults, also has been noted feeding upon flowers prior to aestivation. For this species, the host plant's flowers are only available in early summer and the survival rate of *M. larvatus* over summer and within cages declines with the length of time the insect is required to stay within aestivation (Matthew Smyth, Paul Wilson, pers. comm.). Programs such as these are perhaps the most likely to benefit from supplementary feeding with simple carbohydrates.

Conversely, *M. geographicus* adults, prior to entering aestivation, feed only on the leaves of Paterson's curse (*Echium plantagineum*) even when flowers are available (Paul Wilson, pers. comm.). However, it may still be of benefit to supply green plants grown out of season or to drop the storage temperature.

Red apion adults are entering diapause to endure summer drought and storing them at low temperatures may seem counterintuitive. Entering a state of diapause is, however,a strategy to curtail energy usage in

Table 2. Lifetime reproductive outputs from red apion given water or water and sugars whilst in storage at different temperatures over summer.

Nutrients	Temp. (°C)	% survival[2]	No. of eggs laid[3]	% egg hatch[2]	No. of offspring per initial female	As % of host plant method
Water	5	70.0 ± 5.00 (3)^{c}	18.9 ± 10.18 (19)^{a}	30.0 ± 20.00 (5)^{a}	4.0	15.9
	8	18.3 ± 7.26 (3)^{b}	34.0 (1)^{ab}	85.7 (1)^{ab}	5.3	21.3
	10	16.7 ± 3.33 (3)^{b}	13.0 (1)^{ab}	66.7 (1)^{ab}	1.4	5.8
	15	15.0 ± 5.00 (3)^{b}	(0)	(0)	0.0	0.0
All water		30.0 ± 7.33 (12)	19.4 ± 9.22 (21)	43.2 ± 16.35 (7)	2.5	10.0
Sugars and water	5	96.7 ± 1.67 (3)^{ad}	26.8 ± 9.59 (18)^{a}	1.7 ± 1.67 (8)^{a}	0.4	1.7
	8	98.3 ± 1.67 (3)^{d}	114.8 ± 15.31 (18)^{b}	80.0 ± 4.60 (17)^{b}	90.3	359.9
	10	96.7 ± 1.67 (3)^{ad}	45.8 ± 12.17 (18)^{a}	55.0 ± 16.39 (8)^{b}	24.4	97.1
	15	95.0 ± 2.89 (3)^{acd}	24.8 ± 4.96 (18)^{a}	75.2 ± 13.03 (10)^{b}	17.7	70.7
	25[1]	93.3 ± 3.33 (3)^{acd}				
All sugars + water		96.0 ± 1.00 (15)	53.1 ± 6.98 (72)	59.7 ± 6.35 (43)	30.4	121.1
All capsules		66.7 ± 7.20 (27)	45.5 ± 5.95 (93)	57.3 ± 5.91 (50)	17.4	69.3
Host plant method		72.9 ± 3.60 (14)^{ac}	42.1 ± 13.98 (20)^{a}	81.8 ± 5.62 (10)^{b}	25.1	100.0

[1] Only males set up at 25°C.
[2] Arcsine transformation for ANOVA.
[3] $(x + 0.5)^{0.5}$ transformed.
Means with same letters are not significantly different at 5% (one-way ANOVA, Tukey HSD for unequal *n*).

response to a period of unfavourable environmental conditions regardless of whether the stress is heat or cold and in both cases it is believed to be initiated by suppression of juvenile hormone levels (Tatar & Yin 2001). Although our biotype of red apion has evolved with triggers that initiate the onset of reproductive diapause prior to the onset of summer, other biotypes exist in England (Scott & Yeoh 1996) where they presumably enter a winter diapause to survive. The danger of manipulating the insect's summer temperature is that the selective pressure for entering aestivation may no longer apply so that this beneficial trait gradually disappears in culture. Once released in the field, the selection pressure would be reinstated and this problem could be overcome by periodically adding field-collected individuals to any breeding colonies.

Red apion stored at the lowest temperature (5°C) had good survival but its overall reproductive output was poor due to fecundity and fertility problems. At only a few degrees warmer (i.e. at 8°C), the best reproductive yields occurred. This temperature was 1°C warmer than the calculated lower developmental threshold temperature for immature stages of this insect. Presumably it was warm enough to either permit any necessary development or prevent any permanent damage to the insect's reproductive organs whilst cool enough to reduce somatic senescence to a minimum. Caution should be taken when exposing insects to cold temperatures as even 10 minutes exposure to 2.0°C has been shown to significantly reduce the lifetime fecundity and delay oviposition in the bruchid *Callosobruchus sibinnotatus* (Mbata *et al.* 1998). It was suspected that the short-term exposure to low temperature disrupted normal oocyte maturation.

Our host plant method of storing red apion over the summer, although providing a consistent result both from cage to cage and from year to year, is labour intensive. It requires host plants grown out of season, pests such as aphids, mealy bugs, thrips, spiders, caterpillars and plant pathogens are a problem and there are also issues with either over-watering (insects under pots drown) or under-watering the plants. As biocontrol practitioners, we also have a duty of care to the collaborating farmers that are providing the field release sites. We cannot transfer weeds, pests and diseases to their property. Extracting 500 red apion from the dead and dying food plants, moths, aphids, spiders and other contaminants takes 2–3 hours/cage and has to be done just prior to going to the field site. Releasing red apion from over-summer capsules can be done in the field and takes 5 minutes/capsule. This allows for releases to be made as soon as germination of *E. australis* occurs at the sites, giving red apion the largest possible period of time to breed.

The lack of information on red apion's natural behaviour over summer necessitated the development of novel techniques before the release program could begin in earnest. Enhancements have resulted in methods that have the potential for improving other similar biocontrol programs current or future.

During 2001–02, we conducted a full-scale implementation of the capsule method (18,700 red apion stored and then released), but the adults failed (less offspring produced than adults released). It is possible that local drought conditions were responsible for the poor yield, but experiments are currently under way to investigate if other factors such as slight modifications to the capsule's design and set up procedures (e.g. being directly placed into the capsules rather than being housed with host plants at 15°C for 1 month) or the presence of symbionts (e.g. *Wolbachia*) may have been responsible. The findings of these experiments will be reported upon in another publication.

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GRDC and CSIRO provided the funding for this project. Anthony Johnston assisted with the collection of data. Professor Scott O'Neill and Dr Jeremy Brownlie, University of Queensland, are currently screening our red apion for *Wolbachia* and similar diseases. Thanks to Kathryn Batchelor and Aaron Maxwell for comments on this manuscript.

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Battling the fragrant invader: mass production, application, and implementation of biological control for kahili ginger (*Hedychium gardnerianum*)

Robert C. Anderson and Donald E. Gardner

US Geological Survey, Biological Resources Discipline, Pacific Island Ecosystems Research Center, 3190 Maile Way, St. John 408, Honolulu, Hawaii 96822, USA

Kahili ginger (*Hedychium gardnerianum*) is one of the world's 100 worst invasive species, invading tropical and sub-tropical wet forests in areas where it has been introduced as an ornamental plant. The wilt-causing bacterium *Ralstonia* (=*Pseudomonas*) *solanacearum* has been demonstrated as a viable biological control agent for this weed and has recently been established in the field. This bacterium has significant potential in controlling this weed if effective application and mass production methodology can be developed. To address this need, research into the development of mass-production methodology and field-testing of new application techniques for the biocontrol of kahili ginger with *R. solanacearum* have been initiated in the wet forests of Hawai'i. Three objectives are being investigated in this study: 1) develop and enhance methodology for mass-production of the biocontrol agent; 2) evaluate host resistance among local and international populations of kahili ginger; and 3) evaluate the efficacy of *R. solanacearum*-encapsulated alginate beads and bioherbicide spray. An overview of the kahili ginger biocontrol program, and the results of these investigations, are discussed. In addition, information on technology transfer and implementation is presented.

Using ecological models to assess the efficacy of weed-control measures

Yvonne M. Buckley

NERC Centre for Population Biology, Imperial College, Silwood Park, Ascot, Berkshire, SL5 7PY, UK

Plant and herbivore population models can be used as decision-making tools to enable weed managers to implement successful control measures for troublesome weed populations. Using models we can explore complex interactions within and between populations and incorporate environmental effects inherent in ecological systems, leading to management solutions that were perhaps not intuitively obvious at the beginning of the process. Models of populations of St John's wort (*Hypericum perforatum*), Paterson's curse (*Echium plantagineum*) and scentless chamomile (*Tripleurospermum perforatum*) are used to explore the dynamics of weeds and biocontrol agents and the impacts of various management strategies on the weed population. The importance of density dependence, both its presence and timing, in weed and herbivore dynamics is assessed for both *E. plantagineum* and for the weed alone in *T. perforatum* populations. A complex model can encompass more aspects of the ecology of a particular situation, but it is important that the elements of the model are readily interpretable in terms of the biology of the system; this point is emphasised with reference to a complex individual-based model of *H. perforatum*. Ecological models act as a useful framework for the synthesis and application of our knowledge of population dynamics and interactions of a weed and its management.

The impact of gorse thrips, *Sericothrips staphylinus* (Thysanoptera: Thripidae), ryegrass competition and simulated grazing on the establishment and growth of gorse seedlings, *Ulex europaeus* (Fabaceae)

Jamie T. Davies,[1, 2] John E. Ireson[2] and Geoff R. Allen[1]

[1] School of Agricultural Science, University of Tasmania, GPO Box 252-54, Hobart, Tasmania 7001, Australia

[2] Tasmanian Institute of Agricultural Research, Newtown laboratories, 13 St Johns Ave, Newtown, Tasmania 7008, Australia

The impacts of gorse thrips, ryegrass competition and simulated grazing on the survival and growth of gorse seedlings were assessed in a factorial glasshouse experiment. The shoot dry weight of gorse seedlings was significantly reduced by each individual treatment. Shoot dry weight was reduced by ryegrass competition (96%), simulated grazing (74%) and gorse thrips (57%). Seedling survival was significantly reduced only with treatment combinations that included ryegrass competition plus at least one other factor. When ryegrass competition was the sole treatment, gorse seedling survival was 100%. However, when the ryegrass competition was combined with one additional treatment of either thrips or grazing, survival was reduced to 77% and 67%, respectively. When all three treatments were combined, survival was reduced to 7%. The interactions between treatments and the role of multiple control tactics within an integrated weed management program are discussed.

Seed treatment technology: an attractive approach for delivering *Fusarium oxysporum* "Foxy 2" for the biological control of the parasitic weed *Striga*

Abuelgasim Elzein[1], Juergen Kroschel[1] and Vibeke Leth[2]

[1] Institute of Plant Production and Agroecology in the Tropics and Subtropics, University of Hohenheim (380), D-70593 Stuttgart, Germany

[2] The Danish Institute of Seed Pathology for Developing Countries, Thorvaldsensvej 57, DK-1871 Frederiksberg C, Copenhagen, Denmark

The root-parasitic weed *Striga* constitutes a major biotic constraint to staple food production in the Sahelian and Savannah zones of Africa. The fungus *Fusarium oxysporum* "Foxy 2", isolated from diseased *Striga hermonthica* plants from Ghana, proved to be highly virulent against all developmental stages of the parasite and host specific when its inoculum was propagated on wheat grains or formulated into "Pesta" granules. Thus, the antagonist offers a good prospect for *Striga* control in the future. Coating sorghum seeds with Foxy 2 seems an attractive alternative for minimizing the inoculum amount, establishing the biocontrol agent in the potential infection zone of the host plants, and offering a simple, easy and economical delivery system. Our preliminary work on seed treatment resulted in the selection of appropriate seed-coating materials and a suitable type and form of fungal inoculum. In addition, Foxy 2 survived the seed treatment processing and showed excellent viability on seeds for at least one year of storage after coating. Moreover, the ability of Foxy 2 to colonize or to establish on the root system of the host (sorghum) was also proved, thereby meeting the criteria of being a promising candidate for controlling *Striga* when applied as a seed treatment. The efficacy of treated sorghum seed with Foxy 2 using different coating materials in reducing *S. hermonthica* infestations was evaluated in pot and root chamber trails. The results revealed that the efficacy of seed coating apparently varied according to the type and form of fungal inoculum, as well as with coating material. Coating sorghum seed with dried chlamydospore inoculum homogenized into 20% arabic gum (as adhesive) significantly reduced the number of emerged *Striga* plants by 73–76% compared to the control. In the root

chamber trial, the same treatment caused disease in 77% of the germinated *Striga* seeds and in 100% of attached tubercles. If these results can be confirmed under field conditions, seed treatment might contribute to a more meaningful application of Foxy 2 as an antagonist for *Striga* within an integrated control approach.

Keeping tabs on biological control agents by remote control

Lynley Hayes

Landcare Research, PO Box 69, Lincoln, Canterbury, New Zealand

As the numbers of release sites and biological control agents continue to increase, it becomes impossible for a handful of researchers to keep track of them all. However, at the same time it is critical for the success of biological-control programs that we carefully monitor what is happening in the field. One solution is to make use of trained volunteers. In New Zealand we have, over two decades, built up a network of people throughout the country (mostly local government staff) who can manage biological-control programs for us once agents are established. We are now able to perform many operations (e.g. releasing and redistribution) by remote control. Members of our network are also able to carry out simple monitoring for us if we give them sufficient warning. For this approach to monitoring to be successful, our helpers need to feel confident that they can find the release sites and recognise the agents, and we need to feel sure that the data they send are reliable. We make these things possible by encouraging good record keeping using simple standardised forms, running regular training workshops, spending time with people in the field, and by providing regular newsletters and reference materials. Our network has also helped with a nationwide pheromone trapping operation for two agents that can be difficult to find, and this yielded a lot of useful information about establishment success. Likewise, some of our helpers have put out window traps for us. We have also attempted to go a step further and involve our helpers in trials to assess the impact of one agent. However, we have found that for most people the effort required to maintain and assess even fairly simple impact-assessment plots regularly for several years was too onerous. We believe that assessment trials are probably best left to the experts.

Biological control: an important tool in integrated weed management (IWM) of pasture weeds

R.K. Huwer,[1,2] D.T. Briese,[1] P.M. Dowling,[3] D.R. Kemp,[4] W.M. Lonsdale,[1] D.L. Michalk,[3] M.J. Neave,[1] A.W. Sheppard[1] and T.L. Woodburn[5]

[1] CSIRO Entomology and Cooperative Research Centre for Weed Management Systems, GPO Box 1700, Canberra, ACT 2601, Australia

[2] NSW Agriculture, Tropical Fruit Research Station, PO Box 72, Alstonville, NSW 2477, Australia

[3] NSW Agriculture and CRC for Weed Management Systems, Orange Agricultural Institute, Forest Road, Orange, NSW 2800, Australia

[4] University of Sydney and CRC for Weed Management Systems, Orange, PO Box 883, Orange, NSW 2800 Australia.

[5] CSIRO Entomology and CRC for Weed Management Systems, Private Bag PO Wembley, WA 6014, Australia

Broadleaf weeds, such as nodding thistle, *Carduus nutans* L., Scotch thistle, *Onopordum* spp. (Asteraceae) and Paterson's curse, *Echium plantagineum* L. (Boraginaceae), are a major problem for graziers in high-rainfall grazing areas in south-eastern Australia. Many attempts to control weeds in the past with a single control technique have been successful only in the short-term, and the need for a more

holistic and integrated approach that would result in long-term, sustainable management has become apparent. A combination of biological control, grazing management and herbicides was investigated in an extensive field study in southern New South Wales. During the field trials, we monitored the impact of grazing and herbicide treatments on the weed and biological control agents, as well as on pasture composition. This IWM program was pioneering work in that it is one of the few IWM projects in the world that has a major emphasis on the biological control agents. An important focus of this study was therefore the compatibility and role of biological control in this IWM approach. Results showed that biological control can be successfully established despite limitations by grazing and herbicide treatments. At least at the spatial scale of this study, none of the other control measures impeded the efficacy of the biological-control agents. Management of biological control agents e.g. provision of refugia might be essential. We anticipate that biological control will be an important part of an effective long-term weed management together with herbicide and pasture management strategies.

Developing an integrated management program for kudzu

D. Jewett,[1] K.O. Britton[1] and J.H. Sun[2]

[1] US Forest Service, Southern Research Station, Athens, GA, USA
[2] Chinese Academy of Sciences, Institute of Zoology, Beijing, China

Kudzu is a perennial, semi-woody, climbing legume native to China. Since the late 1800s, it has been introduced deliberately to North America as an ornamental, as forage for livestock, for improving soil, and for preventing soil erosion. By 1946, over 121,406 ha of kudzu had been planted throughout the United States. Presently, extension agents report almost a half-million ha of it in 700 of 3,140 administrative districts from Florida to the Pacific Northwest. Commercial forests occupied by kudzu lose more than US$120 per ha annually, and it may be a reservoir of pathogens responsible for disease outbreaks in row crops. A variety of ways for managing small populations of kudzu exist, including herbicides, mechanical removal, and intensive livestock grazing. No existing strategy yields convenient and economical suppression over large areas, herbicides often are restricted in proximity to aquatic habitats and land of certain propriety (like some national parks), and the relief of areas occupied by kudzu is often considerable, making its eradication inconvenient, dangerous or both. For instances in which herbicide use is ill-advised, alternative strategies for managing kudzu are being considered, including biological control. In China, an abundance of natural enemies prevents, in part, kudzu from becoming either an important economic or environmental liability. Survey of populations there has revealed many insects and pathogens associated with kudzu, including a sawfly and a rust. Preliminary host-range testing of potential biological control agents has begun. Systematic resolution concerning kudzu and related taxa is incomplete, however, and must be refined before selection of biological control agents may proceed. In the field, several different plants are mistaken for kudzu, and it may hybridize with related taxa. Molecular tools for distinguishing among specimens are being tested, and are expected to help professionals match more accurately kudzu with its potential biological control agents.

Biocontrol of *Orobanche* spp. by inundative releases of *Phytomyza orobanchia* (Diptera, Agromyzidae)

Olaf Klein, Jürgen Kroschel and Abuelgasim Elzein

Institute of Plant Production and Agroecology in the Tropics and Subtropics (380), University of Hohenheim, 70593 Stuttgart, Germany

New approaches are necessary to control parasitic weeds of the genus *Orobanche*. The fly *Phytomyza orobanchia* (Diptera, Agromyzidae) is particularly suitable for biological control since it is oligophagous feeding only on *Orobanche* species. In total, of the 140 *Orobanche* spp. described, the occurrence of *P. orobanchia* is reported from 21 species. The use of *P. orobanchia* in biocontrol of *Orobanche* is based on

inundative releases at the time of *Orobanche* emergence. The larvae of *P. orobanchia* mine in *Orobanche* shoots and capsules and intervene at the sensitive reproductive stage of *Orobanche*. Hence, the reduction of *Orobanche* seed production prevents supplementary infestation and dissemination. The advantage of this control approach is its compatibility to all crop/*Orobanche* associations and that it can easily be combined with other control methods. In northern Morocco, the application of *P. orobanchia* in biocontrol of *Orobanche* spp. has been tested from 1995 until 1999. Under natural conditions, 48.9% of *Orobanche* seed capsules are infested by *P. orobanchia*. *P. orobanchia* is parasitized by nine hymenopterous species, but the total parasitization rate does not exceed 8.9% on average. For field releases of *P. orobanchia* adults, a formula for the calculation of the fly number per hectare based on the *Orobanche* infestation level has been developed. Inundative releases of *P. orobanchia* in field cages have shown that the natural efficiency of *P. orobanchia* can be increased considerably. Only 5.3% of viable seeds have been produced in comparison to 62.0% without inundative releases. Seeds are directly destroyed by the mining activity of *P. orobanchia* larvae as well as indirectly by the feeding damage to shoot tissues causing a degeneration of seed capsules. In highly infested fields (> 200 *Orobanche* shoots per m^2), an increase of the *Orobanche* seed bank in the soil could be still observed after inundative releases. In low to medium infested fields, releases of *P. orobanchia* alone are sufficient to reduce the *Orobanche* seed population to an acceptable level. An integrated control approach with tolerant and/or resistant cultivars, combined with mycoherbicides or other control methods is proposed.

Progress on the introduction, rearing and release of the ragwort plume moth, *Platyptilia isodactyla*, for the biological control of ragwort, *Senecio jacobaea*, in Australia

R.M. Kwong,[1,3] D.A. McLaren[1,3] and J.E. Ireson[2,3]

[1] Keith Turnbull Research Institute PO Box 48, Frankston, Victoria 3199, Australia
[2] Tasmanian Institute of Agricultural Research, 13 St. John's Avenue, New Town, Tasmania 7008, Australia
[3] Cooperative Research Centre for Australian Weed Management

Ragwort, *Senecio jacobaea* L. (Asteraceae), is a herbaceous, biennial plant native to Europe and western Asia. It was introduced into Australia during the mid 1800s and now occupies more than820,000 ha in the high rainfall areas of southern Victoria and at least 160,000 ha in Tasmania. Ragwort is an extremely invasive weed in pastures, particularly those grazed by cattle and horses, forestry plantations and natural ecosystems. Biological control of ragwort commenced in Victoria in the 1930s with the release of the cinnabar moth, *Tyria jacobaeae* L. (Lepidoptera: Arctiidae), and in the 1950s, the seed fly, *Botanophila jacobaeae* (Hardy) (Diptera: Anthomyiidae), was released. Neither of these insects established, despite repeated release attempts, probably due to disease, predation by native insects or an inability to adapt to the Australian environment. The flea beetles, *Longitarsus flavicornis* (Stephens) (Coleoptera: Chrysomelidae) and *Longitarsus jacobaeae* (Waterhouse), were introduced into Australia in the late 1970s and 1980s, respectively. In Tasmania, *L. flavicornis* is now widely established on ragwort and has caused significant reductions in plant vigour and density at many sites. In Victoria, flea beetle establishment has been less successful, with populations of *L. flavicornis* persisting only within the Strzelecki Ranges. The ragwort crown-boring moth, *Cochylis atricapitana* (Stephens), introduced in 1987, has established in both Victoria and Tasmania and has been shown to kill ragwort rosettes during autumn. The ragwort plume moth, *Platyptilia isodactyla* Zeller (Lepidoptera: Pterophoridae), is the latest biocontrol agent to be imported and was first released in Victoria in December 1999. This paper describes the release and establishment of *P. isodactyla* in south-eastern Australia.

Herbicide use during *Aphthona lacertosa* flea beetle establishment expedites control of leafy spurge

Rod Lym[1] and Roger Becker[2]

[1] North Dakota State University, Box 5051 Plant Sciences, Loftsgard Hall, Fargo, ND 58105, USA

[2] University of Minnesota, 411 Borlaug Hall, 1991 Upper Buford Circle, St Paul, MN 55108, USA

Aphthona spp. have successfully reduced leafy spurge population densities in the northern plains of the United States. However, establishment of *Aphthona* in some areas has been slow or has failed. In managed pasture/rangeland settings, it is often difficult for farmers to justify a 5–10-year period of no herbicide input to allow establishment of biocontrol agents while foregoing potential forage yield and thus income. The use of herbicides during the initial release of *Aphthona* flea beetles may shorten the time required to reduce leafy spurge population densities below economic impact levels. We studied an integrated management approach combining herbicide use with the establishment of *Aphthona lacertosa* to expedite reducing leafy spurge populations to economic levels. We established trials in North Dakota and Minnesota comparing sites with herbicide input, with or without *Aphthona lacertosa* release. As in the *Aphthona nigriscutis* study conducted in North Dakota by Jeff Nelson, Rod Lym, and Calvin Messersmith, in this study the use of herbicide enhanced control of leafy spurge during the early phase of *Aphthona* establishment, and did not prevent the establishment of *Aphthona lacertosa*. Leafy spurge population densities decreased at a faster rate with the use of herbicides. We propose that the use of herbicides may expedite the control of leafy spurge and gain broader acceptance by ranchers/farmers for management of grazed areas. This integrated approach will still promote the establishment of *Aphthona lacertosa*, thereby providing long-term leafy spurge control and ultimately reducing herbicide input. Concomitantly, higher economic benefits can be maintained to support ranch/farm viability during the initial phases of establishment of the biocontrol agents.

Rearing, redistribution, and dispersal of three biological-control agents for scentless chamomile

Alec McClay

Alberta Research Council, PO Bag 4000, Vegreville, Alberta T9C 1T4, Canada

Three biological-control agents have been released in western Canada against scentless chamomile (*Matricaria perforata* Merat), an annual or short-lived perennial weed native to Europe that is becoming a serious problem in agricultural land in western Canada. The seed weevil *Omphalapion hookeri* and the gall midge *Rhopalomyia tripleurospermi* are well established and dispersing at numerous sites. These two agents are easy to mass-rear in the greenhouse or field cages. The stem weevil *Microplontus edentulus* has proven more difficult to rear and is established only at one site to date. *Omphalapion hookeri* and *R. tripleurospermi* have been provided to users on a fee-for-service basis. The advantages and disadvantages of this approach are discussed.

Assessment of *Dactylaria higginsii* as a post-emergence bioherbicide for purple nutsedge (*Cyperus rotundus*) in bell pepper (*Capsicum annuum*)

J.P. Morales-Payan,[1,2] R. Charudattan,[2] W.M. Stall[1] and J.T. DeValerio[2]

[1] Horticultural Sciences Department, University of Florida, Gainesville, FL 32611-0690, USA

[2] Department of Plant Pathology, University of Florida, Gainesville, FL 32611-0680, USA

In Florida and other tropical and subtropical regions, purple nutsedge (*Cyperus rotundus* L.) is the most troublesome weed in peppers (*Capsicum* spp.) grown in soils without methyl bromide fumigation. *Dactylaria higginsii* has been shown to reduce purple nutsedge growth and competitive ability, but little information is available about how those effects translate into crop yields. Therefore, a field study was conducted to determine the effect of repeated applications of *D. higginsii* on the growth of purple nutsedge and the yield and grade of bell pepper. The results showed that weed-free bell pepper produced the highest yield, and weedy bell pepper without *D. higginsii* treatment the lowest. One application of *D. higginsii* 8 days after weed emergence (DAE) reduced purple nutsedge growth and increased overall bell pepper yield and the proportion of large and extra large fruit, as compared to untreated purple nutsedge-infested pepper. Application of *D. higginsii* twice (8 and 18 DAE) resulted in the same yield of large and medium size fruit as in the weed-free crop, although the yield of extra large ("fancy") fruit was lower than in the weed-free crop. The data indicated that to use *D. higginsii* as an effective post-emergence herbicide, its efficacy per application must be enhanced (i.e. increased fungal virulence, conidia survival, and penetration into nutsedge leaves) and/or more than two applications of this potential bioherbicide are necessary to suppress purple nutsedge interference to acceptable levels (<10% yield loss). The environmental conditions during the study were very adverse to *D. higginsii*, with low humidity and high daytime temperatures. More suppression of purple nutsedge and higher yields are likely to occur following application of *D. higginsii* under more favourable weather conditions.

Synergy of *Pyricularia setariae* with chemical herbicides for control of green foxtail

G. Peng and K.N. Byer

Agriculture and Agri-Food Canada, Saskatoon Research Centre, 107 Science Place, Saskatoon, Saskatchewan S7N 0X2, Canada

Isolates of *Pyricularia setariae* Niskada obtained from green foxtail (*Setaria viridis* (L.) Brauv.) in Canada controlled the weed effectively in the greenhouse when applied at high doses and carrier volumes (1×10^7 spores/mL at 2,000 L/ha). Eight herbicides recommended for control of green foxtail were tested at 1/10 of label rates for potential synergy with the fungus. In the greenhouse, the fungus was applied at 1/5 of the regular rate (2×10^7 spores/mL at 200 L/ha) 48 h after herbicides. Inoculated plants were placed in a dew chamber for 24 h then in a greenhouse for 6 more days before assessment. Herbicides at the reduced rate were only marginally to moderately effective while the fungus was moderately efficacious, reducing plant fresh weight by approximately 54% in comparison to non-treated controls. Significant synergy was observed between the fungus and sethoxydim, imazethapyr, quinclorac, propanil, glyphosate, and glufosinate, with significantly better weed control than achieved by either component alone. Sethoxydim and propanil demonstrated highest synergy with the fungus, exhibiting 45% higher efficacy when compared to either herbicide or fungus alone. When the two pesticides were applied with the fungus to giant foxtail (*S. faberi* Herrm) or yellow foxtail (*S. glauca* (L.) Beauv.), severe damage occurred on these two foxtail species that would otherwise be highly resistant to the fungus. In a field trial in 2002, the fungus and sethoxydim (< label rate) were applied to plots of

green foxtail (4-leaf stage) at 200 L/ha either alone or as a tank-mix. The fungus alone had little effect due to sub-optimal environmental conditions, while the herbicide reduced the fresh weight by approximately 34% when compared to the control. The tank mix of fungus and herbicide, however, resulted in significantly higher disease and approximately 55% fresh weight reduction.

Oviposition preference of the ragwort flea beetle, *Longitarsus flavicornis*, in relation to ragwort, *Senecio jacobaea*, phenology and its implications for biological control

K.J.B. Potter,[1] J.E. Ireson[2] and G.R. Allen[1]

[1] School of Agricultural Science, University of Tasmania, GPO Box 252-54, Hobart, Tasmania 7001, Australia

[2] Tasmanian Institute of Agricultural Research, 13 St Johns Avenue, New Town, Tasmania 7008, Australia

Wick wiping of herbicides in summer to kill flowering ragwort and reduce seed production is the current recommendation for the integration of herbicide use and biological control using the ragwort flea beetle, *Longitarsus flavicornis*, in Tasmania, Australia. Rosettes are undamaged by wick wiping and this enables *L. flavicornis* survival. This recommendation has always been based on the assumed negative impact of boom sprayed herbicides on *L. flavicornis* without the availability of supporting data. Vacuum collections at a site at Franklin, Tasmania, showed that over 80% of adult *L. flavicornis* occurred on rosette rather than bolting ragwort plants. Glasshouse choice trials of *L. flavicornis* oviposition behaviour showed that over 95% of eggs were laid around ragwort rosettes rather than flowering plants. These results now provide supporting evidence for the validity of the current integrated control strategy for ragwort. Reasons for the habitat preference by *L. flavicornis* of rosettes over flowering plants, and the implications for the survival and increase of this biological control agent, are discussed.

Evaluation of *Dactylaria higginsii* as a component in an integrated approach to pest management

E.N. Rosskopf,[1] C.B. Yandoc,[1] J.B. Kadir[2] and R. Charudattan[3]

[1] US Horticultural Research Laboratory, USDA–ARS, 2001 South Rock Road, Fort Pierce, Florida 34945, USA

[2] Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, Selangor, Malaysia

[3] Department of Plant Pathology, 1453 Fifield Hall, University of Florida, Gainesville, Florida 32611, USA

Control of purple and yellow nutsedge (*Cyperus rotundus* and *C. esculentus*) continues to be ranked as one of the greatest problems facing growers in the southern United States. As mandated reductions of the use of methyl bromide are implemented, the area over which nutsedge is considered a major production limitation increases. The competitive ability of nutsedge is significantly decreased with the application of the fungus *Dactylaria higginsii*. A field experiment was designed to use the fungus as a component in an integrated approach to pest management as an alternative to methyl bromide fumigation. A tomato production system utilizing multiple treatment combinations was conducted using fallow season treatment as the main plot and production practice as the sub-plot treatment. Fallow season treatments of *D. higginsii*, glyphosate, and disk fallow were implemented from June to August 2001, and a fall tomato crop was produced in the following season. Significant disease incidence was seen in the fungus-treated plots and no significant difference was found in tomato yield or nutsedge (*Cyperus* spp.) density in the following production season. There was no statistical difference in tomato

yield attributable to fallow season treatments. Overall tomato yield from fumigant/fungus-treated plots was statistically similar to yields achieved in the fumigant/herbicide-treated plots.

Development of *Mycoleptodiscus terrestris* as a bioherbicide for management of the submersed macrophyte, *Hydrilla verticillata*

J.F. Shearer[1] and M.A. Jackson[2]

[1] US Army Engineer Research and Development Center, 3909 Halls Ferry Road, Vicksburg, MS 39180-6199, USA

[2] USDA–ARS–NCAUR, Crop Bioprotection Research Unit, 1815 N. University Street, Peoria, IL 61604, USA

The indigenous fungal pathogen *Mycoleptodiscus terrestris* (Mt) has shown significant potential for use as a bioherbicide for management of the invasive aquatic macrophyte *Hydrilla verticillata*. Liquid culture fermentation methods have been developed that yield stable, effective bioherbicidal propagules of Mt. Under appropriate nutritional conditions, aerated Mt cultures produce high concentrations of vegetative biomass that differentiates to form compact hyphal aggregates that we have termed microsclerotia. Eight-day-old cultures yielded more than 5×10^6 microsclerotia/litre with 50–90% surviving air-drying to less than 4% moisture. Dried Mt microsclerotia germinated both vegetatively and sporogenically upon rehydration, thus improving their potential to infect and kill hydrilla. Sporogenic germination was first evident on the microsclerotia as sporodochia followed rapidly with spore production by day 4 yielding approximately 1.8×10^6 spores/g dried formulation. By day 12, spore counts had increased 10 fold. Applied to hydrilla in 55 litre aquaria, dried Mt formulation reduced hydrilla above ground biomass up to 99% compared to untreated controls.

TAME Melaleuca: the areawide management evaluation of Melaleuca

Cressida S. Silvers and Paul D. Pratt

USDA–ARS Invasive Plant Research Laboratory, 3205 College Avenue, Fort Lauderdale, FL 33314, USA

Melaleuca quinquenervia (common name melaleuca or paper-bark tree) is a myrtaceous tree of Australian origin that has become a noxious weed in Florida, outcompeting native plants and rangeland grasses on approximately 200,000 ha of agricultural, riparian and wetland systems. Melaleuca infestations degrade south Florida's native wildlife habitat, grazing lands and vital waterways that significantly contribute to fisheries productivity, act as nursery sites for fish and crustaceans, regulate run-off quantity and quality, mitigate flooding, and control erosion. Nearly $25 million has been spent over the past decade in managing melaleuca infestations, yet the weed continues to proliferate, particularly on private lands. **T**he **a**reawide **m**anagement **e**valuation of Melaleuca, or TAME Melaleuca, is a multi-agency effort recently established by the US Department of Agriculture's Agricultural Research Service (USDA–ARS) to demonstrate and promote practical, integrated melaleuca management strategies with an emphasis on biological control. In the course of this five-year project, research and demonstration sites will be set up in varied habitats in southern Florida where public and private landowners are highly motivated to manage melaleuca. Project activities include assessing melaleuca's nonindigenous geographic distribution, the impacts of control tactics and the socio-economic factors associated with current and proposed control tactics; researching impacts of control tactics on the weed, interactions among biological control agents, and non-target effects of tactics; and technology transfer. By partnering with federal, state, local and private land managers on these goals, TAME Melaleuca intends to develop a sustainable and integrated melaleuca control program for the long-term control of this invasive weed.

Determining optimal strategies for the establishment of *Pareuchaetes insulata* (Lepidoptera: Arctiidae) on *Chromolaena odorata* (Asteraceae) in South Africa

Lorraine W. Strathie,[1] Wayne A. Parasram,[2] Debbie Muir[3] and Costas Zachariades[1]

[1] ARC–Plant Protection Research Institute, Private Bag X6006, Hilton 3245, South Africa

[2] School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, PO Wits, Johannesburg 2050, South Africa

[3] Department of Water Affairs and Forestry 'Working for Water Programme', c/o Private Bag X6006, Hilton 3245, South Africa

The release of *Pareuchaetes insulata* on *Chromolaena odorata* in South Africa in 2001 marks the first release of this agent worldwide. Since 1970, the congeneric *P. pseudoinsulata* has been released on chromolaena in many countries, with results ranging from non-establishment to widespread, longterm defoliation and suppression of *C. odorata*. These discrepancies are not well understood. In South Africa, *P. pseudoinsulata* was released in 1989 and 1998–9, but did not establish, and another species, *P. aurata aurata*, was released in 1993–4 with the same result. The reasons for non-establishment are unknown, but may include predation, climatic incompatibility, dispersal of the founder population, biotype incompatibility, and/or culturing diseases. These factors are now under investigation in determining optimal strategies for the establishment of *P. insulata*. *Pareuchaetes insulata* was collected in Florida, USA, which closely matches the climate of KwaZulu-Natal province, South Africa, where it was destined for release. The moth is being mass-reared under high standards of hygiene and expertise in a professional insectary. Climate tolerance has been measured in the laboratory and through modelling, and the effects of various predator groups by means of multiple-exclusion field trials. Biotype preference studies have been conducted, as have measurements of adult dispersal. Following poor establishment at several sites, the release strategy has been modified to include larger, long-term releases at fewer sites, with improved initial results. The implications of findings are discussed and strategies recommended for the release and establishment of *P. insulata*.

The post-release larval mortality of the *Chrysanthemoides* leaf roller *Tortrix* sp. in Australia

Anthony Swirepik, Ruth Aveyard and Andrew Sheppard

CSIRO Entomology, GPO Box 1700, Canberra ACT 2601, Australia

The *Chrysanthemoides* leaf roller *Tortrix* sp. was approved for release into Australia in 2001. A release project focused on engaging community participation in the release and redistribution of *Tortrix* sp was commenced in April 2001. Between April 2001 and March 2002, releases of eggs, larvae, pupae and adults were made at 25 sites. Post-release monitoring indicated that *Tortrix* sp. larvae markedly declined in number during the weeks immediately following release, to the point where larvae were undetectable after two months. An experiment to quantify larval mortality and attempt to pinpoint the mechanisms involved in mortality found a significant difference in the survivorship of *Tortrix* sp. released as eggs at the point of hatch onto caged and uncaged plants ((caged low density CLD) 53%, uncaged low density (ULD) 2% survival $p < 0001$), caged high density (CHD), 32.5%, uncaged high density (UHD), 0.86% survival $p < 0001$)). In all treatments, there was a dramatic decline in neonate survivors by week 3 of the experiment (CLD – 49%, CHD – 37%, ULD – 16%, UHD – 17.5%), with a significant difference in larval survivorship between caged and uncaged treatments, $p < 0.0001$. Survivorship then remained constant in the caged treatments for the weeks five and eight samples, while a significant decline was measured in the uncaged treatments, $p < 0.003$. The high initial decline

in larval survivorship in all treatments indicates that there is strong competition between neonate larvae for feeding sites. The additional mortality recorded on uncaged plants may be attributed to predation or larvae leaving the plant on which they were released to escape intraspecific competition. Generalist predators such as spiders and ants appear to have been the primary cause of mortality, while a specialist parasite *Glabridorsum* sp. (Ichneumonidae sub family Cryptinae), was responsible for the death of two individuals. Management options for enhancing the survivorship of larvae after release are discussed.

Using *Aphthona* flea beetles as a biological herbicide to control small patches of leafy spurge

David C. Thompson and Kevin T. Gardner

Department of Entomology, Plant Pathology and Weed Science, New Mexico State University, Box 30003, MSC 3BE, Las Cruces, New Mexico 88003-8003, USA

Leafy spurge, *Euphorbia esula* L., continues to invade new habitats in the United States. Isolated infestations continue to establish at the forefront of expanding populations of this invasive weed. In many areas, control is the responsibility of individual landowners, many of whom are reluctant to use chemical control methods. Although it is commonly accepted that biological control agents acting alone cannot eradicate a host population, they can reduce it to very low levels, especially in the case of inundative strategies. In small isolated populations of leafy spurge, biological control agents can be used as a biological herbicide by collecting or purchasing large numbers of insects from established insectaries. The objective of this study was to determine the potential of using *Aphthona nigriscutis* and *A. lacertosa* flea beetles to control small patches of leafy spurge. Sixteen isolated patches (0.05 to 1.0 hectares) of leafy spurge were selected for study at two upland and one riparian site. Half were "treated" with 160 beetles/m^2 evenly spread over the entire patch; the remaining eight were used as untreated control plots. These beetles functioned very well as a biological herbicide. Reductions in biomass (96.5%) and stem number (87.5%) in the beetle release sites were greater and more consistent on the upland sites when compared with the riparian site (80% biomass and 74% stem density). Roots with live buds were common in the treated plots; but leafy spurge root mass was reduced 52% at the upland sites after the first year and treated plots at the riparian site had 57% fewer roots than the control plots. Stem density and biomass were reduced in control plots when beetles dispersed from the heavily damaged treated plots into them. *Aphthona* beetles rapidly reduced aboveground leafy spurge biomass and stem density after one year and have maintained both at low levels three years after "treatment".

Integrating biological and conventional control methods for control of *Centaurea solstitialis* in central California, USA

Jessica Torrence,[1] Alfred F. Cofrancesco,[2] Joseph M. DiTomaso,[1] Donald B. Joley,[3] Michael J. Pitcairn[3] and Steven R. Bennett[4]

[1] Department of Vegetable Crops and Weed Science, 1 Shields Avenue, University of California, Davis, CA 95616, USA

[2] US Army Corps of Engineers, Environmental Laboratory, 3909 Halls Ferry Road, Vicksburg, MS 39180, USA

[3] California Department of Food and Agriculture Biological Control Program, 3288 Meadowview Road, Sacramento, CA 95832, USA

[4] US Army Environmental Center, Aberdeen Proving Ground, MD 21010, USA

Fort Hunter Liggett is a large military reservation located in the Coast Range of central California. The exotic weed *Centaurea solstitialis* (yellow starthistle) is a major weed on Fort Hunter Liggett land where its presence restricts military training activities, increases fire danger, displaces native vegeta-

tion and threatens endangered species located on the base. The heaviest infestations occur in five different habitats, each requiring specific control methods: stream and river corridors, vernal pools, grasslands with endangered species, oak woodlands, and military use training areas. Management plans incorporating conventional control methods (herbicide applications, burning, and mowing) and releases of biological control agents are described for each habitat. Implementation of these plans was performed in three habitats in study areas of approximately 80–120 hectares. While the management plans were adjusted according to the management goal of the area (e.g. training, increase biodiversity, etc.), all shared the same objective: stop seed production and exhaust the seed bank. The basis of each plan was as follows: burn the area the first year to prevent seed production and encourage germination of the seed bank; apply herbicides the second year to prevent seed production and encourage growth of endemic grasses; during the third year, spot treat areas with yellow starthistle by hand removal or hand application of herbicide. Release biological control agents in untreated areas surrounding the study area to reduce the source of invading seed and to attack plants reinvading the treatment area. This control strategy was modified as needed for each habitat. After three years, the conventional control methods caused a substantial reduction of yellow starthistle, establishment of the biological control agents was successful and movement of the insects into the treatment areas was observed.

Computer-based information systems for accessing information on the management of terrestrial and aquatic invasive plant species

Sherry G. Whitaker,[1] Michael J. Grodowitz,[1] Lavon Jeffers,[2] Sonya F. Lewis[2] and Alfred F. Cofrancesco[1]

[1] US Army Engineer Research and Development Center, 3909 Halls Ferry Road, Vicksburg, MS 39180-6199, USA

[2] DynTel Corporation, US Army Engineer Research and Development Center, 3909 Halls Ferry Road, Vicksburg, MS 39180-6199, USA

There are very many introduced and naturalized terrestrial and aquatic plant species that cause serious problems in many areas of the United States. The development of effective management strategies is directly dependent on access to pertinent and up-to-date information on plant identification, biology, ecology, and applicable management technologies. Unfortunately, because of the tremendous number of species, the collection and summarizing of such information can quickly become overwhelming. While traditional methods of technology transfer (including technical reports, scientific papers, oral presentations, posters, etc.) are adequate, more efficient access is needed. Toward this goal, two computer-based information/expert systems have been developed and recently updated that provide rapid and easy access to up-to-date information on various management and control methods available for particular plant species. These systems include the noxious and nuisance **p**lant **m**anagement **i**nformation **s**ystem (PMIS) and the **a**quatic **p**lant **i**nformation **s**ystem (APIS). These systems are PC-based and operate under the Windows operating system, ensuring a high degree of portability for a wide variety of different computer configurations. The systems contain in-depth textual information as well as numerous photographic quality diagrams and images. Information covered includes plant biology, ecology, identification, and management options, and all operate using sophisticated programming algorithms that allow for easy identification of invasive species or available management options.

Potential for population recovery of an endangered native plant by controlling bridal creeper with rust

A.J. Willis,[1] L. Morin,[2] P.H.R. Moore[3] and R.H. Groves[4]

[1] CSIRO Plant Industry and Cooperative Research Centre for Australian Weed Management, Canberra, Australia. Present address: Office of the Gene Technology Regulator, Department of Health and Ageing, Canberra, Australia
[2] CSIRO Entomology and Cooperative Research Centre for Australian Weed Management, GPO Box 1700, Canberra ACT 2601, Australia
[3] CSIRO Plant Industry, GPO Box 1600, Canberra ACT 2601, Australia
[4] CSIRO Plant Industry and Cooperative Research Centre for Australian Weed Management, GPO Box 1600, Canberra ACT 2601, Australia

The survival of scattered populations of *Pimelea spicata,* a small native shrub, is threatened primarily by continued fragmentation of the Cumberland Plain Woodland, a vegetation type once dominant in a region to the south-west of Sydney. The largest *P. spicata* population within the Sydney Basin is also threatened by the invasion of bridal creeper (*Asparagus asparagoides*), a serious environmental weed of southern Australia introduced from South Africa. Results of two glasshouse experiments are presented to show: 1. the extent of reduction in growth of *P. spicata* by both above- and below-ground competition with bridal creeper; and 2. the extent of reduction in growth and development of bridal creeper by infection with the introduced rust *Puccinia myrsiphylli*. Results of field monitoring of *P. spicata* populations competing with bridal creeper before rust release and some preliminary results post-rust release are also presented. The reduced invasiveness of bridal creeper by the continued impact of the rust potentially provides optimistic grounds for predicting the recovery of the native shrub population in the longer term. The threat of bridal creeper to native plant diversity is thereby reduced. The recent releases of two insects for bridal creeper control in southern Australia may further enhance recovery of a range of native shrubs and herbs, including the endangered orchid *Pterostylis arenicola* in South Australia.

Theme 5:

Evaluation

Evaluating the flow-on effects of the biological control agents for *Ageratina riparia* (mist flower) on plant succession

Jane Barton (née Fröhlich),[1,3] Jonathan Boow,[2,4] Krystian Ragiel,[2,5] Kate Edenborough[2,6] and Kathryn Whaley[1,7]

Summary

Permanent plots were established in an area of forest in northern New Zealand to monitor what plants replaced the weed *Ageratina riparia* (mist flower, Asteraceae) as it came under attack from two deliberately released biological control agents. *Ageratina riparia* is an aggressive and fast-growing weed originating in Central America that has invaded pastures and native forests in the northern half of the north island of New Zealand. Following the successful biological control program against this target in Hawai'i, two natural enemies of the weed, the white smut fungus *Entyloma ageratinae*, and the gall fly *Procecidochares alani*, were introduced into New Zealand in 1998 and 2001, respectively. The permanent plots were established, some with *A. riparia* and some without, in the summer of 1999/2000. All plants within each plot were identified and categorised by origin (i.e. exotic or native), and by taxonomic group (e.g. dicotyledonous, ferns/fern allies). The health and cover of *A. riparia* were also assessed. The plots were reassessed in two subsequent summers. When the plots were first examined there was found to be significantly fewer native plant species in plots with *A. riparia* than in those without it. During the two-year study *A. riparia* cover decreased from 74% to 16% (on average). We attribute this reduction to defoliation by the fungus, as the gall fly has not yet reached the plots. As *A. riparia* cover declined there was an increase in the number of native species (but not of exotic species) in the plots with the weed, relative to those without it. That is, the reduction in *A. riparia* cover appears to be benefiting native plants rather than other exotic weeds.

Keywords: *Ageratina riparia*, mist flower, New Zealand, post-release monitoring, succession

Introduction

Critics of biological control (e.g. Howarth 1991) have said that because only one weed is normally targeted at a time, there is a danger that the target weed will simply be replaced by another unwanted invader. This scepticism is not addressed by studies that demonstrate only that a biological control agent or agents have reduced a weed below a desired threshold. Researchers need to go further and show that the weed has been replaced by more desirable vegetation. In this study we investigate whether *Ageratina riparia* (Regel) R. King and H. Robinson (mist flower) is replaced by more or less desirable vegetation after the introduction of its natural enemies to New Zealand.

Ageratina riparia is a perennial herb or sub-shrub, up to 2 m tall, belonging to the daisy family (Asteraceae). The weed prefers full light but is moderately shade-tolerant. It produces abundant white flowers in the spring which result in numerous wind and water-

[1] Landcare Research, Private Bag 92170, Auckland, New Zealand.
[2] The University of Auckland, Private Bag 92019, Auckland, New Zealand.
[3] Present address: 353 Pungarehu Rd., RD 5, Te Kuiti, New Zealand.
[4] Present address: Auckland Conservancy Office, Department of Conservation, Private Bag 68908, Newton, Auckland, New Zealand.
[5] Present address: 41 Ronaki Rd., Mission Bay, Auckland, New Zealand.
[6] Present address: 7 Rowan Rd., Royal Oak, Auckland, New Zealand.
[7] Present address: Biosecurity Unit, Auckland Regional Council, Private Bag 92012, Auckland, New Zealand
Corresponding author: J. Barton, 353 Pungarehu Rd., RD 5, Te Kuiti, New Zealand <Jane.Barton@ihug.co.nz>.

borne seeds. It is native to Central America but has been moved around the world as an ornamental plant. It is considered a serious invasive weed in many tropical and warm temperate regions of the world including northern Australia, South Africa and Hawai'i. The Hawaiians had a particularly serious problem with the weed and were the first to investigate using biological control as a potential solution (Trujillo 1985).

Ageratina riparia was introduced to New Zealand around 1931 (Webb *et al.* 1988). By the 1990s it had naturalised in a range of habitats (e.g. forest margins, stream banks, pastures and road sides) in the upper half of the north island and was causing considerable concern, especially to government bodies which manage areas of native forest. In 1995 it was decided that biological control would be the best option for tackling the weed. The successful biological control program against *A. riparia* that was conducted in Hawai'i was based on three agents: a white smut fungus *Entyloma ageratinae* Barreto and Evans, a gall fly *Procecidochares alani* Steyskal, and a plume moth *Oidaematophorus beneficus* Yano and Heppner. The fungus and the gall fly were reported to have been the most effective agents in Hawai'i (Fröhlich *et al.* 2000), and are also believed to be complementary in their activity. Consequently, after the relevant authorities had been presented with information on the host range and efficacy of the two agents, they were released in New Zealand; *E. ageratinae* in 1998 and *P. alani* in 2001.

After *E. ageratinae* was released, a small multi-year study was set up in the Waitakere Ranges, an area of native forest near Auckland, to record the direct impacts of the two agents on *A. riparia*, and to monitor the "flow-on" effects of biological control on the surrounding vegetation.

Materials and methods

Thirty-one permanent plots, each of 4 m^2, were established along two walking tracks in the Waitakere Ranges during the summer of December 1999–February 2000 (from here on, the year "1999" will be used for the summer of 1999/2000, and the year "2000" for the summer of 2000/2001 etc.). Plots were within a few meters of cleared walking tracks for two reasons: firstly because *A. riparia* grows abundantly near disturbed areas such as track edges, and secondly for ease of access. Distances between plots varied from less than 1 m to ca. 100 m.

Twenty plots were established along the "Pipeline" (P) walking track. *Ageratina riparia* had a patchy distribution along this track in 1999, and its absence from apparently suitable habitat was taken as evidence that the weed was still spreading in this area. Thus, at this site it was possible to select 10 plots with a reasonably dense cover of *A. riparia* ("+mist flower", hereafter "+MF" plots), and then to pair each of these with a nearby plot with very little, if any *A. riparia* ("–MF" plots). Areas with a reasonably dense cover of *A. riparia* that were adjacent to similar-looking areas with very little *A. riparia* were deliberately targeted for plot placement. However, within these areas the placement of the 2 × 2 m or 1 × 4 m plots was random.

A further 11 plots were established along the "Kura" (K) walking track. This track had a much more extensive cover of *A. riparia* in 1999, with the weed appearing to have colonised almost all of the suitable habitat. Ten plots were established with dense *A. riparia* cover ("+MF" plots). Areas with roughly similar *A. riparia* cover were selected by eye, but within these areas actual plot placement was random. One plot with only a few *A. riparia* seedlings (that were subsequently weeded out) was also set up (near one of the +MF plots), for comparative purposes ("–MF" plot).

Within each of these 31 plots, all plants, including seedlings, were identified to species. These plants were categorised as: exotic or native; dicotyledonous (woody or herbaceous); monocotyledonous (woody or herbaceous); gymnosperms; ferns/fern allies; or, mosses/liverworts.

Percentage cover and size classes of each species were also recorded, but these data will not be discussed here.

Additionally, for the 20 plots containing *A. riparia*, the percentage cover by the weed in each plot was estimated by eye. *Ageratina riparia* health was determined from five plants randomly selected from just outside each +MF plot. These five plants were assessed for: percentage of living leaves showing signs of infection; percentage of attached leaves that were dead; and percentage of stem nodes with regrowth (new leaves developing).

It was intended that the first year's data would be collected before the biological control agents arrived. However, when the plots were set up, the fungus was found to be already present. The capacity of the fungus to spread very quickly meant it was not possible in this study to monitor its direct and indirect impacts by artificially applying it to one of a set of paired, randomised plots. It might have been possible to exclude it with fungicide, but it was not known what side-effects this might have on plant growth and succession.

If *A. riparia* was found to have invaded a "–MF" plot between visits, it was removed.

Changes in the health and cover of *A. riparia* were monitored using simple comparisons of averages. Wilcoxon paired sample tests were used on species presence/absence data for two purposes. Firstly, they were used to distinguish differences between paired +MF and –MF plots on the P track (to determine whether vegetation changes between 1999, 2000 and 2001 were similar or different between plots with and without *A. riparia*). Secondly, they were used to examine changes in vegetation over time in the +MF plots on both tracks.

Results

Direct impacts of the biocontrol agents on the health and cover of *A. riparia*

Between the summers of 1999 and 2001, the proportion of *A. riparia* leaves that were infected by the white smut fungus increased by 44%. Over the same period, the percentage of dead leaves that were attached to *A. riparia* plants initially increased, and then levelled off. Percentage regrowth increased between 1999 and 2000, but then decreased between 2000 and 2001. The estimated cover by *A. riparia* in the plots decreased steadily and dramatically: from 74% to 16% in two years (Table 1).

Indirect impacts of the biocontrol agents, and the presence/absence of *A. riparia*, on other vegetation

The number of exotic species in plots with vs. those without *A. riparia* did not significantly differ for any of the three years on the P track (Fig. 1, Wilcoxon paired sample test, $p > 0.5$ for all three years). Nor was there a significant increase in the number of exotic species in the +MF plots on the K track or the P track during the study (data from 1999 vs. 2001, Wilcoxon paired sample test, $p = 0.1$ for the K track, the number of exotic species in the +MF plots actually declined between 1999 and 2001 on the P track (Fig. 1)).

In contrast, there were significantly fewer native species in plots with *A. riparia* than in those without it on the 'P' track (Fig. 2., Wilcoxon paired sample test, $p = 0.005$). Over time, the number of native species increased in plots with the weed (as its cover decreased) while for unknown reasons they decreased in those without it (where *A. riparia* cover remained unchanged, at zero) (Fig. 2). The increase in native species in the plots with declining *A. riparia* on the P track was so rapid that, within one year, there were no longer significantly fewer native species present in plots with *A. riparia* (Fig. 2., Wilcoxon paired sample test, $0.2 > p > 0.1$). The number of native species appears to be recovering more slowly on the K track, as while there was a slight increase in the mean number of native species in plots with the weed between 1999 (9.5 species) and 2001 (10.4 species), this increase was not statistically significant (data for 1999 vs. 2001, Wilcoxon paired sample test, $0.5 > p > 0.2$). The single plot without *A. riparia* on the K-track still contained many more native species (17 species in 1999, 15 species in 2001) than most of the plots with the weed.

Table 1. *Ageratina riparia* health and cover: average values from the 20 plots with *A. riparia* (+MF) in the Waitakere Ranges (includes data from both the Pipeline and Kura tracks)

Variable	1999	2000	2001
Percentage of living leaves infected by fungus[a]	18	56	62
Percentage of attached leaves that are dead[b]	10	24	23
Percentage of nodes with regrowth[a]	4	13	7
Percentage cover of *A. riparia*[b]	74	49	16

[a] Average from 5 plants × 20 plots.
[b] Average for 20 plots.

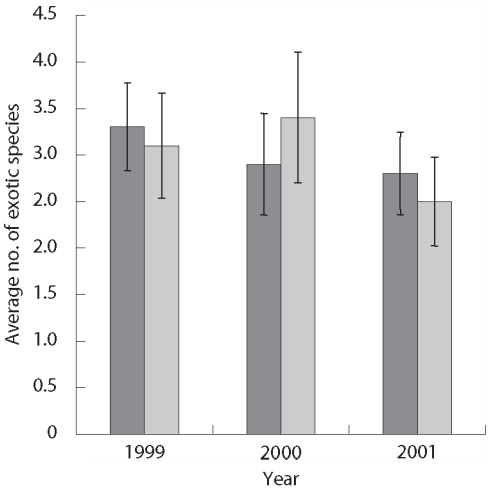

Figure 1. Average numbers of exotic species in plots of the Pipeline track. Dark grey bar = *Ageratina riparia* present; light grey bar = *A. riparia* absent. Error bars indicate the standard error of the mean.

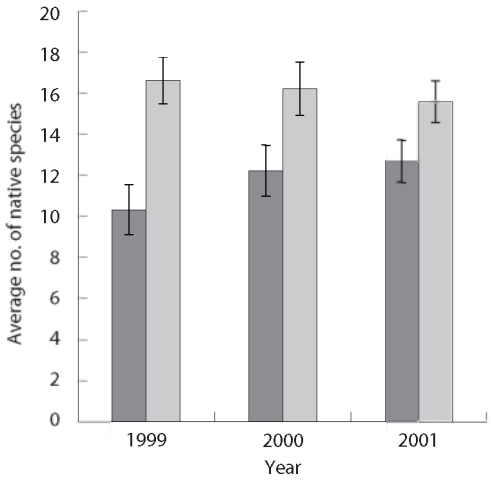

Figure 2. Average numbers of native species in plots of the Pipeline track. Dark grey bar = *Ageratina riparia* present; light grey bar = *A. riparia* absent. Error bars indicate the standard error of the mean.

The plant category that appeared to be most affected by the presence or absence of *A. riparia* was the woody dicotyledonous plants (Table 2). This is the category to which *A. riparia* itself would belong, but it was not included in the species counts. On the P track, there were significantly fewer species of woody dicotyledonous plants (dicots) found in the +MF plots than in the –MF plots in both 1999 and 2000 (Fig. 3, Wilcoxon paired sample test, $0.05 > p > 0.02$ for both years). In 2001 there were still slightly fewer species of woody dicots on average in the +MF compared to the –MF plots (Fig. 3), but this difference was no longer statistically significant (Wilcoxon paired sample test, $0.2 > p > 0.1$). The average number of woody dicots also increased in the +MF plots on the K track between 1999 (average = 3.7 spp.) and 2001 (average = 4.5 spp.), but this change was not statistically significant (Wilcoxon paired sample test, $p = 0.2$). There were eight species of woody dicot in the –MF plot on the K track in 1999, and seven in 2001.

There were consistently fewer species of ferns in plots with *A. riparia* compared to plots without it (Table 2), but this difference was not statistically significant, at least in the plots on the P track, in any of the three years (Wilcoxon paired sample test, 1999 $0.5 > p > 0.2$, 2000 $0.2 > p > 0.1$, 2001 $p = 0.1$). There were also consistently fewer herbaceous monocots when *A. riparia* was present (Table 2). However, the number of herbaceous monocots declined suddenly in the –MF plots between 2000 and 2001 (Table 2), so the species richness in this category appears to have been influenced by something additional to the flow-on effects of the *A. riparia* biocontrol agents.

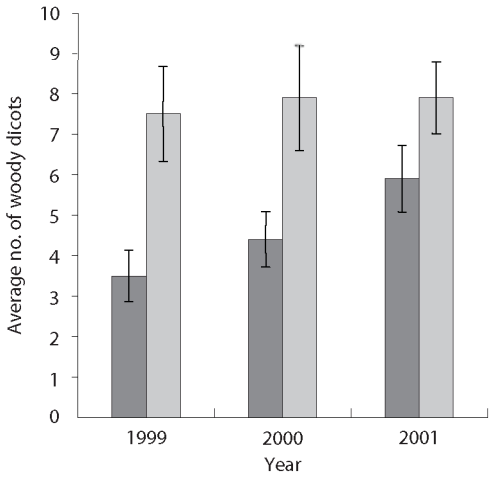

Figure 3. Average numbers of woody dicotyledonous plant species in plots of the 'P' Track. Dark grey bar = *Ageratina riparia* present; light grey bar = *A. riparia* absent. Error bars indicate the standard error of the mean.

Table 2. Average numbers of species in each plant category in plots with (+) or without (–) *Ageratina riparia* for 1999, 2000 and 2001. Data from all 31 plots on both Pipeline and Kura tracks.

Plant category	*A. riparia*	1999	2000	2001
Woody dicots	–	7.1	7.4	7.6
	+	2.8	4.1	4.8
Herbaceous dicots	–	1.1	1.0	0.7
	+	1.2	1.2	1.3
Woody monocots	–	0.7	0.7	0.7
	+	0.8	0.9	0.8
Herbaceous monocots	–	2.8	3.1	2.0
	+	1.6	2.3	1.9
Mosses/liverworts	–	1.8	1.2	1.2
	+	1.1	0.8	0.6
Ferns/fern allies	–	2.4	2.7	2.7
	+	1.4	1.5	1.7

Discussion

Direct impacts of the biocontrol agents on the health and cover of *A. riparia*

In the 20 +MF plots, the proportion of living *A. riparia* leaves that were infected increased to an average of 62% by 2001. Plots established at the nine sites in New Zealand where the fungus was first released showed similar levels of infection (average infection 54% in 2001). The rate of increase in infection appears to have slowed at this site, and infection levels are not expected to increase much further in the future. The percentage of attached *A. riparia* leaves that are dead appears unchanged, at around 23–24%. This trend was also observed at release sites, where the average percentage of dead leaves still attached was 27% in 2000 and 22% in 2001. The usual response of *A. riparia* to defoliation is to produce new leaves at the nodes. Given the large numbers of dead leaves observed in the +MF plots, the percentage of nodes with regrowth was surprisingly low (only 7% in 2001). For comparison, regrowth was observed at an average of 25% of nodes at release sites in 2001.

The extremely rapid reduction in the percentage cover by the weed observed in the plots (from 74% to 16% in two years) was not unique to this site. Cover by *A. riparia* at the nine sites where the fungus was released decreased, on average, from 90% to 35% between 1998 and 2001. Strictly speaking, what the data show is a correlation (and not causation) between the increase in the presence and activity of the fungal biocontrol agent, and a decrease in the cover by target weed. However, given that there was no other obvious reason for the differences in *A. riparia* health observed in the plots between 1999 and 2001 (all disease symptoms could be attributed to the fungus, the gall fly had not yet reached the plots, there had been no changes in management strategy and the plots were examined at the same time of year), in this case it does seem reasonable to attribute the major decline in *A. riparia* cover to

the activity of the white smut fungus alone. As mentioned previously, the fungus was similarly effective in reducing the infestation of *A. riparia* in Hawai'i (Trujillo 1985). This study is ongoing and future results should incorporate the impacts of the gall fly.

Indirect impacts of the biocontrol agent, and the presence/absence of *A. riparia*, on other vegetation

When the 20 plots on the P track were first examined, in 1999, there were found to be significantly fewer native plant species in +MF plots than in –MF plots. In contrast, there was no significant difference between the numbers of exotic plant species (excluding *A. riparia*) between the +MF and –MF plots. This was consistent with the views of land managers familiar with *A. riparia*, that the weed was having a negative impact on the regeneration of native species while not inhibiting other exotics. On the positive side, at least the presence of *A. riparia* did not appear to facilitate the growth of other exotic species.

If *A. riparia* were being replaced by other exotic weeds as its cover decreased, one would expect to see a significant increase in the number of exotic species in +MF plots (where the weed is declining) relative to –MF plots (where cover of the weed has remained at zero). This has **not** been the observed pattern of vegetation change (Fig. 1). There has been no significant increase in the number of exotic species through time in the +MF plots on either the P or the K track. It is the native species that have steadily increased in numbers in the plots where *A. riparia* is present but declining (Fig. 2). It is encouraging that in the first 12 months after the arrival of the first biological control agent, the average number of native species in +MF plots on the P track increased significantly from 10.3 species to 12.2 species per plot (data for 1999 vs. 2000, Wilcoxon paired sample test, $0.05 > p > 0.02$). While the plots with *A. riparia* still have fewer native species than those without it on both tracks, the difference is quickly getting smaller (Fig. 2).

There have been a small number of other studies that have documented vegetation changes associated with biological control. Studies by Huffaker (1951) and Huffaker and Kennett (1959) (cited in Syrett *et al.* 2000) showed a similar result to that reported here. That is, in California, the successful control of *Hypericum perforatum* L. (St John's wort, Klamath weed) by *Chrysolina quadrigemina* (Suffrian) resulted in the replacement of the weed by more desirable forage species, and no long-term increase in other weedy species. In contrast, in Idaho, in many sites *H. perforatum* was mostly replaced by weedy *Centaurea* species (Campbell and McCaffrey, 1991 cited in Syrett *et al.* 2000). This demonstrates that the relative desirability of the vegetation which replaces a weed can vary from place to place. This is not surprising, given that environmental conditions, and the identity and density of other invasive species present, are likely to vary across a weed's range.

Another study similar in purpose to that described here was conducted to assess the probable flow-on effects of biological control of the weed *Hieracium pilosella* (Mouse-ear hawkweed, Asteraceae) (Syrett *et al.* this volume). In that study, plots were set up in the high country of the south island of New Zealand, biological control was simulated by painting herbicide onto *H. pilosella* plants, and then the responses of surrounding vegetation were monitored for 10 years (Syrett *et al.* this volume). It was concluded that the impacts of weed removal were likely to vary between different sites according to soil fertility, environmental conditions and grazing pressure (Syrett *et al.* this volume). At the most hostile sites, *H. pilosella* removal resulted, temporarily, in the undesirable emergence of bare ground. Nevertheless, while the rate of succession, and the identities of the original colonisers, varied between sites, everywhere that *H. pilosella* was "controlled" there was a slow succession towards more desirable vegetation (Syrett *et al.* this volume). It would be interesting to repeat the study on *A. riparia* in another area, perhaps one with more exotic species, to see if differences between areas were as marked as those observed in the *Hypericum perforatum* and *Hieracium pilosella* studies. Fortunately, the environment where *A. riparia* grows is no-where near as inhospitable as that favoured by *H. pilosella*. Thus, while the identity of the plants that first replace *A. riparia* could be expected to differ between sites, it is unlikely that bare ground would ever form as a result of biological control of the weed.

Of the plant categories examined, so far it is the woody dicotyledonous plants that appear to have benefited most from the biological control of *A. riparia*. This is the most species rich group and almost all of the woody dicots encountered were native. By 2001 there were still fewer woody dicots in the plots with *A. riparia* than in the plots without it. However, this gap was rapidly closing and the difference, in the plots on the P track at least, was no longer statistically significant (Fig. 3).

The lower numbers of fern species observed in plots with *A. riparia* compared with plots without it suggest that this category of plants should also benefit from biological control of *A. riparia*. The ferns do not appear to be recovering in diversity as fast as the woody dicots; however, the succession pattern of ferns will be complicated by their complex life-cycle, as their small gametophyte stage would not have been identified in this study. This plant category will be watched with interest in future years.

The herbaceous monocots also behaved differently depending on whether *A. riparia* was present or absent. However, this group showed a less easily explained pattern of increase and decrease in numbers over time

(Table 2). It may be that, overall, in plots both with and without *A. riparia*, woody species are increasing in number at the expense of herbaceous species. This is a typical pattern observed in plant succession in disturbed areas such as near tracks (S. Fowler pers. comm.). Data on this plant category will also be watched with interest in future.

The herbaceous dicots and woody monocots were the only plant categories that appeared to be slightly more diverse in the presence of *A. riparia* than in its absence. However, the differences between +MF and –MF plots were not significant for either of these two plant types.

There is probably a variety of reasons why some plant categories did not show significant differences in diversity between –MF and +MF plots. For example, there were never more than two species of woody monocots or gymnosperms in a plot, so species numbers were probably too low in all plots for any differences to be apparent. Many of the mosses that were recorded were found growing only on fallen logs. Therefore, the presence/absence of a given moss species from a plot may be more influenced by substrate availability than the presence of *A. riparia*. Liverworts were not differentiated at the species level, and this would also have made it difficult to observe patterns in bryophyte succession. Bryophytes were found to be critical to the process of succession in the study on *H. pilosella*, especially in situations where weed removal resulted in bare ground (Syrett *et al.* this volume). However, bryophytes may be less important to succession in the vegetation-rich areas that *A. riparia* prefers.

The results presented here on the indirect impacts associated with the biological control of *A. riparia* were based on data on species presence/absence alone. Data on the percentage cover of each plot by each species, and also on the sizes and numbers of individual plants present, were also collected and should be analyzed and presented in another paper in the near future.

In summary, to date the first of the biological control agents for *A. riparia* appears to be having a positive flow-on effect on native plant succession. That is, in at least this area, there is a strong correlation between a major decline in the cover of the weed and a fairly rapid recovery in the diversity of more desirable native vegetation.

Acknowledgements

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Insect–plant pathogen synergisms for the biological control of rangeland weeds

Anthony Caesar[1]

Summary

Insect–pathogen interactions have remained underutilized and underemphasized until relatively recently. Some studies have now begun to address this by including searches for plant pathogens and their interactions as an integral part of biological-control programs at the outset. Similarly, while climate matching and genetic description of host populations has been incorporated into weed biological-control programs, life-table analysis has remained unexploited. The author has expanded the normal life-table concept to include plant pathogens and, in the case of *Euphorbia esula,* has demonstrated the major contribution of two fungi in causing weed mortality. The propensity of a candidate agent (insect or microbe) to interact should also be considered, and it is recommended that this be a selection factor for candidate natural enemies. Additionally, survival analysis applied to the target weed upon exposure to appropriate combinations of insects and pathogens is also recommended to help assess the potential effectiveness of candidate agents. A protocol is proposed to enable such analyses. Application of one or both of these recommendations could increase success in classical biological control of weeds and reduce associated costs and environmental risks.

Keywords: insect–pathogen interactions, life-table analysis, fungi, *Euphorbia esula*.

Introduction

This paper focuses on some key points presented in a research article recently published (Caesar 2003). The purpose of this paper is to emphasize some practical outcomes the author concluded from that study to have important implications for how classical biocontrol is practised to achieve the goal of controlling exotic, invasive, perennial weeds.

Insect–plant pathogen interactions

Following the initial successes in the annals of weed biological control as described by pioneering researchers who foresaw the necessity and utility of combinations of plant pathogens and insects (Dodd 1940, Wilson 1943) for successful biocontrol, insect–pathogen interactions remained underutilized and underemphasized until revived in a concrete way by Charudattan *et al.* (1978) and Charudattan (1986), who described the role that insect/pathogen interactions can play in the biological control of *Eichhornia crassipes*. Since these milestones, there have been cogent and insightful statements by Hill (1996) and Cullen (1996), both quoted in a review by Hatcher and Paul (2001), recognizing the underutilization of insect–plant pathogen interactions. Some studies have begun to address this by including searches for plant pathogens as an integral part of programs at the outset (Briese *et al.* 2000, De Clerk-Floate *et al.* 2000). Concerning the application of survival analysis to the field of weed biocontrol, there exists a paradox. While climate matching and genetic description of host populations have been borrowed from the field of insect biocontrol, with little apparent impact on weed biocontrol as a science to-date, the comparatively more prominent area of life-table analysis that describes the effects of natural enemies on the target pest has remained unexploited. The author has previously lamented the compartmentalization of plant pathology and entomology from one another as applied to biocontrol of weeds in practice (Caesar 2000) and in theory. Such mutual isolation is illustrated by the author having been, until the writing of this paper, unaware of a long-standing suggestion by McEvoy *et al.* (1990) that life-table analysis should indeed be applied to assessing the

[1] US Department of Agriculture, Agricultural Research Service, Sidney, Montana, USA <caesara@sidney.ars.usda.gov>.

effects of natural enemies of weeds. The present author applied two other procedures residing under the rubric of survival analysis along with life tables: the Kaplan-Meier and Cox proportional hazards procedures which are part of many statistical software packages, such as SAS, SPSS and JMP. This has effectively allowed expansion beyond the normal life-table concept to include plant pathogens and assessment of the propensity of a candidate agent (insect or microbe) to interact as a qualification for candidate natural enemies.

The protocol would proceed as follows: typical minimal levels of a specific plant pathogen capable of causing disease would be determined following their isolation and confirmation of pathogenicity to the target weed. Such pathogens will usually be found attacking the target or related species in its native range. Such pathogens would preferably be found in host tissue damaged by an identifiable insect. A plant pathogen shown to aggressively colonize insect-damaged tissue would be the priority standard, especially if the target weed is a perennial. Others (e.g. Bellows & Van Driesche 1999) have deemed life-table analyses as most appropriate for biennial or perennial weeds, an assertion supported by Caesar (2003) who was the first to use survival analysis to examine the mechanism of biocontrol of *Euphorbia esula/virgata* or any perennial weed. Conversely, such an insect associated with damage resulting in significant colonization by a plant pathogen would become a priority candidate agent. The plant pathogen would be used to infest soil and target plant species planted in the infested soil. The plants would be caged and varying numbers of insects would be applied to the caged plants. Individual plants would be monitored for time to their death as outlined by Caesar (2003). Essentially, the protocol would be a test for a significant, direct interaction leading to specific levels of mortality. However, plant pathogens found to act independently of a promising candidate insect would not be excluded from application of this protocol. Significant interaction of a candidate insect and an apparent highly virulent plant pathogen not associated with damaged tissue could also be determined. Thus, indirect interactions (Conner *et al.* 2000) would be of interest too, especially if the result would be mortality of the target weed. This protocol would obviously require more space and time than has been typically applied in the initial stages of pre-release studies, but increased regulatory scrutiny and the need for fewer, more effective agents would favour a more acute focus on documented impact prior to any further testing.

Advantages

The use of a testing procedure similar to that outlined above can reduce the aggregate costs of present programs. Program costs have been recently estimated at ~US$600,000/agent (McFadyen 1998). For example, of the *ca.* 60 species available for *Veratrum album* biocontrol; potential savings are ~US$6,000,000. This is based on the premise that among this initial number, two-thirds or *ca.* 40 species in this case would be eliminated due to the lack of a sufficiently narrow host range, lack of fecundity or due to other factors, and for example, half the remaining species fail to display a significant interaction with a plant pathogen leading to increased mortality and are thus eliminated. Using the same premise, since *ca.* 40 insect species are available as candidates for biocontrol of *Phragmites australis*, potential savings are approximately US$3,600,000. Finally, with an invasive target weed species most similar to the *Euphorbia* system, *Cardaria draba*, of the *ca.* 60 insect species available for *Cardaria draba* biocontrol, the potential savings are US$6,000,000 or more. There are other advantages in addition to savings in costs and time, such as an increase in the success rate of introduced insect agents, the low rate of which has often been cited (Crawley, 1990) and which the author can confirm from examining data on earlier biocontrol programs. Another potential advantage is the achievement of "value added" to insect releases: a greater expectation of impact and a level of impact that adds as much value as the narrow host range that candidate agents emerging from such tests must demonstrate before release against a perennial weed.

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Bacterial communities associated with a flea beetle used for the biological control of the perennial weed *Euphorbia esula/virgata*

Anthony Caesar[1] and R. J. Kremer[2]

Summary

Using insects principally to control invasive exotic plant species has left 30–50 per cent of all treated sites without impact 10–15 years after insect release. To understand factors possibly affecting the documented need for synergistic interaction of the insects with plant pathogens to cause rapid weed mortality, predominant bacteria associated with the flea beetle *Aphthona flava*, released to control *Euphorbia esula/virgata* in western North America, were isolated and identified by analysis of extracted fatty acid methyl esters (GC-FAME). Two *Euphorbia*-infested sites with differing levels of impact, 8–10 years after insect release, were sampled. One exhibited rapid, sweeping declines in *Euphorbia* density (Knudsen Creek) and the other showed little effect on weed density despite fairly high *Aphthona* populations (Cottonwood). Predominant colony types from 20 live *Aphthona* adults from each site were isolated by serial dilution and plating on 0.3 % tryptic soy broth agar and KB agar. Predominant colony types were selected from each medium and further streaked onto both media. The predominant colony types from each adult were further cultured for the GC-FAME protocol. Using identification confidence levels of at least 0.650, at the Knudsen Creek site, seven of 20 colonies were *Bacillus cereus*, four were coryneform species: *Cellulomonas*, *Corynebacterium*, *Arthrobacter* and *Microbacterium* and others species identified were *Bacillus thuringiensis*, *Pseudomonas putida*, and *Burkholderia cepacia*. Many of these species are known to produce pectinase or cellulase or are low-level plant pathogens. Species from the Cottonwood site were more diverse, including some associated with biocontrol of soilborne plant pathogens: *Stenotrophomonas maltophilia*, *Pseudomonas chlororaphis* and *P. putida*.

Keywords: *Aphthona*, insect–pathogen interactions, flea beetle, pathogenic bacteria, *Euphorbia esula/virgata*.

Introduction

There has been an increasing interest in insect–pathogen interactions (Caesar 2000, Hatcher & Paul 2001) and recognition of the role of such interactions in biocontrol confirmed by recent studies (Martin & Dale 2001, Caesar 2003). The hypothesis addressed in this work is whether the degree of biocontrol activity of the flea beetle *Aphthona flava* on the perennial invasive prairie plant, *Euphorbia esula* L. (leafy spurge), is associated with traits within members of the bacterial community vectored by the beetle. As shown in previous studies, the *Aphthona* beetle typically instigates a synergistic interaction with soil-borne plant pathogens such as *Fusarium* spp., *Rhizoctonia* spp. and *Pythium* spp., which leads to successful biocontrol of *E. esula* (Caesar 2000, 2003). The larvae of the flea beetle injure roots of plants by feeding, providing paths of ingress by plant pathogenic fungi.

The initial thrust of the work was to examine bacterial isolates for hydrolytic enzyme production to determine whether there were trends in enzyme spectra among isolates from beetles recovered at a successful biocontrol site at Knudson Creek, Theodore Roosevelt National Park, North Dakota, USA versus isolates from

[1] US Department of Agriculture, Agricultural Research Service, Sidney, Montana, USA.
[2] US Department of Agriculture, Agricultural Research Service, Columbia, Missouri, USA.
Corresponding author: A. Caesar, US Department of Agriculture, Agricultural Research Service, Sidney, Montana, USA <caesara@sidney.ars.usda.gov>.

a less-successful release site (referred to as Cottonwood). Hydrolytic enzymes were chosen as the traits of interest because of their potential for increasing plant tissue damage as well as conversely acting against soil-borne pathogens through lysis of fungal hyphae.

Another objective of the study was to assess bacterial isolates for hydrolytic enzyme production to determine whether certain isolates vectored by the flea beetle might be minor pathogens. Previous studies by Kremer have documented deleterious rhizobacteria that can damage *E. esula* (Kremer & Kennedy 1996).

Materials and methods

Hydrolytic enzyme activities were tested using published methods. Filter sterilized solutions of 0.25% p-nitrophenyl ß-D-glucopyranoside (pNBG) (Sigma Chemical Co., St Louis MO) (Fahey & Hayward 1983) and 0.25% p-nitrophenyl ß-D mannopyranoside (pNMP) (Sigma Chemical Co., St Louis MO), in pH 7 phosphate buffer were dispensed into sterile 96-well microtitre dishes to give 150–200 µL per well. Bacterial isolates were seeded into the wells and plates were wrapped loosely, but thoroughly, with plastic wrap and incubated at 20°C for 10–14 days. At this time, wells that were yellow due to release of the p-nitrophenyl, indicating enzyme activity for the respective isolate, were scored as positive, while similar wells that remained colourless were scored as negative. Because the pNBG method is not optimal for testing ß-glucosidase activity of fluorescent *Pseudomonas* spp., another test was used which detects the hydrolysis of 0.1% methylumbelliferyl ß-D-glucoside (MUG) by use of long-wave ultraviolet light (Santos *et al.* 1979). To further assay the hydrolytic enzyme's versatility of the isolates, additional tests were conducted on the following substrates: 0.1% 4-methylumbelliferyl N-acetyl ß-D glucosamine (Chitin is a homopolymer of *N*-acetyl-glucosamine) and 0.1% 4-methylumbelliferyl N-acetyl ß-D glucosaminide.

Clearing of coloured substrates on agar media during incubation at 20°C for 10–14 days was used in tests to indicate xylanase (Biely *et al.* 1985) or ß-1,4-glucanase (Scott & Schekman 1980) activity of isolates, respectively. Substrates were 0.2% remazol brilliant blue xylan (4-O-methyl-D-glucurono-D-xylan dyed with remazol brilliant blue R) (Biely *et al.* 1985) and 0.2% Ostazin brilliant red–hydroxyethylcellulose (hydroxyethylcellulose dyed with ostazin brilliant red H-3B) (both Sigma Chemical Co., St. Louis, MO), respectively, in 2YT medium (Sipat *et al.* 1987) with 1.5% agar. Tests for polygalacturonase (Hankin & Lacy 1984) and cellulase (Barros *et al.* 1987) were according to published methods.

Bacterial identifications were based on gas chromatographic analysis of whole cell fatty acid methyl esters with the commercial MIDI system (MIDI, Delaware, USA). Isolates with a similarity index of at least 0.650 were considered to be identified.

Isolates were also assessed for in vitro antibiosis against two soil-borne fungal pathogens of *E. esula*: a *Pythium* spp. isolate and an isolate of *Rhizoctonia solani*. Bacteria were streaked near the edge of Petri dishes containing 0.3% tryptic soy agar, and immediately thereafter agar plugs taken from colony margins of one of the fungi were placed at the opposite side of plates. Plates with these bacterial/fungal pairings were incubated at 20°C and examined for zones of inhibition after 36 hrs. Degree of inhibition was scored as –, +, ++, +++ based on 0, ≤ 1 cm, >1–2 cm and ≥ 3 cm-wide zones of inhibition, respectively.

Results and discussion

This study identified and described some selected phenotypic traits of bacteria isolated from adults of the *E. esula* biocontrol agent *Aphthona* associated with sites showing significant reductions in stand density following the release of the flea beetle and sites where the impact of the beetle was much less. There was a slightly greater extent of hydrolytic enzyme production by predominant bacteria at the highly successful Knudson Creek biocontrol site, compared to the Cottonwood site (Table 1). In vitro antibiosis of the bacteria against *Rhizoctonia solani* and *Pythium* spp. was not a helpful trait in distinguishing the two sets of isolates, since the bacteria from the successful site showed a greater overall degree of in vitro antibiosis. Interestingly, cellulase production was quite common throughout both groups, but eight of nine strains with the heaviest expression (data not shown) were from the Cottonwood site, the less successful one. This raises the question of whether, in the milieu of the plant/microbe/insect interaction, the intensity of hydrolytic enzyme production by bacteria might inhibit plant pathogens by allowing them to successfully compete with fungi in the colonisation of plant tissue and utilisation of leaked complex carbohydrates made available by the insect damage. If competition for nutrients between fungi and bacteria is the effective mechanism explaining the static nature of the plant three-part interaction resulting in a failure to control the weed despite long-term establishment of *Aphthona*, then this might be reflected in lower populations of the pathogen synergists in the rhizosphere soil of *Euphorbia*.

We consider the relevance of describing bacterial communities of adult flea beetles to be based on two premises: 1) that the bacteria carried by the flea beetles may be active participants in the phyllosphere and/or rhizosphere once they are carried passively to the plant, and 2) that the bacteria found on the insects may represent species that predominate in the host plant/insect system. A further possibility is that these bacteria are endemic to the insect or to the plant leaf surface, root zone or perhaps vascular system. The possibility of

Table 1. In vitro antibiosis and hydrolytic enzyme production by bacteria associated with the flea beetle *Aphthona flava* released at two sites, one undergoing rapid population decline of the highly invasive perennial weed *Euphorbia esula* (Knudson Creek site), the other (Cottonwood) exhibiting much slower impact on density following release of the beetle. Tests for enzymes were with chromogenic substrates.

Isolate	**Phenotypic traits of isolated bacteria** [a,b]										
	In vitro antibiosis vs. *Pythium* spp.	In vitro antibiosis vs. *Rhizoctonia solani*	0.25% p-nitrophenyl ß-D-gluco pyranoside test	0.25% p-nitrophenyl ß-D-mannopyranoside	0.1 % 4-methylumbelliferyl N-acetyl ß-D glucosamine	0.1% 4-methyl umbelliferyl ß-D-glucoside	0.1% 4-methylumbelliferyl N-acetyl ß-D glucosaminide	Ostazin Brilliant Red hydroxyethylcellulose	Remazol Brilliant Blue Xylan	Polygalacturonase	Cellulase
Knudson Creek site											
Pseudomonas putida 102	++	+	+	+	+	+	+	+	+	–	–
Bacillus cereus 103	++	–	+	–	–	–	–	–	–	–	+
B. cereus 104	NT	NT	–	–	–					–	+
Arthrobacter oxydans 113	–	–	–	–	–	–	–	–	–	–	+
Bacillus thuringiensis 124	++	+	–	–	+	–	–	+	–	–	+
B. cereus 129	++	+	–	–	+		+	+	–	–	+
B. cereus 154	++	+	–	–	+	+	+	+	–	–	+
Burkholderia cepacia	++	++	–	–	–	–	–	–	–	–	+
Corynebacterium acquaticum	–	–	–	–	–	–	–	–	–	–	–
Cellumonas turbata	–	–	–	–	–	–	–	–	–	–	+
B. cereus 216	NT	NT	–	–	+	–	+	+	–	–	–
Microbacterium liquefaciens	+++	++	–	–	+	–	–	+	–	–	+
Cottonwood Creek site											
Brevibacterium iodinium	–	–	+	+	+	+	+	+	+	–	+
Paenibacillus glucoanalyticus	–	–	–	–	–	–	–	–	+	–	+
Pseudomonas chlororaphis	++	–	+	–	–	+	–	+	+	+	+
Ochrobactrum anthropi	NT	NT	+	–	–	–	–	–	–	–	–
Bacillus thuringiensis kurstakii	+	+	–	–	–	–	–	+	–	–	+
Bacillus cereus	++	+	–	–	+	–	+	–	–	–	–
Pseudomonas putida	–	–	–	–	–	–	–	–	–	–	–
Pseudomonas chlororaphis	++	–	–	–	–	–	–	–	–	–	+
Stenotrophomonas maltophilia	–	–	–	–	–	–	–	–	+	–	–
No match	++	–	+	–	–	–	–	+	–	–	+
No match	–	+	–	–	+	–	–	–	–	–	–
No match	–	+	–	–	–	–	–	+	–	–	–

[a] For in vitro antibiosis tests, degree of inhibition was scored as: (–) = no inhibition; (+) = ≤ 1 cm-wide zone of inhibition; (++) = >1–2 cm-wide zone of inhibition; (+++) = ≥ 3 cm-wide zones of inhibition. NT = Not tested.

[b] For all other tests (–) = trait absent; (+) = trait present; NT = Not tested.

bacteria and other microbes affecting herbivory in some way is not without precedent and could lead to some important contributions to a fuller picture of biocontrol ecology. That the ecology of classical weed biocontrol is justifiably receiving greater attention seems evident by many contributions to these proceedings.

Upcoming studies of the individual tripartite interactions among individual isolates, the flea beetle and *E. esula* will determine what each array of observed traits might confer on the outcome of interactions with the flea beetle and its host.

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Spatially explicit models for weed–biocontrol agent interactions: scentless chamomile as a case study

Tomás de Camino Beck,[1] Alec McClay[2] and Mark Lewis[1]

Summary

Scentless chamomile (*Matricaria perforata*) Mérat is an annual or short-lived perennial weed native to Europe that is becoming a serious problem in agricultural land in western Canada. Three biological control agents have been released in western Canada. The seed weevil *Omphalapion hookeri* and the gall midge *Rhopalomyia tripleurospermi* are well established and dispersing at numerous sites, while the stem weevil *Microplontus edentulus* is only established at a few sites to date. The impact of these insects is difficult to evaluate because of the patchy distribution and fluctuating density of the target weed. We have represented the interactions between scentless chamomile, *O. hookeri*, and *R. tripleurospermi* in a landscape context using a coupled map lattice model. This model incorporates (1) stage-structured population models for the target weed and the two biocontrol agents, (2) dispersal kernels for each of the organisms, and (3) a geographical information system (GIS) landscape layer representing spatial heterogeneity such as land-use patterns. Estimates are available for many of the required parameters. The model will be used to predict the outcome of the weed–biocontrol agent interactions, suggest methods of impact evaluation in the field, and to develop recommendations to optimize release strategies. The model provides a general framework which could readily be adapted to model many weed–biocontrol agent interactions in a spatial context.

Keywords: coupled map lattice, dispersal, modelling, scentless chamomile, spatial ecology.

Introduction

Scentless chamomile (*Matricaria perforata* Mérat, syn. *Tripleurospermum perforatum* [Mérat] M. Lainz, Asteraceae) is an annual, winter annual, or short-lived perennial European weed that has become a major problem in the prairie provinces of Canada (Manitoba, Saskatchewan, Alberta, and north-eastern British Columbia) (Woo *et al.* 1991). It occurs primarily in disturbed or cultivated land, and once established in suitable habitats, it spreads rapidly because of its profuse seed production. Dense populations of scentless chamomile cause significant crop losses (Douglas *et al.* 1991, 1992) and herbicidal control is difficult when plants are beyond the seedling stage (Ali 2000).

The life history of scentless chamomile is plastic. In Canada, seeds germinating by mid-July give rise to annual plants that flower and set seed the same summer (annual life history). Seeds germinating later in the growing season give rise to overwintering rosettes that bolt, flower and set seed the following season (winter annual life history) (Blackshaw & Harker 1997). The overwintered rosettes typically produce larger, multi-stemmed plants that produce large amounts of seed. They are also more difficult to control with herbicides than the summer annual plants. Most plants die after setting seed, but a small percentage may resprout and flower for a second season.

Scentless chamomile was proposed as a target for biological control in Canada in 1989 (Peschken 1989; Peschken *et al.* 1990), and three insect agents have been released and established against it. The seed-feeding weevil *Omphalapion hookeri* (Kirby) (Coleoptera:

[1] Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2G1, Canada.
[2] Alberta Research Council, PO Bag 4000, Vegreville, Alberta T9C 1T4, Canada.
Corresponding author: Alec McClay <alec.mcclay@arc.ab.ca>.

Brentidae) was first released in 1992, and is now established at numerous sites across the prairie provinces (McClay & De Clerck-Floate 1999). The stem-mining weevil *Microplontus edentulus* (Schultze) (Coleoptera: Curculionidae) was first released in 1997 but has only established at a few sites, and the gall midge *Rhopalomyia tripleurospermi* Skuhravá (Diptera: Cecidomyiidae) was first released in 1999 and rapidly established at numerous sites across the prairies (McClay *et al.* 2002; McClay, unpublished).

Scentless chamomile is a suitable target for biological control in that it has no closely related native species in North America, reducing the risk of non-target damage. However, it is a somewhat unorthodox target in that it is a pioneer species which typically forms large flushes of seedlings when a seed source coincides with a soil disturbance. It is not a strong competitor with perennial plants, so if there is no further soil disturbance it tends to be displaced by grasses and other perennials after 3–4 years. In the Parkland region of central Alberta and Saskatchewan, high densities of scentless chamomile are often found around the margins of sloughs (shallow prairie ponds or wetlands), along field edges and roadsides, in farmyards and home sites, along pipeline rights-of-way, and in urban and industrial areas and construction sites (see Bowes *et al.* 1994). These marginal populations probably provide the seed source for dense, localized field-scale outbreaks covering a quarter-section (64 ha) or more of cultivated land, known locally as "white fields". These occur sporadically when scentless chamomile seed is spread though a field by natural dispersal or by farming operations, and other methods of control are not applied in time to prevent flowering and seed set. Scentless chamomile can form the dominant cover in these fields, causing heavy crop losses and creating a large seed bank from which recruitment can occur in future years when conditions are suitable. Seed from both the marginal and "white-field" populations is dispersed naturally by wind and water movement, as well as by human-aided movement of contaminated soil, seed, hay, livestock, farm equipment, and vehicles.

Scentless chamomile populations thus form a shifting mosaic in which large outbreaks occur, fade out and are replaced by new outbreaks elsewhere in the landscape. This poses three problems for biological control:

1. can the biological control agents track these shifting resource patches quickly enough to build up to damaging population levels?
2. how can we identify and evaluate the impact of a biological control agent when, even in the absence of control, the weed populations are transitory?
3. how can biological control agents be selected, and release strategies planned, to maximize the impact of biological control?

Because of the difficulty of conducting experimental evaluations on a large scale, we propose that a simulation model of the interactions between scentless chamomile and its biological control agents on a landscape scale would be a useful tool in understanding the potential for successful biological control in this system.

Materials and methods

Our modelling approach consists of: 1) life history definition for each species (scentless chamomile, *O. hookeri* and *R. tripleurospermi*), 2) matrix model construction, 3) matrix model embedded in a spatial model (coupled map lattice) and 4) simplify the coupled map lattice into a cellular automata.

Because of the high seasonality of scentless chamomile–agent interaction, a matrix model was used. A matrix model summarizes the host's life cycle in a series of transition coefficients that represent the probability of an individual growing from one life stage to the next, and then reproducing. A matrix population model is defined as:

$$\mathbf{n}_{t+1} = \mathbf{B}\mathbf{n}_t \qquad (1)$$

For scentless chamomile, $\mathbf{B}$ is a 3 × 3 projection matrix and $\mathbf{n}$ is a vector $\mathbf{n} = (s,r,f)^T$ with 3 stages. An entry b_{ij} in $\mathbf{B}$ describes the fraction of individuals in stage j that move to stage i. Table 1 describe the transitions in detail. In this matrix model, time occurs in yearly steps, providing a very direct way of describing population dynamics for highly seasonal plants. The total population growth rate λ can be calculated as the dominant eigenvalue of $\mathbf{B}$. Figure 1 shows the life-cycle structure of scentless chamomile and the stage transitions as a graph model. The corresponding projection matrix is defined by:

$$\mathbf{B} = \begin{bmatrix} \sigma_1\sigma_2 & 0 & RF_1 \\ G_2 & 0 & RF_2 \\ hG_1 & H\sigma_3 & hRF_3 \end{bmatrix}$$

We constructed similar models for the biological control agents. For the seed weevil, for example, because the weevil has only one generation per year, the beetle dynamics is modelled by:

$$w_{t+1} = rw_t$$

where w_t is the density of adult weevils at time t, and r is the per capita growth rate. The link to the population model for the host weed is given by r being an increasing function with respect to flower density. That is, when flower density is high, the weevil population growth rate will be maximal. The seed weevil effect in matrix $\mathbf{B}$ is given by making R, the number of seeds per flowering head, a decreasing function of weevil density. The dynamics of the gall midge are included in the model in a similar way.

Thus, interactions between weed and biocontrol agent are represented by terms in the matrix models. For instance, scentless chamomile seed production will be reduced by an amount dependent on the local popu-

Table 1. Parameters for the matrix population model for scentless chamomile.

Parameter	Meaning
R	Seeds per flowering head
s	$\sigma = \sigma_1\sigma_2$, survivorship of seeds from fall to fall
σ_1	Survivorship of seeds from fall to spring
σ_2	Survivorship of seeds from spring to fall
σ_3	Survivorship of rosettes from summer to spring
G_1	Germination fraction seed to flowers (survivorship to summer)
G_2	Germination fraction seed to rosettes (survivorship to fall)
h	Flowering heads per germinated seed
H	Flowering heads per rosette
F_1	Fraction of produced seeds that go to the seed bank
F_2	Fraction of produced seeds that germinate as rosettes
F_3	Fraction of produced seeds that germinate as flowering plants

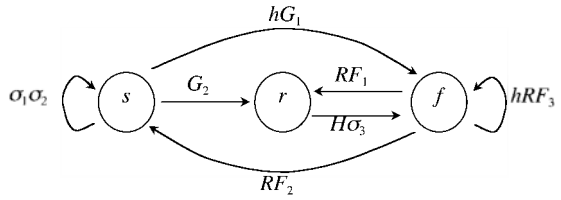

Figure 1. Scentless chamomile stage structure. s ~ seeds in the seed bank, r ~ rosettes and f ~ flowering heads (plants). Transition parameters are described in Table 1.

lation of the seed weevil *O. hookeri*. Conversely, populations of the biological control agents depend on the availability of the required host-plant resources, such as seed heads for *O. hookeri* or rosettes for the overwintering generation of *R. tripleurospermi*.

To place the matrix models in a spatial context, we used a coupled map lattice (CML). A CML is a discrete time and space model where local populations of scentless chamomile are modelled using Equation 1 as cells in a square array, and linked by a dispersal process that moves part of the population in each generation from one cell at y to adjacent or nearby cells at x. There are two important processes occurring, dispersal and demography. The CML model is described by:

$$\mathbf{n}_{t+1}(x) = \mathbf{c}(x) \circ \sum [\mathbf{K}(x,y) \circ \mathbf{B}(y,\mathbf{n}_t(y))]\mathbf{n}_t(y)$$

where **K** is the dispersal matrix, describing the probability of dispersing from y to x; the vector **n** and matrix **B** are the stage vector and projection matrix as in Equation 1, where the matrix may now depend upon location y and have a density-dependence through **n**; and the vector $\mathbf{c}(x)$ represents growth constraints in a given location x. The array of vectors **c** can be linked directly to landscape information using land use/cover maps. The symbol ◦ indicates component-wise multiplication of two matrices or two vectors.

The coupled map lattice model has been implemented as a Windows stand-alone computer program (Fig. 1). This implementation allows a user to specify:

1. an initial spatial distribution for a weed and a biocontrol agent
2. the parameters of the matrix models describing their population growth and interactions
3. their dispersal kernels
4. an underlying landscape layer representing spatial variation in habitat suitability for the weed.

For a desired number of iterations, the program then calculates the populations of the weed and the biocontrol agent in each cell from the matrix model and the dispersal function, and produces a graphical display showing the development of population density of each species over time and space. Sample output for scentless chamomile spread through a hypothetical landscape in the absence of biological control agents is shown in Figure 2.

As a final step, we plan to build an equivalent cellular automaton (CA) model to study the spatial dynamics and pattern in a more general way to derive some rules-of-thumb for decision-making. A cellular automaton is a lattice of cells, each of which can be in a finite number of states, and which evolves in discrete time steps. A uniform set of rules governs the evolution of each cell, based on its current state and that of the cells in its neighbourhood. The state of each cell would represent the populations of the weed and the biological control agents. CA models are computationally simpler than CML models because of the finite number of possible states for each cell, but can reproduce the essential behaviour of the corresponding CML model. CA implementations may thus be more practicable for running scenarios designed to provide guidance on management-related questions such as the optimum size, spacing or location of biological control agent releases.

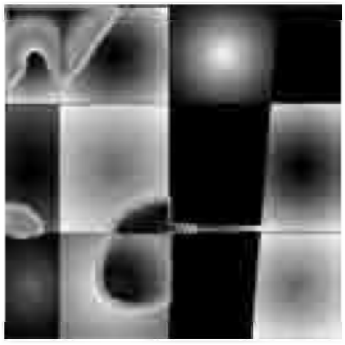

Figure 2. Coupled map lattice model output, illustrating dispersal of scentless chamomile on a hypothetical landscape. The grey levels indicate growing constraints imposed by the landscape. Black represents no growth, and white unrestricted growth. Dotted lines outline areas invaded by scentless chamomile. The simulation was run over a period of 3 years, with a pixel size of 900 m^2.

Data sources

Some of the data to needed to parameterise the matrix and CML models for scentless chamomile and its biological control agents can be estimated from the literature or from previous studies that we have undertaken. A population model for scentless chamomile was developed by Buckley *et al.* (2001) based on the data of Hinz (1999), but this model considers only the winter annual life cycle. Seed predation rates for *O. hookeri* were estimated by McClay *et al.* (1999) at approximately 11 seeds destroyed per weevil completing development. Some field evaluations of the impact of *R. tripleurospermi* have suggested that it has less effect on scentless chamomile than was originally expected, due to compensatory regrowth of the plant (A. McClay, unpublished data). These studies, however, were carried out in the absence of significant competition from other vegetation. Further evaluation should focus on the effects of *R. tripleurospermi* on the performance of scentless chamomile growing in competition with other species. Dispersal rates for *O. hookeri* and *R. tripleurospermi* can be estimated from survey data obtained from the original releases of these species at Vegreville, Alberta. *Omphalapion hookeri* was first released there in 1993 and is currently spreading at a rate of about 2.8 km year^{-1}, while *R. tripleurospermi* was first released in 1999 and is spreading at around 5.2 km year^{-1} (A. McClay, unpublished data). Some spatial distribution data for scentless chamomile are available from field surveys conducted in Saskatchewan (G. Bowes, Saskatchewan Agriculture and Food, personal communication).

Further field and experimental studies are planned to refine the parameter estimates for the scentless chamomile matrix model, evaluate the individual-level impact of the biological control agents on scentless chamomile, and characterize the spatial distribution and temporal persistence of scentless chamomile habitats in infested areas of Alberta.

Results

Figure 2 shows a preliminary result of a simulation of the spread of scentless chamomile under a hypothetical landscape. Parameters for the matrix model are taken from Hinz (1999), and an additional density-dependent seed productivity function is used by fitting a negative exponential function to Hinz's density-dependent experiments. In the simulations, stochastic long-distance dispersal is included.

Discussion

For a perennial weed occupying stable habitats, we expect that individuals and populations of the target weed persist in one area long enough for numerous successive generations of the biological control agent to develop and have a cumulative impact on the weed. In these situations, it may be possible to understand the process of biological control by studying the development of agent populations and their effects on host plant damage, survival, and demography on a local scale. In contrast, a pioneer species like scentless chamomile forms a "moving target", where the appearance and decline of new host patches, and the dispersal processes of both the agents and the weed, must play a major role in their interactions. It is possible that effective use of classical biological control in such a system may require more management involvement, such as periodic re-releases of agents, than is needed in a more "typical" perennial system. We believe that a landscape-scale approach to modelling the interactions between scentless chamomile and its biological control agents, incorporating the kinds of environmental heterogeneity seen in the field, will be a useful tool in understanding the processes involved in biological control of this weed. Such a model may help in evaluating the success of control, indicating data requirements for impact evaluation, guiding management strategies such as selecting the best spacing, distribution, or size of releases of agents, and in selecting further biological control agents for study if it should be determined that more agents are needed.

The models and the computer implementation we are developing can be applied to any weed biocontrol situation where the necessary data are available to describe population processes as a stage-structured model, dispersal as a dispersal kernel, and habitat suitability as a geographical information system (GIS) layer. It may thus be useful in understanding the spatial aspects of weed biocontrol in general and in guiding the development of optimal strategies for its use.

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First results for control of saltcedar (*Tamarix* spp.) in the open field in the western United States

C. Jack DeLoach,[1] Raymond I. Carruthers,[2] Tom L. Dudley,[3] Debra Eberts,[4] David J. Kazmer,[5] Allen E. Knutson,[6] Daniel W. Bean,[7] Jeff Knight,[8] Phil A. Lewis,[9] Lindsey R. Milbrath,[1] James L. Tracy,[1] Nada Tomic-Carruthers,[2] John C. Herr,[2] Gregory Abbott,[10] Sam Prestwich,[10] Glenn Harruff,[11] James H. Everitt,[12] David C. Thompson,[13] Ivan Mityaev,[14] Roman Jashenko,[14] Baoping Li,[15] Rouhollah Sobhian,[16] Alan Kirk,[16] Thomas O. Robbins[1] and Ernest S. Delfosse[17]

Summary

Saltcedars (Tamaricaceae: Tamaricales) are among the most devastating exotic weeds ever to invade western United States riparian ecosystems. The Agricultural Research Service (ARS) began biological-control research in 1986 at Temple, Texas, and in 1998 at Albany, California. Many prospective control insects are reported in the homeland of saltcedar in Eurasia. A leaf beetle, *Diorhabda elongata* Brullé *deserticola* Chen from Fukang, China, and Chilik, Kazakhstan, was released into field cages at 10 sites in Texas, Colorado, Wyoming, Utah, Nevada and California during 1999 and 2000, and into the open field at 7 of these sites in May 2001. It successfully overwintered at five sites north of the 38th parallel in four states, but not in Texas or southern California, presumably because daylength is too short at the southern sites. During the summer of 2002, we observed dramatic defoliation of saltcedar at Lovelock, Nevada; good defoliation at Pueblo, Colorado; and substantial population increases, but not defoliation at Lovell, Wyoming, and Delta, Utah. *Diorhabda* beetles from Turpan (China), Greece, Uzbekistan and Tunisia are active at shorter daylengths and are promising for control in the more southern areas. Predators (ants and birds) have reduced populations at Lovell and Delta, and at Bishop, California, and the control of insects with predator protective behaviours, such as gall formers, may be required in those areas.

Keywords: biological control weeds, *Frankenia,* riparian ecosystems, saltcedar, *Tamarix*.

[1] USDA–ARS, 808 E. Blackland Road, Temple, TX 76502, USA.
[2] USDA–ARS, 800 Buchanan St., Albany, CA 94710, USA.
[3] University of Nevada, Department Environmental & Resource Science, Reno, NV 89512, USA.
[4] US Department of Interior, Bureau of Reclamation, Ecological Research & Investigations Group, Denver, CO 80225, USA.
[5] USDA–ARS, 1500 North Central Ave., Sidney, MT 59270, USA.
[6] Texas A&M University Research & Extension Center, 17360 Coit Road, Dallas, TX 75252, USA.
[7] Department of Vegetable Crops, University of California at Davis, Albany, CA 94710, USA.
[8] State of Nevada, Department of Agriculture, Division of Plant Industry, Reno, NV 89502, USA.
[9] USDA–APHIS, Otis Plant Protection Laboratory, Building 138, Otis ANGB, MA 02542, USA.
[10] USDA–APHIS, 65 South 100 East, Richfield, UT 84701, USA.
[11] USDA–APHIS, 1220 Cole Ave., Helena, MT 59601, USA.
[12] Integrated Farming & Natural Resources Research Unit, USDA–ARS, Weslaco, TX 78596., USA
[13] Dept of Entomology, Plant Pathology & Weed Science, New Mexico State University, Las Cruces, NM 87003, USA.
[14] Entomological Laboratory, Institute of Zoology, National Academy of Sciences of Republic of Kazakhstan & Tethys Scientific Society, Akademgorodok, Almaty C 480 032, Kazakhstan.
[15] Department of Entomology, Nanjing Agricultural University, Weigang, Nanjing, Jiangsu 210095, China.
[16] USDA–ARS, European Biological Control Laboratory, Montferrier-sur-Lez, France.
[17] USDA–ARS, National Program Staff, Beltsville, MD 20705-2350, USA.

Corresponding author: C. Jack DeLoach <jdeloach@spa.ars.usda.gov>.

Introduction

The invasion of riparian ecosystems by exotic saltcedars (*Tamarix* spp.), deciduous shrubs or small trees from the Old World, has caused one of the worst ecological disasters in the history of the western United States. Of the 54 species worldwide (Baum 1978), 10 have been introduced into the United States (Crins 1989). Recent DNA analyses (Gaskin & Schaal 2002) indicate that *T. ramosissima* Ledeb. and *T. chinensis* Lour. and their hybrids are the most widespread and damaging species in the western US, along with *T. parviflora* DC. in California, and *T. canariensis* Willd. and hybrids in some south-western areas. Another species, the large, evergreen, cold-intolerant tree *T. aphylla* (L.) Karst., athel, is a somewhat beneficial shade tree in the desert south-west and hosts different insects in the Old World. Athel is not a candidate for biological control although it is becoming weedy in some areas. Saltcedars displace native riparian plant communities, degrade wildlife habitat (including that of many declining or endangered species), use great quantities of scarce ground water, increase soil salinity and wildfire frequency, and interfere with recreational usage of natural areas. These invasive shrubs increase bank aggradation, narrow and deepen stream channels, and alter water temperature and quality. Saltcedars damage the habitat of many aquatic invertebrates, fish, and riparian animals by eliminating backwaters and open sand and gravel bars, and by changing riffle and bank structure. Native insects and other animals did not evolve with saltcedar and are unable to utilize it as a food resource, except that many pollinating insects (all produced on native plants) visit its flowers (reviewed by DeLoach & Tracy 1997, DeLoach *et al.* 2000).

Saltcedars have many characteristics that enable them to invade and occupy these riparian areas. They produce copious quantities of small windblown or waterborne seeds throughout the growing season and they also can reproduce vegetatively (Everitt 1980). They are deep-rooted, facultative phreatophytes that can utilize either ground water or soil moisture. Thus, they can occupy areas further from the streambanks and use more water across a floodplain than can the shallow-rooted native phreatophytes (Smith *et al.* 1998). Saltcedars are facultative halophytes that can utilize saline groundwater by excreting excess salts through leaf glands. They are tolerant of fire, drought, inundation, livestock or wildlife browsing, and native insects do not damage them. They are tolerant of mechanical controls, and readily resprout from underground stem buds after damage. Saltcedars also interact synergistically with many of the recent human produced ecosystem changes to increase their competitive advantages over native plants (reviewed by DeLoach 1991, DeLoach *et al.* 2000). Saltcedar also has some beneficial values, mostly for controlling streambank erosion (for which it was introduced), a lesser value as an ornamental shrub, and as a maintenance plant for honeybees. Some birds, including the endangered south-western subspecies of the willow flycatcher (*Empidonax trailii* Audubon *extimus* Phillips) discussed below, the white-winged dove (*Zenaida asiatica* (L.)), and other animals use it for cover or to feed on the numerous pollinating insects.

The negative aspects of the saltcedar invasion have alarmed many environmentalists, water users, ranchers, park and wildlife managers and recreationalists, who demand its control. The United States Department of the Interior (USDI) Fish and Wildlife Service (FWS) has become aware of the damage caused by saltcedar to its National Wildlife Refuges and more recently also to many endangered species (including the south-western willow flycatcher) and now also supports biological control. In-depth risk analyses (DeLoach 1991, DeLoach & Tracy 1997, DeLoach *et al.* 2000), including economic analyses (Brown 1989, Zavaleta 2000) have demonstrated that the damage caused by saltcedar far outweighs its few beneficial values. The critical nature of the recent drought in the US south-west has threatened the water supplies of municipalities and of irrigated agriculture, and caused default of water agreements between states and of the water treaty between the United States and Mexico. This has now engendered even more political support for biological control of this invasive plant.

The biological control program, conflicts, and clearances

The low beneficial values of saltcedar, its lack of closely related plants in the Western Hemisphere, and the large number of host-specific and damaging insects that attack it within its native distribution in the Old World, make saltcedar an almost "ideal" weed for biological control. Surveys for natural enemies have been made in Italy, Israel, Iran, India, Pakistan, and Turkey. These searches, together with extensive ecosystem studies in the former Soviet Union and by some of us in China, have revealed over 300 highly specific and damaging insect species as potential biological-control agents. Research began at Temple, Texas, in 1986, with a thorough review of the literature and risk analysis. Overseas testing of control agents then were begun by some of us (Mityaev, Jashenko, Li, Sobhian and Kirk) and testing in quarantine began at Temple in 1992 (see DeLoach *et al.* 1996) and in Albany, California, in 1998 (see Lewis *et al.* 2003a). In March 1994, we submitted a petition to the US Department of Agriculture Animal and Plant Health Inspection Service (APHIS) Technical Advisory Group on Biological Control of Weeds (TAG) asking their recommendation for release of the leaf beetle, *Diorhabda elongata* Brullé, from China and Kazakhstan, into the open field.

However, the listing of the south-western willow flycatcher as federally endangered in February 1995 required consultation with FWS and the preparation of a

biological assessment, which we submitted to FWS Region 2 (Albuquerque, New Mexico) in October 1997. This analysis revealed that the flycatcher utilized saltcedar extensively for nesting habitat in some areas of Arizona but little in other areas, and that other potentially harmful effects of saltcedar reduced reproductive success of the flycatcher to half of that in its native willow habitat (DeLoach & Tracy 1997, DeLoach *et al.* 2000). We then submitted a research proposal to FWS on 28 August 1998. It specified a research phase in which; 1) *D. elongata* could be released into secure field cages at 10 specified sites in different climatic zones in Texas, Colorado, Wyoming, Utah, Nevada and California, all more than 320 km from where the southwestern willow flycatcher nests in saltcedar. The beetles were to be carefully monitored in the cages for one year to determine their overwintering ability, mortality factors, rate of increase, and damage to saltcedar and non-target plants in the cages, and, 2) the beetles then could be released into the open field for a 2-year period, during which the degree and rapidity of control, rate of natural dispersal, and effects on native plant and wildlife communities would be monitored. After this 3-year research period, FWS, ARS and APHIS would review the research results and determine the conditions under which the implementation phase could be carried out. A Letter of Concurrence was issued by FWS on 28 December 1998 (revised 3 June 1999) and an environmental assessment was prepared by USDA–APHIS in February 1999. APHIS issued a Finding of No Significant Impact (FONSI) on 7 July and permits to release in field cages during July 1999.

Meanwhile, the Saltcedar Biological Control Consortium was organized by one of us (DeLoach) in December 1997 to provide coordination between agencies and input, guidance and oversight in the research program from user and environmental organizations. It has met annually since then and now has representatives from some 50 federal and state agencies, universities, and private user and environmental groups (reviewed by Stenquist 2000).

Biology of *Diorhabda elongata deserticola*

The biology of *D. elongata* subspecies *deserticola* Chen from both Fukang, Xinjiang Autonomous Region, China and from Chilik (120 km east-north-east of Almaty), Kazakhstan (Fig. 1A-E) was determined by us in Kazakhstan (Mityaev and Jashenko 1999–2002), in China (Li and Ming 2001–2002), at Temple and Albany, and at the various release sites in the US. Both adults and larvae feed on the foliage of saltcedar and the large larvae also de-bark small twigs causing the distal foliage to die. The adults overwinter and the larvae pupate under litter beneath the trees. In the laboratory, an average female oviposited 194 eggs over a 12-day period. Lewis *et al.* (2003b) measured the duration of each life stage, calculated the optimal net reproductive rate (R_o) of 88.2 times per generation (T) of 39.9 days, and the rate of increase, showing that the population can double each 6.2 days. Field cage studies show a range of population increases by location but a 30-fold increase per generation is not uncommon. The synchronization of the life stages with the normal spring floods may enable the beetle adults and pupae to avoid most flooding mortality while on the soil surface. In Colorado and Wyoming, overwintered adults become active in late April to early May and start ovipositing in early to late May. First-generation larvae are present from mid-May through June and the first-generation adults appear in late June to mid-July. In areas where the daylength is sufficient, the first-generation adults reproduce and the second-generation adults appear from mid August through September. The second-generation adults feed for a while but rarely oviposit, and then overwinter. Heavy population densities, especially of large larvae, can produce severe defoliation in either generation. In the more southern areas, the saltcedar growing season appears to be long enough to allow completion of three, or possibly four, generations.

Host range

Tests conducted at Temple during 1992 and 1993 indicated little survival by larvae or oviposition by adults on any but *Tamarix* plants (DeLoach *et al.* 2003). However, additional testing at Albany during late 1999 indicated substantially more feeding on *Frankenia salina* (Molina) I.M. Johnston than previously had been found on *F. jamesii* Torrey and the endangered *F. johnstonii* Correll (family Frankeniaceae: order Tamaricales) during the 1992–1993 tests at Temple (DeLoach *et al.* 2003). Extensive additional testing of *Frankenia* then was conducted during 2000 at Albany in the laboratory and greenhouse and at Temple in the laboratory (larvae) and in 3 × 3 × 2 m outdoor cages (Lewis *et al.* 2003a), thus postponing open field releases for a year. These tests revealed that larvae of *D. e. deserticola* in the laboratory and greenhouse could develop on *Frankenia* but only at about one-third to one-half the rate as on *Tamarix*. However, multiple-choice tests of adult host-plant selection, between three *Tamarix* (two in each test) and three *Frankenia* plants, and using 95 to 150 adults in the large, outdoor cages under near natural conditions at Temple, demonstrated that adults only rarely selected *Frankenia* for alighting/resting or for oviposition (Lewis *et al.* 2003a).

At Temple, we omitted the *Tamarix* plants in one test in the large outdoor cages, leaving only the three *Frankenia* species and two non-host replacements. This did not increase beetle selection for *Frankenia* and the females laid most of their eggs on the cage walls. In another test in three small (56 × 67 × 122 cm) outdoor cages with the original six plant species, the proportion of adults or eggs on *Frankenia* relative to *Tamarix*

Figure 1. *Diorhabda elongata deserticola* – beetles and damage to *Tamarix*: A) top to bottom – adult female, 1st instar, 3rd instar, adult male; B) adult male; C) 1st and 2nd instar; D) 3rd instar; E) egg mass; F–H) damage to *Tamarix* at Lovelock, Nevada, 2002, second season after release: F) 3rd instars feeding, 13 August; G) damage on 28 August; H) aerial view of damage (brown area) on 9 September; I) damage at Pueblo, Colorado, 1 August 2002.

Figure 2. Saltcedar stand defoliated by *Diorhabda elongata* at Lovelock, Nevada, 28 August 2003, at the end of the third growing season after initial release: 77 ha of tree canopy defoliated within a 190 ha stand of defoliated trees, much of that outside the area of this photograph. All brown shrubs are defoliated saltcedar, green plants are not saltcedar.

plants did not increase. Finally, *D. e. deserticola* beetles from Kazakhstan were tested in a multiple-choice test in the greenhouse at Temple, using 10 test plants (four *Tamarix* and three *Frankenia*, *Salix*, *Atriplex* and *Plumbago*). Host selection for oviposition was not different from the previous tests with the Fukang beetles. These tests demonstrated that *D. e. deserticola* from both Fukang, China and from Chilik, Kazakhstan are safe to release in the field. We expect some feeding and reproduction on *T. aphylla* (athel) but not noticeable damage to this plant. Potted plants of *Frankenia jamesii* in the field cages at Pueblo and of *F. salina* at Bishop were only slightly nibbled by the hundreds of starving adults that flew about in the cage and larvae that fell on the plants during peak populations (Lewis *et al.* 2003a). On *Frankenia*, we expect only occasional attraction to or feeding and oviposition on the plants if they grow adjacent to *Tamarix*. Although possible, we do not expect the beetles to develop self-sustaining populations on *Frankenia*, nor do we expect *Frankenia* to be a sustaining host plant in nature. In parallel with all beetle releases, monitoring of *Frankenia* will be conducted for several years to assess any possible impacts.

Experimental releases and results in field cages: July 1999 to May 2001

We placed *D. e. deserticola* from Fukang into field cages during July and August 1999 at seven sites: on a privately owned ranch near Seymour, Texas; on Bureau of Reclamation land near Pueblo, Colorado; on National Park Service lands near Lovell, Wyoming; on Paiute Indian tribal lands near Schurz, Nevada; on a privately owned farm near Lovelock, Nevada; on Los Angeles County Water District lands near Bishop, California; and on Hunter-Liggett Military Base, near Lockwood, California. Beetles from Chilik were placed in cages on Bureau of Land Management land near Delta, Utah. During the spring of 2000, beetles from Fukang also were placed in cages at Stillwater National Wildlife Refuge (NWR) near Fallon, Nevada and on private land at Cache Creek near Woodland, California. These beetles successfully overwintered in the cages at the eight most northern sites, although only weakly so at Stillwater and Cache Creek. They failed to overwinter at the two most southern sites, at Seymour and Hunter-Liggett. At the six sites where strong overwintering occurred (Pueblo, Lovell, Delta, Lovelock, Schurz and Bishop), the beetles increased to large numbers during the summer and completely defoliated the plants inside the cages during both 1999 and 2000. The two generations of larvae during June and August produced the most damage to saltcedar, such that additional cages had to be established over fresh plants where some beetles were transferred to preserve the culture. After the failure to overwinter at Seymour, the beetles were replaced in the cages in April and May of 2000 and 2001. These beetles reproduced well and the larvae defoliated the plants during June. The first-generation adults emerged in late June but failed to reproduce, entered diapause in early July, and failed to overwinter.

During the summer of 2000, field observations and experiments in the field cages and laboratory at Temple, Dallas, and other field-cage locations, indicated that the most probable cause of the failure to overwinter at Seymour and Hunter-Liggett was the short summer daylengths. Daylength near the origin of these beetles at Fukang (44°17'N) and Chilik (43°33' N latitude) attains a maximum of 15 hours 30 min. Maximum daylength at Seymour (33°35'N) is only 14 hours 21 min and at Temple (31°10'N) is only 14 hours 10 min. Non-ovipositing beetles from the field cages at Temple began ovipositing after 7–10 days when moved to a 16:8 hours (light:dark) photoperiod in the laboratory. Conversely, ovipositing beetles in the laboratory ceased ovipositing after 5–7 days when moved to the field cages. Meanwhile, one of us (Bean) at Albany found, in intensive laboratory studies, that *D. e. deserticola* from Fukang required at least 14 hours 45 min daylength to avoid entering overwintering diapause. Since the beetles in Texas began diapausing in early July, and fall and winter temperatures often are mild, the beetles probably exhausted their fat reserves and starved before saltcedar foliage appeared 8 months later in mid-March (Lewis *et al.* 2003b).

Experimental releases and results in the open field: May 2001 to early spring 2003

The results of the releases into field cages and of the additional testing of the Fukang biotype of *D. e. deserticola* were submitted in a petition to TAG on 25 August 2001 requesting releases into the open field, as allowed by the research proposal of 28 August 1998. TAG recommended approval, FWS concurred, APHIS issued permits, and we made releases of 400 adults at each site, into the open field during May 2001, adjacent to the field cages at the six sites where the beetles had overwintered. However, at Seymour we replaced them in the cages using additional adults sent from Pueblo. Beetles from usually two cages at each site were released at that site – Fukang beetles at all sites except at Delta, Utah, which were Chilik beetles.

At most sites, 20 beetles of mixed sexes were placed in each of 10 sleeve bags over terminal branches outside the cages for 1–2 weeks until they had begun ovipositing, and then the bags were removed. This allowed us to follow development and mortality by knowing where and how many eggs were present. The remaining beetles (*ca.* 400 at each site) were released into the open, except that we retained a small colony in the cages in case the released beetles did not establish and additional releases were needed. Additional

releases were made during the remainder of the year as excess beetles were produced in the cages. Altogether, we released approximately 27,000 adults and larvae at Lovell, Wyoming (six nursery cages had been established there); 6900 adults plus many larvae at Pueblo, Colorado; 15,000 at Delta, Utah (from nine cages); 3500 at Schurz, Nevada; 1650 at Lovelock, Nevada; 4400 larvae and 2000 adults at Bishop, California; and 498 adults at Seymour, Texas. Only low numbers were produced in cages at Cache Creek and Hunter-Liggett, California, and at Stillwater NWR, Nevada, and none were released there, as we felt it best to understand the reasons for these failures and to address them with more appropriate actions or to release other control agents if necessary.

Weekly monitoring of the released beetles indicated variable results at the different sites. At most sites, a few to moderate numbers of eggs, larvae and adults were found throughout the remainder of the summer of 2001, until late August or early September, when no more were found and we assumed they had entered overwintering diapause. A few overwintering beetles were found in the litter beneath the saltcedar trees at some locations. Only small feeding damage was seen at most locations. The most damage was at Pueblo, where the beetles defoliated *ca.* two-thirds of a rather large tree to which they had flown, *ca.* 10 m from the tree on which they had been released.

Similar densities of beetles were found during the spring and early summer of 2002, although they had dispersed over a wider area of *ca.* 50 to 100 m in radius from the release point. Then, when larvae of the second generation reached the third instar in mid-August, we saw extensive damage at some sites. The most spectacular damage was at the Lovelock, Nevada, release site (Fig. 1F, G & H). This site is located in a very large area of monotypic saltcedar in the floodplain of the Humboldt Sink. Essentially the only other vegetation present was a moderate stand of saltgrass growing between and underneath the dense stand of saltcedar trees. Large populations of third-instar and some second-instar larvae were found by one of us (Knight) during the site monitoring on 13 August that were rapidly defoliating the trees. On 28 August, the larvae had almost completely defoliated all trees within an area 100 m in diameter (*ca.* 0.8 ha), centred at the release cage. Although the larvae had eaten perhaps 95–98% of the foliage, the remainder was dead and still hanging from the branches (more so at some other sites). Also, the beetles missed an occasional terminal. Heavy feeding but not defoliation had occurred in an additional concentric ring 50 m wide outside the defoliated area.

The second most severe damage was seen by one of us (Eberts) at Pueblo, Colorado (Fig. 1I). Nearly complete defoliation was seen on *ca.* 25 trees in the centre of the release area, with heavy feeding but not total defoliation out to 50 m from the release point. Fewer trees were attacked here than at Lovelock, but the saltcedar stand here is more open and disperse than at Lovelock.

At Lovell, Wyoming, two of us (Kazmer and Harruff) observed substantial feeding damage but no complete defoliation. Considering the very large numbers of beetles released here (*ca.* 27,000) the damage was less than expected. The most obvious reason was heavy predation by ants, which unfortunately were abundant at the release site or moved in after the releases were made.

At Delta, Utah, two of us (Abbott and Prestwich) found few beetles and no feeding damage at the release site. The centre release point here is in a *ca.* 100 m diameter area of drought/salinity stressed plants, mostly only 1–2 m high, but surrounded by larger, healthier plants at the outer edges of the 10 ha site, on low hills and along the Sevier River; the nursery cages were located across the river just beyond the 178 m radius of the intensive monitoring area. On 1 August 2002, we observed a large swarm of 800+ adult beetles flying about and mating among the larger trees across the river near the cages. By 22 August, these had produced some second and many third instar larvae. However, while we observed, a flock of 10–12 rufous sided towhees (*Pipilo erythrophthalmus*) descended upon and devoured most of the larvae.

At Bishop, California, two of us (Dudley and Carruthers) did not find noticeable damage to plants after release into the open, although defoliation inside the cages had been complete. Two factors seemed to reduce the effectiveness of the beetles. First, we observed some predation by ants and second, laboratory analysis of the beetles by Bean indicated that about half the adults entered diapause in early summer and probably did not survive the winter (also, daylength there was shortened by mountain shadows from the east and west).

A similar situation apparently occurred nearby at Schurz, Nevada. Here also, we (Knight) found that the beetles had increased well in the cages, defoliated the plants, and overwintered in the cages. However, in the field they reproduced little, if any, and apparently failed to overwinter or to establish.

Beetles at Cache Creek, California, and Stillwater NWR reproduced poorly in the cages and were not released in the open. At Cache Creek, the beetles were intended to control *Tamarix parviflora*, which may be a somewhat less-acceptable host plant for them. Other reasons for the poor performance were not identified. At Hunter-Liggitt, no beetles were produced in the cages during the second year and none were released into the open. At Seymour, Texas, the beetles were replenished in the cages, reproduced well, and 498 first generation adults were released into the open on 13 and 26 June, but apparently did not produce a second generation and none were found the following year.

In summary, beetles released at sites north of the 38th parallel where daylength exceeds 14 hours 45 min at least into mid-August, and where predation from ants

or birds was not severe, reproduced well during the first two years in the open field and promise to provide good to excellent control of saltcedar. At some intermediate sites (Bishop, California, and Schurz, Nevada), beetles overwintered in the cages but could not overcome predation in the open field and could not establish. At sites south of the 37th parallel, where daylength did not reach 14 hours 45 min, the beetles failed to overwinter, did not become established, and promise no control.

However, these are only preliminary indications of control, obtained after only two years in the open field. We do not yet know the effect of one severe, late-season defoliation of the plants, the dispersal behaviour of the beetles after defoliation of a stand of saltcedar, or the effects over a period of more than two years. We do not know if the beetles will attack the same damaged plants during each beetle generation and either kill them or only kill part of them, if they will permanently suppress the canopy cover and density of the plants, or if they will let the plants escape to again produce dense stands. At our sites in Pueblo, Colorado, and Bishop, California, two years of severe defoliation in the field cages completely killed some large plants. We do not know if the beetles will be attacked by indigenous parasitoids, how widespread or intense ant or bird predation will be, whether predator populations will increase in response to this new food source, or whether the beetle's dispersal behaviour will evade predators.

Observations by two of us (Mityaev and Jashenko) at a similar severely defoliated site in Kazakhstan in 2001 indicated that severe dieback of most branches occurred, but that most plants resprouted from the base late in the season. At this site, the beetles pupated under the trees they had defoliated. The adults emerged, passed a few days on the defoliated plants, then flew *en masse* to an undamaged part of the stand and began feeding and reproducing there.

Short-daylength beetles discovered

We have discovered beetles with daylength requirements of less than 14 hours 45 min at lower latitudes in Crete and mainland Greece (by Carruthers and Tomic-Carruthers), in Tunisia (by Kirk and Sobhian), and in Turkmenistan (by Myartseva). In China, short-daylength beetles were found (by Li) at Turpan, at about the same latitude as the previously used Fukang and Chilik beetles, but at a much lower elevation, just below sea level. In laboratory tests at Albany (by Bean), these beetles did not enter diapause at 13 hours and some of them not even at 10 hours of light. This indicates that these beetles could establish south of the 38th parallel and perhaps throughout the southern range of saltcedar in the south-western US and northern Mexico. The Crete beetles, placed in a large outdoor cage at Temple during August 2002 and allowed free range inside the cage, emerged in substantial numbers (apparently with very low overwintering mortality), beginning just at bud break on 10 March and continued until mid-April 2003. By early April, they had begun ovipositing vigorously. The growing season in the southern areas is long enough to allow three or even four generations, with consequent increased damage to saltcedar.

Morphological studies by one of us (Tracy) at Temple indicate some differences between beetles we have collected from the above-mentioned areas and with museum specimens from the Mediterranean area to China. The results of these studies are as yet incomplete, but may require further taxonomic separation. All of these beetles, however, appear to be suitable for controlling saltcedar south of the 38th parallel, though perhaps each type only in certain daylength or climatic zones. Preliminary testing of the Crete beetles at Temple and Albany indicate that their host range is similar to that of the Fukang and Chilik beetles, except for somewhat more larval development on *F. salina*. The host range of each morphotype will be determined and approved by APHIS before they are released into open field sites.

The *Diorhabda* beetles alone may not provide satisfactory control in all areas. Especially, different types of control agents may be needed to avoid ant or bird predation. In the Old World, more than 300 species of insects are known to attack saltcedar. We have begun (or finished) testing *ca.* 20 insect species of several different types in France, Israel, Kazakhstan, and China. Several of these have characteristics to avoid predators. Except for a possible seed pathogen in France, no pathogens have been found attacking *Tamarix* within its native range.

Monitoring

The Monitoring Committee of the Saltcedar Biological Control Consortium has prepared detailed plans as required by the Research Proposal to FWS of 28 August 1998. Two years of baseline data have been compiled from the various release sites on the beetle populations, mortality factors, and effects on saltcedar and non-target plants; on the present vegetation composition; and on wildlife (bird species, butterflies, small rodents and bats). Also, differences in insect species, life stages, and abundance between saltcedar and native riparian trees and shrubs, is being measured by two of us (Knutson and Thompson). The monitoring is by far the most time-consuming and expensive part of the project but it is essential to understanding the effects of control on native ecosystems. Monitoring now is required by ARS in all biological control of weeds programs (Delfosse 2000) and was required by FWS in this program because of possible effects on endangered species. Previous and continuing research on remote sensing by one of us (Everitt) promises a good and less-expensive method of monitoring the degree and extent of control (Everitt & DeLoach 1990) and of the recovery of native riparian plant communities following control. Also, a large-scale revegetation

research project is under way by the Ecological Research and Investigations Group, USDI-Bureau of Reclamation, Denver, Colorado.

Postscript

By the end of the third growing season in late August 2003, the Fukang/Chilik biotype of *D. elongata* had begun a rapid and dramatic defoliation of saltcedar at five of the seven release sites north of the 38th parallel. At the best site (Lovelock, Nevada), the beetles had defoliated 0.8 ha of a dense stand of saltcedar in early September 2002 (Fig. 1H), which increase to 4.3 ha in early July 2003, and to 190 ha by early September 2003, along a 5 km reach of the Humboldt River (Fig. 2). By September 2003, several plants had resprouted profusely from the base and occasionally from the upper branches but enough beetles had remained in the stand to defoliate this regrowth. At Pueblo, the beetles were confined to one tree during 2001, had dispersed within a 100 m radius of the release point during 2002, and defoliated *ca.* 40 ha of saltcedar by September 2003. At Delta and Lovell, the beetles overcame bird and ant predation in 2002 to defoliate *ca.* 30 ha and 9 ha respectively by September 2003. At Schurz, the beetles apparently had dispersed beyond the monitoring area in 2002 and were not found but in 2003 they had defoliated *ca.* 15 ha along the Walker River. In the northern area, the beetles failed to establish only at Stillwater NWR, Nevada, and at Cache Creek and Bishop, California.

Additional host-specificity testing by three of us (Herr, Milbrath and Tracy) of the four new biotypes of *D. elongata* received from the Old World demonstrated that they also were safe to release. These were placed in field cages in the southern areas during the summer of 2003, the Crete beetles at five sites in Texas and at one site in New Mexico, and the Tunisian, Uzbekistan, and Turpan, China biotypes at one or two locations each in Texas. The Crete beetles were released into the open field at Seymour, Lake Thomas, and Big Spring, Texas and at Artesia, New Mexico and the Turpan beetles also were released at Seymour. The Crete beetle biotype also was released at Hunter-Liggett in September and at Cache Creek in October 2003. Little is known yet about the rate of kill of the plants but monitoring data from 2004 are expected to begin providing answers.

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Overcoming limits on rust epidemics in Australian infestations of European blackberry

Katherine J. Evans,[1] Louise Morin,[2] Eligio Bruzzese[3] and Richard T. Roush[4]

Summary

Spectacular success in biocontrol of some infestations of the *Rubus fruticosus* aggregate (European blackberry) has occurred since the introduction of *Phragmidium violaceum* to Australia in the early 1980s. When the weather is favourable for the development of rust disease, some biotypes of blackberry are defoliated whereas adjacent infestations remain almost disease free. In determining where biological control of the *R. fruticosus* agg. might be improved, both the weed host and rust pathogen were characterised. Twenty-six *Rubus* clones, comprising a representative sample of the *R. fruticosus* agg. in Australia, were identified by M13 DNA phenotyping and assayed in pathogenicity studies that revealed physiological specialization among different isolates of *P. violaceum*. The virulence and DNA phenotype of each isolate of *P. violaceum*, including 10 isolates imported recently to CSIRO's quarantine facility, are currently being determined using a differential set of 12 *Rubus* clones and AFLP-SAMPL analysis, respectively. The imminent release of additional and well-characterised strains of *P. violaceum* in the Australian environment should enhance the capacity of the rust population to attack this genetically diverse weed. Age-related disease resistance in leaves of *R. anglocandicans*, a widespread taxon of the *R. fruticosus* agg. in Australia, has also been quantified. Given a genetically susceptible blackberry and a virulent rust strain, disease severity will remain low if there is a large percentage of 'old' leaves in the blackberry canopy. Varying rates of shoot growth will contribute to spatiotemporal variation in the disease susceptibility of blackberry canopies. Defoliation of blackberry appears to depend on a continual source of urediniospores to ensure infection of emerging blackberry leaves during active shoot growth. The challenge now is (a) to quantify when pathogen development is synchronised with host growth and (b) to monitor and explain the fate of additional rust strains released in Australia, through an improved understanding of population genetics.

Keywords: biological control, blackberry, disease resistance, *Rubus*, rust.

Introduction

Rubus fruticosus L. agg. (European blackberry) is an aggregate of closely related taxa that have become naturalised in many parts of the world. It is considered an important weed in Australia, New Zealand, South Africa, North America and Chile (Amor *et al.* 1998). Various taxa of the *R. fruticosus* aggregate were introduced to Australia on multiple occasions; records begin in 1842 although earlier importation is likely (Parsons and Cuthbertson 1992). In Australia, taxa of the *R. fruticosus* agg. are vigorous, semideciduous shrubs with perennial roots and biennial stems. There are at least 14 polyploid agamospecies and one diploid, sexual species (*R. ulmifolius* Schott) of the *R. fruticosus* agg. in Australia (Evans

Cooperative Research Centre for Australian Weed Management,
[1] Tasmanian Institute of Agricultural Research, New Town Research Laboratories, 13 St Johns Avenue, New Town 7008, Australia.
[2] CSIRO Division of Entomology, GPO Box 1700, Canberra, ACT 2601, Australia.
[3] Department of Primary Industries, Keith Turnbull Research Institute, PO Box 48, Frankston, Victoria 3199, Australia.
[4] Statewide IPM Program, University of California, Davis CA 95616-8621, USA
Corresponding author: Katherine J. Evans, Tasmanian Institute of Agricultural Research, New Town Research Laboratories, 13 St Johns Avenue, New Town 7008, Australia <Kathy.Evans@dpiwe.tas.gov.au>.

et al. 1998, 1999; K.J. Evans *et al.* unpublished data), all of which are considered to be weeds of national significance (Thorp and Lynch 2000). *R. anglocandicans* A. Newton is the most widespread weedy taxon and various names have been misapplied to this agamospecies, including *R.* affin. *armeniacus*, *R. discolor* and *R. procerus* (Evans and Weber 2003).

In Europe and the Middle East, taxa of the *R. fruticosus* agg. are commonly infected by the rust fungus *Phragmidium violaceum* (Schultz) Winter. The history of introduction of *P. violaceum* to Australia is summarised by Evans *et al.* (2000). Briefly, *P. violaceum* was first reported in the state of Victoria in 1984 following an unauthorized release of unknown origin. Strain F15 of *P. violaceum*, from central France, was released as a biological control agent throughout southern Australia in the summers of 1991 and 1992. This macrocyclic and autoecious rust fungus (Laundon and Rainbow 1969) mainly infects leaves, but occasionally also the petioles, green floral parts, unripe fruits and green stems of the plant. All spore states can form under Australian conditions (Washington 1985).

P. violaceum has had a significant impact on some infestations of European blackberry (Mahr and Bruzzese 1998). Repeated defoliation over five to ten years reduces vegetative spread and allows light to penetrate the thicket, enabling seed from other plant species to grow up through the blackberry. However, *P. violaceum* has had little impact on large areas of European blackberry growing in Australia, with some biotypes escaping severe disease in regions where the weather is mostly favourable for the development of rust epidemics. In this paper, we outline the genetic and environmental factors that limit rust disease development in a given location. We summarise research, conducted by the Cooperative Research Centre for Australian Weed Management since 1996, that has refined our knowledge of these limiting factors and describe potential solutions for improving the biological control of European blackberry in Australia.

Materials and methods

Techniques for identification of M13/*Hae*III-DNA phenotypes of *Rubus* clones and strains of *P. violaceum* were described by Evans *et al.* (1998), Evans *et al.* (2000), and Evans and Weber (2003).

Pathogenicity assays were conducted by detached leaf disk assay (Evans *et al.* 1999) or whole plant assay (K.J. Evans *et al.* unpublished data) under controlled environment conditions. Each collection of blackberry comprised a crown of a single plant or crowns of a single vegetative (clonal) thicket. *Rubus* clones of known DNA phenotype were propagated by taking cuttings from collection plants maintained in a shadehouse at the University of Adelaide. The origin of selected *Rubus* clones is listed in Table 1 and collection details can be obtained from associated voucher specimens deposited at the State Herbarium of South Australia (AD).

For the whole plant assay, *Rubus* clones highlighted in Table 1 and others not reported here were micropropagated *in vitro* then hardened off in potting media in a controlled-environment growth room. When the main shoot had developed at least five fully expanded leaves, the abaxial surface of each compound leaf was inoculated with a fine mist of *P. violaceum* urediniospores, of single-pustule isolates F15, V1, V2 or SA1, suspended in water at a concentration of 0.25 mg/ml. Isolate F15 was collected in central France in 1978 and isolates V1, V2 and SA1 were collected in Australia between 1997 and 1999 (Evans *et al.* 2000). Urediniospore viability was assessed as the percentage spore germination on water agar. The inoculated plants were incubated in a dark dew chamber (Percival Scientific) at 20°C for 24 h prior to being transferred to a glasshouse bench (22–24°C, 14 h photoperiod) for expression of disease symptoms. Eleven days after inoculation, the total number of erumpent and nonerumpent uredinia were counted on the terminal leaflet of each compound leaf and the total number of uredinia per area of leaflet was calculated. Analysis of variance among *Rubus* clones was applied to the number of total uredinia per area of terminal leaflet for the leaf on the shoot that expressed the highest number of uredinia per area of terminal leaflet. The presence of purple flecks on the adaxial surface of the leaflet was also noted. Leaflets without uredinia or very low numbers of uredinia were transferred to Petri plates containing water agar, incubated at 20°C in growth chamber and assessed up to 10 days later to check for delayed symptom expression. *Rubus* clones were classified as resistant if the total number of uredinia per area of terminal leaflet (for the most susceptible leaf on the shoot) was very low and separated statistically from data for susceptible *Rubus* clones.

Results

Forty-nine M13/*Hae*III-DNA phenotypes were identified among 198 collections from the *R. fruticosus* agg. across Australia (Evans *et al.* 1998; K.J. Evans *et al.* unpublished data). Of the 49 M13 DNA phenotypes, 33 phenotypes were correlated to 13 taxa of the *R. fruticosus* agg. and one undetermined taxon (K.J. Evans *et al.* unpublished data). A further 16 DNA phenotypes were undetermined, based on morphology, or determined with only a moderate level of confidence. These undetermined DNA phenotypes are either new biotypes that have evolved in Australia, biotypes that have not yet been recognised and characterised in Europe, or biotypes that no longer exist in Europe.

Disease resistance in the *R. fruticosus* agg. and physiological specialisation among three Australian isolates of *P. violaceum* were identified in detached leaf disk and whole plant assays of 26 *Rubus* clones representing 17 DNA phenotypes and 14 taxa. M13 DNA typing of the rust strains used in these assays confirmed that genetically different rust strains were being tested

Table 1. The *Rubus* differential set comprises representative Australian *Rubus* clones, which have been grouped on the basis of their reaction to three strains of *Phragmidium violaceum* isolated in Australia between 1997 and 1999 (Evans *et al.* 2000) and the reference strain F15.

Rubus DNA phenotype	*Rubus* clone[a]	Origin in Victoria (except clone SR3)	Voucher specimen	*Rubus* taxon according to Evans *et al.* (unpublished data)[c]	*Rubus* taxon according to Amor and Miles (1974)	Host reaction to *P. violaceum* isolates[d]				
						F15	V1	V2	SA1	*Rubus* group[e]
29	EB14	English's Corner, Strzelecki Ranges	AD99811445	*R. cissburiensis* W.C. Barton & Ridd.	*R. cissburiensis*	S	S	R	R	3
25	EB20[b]	Lower Gellibrand	AD99811452/3	*R. erythrops* Edees & A. Newton	*R. rosaceus*	S	S	R	R	3
7	EB9[b]	Highway between Tallangatta and Corryong	AD99811450/1	*R. leucostachys* Schleich. ex Sm.	*R. ulmifolius* hybrids	S	S	R	R	3
6	EB19[b]	Cobden to Port Campbell Road	AD99811454/5	*R. leucostachys* Schleich. ex Sm.	*R. ulmifolius* hybrids	S	S	R	R	3
2	971702	Benambra	unavailable[f]	*R.* sp.	not determined	S	S	R	R	3
14	SR43[b]	Penwortham, South Australia	AD99750231	*R.* sp.	not determined	S	S	R	R	3
37	EB22	Lower Gellibrand	unavailable[f]	*R. laciniatus* Willd. (leaflets divided deeply)	*R. laciniatus*	R	S	S	S	2
37	KE1	Creswick	AD99809203	*R. laciniatus* Willd. (leaflets undivided)	*R. selmeri*	R	S	S	S	2
32	9607[b]	Somerville	unavailable[f]	*R. anglocandicans* A. Newton	*R. procerus*	S	S	S	S	1
21	972101	Foster	unavailable[f]	*R. leucostachys* Foster biotype	*R. ulmifolius* hybrids	S	S	S	S	1
36	961107[b]	Callignee	unavailable[f]	*R. polyanthemus* Lindeb.	*R. polyanthemus*	S	S	S	S	1
28	EB21[b]	Lower Gellibrand	AD99811443/4	*R. vestitus* Weihe	*R. vestitus*	S	S	S	S	1

[a] Code of plants propagated clonally in a shadehouse.
[b] *Rubus* clones assayed using whole plants under controlled environment conditions.
[c] Except for *R. anglocandicans* (Evans and Weber 2003), these names are applied by K.J. Evans, D.E. Symon, M.A. Whalen, J.A. Oliver, J.R. Hosking and R.M. Barker, unpublished data.
[d] R = resistant; S = susceptible.
[e] *Rubus* clones have been grouped according to host reaction to Australian strains F15, V1, V2 and SA1, based on results from assays of 26 *Rubus* clones, representing 17 DNA phenotypes and 14 taxa.
[f] A voucher specimen of a different *Rubus* clone with the same DNA phenotype can be found at the State Herbarium of South Australia (AD).

(Evans *et al.* 2000) The results of the whole plant assays, using selected *Rubus* clones, were correlated with results of the detached leaf assay reported previously. The whole plant assay provided a more realistic assessment of host reaction to a rust isolate, given that individual leaves continue to expand from inoculation to assessment. The *in vitro* propagation technique produced batches of healthy uniform plants, resulting in improved reproducibility of the assay when compared with the detached leaf disk assay. Using information from both types of pathogenicity assay we developed a differential set of *Rubus* clones for characterising the virulence phenotypes of strains of *P. violaceum* (Table 1).

Discussion

Phenotypic plasticity in the *R. fruticosus* agg. is high and DNA markers can identify *Rubus* clones with certainty, including those used in pathogenicity studies with strains of *P. violaceum*. *P. violaceum* strain V1, isolated from western Victoria, produced a susceptible disease response in all *Rubus* clones tested (Table 1) but many questions remain as to why some blackberry biotypes are escaping severe disease at some locations. It may be that a rust strain with the corresponding virulence does arrive on the "resistant" host biotype, but that it arrives too late (Burdon *et al.* 1996); this would delay the initiation of the epidemic and reduce disease levels at critical times in the growing season. Another explanation relates to the fact that blackberry plants exhibit leaf-age-related disease resistance (Evans and Bruzzese 2003), as described below.

Age-related disease resistance

Evans and Bruzzese (2003) have shown that resistance to rust disease increases as blackberry leaves age on a single shoot, following their initial expansion (Figure 1). Consequently, the disease response of a blackberry thicket that is susceptible to rust disease will depend on the age profile of leaves within the plant canopy. Two blackberry biotypes susceptible to a particular strain of *P. violaceum* and growing adjacent to each other may have different growth rates and/or cane densities (Amor 1975). Different growth characteristics result in blackberry canopies with different leaf age profiles and differences in the proportion of the canopy that is susceptible to disease at any given time. Indeed, *P. violaceum* strain V1 was isolated from *Rubus* clone EB19 growing adjacent to *Rubus* clone EB18 and clone EB19 appeared more severely diseased than clone EB18. Both of these *Rubus* clones were characterized as "susceptible" when inoculated with strain V1 under controlled-environment conditions (K.J. Evans unpublished data), which suggests that leaf-age-related disease resistance might have been the factor most limiting rust disease on *Rubus* clone EB18 *in situ*.

Climate and weather

Pigott *et al.* (2003) used GIS tools to develop a map of Victoria that predicts areas of 'no', 'low', 'medium low', 'medium high' or 'high' impact for rust disease. Of the total area that could potentially be infested by blackberry in Victoria (13.4 million hectares), 50% of this area is predicted to be of 'high' or 'medium high'

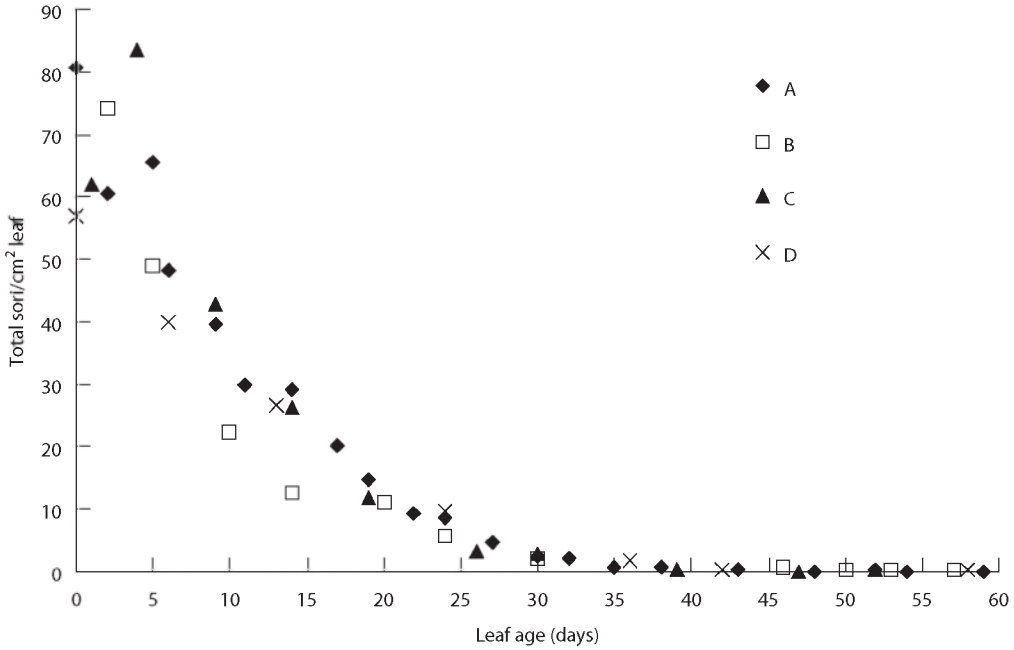

Figure 1. Effect of leaf age on total sori/cm^2 leaf (uredinia plus telia) sampled from four shoots, A, B, C or D, of a single plant of *R. anglocandicans* inoculated with isolate F7 of *P. violaceum* and incubated at 18°C for 20 days prior to assessment. The length of the terminal leaflet of '0 day' leaves at inoculation was at least 3 cm. Reproduced from Evans and Bruzzese (2003).

impact for rust disease. In short, rust disease was predicted to have the highest impact in localities of Victoria where the annual rainfall was greater than 750 mm, where summer rainfall occurred or relative humidity was high, and when the average maximum daily temperatures in January were close to 20°C.

The GIS 'rust impact' model was developed using disease data collected from *R. anglocandicans* only and requires further refinement and validation. However, it illustrates the type of knowledge that is required to make decisions about integrated weed management using biological control. For example, 'no' or 'low' disease impact sites will require other management measures. 'Medium' disease impact sites will require an integrated approach, whereby the biocontrol agent acts to contain the rapid spread of the weed while other control measures can be implemented less urgently when compared with a 'no' impact site. There is also the potential to convert a 'medium' disease impact site to a 'high' impact site by manipulating blackberry growth to reduce the age-profile of the thicket at key times during the growing season.

Temperature is a key environmental factor driving rust epidemics (Evans and Bruzzese 2003). Sub-optimal temperatures increase the generation time or latent period for urediniospores and reduce the rate of blackberry leaf emergence. The development of thermal time growth models for both the pathogen and plant host would improve predictions of where and when disease impact is likely to be greatest.

Conclusions and solutions

When a virulent rust strain is present, defoliation of blackberry appears to depend on a continual source of urediniospores to ensure infection of emerging blackberry leaves during active shoot growth. Age-related disease resistance of blackberry leaves, weather and climate interact to produce severe rust disease when host and pathogen growth is synchronized. Development of host–pathogen growth models will require further study of rust disease epidemiology and taxon-specific growth habits of blackberry.

Ensuring that a virulent rust strain is present at the right time and place cannot be predicted well without further characterisation of fine-scale genetic structure and gene flow of *P. violaceum* in Australia. Given this knowledge gap, we selected additional strains of *P. violaceum* in Europe with the objective of increasing the genetic diversity of *P. violaceum* when these strains are released in Australia. Eight of the ten isolates of *P. violaceum* imported in the CSIRO High Security Quarantine Facility in Canberra in 2002 were cultured and multiplied successfully. These isolates are being characterized for their host specificity (targeted test list), virulence pathotype (using the differential set) and DNA phenotype (M13 RFLP or AFLP-SAMPL, D. Gomez unpublished data).

Exotic *Rubus* spp. have had over 150 years to evolve in Australia, and it is conceivable that new biotypes may have arisen by hybridization or somatic mutation. In theory, the release of additional and genetically different strains of *P. violaceum* should enhance the capacity of the pathogen population to coevolve with its host in Australia, by evolution of new virulence phenotypes through mutation or recombination. Only clonal or recombinant pathotypes that are fit in the new environment, relative to the existing rust population, are likely to survive. The challenge now is to monitor and explain the fate of additional rust strains released in Australia through an improved understanding of the mechanisms of gene flow in *P. violaceum*.

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Interactions between the gorse seed weevil (*Exapion ulicis*) and the gorse pod moth (*Cydia succedana*) explored by insecticide exclusion in Canterbury, New Zealand

A. Hugh Gourlay, Trevor R. Partridge and Richard L. Hill[1]

Summary

The outstanding invasive ability of gorse is strongly related to its large and persistent seed bank. A study of gorse seed predation in England (Hill 1982) suggested that the seasonal abundance of *Cydia succedana* larvae in gorse pods peaked 3 weeks later than that of *Exapion ulicis* and the frequency of co-occurrence in pods was low. It was predicted from this study that the introduction of *C. succedana* to New Zealand in 1992 would not significantly displace *E. ulicis*, and that the combined seed predation by these two agents in spring would be complementary rather than strongly competitive.

To test the accuracy of this predicted outcome, the direct interactions between larvae of these two seed-feeding insects were examined using an insecticide exclusion experiment. Mimic® 70W removed *C. succedana*, but not *E. ulicis* from spring-produced gorse seedpods. The amount of seed attacked by *E. ulicis* in the absence of *C. succedana* was measured and compared to the percentage of spring seed attacked by both insects. The combined effects of the two agents was shown to be greater than either alone.

Keywords: biological control, Mavrik Aquaflow, Mimic 70W, suppression, *Ulex europaeus*.

Introduction

The weedy, leafless, spiny shrub, gorse (*Ulex europaeus* L.), was introduced into New Zealand early in the 19th century as a hedge plant (Bascand 1973, Gaynor & MacCarter 1981). However, it has spread into many other habitats, and today is a major weed of hill country (Blaschke *et al.* 1981, Bascand & Jowett 1982, Hill & Sandrey 1986).

Two seed-feeding biological control agents have been introduced to reduce the amount of seed produced and to slow the rate of spread of gorse. Gorse seed weevil (*Exapion ulicis* [Forster][(Coleoptera: Apionidae]) was imported from Europe and released between 1931 and 1946 (Davies 1928). It is now abundant and widespread (Miller 1970).

The bi-voltine (Emmett 1988) gorse pod moth (*Cydia succedana* (Dennis and Schiffermüller)) (Lepidoptera: Tortricidae) was imported from England in 1989 and released in the early 1990s (Harman *et al.* 1996). It is also now abundant and widespread (Hill & Gourlay 2002).

A study of gorse seed predation in England suggested that *C. succedana* would not significantly displace the gorse seed weevil, and that the combined seed predation by these two agents in spring would be complementary rather than strongly competitive (Hill 1982). Both agents are well established in the Malvern Hills of inland Canterbury in the South Island. A stand of gorse at Jimmy's Knob has been the subject of a two-year study on the interaction of the two agents on gorse seed production (T.R. Partridge, R.L. Hill & A.H. Gourlay, unpublished data). That study showed that both agents were active in spring, and together destroyed virtually all spring-produced seed. Seed

Landcare Research, PO Box 69, Lincoln, New Zealand
[1] Present address: Richard Hill and Associates Ltd, Private Bag 4704, Christchurch, New Zealand
Corresponding author: A.H. Gourlay, Landcare Research, PO Box 69, Lincoln 8152, New Zealand <gourlayh@landcareresearch.co.nz>.

production in autumn was attacked by *C. succedana* alone, and approximately 10% of this seed was destroyed.

There was considerable variation between individual gorse plants in the numbers of seeds being destroyed by each agent, so the nature of the interaction between agents was unknown. In order to examine that interaction more closely, an insecticide exclusion experiment was set up to explore how each agent behaved if the other was removed. The results of that study are reported here.

Methods

Sample sites

Blocks 2 and 3 of the four blocks of the gorse stand used in the original study (T.R. Partridge, R.L. Hill & A.H. Gourlay, unpublished data) were chosen for the insecticide study. Two sprays were chosen for the study. Mimic 70W (700 g L^{-1} tebufenozide) is a moulting-accelerator insecticide specific to Lepidopteran moths. This was applied to remove *C. succedana* but not *E. ulicis* from the spring seeding. The other spray chosen, Mavrik Aquaflow (240 g/litre of tau-fluvalinate), is a broad-spectrum contact synthetic pyrethroid.

Sixty mature, flowering gorse plants were chosen in summer and treatments were randomly assigned. Twenty plants were left unsprayed as controls, 20 plants were sprayed with Mimic 70W, and 20 were sprayed with Mavrik Aquaflow. Spraying commenced in August (early spring) and was continued at 3-weekly intervals until November. Each plant was sprayed to runoff with 1–2 L of field-rate spray mix.

Circular seed trays were placed beneath each selected gorse plant. Each tray was 24 cm in diameter and 11 cm tall. The base was made of shade cloth, allowing water, but not gorse seed, to pass through. The site was visited at 1–2-monthly intervals. During each visit, the flowering and seeding status of each plant was monitored, and the seeds in trays were counted and discarded. When a majority of pods on a plant had ripened (changing colour from green to black), a sample of up to 100 pods was collected from a plant within 1 m of, but not immediately above, the seed tray, and taken to the laboratory for dissection. The total number of seeds, number destroyed in pods attacked by *E. ulicis,* number destroyed by *C. succedana,* and number of seeds destroyed by both agents were recorded. Where damage in the pod was severe, the total was estimated from the number of attachment points. The proportion of seeds destroyed in each pod infested by *E. ulicis*, *C. succedana*, or by both agents, was calculated. From the number of seeds destroyed and the number of seeds falling, the proportion of the seed crop destroyed by each of the agents, or by both together, was calculated.

Results

Gorse flowered twice each year; once in spring (usually October to November) and once in autumn (February to March). The data presented below only represent the spring seed crop, as this was the only time that both insects were active.

Of the 20 unsprayed control plants, only seven produced sufficient seeds for sampling. Of the 20 plants sprayed with Mimic 70W, only eight produced sufficient seed (Table 1). This reduction in replication hampered statistical analysis. In the controls, *C. succedana* destroyed 62% of the seed, while 11% of the seed was destroyed in pods containing both insects. A total of 81% of spring seed was destroyed in the controls.

Mavrik Aquaflow was expected to remove both agents. The study showed that it could not do this, and the results are not presented here.

Mimic 70W significantly reduced the number of seeds destroyed by *C. succedana* ($T = 8.85$, d.f. = 12, $P < 0.01$). It was assumed that Mimic 70W did not affect *E. ulicis* behaviour. At the same time, the percentage of seeds destroyed by *E. ulicis* was significantly greater than in the controls ($T = 5.56$, d.f. = 12, $P < 0.01$). Overall, the total percentage of seeds destroyed by both species in pods sprayed with Mimic 70W was lower than in controls (64% and 81%, respectively), but this difference was not significant ($T = 2.05$, d.f. = 12, $P = 0.063$).

A two-way chi-squared test confirmed that the presence of either *E. ulicis* or *C. succedana* in a gorse seedpod resulted in the likely absence of the other, and that if both species occurred, then *C. succedana* was the more successful ($\chi^2 = 8.34$, d.f. = 1, $P < 0.01$).

Discussion

When *C. succedana* was removed, the proportion of seeds destroyed by *E. ulicis* increased significantly. This indicates that *C. succedana* suppressed weevil activity in unsprayed populations. Results showed a significant under-representation of pods containing both *E. ulicis* and *C. succedana*, and that *C. succedana* was over-represented. The relative behaviour of the two insects suggests that *C. succedana* consumes not only

Table 1 Mean percentage (±SE) of seed in pods destroyed by *Exapion ulicis* and *Cydia succedana*.

Treatment	*C. succedana* alone	*E. ulicis* alone	Both	Total seed destroyed
Mimic ($n = 8$)	4 ± 2	54 ± 5	6 ± 4	64 ± 5
Controls ($n = 7$)	62 ± 7	8 ± 5	11 ± 4	81 ± 6

the seeds within the pod, but may also consume any *E. ulicis* larvae inhabiting the pod. This would explain the over-representation of *C. succedana*, and is probably the mechanism for *E. ulicis* suppression.

Significantly increased seed predation by *E. ulicis* in the absence of *C. succedana* indicates that natural weevil populations will respond to, and compensate for, spatial and temporal variation in the abundance of the moth. Although the increased seed predation was not significant, there is a strong indication that despite the negative effect of the moth on the weevil, the combined effects of the two agents is greater than either alone.

Modelling by Rees & Hill (2001) suggests that with a moderate frequency of large-scale disturbance, and low seedling survival, a reduction in the annual seed crop of 75–85% would be sufficient to cause long-term decline in gorse cover. The results presented here suggest that *C. succedana* and *E. ulicis* are already achieving a reduction in the spring seed crop of this magnitude at this site. In places where autumn seed production contributes little to the annual seed crop, these two agents may already be contributing to a decline in gorse population density, though agent behaviour in these climatic conditions may not support the same levels of seed attack as experienced during our study. The two agents may also be slowing gorse spread into areas where it is not yet present.

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Habitat trade-offs in the summer and winter performance of the planthopper *Prokelisia marginata* introduced against the intertidal grass *Spartina alterniflora* in Willapa Bay, Washington

Fritzi S. Grevstad,[1] Robin W. Switzer,[1] and Miranda S. Wecker[1]

Summary

Spartina alterniflora is invasive in estuaries of the Pacific coast of North America, as well as in Europe, Asia, Australia, and New Zealand. Willapa Bay, located along the southern coast of Washington state, has the largest infestation of invasive *S. alterniflora* and is the site of the first biocontrol program against this grass. The recently introduced biocontrol agent, *Prokelisia marginata* (Delphacidae), has exhibited explosive growth during the summer months, followed by severe declines over the winter. Correlations of quantifiable site characteristics with the growth and decline of 12 released populations reveal the habitat favouring *P. marginata*. Factors favouring population growth during the summer include high host leaf nitrogen and low spider abundance. Winter survival was greatly improved by the presence of intact dead *S. alterniflora* culms throughout the winter. Interestingly, sites favouring *P. marginata* population growth in the summer had the lowest survival over the winter. These correlations and trade-offs suggest possible future strategies for enhancing biocontrol through habitat manipulation.

Keywords: biological control, population growth, *Prokelisia marginata*, *Spartina alterniflora*, winter survival.

Introduction

In the three years since its first introduction for biological control of *Spartina alterniflora* in Willapa Bay, Washington State, the planthopper *Prokelisia marginata* (Delphacidae) has exhibited explosive population growth, demonstrated impacts on the target plant in field cages, and attained local field densities approaching those known to kill the target weed (Grevstad et al. 2003). However, in spite of these encouraging early signs, the long-term persistence and impact of the agent population has been uncertain, due largely to low overwinter survival. The intertidal environment that Spartina invades is particularly harsh during the winter months, with frequent storms and a 2.3 to 3.4 m mean tidal range (Sayce 1988). After two of three initial released populations failed to survive the winter of 2001–02, and the third population only barely persisted, 12 additional release sites were selected, based on their relatively protected locations. By using a larger number of release sites, we hoped to find at least some sites where *P. marginata* populations would expand rapidly and persist year to year. Additionally, by performing periodic population surveys at these sites and quantifying habitat characteristics, we sought to identify habitat factors associated with improved *P. marginata* performance during both the summer and winter months. After one year of following these populations, we have gained important clues as to how to give this biocontrol program the best chance of succeeding.

[1] Olympic Natural Resources Center, University of Washington, Forks, Washington 98331
Corresponding author: Fritzi Grevstad, Spartina Biocontrol Program, 2907 Pioneer Road, Long Beach, WA 98631 <grevstad@u.washington.edu>.

Invasive *Spartina*

Spartina alterniflora, commonly called smooth cordgrass or Spartina, is native and ecologically valued on the Atlantic coast of North America, but it is introduced and a serious environmental threat on the Pacific coast of North America. *S. alterniflora* and the closely related *S. anglica* and *S. townsendii* are also invasive in Europe, China, Australia, and New Zealand (Aberle 1993). This perennial grass invades estuarine intertidal mudflats, which are normally devoid of emergent vegetation, dramatically transforming them into expansive swards of tall dense vegetation. The invasion brings threats to a wide variety of birds, fish, and commercially harvested clams and oysters that rely on the mudflat habitat.

Willapa Bay, a 23,000 hectare estuary along the southern Washington coast, has the most advanced infestation of invasive *S. alternflora*. The plant was accidentally introduced as early as the 1890s during a period when it was used as packing material for oysters shipped from the Atlantic coast (Frenkle and Kunze 1984). The plant was slow to spread until the mid 1900s when an apparent increase in seed production launched the population into a phase of rapid expansion (Sayce 1988; Feist and Simenstad 2000). Aerial photos document a 60% increase in Spartina cover throughout the bay between 1994 and 1997 (Reeves 1999). In 2002, an estimated 2400 solid hectares of *S. alterniflora* plus 2200 hectares of scattered patches were present in Willapa Bay (Wecker et al. this volume).

Novel aspects of the Spartina biocontrol program

Several aspects of the Spartina biocontrol program are unique. First, this is the first use of classical biocontrol against a grass. A lack of projects targeting grasses (Julien and Griffiths 1998) may reflect the fact that weedy grasses often have relatives of economic or ecological importance and tend to be risky targets. This is not the case for *S. alterniflora* in Willapa Bay. As a member of the tribe Chlorideae, *S. alterniflora* has few close relatives in North America and none in coastal areas north of the San Francisco Bay area. Second, the biocontrol program is the first in a marine intertidal environment. This environment has created unique challenges for the biological control program as described in this paper. Third, the use of a planthopper agent is unusual. The only other documented planthopper agent is *Stobaera concinna* (Stål), used against *Parthenium hysterophorus* (L.) and *Ambrosia artemisiifolia* (L.) in Australia (McFadyen 1985; Julien and Griffiths 1998). Finally, this project differs from most classical biocontrol projects in that the targeted weed is invasive in the same country where it is native and the biocontrol agent has likewise been transferred between states rather than between countries. The host specificity testing was nonetheless as rigorous as that used in foreign introductions (Grevstad et al. 2003), including a full review by the Technical Advisory Group on Biological Control of Weeds. In the past, interstate introductions of biocontrol agents have been made without a formal technical review, including one that has been harmful to native plants (Louda and O'Brien 2002).

Prokelisia marginata life history

Prokelisia marginata is native to the Atlantic and Gulf coasts of North America. It also occurs in California, where it may have been introduced in recent decades. *P. marginata* is highly host specific, using only a small number of closely related *Spartina* spp. as hosts (Grevstad et al 2003). In addition to *S. alterniflora*, it can complete development on *S. anglica* and *S. foliosa* (native to California and Mexico). It may also be capable of using the European *S. maritima* and *S. townsendii*, although these species were not included in host range tests. *P. marginata* weakens the plant by ingesting sap from the phloem and also by laying eggs under the leaf surface, causing structural damage and scarring to the leaf. *P. marginata* is known to have three generations per year in its native range and in California (Denno et al. 1996, Roderick 1987) but so far has produced no more than two generations in per year in Willapa Bay. Nymphs pass through five instars before moulting into adults. Overwintering occurs in the nymphal stages. The majority of nymphs pass the winter inside leaf curls of senesced plants (thatch). Some can also be found on short green shoots, which are sparse in winter.

Materials and Methods

Releases of approximately 9000 mixed stage *P. marginata* were made at 12 sites throughout Willapa Bay in late May and early June of 2002. The sites were specifically selected for their perceived winter habitat quality. We selected sites in which at least some of the senesced *S. alterniflora* culms remained intact over the winter. Such sites tended to be in the upper tidal zones, in small backwater sloughs, or otherwise protected from winter storms and wave action. In unprotected and lower tidal zone sites, the Spartina culms typically break off and drift away or become waterlogged and decompose.

Insects used for releases were reared on *S. alterniflora* in a greenhouse during the winter and spring of 2002. The parent stock was collected from Willapa field populations in late fall. In mid-to-late May, the planthoppers were released into field sites by nestling infested rearing plants into a designated 5 × 5 m area of a much larger sward. Most of the planthoppers moved onto nearby field plants within a few days.

The planthopper populations were surveyed at three times: (1) in early July, before any new eggs had hatched; (2) in late September, after one full generation; and (3) in April of the following spring. A gas-powered

insect vacuum converted from a hand-held leaf blower (see Grevstad et al. 2003) was used to sample *P. marginata*. At each release site, insects were vacuumed from the vegetation at 12 sample points in July and September, and at 24 sampling points in the following spring (April). At each sample point an area the size of the intake tube (0.0346 m^2) was thoroughly vacuumed. Sample points were evenly spaced in a grid arrangement within 5 m radius of the release centre. The vacuum bags were brought back to the laboratory, where the numbers of *P. marginata* nymphs and adults from each sample were counted.

During the September and April surveys, the number of spiders in each sample was also noted. To assess the possible influence of variation in plant nitrogen on the *P. marginata* populations, 20 randomly selected leaves (2nd from top) were collected from each site in mid-September. The leaves were dried in a drying oven, ground to a fine powder, and analyzed for nitrogen content. In April, we quantified characteristics of the wintering habitat inside eight 0.25 m^2 quadrats spaced 2 m apart along two transects bisecting the releases area. In each quadrat, we counted the number of new green shoots, measured the height of the tallest shoot, and assessed the percentage of dead culms from the previous year's growth that were still intact and in good condition.

Results

Summer increase

At most sites, population densities increased substantially between the first and the second census (Fig. 1). The average population increase was by a factor of 2.84 ± 0.90. Change in density ranged from a 50% decline to a nearly 12-fold increase. The change in density is an underestimate of the actual reproduction rate because many insects disperse from the initial release area. (In an earlier study, roughly two thirds of the population was found to disperse beyond the immediate release area by the end of the first summer (Grevstad et al. 2003).) The average population density at the end of the summer was 4270 ± 1570 planthoppers per m^2 with a range of 947 to just over 20,000 per m^2. The one site that attained 20,000 per m^2 had nearly four times the density of the next most populous site.

Winter decline

Survival over the winter was low, but better than in previous years. The average fraction surviving from October 2002 to April 2003 was 0.043 ± 0.019. At five sites, no *P. marginata* were recovered in April. The highest level of survival at a site was 0.18. At all but one site, the density of *P. marginata* recovered in the spring was lower than densities measured soon after release in the previous summer. Because some insects dispersed, the decline in density does not necessarily mean a decline in population size.

A striking pattern to arise from these results is that sites where *P. marginata* performed well during the summer had lowest survival during the winter (Fig. 2). Five of the six populations attaining greater than median density appear to have gone extinct, with the extant population surviving at a rate of only 0.43%. In contrast, all of the six populations that attained lower than median fall densities persisted through the winter and the average survival rate was 8.4%.

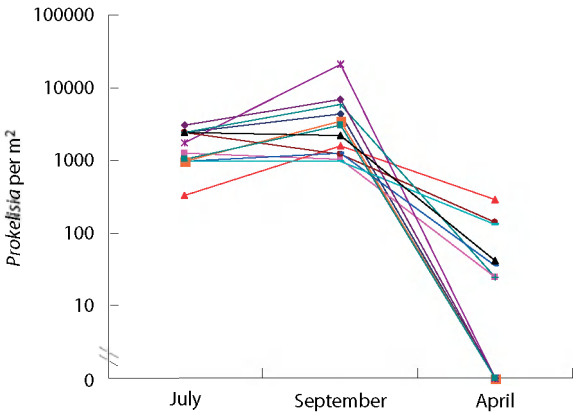

Figure 1. Densities of *Prokelisia marginata* at 12 release sites in July, September, and April after approximately 9000 individuals were released at each site in early June.

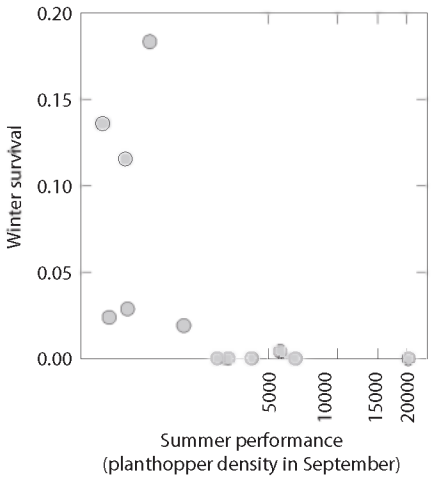

Figure 2. Relationship between winter and summer performance of *Prokelisia marginata* at 12 release sites in Willapa Bay. Winter survival was measured as the ratio of spring to fall *P. marginata* densities. Summer performance was measured as the planthopper density attained by September after release of 9000 individuals at each site in early June.

Site influences

We found clear correlations between *P. marginata* performance and measurable site characteristics. During the summer months, *P. marginata* performance, measured as the density attained by the end of the summer, was positively correlated with leaf nitrogen content (F = 16.5, P = 0.002, Fig. 3a). Leaf nitrogen content at release sites ranged from a low of 1.09% to a high 2.09%. Summer performance was also strongly negatively correlated with spider density (F = 11.8, P = 0.006). Spider densities among sites varied by two orders of magnitude with a range of 22 to 2218 per m^2 in September and a range of 2.4 to 176 per m^2 in April. The outbreak site mentioned above was the site with the highest leaf nitrogen content. It also had the second lowest spider density.

During the winter, increased survival was strongly associated with the presence of intact thatch over the winter (R^2 = 0.62; P = 0.002; Fig. 4a). The level of thatch in the quadrats varied among sites from 0 to 90%, even though all sites had moderate to high levels of thatch in the previous spring when the sites were chosen. Thus, there is variation from year to year in the condition of thatch at particular locations. There appears to be a threshold level of Spartina thatch needed to support *P. marginata* through the winter. Survival was reasonably high at levels of 70% intact thatch or above, but was low or zero at lower levels.

Interestingly, during the winter, the relationship with spider density was reversed from that in the summer (R^2 = 0.45; P = 0.017; Fig. 4b). *P. marginata* survived better at sites where spider densities were high. The likely explanation is that the same conditions that promote *P. marginata* survival also promote spider survival. Predation by spiders does not appear to be a significant mortality factor during the winter. The two other habitat characteristics measured during the spring survey, shoot density and culm height, were not significantly correlated with *P. marginata* survival (Fig. 4c,d).

When only the net result of combined summer population growth and winter declines is considered, i.e. the density of *P. marginata* emerging in the spring, the level of intact thatch was the only factor that significantly influenced *P. marginata* performance (R^2 = 0.62, P = 0.003).

Discussion

Following analyses of the performance of *Prokelisia marginata* at 12 new release sites, the initial challenges imposed by the harsh Willapa Bay environment now appear surmountable. The careful selection of sites that were better protected from wind and wave action, as well as the use of a larger number of varied release locations, provided improved overall performance compared to the first years releases at only three sites. We now also have three easily quantified habitat factors—high leaf nitrogen, low spider density, and the presence of intact thatch over the winter—that can be used to select future release sites for even greater improvement in *P. marginata* performance.

Our results suggest that *P. marginata* should ideally be released into sites that have high nitrogen and low spiders in summer *and* have thatch that remains intact over the winter. But such sites may be hard to come by, as none of our 12 sites had that combination. Instead nitrogen was negatively correlated with thatch condition and spiders were positively correlated with thatch condition. High nitrogen plants and low spider abundance are often found in lower tidal areas and channel banks, where there is greater water flow and better access to nutrients, but where the currents and wave action are likely to break off dead culms during the fall and winter. Also, the taller growth of high nitrogen plants makes them more susceptible to breakage during the fall and winter. As a result of these correlations, populations that had explosive growth during the summer, reaching sampled densities of 20,000 per m^2, went extinct or nearly so during the winter. In the end, the presence of intact thatch was the only single factor

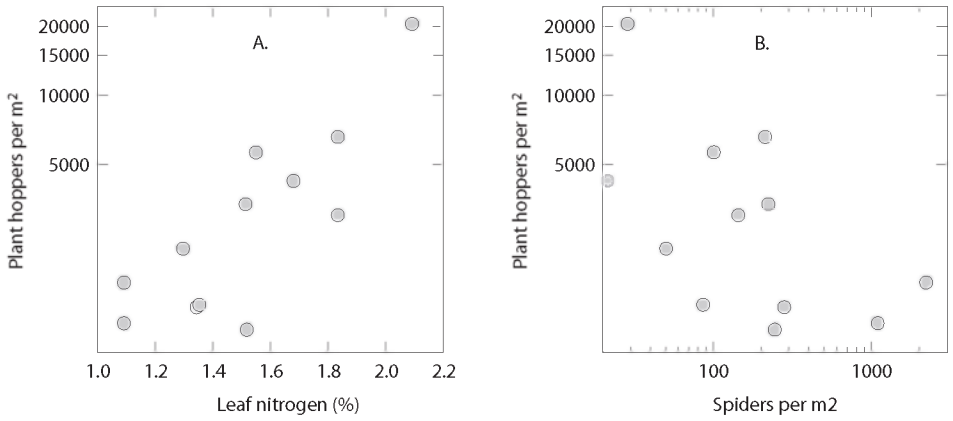

Figure 3. Relationship between *Prokelisia marginata* performance and (A) percent nitrogen content of *S. alterniflora* leaves and (B) spider density.

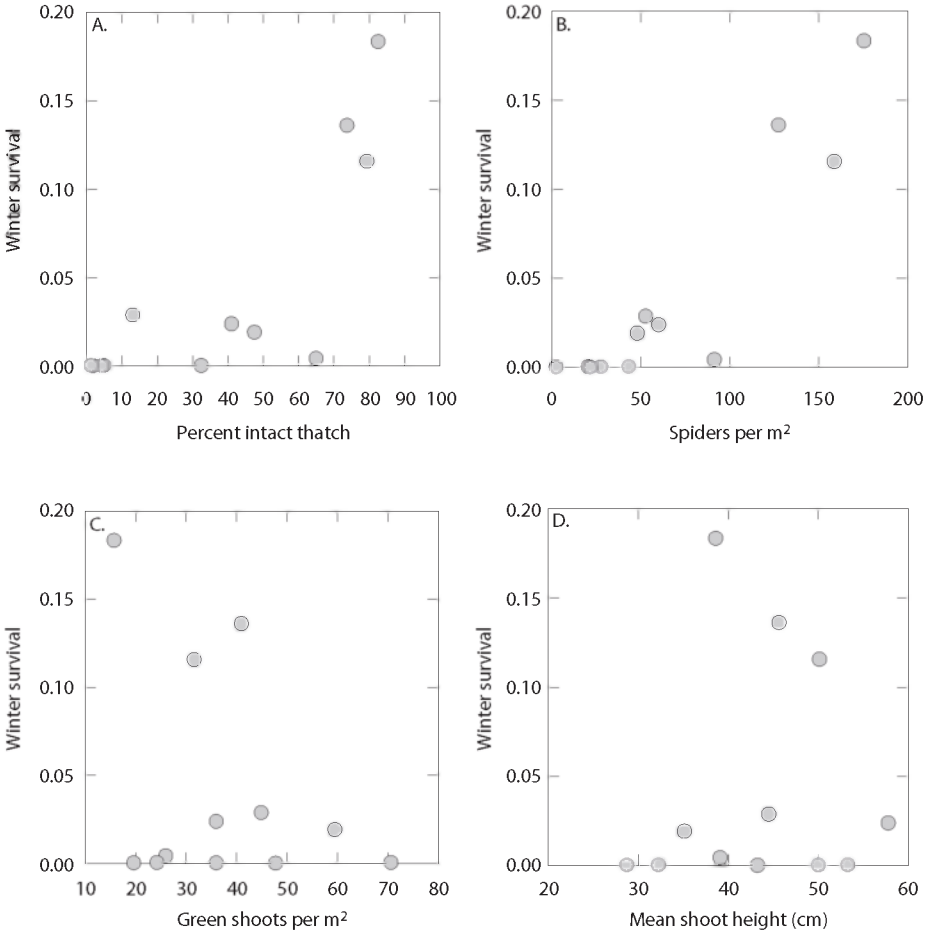

Figure 4. *Prokelisia marginata* winter survival as a function of (A) percentage of Spartina thatch remaining intact, (B) spring spider densities, (C) density of green shoots, and (D) mean tallest shoot height.

that adequately predicted *P. marginata* performance over the full year period.

Newly released populations of *P. marginata* in Willapa Bay seem to be foiled by the spatial separation of superior winter and summer habitat. However, *P. marginata* in its native range has a life history strategy adapted to it. In New Jersey saltmarshes, *P. marginata* reproduces in tall, nitrogen rich plants along channel edges during the summer and then disperses in fall to nearby high marsh Spartina that is more favourable for winter survival (Denno and Grissell 1979). This dispersal also allows *P. marginata* to elude predation by spiders (Denno and Peterson 2000). Such seasonal migration between upper and lower tidal zones has not been observed in Willapa Bay. Instead any dispersal that occurs is not directed toward upper tide zones, and the majority of the planthoppers remain within a few metres of the release area at the onset of winter (Grevstad et al. 2003).

An important difference between east coast and invasive west coast Spartina marshes is that, on the east coast, there are two forms of *S. alterniflora*; a tall form that grows in lower tide zones and near channel edges, and a short stiff form, 10–15 cm tall, that grows in expansive swards in the high marsh. In Willapa Bay, only the tall form of *S. alterniflora* is found and, in all but the most protected areas, it breaks off during winter. Given that there are very large expanses of Spartina in Willapa Bay in areas where *P. marginata* cannot survive the winter and only scattered small areas where it can, it is reasonable to question the potential of *P. marginata* to have widespread impact on the target plant over its full distribution. Perhaps a more likely outcome is that the planthopper will have impacts in some areas but not others.

The results suggest opportunities for habitat manipulation and conservation biocontrol practices to enhance the effectiveness of the biocontrol program. One possibility is to improve *P. marginata* population growth or even create outbreaks through fertilization of Spartina plants in the vicinity of releases. This could be done in sites that had good winter habitat and relatively low spider densities. Fertilization experiments with *P. marginata* have been tried on the east coast with mixed results. Bowdish and Stiling (1998) and Denno et al. (1996) found that fertilizing increased *P. marginata* densities by factors of roughly two and four respectively, while Silvanima and Strong (1991) found initial

increases in abundance that did not persist, and Vince et al. (1981) found no effect of fertilization. Vince et al. (1981) noted higher numbers of spiders in fertilized plots that may have suppressed the planthoppers. Another approach to enhancing biocontrol is to move large numbers of planthoppers from the high reproduction sites when they are abundant in the fall and move them to protected locations to spend the winter. Experiments are needed to determine what kind of sheltering will provide the best winter survival with the least effort. The possibility for doing this on a large scale is not prohibitive. The state and federal agencies currently involved in the Spartina control work have large machines capable mowing and transporting Spartina stems in large quantities.

Acknowledgements

This research was supported by the National Sea Grant Program and the United States Fish and Wildlife Service. We also thank the Washington Department of Natural Resources and the Willapa Wildlife Refuge for airboat transportation, Joe McHugh for site access, and Carol O'Casey for field help during the spring survey.

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Hydrellia pakistanae and *H. balciunasi*, insect biological control agents of hydrilla: boon or bust?

Michael J. Grodowitz,[1] Michael Smart,[2] Robert D. Doyle,[3] Chetta S. Owens,[2] Robin Bare,[2] Christie Snell,[2] Jan Freedman[1] and Harvey Jones[1]

Summary

Of four insect species released in North America for the management of hydrilla (*Hydrilla verticillata*, Hydrocharitaceae), only the two leaf-mining flies *Hydrellia pakistanae* and *H. balciunasi* have become established. While the flies have exhibited impressive range extensions since their first release in 1987, populations at most sites have remained below what was considered damaging. Recently, modest to large increases in fly populations followed by hydrilla declines have been observed at several sites including Lake Seminole, Florida, Coleto Creek Reservoir, Texas, and Sheldon Reservoir, Texas, United States of America (USA). Long-term, large tank experimentation has shown that even modest levels of fly damage can significantly reduce hydrilla biomass (50%) and tuber numbers (25%), apparently by reducing photosynthesis and thereby decreasing plant vigour and production. Field studies have also substantiated these findings where lower numbers of tubers (60%) were observed at sites on Lake Seminole impacted by fly feeding. While more detailed field evaluations are needed, it appears that these agents have the potential to suppress hydrilla populations over the long term. However, a complex of factors can influence their effectiveness, including temperature, plant nutrition, especially protein levels, crowding and the presence of a capable pupal parasite. Further research is needed, including overseas work to identify additional agents and the implementation of new release programs. Based on field surveys, fly releases may increase the likelihood of impact since US release sites now have as much as seven-fold higher fly numbers and associated damage than non-release sites.

Keywords: biological control, *Hydrellia*, *Hydrilla verticillata*.

Introduction

Beginning in 1987, two species of leaf-mining flies in the family Ephydridae were introduced to North America for the management of hydrilla (*Hydrilla verticillata*, Hydrocharitaceae) (Center *et al.* 1997, Grodowitz, *et al.* 1997). The first species, *Hydrellia pakistanae* Deonier, was introduced into Florida and subsequently in Louisiana, Alabama, Georgia, Texas and California, United States of America (USA). Its distribution has expanded considerably, now extending throughout the Florida peninsula, upwards into the Florida panhandle and Georgia, mainly on Lake Seminole, north and west into Alabama, and throughout many locations in eastern and south-eastern Texas. Direct impact to hydrilla by *H. pakistanae* has been observed at several locations mainly in northern Alabama, Texas, and Florida (Grodowitz *et al.* 1995, Grodowitz *et al.* 1999, 2000b), but long-term monitoring for impact has been limited. In many areas, introduced *Hydrellia* spp. population levels and associated damage have been low (Grodowitz 1999, Wheeler & Center 2001). Unfortunately, factors accounting for such low populations have not been quantified, but may include high levels of parasitism, plant nutritional

[1] US Army Engineers Research and Development Center (ERDC), CEERD-EE-A, 3909 Halls Ferry Road, Vicksburg, MS 39180 USA.
[2] US Army Engineers Research and Development Center (ERDC), CEERD-EE-A, Lewisville Aquatic Ecology Research Facility, Lewisville, TX 75056 USA.
[3] Baylor University, Department of Biology, Waco, TX 76798 USA.
Corresponding author: Michael J. Grodowitz <grodowm@WES.ARMY.MIL>.

quality and other forms of predation (Wheeler & Center 1996, Dr Jim Cuda, pers. comm., unpublished data). The three other introduced insect agents have had limited, if any measurable success. These include the closely related leaf-mining fly *H. balciunasi* Bock (Grodowitz *et al.* 1997), the tuber-feeding weevil *Bagous affinis* Hustache and the stem-feeding weevil *B. hydrillae* O'Brien (Grodowitz *et al.* 1995). Of these three species, the only agent to become established has been *H. balciunasi*, but expansion in distribution and population size has been severely limited. Recent surveys have shown the presence of this species at sites in Texas that are substantially removed from the original release sites in Texas.

The purpose of this paper is to review the existing evidence and present new information on the impact of the introduced *Hydrellia* spp. in the US. Information on establishment success, expansion in distribution, population increase and ultimate impact will mainly be directed toward *H. pakistanae*, since much of the current work has focused on this species. The information has been obtained from a variety of published and unpublished research from both controlled experimentation and actual field studies. Limited information on abiotic or biotic factors that could possibly be influential, including plant nutrition and parasitism, is also included.

Results and discussion

Establishment

Since the first release of *H. pakistanae* in North America in 1987, over 3 million individuals have been released at close to 30 different sites from Florida to California. Establishment success has been high, with at least 70% of the release attempts having *H. pakistanae* present six months or longer after terminating the introductions (Center *et al.* 1997). Surveys conducted during 2001 and 2002 at release sites in Louisiana and Texas have shown that establishment success may be higher, since *H. pakistanae* has subsequently been found at sites where it was thought not to have initially established. However, observing the agent after such an extended period subsequent to termination of releases may be due to natural expansion from nearby populations.

Compared to *H. pakistanae*, *H. balciunasi* has shown substantially lower establishment success. For example, establishment success for *H. balciunasi* was only 18% in 1997, nearly four-fold lower than what was observed for *H. pakistanae* (Grodowitz *et al.* 1997). In fact, only two release sites have had verified establishment of this species, both of which are in Texas. Reasons for such low establishment success for *H. balciunasi* are unknown. However, much less effort went into its release in comparison to *H. pakistanae*. For example, *H. balciunasi* was introduced in only 11 sites in two states with less than 300,000 individuals. *Hydrellia balciunasi* was always more difficult to rear than *H. pakistanae* and this may account for the differences in the release effort.

Expansion

Another important measure of success for an agent is its ability to disperse extended distances after initial releases have been discontinued. *Hydrellia pakistanae* has exhibited impressive range expansion since 1987. Considering that this species was released at only about 30 locations in 5 states, it is impressive that it is found in almost every location examined. During surveys conducted in 2000 (Grodowitz *et al.* 2000b), new populations of *H. pakistanae* were located at sites on the Rio Grande near McAllen and Rio Grande City, Texas, that are well over 300 km and 400 km, respectively, from the nearest deliberately established populations. Surveys conducted in 2001 showed that it was present in 50% of non-release sites examined in Louisiana even though it was released in only one isolated system (Lake Boeuf) south-west of New Orleans, Louisiana. Sites examined in Louisiana in 2001 ranged throughout the state and encompassed almost every considerable type of hydrilla habitat. Wheeler & Center (2001) noted the occurrence of *H. pakistanae* in almost every site examined in Florida.

In contrast, *Hydrellia balciunasi* has exhibited only minimal range expansion. As indicated previously, *H. balciunasi* was established in only two Texas sites (i.e. Lake Raven, Huntsville State Park, and Sheldon Reservoir, near Houston) located in the eastern portion of the state. Surveys conducted in the early- to mid-1990s failed to reveal its presence in any other location, even with extensive sampling. However, in 1997, *H. balciunasi* was discovered in locations north and north-east of the original two release locations, often in combination with *H. pakistanae*. These sites include ponds at the Lewisville Aquatic Ecosystem Research Facility (LAERF) and Cypress Springs Lake near the town of Mount Pleasant, Texas. Reasons for its recent expansion are unknown, but offer encouragement for its continued expansion success.

Determining mechanisms for such large expansions is difficult, at best, for these species. First of all, they appear to be relatively weak fliers and are often seen hopping from one resting place to another instead of flying. Human or animal transportation of hydrilla sprigs containing immatures seems plausible, but established sites and associated population size of the introduced *Hydrellia* spp. was minimal during this time, hence the odds of man or animals carrying *Hydrellia* spp. laden sprigs seem unlikely. Additional research is warranted.

Population increase

Another important criterion of success is the ability of the released agents to substantially increase in population size. While large expansion in distribution is desirable, it is often more important to have corre-

sponding increases in population levels to effect control.

In closed, controlled environment systems, *H. pakistanae* populations have been shown to increase significantly (Doyle *et al.* 2003). In this long-term study, examining the impact of herbivory and competition alone and in combination in a 14,000 L tank, large increases in population levels were observed over two growing seasons (Fig. 1). By the end of the 1999 growing season (i.e. October), mean immature numbers exceeded 3000 per kg fresh plant weight (Fig. 1a) and close to 35% leaf damage (Fig. 1b). Similar results were observed in nearby ponds at LAERF, where *H. pakistanae* in small pond systems increased substantially in populations in only one growing season (2002) to almost 5000 immatures per kg (Fig. 1c) with 40% leaf damage (Fig. 1d).

These results are similar to previously reported observations from ponds located at the Tennessee Valley Authority Muscle Shoals, Alabama. There, population levels of *H. pakistanae* reached mean values of almost 7000 immatures per kg with close to 70% leaf damage (Grodowitz *et al.* 1994). Collectively, these data demonstrate that substantial population increases are possible for the introduced leaf-mining flies in experimental or pond situations.

Under field conditions, we see a few sites where substantial population increases have taken place, but we also see very large variations observed from site to site. For example, surveys conducted during 1999 at a variety of release and non-release sites indicated that number of immatures and associated damage varied tremendously; e.g. 3000-fold for immatures and 40-fold for leaf damage (Fig. 2). Reasons for such large variations are unknown but may be related to various abiotic and biotic factors as well as the numbers released into an individual site.

Impact

Larval feeding action directly impacts hydrilla internal leaf cellular material. Hence, it is not surprising that a decrease in light-saturated photosynthesis is positively correlated with increasing leaf damage (Doyle *et*

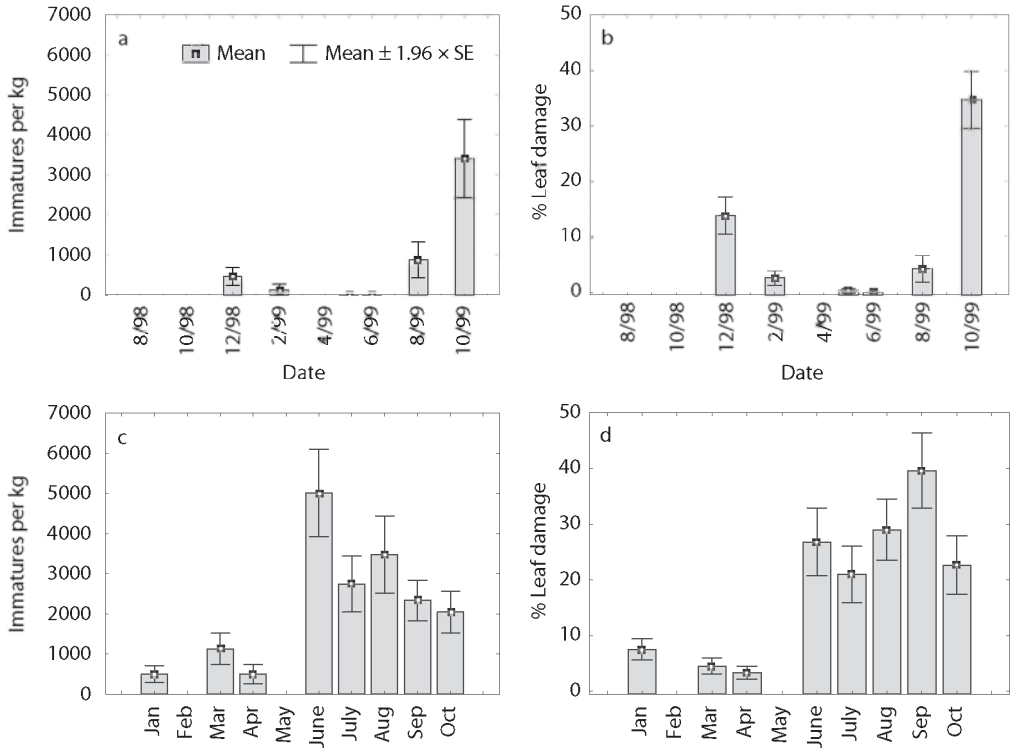

Figure 1. Population increases and associated changes in damage for *Hydrellia pakistanae* reared in closed systems (a and b) and small ponds (c and d). Note the large increases in immatures (a) and associated damage (b) for *H. pakistanae* reared in 14,000 L tanks for two growing seasons (1998 and 1999) as part of an experiment to assess herbivory/plant competition impacts. Only about 400 immatures were introduced into each tank to produce almost 3500 immatures per kg and 35% leaf damage (after Doyle *et al.* 2003). Similarly, substantial increases were observed in small pond experimentation again designed to assess herbivory/plant competition impacts during 2002. Note that in one growing season, immature levels (both *H. pakistanae* and *H. balciunasi*) reached 5000 per kg (c) with 40% leaf damage (d) (unpublished data).

al. 2002). With 10% to 30% leaf damage, the maximum rate of light saturated photosynthesis was reduced 30% to 40% (Fig. 3). At these damage rates, total daily photosynthetic production was estimated to barely balance the daily respiratory needs of the stems. Based on this information, even relatively low leaf damage can be expected to significantly impact hydrilla growth.

Decreases in biomass and tuber number have been observed in several closed-system experiments. Van *et al.* (1998) reported a 30% decrease in biomass with levels of herbivory that resulted in complete defoliation. Wheeler & Center (2001) achieved levels of about 4000 larvae per m^2 within small enclosures that resulted in complete defoliation and significant hydrilla

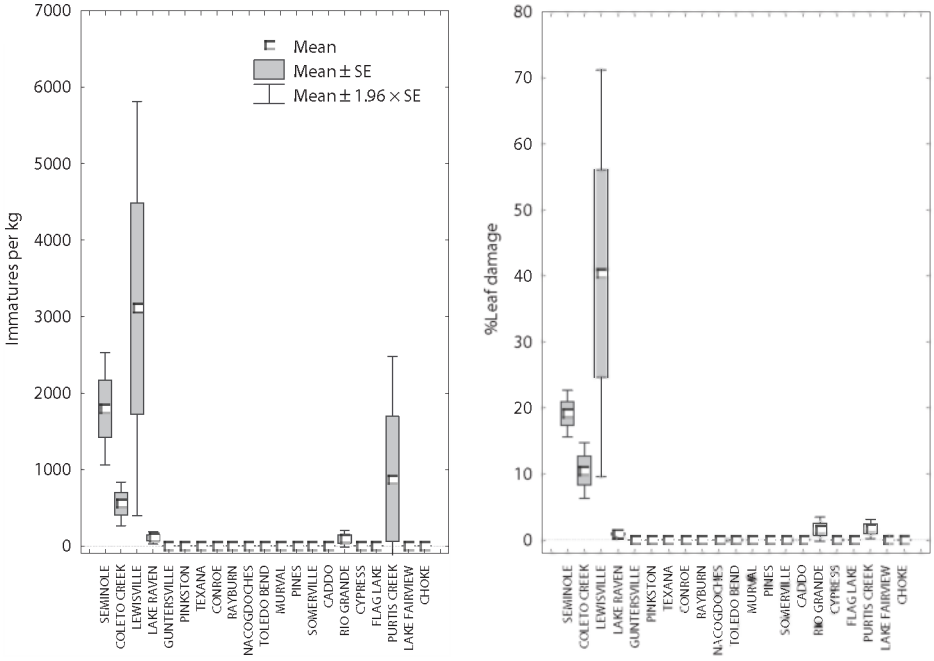

Figure 2. Immatures per kg and percentage leaf damage for release and non-release sites surveyed during 1999 (unpublished surveys). Release sites include Lake Seminole, Florida; Coleto Creek Reservoir, Texas; Lewisville Aquatic Ecosystem Research facility (LAERF) ponds; and Guntersville Reservoir, Alabama. Note large variation from site to site.

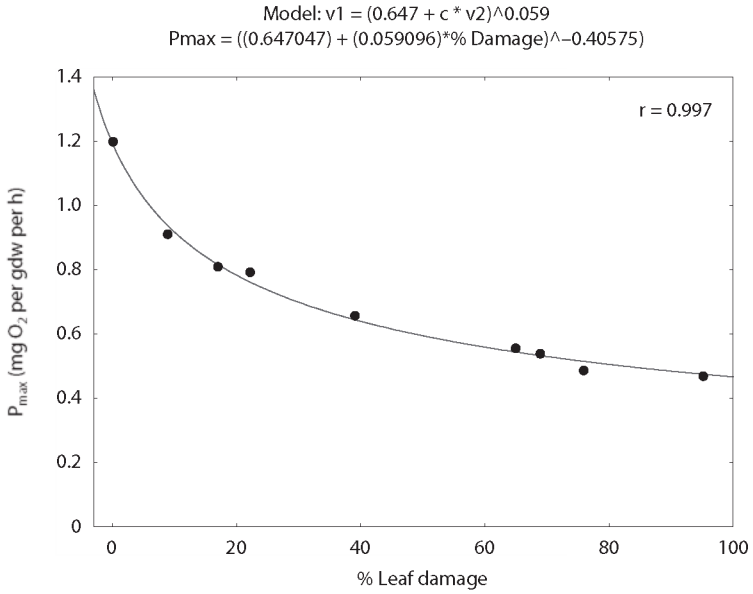

Figure 3. Light-saturated photosynthetic rate (P_{max}) for various amounts of hydrilla leaf damage caused by the feeding action of immature *Hydrellia pakistanae*. Note that maximum rate of light saturated photosynthesis is reduced 30% to 40% for stems having 10% to 30% leaf damage (after Doyle *et al.* 2002).

impact; i.e. no formation of a surface canopy. However, such high levels of herbivory are not typical of what has been observed in the field.

Experiments designed to assess herbivory impact at much lower levels, more similar to what is observed at field locations, also show the same results. Mean hydrilla biomass and tuber number decreased in small, short-term tank experiments by 17% and 40%, respectively with only 15% to 40% leaf damage (Fig. 4; Doyle *et al.* 2002). Similarly, in larger, long-term tank experiments, biomass and tuber number were decreased 45% and 21%, respectively, at herbivory levels more typical of what is observed in the field (Fig. 5).

Impacts to field hydrilla infestations are, quite understandably, harder to assess. However, evidence is now emerging that indicates that long-term, sustained *H. pakistanae* herbivory can significantly impact hydrilla infestations in the field. Hydrilla at several

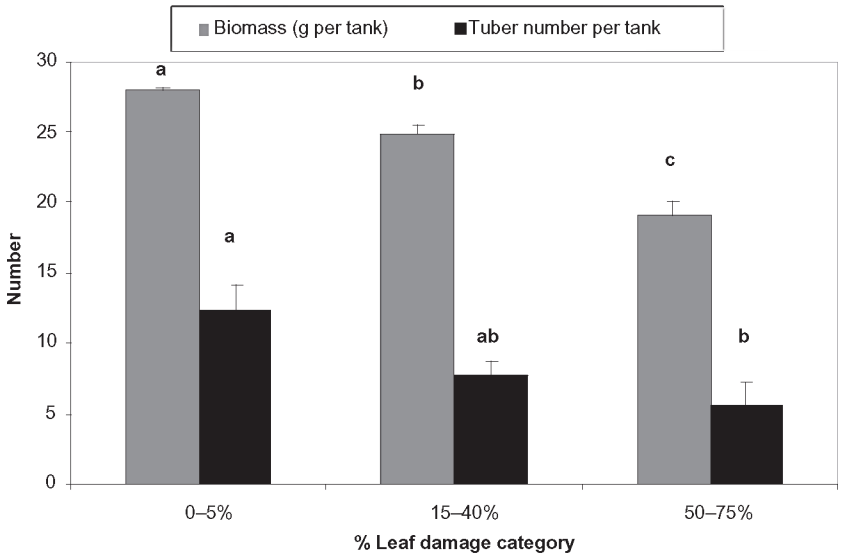

Figure 4. Mean hydrilla biomass (g dry plant material) and tuber number per tank for small, short-term tank experiments measuring herbivory/plant competition impacts to hydrilla. Columns for a specific parameter are not significantly different at $p < 0.10$ if followed by the same letter. Note that significant declines in biomass and tubers occurred with increasing leaf damage (after Doyle *et al.* 2002).

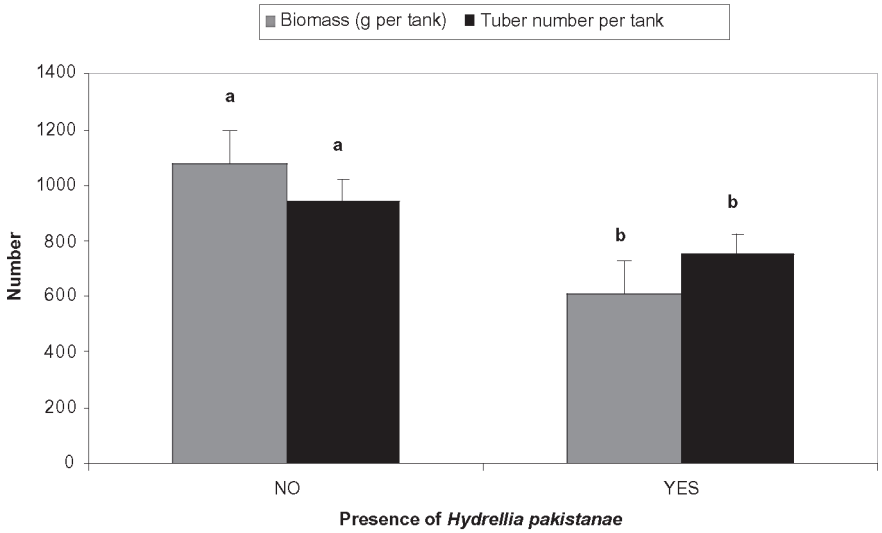

Figure 5. Hydrilla biomass (g dry material per tank) and tuber number per tank over two growing seasons for large (14,000 L), long-term tank experiments designed to assess herbivory/plant competition. Means for a given parameter are significantly different at $p = 0.11$ if followed by different letter. Significant decreases in biomass and tuber number occurred; i.e. 3500 immatures per kg and 35% leaf damage (Figs 1a and 1b) (after Doyle *et al.* 2003).

locations on Lake Seminole, Florida, apparently suffered substantial declines six to seven years after *H. pakistanae* releases were terminated (Grodowitz *et al.* 2003). In the summer of 1999, Lake Seminole managers reported significant declines that were possibly related to *H. pakistanae* feeding action. Surveys conducted in September 1999 showed relatively high field populations of about 2500 immatures per kg with leaf damage approaching 16%; higher than what was reported previously.

Quantitative surveys in November 1999 showed significant reductions in tubers (three-fold) compared to sites with lower insect impact (Grodowitz *et al.* 2003). Also, a strong relationship between tuber numbers and leaf damage with decreased number of tubers associated with higher leaf damage were noted (Fig. 6). The ThreeRiv and Wingates sites served as controls. ThreeRiv was far removed from the original releases and typically showed lower insect numbers and the hydrilla at Wingates was recently recovering from a herbicide treatment and thus exhibited low insect numbers.

The declines were quite evident. Hydrilla present on Lake Seminole prior to insect impact appeared healthy with little evidence of insect feeding (Fig. 7a). Hydrilla grew consistently as a monoculture and appeared lush and fully canopied during the peak of the growing season. However, during 1999, hydrilla was often found growing in association with other native plants and often made up only a small proportion of the total plant community (Fig. 7b). It was noticeably stressed with fewer and smaller leaves.

Figure 7. Hydrilla on Lake Seminole during 1994 (a) and 1999 (b). In 1994, before insect impact, hydrilla grew in monoculture, but by 1999 it was part of a mixed aquatic plant bed and onbviously stressed, coinciding with increased insect populations.

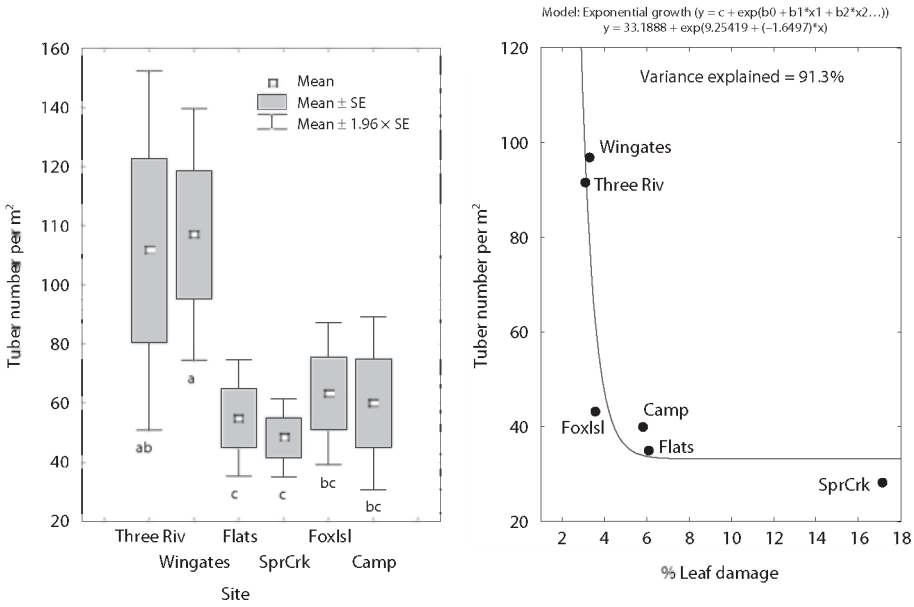

Figure 6. Tuber numbers per m^2 for sites sampled on Lake Seminole during November 1999. Both ThreeRiv and Wingates sites served as controls, since insect numbers and impact were reduced in comparison to the other sites. Means followed by the same letter are not significantly different at $p = 0.05$. Also shown is the exponential growth relationship between tuber numbers and percentage leaf damage. Lower tuber numbers were associated with sites with higher mean leaf damages (after Grodowitz *et al.* 2003).

The following year (2000), more extensive sampling was conducted to assess *H. pakistanae* impact. Insect numbers remained suppressed throughout the year, which is not surprising after the high numbers and damage exhibited in 1999. Still, decreases in species-richness were found related to insect numbers and damage. Using a three-dimensional linear graphing analysis to observe data trends, increases in species-richness (i.e. number of plant species as a measure of diversity) occurred. Species-richness was highest in those samples with high insect numbers and damage; i.e. almost four species for high insect numbers and damage and only one species, hydrilla, in those samples containing minimal insect numbers and damage (Fig. 8).

The presence of higher numbers of broken stem pieces within the canopy is a common occurrence during times of high *H. pakistanae* populations. Apparently, the stem becomes more brittle at the point where several leaves within a single whorl are damaged (unpublished data). With increased stem breakage the canopy is opened and may lead to the recolonization of native species due to increased light penetration below the hydrilla. On Lake Seminole, during September 2000, significant correlations ($p < 0.05$) were observed between species-richness and percentage damaged stems where higher numbers of native plant species occurred in those samples (i.e. species-richness) with highest number of stems containing *H. pakistanae* feeding damage (Fig. 9).

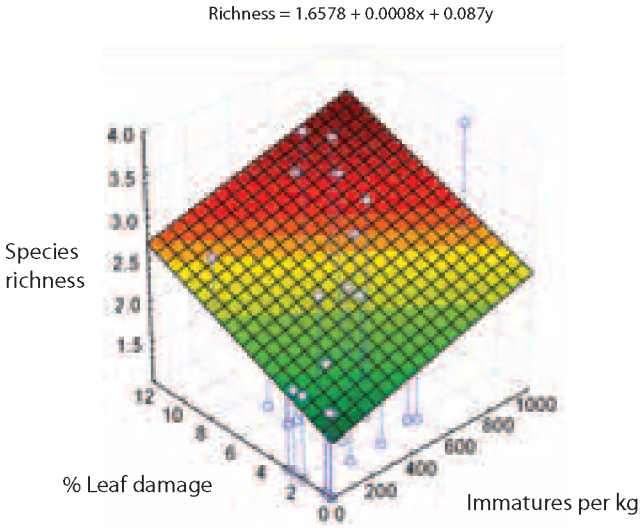

Figure 8. Linear relationship between species-richness and *Hydrellia pakistanae* numbers and leaf damage using a statistical graphing technique to illustrate overall trends.

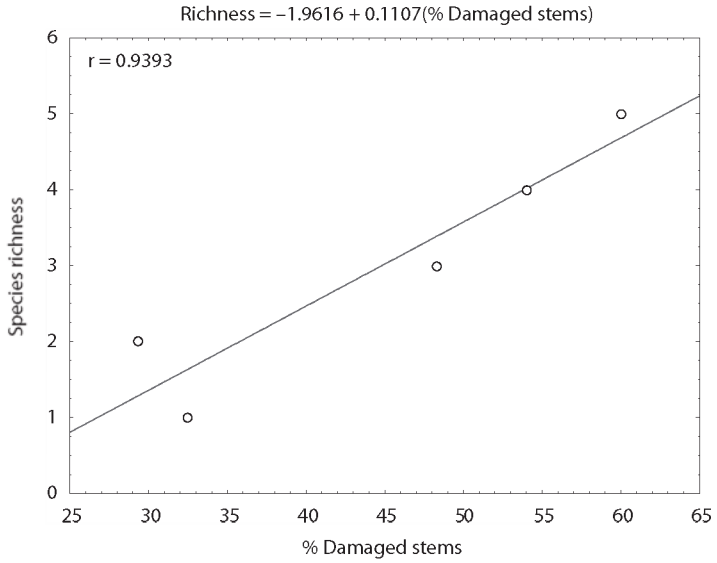

Figure 9. Species-richness and percentage damaged stems for samples collected from Lake Seminole during September 2000. Means were calculated based on each species-richness category. Correlation is significant at $p < 0.05$.

Possible regulators

Based on the evidence presented so far, establishment has been highly successful for *H. pakistanae* and minimally successful for *H. balciunasi.* Range expansion for *H. pakistanae* has been impressive, with populations located upwards of 500 km away from the nearest release sites. *Hydrellia balciunasi*, however, has had only limited range expansion. Population increase for both species is highly variable from field site to field site with higher numbers more typically observed for *H. pakistanae*. Increases have been slow to occur, with some release sites taking from six to eight years for *H. pakistanae* to develop significant populations. Impact, while impressive under controlled conditions (even at relatively low population sizes), is much more variable under field conditions. Nonetheless, verified impact has been observed in at least three sites, including Sheldon Reservoir and Coleto Creek Reservoir, Texas, and Lake Seminole, Florida.

Reasons for limited population increase and impact at field locations, especially in comparison to substantial increases observed for controlled experimentation, are unknown but several reasons appear plausible. Temperature is often cited as a possible limiting factor since the top portion of the hydrilla canopy during the summer months in the southern US can reach 35°C to 40°C relatively rapidly. Unpublished information suggests high larval mortality occurs after temperatures reach 35°C. While this may be an important regulator, it would not be unusual for the larvae to thermoregulate by simply moving from the top portion of the canopy to lower levels during the hotter parts of the day where temperature decreases relatively quickly. Hence, temperature may not be a very important determinant of survival and more research is warranted.

Another possibility is the presence of a pupal parasite, *Trichopria columbiana* Ashmead, commonly found in association with native *Hydrellia* spp., that is now parasitizing both species of introduced *Hydrellia*. While more research is needed, parasitism rates appear to be relatively low for *H. pakistanae* under field conditions, especially in the early part of the growing season and tend to increase roughly proportionally to *H. pakistanae* population increases later in the growing season (Fig. 10). Note that even with 35% parasitism occurring during October 2001, immature numbers were still high; 6000 immatures per kg.

Another important determinant for success under field conditions is plant nutritional composition, especially protein content. Wheeler & Center (1996) showed that *H. pakistanae* larval development was significantly reduced when reared on hydrilla with harder leaf cuticles containing lower nitrogen levels. Recent experiments have shown that hydrilla with low nitrogen content appears to impact not only larval development time but the number of eggs oviposited per female (Fig. 11). Eggs per females were over two-fold higher for larvae reared on hydrilla containing 2.4-fold more protein (as estimated from nitrogen content). However, nitrogen content is only part of the story,

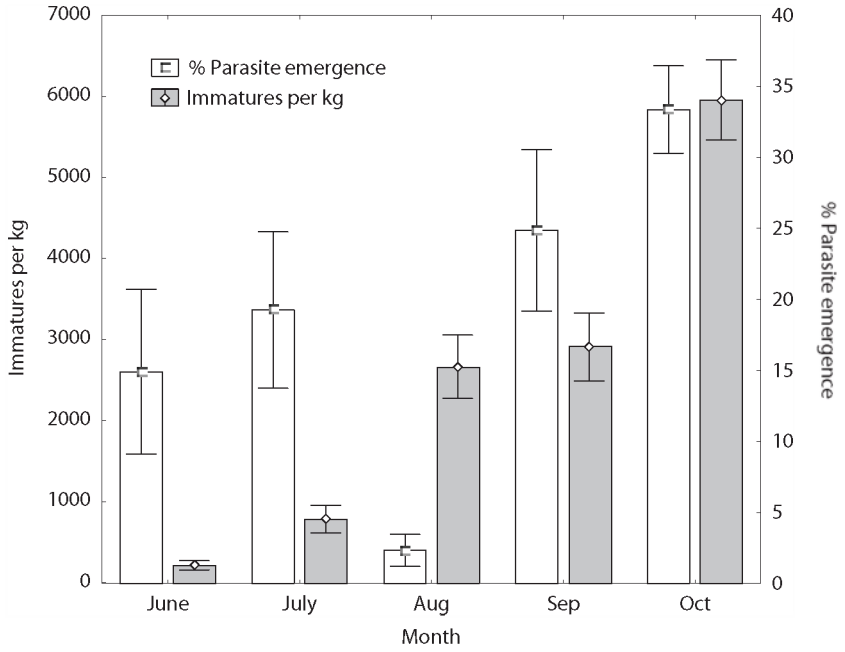

Figure 10. Percentage of parasite emergence from isolated *Hydrellia pakistanae* pupae collected from small ponds in Lewisville, Texas. Also shown is the corresponding number of immatures per kg. Note that parasitism roughly follows population increases with highest immature number and highest parasitism occurring during the latter part of the growing season (unpublished data, C. Snell and M. Grodowitz).

since higher-weight females (typically indicative of having higher egg production) occurred in samples containing higher protein and less crowding as indicated by lower fly emergence. More research is needed to understand the complexities of nutritional composition on *Hydrellia* population increases and associated impact on hydrilla.

One of the more important determinants of population size may be whether or not insects were released into a specific area. Significantly ($p < 0.05$) higher

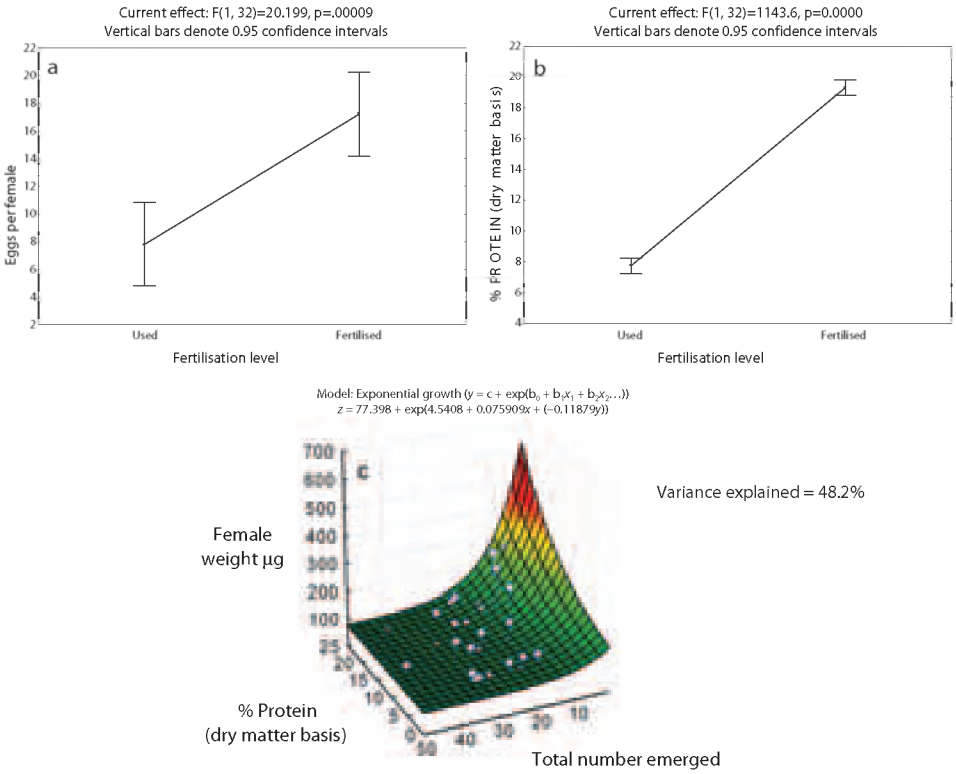

Figure 11. Eggs per female (a) and percentage protein (b) for hydrilla grown in sediment where plants were repeatedly grown in an effort to remove excess nitrogen (Used) and sediment amended with nitrogen (Fertilized; Grodowitz & McFarland 2002). Also, three-dimensional surface plot showing relationship between percentage protein and total number of adults emerged versus female *Hydrellia pakistanae* weight in µg (c).

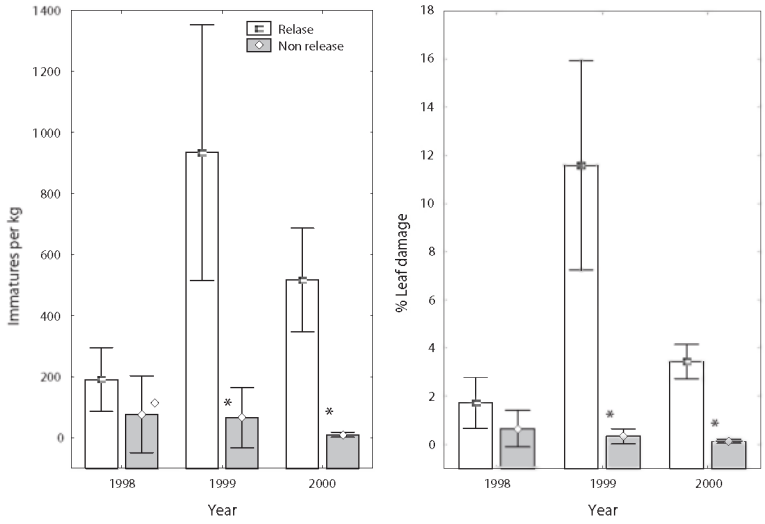

Figure 12. Number of immatures of *Hydrellia pakistanae* per kg and percentage leaf damage for release and non-release sites sampled in 1998, 1999, and 2000. Columns separated by an asterisk are significantly different at $p < 0.05$ level. Note that higher number of immatures and percentage leaf damage occurred for release sites.

numbers of immatures and associated leaf damage were found at sites where immatures were released previously (Fig. 12). This occurred for sites sampled in 1999 and 2000. The difference may be quite dramatic, as in 1999 when immatures were 9-fold greater and leaf damage was 11-fold higher in release versus non-release sites.

It is apparent that additional releases are needed to bolster *Hydrellia* spp. populations. However, since populations have remained low in the field, it has been next to impossible to collect high enough numbers at field sites for adequate additional releases. Also, mass-rearing under greenhouse conditions, while adequate, is prohibitively expensive with each individual costing upwards of US$0.50 (Freedman *et al.* 2001). Recently, a mass-rearing facility developed using small ponds at LAERF has allowed the production of large numbers of flies at low cost. Over the last two growing seasons, over one million flies were produced and released at sites in Texas and Florida at a cost of less than US$0.02 per individual. Such capabilities should allow the release and subsequent increase in field populations at many hydrilla sites across the US.

In summary, it appears that the introduced *Hydrellia* spp. are capable of severely impacting hydrilla. While more research is needed to understand possible population regulators, the use of these agents should be considered operational and used as part of any hydrilla management program.

Acknowledgements

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Development of a supply–demand model to evaluate the biological control of yellow starthistle, *Centaurea solstitialis*, in California

Andrew Paul Gutierrez,[1] Michael J. Pitcairn[2] and Nada Carruthers[3]

Summary

Centaurea solstitialis Asteraceae (yellow starthistle) is an invasive weed from southern Europe that has naturalized throughout western North America. To date, six seed-head insects have been released into western North America. Of these, five have established and three are widespread. New natural enemies that attack the root, stem, and leaves, are currently being examined for use as additional biological-control agents. A physiologically based simulation model of plant growth and population dynamics was constructed to investigate the combined impact of these natural enemies, the presence of positive and negative interactions, and the potential for successful control of this weed. The model examines the energy dynamics of the two-trophic system (plant and natural enemies) and assesses of the relative losses due to different herbivores as modified by weather, plant community competition, and other environmental factors. A basic premise of this approach is that all organisms face the same problems of resource (energy) acquisition and allocation. The model assumes the following energy allocation priority: first to respiration (maintenance costs), then to reproduction and, if assimilate remains, then to growth. The shapes of the acquisition functions and maintenance costs are similar, with the net being the amount of resources available for allocation. These analogies allow us to the use of the same model to describe the dynamics of all interacting species. The importance of this modelling paradigm for evaluating the effectiveness of natural enemies has been demonstrated for several insect biological-control systems (e.g. cassava mealy bug, coffee berry borer, cassava green mite and several whitefly species). This is the first time this modelling paradigm has been used for a weed biological-control system.

Keywords: *Centaurea solstitialis*, simulation model, supply–demand, yellow starthistle.

Introduction

Yellow starthistle (*Centaurea solstitialis* L.) is a noxious invasive weed of Eurasian origin. It infests rangeland, grain fields, roadsides, orchards, natural areas, and wasteland. Once established, yellow starthistle forms impenetrable stands that displace more desirable forage by reducing soil moisture to extremely low levels and by shading out other plants (DiTomaso & Gerlach 2000). It is toxic to horses, but not to cattle or sheep, causing a neurological disorder (equine nigropallidal cephalomalacea) that may cause death if feeding is extensive (Cordy 1978). The plant is an important contaminant of commercial seed and hay, and often establishes itself in alfalfa and cereal grains. The long sharp spines on the flower head are a bane to hikers and greatly reduce the recreational value of grasslands and foothills areas throughout western North America (DiTomaso & Gerlach 2000).

Yellow starthistle was introduced to California in the mid-1800s, probably as a contaminant of alfalfa seed, and since then has successfully colonized much of California (Maddox *et al.* 1985). A recent survey by the California Department of Food and Agriculture in

[1] Division of Ecosystem Sciences, University of California, Berkeley, CA 97410, USA.
[2] California Department of Food and Agriculture, Biological Control Program, 3288 Meadowview Road, Sacramento, CA 95832, USA.
[3] USDA–APHIS–CPHST, Western Regional Research Center, 800 Buchanan Street, Albany, CA 94710, USA.
Corresponding author: Michael Pitcairn <mpitcairn@cdfa.ca.gov>.

2002 found yellow starthistle present in 56 of 58 counties and infesting over 5.6 million hectares (14 million acres) (Pitcairn unpublished data).

Yellow starthistle has been a target for biological control since the 1960s. In total, six insects have been released in California from 1969 through 1992 (Turner *et al.* 1995), and five (three weevil and two fly species) have became established; three are widespread (Table 1). All attack the flower heads and reduce seed production by killing young flower buds or feeding directly on the ovariole tissues and developing seeds. In addition, an unintentionally introduced exotic fly *Chaetorellia succinea* (Costa), which has a strong affinity for yellow starthistle seed heads, has become widespread throughout California (Balciunas & Villegas 1999). Like the other seed-head insects, the larvae of this fly feed on developing seeds in immature seed heads.

Field studies estimate that the attack by these seed-head insects can reduce seed production by as much as 60% (Pitcairn *et al.* 2000, Pitcairn & DiTomaso 2000). Yet, despite this amount of seed destruction, a decline in plant abundance has not been observed (Pitcairn *et al.* 2002). Additional biological-control agents appear to be needed before a reduction in plant density occurs.

Since 1996, there has been a renewed effort to obtain additional biological- control agents for yellow starthistle. A rust disease, *Puccinia jaceae* var. *solstitialis*, which has been studied for several years, has been approved for introduction in 2003. The disease forms pustules on the leaves of the rosette and along the stem of the bolting plant. In the laboratory, it was observed to suppress growth, but its impact on yellow starthistle seed production under California field conditions is unknown. In addition, recent foreign exploration in Turkey and southern Russia has identified at least eight new natural enemies for consideration as biological-control agents: a phytophagous mite and seven insects that attack seedlings, rosettes, root, stem, and seed heads (Table 2).

The objective of classical weed biological control is to introduce the fewest number of exotic natural enemies needed to achieve control. Each release of a biological-control agent adds an increment of unanticipated environmental and economic risk, while also increasing the size, complexity, and redundancy of the plant–herbivore system (McEvoy & Coombs 1999). Before any natural enemy is approved for introduction as a biological-control agent, it must undergo a series of host-specificity tests to determine the risks of attack to non-target plant species. This activity is expensive, requiring at least 3–5 scientist years per control agent.

We are at an opportune point in time to evaluate the suite of newly discovered natural enemies for yellow starthistle. Release of the rust disease was anticipated for spring 2003, but its impact is unknown. For the eight new natural enemies, host testing has begun only for the rosette weevil, *Ceratapion bassicorne* (L. Smith, USDA–ARS, pers. comm.). If we could evaluate and

Table 1. Flower-head insects introduced against yellow starthistle in California.

Species	First year of release	Status
Urophora jaculata Rondani	1969	Failed to establish
Urophora sirunaseva (Hering)	1984	Widespread
Bagasternus orientalis (Capiomont)	1985	Widespread
Chaetorellia australis Hering	1988	Locally established
Eustenopus villosus (Boheman)	1990	Widespread
Larinus curtus Hochhut	1992	Locally established
Chaetorellia succinea (Costa)	Accidental introduction (probably 1991)	Widespread

Table 2. List of potential natural enemies being considered for use as biological-control agents against yellow starthistle.

Scientific name	Common name	Mode of attack
Puccinia jaceae var. *solstitialis*	Rust fungus	Attacks rosette leaves and bolting stems
Ceratapion bassicorne	Rosette weevil	Attacks rosettes and bolting plants
Psylloides chalcomera	Flea beetle	Attacks rosettes and bolting plants
Aceria spp.	Blister mite	Deforms and kills branch tips
Tingis grisea	Lace bug	Attacks leaves and stems
Cyphocleonus morbilosus	Root weevil	Larvae tunnel in roots
Larinus filiformis	Seed-head weevil	Larvae feed on developing seeds
Phytoecia humeralis	Stem beetle	Larvae feed in stems
Lixus scolopax	Stem weevil	Larvae feed in stems
Botanophila turcica	Rosette fly	Attacks young seedlings and rosettes

identify those natural enemies most effective for control prior to testing and introduction, we could substantially lower costs and reduce environmental risks.

To address this issue, we are developing a computer model to simulate the growth and population dynamics of yellow starthistle along with its current and potential natural enemies in California. The objective is to identify life-cycle transitions that are vulnerable to attack and influential on population growth and seed production.

Methods

Physiologically based simulation models have been developed for several agricultural crops: alfalfa (Gutierrez *et al.* 1984), cotton (Gutierrez *et al.* 1977, 1991), Grape (Wermelinger *et al.* 1991), coffee (Gutierrez *et al.* 1998) and cassava (Gutierrez *et al.* 1988, 1999), among others. These models have proven invaluable in helping us understand the trade-offs between herbivore damage and crop yield and how these trade-offs are modified under different environmental conditions (e.g. weather, pest control options and fertilization). The basic premise of this approach is that all organisms face the same problems of resource (energy) acquisition and allocation (Gutierrez 1996). The model assumes an energy allocation priority: first to respiration, then to reproduction and, if assimilate remains, to growth. The shapes of the acquisition functions and maintenance costs are similar across species with the net being the amount of resources available for allocation. These analogies allow us to use the same model to describe the dynamics of all interacting species.

Each organism is assumed to try to satisfy a physiologically based demand for resources via the process of imperfect search, causing the supply obtained to always be less than the demand. Growth, reproduction and survival rates are reduced from the maximum by the supply/demand ratio. In the model, only the units and interpretation of the flow rates differ among species. Hence, in this paradigm, biotic and abiotic factors affect either the supply (production) or the demand (sinks, e.g. fruits) side of the supply/demand ratio. In some cases, both sides may be affected. This supply–demand paradigm simplifies model development and allows assessment of impact to the plant and compensation by the plant in the face of herbivore damage.

Important *supply-side herbivores* include defoliators, sapsuckers, spidermites, nematodes, diseases and others. Defoliators attack leaves and may cause wound-healing losses, but the damage or compensation depends on the age of leaves attacked and the loss rate. The rust fungus kills leaf cells that intercept light, reducing the amount of photosynthesis. Stem-borers may slow the photosynthetic rate by reducing the translocation of water and nutrients, and in the extreme may kill whole plants. These herbivores tend to reduce plant vigour, induce developmental delays, and reduce fecundity.

Demand-side herbivores attack fruit (e.g. sinks), reducing their demand, and reduce seed yield directly. In some cases, the damage may cause premature death of fruit that can alter the demand for photosynthate. Most plants have a reproductive surplus that allows for varying degrees of compensation for such damage. Of crucial importance in compensation is the time and energy lost in the death of fruit. Little time and energy may be lost when small flower buds are killed as sufficient replacement buds may be produced for compensation. Attacks on older fruit may result in considerable losses in time and energy often, precluding compensation.

Dry matter data on growth by yellow starthistle (unpublished) suggest that the allocation of biomass to seed production is relatively large (40–50% of above-ground biomass), and the number of seeds per seed head is large (28–30 on average). All of the natural enemies currently established in California for control of yellow starthistle destroy some or all of the seed in attacked heads. The weevil *E. villosus* affects both sides of the demand ratio. Adult weevils feed on young developing flower heads that die and fail to develop while the larvae feed directly on developing seeds in heads produced later. Field observations show that adult feeding by this weevil will destroy 50–80% of the first crop of flowers and effectively delay peak flowering by 3–4 weeks (unpublished data). Some of the new herbivores considered for introduction may kill the whole plant, affecting the ability of survivors to compensate. These are important dynamics that arise in the yellow starthistle model.

A version of a physiologically based model of the energy dynamics of yellow starthistle and the seed-head feeders has been developed and initial results look encouraging (Fig. 1), but refinement of some model parameters is still required. An advantage of this modelling approach is that the information needed to parameterize the model can be quickly identified and the information gathered efficiently. Good information is available from extensive field studies on yellow starthistle physiology (Maddox 1981), seed germination (Callihan *et al.* 1992, Joley *et al.* 1992, 1997), pollination biology (Maddox *et al.* 1996) and reproductive phenology (Roche *et al.* 1997, M.J. Pitcairn, unpublished data). Initial studies on dry matter allocation in yellow starthistle were performed by A.P. Gutierrez and Hami (unpublished data), while data on plant survivorship and seed compensation under field conditions have been gathered by Pitcairn (unpublished). Field studies of the flowering pattern of yellow starthistle and the attack rates of *Bagasternus orientalis*, *Eustenopus villosus*, *Urophora sirunaseva* and *Chaetorellia succinea* have also been completed (Pitcairn & DiTomaso 2000, Woods *et al.* 2002). What is missing, however, is information on the developmental rate and reproduction as a

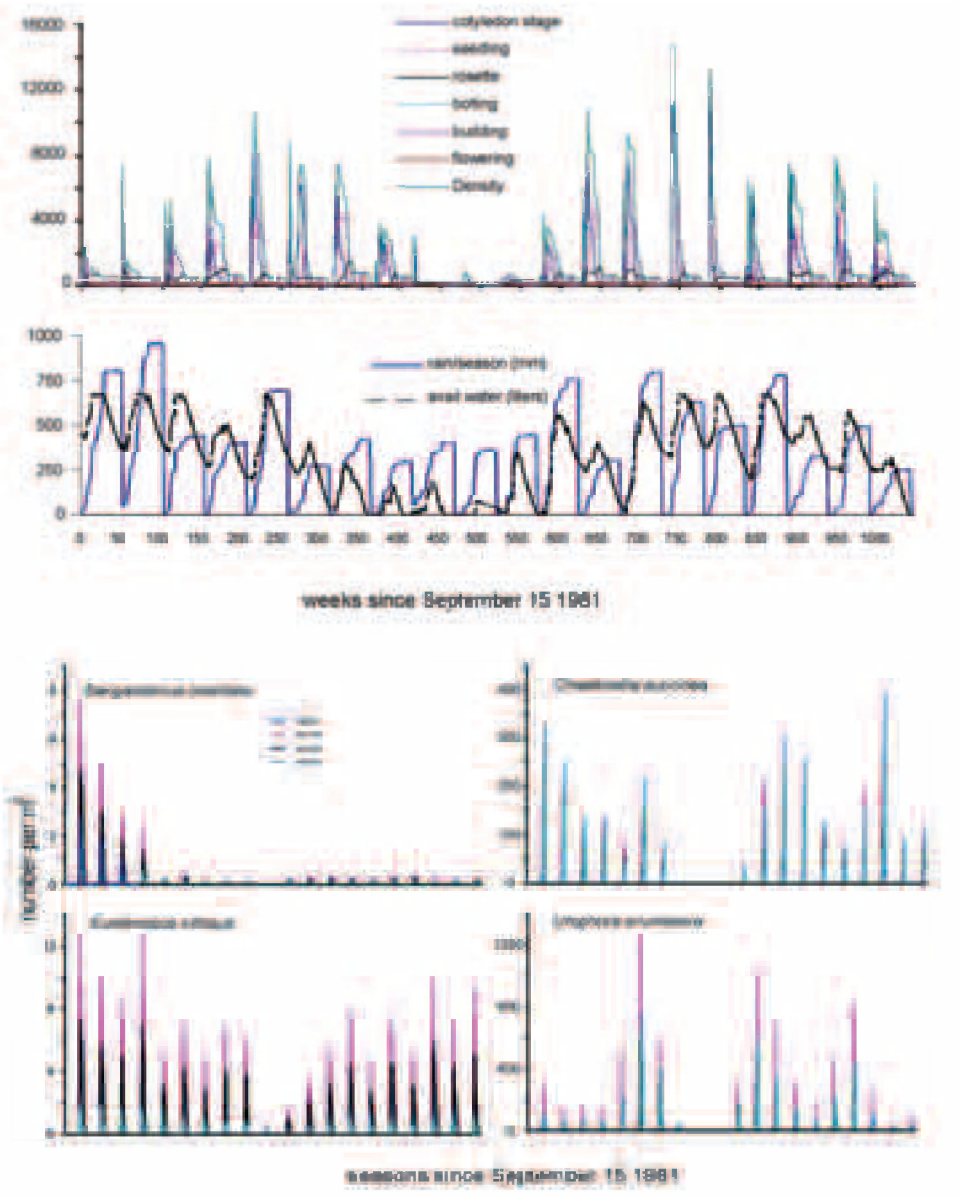

Figure 1. Twenty years (seasons) of simulation of yellow starthistle at Davis with *Eustenopus villosus*, *Bagasternus orientalis*, and *Urophora sirunaseva* and *Chaetorellia succinea* and competition from annual grasses and early-season mortality from an endemic rust: (a) phenology of plant stages, (b) total seasonal rainfall (mm) and available soil moisture above the wilting point (litres per 2 m^3), (c) the dynamics of *B. orientalis* and *E. villosus*, *C. succinea* and *U. sirunaseva*.

function of temperature for three of the four insects now widely established. Nada Carruthers (United States Department of Agriculture–Agricultural Research Service; USDA–ARS) has commenced these studies for *C. succinea* and is cooperating in model development. Information on the other three insects is still lacking.

Overview of the yellow starthistle model

The underlying metabolic pool paradigm and mathematics for the development of physiologically based model is summarized in Gutierrez (1996). The *yellow starthistle* systems model consists of *ns* = 12 linked "*functional populations*" (*n* = 1,...,ns). Yellow starthistle and the natural enemies are poikilotherms, hence time and age are in degree-day units. Nonlinear developmental rate models could be used, but current data were insufficient for these purposes. The plant model consists of age-structured mass dynamics models for leaf (*n* = 1), stem (2) and root (3) tissues as well as fruit mass (4) and numbers (5) (Gutierrez *et al.* 1991) that are linked via the metabolic pool model that predicts daily photosynthetic rates under various plant states and weather and its allocation in priority order to respiration, conversion costs, reproduction and vegetative

growth (see Gutierrez and Baumgartner 1984, Gutierrez *et al.* 1991). Age-structured models are also used to simulate the number dynamics of four introduced natural enemies: *B. orientalis*, *E. villosus*, *C. succinea*, and *U. sirunaseva*. In addition, models incorporating the attack by a hypothetical stem-borer and a rust disease are included. The stem-borer was included because several natural enemies, such as the beetles, *Ceratapion bassicorne* and *Psylloides chalcomera*, and the fly *Botanophila turcica*, have been identified as potential control organisms for yellow starthistle (Table 2). The effects of the pathogenic rust were included as a convex function of daily rainfall intensity. Current information on the biology of the potential biological-control agents is sparse; hence the values in our model are rough estimates. It is hoped that the necessary information on biology will be obtained in studies performed prior to host-specificity testing. Depending on their biology, the insect species emerge in the spring at different times from winter diapause and may produce one or more generations. Diapause occurs over winter, and emergence the following spring making the link between seasons. Here we model the emergence pattern across all cohorts using a Gompertz function (cf. Gutierrez *et al.* 1977). Adults emerging from diapause the previous season are the seed for this year's population.

The model simulates the time-varying mean and variance of developmental times of each cohort in each population throughout the season, tracking the number of individuals in all age classes for each species. One may view all of the seed-head insects as parasites with super and multiple parasitism occurring. In cases of multiple parasitism, *E. villosus* is dominant to all other species, killing them in the process of its development, and the trephiid fly, *C. succinea* appears to be partially dominant to *U. sirunaseva* (unpublished data). Though not too common, cases of successful development of multiple species (*B. orientalis*, *C. succinea*, and *U. sirunaseva*) in the same seed head has been observed (unpublished data).

Conclusion

The plant model integrates the effects of weather and the damage caused by introduced natural enemies, competition with grasses, and the effects of a pathogenic rust. The model is modular in structure and hence combinations of species may be run with simple true–false instructions in the input file over several years and in different ecological zones.

Modelling plant population dynamics is increasingly used to identify weed vulnerabilities and predict the effect of biological-control agents on the population size of the target weed (e.g. *Sesbania punicea* (Hoffman 1990), *Sida acuta* (Lonsdale *et al.* 1995), *Carduus nutans* (Shea & Kelly 1998), *Cytisus scoparius* (Rees & Paynter 1997)). Experience with these models has allowed investigators to make concrete recommendations concerning control measures. Our model validates the observation that herbivores attacking capitula at the rates currently observed in California are not likely to be efficacious. Our analysis suggests that herbivores and pathogens that kill the host plant when it has expended the maximum energy towards growth or that greatly reduce seed production below seedbank replenishment rates may prove to be more successful.

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The mirid *Eccritotarsus catarinensis* is an effective agent against water hyacinth in some areas of South Africa

M.P. Hill[1] and I.G. Oberholzer[2]

Summary

The sap-sucking mirid *Eccritotarsus catarinensis* (Carvalho) (Heteroptera: Miridae) was released against water hyacinth in South Africa in late 1996. This insect has a short generation time and populations can rapidly increase. Adults and nymphs feed gregariously on the leaves of water hyacinth, causing severe chlorosis and stunting of the plants. This agent has been released at 22 sites throughout South Africa. The mirid has established at seven of these sites, failed to establish at eight sites, and the remaining seven sites have not been evaluated. Furthermore it has independently dispersed to at least two additional sites. Although the mirid has established at three high-elevation sites (above 1000 m), which are characterized by cold winters with frost, it is most effective against water hyacinth in more subtropical conditions. At a site in a subtropical region of South Africa, near Durban, KwaZulu-Natal Province, the mirid reduced the infestation of water hyacinth on a 10 ha dam from 100% to less than 10% within 18 months. Although populations of the mirid are negatively affected by wind and rain, it is still an effective agent in tropical and subtropical areas, especially when used in conjunction with the other five natural enemy species released on water hyacinth in South Africa.

Keywords: biological control, Miridae, water hyacinth.

Introduction

The success of biological control initiatives undertaken against water hyacinth in South Africa has been variable, despite the establishment of six natural enemy species (five arthropods and one pathogen) between 1974 and 1996 (Julien and Griffiths 1998). By contrast, successful biological control has been achieved in a relatively short time frame (four years) on Lake Victoria in Uganda, and in Papua New Guinea (using only the two insect agents *Neochetina eichhorniae* Warner and *N. bruchi* Hustache) (Julien 2001). These variable results have been attributed to cold winter temperatures, nutrient enrichment of the aquatic ecosystems and interference from (poorly) integrated control operations (Hill and Olckers 2001). These considerations have prompted the search for additional agents that might be more effective under temperate conditions.

The most recent agent released against water hyacinth in South Africa was the sap-sucking mirid *Eccritotarsus catarinensis* (Carvalho) (Hill *et al.* 1999). This agent was released in 1996 and yet very little quantitative post-release evaluation has been undertaken on the mirid. Here we report on the releases, establishment and impact of the mirid on water hyacinth populations in South Africa.

Materials and methods

The mirids were reared on actively growing water hyacinth plants at a mass-rearing facility in Pretoria, South Africa. Initial studies (Hill *et al.* 2000) showed that the mirids established more successfully if they were released on plants containing eggs, nymphs and adults. Therefore, this agent was released on plants. At least 15 plants were released per site and each plant contained between 200 and 300 mirids (nymphs and adults, the number of eggs were not counted). The

[1] Department of Zoology and Entomology, Rhodes University, PO Box 94, Grahamstown 6140, South Africa.
[2] Weeds Division, ARC-Plant Protection Research Institute, Private Bag X 134, Pretoria 0001, South Africa.
Corresponding author: M.P. Hill <m.p.hill@ru.ac.za>.

plants containing the mirids were then placed into a water hyacinth mat at 22 field sites throughout South Africa.

Results

Establishment of the mirids was confirmed at seven of the 22 release sites, and has not established at eight sites (one of these sites was sprayed with herbicide and another was washed away by severe flooding), and the remaining seven sites have not been evaluated (Table 1). Furthermore, it has independently dispersed to at least two additional sites. Although the mirid has established at three high-elevation sites (above 1000 m), which are characterized by cold winters with frost, it is most effective against water hyacinth in more subtropical conditions.

Clairwood Quarry

Clairwood Quarry is a 10 ha dam situated just south of the city of Durban, KwaZulu-Natal Province (29°54'15"S 30°57'44"E). The quarry is protected on the south-western side by a large rock face which protects the water hyacinth from the prevailing weather fronts. The quarry is fed by run-off from an informal settlement, which is highly eutrophic. Between 1995 and 2000, the quarry was completely covered by water hyacinth (M.P. Hill, pers. obs.). In July 2000, approximately 3 t of water hyacinth infected with the mirid were collected from Hammarsdale Dam and deposited into the quarry. By late August 2000, large brown patches appeared in the water hyacinth mat as a result of heavy feeding damage by the mirids. In March 2001, the entire mat of water hyacinth had broken up and sunk, leaving a small fringe of the weed around the edge of the quarry. This fringe was heavily infected by the mirid. Although there have been fluctuations of the water hyacinth mat at this site, it remains under effective biological control.

The mirids have also dispersed, presumably from the quarry, to another site (Bamboo Canal) some 15 km away, where they are also bringing the weed under control.

Table 1. The release site of the mirid *Eccritotarsus catarinensis* on water hyacinth in South Africa.

Site	Co-ordinates	Description	Date	Establishment
KwaZulu-Natal Province				
Clairwood Quarry	29°54'15"S 30°57'44"E	Subtropical	2000	Yes
Nseleni River	29°48'19"S 30°39'53"E	Subtropical	1996	Yes
Hammarsdale Dam	29°48'20"S 30°39'56"E	Subtropical	1996	Yes
Umlazi River	29°46'05"S 30°29'16"E	Subtropical	1997	Site not revisited
Eastern Cape Province				
Yellowwoods River	32°53'03"S 27°28'19"E	Temperate	1996	No
New Years Dam	33°17'40"S 26°07'20"E	Temperate	1996	No
Kubusi River		Temperate	1999	No
Umtata River	31°35'37"S 28°48'53"E	Temperate	2000	Site not revisited
Western Cape Province				
Breede River		Cool Temperate	1996	No
Robertson		Cool Temperate	1997	Site not revisited
Wolseley		Cool Temperate	1997	Site not revisited
Zeekoeivlei	34°04'40"S 18°31'23"E	Cool Temperate	1999	No
Westlake	34°04'56"S 18°24'55"E	Cool Temperate	1999	Site not revisited
Pardeneiland	33°47'26"S 18°30'27"E	Cool Temperate	2000	Site not revisited
Free State Province				
Schuttes Eiland	26°54'45"S 27°25'20"E	Temperate	1996	Yes
Vaalhardts Weir		Temperate	1999	Site not revisited
North West Province				
Crocodile River	25°39'42"S 27°47'39"E	Warm Temperate	1996	Yes
Gauteng Province				
Bon Accord Dam	25°38'15"S 28°11'01"E	Warm Temperate	1996	Yes
Delta Park		Temperate	1999	No
Marlua Sun Casino		Warm Temperate	1999	Site sprayed
Mpumalanga Province				
Englehardt Dam	23°50'21"S 31°38'14"E	Subtropical	1997	Site Flooded
Yamorna Weir		Subtropical	1999	Yes

Discussion

On its own, the mirid is unlikely to bring water hyacinth under complete control at many sites, especially the more temperate ones. However, in conjunction with the other agents released against the weed, it can be an effective control agent. It appears that the mirid is thermally limited in cooler regions (Coetzee, unpublished data), and is therefore far more effective in tropical and subtropical areas. The success at Clairwood Quarry can be ascribed to the large size of the releases, and the fact that the water hyacinth mat is protected from wind and rain by the quarry wall. The lack of interference from other control options also certainly aided the biological control at the quarry. As Ueckermann and Hill (2000) showed, many of the herbicides used in the control of water hyacinth were toxic to this agent.

The mirid has also been released in Zimbabwe, Zambia, Malawi, Benin and China. Of these releases, establishment has only been recorded on Chiwembe Dam just outside Blantyre, in Malawi.

Clearly, further quantitative post-release evaluations are required to accurately quantify the impact of this agent on water hyacinth, its interaction with the other agents released against this weed, and the link between eutrophication, water hyacinth growth and biological control.

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How important is environment? A national-scale evaluation of a seed-feeding beetle on parkinsonia, a widely distributed woody weed

Rieks Dekker van Klinken[1]

Summary

Environment is an important factor influencing the success of biological control agents, yet its role is still poorly understood. In this paper, I present preliminary results relating to the evaluation of the seed-feeder *Penthobruchus germaini* (Bruchidae) on the woody weed parkinsonia (Mimosaceae: *Parkinsonia aculeata*) across different climatic zones and habitats in northern Australia. The preliminary data and analyses show considerable differences between environments in plant ecology, insect activity and egg parasitism rates, all of which influence total seed loss to predation. It is important to take environmental variation into consideration when designing release and evaluation programs, and when selecting new agents that are to target specific environments. Releases of agents offer valuable opportunities to test and improve our ability to prioritize agents for priority target environments.

Keywords: biological control, Bruchidae, Mimosaceae, legumes, agent prioritization.

Introduction

There is an increasing need for biological control agents that will work in specific environments. Environment-specific agents are required when weeds only occur in a fraction of their potential range (e.g. *Mimosa pigra* in Australia; Lonsdale *et al.* 1995), are widely distributed but are only a high priority target for biological control in specific environments (e.g. parkinsonia), or are under effective management in only parts of their distribution (e.g. Noogoora burr; van Klinken & Julien 2003). The identification of potential biological control agents that will perform well in target environments is therefore a major challenge, and requires a detailed understanding of how environment can influence agent success.

A large study was initiated in 2000 to improve our understanding of the interaction between the environment, weed ecology and various control strategies in northern Australia using the woody weed parkinsonia (Mimosaceae: *Parkinsonia aculeata* L.) as a model system. Parkinsonia is a good system to explore these relationships because it grows in diverse environments, from the arid interior to the wet–dry tropics, and in wetland, riparian and upland habitats. One component of the research is a comparative study of the ecology of parkinsonia, and the performance of existing seed-feeding biological control agents, across the diverse environments in which parkinsonia grows. To achieve this,, a network of permanent, representative study sites were set up throughout the major climatic regions and habitats in northern Australia, and monitored at 4–6 weekly intervals for 2–3 years.

In this paper, I present preliminary data on the national evaluation of the seed-feeding bruchid, *Penthobruchus germaini* (Pic.) (Bruchidae), comparing its performance on parkinsonia in different habitats and climate regions. Of the two seed-feeding agents released against parkinsonia, *P. germaini* is the only one that has become widely established and abundant. The overall objective of the evaluation work was threefold:

- to compare seed losses from predation across different environments

[1] CSIRO Entomology, Long Pocket Laboratories, 120 Meiers Road, Indooroopilly, Queensland 4068, Australia. <rieks.vanklinken@ csiro.au>.

- to determine what factors are responsible for different seed mortality rates in different environments; specific factors considered in this study included host-plant phenology, abundance and phenology of the biological control agent, and parasitoid and predator activity
- to consider whether we can use a better understanding of factors affecting agent performance to select new agents that will do well in the specific environments where they are needed most.

Materials and methods

The plant

Parkinsonia aculeata is a perennial shrub or tree native to the neotropical region. Pods are indehiscent and contain up to 10 seeds each (average *ca* 1.6). Seeds have hard-seeded dormancy and are released if pods decay or are damaged. Pods are relatively unpalatable, and natural dispersal is probably primarily by water, or in mud spread by animals.

The insects

Penthobruchus germaini was introduced into Australia as a biological control agent in 1995 (Julien & Griffiths 1999) and was widely established by at least 1998. Females only oviposit onto mature (or very nearly mature) pods and, rarely, directly onto mature, naked seeds. When there is a choice, *P. germaini* prefers ovipositing on tree-pods over ground pods (van Klinken, unpublished data.). Eggs hatch within 8–9 days, and larvae drill down through the pod and then into the seed. Pupation occurs within the seed and adults emerge by cutting a hole through the seed coat and through the pod wall. Generation times at 30°C are 35–45 days (Briano *et al.* 2002), and several generations can occur in a single season. Although more than one larva can enter a seed, only one adult ever emerges.

Sites

Replicated, permanent study sites were selected to represent the diversity of Australian environments in which parkinsonia is a weed. Data acquisition is costly, and sites were therefore selected as parsimoniously as possibly. Known infestations that could be accessed regularly by trained staff were listed. Sites were chosen along the north–south rainfall gradient from Darwin to Alice Springs and they represented most of the inland climate types in northern Australia (Figure 1). Climatic patterns that are unique to parts of eastern Queensland (in part the result of the Great Dividing Range) were represented by sites there. In each climatic region, sites were selected to represent the main habitats in which parkinsonia occurred. In this paper, I present data from three regions (Table 1, Fig. 1). Climatic data for each region are summarized in Figure 2.

Sampling

In this paper, I present data comparing upland and wetland habitats at semi-humid sites, and data comparing semi-humid, semi-arid and arid sites (upland and riparian sites only; Table 1). The habitat comparison was conducted in 2000–01 (van Klinken, unpublished data). The climate comparison work is ongoing. In this paper, I present pod maturation, pod fall and insect activity data for tree pods (oviposition,

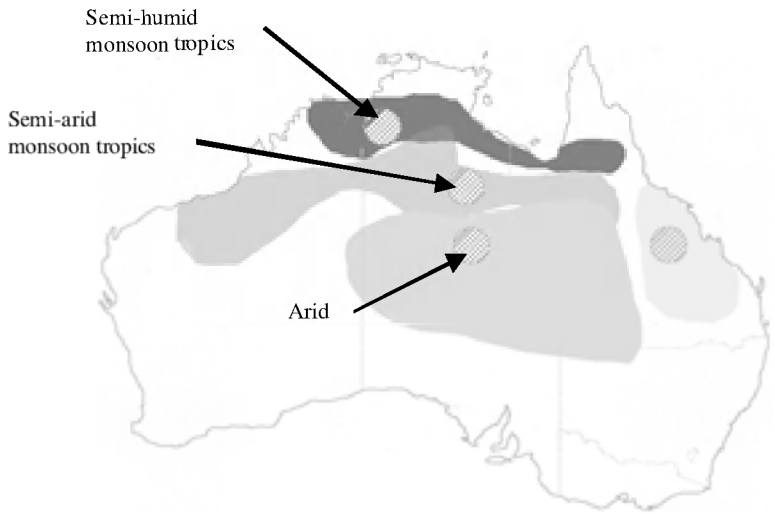

Figure 1. Location of each study site (circles), and the climatically similar areas (shading) which they represent. Shaded areas incorporate all meteorological stations that have a climate match within CLIMEX of 0.7 or more of the study sites (Sutherst & Maywald 1999; rainfall and temperatures equally weighted). Only the three regions for which data are presented in this paper are labelled.

egg parasitism and seed consumption) collected at 4–6 weekly intervals during the 2001–02 podding season. A more complete analysis was awaiting completion of field surveys in 2003 and processing of pod loss data.

Gauze litter traps (diameter 58 cm) were placed under 10 mature, healthy trees at each site, emptied on each visit, and the number of mature seeds within pods subsequently counted. Pod abundance was ranked visually as "no pods", "light" (1–200 pods) and "medium to heavy" (> 200 pods) from a single point 5 m from each of the 10 trees. Data on seed-feeder activity were obtained on each visit by collecting 40 pods from the tree, freezing them within 24 hours of collection to halt development, and examining them individually under the microscope to determine egg abundance, egg parasitism and seed consumption by *P. germaini*.

Results

Habitat comparisons in semi-humid region

Pod availability (for predation by *P. germaini*) in each of the habitats was calculated from data on the timing of pod maturation, pod drop and pod loss (e.g. through inundation, or decay of pods; van Klinken, unpublished data.). Most seeds were available between September and February. However, a greater proportion of the total season's seeds were available to seed-feeders, for longer in upland sites than in wetland sites (Fig. 3a). The main reasons for this were that pods matured more synchronously in upland sites, and ground pods were mostly inundated after January at wetland sites.

Insect activity data were collected throughout 2000–01, but only tree-pod data from early in the season when most pods were still available (October) and late in the season when few pods remained (March) is presented (Table 2). Egg incidence was similar between habitats early in the season. By late in the season, egg incidence had increased considerably, and was much higher in upland sites where most seeds had eggs (Table 2). Egg parasitism was high in both habitats and throughout the season (Table 2).

Total seed loss was calculated from relative pod availability (Fig. 3a) and insect activity on both tree and

Table 1. Sites sampled and the climates and habitats which they represent.

Infestations	Climate	Habitats	Coordinates
Victoria River Region (Auvergne Station)	Semi-humid	Upland (4 sites) Wetland (3 sites)	15°26'S 130°20'E
Barkly Tablelands (Banka Banka Station)	Semi-arid	Upland (3 sites)	18°47'S 134°01'E
Central Australia (Alcoota Station)	Arid	Riparian (3 sites)	22°49'S 134°27'E

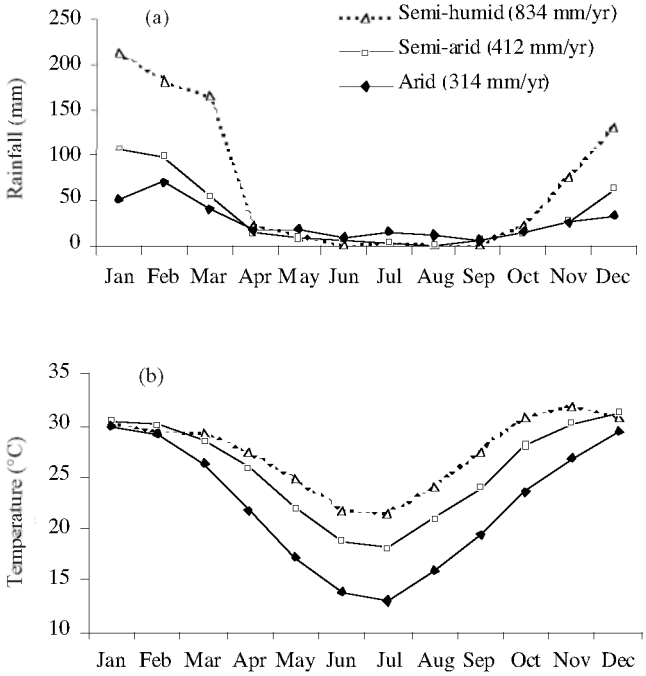

Figure 2. Mean monthly rainfall (a) and temperature (b) in each region. Data are from the closest weather station to field sites within each climatic region.

ground pods (van Klinken, unpublished data.). Total seed loss by the end of the season was twice as high in uplands then in wetlands (Fig. 3b). There was relatively little change in total seed loss after January in either habitat, because relatively few pods were still available for attack after that time (Fig. 3a). The critical factor resulting in differences in total seed loss between habitats was differences in pod availability, not insect activity, which did not differ early in the season when most pods were available.

Climate comparisons

The proportion of trees with pods peaked in November at semi-humid sites, November/December in semi-arid sites and January at arid sites (Fig. 4). Pod maturation was very synchronized at arid sites, in contrast to other regions.

Most pods dropped from October to February, with the main pod drop occurring first at semi-humid sites (*ca* November) and last at the arid sites (*ca* February) (Fig. 5). Pod drop at the semi-arid sites was intermediate, and occurred more gradually. Unfortunately, semi-arid sites were not accessible in January 2001 because of flooding, but better resolution will be obtained in the future. Very few pods remained on trees by late February at any site.

Egg incidence, parasitism rates and consumption rates changed seasonally. Comparative data arejust shown for the period when most pods had matured but were still present on the tree (December at semi-humid and semi-arid sites; January at arid sites) (Table 3). Egg incidence (both as proportion of seeds with eggs and the density of eggs on those seeds) was high at both arid and semi-arid sites and low at semi-humid sites.

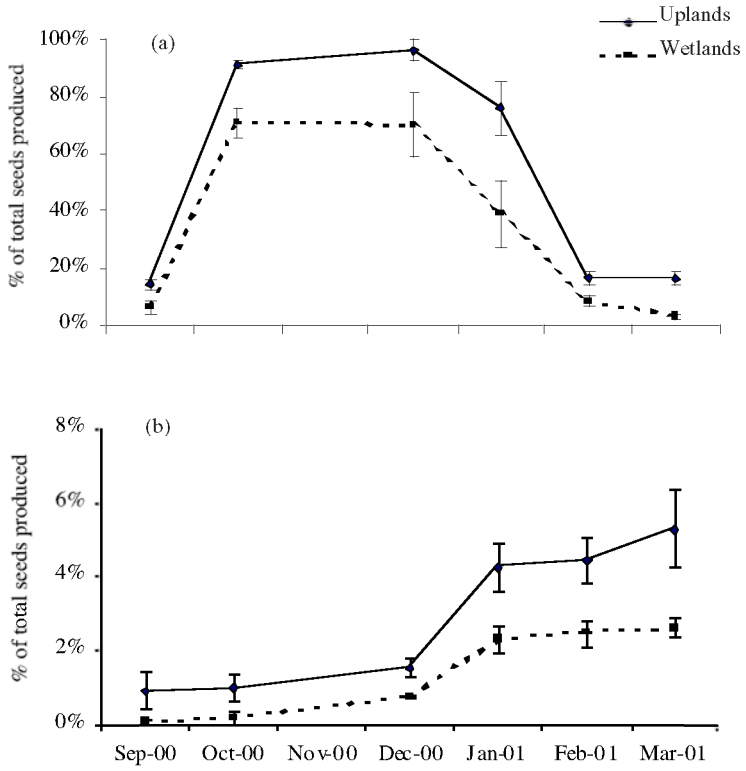

Figure 3. Comparison of pod availability (a) and total seed consumption (b) between habitats in the semi-humid region as a percentage of the total seeds produced in 2000-01 (mean ± SE).

Table 2. Comparison of egg incidence and parasitism rates on tree pods between habitats in the semi-humid region in October, when most pods were available to seed-feeders, and late in the season (March) when few pods remained (mean ± SE).

Habitat	October 2000			March 2001		
	Seeds with eggs	Eggs/seed with eggs	Eggs parasitized	Seeds with eggs	Eggs/seed with eggs	Eggs parasitized
Uplands	19.2 ± 5.2%	1.2 ± 1.1	68.9 ± 2.1%	94.2 ± 4.6%	6.0 ± 1.1	66.3 ± 3.1%
Wetlands	14.1 ± 5.0%	1.3 ± 1.2	86.3 ± 1.8%	57.1 ± 8.7%	2.9 ± 1.2	63.0 ± 6.3%

Conversely, egg parasitism was highest in semi-humid sites and lowest in arid sites. Maximum seed consumption within a sample was very low in semi-humid sites compared with other climatic regions.

Discussion

Penthobruchus germaini has broad ecological requirements as beetle activity was high in all climate regions and in both upland and wetland habitats. However, parkinsonia phenology, and seed-feeder and parasitoid abundance and activity, clearly differ greatly between habitats and climatic regions.

In the semi-humid region, differences in pod-availability between habitats resulted in significant differences in total seed consumption between habitats. Differences in insect activity between habitats was not so important, because they only occurred later in the season when few pods remained. Parasitism rates did not differ significantly with habitat (van Klinken, unpublished data).

In contrast, pod availability, insect activity and parasitism all differed considerably between climatic regions. The true significance of these patterns will become clear once total seed consumption rates are calculated. In any case, egg parasitism is likely to have

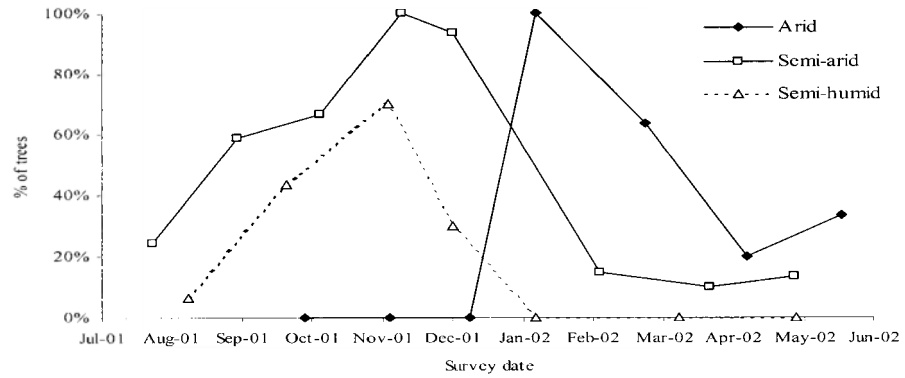

Figure 4. Mean percentage of trees in each climatic region with medium to heavy loads of mature pods.

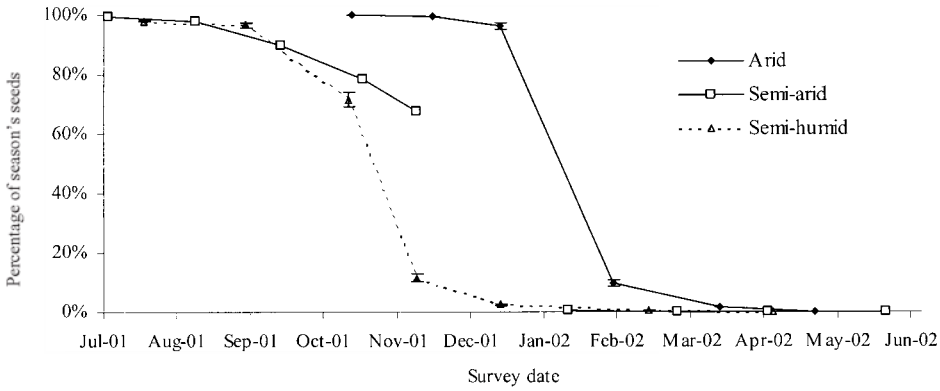

Figure 5. Mean percentage (± SE) of seeds produced in each region during the 2001–02 season that remained on the tree.

Table 3. Comparison between climatic regions of egg incidence, egg parasitism rates and maximum seed consumption rates within a sample. Egg incidence and parasitism rates are for when most pods were available to seed feeders (January at arid sites, December at other sites) (mean ± SE).

Climate	Seeds with eggs	Eggs/seed with eggs	Eggs parasitized	Maximum consumption
Semi-humid	17.4 ± 3.3%	1.1 ± 0.0	67.2 ± 1.3%	5.2% (Jan)
Semi-arid	48.8 ± 6.4%	1.8 ± 0.1	42.8 ± 10.2%	45.4% (Mar)
Arid	64.7 ± 10.8%	1.5 ± 0.1	2.5 ± 0.7%	44.2% (Feb)

an important bearing on consumption rates, especially in the semi-humid region. High parasitism in semi-humid regions is likely to be further exacerbated by relatively low rates of multiple oviposition by the beetle, especially early in the season (Table 3).

Any future agents for parkinsonia will probably need to be targeted at more mesic environments, where parkinsonia appears to pose the greatest threat. If further seed-feeders are to be considered, then their vulnerability to egg parasitism certainly needs to be considered. Species that oviposit on the exterior of pods, such as *P. germaini*, are probably particularly vulnerable. The same applies if considering bruchid seed-feeders in other biological control programs in northern Australia. Another consideration is the duration that seeds are available for predation by seed-feeders. High seed predation is likely to be harder to achieve in wetlands, where the window of time in which seeds are available is briefest. This is particularly true for multivoltine insects, such as *P. germaini*, that are likely to require more then one generation to cause high overall seed mortalities.

Environment (be it climate or habitat) is clearly important in determining seed consumption rates (and ultimately impact and agent effectiveness) through its effect on host availability, the seed-feeder, and parasitoids. It is important to take such factors into consideration when designing release and evaluation programs, especially to ensure the full range of environmental conditions are represented when selecting release and evaluation sites. The effect of environment also needs to be considered more explicitly when prioritizing potential biological control agents to work in specific environments, especially if we are to move on from a "hope for the best" strategy. There have already been some excellent attempts at predicting the interaction between the environment and agent performance (e.g. Scott 1992, Julien *et al.* 1995, McClay 1996, Byrne & Hill 2004), but the science still needs considerable development in this area (van Klinken *et al.* 2003a,b). All releases offer an excellent opportunity to test and improve our ability to make sound predictions of agent performance in specific environments, and therefore to improve our agent prioritization process.

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Biological control of *Carduus* thistles in Virginia—a long-term perspective, three decades after the release of two exotic weevils

L.T. Kok, T.J. McAvoy and W.T. Mays[1]

Summary

One major deficiency in biological control is the lack of long-term post establishment studies on exotic biological control agents. Host specificity concerns and non-target impacts have raised questions about past decisions on the release of biological control agents that are not monophagous. One such example is *Rhinocyllus conicus* released for control of *Carduus nutans*, musk thistle, in 1969 in Virginia. With the release of *Trichosirocalus horridus* in 1974 for control of *C. nutans* and *Carduus acanthoides,* plumeless thistle, we have studied the establishment and impact of these two weevils for about three decades. Five sites with releases of *R. conicus* and *T. horridus* between 1969–75 that were monitored annually until 1991 were revisited the past four years to obtain a long-term perspective of the weevils' impact and current status of vegetation at these sites. Thistle density, associated plant species, and their coverage were determined pre- and post-release of weevils. Data on target and non-target plants, and percent coverage of current vegetation support and reinforce pre-release expectations. *Carduus* thistles are no longer the dominant species at these five sites. *C. nutans* control has been complete, declining from 50% coverage to zero in 2002 (mean density from 6.5/m^2 to 0) in the three sites infested with *C. nutans*. *C. acanthoides* declined from 48.8% coverage to 3.1% (mean density from 9.5 to 0.9/m^2), but persists in 3 of 4 sites, covering < 9% of its most extensive site in 2002. *Cirsium discolor*, the only native *Cirsium* species found in our study, remains in small numbers in two sites despite establishment of both biological control agents. Current dominant plant species at these sites, are orchard grass (*Dactylis glomerata*), Kentucky blue grass (*Poa pratensis*), fescue (*Festuca arundinacea*), broom sedge (*Andropogon virginicus*), and white clover (*Trifolium repens*).

Keywords: *Carduus nutans, Carduus acanthoides, Rhinocyllus conicus, Trichosirocalus horridus,* long-term impact, target and nontarget plants, percent coverage.

Introduction

Biological control has often been touted as a long-term solution to pest problems. By reestablishing the old association of predator/prey in the target area, classical biological control successes have been well documented in a number of weeds (Julien & Griffiths 1999). Yet, in spite of the importance of long-term impact, much of the reports in biological control have been based on short-term studies. This is not due to a lack of interest by the investigators, but more to the lack of support for funding post-establishment studies. Hence, one clear deficiency of biological control literature is the lack of long-term evaluations.

Carduus nutans L. (musk thistle) and *Carduus acanthoides* L. (plumeless thistle) are introduced Eurasian noxious weeds in pastures and rangelands in North America (Kok 1978). Biological control of *Carduus* thistles in the USA was initiated in 1956, and in 1969 the first insect imported from France, *Rhinocyllus conicus* Froel. (Coleoptera: Curculionidae), was released for *C. nutans* control (Dunn 1978). Virginia was one of three states involved in the initial release of *R. conicus* (Kok 1974) and successful establishment

[1] Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0319, U.S.A.
Corresponding author: L.T. Kok <ltkok@vt.edu>.

and impact of the weevil on the target weed soon followed (Kok & Surles 1975). This was followed by release of a second weevil from Italy, *Trichosirocalus horridus* (Panzer) (Coleoptera: Curculionidae), in 1974 for *C. nutans* and *C. acanthoides* control (Kok & Trumble 1979). Both weevils soon became established on *C. nutans* in the state due to additional releases that were made by relocating adult weevils from the initial established sites. Subsequent successes at the release sites have been documented over the years (Kok 1986, 1998, 2001, Kok & Mays 1991).

Two major issues relating to the use of exotic biological control agents that are not monophagous have surfaced. Ecological risk (Simberloff 1986, 1992, Howarth 1991, Simberloff & Stiling 1996, Stiling & Simberloff 1999) and non-target effects (Louda *et al.* 1997, 1998, Johnson & Stiling 1998, Boettner *et al.* 2000, Follet & Duan 1999, Stiling & Simberloff 1999, Louda & Arnett 2000, Wajnberg *et al.* 2001) have received much attention and debate in recent years. Questions about the decision to release *R. conicus* led to the retesting of host specificity of naturalized populations of this insect in the USA (Arnett & Louda 2002). They concluded that its behaviour or host preference has not changed since its original testing (Zwölfer & Harris 1984) in the 1960s.

Another aspect that has received little attention is the long-term benefits of the released biological control agents on target and non-target plants, and the resulting vegetation after successful biological control of the target weed. Data on current vegetation in undisturbed target areas need to be documented to provide answers to this basic question. What is the replacement vegetation as a result of successful biological control of weeds? We attempt to answer this question in Virginia. After about 20 years of continuous monitoring at release sites, we discontinued the annual evaluations in 1991 when vegetation stabilized and had not changed for several years. During the past four years, we revisited five selected sites to examine the status of plant diversity resulting from successful biological control. These sites were infested by *C. nutans*, *C. acanthoides*, or by both weeds, and were selected because they were large farms with little or no management changes or land development.

Materials and methods

Five large farm sites maintained as livestock pasture and infested with one or more thistle species were selected. These sites were selected on the basis of their stability of ownership and availability for our use. They are located in three counties: Farrier and Lester in Giles County, Belspring and Dublin Arsenal in Pulaski County, and Copper Creek in Russell County (Fig. 1). Copper Creek and Farrier were *C. acanthoides* sites, Dublin Arsenal was a *C. nutans* site, and Belspring and Lester were mixed sites with both *C. nutans* and *C. acanthoides*. Of the five sites, *C. nutans* was present in three sites and *C. acanthoides* in four sites. Pre- and post-weevil establishment density of thistle and nontarget plants, and coverage of individual plant species at each site were determined. Cattle grazed all the study sites.

When the study was initiated, baseline data on thistle density and coverage were obtained by counts from 10 marked 16 m × 1 m permanent parallel line transects. This was continued annually until 1991. In 1999 four 50 m long and 1 m wide transects were marked with 1 inch PVC pipes driven into the ground. The four transects were parallel to each other and spaced 10 m apart. Three 1 m^2 plots were located in each 50 m transect for a total of 12 × 1 m^2 plots at each site. These plots were located at the two ends of each transect and in the center of each transect.

In May of each year from 1999 to 2002, the number of plant species and percent cover of each species within the four 50 m transects were recorded. Senescent flower heads were collected in July and caged in the laboratory to collect emerging adult *R. conicus*. The flower heads were placed in paper bags, 100 per bag,

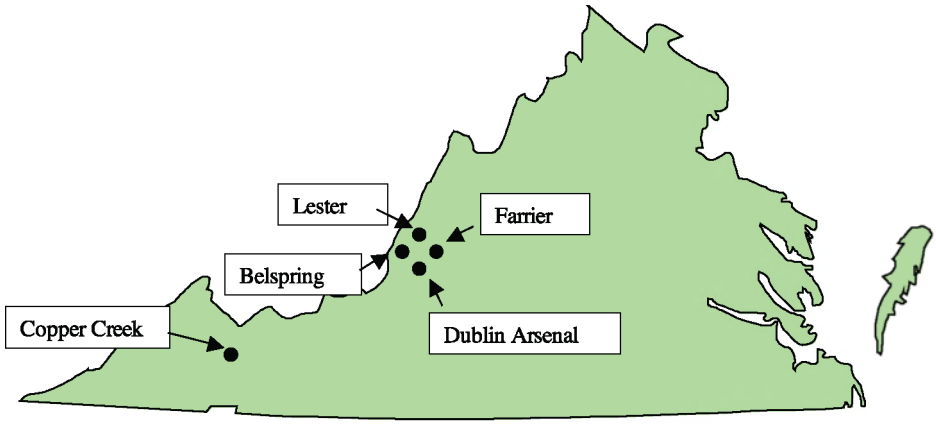

Fig. 1. Location of five selected thistle sites in Virginia.

and stapled closed. The total number of *R. conicus* adults that emerged was tallied in the autumn. Presence of *T. horridus* was based on rosette infestation and counts of emerging adults found on thistle plants in June.

Results and discussion

Thistle density and coverage

Total *Carduus* spp. coverage before weevil release ranged from 50 to 90% (Table 1). At the Dublin Arsenal site, *C. nutans* was the dominant plant species with about 50% coverage followed by tall fescue (40%) and orchard grass (10%). The Belspring and Lester sites were a mixture of *C. nutans* and *C. acanthoides*, with *C. nutans* (60%) being the dominant plant species in Belspring, followed by *C. acanthoides* (30%) and tall fescue (10%). Lester site had 85% thistle (*C. acanthoides* 45%, *C. nutans* 40%) and 15% tall fescue. Copper Creek and Farrier were *C. acanthoides* sites with 50% and 70% thistle coverage at each site, respectively, and the remaining 50% and 30% consisting of Kentucky blue grass, tall fescue, orchard grass, and white clover. Besides *C. nutans* and *C. acanthoides*, there were two other *Cirsium* thistle species: *Cirsium discolor* (Muhl. Ex Willd.) Spreng. (field thistle) and *Cirsium vulgare* (Savi) Tenore (bull thistle). The three *C. nutans* sites declined from a mean coverage of 50% to practically zero (0.01% due to a few isolated plants) by 2002, while the four *C. acanthoides* sites declined from a mean of 48.8% to 3.1% coverage (Table 1). Although lower in density and coverage, *C. acanthoides* persists in three of its four sites (Tables 1 and 2). Copper Creek and Farrier, which were originally *C. acanthoides* sites, have higher densities and coverage of *C. acanthoides* than the third site, Lester. During the past four years, the Copper Creek site had the highest density of *C. acanthoides* (mean 3.4, range 2.1 to 4.7/m^2) from 1999 to 2002, and the largest percentage of the area covered (mean 9.9, range 6.8 to 15.8%) compared with

Table 1. Thistle density and coverage pre and post weevil release.

County: site[2]	Spp.[a]	Thistle density (No./m^2)			% Thistle coverage		
		Pre-weevil release	2002	% reduction	Pre-weevil release	2002	% reduction
Pulaski:							
Belspring	M[b]	4.9	0	100	60	0	100
	P	3.7	0.03	91.9	30	0.4	98.7
Pulaski:							
Dublin Arsenal	M	2.3	0	100	50	0.05	99.0
Giles:							
Farrier	P	12.5	2.0	87.5	70	2.3	96.7
Giles:							
Lester	M	12.4	0	100.0	40	0	100
	P	16.2	0.4	97.5	45	1.1	97.6
Russell:							
Copper Creek	P	5.7	1.3	77.2	50	8.3	83.4

[a] M = musk thistle; P = plumeless thistle; *R. conicus* released in 1969/70 and *T. horridus* released in 1974/75.
[b] Non-thistle coverage: Belspring 10% fescue; Dublin 40% fescue, 10% orchard grass; Lester 15% fescue; Farrier and Copper Creek 30% and 50% mixture of Kentucky bluegrass, fescue, orchard grass and white clover, respectively.

Table 2. Mean ± SD % coverage* of dominant replacement vegetation at five thistle sites in Virginia, 1999 to 2002.

Plant species	Site				
	Belspring	Copper Creek	Dublin Arsenal	Farrier	Lester
Festuca arundinacea (tall fescue)	59.6 ± 12.1	17.5 ± 4.5	65.6 ± 8.8	33.6 ± 21.4	34.3 ± 7.9
Dactylis glomerata (orchard grass)	0	10.4 ± 7.4	9.1 ± 6.8	33.2 ± 30.8	8.9 ± 9.3
Poa pratensis (Kentucky blue grass)	0	29.0 ± 9.9	4.5 ± 6.3	16.8 ± 18.7	30.7 ± 13.5
Trifolium repens (white clover)	1.0 ± 1.6	9.7 ± 5.8	5.5 ± 3.7	19.4 ± 3.7	38.9 ± 6.5
Andropogon virginicus (broom sedge)	27.2 ± 16.2	0.4 ± 0.9	4.2 ± 4.8	0	0
Panicum sp. (panic grass)	0	9.0 ± 6.0	3.3 ± 5.8	0	0
Thistle spp.					
Carduus nutans (musk thistle)	0	0	0.01 ± 0.01	0	0
Carduus acanthoides (plumeless thistle)	0.4 ± 0.3	9.9 ± 4.0	0	5.9 ± 5.4	1.1 ± 1.3
Cirsium vulgare (Bull thistle)	0.1 ± 0.1	0	0	0	0.1 ± 0.1
Cirsium discolor	0	0.1 ± 0.1	0.1 ± 0.1	0	0
No. of non thistle species per sq. m	4.6 ± 1.2	13.0 ± 2.4	8.6 ± 1.4	5.5 ± 1.0	5.6 ± 1.7
Total # plant species	24	37	31	12	17

* Due to overlap of vegetation, sum may exceed 100% coverage.

the other thistle species (Table 2). The Farrier site has consistently been the most overgrazed of all sites, and establishment of *T. horridus* was hindered for several years until the owner was persuaded to leave an area cordoned off from cattle grazing. Thistle density declined after that, as *T. horridus* increased. In the intervening years when the site was not visited, overgrazing resumed and the *C. acanthoides* recovered. Since 1999, with mowing by the owner, and the reduction of grazing, thistle density has declined again. Grazing pressure continued to be fairly high at the Lester site, which still has some *C. acanthoides* but not *C. nutans*. Of the four sites with initial stands of *C. acanthoides*, thistle coverage in 2002 was 8.3% at Copper Creek, < 3.0% at the Farrier and Lester sites, and only a few plants in the Belspring site (Table 1).

No recurrence has occurred at the *C. nutans* sites where control has been sustained. Except for brief periods when soil was disturbed followed by resurgence of thistles, *C. nutans* has been under control. The two *Cirsium* species (*C. discolor* and *C. vulgare*) are spotty and are of minor importance in all sites. *Cirsium discolor*, the only native thistle species found in small numbers in Copper Creek and Dublin Arsenal, has maintained itself in these two sites despite the establishment of the biological control agents. The two *Cirsium* species bloom later than the *Carduus* species and subsequently avoid oviposition by *R. conicus*.

Plant diversity

Mean percent coverage (1999–2002) of replacement vegetation at the sites shows that the number of plant species ranged from 12–37 during the past four years (Table 2). At Farrier, there are 12 plant species. Dominant plants in descending order are *Festuca arundinacea* Schreb. (tall fescue), *Dactylis glomerata* L. (orchard grass), *Trifolium repens* L. (white clover) and *Poa pratensis* L. (Kentucky blue grass). Tall fescue and orchard grass cover more than 30% while *C. acanthoides* covered 2.3% in 2002 and a mean of 5.9% during the past four years. At Belspring, of 24 plant species recorded, two plants that dominate the pasture are tall fescue (59.6%) and *Andropogon virginicus* L. (broom sedge) (27.2%). *C. nutans*, the dominant species initially, and *C. vulgare* were absent in 2002. At the *C. nutans* site in Dublin, 31 species of plants were found. Tall fescue, with 65.6% coverage, was clearly dominant. Other plants species were orchard grass, white clover, Kentucky blue grass, and broom sedge, with each covering < 10%. The four-year average showed 0.01% coverage due to a few occasional *C. nutans*, but no *C. nutans* were present in 2002. At Copper Creek, of 37 plant species recorded, the dominant species were Kentucky blue grass, tall fescue, and orchard grass, all exceeding 10% coverage. *C. acanthoides* ranked fourth with 9.9% coverage (Table 2). This site had the greatest number of plant species, with none exceeding 30% coverage. Grazing pressure at Cooper Creek has been low and may have contributed to the greater plant diversity. At Lester, there were 17 plant species with white clover, tall fescue, and Kentucky blue grass exceeding 30% coverage. *C. acanthoides* and *C. vulgare* were present in 1999 and 2000, but not in the past two years. Plant diversity has increased and species richness has recovered with the reduction of thistles at all five sites. Overall, the six most dominant plants were tall fescue, Kentucky blue grass, orchard grass, white clover, broom sedge and panic grass. All except broom sedge are desirable livestock pasture species.

Populations of *T. horridus* and *R. conicus*

The thistle weevil populations have also declined from their previous peaks, but are still present to suppress the thistles. *Trichosirocalus horridus* has been established at these sites for over 20 years and *R. conicus* for over 25 years. Both weevils prefer *C. nutans* to *C. acanthoides* and this is confirmed by the control of *C. nutans* in the three sites where it once dominated. However, *C. acanthoides* is still present in three of the four original sites, but at much lower densities. Although the number of weevils has also declined with thistle density and coverage, sufficient populations of both weevils remain at the sites with thistles to maintain the thistle equilibrium at a low level. In 2002, adult *R. conicus* per head was 0.57 ± 0.27 at Copper Creek, 0.68 ± 0.43 at Farrier, and 0.42 ± 0.30 at Lester (n = 600 heads), and adult *T. horridus* per plant was 0.44 ± 0.08 at Copper Creek, 0.24 ± 0.08 at Farrier, and 0.14 ± 0.08 at Lester (n = 100 plants). Copper Creek had significantly more adult *T. horridus* per plant than Lester ($P < 0.05$). *C. nutans* was not found in these three sites during the past four years. *R. conicus* has had to adapt solely to *C. acanthoides* and it is possible that *R. conicus* has been able to delay its oviposition cycle to avail itself of the later blooming *C. acanthoides* flower heads. During the whole duration of our study, *T. horridus* has been found on *C. nutans*, *C. acanthoides*, *C. vulgare* and *C. discolor*, and *R. conicus* has been found on *C. nutans*, *C. acanthoides* and occasionally on *C. vulgare*. These are within expectations based on their host-specificity screening. They have reduced thistle density and coverage as intended, resulting in increased plant diversity but have not produced any major surprises in Virginia.

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Exploring interactions between cultural and biological control techniques: modelling bitou bush (*Chrysanthemoides monilifera* ssp. *rotundata*) and a seed fly (*Mesoclanis polana*)

Darren J. Kriticos,[1,3] Rachel M. Stuart[1,2] and Julian E. Ash[2]

Summary

Weed seed-production and seedbank dynamics have been a focus of attention for many biological control campaigns. This interest has perhaps been promoted by the recognition of the important role of weed seed dynamics in annual cropping systems, and frequent observations that seed production is markedly increased in ranges into which a plant is introduced, compared with rates in its native range.

Seeds are the means by which most higher-order perennial plants disperse, and reestablish following disturbance. The role and importance of seeds in the population dynamics of weed populations depends upon factors such as successional state of the invaded vegetation association, the disturbance frequency, plant age at maturity, seed decay rate, and self-thinning patterns. The role of seeds and their predators in maintaining a plant population may be minimal, and decreasing the rate of seed production and the size of the seedbank may have only minor impacts on the population dynamics of perennial weeds.

The interactions between cultural management techniques for bitou bush and its seed fly were explored using a process-based population dynamics model. The role of the seed fly in reducing the invasive potential of bitou bush and modifying the population reestablishment rates following disturbance were studied. The seed fly has substantially reduced seed production, but the effect of the fly on canopy cover of bitou bush and on its invasion potential appears negligible. These findings highlight the importance of using models to explore beyond the immediate effects of an agent on its host to gauge its ultimate impact on the weed population, and to better understand the interactions between cultural and biological control processes.

Keywords: DYMEX, integrated weed management, population model, seedbanks, seed-feeder.

Introduction

Bitou bush, *Chrysanthemoides monilifera* ssp. *rotundata* (Asteraceae) is a noxious invasive weed within most temperate coastal areas of the eastern Australian mainland. The success of bitou bush as an invasive species has been attributed to its ability to outcompete and swamp other species in the community by its seed production and seedbank. Since 1989, bitou bush has been a target for biological control. *Mesoclanis polana* (Tephritidae), a native seed-fly predator of bitou bush in South Africa, has been established since 1996 within Australia. By feeding on the developing ovary of bitou bush fruits, *M. polana* larvae could reduce greatly the number of viable seed that enters the seed bank or is dispersed.

[1] CSIRO Entomology and CRC for Weed Management Systems, GPO Box 1700, Canberra, ACT 2601, Australia.
[2] Australian National University, School of Botany and Zoology, Canberra, ACT 2601, Australia.
[3] Corresponding author: Darren Kriticos <Darren.Kriticos@csiro.au>.

Following releases in northern New South Wales (NSW), *M. polana* spread rapidly in Australia, extending into more southerly latitudes than in its native range in South Africa. However, despite the rapid nature of the invasion by *M. polana*, impact studies have indicated that bitou bush seed destruction rates have been generally low, ranging from 23% to 31% across the latitudinal range of bitou bush in Australia (Stuart *et al.* 2002). These rates are well below the 95% seed destruction levels thought necessary to achieve satisfactory control of bitou bush in the absence of other control techniques (Noble and Weiss 1989).

A population dynamics model (B2) was previously built using DYMEX™ (Maywald *et al.* 2004) to simulate the population dynamics and management of bitou bush at Moruya, on the south coast of NSW (D.J. Kriticos, unpublished data). The parameterization of B2 relied mostly on observations made by Weiss (1983) at Moruya, and included a mixture of growth index and chronologically based life processes. A review of this model (Kriticos & Groves 2000) argued that the model should be verified at sites apart from Moruya, and *M. polana* should be included. We could then explore the interaction between cultural control techniques and the best established biological control agent.

B2 was adapted so that it could simulate populations of bitou bush growing at different latitudes along the NSW coastline, and included modules describing the life cycle of *M. polana* and its interaction with bitou bush (Stuart *et al.* 2002). The adaptations required that the model life processes were reformulated from a weekly to a daily time scale to accommodate the faster rate of processes involved in the insect life cycle compared with that of bitou bush. The resulting model was named B2MP. To our knowledge B2MP is the first cohort-based process-driven population dynamics model built to simulate the dynamics of a weed species and its biological control agent (Barlow 1999).

In this study, B2MP is used to simulate the interactions between cultural techniques (fire and herbicide), and the introduced biological control agent *M. polana* with a view to understanding the ultimate impact of *M. polana* on bitou bush population dynamics, and identifying guidelines for integrated management of bitou bush.

Materials and methods

The model

B2MP (Stuart *et al.* 2002) is a process-based population dynamics model built using DYMEX™ (Maywald *et al.* 2004). The model includes life cycles for bitou bush and *M. polana* (Fig. 1), ignoring the presence of other vegetation. The modelled interactions between bitou bush and *M. polana* include density-dependent feedback on oviposition and larval survival rates (Fig. 2). The development and survival of *M. polana* larvae are related to the growing conditions of bitou bush through the growth index, which is based on temperature and modelled soil moisture.

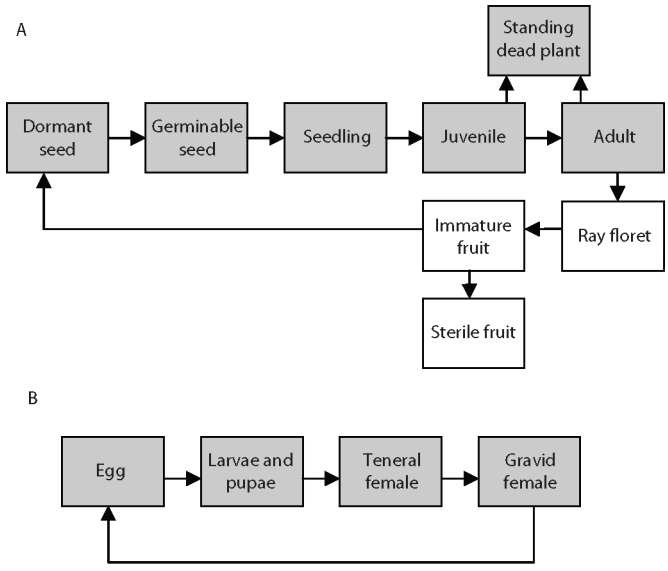

Figure 1. Life cycle diagrams of (A) bitou bush and (B) *Mesoclanis polana* included in the B2MP model. Unshaded boxes indicate life stages that are endostages – stages that are "within" (and therefore dependent upon) another stage – in this case, the adult bitou bush plant. Endostages are removed from the simulation whenever the cohorts containing those stages are removed through stage transfer or death.

B2MP includes an immigration function for *M. polana*. When enabled, the immigration process introduces two teneral females on each day that the following conditions are met: there are no other female flies, the temperature is suitable for flight (>11°C), and there is at least one bitou bush ray floret. This function was introduced to deal with a scaling problem where there are insufficient flowers and seeds available in a 1 ha patch at certain times of the year in order to support a population of *M. polana*. Similarly, without such an immigration process, post-fire populations of *M. polana* would not reestablish.

Herbicide application and fires of various intensities are the two cultural control techniques included in B2MP. Herbicide kills seedlings and immediately removes them from the simulation. Juvenile and adult plants are moved into the standing dead plant category which maintains their contribution to canopy cover and therefore suppression of seedling development and survival until they decay. Herbicide is assumed to have no direct effect upon fly populations. The death of adult plants removes the reproductive endostages (Fig. 1), which in turn kills *M. polana* larvae and pupae.

The effects of each fire depends upon its intensity (class 1 fires have a negligible effect on plants or the seedbank, whilst class 3 fires clear all standing plant material and reduce the seedbank). Moderate or high intensity fires are assumed to kill eggs, larvae and pupae of *M. polana.* Adult flies are also removed from the simulation following moderate to high intensity fires on the assumption that fires prompt them to migrate out of the simulation zone. Fly populations can then reestablish from small numbers of immigrant females. Following fires, a nutrient pulse is added to a soil fertility module. This is used to increase the growth rate of bitou plants for up to two years. For simplicity, the amount of the fertility pulse is always set to top up the nutrient store to capacity following each fire. It is likely that the nutrients added to the soil are the non-volatile components of plants, and so the mechanism is thought to be adequate, even under repeated fires. In B2MP, the intensity of fires is a user-defined parameter. Simulations that include high frequency, high intensity fires should therefore be avoided as it is unreasonable to expect that the plant community could support repeated high intensity fires without time for the standing biomass to accumulate sufficient fuel.

Model validation

Comprehensive validation of a complex process-based simulation model such as B2MP, involving a long-lived perennial plant, is impossible. Instead, it is necessary to undertake a diffuse validation process, gradually building up confidence in the model by comparing model results with field and experimental evidence (Starfield & Bleloch 1991). Stuart (2002) compared model predictions of flower production with field observations for five sites across the range of bitou bush in Australia; the level of agreement in terms of seasonality and intensity of flowering was quite acceptable. The close linkage between plant growth and flowering in bitou bush makes this a good state variable with which to assess the overall behaviour of the model. Otherwise, the model appears to behave in accord with field observations in terms of plant growth rates, maturation rates, seedling recruitment patterns, seedbank dynamics etc. Whilst the annual attack rate of seeds at all sites compared favourably with field observations, during the winter months (August to October), predicted attack rates were much higher than field observations. The reasons for the discrepancy are unknown, but sugges-

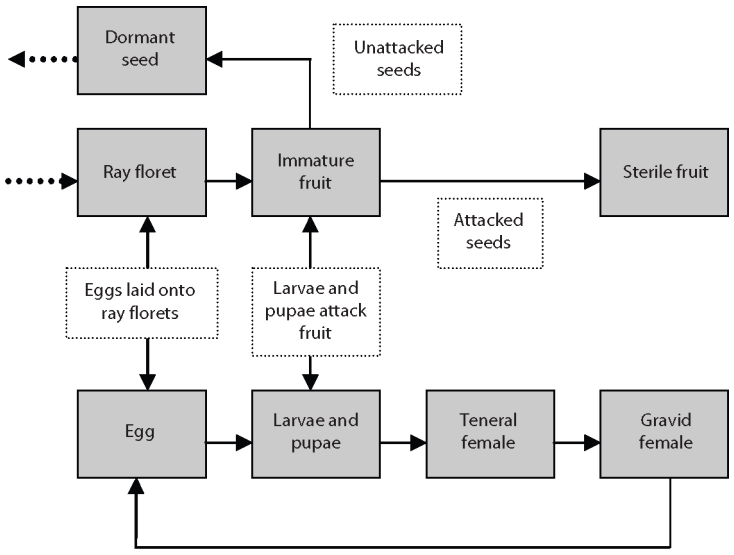

Figure 2. A schematic diagram of the interactions between the bitou bush and *Mesoclanis polana* life cycles used in B2MP.

tions include reduced oviposition rates under cool or clouded conditions, or the use of an index of oviposition site finding efficiency that is inappropriate at low flower densities. Fortunately, the net effect of this discrepancy on the overall model behaviour is minimal because the seed-production rates during this period are minimal.

The transient nature of the bitou bush seedbank is well illustrated in B2MP with large numbers of seedlings germinating and then, in the presence of competition from larger established plants, the seedlings succumb to competition.

Model simulations

For comparative purposes, all simulations were initialized in the same manner, starting on the 1st January 1995 with 1200 juvenile plants and 2000 adult plants. This choice gave some age structure to the population and was based upon previous experience that indicated that site occupancy by bitou bush is rapid, and once the canopy has closed, a dynamic equilibrium seedbank was rapidly established. Two thousand adult flies (one per 5 m^2) were introduced in April 1995. Treatments were then imposed in 1998, leaving time for significant initialization artefacts to wash out of the system prior to imposing management treatments.

High intensity fires were applied in late Summer (March), when temperatures had started to wane. Similarly, herbicide was applied in Autumn (late April) when bitou bush growth is generally strong, to facilitate translocation of the herbicide.

Weather data for the simulations were obtained from the Queensland Department of Natural Resources and Mines silo datdrill website <http://www.nrm.qld.gov.au/silo/>.

The simulations included firstly a partial factorial combination of *M. polana* presence, fire event and herbicide application. After considering the results of these simulations, the effects of herbicide followed by a fire, and the combination of *M. polana* and a generic vegetative control agent were simulated.

All simulations that included fly populations also included fly immigration processes; on any day that flower buds were present and flies were absent from the simulation, two teneral flies are introduced into the study population.

Results

No control

In the absence of any management disturbance or biological control effects, bitou bush rapidly attains canopy closure (100% canopy cover) and a dynamic equilibrium seedbank size that accords with the field observations (Fig. 3). The adult plants subsequently undergo self-thinning as remaining plants increase in size. Seedlings emerge and die due to competition with adult plants before they reach juvenile size (not shown).

Biological control – *M. polana*

The inclusion of *M. polana* in the simulation (Fig. 4) has a negligible effect on the population dynamics of bitou bush due to the limited seed damage rate and high seedling mortality due to asymmetric competition between seedlings and adults.

Fire

In the absence of *M. polana,* a high intensity fire applied in March 1997 removed the adult bitou bush plants and a proportion of the seedbank (Fig. 5). Within a month of the fire, the remaining seedbank produced a carpet of seedlings that closed the canopy. The growth rate of the seedlings in the high nutrient post-fire conditions was sufficient for them to mature within one and a half years of germinating. *Mesoclanis polana* had a negligible effect on the post-fire population dynamics of

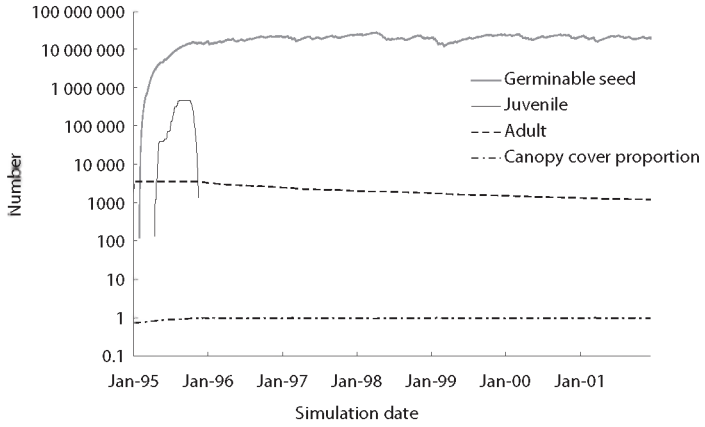

Figure 3. Simulated population dynamics of bitou bush at Moruya, without management disturbance and in the absence of *Mesoclanis polana.*

bitou bush (Fig. 6). Density-dependent self-thinning processes act to buffer the effect of a reduction in the size of the post-fire seedling flush.

Herbicide

Herbicide applied in late April 1997 killed the standing adult bitou bush plants, creating standing dead plant material that reduced the vigour of the seedlings and juveniles that developed subsequently (Fig. 7). In the four years it took for the first post-herbicide adult plants to develop, the seedbank was depleted due to the suspension of inputs and the continuation of the germination and seed decay processes. The presence of flies had a negligible effect on these processes (Fig. 8).

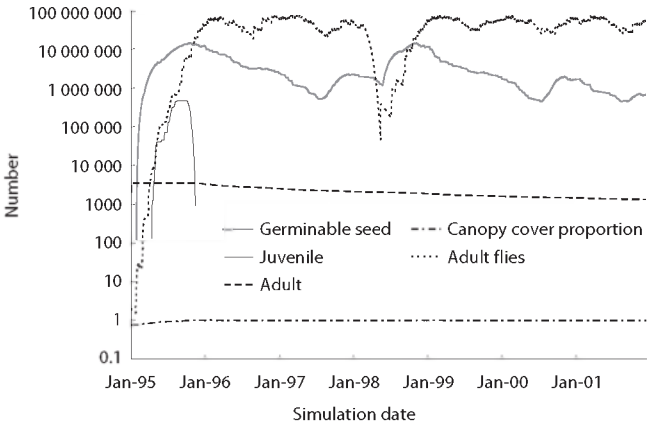

Figure 4. Simulated population dynamics of bitou bush and *Mesoclanis polana* at Moruya without cultural management disturbance.

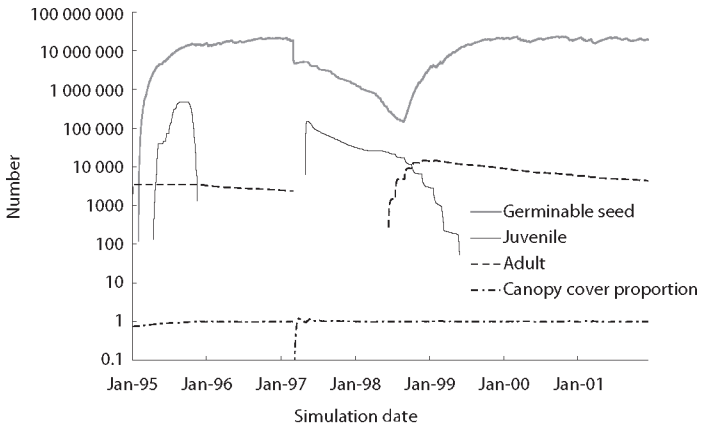

Figure 5. Simulated population dynamics of bitou bush at Moruya in the absence of *Mesoclanis polana* with a fire in March 1997.

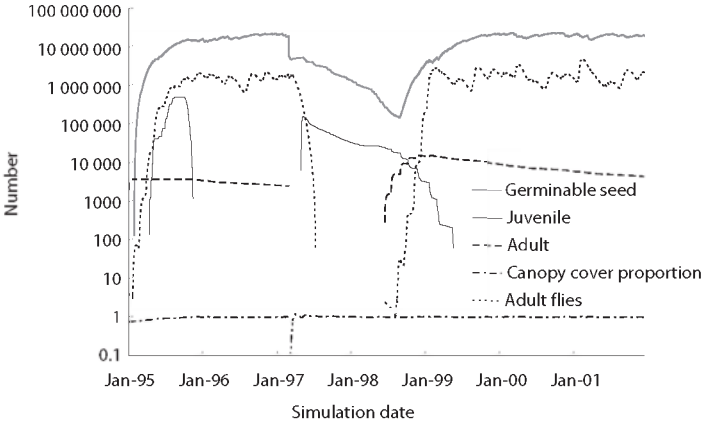

Figure 6. Simulated population dynamics of bitou bush and *Mesoclanis polana* at Moruya with a fire in March 1997.

Except for continued presence of adults immediately following the herbicide application, flies are absent when there are no flowers present (i.e. no adult plants present).

Herbicide and fire

Applying a herbicide to the population in April 1997, and then applying a fire in March 2000 after the seedbank had been depleted, resulted in the elimination of bitou bush from the simulation (Fig. 9).

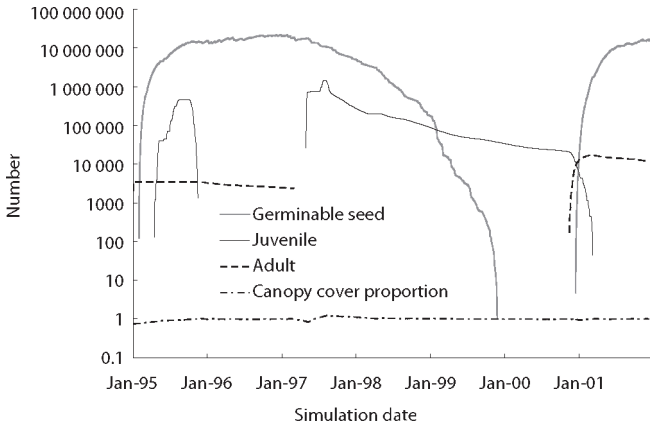

Figure 7. Simulated population dynamics of bitou bush at Moruya in the absence of *Mesoclanis polana* with herbicide applied in April 1997.

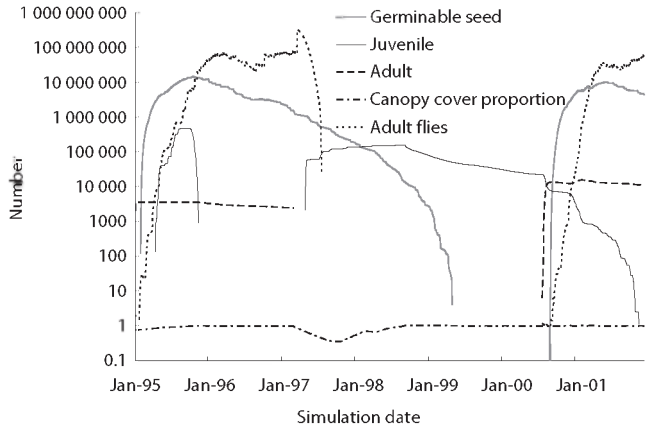

Figure 8. Simulated population dynamics of bitou bush and *Mesoclanis polana* at Moruya with herbicide applied in April 1997.

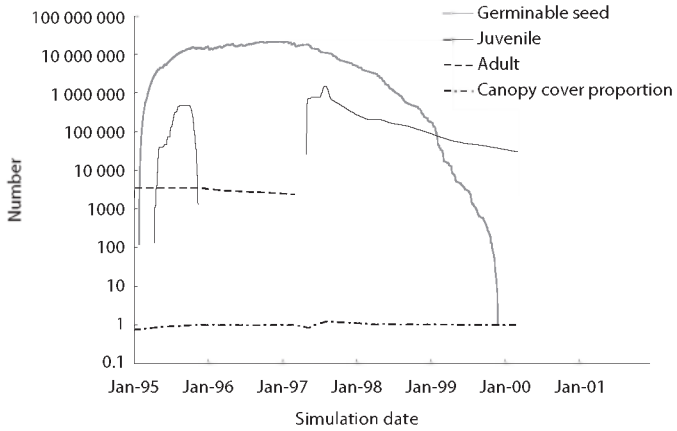

Figure 9. Simulated population dynamics of bitou bush and *Mesoclanis polana* with a herbicide applied in April 1997 and a fire in March 2000.

Biological control – generic foliage feeder

The inclusion of a generic vegetative biological control agent as a complement to *M. polana* in the simulation had a marked effect on the seasonal pattern of bitou bush canopy cover, though no effect on the number of adult plants (Fig. 10). The generic agent reduced the number of *M. polana* present due to reduced flowering and seeding.

Discussion

Integrated weed management (IWM) has become something of a catchcry amongst weed scientists and managers. As with the term *weed*, the definition of IWM is problematical because it is subjective; varying widely depending upon the weed management context and the perspective of the user. However, the basic notion of IWM includes recognition that single weed management techniques are unlikely to be sufficient to achieve satisfactory control, and that different techniques may have interactive effects.

Bitou bush has a *transient* seedbank *sensu* Begon *et al.* (1996) and a short to moderate maturation period depending upon nutrient conditions. Following fires, the maturation period is 1–2 years at Moruya, and in the order of 5–6 years in the absence of a fire-induced nutrient pulse. From Figure 9, it appears that there may be an opportunity to usefully combine a herbicide treatment with a fire or a second herbicide application several years later when the seedbank has been depleted, but juvenile plants have not yet matured. As a follow-up to a herbicide application, fire has the advantage that a portion of any remaining seed may be killed by the fire. If fire is used, the nutrient-enhanced seedbed could also support restoration plantings and sowings. At northern sites, the window of few or no seeds of bitou bush combined with juvenile plants may be narrower, or may not exist due to higher growth rates of bitou bush plants. A set of experiments to test this hypothesis across several sites would be instructive.

As a seed fly, *M. polana* can only act upon mature bitou bush plants. Its role in the biological control program is therefore confined to suppressing seed dispersal from existing mature stands of bitou bush, and reducing the size of the seedbank beneath such stands. The effect of *M. polana* may be to widen any gap between the depletion of the seedbank and the recommencement of flowering in a patch following herbicide application (Figs 7 and 8). If such an effect is real, then it appears to be a small benefit. B2MP ignores any increase in soil fertility due to litter fall following herbicide application and, consequently, any potential increase in seedling growth rates, and hence rate of attainment of maturity. This may act to diminish any potential management window between the depletion of seedbank and attainment of maturity in the bitou bush population.

A 23–31% reduction in seed production is unlikely to lead to similar reductions in the rate of invasion from occupied patches. Reduced seed production may lead seed dispersers to forage more extensively, and there is a large degree of density-dependent reduction in site invasion rates due to clumped dispersal of seed under perch trees employed by currawongs and fox dung sites (Weiss 1983).

From Figure 10, it appears that biological control agents that open up the bitou bush canopy periodically offer hope that other (native) species may invade and occupy that space. Such modelling indications support ongoing efforts to get *Comostolopsis germana* and *Tortrix* spp. established in the field.

This study raises the question: what level of fitness reduction by biological control agents is necessary for bitou bush's competitors, such as *Acacia longifolia* to gain an advantage? Is there a competitive crossover

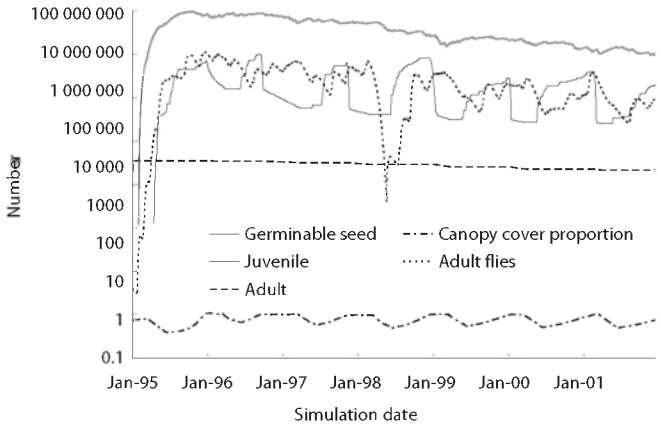

Figure 10. Simulated population dynamics of bitou bush with *Mesoclanis polana* and generic biological control agent that attacks the leaves of bitou bush, removing 0.5% of the canopy per day for the three month active period during autumn.

point where whole plant fitness would lead to a rank reversal in plant community composition between bitou bush and *A. longifolia*. The fact that *A. longifolia* outcompetes bitou bush in South Africa (Henderson 2001) suggests that such a point does exist. The corollary of this question is whether this crossover point can be achieved with herbivores and pathogens of bitou bush which have so far been identified as potential biological control candidates. The lack of consideration of the presence of other vegetation means that it is impossible using B2MP in its current form to consider whether control techniques could affect the competitive relationship described by Weiss (1983) between *A. longifolia* and bitou bush in such a manner that *A. longifolia* could either resist invasion by bitou bush, or reinvade sites dominated by bitou bush.

Acknowledgements

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Bugs offer sustainable control of *Mimosa invisa* and *Sida* spp. in the Markham Valley, Papua New Guinea

Lastus S. Kuniata and Kaile T. Korowi[1]

Summary

A number of exotic weeds are a serious problem to the cattle industry in Papua New Guinea (PNG). They can displace pastures and native plants as new areas are colonized and can also become a nuisance to the local inhabitants. Both *Mimosa invisa* and *Sida* spp. are native to Mexico/Central America, but are now widespread in most areas of PNG, and have caused serious problems in the Markham Valley, especially following the 1997 drought. Control of such broadacre weeds with herbicides is difficult and environmentally risky. A psyllid, *Heteropsylla spinulosa*, was introduced in 1993 to control *M. invisa,* which has now become established and is exerting excellent control. Similarly, a chrysomelid beetle, *Calligrapha pantherina* was introduced in 2000 and again this agent had provided excellent control of *Sida* spp. infestations. Attempts have been made to distribute these agents widely in PNG, and in most cases have proved successful. The application of nitrogen to the plants before the release of biocontrol agents has had an indirect effect on insect numbers. Strategies for dealing with weed outbreaks following severe dry seasons are also discussed.

Keywords: *Calligrapha, Heteropsylla*, drought, *Mimosa*, nitrogen, psyllid, *Sida* spp.

Introduction

Exotic weeds cause much stress on agricultural systems as well as the wellbeing of rural people in Papua New Guinea (PNG). In agriculture, losses can be high due to direct crop losses and increased expenditure on control. For small farmers, this may cause complete farm failure. Certain aquatic weeds, such as salvinia and water hyacinth, have had serious impacts on local people along the Sepik River in the past (Room & Thomas 1985, Julien *et al.* 1999). The terrestrial weeds *Mimosa invisa,* giant sensitive plant (GSP) and *Sida* spp. have also had similar impacts on the livelihood of the rural people, but were most serious on the cattle industry in PNG (Kuniata 1994, 2001).

The control of such widespread weed species requires sustained efforts and a constant supply of limited resources. Biological control offers sustainable control and is also safe to people and the environment. However, as a prerequisite to a successful program, the biology and ecology of both the agent and target weed species need to be studied. In this paper we discuss the classical biological control cases achieved recently for GSP and *Sida* spp. in PNG. Strategies for dealing with weed explosions following droughts, especially in the Ramu–Markham valleys, are also discussed.

Giant sensitive plant, *Mimosa invisa* Mart. ex Colla (Mimosaceae)

GSP, *M. invisa,* has become a serious weed in many parts of South-East Asia and the Pacific Islands, including PNG (Verdcourt 1979) and Australia (Holm *et al.* 1977). It is now widespread in coastal and island areas, is spreading into the Highlands of PNG, and has been a major weed of agriculture, pastures, wastelands and roadsides. In some places, the dense cover of GSP affected rural people too. In 1991, up to 40% of grazing land owned by Ramu Sugar Ltd in the Markham Valley was infested with GSP, with most of these areas useless for cattle grazing.

It is difficult to estimate economic losses and the cost of control of GSP for the whole of PNG. Kuniata

[1] Ramu Sugar Limited, PO Box 2183, LAE 411, Papua New Guinea. Corresponding author: Lastus S. Kuniata <lkuniata@ramusugar.com.pg>.

(1994) reported from cattle properties owned by Ramu Sugar Ltd in the Ramu/Markham valleys in Madang/Morobe provinces that up to US$130,000 annually was spent on chemical control and slashing of this weed. On its sugarcane estate, up to three hours downtime per day/harvester was experienced as a result of GSP interference with normal sugarcane harvesting (green cane). Persistent herbicides such as 2,4-D used for GSP control are not only a hazard to people handling them, but also can contaminate the environment and cause pesticide residues in animal products.

The psyllid, *Heteropsylla spinulosa* Muddiman, Hodkinson and Hollis, is a native of Central America and is probably confined to *M. invisa* as a host plant (Muddiman *et al*. 1992). Of about 100 plant species tested by Wilson and Garcia (1992), *H. spinulosa* developed successfully only on *M. invisa,* indicating its high specificity to this host. Several attempts were made in 1992/93 to introduce this biocontrol agent from Charters Towers, Queensland. It was in 1993 that a colony was released from post-entry quarantine at Laloki Research Station, Port Moresby (Kuniata & Korowi 2001). Colonies were reared in cages at Ramu Sugar estate, some of which were treated with urea fertilizer. Large numbers of insects and severe damage was observed in fertilized cages compared with the unfertilized ones (Figure 1). These observations highlighted the need for nitrogen application in GSP to assist in the establishment of the psyllid and, therefore, this was recommended for all field releases.

In long-term monitoring sites at Gusap-Ramu Sugar plantation, significant reductions in infestations of GSP have been observed since 1991 (Table 1, Figure 2). Ground cover infested with GSP declined from 100% in 1991 to less than 5% in two years following the release of the psyllid. Similarly, the prolific seed production observed in 1991 had been reduced to less than 20% in 2001. In 1998, the psyllid's effect on GSP was delayed due to the severe drought of 1997, thus giving a slight increase in the infestations of the weed. However, the psyllids came back very strongly in 1999 and effectively controlled the GSP, reducing it to minor status in pastures in the Markham–Ramu valleys.

Releases of the psyllids have been made in New Ireland, New Britain, East Sepik, Central, Western Highlands and Sandaun provinces in PNG. The psyllids naturally spread into Morobe, Madang and the Eastern Highlands. In all these areas, good control of GSP has been observed. Land previously infested by GSP on properties owned by Ramu Sugar Ltd in the Markham Valley has been reclaimed for cattle production.

"Broom stick", *Sida* spp. (Malvaceae)

Species of *Sida* are common weeds of disturbed areas, infesting crops, pastures and roadsides in many parts of the world, including PNG (Holm *et al*. 1977). In pastoral areas, they can become serious weeds, especially in areas frequented by cattle, particularly their feeding and drinking sites. Overgrazing of pastures can also result in Sida becoming a dominant weed species. Following the 1997 drought, up to 80,000 ha in the Markham–Ramu valleys in PNG were infested with *Sida* spp. Monospecific stands of plants up to 1.5 m high were observed. As a result of these infestations, culling of animals was carried out on a number of properties in the Markham Valley including up to 800 animals at Leron ranch owned by Ramu Sugar Ltd.

During a visit to Darwin in 1994, large tracts of *Sida acuta* Burman f. and *S. rhombifolia* Linnaeus were observed damaged by a chrysomelid beetle, *Calligrapha pantherina* Stål. Damage to juvenile and mature Sida was quite severe and provided excellent control of the weed. As a result of this excellent

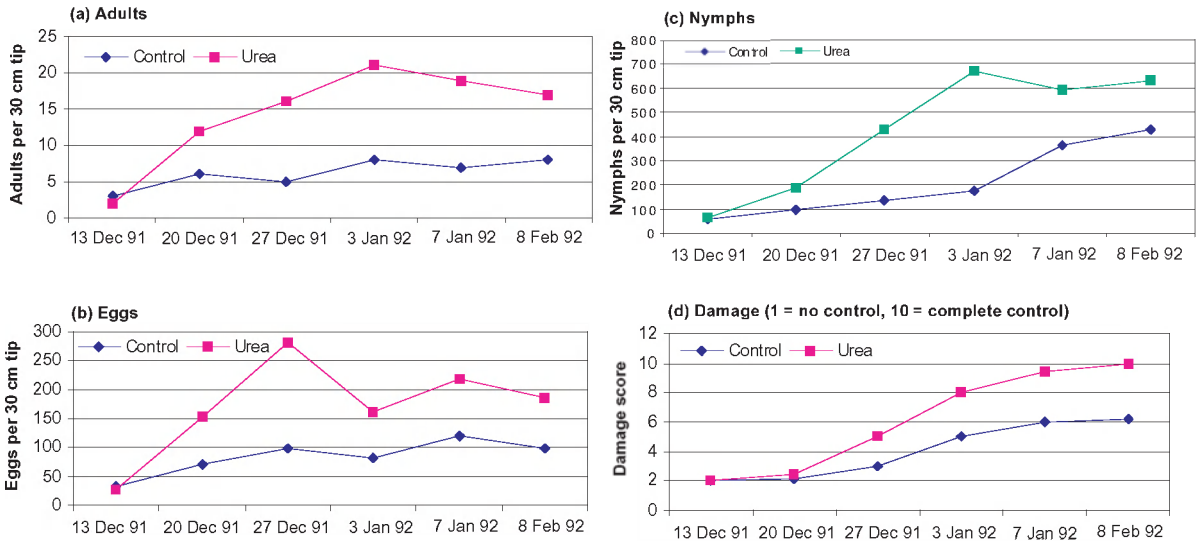

Figure 1. Effect of nitrogen on *Heteropsylla spinulosa* (a) adults, (b) eggs, (c) nymphs and (d) damage in GSP.

performance in Northern Territory, this biocontrol agent was selected for introduction into PNG (Kuniata 2001).

In general, females can lay up to 1800 eggs over a 6-month period, which is about 28–147 eggs/week (Forno *et al.* 1992). There are four larval stages with a total duration of 7–14 days. The mature larvae burrow into the topsoil to pupate, taking 7–10 days to do so. The adults can live for up to 6 months and for up to 15 days without food. This agent is reported to be highly specific on *Sida acuta* and *S. rhombifolia*.

The 1997 drought exacerbated the Sida problem in Papua New Guinea, especially in the Markham Valley and a program to obtain and release the beetle was funded by the Cattleman's Association (Ramu Sugar Ltd, Zifasing cattle ranch and Sulikon Farming). A visit to Darwin to collect this biocontrol agent was made in early December 1999. A total of 740 adults were hand collected and brought back to Papua New Guinea, and these underwent post-entry quarantine at Laloki Research Station. The National Agricultural Research Institute provided facilities and personnel for the post-entry quarantine.

The initial releases were made in February 2000 under a 2 m × 2 m × 1.5 m screened cage at Gusap Ranch. The cage was surrounded by about 10 ha of dense *Sida* spp. cover. Within 7 days, the Sida inside the cage was exhausted and, therefore, the cage was lifted to allow the insects to disperse. Monitoring sites were established in sites where releases were made. The effect of the biocontrol agent was monitored by subjective assessment of ground cover where a score of 1 is no Sida and 10 is completely covered. Weed density was assessed using 50 cm × 50 cm quadrats. By April 2000, the agent was already inflicting severe damage on both young and mature Sida in the release areas, and by June 2000 these infestations of Sida were under control and remained very low up to end of 2001 (Figure 3). Young Sida plants were severely defoliated and stems heavily chewed. As a result, plant mortality was high in the release site, especially during the dry season (May to September). Mature Sida plants were completely stripped of their leaves and

Table 1. The effect of *Heteropsylla spinulosa* on *Mimosa invisa* seed production at Ramu, Papua New Guinea; field releases of the psyllid began in February 1993.

Season	No. of sites	Ground cover score[a]	No. of clusters/m^2	No. of seeds/cluster	Est. no. of seeds/ m^2
1991	40	5.9	300	55	16,630
1992	40	5.8	242	61	14,860
1993	75	5.2	126	36	4,580
1994	44	3.4	57	9	530
1995	51	1.8	50	8	415
1996	36	1.6	106	7	439
1997	43	1.8	31	10	326
1998	43	3.8	128	26	3,546
1999	16	1.0	21	6	125
2000	16	1.0	4	3	13
2001	12	1.0	6	3	18
2002	12	1.0	<1	1	6

[a] Score: 1 = 0–1%, 2 = 1–5%, 3 = 5–25%, 4 = 25–50%, 5 = 50–75%, 6 = 75–100% ground cover.

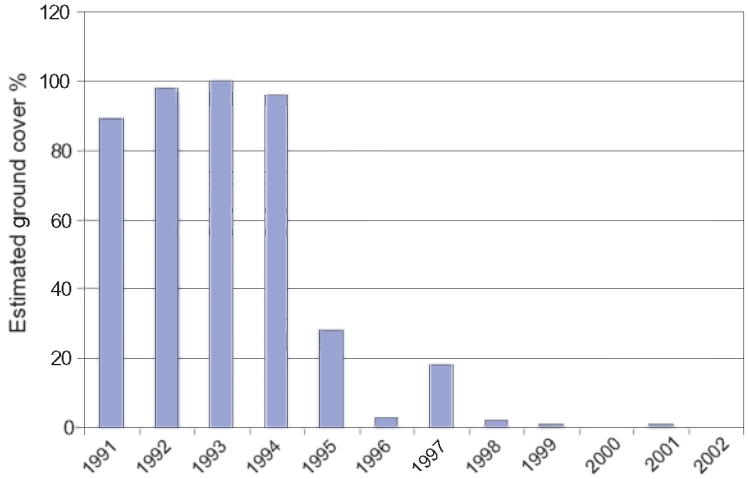

Figure 2. Summary of *Mimosa invisa* cover at a long-term monitoring site at Ramu Sugar plantation and Gusap ranch.

of other green tissue from stems. This allowed pasture and other weeds to invade and overtake Sida.

In April 2001, an exclusion trial was established at Gusap ranch to study the effect of *C. pantherina* on semi-mature Sida (up to 50 cm high, but not flowering) and other weed species. Permethrin was applied at 250 g active ingredient/ha at 2-week intervals to control the insects in the sprayed plots while the unsprayed plots were not sprayed. In the sprayed plots, Sida ground cover increased and was 100% for the rest of the trial period, while other weed species cover declined and remained low (Figure 4). There was severe defoliation in the unsprayed plots and cover was less than 1% from December 2001. Ground cover for other weeds and pasture species then increased, reaching 100% by January 2002. Sida densities remained higher and continued to increase in the sprayed compared to unsprayed plots (Figure 5). By the end of December 2001, much of the mature Sida had died following the dry season. Plant densities observed in early 2002 were lower than those seen in 2001, but the trends were similar, with the sprayed having more plants than in the unsprayed plots. These results strongly indicate the potential of *C. pantherina* for controlling Sida infestations in PNG. Severe infestations can be brought under effective control within 12 months.

A field trial was established in 2002 to study the effect of nitrogen application on populations of *C. pantherina*. It was clear from the results that application of nitrogen had an indirect effect on the biocontrol agent, with significantly higher numbers of egg masses, larvae and adults found in the fertilized plots than in the unfertilized treat-

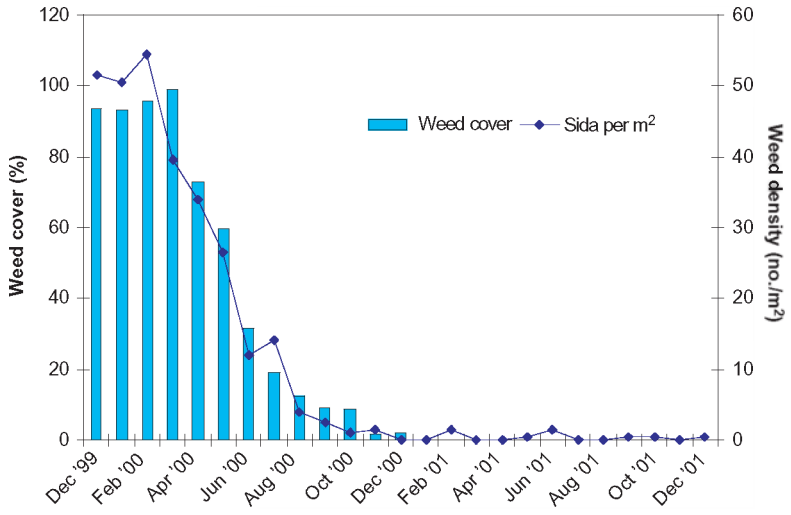

Figure 3. Summary of Sida weed cover and density observed in long-term monitoring sites at Gusap ranch, Markham Valley. Most of the plants were young ones, less than 50 cm high.

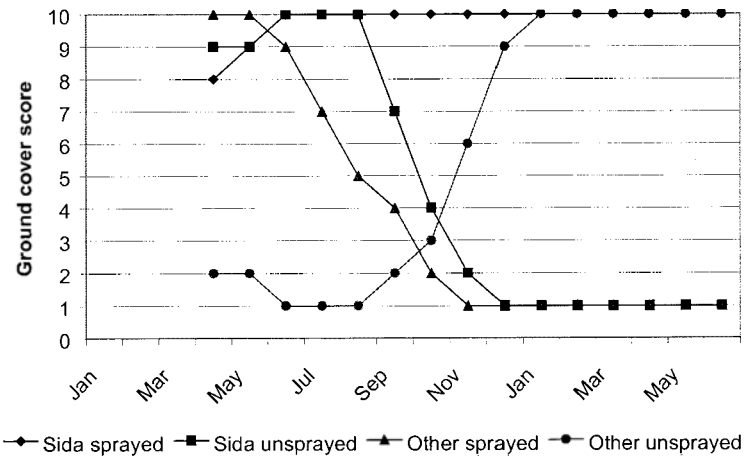

Figure 4. *Sida* spp. and other weed species cover in insecticide-sprayed and unsprayed plots to assess the effect of *Calligrapha pantherina*. The observations were made in mature Sida at Gusap ranch from April 2001 to June 2002. A score of 1 means no effect while a 10 means 100% of land area covered.

ments (Table 2). Adult numbers increased rapidly in a week after fertilizer application, followed by oviposition and appearance of larvae. The insect numbers in the unfertilized plots declined over the duration of the trial. These results further highlighted the need for fertilized plants to provide the high-quality plants required by the insects. Therefore, fertilizer application has been recommended for all new releases of *C. pantherina*.

Strategies for dealing with weed explosions

There is a pronounced dry season from May to September in the Ramu–Leron areas of the Markham Valley. Sometimes the dry period can extend to the end of November, as was the case in the 1987, 1993, and 1997 droughts. Coupled with these dry periods, frequent burning of the grasslands can also affect the establishment of the biocontrol agents. Weed explosions occur following the first rains in September and large areas can be affected. Often the biocontrol populations are too low at this time to provide adequate control of the weeds.

Kuniata (1994) observed that application of nitrogen to GSP indirectly increased the psyllid populations and severe damage was inflicted in the plants sooner than in unfertilized plots (Figure 1). Similar studies done with *Sida* spp. showed a high number of *C. pantherina* insects were found in fertilized plots than in unfertilized plots. Therefore, it is standard practice that some nitrogenous fertilizer is applied to GSP and *Sida* spp. before the inoculation of the biocontrol agents.

The gradual invasion of weeds such as *Sida* spp. in pastures may not pose any problems for the ability of the biocontrol agents to colonize and maintain an equilibrium situation. However, in the Markham Valley, "recreational" burning of large areas of grassland is often done and severe droughts such as the one experienced in 1997 can cause severe weed explosions, especially after the first rains. Large areas can be infested at once, while the biocontrol agents may be slow to provide adequate control of the weeds. This has been observed for *H. spinulosa* in 1997/98, with poor control achieved on GSP (Table 1, Figure 2).

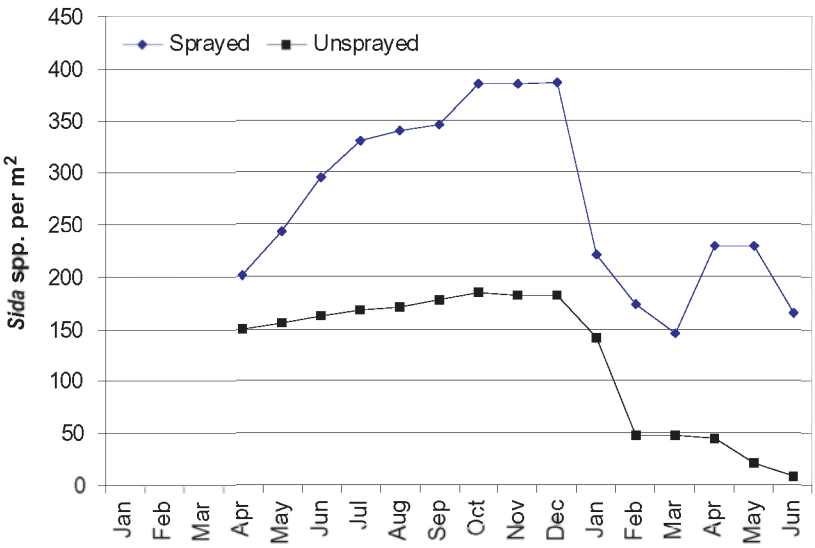

Figure 5. Density of mature *Sida* spp. observed in exclusion trials to assess the effect of *Calligrapha pantherina*.

Table 2. Summary of *Calligrapha pantherina* numbers in nitrogen fertilized and unfertilized plots (no./m^2).

Date (2002)	Nitrogen applied (40 kg /ha)			Unfertilized		
	Egg masses	Larvae	Adults	Egg masses	Larvae	Adults
12 Apr[a]	2	36	91	3	69	75
19 Apr	24	59	143	2	30	48
2 May	2	151	152	2	45	38
17 May	1	33	19	1	17	20
23 May	1	40	45	0	9	31
Total	29	320	449	7	169	212

[a] Pre-treatment counts.

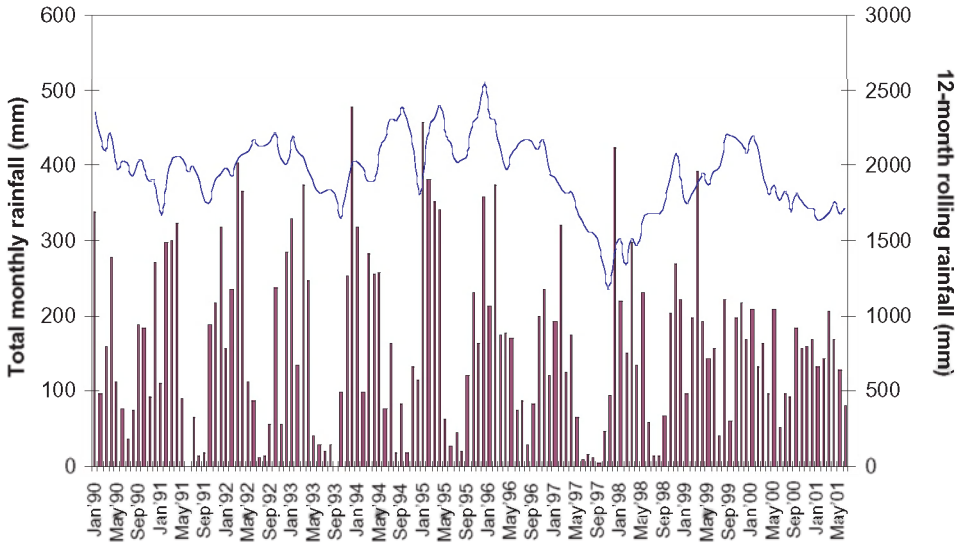

Figure 6. Summary of monthly and 12-monthly rolling total rainfall to end of June 2001.

Plots of *M. invisa* and *Sida* spp. were established on Ramu Sugar plantation to maintain sufficient numbers of the biocontrol agents during the dry season (July to September). Insects from these plots were used to make inoculation releases once the rains returned in September/October. We used a 12-monthly rolling total rainfall as a guide to determine whether the dry season would be severe, so that plots of Sida and GSP could be established (Figure 6). If the 12-month rolling total rainfall continues to decline from June, this prompts the establishment of these plots.

Following the successful use of nitrogen fertilizer, it has also been recommended that plots of *Sida* spp. or *M. invisa* are fertilized at 40 kg N/ha before the agents are released in the field, to increase the insect numbers so as to be able to cope with weed explosions.

Large numbers of *C. pantherina* can be obtained by breeding in the laboratory. Adult beetles are confined in cages with fresh leafy shoots of Sida. Eggs are removed and fresh plant material is replenished daily. These eggs are allowed to hatch in the laboratory and then released in the field as stage 3–4 larvae. Up to 20,000 eggs per week can be obtained if 800–1000 adults are used. Release of larval stages rather than the adults may assist the insects to keep together, enabling them to more easily find their "mates". The adults are replaced every month with field-collected insects to maintain a continuous supply of eggs.

Conclusion

Exotic weeds have become important constraints in agricultural production in PNG and new species are continuing to appear. The most recent arrivals are Noogoora burr (*Xanthium strumarium* L.) and Siam weed (*Chromoleana odorata*). These are now present in the Markham Valley and will become very important in the near future as infestations spread, especially for siam weed. Although herbicides can be used against these weeds, they are often expensive, pose health risks to people, and could contaminate the environment. The recent successes achieved in the biocontrol of GSP and *Sida* spp. in PNG are further cases of classical biological control. Such successes can be achieved at relatively low costs.

Acknowledgements

We thank Ramu Sugar Ltd for financial support. Queensland Department of Primary Industries supplied the psyllids, while the *C. pantherina* was provided by Northern Territory Department of Primary Industries and Fisheries.

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A decade of biological control of *Acacia saligna* in South Africa, using the gall rust fungus, *Uromycladium tepperianum*

C.L. Lennox, M.J. Morris, C. van Rooi, M. Serdani, A.R. Wood, A. Den Breeÿen, J.L. Markram and G. Samuels[1]

Summary

Acacia saligna, introduced into South Africa from south-western Australia in the 1800s, was until recently regarded as the most important invasive weed in the Cape Floristic Region. Host specificity testing established that the *A. saligna* genotype of the Australian gall rust *Uromycladium tepperianum* was suitably specific for use as a biological control agent in South Africa, and permission for release was approved in 1987. The pathogen was established at 200 sites throughout the range of the weed between 1987 and 1989. This paper describes the effect of the rust on *A. saligna* populations and changes in the population levels of the pathogen from 1991 to 2001 at eight of the release sites. Disease severity was low in 1991 but increased rapidly thereafter at most sites. By 1993, almost 100% of the trees were infected at most sites. By 2001, tree densities were reduced by 83–95% compared to 1991. Most of the old trees were killed, and regenerating seedlings infected. Numbers of living trees in smaller size classes declined more rapidly than in larger size classes, but trees of all ages eventually died. After initial increases, seed numbers tended to stabilize although fires reduced the seed numbers considerably at certain sites. In many areas, dead *A. saligna* trees are being replaced by fynbos, other weeds and grasses. The recently introduced seed-destroying agent *Melanterius compactus* will enhance the control of this weed. In biological control terms, the vegetative parts of *A. saligna* are considered to be under complete control.

Keywords: *Acacia saligna*, classical biological control, gall rust, South Africa, *Uromycladium tepperianum*.

Introduction

Acacia saligna (Labill.) H.L. Wendl. (Port Jackson willow), a small willow-like evergreen shrub or tree was introduced into South Africa from south-western Australia in the mid 1800s to stabilize sand dunes in coastal areas. This tree has become a serious environmental weed, invading fynbos, woodlands, coastal dunes, roadsides and watercourses. MacDonald & Jarman (1984) regarded *A. saligna* as the most troublesome invasive alien weed in the Cape Floristic Region of South Africa. According to the March 2001 amendment to "The Conservation of Agricultural Resources Act" (Act No. 43 of 1983), *A. saligna* is classified as a declared invader (category 2). Category 2 plants may not occur on any land or inland water surface other than a demarcated area or a biological control reserve.

The gall-forming rust, *Uromycladium tepperianum* (Sacc.) McAlp. is highly destructive to *A. saligna* in south-western Australia. This rust was selected as a potential biological control agent and extensively tested for host specificity (Morris 1987). Once it was established that the *A. saligna* genotype of *U. tepperianum* was suitably specific for use as a biological control agent in South Africa (Morris 1987), permission for release was approved and the first release took place in 1987 (Morris 1991). By 1997, the pathogen had become established at nearly 200 sites where it had been released, and wind had

[1] ARC–PPRI Weeds Research Division, Private Bag X5017, Stellenbosch 7599, South Africa.
Corresponding author: C. Lennox <vredcl@plant3.agric.za>.

dispersed the fungus throughout the range of the weed (Morris 1997).

Materials and methods

The effect of the pathogen on *A. saligna* populations and changes in the population levels of the pathogen were measured annually from 1991 to 2001 at eight of the sites inoculated during 1988 and 1989. The method used is described by Morris (1997).

Results and discussion

Disease severity, shown by the mean numbers of galls per tree, was relatively low in 1991 but increased rapidly thereafter at most sites. By 2001, tree densities were reduced by 83–95% compared to 1991. Regenerating seedlings were included in these counts. Most of the old trees were killed and many of the remaining trees are new seedlings, which are now also infected. The number of living trees in the smaller size classes declined more rapidly than in the larger size classes, but trees of all ages eventually died. In 2001, the mean percentage trees infected per site ranged from 16.8 to 100%, with a mean of 81.35%. The mean number of galls per infected tree in the largest tree size increased from 21.05 in 1991 to 169.34 in 2001. The number of seeds recovered from soil samples varied greatly depending on the history of the site. During the period 1991–1995, mean seed numbers per site increased from 37,497 to 47,386 seeds/m^2. Thereafter, soil seed numbers tended to decrease. The mean seed number in 2001 was 25,554 seeds/m^2. At certain sites the occurrence of fires was seen to reduce seed numbers considerably.

This long-term study has shown that the gall rust has had a major impact on *A. saligna* populations in South Africa during the past decade. In biological control terms, the vegetative part of the weed has been brought under complete control (Morris 1999). Although seeds are still being produced, the numbers are now considerably reduced and new emerging seedlings rapidly become infected. It is envisaged that the recent release of the seed-feeding weevil, *Melanterius compactus*, on *A. saligna* in South Africa will further enhance the biological control of this alien invasive plant. In many areas, fynbos and native grasses are replacing dead *A. saligna* trees and the challenge now is to ensure that these areas are not simply recolonized by other invasive plants.

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The successful biological control of *Azolla filiculoides* in South Africa: an economic perspective

A.J. McConnachie,[1] M.P. Hill,[2] M.J. Byrne[1] and M.P. de Wit[3]

Summary

Azolla filiculoides Lamarck (Pteridophyta: Azollaceae) (red waterfern) is one of the five main aquatic weeds in South Africa. This fern is native to South America and was first recorded in South Africa in 1948. A combination of phosphorous-rich waters and lack of natural enemies led to its inevitable spread to over 150 recorded localities throughout the country. Dense mats of the weed (up to 30 cm thick) severely degraded aquatic ecosystems and impacted all aspects of their utilization. The failure of mechanical control and the risks associated with chemical control in the aquatic environment made *A. filiculoides* an ideal candidate for biological control in South Africa. A frond-feeding weevil, *Stenopelmus rufinasus* (Coleoptera: Curculionidae), was released in December 1997. Here we report on the post-release evaluation of this insect five years after its initial release, with particular emphasis on the costs and benefits of the study. To date, *S. rufinasus* has been released at 112 sites throughout South Africa. The weevil has been responsible for clearing 91 of these sites completely. The remaining 21 were either washed away during flooding, not revisited, or in the early stages of control. Within three years, the weevil reduced the weed population to the point where it was no longer considered a problem in South Africa. The cost savings (per user) resulting from the biological control program included a reduction of on-site damage caused by the weed to the value of US$589 per hectare per year. The average cost per hectare per year for the biological control program for the period 1995–2000 amounted to US$278. These historic costs and benefits were adjusted to constant year 2000 values. The predicted spread of the weed was calculated on the basis of a sigmoid-curve rate of spread model. The net present value (NPV) of the program was calculated from 1995 onwards and discounted at 8%. This resulted in a NPV of US$1093 per hectare and US$206 million for South Africa as a whole. For the year 2000, the benefit–cost ratio was calculated at 2.5:1, increasing rapidly to 13:1 in 2005 and 15:1 in 2010 as the annual costs of the biological control program are expected to decrease. These indicators reinforce the overall economic viability of biological control. Long-term monitoring is still required to determine the dynamics of weed resurgence and weevil location. The findings of this post-release evaluation are important for other countries (e.g. Australia, United Kingdom) that have infestations of *A. filiculoides*.

Keywords: benefit–cost analysis, benefit–cost ratio, net present value, post-release evaluation, red waterfern.

Introduction

South Africa has 13 aquatic plant species which have been declared either as invaders or as weeds (Henderson & Cilliers, 2002). These weeds invade dams, rivers and wetlands in both urban and rural environments. *Azolla filiculoides* Lamarck (Pteridophyta: Azollaceae) (red waterfern), an aquatic fern, is regarded

[1] University of the Witwatersrand, School of Animal, Plant and Environmental Sciences, Ecophysiological Studies Research Programme, Johannesburg, Wits, 2050, South Africa.

[2] Rhodes University, Department of Zoology and Entomology, Grahamstown, 6140, South Africa.

[3] Council for Scientific and Industrial Research (CSIR), Environmental and Resource Economics, Environmentek, PO Box 395, Pretoria, 0001, South Africa.

Corresponding author: A.J. McConnachie, University of the Witwatersrand, School of Animal, Plant and Environmental Sciences, Ecophysiological Studies Research Programme, Johannesburg, Wits, 2050, South Africa <Rietamc@plant2.agric.za>.

as one of the top five invasive weeds in South Africa. First recorded in the Oorlogspoort River (Colesburg, Northern Cape Province) in 1948, *A. filiculoides* is thought to have been introduced as an ornamental fish-pond plant (R. Randall, Cape Nature Conservation, Sedgefield, Eastern Cape, South Africa, pers. comm.). Hill (1998a) proposed that the lack of natural enemies, human and waterfowl movement between water bodies, and phosphorus-enriched waters facilitated the spread and establishment of *A. filiculoides* in South Africa. At the peak of its invasion in 1998, the weed was recorded at 152 sites in South Africa (Henderson 1999).

Dense mats (5–30 cm thick) of *A. filiculoides* deleteriously affect the biodiversity of aquatic ecosystems (Gratwicke & Marshall 2001). In addition, the weed has increased the siltation rates of rivers and dams, reduced the quality of water for domestic and agricultural use, clogged irrigation canals and pumps, and has led to the drowning of livestock that were unable to differentiate between pasture land and weed covered water bodies (Hill 1997).

On the grounds of insufficient research and risk involved, Ashton (1992) recommended that biological control of *A. filiculoides* not be considered. However, in view of the expense, risk and variable results of chemical control programs, and the difficulties associated with mechanical control, biological control was seen as the only viable long-term control option for this invasive weed (Hill 1997, McConnachie *et al.* 2003a). The frond-feeding weevil, *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) was imported from Florida (USA) in 1995 and, following host-specificity screening (Hill 1998b), was released in December 1997.

Biological control is generally deemed successful when the target plant population is significantly reduced and no additional control methods are required. Forno and Julien (2000) reported on methods for measuring the success of biological control agents that have been released on weeds. These range from simple descriptive methods proposed by Hoffmann (1995) and Laing and Hamia (1976), which rate success from negligible to complete, to the more complex methods of Moran and Zimmermann (1984), or a combination of qualitative and quantitative methods (Julien 1997). Julien (1997) also includes the less frequently used method of economic evaluation in describing agent success.

Initial successes of *S. rufinasus* on *A. filiculoides* were reported on at the 10th International Symposium on Biological Control of Weeds (McConnachie *et al.*, 2000). In this paper, we report on various aspects of the post-release evaluation of this insect five years after its initial release, concluding with an economic evaluation of the study.

Materials and methods

Predictive thermal modelling

We undertook a series of laboratory trials to investigate the thermal physiology of the weevil. Results were used to predict areas in South Africa where *S. rufinasus* might not establish on *A. filiculoides* because of extremes in climate. Thermal parameters were measured according to the methods of Mitchell (1993), Klok and Chown (1997) and McClay and Hughes (1995). These included critical thermal minima (CT_{MIN}) and maxima (CT_{MAX}), lower (LLT_{50}) and upper (ULT_{50}) temperatures, and developmental rates. The thermal parameters were incorporated into the CLIMEX (CSIRO © 1999) model (CLIMEX programme ver. 1.1), and a predictive distribution map was generated.

Cage impact assessments

Trials were conducted in field cages (0.5 × 0.5 × 0.5 m) at five different sites during summer 1999 and winter 2000. Two samples (0.0015 m^2 each) of the weed were taken from each of the cages once a week until the weevil had controlled the weed. One of the samples was hand-sorted to determine the number of eggs, larvae, pupae and adults present. The other sample was oven-dried to obtain a measure of plant vigour. These methods are fully explained in McConnachie (2003).

Field impact assessment

Stenopelmus rufinasus was mass reared and released at 112 *Azolla*-infested sites around South Africa between 1997 and 2002. Batches of 100 weevils were released at each site. Where possible, sites were visited twice anually. When site visits were not feasible, telephonic contact was maintained with the respective landowners to ascertain the status of the weed. A record was kept of weevil establishment and the impact of the weevils on the weed (i.e. changes in area of the water body covered, time taken for the weed to disappear, re-appearance of the weed and recolonisation by the weevil). The effects of the weevils were recorded using "before" and "after" fixed point photographs at 20 sites (see McConnachie *et al.* 2003b).

Weevil dispersal

Intra-site dispersal of the weevil was investigated by growing *A. filiculoides* in stainless steel trays (2.2 × 1.0 × 0.1 m) under glasshouse conditions. Ten evenly spaced transect lines were run along the length of each tray. Thirty pairs of weevils were released at one end of each tray (three pairs per transect). The numbers of each of the weevil life stages were counted in 40 × 40 mm quadrats at 100 mm intervals along each transect line every three days. Inter-site dispersal between *Azolla* sites was recorded twice annually during field site visits (see above). New dispersal localities were recorded

(coordinates) and the distance to the nearest release site was estimated.

Economic assessment

A full discussion on the methodology of the economic evaluation can be found in McConnachie *et al.* (2003a), but a summary follows. A questionnaire was completed with 30 randomly selected water users. The questionnaire requested information on the direct costs of the weed to the respondent, the estimated surface area of the respondents' water bodies and percentage infested, as well as the duration of the infestation. The average cost per hectare per year of the weed per respondent was calculated from the questionnaire. As a result of biological control these are costs foregone (or benefits of control). The costs to develop the biological control agent, including salaries, overheads, and operational costs were obtained from the Plant Protection Research Institute, Pretoria. Both the benefits and costs of control were adjusted using Statistics South Africa's most recent producer price index and expressed in constant, year 2000 South African Rands (ZAR). All amounts were converted to United States dollars (US$) at a ZAR/US$ exchange rate of 10:1. Average costs and benefits per hectare were then calculated for the period 1995–2000. Using a sigmoid curve rate-of-spread model (see Van Wilgen *et al.* 2003), the area estimated to be invaded by the weed with and without biological control in the future was calculated. Finally, the assumptions were made that (a) the value of future benefits would increase at 3% per annum, and (b) that the future costs of control will be 20% of the average costs during the period 1995–2000 – conservatively high for *A. filiculoides*, but one used as a proxy for the costs of maintaining biological control on different alien species in the future (Van Wilgen *et al.* 2003).

Results and discussion

Predictive thermal modelling

The thermal parameter values for *S. rufinasus* were incorporated into the CLIMEX model in the form of various climatic indices (see McConnachie 2003). Annual ecoclimatic indices (EI) were derived using these indices and meteorological data from 134 localities in South Africa. The EI describes the climatic favourability of a given location for *S. rufinasus*. The EI is scaled between 0 (totally unsuitable) and 100 (optimum). The predictive distribution plot for South Africa using the CLIMEX model shows a high probability of the weevil being able to establish throughout the country (Fig. 1).

Cage impact assessments

Both summer (Fig. 2a) and winter (Fig. 2b) cage trials initially showed an increase in plant vigour. However, once the weevil numbers increased sufficiently, a rapid decline in plant vigour was observed. The difference in clearance time of summer (seven weeks) and winter (14 weeks) cage trials clearly illustrates the effect of temperature on the developmental rate of the insects. Nonetheless, *S. rufinasus* is capable of locally eradicating *A. filiculoides* even under winter conditions (with minimum temperatures reaching –5°C on occasions). These findings support the predictions of the CLIMEX model.

Figure 1. CLIMEX generated map (including microclimatic effects) of the predicted distribution of *Stenopelmus rufinasus* in South Africa. Areas of the circles are proportional to the suitability of each location. The present distribution of the weevil is shown (grey transparent area).

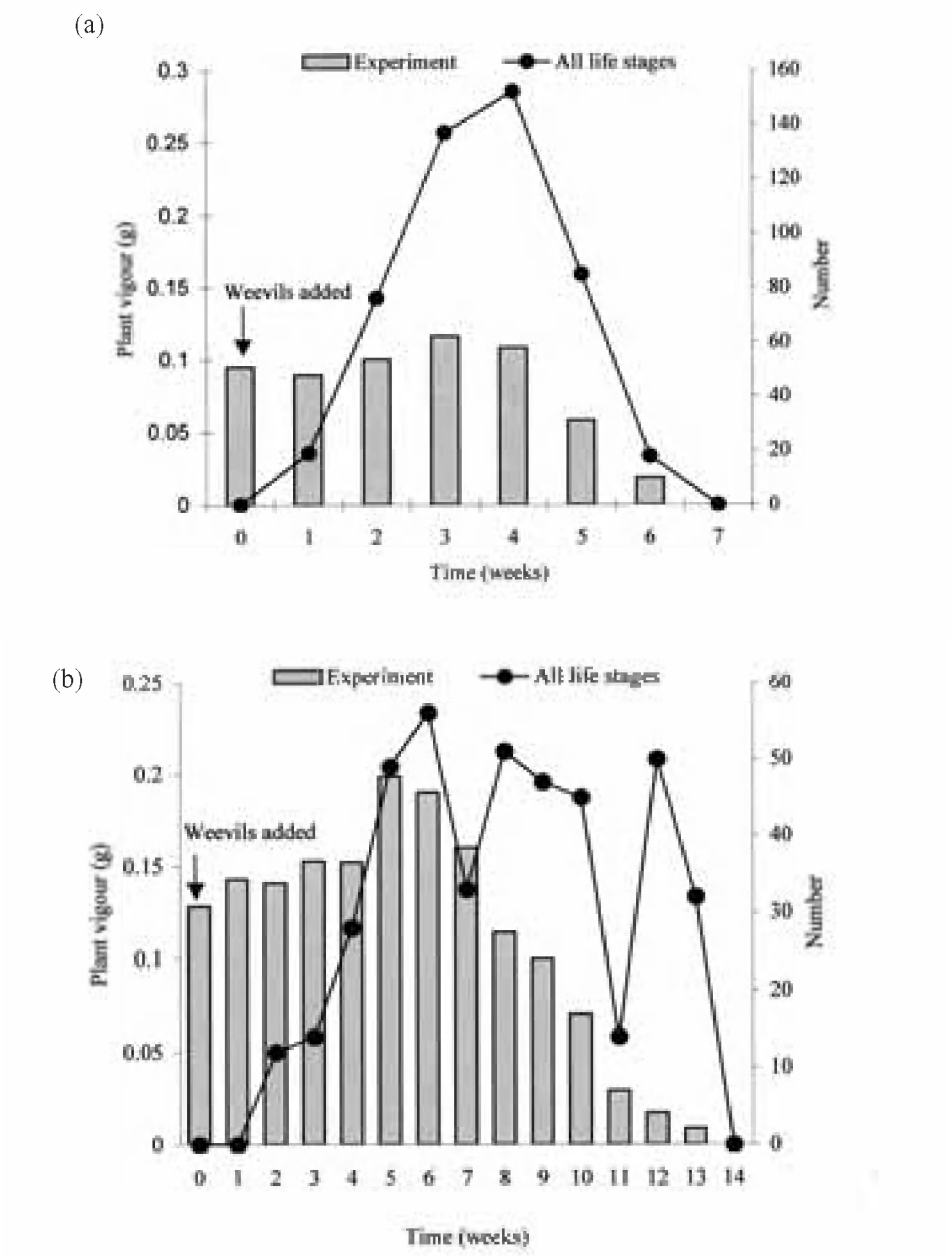

Figure 2. Comparative plots of *Azolla filiculoides* dry weight versus *Stenopelmus rufinasus* total life stages: (a) summer trial; (b) winter trial.

Field impact assessment

Over 24,700 weevils were released at 112 *Azolla* sites throughout South Africa (Fig. 3). *Stenopelmus rufinasus* caused local extinctions at 81% of these sites (Fig. 4). The weevil has not failed at a single site, as lack of control was caused by other factors such as flooding. The surface area of the weed controlled totalled 203.5 ha. On average, infested sites were controlled in 6.9 (± 4.3) months (Table 1). The weed recolonised itself at 22 of the sites (Fig. 4), either through spore germination or waterfowl movement, but the weevils subsequently located all of these and successfully caused local extinction of the weed at 18 sites.

Weevil dispersal

Intra-site dispersal and oviposition of *S. rufinaus* occurs in a wave-like manner (Fig. 5a–d). After three days adults had moved only as far as 30 cm from the release point, and oviposition occurred only 20 cm into the tray (Fig. 5a). After six days, adults had moved 70 cm from the release point (Fig. 5b). Oviposition, however, tapered off further from the release point. After 12 days, adults dispersed along the entire length of the growth tray, a distance of 2.2 m (Fig. 5c). The rapid decline in oviposition was followed by a rapid increase in oviposition, followed by another decline. This finding is hypothesised to be as a result of resource provisioning by the adult females to ensure first

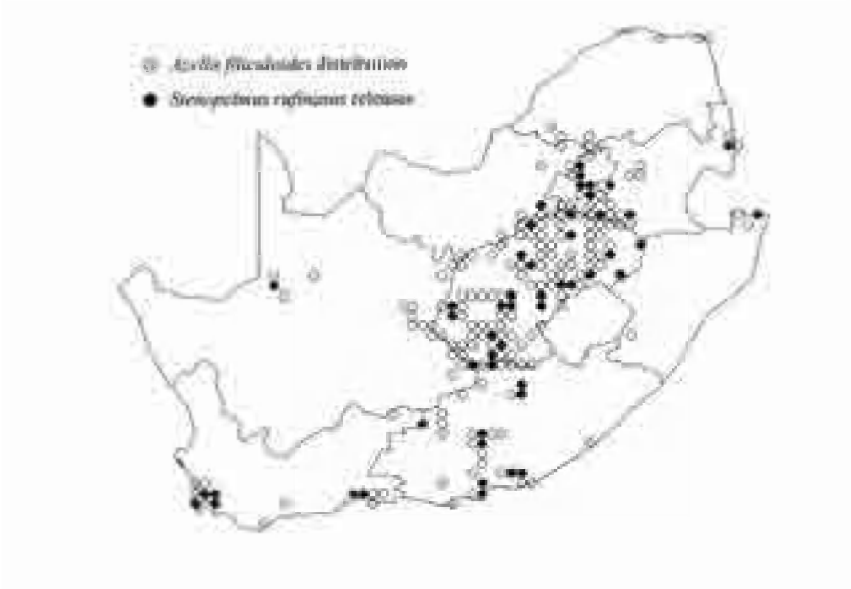

Figure 3. Distribution of *Azolla filiculoides* in South Africa and release localities of *Stenopelmus rufinaus*.

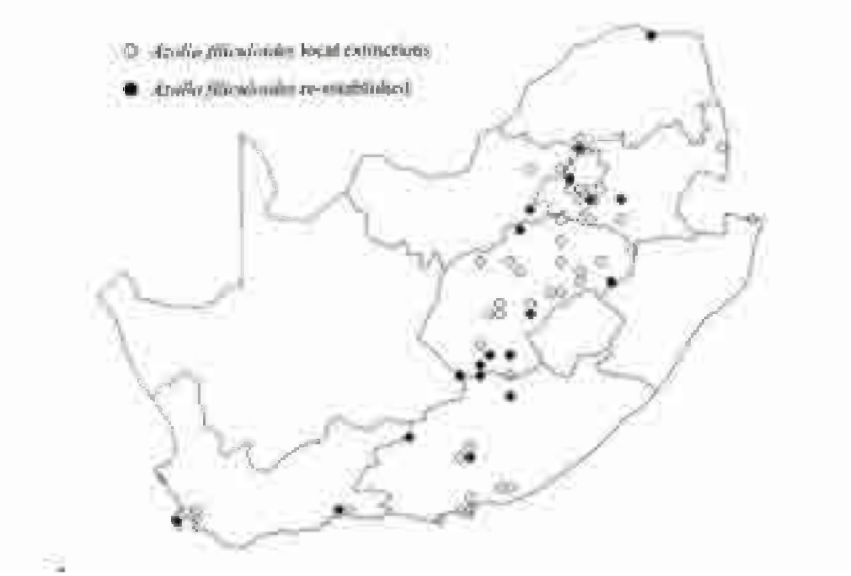

Figure 4. Localities of *Azolla filiculoides* local extinctions and reestablishments in South Africa.

Table 1. Records of *Azolla*-infested water bodies in southern Africa where *Stenopelmus rufinasus* has been released, showing the success rate and time to reach control.

Province/country	No. of weevils released	Area of *Azolla* cleared (ha)	Mean time to control (months ± S.D.)
Eastern Cape	4000	76.3	4.6 ± 3.4
Free State	8500	49.1	7.4 ± 3.8
Gauteng	4600	19.0	7.1 ± 4.9
KwaZulu Natal	500	3.0	7.4 ± 0.0
Limpopo	400	6.5	11.8 ± 7.2
Mpumalanga	1600	16.6	5.5 ± 4.2
Northern Cape	600	11.0	9.0 ± 4.8
Western Cape	4200	15.0	6.4 ± 4.8
Chiredzi (Zim.)	300	7.0	10.8 ± 0.0
Summary	24700	203.5	6.9 ± 4.3

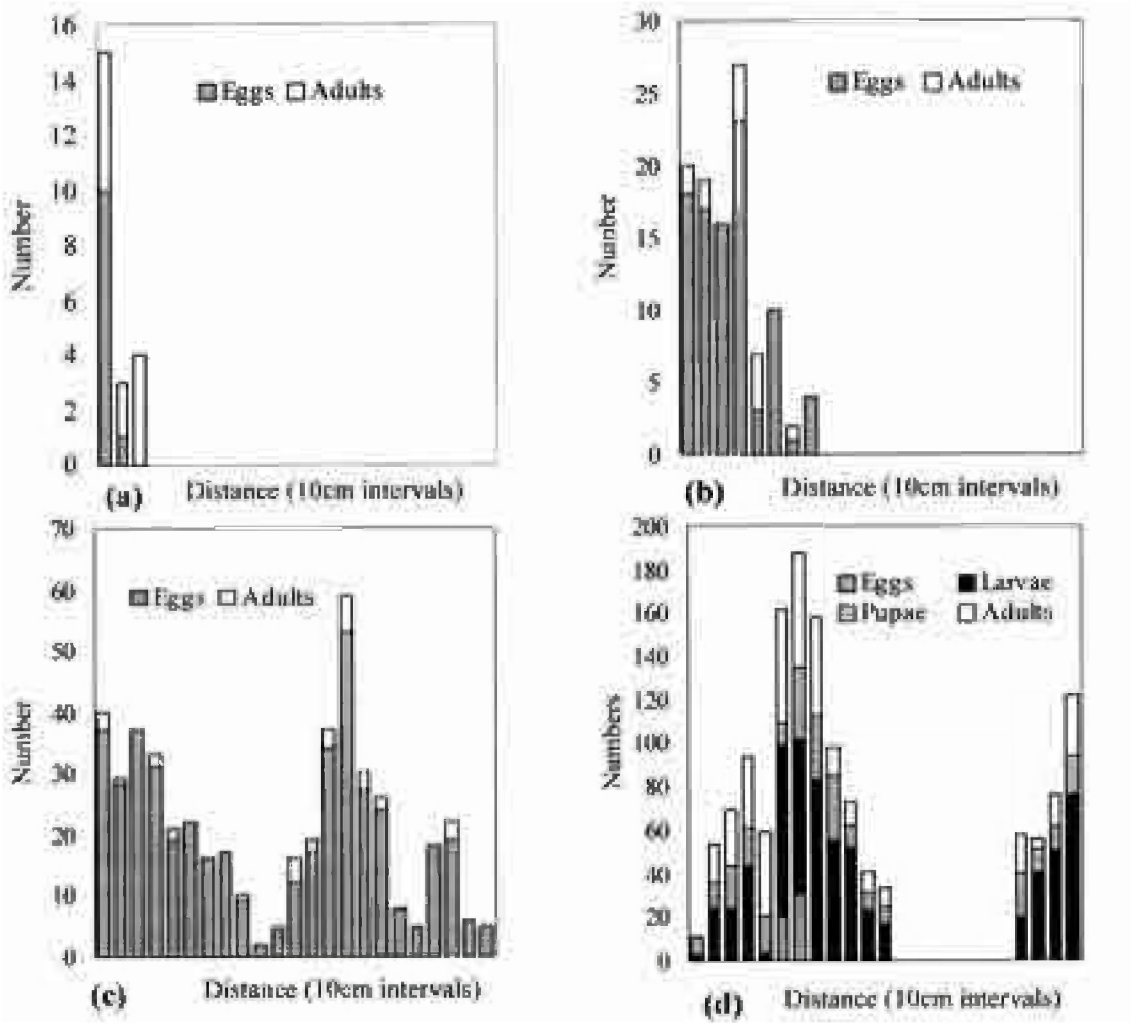

Figure 5. Intra-site dispersal of *Stenopelmus rufinasus* in a mat of *Azolla filiculoides* after (a) 3 days, (b) 6 days, (c) 9 days, (d) 30 days.

generation larvae have good quality food. Inter-site dispersal of the weevil was recorded from field data (Table 2). Dispersal distances of up to 350 km were recorded, ranging in time from 1–2 years after the initial release.

Economic assessment

Of the 30 respondents affected by *A. filiculoides*, 71% were involved with farming, 24% were recreational users, and 5% were municipal users. Based on year 2000 data, the cost savings (per user per hectare) resulting from the biological control program included a reduction of on-site damage caused by the weed to the value of US$589 per hectare per year. The average cost per hectare per year for the biological control program for the period 1995–2000 amounted to US$278, excluding investment costs of USD$7700 in 1995. The net present value (NPV) of the program was calculated from 1995 onwards and discounted at 8%. This resulted in a NPV of US$1093 per hectare and US$206million for South Africa as a whole. For the year 2000, the benefit–cost ratio was calculated at 2.5:1, increasing rapidly to 13:1 in 2005 and 15:1 in 2010 as the annual costs of the biological control program are expected to decrease. These indicators reinforce the overall economic viability of biological control.

Conclusion

Results obtained at the beginning of this five-year study, from the predictive thermal modelling and cage impact assessment, suggested that the establishment and impact of *S. rufinasus* would not be limited by temperature. Field impact data bore testimony to this hypothesis, with local extinctions of *A. filiculoides* occurring in climatically diverse regions of South Africa. Reestablishment of the weed was also countered by effective dispersal of the weevil. The positive benefit–cost ratio and NPV obtained in this study rank favourably with other such projects (McConnachie *et al.* 2003a). Presently, no additional control methods are required to control *A. filiculoides*, thus highlighting the success of this project. Long-term monitoring is still required, however, to further determine the dynamics of weed resurgence and weevil location, as well as the effect of parasitism on the weevil.

Table 2. Records of intersite dispersal of *Stenopelmus rufinasus* in South Africa.

Release locality	Date	Dispersal locality	Date	Dispersal distance (Km)[a]
Clocolan	12/98	Westminster	02/99	30
Wits	01/98	Walkerville	02/99	50
Wits	01/98	Parkview	12/98	10
Sunset Dam	11/98	Bethal	07/00	200
Great Fish River	01/99	Cradock	01/10	15
Orpen Dam	11/98	Engelhart Dam	11/99	15
Orpen Dam	11/98	Jackalbessie	11/99	17
Wits	01/98	Delta Park	07/99	15
Durbanville	02/98	Faure	01/99	15
George	03/98	George	02/99	20
Villiers	02/98	Villiers 1	05/00	20
Villiers	02/98	Villiers 2	05/00	20
Magaliesburg	11/99	Pecanwood	05/00	20
Benoni	09/98	Benoni	08/00	10
Wits, Jhb	01/98	Marievale	02/99	100
Marquard	01/99	Sandsloot	11/00	10
Bloemfontein	09/98	Bloemfontein	11/00	1
Zeekoeivlei	02/98	Park Island	12/00	5
Bloemfontein	09/98	Bloemfontein	12/00	45
Orpen Dam	11/98	Hendrina	05/00	200
Bethulie	09/98	Bethulie	11/00	10
Harrismith	02/99	Bluff Nature Reserve	12/00	350

[a] Approximate distances

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Biotic suppression of invasive weeds in Washington state: a half-century of progress

Gary L. Piper[1]

Summary

Washington state has long employed natural enemies for the management of invasive aquatic and terrestrial plant species infesting state, federal, tribal, and privately owned water bodies and lands. During the last 55 years, a total of 72 natural enemies have been used for the biological control of 30 nonindigenous weeds. Over 85% of the organisms deployed have been insects, the remaining bioagents consisting of fungi, mites, and nematodes. Of these accidental and intentional introductions, 81% have readily established and yielded various levels of suppression against 27 annual, biennial, and perennial weeds. In recent years, populations of Dalmatian toadflax, *Linaria genistifolia* ssp. *dalmatica*, diffuse knapweed, *Centaurea diffusa*, and other undesired plant species have been reduced by a diverse assemblage of biotic agents.

Keywords: biological control, failure, history, invasive weeds, success, Washington state.

Introduction

Humans have altered the composition of the Earth's vegetation for several millenia through their deliberate or accidental dispersal of plant species beyond their native ranges (Williams 1980, di Castri 1989). Unfortunately, many of these introduced species have become highly invasive or "weedy" in their new ranges and have caused severe economic, ecological, and human health impacts over a wide range of agricultural and nonagricultural environments by displacing native plant species and diminishing biodiversity (Kummerow 1992). Currently, there are over 2000 invasive weed species that have established on private, state, tribal, and federal lands in the United States (Anon. 1999). These unruly weeds represent a major factor in the management of all land and water resources, especially in the western United States.

The state of Washington is located in the extreme north-western corner of the continental United States. It is bounded by the Canadian province of British Columbia to the north, the Pacific Ocean to the west, and the states of Oregon and Idaho to the south and east, respectively. The state is divided into 39 counties which encompass an area of 184,674 sq km or 17.2 million ha. Of this land area, approximately 5.1 million ha (29.7%) are publicly owned and 12.1 million ha (70.3%) are owned by private individuals, corporations, and tribal entities. Rangeland and forestland cover almost 75% of the state, with rangeland accounting for 2.8 million ha and forests occupying 2.2 million ha (WRC/WCC 1986). Agriculture, especially the range-based livestock industry, and forestry enterprises – production, processing, and marketing – is worth $28 billion, or about 12% of Washington's economy (Hasslen & McCall 2002).

The suppression of undesirable plant populations has long been associated with the protection of the state's valued croplands, rangelands, and forests, and is essential if agriculture is to be be sustained into the 21st century and beyond. The majority of Washington's weeds are exotic introductions and, as such, are degrading resource areas they occupy and changing forever the native character of the state. Cost-effective management of weeds by eradicating new introductions and preventing further spread of established species serves to maintain weed-free land for crop production and preserve the multiple-use potential of grazing lands. It is important to note too that weeds not only threaten the state's agricultural base but also its waterways, recreational lands, property values, public health and safety, and the general ecological health and animal and plant diversity of its native ecosystems as well.

[1] Department of Entomology, Washington State University, Pullman, WA 99164, USA <cfabich@wsu.edu>.

Washington's noxious weed law (Chapter 17.10 RCW) is one of the most comprehensive and systematic of such laws in the United States. The law recognizes that weed control is the responsibility of the landowner. To ensure a state-wide effort and compliance at the local level, the law established a network to share the responsibility for weed suppression activities. The Washington State Noxious Weed Control Board (WSNWCB) and the Washington State Department of Agriculture (WSDA) work cooperatively with 37 county weed control boards, 11 weed districts, and other allied state and federal agencies. The WSNWCB annually develops and adopts a state noxious weed list. This list categorizes non-native, invasive plant species into three major classes: A, B, and C – according to the seriousness of the threat they pose to the entire state or a region thereof. Class A weeds are species with a very limited distribution or are unrecorded in Washington and represent a serious threat to the state. Their control or eradication is mandated by law. Class B weeds are of limited distribution or are unrecorded in a region of the state and pose a serious threat to the region. In regions where they are threatening, Class B-designates must be controlled. These species may be too abundant in other regions for control to be practical or realistic. Class C weeds are species that are widely established in the state. County weed control boards and districts may seek control of Class C species depending on the local threats they pose and the feasibility of suppression. There are presently 30 Class A, 62 Class B, and 30 Class C weeds on the state noxious weed list (WSNWCB 2003).

Washington state has adopted the integrated weed management (IWM) approach for dealing with many of its identified problem plants. IWM involves the deliberate selection, artful integration, and application of cost-effective, environmentally safe, and sociologically acceptable practices for undesirable plant suppression (Piper 1992, 2003). The goal of IWM is optimization of production/protection of a weed-afflicted ecosystem through the concerted use of scientific knowledge, preventive tactics, monitoring procedures, and application of diverse control methodologies. Weed suppressive methods are categorized as being preventive, physical, managerial, chemical, and biological (Ross & Lembi 1985). Of these, biological control has experienced a high level of acceptance and widespread utilization throughout Washington State because of the alien nature of the invasive weed flora. Biological control involves the intentional deployment of various naturally occurring organisms such as insects, mites, vertebrate animals, and plant pathogens to destroy or effectively diminish established exotic weed populations. Excellent reviews of the procedures followed by practitioners of biological control are provided by Wilson (1964), Frick (1974), Andres *et al.* (1976), Schroeder (1983), Harley & Forno (1992), McFadyen (1998) and Clark *et al.* (2003). In Washington, 1 Class A, 19 Class B and 6 Class C weeds have been targeted for biological control. Biological control agents have also been introduced against cornflower (*Centaurea cyanus* L.), Russian thistle (*Salsola kali* L.), common mullein (*Verbascum thapsus* L.), and moth mullein (*V. blattaria* L.), species not currently included on the state list. Only 21% of the weeds on the state list have had natural enemies introduced against them so many species are still opportune targets for biologically based control efforts.

Historical perspectives

Anyone involved with biological control of weeds program activities soon learns that the successful control of a target plant is the end result of numerous collaborative interactions between individuals at the local, state, national, and international levels (Goeden 1993). This cooperative undertaking is important during all phases of a program: project selection, natural enemy survey and discovery, biological and host-range studies, importation, release, and establishment, and evaluation. Western North America has been a highly active centre of biological control of weeds research and program implementation for decades (Nowierski 1985). Researchers in California, Idaho, Montana, New Mexico, Oregon, Utah, Washington and Wyoming, along with those in Canada, have forged a strong partnership to facilitate the biological suppression of many weeds of regional importance. Consortia of researchers and other interested parties generate the long-term funding that is typically required to ensure eventual biological control agent acquisition and delivery against undesirable plant species.

In the western United States, biological control of weeds programs are administered differently on a state-by-state basis. In some states, primary responsibility rests with the state department of agriculture or with university scientists; in other states the responsibility is shared by both entities. United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA-APHIS-PPQ) and/or USDA Agricultural Research Service (USDA-ARS) personnel also frequently collaborate with state or university individuals on bioagent introduction and redistribution efforts. In Washington, I serve as the university specialist in biological weed control and am responsible for implementing and directing program efforts state-wide, with assistance from USDA–APHIS–PPQ, USDA–ARS, WSDA, WSNWCB, and county weed control boards/districts or other interested parties. This multiagency coordinated effort ensures that owners/managers of weed-affected lands within Washington will benefit from biological control program activities. However, this type of organized effort has been in place for only about 25 years. Before the creation of my university position, biological control of weeds activity in the state was unfo-

cused, not vigorously pursued, and consequently its potential as a plant management method was unappreciated by the general public. Insects that had been approved as weed control agents in the United States were often made available to university entomologists or county agricultural extension agents in Washington by University of California (Berkeley) or USDA–ARS entomologists (Albany, California) and released in a few localities against various weed species (Johansen 1957). Unfortunately, very little time and effort was subsequently expended on intrastate redistribution and impact evaluation activities.

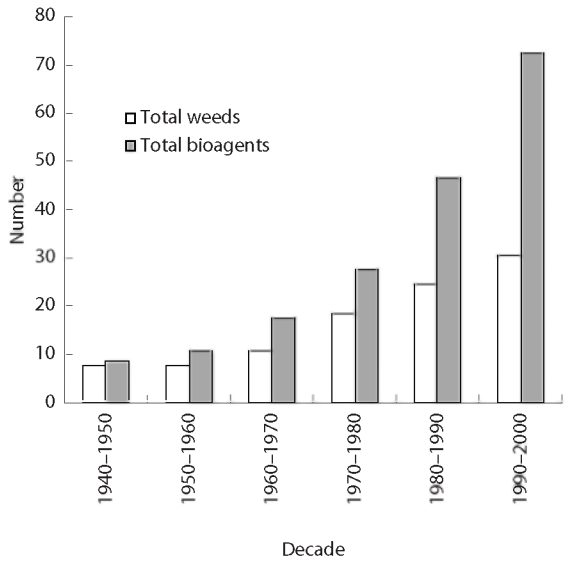

Figure 1. Cumulative summary by decade of the number of new weed targets and bioagents released in Washington.

A concerted biological control of weeds program effort was initiated in Washington in 1948 with the purposeful introduction of *Chrysolina hyperici* (Forster) and *C. quadrigemina* (Suffrian) (Coleoptera: Chrysomelidae) for the suppression of the rangeland weed St John's wort (*Hypericum perforatum* L.) (Johansen 1957, Piper 1985). Other weeds reported to be under attack by accidentally introduced natural enemy species during the 1940–1950 era included common and moth mullein (*V. thapsus* and *V. blattaria*), Dalmatian toadflax (*Linaria genistifolia* spp. *dalmatica* (L.) Maire and Petitmengin), yellow toadflax (*L. vulgaris* Mill.), gorse (*Ulex europaeus* L.), and Scotch broom (*Cytisus scoparius* (L.) Link (Fig. 1). No new weeds were worked on during the 1950–1960 time interval. Between 1960 and 1970, Canada thistle (*Cirsium arvense* (L.) Scop.), puncturevine (*Tribulus terrestris* L.), and tansy ragwort (*Senecio jacobaea* L.) represented new weeds selected for bioagent releases (Fig. 1). A notable increase in program activity during the late 1970s was linked to the establishment of the biological control of weeds specialist position. Importation efforts were begun against eight additional non-indigenous plant species at that time (Fig. 1). These included leafy spurge (*Euphorbia esula* L.), black (*Centaurea nigra* L.), brown (*C. jacea* L.), diffuse (*C. diffusa* Lam.), meadow (*C. pratensis* Thuill.) and spotted knapweed (*C. maculosa* Lam.), rush skeletonweed (*Chondrilla juncea* L.) and Russian thistle (*S. kali*). During the following decade, cornflower (*C. cyanus*), bull (*Cirsium vulgare* (Savi) Tenore), musk (*Carduus nutans* L.) and plumeless thistle (*C. acanthoides* L.), poison hemlock (*Conium maculatum* L.), and Russian knapweed (*Centaurea repens* L.) were designated for biological control (Fig. 1). From 1990 to the present, six more weed species have been selected as biocontrol targets. These include field bindweed (*Convolvulus arvensis* L.), Mediterranean sage (*Salvia aethiopis* L.), purple loosestrife (*Lythrum salicaria* L.), smooth cordgrass (*Spartina alterniflora* Loisel.), yellow nutsedge (*Cyperus rotundus* L.), and yellow starthistle (*Centaurea solstitialis* L.). During the last three decades, the number of new weeds earmarked for biocontrol within the state increased by 66%. Of this mix of exotic plant targets, there are 17 perennials, 9 biennials, and 4 annuals. Ninety-three percent of these occupy terrestrial habitats, the only semi-aquatic or aquatic species being *L. salicaria* and *S. alterniflora*.

During the past 55 years, 72 natural enemies have been either accidentally (10 species) or intentionally (62 species) introduced for the suppression of these weedy plants (Fig. 1). Of these organisms, 63 are insects, 5 are fungi, 3 are mites, and one is a nematode. Within the class Insecta, 37 or 59% of the bioagents belong to the order Coleoptera, with the families Curculionidae and Chrysomelidae being represented by 19 and 11 species, respectively. Of the remaining insect species, 13 (20.5%) belong to the order Diptera, 12 (19%) to the order Lepidoptera and one (1.5%) to the order Homoptera. The fungi are all *Puccinia* spp. (Uredinales: Pucciniaceae). Within the order Acari, two mites belong to the family Eriophyidae and one to the family Tetranychidae. The nematode used as a bioagent is *Subanguina picridis* Kirjanova & Ivanova (Nematoda: Tylenchidae). Of this diverse array of organisms, 81% have readily established; the fate of another 13% has not yet been fully ascertained. Remarkably, only 6% of all introduced agents failed to establish. Lack of establishment has been noted for *Aceria malherbae* Nuzzaci on field bindweed, *Altica carduorum* Guérin-Méneville on Canada thistle, *Hyles euphorbiae* (L.) on leafy spurge, *Microlarinus lareynii* (DuVal) and *M. lypriformis* (Wollaston) on puncturevine, *Pterolonche inspersa* Staudinger on diffuse and spotted knapweed, *Puccinia canaliculata* (Schweinitz) Lagerh. on yellow nutsedge, *S. picridis* on Russian knapweed, and *Zeuxidiplosis giardi* (Kieffer) on St John's wort.

Program successes

Using the terminology of Hoffman (1995), complete control success achieved by employing bioagents in Washington has been achieved against only two weeds, *L. salicaria* and *S. jacobaea*. Of the remaining 28 target weeds, substantial success has been recorded against 20 of them and negligible success documented against eight, yielding an overall program success rate of 73%. Biological control of weeds program efforts in the state up until the mid-1980s were previously discussed by Piper (1985). Since then, notable success has been or is being achieved in the natural enemy-induced suppression of populations of several non-indigenous plant species, two of which are profiled herein.

Dalmatian toadflax, *Linaria genistifolia* spp. *dalmatica*

Dalmatian toadflax, a plant of Eurasian origin, was intentionally introduced into Canada and the United States in the mid-1890s for its ornamental and medicinal value (Alex 1962). It quickly escaped from its garden confines and spread to infest North American farmland, pastures, rangeland, and transportation rights-of-way where it has become a liability. Dense populations of the short-lived herbaceous perennial weed occur in Washington. Vegetative reproduction can give rise to patches of the weed that can persist at a site for many years (Robocker 1974). The plant is also a prolific seed producer, a several-year-old plant often producing up to a half million seeds, many of which may remain viable in the soil for nearly a decade (Robocker 1974).

The first biological control program targeting this weed was begun in 1960. Field surveys for arthropods associated with the plant were contracted for by the then Canada Department of Agriculture (now Agriculture and Agri-Food Canada) and performed by scientists affiliated with the then Commonwealth Institute of Biological Control (subsequently the International Institute of Biological Control, and now CABI Bioscience) (Harris & Carder 1971, Harris 1984). A complex of insects was discovered, and one of them, the flower and foliage-feeding moth, *Calophasia lunula* (Hufnagel) (Lepidoptera: Noctuidae), was evaluated and eventually released in Canada and the United States (Harris & Carder 1971, Piper 1985). Although well-established in a number of areas of Washington, it has not significantly diminished weed population abundance.

Foreign exploration and host-specificity studies on Dalmatian toadflax bioagents were continued during the 1980s and 1990s (Rees *et al.* 1996.). This effort resulted in the eventual approval for release in North America of the flower-feeding beetle *Brachypterolus pulicarius* (L.) (Coleoptera: Nitidulidae), root-boring moths *Eteobalea intermediella* Riedl and *E. serratella* Treitschke (Lepidoptera: Cosmopterygidae), root-galling weevil *Gymnetron linariae* Panzer, seed-attacking weevils *G. antirrhini* (Paykull) and *G. netum* (Germar), and stem-boring weevil *Mecinus janthinus* Germar (Coleoptera: Curculionidae). Of these natural enemies, *M. janthinus* has proven to be the most damaging thus far.

Extensive feeding by *M. janthinus* adults on succulent foliage and stems of the plant during the spring results in the death of stem terminals, thus greatly inhibiting potential flower development and seed formation. Numerous eggs are laid in the stems, which the larvae mine within for short distances. Their feeding impairs plant vigour by reducing carbohydrate supplies, causes premature wilting of stems when xylem vessels are severed, and suppresses flower-bud formation. A 2001 survey (G.L. Piper, unpublished data) of several Washington counties bordering Canada indicated the occurrence of the insect as a consequence of its immigration into the United States from nearby British Columbia release sites where it first established during the mid-1990s (De Clerck-Floate & Harris 2002). Large weevil populations have successfully suppressed Dalmatian toadflax stands at some sites both in Canada (De Clerck-Floate & Harris 2002) and Washington. Extensive intrastate redistribution of this highly effective bioagent into other weed-plagued counties commenced in 2002 and will be continued into the foreseeable future. Additional natural enemy species will be released against the weed as they become available.

Diffuse knapweed, *Centaurea diffusa*

Diffuse knapweed, a Eurasian biennial or short-lived perennial accidentally introduced into Washington during the early 1900s as an alfalfa seed contaminant, has since become one of the most serious rangeland and forest weeds in the state (Roché & Roché 1988). The plant's seed output is enormous and its infestations are extensive. Efforts to biologically suppress the weed have been underway since 1973 in the United States and have culminated in the introduction and release of 10 seed head- and root-infesting organisms (Story & Piper 2001). Of these, the curculionid *Larinus minutus* Gyllenhal is unequivocally the most destructive bioagent established thus far in Washington.

During the spring and early summer, large populations of adult weevils feed on the foliage, shoots, and immature flower buds of plants. Such feeding may lead to outright mortality, especially of seedling and rosette stage plants, or, if attacked bolted plants survive, pronounced stunting and flower head deformation typically result. Plants that escape extensive feeding injury are selected by the females for oviposition. Eggs are deposited among the pappus hairs and the emergent larvae consume developing seeds and receptacle tissue within the heads. In areas where *L. minutus* has become well-established, the larvae often destroy every seed head in a stand of diffuse knapweed. It readily survives in most sites where it is introduced and attains large population densities very rapidly, being capable of severely impacting *C. diffusa* populations within three

to five years after release. The weevil also possesses an excellent dispersal capability, enabling it to quickly locate and colonize new weed infestations (Whaley 2002). At numerous sites in Washington, diffuse knapweed stand densities have been reduced by 95% or more by this bioagent alone.

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Impact of biological control agents on *Centaurea diffusa* (diffuse knapweed) in central Montana

Lincoln Smith[1]

Summary

Centaurea diffusa (diffuse knapweed) is a major weed in semi-arid regions in the north-western United States. Investigations on biological control began in the 1960s and have resulted in the release of 13 species of insect biological control agents (most of which also attack spotted knapweed: *C. stoebe* subsp. *micranthos* [often reported as *C. maculosa*]). In central Montana, three capitulum-feeding insects (*Urophora affinis*, *U. quadrifasciata* and *Larinus minutus*) and three root-feeding insects (*Sphenoptera jugoslavica*, *Agapeta zoegana* and *Cyphocleonus achates*) have become well established. Populations of diffuse knapweed have rapidly declined at study sites at two locations in the presence of high densities of biological control agents. *Larinus minutus* and the *Urophora* species infested up to 62% and 59% of capitula, respectively. *Cyphocleonus achates*, *A. zoegana* and *S. jugoslavica* infested up to 64%, 57% and 31% of roots, respectively. By the summer of 2000, some study sites had no mature plants that could be sampled. Impacts of these insect populations on seed production and plant survivorship are discussed.

Keywords: biological control, *Centaurea diffusa*, diffuse knapweed, rangeland, success.

Introduction

Centaurea diffusa Lam. (Asteraceae), diffuse knapweed, is an important invasive weed in semi-arid regions of the north-western continental United States and south-western Canada (Harris & Cranston 1979, Maddox 1979, Sheley *et al.* 1998, Roché & Roché 1999). The plant presumably originated in Eurasia, and the first North American specimens were discovered in 1907 in alfalfa fields in Washington State (Howell 1959). Since then, the plant has spread exponentially (Fig. 1), and infested 1.4 million ha by 2000 (Duncan 2001). It is an important weed in the states of Colorado, Idaho, Montana, Oregon and Washington, and it is designated as noxious in 13 states and four Canadian provinces (Rice 2000).

This plant, and its close relative *Centaurea stoebe* L. subsp. *micranthos* (Gugler) Hayek (spotted knapweed; often reported in the literature as *C. maculosa* Lam. or *C. biebersteinii* DC. [Ochsmann 2001]) have been targets of biological control for over 40 years (Piper & Rosenthal 1995). Thirteen species of insect biological control agents have been introduced (Table 1; Müller-Schärer & Schroeder 1993, Rees *et al.* 1996, Story & Piper 2001). These species were multiplied and distributed by USDA–ARS, APHIS and Forest Service; USDI–BLM; and by state departments of agriculture, university personnel, and county agents. All of these species have established to some extent, and about half of them have become abundant in at least some regions (Story & Piper 2001; E.M. Coombs, pers. comm.).

Diffuse knapweed populations recently appear to be declining at many sites in Colorado, Montana, Oregon and Washington (Seastedt *et al.* 2003, personal observation, G.L. Piper, E.M. Coombs and R.F. Lang, personal communication). However, because of limited resources and general emphasis on releasing and distributing agents, rather than on investigation, we lack quantitative documentation of the recent impact of these agents. I arrived in Montana just as the insect populations were beginning to impact *C. diffusa* populations and here report the partial results of two years'

[1] USDA-ARS Western Regional Research Center, 800 Buchanan Street, Albany, CA 94710, USA <lsmith@pw.usda.gov>.

observations at two locations that were previously heavily infested by this weed.

Methods

Studies were conducted at two locations in Fergus County, Montana, where agents had previously been released. Both are in habitat currently (or historically) dominated by ponderosa pine (*Pinus ponderosa* P. & C. Lawson) at about 1000 m elevation with annual precipitation of about 350 mm. The Eickhoff site is a grass meadow used for cattle grazing, but was enclosed by a fence in 1990 to protect it as a biological control release site. The Shannon site was historically excavated for gravel. It is gradually reverting to rangeland and deciduous forest and is grazed by cattle. Releases of *Agapeta zoegana*, *Cyphocleonus achates*, *Larinus minutus*, *L. obtusus*, *Pterolonche inspersa*, *Sphenoptera jugoslavica*, *Terellia virens*, *Urophora affinis*, and *U. quadrifasciata* were made between 1990 and 1997 at the Eickhoff site (see also Table 1). At the Shannon site, the same species were released over the same period, with the addition of *Bangasternus fausti*, and omission of *Urophora* spp., *L. obtusus*, and *T. virens*. Releases generally comprised 50 to 300 insects except that about 10,000 *Urophora* spp. were released in 1990. By 1995, seed head weevils, primarily *L. minutus*, were being collected at the Shannon site for redistribution.

Permanent transects were established at the two locations and permanent positions for Daubenmire frames (20 cm × 50 cm) were marked along the transects at 5 m intervals at Eickhoff and at 1 m intervals at Shannon, where tree clumps interfered with long continuous transects. In the second year (1999), data were collected from additional nearby transects. Numbers of mature plants, rosettes, and bolts (stems

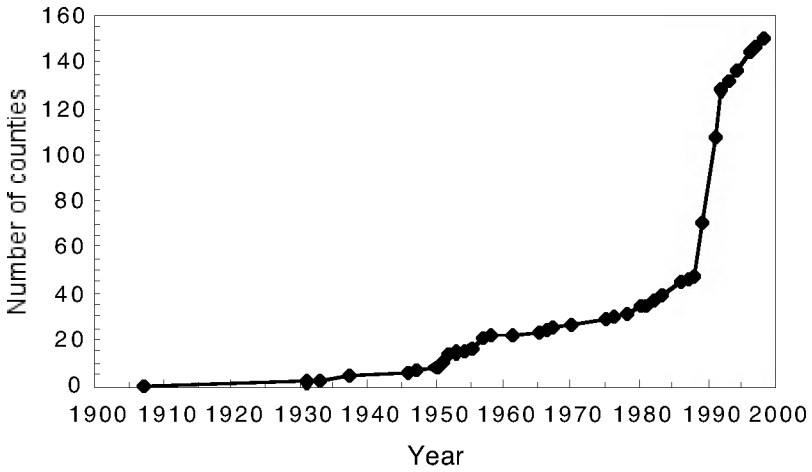

Figure 1. Rate of spread of *Centaurea diffusa* in the north-western United States (data from Rice 2000).

Table 1. Biological control agents released in North America to control *Centaurea diffusa* and *C. stoebe*.

Species	Order: family	First introduction[a]	Releases at study sites[b]	
			Shannon	Eickhoff
Seed-head insects				
Bangasternus fausti	Col.: Curculionidae	1990	91,92,94	
Chaetorellia acrolophi	Dip.: Tephritidae	1992		
Larinus minutus	Col.: Curculionidae	1991	91,92	96
Larinus obtusus	Col.: Curculionidae	1993		95
Metzneria paucipunctella	Lep.: Gelechiidae	1980		
Terellia virens	Dip.: Tephritidae	1992		96
Urophora affinis	Dip.: Tephritidae	1973		90
Urophora quadrifasciata	Dip.: Tephritidae	1981		90
Root-feeding insects				
Agapeta zoegana	Lep.: Tortricidae	1984	90,96	96,97
Cyphocleonus achates	Col.: Curculionidae	1988	95,97	96,97
Pelochrista medullana	Lep.: Tortricidae	1984		
Pterolonche inspersa	Lep.: Pterolonchidae	1986	90,92,96,97	97
Sphenoptera jugoslavica	Col.: Buprestidae	1980	96,98	90

[a] First introduction of the species to the United States (Rees *et al.* 1996).

[b] Year of release omitting first 2 digits (e.g. 90 = 1990).

with flowers) were recorded in August 1998 and 1999. To estimate insect attack rates, seed heads were collected in October 1998 and roots in June 1999 from haphazardly chosen plants at uniformly spaced intervals adjacent to the transects. Seed heads were held in a refrigerator and dissected during the winter to determine insect infestation, and roots were dissected immediately in the field. Insect identifications were based on morphology of immature stages except for *L. minutus*, which was sometimes based on exit hole and characteristic flower-head damage.

Results and discussion

Seed heads were heavily infested at both sites: 99% at Shannon and 59% at Eickhoff (Table 2). The most abundant seed-head insects were *L. minutus* at Shannon and *Urophora* spp. at Eickhoff (primarily *U. affinis*, which tends to displace *U. quadrifasciata* under competition [Berube 1980]). A large proportion of roots were damaged at the two sites: 74% at Shannon and 69% at Eickhoff. Some roots were infested by more than one insect and sometimes by more than one species. *Cyphocleonus achates* was the most abundant root insect at both sites. This weevil appeared to directly kill some plants at the time they began to bolt because the mature larvae had girdled the vascular tissue from the inside.

The knapweed population drastically decreased during the course of this study (Table 3). At Eickhoff, the knapweed population had already decreased substantially below historical levels, and grasses had become a dominant component of the plant community. Grasses made up a much smaller proportion of the canopy at the Shannon site, which is very gravelly, yet the knapweed population decreased to levels similar to those at Eickhoff. Grass species at both sites included *Agropyron smithii* (western wheatgrass), *A. spicatum* (bluebunch wheatgrass), *Poa pratensis* (Kentucky bluegrass), *Stipa comata* (needle-and-thread), and *S. viridula* (green needlegrass) (McGregor *et al.* 1986). The Eickhoff site also had *Festuca idahoensis* (Idaho fescue), *Bouteloua gracilis* (blue grama), and *Deschampsia cespitosa* (tufted hairgrass). There were no nearby sites that were not infested with insects for comparison, so these data do not prove that the insects caused this reduction. However, *C. stoebe* populations being studied in the same region showed no decline during this period (unpublished data).

Table 2. Proportion of *Centaurea diffusa* roots and seed heads infested by insects at two sites in Fergus County, Montana.

	Infestation rate	
	Shannon	Eickhoff
Roots (June 1999)		
Agapeta zoegana	20%	17%
Cyphocleonus achates	52%	29%
Sphenoptera jugoslavica	2%	12%
Root damage	74%	69%
No. plants sampled	86	99
Seed heads (Oct. 1998)		
Larinus minutus	62%	10%
Urophora spp.	37%	49%
No. seed heads sampled	145	299

Centaurea diffusa populations recently appear to be declining at many sites in Colorado, Montana, Oregon and Washington, where insect biological control agents are abundant (Seastedt *et al.* 2003, personal observation, G.L. Piper, E.M. Coombs and R.F. Lang, pers. comm.). This decline has been attributed primarily to the impact of high densities of the two *Urophora* flies, *L. minutus* and *S. jugoslavica*. In earlier studies in British Columbia, the *Urophora* flies greatly reduced seed production, but generally not enough to provide adequate control (Cloutier & Watson 1989, Myers *et al.* 1989). Although *S. jugoslavica* is widespread in British Columbia, Oregon and Washington, it appears to be unable to control the weed by itself (Powell & Myers 1988, Powell 1989). The impact of *L. minutus* adults

Table 3. Decrease of diffuse knapweed at two locations in Fergus County, Montana.

	Density[a]		
	1998	1999	Reduction[b]
Eickhoff location			
Bolted plants	8.3 ± 3.6	2.3 ± 0.8	72% *
Bolts	10.3 ± 4.4	2.5 ± 1.0	76%
Rosettes	10.7 ± 3.4	3.5 ± 2.2	67%
No. quadrats	6	10	
Shannon			
Bolted plants	24.3 ± 3.6	2.4 ± 0.8	90% **
Bolts	38.6 ± 4.4	3.6 ± 1.0	91% **
Rosettes	31.0 ± 3.4	0.8 ± 2.2	98% **
No. quadrats	12	60	

[a] Mean ± SE per Daubenmire frame (0.1 m^2) on permanent transects.
[b] One-way ANOVA test of difference between years, * = $P < 0.05$, ** = $P < 0.001$.

feeding on rosettes in the spring is suspected to be a deciding factor in the decline of *C. diffusa* in some areas (G.L. Piper and R.F. Lang, pers. comm.), although this has not been proven. Seastedt *et al.* (2003) found that in central Colorado within 4 years of the initial releases, *C. achates* infested about as many *C. diffusa* plants as *S. jugoslavica*, despite releasing seven times more of the latter species. *Cyphocleonus achates* was originally expected to attack primarily *C. stoebe* (Stinson *et al.* 1994), but it is clearly an important agent for *C. diffusa*, at least in habitats that have a sufficiently warm summer to allow the insect to emerge early enough for it to exploit its long oviposition period. *Centaurea diffusa*, which is usually a monocarpic biennial, generally has a much smaller root diameter than *C. stoebe*, which is a perennial (Story *et al.* 2001), so the weevil is likely to have much more impact on this plant than on *C. stoebe*.

Unfortunately, the apparently widespread success of biological control agents to control *C. diffusa* lacks rigorous documentation of the declines in weed density and hard data showing the direct impact of the agents on the weed population. Further study of these agents would be useful to gain a better understanding of why some became abundant while others did not, and which ones contributed to the decline of the weed population. Many of these agents also attack *C. stoebe*, although this weed still appears to be uncontrolled in this region.

Acknowledgements

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Simulated biological control of *Hieracium pilosella* at two sites in the Mackenzie Basin, New Zealand

Pauline Syrett, Lindsay Smith, Colin Meurk and Trevor Partridge[1]

Summary

Biological control of *Hieracium pilosella*, an invasive weed in modified and native grasslands, has been controversial in New Zealand. The increase in *H. pilosella* is associated with vegetation degradation through over-grazing, and without this plant it has been suggested that soil erosion will accelerate. This study aimed to evaluate the consequences of simulated biological control at two sites with differing levels of degradation, in the presence and absence of grazing by sheep and rabbits. Small patches of *H. pilosella* were repeatedly suppressed by painting glyphosate herbicide onto leaves, effectively killing *H. pilosella* without removing dead material or affecting other plants. Plant species cover was measured annually. Vegetation responses over a 10-year period varied between sites, and according to the grazing regime. At the more degraded site, without grazing, *H. pilosella* declined in control plots, litter and bare ground increased initially in treatment plots, but bare ground was colonised by cryptogams. Recovery was slower in the presence of grazing. At the less degraded site, without grazing, *H. pilosella* increased significantly in control plots with an associated decline in native and other adventive species. In treatment plots, the initial replacement of *H. pilosella* by litter and bare ground led to increases in other plants and in cryptogams. The effect was similar under grazing, except that bare ground was colonised more slowly. We conclude that biological control outcomes will vary with site. At less degraded sites, competing vegetation is likely to replace *H. pilosella* as it comes under biological control, in a process resembling secondary succession. However, at degraded sites, where environmental conditions are harsh, removing the dominant *H. pilosella* is likely to increase bare ground, initiating primary succession through slow colonisation by cryptogams. Therefore, the eventual outcome of biological control is likely to vary between sites depending on factors such as the degree of soil degradation, environmental conditions and land management.

Keywords: grazing, *Hieracium pilosella*, plant succession, rangeland weed, simulated biological control.

Introduction

Over the past two or three decades, *Hieracium pilosella* L. and other weedy hawkweeds (*Hieracium* species, Asteraceae) have spread rapidly through tussock grassland areas of the South Island of New Zealand (Scott *et al.* 1988, Hunter 1991, Treskonova 1991, Rose *et al.* 1995, Johnstone *et al.* 1999). There has been considerable debate as to the cause of the increase in abundance of this weed. While Scott (1984) declared *Hieracium* species to be aggressive invaders, even of undisturbed areas, Treskonova (1991) attributed the increase in abundance of *H. pilosella*, and the decline in diversity of native species, to grassland degradation through overgrazing by exotic herbivores and pastoral farming. However, neither of these explanations adequately accounted for the patterns of invasion observed by Rose *et al.* (1995). These authors suggested that interactions between environmental factors, including disturbance, and species composition, were likely to influence vegetation change. Johnstone *et al.* (1999) attributed the success of *Hieracium* invasion to a combination of, and interaction between, "general exotic invasion", changes in land management, and metapopulation dynamics possibly facilitated by genetic adaptation.

[1] Landcare Research, PO Box 69, Lincoln, New Zealand.
Corresponding author: P. Syrett, Landcare Research, PO Box 69, Lincoln, New Zealand <rockview@actrix.co.nz>.

Biological control for *H. pilosella* was first proposed by Scott (1984), but it was not until the Hieracium Control Trust was formed in 1992 that funds were made available for the project. The trust was set up by a group of concerned farmers keen to develop effective control measures for *Hieracium* species. The first agent to be investigated was the rust fungus *Puccinia hieracii* var. *piloselloidarum* (Morin & Syrett 1996). A suite of possible insect control agents was studied concurrently (Syrett *et al.* 1996). There was real concern among ecologists that successful biological control of *H. pilosella* might result in increased vegetation degradation in areas where there was already substantial bare ground and the weed comprised the dominant plant cover. For this reason, a manipulative experiment was designed to investigate whether biological control of *H. pilosella* would be more likely to be beneficial in restoring desirable native and adventive plant species or to result in increased degradation.

Materials and methods

The trial was conducted at two sites in the intermontane Mackenzie Basin, in South Canterbury, on the South Island of New Zealand: Maryburn (44° 09' 06.7" S, 170° 20' 49.5" E, 548 m altitude, 600 mm mean annual rainfall) and Sawdon (44° 04' 36.8" S, 170° 29' 11.0" E, 670 m altitude, 550 mm mean annual rainfall). At each site, treatments were applied within each of two 75 × 50 m blocks. One was fenced to exclude grazing animals, both sheep and rabbits, and the other was open to grazing animals. Measurements began in 1993. The size of treatment plot selected was 0.2 × 0.2 m so as to allow consistent estimates of cover for predominantly small, low-growing plant species. The cover of *H. pilosella* was 40–60% in the treatment plot. It was judged that an "effective" biological control agent should be able to completely kill this amount of weed. *Hieracium pilosella* was suppressed in treatment plots by applying glyphosate herbicide (10% Roundup®, 360 g/litre glyphosate) by hand with a paintbrush to all plant material within the treatment area. Treatments were replicated four times in each block. Control plots were 0.5 × 0.5 m and replicated eight times in each block. Treatments were reapplied annually. Visual estimates of plant species cover were made once a year by a skilled observer (CM). The 1993 measurements were taken before the treatments were applied.

Plant species were allocated to one of four categories, and total percentage cover was calculated for each category. These were *Hieracium* species (mostly *H. pilosella*), adventive vascular plants, native vascular plants and cryptogams (lichens and mosses) as well as bare ground and litter. Cover values were proportionally standardised to 100% because multiple strata were measured in some cases. Linear regression of cover over time was used to test whether changes through time were statistically significant.

Results

At the Maryburn site, in the control block protected from grazing, *Hieracium* increased, replacing bare ground and native vascular plants, which declined (Fig. 1, Table 1). When *Hieracium* was removed, it was replaced by native plants and cryptogams. There was an initial increase in litter followed by a short-lived burst of adventive vascular plants. This was mostly the adventive grass, *Anthoxanthum odoratum*. In the grazed control plot, a significant decline in native plants was recorded, with a corresponding increase in *Hieracium* and cryptogams, although these increases were not significant (Table 1). When *Hieracium* was removed, native plants increased significantly, with a significant decline in litter.

At Sawdon, the opposite effect was observed in the control plots to that at Maryburn (Fig. 2). In the control block protected from grazing, *Hieracium* declined and was replaced by native plants and cryptogams. A similar pattern was observed in the control grazed block, where again *Hieracium* declined while native plants and cryptogams increased. When *Hieracium* was removed in the ungrazed block, however, only cryptogams increased to occupy the resulting bare ground. In the grazed block, colonisation of bare ground by cryptogams was very slow.

Discussion

Colonisation of bare ground created by *Hieracium* removal was slow: after 10 years the eventual outcome is unclear. In artificially created gaps in a sward of alpine vegetation in New Zealand, Lloyd *et al.* (2003) found that after 12 years the species composition of gaps was still in an early stage of succession. Although the Mackenzie plots contain a mixture of adventive and native species and are not at a comparable altitude, the environment is similarly harsh. Nevertheless, the change in vegetation following suppression of *Hieracium* at Maryburn is an encouraging response to simulated biological control. *Hieracium* is replaced by more desirable species, and this outcome is realised both under grazing and when vegetation is protected from grazing. The response is slower under grazing, however, and the increase in bare ground was maintained (Fig. 1). It is important to remember that grazing is the normal state for these grasslands, and that the level of grazing during this trial was low. At Sawdon, removal of *Hieracium* resulted in a substantial increase in bare ground initially, a situation that was maintained throughout the trial in the grazed block (Fig. 2). Although cryptogams increased in response to *Hieracium* removal, there was no significant increase in vascular plants, either native or adventive (Table 1). If biological control agents were to suppress *Hieracium* to a similar level, it is likely that there would be an increase in bare ground with an associated increased risk of soil erosion, at least at degraded sites such as Sawdon.

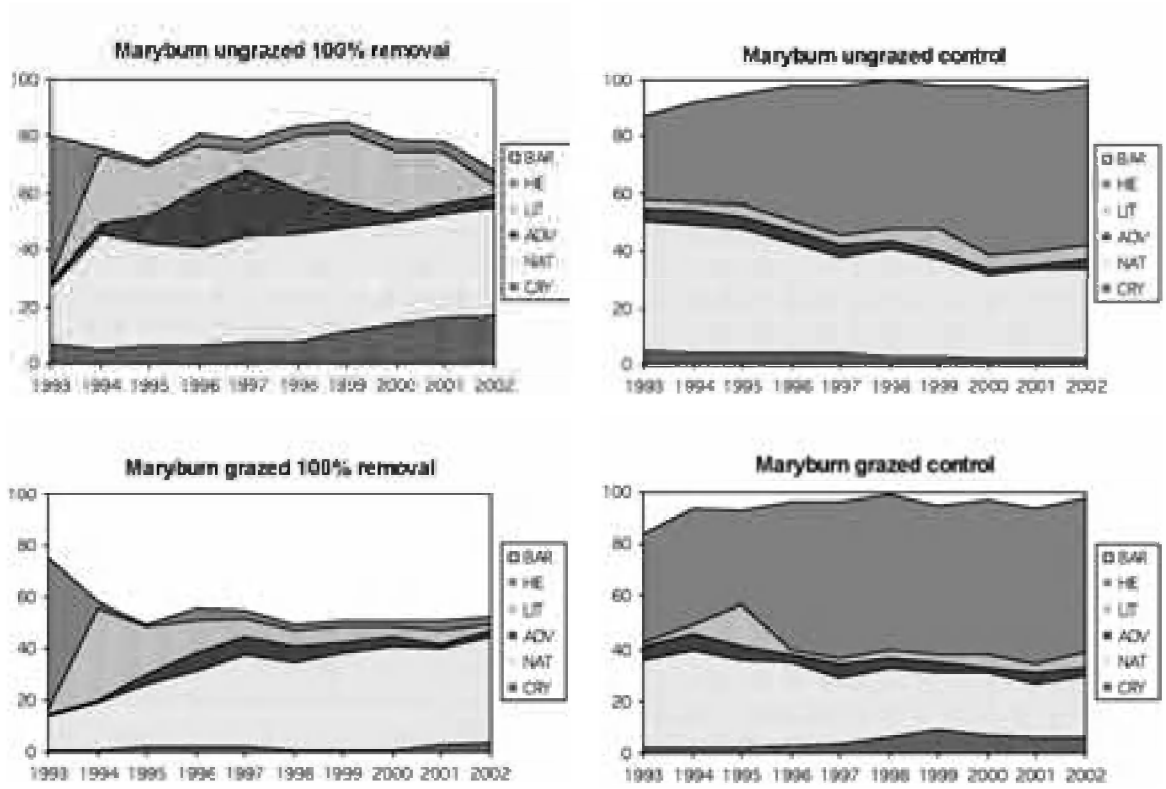

Figure 1. Proportional percentage cover of bare ground (BAR), *Hieracium* species (HIE), Litter (LIT), adventive vascular plants (ADV), native vascular plants (NAT) and cryptogams (CRY) from 1993 to 2002 in grazed and ungrazed blocks at Maryburn, *Hieracium* removed in treated plots.

Table 1. Levels of significance of increasing or decreasing trends in percentage cover.

Location	*Hieracium*	Grazing	Plots	Bare	Litter	Hieracium	Adventive	Native	Cryptogam
Sawdon	control	none	7	0.23 –	0.51 +	0.006** –	0.14 +	0.044* +	0.034* +
	removed	none	3	0.032* –	0.43 –	0.18 +	0.69 +	0.09 +	0.007** +
	control	grazed	7	0.64 –	0.43 +	0.046* –	0.38 +	0.048* +	0.022* +
	removed	grazed	3	0.18 –	0.13 –	0.21 +	0.36 +	0.57 –	0.032* +
Maryburn	control	none	7	0.040* –	0.62 +	0.003** +	0.19 –	0.011** –	0.001** –
	removed	none	3	0.21 –	0.39 –	0.092 +	0.53 +	0.039* +	0.044* +
	control	grazed	7	0.17 –	0.73 –	0.20 +	0.26 –	0.016* –	0.078 +
	removed	grazed	3	0.98 –	0.033* –	0.35 +	0.41 –	0.0017** +	0.25 +

* significant at $P < 0.05$
**significant at $P < 0.01$

The contrasting situations at the two sites suggest different processes taking place following the removal of *Hieracium*. These processes may be attributable to different environmental conditions prevailing at the two locations. At Maryburn, there is an immediate colonisation by vascular plants, which is suggestive of secondary succession following disturbance. At Sawdon, the colonisation is slow, and initiated by cryptogams, suggesting a primary succession. Vegetation response patterns similar to those at Maryburn are typical responses to species removal, while those at Sawdon are unusual and extreme (Austin 1981, Silander & Antonovics 1982, Partridge 1992). The extreme environmental stresses at Sawdon, and its degraded, eroding soils have probably been the main causes of the different vegetation changes recorded at this site.

It is possible that under conditions where *Hieracium* suffers extreme stress, such as at Sawdon, a biological control agent might also be expected to perform poorly, but the little experimental evidence available so far contradicts this intuitive hypothesis. One of the biological control agents for *H. pilosella*, the gall wasp *Aulacidea subterminalis* Niblett, has been shown in a glasshouse experiment to suppress stolon growth of *H. pilosella* under both nutrient and water stress (Klöppel *et al.* 2003). However, even under stressed conditions, stolon production was substantially greater than that observed in the field at extreme sites, and the combined effect of both stresses (which might be expected to have

a greater impact on stolon development) was not tested. It is assumed that the wasp does require plants with healthy stolon development for its survival, as normally eggs are laid in stolon tips and larvae develop in galls induced at the end of the stolons.

Table 2 summarises key conditions and outcomes in *Hieracium*-infested grasslands likely to result at the extremes observed in the trial described here. If it is assumed that successful biological control of *H. pilosella* will result in substantial reduction in cover of the weed, then it is likely that native and adventive plant species will increase at less-degraded sites under moderate environmental conditions. However, at degraded sites and under harsh conditions, an increase in bare ground is likely and an initiation of primary succession through cryptogams will precede replacement by more desirable vascular plant species. Rates of succession are likely to be slower under grazing, with increased risk of erosion.

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We thank Chris Frampton for applying his considerable expertise to achieving an appropriate experimental design, to the landowners Rob Allen at Sawdon Station and Martin Murray at Maryburn Station, for providing access and continued interest in the project, and to the New Zealand Foundation for Research, Science and Technology for funding under contract no. C09X0020.

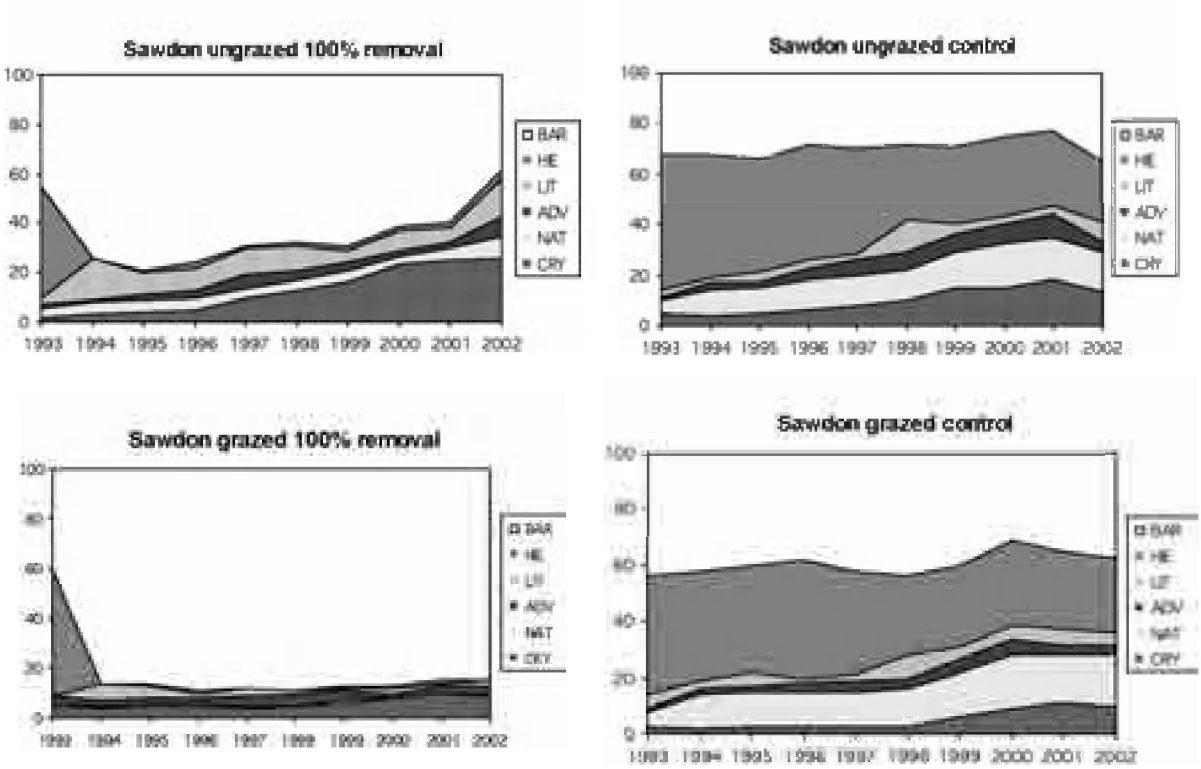

Figure 2. Proportional percentage cover of bare ground, litter, *Hieracium* species, adventive vascular plants, native vascular plants and cryptogams from 1993 to 2002 in grazed and ungrazed blocks at Sawdon, *Hieracium* removed in treated plots.

Table 2. Projected outcomes from extremes of soil, climatic and management conditions observed at two sites; Maryburn and Sawdon in the Mackenzie Basin.

Soil	Climate	Management	Outcome	Performance of biological control agent	Effect
Highly degraded	Higher altitude, harsher climate	Grazing	Bare ground, stressed hawkweed	Poor	Bare ground
				Good	Increased bare ground
Less degraded	Lower altitude, less harsh climate	No grazing	Less bare ground, unstressed hawkweed	Poor	Hieracium continues to replace desirable plants
				Good	Suppression of hieracium benefits native and adventive plants

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Understanding variability in the effectiveness of a classical biological control agent: the importance of the timing of density dependence in the agent life cycle

John R. Wilson,[1,4] Mark Rees[2] and Obinna Ajuonu[3]

Summary

Water hyacinth (*Eichhornia crassipes*) has been successfully controlled in many locations by the weevils *Neochetina eichhorniae* and *N. bruchi*. However, these classical biological control agents are not universally successful. We use population models of the plant–herbivore interaction to explain this variation. In particular, we explore the effect of the timing of density dependence in the life cycle of the control agent. We argue, from experimental work and modelling, that water hyacinth weevils suffer density-dependent mortality before they cause damage to the plant. This, combined with the developmental delay in producing individuals that cause damage to the plant (about 30 days), will mean that the herbivore pressure is slow to respond to changes in the plant population. Consequently, the levels of control in regions where there are regular disturbances (e.g. frost) will be significantly lower than in other regions.

Keywords: density dependence, plant–herbivore models, population regulation, water hyacinth.

Introduction

Water hyacinth causes serious economic, environmental and health problems across the tropics by overgrowing waterways. The use of classical biological control agents (*Neochetina eichhorniae* and *N. bruchi*) has produced noticeable control in some locations, but there is a great deal of variation in the level of control (Julien *et al.* 1999). In order to understand this, we use mathematical models and experiments to address the following questions:

- What regulates the weevil population dynamics in the introduced ranges?
- What is the effect of nutrients on the level of control?
- What will be the effect of integrated management on the weevils?

The life history of both weevil species has been well studied (for a review see Julien *et al.* (1999)). Adults feed on the surface of the leaves and petioles and tend to lay eggs in young leaf tissue. The larvae undergo three instars. The first two instars feed predominately inside the leaf in which they were laid, while the third instar feeds on the root-stock. Pre-pupae form a cocoon using root hairs, and emerge a couple of weeks later. The life cycle takes around 60–100 days.

Several models have been used to describe water hyacinth management (Ewel *et al.* 1975, Mitsch 1976, Lorber *et al.* 1984, Musil & Breen 1985, Akbay *et al.* 1991, Gutiérrez *et al.* 2000). Only one model, however, explored the effect of biological control agents, but this project finished before key features, such as the effect of nutrients, could be incorporated (Akbay *et al.* 1991). We describe the plant–herbivore interaction using a classic two species population model (May 1974, Caughley & Lawton 1981), and a more complicated

[1] NERC Centre for Population Biology and CABI Bioscience, Imperial College at Silwood Park, Ascot, Berkshire SL5 7PY, UK.
[2] Department of Biology and NERC Centre for Population Biology, Imperial College at Silwood Park, Ascot, Berkshire SL5 7PY, UK.
[3] International Institute of Tropical Agriculture, IITA/ BCCA, B.P. 08-0932, Cotonou, Bénin.
[4] Present address: Riversdale, Lochwinnoch Road, Kilmacolm, Renfrewshire, PA13 4DZ, UK.
Corresponding author: John R. Wilson <john.wilson@safe-mail.net>.

(but still analytically tractable) stage-structured model (unpublished data).

Classic plant–herbivore model and the effect of nutrients

Caughley and Lawton (1981) used the following model to describe the control of prickly-pear cacti *Opuntia* spp. by the moth *Cactoblastis cactorum*. The plant density is B and the insect population density Ω,

$$\frac{dB}{dt} = rB\left(1-\frac{B}{K}\right) - cW\left(\frac{B}{B+h}\right)$$
$$\frac{dW}{dt} = r_w W\left(1-j\frac{W}{B}\right) \qquad \text{Equation 1}$$

In the model, the plant population shows logistic growth. This provides an excellent description of the growth of water hyacinth in experimental systems (Fig. 1). A full description and parameterization for the water hyacinth/weevils system will be presented elsewhere.

Both the weed and the control agent grow faster in higher nutrient conditions, with nitrogen the most common limiting factor (Sastroutomo *et al.* 1978, Musil & Breen 1985, Imaoka & Teranishi 1988, Reddy *et al.* 1989, 1990, Reddy *et al.* 1991, Carignan & Neiff 1994, Heard & Winterton 2000). Heard & Winterton (2000) showed that nutrients have a large effect on the interaction between the weevils and water hyacinth and that, over short periods, control is reduced at higher nutrient levels. To assess how equilibrium conditions are affected, we reviewed the literature to derive a relationship between nutrients and the parameters of the model.

In the absence of weevils, the model is good at predicting the growth rate of water hyacinth, but, as would be expected from theory, the intrinsic growth rate is much higher than the rate of increase in area covered (Higgins & Richardson 1996).

In the presence of weevils, the equilibrium density of the plant is predicted to increase with nutrients (Fig. 2). However, control is faster at higher nutrient levels (Fig. 3). Therefore, the level of control observed in the field may not decline with increasing nutrients, and so there is no simple qualitative relationship between level of control and eutrophication.

The initial model was very sensitive to changes in the rate of damage, *c*, but this parameter was poorly defined and dependent on assumptions that were difficult to justify. To make parameters biologically intuitive, and to include more detail of the weevil's life cycle, the model was adapted to include stage structure.

Stage-structured plant–herbivore models and the position of density dependence

In the stage-structured models, the weevil's life cycle is split into stages. Within each stage all individuals have the same vital rates (Gurney *et al.* 1983). This allows a model to include more details of the biology of the system, e.g. which stage of the insect damages the plant. Third instar larvae can cause major damage directly by feeding on the rhizome, or indirectly by

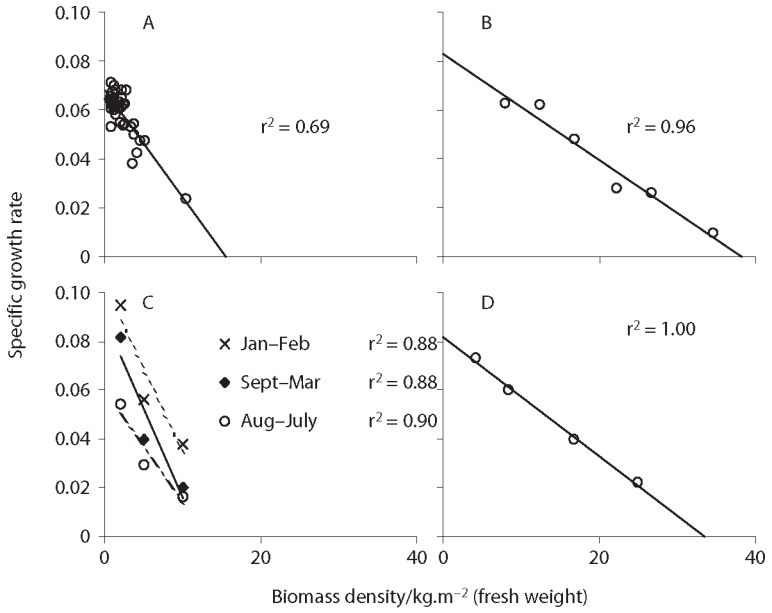

Figure 1. Specific growth rate against biomass density from experimental studies. A) Japan (Imaoka & Teranishi 1988); B) Florida, USA (Reddy & DeBusk, 1984); C) Argentina (Fitzsimons & Vallejos 1986); D) Florida, USA (Debusk *et al.* 1981).

facilitating the entry of pathogens. In comparison, adult feeding is thought to have little impact, unless at very high densities. Therefore, in the stage-structured models we assume that only the larval stages have an effect on the plant.

We consider three separate models, each model having a different effect of the plant on the insect, i.e. the models differ in the mechanism regulating the population size of the weevils. In the first case, density dependence occurs through adult migration, in the second model larval survival is density dependent, and finally density dependence is assumed to occur in the first and second instar larvae and only third instar larvae cause damage.

All the models predict the weevil population has a greater ratio of larvae to adults than is seen in the field, but the model where density dependence occurs in the larval stages gives the prediction closest to observed values (unpublished data). It is also the only model where stability varies strongly across the range of realistic parameters, i.e. it can predict a low stable equilibrium. In short, the model where population regulation occurs early in the weevil's life cycle gives the best description of observed dynamics. To test this, and to measure the strength of density dependence, we experimentally manipulated larval densities.

Experiment

The experiment was designed to test the effect of the number of eggs per plant and host-plant quality on the

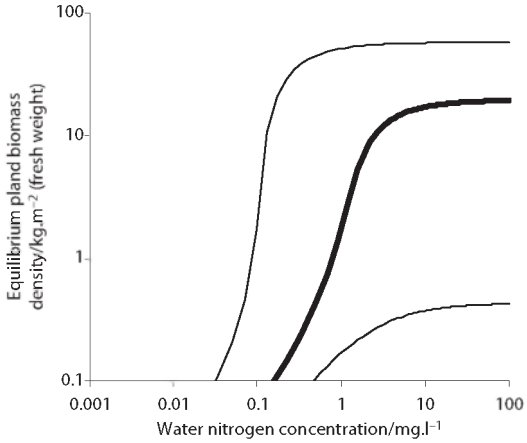

Figure 2. The effect of water nutrient conditions on the predicted equilibrium plant biomass density as predicted from the Caughley and Lawton model (1981). The bold line is the best estimate for the rate of feeding, c, and the thin lines are the upper and lower estimates for the rate of feeding.

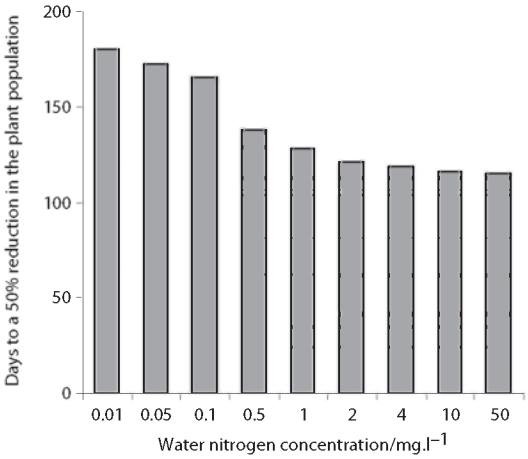

Figure 3. The effect of nutrients on the speed of control as predicted from the Caughley and Lawton model (1981). The system was initiated with 0.05 weevils m^{-2} and the plant population at its carrying capacity, K.

development of *N. eichhorniae* (unpublished data). Five-day-old eggs were inserted into plants grown in water of two different concentrations of nitrate-nitrogen (0–0.4 mg/L or 1–4 mg/L). The insects were left to develop until 25 or 45 days old before the plants were destructively harvested. Based on literature values (Julien *et al.* 1999), these development times correspond to sampling late second instar larvae and late third instar/pupae.

The key result was density-dependent mortality operating on early larvae (Fig. 4). The strength of this density-dependent mortality was not found to change between the harvest dates, i.e. during the third instar.

Discussion

Center (1987) showed that the dispersion patterns of larvae within water hyacinth shoots in the field were such that leaf senescence could be an important factor in larval mortality. First and second instar larvae do not tend to migrate from the petioles in which they were laid, whereas third instars frequently move between petioles and even between plants. This greater mobility would make them less subject to competition for food, and less likely to be stranded in a dying leaf (as was observed during the leaf dissections). Larvae that tunnelled up the petiole towards the leaf were sometimes unable to tunnel back down as other larvae had destroyed the lower part of the petiole. Although leaf production rate was not affected by egg density, the likelihood of a leaf dying prematurely would be expected to increase with increasing larval density. Therefore, the density-dependent mortality observed may be because the probability of being stranded in dead and dying leaves increases with increasing larval density.

This has two important implications for management. First, control methods that disrupt leaf dynamics, e.g. foliar herbicides, will be expected to disrupt weevil populations. Second, the weevils will be slow to respond to changes in the plant population, and so the weed may be expected to out-grow herbivore pressure following winter or after mechanical control. Clearly, such issues of timing could not have been captured using the classic model. Successful integration of classical biological control with other control options, at least for water hyacinth, requires knowledge of the details of the mechanism that regulates the population dynamics of the control agent.

Acknowledgements

This work was funded by a Natural Environment Research Council (NERC) grant and the International Mycoherbicide Programme for *Eichhornia crassipes* Control in Africa (IMPECCA) Program, which was funded by the Danish International Development Agency (DANIDA) through the Environment, Peace and Stability Facility (EPSF).

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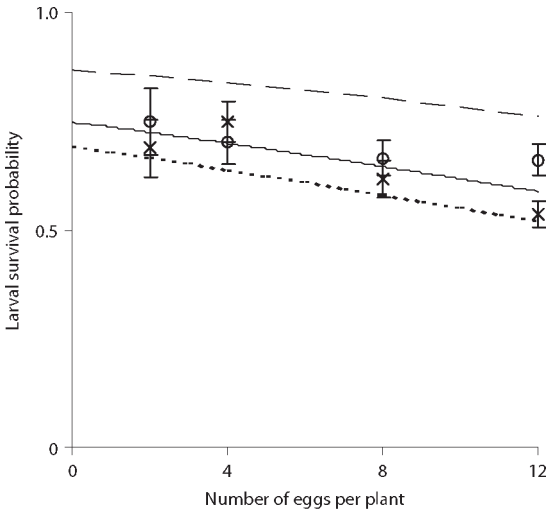

Figure 4. The effect of egg density on larval survival for 25-day-old larvae. Larvae from low nutrient plants are x, and from high nutrient plants are o, with standard error bars shown. The fitted relationships are for plants of initial weight 40 g (bottom line/lowest initial weights), 160 g (middle line/experimental average), and 300 g (top line/highest initial weight).

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Development of bioherbicides for rice weeds in Vietnam

B.A. Auld,[1] H.M. Trung,[2] D.V. Chin,[3] N.V. Tuat,[2] S.D. Hetherington,[1] H.L. Thi[3] and H.M. Thanh[2]

[1] NSW Agriculture, Orange Agricultural Institute, Forest Road, Orange NSW 2800, Australia
[2] National Institute of Plant Protection, Chem-tu Liem, Hanoi, Vietnam
[3]Cuu Long Rice Research Institute, Omon, Cantho, Vietnam

The two major grass weeds of rice in Vietnam, *Leptochloa chinensis* in the Mekong Delta and *Echinochloa crus-galli* in the Red River Delta, are targets for bioherbicides being developed in an Australian Centre for International Agricultural Research-funded project. The project has now entered the field evaluation phase. In the Mekong Delta, the fungus *Setosphaeria rostrata* has proven effective in controlling *L. chinensis* in repeated field experiments. Techniques for mass production of the fungus are currently being investigated. In the Red River Delta, the fungus *Exserohilum monoceras*, although promising in screen house experiments on *E. crus-galli*, has not been as successful in field experiments. The same fungus has proven effective in the field in Japan. Reasons for the reduced effectiveness of the fungus under field conditions in Vietnam are discussed and further experiments are suggested.

Potential of the petiole-galling weevil, *Coelocephalapion camarae*, to markedly improve biocontrol of *Lantana camara*

J.-R. Baars,[1] F. Heystek[1] and M.P. Hill[2]

[1] ARC–Plant Protection Research Institute, Private Bag X134, Pretoria 0001, South Africa
[2] Rhodes University, Department of Zoology and Entomology, PO Box 94, Grahamstown 61403, South Africa

Biocontrol of *Lantana camara* L. is hampered in some areas by the leafless condition that the plant periodically experiences in response to frost, drought or cold, dry winters. One aspect of the South African strategy against this noxious weed is therefore to select candidate biocontrol agents that have the potential to bridge periods of leaflessness. An apionine, recently described as *Coelocephalapion camarae* Kissinger (Coleoptera: Brentidae), was collected from *L. urticifolia* in Mexico and evaluated in quarantine. The small, robust adults of this weevil are long-lived and may be able to bridge periods of leaflessness. The adults chew shot-holes into the leaves and the female inserts an egg into a suitable petiole, where the larva emerges and mines the vascular tissue, inducing gall formation. This disrupts translocation of photosynthates to the roots, and at sufficient galling levels, causes root growth to cease. The oviposition requirements of the female reduce the potential for non-target impact, as only few, related, indigenous plants proved suitable, but inferior, for larval development. Few provided suitable oviposition sites. Different varieties of *L. camara* naturalized in South Africa proved equally suitable for oviposition preference and development. Due to the high impact and potential to sustain population levels during leafless periods, *C. camarae* is expected to establish throughout South Africa and markedly improve the success of lantana biocontrol.

The influence of herbivory by the mirid *Eccritotarsus catarinensis*, on the competitive ability of water hyacinth

J. Coetzee,[1] M.P. Hill[2] and M.J. Byrne[1]

[1] University of the Witwatersrand, School of Animal, Plant and Environmental Sciences, Ecophysiological Studies Research Programme, Johannesburg, Wits 2050, South Africa
[2] Rhodes University, Department of Zoology and Entomology, Grahamstown 6140, South Africa

Eccritotarsus catarinensis is a relatively new biological control agent, approved for use against water hyacinth in South Africa. As one of five arthropods now released against the weed, this study was undertaken to evaluate the efficacy of an additional agent against water hyacinth. Studies on the effects of biological control agents are usually limited to the direct effects of the agent on the target weed and the potential direct effects of the agents on indigenous species related to the target weed. A fundamental justification for using biological control agents to suppress invasive plants is that, by weakening the invader, indigenous species may gain a competitive advantage. Under high nutrient conditions, water hyacinth has been found to be a superior competitor over other aquatic macrophytes, such as water lettuce. Because of its plastic growth form, water hyacinth easily outcompetes water lettuce by overshading and removing nutrients from the system. Previous studies have shown that differential pressure from herbivores can shift the competitive balance between plant species, in favour of the weaker competitor. An addition-series experiment was conducted to determine the effect of herbivory by the mirid on the competitive interactions between water hyacinth and water lettuce. Our results show that water hyacinth remains the superior competitor over water lettuce, in spite of feeding damage from the mirid. However, under low nutrient conditions, the mirid had the greatest effect on water hyacinth's competitive ability. Unfortunately, water hyacinth presents the greatest problem in eutrophic sites in South Africa. With these data, the value of *Eccritotarsus catarinensis* as an additional agent against water hyacinth is considered.

What is "success" in biological control?

Ernest S. Delfosse

USDA–ARS–NPS, 5601 Sunnyside Avenue, Room 4-2238, Beltsville MD 20705-5139, USA

Success in biological control is usually measured in terms of some degree of perceived management of the target species. This is an important aspect to measure, but is often so qualitative as to be almost meaningless, and does not reflect fully the accomplishments of a biological-control program. I propose that there are six primary components of "success" in biological control that should be measured: political, scientific, economic, social, legal, and environmental success. Political success includes initiation, visibility, action, apparent management of target weed, increased support for systematics, and philosophical support from nontraditional groups. Scientific success includes establishment of agents, experiments based on testing refutable hypotheses, results used to improve predictability and contribute to ecological theory, conforming to highest ethical standards, exploring the physiological versus ecological host range, and use of risk analysis. Economic success includes factors such as acceptable benefit/cost ratios, development of meaningful metrics of "management," and involving economists early in program development. Social success factors include scientific programs that identify and address social needs, involving appropriate societal groups (scientists, environmentalists, states, politicians, the private sector, and other special interests), increasing communication through extension and technology transfer, and involving sociologists early in program development. Legal success is almost never achieved, and includes factors such as knowledgeable lawyers being involved in development of workable laws and regulations for biological control, and having laws and guidelines that are science-based. Environmental success includes long-term pest management that is low-input and energy-conserving, maintains or increases biological diversity, and results in global decrease in pesticide risk. I propose a working hypothesis that all six components of "success" are important, but must feed directly and deliberately into "political success", or long-term benefits from all components will be lost. Data from biological control programs are presented to test this hypothesis.

Establishment and impact of *Falconia intermedia* (Hemiptera: Miridae) on *Lantana camara* (Verbenaceae) in South Africa

F. Heystek[1] and T. Olckers[2]

[1] ARC–Plant Protection Research Institute, Private Bag X134, Pretoria 0001, South Africa

[2] ARC–Plant Protection Research Institute, Private Bag X6006, Hilton 3245, South Africa

The lantana mirid, *Falconia intermedia*, was first released in South Africa in 1999. Releases were made throughout the country, but the largest numbers were released in the Limpopo, Mpumalanga and KwaZulu-Natal provinces where lantana is most problematic. The mirid established at several sites in these provinces, but failed to establish at sites in the North-West and Gauteng provinces. Releases in the Eastern Cape were made only recently. Establishment failure is ascribed to: climatic incompatibility (too cold or dry), low release numbers (predation), insecticide drift and varietal resistance in the target weed. Where established, the mirids rapidly built-up large populations and spread through infestations in a wave action. At these population levels, the mirids caused severe chlorosis of the leaves and leaf drop, resulting in the mirid population crashing. Following regrowth of the plants, the mirid populations increased rapidly. Severe damage to seedlings was also observed. Some temporary spillover onto indigenous *Lippia* species has been observed when high population densities were reached on adjacent lantana. At sites where the mirid established well, there was a marked reduction in fruit-heads produced. The impact that *F. intermedia* has on lantana will be limited to climatically suitable areas, and will vary according to site conditions and over time. This confirms the limitations of leaf-feeding agents for control of a plant that is sometimes deciduous.

Ecology of *Megastigmus aculeatus* (Hymenoptera: Torymidae), a seed parasitoid of *Rosa multiflora* in Iowa, USA

Laura C. Jesse and John J. Obrycki

Department of Entomology, Iowa State University, Ames, IA 50011 515/294-1999, USA

Rosa multiflora Thunb. (Rosaceae) currently infests 18.2 million hectares of pastures and nearby forested areas in the eastern United States. A natural enemy that has the potential to reduce the reproductive output of *R. multiflora* is *Megastigmus aculeatus* (Hymenoptera: Torymidae). Female *M. aculeatus* lay their eggs directly in the developing *R. multiflora* seeds within the hip (the fleshy fruit containing the seeds), the larvae feed within the seed and over-winter within the seed. *Megastigmus aculeatus* may reduce the spread of *R. multiflora* by seed dispersal if it destroys a high percentage of seeds. Natural infestation rates of *R. multiflora* hips collected in Virginia and North Carolina ranged from 25 to 42%. The objectives of this study were to determine 1) if *M. aculeatus* occurs in Iowa, 2) levels of *M. aculeatus*-infested seeds in southern, eastern, and north-eastern Iowa, and 3) if *M. aculeatus* reduces the number of viable *R. multiflora* seeds per hip. Rosehips were collected from 49 sites in Iowa in fall–winter 2001–2002. Twenty hips from each site were dissected and we recorded the number of seeds attacked by *M. aculeatus*, the number of viable, undeveloped (dwarf), and black (non-viable) seeds within each hip. We found at least one *M. aculeatus* larva at 63% of the sites sampled. The majority of hips contained no *M. aculeatus* larvae (73%). In the hips that were attacked, there were 1–7 *M. aculeatus* larvae per hip; 52% of hips contained 1 larval-infested seed. There were 2,287 seeds in hips containing at least one wasp larva, of these seeds 21% contained a larva, 13% appeared viable, 24% non-viable, and 42% were undeveloped. There were 6,091 seeds in hips containing no wasp larvae, of these seeds 20% appeared viable, 31% non-viable, and 49% were undeveloped.

Spatial distribution and seasonal life history of *Aceria malherbae* (Acari: Eriophyidae) on *Convolvulus arvensis* in Montana, USA

J.L. Littlefield

Montana State University, Department of Entomology, 333 Leon Johnson Hall, Bozeman MT 59717 USA

Field bindweed, *Convolvulus arvensis* L. (Convolvulaceae), is one of the most aggressive, perennial weeds of grain-producing areas of North America. To control this weed, the leaf-galling mite, *Aceria malherbae* Nuzzaci (Acari: Eriophyidae) has been utilized. The phenology and spatial distribution of the mite were followed for a three-year period (2000–2002) in central Montana. Stems emerged late in May and continued to emerge until mid-July. Thereafter, numbers generally declined due to senescence caused by dry conditions. In contrast, the number and percent of infested stems increased during the summer. Approximately 20 to 27% of stems were infested in early spring. By late August, between 39 and 62% of the remaining stems were infested. No significant declines in stem densities were observed, although densities varied within the season depending upon moisture, as well as among years. Leaf production generally increased during the summer. In early June, 9 to 13% of leaves were infested, whereas by early autumn, 20 to 42% of leaves present were infested. Greater numbers of galls were observed on leaves in the upper stem crown. In 2000, mean mite populations per gall increased during the season, whereas in 2001 and 2002, populations peaked in July and then decreased. Mites dispersed to root buds during the drier parts of the summer, but were also located on buds throughout the season. Both bindweed stems and infested stems were spatially aggregated within plots. This aggregation, although somewhat consistent from year to year, varied throughout the season and among years. Weak associations between stem densities and the presence of infested stems were noted. These associations did not reflect the intensity of mite infestation. Slight microhabitat differences may exist which would influence the success of the mite to overwinter and to repopulate plants the following year.

Phomopsis amaranthicola as a post-emergence bioherbicide in peppers (*Capsicum annuum* and *C. frutescens*) and eggplant (*Solanum melongena*)

J.P. Morales-Payan,[1, 2] R. Charudattan,[2] W.M. Stall[1] and J.T. DeValerio[2]

[1] Horticultural Sciences Department, University of Florida, Gainesville, FL 32611-0690, USA

[2] Department of Plant Pathology, University of Florida, Gainesville, FL 32611-0680, USA

Pigweeds (*Amaranthus* spp.) are among the most abundant weeds occurring in vegetable crops throughout the world. Biological suppression of pigweeds is desirable in organic and/or conventional production systems in which selective chemical herbicides are lacking, limited or not efficacious. In several field experiments, the fungus *Phomopsis amaranthicola* was evaluated as a post-emergence bioherbicide to control *Amaranthus lividus* in bell pepper (*C. annuum*), and *A. dubius* in Caribbean-bonnet pepper (*C. frutescens*), and eggplant (*S. melongena*). In all experiments, the fungus was sprayed at run-off volume on the weed/crop canopy at a rate of 1.0–1.5 million conidia per mL. Pigweeds that survived inoculation with *P. amaranthicola* were allowed to interfere with the crops season-long. In eggplant and Caribbean-bonnet pepper, spraying *P. amaranthicola* 10 days after weed emergence (DAE) caused about 30% mortality in different population densities of *A. dubius*, and resulted in yield loss reductions of about 25% in pepper and 16% in eggplant, as compared with the untreated weedy crops. In the bell pepper experiments, the results were similar when using a *Psyllium* mucilloid or an agricultural oil (PCC-588) as a surfactant in the spraying mix. In bell pepper, two applications of *P.*

amaranthicola (10 and 20 DAE) were more effective than one application (10, 20, 30, or 40 DAE) in suppressing *A. lividus* growth and interference with the crop. When *P. amaranthicola* was applied more than twice, improvements in pigweed control and pepper yield were negligible. Maximum weed mortality, growth suppression, and yield-loss reduction in these crops were obtained with one or two early applications of the fungus (10 DAE in eggplant and Caribbean-bonnet pepper and 10 and 20 DAE in bell pepper). Further enhancement in the efficacy of *P. amaranthicola* as a post-emergence bioherbicide may be possible through the use of improved formulations.

Paterson's curse crown weevil (*Mogulones larvatus*) impacts in north-eastern Victoria, Australia

T. Morley,[1] D.A. McLaren,[1,2] K. Roberts[1] and J. Bonilla[1]

[1] Department of Natural Resources and Environment, Keith Turnbull Research Institute, PO Box 48, Frankston 3199, Australia

[2] Cooperative Research Centre for Australian Weed Management

The Paterson's curse crown weevil, *Mogulones larvatus* was first released in Victoria in 1993 and has successfully established on *Echium plantagineum.* At Euroa, in north-eastern Victoria, an insecticidal exclusion technique was used protect *E. plantagineum* from attack by *M. larvatus,* enabling assessment of the weevil's biological control impacts. The effects of the insecticide treatment varied between trials and sampling times. In one case, *E. plantagineum* cover in treated plots was observed to be 53% greater than in control plots, with concurrent decreases of grass and clover. Higher *E. plantagineum* plant density and greater plant size in treated compared to control were also observed.

The effect of nutrient-rich water on the biological control of water hyacinth

I.G. Oberholzer[1] and M.P. Hill[2]

[1] ARC–Plant Protection Research Institute, Private Bag X134, Pretoria 0001, South Africa

[2] Department of Zoology and Entomology, Rhodes University, Grahamstown 6140, South Africa

The effect of nutrient rich water on the biological control of water hyacinth was investigated at a field site and under laboratory conditions. Over an 18-month period, water hyacinth was sampled at three sites at Hammarsdale Dam (Kwa-Zulu Natal Province, South Africa), a dam where the weevil, *Neochetina eichhorniae* Warner was released in 1974. Additional agents, *Neochetina bruchi* Hustache (Coleoptera: Curculionidae), *Eccritotarsus catarinensis* (Carvalho) (Heteroptera: Miridae), *Niphograpta albigutallis* Warren (Lepidoptera: Pyralidae) and the mite *Orthogalumna terebrantis* Wallwork (Acarina: Galumnidae) were also released during the mid 1990s. Water hyacinth growth parameters and biological control agent population dynamics varied significantly between the three sites and this was positively correlated with differences in nitrate (NO_3^-), nitrite (NO_2^-) (N) and phosphorus (P) concentrations between the sites. In the laboratory, the effect of three natural enemies, *Neochetina eichhorniae, N. bruchi* and *Eccritotarsus catarinensis*, was quantified on water hyacinth growing in water with six different nutrient (N and P) concentrations. The results showed that the higher the nutrient concentrations, the less effective the biological control. This study reconfirms the importance of nutrient control in the long-term biological control of water hyacinth.

Establishment and impact of the lace bug *Gargaphia decoris* released against the invasive tree *Solanum mauritianum* in South Africa

T. Olckers[1] and W.D. Lotter[2]

[1] ARC–Plant Protection Research Institute, Private Bag X6006, Hilton 3245, South Africa
[2] Sappi Forests, PO Box 13124, Cascades 3202, South Africa

Biological control of *Solanum mauritianum* Scopoli, a major environmental weed in the high-rainfall regions of South Africa, was initiated in 1999 with the release and subsequent establishment of a leaf-sucking lace bug, *Gargaphia decoris* Drake (Tingidae). Post-release evaluations have focused on the seasonal population dynamics of *G. decoris* and the impact of feeding damage on the growth and reproduction of the weed. However, expectations that *G. decoris* would become a very successful agent and cause extensive damage in the field have so far not been realized. The lace bug has failed to establish at the majority of release sites, largely because of interference from generalist predators and possibly adverse climatic conditions. Also, in the colder, high-altitude regions of South Africa, where releases of *G. decoris* have been the most successful, there is a lack of synchrony between high insect population densities and the phenology of the weed. Populations of *G. decoris* decline drastically during the winter months, recover slowly during spring and reach high densities only at the end of summer and during autumn, ensuring that the weed suffers insufficient stress during the growing season. The moderate levels of damage recorded so far have thus been insufficient to adversely affect the considerable growth rate and reproductive output of *S. mauritianum* plants. New genetic stocks of *G. decoris*, recently imported from colder high-altitude areas in Brazil, may be better adapted to these climatic conditions than are the original stocks that were imported from warmer areas in Argentina and may thus prove more successful.

Effects of site characteristics on establishment of *Larinus minutus* (Coleoptera: Curculionidae), a capitulum-infesting weevil of diffuse knapweed, *Centaurea diffusa* (Asteraceae), in north-central and eastern Washington State, USA

Daro G. Palmer and G.L. Piper

Department of Entomology, Washington State University, Pullman
WA 99164-6382, USA

Programs for the biological control of weeds are generally based on redistributing insects from a nursery site to new sites within the same region, with the expectation that the natural enemies will establish. However, a variety of factors may play a role in whether or not an insect release will be successful in terms of establishment. Studies have shown that specific site factors and practices are important to consider, but studies must be conducted on an individual insect basis. The lesser knapweed weevil, *Larinus minutus* Gyllenhal, is generally known to thrive in hot areas with sandy, well-drained soils. This insect's plant host, diffuse knapweed (*Centaurea diffusa* Lamarck), is not limited in its distribution by these criteria, making it important to identify site factors and practices that result in increased success of establishment of the insect or determine if other bioagents may be more suitable for release. Weevil establishment was evaluated at multiple release sites in Washington in 2002. The factors evaluated included: 1) release size, 2) soil type, 3) slope aspect, 4) percentage slope, 5) percentage canopy, 6) annual precipitation, 7) elevation, 8) land use type, 9) disturbance, 10) forest structure, 11) site topography, and 12) infestation size and shape.

Impacts on gorse (*Ulex europaeus*) seed production of two biological control agents, gorse seed weevil (*Exapion ulicis*) and gorse pod moth (*Cydia succedana*), in Canterbury, New Zealand

Trevor R. Partridge[1] A. Hugh Gourlay[1] and Richard L. Hill[1, 2]

[1] Landcare Research, PO Box 69, Lincoln, Canterbury, New Zealand
[2] Richard Hill & Associates, PB 4704, Christchurch, Canterbury, New Zealand

The impact of two seed-feeding insects, gorse seed weevil (*Exapion ulicis*) and gorse pod moth (*Cydia succedana*), on seed production and seed-fall of gorse (*Ulex europaeus*) was examined at Jimmy's Knob, Canterbury, New Zealand. Seeds per pod were counted and loss of seed from infested pods attributed to the two biological control agents. Seed-fall was determined from seed collected in trays beneath the plants. Thus, a seed budget on a per area basis was calculated. At the site, gorse generally flowered once in spring and again in autumn, but with significant local variation. Two blocks of gorse in the stand produced only spring seed, another produced seed only in autumn, and an intermediate produced seed in both seasons. Spring seeding tended to occur on the same plants each year, while autumn seeding tended to occur on different plants in different years. Seed-fall differed by blocks: spring-produced seed accumulated under a few bushes with little seed falling in between, while the heavier autumn seed crop was well distributed. Spring-produced seed was attacked by both agents, with virtually all seed being destroyed. Autumn-produced seed was attacked only by *Cydia*, with about 10% of the seed being destroyed. Insecticide treatment to remove *Cydia* showed that *Exapion* was able to increase its seed destruction in the absence of *Cydia*. Because spring and autumn produced seed was attacked differently, variable seeding behaviour requires study over a longer time and in other areas because of its important implications for biocontrol and land-management practices.

Indirect impacts of herbivory by *Oxyops vitiosa* on the reproductive performance of the invasive tree *Melaleuca quinquenervia*

Paul D. Pratt and Ted D. Center

USDA–ARS Invasive Plant Research Laboratory, 3205 College Ave., Fort Lauderdale FL 33314, USA

Impacts of herbivores are dependent, in part, on the compensatory abilities of their host plants in response to feeding damage. Predicting how a plant will respond to herbivory is complex, but often related to the timing and type of herbivory, plant competition, and nutrient availability. The invasive tree *Melaleuca quinquenervia*, for instance, is a long-lived perennial that is competitively superior to native vegetation and occurs in the nutrient-rich wetlands of southern Florida. The introduced weevil *Oxyops vitiosa* Pascoe (Coleoptera: Curculionidae) is a host-specific natural enemy of *M. quinquenervia* and feeds exclusively on developing foliage at branch apices, which consist of <1% of the plants total biomass. The primary goal, and therefore the measure of success, for introducing *O. vitiosa* was to reduce the plant's reproductive capacity. When considering the seemingly ideal growing conditions in the weed's adventive range and the level of feeding damage, we questioned if the indirect effects of herbivory by *O. vitiosa* negatively affects the reproductive potential of *M. quinquenervia*. When comparing plant reproductive performance in replicated melaleuca stands, trees incurring four consecutive years of damage by *O. vitiosa* had a lower probability of flowering across the entire range of tree sizes evaluated. Assuming tree size is correlated with age, herbivory also delayed the reproductive maturity of saplings. Similarly, herbivory influenced biomass allocation, with damaged trees producing more secondary (terminal) branches and fewer fruit than undamaged trees. In a separate weevil exclusion experiment with uniform tree sizes, a single herbivory event resulted in an 80% reduction in the number of flowers produced per tree; however, seed viability (germinability + dormancy) from the few fruits that developed on damaged trees was not

different when compared with undamaged controls. These findings suggest that although *M. quinquenervia* grows under highly favourable conditions, the invasive tree undercompensates reproductively in response to herbivory by *O. vitiosa*.

Biocontrol of hawkweeds in New Zealand, 10 years on

L. Smith, C. Horn and P. Syrett

Landcare Research, PO Box 69, Lincoln, Canterbury, New Zealand

Five hawkweed species (Asteraceae: *Hieracium pilosella, H. praealtum, H. caespitosum, H. lepidulum, H. aurantiacum*) have been identified as invasive over large areas of hill and high country in New Zealand. Hawkweeds have reduced agricultural and conservation values by replacing both exotic pasture and native plant species. Chemical and physical control is difficult, non-specific, and often costly. Similarly, some management techniques at best only slow the spread of these weeds. In 1993, a farmer group, the Hieracium Control Trust (HCT), was formed to fund research into biological control of hawkweeds, providing an excellent example of a community-based end user group working in partnership with scientists to better manage a weed problem. Their achievements include the introduction of a rust (*Puccinia hieracii* var. *piloselloidarum)* isolated from European collections, and four insect agents. The first insects to be successfully tested for host specificity, imported, reared, and released were the gall wasp (Cynipidae: *Aulacidea subterminalis*) and plume moth (Pterophoridae: *Oxyptilus pilosellae*), both released in 1999. Two further species were released in 2002: the gall midge (Cecidomyiidae: *Macrolabis pilosellae*) and the root hover fly (Syrphidae: *Cheilosia urbana*). Release of the crown-feeding hover fly (Syrphidae: *Cheilosia psilophthalma*) was planned for 2003–04. All agents attack *H. pilosella,* with some also attacking various combinations of other introduced hawkweeds present in New Zealand. Insects have been released at more than 70 sites throughout New Zealand, with further releases planned. Procedures are in place to monitor their establishment and impact on target and non-target vegetation. The success of the HCT has led to the formation of other land manager/scientist partnerships for biological control of Californian thistle (*Cirsium arvense*) and Scotch broom (*Cytisus scoparius*). The activities of these groups illustrate the increasing importance of community leadership in seeking science-based, practical solutions to New Zealand weed problems.

Biological control of *Salvinia molesta* in the United States

Philip W. Tipping and Ted D. Center

USDA–ARS Invasive Plant Research Laboratory, Ft. Lauderdale, FL 33314, USA

Giant salvinia, *Salvinia molesta*, is one of the world's worst aquatic weeds. It has invaded freshwater ecosystems on four continents, with the latest invasion occurring in North America in 1998. The plant is now well established in the states of Texas and Louisiana, and it is here where the first biological control efforts were focused using the almost universally successful insect agent *Cyrtobagous salviniae*. These weevils were first collected from common salvinia, *S. minima*, growing in Florida and then released on *S. molesta* in Texas in June 1999. Additional releases were made through 2000 in a number of sites, but a series of natural and man-made disruptions made evaluations impossible. Genetic comparisons of the D2 gene region from weevils collected in Florida and Australia found enough base pair differences to question the taxonomy of the Florida weevils, resulting in a halt to further releases. Resolving permit issues to release *C. salviniae* from Australia (ex Brazil) took about 16 months until October 2001 when weevils were finally released at all new sites. No additional releases were conducted that year because of the oncoming winter. In March 2002, adults were found at some release sites, indicated their ability to survive in areas where below freezing air temperatures were present for 24–72 hours. Repeated detections of weevils, including teneral adults, have been made at all release sites during 2002. Significant damage to *S. molesta* is evident at most release sites and the damage appears to be spreading out from the release points. While it is too soon to verify establishment of the insect and fully evaluate its impact, the project is progressing positively and may enable the United States to join the list of other countries that have used biological control to suppress giant salvinia.

Release strategies and associated factors affecting the establishment of four rust fungi introduced into Australia between 1991 and 2001 for the biocontrol of *Parthenium hysterophorus, Cryptostegia grandiflora* and *Lantana camara*

Allan Tomley,[1] Harry Evans,[2] Carol Ellison,[2] Marion Seier,[2] Sarah Thomas[2] and Djamila Djeddour[2]

[1] Alan Fletcher Research Station, PO Box 36, Sherwood, Queensland 4075, Australia

[2] Weed Biological Control Programme, CABI Bioscience UK Centre (Ascot), Silwood Park, Buckhurst Road, Ascot, Berks SL5 7TA, UK

Four pathogens were imported into Australia for the biocontrol of *Parthenium hysterophorus* (parthenium weed), *Cryptostegia grandiflora* (rubber vine) and *Lantana camara* (lantana) over a 10-year period from 1991 onwards. The Mexican rusts *Puccinia abrupta* var. *partheniicola* and *Puccinia melampodii* were imported for the biocontrol of parthenium weed in 1991 and 2000, respectively. *Maravalia cryptostegiae* was introduced from Madagascar to control rubber vine in 1994 and *Prospodium tuberculatum* from Brazil was released in 2001 against lantana. Prevailing weather, particularly rainfall, at the time of and after release has had a significant impact on the release strategies used and subsequent target impact. In Queensland, 2002 has been one of the driest years since records began, and abnormally dry conditions since 1990 have hindered the progress of the projects described.

Parthenium weed. Parthenium weed grows in sub-coastal central Queensland, which has had below average rainfall since 1990. *Puccinia abrupta* var. *partheniicola* requires cool, moist conditions and was first released in 1991. Due to prolonged dry weather, repeated releases over a six-year period were required to gain establishment. Field inoculations were carried out opportunistically on actively growing parthenium weed after rainfall, or on plants in irrigated nursery sites. Inoculations were made by applying dry spores to the foliage or by deploying infected bait plants amongst patches of the weed. Throughout the host range south of Clermont, sporadic outbreaks of *P. abrupta* now occur following moist conditions in autumn and winter. Damage overall is minimal in most years. However, plants are stunted and weakened by heavy infection which accompanies optimum moisture levels. Releases of *P. melampodii*, which requires warm moist conditions, began in late 1999 and are continuing in areas with adequate rainfall. As a result of recent drought conditions, the rust has failed at some sites where it appeared to have been established, and further releases were required. As the spores of this rust cannot be collected and stored, field inoculations are made by deployment of infected bait plants. *P. melampodii* is established along the Burdekin River near Charters Towers, and near Rockhampton and Rolleston. When moisture levels are adequate, this rust has the potential to inflict severe damage to its host, causing stunted growth and reduced seed production

Rubber vine. In Queensland, most rubber vine grows in the far north, which has defined wet and dry seasons. As the optimum period of leaf wetness for rubber vine rust (*Maravalia cryptostegiae*) is 12 hours, the best time for field inoculation was at the height of the wet season. A light aircraft was used to visit remote sites that could not be accessed by motor vehicle. At release sites, rubber vine plants were inoculated by spraying spore-suspensions onto the foliage with a petrol-powered knapsack-misting machine which was carried on board the aircraft. Since the rust was released in 1995, relatively good rainfall has been received in far north Queensland and the rust has spread throughout the host range. Target damage is spectacular in wet years, with prolonged and severe defoliation accompanied by reduced flowering, seed production and significant stem dieback. However, record drought conditions in north Queensland in 2002 have correspondingly reduced target damage.

Lantana. Lantana is widely distributed along the eastern seaboard of Australia, from north of Cairns to the Victorian border. Releases of the rust *Prospodium tuberculatum* began in late 2001 and are continuing. As drought conditions during 2002 severely restricted the program, only opportunistic releases were made at sites with adequate rainfall. Due to the dry conditions, several inoculations have been required at some sites to achieve infection. Field inoculation is carried out by applying spores mixed with water or powdered talc to the undersides of the leaves. So far, the rust appears to be established at 16 of 80 releases sites in New South Wales and Queensland.

Efficacy and epidemiology of an oil-based formulation of *Colletotrichum gloeosporioides* used as a bioherbicide against *Hakea sericea*

C. van Rooi and A.R. Wood

ARC–PPRI, Weeds Research Division, Private Bag X5017, Stellenbosch 7599, South Africa

Hakea sericea is an alien invasive tree in many parts of the Western and Eastern Cape provinces of South Africa. Although it has been brought under control in many areas by a combination of biological and mechanical control, it still remains a serious threat to native vegetation in certain parts of this country. A South African form of the fungus *Colletotrichum gloeosporioides* causes a serious disease of *H. sericea*. The most characteristic symptoms of the disease are stem cankers and gummosis. Seedlings, induced to germinate by fires, are the stage of the weed most susceptible stage to infection. Fungal-colonized wheat bran was developed as an application method targeting seedlings, but was not feasible or cost-effective on a large scale (dense stands >10 ha in size). A practical method of introducing the fungus to newly germinated seedlings over a large area is needed to maximize control. An oil-based ultra-low volume (ULV) formulation of the fungus has recently been developed. Its efficacy under field conditions and the environmental limits and optimal conditions of infection of this oil formulation are discussed. These include the effect of temperature on growth and infection, comparison of minimum dew period of the formulation and of an aqueous spore solution, and the effect of a dry period between inoculation and dew period. The results indicate that this oil-based ULV formulation would give effective control of *H. sericea* over a large area.

Workshop Summaries

Bioherbicides: the next generation

Graeme W. Bourdôt[1]

Summary

At this sixth meeting of the International Bioherbicide Group, twenty one papers were presented summarising work being conducted in laboratories around the world investigating the potential and application of plant pathogens as biological herbicides (listed below). The title of the workshop "The Next Generation" reflected a need to find ways of overcoming the constraints that have resulted in only two or three new bioherbicide products reaching the market following the early successes of Collego and Devine in the late 1970s. Professor Alan Watson of McGill University, Canada, asked "When will we be successful? Can we solve the formulation and production problems? Can we increase the virulence of our bioherbicides? Can we satisfactorily answer all the regulatory questions? and Can we raise the capital (1–2 million dollars) to complete registration requirements and launch a bioherbicide product?" Lively debate was held around these and other issues in a general discussion following the formal presentations by participants.

Choosing the right market niche and the right organism were considered to be vital for the successful application of plant pathogens as biological herbicides. Further information on the research activities of members of the International Bioherbicide Group is available at <http://ibg.ba.cnr.it>.

Abstracts

Battling the fragrant invader: mass production, application, and implementation of biological control for kahili ginger *(Hedychium gardnerianum)*
R. Anderson

Biological control of aquatic weeds of rice in Australia using *Rhynchosporium alismatis*
G.J. Ash, E.J. Cother, F.G. Jahromi, W. Pitt, V.M. Lanoiselet & S. Ciquet

WOW emulsion formulation for bioherbicides
B.A. Auld

Evaluation of *Phoma macrostoma* for control of broadleaf weeds in turfgrass
K.L. Bailey, J. Derby & S. Falk

Microbes and microbial products for biological control of parasitic weeds
Boari, M. Vurro, M. A. Abouzeid, M.C. Zonno & A. Evidente

Tobacco mild green mosaic virus: a virus-based bioherbicide
R. Charudattan, M. Elliott, J.T. DeValerio, E. Hiebert, & M.E. Pettersen

Microencapsulation: an answer to the formulation quandary?
T. Chittick, G.J. Ash, R.A. Kennedy & J.D.I. Harper

Evaluation of *Ascochyta caulina* for biological control of *Chenopodium album*
R. Ghorbani, C. Leifert & W. Seel

Survey of diseases of alligator weed in eastern Australia for their bioherbicide potential
B.R. Hennecke, R.L. Gilbert & B.A. Auld

Evaluating *Fusarium tumidum* and *Chondrostereum purpureum* as mycoherbicides for gorse
G.A. Hurrell, G.W. Bourdôt, J. Barton (née Fröhlich) & A. Gianotti

Evaluation of the efficiency of *Cercospora caricis* for control of purple nutsedge
S.C.M. Mello & E.A. Teixeira

Phomopsis amaranthicola as a post-emergence bioherbicide in peppers *(Capsicum annuum* and *C. frutescens)* and eggplant *(Solanum melongena)*
J.P. Morales-Payan, R. Charudattan, W.M. Stall & J.T. DeValerio

Assessment of *Dactylaria higginsii* as a postemergence bioherbicide for purple nutsedge *(Cyperus rotundus)* in bell pepper *(Capsicum annuum)*
J.P. Morales-Payan, R. Charudattan, W.M. Stall & J.T. DeValerio

Interactions of *Pyricularia setariae* with herbicides for control of green foxtail
G. Peng & K.N. Byer

International Mycoherbicide Programme for *Eichhornia crassipes* control in Africa (IMPECCA)
R.H. Reeder

[1] New Zealand Pastoral Agriculture Research Institute Ltd, PO Box 60, Lincoln, Canterbury, New Zealand <graeme.bourdot@agresearch.co.nz>.

Centres of origin: do they exist, can we identify them, does it matter?

A.S. McClay,[1] M.D. Crisp,[2] H.C. Evans,[3] T. Heard,[4] R.A. Hufbauer,[5] T.-K. Qin[6] and R. Shaw[3]

Introduction

This workshop assessed the importance and feasibility of identifying "centres of origin" as a guide to selecting areas for survey for classical biocontrol agents for invasive weeds. After an introduction to the issues (McClay), presentations were made on biogeographical methods and problems associated with the centre-of-origin concept (Crisp) and the role of molecular genetic methods in identifying areas of origin (Hufbauer). Two case histories were presented, on the identification of the geographical origin of a wax scale insect (Qin) and of the invasive shrub *Ligustrum robustum* (Evans, Shaw). These presentations were followed by a general discussion (recorder Tim Heard).

Terms and concepts

It is important to distinguish some of the concepts used in relation to "centres of origin" and as guides to selecting areas to survey for biological control agents. The "native range" is the area where a species occurs without having been introduced, deliberately or accidentally, by humans. The "centre of origin" of a species (or higher taxon) is the range that the taxon occupied when it first separated from its sister group. This may or may not be a smaller area than its present native range. The "source of introduction" or "provenance" is the location within the native range from which a founding population was introduced into the exotic range. A "centre of diversification" is an area containing a high diversity of native species closely related to the target weed; this may or may not be an indication of the area in which the target species itself originated, depending on the phylogenetic relationship between the target weed and the other species involved.

Biogeographic methods are available for inferring centres of origin. However, it should be noted that the vicariance school rejects the very notion of a "centre" of origin (Craw et al. 1999, Nelson & Ladiges 2001). Speciation theory suggests that some species evolve from widely occurring meta-populations, rather than from a localized site or "centre". It is difficult to distinguish these alternatives retrospectively, as indicated by the controversy over whether *Homo sapiens* originated over a broad front or in an African centre. Moreover, species' ranges expand and contract with time, and present distribution is not a reliable indicator of that in the distant past (Losos & Glor 2003). The Progression Rule (Brundin 1988, Hennig 1966), a long-standing approach that is still in use, makes the fallacious assumption that a species-poor ("basal") sister taxon occupies the centre of origin from which migration proceeded to the area occupied by its species-rich ("derived") sister taxon (Platnick 1981). Other methods for inferring ancestral areas using a phylogeny have been proposed by Bremer (1992), Page (1994), and Ronquist (1997). The last two attempt to trade off vicariance against jump dispersal and involve difficult decisions about the relative weight (probability) of different events, such as vicariance, dispersal, speciation and extinction. Although some data sets will give unambiguous answers to the area of origin using these methods, in many cases the area of origin cannot be resolved. It was suggested that weed biologists should ask whether it is important to find the (perhaps mythical) centre of origin of the host taxon, or whether they should sample the native range as widely as possible in a direct search for novel biocontrol agents. If the purpose is to identify the native range of a weed, this is better addressed by the methods of population genetics than by biogeographic methods.

The modes of speciation discussed above (see also Levin 2000) will influence patterns of molecular variation used to detect potential areas of origin of species. Speciation via vicariance is likely to lead to greater genetic variation than speciation via dispersal or sympatric speciation. The ability to detect the geographic location in which an organism speciated will depend upon the mode of speciation, the subse-

[1] Alberta Research Council, PO Bag 4000, Vegreville, Alberta, Canada T9C 1T4.
[2] Australian National University, Division of Botany & Zoology, Canberra ACT 0200, Australia.
[3] CABI Bioscience, Silwood Park, Ascot SL5 7TA, Berkshire, UK.
[4] CSIRO Entomology, 120 Meiers Road, Indooroopilly, Queensland 4068, Australia.
[5] Department of Bioagricultural Science and Pest Management, Colorado State University, Fort Collins, CO 80526, USA.
[6] Plant Biosecurity Australia, Plant Biosecurity MAB, AFFA, GPO Box 858, Canberra ACT 2601, Australia.

quent geographic range and history of the species, the samples obtained for analysis, and the markers used. It is critical to obtain samples of the species of interest from across the native range.

Phylogeography is the field of study concerned with mapping of lineages of populations and closely related species through time and geographical space (Avise 2000). In reconstructing phylogeographic relationships, the most useful markers are ordered markers such as sequence data. By knowing the location of samples of the species of interest, plus the location of closely related species, it is possible to infer a common geographic region at the base of the lineage. Because gene trees may not always represent the history of a species, it is key to obtain sequences from more than a single gene to determine if there is concordance between them.

Qin et al. (1994) used cladistic and biogeographic analyses to identify the geographic origin of an insect pest, the wax scale *Ceroplastes sinensis*. Similar analyses may be useful for some cosmopolitan weeds. Shaw and Evans used molecular methods to identify Sri Lanka as the provenance of the invasive privet *Ligustrum robustum* ssp. *walkeri* in La Réunion, but this area had a limited range of host-specific agents. It was felt that surveys in the area of origin, if this could be identified, might increase the pool of candidate agents.

Conclusion

The discussion covered a variety of topics including the geographic origins of alligatorweed, water hyacinth, parkinsonia, parthenium, melaleuca, and climbing fern. Practitioners appeared to feel that the attempt to determine the centre of origin was worthwhile as a way of prioritizing areas for survey, despite some of the biogeographic problems surrounding the concept. In some cases it may be more appropriate to say that the goal is to identify the native range, rather than the centre of origin. It is clear that these issues need to be further explored in order to provide practical guidance in the selection of survey areas, and the participants in the workshop hope to do so in a future paper.

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Agents that reduce seed production – essential ingredient or fools folly?

Rieks D. van Klinken,[1] Darren Kriticos,[2] John Wilson[3,4] and John Hoffmann[5]

Introduction

Seed and flower feeders are routinely used in the biological control of weeds, with the aim of reducing a weed's invasiveness. In many cases these agents are used in preference to agents that attack the vegetative parts, either to avoid conflicts of interest (where vegetative parts of the plant are of value) or because they are frequently host-specific and easy to rear. Although some reproductive feeders have been used very effectively in weed biological control, debate continues about whether our expectations for flower and seed feeders are realistic. Some of the uncertainty stems from the lack of case studies to quantify the effect of the agents on the population dynamics of the target weeds.

A workshop, with 32 attendees, was held to investigate issues surrounding the use of reproductive feeders as biological control agents. Specific aims were to identify the role of flower and seed feeders in biological control, examine evidence for impact, and to discuss means of obtaining further evidence of impact, especially for reduced rates of spread. Attention was focused on insects and pathogens (here nominally included as "feeders") that specifically target buds, flowers or seeds. Natural enemies, such as gall-formers and defoliators that indirectly impact on reproduction were not considered because their impacts are too easily confounded with other effects such as reduced growth rates. Below we summarize some of the general themes that were explored during the workshop, and synthesize the contributions made by workshop participants.

[1] CSIRO Entomology, Long Pocket Laboratories, 120 Meiers Road, Indooroopilly, 4068, Queensland, Australia <rieks.vanklinken@csiro.au>.
[2] CSIRO Entomology and CRC for Weed Management Systems, GPO Box 1700, Canberra, ACT, 2601, Australia.
[3] NERC Centre for Population Biology and CABI Bioscience, Imperial College at Silwood Park, Ascot, Berkshire SL5 7PY, U.K.
[4] Present address: Riversdale, Lochwinnoch Road, Kilmacolm, Renfrewshire, PA13 4DZ, U.K.
[5] Zoology Department, University of Cape Town, Rondebosch 7700, South Africa.

Possible impacts

The direct effects of reproductive feeders include: reduced seed production, increased seed mortality, altered seed quality (e.g. weight, dormancy characteristics), altered seed dispersal characteristics (e.g. *Rhinocyllus conicus* deforms inflorescences and prevents detachment of undamaged seeds from pappus), and altered timing of seeding. These direct effects can potentially result in a wide range of impacts on weed populations, including:

- reduced density of seedlings and/or mature plants
- reduction in the distributional range of the weed
- altered age structures
- reduced population growth rates (e.g. longer periods required to form dense stands in newly invaded areas)
- slower rate of range expansion
- slower rates of reinvasion following disturbance (such as from clearing)
- reduced competitiveness, allowing greater opportunities for desirable plant species to compete with weed
- increased effectiveness of other control agents or control methods.
- more effective management opportunities such as monetary savings, and less habitat disturbance when other control options are exercised (Moran *et al.* 2004)

What types of plant are good targets for reproductive feeders?

The discussion surrounding the identification of these impacts centred on the life history traits of the plant and the invasion/reinvasion phase following disturbance under which the above impacts may be observed. As a result, we attempted to explore factors that indicated weeds that were better targets for biological control using agents that disrupt reproduction. Some of the factors that were identified as favouring the chances of achieving some success with reproductive feeders include:

- low plant fecundity (e.g. a plant with a few large seeds as opposed to many small seeds)
- long maturation period

- limited ability to undergo asexual reproduction
- low seed viability
- limited seed longevity (i.e. limited dormancy)
- density dependent dispersal of seeds
- seed, rather than micro-site, limitations on seedling recruitment rates
- low flower:seed ratio (at least for flower feeders feeding on annual species; there was uncertainty whether this is important for perennial species)

No agreement could be reached about the importance of the following:

- dispersal mechanisms
- duration of seed availability on the host plant
- frequency of disturbance in the weed's habitat (disturbance frequency is an environmental factor whose importance depends on the life history characteristics of the agent)

What types of insects or pathogens are good/bad agents?

This was identified as a large, potentially fruitful area of inquiry. However, it was not dealt with in detail during the workshop.

Demonstration of impact on rate of spread

There are already some dramatic examples of high seed predation resulting in significant decreases in plant populations (e.g. Louda & Potvin 1995, Hoffmann & Moran 1998). Such impacts are relatively easily verified, and provide evidence for the potential of reproductive feeders in biological control. However, reduced rate of spread (including range expansion, formation of thickets in new areas and rates of recovery of infestations) due to reproductive feeders have not yet been demonstrated for any weed, even though it is expected to occur. Reduced rate of spread is a highly desirable outcome of biological control, so significant impact by biological control agents may therefore not be getting acknowledged.

One of the reasons for the impact of reproductive feeders on rates of spread not being studied may be difficulties in demonstrating impact. However, the workshop identified at least two potential approaches:

- *Comparison of spread rates before and after the release of the agent*. Limitations include a requirement for long-term survey data, and potential confounding effects of other factors that might be altering spread rates (e.g. vertebrate herbivores; changing land uses; variable climate) and factors affecting recruitment success (e.g. disturbance regimes).
- *Modelling of dispersal*. Models offer the most likely method for estimating the impact of reproductive feeders on rates of spread. By integrating the available knowledge on the population dynamics of the plant, establishment rates of the plant, damage functions of the agent and the population dynamics of the agent, the model can project the likely impact of the agent on rates of spread. For the same reasons that the direct measurement of the effects are difficult to measure due to confounding effects, such models are likely to be difficult to comprehensively validate.

A priority is to identify systems where biological control agents are likely to be having an impact on rates of spread, and where such impact is likely be relatively easy to demonstrate. Some systems, such as long-lived woody weeds that occur in arid systems where major dispersal and recruitment events are episodic are likely to be especially difficult (Kriticos *et al.* 1999).

Conclusions

Reproductive feeders can potentially impact upon weeds in diverse ways. It is important that any significant impact is clearly demonstrated, so that the benefits of biological control can be acknowledged. Reduction in rates of spread is one impact that may be occurring, has not yet been clearly demonstrated, and is likely to be difficult to quantify. Research into techniques to quantify impacts of reproductive agents on the rate of spread of weeds will be challenging and useful.

Overall, relatively few flower or seed-feeding biological control agents appear to have a significant impact on weed populations. The process of identifying suitable targets for reproductive feeders, and identifying suitable agents, has received relatively little attention, and remains a fruitful area of inquiry. Given the costs and risks of importing agents, research to provide guidelines for the appropriate use of reproductive feeders should be a high priority.

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Symposium Synopsis

Synopsis: the long and winding road

Jim Cullen[1]

Introduction

Biological control of weeds is a steadily evolving science, steering its way round bureaucratic obstacles, funding crises and through the complexities of uncertainties, prediction and probabilities of ecological systems, both old and new, and coevolution, variability, and behaviour. In this synopsis I will try to review briefly the content presented in this symposium and draw out future directions – the next steps in the evolution of our science.

There are a number of procedures involved in a biological control program, procedures under steady improvement as the associated science progresses and we can see where there has been some progress. These will be considered under a small number of groupings similar to, but not constrained by, the main themes of the symposium. There will also be a need to consider what has been achieved in a more general sense and what influence this might have on the future.

Agent selection

The parsimonious approach advocated by McEvoy.[2] only importing effective and damaging agents, is being more generally accepted, but depends critically on the ability to predict effectiveness, and McEvoy emphasized the need to look at whole systems to achieve this. On this theme of effectiveness, Evans & Ellison examined the relative merits of old and new associations, a question originally posed by Hokkanen & Pimentel (1984). The general conclusion was that, while examples of effective old and new associations existed, the concept of closely coevolved natural enemies was still one of the most valuable guiding principles. Goolsby's work on *Floracarpus* was a nice example of the modern approach to matching the natural enemy to the exact host genotype, and Hufbauer presented some recent advances in technology in tracing the origins of invasive weeds. However, the original debate about old and new associations is almost certainly being considered at too superficial a level, and plant pathologists in particular will no doubt concentrate more on the presence and absence of resistant and susceptible genes and their evolution. We can anticipate some interesting work in the application of this more fundamental genetic approach to insect–plant interactions in the future.

However, approaches are always likely to be determined by resources, time frames and feasibility. The better effectiveness can be predicted, the easier it will be to combine necessary pragmatism with good science and be effective, economical and accurate.

In terms of targets, grasses emerged from the shadows as subjects of serious consideration for classical biological control, with *Sporobolus* spp. (Witt & McConnachie), *Nasella* spp. (Anderson *et al.*) and *Spartina* (Wecker *et al.*), each at different stages from review of possibilities to evaluation of natural enemy impact.

Risk analysis and host specificity

Singer's keynote presentation was inspiring, entertaining and thought-provoking, but translation of erudite studies on host acceptability by insects to everyday practice in biological control is not easy. Practitioners do, however, need to remain in contact with theoretical developments so that their methodology does not fall short.

Dealing with genetic variation in the agent under study received increased emphasis with Haines *et al.* pointing out the problems of not taking it into sufficient account. Its frequency and scope in an agent and the consequent probabilities of possible outcomes need to be carefully considered. This clearly increases the complexity of the assessment and places more emphasis on developing the discipline of risk analysis to deal with uncertainties in a standard manner.

Singer also raised the question of whether an opportunity was being lost by ignoring the possibility of using more-specialized biotypes or subspecies among a more generalist insect species; an area not new to plant pathologists. The basis for and stability of such specialization becomes critical.

Variation in the target weed is a recurring theme, but still has the capacity to surprise people. Strong referred to the concept of "self-defeating biological control", where selection pressure on one form of a weed by an agent may simply lead to its replacement by another more-resistant form. Urban *et al.* (poster) for lantana,

[1] CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia email: <jim.cullen@csiro.au>.

[2] Unless otherwise indicated, authors cited in the text refer to presentations (or posters) at the symposium. Not all the work cited appears as full papers in these proceedings.

and Evans *et al.* (poster) for blackberry, provided examples of studies where variation in the host is critical to the conduct of control programs.

The delineation of host range in relation to phylogeny is benefiting from the increasing application of modern technology, and Kelch & McClay presented an informative example of this approach. In the continual emphasis on improving testing procedures to produce more realistic and reliable results, Heard & Segura demonstrated that open field tests are not always the answer. Amidst the push for more standardized procedures that can be understood and accepted by regulatory agencies, flexibility remains of key importance, with testing tailored to agent behaviour, environment and whatever constraints are operating.

Given the controversy in recent years (Louda *et al.* 1997), consideration of non-target effects needed to be on the agenda, but there would be few workers now unaware of the issue. Non-target effects clearly need to be avoided, and recent history suggests that biological control practitioners are doing so (Pemberton, Fowler *et al.*). There remains a critical need to follow up any possibilities and understand them (Snell & McLaren (poster), Hight (poster)), and we will no doubt see more. In analysing any such situations, it is also critical that a distinction be made between damage to non-target species that may or may not have any consequences, and real impact on the population dynamics and possible survival of the species (Baker *et al.*) (also see Willis *et al.* 2003).

Integration and management

Paynter & Flanagan pointed out that, with "partial success" being the commonest outcome (55%) of biological control programs, there is a fertile field for improving the outcome by aiding and abetting the control system. However, to do so effectively requires a good knowledge of the whole system, well demonstrated by Paynter & Flanagan for *Mimosa pigra* and by Erickson & Lym for *Aphthona* spp. on *Euphorbia esula*, leafy spurge. To some extent, this parallels the plea of McEvoy to understand the system in order to predict the effectiveness of an agent in the first place.

Caesar questioned whether an opportunity is being missed with regard to insufficient study of the integration of pathogens and arthropods, while Wecker *et al.* added the socio-economic dimension, whose importance is increasingly being recognized (see below).

Evaluation

The evaluation session again demonstrated that evaluation has several different connotations. Modelling is seen as an important tool, but is generally aimed at analysis of the system to help explain it and try to improve the level of control. McEvoy pointed out that models are getting better as they get closer to reality and, in time, their utility in generating more general assessments of the outcome of a program and the key elements on which it might depend, will be valuable both for evaluation and for improving predictability.

The need for good data describing the outcome of a biological control program was emphasized from the Chair (Judy Myers), particularly population data or quantitative data in terms of cover or biomass where these are relevant. These also become the basis for good economic evaluation, of which there is still a serious lack (also referred to by Sheppard *et al.*). Presentations by Kuniata & Korowi and by McConnachie were the exceptions rather than the rule, but demonstrated the power of good data.

Alternatively or additionally, outcomes in terms of biodiversity improvement are also valuable, but few and far between. Barton *et al.* and Willis *et al.* (poster) provided preliminary examples, while Schooler *et al.* (poster) presented some important and necessary data on biodiversity loss due to invasive weeds, thus providing a basic parameter for later comparison.

Status

A number of successes were reported, particularly against aquatic weeds; biological control programs against water hyacinth, salvinia, water lettuce, and now *Azolla filiculoides*, red water fern, (McConnachie) have been successful in several regions of the world. *Mimosa invisa* and *Sida* spp. have been well controlled in PNG (Kuniata & Korowi), and it appears that *Mimosa pigra* in Australia, via an integrated program (Paynter & Flanagan), and possibly *Hydrilla* in parts of the USA (Grodowitz *et al.*), are heading that way. There were also some initial dramatic results on *Tamarix* spp., saltcedar (DeLoach *et al.*). At the same time it is apparent that steady "progress with the process" is occurring and, if some of the opportunities in the 55% partial successes result in more complete successes, the overall scene looks promising.

However, Sheppard *et al.* managed to ring a number of alarm bells, pointing out that compared with 461 papers on the benefits of biological control, 1685 had been published on non-target effects, producing increasing concern in the community, a proliferation of bureaucracy and an overall slowing of the process, leading to funding concerns. Is biological control being swept along by a range of perceptions and unsympathetic officialdom to somewhere it doesn't want to be? This situation suggests a need to pay greater attention to the societal context of biological control.

Strong pointed out that biological control, being an applied science, is driven by diverse economic and environmental interests, whether agricultural, ecosystem health related, conservation or aesthetics. He also made the point that socio-economics is extremely important, but seemed to have little emphasis in the symposium program. In fact, its presence was some-

what cryptic. The workshop on "Where biocontrol is heading in the 21st century", conducted by Rachel McFadyen, was clear on the need to engage with stakeholders; the community, regulators, politicians and the need to inform, involve and educate. Community understanding and involvement was also clearly an essential part of the Spartina program (Wecker *et al.*), the involvement in the Working for Water program in South Africa (Gillespie *et al.*, Hill & Julien) and the delivery programs of the Cooperative Research Centre for Australian Weed Management (Kwong, Batchelor *et al.*, Swirepik *et al.*). There is clearly a need to engage and inform the community, despite the difficulties. It is also essential to involve the critics, the sometimes distant academics and the bureaucracy, and communicate the benefits. To do this, there is no substitute for data. Biological control is dependent on society for its mandate to proceed.

Consequences and conclusions

Given the context, including the increasing cost of complying with regulatory systems and simply doing the job better, the need to justify introductions becomes more critical. This comes back to some of McEvoy's initial comments on a parsimonious approach and therefore better predicting outcomes. There were many contributions on the effects of particular agents and the factors that influence them that may need to be taken into account, but the need is to make predictions that can be tested (McFadyen & Spafford Jacob, van Klinken).

Biological control deals with enormous complexity (Strong) and the demands to unravel at least some of the complexity are increasing. Does biological control have the capacity to understand sufficient of the big picture to be able to continue the way it has? Probably not, on its own. Natural systems cannot be understood simply by understanding some individual parts. Perhaps new insights are necessary, involving complex systems science.

For the scientist, prediction of effectiveness is still the Holy Grail. This symposium emphasized this again and explored further some approaches to help the quest. The satisfaction and rewards from successful programs were again apparent, while there is no lack of scientific and societal challenges to keep the research fascinating and the application occasionally frustrating.

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Author index

Keyword index

Delegate address list

Given Name	Surname	Address	Email
Freda	Anderson	CERZOS—Universidad Nacional del Sur, Camino de la Camindonga, Km 7, 8000 Bahia Blanca, Argentina	anderson@criba.edu
Joel	Armstrong	CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia	joel.armstrong@csiro.au
Bruce	Auld	NSW Agriculture, Orange Agricultural Institute, Forest Road, Orange, NSW 2800, Australia	bruce.auld@agric.nsw.gov.au
Ruth	Aveyard	CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia	ruth.aveyard@csiro.au
Karen	Bailey	Agriculture & Agri-Food Canada, 107 Science Place, Saskatoon, SK S7N OX2, Canada	baileyk@agr.gc.ca
John L. (Lars)	Baker	Fremont County Weed And Pest, 450 N. 2nd Street, Room 315, Lander, WY 82520, USA	larbaker@wyoming.com
Joe	Balciunas	USDA-ARS, Exotic & Invasive Weed Research Unit, Albany, California USA	joe@pw.usda.gov
Jane	Barton	Contractor to Landcare Research, 353 Pungarehu Road, RD 5, Te Kuiti, New Zealand	jane.barton@ihug.co.nz
Kathryn	Batchelor	CSIRO, Private Bag 5, PO, Wembley, WA 6913, Australia	kathryn.batchelor@csiro.au
Daniel	Bickel	Australian Museum, 6 College Street, Sydney, NSW 2010, Australia	danb@austmus.gov.au
Andrew	Bishop	Dpt Primary Industries, Water & Environment, PO Box 303, Devonport, TAS 7310, Australia	Andrew.Bishop@dpiwe.tas.gov.au
Dana	Blumenthal	USDA-ARS Rangeland Resources Research Unit, 1701 Center Avenue, Fort Collins, CO 80526-2083, USA	dblumenthal@npa.ars.usda.gov
Graeme	Bourdot	AgResearch, Gerald Street, Lincoln, New Zealand	graeme.bourdot@agresearch.co.nz
Susan	Boyetchko	Agriculture & Agri-Food Canada, 107 Science Place, Saskatoon, SK S7N 0X2, Canada	boyetchkos@agr.gc.ca
David	Briese	CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia	david.briese@csiro.au
Bradley	Brown	CSIRO Entomology, 120 Meiers Road, Indooroopilly, QLD 4068, Australia	Bradley.Brown@csiro.au
Bill	Bruckart	USDA-ARS, FDWSRU, 1301 Ditto Avenue, Fort Detrick, MD 21702-5023, USA	bruckart@asrr.arsusda.gov
Yvonne	Buckley	NERC Centre For Population Biology, Imperial College, Silwood Park, Ascot, Berkshire SL5 7PY, UK	y.buckley@ic.ac.uk
Marcus	Byrne	Animal, Plant and Environmental Sciences, University of Witwatersrand, Johannesburg 2050, South Africa	marcus@gecko.biol.wits.ac.za
Anthony	Caesar	USDA, ARS, 1500 N Central Avenue, Sidney, MO 59270, USA	caesara@sidney.ars.usda.gov
Ludovit	Cagan	Slovak Agricultural University, A. Hinku 2, Nitra, 949 76, Slovak Republic	ludovit.cagan@uniag.sk
Peter	Caley	CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia	peter.caley@csiro.au
Seona	Casonato	Landcare Research, Mt Albert Research Centre, 120 Mt Albert Road, Auckland, 1030, New Zealand	casonatos@landcareresearch.co.nz
Ted	Center	USDA, ARS, IPRL, 3205 College Ave, Fort Lauderdale, FL 33314, USA	tcenter@saa.ars.usda.gov
Richard	Chan	CSIRO Entomology, 120 Meiers Road, Indooroopilly, QLD 4068, Australia	Richard.Chan@csiro.au
Raghavan	Charudattan	University Of Florida, Plant Pathology, P.O. Box 110680, Gainesville, FL 32611-0680, USA	rc@mail.ifas.ufl.edu
Matthew	Cock	CABI Bioscience Switzerland Centre, Rue Des Grillons 1, Delemont, CH-2800, Switzerland	m.cock@cabi-bioscience.ch
Phil	Cowan	Landcare Research, Private Bag 11052, Palmerston North, 5301, New Zealand	cowanp@landcareresearch.co.nz
Jim	Cullen	CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia	jim.cullen@csiro.au
Jamie	Davies	Tasmanian Institute of Agricultural Research, 13 St Johns Avenue, New Town, TAS 7008, Australia	jamie.davies@dpiwa.tas.gov.au

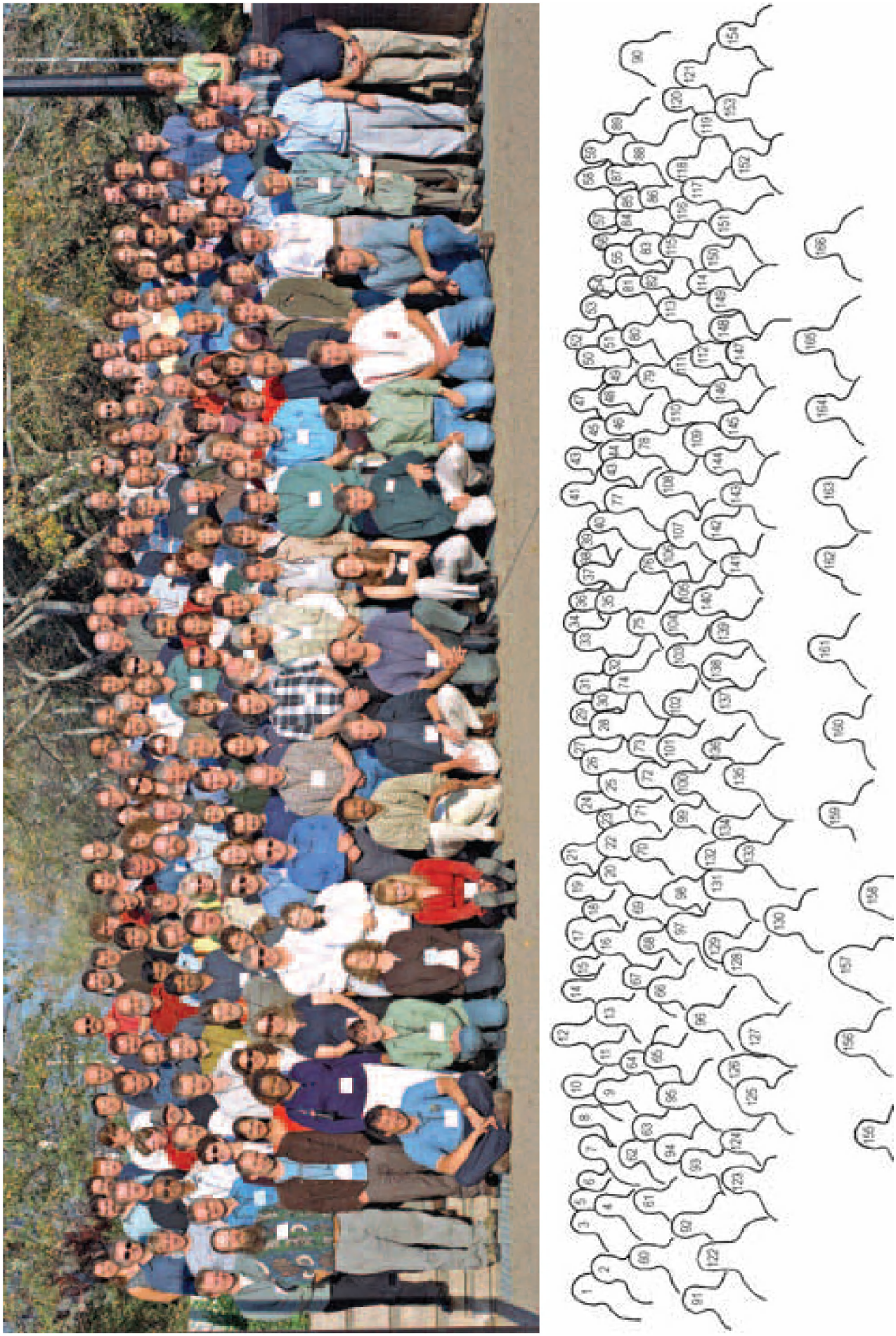
Given Name	Surname	Address	Email
Michael	Day	Alan Fletcher Research Station, PO Box 36, Sherwood, QLD 4075, Australia	Michael.Day@nrm.qld.gov.au
Ernest	Delfosse	USDA-ARS, 5601 Sunnyside Avenue, 4-2238, Beltsville, MD 20705, USA	esd@ars.usda.gov
Alana	Den Breeyan	ARC–PPRI, Vredenburg Research Station, Polkadraai Avenue, Stellenbosch, 7599, South Africa	vredadb@plant3.agric.za
Kunjithapatham	Dhileepan	Alan Fletcher Research Station (QDNR&M), 27, Magazine St., Sherwood, QLD 4075, Australia	dhileepan_k@nrm.qld.gov.au
Marcelo	Diniz Vitorino	Blumenau Regional University, 83 Campus II, Itoupava/Blumenau, Santa Catarina 89030-080, Brazil	diniz@furb.br
Djamila	Djeddour	CABI Bioscience, Silwood Park, Buckhurst Road, Ascot, Berkshire SL5 7TA, United Kingdom	d.djeddour@cabi.org
Margarita	Dolgovskaya	Zoological Institute, Russian Academy Of Sciences, Universitetskaya Nab. 1, St.Petersburg, 199034, Russia	polycest@zin.ru
Liz	Dovey	South Pacific Regional Environment Programme, PO Box 240, Apia, Samoa	lizd@sprep.org.ws
Carol	Ellison	CABI Bioscience, Silwood Park, Buckhurst Road, Ascot, Berkshire SL5 7TA, UK	c.ellison@cabi.org
Abuelgasim	Elzein	University of Hohenheim, Institute of Plant Production & Agroecology, D-70593 Stuttgart, Germany	gasim@uni-hohenheim.de
Harry	Evans	CABI Bioscience, Silwood Park, Buckhurst Road, Ascot, Berkshire SL5 7TA, UK	h.evans@cabi.org
Stuart	Falk	The Scotts Company, 14310 Scottslawn Road, Marysville, OH 43041, USA	stuart.falk@Scotts.com
Giovanni	Fichera	CSIRO Entomology, Long Pocket Laboratories, 120 Meiers Road, Brisbane, QLD 4068, Australia	gio.fichera@csiro.au
Simon	Fowler	Landcare Research, Mt Albert Research Centre, 120 Mt Albert Road, Auckland 1030, New Zealand	fowlers@landcareresearch.co.nz
Kylie	Galway	CRC/Landcare, 18 Kuringal Drive, Ferny Hills, QLD 4055, Australia	kepnz@optusnet.com.au
Daniel E	Gandolfo	USDA-ARS-SABCL, Bolivar 1559, Hurlingham, B1686EFA, Argentina	gandolfo@mail.retina.ar
Esther	Gerber	CABI Bioscience, Rue Des Grillons 1, Delmont, 2800, Switzerland	e.gerber@cabi-bioscience.ch
Magen	Geyer	CSIRO Entomology, TERC, PMB 44, Winnellie, NT 0821, Australia	magen.geyer@csiro.au
Alison	Gianotti	Landcare Research, Mt Albert Research Centre, 120 Mt Albert Road, Auckland 1030, New Zealand	gianottia@landcareresearch.co.nz
John	Goolsby	USDA-ARS-ABCL, 120 Meiers Rd., CSIRO Long Pocket Laboratories, Indooroopilly, QLD 4068, Australia	john.goolsby@csiro.au
Hugh	Gourlay	Landcare Research, PO Box 69, Lincoln, Christchurch, New Zealand	gourlayh@landcareresearch.co.nz
Blair	Grace	D.I.P.E, PO Box 30, Palmerston, NT 0831, Australia	blair.grace@nt.gov.au
Rowena	Gray	Keith Turnbull Research Institute, PO Box 48, Frankston, Victoria 3199, Australia	raelene.kwong@nre.vic.gov.au
Fritzi	Grevstad	University Of Washington, 2907 Pioneer Road, Long Beach, WA 98631, USA	grevstad@u.washington.edu
Michael	Grodowitz	US Army Corps of Engineers, 3909 Halls Ferry Rad, ERDC- CEERD-EE-A, Vicksburg, MS 39180, USA	grodowm@WES.ARMY.MIL
Richard	Groves	CSIRO Plant Industry and CRC Weeds, GPO Box 1600, Canberra, ACT 2601, Australia	richard.groves@csiro.au
Melanie	Haines	Lincoln University, PO Box 69, Ecology and Entomology Unit, Canterbury, New Zealand	hainesmz@lincoln.ac.nz
Helen	Harman	Landcare Research, Mt Albert Research Centre, 120 Mt Albert Road, Auckland 1030, New Zealand	harmanh@landcareresearch.co.nz
Lynley	Hayes	Landcare Research, PO Box 69, Lincoln, Christchurch, 8152, New Zealand	hayesl@landcareresearch.co.nz
Tim	Heard	CSIRO Entomology, 120 Meiers Road, Indooroopilly, QLD 4068, Australia	tim.heard@csiro.au
Bertie	Hennecke	University of Western Sydney, Hawkesbury Campus, Locked Bag 1797, Penrith South, NSW 1797, Australia	b.hennecke@uws.edu.au

Given Name	Surname	Address	Email
Maria Cristina	Hernandez	USDA-ARS-SABCL, Bolivar 1559, Hurlingham, B1686EFA, Argentina	crisher@speedy.com.ar
Stephen	Hight	USDA, ARS, 1650 Summit Lake Drive, Lakeside Building, Tallahassee, FL 32317, USA	hight@nettally.com
Martin	Hill	Rhodes University, Department of Zoology and Entomology, P.O. Box 94, Grahamstown, 6140, South Africa	m.p.hill@ru.ac.za
Richard	Hill	Richard Hill & Associates, Private Bag 4704, Christchurch, New Zealand	hillrl@xtra.co.nz
John	Hoffmann	University of Cape Town, Zoology Department, Rondebosch, 7600, South Africa	hoff@botzoo.uct.ac.za
Royce	Holtkamp	NSW Agriculture, Weeds Biological Control Unit, RMB 944 Calala Lane, Tamworth, NSW 2340, Australia	royce.holtkamp@agri.nsw.gov.au
Shane	Hona	Landcare Research, Mt Albert Research Centre, 120 Mt Albert Road, Auckland 1030, New Zealand	honas@landcareresearch.co.nz
Ruth	Hufbauer	Colorado State University, Dept. Of Bioag. Sci. And Pest Mgmt., Fort Collins, CO 80523-1177, USA	hufbauer@lamar.colostate.edu
Geoff	Hurrell	AgResearch, Gerald Street, Lincoln, New Zealand	geoff.hurrell@agresearch.co.nz
Ruth	Huwer	NSW Agriculture, CTH, PO Box 72, Alstonville, NSW 2478, Australia	ruth.huwer@agric.nsw.gov.au
Fiona	Impson	University of Cape Town, Zoology Department, Rondebosch, 7600, South Africa	vredfi@plant3.agric.za
John	Ireson	Tasmanian Institute of Agricultural Research, 13 St John's Avenue, New Town, TAS 7008, Australia	john.ireson@dpiwe.tas.gov.au
Dennis	Isaacson	Oregon Department of Agriculture, 303 NW 31st Street, Corvallis, OR 97330-5154, USA	tansy1942@yahoo.com
Laura	Jesse	Iowa State University, 4 Insectary, Ames, IA 50011, USA	lrahnsen@iastate.edu
Darryl	Jewett	USDA, Forest Service, 320 Green Street, Athens, GA 30602, USA	djewett@fs.fed.us
Mic	Julien	CSIRO Entomology, 120 Meiers Road, Indooroopilly, QLD 4068, Australia	mic.julien@csiro.au
Doug	Luster	USDA-ARS, FDWSRU, 1301 Ditto Avenue, Fort Detrick, MD 21702-5023, USA	luster@ncifcrf.gov
L. T.	Kok	Virginia Tech, Department Of Entomology, Virginia Tech, Blacksburg, VI 24061-0319, United States	ltkok@vt.edu
Kaile	Korowi	Ramu Sugar Limited, P.O. Box 2183, Lae 411, Papua New Guinea	lkuniata@ramusugar.com.pg
Darren	Kriticos	CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia	Darren.Kriticos@csiro.au
Francesca	Lecce	BBCA Onlus, Largo S. Stefano 3, Anguillara Sabazia, 00061, Italy	flecce@e-bbca.net
Cheryl	Lennox	University of Cape Town, Zoology Department, Rondebosch, 7600, South Africa	Vredcl@plant3.agric.za
Jeffrey	Littlefield	Montana State University, Department Of Entomology, PO Box 173020, Bozeman, MT 59717-3020, USA	JeffreyL@Montana.edu
Mark	Lonsdale	CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia	Mark.lonsdale@csiro.au
Wayne	Lotter	SAPPI Forests, PO Box 13124, Cascades, Pietermaritzburg, 3202, South Africa	wayne.lotter@za.sappic.com
Rodney	Lym	North Dakota State University, Plant Sciences Dept., P. O. Box 5051, Fargo, ND 58105, USA	Rod.Lym@ndsu.nodak.edu
Alec	McClay	Alberta Research Council, PO Bag 4000, Vegreville, T9C 1T4, Canada	alec.mcclay@arc.ab.ca
Andrew	McConnachie	Plant Protection Research Institute, Private Bag X134, Pretoria 0121, South Africa	Rietamc@plant2.agric.za
Peter	McEvoy	Oregon State University, Department of Entomology, Corvallis, OR 97331-2907, USA	mcevoyp@science.oregonstate.edu
Rachel	McFadyen	CRC for Australian Weed Management, PO Box 36, Sherwood, QLD 4075, Australia	rachel.mcfadyen@nrm.qld.gov.au
Fernando	McKay	USDA-ARS-SABCL, Bolivar 1559, Hurlingham B1686EFA, Argentina	fermc@ciudad.com.ar

Given Name	Surname	Address	Email
David	McLaren	Keith Turnbull Research Institute, PO Box 48, Frankston, VIC 3199, Australia	david.mclaren@nre.vic.gov.au
Jeff	Makinson	CSIRO Entomology, 120 Meiers Road, Indooroopilly, QLD 4068, Australia	Jeff.Makinson@csiro.au
George	Markin	United States Forest Service, MSU, 1648 S. 7th Avenue, Bozeman, MT 59717, USA	gmarkin@fs.fed.us
Catherine	Mathenge	UCT/ARC, GPO Box 2486, Sydney, NSW 2001, Australia	Wambui@mailcity.com
Aaron	Maxwell	CSIRO Entomology, Private Bag 5, PO, Wembley, WA 6913, Australia	aaron.maxwell@csiro.au
Glynn	Maynard	OCPPO/AFFA, GPO Box 858, Canberra, ACT 2601, Australia	glynn.maynard@affa.gov.au
Julio	Medal	University of Florida, PO Box 110620, Building 970, Gainesville, FL 32611, USA	medal@maif.ifas.ufl.edu
David	Minkey	University of Western Australia, 25 Broome Street, Nedlands, WA 6009, Australia	dminkey@agric.uwa.edu.au
Joslin	Moore	CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia	joslin.moore@csiro.au
Patrick	Moran	USDA, ARS, Beneficial Insects Research Unit, 2413 E. Hwy 83, Weslaco, TX 78596, USA	pmoran@weslaco.ars.usda.gov
Louise	Morin	CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia	louise.morin@csiro.au
Tom	Morley	Keith Turnbull Research Institute, PO Box 48, Frankston, VIC 3199, Australia	Tom.Morley@nre.vic.gov.au
Rangaswamy	Muniappan	Agricultural Experiment Station, University Of Guam, Mangilao, Guam 96923, USA	rmuni@uog9.uog.edu
Judith	Myers	Univ. British Columbia, Dept. Zoology, 6270 University Blvd., Vancouver, BC V6T 1Z4, Canada	myers@zoology.ubc.ca
Joseph	Neal	North Carolina State University, Dept of Horticultural Science, Box 7609, Raleigh, NC 27695-7609, USA	jcneal@unity.ncsu.edu
Pam	Neame	Consec—Conference Management & Secretariat Services, PO Box 3127, ACT 2617, Australia	pamela.neame@consec.com.au
Michael	Neave	CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia	mick.Neave@csiro.au
Hernan	Norambuena	Inst^{o} de Investigaciones Agropecuarias, C^{o} Regional de Investigacin Carillanca, Casilla 58-D, Temuco, Chile	hnorambu@carillanca.inia.cl
Terry	Olckers	Plant Protection Research Institute, Private Bag X6006, Hilton, 3245, South Africa	ntto@natal1.agric.za
Jose	Pedrosa Macedo	FUPEF/UFPR, Rua Cambara, 50, Curitiba, Parana 80030-380, Brasil	johpema@netpar.com.br
Daro	Palmer	Washington State University, Department Of Entomology, PO Box 646382, Pullman, WA 99164-6382, USA	palmer@coopext.cahe.wsu.edu
William	Palmer	Alan Fletcher Research Station, PO Box 36, Sherwood, QLD 4075, Australia	palmerwa@nrm.qld.gov.au
Quentin	Paynter	CSIRO Entomology, PMB 44, Winnellie, NT 0822, Australia	quentin.paynter@csiro.au
Robert	Pemberton	USDA, ARS, IPRL, 3205 College Ave, Fort Lauderdale, FL 33314, United States Of America	bobpem@saa.ars.usda.gov
Gary	Peng	Agriculture And Agri-Food Canada, 107 Science Place, Saskatoon, SK S7N 5B7, Canada	pengg@agr.gc.ca
Bill	Pettit	CSIRO Entomology, TERC, PMB 44, Winnellie, NT 0821, Australia	bill.pettit@csiro.au
Gary	Piper	Washington State University, Department of Entomology, PO Box 646382, Pullman, WA 99164-6382, USA	cfabich@wsu.edu
Michael	Pitcairn	CDFA, Biological Control Program, 3288 Meadowview Road, Sacramento, CA 95832, USA	mpitcairn@cdfa.ca.gov
Karina	Potter	University of Tasmania, 13 St .John's Avenue, New Town, Hobart, TAS 7098, Australia	karinapotter@dpiwe.tas.gov.au
Paul	Pratt	USDA, ARS, IPRL, 3205 College Ave, Fort Lauderdale, FL 33314, United States Of America	prattp@saa.ars.usda.gov
Matthew	Purcell	CSIRO Entomology, 120 Meiers Road, Indooroopilly, QLD 4068, Australia	matthew.purcell@csiro.au

Given Name	Surname	Address	Email
Ting-Kui	Qin	Biosecurity Australia, AFFA, GPO Box 858, Canberra, ACT 2601, Australia	
Alain	Roques	INRA, Ardon- BP 20619, Olivet, 45166, France	Alain.Roques@orleans.inra.fr
Sathyamurthy	Raghu	CRCAWM and QDNR&M, Alan Fletcher Research Station, Sherwood, QLD 4075, Australia	raghu.s@nrm.qld.gov.au
M.	Ramamoorthi	Centre for Plant Conservation	
Brian	Rector	USDA-ARS, European Biological Control Laboratory, 34988 St Gely Du Fesc Cedex, France	
G.V.P.	Reddy	Agricultural Experiment Station, University Of Guam, Mangilao, Guam 96923, USA	reddy@guam.uog.edu
Ren	Sforza	USDA-ARS European Biological Control Laboratory, 34988 St Gely Du Fesc Cedex, France	rsforza@ars-ebcl.org
Jean Louis	Sagliocco	Keith Turnbull Research Institute, PO Box 48, Ballarto Road, Frankston, VIC 3199, Australia	JeanLouis.Sagliocco@nre.vic.gov.au
Shon	Schooler	Oregon State University, 2046 Cordley Hall, Oregon State University, Corvallis, OR 97331, USA	ssschooler@yahoo.com
John	Scott	CSIRO, Private Bag 5, PO, Wembley, WA 6913, Australia	john.k.scott@csiro.au
Ricardo	Segura	CSIRO Entomology, A. Carlon No. 5, Ejido 1 De Mayo, A.P.14, Boca Del Rio, Veracruz 94297, Mexico	Ricardo.Segura@csiro.au
Marion	Seier	CABI Bioscience, Silwood Park, Buckhurst Road, Ascot, Berkshire SL5 7TA, UK	m.seier@cabi.org
Richard	Shaw	CABI Bioscience, Silwood Park, Buckhurst Road, Ascot, Berkshire SL5 7TA, UK	r.shaw@cabi.org
Judy	Shearer	US Army Corps of Engineers, 3909 Halls Ferry Rad, ERDC- CEERD- EE- A, Vicksburg, MS 39180, USA	shearej@wes.army.mil
Andy	Sheppard	CSIRO, Campus International De Baillarguet, 34980 Montferrier-Sur-Lez, France	andy.sheppard@csiro-europe.org
David	Simelane	Plant Protection Research Institute, Weeds Division, P/Bag X134, Pretoria 0001, South Africa	rietdos@plant2.agric.za
Michael	Singer	University of Texas, Integrative Biology, Univ Of Texas, Austin, TX 78712, USA	sing@mail.utexas.edu
Kate	Smith	CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia	kate.smith@csiro.au
Lindsay	Smith	Landcare Research, PO Box 69, Lincoln, New Zealand	smithl@landcareresearch.co.nz
Lincoln	Smith	USDA-ARS Western Regional Research Center, 800 Buchanan Street, Albany, CA 94710, USA.	lsmith@pw.usda.gov
Matthew	Smyth	CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia	matthew.smyth@csiro.au
Kelly	Snell	Keith Turnbull Research Institute, PO Box 48, Ballarto Road, Frankston, VIC 3199, Australia	Kelly.Snell@nre.vic.gov.au
Alejandro	Sosa	USDA-ARS-SABCL, Bolivar 1559, Hurlingham, B1686EFA, Argentina	alejsosa@speedy.com.ar
Helen	Spafford Jacob	University of Western Australia, School of Animal Biology, 35 Stirling Hwy, Crawley, WA 6009, Australia	hsjacob@agric.uwa.edu.au
Peter	Stahle	DPS Strategy Pty Ltd, 84 Richmond Terrace, Richmond Victoria 3121, Australia	
Margaret	Stanley	Landcare Research, Mt Albert Research Centre, 120 Mt Albert Road, Auckland 1030, New Zealand	stanleym@landcareresearch.co.nz
Donald	Strong	University of California, Davis, Section of Evolution and Ecology, Davis, CA 95616, USA	drstrong@ucdavis.edu
B.	Srinivasan	Centre for Plant Conservation	
Anthony	Swirepik	CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia	anthony.swirepik@csiro.au
Pauline	Syrett	Landcare Research, 14 Rockview Place, Christchurch, 8008, New Zealand	rockview@actrix.co.nz
Dianne	Taylor	CSIRO Entomology, 120 Meiers Road, Indooroopilly, QLD 4068, Australia	dianne.taylor@csiro.au

Given Name	Surname	Address	Email
Thierry	Thomann	CSIRO, Campus International De Baillarguet, 34980 Montferrier-Sur-Lez, France	thierry.thomann@csiro-europe.org
David	Thompson	New Mexico State University, Box 30003, MSC 3BE, Skeen Hall #N220, Las Cruces, NM 88003, USA	dathomps@nmsu.edu
Philip	Tipping	USDA/ ARS/ IPRL, 3205 College Avenue, Fort Lauderdale, FL 33314, USA	ptipping@saa.ars.usda.gov
Allan	Tomley	Alan Fletcher Research Station, PO Box 36, Sherwood, Brisbane, QLD 4075, Australia	allan.tomley@nrm.qld.gov.au
Peter	Toth	Slovak Agricultural University, A. Hinku 2, Nitra, 949 76, Slovak Republic	petery@nextra.sk
Carlo	Tronci	BBCA Onlus, Largo S. Stefano 3, Anguillara Sabazia, 00061, Italy	ctronci@e-bbca.net
Rieks	Van Klinken	CSIRO Entomology, TERC PMB44, Winnelle, Darwin, NT 0822, Australia	reiks.vanklinken@csiro.au
Cicelia	Van Rooi	ARC–PPRI, Vredenburg Research Station, Polkadraai Avenue, Stellenbosch, 7599, South Africa	vredcvr@plant3.agric.za
Janine	Vitou	CSIRO, Campus International De Baillarguet, 34980 Montferrier-Sur-Lez, France	janine.vitou@csiro-europe.org
Mark	Volkovitsh	Zoological Institute, Russian Academy of Sciences, Universitetskaya Nab 1, St Petersburg, 199034, Russia	polycest@zin.ru
Maurizio	Vurro	CNR—Institute Of Sciences Of Food Production, Viale Einaudi 51, Bari, 70125, Italy	maurizio.vurro@ispa.cnr.it
Nick	Waipara	Landcare Research, Mt Albert Research Centre, 120 Mt Albert Road, Auckland 1030, New Zealand	waiparan@landcareresearch.co.nz
Linnea	Wang	Norwegian Crop Research Institute, Plant Protection Center, Hgskoleveien 7, s, 1342, Norway	linnea.wang@planteforsk.no
Alan	Watson	McGill University, Dept. Plant Science, 21, 111 Lakeshore Road, Montreal, Quebec H9X 3V9, Canada	alan.watson@mcgill.ca
Hilary	Webb	Manawatu-Wanganui Regional Council, Marton Service Centre, P O Box 289, Marton, New Zealand	hilary.webb@horizons.govt.nz
Miranda	Wecker	University Of Washington, PO Box 160, Naselle, WA 98638, USA	mwecker@willapabay.org
Timothy	Widmer	USDA-ARS European Biological Control Laboratory, 34988 St Gely Du Fesc Cedex, France	tlwidmer@ars-ebcl.org
Anthony	Willis	Office of the Gene Technology Regulator, MDP 54, PO Box 100, Woden, ACT 2606, Australia	tony.willis@helath.gov.au
John	Wilson	CSIRO Entomology, 120 Meiers Road, Indooroopilly, QLD 4068, Australia	john.wilson@csiro.au
Julia	Wilson-Davey	Manaaki Whenua Landcare Research, PO Box 69, Lincoln, New Zealand	wilson-daveyj@landcareresearch.co.nz
Chris	Winks	Landcare Research, Mt Albert Research Centre, 120 Mt Albert Road, Auckland 1030, New Zealand	winkc@landcareresearch.co.nz
Toni	Withers	Forest Research, PB 3020, Rotorua, New Zealand	toni.withers@forestresearch.co.nz
Arne	Witt	Queensland Department of Natural Resources & Mines, PPRI, P/Bag X134, Pretoria 0001, South Africa	rietaw@plant2.agric.za
Ken-Ichi	Yamaguchi	Minami Kyushu University, 11609 Minami Takanabe, Takanabe-Cho, Miyazaki-Ken 884-0003, Japan	yamaguchi-nel@siren.ocn.ne.jp
Paul	Yeoh	CSIRO, Private Bag 5, PO, Wembley, WA 6913, Australia	paul.yeoh@csiro.au
Costas	Zachariades	ARC-PPRI, Private Bag X6006, Hilton, 3245, South Africa	ntczs@natal1.agric.za
Miguel	Zapater	University of Buenos Aires, Av. San Martin 4453, Buenos Aires, 1417, Argentina	mmzapater@arnet.com.ar
Wenming	Zhang	Alberta Research Council, Hwy. 16A, 75 Street, PO Bag 4000, Vegreville, AL T9C 1T4, Canada	zhangw@arc.ab.ca
Ryan	Zonneveld	CSIRO Entomology, 120 Meiers Road, Indooroopilly, QLD 4068, Australia	Ryan.Zonneveld@csiro.au



1	Magen Geyer
2	David Briese
3	Peter Turner
4	Phil Tipping
5	Tony Caesar
6	Jeff Makinson
7	Not identified
8	Gary Peng
9	Bill Pettit
10	Blair Grace
11	Matthew Purcell
12	Royce Holtkamp
13	Bruce Auld
14	Maurizio Vurro
15	Lincoln Smith
16	Daniel Gandolfo
17	Susan Boyetchko
18	Terry Olckers
19	John Lester
20	John Hoffmann
21	Doug Luster
22	Djamila Djeddour
23	Yvonne Buckley
24	John (Lars) Baker
25	Esther Gerber
26	Peter McEvoy
27	G.V.P. Reddy
28	David Thompson
29	Miguel Zapater
30	Judy Myers
31	Michael Day
32	Paul Pratt
33	Richard Groves
34	Costas Zachariades
35	Mic Julien
36	Rieks Van Klinken
37	Bill Bruckart
38	Kaile Korowi
39	David Minkey
40	Ryan Zonneveld
41	Michael Pitcairn
42	Joe Balciunas
43	Bill Palmer
44	Carlo Tronci
45	Margarita Dolgovskaya
46	Dennis Isaacson
47	Schon Schooler
48	Michael Grodowitz
49	Laura Jesse
50	Ernest (Del) Delfosse
51	Stuart Falk
52	Andrew Bishop
53	Seona Casonato
54	Ruth Aveyard
55	Cheryl Lennox
56	Kelly Snell
57	Melanie Haines
58	Brian Rector
59	Ren Sforza
60	Bradley Brown
61	Rangaswamy Muniappan
62	Karen Bailey
63	Graeme Bourdot
64	David McLaren
65	Mark Lonsdale
66	Kunjithapatham Dhileepan
67	S. Raghu
68	Peter Toth
69	Alain Roques
70	Alec McClay
71	Alan Watson
72	Allan Tomley
73	Linnea Wang
74	Chris Winks
75	Hernan Norambuena
76	Dianne Taylor
77	John Scott
78	Jim Cullen
79	Anthony Swirepik
80	John Goolsby
81	Joe Neal
82	Patrick Moran
83	Maria Cristina Hernandez
84	Alejandro Sosa
85	Janine Vitou
86	Fernando McKay
87	Andy Sheppard
88	Thierry Thomann
89	Tim Widmer
90	Fritzi Grevstad
91	Tony Willis
92	Tim Heard
93	Kate Smith
94	Don Strong
95	Marcus Byrne
96	Bob Pemberton
97	Not identified
98	Judy Shearer
99	Miranda Wecker
100	Hugh Gourlay
101	Richard Chan
102	Marion Seier
103	Shane Hona
104	Fiona Impson
105	Julia Wilson-Davey
106	Alana Den Breeyan
107	Helen Harman
108	Lindsay Smith
109	Rachel McFadyen
110	Alison Gianotti
111	Helen Spafford Jacob
112	Carol Ellison
113	Ted Center
114	Liz Dovey
115	Martin Hill
116	Jamie Davies
117	Richard Shaw
118	Harry Evans
119	Phil Cowan
120	Not identified
121	Peter Caley
122	Stephen Hight
123	Aaron Maxwell
124	Not identified
125	Catherine Mathenge
126	Frieda Anderson
127	Glynn Maynard
128	Geoff Hurrell
129	Dana Blumenthal
130	Ruth Huwer
131	Jeffrey Littlefield
132	Margaret Stanley
133	Peter Stahle
134	Andrew McConnachie
135	Daro Palmer
136	Raelene Kwong
137	Gary Piper
138	Mike Singer
139	Jose H. Pedrosa-Macedo
140	Gio Fichera
141	Ricardo Segura
142	Toni Withers
143	Quentin Paynter
144	John Wilson
145	Rodney Lym
146	Kathryn Batchelor
147	Julio Medal
148	Karina Potter
149	Bertie Hennecke
150	Wenming Zhang
151	Matthew Cock
152	L.T. Kok
153	Simon Fowler
154	Richard Hill
155	Paul Yeoh
156	Jane Barton
157	Louise Morin
158	Lynley Hayes
159	Abuelgasim Elzein
160	Jean-Louis Sagliocco
161	Tom Morley
162	Ruth Hufbauer
163	John Ireson
164	Matthew Smyth
165	Arne Witt
166	Mick Neave

Apologies for errors and ommissions. Eds.