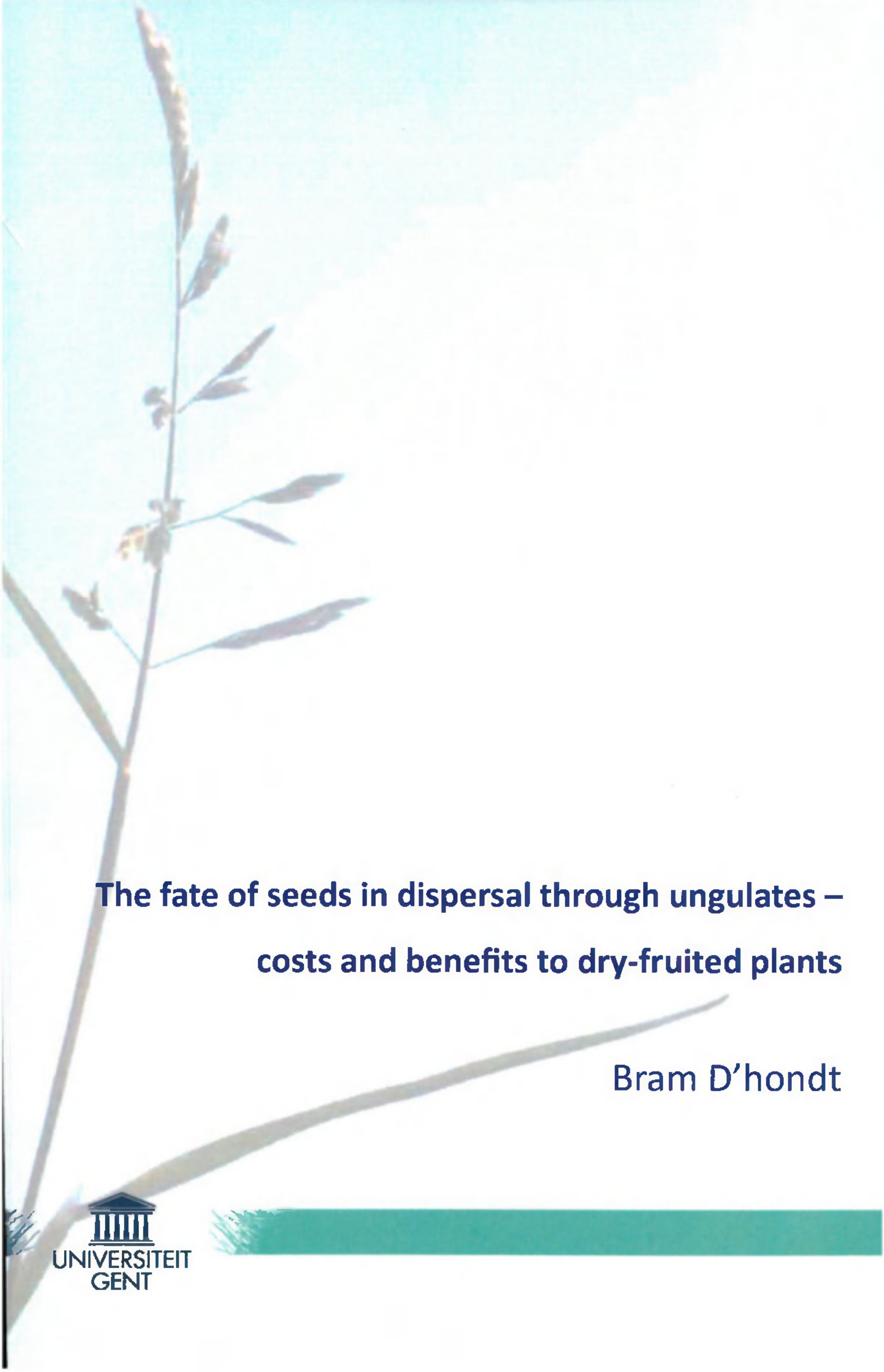

The fate of seeds in dispersal through ungulates – costs and benefits to dry-fruited plants

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UNIVERSITEIT
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Universiteit Gent

Faculteit Wetenschappen

Academiejaar 2010-2011

THE FATE OF SEEDS IN DISPERSAL THROUGH UNGULATES – COSTS AND BENEFITS TO DRY-FRUITED PLANTS

HET LOT VAN ZADEN BIJ VERBREIDING DOOR HOEFDIEREN – KOSTEN EN BATEN VOOR PLANTEN MET DROGE VRUCHTEN

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Thesis submitted in fulfilment of the requirements for the degree of Doctor (Ph.D.) in Science

Proefschrift voorgedragen tot het bekomen van de graad van Doctor in de Wetenschappen

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Date of public defence: 6 May 2011

D'hondt B. 2011. The fate of seeds in dispersal through ungulates – costs and benefits to dry-fruited plants. PhD thesis. Ghent University, Ghent (Belgium).

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This research was funded by a grant of the Research Foundation – Flanders (FWO).

Acknowledgements

My first debt of gratitude in these acknowledgements is owed to the people that have guided me into and throughout this thesis. To my scientific supervisor, Maurice Hoffmann, for his continuing interest in both big beasts and small seeds have spurred many students' interests in plant ecology. Equally, to Beatrijs Bossuyt, Dries Bonte and Rein Brys, who have learned me 'the ropes' of academic science, and without whom this thesis might not have succeeded.

Sincerely, very sincerely, I wish to thank all of my present and former colleagues at TEREC (Terrestrial ecology research unit) and LIMNO (Limnological research unit), with whom *work* hardly seemed *work* at all. Amittedly dull –though fair...– I will list you in alphabetical order: thank you, Angelica, Annelies, Arthur, Ataollah, Boris, BramV, Carl, Céline, Charlie, Davy, Debbie, Dieudonné, Eddie, Els, Eva, nonkel Fon(d)s, Frederik, Greet, Hanski, Helena, Jean-Pierre, Jeroen, Joeri, Johan, Katrien, K. (L.), Leen, Liesbeth D, Liesbeth W, Luc, Lucien, Lynda, Mars, Nathalie, Nele, Pejman, Reza, Steven, Sudhi, Tanja, Thijs, Tom, Toon, Valérie, Vanessa, Viki & Wouter. No better symbol of the cordial collegiality than the ninth-floor coffee table: many BRAVILOR®/ BONAMAT®-heated coffee cups and not-so-cool beers it has borne, many highly scientific and not-so-scientific discussions it has heard... Though many bar tables have undergone similar fates, this truly was the center stage of terec. Most certainly, times with terec will be missed!

Recently, I was fortunate to extend my collegial circle to "the second floor". In an equal respect, I therefore wish to thank the people from the Pteridology, Spermatophyte and Mycology groups for allowing me to join their ranks.

Next, I would like to thank my fellow biologists from this same university (with a special mention to my fellow botanists –the five of them), with who I spent many great times, and have left me some good friends. More than just a friend, to Marijke, who by herself reminds me of this excellent (study) choice daily.

In a wider perspective, then, I wish to thank *all* of my friends that make living in Ghent so enjoyable. Whenever this city is termed lively, engaged and creative, the reference is actually made to you! (Please, do feel addressed.)

A very sincere gratitude also goes to my parents, not primarily for their sporadic and selfless help throughout the past five years, but –more profoundly–, for always having been highly supportive for the interests of their offspring. In my first attempts of scientific experimentation, both of my brothers, David and Bart, proved the perfect allies.

Given the topics of this thesis, to my friends and colleagues from Natuurpunt inZICHT. Although the efforts put in our reserve were often very time-consuming, studying ecology would not be half as rewarding when not applied in nature. Kindly, the back cover photograph was provided by Erwin Derous.

Many of the work performed for this study would have been impossible without the help from other people and institutions. In this respect, I am especially indebted to: Frank Broucke, Eric Cosyns, Ward Vercruysse, Tanja Milotic, Sam Provoost, Marc Leten, Gerard Stroo, Franky Danneels, Leen De Laender, Nathasja De Couck, Valerie Verstraeten, Sophie D'hondt, Bert Delanoeije and his team, the Research Institute for Nature and Forest (INBO), the people from the Flemish Marine Institute (VLIZ), Agency of Nature and Forest (ANB), and Réserves Naturelles de France.

I hope not to have missed anyone.

Again, thank you!

For science to be good, it should protrude in popular books. I here chose to let every chapter precede by a quote from the recent book of Jonathan Silvertown (2009), whose work has seeded and proceeded an interest in plant ecology among many –including myself.

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"A seed hidden in the heart
of an apple is an orchard
invisible. [WELSH PROVERB]"

An Orchard Invisible: 3

CHAPTER 1

General introduction

Seeds play a key role in the life of flowering plants. Consisting of an embryonic plant within a protective covering of maternal tissue, seeds link the parental generation with that of the offspring. Germination of seeds, however, is both irreversible and perilous: once started, the emerging plant is irrevocably committed to growth or death (Fenner and Thompson 2005). Therefore, any seed attribute that affects the chances of successful recruitment matters greatly to the plant.

Two processes can be distinguished in this respect: dormancy and dispersal. **Dormancy** refers to the process in which seeds are prevented from germinating (Baskin and Baskin 2001). This can be due to inadequate environmental conditions, but importantly (and more essentially), this also refers to conditions in which seeds are able to germinate but refrain from doing so. Dormancy is thus all about proper timing of germination. **Dispersal**, on the other hand, refers to the spatial relocation of seeds, determining where seeds end up for germination (Cousens *et al.* 2008). The focus of this thesis is primarily concerned with the latter process, and in this General introduction I therefore start with an elaboration of dispersal in general.

Dispersal

As dispersal, in its broadest sense, applies to organismal movement, it forms a central component of both the fitness of individuals, the dynamics of populations, and the distribution of species (Bowler and Benton 2005). Since these entail very divergent scales, the implications of dispersal are extremely wide-ranging. For plants alone, Schupp *et al.* (2010) list dispersal to be prominent in recent studies on recruitment limitation, gene flow, metapopulation dynamics, historic and future plant migration (cf. climate change), evolutionary trade-offs, the structure of interaction networks, scale dependence of ecological processes, maintenance of biodiversity, structuring of species–area curves, ecological consequences of habitat fragmentation, plant invasions, ecological restoration, the effectiveness of corridors for conservation, and –as the authors note drily– "more".

Because of this appreciation, dispersal itself has been the centre of much theoretical work concerned with the question of *why* organisms disperse (reviews by Bowler and Benton 2005, Cousens *et al.* 2008). The potential driving forces that have been identified include kin selection (dispersal to reduce competition between kin at the site of birth), inbreeding avoidance (dispersal to avoid breeding between relatives), and environmental stochasticity (including population dynamics and habitat turnover).

At the basis of these processes lie decreases in fitness, or **costs**, to those individuals that do not disperse. Nonetheless, those individuals that dó disperse become confronted with (other) costs as well. The relative costs that become charged to organisms therefore lead to the adoption of particular strategies of dispersal in habitats (Southwood 1977). A focus on costs and benefits thus forms a natural and useful conceptual base to disentangle the complex nature of dispersal, with direct implications for its evolutionary ecology as well.

As the causes and consequences of dispersal clearly extend beyond the mere movement of organisms, researchers mostly regard 'dispersal' as to include both the stages of emigration (from the natal site), movement (transfer) and immigration (into the new site of residence). This multi-phase definition is adopted in this thesis, too. Each of these phases comes with costs, which can be of several types and are often specific to particular modes of dispersal. I will therefore get back at these after having introduced seed dispersal and ungulate endozoochory.

Seed[*] dispersal

Being sessile organisms, plants mostly rely on external vectors to disperse their seeds. Numerous vectors have been identified, both abiotic (wind, water) and biotic. Of the latter, frugivorous animals (birds and mammals) and ants probably are the predominant examples.

Some shapes and structures of seeds clearly promote dispersal through a particular vector, and traditional schemes that accord form with function in dispersal are therefore widely established: plumes and wings are attributed to dispersal by wind, fleshy tissues to dispersal by frugivores, elaiosomes to

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[*] 'Seed' will be used throughout this work as a generic term for any individual propagule of generative origin from angiosperm plants. This can be a true seed, fruit or mericarp.

dispersal by ants, *etc.* (Van der Pijl 1969, Bouman *et al.* 2000, Bonn and Poschlod 1998, Poschlod *et al.* 2005). However, ecological research shows that classifications based on morphological syndromes can be misleading. Assumedly wind-dispersing species, for instance, often exhibit worse wind dispersal potential than species classified otherwise (Tackenberg *et al.* 2003). Seeds of a particular species may also be successfully dispersed by (many) more means than the one implied by morphology (cf. Higgins *et al.* 2003).

Howe and Smallwood (1982) distinguished three main reasons plants apply dispersal for in nature. Touching on the fundamental drivers for dispersal mentioned above, these include the 'escape' from detrimental effects in the maternal environment and the 'colonization' of unstable hatibats. Stressing benefits at the target site rather than costs at the natal site, these authors also introduced 'directed dispersal' to be applied, i.e. when seed movement occurs non-randomly towards specific sites with a disproportionally high chance for establishment. Classic examples of the latter include dispersal of mistletoes by birds, and of elaiosome-seeded plants to anthills, but Wenny (2001) advocated that directed dispersal might be more common in nature than is often believed.

Endozoochory

Ungulates are considered important vectors in dispersal too (Ridley 1930). On the one hand, these mammals move seeds externally in their fur and on hooves (epizoochory; e.g. Fischer *et al.* 1996, Heinken and Raudnitschka 2002, Couvreur *et al.* 2004). On the other hand, seeds become ingested while feeding and eventually excreted. This is referred to as **endozoochory**[*,†]. Although the ungulates[‡] as a group include many species that feed selectively on fruits (e.g. rhinoceros [Dinerstein 1991], giraffe [Miller 1994], roe deer [Tixier and Duncan 1996]), the focus is put here on equids and ruminants that feed on grass and roughage (*sensu* Hofmann 1989; e.g. horse, sheep, cattle, red deer…). In essence, these animals form a

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[*] The suffix –chory refers to dispersal; from 'chorein' (Greek), 'to wander' (Van der Pijl 1969).

[†] Although 'endozoochory' does neither specifiy the animal vector nor plants involved, it is mostly restricted throughout this thesis to denote endozoochory of dry-fruited plants by ungulates.

[‡] 'Ungulate' here refers to any member of the orders Perissodactyla and Cetartiodactyla with exclusion of the Cetacea (Huffman 2010).

functional group of larger-sized mammals that forage on the foliage of plants with graminoid and herbaceous life-forms, and any seed ingestion from such plants is therefore unintentional from the animal's perspective.

Although they involve a somewhat distinct set of species, endozoochory appears to outweigh epizoochory for most herbivores in both the number of seeds and species dispersed (Couvreur *et al.* 2005; Mouissie *et al.* 2005a). Indeed, hundreds of plant species have been recorded as viable seeds in dung from free-ranging ungulates (see Appendix 1A), and estimates on the number of seeds dispersed in this way suggest it to be of considerable significance (see Appendix 1B).

A grazer grazing grassland: an opportunity for dry-fruited forbs and grasses to have their seeds dispersed by endozoochory

Most grasses and forbs which constitute the diets of these ungulates are characterized by fruits and seeds that lack obvious attributes for dispersal, being dry, small and overall inconspicuous. Indeed, this so-called 'unassisted' dispersal syndrome is the predominant seed syndrome in open, disturbed communities which are frequented by many of these animals (Eriksson and Jakobsson 1999).

Since the animal guild described above includes many species of livestock, endozoochory might have played a very important and common role for maintaining plant diversity in past agricultural landscapes (Bonn and

Poschlod 1998, Poschlod and Bonn 1998). Now, for Western and Central Europe, low-pressure grazing in heterogeneous and species-diverse habitats has become largely restricted to nature reserves, where it is commonly applied as a management measure (WallisDeVries *et al.* 1998).

The ecology of endozoochory

Endozoochory inherently is a complex mode of dispersal. It refers to a cycle in which one generation is linked to the next through a series of stages that pertain to seed uptake, movement, deposition and establishment (Fig. 1-1). Each of these stages involves potential failure and mortality, and seeds that enter the cycle therefore face a particularly uncertain fate (Chambers and MacMahon 1994). Here, we briefly review each of these stages according to our current understanding (also see the extensive review of Bonn and Poschlod 1998).

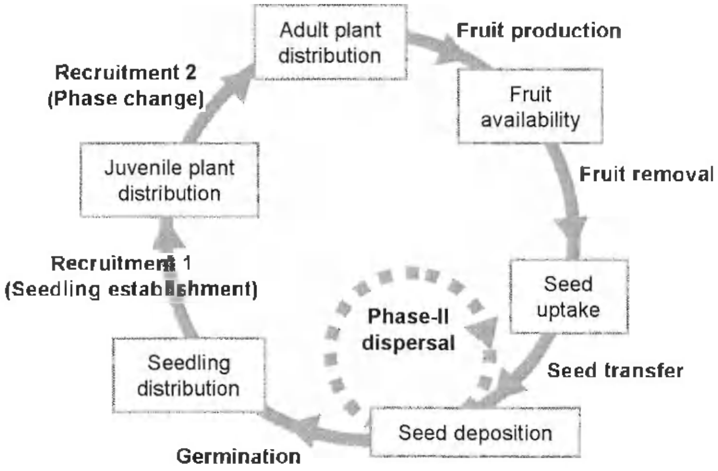

Figure 1-1. The seed dispersal cycle for endozoochory, as modified from Wang and Smith (2002). A distinction is made between patterns (in boxes) and processes (in bold). The dashed line forms an 'optional' route.

From seed uptake to seed deposition

The processes that act between seed ingestion and excretion make up the centre stage of endozoochory: here, the ungulate vector comes into play and helps determining *whether*, *when* and *where* seeds will be deposited.

Surviving the digestive tract

From an ecological perspective, the most important effect brought about in seeds after ingestion is mortality due to chewing or digestion. This mortality is most directly illustrated from experiments in which seeds have been fed to captive animals. Many such experiments have been performed, but mostly from agronomical interests and involving a low number of plant species (an exhaustive literature survey is given in Appendix 1C). However, it is readily clear from these studies that mortality rates vary from 0 up to 100 %, though with mortality being substantial (> 50 %) in most of the cases.

These mortality rates depend on both the plant and animal species (Cosyns *et al.* 2005a). The question of how these mortality rates are affected by seed traits has been addressed only a few times, in feeding experiments that involved a sufficiently broad range of plant species (Gardener *et al.* 1993a, 1993b, Bonn 2004, Mouissie *et al.* 2005b, Cosyns *et al.* 2005a, and Peco *et al.* 2006). However, the results of these studies appear conflicting, and a re-assessment of this interaction is therefore needed. This will be extensively discussed below.

When deposition?

The time taken from ingestion to excretion of seeds is also best derived from feeding experiments (cf. Appendix 1C). The frequency distribution of passage times from a single seed cohort is typically peaked and skewed towards longer times, with peaks of excretion mostly falling between one and three days in domesticated ungulates (Bonn and Poschlod 1998, Cousens *et al.* 2008). As an illustration, Cosyns *et al.* (2005a) noted mean retention times of 49, 55, 56 and 70 hours in cattle, horse, sheep and donkey, respectively, while Mouissie *et al.* (2005b) found the median retention time to be 25 hours in fallow deer. It has traditionally been assumed that mean retention times are positively related to increasing body mass in herbivores (Illius and Gordon 1992), but Clauss *et al.* (2007) recently reviewed and rejected this relation.

Where deposition? – the macro-environmental setting

Where seeds become deposited is of considerable importance to the plant, as conditions become set that might determine failure or success in later stages (germination, establishment or flowering).

The bee-line distance that a seed moves from its source has always been a focal issue in dispersal research. In endozoochory, this depends on the time taken for it to be defecated, combined with the rate and pattern of animal movement (Cousens *et al.* 2008). Obviously, ungulates can move large distances between ingestion and excretion, and dispersal distances therefore readily amount to several kilometres (Pakeman 2001, Vellend *et al.* 2003, Will and Tackenberg 2008). Ungulates therefore are important vectors for long-distance dispersal (landscape to regional scales, 10^2-10^5 m; Nathan *et al.* 2008a).

Apart from distance, a second determining aspect is the identity of the environment where seeds end up in. Some animal dispersers have habitual latrine sites. For instance, rhinoceroses in Nepal are known to deposit seeds of *Trewia* in latrines (Dinerstein and Wemmer 1988), and tapirs perform the same with the palm species *Maximiliana maripa* in Amazonia (Fragoso 1997). In both cases the seeds have an increased chance for establishment in these sites, either through the arrival in favourable growing conditions, or through the escape from predators (cf. Howe and Smallwood 1982, above). Whether domestic herbivores, such as horses, establish latrines is apparently dependent on the environment (Lamoot *et al.* 2004). Seed deposition patterns at the habitat level are therefore less predictable, and largely unexplored, for these animals. Nonetheless, this forms a crucial qualitative component of the effectiveness of ungulates as dispersal vectors (Schupp 1993, Schupp *et al.* 2010).

Where deposition? – the micro-environmental setting

Well predictable, on the other hand, is that seeds are deposited along with faecal material. This creates a gap in the established vegetation, which should provide seeds with enhanced opportunities for recruitment. Indeed, gap dynamics are considered a major driving force in grassland communities (Bullock 2002).

Within this micro-environmental setting, the dung brings forth a variety of effects on the surrounding biotic and abiotic environment (listed by Dai 2000 and Gillet *et al.* 2010). The early life stages of the plants from within the dung pellets also become affected. First, the physico-chemical properties of the pat can influence germination and early root growth through either a 'manuring' (stimulating) or phytotoxic (inhibiting) effect (e.g. Hoekstra *et al.* 2002, Carmona *et al.* 2010). Second, ambient environmental conditions can influence seed germination/establishment indirectly by bringing about changes in the dung pat matrix. The underlying soil type, seed position in a pat, and dung pat moisture status (with the formation of crusts due to drying being particularly adverse) all have been found to influence direct short-term germination from pats (Akbar *et al.* 1995).

Apart from these outside effects, the distribution of the seeds themselves can affect germination and seedling establishment. Seed patterning in dung pats is highly concentrated and this may increase density-related competition among seedlings from different or from the same species (Loiselle 1990). Seeds may even be closely related to each other, since seeds become ingested from the same mother plant or neighbouring plants in groups (cf. kin competition, above). On the other hand, it should be borne in mind that many plant species spread germination through time by means of dormancy, which helps to escape competition in simultaneously dispersed seed cohorts (Baskin and Baskin 2001).

Despite the presumed effects of nutrient enrichment and competition introduced here, Eichberg *et al.* (2007) paradoxically found the few species to eventually establish as fruiting adults from sheep dung in an inland dune system, to be low-competitive species fom resource-poor environments. They argue that the harsh micro-environmental conditions involved let pass only stress-tolerant species, primarily because of water limitation.

Over time, the dung matrix erodes, with a guild of invertebrate animals playing a role in this process (including earthworms, beetles...; Gobat *et al.* 1998). This can happen well after or before the fate of endozoochorous seeds is sealed.

Phase-II dispersal

After seeds have been deposited in excrements, dung-dependent or dung-visiting fauna may interact with the seeds in various ways. Seeds may become predated. Or diplochory might occur, i.e. the process in which seeds go through two dispersal phases, each including a different vector (Vander Wall and Longland 2004). Phase-one dispersal, here by the ungulate, is followed by phase-two dispersal[*]. Note that seeds might become lost, or survive, during predation, and any seed predator may thus also act as a (phase-two) disperser. Since effects of former stages can become overruled, predation or phase-two dispersal of seeds can have a decisive effect on plant patterning.

With regard to frugivorous (phase-one) dispersers, it is widespread in tropical regions for seeds to be removed from excrements by rodents (Vander Wall and Longland 2004). This is also anecdotically reported from temperate regions (Bermejo *et al.* 1998). Cases where seeds are removed from excrements by ants are known too (e.g. Levey and Byrne 1993, Aronne and Wilcock 1994). Janzen (1982a) reported on the removal of seeds from a tropical forest tree by spiny pocket mice from horse dung.

Such studies on post-deposition seed-animal interactions appear notably biased towards frugivorous dispersers and/or (sub)tropical regions, which Manzano *et al.* (2010) attribute to the relative conspicuity of the (larger-sized) seeds and predators involved. These authors advocate that the same interactions nonetheless also apply to small-sized seeds from herbivorous dispersers' faeces outside the tropics, though these go apparently unnoticed. In Meditteranean pastures, their exclusion experiment indicated one out of three seeds from sheep dung to be predated through the dung-collecting behaviour of harvester ants (Manzano *et al.* 2010).

Dung beetles (coprophilous Scarabaeidae, Janssens 1960) use dung for feeding and breeding, and for this, they manipulate excrements in various ways (Hanski and Cambefort 1991a). Dung beetles do not consume seeds[†], and some manipulations therefore effectuate phase-II dispersal. Again, this interaction is best known for frugivorous phase-one-dispersers from tropical regions, which has been extensively reviewed by Andresen and Feer (2005). Here, seed burial by beetles mostly increases survival of seeds through escape from rodent predation, but at the same time, hampers seedling emergence. In their study from an Amazonian rain forest, Andresen and

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[*] Phase-two dispersal is a special case of 'secondary dispersal' (Vander Wall & Longland 2004), the latter also applying to cases in which the new vector is the same as from the first stage. Since it is unlikely for a seed to become ingested by an ungulate shortly after deposition, secondary dispersal *sensu strictu* is considered not to apply. Less unlikely, ungulates could act as phase-II dispersers themselves if seeds are ingested that have been dispersed by e.g. wind but were retained in plant foliage.

[†] A deviant case, the Mediterranean *Thorectes* buries and feeds upon entire acorns, thereby acting as a predator, but sometimes also as a seed disperser (Pérez-Ramos *et al.* 2007).

Levey (2004) found the net effect to be positive. Phase-II dispersal by dung beetles in the temperate region, however, has never been studied before.

From seed to seedling: germination

Studies in which emerging seedlings from dung were counted and contrasted between field and greenhouse samples (Mouissie *et al.* 2005a, Cosyns *et al.* 2006, Eichberg *et al.* 2007, Pakeman and Small 2009), indicate that only a (very) low proportion of the available seeds germinate *in situ* from dung during the first months after deposition; often about 5 % or less. *So, what is happening to the other seeds?*

Seedling emerging from a pellet

First, requirements for dormancy break (scarification) or germination of seeds might not have been met, leaving seeds dormant or ungerminated during the study. Indeed, Dai (2000) sampled cattle dung pats of different ages in a Swedish limestone grassland, and clearly showed that pats hold seeds readily for more than three years. Illustratively, he refers to the pats as a 'dung seed bank'. Also, Traba *et al.* (2003) followed the vegetation of dung-enriched plots over four years, and found new species to appear up to two years after application, as did Eichberg *et al.* (2007). Finally, Malo and Suárez (1995a) found that germination from the legume *Biserrula pelecinus* from cattle dung was spread over (at least) four years. Such results have important implications for endozoochory research, since they illustrate endozoochory to be particularly cryptic in the field as germination is spread over long time frames. Second, seeds may leave the dung pat and fall outside the observer's scope, for instance through dung degradation (with incorporation of seeds into the soil seed bank) or through phase-II dispersal (see above). Third, seeds may die. This can happen before or after germination, for instance, through intense seedling competition or seed predation (see above).

Growing up: from seedling to adult

Accounts of plants that close the cycle successfully through establishment and flowering are scarcely found in literature. Eichberg *et al.* (2007) found 12 out of 148 seedlings on sheep dung to set seeds within two years. Mouissie *et al.* (2005a) recorded 7 out of 34 dung-borne species to produce flowers in their two-year observations. Traba *et al.* (2003) do not provide figures, but fruiting is suggested in their study as well.

Costs – How much for endozoochory?

As introduced above, dispersal comes with costs. Four types of costs can potentially be distinguished in any means of dispersal (Bonte *et al.* unpub.): *energetic costs* (e.g. loss of metabolic energy, tissue production), *time costs* (time invested in dispersal that cannot be invested in other activities), *opportunity costs* (if some advantage of the natal environment is given up in the novel environment), and *risk costs* (mortality or damage).

Several costs were already encountered when discussing the several stages of endozoochory above. An overview of these is given in Table 1-1. Only the most pronounced and direct costs are listed in this table. Note that costs associated with phase-II dispersal are not included explicitly; the listed costs for the stages of germination and recruitment also apply to phase-II dispersal (the comparison then being with phase-I dispersed seeds). This costs account will be further discussed in the General discussion.

If so many seeds enter the cycle but few complete it, as data suggests, doesn't this mean that the cycle is extremely costly? Consider, however, that "the average plant leaves on average just one descendant" (Harper 1977), and that high mortality rates are therefore 'acceptable' for any dispersal vector (Fenner and Thompson 2005, also see Baeten *et al.* 2009)!

Table 1-1. An illustration of the costs of ungulate endozoochory

Stage[1]	Cost type	Comments
Fruit production	Energetic cost	Maternal tissue investment (considered minimal for 'unassisted' seeds)
Fruit removal	Risk cost (mortality/damage)	Pre-dispersal seed predation, foliage destruction in adult plant, seed destruction during ingestion (chewing)
Seed transfer	Risk cost (mortality)	Digestion/destruction (mostly high)
Germination	Time cost	The new environment may inflict seeds to remain dormant longer
Germination	Risk cost (mortality)	Post-transfer seed predation
Recruitment 1: seedling establishment	Risk cost (mortality/damage)	Seedling predation, density dependent competition
Recruitment 1: seedling establishment	Opportunity cost	Lesser habitat quality
Recruitment 2: phase change (flowering)	Opportunity cost	Lesser habitat quality

[1] According to the processes from the seed dispersal cycle. Please refer to Fig. 1-1.

Most generally, dispersal can be selectively advantageous when the fitness benefits of moving to a new patch exceed the fitness costs of movement (Southwood 1977, Bowler and Benton 2005). The paradigm of costs and benefits thus allows us to elaborate on a key question of endozoochory, namely, whether its significance extends beyond the anecdotal (i.e., a rare and accidental event without noticeable consequence), to a process with fundamentals that are similar to wind and bird dispersal (i.e., a common and selectively advantageous event of considerable importance).

Ideally, a full account of costs and benefits could learn us *why* plants should evolve to maximize endozoochory. Importantly however, it cannot tell us *whether* they really did, or do, so. These convey questions of evolution.

The evolution of endozoochory

'Evolution', as a discipline, is somewhat twofold, covering the study of biological change over the course of generations (*micro*-evolution), as well as over geological time scales (*macro*-evolution; Reeve and Sherman 1993).

The evolutionary history of vertebrate seed dispersal

Tiffney (2004) provides an outline of vertebrate seed dispersal through geological times. Seeds first appeared in the Devonian (± 416-359 Mya) and by the Carboniferous (± 359-299 Mya), many of the morphologies associated with major dispersal modes were already established in seeds: winged, plumose, even spinose and fleshy seeds. True herbivorous land vertebrates, on the other hand, did not establish until the Permian (± 299-251 Mya). This remarkable antedating of fleshy seeds to plant-eaters suggests that fleshy tissues did not originate for dispersal or that dispersal was through omnivores or carnivores[*]. With the rise of herbivorous vertebrates, seed ingestion (dispersal?) probably became more commonplace quickly. The oldest evidence of endozoochory of any kind comes from two similar, Late-Permian fossils of gut cavities holding seeds; surprisingly, this animal (*Protorosaurus*) has a carnivorous dentition and the seeds appear to lack any morphology for vertebrate consumption (Tiffney 2004).

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[*] Omni-/carnivores that maybe mistook these tissues for carrion (as hypothesized by Tiffney 2004). It might be noted that this author does not consider amphibians or fish as seed dispersal vectors.

Of course, the leading actors of this thesis did not appear on earth until much later. Jacobs *et al.* (1999) extensively discussed the origin of Poaceae and grazers. Grasses originated during the latest Cretaceous or during the subsequent Paleogene (± 65-23 Mya). Most herbivorous mammals were folivores or frugivores during this prevalently forested period, but over time, first browsers and then grazers arose. Eventually, the Miocene (± 23-5 Mya) saw a radiation of grasses and annual/biennial forbs, as well as a radiation of even- and odd-toed ungulates, as cooling and drying climates led to the formation of open, grass-dominated communities (Jacobs *et al.* 1999; Tiffney 2004; Kürschner *et al.* 2008). A gut cavity fossil of a Miocene rhinoceros holding grass seeds probably forms the oldest fossil of ungulate endozoochory (Voorhies and Thomasson 1979).

Janzen (1984)

In his 1984 paper, Janzen explicitly drew attention to the fate of seeds in endozoochory. He emphasized both its ubiquity in nature and its potential benefits, especially in past landscapes, and then put forth a series of hypotheses concerning its ultimate causation. He proposed that endozoochory (had) selected for particular plant attributes, primarily with regard to seeds. Moreover, he hypothesized that endozoochory was beneficial to such a degree, that many forbs and grasses also adjusted their vegetative parts and phenology to promote ingestion. Plant foliage then functionally acts to attract and reward herbivores, just as fleshy fruit tissues attract and reward frugivores. He listed a suite of seed traits that would expectedly arise under such scenario, many of which are found among extant grasses and herbs.

Collins and Uno (1985) commented on these views, particularly questioning the benefits of endozoochorous dispersal in prairies. Dinerstein (1989) looked for the suggested seed syndrome in Asian lowland grasslands, but rejected its importance. On the other hand, Quinn *et al.* (1994) pinpointed a suite of plant attributes that are apparently derived and clearly adaptive in endozoochory for two major North American prairie grasses. Other than these, the foliage-as-fruit hypothesis hasn't been addressed explicitly, though it spurred much of the above research on seed fate in endozoochory.

Micro-evolution – Evolution on 'ecological' timescales

Vectors of dispersal have the potential to select for particular seed attributes[*]. For dispersal modes that are associated with conspicuous seed traits, principal evolutionary–ecological underpinnings are relatively well established. For instance, Siepielski and Benkman (2007a, 2010) elaborated on the seed attributes which nutcrackers (as dispersers) and squirrels (as predators) select for in pines, demonstrating phenotypic shifts in seed traits. Cheptou *et al.* (2008) analysed the evolvability and intensity of selection in urban populations of wind-dispersing *Crepis sancta*, and illustrated rapid evolution in the proportion of pappus-bearing fruits. Manzaneda *et al.* (2009) showed how ants select for certain seed sizes in an ant-dispersing plant, but also how this conflicted with selection in later life stages.

A similar focus on phenotypic shifts in seeds under ungulate endozoochory would likewise be the most powerful approach to study its selective significance. This has never been performed, however. Among other reasons, this is due to the lack of obvious seed traits that promote endozoochory. Part of this thesis therefore attempts to identify and elaborate on such traits.

Endozoochory research – a brief history

The study of seed transport by herbivores has a long tradition (Bonn and Poschlod 1998[†], Poschlod *et al.* 2005). In the 18th century, students of Linnaeus have fed over 800 plant species to ungulates, probably being the first to experiment on ungulate endozoochory. Alike, the Austrian botanist Anton Kerner von Marilaun published a monumental series on the natural history of plants in the 1890's (Kerner von Marilaun 1902). One of his chapters dealt with dispersal, and here, he referred to endozoochory as "a matter of dispute amongst botanists". He reports on an experiment in which 250 plant species were fed to various animal species, among which were horse, cattle and pig. Though at least a few seeds apparently passed uninjured through these ungulates, it was concluded that "the number of the

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[*] Violle *et al.* (2007) provide useful definitions for the functional ecology of plants. A 'trait' is defined as any morpho-/physio-/phenological feature measurable at the individual without reference to the environment; an 'attribute' is the value or modality taken by a trait in a given environment.

[†] These authors provide a very extensive and worthwile review on the history of dispersal research in general.

seedlings so obtained was scarcely appreciable" so that "very few words will suffice". Obviously, I do not adopt the latter opinion.

These early botanical interests are remarkable, since later reports primarily stemmed from an increased agronomical awareness on the dispersal of weed seeds through livestock and manure. Lawsuits had even been filed against people who sold livestock that infested the buyer's fields with weeds (Burton and Andrews 1948). On the other hand, Suckling (1952) makes reference of an agricultural practice in Germany where sheep were deliberately used to seed white clover into new pastures (also see Bonn and Poschlod 1998: 198). Although it can be reasonably assumed that seed 'contamination' was recognized by farmers (much) earlier already, a *continual reporting of observations* (e.g. Adams 1907, Beach 1909, Hills and Jones 1911 in Burton and Andrews 1948), as well as experiments on these matters (see Appendix 1C), initiated in the beginning of the 20th century. Treatises on endozoochory from a botanical/ecological perspective were much scarcer during most of the century (e.g. Müller-Schneider 1954, and earlier of his work cited herein).

The second half of the 20th century saw an increased research interest in the biology of plant-animal interactions, as exemplified by the coining of the term co-evolution (Ehrlich and Raven 1964). Dispersal processes were granted a fair degree of attention in this respect, but it wasn't until the 1980's that D.H. Janzen introduced herbivore endozoochory of dry-fruited plants in these perspectives (Janzen 1983). In 1984, he published a seminal paper in which he emphasized the potential benefits provided to plants through endozoochory, and hypothesized that evolution had occurred, not only in seeds to facilitate such dispersal, but also in the plant's vegetative parts to promote ingestion at fruiting (Janzen 1984, above).

In recent decades, endozoochory received considerable attention from plant ecologists (e.g. Welch 1985, Malo and Suárez 1995a, 1995b, Pakeman *et al.* 1998). Illustratively, three Ph.D. projects on the matter were finished almost simultaneously, little more than 100 years after Kerner von Marilaun's work (Bonn 2004, Cosyns 2004, Mouissie 2004). These advances fitted in with a general increase of interest in seed dispersal, which has now become a key topic in plant ecological research (Bullock *et al.* 2002, Fenner and Thompson 2005, Bullock and Nathan 2008; Schupp *et al.* 2010 illustrate an increase from <25 to nearly 500 papers published yearly over the past three decades). Many of these recent advances were inspired by both problems and practice from nature conservation, such as dispersal limitation and grazing management. More recently, this includes the spread of alien species in (semi)natural areas through endozoochory (e.g. Quinn *et al.* 2010, Törn *et al.* 2010), somewhat paralleling the concerns of agronomists from a century ago!

Thesis aims and outline

The aims of this study are twofold. As illustrated, seed fate pathways are complex, and many stages of them still need to be explored or substantiated further (see 'The ecology of endozoochory'). Therefore, the first part of this thesis wishes to contribute to our ecological understanding of endozoochory.

The stages that have already been studied could be integrated into a model that covers endozoochory as a whole. This would not only allow us to evaluate our understanding of how these stages interact, but also to use that model to explore new patterns of endozoochory. As described in **Chapter 2**, we therefore constructed and tested a data-driven computer simulation model for endozoochory in heterogeneous landscapes. By means of this model, we then evaluated the spatial seed deposition pattern obtained by grazers in these landscapes, as this poses a key –but largely unexplored– component of the effectiveness of seed dispersal.

In Chapter 2, endozoochory is treated from the stage of seed ingestion up to seed deposition (cf. Fig. 1-1). Chapter 3 then starts where Chapter 2 has ended.

Indeed, after deposition, the fate of a dung-borne seed is still uncertain since seeds may interact with their environment in various ways. These may include biotic interactions with invertebrate and vertebrate mammals, and it has been suggested that these go largely unnoticed in temperate ecosystems (see above). In an experiment, we focused on the particular role of dung beetles in this respect. These insects might provide seeds with phase-two dispersal, and could hence have a decisive effect on the seeds' chances of recruitment. I report on this experiment in **Chapter 3**.

In Chapters 2 & 3, endozoochory is approached from the *process*' perspective. Here, the primary focus is on the costs charged and the benefits gained. This is different from Chapters 4 & 5, where the emphasis is on the behaviour of the *seeds* themselves. Though costs and benefits play a key role here as well, it does form a shift of focus the reader might want to be aware of.

Doing so, chapters 4 & 5 fit in with the second main aim of this thesis, i.e. to explore the evolutionary significance of endozoochory by ungulates to dry-fruited plants on ecological timescales (see 'The evolution of endozoochory'). Following examples from other dispersal systems, I try to detail a system for studying the evolutionary ecology of endozoochory in a micro-evolutionary sense. Simply, I elaborate on the three prerequisites that are needed for natural selection to be able to occur (cf. Darwin 1859). First, a phenotypic trait is needed that directly determines performance of seeds in endozoochory. Second, this trait should vary among individuals within a species, and third, this trait should be heritable.

> In **Chapter 4**, I report on an experiment in which predetermined amounts of seeds were fed to cattle in order to estimate their rates of mortality during digestion. Although such feeding experiments have been performed before (Appendix 1C), additional data are useful because a very specific and important cost of endozoochory is charged in this stage (Table 1-1). More profoundly, however, we performed this experiment to re-assess suggested effects of seed size and shape in the process, since these appear inconsistent from literature. This is done in order to explore the suitability of these simple seed traits for the micro-evolutionary objectives stated above.
>
> From Chapter 4, I will conclude that the quantitative seed traits investigated are not suited for the aims stated, and water-impermeability of seeds might be used instead. For it to be of relevance, a better understanding of this latter trait is needed, and this forms the focus of Chapter 5.
>
> In **Chapter 5**, the *in situ* variation and heritability of seed water-impermeability in *Trifolium repens* is investigated. Though endozoochory forms the reason to these investigations, the parameters under study are

essentially unrelated to dispersal. Instead, the results are interpreted in combination with an experiment on their demographical significance.

A graphical outline of how the individual thesis chapters fit in conceptually with the endozoochorous cycle is provided in Fig. 1-2.

In **Chapter 6**, we eventually put the results of these chapters in perspective, further discussing the ecological and evolutionary significance of endozoochory to dry-fruited plants.

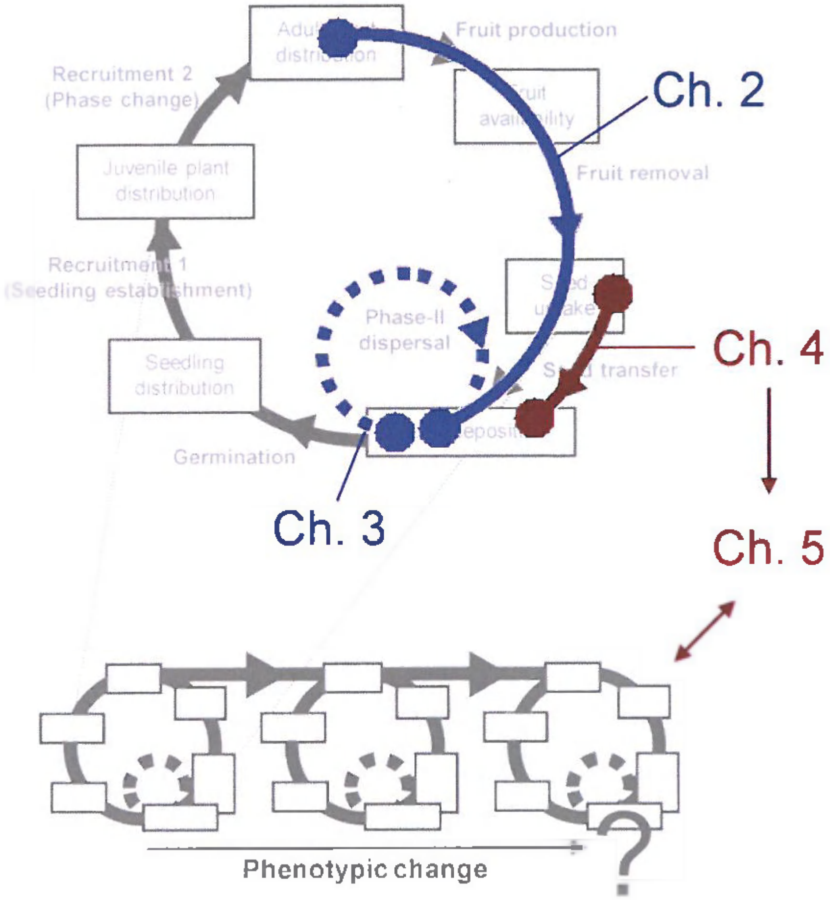


Figure 1-2. A conceptual outline of the thesis. The endozoochorous cycle from Fig. 1-1 is used as a template. For the chapters in blue, the emphasis is on the *processes* involved: Chapter 2 spans the processes from fruit production until seed deposition, while Chapter 3 focuses on subsequent phase-II dispersal. For the chapters in red, the focus is on the behaviour of *seed attributes*. Chapter 4 tests the role of seed traits during transfer. Chapter 5 elaborates hereon in an attempt to meet the question of phenotypic evolution through endozoochory. Below, successive cycles are linked to illustrate its successive action through time.

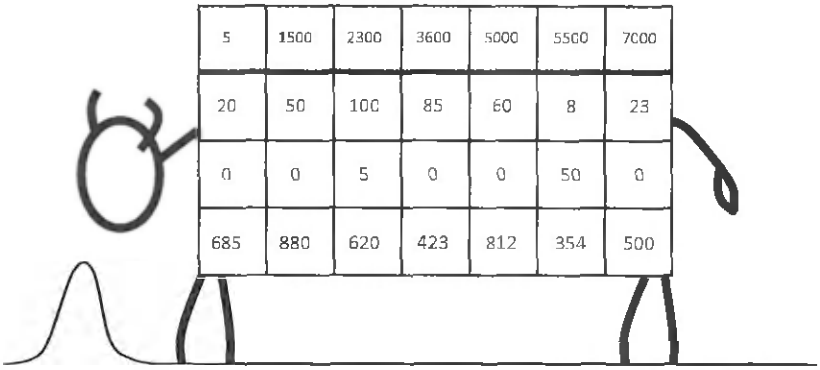
« Like the realtor said, only
three things matter :
location, location, location. »

An Orchard Invisible: 91

CHAPTER 2

A data-driven simulation model of endozoochory by herbivorous mammals – do grazers provide seeds with directed dispersal?*

Bram D'hondt, Sophie D'hondt, Dries Bonte, Rein Brys
and Maurice Hoffmann



5	1500	2300	3600	5000	5500	7000
20	50	100	85	60	8	23
0	0	5	0	0	50	0
685	880	620	423	812	354	500

Cattle grazing: putting endozoochory into mathematics

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[*] Adapted from: D'hondt, B., D'hondt, S., Bonte, D., Brys, R. and Hoffmann, M. A data-driven simulation model of endozoochory by herbivorous mammals – do grazers provide seeds with directed dispersal in heterogeneous landscapes? *Journal of Universal Rejection* ∞: 120-110.

Abstract

Large herbivorous mammals act as vectors of dispersal for many forbs and grasses through ingestion and subsequent excretion of seeds (endozoochory). As endozoochory involves both plant-, animal- and landscape-specific attributes, it is inherently complex and highly data-demanding to study. Accordingly, seed deposition patterns in heterogeneous landscapes have hardly been examined, though these are essential for evaluating the effectiveness of dispersal. We firstly constructed a spatially explicit simulation model for endozoochory, and parameterized it for dispersal by cattle of 25 plant species in a coastal dune landscape. Model output was then compared with field observations of the number of seeds dispersed. Second, we applied the model to assess the spatial pattern of seed deposition among vegetation types in this landscape, with a special emphasis on whether grasslands plants are transferred towards suitable habitat in a directed manner ('directed dispersal'). We additionally examined how altered habitat availability affects these patterns, and we included tentative simulations of wind dispersal as a reference. The observed numbers of seedlings that germinated from field-collected dung pats matched the model predictions closely. This indicates that patterns of endozoochory can to some extent be forecasted if sufficient contextual detail is included. Simulations demonstrated seeds to have their highest probability of being deposited in grassland vegetation. Since this probability was higher than would be expected from random dispersal, grassland plants are thus subject to directed transfer. The extent of this, however, depended on the availability of grassland in the landscape. Nonetheless, compared to wind dispersal, herbivores moved more seeds towards unsuitable habitat. Our model therefore suggests the main contribution of grazers to be in the combined action of directed and long-distance seed transfer, and not in directed transfer *per se*.

Keywords: dispersal, seed dispersal, zoochory, ungulates, grazing, seed shadow, seed rain, seed dispersal effectiveness, long-distance dispersal, spatial heterogeneity

Introduction

Many plant species have a spatio-temporal distribution which is dynamic on both a local and regional scale (Freckleton and Watkinson 2002). The dispersal of seeds is an essential aspect of these dynamics, enabling gene flow among established populations and colonization of new habitat patches (Cousens *et al.* 2008). Most plants rely on external vectors to disperse their seeds, and the understanding of these processes has grown to a central issue in plant ecological research over recent decades (e.g. Bullock *et al.* 2002, Fenner and Thompson 2005, Bullock and Nathan 2008).

An important, but less understood mode of dispersal is zoochory by large mammalian grazers (notably ungulates). Apart from seed transport in fur and on hooves (epizoochory; Fischer *et al.* 1996, Couvreur *et al.* 2004), these animals disperse seeds through ingestion and subsequent excretion (endozoochory). Indeed, various studies have shown considerable seed numbers from numerous herbaceous and graminoid plants to be viably present in dung from free-ranging ungulates (e.g. Malo and Suárez 1995b, Pakeman *et al.* 2002, Myers *et al.* 2004, Cosyns *et al.* 2005b, Jaroszewicz *et al.* 2009). Although they involve a somewhat distinct set of species, endozoochory appears to outweigh epizoochory for most herbivores in both the number of seeds and species dispersed (Couvreur *et al.* 2005, Mouissie *et al.* 2005a).

Endozoochory is inherently a complex mode of dispersal, comprising a succession of stages that pertain to the availability, uptake, movement and deposition of seeds (Wang and Smith 2002). Each of these stages involves components from the movement ecology of the plant or the animal vector (Nathan *et al.* 2008b). However, features of the landscape are inherent components of organismal movement as well (environmental factors *sensu* Nathan *et al.* 2008b, Damschen *et al.* 2008), since they influence, for instance, plant abundance and animal behaviour. Endozoochory should therefore necessarily be regarded as a context-dependent process in which both plant, animal and landscape attributes should be integrated to understand the process as a whole. This complexity and high demand of data thus make endozoochory a laborious process to study. One way of handling this is through ecological simulation, i.e. by bringing systems *in silico* (Peck 2004, 2008). To date, however, few efforts have been made for data-driven, spatially explicit simulation approaches of endozoochory by herbivores. In a notable exception, Will and Tackenberg (2008) compiled data from various sources into a simulation model on zoochory by ungulates and elaborated on the relative importance of plant and animal traits. However, their model (as presented) primarily applies to the regional level (e.g. exchange of seeds among landscape fragments), since little landscape detail is implemented on the local level (such as plant and habitat distribution within fragments). Moreover, their model was not operationally validated; for instance, through a comparison with independent observations from the field (Rykiel, 1996).

For a seed to benefit from dispersal, it should be transferred to a site where conditions are suited for emergence and recruitment (Duncan *et al.* 2009). Spatial patterns of seed deposition are therefore of great interest for assessing the effectiveness of endozoochory (Schupp 1993, Schupp *et al.* 2010). Observational data on seed deposition patterns in ungulate endozoochory are generally scarce. By quantifying dung deposition by rabbits, deer and cattle in a Mediterranean wooded pasture system, Malo *et al.* (2000) documented considerable variation in the amount of seeds deposited per herbivore species among and within plant communities. From a comparative survey of the vegetation composition and the seed content of dung in a landscape comprising salt-marshes and sand dunes, Bakker *et al.* (2008) concluded that seeds from each community are dispersed into the other, but do not establish there.

By definition, if seeds arrive in a habitat with a probability that equals the proportional surface of that habitat within the landscape, dispersal is random. On the other hand, if the arrival probability for suitable habitat is higher than expected by chance, seeds benefit from 'directed dispersal' provided by that vector (Howe and Smallwood 1982, Wenny 2001). Non-random terrain use of an animal disperser in heterogeneous environments can result in such disproportionate arrival. Although this has been addressed for endozoochory by frugivorous and scatter-hoarding birds (e.g. Wenny and Levey 1998, Gómez 2003; also see Deckers *et al.* 2008), directed dispersal has to our knowledge never been investigated for herbivorous mammals.

Grazers allow early- and mid-successional vegetation stages to persist through effects of feeding, treading and nutrient redistribution (Van Wieren 1995, Olff and Ritchie 1998, Olff *et al.* 1999). Moderate grazing by livestock (cattle, horse, sheep) is therefore frequently applied in nature conservation practice (WallisDeVries *et al.* 1998). This also is the case in many temperate coastal reserves, where without management, species-rich dune grasslands quickly become suppressed by shrubs (notably by *Hippophae rhamnoides* L.; Isermann *et al.* 2007, Provoost *et al.* 2011). Since dune grasslands and scrub typically form part of highly dynamic (coastal) landscapes that exhibit a high variation in vegetation, such grazed reserves might provide good systems for studying the contribution of herbivores to plant dynamics in heterogeneous environments.

One of such reserves is Westhoek-South, which is located in the westernmost tip of Belgium (51°04'50''N, 2°33'50''E) and about 60 ha in size. Since 1998, the site is fenced and stocked with Scottish Highland cattle and Shetland ponies (3 and 16 individuals in 2008, respectively; Leten *et al.* 2005). In this work, we took advantage of a large body of data obtained from this reserve to evaluate the contribution that grazers make to seed dispersal in heterogeneous landscapes. Our aims were firstly to construct, parameterize and validate a mechanistic simulation model for endozoochory by cattle. We then applied the model to assess how seeds are redistributed within the terrain, and how this is affected by landscape changes. Here, a special emphasis is given on whether seeds are transferred in a directed manner towards suitable habitat or not.

Methods

First, we constructed an individual-based and spatially explicit model (Cousens *et al.* 2008, Couvreur *et al.* 2008). In short, a single animal is introduced in a two-dimensional landscape, in which it moves, and ingests, digests and defecates seeds. This model was subsequently parameterized for endozoochorous dispersal of 25 plant species by cattle in Westhoek-South. The plants comprised dry-fruited forb and graminoid species (Table 2-1). We then validated the parameterized model's output on the expected number of seeds that are dispersed per defecation, by comparing these with field-based observations (cf. Rykiel 1996).

Second, we used the parameterized model to assess the pattern in which viable seeds are deposited among the site's main vegetation types, or, in other words, the probability for seeds to end up in a particular vegetation. We also quantified levels of directed seed transfer, defined as the probability for a dispersing seed to end up in suitable vegetation relative to the proportional surface area of that vegetation in the landscape (Spiegel and Nathan 2010). This yields a measure for the deviance from random transfer (transfer away from suitable habitat at <1, random transfer at =1, directed transfer at >1). As seed transfer is seldom random in nature, however, we also included tentative simulations of wind dispersal (anemochory) as a reference.

As no data on emergence and establishment of species among vegetation types is available, we operate under the assumption that the most suitable vegetation type for a species is the one where we found it most frequently fruiting (data in Appendix 2A). For most of the species, this is the grassland (Table 2-1), and we therefore elaborate on this vegetation type in particular. If the assumption should prove invalid in some species, modeled seed patterns would still hold, though a reinterpretation of their suitability to species would be needed.

Model structure

The model's structure is extensively discussed in Appendix 2A. Here, only a summary is given. The model code is provided in Appendix 2B.

After having introduced the animal into the landscape, the model proceeds in steps. Each stap corresponds to a repositioning of the animal from one cell to a neighbouring cell. Steps are repeated until the pre-set duration is reached.

Landscape – The landscape is a grid of which each cell is assigned to one of several vegetation types.

Animal movement – Cell-to-cell movement of the animal accords with a constrained random walk. The movement constraint is based on the combination of the animal's preference among vegetation types (which it is facing in the neighbouring cells), and its internal propensity to change direction (irrespective of the environment). The length of stay in a cell is variable.

Grazing / seed ingestion and digestion – The animal grazes parts of its path while moving. When grazing a cell, a certain quantity of seeds from each of the set of study plant species becomes available to the animal. A portion of these seeds is subsequently ingested, proportional to the time spent in the cell and to the forage value of the species. The mortality of ingested seeds that is due to destruction or digestion by the animal is included in this step.

Defecation / seed excretion – From time to time, the animal defecates along its path. The probability for a seed to be either excreted or retained at defecation, is determined by the time that has passed since its ingestion. This relationship is given by a cumulative lognormal probability density function (Rawsthorne *et al.* 2009).

Table 2-1. The plant species for which the model was parameterized. Per species, the presumed vegetation type suitable for establishment is given (S: scrub; F: forest; G: grassland; Rg: rough grassland; D: dune; W: wetland)

Agrostis capillaris L.	G	*Juncus articulatus* L.	G
Agrostis stolonifera L.	G	*Juncus bufonius* L.	G
Carex arenaria L.	D	*Juncus inflexus* L.	Rg
Carex flacca Schreb.	G	*Mentha aquatica* L.	G
Carex hirta L.	G	*Plantago major* L.	G
Carex oederi Retz.	G	*Poa annua* L.	G
Cerastium fontanum Baumg.	G	*Poa pratensis* L.	G
Cerastium semidecandrum L.	D	*Poa trivialis* L.	F
Galium aparine L.	S	*Trifolium campestre* Schreb.	G
Galium mollugo L.	W	*Trifolium dubium* Sibth.	G
Galium palustre L.	G	*Trifolium pratense* L.	G
Galium uliginosum L.	G	*Trifolium repens* L.	G
Galium verum L.	S		

Table 2-2. The distinguished vegetation types with some representative plants, their surface area (proportional to the entire area), and the coefficient of vegetation preference for cattle

Vegetation	Representative plants	Surface	Pref. coeff.
Scrub	*Hippophae rhamnoides* L., *Ligustrum vulgare* L., *Salix repens* L.	0.46	0.12
Forest	*Populus* spp., *Alnus glutinosa* (L.) Gaertn.	0.19	0.28
Grassland	various perennial grasses, numerous forb species	0.13	1.00
Rough grassland	*Calamagrostis epigejos* (L.) Roth, *C. canescens* (Weber) Roth, otherwise low diversity of forbs	0.10	0.63
Dune	*Carex arenaria* L., winter annuals, mosses, lichens	0.10	0.25
Wetland	almost monospecific stands of *Iris pseudacorus* L.	0.02	0.14

Model parameterization

Parameterization of the model is extensively discussed in Appendix 2A. Here, only a summary is given.

Landscape – We used the vegetation map of Westhoek-South from Ebrahimi (2007), who visually interpreted aerial images of the area. Some minor changes were performed, such as an update of the fencing and some vegetation polygons, before converting the map to a grid. Each grid cell corresponded to 1 m^2 in the field. Six vegetation types were distinguished in the landscape: scrub, forest, grassland, rough grassland, dune and wetland (Table 2-2). (Also note that the distinction between habitats is overall rough and their names are essentially arbitrary. Assignment of species to particular habitats therefore should be considered approximate.) The integer landscape is shown in Fig. 2-1a.

Animal movement – We used data from Lamoot *et al.* (2005) to determine the animal's preference for each vegetation type. They extensively recorded the behaviour of individual Highland cattle within this reserve in six-hour observation sessions, which included mapping its position every 15 min. We used these positions (275 in total, from 13 sessions) to calculate 'vegetation preference coefficients' according to Couvreur *et al.* (2008): the number of observed positions in each vegetation type was divided by that vegetation type's area, and these numbers were then rescaled by dividing them by the highest value (Table 2-2). Values for 'direction preference coefficients' (reflecting the animal's propensity to change direction) were chosen arbitrarily due to a lack of data: in a homogeneous environment, the animal changes its direction (45° or 90°) in about one out of three steps. The duration for which the animal stays in a cell is drawn randomly from a vector of data values (length of stay per m^2) which were also derived from the positional data of Lamoot *et al.* (2005). From their data, we moreover calculated that cattle do not have habitual latrine sites in the area, but instead perform random excretions with respect to vegetations. This is included in Appendix 2C.

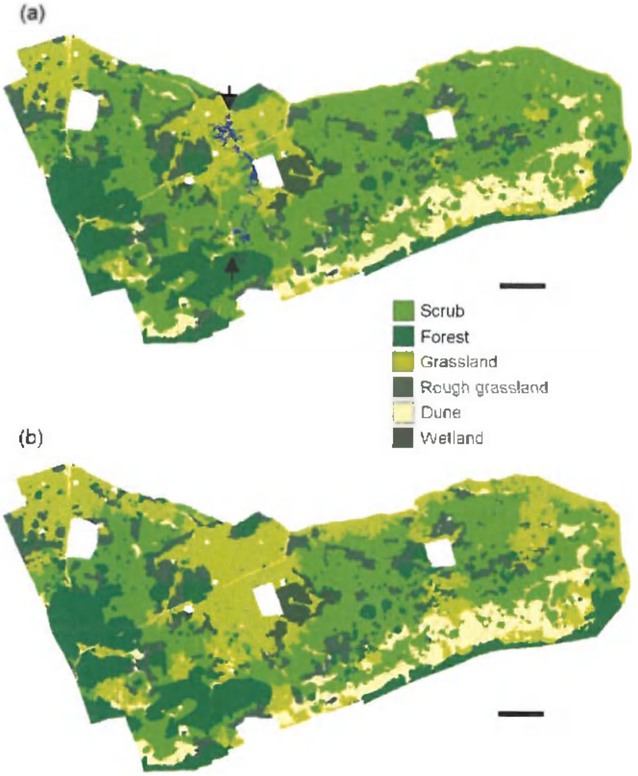


Figure 2-1. Vegetation maps of the study area. (a) The integer map. A simulated twenty-four hours' path is plotted (line, ends indicated by arrows). (b) A manipulated version, in which grassland vegetation is doubled at the expense of scrub. Scale bar = 100 m.

Grazing / seed ingestion and digestion – In the study area, Highland cattle graze about 30.2 % of their time in summer (Lamoot *et al.* 2005). The animal therefore grazes along this proportion of its path in the model. We determined the study species' availability of seeds for ingestion by sampling 25 to 34 plots per vegetation type during the summer of 2008 (1 m^2 plots). Using species-specific protocols, we estimated the number of seeds from each of the species in each of the plots. We counted the number of inflorescences per plot (or lower levels, e.g. fruits, if convenient), and then hierarchically sampled the missing levels (e.g. seeds per fruit) to obtain these estimates. Values were included in separate vectors for every species/vegetation type combination, from which values are drawn randomly at grazing. (The results of these are also included in Appendix 2A.) Species-specific forage values were derived from the BiolFlor database (based on Briemle *et al.* 2002). On a 0-to-1 scale, these numbers reflect the species' palatability to livestock.

In previous work, we determined species-specific mortality rates that are due to animal destruction, by feeding known amounts of seeds from various plant species to cattle (D'hondt and Hoffmann 2011). Twelve of the species used in that study were also included here, and we therefore adopted the raw values for them into vectors. As intra-generic differences in mortality rate are generally low (D'hondt and Hoffmann 2011), we lumped the raw values from congeneric species for the remaining thirteen species. The model randomly draws values from these vectors at ingestion.

Defecation / seed excretion – A vector of observed time intervals between two defecation events was derived from the behavioural data of Lamoot *et al.* (2005). After each defecation, a new value is drawn from this vector. Parameters for the function describing the probability for a seed to become excreted at defecation, are provided by D'hondt and Hoffmann (2011). For instance, seeds have a 50 % chance of being excreted within 29 h after ingestion, and within 106 h, 99.99 % of them are excreted. The retention pattern is considered the same for all species.

Model validation for dispersed seed numbers

We used field observations to validate the expected number of seeds that become dispersed per defecation as obtained from the model. Concurrently with the determination of seed availability (summer 2008), we collected 33 cattle pats *in situ*. These samples were subsequently stratified for 41 days at ± 7.9 °C, then mixed through a shallow layer of sand and potting soil (1:1 volume) in trays (45 x 40 x 10 cm), and left to germinate in a greenhouse for 12 months. Throughout this period, all seedlings were identified and removed.

Importantly, the model essentially yields numbers of viable seeds in dung pats, while from the greenhouse we obtained numbers of seedlings germinating from pats. Since not all viable seeds germinate from dung in the greenhouse, a correction is needed to allow for a comparison of model predictions and greenhouse observations. In our feeding experiment (Chapter 4, D'hondt and Hoffmann 2011), we included a treatment in which seeds were germinated on a mixture of sand and potting soil, and a treatment in which seeds from the same batch were germinated on a mixture of sand, potting soil and cattle dung; combining both yielded species-specific factors for this correction (these can be found from Appendix 4C).

We ran the model 20 times for a simulated twenty-four hours' period. Animal introduction was forced to be in the grassland (see below). A non-parametric Spearman rank correlation was used to confront the mean number of seedlings from the model and the greenhouse samples.

Patterns of seed deposition

Before inferring on spatial seed deposition patterns, we firstly checked whether simulations accurately reflected the observed animal movement.

We then firstly assessed the deposition pattern for the integer landscape of our study site. Second, we manipulated the vegetation map to test how this distribution is affected by the availability of grassland. We increased the proportional surface area of grassland by gradually extending the margins of existing grassland patches at the expense of neighbouring vegetation (simulating successful grazing management or mechanical removal). At first, scrub was turned into grassland, but when scrub area equalled that of woodland (the next most abundant non-grassland vegetation), both were collectively converted until equation with rough grassland, and so on. In this way, coexistence of the different vegetation types was assured in all landscape scenarios (see Fig. 2-1b). Vice versa, decrease of grassland was simulated by extending ranges of neighbouring vegetation at the expense of grassland.

We ran the model 100 times for a simulated 3 days' period in each landscape scenario. The animal was always introduced on a fixed position in the grassland since random introductions would bias results.

Endozoochory *vs* wind dispersal

We tentatively included simulations of wind dispersal for comparison with endozoochory. In each of the landscape scenarios, we took 10000 random positions in the grassland. From each position, one seed was allowed to disperse. Its direction of dispersal was chosen randomly, while the dispersal distance was drawn from an exponential distribution with a mean of either 1, 5, or 25 m. Using this one-parameter distribution provides a simple means of generating anemochorous seed rains (Willson 1993, Gros *et al.* 2006).

Results

Model validation for dispersed seed numbers

Sixteen of the study species emerged from the dung samples obtained from the field. Statistics from these are listed in Appendix 2D. Species means of the number of seedlings from the model (213 simulated pats) matched those obtained from the dung samples relatively closely (Spearman rank correlation coefficient = 0.693, $P < 0.001$, Fig. 2-2).

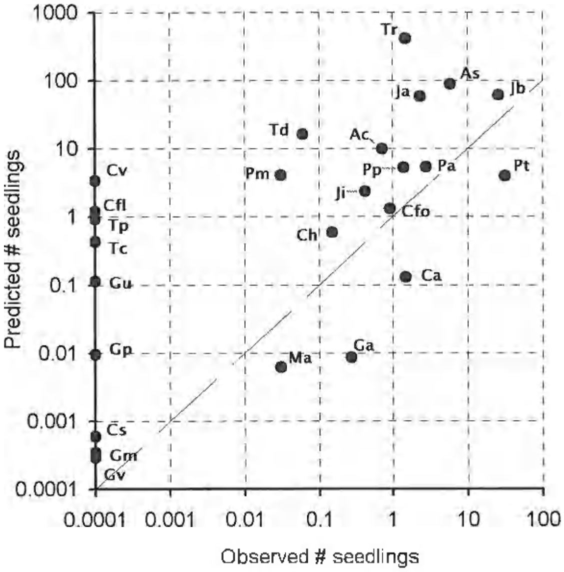


Figure 2-2. The mean number of seedlings in dung pats for each of the 25 species, as observed from the germination experiment in the greenhouse and as predicted by the model. The scales are logarithmic; 0.0001 is added to the values. The line depicts X equalling Y. Species abbreviations consist of the first letters of genus plus species; please refer to Table 2-1.

Patterns of seed deposition

The relation between the observed and simulated terrain use of animals with respect to vegetations is shown in Fig. 2-3. As there is a good correspondence, the model is appropriate for questions concerning the directionality of dispersal towards particular vegetation types. (Although a slight overestimation for scrub, as opposed to that for forest and rough grassland, may be taken into account.) The model performed less well with respect to distances covered in animal movement, and the model is therefore not suited to infer on seed dispersal distances. This is included in Appendix 2C.

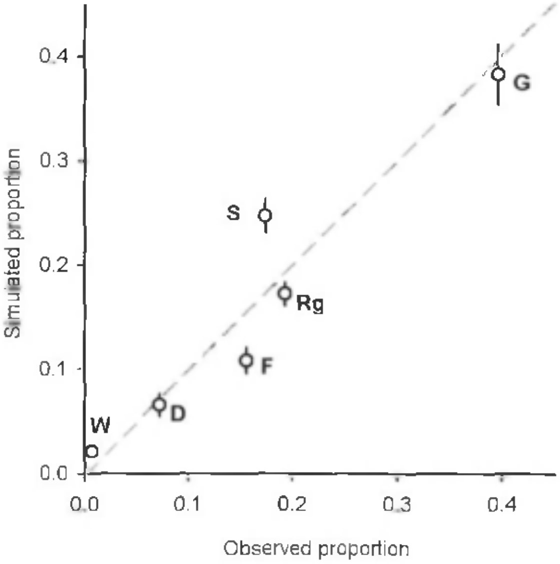


Figure 2-3. Correspondence between the observed and simulated terrain use of animals with respect to vegetation types. 'Observed' refers to the proportion of observed positions in the respective vegetation types. 'Predicted' refers to the proportion of the simulated animal paths in those types. The results (mean ± s.e.) are shown for 100 runs (the same as in Fig. 2-4). S: scrub; F: forest; G: grassland; Rg: rough grassland; D: dune; W: wetland. The line depicts X equalling Y. Pearson correlation of the data: $\rho = 0.953$, $P = 0.003$.

The distribution of endozoochorously dispersed seeds among vegetation types, or, the probability for a dispersing seed to end up in each vegetation type, is shown in Fig. 2-4 for the integer landscape. This pattern appeared to be the same for each species, irrespective of its occurrence pattern in the area. Seeds apparently had the highest chance to end up in the grassland and the lowest in the wetland. The probability to end up in scrub was lower than expected from the scrub surface. This thus suggests that cattle transfer scrub species (e.g. *Galium aparine*, cf. Table 2-1) away from suitable habitat. The chance of reaching grassland was three times higher than expected (Figs 2-4 and 2-5b), and grassland species are thus provided with directed transfer.

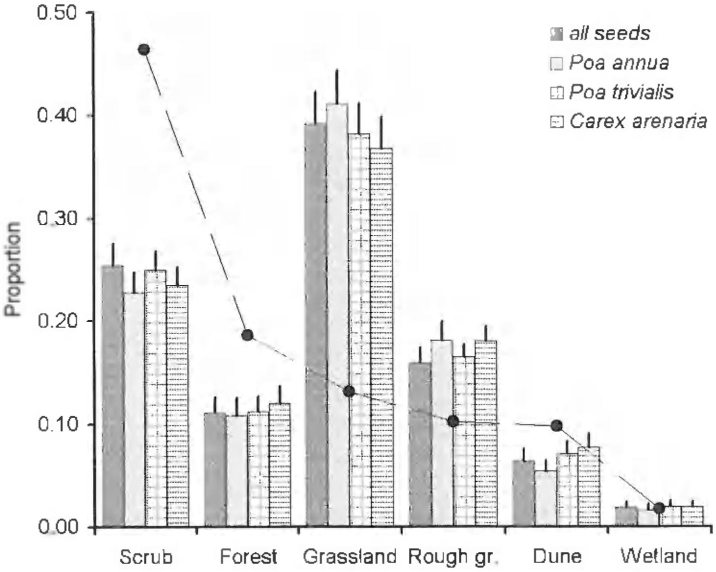


Figure 2-4. The proportion of seeds that end up in each vegetation type, as modelled for the integer landscape (mean ± s.e. of 100 runs). The proportional surface area for each vegetation type is given by closed dots. Three exemplary species are included: *Poa annua*, *P. trivialis* and *Carex arenaria* are considered grassland, forest and dune species, respectively.

As demonstrated from the manipulated landscapes, the probability for a seed to end up in grassland related positively to the surface area of grassland (Fig. 2-5a). With increasing area, this probability became the highest of the six vegetation types when its area was about 8 % of the total surface, and from ± 22 % on, this probability exceeded the chance of ending up in non-grassland vegetation (i.e. all other vegetation types taken together). This probability was higher than the surface area of grassland, suggesting directed transfer, in all scenarios. However, the level of directed transfer decreased with increasing surface of grassland (Fig. 2-5b). The probability to be deposited in grassland and the level of directionality thus show opposite patterns with changing levels of grassland area.

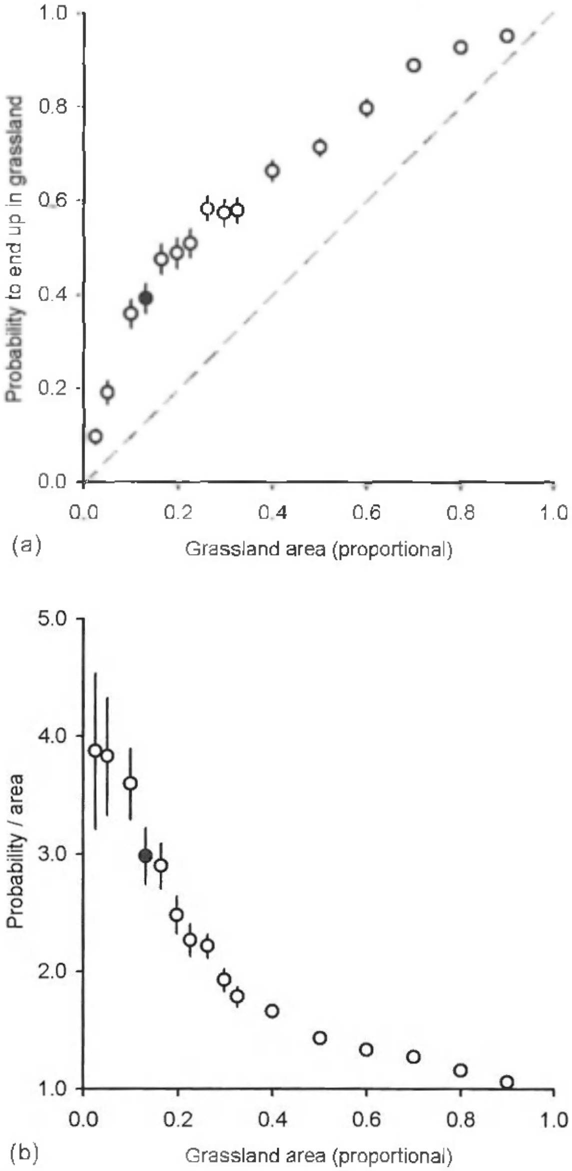
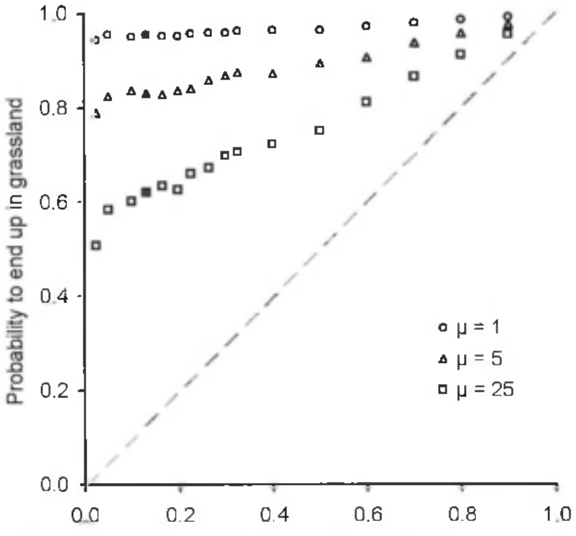
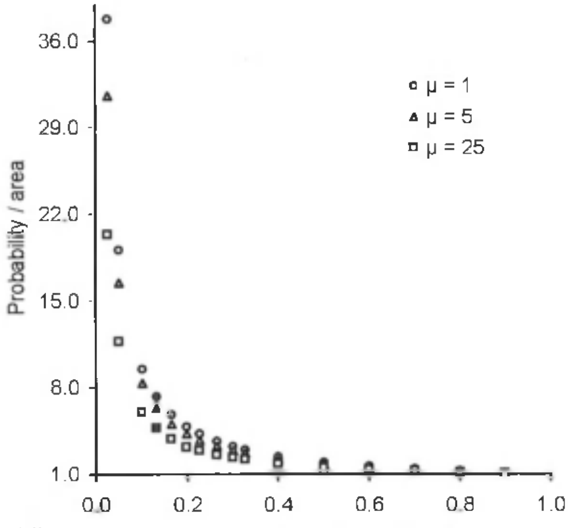


Figure 2-5. (a) The probability for seeds to end up in grassland through endozoochory, as a function of grassland surface (mean ± s.e. of 100 runs). (b) The level of directed transfer in endozoochory, as a function of grassland surface. When the probability to end up in grassland equals its area (Y = 1.0), dispersal is random.



(c)



(d)

Figure 2-5, *continued*. Panels (c) and (d) provide the same measures, through wind dispersal. μ is the parameter of the exponential distribution used in simulation (mean dispersal distance). Closed symbols refer to the integer vegetation map, open symbols refer to manipulated maps.

Endozoochory *vs* wind dispersal

Seed dispersal distances obtained through the tentative wind dispersal simulations are given in Fig. 2-6 (basically, these reflect the implemented exponential distributions). The probability to end up in grassland was also positively related to the surface area of grassland (Fig. 2-5c). In any given landscape, this probability decreased with increasing wind dispersal capacity. The ratio of this probability to the area of grassland also related negatively to grassland availability (Fig. 2-5d). Moreover, the results suggested that directed transfer is stronger in wind dispersal than in endozoochory (Fig. 2-5b *vs* 2-5d). (However, the use of 'directed' might be inappropriate in terms of wind dispersal, as is discussed below).

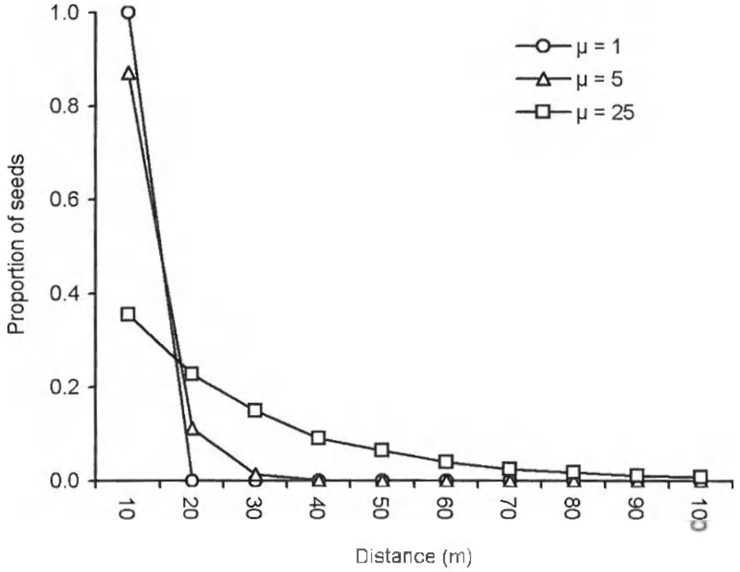


Figure 2-6. Frequency distribution of dispersal distances obtained through the tentative wind dispersal simulations. μ is the parameter of the exponential distribution used in simulation (mean dispersal distance). Simulations are performed in the integer vegetation map. X axis labels are upper boundaries.

Discussion

This is the first study that validated a data-driven simulation of endozoochorous dispersal by large herbivorous mammals (cf. Couvreur *et al.* 2008 for epizoochory; Russo *et al.* 2006 for frugivores). A good agreement was found between field observations on the number of seeds that are dispersed by cattle in Westhoek-South, and model predictions hereof, showing that endozoochory can to some extent be forecasted if sufficient contextual detail is included. Though the correlation involved a significant scatter and tended to overestimate, we nonetheless believe this already is a substantial realization when considering its real world complexity. As is the case with any model, more detail could be added, of course. In *Trifolium*, for instance, digestion-resisting seed attributes are known to vary *in situ* (Chapter 5, D'hondt *et al.* 2010; note *T. repens* to be the most highly overestimated species in Fig. 2-2).

Spatial patterns of seed deposition constitute a major component of a vector's effectiveness (Schupp 1993). In our case-study, cattle deposited seeds in each of the considered vegetation types, which agrees with the observations of Malo *et al.* (2000) and Bakker *et al.* (2008). The probability for a dispersing seed to end up in a particular vegetation type appeared to be similar for all species, which can be explained by the fact that the time from seed ingestion to excretion largely exceeds temporal intervals with which vectors cross vegetation type boundaries in this small-scaled heterogeneous landscape. Since such probabilities are affected by their surface areas, they will largely vary among (spatially) or within landscapes (temporally). As large grazers prefer grasslands disproportionally more than any other vegetation, transfer towards grassland is nonetheless always directed. (By definition, however, transfer is not directed but random when grassland is the sole vegetation. Therefore, $Y \rightarrow 1$ in Fig. 2-5b.) The directedness is strongest when the proportion of grassland is low. However, the actual number of seeds reaching grassland is considerably small under such circumstances.

So far, we have been considering quality of deposition at the level of the vegetation. On a smaller scale, seeds end up in a nutrient-rich gap through endozoochory (the dung pat, Gillet *et al.* 2010). The direct effects of these conditions on seed viability, germination and seedling establishment are barely known (but see Carmona *et al.* 2010). Although several studies suggest a high incidence of pre- or post-germination mortality under field conditions (Cosyns *et al.* 2006, Mouissie *et al.* 2005a, Pakeman and Small 2009), seeds might also postpone germination and become incorporated into the soil seed bank with dung effects eroding over time (Malo *et al.* 1995, Gittings *et al.* 1994). Seeds in dung pats can also become secondarily dispersed; for instance, by dung beetles in late summer (Chapter 3, D'hondt *et al.* 2008). The concentrated pattern in which seeds are deposited by grazers may increase density-related competition among seedlings, and Spiegel and Nathan (2010) therefore recently argued for an inclusion of this component into the framework of directed dispersal.

Because we lack a general comprehension of the post-deposition fate for endozoochorously dispersed seeds, we restricted our model to deposition at the vegetation level, which sets the stage for these lower-level processes. Indeed, even abiotically dispersed seeds face complex fates after dispersal. Our model suggested seed transfer by wind to be more 'directed' than endozoochory. It should be noted, however, that terming these anemochorous events as 'directed dispersal' (as implied by our mathematical formulation) might be inappropriate. The higher-than-random chances of reaching suitable habitat for these seeds simply reflect the shorter distances travelled by them, with most seeds falling near the mother plant. Whereas definitions of 'directed dispersal' suggest that habitat patch boundaries become crossed (although this is rarely stated explicitly, cf. Wenny 2001). Indeed, the majority of forb species lack capacities to disperse by wind significantly far (Tackenberg *et al.* 2003).

If seeds end up close to the mother plant, e.g. through localized wind dispersal, plants might experience increased levels of conspecific competition (Cheplick 1992), host-specific enemies (Nathan and Casagrandi 2004) or inbreeding (Roze and Rousset 2005). Endo- and epizoochory, on the other hand, move seeds over much larger distances, allowing seeds to escape the maternal environment easily (Pakeman 2001, Vellend *et al.* 2003, Will and Tackenberg 2008, Couvreur *et al.* 2008; see Appendix 2C for output from our model). Therefore, it remains hard to evaluate the demographic result of these dispersal modes, i.e. to what extent transfer also results in succesful dispersal. If distance-related demographic effects do not play an important role, then wind and herbivores provide seeds with complementary benefits in terms of distance and habitat suitability. While wind provides succesful short-distance dispersal, herbivores disperse seeds over large distances with directed dispersal being relatively costly (as compared to wind). The main

« a “seed bank” … isn’t the
kind of bank anyone would
want to invest their money
in. … (T)his is a bank regularly
robbed by rodents and
riddled with fungi »

An Orchard Invisible: 115

CHAPTER 3

Dung beetles as secondary seed dispersers in a temperate grassland[*]

Bram D'hondt, Beatrijs Bossuyt, Maurice Hoffmann and Dries Bonte

Onthophagus similis SCRIBA, a tunneler

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[*] Adapted from: D'hondt, B., Bossuyt, B., Hoffmann, M. and Bonte, D. (2008) Dung beetles as secondary seed dispersers in a temperate grassland. *Basic and Applied Ecology* 9: 542-549.

Abstract

The two-phase dispersal event in which dung beetles move seeds after endozoochory is often assumed to be advantageous for plant regeneration. Because seeds are expected to end up in favourable and safe germination sites, it is considered as an example of directed dispersal. However, literature so far is restricted to tropical rain forest ecosystems, while data for temperate regions are lacking. In this study, the effect of dung beetles on seedling establishment of endozoochorously dispersed seeds is evaluated for a temperate grassland ecosystem. We performed a field experiment in which cages excluded dung beetles from horse and cattle dung samples with mixed-in grass seeds. Seed germination from these samples was significantly higher than that from samples which were accessible to dung beetles. This indicates that the effect of dung beetles on short-term seedling establishment was negative, which contrasts with the patterns found for large-seeded species used in tropical studies. This is most likely attributed to the lack of roller species and the larger depth at which tunneling *Geotrupes* species bury seeds.

Keywords: seed dispersal, secondary dispersal, diplochory, seed burial, *Agrostis*, *Poa*

Introduction

Acting as dispersal agents, animals may take plant seeds to non-random microhabitats that are well-suited for establishment and growth. This process, referred to as 'directed dispersal', was distinguished by Howe and Smallwood (1982) as one of three major advantages of seed dispersal, and is assumed to be more common than formerly thought (Wenny 2001). Directed dispersal is considered to be a key step in diplochory (Vander Wall and Longland 2004), in which seeds are dispersed in two successive phases, each involving a different dispersal agent. According to Vander Wall and Longland (2004), the benefits offered by the different phases differ: second phase dispersers (e.g. ants, rodents, corvids) often take seeds to microsites that are more suitable for establishment than those reached by phase one dispersers (e.g. wind, herbivores). One particular case of diplochory occurs when dung beetles act as secondary dispersers of seeds contained in vertebrate dung.

Within the dung beetles (coprophilous species of the Scarabaeidae subfamilies Aphodiinae, Geotrupinae and Scarabaeinae [classification following Janssens 1949, 1960]), three functional groups are distinguished: dwellers, tunnelers and rollers (Cambefort and Hanski 1991). Aphodiinae are predominantly dwellers: these small-sized beetles (generally less than 10 mm in length) eat their way through the dung and deposit their eggs without constructing a nest or chamber. Geotrupinae and most Scarabaeinae are tunnelers: they dig a more or less vertical tunnel beneath a dung pat and move the dung to the shaft base. Many Scarabaeinae, specified as rollers, construct a dung ball and transport this over a distance prior to burying it (telecoprid nidification *sensu* Bornemissza 1969). Dung beetles do not eat seeds (Andresen and Feer 2005), so tunnelers and rollers may contribute to dispersal by moving and burying seeds along with the dung.

So far, studies on secondary seed dispersal by dung beetles and its influence on plant regeneration almost exclusively focused on tropical rain forest ecosystems. In a review, Andresen and Feer (2005) concluded that burial has both a positive impact through lower seed predation and a negative impact through decreased seedling emergence. Little is known, however, about the net outcome of these antagonistic effects. Only Andresen and Levey (2004) really followed the fate of seeds placed in dung until seedling establishment in a (Central Amazonian) rain forest ecosystem. Their results, concerning 11 large-seeded tree species and howler monkey dung, indicated that seeds buried by dung beetles were on average twice as likely to become seedlings than unburied seeds. In the only study investigating dung beetle mediated seed dispersal outside tropical regions we know about, Wicklow *et al.* (1984) found a subtropical roller species (Kohlmann 1991) to facilitate seedling establishment of a prairie grass species in North America.

More studies are needed to ascertain if this potential positive effect also holds for other climate regions and ecosystems (Andresen and Feer 2005), because it is reasonable to assume that important differences in the dung beetle communities or the plant species involved may lead to deviant patterns. For instance, at lower latitudes dung beetle communities are dominated by rolling or tunneling Scarabaeinae, whereas the northern temperate regions are characterized by a dominance of the dwelling genus *Aphodius* (Aphodiinae), normally accompanied by one or a few species of tunneling Geotrupinae (Hanski 1991).

In this study, we aim to test the hypothesis that dung beetle activity has an overall positive effect on successful germination of seeds present in dung in a temperate coastal dune ecosystem. A field experiment was constructed, in which the effect of dung beetle presence on short-term seedling establishment from horse and cattle dung was tested. This 'main experiment' was supplemented by some supplementary experiments, i.e. controls to evaluate the main setup in detail and a study of the dung beetle fauna present within.

Material and methods

Study area and local dung beetle assemblage

The field experiment was set up in the nature reserve 'Westhoek' (Belgium, 51°04'50"N–2°34'19"E), consisting of over 340 ha of coastal dune landscape. It is characterized by a spatially heterogeneous mosaic of open dunes, grey dunes, grassland, shrub and woodland. Since 1996, part of the area is grazed by cattle and several equine breeds, restoring grazing activities previously maintained by livestock. In a recent study of the associated dung beetle fauna, 15 species were found (Struyve 2002), 12 of which belong to the dweller genus *Aphodius* ILLIGER. *Onthophagus similis* SCRIBA, *Geotrupes niger* MARSHAM and *Geotrupes spiniger* MARSHAM were the tunneler species found. No roller species were present in the area. Hence, functionally, the overall dung beetle fauna can be considered as typical of a temperate ecosystem (Hanski 1991).

Main experiment

At each of two grassland sites within the study area (400 m apart), the effect of dung beetle activity on seed germination was assessed using the same experimental randomized block design. The sites consisted of preliminary mown grasslands located within livestock-excluding fences, the surroundings of which were grazed by cattle as well as ponies.

One site is distinctly moister than the other: from September 2005 to April 2006, ground water fluctuated between 0.2 and 1.0 m beneath the surface at the moist site and between 1.5 and 2.1 m at the dry site (De Becker *et al.* unpub.).

Perpendicular to the prevailing moisture gradient at each site, six strips of 1 m width were delimited, which contained (among controls, see below) eight sod-cut plots (50 × 50 x 10 cm, 25 cm inter-plot spacing), on which treated dung samples were deposited, the treatments being allocated at random within a strip (block).

Dung was collected from horses (Konik) and cattle (Galloway) that were fed a supposedly seed-free diet in captivity. In September 2005, two-litre samples were deposited on the field plots, and simultaneously, 100 seeds, either from *Agrostis capillaris* L. or *Poa pratensis* L., were mixed into each sample. Dispersules were on average 1.60 mm in length for *A. capillaris* and 2.78 mm for *P. pratensis* (n = 20 each, glumes included). Cosyns *et al.* (2005b) found both grasses to be viably present in dung of cattle, horses and ponies in the 'Westhoek' reserve. Period of seed set is August to October for the former, and August for the latter (Grime *et al.* 1988).

After deposition in the field, half of the dung samples were caged ('closed cage'), making the dung inaccessible to dung beetles (1.25 mm mesh). The others were equipped with an 'open cage' that covered the top and southern side (cf. sunlight) of the plot but left all other sides open, enabling dung beetles to gain access to the samples easily.

During September and October 2005 –the months in which dung samples were subject to dung beetle colonization– precipitation rates were normal, but temperatures were exceptionally high (in September, mean temperature was 16.0 °C instead of 14.2 °C, October: 14.4 °C instead of 10.6 °C; recorded at ± 20 km off; Malcorps 2005a, 2005b).

About 73 days after deposition, in November–December 2005, the number of seedlings of either *A. capillaris* or *P. pratensis* was counted on each dung pat once. Seedlings were removed after counting. An additional count was conducted in the following spring (April 2006), but very few seedlings could be added. Visual traces of dung beetle activity were recorded, particularly of tunneling by *Geotrupes*, which was recognized by the presence of tunnel entrances surrounded by bare sand.

Germination controls

To test whether the obtained dung was free from seeds, a greenhouse control experiment was performed in which germination from six untreated dung samples per herbivore type (2 l) was monitored. These were deposited on a sand–peat substrate mixture, subjected to a 16 h light : 8 h dark regime, and watered manually (September–December 2005).

In order to test the used grass seeds for their viability, we determined germination of 6 times 100 seeds per species in two controls: once in the field and once in the greenhouse. In both, seeds were sown in sand–peat substrate (which, in the field, was spread out within cageless sod-cut plots; September–December).

Moreover, the effect of both 'open' and 'closed cage' constructions on germination conditions was tested in the greenhouse by sowing seeds in the same substrate, with six replicates for every combination of cage type and plant species (September–December).

Dung beetle fauna

To assess the species composition of the dung beetle community in the study area, six horse dung and six cattle dung samples were deposited at both sites and contiguously encircled by five pitfall traps per sample (65 mm diameter, ± 80 mm spacing between). Half of these samples were fitted with 'open cages' while the other remained cageless, in order to determine whether the dung beetle fauna visiting the 'open cages' was representative of the cageless, i.e. natural situation.

During a 63-day period (mid-September to mid-November 2005), all Scarabaeidae were gathered, identified up to the species level (Janssens 1960) and classified as tunnelers (*Geotrupes* spp., *Onthophagus* spp.) or dwellers (*Aphodius* spp.).

Data analysis

In the main experiment, DUNG TYPE, PLANT SPECIES, SITE and CAGE TYPE were subsequently used as fixed factors in two separate Mixed Models, in each case with seedling numbers ('successful germination') as the dependent variable. In the first, 'open' and 'closed cages' were the CAGE TYPE levels, while in the second analysis the 'open cages' group was split up in two: with and without traces of tunnelers. In both models, the correction for location of a plot according to the prevalent moisture gradient was made by incorporating the position of the strip it was in as a random factor (AR(1)-structured; Littell *et al.* 1996). We started from the full model, and used a backward procedure by stepwise eliminating non-significant contributions to reach the reduced models presented here.

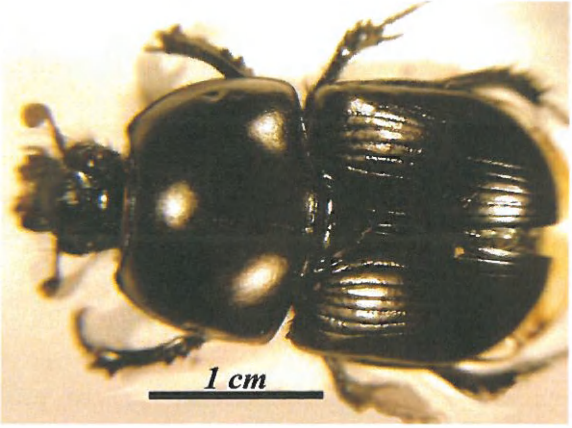
To analyse the dung beetle fauna gathered in the pitfalls, we used General Linear Models to evaluate densities (instead of Mixed Models, as the corrections for location proved not to contribute significantly) and an ordination technique to evaluate composition. In the linear models, the total number of dung beetles per sample and the numbers of dwellers and tunnelers were used as dependent variables. The fixed factors CAGE TYPE, DUNG TYPE, SITE (and interactions) were used as independent variables. Species composition was analysed by detrended correspondence analysis (DC analysis), following suggestions of McCune and Grace (2002) on the determination coefficient (r^2). Rare species (occurrence in less than 3 out of 24 samples) were removed in advance, and densities were converted to relative numbers (percentage individuals of species *x*, occurring in sample *y*). We then tested whether the coordinates on the axes that represented the highest proportion of variation, were significantly different between the levels within each factor, using Wilcoxon two-sample tests.

Except for the DCA, which was done using Pc-Ord 4.26 (McCune and Mefford 1999), statistical analyses were executed with the SAS software package (SAS Institute 2003).

Results

Dung beetle fauna

Using the pitfall traps distributed among the 24 dung samples (12 per site), 650 Scarabaeidae individuals were caught, belonging to 12 species and 3 genera. The three most abundant species were *Aphodius contaminatus* HERBST (46 % of all captured individuals), *A. foetens* FABRICIUS (27 %) and *G. spiniger* MARSHAM (11 %). The only other tunnelers, *O. similis* SCRIBA (1 %) and *G. niger* MARSHAM (<0.2 %), were far less numerous. Full data is provided in Appendix 3A.



Geotrupes spiniger MARSHAM, a large beetle

Numbers of tunnelers were significantly higher for the dry site. The opposite was true for dwellers: higher numbers were found at the moist site. The total number of dung beetles did not differ between the two sites (Table 3-1; Fig. 3-1). Apparently, at the moist site the open cages had a repulsive effect on some dweller species (as opposed to the dry site), resulting in a significant interaction of these factors in the dweller, and concomitantly, the total beetle analysis. Also, when visiting horse dung, tunnelers seemed to prefer the open-caged samples, whereas visiting cow dung, cageless pats were preferred. Moreover, the three-way interaction proved significant.

Table 3-1. Numbers of cases (*N*), degrees of freedom (df), *F*-statistics (*F*) and *p*-values (*p*) of the General Linear Models testing for the effects of Cage type, Dung type and Site on the total number of individuals caught per dung sample

Source of variation	Dwellers + tunnelers				Dwellers			Tunnelers*		
	N_1, N_2	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*
Cage type	12, 12	1	1.45	0.247	1	2.22	0.156	1	0.31	0.586
Dung type	12, 12	1	2.28	0.151	1	1.77	0.202	1	2.78	0.115
Site	12, 12	1	2.11	0.166	1	7.08	0.017	1	19.79	<0.001
Cage type × Dung type		1	1.13	0.304	1	3.03	0.101	1	5.58	0.031
Cage type × Site		1	6.91	0.018	1	7.59	0.014	1	0.95	0.345
Dung type × Site		1	0.23	0.641	1	0.90	0.356	1	3.27	0.090
Cage type × Dung type × Site		1	13.01	0.002	1	12.80	0.003	1	4.95	0.041

* The horse dung sample on the dry site showing the highest tunneler number skewed the residual distribution to be non-normal (Shapiro-Wilk's test, $p < 0.001$). Nonetheless, the GLM is not altered when excluding this outlier (with regard to [non-]significant factors).

In the DC-analysis, only the first axis represented a substantial portion of the variance ($r_1^2 = 0.361$, gradient length = 3.241). The Wilcoxon two-sample test on the sample coordinates of this axis supported the significant segregation between sites ($Z = -2.859$, $p = 0.004$; Fig. 3-2). This supported the use of Site as a fixed factor in the analysis of the main experiment: the one site represented a high tunneler presence, the other a low tunneler presence. Moreover, species composition differed between cattle and horse dung ($Z = -2.051$, $p = 0.040$; Fig. 3-2), due to apparent preferences of some *Aphodius* species (e.g. *A. sphacelatus* Panzer was found almost exclusively in the former, *A. contaminatus* in the latter).

Densities of dung beetles did not differ between dung samples that were equipped with open cages and cageless samples (Table 3-1), nor did the species composition ($Z = 0.664$, $p = 0.507$; Fig. 3-2).

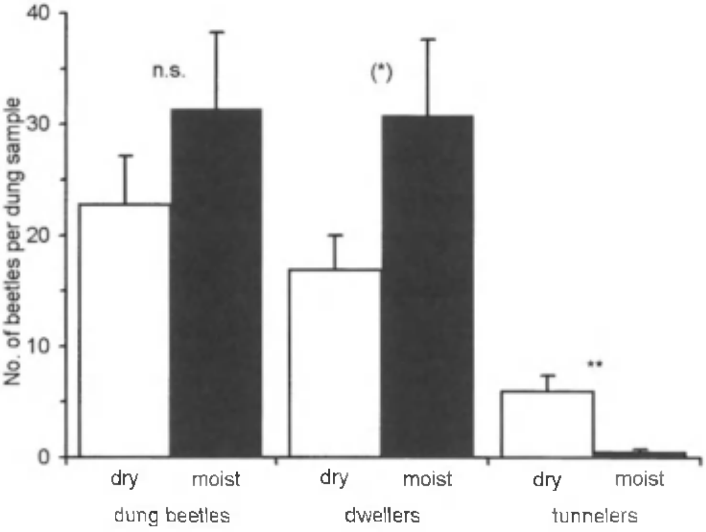


Figure 3-1. Mean number (+ s.e.) of dung beetle individuals caught in the pitfalls during the study period, separated in functional groups (dwellers plus tunnelers, and both separately). n.s.: non-significant. (*): 0.10 > p > 0.05. **: p < 0.01 (for differences between sites).

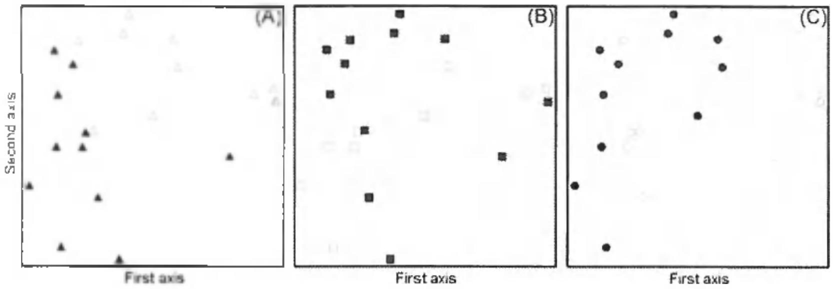


Figure 3-2. DC-analysis ordination diagram. In (A), an overlay is made for the sites. Species composition significantly differs between the dry (▲) and moist site (Δ). In (B), an overlay is made for the cages: no significant difference was found between dung samples without cages (■) and those with open cages (□). In (C), the overlay is for dung type: horse dung samples (●), and cattle dung samples (○). The coefficients of determination (r^2) equal 0.36 and 0.19 for the first and the second axis, respectively; the comparisons apply to the first axis only.

Germination controls

The sites themselves apparently influenced germination differently: within the closed cages on the dry site, it was lower compared to closed cages on the moist site ($F = 11.39$, df = 1, $p = 0.002$, full model on closed cages data). Presumably, this is due to differences in soil moisture availability.

To a certain extent, the used dung was contaminated with seeds[*]: on average, 16.33 ± 9.16 alien seedlings germinated from each horse dung sample ($n = 6$), of which 1.67 ± 1.97 individuals could be attributed to *Poa* spp. and 0.88 ± 0.98 to *Agrostis* spp. From these data, it was calculated that approximately 2.6 % of the *Agrostis* seedlings and 4.0 % of the *Poa* seedlings counted in samples of the main experiment actually concerned contaminations. From the cattle dung samples, 1.33 ± 1.21 alien seedlings emerged ($n = 6$), none of which were *Poa* or *Agrostis* species. Since the contamination degree was less than 5 % for horse dung, and 0 % for cattle, its effect was not taken into account in further analyses.

Seeds of *A. capillaris* and *P. pratensis* were sufficiently viable, as shown in the germination controls with seed deposited in sand–peat substrate in a greenhouse (*A.c.* 91.17 ± 2.86 %; *P.p.* 84.50 ± 6.53 %; $n = 6$ each) and in field conditions (*A.c.* 61.58 ± 7.28 %; *P.p.* 62.00 ± 8.21 %; $n = 12$ each).

CAGE TYPE ('open' or 'closed cage') had no effect on germination success in controlled greenhouse conditions with the sand–peat substratum ($F = 0.095$, df = 1, $p = 0.760$).

Main experiment

For *A. capillaris* and *P. pratensis* sown in the dung samples, germination varied from 2 to 66 seedlings per dung sample (from 100). There was also large variation in the degree to which dung beetles had processed the dung: some samples were strongly fragmented and exhibited many dung beetle traces, while others were left intact.

Showing no significant contributions, four- and three-way interactions were stepwise eliminated from the full models, resulting in the reduced models presented in Table 3-2. Using all samples in the analysis, successful germination turned out to be significantly higher under closed cages than under open cages (Table 3-2; Fig. 3-3). Relative to this, the difference

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[*] This could be contaminations from the feed, as well as from other sources (e.g. floor cover?).

between closed cages and the open-caged samples that showed tunneler traces increased, whereas the difference between closed cages and open samples that showed no traces decreased (Table 3-3; Fig. 3-3). Thus, animals visiting open cages clearly brought about a substantial decline in seedling establishment, and this is especially true for tunnelers. Also, in both approaches, successful germination turned out to be higher on the moister than on the drier site (Table 3-2; Fig. 3-3). Overall, *P. pratensis* germinated better than *A. capillaris*, and germination from horse dung was higher than from cattle dung (Table 3-2), which is likely due to differential nutrient requirements from the plant species, and dung textures (loose *vs* compact), respectively.

Table 3-2. Numbers of cases (*N*), degrees of freedom (df; numerator, denominator), *F*-statistics (*F*) and *p*-values (*p*) of the Mixed Models testing for the effects of Cage type, Dung type, Plant species and Site on the number of seeds germinating from dung

Source of variation	Including all open cages (analysis 1)				Splitting the 'open cage' level up in those with and those without tunneler traces (analysis 2)			
	N_1, N_2	df	*F*	*p*	N_1, N_2, N_3	df	*F*	*p*
Cage type	48, 48	1, 75	79.15	0.000	48, 31, 17	2, 71	48.49	0.000
Dung type	48, 48	1, 75	9.68	0.003	48, 48	1, 71	13.35	0.001
Plant species	48, 48	1, 75	46.18	0.000	48, 48	1, 71	32.85	0.000
Site	48, 48	1, 10	6.10	0.033	48, 48	1, 10	5.62	0.039
Cage type × Dung type		1, 75	11.45	0.001		2, 71	9.83	0.000
Cage type × Plant species		1, 75	1.13	0.291		2, 71	1.36	0.263
Cage type × Site		1, 75	6.72	0.011		2, 71	1.34	0.268
Dung type × Plant species		1, 75	3.34	0.072		1, 71	6.50	0.013
Dung type × Site		1, 75	1.51	0.223		1, 71	0.17	0.682
Plant species × Site		1, 75	1.31	0.255		1, 71	1.56	0.215
	AR(1): estimate = 0.9108, *Z* = 3.13, *p* = 0.002				AR(1): estimate = 0.9157, *Z* = 3.27, *p* = 0.001			

Table 3-3. Results of the multiple comparison procedure using Tukey–Kramer adjustment

	Closed cages (1)	Open cages	
		With tunneler traces (2)	Without tunneler traces (3)
(1)		<0.0001	0.0210
(2)	<0.0001		0.0008
(3)	0.0210	0.0008	

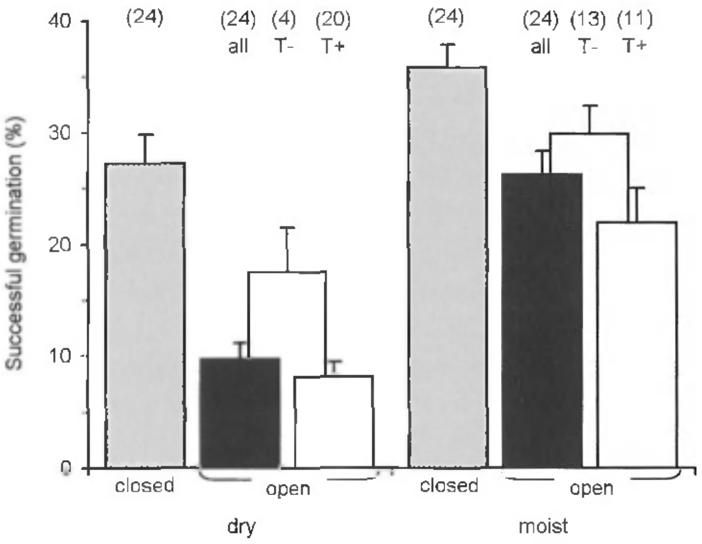


Figure 3-3. The mean number (+ s.e.) of seedlings counted in the dung samples as a function of Site and Cage type. Numbers are for *Agrostis capillaris* and *Poa pratensis* combined. Grey bars represent 'closed cages'. Whereas black bars represent all the 'open cages', white bars represent the 'open cages' without (T–) and with (T+) tunneler traces (thus, the combination of white bars result in the black). The numbers of samples are enclosed within brackets at the top.

Discussion

Our results show that dung-visiting fauna had a negative effect on short-term, *in situ* germination for the two small-seeded species in a temperate grassland situation, and this was especially true for tunnelers. Since cage type did not affect germination conditions, and the open cages allowed access of a dung beetle community representative of the study area (both in numbers and composition), this effect can be assumed representative of natural situations.

However, apart from considering this direct effect of seedling emergence, it should be borne in mind that dung beetles might contribute to the soil seed bank if seeds are retained in the brood burrow and remain dormant there. Indeed, seeds of *P. pratensis* are known to stay viable for more than 20 years, even at great burial depths (Goss 1924). Then germination could occur successfully later on in time, for instance, as a result of soil disturbances. If this applies to a substantial number of seeds, the interaction could nonetheless be positive. The outcome on the long term might thus determine whether the event under study here can indeed be considered "directed", since this requires sites to be "especially favourable for survival" (Wenny 2001). Seed banks could be such sites, although this is to some extent arguable, since favourable sites must be out there that do not enforce seeds to remain dormant. That is, a time cost is charged to the seeds in this second-phase dispersal (see General introduction).

On the short-term scale, our experimental results clearly contrast the positive outcome found by Andresen and Levey (2004), who conducted the only field study following dung beetle mediated dispersal up to the point of seedling establishment until now. Evidently, differences in the dung beetle community, predator guild and/or plant species involved led to deviant patterns.

First, one of the possible dung beetle effects listed by Andresen and Feer (2005) is the reduction of seed clumping in a dung pat, and hence of seedling competition. However, this implies that seed movement by dung beetles also includes a horizontal component, which only is obvious in telecoprid nidification. Since rollers are abundant in tropical regions (cf. Andresen and Levey 2004) but absent from typical assemblages of northern temperate regions (Hanski 1991), this presumed positive effect does not occur in the latter.

Second, dung processing may also affect the microclimate that seeds experience. In the case of tunnelers, buried seeds may, for instance, experience more humidity and less light relative to ground level. This may positively as well as negatively affect the probability of germination, depending on the particular requirements of each species (cf. Wicklow *et al.* 1984). Because of dweller activities, seeds may end up in a more favourable mixed dung–soil substrate (see Holter 1977). For *A. capillaris* and *P. pratensis*, microclimatic effects were not considered in detail here, although a comparison between germination successes in dung (closed cages) and in sand–peat substratum hints dung to inhibit germination for both species.

Third, seed burial decreases the risk of seed predation as compared to unburied seeds. Indeed, seed removal by rodents after endozoochory is known for large-seeded plant species of tropical forests (Vander Wall and Longland 2004) and, anecdotically, of temperate forests (e.g. Bermejo *et al.* 1998 [from bear faeces]; Page *et al.* 2001 [raccoon faeces]). In the case of ungulates ranging in temperate grassland systems, however, this as yet remains to be quantified. Although these animals mainly disperse dry, inconspicuous, usually small-sized fruits or seeds (Cosyns *et al.* 2005b, Malo and Suárez 1995b, Pakeman *et al.* 2002), suchlike seeds are known to suffer high post-dispersal predation rates, too (Hulme and Benkman 2002). For instance wood mice (*Apodemus sylvaticus*) and harvest mice (*Micromys minutus*) –both known to occur within the 'Westhoek' reserve (Smeers 2001)– are known to forage for grass seeds in Western-European grasslands (Edwards and Crawley 1999). These, as well as any seed or seedling predating group –e.g. insects, birds...– could be responsible for the losses witnessed under open cages. However, the experimental setup did not allow to quantify any of their contributions.

Last, Andresen and Feer (2005) state that "deep burial can also have a negative effect on seedling recruitment by preventing elongating seedlings from reaching the surface". To evaluate this, we must consider the behaviour of *G. spiniger* MARSHAM, by far the most abundant tunneler in this study. This species constructs brood burrows consisting of a vertical shaft leading to a few horizontal tunnel-like brood chambers, each of the latter being filled with dung to make a horizontal sausage-shaped brood mass (about 100–150 mm in length and ca. 28 mm in diameter [Ampe 2003, Klemperer 1979]) which provides food for the future larva. These brood chambers are found between 10 and 30 cm depth (as measured within the study area by Ampe

2003), depths at which immediate germination of the dung seed content is unlikely to occur (e.g. Grundy *et al.* 2003). The statement of tunnelers having a negative effect on germination is unequivocally shown by our results: in a comparison of both analyses, the difference between open and closed cages types increased when excluding the samples not visited by tunnelers (Fig. 3-3). Moreover, the between-site differences in tunneler numbers seemed to contribute to the between-site differences in germination: on the moist site, germination was less hampered by tunneler activity than on the dry site (significant interaction of SITE and CAGE TYPE for the analysis including all cages; Table 3-2, Fig. 3-3). Indeed, tunnelers (almost exclusively *G. spiniger*) were more numerous at the drier site than at the moister site, which is most likely due to hydrological conditions: ground water level comes within reach of the shaft depths during the developmental stage of *G. spiniger* (September–March; Kühne 1995) at the moist site, while it does not at the dry site. The contrasting pattern of dweller numbers can subsequently be attributed to the competitive hierarchy underlying the assemblage (see Hanski and Cambefort 1991b).

A glimpse of an open-caged sample, in which bare sand and a shaft opening reveal the presence of *Geotrupes*

In conclusion, the negative short-term effect of tunnelers witnessed in this study is most likely attributed to deep burial. Whether burial 'rescues' seeds from being predated (cf. tropical studies) by means of seed bank enrichment, or is deleterious, remains to be quantified. Also, within one region, contrasting small-scale patterns may occur. If *Geotrupes* species were absent, and small *Onthophagus* species were present, for instance, the overall effect on immediate establishment might as well be positive since the latter bury dung to depths that might prove favourable to seedlings. However, since the dominance of *Aphodius* species is complemented with one or a few deep-tunnelers in many habitats, e.g. *Typhoeus typhoeus* LINNÉ in heathlands (Brussaard 1985) and *Geotrupes stercorosus* SCRIBA in deciduous forests (Rembialkowska 1982), the effect of dung beetle activity described here, might prove to hold elsewhere.

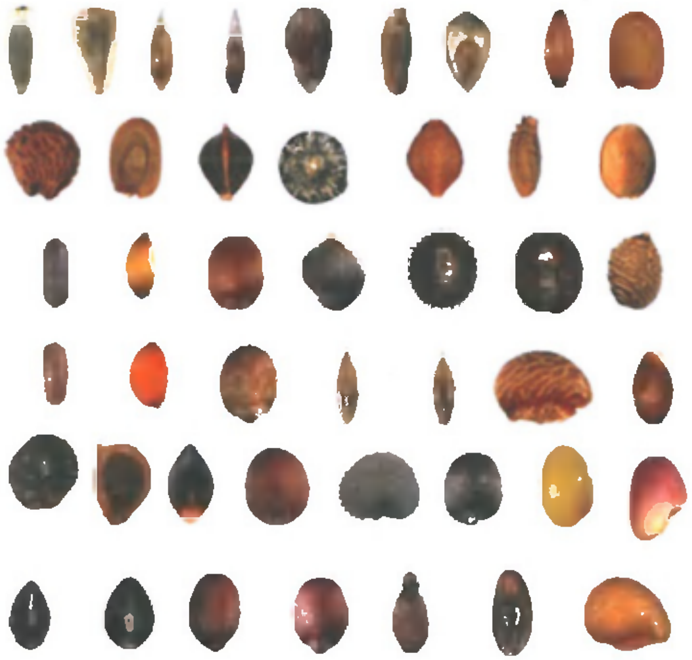
« trees sacrifice a fraction of
their seeds as a taxi fare in
exchange for the dispersal of
the remainder »

An Orchard Invisible: 83

CHAPTER 4

A reassessment of the role of simple seed traits in mortality following herbivore ingestion[*]

Bram D'hondt and Maurice Hoffmann



A variety of seeds, from species used in this experiment (photographs by Cappers *et al.* 2006)

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[*] Adapted from: D'hondt, B. and Hoffmann, M. (2011) A reassessment of the role of simple seed traits in mortality following herbivore ingestion. *Plant biology* 13 (Suppl. 1): 118–124.

Abstract

Grazing mammals are regarded as major vectors in seed dispersal of grassland plants through seed ingestion and subsequent excretion (endozoochory). The (evolutionary) ecology underlying this dispersal mode is relatively poorly understood as we have limited data, among others, on how seed attributes perform in this process (and could thus be selected for). Regarding mortality due to digestion, contrasting patterns found on the role of simple seed traits seem partly due to inadequate comparative-analytical methods. We conducted a feeding experiment in which controlled seed quantities from a large number of grassland herbs and grasses were fed to cattle (48 species in total). Seed mass, length and shape measurements were related to mortality rates using phylogenetically independent contrasts, which account for taxonomical interdependence. For a subset of the plant species, seed coat thickness was tested as well. The proportion of seeds surviving the digestive tract was generally low, though high in some. Several species even showed increased germination. Neither seed size, shape, nor seed coat thickness correlated significantly with mortality. Other structural traits of the seed coat are likely to overrule these simple seed traits, as illustrated by high survival rates of species that are characterized by water-impermeable seeds. As the latter trait holds some interesting properties with respect to intraspecific variation and genetics, it could open up perspectives for inference on any evolutionary consequences for this type of dispersal.

Keywords: seed dispersal, endozoochory, zoochory, ungulates, foliage is the fruit, phylogenetically independent contrasts, hardseededness, physical dormancy

Introduction

Plant propagules serve to ensure the continuation of populations through time and space. In nature, seeds –broadly defined as any propagules of generative origin (Cousens *et al.* 2008)– disperse via a vast range of mechanisms, involving both abiotic and biotic vectors. Among the latter, herbivorous mammals are regarded as a major group of dispersers. Indeed, numerous grazers from (semi-)natural systems are known to successfully, and more or less continuously, disperse seeds through either attachment to fur and hooves (epizoochory), or through abundant ingestion of seeds with the foliage they feed on (endozoochory; Couvreur *et al.* 2005). Observations of endozoochory go back a relatively long time (e.g. Kerner von Marilaun 1902, others in Ridley 1930), and now from temperate grasslands alone hundreds of plant species are known to show viable seeds in herbivore dung (e.g. Malo and Suárez 1995b, Pakeman *et al.* 2002, Myers *et al.* 2004, Cosyns *et al.* 2005b; Mouissie *et al.* 2005a).

Through dispersal, vectors hold the potential to select for particular seed traits and attributes (*sensu* Violle *et al.* 2007). For dispersal by e.g. birds, wind or ants, principal evolutionary-ecological underpinnings are relatively well-established, reflecting a thorough understanding of plant-vector interactions (e.g. Vander Wall 2001, Cheptou *et al.* 2008, Manzaneda *et al.* 2009). Drawing from observations of dung-borne seeds and from diverse theoretical considerations, Janzen (1984) was the first to suggest herbivorous mammals (grazers) as a selective force on seed attributes, too. Although his ultimate views of a widespread endozoochorous syndrome were soon countered by a lack of empirical support (Collins and Uno 1985), the idea that grazers can at least in some cases significantly exert selection, seemed launched and accepted (e.g. Quinn *et al.* 1994, Malo and Suárez 1995b, Cousens *et al.* 2008). This might be premature, however, as our understanding of the seed-grazer interaction, a.o. the traits that function in the process and hence might be selected for, is still relatively poor.

Of the several stages involved in endozoochory (Wang and Smith 2002), seed mortality due to digestion is recognized as a severe bottleneck. Former feeding trials in which mortality rates were related to seed characteristics yielded contrasting results. Smaller-seeded species tended to show lower mortality rates than larger-seeded species in the feeding trial of Mouissie *et al.* (2005b), whereas the opposite was found by both Peco *et al.* (2006) and

Cosyns *et al.* (2005a). Bonn (2004) found no correlation with seed mass in both of her feeding experiments. For seed shape, a negative (Bonn 2004, Mouissie *et al.* 2005b), neutral (Bonn 2004, Peco *et al.* 2006), and positive effect (Cosyns *et al.* 2005a) were found.

Since seed size and weight are considered very important plant traits also for plant fecundity, recruitment and (other means of) dispersal (Harper 1970, Eriksson and Jakobsson 1999), a resolution of these inconsistencies is desirable. At least a part of these inconsistencies might come from a statistical problem suffered by these analyses, obscuring interpretation and comparison. Basically, seed attributes are considered across species; its results can be extrapolated beyond the studied species only when they properly represent the composition of the targeted species pool. When one wants to interpret the relationship in a more causal sense, however, caution is called for (Silvertown and Dodd 1996). Because species are related to each other through common descent, they cannot be regarded as independent data points, an essential for relevant statistical analysis (Garland *et al.* 2005). 'Comparative methods', evaluating functional relationships from species comparisons (Harvey and Pagel 1995), accordingly made progress in recent decades by constructing several methods that control for interspecific affinities. Of these, the method of phylogenetically independent contrasts (PIC) was the first to be elaborated and to be widely applied (see Garland *et al.* 2005 for a review).

There is, moreover, still a scarcity of basic empirical data on plant species' gut passage data. In their review, Will and Tackenberg (2008) recently stressed and advocated that continued contributions from feeding experiments would be of great value.

We performed an experiment determining mortality rates of seeds fed to cattle for 48 plant species, the highest number included in any *in vivo* assay so far (cf. Appendix 1C). Specifically, we aim (i) to contribute to the limited data of plant species performances in endozoochory, (ii) to reassess the role of simple seed traits using proper comparative-analytical tools, and (iii) to ultimately identify a system (i.e. a taxon and trait) that holds the potential to infer on endozoochory-imposed seed evolution. The simple seed traits under study primarily relate to the size and shape of seeds. Tentatively, we also investigated the thickness of the seed coat for a subset of species.

Methods

We assessed mortality rates by feeding known numbers of seeds to farm cattle. Note that, when referring to our numbers, we express them not as the percentage of seeds dying, i.e. mortality rate, but as the percentage of seeds surviving digestion ('survival rate'). This is because germination of ingested seeds is corrected for germination under normal conditions (without ingestion), yielding a number that can exceed 100 % (when gut passage increases germination).

Species selection and seed attributes

Although they inherently do not represent wide ranges of seed attributes (see Discussion), the selection was restricted to herbs and graminoids from open habitats (mostly grasslands) for several reasons. First, these species can be considered the ones for which mortality rates are the most relevant, as they are relatively prone to consumption by grazers on a regular basis. Second, we are concerned about the effects of small –rather than large– changes in seed attributes on mortality, given our objective to identify a trait or traits with which the selective strength of endozoochory could be assessed. For such a trait, its relationship with mortality should also hold over small scales.

We obtained seeds of 48 plant species from commercial sources (Table 4-1). Provided their availability, priority was given to species that have been found as viable seeds in herbivore dung from (semi)natural habitats (Cosyns 2004; also see Appendix 1A). As defined above, in the text "seeds" refer to functionally analogous structures (true seeds, fruits or mericarps) rather than morphologically homologous structures (Table 4-1).

Single-seed mass was assessed by weighing three samples of a known number of air-dried seeds (500 or 1000 seeds, 0.01 mg precision). Before measuring dimensions, we conducted manipulations in some species (Table 4-1): open and loose structures, such as glumes in grasses, perigynia in *Carex* and pericarps in *Geranium*, were removed. The assumption is that these readily disintegrating structures are unlikely to affect survival, while they would greatly bias trait measurements, and thus, correlations. Dimensions (length, width and height) were measured on 10 randomly selected seeds per species using stereoscopic binoculars (25 µm precision). Seed length is defined as the longest of the three dimensions. Seed shape is expressed according to Thompson *et al.* (1993), as the variance in seed dimensions after dividing each dimension by length. We multiplied this measure by 100 for practicability.

Table 4-1. Species included in the experiment, propagule type (prop.), and their measured dimensions (mean and standard error; n = 3 for mass, n = 10 for shape and length). An indication is made of those species that are included in Cosyns' (2004) overview of species recorded from herbivore dung ('+'). Nomenclature based on Lambinon *et al.* (1998).

species name	prop.	mass (mg)	length (µm)	shape	+
Achillea millefolium L.	F	0.122 (0.001)	1590 (57)	12.60 (0.25)	+
Achillea ptarmica L.	F	0.233 (0.006)	1680 (53)	9.96 (0.22)	+
Agrostis capillaris L.	F*	0.069 (0.003)	905 (24)	8.65 (0.31)	+
Agrostis stolonifera L.	F*	0.122 (0.001)	1068 (25)	8.74 (0.19)	+
Artemisia campestris L.	F	0.090 (0.004)	1028 (41)	8.25 (0.38)	
Artemisia vulgaris L.	F	0.136 (0.001)	1415 (44)	10.92 (0.18)	+
Briza media L.	F*	0.885 (0.007)	1498 (43)	6.58 (0.23)	+
Briza minor L.	F*	0.838 (0.024)	1510 (31)	5.35 (0.35)	
Campanula rotundifolia L.	S	0.079 (<.001)	848 (20)	5.74 (0.27)	+
Cardamine hirsuta L.	S	0.116 (<.001)	958 (24)	9.09 (0.62)	+
Cardamine pratensis L.	S	0.419 (0.004)	1483 (53)	7.00 (0.49)	+
Carex flacca Schreb.	F*	0.805 (0.013)	1540 (37)	2.35 (0.16)	+
Carex vulpina L.	F*	1.495 (0.006)	2070 (43)	7.89 (0.23)	
Cerastium dubium (Bast.) Guépin	S	0.095 (<.001)	660 (18)	2.52 (0.24)	
Cerastium fontanum Baumg.	S	0.123 (0.001)	745 (14)	3.53 (0.30)	+
Chenopodium album L.	$F^{1,*}$	0.616 (0.003)	1200 (33)	4.27 (0.22)	+
Chenopodium bonus-henricus L.	$F^{1,*}$	1.876 (0.014)	1670 (43)	2.63 (0.15)	+
Galium aparine L.	Mc	4.705 (0.011)	2333 (53)	1.72 (0.17)	+
Galium verum L.	Mc	0.349 (0.002)	1040 (40)	2.21 (0.25)	+
Geranium molle L.	S*	1.265 (0.033)	1485 (32)	2.38 (0.21)	+
Geranium robertianum L.	S*	1.701 (0.008)	1908 (28)	4.74 (0.19)	+
Helianthemum nummularium (L.) Mill.	S	0.961 (0.011)	1573 (39)	4.14 (0.43)	+
Hypericum perforatum L.	S	0.131 (<.001)	1160 (15)	7.83 (0.18)	+
Hypericum tetrapterum Fries	S	0.037 (<.001)	750 (27)	7.46 (0.38)	+
Juncus bufonius L.	S	0.022 (0.001)	413 (9)	2.17 (0.14)	+
Juncus effusus L.	S	0.017 (0.001)	463 (24)	5.88 (0.45)	+
Mentha aquatica L.	Mc	0.141 (0.003)	890 (12)	2.49 (0.15)	+
Mentha pulegium L.	Mc	0.089 (<.001)	675 (19)	2.85 (0.19)	+
Myosotis arvensis (L.) Hill	Mc	0.296 (0.001)	1405 (28)	5.23 (0.13)	+
Myosotis scorpioides L.	Mc	0.437 (0.001)	1603 (69)	4.78 (0.33)	+
Origanum majorana L.	Mc	0.236 (0.001)	1013 (18)	3.61 (0.18)	
Origanum vulgare L.	Mc	0.077 (<.001)	765 (19)	4.28 (0.23)	
Pimpinella saxifraga L.	Mc	0.396 (0.003)	1480 (58)	6.53 (0.31)	

Table 4-1, *continued*.

Plantago lanceolata L.	S	2.094 (0.027)	2780 (66)	9.24 (0.23)	+
Poa annua L.	F*	0.382 (0.008)	1383 (31)	6.34 (0.37)	+
Poa pratensis L.	F*	0.320 (0.006)	1538 (52)	9.43 (0.27)	+
Potentilla argentea L.	F	0.135 (0.002)	1040 (10)	4.32 (0.19)	
Potentilla tabernaemontani Aschers.	F	0.581 (0.007)	1568 (30)	4.63 (0.09)	+
Primula vulgaris Huds.	S	0.975 (0.002)	1483 (62)	2.88 (0.39)	
Ranunculus bulbosus L.	F	3.146 (0.039)	2995 (63)	8.22 (0.25)	+
Rumex acetosa L.	F	0.883 (0.017)	1988 (41)	4.43 (0.31)	+
Rumex obtusifolius L.	F	1.595 (0.015)	2108 (68)	3.83 (0.25)	+
Silene vulgaris (Moench) Garcke	S	0.949 (0.021)	1380 (42)	2.56 (0.25)	
Thymus pulegioides L.	Mc	0.131 (0.003)	693 (24)	1.62 (0.17)	+
Thymus serpyllum L.	Mc	0.122 (0.001)	713 (12)	1.52 (0.17)	
Trifolium campestre Schreb.	S	0.668 (0.010)	1125 (45)	4.91 (0.46)	+
Trifolium pratense L.	S	1.385 (0.009)	1730 (60)	3.75 (0.26)	+
Viola arvensis Murray	S	0.684 (0.006)	1718 (24)	5.35 (0.19)	+
all		0.688 (0.057)	1355 (26)	5.36 (0.13)	

S, seed; F, fruit (*without exocarp); Mc, mericarp. Propagules marked with an asterisk were manipulated before measuring length and shape (see text for details).

Seed coat thickness

For 21 of the species, listed in Appendix 4A, we tentatively measured the thickness of the 'seed' coat through microscopic sections (De Laender 2008).

A single seed of each species was cut longitudinally with a scalpel. One half of the seed was then fixed in FAA solution, embedded in Technovit 7100 and stained with toluidine blue O (protocol according to Leroux *et al.* 2007). The sections were photographed under a light microscope, and these images were subsequently used for seed coat thickness measurements using ImageJ software. Per section, min. 15 to max. 36 measurements of the seed coat were taken, distributed over the entire seed section circumference.

A microscopic section through a seed of *Campanula rotundifolia*

Survival rate

Seed-to-feed quantities were measured using seed mass; for each species these quantities comprised about 5000 seeds per individual animal (due to limited supplies 3400 or 3000 for some). In a pack of 14 cows, tied individually in a stable, each species was randomly attributed to seven animals, with the sole restriction of keeping congeneric plant species separate in order to facilitate later seedling identification. As such, an individual animal was fed between 21 and 27 species.

The animals were adult (three to four years old) cows of the Belgian Blue breed, weighing about 550-600 kg. Their standard diet mainly consisted of maize silage, pressed beet pulp, and hay; being a potential source of seed contamination, the latter was excluded about seven days before the experiment. One hour before feeding, dung samples were collected in order to (among others, see below) control for contamination.

The seeds were mixed through the silage and applied to all animals simultaneously during morning feeding. All dung was collected 6, 11, 24, 35, 48, 72, 96, 120 and 144 hours thereafter. Per collection the excrements were weighed and mixed for each individual animal. From these, samples were taken and weighed (± 475 g). These samples were stratified for 34 days at ± 4.5 °C, then mixed through a shallow layer of potting soil-sand mixture (1:1) in trays (45 x 40 x 10 cm), and left to germinate in a greenhouse for 8 months under a natural light regime (April-December 2007). Samples were watered manually.

As a control, we added seeds to dung samples that were collected one hour before feeding (non-ingested control; 100 seeds per species, 9-14 spp. per sample, again with congeneres kept separate, five samples per species). Apart from this, these samples experienced exactly the same treatments as the ones containing ingested seeds.

Per plant species and per individual animal, the survival rate was estimated as follows (Cosyns *et al.* 2005a),

$$\sum_{i=\mathrm{int}1}^{i=\mathrm{int}9} n_i \times (W_{Di}/W_{di}) \Big/ V \times (W_{sb}/W_s) \quad \text{(Eq. 1)}$$

where W_{Di} is the weight of the dung produced by the animal in the time interval passed. W_{di} is the weight of the dung sampled from it (± 475 g). The number of seedlings found germinating from this sample is denoted n_i. After doing the summation over all nine time intervals, the numerator yields an estimation of the number of viable seeds excreted by the animal. As W_s is the weight of a single seed (assessed beforehand) and W_{sb} denotes the weight of the seed batch fed to the animal, W_{sb}/W_s equals the number of seeds fed to the animal (mostly ± 5000). V is the mean proportion of seedlings germinating from the (five) control samples containing non-ingested seeds. Its insertion controls for the combined effect of intrinsic viability of the seeds and any possible effects of the dung environment on germination.

Data analysis

As noted in the Introduction, multi-species analyses concerned with functional relationships should control for species interdependence. In the method of phylogenetic contrasts, this is done on the basis of a (phylogenetic) tree. Given the tree, i.e. given the ways in which species are related to each other, the method circumvents taxonomical bias by comparing ("contrasting") taxa in a pairwise manner both at the tips of the tree and at internal nodes. The contrasts themselves are independent from each other.

For the phylogeny of our species (Fig. 4-1a), we used the widely accepted APGII system (Stevens 2001 onwards) for both the assignment of genera to families and the topology of families. For two families additional sources were needed. We used the consensus trees published in Walker *et al.* (2004) and Quintanar *et al.* (2007) for the Lamiaceae and Poaceae, respectively. All branch lengths were arbitrarily set to 1.0 (unit).

Contrasts are calculated as elaborated in Felsenstein's (1985) seminal paper. On these contrasts, linear regression is performed following the procedure set out by Garland *et al.* (1992), with intercept being zero. The unit branch lengths satisfy the conditions set by these authors. The three polytomies are considered soft, and the number of degrees of freedom in significance testing are lowered accordingly (42 d.f.; Purvis and Garland 1993). The analysis was executed using the PDAP module (Midford *et al.* 2005) implemented in Mesquite software (Maddison and Maddison 2009).

As contrasts are calculated on specific means, information is lost on intraspecific variation in seed survival. For each node of the tree, we tested for differences between both sides of the branching using standard permutation tests at the 0.10-level (2000 randomisations). These provide us with an idea of those contrasts that are most informative.

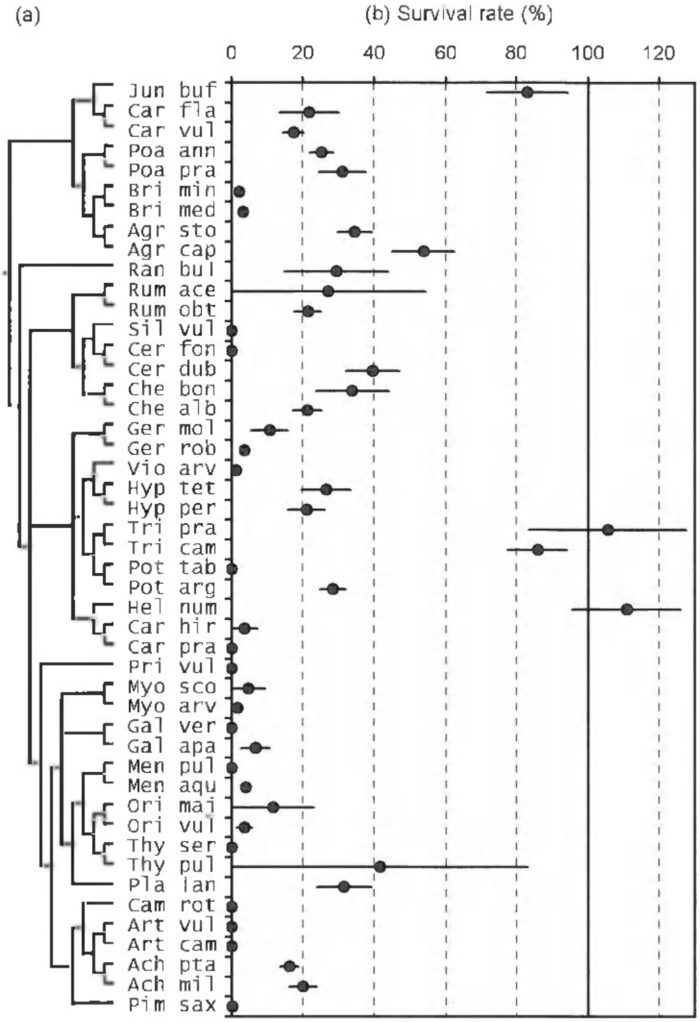

Figure 4-1. (a) Working phylogeny of the species used in the experiment. Branches are not drawn to scale. (b) Mean (± s.e.) proportion of seeds that survived ingestion by cattle (survival rate from Eq. 1). Species names are abbreviated (see Table 4-1). *Juncus effusus* is omitted; see text for details.

Results

From the study species, a total of 3507 seedlings emerged from the dung samples with ingested seeds (126 samples), and 3948 from the dung samples with non-ingested seeds (20 samples). Neither the sand-potting soil substrate, nor the dung samples themselves contained any relevant seed contamination; some did occur from the external environment, but all belonged to non-included taxa (e.g. *Oxalis*, *Epilobium*, *Betula*).

A snapshot of a sample from the greenhouse, collected 35 hours after feeding

More than 90 % of the seeds was excreted within 48 hours after feeding and only a negligible fraction was found from the last time interval sampled (Fig. 4-2). As retention parameters are considered pivotal in spatially explicit approaches of animal seed dispersal (e.g. Vellend *et al.* 2003, Will and Tackenberg 2008), results of a lognormal fit to these data are included in Fig. 4-2 (Rawsthorne *et al.* 2009).

Specific retention curves are shown in Appendix 4B. No species-specific temporal patterns were observed: nearly all of the species showed their maximal excretion from the 24- or 35-hour collection. The few exceptions had very few emerging seedlings, except for *Carex vulpina*, which showed a clear maximum from the 48-hour collection.

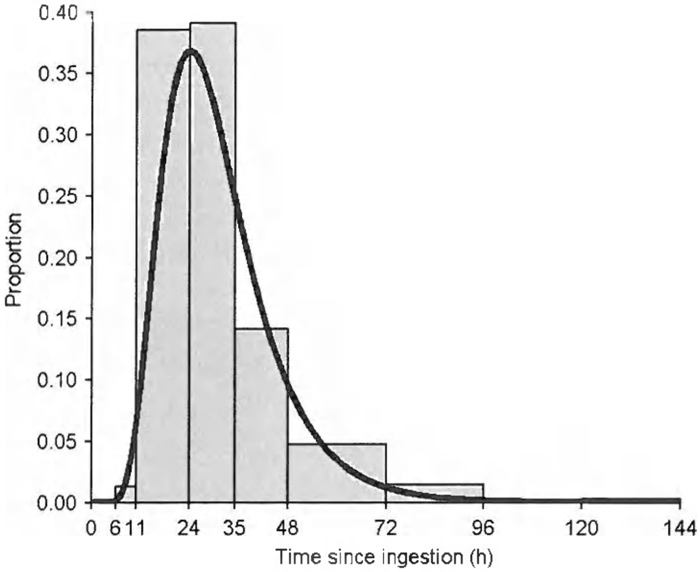

Figure 4-2. Excretion by cattle of seeds over time as shown from the germination trial. Bars represent the experimentally assessed proportion of seeds excreted. All species were pooled. A lognormal curve was fitted to the data (cf. Rawsthorne *et al.* 2009), the parameters are $\mu = 3.35$ and $\sigma = 0.42$.

Specific survival rates are shown in Fig. 4-1b. The exact numbers can be found in Appendix 4C, together with the germination rates from the non-ingested control. The species showing the highest proportions of seeds surviving ingestion were *Helianthemum nummularium*, *Trifolium pratense*, *T. campestre*, and *Juncus bufonius*. For the first two species mentioned, digestion even appeared to stimulate germination as survival rates exceeded 100 %. [*Juncus effusus* did not germinate from the control, while it did germinate from the ingested seeds with very low incidence. This would suggest a positive effect of ingestion on germination, but as this cannot be formalized mathematically (a division by zero; Eq. 1), it is omitted from further analysis.]

From the 19 congeneric species pairs, *Agrostis*, *Cardamine*, *Cerastium*, *Galium*, *Mentha*, *Myosotis*, *Potentilla* and *Thymus* yielded significant within-genus differences (permutation tests, 0.10-level). Except for *Agrostis* and *Myosotis*, in each pair one species showed zeros only.

Results of the seed mass, length, and shape measurements are included in Table 4-1. Results of the measurements of seed coat thickness are provided in Appendix 4A; they are discussed in more detail by De Laender (2008).

When our data is subjected to a correlation analysis across species without phylogenetic control, non-significant relationships emerge: survival-log(mass): Pearson's ρ = 0.045, P = 0.76; survival-length: ρ = 0.017, P = 0.91; survival-shape: ρ = -0.045, P = 0.76; survival-seed coat thickness: ρ = -0.023, P = 0.92.

With phylogenetic control, there also appeared no significant impact of each of the three studied dimensional seed traits (mass, length and shape) on seed survival rates of the species (Fig. 4-3). Within Fig. 4-3a, a point above the horizontal axis represents a contrast in which the heavier-seeded taxon of the pair showed the higher survival, whereas a point below the horizontal axis represents a contrast in which the lighter-seeded taxon performed better. Overall, the points counterbalance both in number and magnitude, and no overall effect is observed. The same reasoning applies to Fig. 4-3b and 4-3c. Also for seed coat thickness, no significant impact was found (Fig. 4-4).

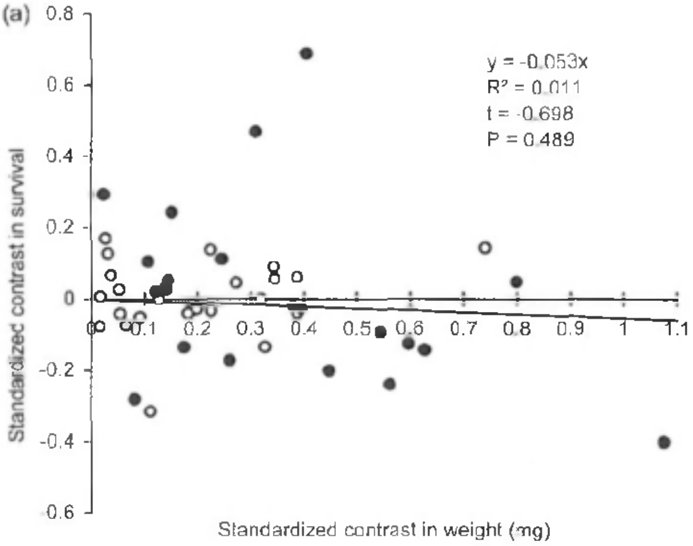

Figure 4-3. Bivariate scatter plot of standardized independent contrasts in seed survival rate and (a) log-transformed seed weight. *(Continued on next page.)*

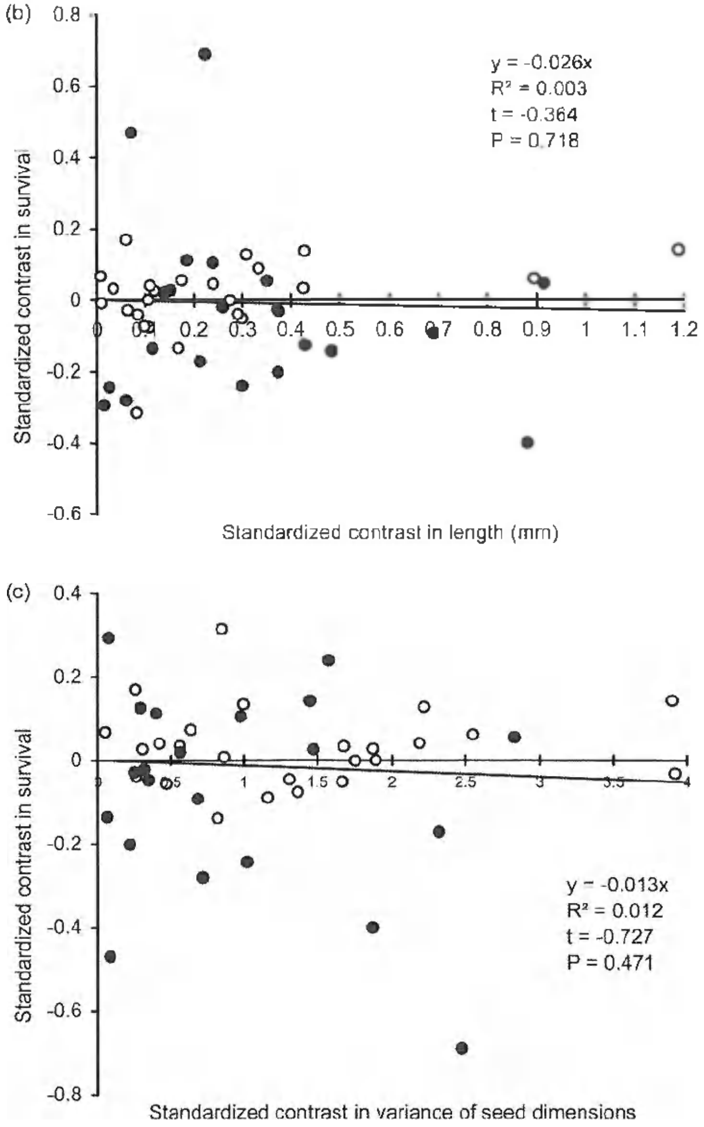

Figure 4-3, *continued*. Bivariate scatter plot of standardized independent contrasts in seed survival rate and (b) seed length and (c) seed shape. Statistics are from least squares regressions (through origin, see Garland *et al.* 1992; number of contrasts = 46, degrees of freedom = 42). Contrasts that proved significant in the permutation tests are given as filled dots; those that did not as open dots.

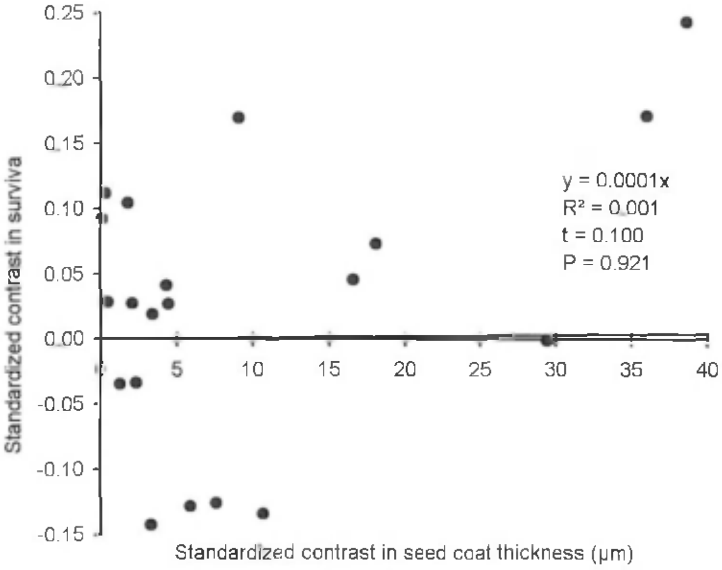

Figure 4-4. Bivariate scatter plot of standardised independent contrasts in seed survival rate and seed coat thickness. Statistics are from least squares regressions (through origin, see Garland *et al.* 1992; number of contrasts = 20, degrees of freedom = 16).

Discussion

The proportion of seeds surviving ingestion differed profoundly between species: most species show high mortality rates (up to 100 % of the seeds ingested) and a decreasing number of species show lower mortality rates (down to 0 %). Overall, this corresponds to the patterns found by others (references below). Note that the increased germination rates observed in *Helianthemum nummularium* and *Trifolium pratense* should not be mistaken with mortality rates, which are essentially zero.

Several large-scale feeding experiments have already been performed (e.g. Gardener *et al.* 1993a, Cosyns *et al.* 2005a, Bonn 2004, Peco *et al.* 2006). Their analyses consisted of looking for correlations across species. This is ecologically meaningful, since these cross-species analyses may learn us whether, for instance, the smaller-seeded species from a species pool are more likely to survive ingestion than others (given a negative correlation was found; e.g. as in the feeding experiment of Mouissie *et al.* 2005b).

However, this lower chance may well be due to the smaller-seeded species actually being grasses and these happen to disperse better –for some reason unknown. It is in these matters that phylogenetically controlled analyses perform better[*]. When one applies a contrasting perspective to the data of Mouissie *et al.* (2005b), for instance (e.g. by considering the pattern only within the Asteraceae or the graminoids), the data clearly suggests no effect. This is supported by subjecting their data to PIC analysis. So, taxonomical interdependence can clearly affect analyses of relationships among the involved traits significantly. When feeding experiments strongly differ in taxonomical coverage, this could thus lead to different conclusions.

Through a different approach, i.e. through a correlation of seed abundance in the vegetation and in dung, Bruun and Poschlod (2006) also concluded neither seed size nor shape to affect survival.

A reflection could be made on the attribute ranges considered here. In nature, seed size varies enormously, over 10 orders of magnitude (± 10^{-3}-10^{7} mg; Harper *et al.* 1970), with species from fields, pastures, forest clearings, and other habitats that are inviting to grazers comprising a subset hereof (± 10^{-2}-10^{1} mg; Salisbury 1942; Lord *et al.* 1995). From these wide ranges, it is up to the experimenter to choose which range is considered most relevant, with any claim of an "effect of size on mortality" restricted to that range. The studies mentioned above all stay in the lower range (± 10^{-2}-10^{2} mg). We cannot rule out size to have an effect beyond this range (10^{3} mg up to the maximal size animals are able to ingest) as wide-scale studies herein are lacking. We stuck to the lower range, too, on grounds stated in the introduction, although frequent ingestion of large (dry) seeds by grazers might be of relevance in the (sub)tropics (e.g. Janzen 1982b).

A second reflection, note that we have selected a modern breed of cattle in our feeding experiment. These individuals are normally fed by sileage and are adapted to high protein food. This differs from other breeds, including those commonly applied in nature management (or ancient breeds, see Poschlod *et al.* 2005), which are used to fibrous hay instead.

If neither size nor shape show clear relationships with species' performances, which traits could do so?

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[*] As Garland *et al.* (2005) rightly notice, however, even phylogenetic comparative methods are inherently correlational and cannot demonstrate causality of relationships alone. Only experiments can do this.

In this study, we have tentatively measured and included seed coat thickness in our tests, but this variable proved insignificant. This accords with similar attempts of Bonn (2004). It must be borne in mind, however, that the thickness of seed coats is not an easily measurable trait. Seed coats possess numerous textural and structural complexities (including local thickenings), and on the anatomical level, multiple cell layers are involved (for instance, near the micropyle, four epidermal layers are found instead of two; Werker 1997). Its use as a trait might therefore be too simplified.

Reconsidering the two genera performing best (Fig. 4-1b), they have one striking seed attribute in common: their seeds are known to be physically dormant (a feature found in only sixteen families; Baskin *et al.* 2000, Baskin and Baskin 2001, Baskin *et al.* 2006). In essence, this means that seeds are water-impermeable when shed. Its favourable effect is confirmed in several studies on dispersal of Fabaceae and Cistaceae by ungulates. Suckling (1952) fed both impermeable and permeable *Trifolium repens* seeds to sheep, but could only retrieve seed(ling)s from the impermeable seed lot. Fukuda *et al.* (1998) placed seeds from the same species in the rumen of fistulated cattle and followed their fates: mortality of water-impermeable seeds was very low during the 14 days of study, whereas permeable seeds were generally digested within 24 hours. Also, Gardener and colleagues found a near-to-perfect correlation between the fractions of seeds surviving a stay in the rumen and the initial impermeable seed content both within and among legume species (Gardener *et al.* 1993a, 1993b; also see Janzen 1981). Ramos *et al.* (2006) found seeds from several Cistaceae to pass the alimentary tract of sheep intact (also see Malo and Suárez 1998). The observation of increased germination rates in these species is then linked to a well-timed loss of dormancy (Baskin and Baskin 2001). If one considers this trait, the benefits of having a water-impermeable barrier are clear since it presumably are the digestive fluids –containing proteolytic enzymes and bacteria– that pose the severest threat to seeds.

However, water-impermeability alone cannot account for high survival following ingestion. Indeed, *Geranium* spp. performed less well in the experiment, despite them being physically dormant, too (Baskin *et al.* 2000). Then again, their seeds are considerably more fragile than those of *Trifolium* and *Helianthemum* (as can be tested by rupturing seeds with a forceps; Rodgerson 1998). Seeds are also subject to mechanical stress within the animal (e.g. exposure in chewing), and seed toughness can therefore be expected to play an important role as well. Mortality rates are thus very likely to be the result of several traits acting together, which advocates a multivariate approach in analyses that aim at a full comprehension of the process.

Accordingly, it is probable that other physical, structural, anatomical and/or textural traits pertaining to the seed surface or seed coat are likely to overrule simple traits such as seed size, shape or even coat thickness in explaining mortality rate. We lack knowledge on the effect of suchlike attributes for dry-fruited herbs until this date, whereas for fleshy-fruited shrubs and trees they are more often dealt with (e.g. in ornithochory; Traveset 1998, Traveset *et al.* 2008). So far, we have no indications on what might have caused the significant intrageneric contrasts observed in *Agrostis, Cardamine*, *Cerastium*, *etc.*

When excluding the physically dormant species from our analyses, seed mass related negatively to survival rate (P = 0.019). This is, however, due to the extreme values in *Juncus bufonius;* further exclusion of this species renders the relationship back to non-significant. Interestingly, Peco *et al.* (2006) found this species to have "highly water-impermeable seeds", thus making it functionally similar to the physically dormant species.

As outlined in the Introduction, an ultimate objective would be to identify a seed trait that holds potential to infer on seed evolution through endozoochory. For this purpose neither size (weight or length) nor shape and seed coat thickness seem suitable since no univocal effects are observed, at least not on a small scale. Water-impermeability might prove a better candidate, since it satisfies three pivotal conditions: it closely relates to survival following ingestion in several taxa (accounts mentioned above), it is known to hold intraspecific variation (Baskin and Baskin 2001), and it has at least some of its parameters genetically based (e.g. Nair *et al.* 2004, Boersma *et al.* 2007). If grazers can induce seed evolution, this might be the trait to focus on in future research.

«That's why the seed banks
are full of grounded
offspring, wrestling but
unable to escape their
maternal bonds till the hour
and the clime she has
chosen.»

An Orchard Invisible: 117

CHAPTER 5

The incidence, field performance and heritability of non-dormant seeds in white clover (*Trifolium repens* L.)*

Bram D'hondt, Rein Brys and Maurice Hoffmann

A white clover inflorescence, marked for follow-up

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* Adapted from: D'hondt, B., Brys, R. and Hoffmann, M. (2010) The incidence, field performance and heritability of non-dormant seeds in white clover (*Trifolium repens* L.). *Seed Science Research* 20: 169-177.

Abstract

Mature seeds of many legume species are normally characterized by water-impermeable seed coats, a form of physical dormancy. However, observations suggest that the incidence of mature but permeable (non-dormant) seeds is sometimes substantial. Yet, the ecological processes associated with this variation have received little attention by plant ecologists. Nonetheless, it could be of relevance also to dispersal since this seed trait is known to affect performance in ungulate endozoochory.

In white clover (*Trifolium repens*), we therefore studied: (1) the occurrence of initially permeable seeds in wild populations; (2) the relative performance of non-dormant and dormant seeds in plant establishment and reproduction in a field-sown experiment; and (3) the extent to which the trait is affected by air humidity and plant genotype in a greenhouse experiment.

No less than 35 % of all viable seeds from the wild populations proved to be water permeable at maturity. The proportion of permeable seeds within inflorescences ranged from 0 to 100 %. In the field-sown experiment, autumn-germinated non-dormant seeds had almost equally good chances of establishing as spring-germinated dormant seeds. Due to a marked head start in growth, the former yielded more flowers (and thus seeds) in the first flowering season. However, the greenhouse experiment proved that variation in the proportion of permeable seed between inflorescences represented a plastic response to humidity conditions during seed ripening, rather than variation among clones (broad-sense heritability ≤ 0.025). Thus the trait is not easily subject to selection.

Keywords: clonal repeatability, Fabaceae, genotype by environment, germination, hardseededness, softseededness, water impermeability

Introduction

Several mechanisms have been identified by which plant seeds are dormant when shed (Baskin and Baskin 2004). In physical dormancy, seeds are dormant due to impermeability of the seed or fruit coat to water, acquiring germinability only when this barrier is broken (Rolston 1978). Sixteen families are known to have species with physical dormancy (Baskin *et al.* 2000, 2006), of which the legume family (Fabaceae) is the largest and most important. Seed impermeability in legumes is also often termed 'hard-seededness', impermeable seeds being referred to as 'hard seeds' (Taylor 2005).

Baskin and Baskin (2001) outlined several theoretical scenarios in which seed dormancy is an adaptive feature. However, these scenarios are not mutually exclusive. In one of these, dormancy serves to prevent germination when conditions are unfavourable for seedling establishment, and it is this scenario that is considered to pertain to physical dormancy the most. Physically dormant species usually have defined germination periods in well-suited seasons, such as at the start of the warm season in typically temperate conditions (Roberts and Boddrell 1985, Van Assche *et al.* 2003), or after the hot summer in Mediterranean climates (Norman *et al.* 1998, 2005). Physical dormancy is indeed important relative to other dormancy types in vegetation that is subject to distinctly dry and wet periods, which are unfavourable and favourable for establishment, respectively. In a second scenario, the benefit of dormancy is in the timing of germination, so that fitness of the resulting plant is maximized. Indeed, several studies on various species have shown an increased seed production in either early-, or late-germinated plants within seasons (listed by Baskin and Baskin 2001). Thirdly, dormancy may act to reduce the risks associated with temporal habitat unfavourability by forming soil seed-banks that persist over several years (Venable and Brown 1988, Snyder 2006).

In legumes, water impermeability of seeds is acquired through dehydration during seed ripening (Quinlivan 1971). Water at first evaporates through the seed coat, but as the internal moisture content drops, the coat becomes impermeable, and further evaporation occurs via the seed hilum. The hilum then acts as a unidirectional valve that allows evaporation when external conditions are dry, but prevents water from entering when conditions are moist (Hyde 1954).

Legume seeds are normally considered to become shed in this water-impermeable, dormant state (Taylor 2005). However, studies that considered legume seed impermeability at the plant level indicated that seeds are sometimes permeable to water, thus non-dormant at maturity. Several treatises on *Trifolium subterraneum*, for instance, included the initial proportion of dormant seeds as a variable of interest. Nichols *et al.* (2009) recorded about 60 % of the seeds to be permeable at harvest (at plant senescence) in their experiment, while Smith (1988) noted proportions from 18 to 91 % for various line–year combinations 10 d after harvest. Piano *et al.* (1996) and Taylor (2005) reported similar findings. Cowan *et al.* (1997) investigated initial seed impermeability in other, non-geocarpous clover crops grown in controlled conditions and reported levels <10 % permeable in *T. repens* and *T. nigrescens*, but higher levels in *T. occidentale* (up to 61 %). The initial degree of dormancy has, however, more rarely been described for spontaneous populations. In a New Zealand pasture system, Chapman and Anderson (1987) found about 25 % of the seeds in *T. repens* to be non-dormant. Although not tested, some of these studies attributed the incidence of permeable seeds to coincidently high levels of moisture during seed maturation (due to rainfall, poor soil drainage or extended irrigation), which would prevent the seeds from drying, and thus from becoming dormant.

Although it might be an important phenomenon in natural populations, this intra-specific variation has never been dealt with explicitly by plant ecologists. Nonetheless, this may be of clear value also in the study of endozoochorous seed dispersal, because from literature, it appears that water-impermeable seeds have increased chances of surviving passage through the alimentary tract of ungulates (as compared to water-permeable seeds). This is most directly shown by experiments from Suckling (1952), Janzen (1981), Gardener *et al.* (1993a, 1993b), Fukuda *et al.* (1998) and Malo and Suárez (1998); also see Baskin and Baskin (2001). If genetically based *in situ* variation exists in this trait, then endozoochory might impose evolution on this trait through selection of the impermeable-seeded genotypes. That is, *if* endozoochorous dispersal is of significant selective advantage.

In this study, we therefore raise and address the following three questions concerning legume non-dormancy. (1) To what extent do wild plants show variation in initial non-dormancy? (2) To what extent is this variation genetic? (3) What significance, if any, do non-dormant seeds have for plant fitness? As a study species, we chose white clover (*Trifolium repens* L.), an ubiquitous and clonal species that lends itself easily for these practical purposes. First, we recorded the degree of initial impermeability in spontaneous populations. We secondly performed a greenhouse experiment to determine how ambient humidity and plant genotype affect the attribute in this species. Finally, we assessed experimentally the relative importance of permeable and impermeable seeds in contributing to plant establishment and reproduction.

White clover is a widespread legume that can be found in various habitats; most frequently in conditions that are grazed, mown or kept open otherwise (Burdon 1983). It is a perennial species, the main mode of regeneration of which is through the production of stolons. Yet yearly, new recruits are added to populations through seeding (Barrett and Silander 1992). The inflorescence is a racemose head containing about 30 flowers, each of which usually yields a three- to six-seeded pod. When a flower is pollinated, the pedicel recurves and allows for an easy indication that seed development has started (Hyde *et al.* 1959). Developing seeds become viable some days before acquiring dormancy (about 16 and 24 days after pollination, respectively; Hyde *et al.* 1959). For determining the proportion of dormant seeds, it is therefore important to collect seeds sufficiently late (in effect, at least 24 d after pollination). *T. repens* was included in the experiments of Suckling (1952), Gardener *et al.* (1993a, 1993b) and Fukuda *et al.* (1998), all concluding water-impermeability to positively affect survival in the alimentary tract of ungulates.

Materials and methods

Impermeability and viability testing

The general protocol used to test seeds for impermeability and viability is explained in this paragraph, since this applies to each of the three experiments explained below.

To determine the initial proportion of impermeable (to viable) seeds for a given head, all seeds were dissected from the head the day after collection. Seeds were tested for water impermeability by placing them on filter paper (Whatman GF/A, 90 mm; Whatman International Ltd, Maidstone, Kent, UK) in Petri dishes with 3.5 ml of water at 22 °C, and recording them for 3 d. If a seed is permeable, it imbibes water and swells, or germinates (ISTA 2008). Imbibed seeds were subsequently submersed in a tetrazolium solution (1.0

%) to test for viability (Peters 2000). At the end of the 3-day period, all remaining impermeable seeds were superficially cut with a scalpel near the distal end of the cotyledons to make them permeable, and tested for viability.

Field occurrence of impermeability

In July 2008, we selected white clover plants at six sites in the province of West-Flanders (Belgium; Table 5-1). At each site we randomly selected 10 (20 at one site) flowering patches that were at least 10 m apart. We marked 6–10 heads per patch by tying colourful strings to the peduncles. Collections were performed 26 d later. Priority in marking was given to heads in which all flower pedicels were recurved, but had not yet turned brown. Otherwise, heads were allowed to have at most four flowers not yet recurved (although this was rarely the case; these flowers could slightly bias impermeability proportions due to incomplete development at collection). Some patches were lost during the period from marking to collection (for example, due to grazing), and we eventually analysed 107 heads from 59 patches for seed viability and water impermeability (Table 5-1). Since densities of white clover genotypes can be high at small scales (Cahn and Harper 1976), no inference on individual clones can be drawn from this part of the study.

To tentatively check whether seed impermeability is related to levels of precipitation, we consulted climatological statistics for the seed-ripening period from the ten weather stations nearest to the six-site centroid (Malcorps 2008; stations of the Belgian Royal Meteorological Institute, all within 100 km from the centroid).

Table 5-1. Description of the sites at which the degree of initial impermeability in mature white clover inflorescences was recorded, and the number of flower heads investigated

Site name	Latitude, longitude	Description (soil type)	heads (patches)
De Panne (DP)	51°04′54″N, 02°33′27″E	Extensively grazed coastal dune complex (calcareous sandy soil)	25 (18)
Loppem (L)	51°07′36″N, 03°11′14″E	Meadow (acidic sandy soil)	15 (6)
Oostduinkerke (O)	51°07′18″N, 02°41′53″E	Grazed pasture (calcareous sandy soil)	12 (5)
Westkapelle (W)	51°19′28″N, 03°20′12″E	Grazed pasture (clay soil)	17 (10)
Zedelgem (Ze)	51°07′34″N, 03°08′27″E	Residential lawn and road verge (acidic sandy soil)	11 (10)
Zuienkerke (Zu)	51°16′38″N, 03°09′12″E	Road verges (clay soil)	27 (10)

Genotype by humidity

To assess whether variation in the proportion of impermeable seeds from heads is caused by differences in humidity, or by different genotypes, we conducted an experiment under controlled climatic conditions of light, temperature and humidity.

In May 2008, we isolated nine adult white clover ramets from three natural sites. The distances between ramets within sites were at least 30 m. They were grown in a glasshouse, and cuttings were propagated from them. In May 2009, after open pollination by wild insects, six ramets from each of these clones (54 plants in total) were transferred to the climatically controlled greenhouses for ripening. Since physical dormancy is a feature of the seed coat, it is maternally determined; therefore, no controlled hand-pollination was needed. Two ramets per clone were allowed to ripen under one of three levels of relative air humidity (RH), denoted as 'wet' (median relative humidity 99.9 %, minimum 89.0 %), 'mesic' (median 94.3 %, minimum 61.1 %) and 'dry' (median 78.3 %, minimum 46.5 %); RH was recorded by two loggers per treatment, 9300 measurements per logger. Since the desiccation of developing seeds, and with it, the acquisition of water impermeability, is an irreversible response to decreases in moisture, it is important to note that the minimum might be a better measure for humidity than the median.

After ripening in the greenhouses, heads were collected from each ramet (105 in total, 25–32 d after translocation from the glasshouse), and their seeds were tested for impermeability and viability.

Performance of permeable versus impermeable seeds

Once dormancy is broken, germination requirements are usually readily met in white clover. Seeds that are non-dormant when shed in autumn are consequently expected to germinate that same season. Dormant seeds, on the other hand, usually germinate in spring (Roberts and Boddrell 1985, Van Assche *et al.* 2003). In a field-sown experiment, we compared the field performance of non-dormant (autumn-germinating) and dormant (spring-germinating) seeds in germination, seedling establishment and first-year flowering.

The experiment was set up on the boundary of a sward and fallow vegetation at the 'Loppem' site (Table 5-1). The sward represented a habitat suitable for white clover: it was mown twice a year and was dominated by *Lolium perenne*, *Poa pratensis* and white clover. The fallow was former arable land, at mid-successional stage (± 75 cm vegetation height; saplings were lacking at the scale of the experiment). It was dominated by *Juncus effusus* and *Holcus lanatus*. Being a light-demanding species, this habitat is not optimal for adult white clover though potentially encountered by young plants, as germination itself is considered independent of light (Van Assche *et al.* 2003).

We harvested seeds for the experiment in the vicinity of the site in summer 2008, and separated permeable from impermeable seeds. The installation of plots (see below) consisted of sod-cutting a turf of 50 × 50 cm (10 cm in depth), turning it upside down (to make it free from competition), then sod-cutting the central 30 × 30 cm again and replacing it by a mixture of sand and potting soil (1:1 volume). After the introduction of seeds, plots were caged to exclude herbivores (1.25 mm mesh).

In order to appropriately compare establishment from autumn- and spring-germinating seeds, it is necessary to determine the exact timing of natural spring germination at the site. Along the sward– fallow boundary, we delimited five blocks, each comprising one plot in the sward and one in the fallow. Each of these plots was provided with 20 dormant seeds in autumn (1 October). They were buried superficially (<1 cm) in open plastic tubes (13 mm diameter) to facilitate retrieval, and seedling emergence was monitored on a monthly basis. At the end of the study period, we exhumed the non-germinated seeds and determined their viability.

We installed six blocks, each comprising two plots in the sward and two in the fallow, alternating with the blocks of the former experiment. Within each of these vegetation/block-combinations, one plot, chosen randomly within the pair, was provided with freshly imbibed seeds in autumn (representing naturally non-dormant seeds; sown on 5 September; 26 seeds per plot). The other plot was provided with imbibed seeds that had been artificially scarified in the following spring (mimicking naturally dormancy-released seeds; the sowing date was therefore determined by the previous experiment and was set to 15 April; 26 seeds per plot). Scarification of the latter was performed by nicking the seed coat near the cotyledons the day before sowing. Seeds were buried superficially (<1 cm), and seedling emergence and survival were monitored monthly. The first-year reproductive output from these recruits was recorded by counting the number of inflorescences produced during the summer of 2009. In August, we additionally marked ten heads from both the sward and the fallow (chosen randomly), and collected them 35 d later to test the seeds for viability and impermeability.

We manually controlled for weeds within plots throughout the study. Four control plots without introduced seeds were monitored for external contamination (one at each end of the set-up); however, no background germination occurred. Temperature and relative air humidity were recorded hourly at ground level by loggers. A scheme of the experimental set-up from the field is provided in Appendix 5A.

Statistical analyses

To test whether mean proportions of impermeable to viable seeds from heads differed significantly between the six sites studied, a generalized linear model (Kutner *et al.* 2005) was constructed in which SITE was entered as a fixed factor, and PATCH as a nested random factor. A Spearman rank correlation was performed to test for an interdependence between the number of viable seeds in a head and the proportion of impermeable seeds.

To test whether humidity and genotype affected impermeable seed proportions, we used a generalized linear model on the data from the greenhouse experiment. The proportion of impermeable to viable seeds from heads was the response variable. HUMIDITY was entered as a fixed factor (three levels), the CLONES as a random factor (nine levels), and their interaction as a random factor. By considering the variance that is due to the clones relative to the total (phenotypic) variance, a measure termed the 'clonal repeatability' was determined (Falconer and Mackay 1996). This measure can include some environmental effects (those that are transmitted from the original plant to all derived ramets), but sets the upper limit for the trait's broad-sense heritability, which is the proportion of the phenotypic variation that represents genotypic variation.

For the experiment on dormancy loss, we tested the effect of the VEGETATION (sward versus fallow) on seed germination using a generalized linear model. As this experiment employed repeated measurements of plots over time, the within-plot correlation among the subsequent dates of monitoring was accounted for (marginal model; Fitzmaurice *et al.* 2004). For the second experiment (initially non-dormant versus dormancy-released seeds), we disconnected seed germination from seedling survival: germination was given by the first census (baseline), while survival was given by the subsequent censuses corrected for the baseline. Germination percentage was treated in a generalized linear model, with VEGETATION (sward versus fallow) and SOWING DATE (autumn versus spring, representing the non-dormant and dormant seeds, respectively) as fixed effects. Seedling survival was treated in a general linear model with the same effects as well as an assumedly linear effect of TIME; monitoring dates accounted for within-plot correlation among repeated measurements. For the proportion of dormant (to viable) seeds from heads, a comparison between both VEGETATION types was made using a simple generalized linear model.

All statistical analyses were executed using SAS 9.2 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Field occurrence of impermeability

The summer of 2008 proved relatively wet compared to previous summers. Precipitation levels in July reached 0.8–1.6 times the July means of the weather stations, which were 'normal' anomalies for most stations (Malcorps 2008). In August, precipitation amounted to 1.1–2.3 times the August means, which was classified as 'abnormal' for most stations, meaning that similar or higher precipitation levels are, on average, observed once every 6 years.

Vivipary in *T. repens*: non-dormancy can lead seeds to germinate within flowers

From 107 inflorescences, we obtained 6536 seeds that proved viable (on average 61 per head, standard deviation = 38). No less than 35 % of these seeds imbibed water during the 3-day test period (mostly during the first night). The proportion of permeable seeds varied greatly among heads (Fig. 5-1): some heads only contained permeable seeds, others only contained impermeable seeds, and others contained intermediate levels of both. This range from 0 to 100 % was observed at all but one site; site means were nevertheless significantly different (Table 5-2), ranging from 46 to 87 %. In some heads, we observed vivipary as seedlings emerged in or from pods.

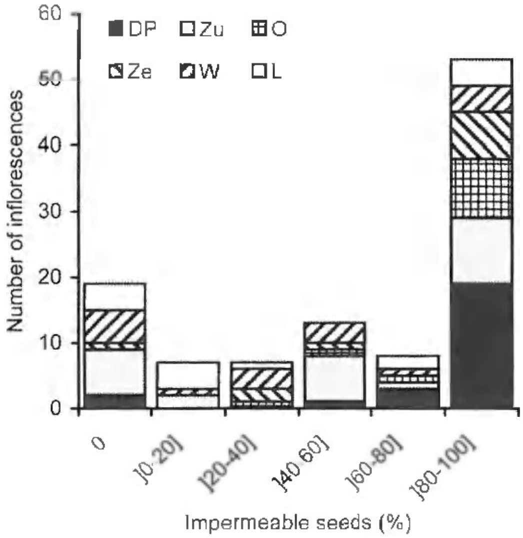

Figure 5-1. Frequency distribution of the proportion of impermeable (to viable) seeds held within mature white clover inflorescences (n = 107). Different shading refers to the six sites studied; see Table 5-1 for abbreviations.

Table 5-2. Output from the generalized linear mixed model from the field survey.

Type-III tests of fixed effects

Effect	df (num., den.)*	*F*	*p*
Site	5, 46.06	2.71	0.032
	* with Satterthwaite correction		

Covariance parameter estimates

Covariance parameter	Estimate	S.e.
Patch(site)	1.498	0.603
Residual (vc)	24.572	4.517

There was no significant correlation between the number of seeds from heads and their impermeable proportions (*P* = 0.343).

Genotype by humidity

Humidity had a marked effect on seed impermeability (Table 5-3). As shown in Fig. 5-2, heads that ripened under wet conditions contained only permeable seeds, while those that ripened under dry conditions contained few permeable seeds. The mesic treatment yielded intermediate levels of both. A test of the covariance parameters indicated that the clone-by-humidity interaction did not significantly differ from zero (cf. Table 5-3, χ^2 = 1.97, *P* = 0.160). We therefore omitted it from the model, the results of which are provided in Appendix 5B. The very low (yet significant) clonal variance component then yielded a clonal repeatability estimate of 0.025. This also is the maximal broad-sense heritability, and the observed phenotypic variation thus almost purely represented phenotypic plasticity within genotypes.

There was a significant correlation between the number of seeds from heads and their impermeable proportions: higher seed numbers coincided with higher fractions of dormant seed (P < 0.001).

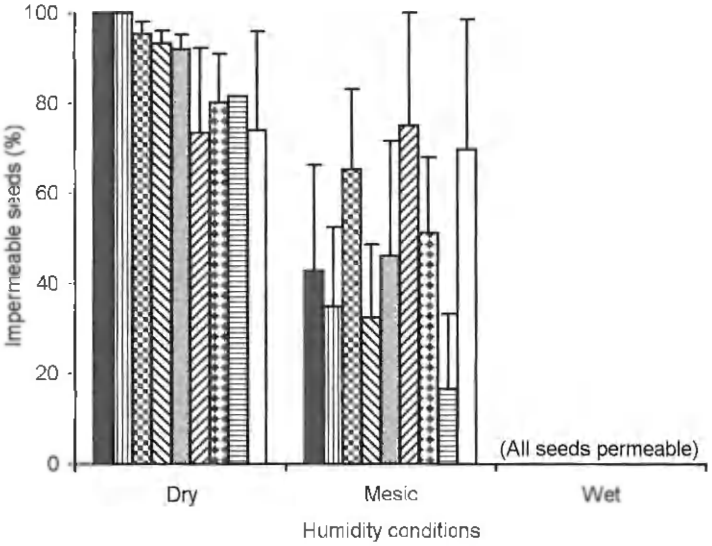

Figure 5-2. The proportion of impermeable (to viable) seeds held within mature white clover inflorescences in relation to humidity and genotype (average ± s.e.). Each shading refers to one of nine genetically distinct clones. Ramets of these were grown under either dry [minimum relative air humidity (min. RH) 46.5 %], mesic (min. RH 61.1 %) or wet (min. RH 89.0 %) conditions.

Table 5-3. Output from the generalized linear mixed model from the genotype-by-humidity experiment

Type-III tests of fixed effects

Effect	df (num., den.)*	*F*	*p*
Humidity	2, 26.76	8.27	0.002
	* with Satterthwaite correction		

Covariance parameter estimates

Covariance parameter	Estimate	S.e.
Clone	0	.
Clone x humidity	0.436	0.321
Residual (vc)	10.258	1.526

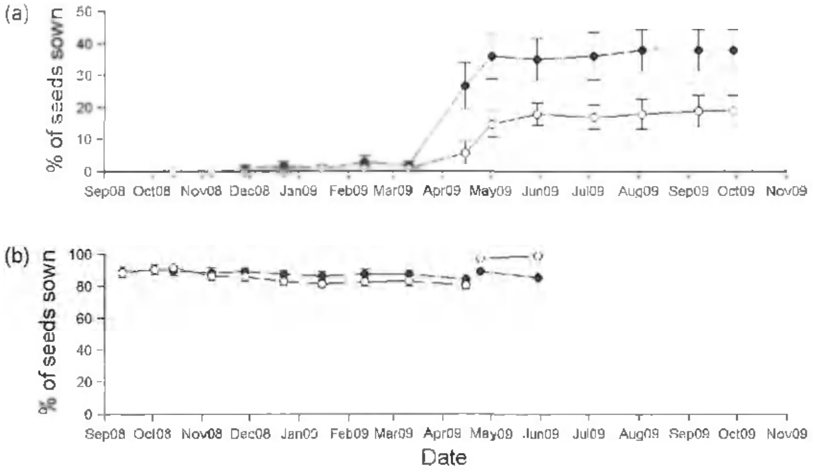

Figure 5-3. (a) The percentage of emerged seedlings from dormant seeds that were sown in autumn 2008 (average ± s.e.). Closed dots, sward vegetation; open dots, fallow vegetation. (b) The percentage of emerged seedlings from non-dormant seeds that were sown in autumn (left set of lines, from September on), and from scarified seeds that were sown in following spring (right set of lines, from April on; average ± s.e.). Closed dots, sward vegetation (the outlier plot is not included, see text); open dots, fallow vegetation.

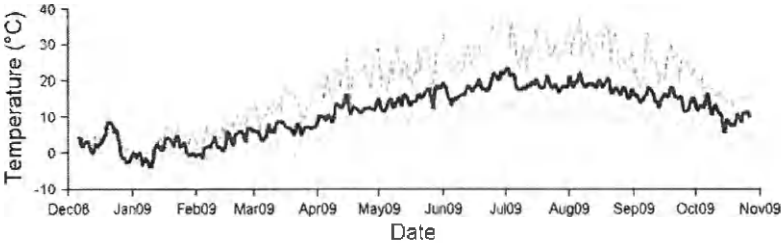

Figure 5-4. Daily minimum, mean and maximum temperatures recorded at the study site (bold grey, black and thin grey lines, respectively). Data is from the sward vegetation.

Performance of permeable versus impermeable seeds

Of the 200 dormant seeds that were introduced in the field experiment, 58 (29 %) emerged during the study (Fig. 5-3a), with a clear upsurge between mid-March and mid-April. March 15 was the first day on which the temperature range spanned the 6–15 °C interval in each vegetation (Fig. 5-4), giving further support to these numbers as a cue for germination in the species (see Van Assche *et al.* 2003). Significantly higher germination percentage was observed in the sward than in the fallow (χ^2 = 4.08, P = 0.043). We retrieved 129 of the non-germinated seeds at the end of the study (91 %), which all proved to be alive and dormant.

Germination percentages of the introduced non-dormant seeds were high for both the autumn- and spring-sown seeds and in both vegetation types (Fig. 5-3b). One sward plot was lost because of damage to the seedlings by an unidentified invertebrate. Despite the high germination, significant effects of VEGETATION (fallow > sward), SOWING DATE (spring > autumn) and their interaction were seen, since germination of the spring-sown seeds in the fallow was nearly complete (Table 5-4; the outlier plot excluded).

Table 5-4. Output from the generalized linear model on seed germination from autumn- and spring-sown (non-dormant) seeds. Cf. Fig. 5-3b

LR statistics for type-III analysis

Effect	df	χ^2	p
Vegetation	1	5.05	0.025
Sowing date	1	6.31	0.012
Vegetation × sowing date	1	6.03	0.014

Seedling survival from these seeds proved to be high as well (Fig. 5-3b). For the autumn-sown seeds, the effect of TIME, or in other words, winter mortality, was nevertheless significant (Table 5-5). No effect of VEGETATION was found, nor of its interaction with TIME.

Table 5-5. Output from the general linear model on seedling survival from autumn- and spring-sown (non-dormant) seeds. Cf. Fig. 5-3b

Type-III tests of fixed effects

Effect	df (num, den)*	F	p
Vegetation	1, 116	0.07	0.787
Time	1, 116	10.97	0.001
Vegetation × time	1, 116	1.77	0.186
	* with Satterthwaite correction		

Although we did not quantify vegetative regeneration of the recruits, stolon growth appeared markedly faster in the sward than in the fallow. For the autumn-germinated plants, stolon growth commenced in winter and was so extended by April that it became too difficult to discriminate between intertwined individuals in plots. For the spring-germinated plants, stolon growth was even more rapid, and within 2 months censuses were ceased for the same reason. Due to this marked head start in growth, plants from autumn-germinated seeds developed inflorescences sooner, and, eventually, more of them, than plants from spring-germinated seeds in the same vegetation (Fig. 5-5). Being a suitable habitat for adult white clover, the sward yielded more flowers per individual than the fallow.

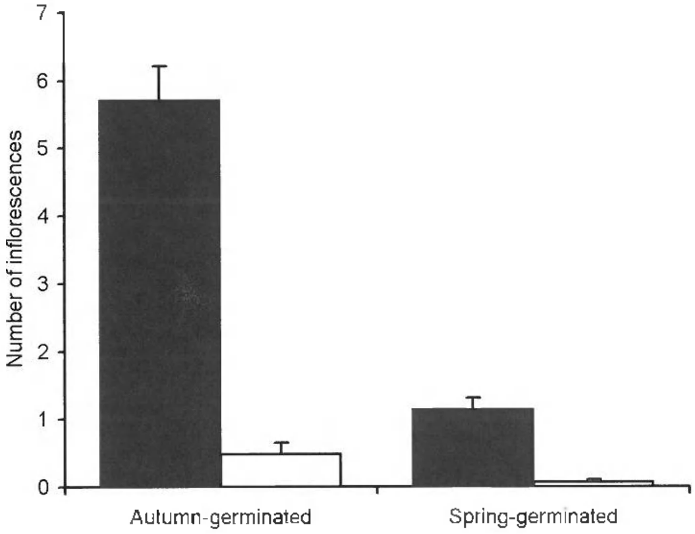

Figure 5-5. The average number of inflorescences produced per individual plant during summer 2009 (± s.e.). Closed bars, sward vegetation; open bars, fallow vegetation. The plants correspond to those from Fig. 5-3b.

During the period from marking inflorescences of these recruits to fruit collection in summer 2009, the minimal relative humidity recorded at ground level was 34.7 % (median 89.1 %) in the sward and 70.0 % (median 96.5 %) in the fallow. The newly ripened seeds were markedly dormant: of the 1566 viable seeds tested (from 20 heads), only 93 (6 %) were permeable. For most heads, over 95 % of the seeds were impermeable (range: 38–100 %), with no significant difference between vegetation types ($P = 0.498$).

Discussion

In normal summer conditions, white clover develops seeds of which the great majority are water impermeable at maturity (cf. summer 2009). However, if environmental conditions during seed ripening are relatively wet, such as during the summer of 2008, a significant proportion of seeds might be prevented from becoming dormant. Since climatological records indicate similar anomalies to occur once every 6 years or so, we suggest that legume populations (from higher latitudes or altitudes, for example) might be confronted with non-dormancy more often than is generally assumed.

As humidity conditions can vary through space and time even at small scales, the variation witnessed in initial seed permeability most likely reflects such microclimatic variation. The position of a ripening head relative to surrounding foliage (own or alien), for instance, will be a major determinant for its level of dormancy in the field, in combination with the prevailing weather conditions. Larger-scale patterns are then likely to be found when consistently associated with such conditions (e.g. short, sunlit *vs* high, dense vegetations). In legume cultivation, sufficient attention should accordingly be given to homogenize and follow humidity conditions at the level of the inflorescences, where a sufficient degree of drying should be ensured to obtain good seed yields (*cf.* Clua and Gimenez 2003, Taylor 2005). Although humidity at ground level during seed ripening in the fallow vegetation approximated the moist treatments from the greenhouse, dormant seeds were produced. We assume this is due to small-scale differences, as humidity was not measured at the height of inflorescences (where the minimal humidity presumably approached that of the sward).

In our opinion, non-dormant seeds have been overlooked in demographic studies on legumes. For instance, Chapman (1987) reported a germination peak in autumn and a smaller one in spring from fallen heads in a set of New-Zealand pasture populations of white clover. Although not indicated by the author, these peaks very likely represent initially permeable and impermeable seeds, respectively (cf. Chapman and Anderson 1987, introduced above). In the lawn population studied by Barrett and Silander (1992), autumn- and spring-germinated seedlings were recorded, too, the former associated with an increase in the number of worm cast mounds. Unfortunately, no details are provided on characteristics of flowering and seeding, and it is thus not clear whether the autumn cohort comes from occasionally germinated dormant seeds, or from newly produced non-dormant seeds. In a demographic study on a meadow population of red clover (*Trifolium pratense*), Sakanoue (2002) also reported two cohorts, and actually concluded the population to be mainly derived from autumn-germinated seeds. He attributed the occurrence of autumn germination to the timing of haymaking before the development of dormancy (Sakanoue 2004), although it seems likely that at this high-rainfall site mature seeds that were prevented from becoming dormant contributed as well.

Above, we introduced several scenarios in which seed dormancy is an adaptive feature. In the first, dormancy serves to prevent germination in unfavourable conditions for seedling establishment. In our field experiment, however, we found that non-dormant and dormant seeds had equally high chances of establishing. In the second scenario, the merit of dormancy is to maximize seed production of the resulting adult. In our experiment, however, plants from non-dormant seeds yielded more flowers than plants from dormant seeds in their first year. Of course, this only is a short-term difference in fecundity since seed production is expected to be equal from the second year on. Nonetheless, dormancy does not appear to be adaptive under each of the scenarios in our study system, despite the seeds of this species being (modally) dormant. How could this apparent paradox be explained?

Supposing that non-dormancy is beneficial over dormancy, our results show that selection for non-dormancy would be restricted since the trait's heritability is nearly zero. A similar situation was found by Arthur *et al.* (1973) for *Papaver dubium*. In this species, autumn-germinated recruits were more successful in the population than spring-germinated recruits, but the very low heritability of timing of germination prevents selection of the former. Although in our greenhouse experiment, genotypic variation did not contribute to phenotypic variation, this does not necessarily mean that the attribute is independent of genetic mechanisms, however (Pigliucci 2001). In forage lupins (*Lupinus* spp.), for instance, a recessive allele has been identified which results in 100 % permeable seed proportions at maturity (Arrieta *et al.* 1994, Boersma *et al.* 2007). Permeable-seeded lines are also known for *Lens* (Ladizinsky 1985), *Vicia* (Ramsay 1997) and *Ornithopus* (Taylor 2005) but, to our knowledge, not for *Trifolium*.

The results from the field experiment might suggest that mother plants use environmental cues during seed maturation for post-dispersal conditions. I.e., non-dormant seeds are yielded in wet conditions, when seedling survival is likely to be high, whereas dormant seeds are yielded in dry conditions, when survival is likely to be low. This might suggest phenotypic plasticity to be adaptive (Dudley 2004). However, whether each of these phenotypes really is optimally appropriate for its environment, is also dependent on the fitness outcome of the dormant phenotype under wet conditions.

Indeed, there are other potential benefits linked to dormancy, such as the escape from crowding or sib competition, or the spreading over several seasons of risks associated with germination (Venable and Brown 1988). The latter scenario in particular has received much attention, especially for annuals in 'risky' environments. Annual legumes from the Mediterranean, for instance, are well known to form seed banks with high levels of seed carry-over between years (Russi *et al.* 1992, Ehrman and Cocks 1996, Norman *et al.* 2005). *Trifolium* is considered to originate from the Mediterranean basin and many annuals constitute its basal clades (Ellison *et al.* 2006), suggesting that such long-term dormancy might be ancestral in the genus. Most of the dormant seeds in our field experiment also did not germinate in their first year. In more marginal environments such as the subtropics, perenniation in white clover may not occur every year (Blaser and Killinger 1950). As the addition of new seedling recruits then becomes particularly important, we would expect the risk-reducing aspect of its dormancy to be of increased significance in these conditions.

Moreover, it can be expected that permeable legume seeds are relatively susceptible to fungal attack both before and after dispersal (cf. Roy *et al.* 1994). This is possibly less relevant for predation by arthropods, which mostly attack immature seeds for egg deposition (for instance, seed weevils; Freeman 1967), but data for this are lacking. We found high fractions of permeable seeds to come with small seed numbers in the greenhouse. Here, we used insecticides to control spider mite infection on the leaves and stems, but not fungicides, which might have given increased chances for fungi to infest seeds within this dense aggregation of plants. If infection causes seeds to test as unviable, this might have biased the results from the greenhouse experiment in favour of impermeable seeds. On the other hand, since the variables consist of compounding variables (i.e. X and Y are related), there are increased chances for a type-I error to be involved in this correlation (Atchley *et al.* 1976).

Dormancy can be closely linked to dispersal in legumes: when ingested by herbivores, water-impermeable seeds survive the digestive tract, while permeable seeds do not (references in the Introduction). This direct, positive effect of dormancy on fitness has been suggested as a system for studying endozoochory-imposed evolution in Chapter 4. Particularly, a significant interaction from the clone-by-humidity experiment would have been particularly useful in this respect, since this would mean that for a given environment, (genetically based) variation exists between individuals in their numbers of impermeable seeds. If grazers would occur in that environment, those clover plants with higher numbers could become selected over those with low numbers through endozoochory. Unfortunately, although variation in this trait appears to be potentially substantial, its use for these aims is limited due to the low heritability.

Of course, we cannot expect our field experiment to have given us an unbiased representation of natural white clover establishment, as this was a one-year/one-site study and involved experimental manipulations such as the reduction of competition (Clatworthy 1960 in Harper 1977) and herbivory. The estimates of winter mortality in the study of Barrett and Silander (1992), for instance, were much higher than ours. As these authors have shown, even small-scale differences relating to site attributes and plant condition can affect winter performance. Yet, demographic studies such as those described above support the notion that real populations do, at least in some cases, effectively show establishment from autumn-germinated, initially non-dormant seeds.

A nightly view on the greenhouses of the Flemish Marine Institute (Wenduine)

« The story of seeds, in a
nutshell, is a tale of
evolution. »

An Orchard Invisible: 4

CHAPTER 6

General discussion

The ecology of endozoochory

In this part, I pick up some of the stages that together constitute endozoochory (as introduced in Chapter 1). I put some additional reflection on each of these, including on their costs and on the challenges still posed by them.

From seed uptake to seed deposition

Seed uptake

Not discussed in the General introduction, the uptake of seeds probably is among the least-known aspects of endozoochory. This primarily applies to the number of seeds available to animals, the rate at which seeds become ingested, the selectivity with which plants (seeds) become ingested, potential seed spill during ingestion, *etc.* Clearly, the number of seeds ingested is nonetheless of crucial importance in quantifying the importance of endozoochory.

In our field survey of local seed availability (Chapter 2), we often encountered plants of which the flowers had many undeveloped or unripe embryos, or of which the capsules had already shed half of their seeds, *etc.* Hence, the availability of seeds to grazers at a particular moment during the fruiting season (Table 2A-2 in the Appendices) was often much lower than would be expected from the mere cover or abundance of plants. We therefore believe that using idealized figures on the reproductive capacity of plants causes overestimation on endozoochorous seed numbers. Due to lack of data, Will and Tackenberg (2008) based the local production of seeds in their model on such numbers, though their sensitivity model clearly showed this to be one of the (three) most decisive plant-related variables.

Given a certain quantity of seeds available, it is highly unclear what portion becomes ingested by an animal during grazing. This was nonetheless also identified as a major decisive parameter by Will and Tackenberg (2008). Due to this lack of data, the ingestion proportion was necessarily included with some degree of arbitrariness in both their model and ours. A high degree of random variation is to be expected in these proportions (e.g. surrounding vegetation, individual diet preference), though certainly some degree of systematic variation must exist (e.g. plant architecture, biting behaviour [Harper 1977: 421]). Therefore, this would certainly be an interesting topic in further endozoochory research.

Surviving the digestive tract

Mean percentages of seed survival following ingestion are provided in Chapter 4 for many species. These percentages are often assumed to represent constant, species-specific characteristics. However, when comparing the results of (the very few) species that have been included in more than one feeding experiments, these seem not to be consistent (Table 6-1). These differences show that seed survival is very variable, and many factors that might contribute variation in these figures can be assumed: animal breeds, animal group dietary habits, individual animal condition, among-batch variation in seeds (for instance, in their viability, states of dormancy, *etc.*), experimental methodology... In Appendix 1C, we have tried to collect all feeding experiments from literature, which could form a solid base for an exhaustive (meta-)analysis on seed survival variability and patterning.

Table 6-1. An overview of the shared species from the feeding experiments of D'hondt and Hoffmann (2011; Chapter 4), Bonn (2004) and Cosyns *et al.* (2005a). In each study, a given number of seeds was fed to cattle; figures denote the proportion of seeds that survived ingestion

	Chapter 4 avg (s.e.)	*Bonn (2004)* avg (s.e.)	*Cosyns et al. (2005a)* avg (s.d.)
Achillea millefolium	20.1 (3.9)	16.3 (1.9)	
Agrostis capillaris	53.8 (8.7)		15.7 (3.7)
Galium verum	0.0 (0.0)		0.0 (0.0)
Pimpinella saxifraga	0.2 (0.2)	0.3 (0.1)	
Plantago lanceolata	31.6 (7.53)		4.1 (2.5)
Poa pratensis	31.6 (6.6)		9.4 (2.4)
Thymus pulegioides	41.7 (41.7*)	1.5 (0.2)	1.6 (1.6)

* Note the very high distortion in this species (Fig. 4-1b).

From Chapter 4, we suggested seed structural, textural, anatomical (e.g. seed surface topography; cf. Werker 1997) and/or physical traits (e.g. seed toughness; cf. Rodgerson 1998) to override simple dimensional traits (such as size or shape) in explaining mortality rates. We believe this reflects the nature of feed digestion in ruminants. Commensal bacteria, and –to a lesser degree– free enzymes, effectuate plant tissue digestion within the animal (Akin *et al.* 1974). Since these bacteria have trouble attaching to cuticles or lignified plant tissues (which are entirely resistant to digestion), ruminal bacteria gain access to digestible inner-tissues through stomata, lenticels or damaged areas. Thus, most plant fragments are digested from the inside out (McAllister *et al.* 1994). Damage of tissue is facilitated through rumination (Péréz-Barbería and Gordon 1998).

As suggested, seeds that are impermeable even to water therefore have the best chances of passing through the intestinal tract alive[*]. That is, if seeds succeed in remaining impermeable throughout (most of the) tract. Therefore, an impermeable seed's ability to resist damage may be an equally important precondition for success. For instance, we found *Geranium* spp. in our experiment to exhibit low survival, despite them being known as having impermeable seeds (Meisert 2002). In these species, seeds appeared relatively less tough than those of other species with impermeable seeds (*Trifolium* and *Helianthemum*). Moreover, *Geranium* seeds have stomata on their surface which might provide easier intrusion sites for digestive fluids or bacteria (Werker 1997).

Traveset (1998) suggested seed coat thickness to be a decisive trait in endozoochory by frugivorous birds. In our tentative test of seed coat thickness in Chapter 4, we found no relation with mortality rates. Even in birds, the evidence is somewhat controversial, since Soons *et al.* (2008) found seed coat thickness to be only of minor importance in determining intact passage through the guts of ducks. Note that the overall seed coat thicknes rarely is a reliable proxy for precluding intrusion of digestive agents from the animal, since water entrance primarily is through gaps (Werker 1997, Baskin *et al.* 2000). Seed coats form relatively complex structures, and measurements of thickness are therefore not that straightforward (Chapter 4).

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[*] In Poschlod & Bonn (1998), it is mentioned that hard seed coats are less sensitive to the toxic effects of ammonia, which may positively affect survival in manure.

throughout summer, and probably there is much variation in their abundance among years. As Ramos *et al.* (2006) noticed, variables as subtle as intact *vs* crumbled sheep dung pellets can have a decisive effect on the establishment of seedlings! (iii) It is inherently difficult to summarize and include all costs from the process, as some of these are very subtle (e.g. costs of dispersal away from areas of local adaptation), or only apply to a subset of seeds (e.g. those that become secondarily dispersed), *etc.*

To evaluate the overall significance of endozoochory to plants, it is moreover important to define an appropriate null model with which to compare endozoochory. This is rarely made explicit. The most obvious (ubiquitous) vector in nature is wind, and this is why we included anemochory in our simulations in Chapter 2. Different vectors are likely to pose different costs and benefits to plants, and a continued effort to research all of these vectors and their demographical consequences is therefore needed.

Table 6-2. Costs of ungulate endozoochory, as contributed from Chapters 2, 3 & 4.

Stage(s)	**Cost type**	**Comment**	**Estimated cost for an ‘unassisted’ seed in endozoochory**
[Ch. 2] From germination to flowering	Opportunity cost/risk cost[1]	Transfer towards lesser-quality habitat	Landscape-dependent, higher than compared to wind
[Ch. 3] Secondary dispersal	Risk cost (mortality)/time cost[2]	Burial by dung beetles	Potentially high (mortality/enforced dormancy)
[Ch. 4] Seed transfer	Risk cost (mortality)	Digestion or destruction by the ungulate vector	Mostly high

[1] Depending on the extent to which the individual’s fitness is decreased in the novel habitat (cf. Table 2A-2 in the Appendices).

[2] Depending on whether seeds remain dormant in the soil, or die.

Working backward

Wang and Smith (2002) distinguished two main approaches to study animal-mediated seed dispersal. Researchers working 'forward' look at dispersal as it happens, following seed fate through its successive stages (fruit removal, animal behaviour, germination, *etc.*). Most studies of endozoochory (including the ones in Chapters 2, 3 and 4) adopted this approach.

Dispersal can also be approached in a 'backward' manner by studying the results of dispersal (Wang and Smith 2002, Poschlod *et al.* 2005). This includes molecular-genetic studies that measure gene flow among populations, or match offspring with parents. Willerding and Poschlod (2002) and Honnay *et al.* (2006) studied the population genetic structure of, respectively, *Bromus erectus* and *Anthyllis vulneraria* in a German and Belgian landscape which are managed through sheep herding. While sheep were found to be of minor importance in the former study, the latter authors suggest roaming livestock to have significantly exchanged seeds among fragments. Phillip Kollmar (unpub.) showed that sheep performed a significant mixing of *Medicago minima* populations along transhumance herding routes in eastern France, relative to off-route populations that are spatially similarly structured. These three species are expectedly dispersed on the animals' outside, although the contributions of epi- and endozoochory cannot be teased apart. On the other hand, Vickery *et al.* (1986) could attribute monkey flower seeds from deer scats to a population a kilometer away through genetic analysis.

Where dispersal meets dormancy

Water-impermeability of seeds is essentialy considered to be a form of dormancy ('physical dormancy'*; Baskin and Baskin 2004). However, there are several interesting aspects of this type of dormancy from the perspective of endozoochorous dispersal. First, from literature, water-impermeable seeds appear to be of substantial significance in surviving the alimentary tract after ingestion, provided that they are sufficiently tough not to be

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* Physical dormancy is also very commonly referred to as 'hardseededness'. I refrain from using this, since it is better used for the hardness of seeds, which is essentially independent from impermeability to water (see Werker 1997: 305).

damaged during the digestive process[*]. This appears to apply primarily to two families: Fabaceae and Cistaceae. Second, because these seeds can be expected to remain dormant after deposition for decades, allowing all biotic and abiotic effects of the dung environment to pass, deposition may be similar to that of non-endozoochorous dispersal. Therefore, a full account of costs and benefits might be easier to make in these groups.

Fabaceae

The Fabaceae (the legumes) are a very widespread and diverse plant family. Physical dormancy is ubiquitous in the family and not surprisingly, herbaceous legumes from grasslands are therefore commonly encountered in studies of dung seed banks (*Anthyllis, Biserrula, Coronilla, Cytisus, Hippocrepis, Lotus, Medicago, Melilotus, Ononis, Trifolium, Trigonella, Vicia*; for references, see Appendix 1A).

Legume seeds are often easily observed in faecal pellets. Here, a *Trifolium* seed (nature reserve D'Heye, fall)

Several studies on legumes give unambiguous evidence that seed water-impermeability accounts for survival during digestion. Suckling (1952) fed both impermeable and scarified *T. repens* seeds to sheep, but could only retrieve seed(ling)s from the impermeable seed lot. Fukuda *et al.* (1998) placed several seed types of the same species into the rumen of fistulated cattle and followed their fates. Mortality of water-impermeable seeds was very low during the 14 days of study, whereas permeable seeds were generally digested within 24 hours. They even provided a mathematical model of scarification and mortality dynamics within the rumen through time. Gardener and colleagues found a near-to-perfect correlation between

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[*] Or better: "... provided that they are sufficiently tough not to be scarified too early during digestion."

the fractions of seeds surviving a stay in the rumen and the initial impermeable seed content both within and among legume species (Gardener *et al.* 1993a, 1993b).

Interestingly, some legumes apparently exhibit endozoochory-promoting attributes that extend beyond water-impermeable seeds. Consider, again, white clover (*Trifolium repens*). This species is bound to open habitats, which are grazed under natural conditions. Its nutritious foliage is highly preferred by grazers (Ridout and Robson 1991, Horadagoda *et al.* 2009, Rutter 2010, but see Newman *et al.* 1991, Archer 1973 in Harper 1977), and it copes with grazing very well due to its creeping habit and rapid regrowth after partial defoliation. On the other hand, plants can produce cyanide compounds which deter small, non-disperser herbivores such as snails and voles (Harper 1977: 415, Hayden and Parker 2002). Inflorescences are raised above the foliage, where the fruits ripen and become abundantly ingested. Contrary to many other *Trifolium* spp., the fruit and calyx of *T. repens* do not show obvious dispersal features, and the wind dispersal potential is very weak (Tackenberg 2001). The species is nutrient-tolerating, and seeds that germinate soon after deposition readily establish as seedlings. If to any means of dispersal, white clover therefore appears particularly apt to endozoochory.

Trifolium repens (beneath arrow) and *Poa* sp. colonizing barren terrain through endozoochory by cattle. (Photo: Simon Brandt.)

Cistaceae

The Cistaceae are a medium-sized family of shrubs and herbs with the main centre of diversity in the Mediterranean Basin (Thanos *et al.* 1992). Malo and Suárez (1998) found an extremely high number of *Cistus* seeds to be dispersed by red deer (see Appendix 1B). These same authors also encountered *Tuberaria* seeds in dung from various herbivores (Malo and Suárez 1995b), while others reported *Helianthemum* to be dispersed endozoochorously (e.g. Müller-Schneider 1954, Dai 2000, Cosyns *et al.* 2005b).

Ramos *et al.* (2006) studied several stages of seed dispersal by sheep in six Cistaceae spp. They found (i) recovery of intact seeds after gut passage to be overall high, (ii) gut-passage to increase germination up to seven-fold relative to non-ingested seeds, (iii) seedling emergence from dung-borne seeds to be similar or higher than emergence from non-dung-borne seeds, and (iv) survival of emerged seedlings not to be reduced by dung. Overall, the 'probability of recruitment' was increased by seed consumption. To my thinking, however, higher germination percentages of gut-passed seeds relative to non-ingested seeds are somewhat misleading in this respect, since the latter most certainly remain dormant longer. Consumption therefore provides 'faster' recruitment in these cases, but on the long term, not necessarily 'increased' recruitment.

Our indigenous species, *Helianthemum nummularium*, is a prostrate grassland plant that additionally has a mucilaginous seed coat exterior (Werker 1997). Probably, this serves in sticking to legs of animals that tread on them. If so, then common rockrose might be considered a pre-eminently zoochoroous species, dispersed on both the inside and outside of grazers.

Other families

Physical dormancy is found in only 16 plant families (Baskin *et al.* 2000, 2006). For open communities of temperate regions, these not only are the Fabaceae and Cistaceae, but also the Geraniaceae and Malvaceae.

In their extensive study from a Mediterranean dehesa, Malo and Suárez (1995b) found significantly more Geraniaceae (*Erodium* spp., *Geranium* spp.) to be dispersed by deer than was expected from the species in the area (which also applied to the Fabaceae). *Erodium* and *Geranium* were also encountered in various other surveys, though mostly in small numbers (e.g. Müller-Schneider 1954, Cosyns and Hoffmann 2005, Cosyns *et al.* 2005b, Bruun and Poschlod 2006, Schmidt *et al.* 2004, Eichberg *et al.* 2007, Kuiters and Huiskes 2010). Indeed, we found low survival rates in *Geranium*, which is discussed above. Moreover, it should be borne in mind that these species dispose of a clear ballistic dispersal syndrome. Maybe, seeds are sometimes shot away but then become retained in surrounding foliage, after which they become ingested by herbivores. The latter then act as phase two dispersers.

Malva spp. form a rare constituent of the dung seed bank (Müller-Schneider 1954, Malo and Suárez 1995b, Kuiter and Huiskes 2010), which may be due to its infrequent occurrence in grazed habitats in most regions. Nevertheless, Schoenbaum *et al.* (2009) found it to be one of the most abundant species in sheep dung from a Mediterranean grazing system. No data are available on the seed's potential performance in endozoochory.

Evolutionary perspectives

The diffuse mutualism of dispersal

Particular disperser species generally exert low selection intensity on particular plant species. Herrera (1985) lists several reasons why. First, plant-disperser mutualisms are generally **diffuse**: each plant species is dispersed by several animal species, and each animal species disperses several plant species. Indeed, Bascompte and Jordano (2007) illustrated that most interactions in plant-frugivore networks involve a weak dependence of the one on the other. Second, the full cycle as shown in Fig. 1-1 is commonly associated with very high environmental (non-heritable) variation (see above), likely setting low upper limits to the selective pressures that dispersers can exert on plants. Alcántara *et al.* (2007), for instance, demonstrated that the pressure exerted by ant dispersers on seed size in the elaiosome-bearing *Helleborus foetidus* is highly variable in space, and that their pressure is insufficient to explain the trait's current attributes in populations unless ants are abundant. Third, non-disperser organisms may be decisive in selecting for plant traits associated with seed dispersal. As an example, Siepielski and Benkman (2007b) demonstrated that pre-dispersal seed-predating squirrels constrain the evolution of dispersal by corvids in pines. Considering our treatise, all three drawbacks clearly also apply to endozoochory of dry-fruited plants.

Plant-disperser coevolution is therefore to be considered coarse, between blocks of species, and over long time scales, with particular disperser species replacing previous ones through evolutionary time, at best (Herrera 1985, Tiffney 2004).

The case of legumes

In his seminal treatise, Janzen (1984) put an accent on past landscapes (prehistoric to geologic timescales), providing dialectic reasoning on how grazers and dry-fruited plants would have interacted through time. However, there exists some vagueness in these sketches. Taking his ideas at heart, I here try to perform the exercise for the case of legumes.

As mentioned in the General introduction, the Paleogene (± 65-23 Mya) saw a gradual evolution of foli- and frugivorous mammals to browsers and, eventually, grazers (Jacobs *et al.* 1999). Recent interpretations place the origin of legumes in this same period (Doyle and Luckow 2003). Interestingly, many extant legume trees possess a so-called 'megafaunal' fruit syndrome (Guimarães *et al.* 2008). Their large, fibrous, indehiscent pods are strikingly un-fit for present-day dispersers, though suit the once omnipresent but now largely extinct megafauna of browsing proboscideans, giant ground sloths, and many others. Such megafaunal dispersal is extant in Africa and Asia (involving elephants [e.g. Miller 1996] and rhinoceros [e.g. Dinerstein 1991]), but outside these continents, these traits are to be considered ghosts of past mutualisms (Johnson 2009). Well-described examples of such anachronistic legumes include the Mediterranean *Anagyris* (Valtueña *et al.* 2008) and the North-American *Gymnocladus* (Zaya and Howe 2009). In effect, the latter genus effectively dates back as far as 50 Mya (Doyle and Luckow 2003). The syndrome can even be found much more widely: Guimarães *et al.* (2008) add 24 species from Brazil alone. If megafauna was dispersing pods early from the history of legumes on, then maybe the (climate-driven) evolution from these browsers to grazers paralleling that from woody to herbaceous legumes throughout the Paleogene, allowed for a continuation of this mutualism? Then, ungulates dispersing clover-like legumes –first seen in the Miocene (23-5 Mya)* – is in effect to be regarded as the current representative of a long-held relation! Albeit a diffuse one...

Some householding: are plants "adapted" to endozoochory?

Janzen (1984) hypothesized that, for a number of plant species, several phenological, compositional and structural traits, both in the vegetative parts and in the seed, have been selected for through endozoochory. These traits

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* The legume clade including genera of interest such as *Medicago*, *Trifolium*, *Lathyrus* and *Vicia*, is considered to have originated near the start of the Miocene, at 24.7 ± 2.3 Mya (Lavin *et al.* 2005, Ellison *et al.* 2006). Also most major divergences in the Cistaceae occurred in this epoch (Guzmán & Vargas 2009).

include attractive (nutritive) foliage; retention of seeds by foliage; a synchronization of seed maturation and foliage attractiveness; and small, hard, digestion-resistant seeds. Such attributes have widely been adopted as referring to "adaptations" to endozoochory (e.g. Malo and Suárez 1995b: 249; Bonn 2004: 79; Cosyns and Hoffmann 2005: 12; Couvreur *et al.* 2005: 38; Mouissie *et al.* 2005a: 549, 555; Mouissie *et al.* 2005b: 284, 288). Ironically, however, Janzen (1984) never used this wording himself.

Indeed, adaptation can be "a slippery concept" (Reeve and Sherman 1993), and its use therefore needs careful attention. The latter authors reviewed the variety of definitions of adaptation in literature, and pinpointed the problems associated with them. Most definitions assume a historical element, which often involves the requirement that traits are built by natural selection for some role. These definitions are difficult to apply, however, because they confound product with process (which is not satisfactory), processes hardly leave good fossil records, and both traits and roles are particularly elastic concepts (examples provided by the authors). Therefore, Reeve and Sherman (1993) proposed a workable, non-historical definition of adaptation, as "a phenotypic variant that results in the highest fitness among a specified set of variants in a given environment".

If we assume an environment in which seeds are destined to become ingested by ungulates, then an impermeable-seeded variant will have a higher fitness than a permeable-seeded variant. Therefore, seed impermeability is an adaptation to endozoochory in this environment under the definition of Reeve and Sherman (1993), whereas small seed size, for instance, is not (Chapter 4). Real environments and the fitness consequences that come with endozoochory, of course, are much more complex (see above). As we have not yet resolved the net balance of costs and benefits, it is still somewhat precarious to claim traits as "adaptations", even under this simple definition.

The lack of obvious dispersal attributes in the majority of seeds is intriguing, and Willson (1993) reviewed several alternative hypotheses that exist for this observation. Seeds may, for instance, lack a dispersal mechanism just because of various trade-offs in recruitment (e.g. Eriksson and Jakobsson 1999) or because of selective forces other than dispersal (e.g. dormancy, predation). Here, Janzen's hypothesis has to withstand severe competition as some of the alternative hypotheses are clearly more parsimonious.

Evolution on 'ecological' timescales

Part of this thesis was concerned with an attempt to meet the question of whether endozoochory poses any micro-evolutionary significance to seeds from dry-fruited plants. For this, seed water-impermeability in legume seeds was explored. However, this seemed to be a dead end, since no genetic component was observed in the *in situ* variation of this trait in *Trifolium repens*, which thus obstructs any potential shift.

However, as discussed in Chapter 5, several legume species are known to have permeable-seeded lines, and some species are even known to consistently have permeable seeds (e.g. *Lupinus albus* [Werker 1997], *Ebenus creticus* [C. Thanos, pers. comm.]). It could therefore be nonetheless worthwile to study these species' occurrence patterns or allele frequencies; for instance, in a comparison of historically grazed and non-grazed terrains.

Further research

Throughout this General discussion, I have already pinpointed several caveats in our understanding of ungulate endozoochory. Here, I further elaborate on these and other suggestions for continued research on endozoochorous dispersal.

As mentioned above, the *in situ* significance of endozoochory can be studied using 'backward' approaches (Wang and Smith 2002), though to date they have been applied possibly too little. Complementary to the population-genetic studies on epizoochorous species (Willerding and Poschlod 2002, Honnay *et al.* 2006), studies that determine gene flow in species with high endozochorous capacities might help clarify the importance of this dispersal mode to plant (meta)population dynamics (also see Vickery *et al.* 1986).

Several ecologists (including myself) have attempted to understand the behaviour of seeds *within* the ungulate vector. It is, however, clear that this forms a complex interaction which is determined at the microscopic scale. Therefore, more thorough research on this topic, with sufficient seed anatomical and animal physiological detail, might contribute to endozoochory research greatly.

In that same vein, passage through ungulate guts must leave some sort of traces on the seed coat or surrounding structures? If such anatomical, biochemical or microbiological traces of endozoochory could be identified (e.g. tissue damage, microbial remains...; cf. Simao Neto *et al.* 1987), retrieved seeds or seed fragments from near the seedling's hypocotyl could tell away their mode of dispersal. In effect, dung-borne seedlings are a common sight in fall in grazed areas, but it is very hard to distinguish between seedlings that reached these gaps endozoochorously and those that did by wind (or other means). In general, various methods should be explored and advanced in studying endozochory (Wang and Smith 2002; Bullock *et al.* 2006; e.g. Levey and Sargent 2000, Traveset *et al.* 2008).

As discussed above, both the start and the end of the endozoochorous cycle are relatively ill-known. They are nonetheless crucial to evaluate its significance, as they quantify the amount of seeds entering the cycle and the number of seeds succesfully completing the cycle, respectively.

Probably, many auto-ecological interactions between particular plant species and (particular) animal vectors remain to be explored, with respect to how their phenological, morphological, physiological... traits impact endozoochorous dispersal. An interesting taxon in this respect may be *Urtica*. Despite being seemingly unpalatable, *U. dioica* has been found abundantly in dung by several researchers (Cosyns *et al.* 2005b, Mouissie *et al.* 2005a, also in our field-collected samples from Chapter 2). Maybe, the plant at first puts its trichomes into battle against herbivores, but by the end of the season, makes the best of a bad deal? This fits in with Janzen's (1984) most intriguing, but controversial, hypothesis; i.e. whether plants adjust their vegetative parts in response to grazers and seed dispersal provided by them*.

Legumes (Fabaceae) form a species-rich, morphologically diverse and widespread group of grassland plants, and the impermeability of their seeds is an easy-to-measure trait which relates directly to endozoochorous performance. Therefore, they might prove to be a rewarding subject not only for auto-ecological, but also comparative studies concerning their interactions with grazers. The study area of interest would then be the Mediterranean, not only because a high diversity of herbaceous legumes is found here, but also because this region is characterized by long-held grazing traditions.

Also in a macro-evolutionary context, by compiling information on seeds/fruits, climate and potential mammal dispersers through time, the likelihood of a 'diffuse mutualism' in the legume family could be substantiated (cf. Eriksson *et al.* 2000).

Moreover, the genome of *Medicago truncatula* is currently being sequenced as a model legume (Cook 1999), and this may open new

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* *Urtica* is currently being studied in a master thesis study at Ghent University.

perspectives for advanced research on dispersal and dormancy in legume seeds (Bolingue *et al.* 2010).

Due to the tremendous complexity that is inherent to (many) seed dispersal systems, continued efforts will be needed to also approach the costs/benefits balance in more theoretical ways. This can be performed through analytical models, or through simulation models in spatially realistic contexts. In this way, different vectors in dispersal can be contrasted to each other, yielding insights in how they affect plant population dynamics and evolution differently.

The consequences of endozoochory for plant conservation have not been treated explicitly in this thesis. Due to the context-dependency associated with many aspects from the process, the degree to which particular plant species make use of endozoochory is subject to substantial variation. Indeed, while Mouissie *et al.* (2005a) found mainly nitrophilous species to be dispersed by cattle, Eichberg *et al.* (2007) found only rare stress-tolerant species to eventually establish from sheep dung. However, grazers nonetheless provide seeds with a natural way of bridging middle to large distances. This gene flow can be of considerable importance for many plants in the face of habitat fragmentation, even if succesful recruitment is rare (Trakhtenbrot *et al.* 2005). Given its potential benefits, further research efforts on endozoochory would therefore continue contributing to plant and landscape conservation biology.

CHAPTER 7

Summary

Seeds are essential to plants. Consisting of an embryonic plant within a protective layer of maternal tissue, they serve the continuation of plants across generations.

Seed **dispersal** is the process in which seeds become displaced In nature, this happens in numerous ways, either abiotically (through wind or water) or biotically. Classic examples of animals serving as dispersal vectors include frugivorous birds or mammals, and ants. Large, herbivorous (foliage-eating) mammals, notably ungulates, also contribute to the dispersal of seeds. This is *done either through the adhesion of seeds in the fur and on legs* (epizoochory), or through the ingestion and subsequent excretion of seeds through feeding (**endozoochory**).

Although the latter process has been recognized for some time already – for instance, because of agronomical concerns–, it was granted attention by ecologists only relatively recently. From detailed observations, several hundreds of plant species proved to have viable seeds in the excrements of ungulates, often in large numbers. However, endozoochory is inherently very complex because (among other factors) it is determined by many constituent processes and patterns: seed production, seed ingestion, transfer, excretion, processes following deposition, germination and establishment... The fate of these seeds is therefore highly uncertain.

Also, it is unclear if ungulate endozoochory poses a selective force on the concerned plants and their seeds. There have been substantiated suggestions which pose that selection has been acting to such a degree, that grassland plants have adjusted their traits in response to facilitate this means of dispersal (e.g. small, inconspicuous seeds, palatable foliage,...). On the other hand, endozoochory might not be more than a rare and accidental fact without any significant result. Until today, this particularly challenging question has hardly been examined.

This thesis studies endozoochory, notably of herbaceous plants with dry (not fleshy) fruits by large grazers in temperate regions. The aims of this thesis are two-fold. On the one hand, we want to contribute to our ecological understanding of this dispersal process. For this, the focus is on previously unstudied patterns and processes. In addition, we try to explore the evolutionary-ecological meaning of endozoochory, by looking for a study system with which its selective strength can be determined more efficiently.

In **Chapter 2**, we integrate various available data on aspects that constitute endozoochory, from *in situ* seed production up to dung deposition, into a computer simulation model of seed dispersal. In short, the model introduces an individual animal into a two-dimensional landscape, in which it then moves, and ingests, digests and defecates seeds. We parameterized the model for the (actual) situation of endoochorous seed dispersal of 25 herbaceous plant species by Highland catle in the Westhoek-South nature reserve. For this parameterization, we used data from existent descriptive and experimental studies, and we collected new data (data concerning the landscape, seed availability in the field, plant ingestion, digestion, excretion and animal movement). We tested this model by germinating dung samples collected from the field and counting the seedlings emerging from them; this proved to result in a good correlation with the seedling numbers predicted by the model. Subsequently, we used the model to predict the spatial pattern in which seeds become distributed within the landscape, as this forms a key element in evaluating the effectiveness of ungulates as vectors in dispersal. We performed this mainly with respect to the vegetation types seeds end up in (given their suitability for plant establishment to particular species). Indeed, herbivores might displace seeds to suitable habitat more often than could be expected from random dispersal, a pattern known as 'directed dispersal'. Our model pointed out that cattle disperse seeds from grassland plants to suitable habitat in a directed manner, but that this does not hold for plants from other vegetations, e.g. from scrub. Simulations of landscape change moreover showed that the less grassland available, the stronger 'directed dispersal' acts. Moreover, simulations of wind dispersal showed that wind transfers seeds to suitable habitat more directed than grazers do, simply because seeds are transported over much shorter distances. (Most forbs and grasses have very limited wind dispersal capacities.) Grazers dó have the benefit of transferring seeds over longer distances, but apparently, this comes with a high cost of seeds being deposited in unsuitable habitat.

Even if seeds are deposited in apparently suitable habitat, numerous processes after deposition may act to determine the fate of seeds significantly. For instance, seeds can become predated or they can become displaced again. The latter is known as 'diplochory' (dispersal in two phases), and has mainly been studied for large-seeded plant species from tropical regions. By means of the experiments which are discussed in **Chapter 3**, we determined the role of dung beetles (coprophilous Scarabaeidae) in determining the fate of small seeds following endozoochorous deposition in a temperate grassland. In the main experiment, we mixed seeds from two grasses (*Agrostis capillaris* and *Poa pratensis*) with dung from cattle and horses, and subsequently deposited these samples on two sites within the Westhoek-South nature reserve. For half of these samples, the accessibility to dung beetles was prevented, which allowed us to determine their effect on the short-term establishment of seedlings. This proved to be clearly negative, contrary to the patterns found from tropical regions. This can be mainly attributed to the differences in nesting behaviour among beetles. The displacement of seeds by tropical dung beetles often includes a horizontal component, through which seeds escape density-dependent competition and predation. Seeds in our study system were –at best– buried in tunnels beneath the dung pat. This hampers establishment, and in this case, seeds appear to be better off with one disperser instead of two.

In **Chapter 4** we focus on the fate of seeds after they have been ingested by grazers. Indeed, destruction in the chewing apparatus and the alimentary tract of animals poses a severe bottleneck to seeds. A central question in this respect is which seed traits are determining for survival, and thus increase the chances for successful endozoochory. Therefore, we performed an experiment in which we fed seeds from 48 plant species to cattle. By collecting dung samples and allowing these to germinate, we could calculate what proportions of seeds were lost in the process. Although such experiments have been performed before, this study included the highest number of plant species so far. Moreover, these experiments yielded conflicting results concerning the role of seed traits in the process. This can be partially attributed to a statistical problem suffered by these studies: because all plant species are related to each other in some degree, they cannot be considered independent in statistical analysis. It can be shown that this also applies to the correlation of seed traits and mortality involved in such studies, and we therefore adopted a control for phylogenetic affinities instead. We found that neither the size nor shape of seeds account for differential mortality. The water-impermeability of seeds appears to hold better explanatory power in this respect, although a side condition is that seeds should be tough enough to resist fractures in the seed coat. In general, we expect from the results of this experiment as well as existing literature, that the importance of anatomical, structural, compositional and physical seed characteristics outweigh those of simple morphological traits.

Water-impermeability of seeds is a binary and easy-to-measure trait. However, it only occurs in a limited number of plant families, of which the legumes (Fabaceae) and the rockrose family (Cistaceae) are the most important in the face of endozoochorous dispersal (representatives of both are abundantly fed upon by grazers). Water-impermeability is a form of **dormancy** in these families: the process in which seeds postpone germination in time (i.e. the temporal complement of dispersal). In order to learn more on the role of this so-called 'physical dormancy', we performed a series of experiments on white clover (*Trifolium repens* L., Fabaceae). These are discussed in **Chapter 5** of this thesis. In summer 2008, a descriptive field study among six sites showed that the proportion of water-impermeable seeds within this species' inflorescences (heads) varied from 0 to 100 %. In effect, this means that mother plants bring forth both autumn-germinating seeds (those that are permeable or non-dormant) and spring-germinating seeds (those that are impermeable or dormant). The demographical consequences of this were investigated in more detail in a field experiment. From this, winter mortality among autumn-germinated plants proved to be limited, and these therefore obtained a head start in growth and flowering (which was more abundant) during the first summer. From a greenhouse experiment, the witnessed variation in the proportion of water-impermeable seeds within fruiting heads appeared to be a consequence of the ambient humidity during riping, and not of genetic consequences among individuals.

The results of Chapters 2 and 3 acknowledge that endozoochory is a particularly complex process. The degree to which seeds reach suitable habitat in a directed manner apparently is highly dependent on the landscape (Chapter 2; this chapter nonetheless shows that the process is to some degree predictable as well). If tunneling dung beetles are present, these can affect the chances of seeds negatively, although this is not equally pronounced on different sites (Chapter 3). For the other constituent processes, too, many complications are known. In addition, studies on endozoochory are complicated by its cryptic nature: the seeds involved are small and can remained ungerminated for long. Therefore, small spatial scales need to be followed over long time frames. For these reasons, the fate of endozoochorous seeds remains difficult to evaluate.

Consequently, the selective strength of endozoochory for dry-fruited plants also remains unclear. In this thesis, we emphasized the positive effect of water-impermeability in endozoochory (cf. Chapter 4), and showed that this trait varies *in situ* (Chapter 5). One could state that this satisfies two essential conditions for a trait to evolve under natural selection (i.e. a fitness-enhancing trait, which holds intraspecific variation). However, we also showed that the *in situ* variation is not the consequence of genetic differences (a third essential condition), but the consequence of environmental differences, and this thus stresses this trait's use as an *evolutionary-ecological* study *system. Nonetheless, this possibility is not* entirely excluded, since a genetic basis for this variation is confirmed in several other legumes (e.g. *Lupinus*, *Vicia*). (However, the extent of the *in situ* variation, and their relationships with grazers, needs further investigation.)

What is considered a seemingly intimate relationship between seeds and their dispersal vectors by many, such as that between birds and berries, actually concerns a rather 'diffuse' mutualism. Indeed, the relationship is between a set of plant species and a set of vectors (not: one-to-one), and takes place over long evolutionary time scales in which dispersers come and go. The here-studied form of endozoochory probably should be considered alike. Take legumes as an example. Very probably, early legume taxa already had their pods dispersed by a now extinct (mega)fauna. Gradually, herbaceous legumes arose on earth, paralleled by the rise of modern herbivorous grazers, and this could have allowed a continued existence of the legume-herbivore interaction. Albeit diffuse.

CHAPTER 8

Samenvatting

Zaden zijn voor planten van essentieel belang. Bestaande uit embryonale plantjes die door een laagje moederweefsel worden beschermd, verzorgen zij de voortzetting van planten overheen generaties.

Zaad**dispersie**, of –verbreiding[*], is het proces waarin zaden ruimtelijk worden verplaatst. Dit gebeurt in de natuur op talloze manieren, zowel abiotisch (door wind of water) als biotisch. Klassieke voorbeelden van dieren die als verbreidingsvectoren optreden, zijn vruchtetende (frugivore) vogels of zoogdieren, en mieren. Grote, loof-etende (herbivore) zoogdieren, met name grazende hoefdieren, dragen niettemin ook tot de verbreiding van zaden bij. Zij doen dit enerzijds via aanhechting van zaden in de vacht en aan poten (epizoöchorie), en anderzijds via opname en daaropvolgende uitscheiding van zaden met het voedsel (**endozoöchorie**).

Hoewel dit laatste proces sinds geruime tijd wordt (h)erkend, bv. vanuit landbouwkundige belangen, kwam het proces slechts relatief recent onder aandacht van ecologen. Uit nauwgezette obervaties blijken vele honderden plantensoorten levensvatbare zaden te tonen in de uitwerpselen van deze dieren, en dikwijls in grote aantallen. Evenwel blijkt het proces bijzonder complex van aard te zijn, onder andere omdat het door vele deelprocessen en –patronen wordt bepaald: zaadproductie, zaadopname, transfer, uitscheiding, processen na uitscheiding, kieming en vestiging... Het lot van deze endozoöchore zaden is dan ook verre van beslist.

Hiermee gepaard is het onduidelijk hoe de selectieve kracht van endozoöchorie door grote grazers op de betrokken planten en hun zaden moet worden begrepen. Er zijn onderbouwde suggesties die stellen dat selectie zodanig sterk en algemeen is (geweest) dat graslandplanten kenmerken hebben verworven ten voordele van deze dispersiewijze (bv.

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[*] Bouman *et al.* (2000) argumenteren dat het gebruik van 'verbreiding' moet worden vermeden omdat dit een germanisme betreft, en gebruiken 'verspreiding' in de plaats. Naar mijn mening is dit argument niet bezwarend genoeg, temeer omdat dit laatste alternatief gemakkelijk kan worden verward met het *patroon* van ruimtelijk voorkomen.

kleine, onopvallende zaden; eetbaar loof...). Aan de andere kant zou endozoöchorie niet meer kunnen zijn dan een zeldzaam en toevallig gegeven zonder merkelijk gevolg. Deze bijzonder uitdagende vraagstelling is tot op vandaag amper onderzocht.

Dit proefschrift bestudeert endozoöchorie, en wel van kruidachtige planten met droge (en niet: vlezige) vruchten door grote grazers in gematigde streken. Het doel van het proefschrift is tweeërlei. Enerzijds willen we een bijdrage leveren aan ons ecologisch begrip van dit dispersieproces. Hiervoor worden patronen en processen bestudeerd die voorheen nog niet of amper zijn behandeld. Daarnaast trachten we een lans te breken voor evolutionair-ecologisch onderzoek naar endozoöchorie, en wel door op zoek te gaan naar een studiesysteem waarmee diens selectieve kracht efficiënter kan worden bepaald.

In **hoofdstuk 2** integreren we bestaande resultaten omtrent de verschillende deelaspecten van zaadbeschikbaarheid tot –depositie, in een computergestuurd simulatiemodel van endozoöchorie. Kort omschreven introduceert het model een individueel dier in een landschap, dat vervolgens beweegt en zaden opneemt en afzet. We parametriseerden dit model voor de (bestaande) situatie van endozoöchore zaaddispersie van 25 kruidachtige plantensoorten door Hooglandrunderen in het Vlaams natuurreservaat 'Westhoek-Zuid'. Hiervoor werd data gebruikt uit beschrijvend en experimenteel onderzoek, alsook nieuwe data verzameld (data m.b.t. het landschap, zaadproductie in het veld, differentiële opname, vertering, uitscheiding & dierbeweging). We testten dit model door in het veld meststalen te verzamelen en te laten kiemen, en vonden een goede overeenstemming met de aantallen kiemplanten die door het model werden voorspeld. Vervolgens gebruikten we het model om het ruimtelijk patroon te voorspellen waarin zaden in het landschap worden verbreid, en wel met betrekking tot de verschillende vegetatietypes in het landschap. Het is voor planten immers van belang in gunstig habitat te belanden, waar zij een verhoogde kans op vestiging hebben. Dit staat gekend als 'gerichte dispersie'. Ons model wees uit dat de runderen planten van graslanden effectief gericht naar gunstig habitat verplaatsen, maar dat dit voor planten van bv. struwelen niet het geval is. Gesimuleerde landschapsveranderingen toonden bovendien aan dat, hoe minder grasland aanwezig, hoe sterker deze gerichte transfer is. Simulaties van windverbreiding toonden dan weer aan dat de wind zaden veel gerichter dan grazers in verondersteld gunstig habitat deponeert, eenvoudigweg omdat zaden over veel kortere afstanden worden verplaatst. (De meeste kruidachtige planten hebben zeer beperkte windverbreidingscapaciteiten.) Grazers bieden wél het voordeel dat zaden over grote afstanden worden verplaatst, maar kennelijk komt dit met een grote kost van zaden die in ongunstig habitat eindigen.

Zaden mogen dan al worden afgezet in gunstig habitat, na depositie kunnen tal van processen het lot van zaden bepalend sturen. Zo kunnen zaden door dieren worden gepredeerd of opnieuw worden verplaatst. Dit laatste staat gekend als 'diplochorie', en is voornamelijk bij grootzadige plantensoorten uit tropische regio's bestudeerd. Aan de hand van de experimenten die in **hoofdstuk 3** worden beschreven, gingen wij na welke rol mestkevers (coprofiele Scarabaeidae) spelen in het lot van kleine zaden na endozoöchore depositie in een gematigd grasland. In het voornaamste experiment werden zaden van twee grassen, *Agrostis capillaris* en *Poa pratensis*, gemengd met mest van runderen of paarden, en vervolgens uitgestald op twee sites binnen het Vlaams natuurreservaat 'Westhoek-Zuid'. Toegang voor mestkevers werd voor de helft van de stalen onmogelijk gemaakt, waardoor kon worden nagegaan wat hun effect was op de kortetermijnsvestiging van zaailingen. Deze bleek duidelijk negatief, tegengesteld aan de patronen die voor tropische regio's zijn beschreven. Dit valt in eerste instantie te wijten aan de verschillen in nestgedrag tussen kevers. De verplaatsing van zaden door tropische mestkevers houdt dikwijls een horizontale component in, waardoor zaden aan densiteitsafhankelijke competitie en predatie ontsnappen. In ons studiesysteem werden zaden – hoogstens– in tunnels onder de mest ingegraven. Dit belemmert vestiging, en in dit geval blijken zaden dus beter af met één dispersievector dan met twee.

In **hoofdstuk 4** spitsen we ons toe op het lot van zaden nadat deze door grazers zijn opgenomen. De vernieling van zaden in het kauwapparaat en de vertering in het maagdarmstelsel stelt zaden immers voor een duidelijke bottleneck. De centrale vraagstelling hierbij is welke zaadkenmerken bepalend zijn voor overleving, en dus de kans op succesvolle endozoöchorie verhogen. Wij voerden daarom een experiment uit waarbij we in totaal 48 plantensoorten voedden aan runderen. Aan de hand van opgekiemde meststalen kon worden berekend welke proporties van de zaden in het proces verloren waren gegaan. Hoewel dergelijke experimenten al eerder zijn uitgevoerd, omvatte deze studie het hoogste aantal plantensoorten totnogtoe. Bovendien bleken deze experimenten tegenstrijdige resultaten op te leveren wat betreft de rol van zaadkenmerken in het proces. Dit kan ten dele worden verklaard door een statistisch probleem bij dergelijke studies: omdat plantensoorten steeds in meer of mindere mate met elkaar verwant zijn, kunnen zij in analyses niet als onafhankelijk worden beschouwd. Dit heeft aantoonbaar tot foutieve conclusies geleid wat betreft verbanden tussen zaadkenmerken en overleving, en wij maakten daarom gebruik van een controle voor fylogenetische verwantschap. Wij vonden dat noch de grootte of het gewicht van zaden, noch hun vorm, verschillen in overleving verklaarden. Een beter verklarend kenmerk lijkt de waterondoordringbaarheid van zaden te zijn, hoewel als randvoorwaarde moet gelden dat zaden stevig genoeg moeten zijn om breuken in de zaadhuid te weerstaan. In het algemeen vermoeden wij op basis van de resultaten van dit experiment en de bestaande literatuur, dat het belang van anatomische, structurele, compositionele en fysieke zaadkenmerken dat van eenvoudige morfologische kenmerken overstijgt.

Waterondoordringbaarheid van zaden is een binair en eenvoudig te meten kenmerk. Het komt echter slechts bij een beperkt aantal plantenfamilies voor, waarvan de vlinderbloemigen (Fabaceae) en de zonneroosjesfamilie (Cistaceae) de meest noemenswaardige zijn in het licht van endozoöchore verbreiding (vertegenwoordigers van beiden worden veelvuldig door grazers gegeten). Waterondoordringbaarheid vormt bij deze families een vorm van **dormantie**, wat het proces is waarmee zaden kieming in de tijd uitstellen (het temporele complement van dispersie). Om meer over de rol van deze zogeheten 'fysieke dormantie' te weten te komen, voerden wij een reeks experimenten uit op witte klaver (*Trifolium repens* L., Fabaceae). Deze worden in **hoofdstuk 5** van dit proefschrift beschreven. In de zomer van 2008 wees een beschrijvende veldstudie overheen zes sites uit dat de proportie van waterondoordringbare zaden in vruchthoofdjes varieert van 0 tot 100 %. Dit betekent concreet dat moederplanten zowel herfstkiemers (de doordringbare of niet-dormante zaden) als lentekiemers voortbrengen (de ondoordringbare of dormante zaden). De demografische gevolgen hiervan werden in een veldexperiment verder uitgeklaard. Hieruit bleek wintersterfte bij herfstkiemers gering te zijn, waardoor deze met een groeivoorsprong sneller en abundanter dan lentekiemers tot bloei kwamen in de eerste zomer. Uit een serre-experiment bleek dan weer dat de vastgestelde variatie in de proporties ondoordringbare zaden in vruchthoofdjes een gevolg is van de heersende vochtigheid tijdens zaadrijping, en niet van genetische verschillen tussen individuën.

De resultaten van hoofdstukken 2 en 3 onderschrijven dat endozoöchorie een bijzonder complex proces is. De mate waarin zaden gericht in geschikt habitat terechtkomen blijkt sterk af te hangen van het landschap (hoofdstuk 2; uit dit hoofdstuk blijkt echter ook dat het proces tot op zekere hoogte voorspelbaar is). Indien tunnelgravende mestkevers aanwezig zijn, kunnen deze de kansen van zaden negatief beïnvloeden, hoewel dit niet op elke site even sterk bleek te gelden (hoofdstuk 3). Ook voor de andere deelprocessen zijn vele complicaties bekend. Bovendien worden studies naar endozoöchorie bemoeilijkt door het cryptische karakter ervan: de betreffende zaden zijn klein en kunnen lang ongekiemd blijven, waardoor kleine ruimtelijke schalen over lange tijdsvensters moeten worden gevolgd. Hierdoor blijft de levenswandel van endozoöchoor verbreide zaden nog steeds moeilijk te vatten.

Hiermee gepaard, tasten we ook wat betreft de selectieve kracht van endozoöchorie voor droogvruchtige planten nog steeds grotendeels in het duister. In dit proefschrift werd erop gewezen dat de waterondoordringbaarheid van zaden endozoöchoor succes positief beïnvloedt (hoofdstuk 4), én dat dit kenmerk *in situ* varieert (hoofdstuk 5). Men zou kunnen stellen dat hiermee twee basisvoorwaarden zijn voldaan voor een kenmerk om te kunnen evolueren onder natuurlijke selectie (nl. een fitness-bepalend kenmerk, en variatie hierin tussen individuën). Echter, we toonden ook aan dat deze *in situ* variatie niet het gevolg is van genetische verschillen, maar van verschillen in de omgeving, en dit zet het gebruik van dit kenmerk als een evolutionair-ecologisch studiesysteem dus onder druk. Niettemin is deze mogelijkheid niet geheel uitgesloten, aangezien een genetische basis voor deze variatie bij sommige andere vlinderbloemigen wél is bevestigd (vb. *Lupinus*, *Vicia*[*]).

Wat door velen als een ogenschijnlijk intieme relatie tussen zaden en hun verbreidingsvectoren wordt aanzien, zoals dat tussen een vogelsoort en een boom met vlezige vruchten, betreft eigenlijk een eerder 'diffuus mutualisme'. De relatie is tussen een blok van plantensoorten en een blok van vectoren (niet: één-één), en vindt plaats over lange evolutionaire tijdsschalen waarin vectoren komen en gaan. Ook de hier bestudeerde vorm

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[*] Maar de grootte van de *in situ* variatie en de relaties van deze taxa met grazers moet nog verder worden uitgezocht.

van endozoöchorie valt mogelijks zo te begrijpen. Het heeft er alle schijn van dat de vroeg(st)e vlinderbloemigen hun grote peulen door een nu grotendeels uitgestorven megafauna zagen verbreid. Door wijzigingen in het klimaat ontstonden geleidelijk de kruidachtige vlinderbloemigen, en ook moderne, herbivore grazers. Endozoöchore zaadverbreiding was dus reeds vóór het ontstaan van beide groepen een feit, en heeft wellicht onophoudelijk bestaan –zij het als een diffuse interactie.

CHAPTER 9

Appendices

Appendix 1A – Endozoochory qualified

Below, Table 1A presents a tentative list of studies that have surveyed the seed content of dung from free-ranging ungulates. It is not intended to present an exhaustive list of all such studies; in effect, many more could be added.

Given are the short references (note : these have not been additionally included in the list of References in Chapter 10), the animal species involved, an indication of the number of plant species involved, and a brief habitat description (with two-digit country codes).

Appendix 1B – Endozoochory quantified

Below, Table 1B reproduces the (scarce) estimations from literature of endozoochorous seed dispersal rates, i.e. the number of seeds transferred per time unit by individual, free-ranging ungulates.

Given are the short references (note : these have not been additionally included in the list of References in Chapter 10), the animal species involved, the seed dispersal rate estimates, and a brief habitat description (with two-digit country codes).

Appendix 1C – Seed feeding experiments

Below, Table 1C presents an overview of studies in which seeds have experimentally been fed to ungulates. Given are the short references (note : these have not additionally been included in the list of References at the end *of this thesis) and a short description.*

Contrary to the former, this Appendix is intended to provide an exhaustive list of all such feeding experiments. Nonetheless, some additional references might be found from the literature study of Bonn and Poschlod (1998).

Table 1A. Endozoochory qualified. See above.

authors	year	source	animal sp. [# plant species]	habitat (country)
Muller-Schneider	1954	Vegetatio 5: 23-28	cattle [?], goat [?], sheep [?]	mountain pastures (CH)
Welch	1985	J Appl Ecol 22: 461-472	cattle [?], red deer [?], sheep [?]	heather moorland (GB)
Malo and Suárez	1995	Oecologia 104: 246-255	fallow deer [67], red deer [66], cattle [78]	dehesa (ES)
Dai	2000	J Veg Sci 11: 715-724	cattle [26]	alvar limestone grassland (SE)
Pakeman et al.	2002	Fun Ecol 16: 296-304	sheep [21]	several (GB)
Traba et al.	2003	Rest Ecol 11: 378-384	cattle [53]	Mediterranean grassland (ES)
Schmidt et al.	2004	Eur J For Res 123: 167-176	roe deer [36], wild boar [51]	forest (DE)
Myers et al.	2004	Oecologia 139: 35-44	white-tailed deer [72]	mixed deciduous forest (US)
Mouissie et al.	2005	Bas Appl Ecol 6: 547-558	cattle [56], horse [35], sheep [31]	landscape mosaic (NL)
Cosyns et al.	2005	Plant Ecol 178: 149-162	horse [90, 67], cattle [67, 82]	coastal dune landscape (BE)
Cosyns and Hoffmann	2005	Bas Applied Ecol 6: 11-24	horse [106]	several coastal dune landscapes (BE)
Bruun and Poschlod	2006	Oikos 113: 402-411	cattle [57]	moist meadow (DE)
Bråthen et al.	2007	Ecography 30: 308-320	reindeer [20]	low alpine vegetation (FI)
Eichberg et al.	2007	Flora 202: 3-26	sheep [28]	inland sand ecosystem (DE)
Eycott et al.	2007	Oecologia 154: 107-118	red deer & fallow deer [96], roe deer [40], Reeves's muntjac [31]	forest mosaic (GB)
Baraza and Valiente-Banuet	2008	J Arid Env 72: 1973-1976	goat [>5]	semiarid thornscrub (MX)
Bakker et al.	2008	Plant Ecol 197: 43-54	cattle [60]	salt-marshes and dunes (NL)
Bruun et al.	2008	Oecologia 155: 101-110	muskox [21]	arctic (GL)
Schoenbaum et al.	2009	Crop & Pasture Sci 60: 675-683	sheep [35]	agricultural landscape (IL)
Vignolio et al.	2010	Ann Bot Fenn 47: 14-22	cattle [41]	pampa grasslands (AR)
Kuiters and Huiskes	2010	Appl Veg Sci 13: 163-172	sheep [72]	calcareous grasslands (NL/BE)
Davis et al.	2010	Biol Invasions 12: 1079-1092	hog deer [42]	several (AU)

Table 1B. Endozoochory quantified. See above.

authors	year	source	animal sp. [#]	habitat
Malo and Suárez	1995	Oecologia 104: 246-255	fallow and red deer [20 000 seeds/day], cattle [300 000 seeds/day]	dehesa (ES)
Bonn and Poschlod	1998	several references in *Ausbreitungsbiologie der Pflanzen Mitteleuropas*: 202-203	cattle [6000 seeds/day; 903 760 seeds/season; > 10 000 seeds/day; 3000 seeds/day]	-
Malo and Suárez	1998	Ecography 21: 204-211	red deer [> 24 000 *Cistus ladanifer*/day in summer]	dehesa (ES)
Schmidt et al.	2004	Eur J For Res 123: 167-176	roe deer [38 seeds/day], wild boar [12 seeds/day]	forest (DE)
Myers et al.	2004	Oecologia 139: 35-44	white-tailed deer [10 seeds/m²/year]	mixed deciduous forest (US)
Mouissie et al.	2005	Bas Appl Ecol 6: 547-558	cattle [2 600 000 seeds/year], horse [500 000 seeds/year], sheep [40 000 seeds/year]	landscape mosaic (NL)
Cosyns et al.	2005	Plant Ecol 178: 149-162	horse [1 200 000 and 291 000 seeds/summer], cattle [1 200 000 and 382 000 seeds/summer]	coastal dune landscape (BE)
Eycott et al.	2007	Oecologia 154: 107-118	red deer & fallow deer [750 seeds/day], roe deer [86 seeds/day], Reeves's muntjac [13 seeds/day]	forest mosaic (GB)
Davis et al.	2010	Biol Invasions 12: 1079-1092	hog deer [> 130 000 seeds/day]	several (AU)

Table 1C. Seed feeding experiments. See above

authors	year	source	remark
Kerner v. Marilaun	1898	Natural history of plants	several spp. fed to horse, cattle & hogs. Lacks quantification
Kempski	1906	PhD (172pp.)	cited in Bonn (2004)
Korsmo	1911	Tidsskr. Nor. Landburk 18: 223-230	cited in Burton and Andrews (1948): 6 spp. fed to horse, cattle & hogs
Milne	1915	Agric. J. India 10: 353-369	wheat & grass sp. fed to cattle
Harmon and Keim	1934	Journal of the American society of agronomy 26: 762-767	cited in Burton and Andrews (1948)
Atkeson et al.	1934	Journal of the American society of agronomy 26: 390-397	cited in Burton and Andrews (1948)
Burton and Andrews	1948	Journal of Agricultural Research 76: 95-103	7 grass and 1 legume spp. fed to cattle
McCully	1951	Journal of Range management 4: 101-106	1 rose sp. fed to cattle.
Lehrer and Tisdale	1956	Journal of range management 9: 118-122	6 spp. fed to sheep & rabbit
Lennartz	1957	Zeitsshrift Acker-Pflanzenbau 103: 427–453	17 plant spp. fed to cattle
Kirk and Courtney	1972	Proceedings of the 11th British Crop protection conference 11/1972: 226-233	cited in Bonn (2004)
Yamada and Kawaguchi	1972	Journal of Japanese Society of Grassland Science 18: 8-15	5 spp. fed to cattle
Özer and Hesimoglu	1977	Journal of Turkish phytopathology 6: 105-110	cited in Bonn (2004)
Takabayashi et al.	1979	Japan Agricultural Research Quarterly 13: 204-207	3 spp. fed to cattle
Özer	1979	Weed Research 19: 247– 254	10 spp. fed to sheep
Janzen	1981	Ecology 62: 593-601	1 legume sp. fed to horse.
Janzen	1982	Oikos 38: 150-156	1 legume sp. fed to horse & cattle.

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Simao Neto et al	1987	Australian journal of experimental agriculture 27: 239-246	6 spp. fed to cattle, sheep & goats.
Simao Neto and Jones	1987	Australian Journal of experimental agriculture 27: 247-251	2 grass & 4 legume spp. subjected to in sacco (cattle) & in vitro digestion
Jones and Simao-Neto	1987	Australian journal of experimental agriculture 27: 253-256	4 spp. fed to sheep
Norton et al	1989	Tropical grasslands 23: 219-224	1 sp. (4 different seed lines) fed to cattle
St. John-Sweeting & Morris	1990	Proceedings of the 9th Australian weeds conference	6 spp. fed to horse
Thomson et al.	1990	Journal of agricultural science 114: 295-299	legumes fed to sheep
Blackshaw and Rode	1991	Weed Science 39: 104–108	several species subjected to cattle digestion
Barrow and Havstad	1992	Journal of arid environments 22: 395-399	4 spp.fed to cattle
Russi et al.	1992	Journal of applied ecology 29: 772-778	3 clover species fed to sheep
Lacey et al.	1992	Weed technology 6: 599-602	cited in Bonn (2004)
Gardener et al.	1993	Journal of applied ecology 30: 75-85	47 legume & grass spp. subjected to semi-vivo cattle ingestion
Gardener et al.	1993	Journal of applied ecology 30: 63-74	10 legume and 8 grass spp. fed to cattle
Wallander et al.	1995	Journal range management 48: 145-149	1 sp. fed to mule deer and sheep
Shayo and Uden	1998	Tropical grasslands 32: 195-200	4 shrub spp. fed to cattle, sheep & goat
Ghassali et al	1998	Experimental agriculture 34: 391-405	legume spp. fed to sheep
Armke and Scott	1999	Texas Journal of Agriculture and natural resources 12: 28	6 spp. fed to cattle
Ide et al	1999	Grassland science 45: 157-162	3 grass spp. fed to cattle
Doucette et al.	2001	Journal of range management 54: 575-581	7 plant spp. fed to cattle
Gökbulak	2002	Grass and forage science 57: 395-400	6 spp. fed to bison

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Razanamandranto et al.	2004	Flora 199: 389-397	4 woody spp. fed to cattle & sheep
Bonn	2004	PhD: 77-121 (Ch. 5)	14 and 12 spp. fed to cattle & sheep
Cosyns et al.	2005	Journal of ecology 93: 353-361	19 spp. fed to sheep, cattle, rabbit, horse & donkey
Manzano et al.	2005	Seed science research 15: 21-28	5 shrub spp. fed to sheep
Mouissie et al.	2005	Functional ecology 19: 284-290	25 spp. fed to fallow deer
Gökbulak	2006	OnLine Journal of Biological Sciences 6: 23-27	2 grass spp. fed to cattle
Peco et al.	2006	Acta oecologica 30: 269-275	20 spp. subjected to simulated sheep ingestion
Ramos et al.	2006	Plant ecology 185: 97-106	6 Cistaceae spp. fed to sheep
Pakeman and Small	2009	Basic and applied ecology 10: 656-661	12 spp. fed to sheep
D'hondt and Hoffmann	2011	Plant biology	47 spp. fed to cattle

Appendix 2A – Model overview, design concepts, and details

In this Appendix, the model from Chapter 2 is described in full detail following the ODD (Overview/ Design concepts/ Details) protocol for agent-based models (Grimm *et al.* 2006, 2010). Given the purpose of the model to accommodate much empirical data from different sources, the 'Input data' paragraph is elaborated on relatively thoroughly. Parts of this overview may found to be somewhat redundant with Chapter 2, but such overlap is unavoidable as this Appendix aims at being complete and fully comprehensible in itself.

A. Purpose
B. Entities, state variables and scales
C. Process overview and scheduling
D. Design concepts
  1. Basic principles
  2. Emergence
  3. Stochasticity
  4. Observation
E. Initialization
F. Input data
  1. Landscape: vegetation map
  2. Animal movement: lengths of stay in cells
  3. Animal movement: vegetation preference coefficients
  4. Animal movement: direction preference coefficients
  5. Grazing: cell probability for becoming grazed
  6. Grazing: local seed availability
  7. Grazing: plant species' forage values
  8. Grazing: seed survival probabilities following ingestion
  9. Defecation: time intervals between excretions
  10. Defecation: seed excretion probabilities
G. Submodels
  1. Animal movement
  2. Grazing / seed ingestion and digestion
  3. Defecation / seed excretion

A. Purpose

The purpose of this simulation model is to better understand the quantitative aspects underlying endozoochorous seed dispersal by ungulates in single landscapes. The model is explicitly constructed to accommodate and integrate the limited data we have on (only some of) the individual processes and patterns from landscapes, plants and animals that –together– constitute endozoochory in the field. Given the relatively large body of data on both the terrain, vegetation and grazers of the Westhoek-South reserve (Belgium), model parameterization primarily examines this case in particular. Nonetheless, the model could easily be adjusted to accommodate other, including more generic, data.

B. Entities, state variables and scales

The model simulates a continuously vegetated landscape in which an individual animal moves and disperses seeds from various plant species. Model runs are intended to simulate periods of several days.

The landscape is a grid of cells, each of which corresponds to 1 m^2 in the field. Each cell is characterized by its grid coordinates, and one of six major vegetation types (forest, scrub, grassland, rough grassland, dune, and wetland; see Table 2-2). A grid cell can harbour a certain amount of seeds from each of twenty-five plant species (see Table 2-1). The amount of seeds in a cell depends on both the plant species and the vegetation type in that cell.

The animal is characterized by several state variables. These include its position (grid cell coordinates), an indication of how long it is staying in that position (a countdown timer), and the direction is was moving in. In addition –and of central importance to the model– the animal is characterized by its 'gut contents', i.e. the number of viable seeds that are contained in the animal and awaiting excretion.

C. Process overview and scheduling

After having introduced the animal into the landscape, the model proceeds in steps. Each step corresponds to a repositioning of the animal from the cell it was in to one of its neighbouring cells. The length of stay in the new cell, and thus the time interval associated with a model step, is therefore variable (and in the order of several seconds to minutes). Movement occurs through the interplay of two parameters: the animal's preference to move through the vegetation types it is facing, and its internal propensity to change direction. Details are provided below ('Animal movement' submodel).

After arrival, there is a chance for the new cell to become grazed. If so, seed amounts for each of the plant species are attributed to that cell, and subsequently, the animal ingests and digests some portion of these seeds ('Grazing / seed ingestion and digestion' submodel). Apart from that, defecation may occur in the new cell. If so, some portion of the seeds from within the animal becomes excreted ('Defecation / seed excretion' submodel).

Steps are repeated until the pre-set duration to be simulated is reached. A brief summary of the main model variables is included in Table 2A-1.

D. Design concepts

1. Basic principles

This model treats endozoochory as an assembly of processes in which either landscape, animal or plant attributes (or a combination thereof) play a role (cf. the seed dispersal cycle of Wang and Smith 2002). Some of these processes have been studied in their own respect, and the findings of such studies are therefore contained in the model (as data or parameters).

2. Emergence

Patterns of endozoochory should then emerge from the interaction of the individual processes and patterns implemented. A major output, for instance, is the number of seeds that are dispersed through endozoochory. This emerges from the distribution of vegetation types, the availability of seeds within vegetations, animal visitation rate, plant forage value and seed resistance to digestion. Some of these aspects are affected by a subset of attributes themselves, e.g. animal visitation rate emerges from animal movement and landscape configuration. The spatial pattern in which seeds are dispersed (a major focus in Chapter 2) emerges from animal and landscape attributes.

3. Stochasticity

A great deal of stochasticity is included in the parameterized model. Firstly, values for most of the model variables are drawn randomly from structures holding raw data (see Table 2A-1). Note that this approach avoids making assumptions on parameter distributions. Secondly, several binary decisions in the model are based on probabilities; e.g. whether a new cell will be grazed or not, whether a given seed from within the animal will be excreted or not, *etc.* It is thus extremely unlikely that model runs will ever yield the exact same results, and for this reason, multiple model runs are performed for inference.

4. Observation

Apart from the internal state of the landscape and the animal, data that can be retrieved from the model at any point during simulation include a log of the animal's path, and of the seeds contained in all defecations.

The seed content of simulated defecations is used directly for validation with independent observations (Rykiel 1996). Also, we tested whether the submodel for animal movement yielded simulated paths which correspond to initial observations well; this is discussed in Appendix 2C.

If animal path simulations prove to be realistic, model output can be confidently used for ecological inference on spatial patterns of seed deposition.

E. Initialization

In our runs, the animal was always introduced on a fixed position in the grassland vegetation. Since the animal starts off 'on an empty stomach', each run includes a preparatory period of five simulated days that are considered irrelevant.

Table 2A-1. An overview of the model variables that needed parameterization. An indication is given on how they are treated in the model. See text for details (paragraphs F.1. to F.10.).

F.1.	Vegetation map	fixed matrix
Animal movement		
F.2.	Length of stay in cells (minutes)	random draw from vector
F.3.	Preference coefficient for vegetation type x	fixed for x
F.4.	Propensity to change direction	fixed
Grazing / seed ingestion & digestion		
F.5.	Probability for new cell to become grazed	fixed
F.6.	# seeds of plant sp. x in a cell of vegetation type y	random draw from vector for x/y-combination
F.7.	Forage value of plant sp. x	fixed for x
F.8.	Probability for a seed of plant sp. x to survive ingestion	random draw from vector for x
Defecation / seed excretion		
F.9.	Time interval between two excretion events (minutes)	random draw from vector
F.10.	Probability for seed to be excreted as function of time (min)	fixed function

F. Input data

1. Landscape: vegetation map

A vegetation map of Westhoek-South was compiled by Ebrahimi (2007), who visually interpreted aerial images of the area that were made in 2004. We adopted this map, although some adjustments were needed. Some parts of the area had been fenced recently, and we therefore entered these in the map. We also lumped some minor vegetation types (e.g. 'white' and 'grey' dunes), and redefined some doubtful vegetation polygons after visits in the field. We opted for the landscape to consist of the six vegetation types mentioned above. Then, the map was converted to a numerical grid with a cell size of 1 m^2.

This resulting matrix consisted of 685 ×1391 elements, of which 559448 represented vegetated cells accessible to the animal. The integer landscape is visualized in Fig. 2-1.

2. Animal movement: lengths of stay in cells

We opted for the animal's path to be a continuous linkage of neighbouring cells, and therefore, we let a model step coincide with a (spatial) cell-to-cell step of the animal. The time interval associated with a model step then reflects the length of stay in that particular cell of 1 m^2.

Lamoot *et al.* (2005) extensively recorded the behaviour of individual Highland cattle at the Westhoek-South reserve in six-hour observation sessions, which included mapping its position every 15 minutes. We digitalized these positions (275 in total, from 13 sessions) to determine the animals' speed, through calculations of the spatial and temporal intervals between successive observations. The duration for which the animal resides in a cell of 1 m^2 then ≈ speed-1. These values, however, need to be weighed according to their spatial component (e.g. one observation of 2 m/s transforms into two observations of 0.5 s/m). For speed observations of 0 m/s (i.e. when cattle positions remained unchanged over successive observations), no time expenditure values could be calculated. Here, the full duration for which the animal stayed at that position was taken instead.

These calculations resulted in 7519 time interval values. A frequency distribution of these is provided in Fig. 2A-1. The values are contained in a vector from which the model randomly draws values when needed.

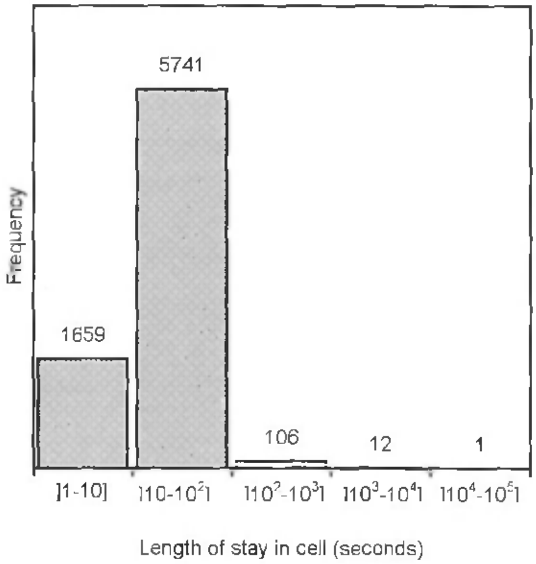


Figure 2A-1. Frequency distribution of the values for the time spent per cell (1 m^2).

3. Animal movement: vegetation preference coefficients

The same positional data from Lamoot *et al.* (2005) was used to calculate coefficients for the animal's preference for each vegetation type (cf. the model of Couvreur *et al.* 2008). By digitalizing their positions on our vegetation map, we were able to calculate the number of observed positions in each vegetation type. These numbers were then divided by the vegetation types' surface areas, and subsequently rescaled by dividing them by the highest value.

The resulting vegetation preference coefficients are given in Table 2-2. These remained unchanged during simulation.

4. Animal movement: direction preference coefficients

When movement is exclusively based on vegetation preference coefficients, the animal more or less resides near its position of introduction. In order to 'force' some directionality into movement –to make the animal explore new parts of the terrain– a direction coefficient was introduced. This coefficient stimulates the animal to continue moving in the direction it was in previously, and thus represents a propensity that is internal to the animal and independent from the environment.

Unfortunately, such a coefficient could not be derived from the positional data of Lamoot *et al.* (2005), as it is impossible to tease apart such internal behaviour from the effect of the landscape in this heterogeneous environment. For that reason, we used arbitrary values instead. Coefficients were set to 1.0 (straight movements), 0.2 (slight turns) and 0.0 (sharp turns). *In a homogeneous environment, these values result in the animal to turn in* about one out of three steps. Their functioning is illustrated ('Animal movement ' submodel) and evaluated in Appendix 2C.

5. Grazing: cell probability for becoming grazed

The probability for a cell to be grazed was fixed at 0.302; this is the time Highland cattle spend grazing at Westhoek-South in summer (as published by Lamoot *et al.* 2005).

6. Grazing: local seed availability

To quantify the availability of seeds at the scale of grid cells (1 m^2), we randomly located plots (1 m^2) in each of the vegetation types during summer 2008. We then determined the total number of seeds present within the plot, using species-specific protocols. The protocol consisted of counting the number of infructescences per plot (or lower levels, e.g. fruits, if convenient), and then hierarchically sampling the missing levels down to the individual seeds (e.g. fruits per infructescence, seeds per fruit...). Estimates were then made on the seed quantities within each of the plots for each of the species.

For each plant species/vegetation type combination, these values were contained in vectors (6 vegetation types × 25 plant species, 25-34 values per vector). Table 2A-2 provides summary statistics for each of them. The model randomly draws values from these vectors when needed.

Table 2A-2. Summary statistics for the resulting vectors from the field survey on local seed production. Per vegetation type, the number of studied plots (1 m^2) is indicated between brackets. Frequency (F) is the number of plots in which the species was found fruiting; minimum (N), mean (A) and maximum (X) values only apply to those plots. Figures for the vegetation type where we found it most frequently fruiting are given in grey.

		Scrub	Forest	Grassland	Rough grass.	Dune	Wetland
		(n = 34)	(n = 29)	(n = 34)	(n = 25)	(n = 30)	(n = 30)
Agrostis	*capillaris*	F= 3	F= 0	F= 8	F= 0	F= 0	F=0
		N= 18.5		N= 37.0			
		A= 61.7		A= 376.9			
		X= 92.5		X= 1258.0			
Agrostis	*stolonifera*	F= 2	F= 2	F= 31	F= 3	F= 0	F= 15
		N= 261.2	N= 65.3	N= 65.3	N= 65.3		N= 65.3
		A= 457.1	A= 261.2	A= 2826.9	A= 631.2		A= 278.6
		X= 653.0	X= 457.1	X= 11493	X= 1436.6		X= 914.2
Carex	*arenaria*	F= 11	F= 0	F= 0	F= 5	F= 19	F= 0
		N= 27.0			N= 53.0	N= 52.0	
		A= 217.3			A= 86.2	A= 231.5	
		X= 1064.0			X= 182.0	X= 728.0	
Carex	*flacca*	F= 0	F= 0	F= 4	F= 2	F= 0	F= 0
				N= 119.0	N= 18.0		
				A= 231.8	A= 180.0		
				X= 358.0	X= 342.0		
Carex	*hirta*	F= 0	F= 0	F= 8	F= 0	F= 0	F= 0
				N= 14.0			
				A= 191.1			
				X= 795.0			
Carex	*oederi*	F= 0	F= 0	F= 2	F= 0	F= 0	F= 0
				N= 57.6			
				A= 2563.2			
				X= 5068.8			
Cerastium	*fontanum*	F= 1	F= 0	F= 22	F= 3	F= 2	F= 0
		N= 21.6		N= 10.8	N= 54.0	N= 10.8	
		A= 21.6		A= 236.6	A= 205.2	A= 27.0	
		X= 21.6		X= 831.6	X= 432.0	X= 43.2	
Cerastium	*semidec.*	F= 0	F= 0	F= 0	F= 0	F= 1	F= 0
						N= 22.0	
						A= 22.0	
						X= 22.0	

Table 2A-2, *continued*.

Galium	*aparine*	**F= 11**	F= 10	F= 0	F= 7	F= 0	F= 10
		N= 20.0	N= 2		N= 12.0		N= 142.0
		A= 477.1	A= 133.4		A= 278.6		A= 1034.4
		X= 2366.0	X= 700.0		X= 784.0		X= 3614.0
Galium	*mollugo*	F= 1	F= 1	F= 0	F= 0	F= 0	**F= 1**
		N= 32.3	N= 28				**N= 119.0**
		A= 32.3	A= 28				**A= 119.0**
		X= 32.3	X= 28				**X= 119.0**
Galium	*palustre*	F= 0	F= 0	**F= 11**	F= 1	F= 0	F= 3
				N= 1.3	N= 159.0		N= 1.3
				A= 61.6	A= 159.0		A= 3.1
				X= 320.2	X= 159.0		X= 4.0
Galium	*uliginosum*	F= 1	F= 0	**F= 6**	F= 1	F= 1	F= 6
		N= 105.0		**N= 4.6**	N= 62.0	N= 66.0	N= 21.0
		A= 105.0		**A= 1335.7**	A= 62.0	A= 66.0	A= 92.4
		X= 105.0		**X= 7855.7**	X= 62.0	X= 66.0	X= 148.8
Galium	*verum*	**F= 1**	F= 0	F= 0	F= 0	F= 0	F= 0
		N= 20.0					
		A= 20.0					
		X= 20.0					
Juncus	*articulatus*	F= 0	F= 0	**F= 23**	F= 0	F= 0	F= 0
				N= 41.4			
				A= 7095.0			
				X= 30070			
Juncus	*bufonius*	F= 0	F= 0	**F= 21**	F= 0	F= 0	F= 0
				N= 157.5			
				A= 6675.0			
				X= 30330			
Juncus	*inflexus*	F= 0	F= 0	F= 1	**F= 2**	F= 0	F= 0
				N= 6498.0	**N= 1314.0**		
				A= 6498.0	**A= 1665.0**		
				X= 6498.0	**X= 2016.0**		
Mentha	*aquatica*	F= 0	F= 0	**F= 2**	F= 0	F= 0	F= 1
				N= 56.0			N= 244.0
				A= 286.0			A= 244.0
				X= 516.0			X= 244.0
Plantago	*major*	F= 0	F= 0	**F= 11**	F= 0	F= 0	F= 0
				N= 39.6			
				A= 1913.6			
				X= 17017			
Poa	*annua*	F= 0	F= 0	**F= 6**	F= 0	F= 0	F= 0
				N= 19.4			
				A= 433.3			
				X= 1280.4			

Table 2A-2, *continued.*

Poa	*pratensis*	F= 1	F= 0	F= 3	F= 1	F= 0	F= 1
		N= 31.8		N= 31.8	N= 127.2		N= 63.6
		A= 31.8		A= 180.2	A= 127.2		A= 63.6
		X= 31.8		X= 349.8	X= 127.2		X= 63.6
Poa	*trivialis*	F= 4	F= 10	F= 9	F= 7	F= 0	F= 6
		N= 14.6	N= 29.2	N= 14.6	N= 14.6		N= 14.6
		A= 124.1	A= 178.1	A= 30.8	A= 56.3		A= 119.2
		X= 248.2	X= 584.0	X= 87.6	X= 146.0		X= 350.4
Trifolium	*campestre*	F= 0	F= 0	F= 1	F= 0	F= 0	F= 0
				N= 61.0			
				A= 61.0			
				X= 61.0			
Trifolium	*dubium*	F= 0	F= 0	F= 7	F= 0	F= 0	F= 0
				N= 20.2			
				A= 539.1			
				X= 2541.0			
Trifolium	*pratense*	F= 0	F= 0	F= 2	F= 0	F= 0	F= 0
				N= 196.2			
				A= 286.6			
				X= 377.0			
Trifolium	*repens*	F= 1	F= 0	F= 29	F= 0	F= 0	F= 0
		N= 53.2		N= 12.6			
		A= 53.2		A= 1503.5			
		X= 53.2		X= 4924.9			

7. Grazing: plant species' forage values

Not every species becomes ingested at the same rate due to diet preferences of the animal, and we therefore chose to include the latter as a plant-specific variable in the grazing submodel. Based on various palatability characteristics, Briemle *et al.* (2002) defined forage values for livestock for a wide set of grassland plant species. We adopted these forage values (available from the BiolFlor database; Klotz *et al.* 2002), assuming that they relate directly to the ingestion of plant material. From their ordinal 1-to-9 scale, values were rescaled on a 0-to-1 interval and then squared to stretch differences among species.

Table 2A-3 lists the resulting values for each of the plant species. These remained fixed throughout model simulations.

Table 2A-3. Forage values of the study species.

Agrostis capillaris	0.391	*Juncus articulatus*	0.016
Agrostis stolonifera	0.141	*Juncus bufonius*	0.016
Carex arenaria	0.016	*Juncus inflexus*	0.016
Carex flacca	0.250	*Mentha aquatica*	0.016
Carex hirta	0.063	*Plantago major*	0.063
Carex oederi	0.063	*Poa annua*	0.391
Cerastium fontanum	0.141	*Poa pratensis*	1.000
Cerastium semidecandrum	0.141*	*Poa trivialis*	0.563
Galium aparine	0.016	*Trifolium campestre*	0.563
Galium mollugo	0.141	*Trifolium dubium*	0.563
Galium palustre	0.141	*Trifolium pratense*	0.766
Galium uliginosum	0.141	*Trifolium repens*	1.000
Galium verum	0.141		

* The forage value for this species was missing in the BiolFlor database. We therefore adopted the value of *C. fontanum* for it.

8. *Grazing: seed survival probabilities following ingestion*

Mortality of seeds due to chewing or digestion by the animal is a major bottleneck for seeds in endozoochory. We have previously determined species-specific mortality rates by feeding known amounts of seeds from various plant species to cattle (D'hondt and Hoffmann 2011). Twelve of the species used in that study were also included in the model, and we therefore adopted the raw values for them (n = 7). As intra-generic differences in mortality rate are generally low (D'hondt and Hoffmann 2011), we lumped the raw values from congeneric species for the remaining thirteen species (n = 14).

Seed survival probabilities can be consulted in the abovementioned article. Per species, these values were lumped into a vector from which the model randomly draws values when needed.

9. *Defecation: time intervals between excretions*

In the study of Lamoot *et al.* (2005) on Highland cattle in Westhoek-South, defecations were included in the behavioural monitoring protocol. From their data, the time that passed between two consecutive defecation events could easily be calculated.

In total, 37 time intervals were obtained; a frequency distribution of their values is provided in Fig. 2A-2. These time intervals were put together in a vector, from which the model randomly draws values when needed. After each defecation. A new value is drawn after each defecation.

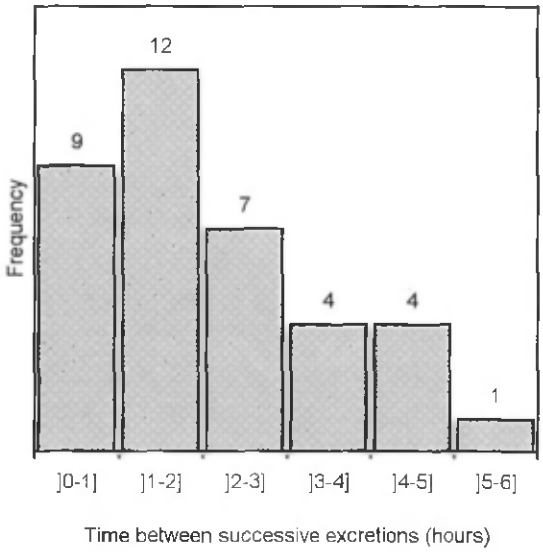


Figure 2A-2. Frequency distribution for the time interval between successive excretions.

10. Defecation: seed excretion probabilities

Rawsthorne *et al.* (2009) advocated that two-parameter lognormal distributions provide both easy and reliable fits to gut passage data. In our feeding experiment (D'hondt and Hoffmann 2011), we accordingly derived these parameters for grassland plant seeds ingested by cattle: $\mu = 7.45$, $\sigma = 0.42$. Together, these values determine the probability for a given seed to be excreted after ingestion (Fig. 2A-3). Based on our results, the excretion pattern is considered the same for all species. μ and σ remained fixed throughout all simulations.

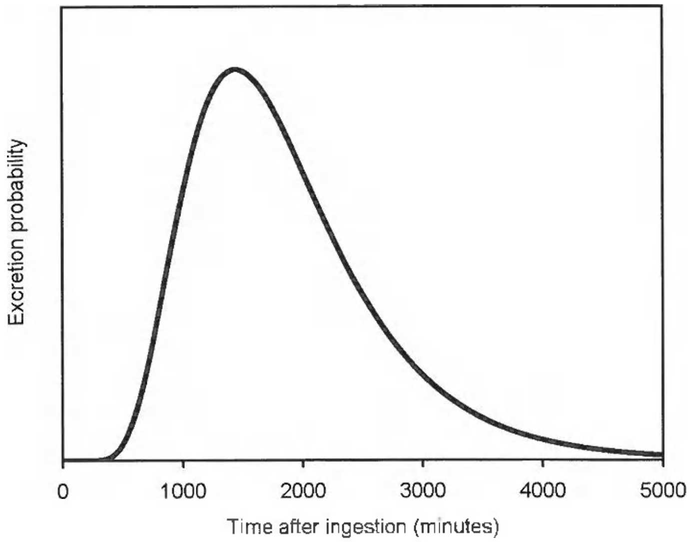


Figure 2A-3. The lognormal probability density function representing the chance for a seed to be excreted as a function of time (excretion pattern; $\mu = 7.45$, $\sigma = 0.42$).

G. Submodels

All modelling was performed in the array-based computing environment MATLAB (The Mathworks 2010; also see the model of Couvreur *et al.* 2008). The model source code (in M format) is provided in Appendix 2B.

1. Animal movement

The animal moves through the landscape according to a constrained random walk, from its resident cell to one of the (eight) neighbouring cells. The movement constraint is based on the combination of the animal's preference among vegetation types, and its propensity to change direction. This is performed through the vegetation preference coefficients (F.3.), and the direction preference coefficients (F.4.), respectively. As illustrated in Fig. 2A-4, both coefficients are multiplied to represent the overall probability for a neighbouring cell to become accepted as the next cell of residence. The procedure then consists of randomly drawing neighbouring cells and testing these, until one of them gets accepted. The length of stay in the new arrival cell is drawn randomly from the vector of time interval values (F.2.).

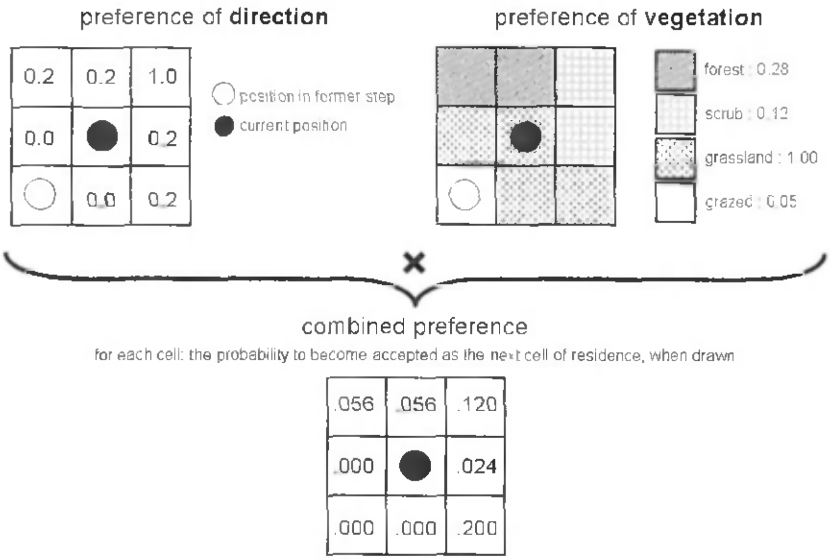


Figure 2A-4. An illustration of the animal movement submodel. The closed dot represents the current position of the animal on the grid, while the open dot represents the position of the animal in the previous step. Upper left: the direction preference coefficients for the neighbouring cells. Upper right: the vegetation types and their associated vegetation preference coefficients. Both coefficients are multiplied. In this example, the choice is most strongly between straightly entering the scrub, or staying in the grassland through a right turn.

2. Grazing / seed ingestion and digestion

After having arrived in a new cell, the animal may or may not graze that cell; the probability for a cell to become grazed is fixed (F.5.).

If grazed, a series of actions is undertaken. First, seed quantities are assigned to the new cell for each of the plant species through random draws from the respective vectors (F.6.). Then, a certain portion of those seeds become ingested by the animal. This portion is proportional to the time spent in the cell on the one hand (F.2.), and to the forage value of the species on the other (F.7.). A portion of these ingested seeds moreover become destroyed, representing (momentaneous) mortality because of seed destruction/digestion (F.8.).

These actions (local seed production, differential ingestion, digestion) are implemented in a single step. Mathematically spoken: for each species, the number of seeds ingested = the local seed availability × (the time spent in that cell / the maximum grazing time spent in any cell) × the species' forage value × the species' probability to survive digestion. The newly ingested seeds are then added to the 'gut' matrix of the animal, as is illustrated in Fig. 2A-5.

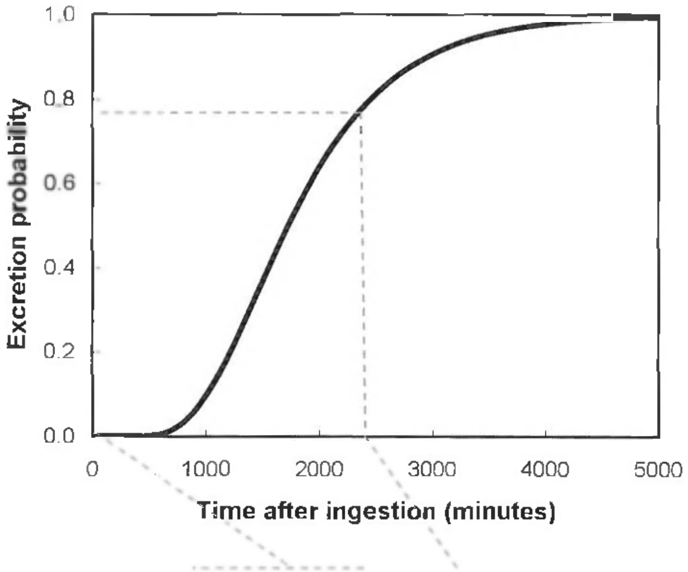
$$\begin{bmatrix} \mathit{0.3} & \mathit{1.5} & \mathit{3.0} & \cdots & \mathit{2400.0} \\ 0 & 0 & 5 & \cdots & 7 \\ 136 & 54 & 23 & \cdots & 0 \\ 0 & 0 & 0 & \cdots & 1200 \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ 2 & 0 & 0 & \cdots & 11 \end{bmatrix} \rightarrow \begin{bmatrix} \mathit{2.0} & \mathit{2.3} & \mathit{3.5} & \mathit{5.0} & \cdots & \mathit{2402.0} \\ 10 & 0 & 0 & 5 & \cdots & 7 \\ 10 & 136 & 54 & 23 & \cdots & 0 \\ 0 & 0 & 0 & 0 & \cdots & 1200 \\ \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\ 0 & 2 & 0 & 0 & \cdots & 11 \end{bmatrix}$$

Figure 2A-5. A simplified example of the animal 'gut matrix', and its dynamics during grazing. Left: in this two-dimensional matrix, column headings give the time that has passed since seeds were ingested (in minutes). Other matrix elements refer to seed numbers, with each row corresponding to a different species. Left to right: with each model step, the elapsed time is added to the column headings. If seeds are ingested, a new column is added to the left. In this example, the most recent step spanned two minutes, and several new seeds have been ingested.

3. Defecation / seed excretion

While moving through the landscape, a countdown timer keeps track of the time left for the next defecation to occur. After each defecation, a new value is drawn from that particular time interval vector (F.9.).

The probability for each seed from within the animal to be either excreted or retained at defecation, is determined by the time that has passed since its ingestion. This relationship is given by the cumulative lognormal probability density function which is defined by μ and σ (F.10.). As is illustrated in Fig. 2A-6, the retained and excreted fraction are easily calculated from the animal 'gut matrix'. The excreted fraction then forms the seed content of the defecation (dung pat), and is added to an output matrix.



$$\begin{bmatrix} \mathit{2.0} & \mathit{2.3} & \mathit{3.5} & \mathit{5.0} & \cdots & \mathit{2402.0} \\ 10 & 0 & 0 & 5 & \cdots & 7 \\ 10 & 136 & 54 & 23 & \cdots & 0 \\ 0 & 0 & 0 & 0 & \cdots & 1200 \\ \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\ 0 & 2 & 0 & 0 & \cdots & 11 \end{bmatrix}$$

Figure 2A-6. Exemplified dynamics of the animal 'gut' matrix during excretion. The plot is the cumulative version of Fig. 2A-3. The matrix is explained in Fig. 2A-5. For example, 79 % of the seeds from the latter column will be excreted at this defecation, the remainder will be retained in the animal.

Appendix 2B – Model code

Below, the M code (for MATLAB; The Mathworks 2010) for single runs is given. Please refer to specialized literature for technical details.

```
% The endozoochory model. D'hondt (2011).

% LEXICON: WHZ = Westhoek-South, DUUR = Duration, FV = Forage
% Value, RPOS = Row Position, CPOS = Column Position, VDIR =
% Vertical Direction, HDIR = Horizontal Direction, T = Time, E =
% Time Since Last Excretion, EXCTIME = Excretion Time Intervals,
% PAD = Path, KOE = Cow, M = Patty Matrix, NRPOS = New Row
% Position, NCPOS = New Column Position, TRANSPROB = Transition
% Probability, PERMCOEFF = Permeability Coefficient, LOCPROD =
% Local Seed Production, RETFRAC = Retained Fraction, EXCFRAC =
% Excreted Fraction

load WHZ.mat            % Loads the vegetation map
WHZint = WHZ;
DATA_MOVEMENT           % Loads movement data from other script
DATA_SEEDPRODUCTION     % Loads seed production data ~
DATA_SURVIVAL           % Loads seed survival data ~
DATA_EXCRETION          % Loads seed excretion data ~
DATA_FORAGINGVALUE      % Loads species forage values ~
DAYS = 3;               % One run simulates x days
DUUR = (DAYS+5)*24*60;% 5 days 'entrance' are added

tic

[RPOS CPOS] = introduce(WHZ); % = Random intro (other script)

VDIR = round(3*rand-1.5);
HDIR = round(3*rand-1.5);
T = 0;
E = 0;
E_THRESHOLD = EXCTIME(ceil(rand*length(EXCTIME)));

PAD = [T E RPOS CPOS 0];
KOE = [0; zeros(26,1)];
M = [T; WHZint(RPOS,CPOS); zeros(26,1)];

MU = 7.4487; SIGMA = 0.4219;

%% %% %% %% MAIN LOOP start

while T <= DUUR

%% MOVEMENT PROCEDURE

NRPOS = RPOS + round(3*rand-1.5);
NCPOS = CPOS + round(3*rand-1.5);

switch sum([NRPOS == RPOS + VDIR , NCPOS == CPOS + HDIR])
    case 2; TRANSPROB = 1.0;
    case 1; TRANSPROB = 0.2;
```

```
     case 0; TRANSPROB = 0.0;
end

RANDOMNUMBER = rand;

while ((NRPOS == RPOS) && (NCPOS == CPOS)) || ...
WHZ(NRPOS,NCPOS)==0 ||  ...
RANDOMNUMBER>TRANSPROB*PERMCOEFF(WHZint(NRPOS,NCPOS));
NRPOS = RPOS + round(3*rand-1.5);
NCPOS = CPOS + round(3*rand-1.5);
switch sum([NRPOS == RPOS + VDIR , NCPOS == CPOS + HDIR])
     case 2; TRANSPROB = 1.0;
     case 1; TRANSPROB = 0.2;
     case 0; TRANSPROB = 0.0;
end
RANDOMNUMBER = rand;

% It is inevitable that KOE wil sometimes run stuck.
if toc > 40;
disp('Run interrupted due to time-out. KOE appeared stuck.');
     PAD=0; KOE=0; M=0;
     return;
end

end

VDIR = NRPOS - RPOS;
HDIR = NCPOS - CPOS;
RPOS = NRPOS;
CPOS = NCPOS;

%% RESIDENCE PROCEDURE

TS = TIMESPENT(ceil(rand*length(TIMESPENT)));
T = T + TS;
E = E + TS;
PAD = [PAD ; T E RPOS CPOS TS];
KOE(1,:) = KOE(1,:) + TS;

switch rand < 0.302   % Grazing
     case 1
          MAKE_LOCPROD_AND_SURV; % Calls ‘seeds’ (other script)
          INGEST = (TS/10.439).*LOCPROD.*FV.*SURV;
          KOE = [[TS;INGEST] KOE];
end

while sum(round(KOE(2:end,end)))==0 && size(KOE,2)>1
     KOE(:,end)=[];
end

%% EXCRETION PROCEDURE

if E >= E_THRESHOLD

EXCFRAC = ones(size(KOE));
RETFRAC = ones(size(KOE));
for i = 2:size(EXCFRAC,1)
     EXCFRAC(i,:) = logncdf(KOE(1,:),MU,SIGMA);
end
```

```
RETFRAC(2:end,:) = 1-EXCFRAC(2:end,:);

M = [M [T ; WHZint(RPOS,CPOS); zeros(size(M,1)-2,1)]];
for i = 3:size(M,1);
    M(i,end) = KOE(i-1,:)*EXCFRAC(i-1,:)'; % Matrix product
end

KOE = KOE.*RETFRAC;

E = 0;
E_THRESHOLD = EXCTIME(ceil(rand*length(EXCTIME)));

end

WHZ(RPOS,CPOS)=7;

end
%% MAIN LOOP stop
%% FINISH UP

% OPTIONAL: delete the first five days in PAD and M

while size(PAD,1) >= 1 && PAD(1,1) <= 7200;
PAD(1,:) = [];
end
if isempty(PAD);
disp('Note: no PAD constructed (path lasted <5 days).');
end

while size(M,2) >= 1 && M(1,1) <= 7200;
M(:,1) = [];
end
if isempty(M);
disp('Note: no M constructed (path lasted <5 days).');
end

toc
```

Appendix 2C – More on model validation and assumption

This appendix provides more information on the model's validity for inferring spatial seed deposition patterns, and on the assumption of cattle latrine behaviour.

A. Validaty of animal path simulations

Since an important focus of the article is put on the spatial pattern of seed deposition, a validation of simulated animal paths was needed. We performed this with respect to two variables: (i) the relative proportion of the vegetation types visited along paths, and (ii) the global bee-line distances covered by the animals.

For the relative proportion of visited vegetations along paths, model results can be correlated back with the observed positions of Highland cattle in Westhoek-South of Lamoot *et al.* (2005). This is performed in Chapter 2: there appeared to be a good correlation between observed and simulated paths, making the model suitable to infer on seed deposition patterns with respect to vegetation types (directed dispersal).

The bee-line distances travelled by animals in simulation corresponded less satisfactorily to the observations. As can be seen from Figs. 2B-1 and 2B-2, observed displacements after several hours easily exceeded 100 metres, while in simulation, this was rarely the case. Therefore, the model is not suited for inferring on dispersal distances. Probably, complex processes not taken into account here lie at the basis of higher distances covered by animals in reality (e.g. social behaviour, drinking water access, disturbances, explorative behaviour...).

Nonetheless, we extracted dispersal distances from our simulations for illustration. The resulting frequency distribution of bee-line seed dispersal distances is given in Fig. 2B-3. When taking into account the severe underestimation of distances covered by the animals, it is clear that seed dispersal distances highly outweigh those obtained by wind dispersal.

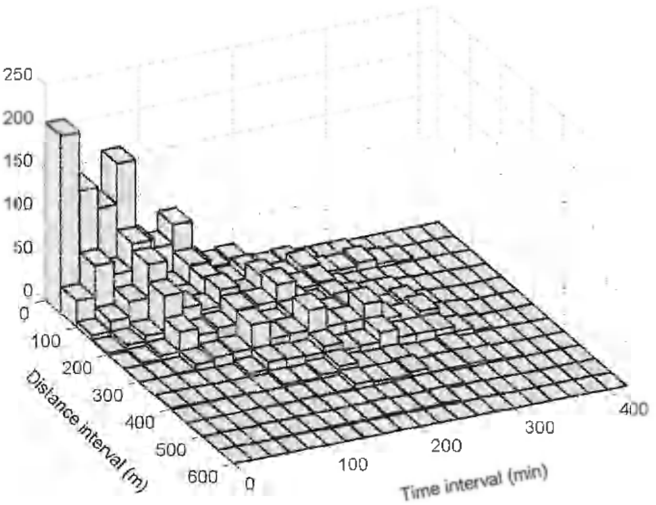


Figure 2B-1. Frequency distribution of the spatial and temporal components of observed cattle displacements in Westhoek-South. Distances refer to bee-line distances, and time intervals to the time needed for those distances to be covered.

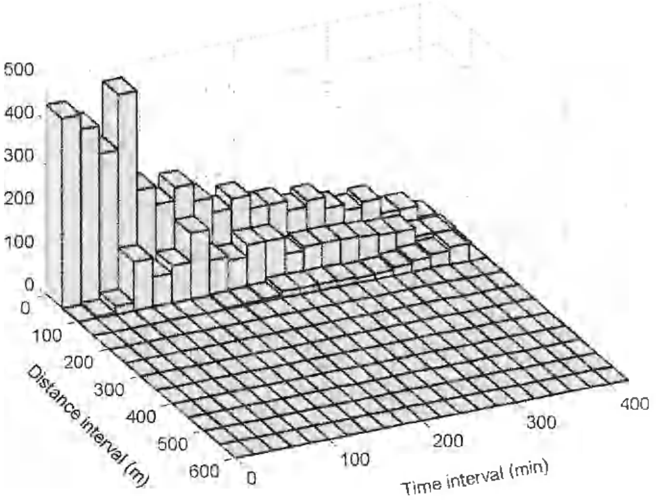


Figure 2B-2. Same as in Fig. 2B-1. Now, data is based on a simulated path from the integer landscape.

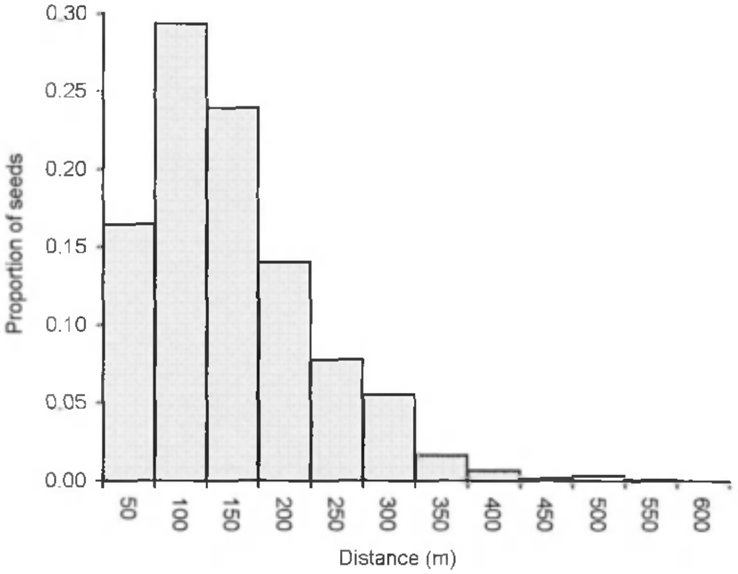


Figure 2B-3. Frequency distribution of seed dispersal distances obtained from the model (methodology according to Vellend *et al.* 2003). Data is from 100 six-day runs.

B. Do cattle perform latrine behaviour?

If cattle have habitual latrine sites, seeds might have a pronounced probability to end up at the particular vegetations these sites are in. Therefore, we tested for random defecations among habitats according to Lamoot *et al.* (2004), who addressed this question for free-ranging horses. If a strong positive relation is found between the grazing time and number of defecations within a set of complementary vegetation types, this would indicate that latrine areas are not formed.

We used 192 hours of observation, including of grazing behaviour and defecation, on Highland cattle at our site (data from Lamoot *et al.* 2005). Seventeen vegetation types were distinguished.

There was a tight positive relation between the grazing time and number of defecations within the vegetations (Fig. 2B-4). As animals exhibit no preference for particular vegetation types at defecation, defecation can thus be modelled to occur randomly along an animal's path.

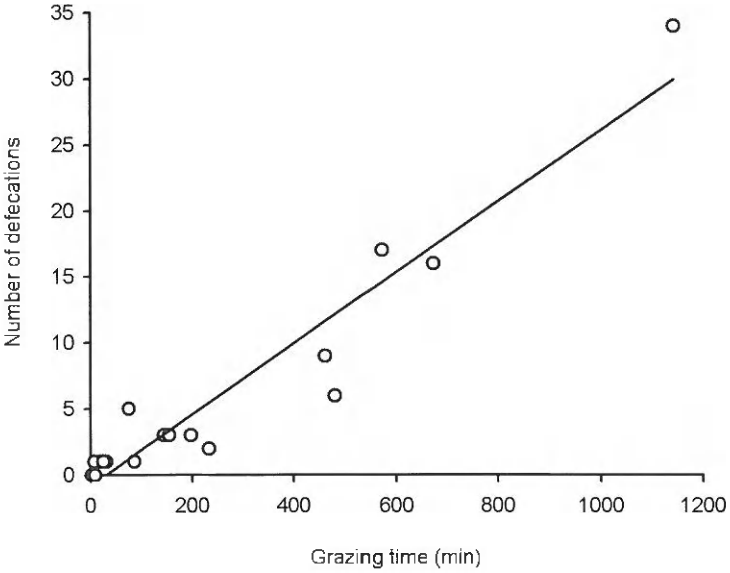


Figure 2B-4. The time spent by Highland cattle to grazing in each of 17 distinguished vegetation types at Westhoek-South, and the number of defecation events in them. The regression line is shown (R^2=0.92).

Appendix 2D – Seedling emergence from the greenhouse

Table 2D. Statistics of seedling emerge from the dung pats in the greenhouse. Given are the species identity, the frequency (out of 33 samples), and the mean, standard deviation (s.d.), minimum (min.), maximum (max.) and total number of seedlings observed. Only the species relevant for this study are listed (the others have been collected with less detail).

Species	Freq.	Mean	S.d.	Min.	Max.	Total
Agrostis capillaris	5	0.727	2.541	0	13	24
Agrostis stolonifera	12	5.818	22.419	0	128	192
Carex arenaria	16	1.485	2.425	0	9	49
Carex flacca	0	.	.	.	.	
Carex hirta	4	0.152	0.442	0	2	5
Carex oederi	0	.	.	.	.	
Cerastium fontanum	13	0.909	1.588	0	7	30
Cerastium semidecandrum	0	.	.	.	.	
Galium aparine	5	0.273	0.801	0	4	9
Galium mollugo	0	.	.	.	.	
Galium palustre	0	.	.	.	.	
Galium uliginosum	0	.	.	.	.	
Galium verum	0	.	.	.	.	
Juncus articulatus	22	2.303	2.878	0	11	76
Juncus bufonius	29	25.636	55.343	0	300	846
Juncus inflexus	4	0.424	1.768	0	10	14
Juncus subnodulosus	6	0.242	0.561	0	2	8
Mentha aquatica	1	0.030	0.174	0	1	1
Plantago major	1	0.030	0.174	0	1	1
Poa annua	19	2.727	4.033	0	17	90
Poa pratensis	21	1.394	1.903	0	9	46
Poa trivialis	31	31.576	25.204	0	92	1042
Trifolium campestre	0	.	.	.	.	
Trifolium dubium	1	0.061	0.348	0	2	2
Trifolium pratense	0	.	.	.	.	
Trifolium repens	14	1.485	2.279	0	8	49

Appendix 3A – Dung beetle fauna

Table 3A. Pitfall trap capture data. Given are the number of beetles captured. The frequency (number of plots) is given between square brackets.

Species	Moist site		Dry site		Total
	Horse dung [6]	Cattle dung [6]	Horse dung [6]	Cattle dung [6]	[24]
Aphodius contaminatus	215 [6]	81 [6]	2 [2]	4 [4]	302 [18]
Aphodius fimetarius	0 [0]	2 [2]	0 [0]	2 [2]	4 [4]
Aphodius prodromus	1 [1]	1 [1]	0 [0]	0 [0]	2 [2]
Aphodius rufipes	2 [1]	2 [1]	47 [4]	5 [3]	56 [9]
Aphodius rufus	0 [0]	0 [0]	3 [2]	0 [0]	3 [2]
Aphodius foetidus	0 [0]	4 [2]	13 [5]	4 [3]	21 [10]
Aphodius sphacelatus	0 [0]	8 [4]	0 [0]	0 [0]	8 [4]
Aphodius sticticus	0 [0]	0 [0]	2 [2]	0 [0]	2 [2]
Aphodius foetens	3 [3]	51 [6]	40 [6]	80 [6]	174 [21]
Geotrupes niger	0 [0]	0 [0]	1 [1]	0 [0]	1 [1]
Geotrupes spiniger	1 [1]	3 [2]	42 [6]	23 [5]	69 [14]
Onthophagus similis	2 [2]	1 [1]	5 [4]	0 [0]	8 [7]

Appendix 4A – Seed coat thickness

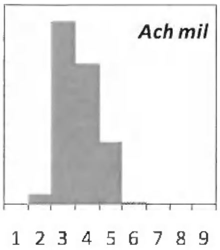
Table 4A. Measurements of 'seed' coat thickness, as performed by De Laender (2008). Per species, the measurements apply to a single seed.

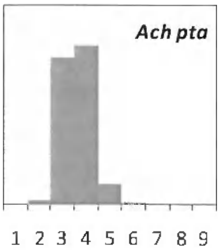
species	n	mean (µm)	s.d.	s.e.
Achillea millefolium	19	23.878	4.862	5.478
Achillea ptarmica	20	20.935	5.100	4.681
Agrostis stolonifera	24	5.035	1.719	1.028
Artemisia campestris	20	19.080	4.350	4.266
Carex vulpina	20	91.796	10.702	20.526
Cerastium fontanum	20	23.161	9.458	5.179
Chenopodium bonus-henricus	15	32.507	3.919	8.393
Galium aparine	36	20.538	11.214	3.423
Geranium molle	26	77.665	27.680	15.231
Hypericum tetrapterum	33	22.437	8.691	3.906
Mentha aquatica	20	37.789	15.731	8.450
Mentha pulegium	18	37.100	4.684	8.745
Myosotis arvensis	21	79.298	23.018	17.304
Pimpinella saxifraga	24	14.354	18.338	2.930
Poa annua	18	11.336	3.726	2.672
Poa pratensis	32	17.424	5.491	3.080
Potentilla tabernaemontani	20	109.710	29.199	24.532
Primula vulgaris	30	35.422	18.484	6.467
Rumex acetosa	30	29.680	12.082	5.419
Silene vulgaris	28	36.343	12.066	6.868
Viola arvensis	15	28.196	7.736	7.280

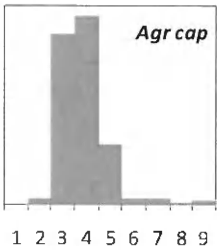
Appendix 4B – Specific retention curves

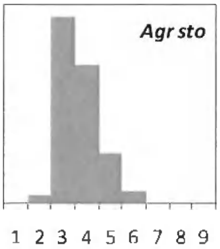
Frequency distributions of the relative proportion of seeds excreted per time interval, as derived from the feeding experiment.

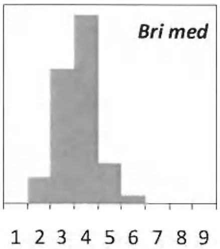
Species are abbreviated, please refer to Table 4-1. Only species of which viable seeds were excreted are shown. Time intervals are coded as follows: (1): between 0 and 6 hours after feeding, (2): 6-11 hours, (3): 11-24 hours, (4): 24-35 hours, (5): 35-48 hours, (6): 48-72 hours, (7): 72-96 hours, (8): 96-120 hours, (9): 120-144 hours.

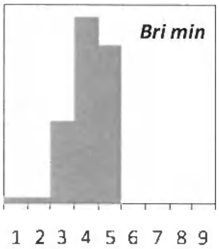


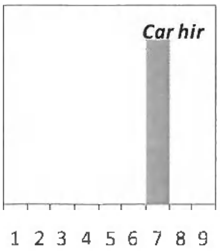


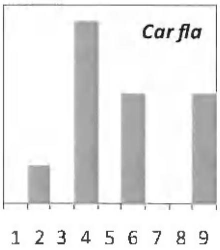


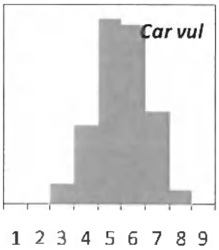


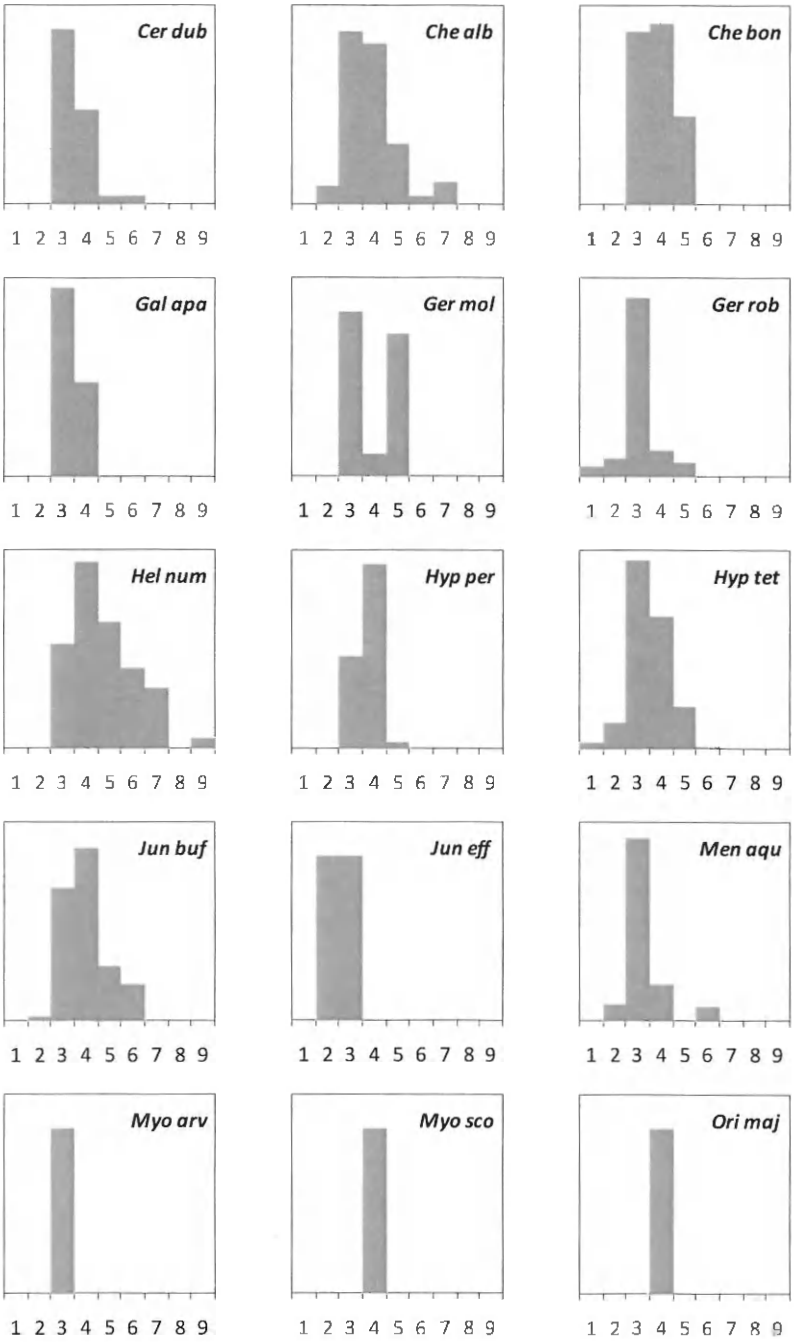


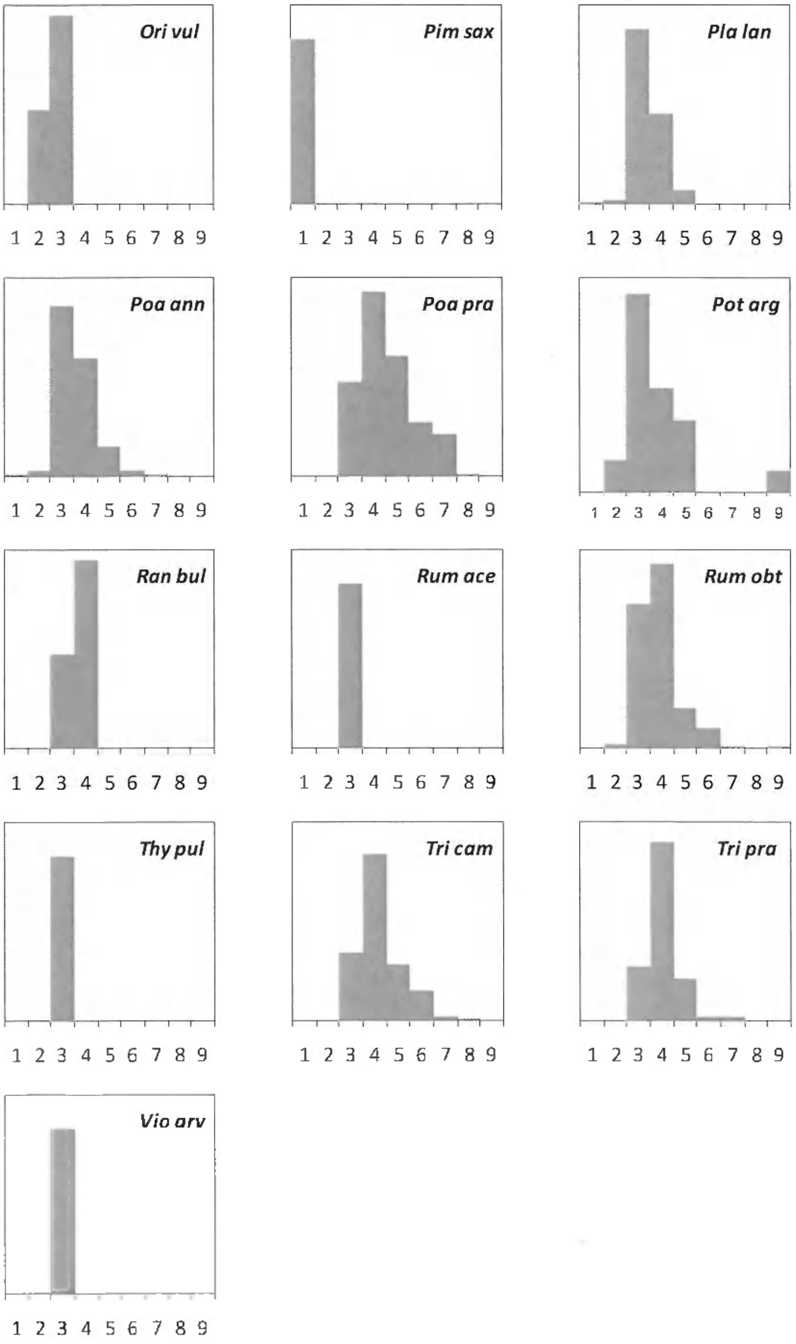









Cer dub
1 2 3 4 5 6 7 8 9
Che alb
1 2 3 4 5 6 7 8 9
Che bon
1 2 3 4 5 6 7 8 9
Gal apa
1 2 3 4 5 6 7 8 9
Ger mol
1 2 3 4 5 6 7 8 9
Ger rob
1 2 3 4 5 6 7 8 9
Hel num
1 2 3 4 5 6 7 8 9
Hyp per
1 2 3 4 5 6 7 8 9
Hyp tet
1 2 3 4 5 6 7 8 9
Jun buf
1 2 3 4 5 6 7 8 9
Jun eff
1 2 3 4 5 6 7 8 9
Men aqu
1 2 3 4 5 6 7 8 9
Myo arv
1 2 3 4 5 6 7 8 9
Myo sco
1 2 3 4 5 6 7 8 9
Ori maj
1 2 3 4 5 6 7 8 9


Ori vul
1 2 3 4 5 6 7 8 9
Pim sax
1 2 3 4 5 6 7 8 9
Pla lan
1 2 3 4 5 6 7 8 9
Poa ann
1 2 3 4 5 6 7 8 9
Poa pra
1 2 3 4 5 6 7 8 9
Pot arg
1 2 3 4 5 6 7 8 9
Ran bul
1 2 3 4 5 6 7 8 9
Rum ace
1 2 3 4 5 6 7 8 9
Rum obt
1 2 3 4 5 6 7 8 9
Thy pul
1 2 3 4 5 6 7 8 9
Tri cam
1 2 3 4 5 6 7 8 9
Tri pra
1 2 3 4 5 6 7 8 9
Vio arv
1 2 3 4 5 6 7 8 9

Appendix 4C – Germination percentages

Table 4C. Germination rates of non-ingested seeds (control, n = 5), and survival rates of ingested seeds (n = 7)

	control		survival rate	
species name	mean	s.e.	mean	s.e.
Achillea millefolium L.	55.20	1.28	20.12	3.86
Achillea ptarmica L.	48.40	3.14	16.22	2.61
Agrostis capillaris L.	50.20	5.89	53.78	8.67
Agrostis stolonifera L.	69.40	2.36	34.67	4.75
Artemisia campestris L.	1.60	0.40	0.00	0.00
Artemisia vulgaris L.	1.60	0.51	0.00	0.00
Briza media L.	61.60	4.17	3.29	1.14
Briza minor L.	47.20	2.44	2.17	0.48
Campanula rotundifolia L.	5.40	1.36	0.00	0.00
Cardamine hirsuta L.	2.40	1.17	3.67	3.67
Cardamine pratensis L.	4.40	1.75	0.00	0.00
Carex flacca Schreb.	1.60	0.51	22.00	8.29
Carex vulpina L.	17.00	4.44	17.54	2.86
Cerastium dubium (Bast.) Guépin	11.20	0.49	39.69	7.37
Cerastium fontanum Baumg.	0.60	0.40	0.00	0.00
Chenopodium album L.	13.80	3.06	21.36	4.06
Chenopodium bonus-henricus L.	6.40	2.01	34.01	10.13
Galium aparine L.	7.40	1.69	6.74	4.05
Galium verum L.	3.40	0.98	0.00	0.00
Geranium molle L.	3.60	1.50	10.80	5.09
Geranium robertianum L.	22.20	4.19	3.58	0.59
Helianthemum nummularium (L.) Mill.	5.40	1.50	110.85	15.15
Hypericum perforatum L.	5.80	1.46	21.13	5.22
Hypericum tetrapterum Fries	17.20	2.63	26.60	6.94
Juncus bufonius L.	8.40	2.23	83.07	11.33
Juncus effusus L.	0.00	0.00	n/a*	n/a*
Mentha aquatica L.	25.80	4.79	4.03	1.02
Mentha pulegium L.	1.20	0.58	0.00	0.00
Myosotis arvensis (L.) Hill	3.60	1.17	1.63	1.63
Myosotis scorpioides L.	1.80	0.49	4.77	4.77
Origanum majorana L.	0.20	0.20	11.60	11.60
Origanum vulgare L.	7.00	1.45	3.65	2.10
Pimpinella saxifraga L.	10.40	1.99	0.18	0.18
Plantago lanceolata L.	45.60	5.41	31.64	7.53

Table 4C, *continued.*

Poa annua L.	60.20	3.69	25.42	3.39
Poa pratensis L.	52.80	2.67	31.26	6.62
Potentilla argentea L.	14.40	2.87	28.50	3.58
Potentilla tabernaemontani Aschers.	0.60	0.40	0.00	0.00
Primula vulgaris Huds.	0.20	0.20	0.00	0.00
Ranunculus bulbosus L.	0.60	0.40	29.57	14.51
Rumex acetosa L.	0.20	0.20	27.19	27.19
Rumex obtusifolius L.	65.00	2.21	21.49	3.74
Silene vulgaris (Moench) Garcke	0.20	0.20	0.00	0.00
Thymus pulegioides L.	0.20	0.20	41.56	41.56
Thymus serpyllum L.	0.40	0.24	0.00	0.00
Trifolium campestre Schreb.	17.80	1.53	85.92	8.28
Trifolium pratense L.	2.80	0.86	105.48	22.07
Viola arvensis Murray	7.20	2.15	1.36	1.36

* Since the control germination rate was equal to zero, the survival rate estimate is undefined for this species; however, two seedlings emerged from dung samples containing ingested seeds.

Appendix 5A – Experimental field set-up

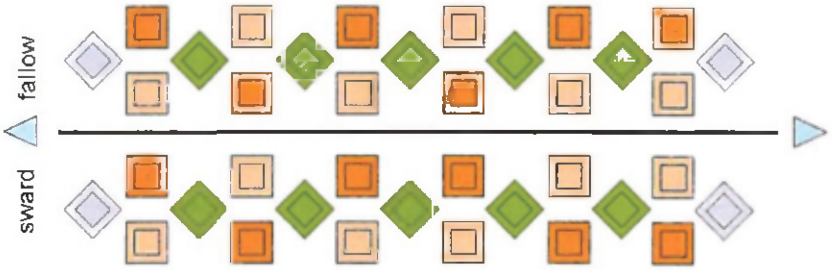


Figure 5A. An overview of the set-up used in the *Trifolium repens* field experiment. Each square represents a plot. Orange plots make part of the experiment on recruitment from non-dormant (light orange) and dormant (dark orange) seeds. The green plots make part of the experiment on the natural timing of dormancy break; they alternate with the plots of the former experiment in order to cover the same grounds. The grey plots are controls (empty).

Appendix 5B – Clonal repeatability estimate

Table 5B. Output from the generalized linear mixed model from the genotype-by-humidity experiment, after omission of the interaction factor (clone × humidity). Compare to Table 5-3

Type-III tests of fixed effects

Effect	df (num., den.)*	*F*	*p*
Humidity	2, 102	16.36	<0.0001
	* with Satterthwaite correction		

Covariance parameter estimates

Covariance parameter	Estimate	S.e.	
Clone	0.298	0.298	(see below)
Residual (vc)	11.438	1.666	

Test of covariance parameter

Covariance parameter	df	Chi-square	*p*
Clone	1	2.98	0.042

Estimate of clonal repeatability: 0.298/(0.298+11.438) = 0.025.

CHAPTER 10

References

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« Honestas super omnia. »

~~« Veritas super omnia. »~~

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D'hondt S. (2009) Endozoochory by large grazers: a modelling approach. (In Dutch.) Master thesis in Biology. Ghent University, Ghent (BE).

Vansteenbrugge L. (2009) Potential endozoochorous seed dispersal by foxes (*Vulpes vulpes* L.) in Flanders. (In Dutch.) Master thesis in Biology. Ghent University, Ghent (BE).

Teaching

Instructor (assistant), practicals of botany (botany course), Bachelor programme of Pharmaceutical Sciences. Fall 2010.

Instructor (assistant), practicals of botany (botany course), Bachelor programme of Bioscience Engineering. Fall 2010.

Instructor (assistant), practicals of botany (advanced botany course), Bachelor programme of Biology. Fall 2010.

Supervisor/instructor, field course in biology, Bachelor programme of Biology. Spring 2007, 2008, 2009.

Supervisor/guide, (various) biological excursions. Bachelor programme of Biology. Spring 2007, 2008, 2009.

Publications

Publications in international peer-reviewed journals:

D'hondt B., D'hondt S., Brys R., Bonte D., Hoffmann M. (subm.) A data-driven simulation model of endozoochory by large herbivores demonstrates low levels of directed dispersal in heterogeneous landscapes.

D'hondt B., Vansteenbrugge L., Van Den Berge K., Bastiaens J., Hoffmann M. (2011) Scat analysis reveals a wide set of plant species to be potentially dispersed by foxes. Plant ecology and evolution 144: 106-110.

D'hondt B., Hoffmann M. (2011) A reassessment of the role of simple seed traits in survival following herbivore ingestion. Plant biology 13 (Suppl. 1): 118–124.

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D'hondt B., Bossuyt B., Hoffmann M. & Bonte D. (2008) Dung beetles as secondary seed dispersers in a temperate grassland. Basic and applied ecology 9: 542-549.

de la Peña E., D'hondt B., Bonte D. (in press) Landscape structure, dispersal and the evolution of antagonistic plant-herbivore interactions. Ecography.

Bonte D., Breyne P., Brys R., de la Peña E., D'hondt B., Ghyselen C., Vandegehuchte M., Hoffmann M. (in press) Landscape dynamics determine the small-scale genetic structure of an endangered dune slack plant species. Journal of Coastal Research.

Publications in national peer-reviewed journals:

D'hondt B., Vansteenbrugge L., Van Den Berge K., Bastiaens J., Hoffmann M. (2011) Vossen als verbreiders van plantenzaden. Natuur.Focus 10: 4-10.

Contributions to scientific meetings

Oral presentations:

D'hondt B. (2007) Aspects of endozoochory: the fate of seeds during and after ingestion. In: Becker *et al.* (eds.) Verhandlungen der Gesellschaft für Ökologie 37: 295. Verlag Die Werkstatt, Göttingen. [published abstract, 37th annual GfÖ conference, Sep'07, Marburg (DE)]

D'hondt B., Bossuyt B., Hoffmann M. & Bonte D. (2008) Ungulates as seed dispersers in plant conservation: what do dung beetles do? – In: anonymous. Abstracts of the young botanists day 2007, "Plant conservation in theory and practice". Belgian Journal of Botany 141 (2): 185-203. [published abstract, young botanists day, 23 Nov'07, Bailleul (F)]

D'hondt B., Hoffmann M., Bossuyt B. & Bonte D. (2007) Dung beetles as secondary seed dispersers in a temperate grassland. Dispersal meeting, 6-7 december 2007, Paris (F).

D'hondt B., Leroux O. & Hoffmann M. (2008) De overleving van plantenzaden bij vertering door grote herbivoren: de rol van anatomische kenmerken. Necov wintersymposium, 7-8 februari 2008, Antwerpen (BE).

D'hondt B. & Hoffmann M. (2007) The herbivore-seed interaction: weak support for Janzen's idea. In: Stadler J. *et al.* (eds.) Verhandlungen der Gesellschaft für Ökologie 38: 417. [published abstract, 38th annual GfÖ conference, 15-19 Sep'08, Leipzig (DE)]

D'hondt B. & Hoffmann M. (2009) Can herbivorous mammals select for seed attributes through endozoochory? Young botanists day, 24-25 november 2009, Brussel (BE).

D'hondt B., D'hondt S., Hoffmann M. (2010) Verbreiding van kleine zaden door grote dieren: een modelmatige aanpak voor endozoöchorie door grazers. Starters in het natuuronderzoek, 18 maart 2010, Brussel (BE).

D'hondt B., Hoffmann M. (2010) Where dispersal meets dormancy: "hard" seed dispersal by herbivorous mammals. In: Pendleton *et al.* (eds.) Proceedings of the Seed Ecology III Conference – The Third International Society for Seed Science Meeting on Seeds and the Environment: 34-35. ISSS. [published extended abstract, Seed Ecology III Meeting, 20-24 June 2010, Salt Lake City (USA)]

Poster contributions:

D'hondt B., Hoffmann M. & Bossuyt B. (2007) Diplochorie: wordt de kieming van endozoöchoor verbreide zaden beïnvloed door mestkeveractiviteiten? Necov wintersymposium, 17-19 januari 2007, Nijmegen (NL).

D'hondt B., Hoffmann M. & Bossuyt B. (2007) Evolutionary adaptations of dry-seeded plant species to endozoochorous dispersal. Doctoraatssymposium faculteit wetenschappen, 24 april 2007, Gent (BE).

D'hondt B. (2008) Janzen's 'foliage is the fruit' hypothesis: a bridge too far? Symposium of the Spanish association of terrestrial ecology, 21-23 april 2008, Palma de Mallorca (ES).

D'hondt B., De Laender L. & Hoffmann M. (2008) Is "softseededness", rather than hardseededness, the norm in temperate legume populations? Young botanists day, 24 november 2008, Brussel (BE).

D'hondt B., D'hondt S. & Hoffmann M. (2009) How good do we know endozoochory by herbivorous mammals? – A simulation model and its evaluation. Organisms on the move: on the ecology and evolution of dispersal. 4th meeting of the international dispersal workgroup, 14-15 september 2009, Gent (BE).

D'hondt B., D'hondt S., Bonte D., Brys R., Hoffmann M. (2010) Contrasting benefits of seed dispersal by large herbivores and wind: distance vs. directed dispersal. Young botanists day, 19 november 2010, Meise (BE).

Scientific awards

'Best Student Oral Presentation'. Awarded by: the International Society for Seed Science & the Seed Ecology III Meeting Organizing Committee, 24 June 2010, Salt Lake City (USA).

'Pièrre Verkerk Award for Best Master Thesis, Ma2 Biology – Botany'. Awarded by: educational board of Biology, 4 July 2006, Ghent (BE).

'Dodonaea Award for the Most Creditable Student of Ma2 Biology – Botany'. Awarded by: the Royal Society of Natural History Dodonaea, 6 July 2006, Ghent (BE).

Shortlisted for 'Jacques Kets Award'. Granted by: the Royal Zoological Society of Antwerp & Parc Paradisio, 17 November 2007, Antwerp (BE).

« Hence, a traveller should be
a botanist, for in all views
plants form the chief
embellishment. »

from: *The Voyage Of The Beagle*

ISBN 978-94-9069-570-5
9 789490 695705 >
UNIVERSITY press