

## **Feeding and oviposition preferences of *Monochamus galloprovincialis* on its main hosts *Pinus sylvestris* and *Pinus pinaster***

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### **ABSTRACT**

Considering the key role of *Monochamus galloprovincialis* (Olivier) (Coleoptera: Cerambycidae) in the dispersion of the pine wood nematode, *Bursaphelenchus xylophilus* (Steiner & Bühner) Nickle (Nematoda: Aphelenchoidea), in Europe, defining the host spectrum and preference of *M. galloprovincialis* is of primary importance for forest management. Therefore, comparative studies under laboratory conditions were performed in order to define the feeding and oviposition preferences of *M. galloprovincialis* adults as well as the larval development on *Pinus sylvestris* and *Pinus pinaster*. Both nutrition and oviposition were significantly higher on *P. sylvestris*. However, no difference was found when comparing larval survival on the two *Pinus* species even though larvae attended the fourth instar earlier when reared on *P. sylvestris* than on *P. pinaster*. The results of this study reinforce the suspicions of a future rapid propagation of the nematode, by *M. galloprovincialis*, into the Pine forests all over Europe.

KEYWORDS: *Pinus sylvestris*, *Pinus pinaste*, *Monochamus galloprovincialis*, host preference.

### **Introduction**

*Monochamus galloprovincialis* (Olivier 1795) (Coleoptera: Cerambycidae), widely known as the pine sawyer, feeds and develops mainly on *Pinus* tree species (Picard 1929, Portevin 1934, Hellrigl 1971, Villiers 1978, Sama 2002). This species was considered as a secondary forest pest. Nonetheless, a Pest Risk Analysis on the European Union territory recognized *M. galloprovincialis* as potential vector for the Pine Wood Nematode (PWN)

*Bursaphelenchus xylophilus* (Steiner & Bühner) Nickle (Nematoda: Aphelenchoidea) in Europe (Evans et al. 1996). This nematode is responsible of the Pine Wilt Disease (PWD) and has already devastated hundreds of millions of hectares of pine stands in Asia (Mamiya 1988). Following the introduction of *B. xylophilus* in Portugal in 1999 in *Pinus pinaster* (Aiton) stands, early records mentioned its association with *M. galloprovincialis* confirming its role as a vector of the nematode (Sousa et al. 2001, Sousa et al.

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2002). *M. galloprovincialis* adults transport and transmit the PWN during their obligatory sexual maturation nutrition on healthy pine trees and during their oviposition on weakened or recently dead trees. These two periods of the vector's life cycle correspond to two different dispersal periods conditioned by research of adequate hosts and consequently favour the nematode's dispersion.

Considering its role in the dispersion of the nematode, defining host spectrum and preference of *M. galloprovincialis* is of primary importance for forest management. In Portugal only *P. pinaster* has been found to be infested by the nematode, although other *Pinus* species (*P. pinea* L. and *P. halepensis* Miller) grow in the infested zone (Mota et al. 1999, Naves et al. 2006). *P. sylvestris* L. is the most frequent host of *M. galloprovincialis* in Northern and Central Europe while its Southern and Mediterranean populations are more frequently collected on *P. pinaster* and *P. halepensis* (Hellrigl 1971, Francardi et al. 2000). A recent host choice experiment under laboratory conditions showed that Portuguese populations of *M. galloprovincialis* exhibit preferences for *P. sylvestris* among several pine species for nutrition, although the absence of preference among host species tested as oviposition substrates demonstrated that multiple hosts can be adequate for oviposition (Naves et al. 2006).

The southern limit of the *P. sylvestris* range is on the Iberian Peninsula (Mirov 1967, Richardson and Rundel 1998) while *P. pinaster* is a Western Mediterranean species occurring up to the Atlantic coasts of France and its Northern limits are beneath Northern France. The majority of conifer trees composing the French forests are *P. pinaster* (1.4 Mha) and *P. sylvestris* (1.1 Mha). Furthermore, in France both the Mediterranean form, *M. g. galloprovincialis*,

and the more Northern European form, *M. g. pistos* (Germar 1818), two potential vectors of the PWN, were observed attacking several pine species (Villiers 1978, Vives 2000) generating a great risk for the country in case of an eventual introduction of the PWN on its territory. The adequate climatic conditions for all three partners of the PWD (nematode, hosts and vectors) in France would make it a pathway allowing the propagation of the PWD from the Iberian Peninsula to the rest of Europe. Therefore, a surveillance network and management strategies, with particular attention on the areas where the insect and its preferable hosts exist, should be developed.

Owing to former observations of feeding preference for *P. sylvestris* and considering the large distribution of *P. sylvestris* in Northern Europe, we investigated whether French individuals of *M. galloprovincialis* exhibit significant preferences between the two most common *Pinus* species in France, *P. pinaster* and *P. sylvestris*, when given the choice for nutrition and oviposition. Experiments under laboratory conditions were conducted in order to gather information on the insects' ability to adapt to other host species than their larval host during feeding and oviposition. We also compared the larval performances between the two pine species.

## Materials and Methods

### Experimental device

All *M. galloprovincialis* adults used in this study originated from a laboratory population (1<sup>st</sup> and 2<sup>nd</sup> generation) reared on *P. sylvestris*. No tests were performed with adults originated from *P. pinaster* due to insufficient number of insects originated from this *Pinus* species. All beetles emerged in June 2006. Pine branches and logs used for feeding and oviposition preference assay were collected from the Trois Pignons forest

(Fontainebleau France, 48°24' N and 02°33' E). Larger branches (diameter:  $5 \pm 0.1$  cm) of healthy-looking *P. sylvestris* and *P. pinaster* (trees about 40years old) were collected at the end of June 2006 cut into logs and brought to the laboratory as oviposition substrates (Table 2). Young shoots and thinner branches (up to 3 cm diameter) were also brought to the laboratory as feeding substrates. Branches for nutrition were put in containers whose bottom was filled with water. Extremities of logs were sealed with paraffin to prevent desiccation and kept at 4°C until use. Both nutrition and oviposition experiments occurred at 21°C and under a photoperiod of 12:12 (L:D) in the laboratory.

For the feeding preference test, between *P. sylvestris* and *P. pinaster*, 38 adults (21 males and 17 females), which body length was previously measured, were placed separately in plastic boxes (26.5x13.5x7.5cm) in which a 5cm diameter hole had been prepared and covered with 1 mm mesh tulle. Each adult was provided with about the same quantity of *P. sylvestris* and *P. pinaster* branches, needles and sometimes cones. The two pines material was placed separately at the two extremities of the box. Moistened paper was also added in the boxes. After 36 h (36hA) branches, needles and cones were replaced by new ones and were checked out for feeding wounds. The number of needles cut down and the total length of needles eaten were recorded. The feeding activity on the new substrate was also checked by measuring bites surface after additional 36 h (36hB). The wounds were photographed with a Canon PowerShot A80 digital camera and they were measured on the pictures using ImageJ 1.32j.

Test for oviposition preference between *P. sylvestris* and *P. pinaster* was performed with the females that had fed either only on *P. sylvestris* or on both *P. sylvestris* and *P.*

*pinaster* (females from the feeding preference test) during their maturation nutrition for twenty days. Each female was kept with one male (same males from the feeding preference test) in a plastic box (89x38x29cm) covered in its whole surface by a plastic net (1mm mesh) in the presence of a *P. sylvestris* and a *P. pinaster* log as oviposition substrate. Some shoots of both *Pinus* species were also added for nutrition. The logs were placed to the opposite ends of the box, separated by the shoots. The adults were placed in the middle of the box and were allowed to circulate freely in it and lay eggs during 48 h. Logs were finally removed and checked for the occurrence of oviposition scars (slits), under a binocular lens. The number of slits with and without jelly was recorded. Oviposition scars with jelly, secreted by the females during oviposition procedures, were considered to contain at least one egg according to Anbutsu and Togashi (1997). They proposed, for *M. alternatus*, that jelly was a reliable prediction of egg deposition. Slits without jelly were inspected for the presence of eggs. The logs supporting eggs were placed in plastic containers (53cm high and 47.5cm in diameter) covered with tulle for aeration and protection from other woodborers attacks. Containers were stored outdoor during larval development, and sheltered from rain to avoid excessive fungal development. At the end of February, when most larvae had completed their development (Togashi 1991, Togashi et al. 1994, Naves 2007), logs were debarked and sliced to check for the occurrence of the different larval instars. Live larvae were also weighted and their body length was measured. After dissection the head capsule length and width were measured and, according to Koutroumpa et al. (2008), used to determine the larval instar. The number of all larvae found alive, besides the fourth instar (final instar) larvae, (A), was

withdrawn from the total number of eggs laid (E). Therefore, the survival for the fourth instar larvae (SL4) was the number of the fourth instar larvae found alive (AL4) divided by (E-A) and this separately by *Pinus* species.

$$SL4 = AL4 / E - A$$

### Statistical analysis

The effect of sex on the adult nutrition (consumption of bark surface and quantity of needles eaten as well as number of needles cut down) was tested with Mann Whitney U statistical test while for the effect of the two 36h experiments (A and B) a Wilcoxon test was used. All paired tests (adults' nutrition and females' oviposition preference, surface and volume of the logs as well as egg density on the two host species) were conducted with the Wilcoxon's test and all unpaired (larval size, survival and head capsule width differences between *P. sylvestris* and *P. pinaster*) with Mann Whitney's U statistical test. The Pearson's correlation test was used to check for relationships between adult size and consumption parameters. It was also used to check for relationships among logs' parameters (surface and volume) and oviposition rate, larval survival and size variation among the different instars. Differences were considered significant for  $P \leq 0.05$ . Values are presented with their standard error. All statistical tests and calculations were performed with GraphPad InStat version 3.00 (Motulsky 1999).

## Results

### Feeding preference test

Fifteen individuals (41.7%) fed only on *P. sylvestris* the first 36h, thirteen (36.1%) fed only on *P. pinaster* and seven (19.4%) fed on both species. Only one individual failed to feed in the 36hA. In 36hB experiment most insects continued to feed

on the same host as in the 36hA. However, the percentage was higher for those that had started their nutrition on *P. sylvestris*; 73.3% continued feeding on *P. sylvestris* versus 38.5% that continued to feed on *P. pinaster*. 42.8% of the adults that had no special preference for a host species, at 36hA, continued their nutrition on both of them. The remaining insects consumed either *P. sylvestris* or *P. pinaster* (28.6% each). When the first contact with a host species (36hA) was not considered almost half of the adults fed on *P. sylvestris* (47.2%) while about the same number fed on *P. pinaster* or both pine species (25% and 27.8% respectively). Two males did not feed at all during the 72h and were excluded from analyses. For the two sets of experiments (36hA and 36hB), no significant difference between *P. sylvestris* and *P. pinaster* was found for males and females that fed on *P. sylvestris* and *P. pinaster*, considered together or separately. The Mann Whitney test showed no effect of the sex on any of the tested parameters (bark surface, quantity of needles eaten and number of needles cut down) and this for any of the two pine species tested and for the two experiments (36hA and 36hB). Therefore, the rest of the analyses was continued with males and females grouped together. The Wilcoxon test showed no significant difference between the two 36h experiments (A and B), except for the length of the *P. sylvestris* needles eaten that was higher for the 36hB (mean 36hA:  $3.6 \pm 1.1$  and 36hB:  $7.3 \pm 1.9$ ,  $P = 0.02$ ). Consequently, to test the differences in nutrition on the two host species, the two experiments A and B were grouped together for the number of needles cut down and the bark volume consumption but were analyzed separately for the needles' length consumption. These analyses showed very significant statistical differences in the number of needles cut down and the bark consumed volume between the two *Pinus* species (Table 1). No

significant difference was found to the quantity of pine needles consumed.

Male and female adults had similar sizes (males:  $18.0 \pm 1.42$  mm and females  $19.5 \pm 1.66$  mm) and were, therefore, grouped for the following analyses. No correlation was evident between the adult size and the consumption of the different parameters tested (bark surface, quantity of needles eaten and number of needles cut down), for the two consecutive 36h and for the two pine species.

### Oviposition preference test

A total of 601 eggs were laid, 202 on *P. pinaster* and 399 on *P. sylvestris*. No significant differences of oviposition rate and larval survival were found between the females that fed only on *P. sylvestris* or on both pine species during their maturation nutrition. Data were then pooled for further analyses.

A significant difference, in favor of *P. sylvestris*, was found for the number of eggs laid (66.6% on *P. sylvestris* and 33.4% on *P. pinaster*) and for the number of larvae found alive, but no significant difference was found for the larval survival per log between

*P. sylvestris* and *P. pinaster* (Table 2). The number of eggs laid was correlated with the number of larvae found alive (Fig. 1). The survival was negatively related to the egg density in the logs (Fig. 2) but the density did not vary with the *Pinus* species. Furthermore, significant difference was found for the surface and the volume of the logs corresponding to the two hosts (Table 2). Surface and volume of the logs were not related to the number of eggs laid and of larvae found alive in the two host species.

No significant difference was found between head capsule width of the third and fourth instar larvae between the two pine species. However, a difference in the body length and the weight of the fourth instar larvae was found in favor of *P. pinaster* (Table 2). As a *P. pinaster* log was found to be much bigger than the rest of the logs, and contained only big fourth instar larvae, it was withdrawn from the analyses. The differences between logs were then not significant and no correlation was found between logs size and the length and weight of the fourth instar larvae.

TABLE 1. Mean consumption on the two conifers *P. sylvestris* and *P. pinaster* for the 36 *M. galloprovincialis* adults tested twice 36h (A and B).

Species	Bark surface (cm <sup>2</sup> )		Needles cut down		Needles length eaten (cm)	
	36h (A and B)		36h (A and B)		36hA	36hB
<i>P. sylvestris</i>	$0.7 \pm 0.2$		$15.6 \pm 3.1$		$3.4 \pm 1.1$	$7.3 \pm 1.9$
<i>P. pinaster</i>	$0.15 \pm 0.06$		$3.8 \pm 0.7$		$8.4 \pm 2.9$	$5.3 \pm 1.4$
<i>P</i> value*	0.0009		0.0027		NS	NS

\**P* values are given for statistically significant results (NS=non significant)

TABLE 2. Mean and *P* values of log parameters, eggs laid, number of larvae found alive and their survival on *P. sylvestris* and *P. pinaster*, as well as fourth instar larvae (*L*<sub>4</sub>) body length and weight when developed in these two different hosts.

	<i>P. sylvestris</i>	<i>P. pinaster</i>	<i>P</i> *
Log surface	610.8 ± 17.4	560.7 ± 21.0	0.0003
Log volume	822.9 ± 48.9	696.9 ± 59.9	<0.0001
Eggs laid	8.9 ± 1.0	4.4 ± 1	0.0041
Larvae alive	3.2 ± 0.3	1.6 ± 0.2	0.0019
Survival	0.47 ± 0.06	0.43 ± 0.06	NS
<i>L</i> <sub>4</sub> body length	25.17 ± 0.52	28.67 ± 1.16	0.0075
<i>L</i> <sub>4</sub> body weight	0.25 ± 0.01	0.35 ± 0.03	0.0093

\**P* values are given for statistically significant results (NS=non significant)

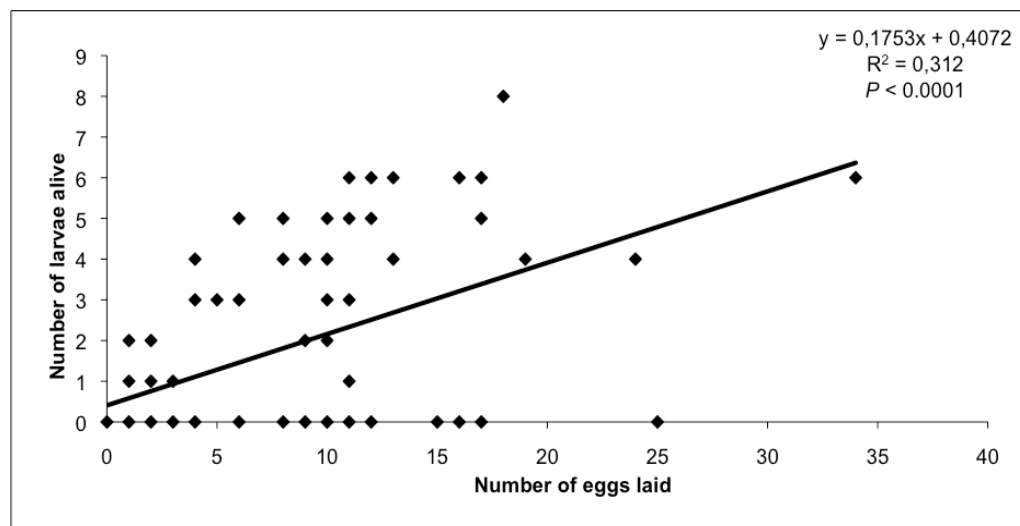


FIG. 1. Correlation of number of eggs laid per log and of the number of larvae found alive in each of these logs.

Larvae developing in *P. sylvestris* and *P. pinaster* differed in their development speed (Fig. 3). More larvae reached the L4 instar when developed in *P. sylvestris* than larvae in *P. pinaster*. The survival ratios for the fourth instar larvae were 0.28 and 0.18 for *P. sylvestris* and *P. pinaster* respectively.

## Discussion

Our experiment showed that although adults of *M. galloprovincialis* consumed both *P. sylvestris* and *P. pinaster*, they preferred bark and needles from *P. sylvestris*. Furthermore, there was a preference for ovipositing on this host species. Our results are consistent with these of Naves et al. (2006) showing also a feeding preference for *P. sylvestris* even though their adults had emerged from *P. pinaster*. However, he found no significant difference in the choice of oviposition substrate. Therefore, *P. sylvestris* might be the preferred host for maturation feeding of *M. galloprovincialis* whereas the oviposition might have been influenced by the larval host species. Attention should be given when these results are reflected on natural environment.

Concerning larval final instar survival in the two pine species logs, at the end of winter that corresponds to the end of the diapause (Togashi 1991, Togashi et al. 1994, Naves 2007), we found no significant difference in survival rates between larvae developing in *P. sylvestris* and in *P. pinaster*. As the number of eggs laid was correlated with the number of larvae found alive, the number of alive larvae was higher in *P. sylvestris* than in *P. pinaster* since the number of eggs laid was higher in this species. Furthermore, delayed development was obvious for larvae developing in *P. pinaster*. This result suggests that development of the *M. galloprovincialis* individuals under study was more affected

by the host species than development of the Portuguese individuals that showed no difference in their emergence time between the two conifers (Naves et al. 2006). However, it has been shown that adult emergence occurs within a narrow period (61 days) even though all four larval instars are present in spring (Naves 2007, Koutroumpa et al. 2008). As the larval development was controlled before adult emergence, we could not observe if delayed larvae in *P. pinaster* logs would have emerged as adults at the same time as larvae in *P. sylvestris*, supporting the founding of simultaneous emergence in Koutroumpa et al. (2008).

The size of *M. galloprovincialis* last instar larvae was not significantly different between *P. sylvestris* and *P. pinaster*, even though it appeared bigger when developed in *P. pinaster* logs, and seemed to be more influenced by the size of the log. In contrast, the Portuguese individuals of *M. galloprovincialis* that emerged after developing in *P. pinaster* logs had a significantly more important size compared to those that developed in *P. sylvestris* (Naves et al. 2006).

Our findings, under laboratory conditions and those of Naves et al. (2006) on the same species, differ from observations in the field (personal observations, Hellrigl 1971) that indicate preference of Southern populations of *Monochamus* for *P. pinaster* and Northern ones for *P. sylvestris*. Therefore, additional factors such as temperature or humidity could interact with pine species in the distribution pattern of this insect. According to Villiers (1978) the Cerambycidae beetles need rather hot summers for their development and they can easily support cold winters. This is probably the reason why the North-East of France has a more diversified fauna than the North-West

(Villiers 1978). Considering the two European forms previously described within

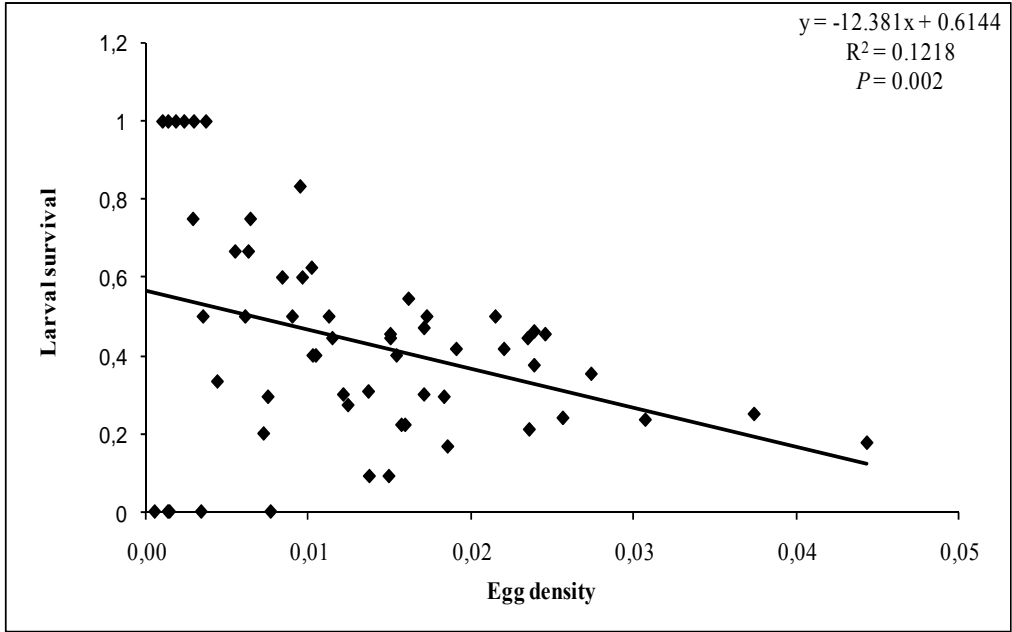


FIG. 2. Correlation of egg density on the logs and survival rate per log.

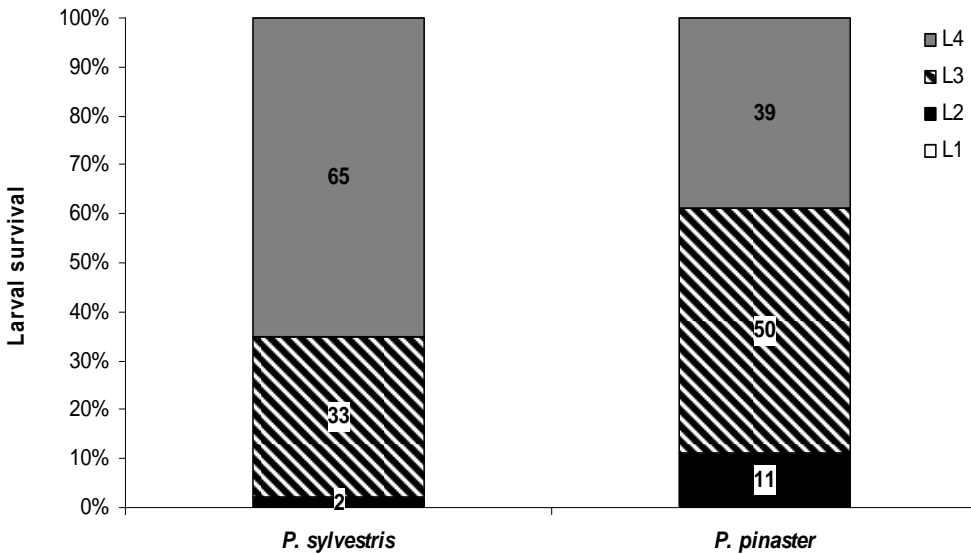


FIG. 3. Percentage of *M. galloprovincialis* larvae found alive in *P. sylvestris* and *P. pinaster* for each larval instar (L<sub>1</sub>, L<sub>2</sub>, L<sub>3</sub> and L<sub>4</sub>).



*M. galloprovincialis*, the Mediterranean one *M. g. galloprovincialis* was mainly found in France at low altitude, whereas *M. g. pistor* was mainly observed at higher altitude and in Northern Europe (Hellrigl 1971). The geographical distribution of the form *M. g. galloprovincialis* coincides more with the one of its Mediterranean hosts, even though when in contact with *P. sylvestris* (this study) its preference for this *Pinus* species is clearly declared. Even though a preference for *P. sylvestris* was evident by our results, the host species does not seem to restrict this insect species. These results together with the global warming during the last decades could have dangerous impact on the geographical range expansion of *M. galloprovincialis*, from Mediterranean populations to more Northern ones. As already observed for other insects such as the pine processionary moth *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae) (Battisti et al. 2005), two are the possible scenarios. The first implies shifting of its range boundaries following up northwards the range of its principal host species. The impact of climate change has been studied for 67 forest tree species in France and it has been found that the extension of the Atlantic and the Mediterranean species is possible while a regression of the mountain species range is expected (CARBOFOR project 2004). Considering the plasticity in feeding of *M. galloprovincialis*, as shown in this study, the second pattern would be a probable adaptation on new hosts. Furthermore, the risks of *Monochamus* populations' expansion to other secondary hosts such as *P. pinea* or *P. nigra* (Naves et al. 2006) would be very important in the case of expansion of the PWD, which is usually followed by outbreaks of the *Monochamus* populations. This was the case in Japan after the introduction and installation of the PWN

on its territory during the second world war (Mamiya 1988, Takasu et al. 2000).

Facts such as global warming and expansion of the PWD range in Portugal (Sousa personal communication) in combination with the results of this study underline the dangerousness of this insect for the conifer forests and the importance of these results in the surveillance and management of the disease.

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**Προτιμήσεις στη διατροφή και ωτοκία του *Monochamus galloprovincialis* (Coleoptera Cerambycidae) πάνω στους βασικούς του ξενιστές *Pinus sylvestris* και *P. pinaster***

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**ΠΕΡΙΛΗΨΗ**

Θεωρώντας τον ρόλο του *Monochamus galloprovincialis* κλειδί στην εξάπλωση του νηματώδη του πεύκου στην Ευρώπη, η διαλεύκανση του εύρους των ξενιστών και των προτιμήσεων του *M. galloprovincialis* σε αυτούς είναι πρωτεύουσας σημασίας για την προστασία του δάσους. Συγκριτικές μελέτες σε συνθήκες εργαστηρίου διεξήχθησαν με σκοπό την διευκρίνηση των διατροφικών και ωοθετικών προτιμήσεων των ενηλίκων αυτού του εντόμου καθώς και της ανάπτυξης των προνυμφών του στα *P. sylvestris* και *P. pinaster*. Η διατροφή και η ωοτοκία βρέθηκαν στατιστικά πιο σημαντικές στο *P. sylvestris* αλλά καμία διαφορά δεν βρέθηκε στην επιβίωση των προνυμφών στα δύο είδη πεύκου. Οι προνύμφες έφτασαν στην 4<sup>η</sup> ηλικία νωρίτερα στο *P. sylvestris* από ότι στο *P. pinaster*. Τα αποτελέσματα της παρούσης μελέτης ισχυροποιούν τις υποψίες για μια γρήγορη μελλοντική εξάπλωση του νηματώδη σε ολόκληρη την Ευρώπη.