Could *Medauroidea extradentata* (Brunner von Wattenwyl 1907) survive in Provence (France)?

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Abstract
Several cases of accidental introduction of insects far away from their biotope have already been reported. Could *Medauroidea extradentata*, a stick insect originally from the district of Annam, Vietnam, survive in Provence (France)? To answer this question, two sets of experiments were performed. In the first one, specimens were put together with five plants growing only in the Mediterranean areas (bear's breeches (*Acanthus mollis*), almond tree (*Prunus dulcis*), fig blanche d'Argenteuil variety (*Ficus carica*), fig violette de Solliès variety (*Ficus carica*), olive (*Olea europaea*), Aleppo pine (*Pinus halepensis*)) to investigate their adaptability to these diets. In the second set, the egg survival rate when exposed to moderate cold was investigated. In view of the results, *M. extradentata* could survive in Provence, since it was possible to breed specimens on almond trees and on the two fig trees, while half of the eggs have survived the cold.

Key words
Stick insects, walkingsticks, Phasmid, *Medauroidea extradentata*, breeding, Provence, acclimatization

Introduction
The case of species which acclimatise to non-native regions after an accidental importation is unfortunately quite common. *Carausius morosus* (Sinéty 1901) (native of southern India (Westmark, 2007)), a very common breeding stick insect, is a perfect example of this, having notably acclimatised in the United States, the Azores and South Africa (Baker, 2015). It is also possible to mention the palmivorous butterfly *Paysandisia archon* (Burmeister 1880) in the Var (France) in 2001 coming originally from South America (André & Tixier Malicorne, 2013) or the spotted aphid *Therioaphis maculata* (Buckton 1899), endemic from Central Asia and Southern Europe, which colonized New Mexico (United Sates) in 1954 (Kindler, 1967 ; Bournoville, 1977 ; Haymaker, 1999). In 2013, Siaud discussed the possible acclimatization in Provence of *Macrothele calpeiana* (Walckenaer 1805), a tarantula native to Andalusia in southern Spain imported with Spanish olive trees at Cuges-les-Pins (Siaud & Raphaël, 2013).

France has, among its insects, only three morphologically similar species of stick insects, out of the 3196 described in the world (Brock et al., 2018): the French Stick Insect *Clonopsis gallica* (Charpentier 1825), the Mediterranean Stick Insect *Bacillus rossius* (Rossi 1790) and the Spanish Stick Insect *Pijnackeria masettii*[1] (Scali, Milani & Passamonti 2013) (Lelong, 1995b ; Baliteau, 2005 ; Lelong, 2014 ; Mûller, 2015). Of these three species, *B. rossius* and *P. masettii* live only in the Mediterranean area between the Italian border and the Spanish border on a broad band of about 50 km, while *C. gallica* is found in the Mediterranean arc and in the western half of a line going from Montpellier to Le Havre (Lelong, 1995b). Their eggs are perfectly capable of surviving the winter with only their chorion (Baliteau, 2005) for protection and will hatch the following spring or a year later (Lelong, 1995b ; Baliteau, 2005). The eggs of the French Stick Insect can even survive unprotected in temperatures of -5 °C (Lelong, 2014). On the other hand, none of the adults of the *C. gallica* and *P. masettii* species are able to survive the winter without dying, whereas those of *B. rossius* can survive (Lelong, 1995b). The diet of the French Stick Insect and of the Mediterranean Stick Insect is composed of Bramble (*Rubus fruticosus* (L. 1753)), Spiny Sloe (*Prunus spinosa* (L. 1753)), Wild Rose (*Rosa* sp. (L. 1753)) and almond (*Prunus dulcis* (Mill.) D.A. Webb 1967) (Baliteau, 2005) while *P. masettii* feeds exclusively on *Dorycnium pentaphyllum* (Scop. 1772) (Lelong, 1995b ; Lelong, 2014).

[1] Until 2009, it was described under the binomial name *Leptynia hispanica* (Bolivar 1878).

Insects appeared on Earth 479 million years ago (Rausher, 2015). The discovery in the Jehol deposit in Inner Mongolia (China) of three fossils of *Cretophasmomima melanogramma* dating from the lower Cretaceous is a evidence of the presence of stick insects on Earth for more or less 126 million years (Wang et al., 2014). The genus *Medauroidea* belongs to the order Phasmatodea (or Phasmida), in the suborder Verophasmatodea, infraorder Anareolatae, super-family Phasmatidae, family Phasmatinae, subfamily Clitumninae and the Medaurini tribe. *Medauroidea extradentata* (Brunner von Wattenwyl 1907) is a stick insect native to the Annam district in southern Vietnam (Lelong, 1995a ; Boucher & Varady-Szabo, 2005), better known as Annam stick insect in English ("Vietnam stick insect" as common name in French). This stick insect has been renamed several times since its discovery, but is named *Cuniculina imbriga* by some authors especially on the Internet (Olive et al., 2016). Trade of this stick insect is completely legal in France (OPIE, 2015 ; Arthropodia, 2016). Females can be up to ten centimeters long and only seven for the males, which seldom occur, and live for 12 to 14 months (Westmark, 2007). *M. extradentata* is easy to breed and accepts a large number of plants for feed, in particular, bramble (*Rubus fruticosus* (L. 1753)), raspberry (*Rubus idaeus* (L. 1753)) or hazel (*Corylus maxima* (Mill. 1768)). It can adapt in case of shortage as already demonstrated (Olive et al., 2016). There are no different dietary preferences at the different instars of this stick insect.

The study focuses on the themes mentioned above, and the purpose of this note is to show the possible acclimatization of the *M. extradentata* species in Provence (France). Although there is no known case of adaptation of this species outside its natural environment, with the current global transit[2], an invasion could happen, like the other commonly reared stick insect, *C. morosus*. A number of experiments have been carried out, both in terms of adaptation to typically Mediterranean diets, and in terms of cold resistance. The bear's breeches (*Acanthus mollis* (L. 1753)), the almond tree (*Prunus dulcis* ((Mill.) D. A. Webb 1967)), the fig blanche d'Argenteuil variety (*Ficus carica* (L. 1753)), the fig violette de Solliès variety (*Ficus carica* (L. 1753)), the olive tree (*Olea europaea* (L. 1753)) and the Aleppo pine (*Pinus halepensis* (Mill. 1768)) were tested and compared to a control group fed exclusively with bramble. This latter, but also the wild rose and the blackthorn are common diets for the French Stick Insect, the Mediterranean Stick Insect, and the other species studied. The almond tree was chosen because it is also food for some french stick insects. The other plants have been tested because they grow exclusively in the Mediterranean. If *M. extradentata* can feed with one or more of these diets, this would increase its chances of survival in Provence. In addition, the cold resistance of eggs has also been tested. These first tests are described in this note and others will be conducted later on over a longer period and repeated, in order to assess the ability to perform a complete lifecycle. The goal is simply to be able to answer the question of the title, without considering its potential to impact the indigenous stick insects.

Materials and Methods

Experiments were conducted in Marseilles (France) [43°18'47"N 5°28'15"E] and in Gembloux (Belgium) [50°34'15"N 4°41'50"E] (Google Earth) in perforated polypropylene boxes of 1180 cm^3 (15 cm (l) x 10.5 cm (w) x 7.5 cm (h)), carefully washed with green Dreft® (brand Procter & Gamble) and thoroughly rinsed with warm water between each experiment. The stick insects were kept at room temperature (32 ± 2 °C for Marseilles and 22 ± 3 °C for Gembloux), the lighting corresponding to the natural light cycle and the relative humidity was approximately 65 % in both experimental areas. The number of specimens used in each experiment was randomly selected by the module Study on Random V 1.6 of the software Gabriel Data Analysis and the curves were analysed by the module Two variables statistic V 1.35 of the same software (Olive, 2016). Stick insects were measured from the tip of the abdomen to the base of antennae with a Stanley brand ruler. *M. extradentata* insects originated from the Hexapoda breeding facility located in Belgium (Insectarium "Jean Leclercq", Rue de Grand-Axhe 45, B-4300 Waremme).

Preliminary feeding tests with Mediterranean plant leaves

Preliminary feeding trials with bear's breeches, almond, clementine tree, fig blanche d'Argenteuil variety, fig violette de Solliès variety, medlar, olive tree were made by placing two specimens, whose size

[2] For example, the authors live in Belgium and spend their holidays in the south of France.

was chosen randomly, in the presence of a leaf surrounded by aluminum foil so that the insects did not have access to water. The tests were stopped as soon as it was certain that the leaf had been eaten or that the leaf was beginning to be inedible.

The same methodology was used with bear's breeches for a later test to check if this stick insect can eat leaves of this plant.

Survival on the leaves of Mediterranean plants (4th August to 1st December 2015)
This study is based on a previously described protocol (Olive et al., 2016). A random number of specimens, which the size was also chosen at random, was placed in 6 boxes with a leaf of different Mediterranean plants (bear's breeches, almond tree, fig blanche d'Argenteuil variety, fig violette de Solliès variety, olive tree or pine) whose petiole is held in moist paper towels surrounded by a sheet of aluminium foil, so the leaf is fed with water without the stick insects having access to it (Figure 2-B). The leaves were changed on the 5th, 8th and 12th days (Phase 1). This first phase was carried out in Marseilles (France) while the following phases were carried out at Gembloux (Belgium). At the end of the 15th day, the leaves of Mediterranean plants were replaced by leaves of *Rubus idaeus* which were changed on the 5th, 8th and 12th following days (Phase 2). This second phase also lasted 15 days. The specimens were also followed for 91 days more to achieve an experience of 120 days (Phase 3). Phase 4 consisted of observing the stick insects for another 150 days. The results were compared to a control group, which under the same conditions was only fed on bramble or raspberry.

Cold survival of eggs (12th September 2015 to 30th April 2016)
A total of 12 eggs of an adult female, selected from the main breeding tank, were recovered after three and a half days of laying. Six eggs were kept at room temperature (ERT), while the other six were put in a refrigerator at 9 ± 1 °C (EC). After 21 days, the latter were placed next those named ERT. Their development was followed for nine months. All specimens born were fed on bramble or raspberry. Since it was not always possible to see all developmental instars, a measurement at a fixed date has been preferred. These measurements were carried out in Gembloux.

Results and discussion
The first experiment was to observe the survival of specimens on typically Mediterranean plants, that is to say, fed for 15 days only with bear's breeches, almond tree, fig blanche d'Argenteuil variety, fig violette de Solliès variety, olive tree and Aleppo pine. Table 1 shows the evolution of the different specimens of *M. extradentata* over time for each plant and for the control group. The high number of deaths found in the first phase with *A. mollis* is not consistent with what was expected. In fact, leaves of this plant were eaten either during a preliminary test or during a subsequent verification carried out from the 2nd to the 6th January 2016 (Figure 1-A and 1-B). No explanation was found for this observation, but two assumptions can be made. The first is related to the quality of the leaves picked. The leaves harvested in August were less lush and more elastic. The second hypothesis is related to the size of the specimens participating in the experiments. For all experiments, the number and the size of each specimen present are totally random. However, in the case of the first and last experiments, there was at least one specimen whose size was greater than the largest of the August's experiment (4.40 cm (August 2015), 6.70 cm (early) and 8.90 cm (late)). From the fifth day of the first test the authors were challenged by the inexplicable disappearances of the smallest *A. mollis* and *F. carica* violette de Sollies variety phasmids, thought only to be accounted for by incomprehensible escapes. Figure 1-C provides a more plausible explanation for this observation ; the specimens of the species studied, *M. extradentata,* are liable to cannibalism during severe food and water shortage (Wikimini, 2015). The first deaths were recorded on the sixth and tenth days for the olive tree and the Aleppo pine respectively, although in the case of the olive tree a death was found the day after the beginning of the test, but after examination of the corpse, in poor condition, this one has been excluded from the results. For these two plants, olive tree and pine, none of the ten (twice five) starting specimens survived the first phase of this experiment. It is clear that neither the olive tree nor the Aleppo pine allow the survival of the *M. extradentata* species. On the other hand, this is not the case for the tests carried out in the presence of

	A. mollis	*P. dulcis*	*F. carica*[b]	*F. carica*[c]	*O. europaea*	*P. halepensis*	control group
Start phase 1 Day 1	1.30 ; 1.35 ; 1.50 ; 2.60 ; 2.80 ; 4.40 a: 2.33	1.25 ; 1.30 ; 1.40 ; 2.40 ; 2.40 a: 1.75	1.30 ; 1.35 ; 2.50 ; 3.40 a: 2.14	1.20 ; 1.25 ; 1.25 ; 1.40 ; 3.90 ; 5.30 a: 2.38	1.20 ; 1.25 ; 2.15 ; 2.40 a: 1.75	1.15 ; 1.25 ; 2.50 a: 1.63	1.15 ; 1.25 ; 1.25 ; 1.25 ; 1.35 a: 1.25
Start phase 2[a] Day 15	1	5	4	5	0	0	5
Start phase 3 Day 29	-	1.80 ; 1.95 ; 2.00 ; 2.90 ; 3.50 a: 2.43	1.70 ; 1.95 ; 3.60 ; 3.75 a: 2.75	1.45 ; 1.70 ; 1.70 ; 3.55 ; 6.20 a: 2.92			1.90 ; 2.00 ; 2.03 ; 2.13 ; 2.65 a: 2.14
End phase 3 Day 120		4.60 ; 5.30 ; 5.60 ; 6.30 ; 6.90 a: 5.74	3.70 ; 5.10 ; 5.40 ; 7.60 a: 5.45	3.50 ; 4.6 ; 6.1 ; 9.3[d] a: 5.88			5.80 ; 6.90 ; 7.70 ; 7.80 ; 8.00 a: 7.24

[a] number of survivors

[b] blanche d'Argenteuil variety

[c] violette de Solliès variety

[d] the smallest specimen, which never moulted, died on the 56th day

Table 1. Size in centimeters of the specimens during the different phases of feeding with Mediterranean plants. Days are counted from the beginning of the experiment. a represents the average of the cell.

P. dulcis, *F. carica* blanche d'Argenteuil variety and *F. carica* violette de Solliès variety. Figure 2 clearly shows the areas eaten for these three plants and Table 1 describes the evolution over four months of the different specimens involved. It is clear that these three diets can perfectly allow a normal development of the specimens of the studied species. The change in the number of specimens in the case of the violette de Solliès variety fig tree calls for a number of comments since there have been two losses. As already mentioned above, there was a case of cannibalism on the fifth day. In addition, the smallest surviving specimen in the first two phases died on day 56 without moulting. These two events were not observed either with the almond leaves or with the fig leaves of the blanche d'Argenteuil variety, where all the specimens survived. Although the experiment was stopped after 120 days, a follow-up of more than 150 additional days (Phase 4) showed that the 12 stick insects were always healthy and all became adults, all females giving viable eggs. It should be noted, however, that if the control group was initially the smallest average size in the series, after 120 days, this control group became the largest, from 1.25 to 7.24 cm (a factor of 5.80), largely exceeding the three groups tested as shown in Table 1. The same remark can be made when analyzing the averages of Mediterranean plants, the average of specimens fed with almond tree being multiplied by 3.28 (from 1.75 cm to 5.74 cm) whereas for both varieties of fig the multiplication factor is only 2.55 and 2.47 respectively for the blanche d'Argenteuil variety and the violette de Solliès variety. Survival on *Ficus carica* leaves is not so surprising, as it is well known that it is possible to breed *M. extradenta* on *Ficus benjamina* (L. 1767) (Calvin & Lange, 2010 ; da Silva et al., 2012) which are both of the same genus, the first having the vernacular name "common fig tree" and the second "weeping fig tree".

The second experiment was to test the resistance to moderate cold of the eggs. The methodology of Potvin was not used (Potvin, 1996), but instead, a female was isolated for almost four days and its eggs, twelve in all, were then harvested and divided into two equal groups. The first one was kept at room temperature (ERT), that is to say at about 22 °C, while the second one, named EC, was placed 21 days in a refrigerator at 9 °C and then at room temperature. This value of 9 °C was chosen because it is accessible in Provence to eggs, even in winter in protected places. All ERT specimens were born between days 63 and 67, while only half of the ECs hatched between the 100th and the 106th day, and after 200 days, the last three ECs were declared unsustainable. There is therefore a lag of about

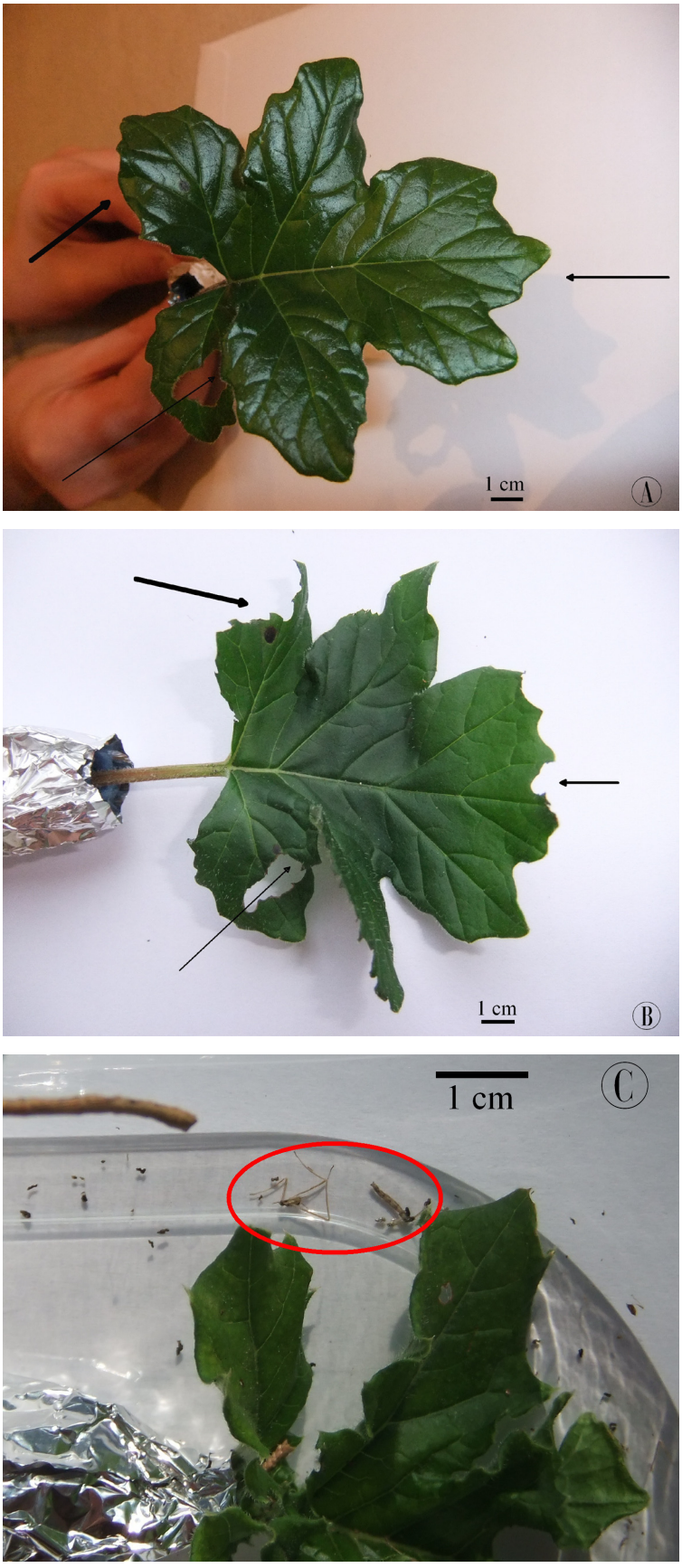

Figure 1. Tests with *A. mollis*. A: leaf at the beginning of the posterior experiment carried out from 2nd to 6th January 2016. The thickness of the line of the arrows shows the certainty that the place has been eaten. B: leaf at the end of the posterior experience. C: cannibalism (in the center of the oval) on the 9th day between specimens of *M. extradentata* in case of shortage of diet and water. G. Olive.

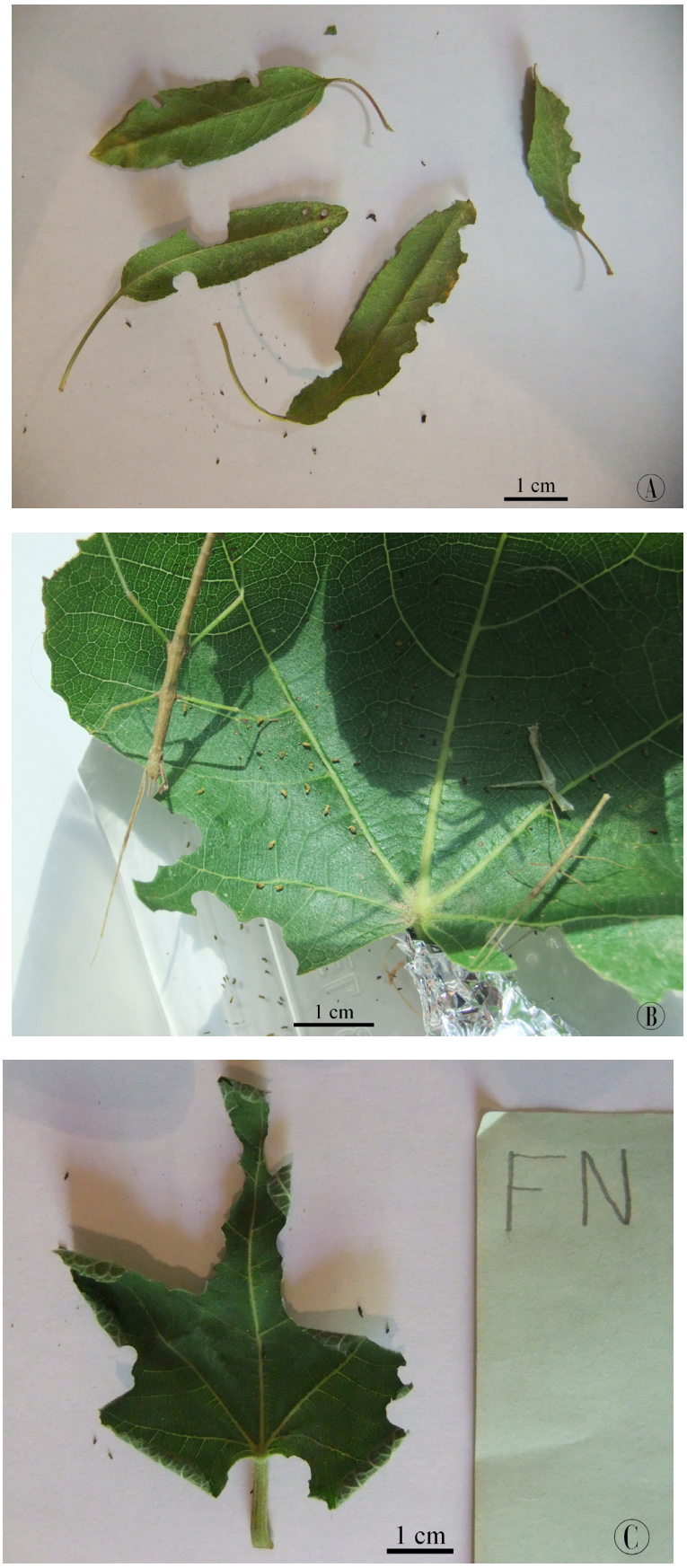

Figure 2. Leaves' surface eaten among the various Mediterranean plants allowing the survival of the species *M. extradentata*. A: *P. dulcis*. B: *F. carica* blanche d'Argenteuil variety. C: *F. carica* violette de Solliès variety. G. Olive.

	Eggs kept only at room temperature (ERT)	Eggs having been 21 days at 9 °C and then at room temperature (EC)
Number of days (and size (cm)) for birth	63 (1.20 ; 1.20 ; 1.25) 66 (1.15 ; 1.20) 67 (1.25) a: 64.7 (1.21)	100 (1.20) 106 (1.15 ; 1.20) a: 104.0 (1.18)
Number of days for first moult	101	133
Size (cm) at day 130	2.85 ; 2.90 ; 3.6 ; 3.65 ; 3.70 ; 3.90 a: 3.43	1.60 ; 1.70 ; 2.15 a: 1.82
Size (cm) at day 155	3.80 ; 4.60 ; 4.70 ; 4.90 ; 4.90 ; 5.10 a: 4.67	2.50 ; 2.65 ; 2.80 a: 2.65
Size (cm) at day 172	4.70 ; 4.80 ; 4.80 ; 6.10 ; 6.20 ; 6.25 a: 5.48	2.60 ; 2.80 ; 3.60 a: 3.00
Size (cm) at day 193	6.10 ; 6.10 ; 6.20 ; 7.70 ; 7.90 ; 8.05 a: 7.01	3.40 ; 3.40 ; 3.75 a: 3.52
Size (cm) at day 218 (sex)	6.80 (M) ; 6.80 (M) ; 7.00 (M) ; 9.20 (F) ; 9.30 (F) ; 9.50 (F) a: 8.10	5.60 (F) ; 6.25 (M) ; 6.40 (F) a: 6.08
Size (cm) at day 242 (sex)	a: 8.10	8.00 (F) ; 7.30 (M) ; 7.10 (F) a: 7.47
Size (cm) at day 270 (sex)	a: 8.10	10.10 (F) ; 7.30 (M) ; 10.80 (F) a: 9.40

Table 2. Summary of the cold resistance of the eggs. Days are counted from the day of egg collection. a represents the average of the cell.

40 days which is almost found during the first moult (Table 2). It also seemed important to see if the incubation temperature of the eggs had an influence on the development of young stick insects after their birth. Figure 3 shows that the temperature influences the growth of the young during the first 90 days of life, but then the difference disappears since the 110th day. In such cases, the analysis, based on linear adjustments[3], indicates that in the first part, with a slope of 0.04368 (Equation 1), the ERT specimens grow 1.6 times faster than the EC specimens with a slope of only 0.02673 (Equation 4). On the other hand, after that, the difference decreases with 0.04568 (Equation 2) and 0.03873 (Equation 5) respectively, and in terms of the average size, the ECs catch up with the ERTs (Figure 3) and even exceed them. A closer look shows that, in fact, the growth curve for ERT follows the Herschell-Bulkley law (Equation 3). Equations 1 to 5 give the evolution of the mean size of the specimens according to the number of days of life (Figure 3), whereas the equations 6 to 9 give this evolution according to the number of days of experimentation (Figure 4).

$$\text{Size ERT}_{90\text{ days}}\text{ (cm)} = 0.94222 + 0.04368 \times \text{NumberDaysLife} \text{ ; } r = 0.98717 \quad \text{Eq. 1}$$

$$\text{Size ERT (cm)} = 0.83830 + 0.04568 \times \text{NumberDaysLife} \text{ ; } r = 0.99008 \quad \text{Eq. 2}$$

$$\text{Size ERT (cm)} = 1.19383 + 0.000905 \times \text{NumberDaysLife}^{1.32246} \text{ ; } r = 0.99845 \quad \text{Eq. 3}$$

[3] Without forgetting that in the case of insects, the experimental values are discrete values by the principle of the moults.

Size $EC_{90\ days}$ (cm) = 1.18306 + 0.02673 × NumberDaysLife ; r = 0.99772 Eq. 4
Size EC (cm) = 0.79549 + 0.03873 × NumberDaysLife ; r = 0.94262 Eq. 5

Size ERT (cm) = -2.11689 + 0.04568 × NumberDaysExp ; r = 0.99008 Eq. 6
Size ERT (cm) = 0.14976 + 0.000866 × $NumberDaysExp^{1.69776}$; r = 0.99816 Eq. 7

Size EC (cm) = -3.23213 + 0.03873 × NumberDaysExp ; r = 0.94262 Eq. 8
Size $EC_{90\ days}$ (cm) = -3.19647 + 0.16460 × $NumberDaysExp^{0.70517}$; r = 0.99810 Eq. 9

The 218th day line in Table 2 shows that the cold does not eliminate any gender, thus allowing optimal survival of this species. The experiment was stopped when all surviving specimens reached the adulthood.

Conclusion

Thanks to the two experiments carried out, it is possible to answer the question of the title. The species *M. extradentata* could effectively live in Provence. Indeed, this species already accepts a large number of not typically Mediterranean diets (also present in the south of France) such as bramble, raspberry or even hazel. In addition, and this has just been demonstrated in this note, these stick insects can survive on Provençal plants, such as almond and fig, whether white or black variety. With the strong adaptability that this stick insect has shown, this species could become invasive during an accidental introduction. Furthermore, and this has just been highlighted in this work, the eggs are able to survive to the winter at relatively mild temperatures, as is it the case today with global warming.

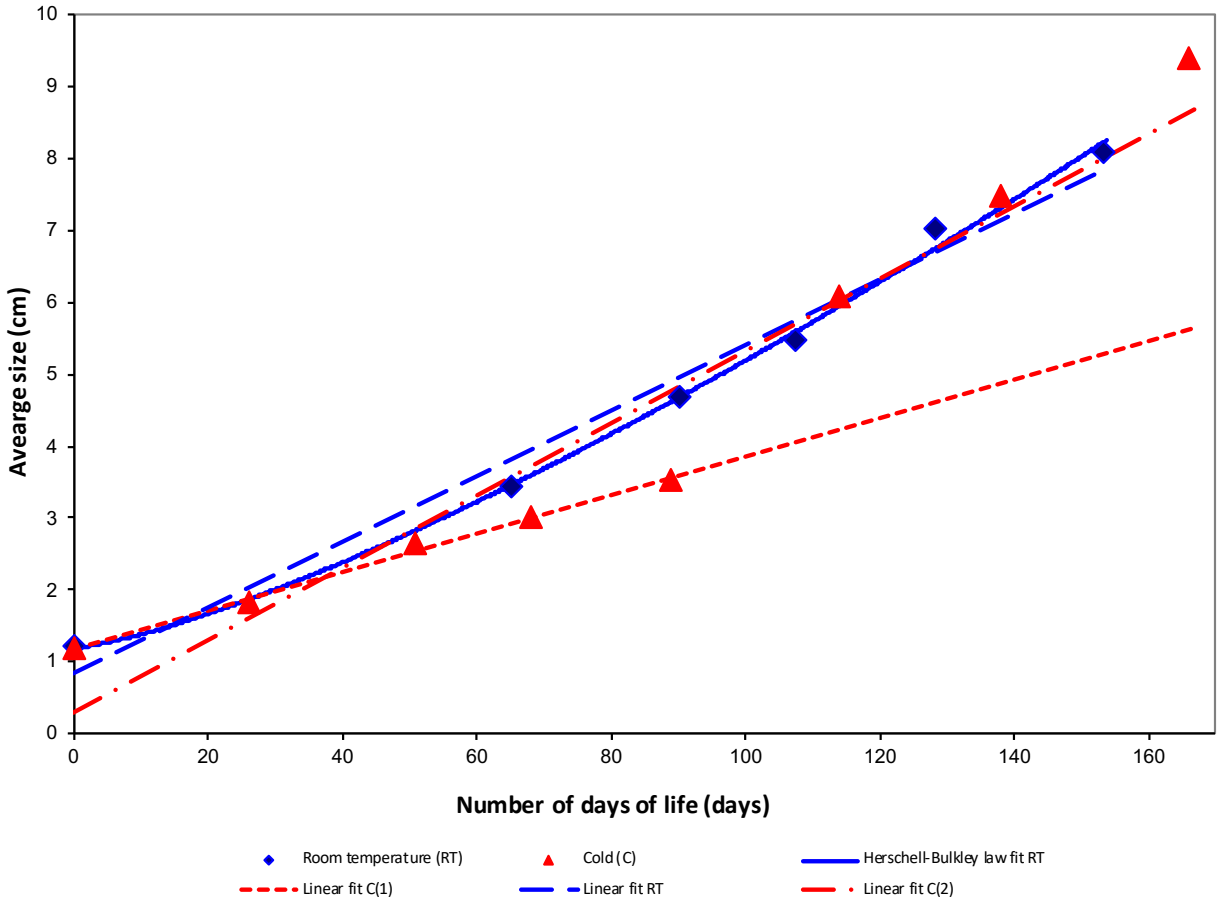

Figure 3 . Evolution of the average size of the specimens according to the number of days of life of the specimens for ERT and EC. Days are counted from the day of birth. C(1) corresponds only of the first 90 days of life of ECs. C(2) corresponds of all EC life days.

These tests will be repeated over longer periods to study, for example, whether specimens living on one of the plants mentioned, but also those which are derived from eggs having been placed in the cold, have the capacity to perform a complete cycle, if the eggs laid by adults are viable while comparing also their size compared to a control group. In addition, other plants, including *Dorycnium pentaphyllum*, and other colder temperatures will be tested. Indeed, despite all our efforts, it was impossible to find *D. pentaphyllum* in the study area.

Moreover, thanks to this communication, the number of diets accepted by this species has been increased, since it is able to feed on almond and fig leaves but also bear's breeches to a lesser extent.

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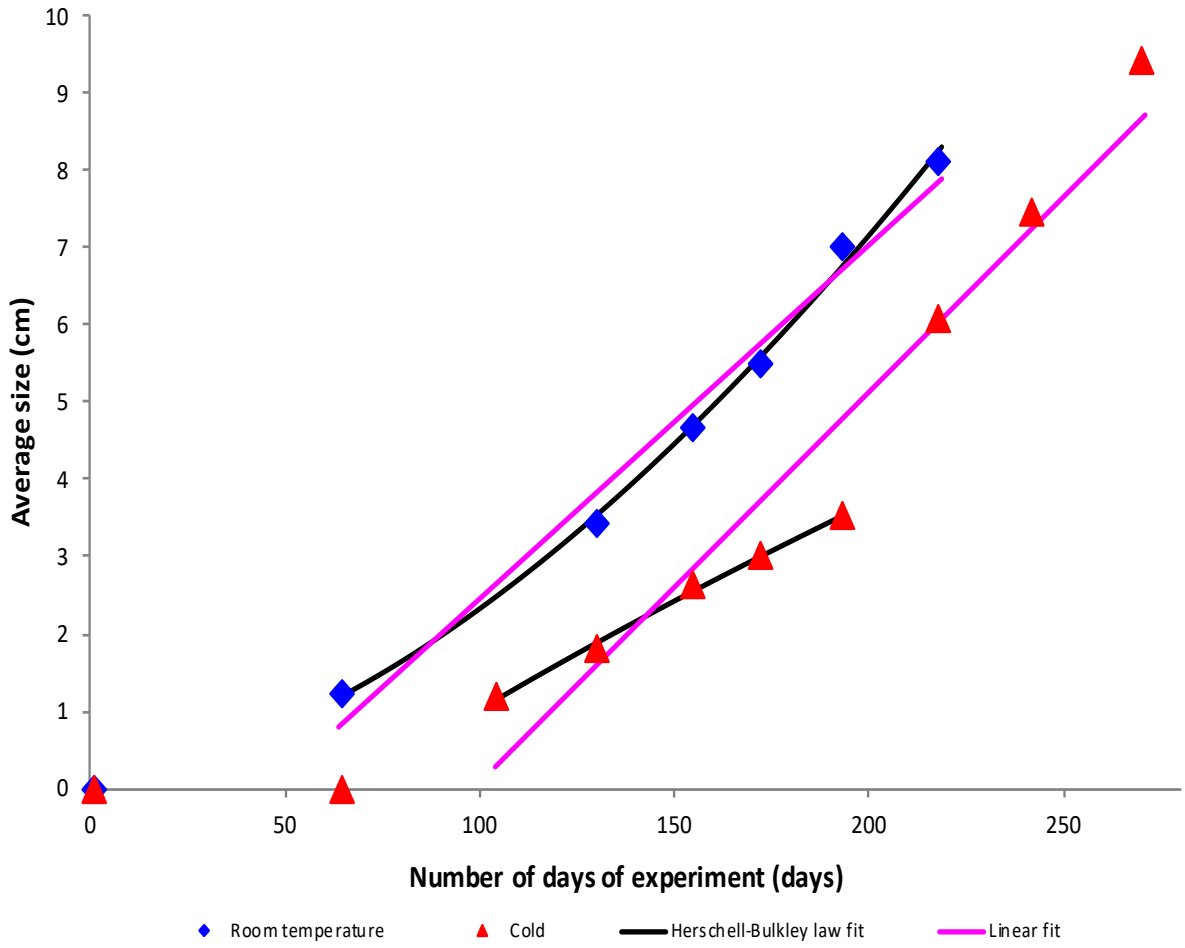

Figure 4. Evolution of the average size of the specimens as a function of the number of days of experiment for ERT and EC. Days are counted from the day of egg collection.

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