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Biology of *Monochamus galloprovincialis* (Coleoptera, Cerambycidae) in the Pine Wilt Disease Affected Zone, Southern Portugal

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Abstract. The biology of *Monochamus galloprovincialis*, which is the vector of the pathogenic nematode *Bursaphelenchus xylophilus*, was studied in the pine wilt disease-affected zone south of the Tejo River, Portugal. Insects required ten to thirteen months to develop and had a single generation per year. The emergence pattern during 2001 to 2004 occurred from May to September with a peak in July, being consistent with the captures of attractive traps (flight curve). Emergencies occurred slightly earlier for males than for females, with a global sex ratio of 0.48. Females laid eggs on trap-trees from May to August and the young larvae had an extremely fast development before tunnelling into the xylem, where they passed the winter. The number of larval instars could not be defined by the frequency distribution of head-capsule width measurements. Developmental success and adult dimensions differed between tree sections, being higher for the trunk. Mortality was generally low for all developmental instars and the within log generation survivorship from egg to adult was 53%. The most important identified mortality agents were the fungi *Beauveria bassiana* and the parasitic wasp *Cyanopterus flavator*. Results are discussed in view of the current strategies to control the insect and the possible existence of a winter dormancy affecting the insect's larvae.
Key words: Pine sawyer; life cycle; emergence; seasonal development; mortality

Biologia do *Monochamus galloprovincialis* (Coleoptera, Carambycidae) na Região Afectada pelo Nemátodo da Madeira do Pinheiro em Portugal

Sumário. A biologia do *Monochamus galloprovincialis* em pinheiro bravo *Pinus pinaster* foi estudada na região da península de Setúbal, Portugal, onde este insecto é o vector do nemátodo da madeira do pinheiro *Bursaphelenchus xylophilus*. O desenvolvimento larvar durou cerca de dez-treze meses, com uma única geração anual. Entre 2001 e 2004 o período de emergência ocorreu entre Maio e Setembro com um pico em Julho, sendo consistente com o padrão de capturas de insectos em voo por armadilhas iscadas com atractivos (curva de voo). Os machos emergiram ligeiramente mais cedo que as fêmeas, verificando-se um sex ratio global de 0.48.

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No terreno, foram encontradas posturas entre Maio a Agosto, tendo-se constatado que as larvas recém-eclodidas tiveram um desenvolvimento muito rápido durante os meses de Verão, permanecendo em galerias na madeira durante o Inverno. Não foi possível discriminar o número de estádios larvares desta espécie através da distribuição de frequência das medidas da cápsula cefálica. A taxa de sobrevivência e as dimensões dos adultos diferiram consoante as zonas de desenvolvimento na árvore, tendo sido maiores para os insectos provenientes do tronco. No geral, a mortalidade foi baixa para todos os estádios de desenvolvimento estudados, com taxa de sobrevivência generacional (do ovo ao adulto) de cerca de 53%. Os factores de mortalidade mais importantes foram o fungo *Beauveria bassiana* e o parasitóide larvar *Cyanopterus flavator*. Os resultados obtidos são analisados face às estratégias de controlo do insecto actualmente implementadas e à possibilidade de existir uma fase de dormência larvar associada às larvas de *M. galloprovincialis*.
Palavras-chave: Longicórnio do pinheiro; ciclo de vida; padrão de emergência; desenvolvimento sazonal; factores de mortalidade

Biologie de *Monochamus galloprovincialis* (Coleoptera, Cerambycidae) dans la Région Affectée par le Nématode du Pin au Portugal

Résumé. La biologie de l'insecte *Monochamus galloprovincialis* sur le pin maritime *Pinus pinaster* a été étudiée dans la région de la péninsule de Setúbal, au Portugal, où cet insecte est le vecteur du nématode du pin *Bursaphelenchus xylophilus*. Le développement larvaire a duré environ dix - treize mois, avec une seule génération annuelle. Entre 2001 et 2004 la période d'émergence se faisait entre mai et septembre avec un pic en juillet, en cohérence avec les captures d'insectes en vol par des pièges avec attrayants (courbe de vol). Les mâles ont émergé légèrement plus tôt que les femelles, avec un sex-ratio de 0.48. Sur le terrain, ont été trouvées des pontes de mai à août et les jeunes larves ont eu un développement très rapide pendant les mois d'Été, effectuant plus tard des galeries dans le bois où elles sont abritées en Hiver. Il n'a pas été possible de déterminer le nombre de stades larvaires de cette espèce à travers la distribution de fréquence des mesures de la capsule céphalique. Le taux de survie et les dimensions des adultes ont différé selon les zones de développement dans l'arbre, ayant été plus grand pour les insectes provenant du tronc. En général, la mortalité a été basse pour tous les stades de développement étudiés, avec un taux de survie de l'œuf à l'adulte de 53%. Les facteurs de mortalité les plus importants ont été le champignon *Beauveria bassiana* et le parasitoïde larvaire *Cyanopterus flavator*. Les résultats obtenus sont analysés face aux stratégies de contrôle de l'insecte actuellement mis en œuvre et à la possibilité d'une phase de dormance larvaire associée aux larves de *M. galloprovincialis*.
Mots clés: Longicorne du pin; cycle de vie; courbe d'émergence; développement saisonnier; facteurs de mortalité

Introduction

The genus *Monochamus* contains about 150 species widespread throughout North America, Europe and Asia, being particularly numerous in Africa (HELLRIGL, 1971). In Europe occur five native species of which the *M. galloprovincialis* (Olivier) was recently found to be the vector of the pathogenic pine wood nematode *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle, which was recently introduced into Portugal (SOUSA *et al.,* 2001).

According to FRANCARDI and PENNACCHIO (1996), the pine sawyer *M. galloprovincialis* has one generation per year in Italy, although in colder regions of central and northern Europe the majority of the insects require two years to complete their development (TOMMINEN, 1993). To reproduce the females need stressed, dying or recently killed trees and freshly felled timber (HELLRIGL, 1971) being pines the most important hosts (e.g., HELLRIGL, 1971; FRANCARDI and PENNACCHIO, 1996; FRANCARDI *et al*., 1998). The eggs are deposited in slits excavated by the females in the bark of suitable hosts and the newly-hatched larvae develop in individual galleries in the phloem before entering into the xylem, where they built a linear gallery ending in a pupal chamber from where the adults emerge through a perfectly round-shaped hole (HELLRIGL, 1971; FRANCARDI and PENNACCHIO, 1996). The number of larval instars is unknown for this species.

Before the introduction of *B. xylophilus* in Europe the pine sawyer *M. galloprovincialis* was generally looked upon as a secondary forest insect and its biology and ecology had never been thoroughly studied, with the exception of the classic paper of HELLRIGL (1971) and the works of FRANCARDI and PENNACCHIO (1996) and FRANCARDI *et al*. (1998) in Italy. Nevertheless, detailed biological knowledge is necessary to develop pest management programmes against the pine sawyer in Portugal, in order to prevent the dissemination of wilt disease and to efficiently control the pine wood nematode and its vector. The objective of this paper is to study some aspects of the biology of *M. galloprovincialis* in the pine wilt disease--affected zone in Portugal, namely the number of annual generations, seasonal development, pattern of adult emergence and its variation throughout the years, determination of the number of larval instars and to evaluate, both quantitatively and qualitatively, the mortality factors affecting the eggs, larval and pupal stages.

Materials and methods

Study 1 - Emergence of adult *M. galloprovincialis*

Between four and nine pine trees with *M. galloprovincialis* were cut every year from 2001 to 2004 during January and February on Tróia peninsula (38º28'N; 8º53'W), Portugal. The trees were selected from adult maritime pines (*Pinus pinaster* Aiton) which had died from *B. xylophilus* infection the previous year. After being cut the trunk and branches were carefully analyzed and all segments thought to contain *M. galloprovincialis* larvae were divided into approximately 60cm-long bolts which were placed in black plastic containers (approximately 0.6m^3) covered at the tops with semi-transparent cloth meshes and kept on a shaded place with ambient temperature at Tróia. The wood material from each tree was divided according to its origin and maintained isolated: the canopy was divided into three-thirds and its branches separated as "upper", "mid" and "lower", while the wood from the trunk was divided as "upper trunk" (bolts with a diameter below 9cm) and "lower trunk" (diameter above 9cm). Each year from April to October all the containers were observed at least twice a week (although most frequently between three and four times each week), collecting all the emerging beetles which were sexed, weighted and measured (length of the right elytra).

Additionally, from May to October 2001 trap-trees were created at Tróia to study the adult's emergence pattern from different-aged pines. Each month two healthy adult *P. pinaster* trees were randomly selected and manually debarked with an axe at a height of 1.5 meters, in order to interrupt the sap flow and gradually debilitate the standing trees, making them attractive to breeding *M. galloprovincialis* females in the stand. In November 2001 the trees were cut, divided and stored until the insect's emergence in 2002 as previously described.

The sex ratio of emerging beetles was calculated as ♀/(♂ + ♀). Daily temperatures for Tróia (period of 2002-2004) were obtained from a local weather station with hourly readings, while in their absence for the year 2001 the records from the Setúbal weather station (1.5 Km north) were used.

Study 2 - Flight period of *M. galloprovincialis*

The insects captured on the traps set by the Portuguese authorities (Prolunp--AFN) to control the pine sawyer during 2001 to 2003 were used to study the flight pattern *M. galloprovincialis* in Setúbal peninsula. Between 140 and 180 traps were dispersed through the whole *B. xylophilus*-affected zone from late April to October every year, and two different trap designs were used: either large metal containers filled with recently-collected pine debris of maritime pine (which were replaced periodically), the top of which was covered with fishing net with transparent and non-smelling entomological glue where the flying insects got stuck when trying to reach the pine material; and transparent interception traps baited with ethanol which were placed on the crown of healthy pine trees. Furthermore, a group of 30 multi-funnel traps baited with ethanol and turpentine were also set on Tróia peninsula during the three years. All the captured insects were collected twice or three times a week and stored in alcohol to be identified in the laboratory later.

Study 3 - Seasonal development in relation to oviposition time

On five distinct occasions (23 May, 18 June, 13 July, 13 August and 13 September 2001) 12 recently-cut maritime pine logs (approximately 30cm length, 5-7 cm diameter) were placed under laboratory conditions on a large wooden box containing between six and eleven breeding couples of *M. galloprovincialis*. Female beetles were allowed to freely breed on the logs for two days after which the material was removed and kept on a shaded location with ambient temperature and humidity near the EFN Labs at Oeiras. A log from each week's exposure was randomly analysed after 1, 2, 3, 4, 6, 8, 12, 16, 20, 24 and 28 weeks with the insects. Sampled logs were debarked to determine the pine sawyer's developmental stages present and if the larvae had penetrated into the xylem the galleries were open using a vertical electric saw. The 12th log from each date was left untouched at ambient temperature to follow adult emergence, being checked every two days from April to October 2002. At the end of the emergence period (October 2002) these final logs were also dissected.

Study 4 - Determination of the number of larval instars

Head capsule width measurements were used to determine the number of larval instars for *M. galloprovincialis.* Larvae of all sizes and ages were periodically collected from both sub--cortical and within-wood galleries of several dozen *P. pinaster* trees cut in Tróia from 2001 to 2003. After collected, the larvae were maintained in alcohol until their identification in the laboratory (ŠVÁCHA, 2001), after which the dorsal view of the larva's head was photographed with a digital Leica camera attached to a binocular microscope. The widest section of the larvae's epicranium was measured using the "Measure" module of the QWin software programme (Leica Imaging Systems, Wetzlar, Germany). Three measurements were made for each head capsule and the mean of the values recorded.

Study 5 - Mortality of the immature stages

Study 5.1 - Mortality of eggs and sub-cortical larvae

Six adult *P. pinaster* trees were cut on the 24th of June 2004 on Comporta and divided into 28 similar logs with a length of approximately 50 cm and 8-11 cm diameter. After being kept for eleven days on the laboratory, the logs were randomly placed on large wooden boxes containing between three and four adult couples of *M. galloprovincialis* no less than 20 days old. After three days with the insects the logs were removed and their bark carefully analysed for egg pits, which were marked with white ink correct fluid. On the 9th of July the logs were taken to an adult *P. pinaster* stand at Comporta, where they were stuck with ropes on the lower canopy of three adjacent healthy maritime pine trees. After 1, 2, 3, 4, 6, 8 and 10 weeks at ambient conditions four of the logs were randomly selected on each occasion and taken to the laboratory, where they were debarked to observe the frequency and condition of the insect's developmental stages. The air temperature and precipitation (hourly records for both) were locally recorded from July 9 to September 17th 2004 using a WatchDog™ Data Shuttle (Spectrum Technologies).

Study 5.2 - Mortality of mature larvae, pupae and pre-emerging adults

To study the mortality affecting the mature larvae, pupae and pre-emerging adults, the pine bolts from which the *M. galloprovincialis* emerged in 2001 and 2002 (Study 1) were individually measured (length and diameter) and dissected after the adults had emerged. For each bolt its location on the tree, the number of larvae that tunnelled into the wood and number of emerged adults were recorded. Larval galleries without emergence were dissected with an axe and an electrical vertical saw to determinate the fate of the larvae. When dead larvae were detected they were observed under a binocular microscope to determinate the cause of mortality, and if fungal filaments were present they were isolated, cultured on potato dextrose agar (PDA) plates, purified and stored in a refrigerator for identification. Larvae suspected to be parasitized were kept isolated on a plastic Petri dish at ambient temperature until emergence of the responsible agent.

Statistical analysis

Results were analysed through the nonparametric Kruskal-Wallis test or analysis of variance (ANOVA) followed by a Tukey HSD test to compare significant means. In all cases $p \leq 0.05$.

Values are presented as means ± SEM in the text and tables unless otherwise stated. Statistical analyses were carried out using the software Statistica 6 (StatSoft, Inc. 2003).

Results

Study 1 - Emergence of adult *M. galloprovincialis* at Tróia peninsula

All insects required at least one year to develop, with emergencies taking placed during a defined period in late spring/early summer and which usually prolonged for two/three consecutive months. During the four-year study, the earliest individuals appeared in mid--May while the last ones were captured in early September, although the emergence peak systematically occurred in July (Figure 1; Table 1). Nevertheless, the emergence pattern differed between the years and the beetles emerged signify-cantly sooner during 2003 and 2004 than in 2001 and 2002 (F= 198.0; df= 3; P<0.05), being the mean daily tempera-ture of the last two years also higher than on 2001 and 2002 (F= 2.99; df= 3; P=0.031).

With the exception of the year 2004, the adult males emerged always earlier than the females, although only in 2001 the difference was statistically significant (F= 21.3; df= 1; P≤0.005). The sex ratio of 2112 adult beetles collected at emergence was slightly biased towards males, resulting in an overall ratio of 0.48.

Although the timing of beetle emergence varied between tree-traps (Kruskal-Wallis test: χ^2 = 45.72, d.f. = 4, P ≤0.005), it was apparently unaffected by the timing of the trap-tree in the terrain (Figure 2). The September and October tree-traps were not colonised by ovipositing female *M. galloprovincialis*.

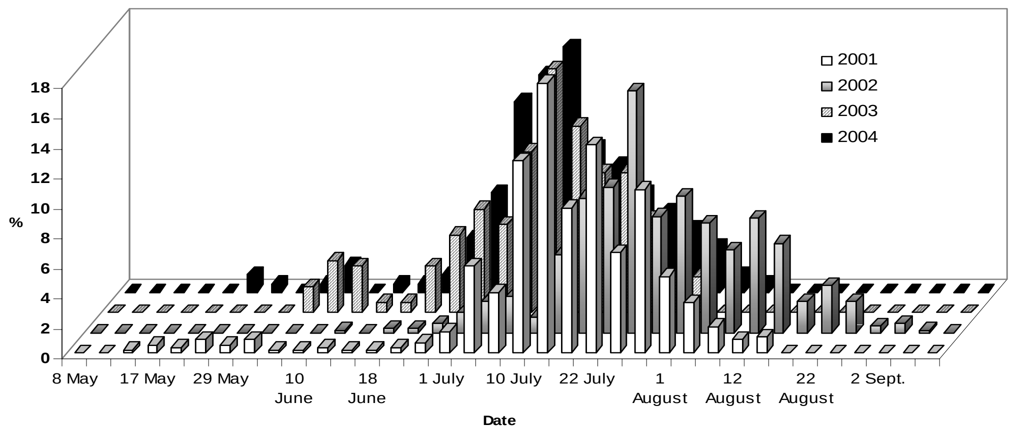

Figure 1 - Emergence (as percentage of each year) of *M. galloprovincialis* in Tróia during 2001-2004

Table 1 - Emergences of both sexes of adult *M. galloprovincialis* in Tróia, duration of emergence (in days) and number of emerged insects in 2001-2004

Year:	2001		2002		2003		2004	
Sex:	**m**	**f**	**M**	**f**	**m**	**f**	**m**	**f**
First emergence	17 May	14 May	18 June	14 June	4 June	4 June	25 May	25 May
10% emergence	1 July	4 July	11 July	14 July	16 June	18 June	29 June	25 June
50% emergence	14 July	20 July	25 July	27 July	8 July	10 July	9 July	9 July
90% emergence	30 July	1 Aug.	15 Aug.	15 Aug.	20 July	23 July	24 July	25 July
Last emergence	14 Aug.	14 Aug.	5 Sept.	2 Sept.	1 Aug.	11 Aug.	4 Aug.	8 Aug.
Emergence duration	89	92	79	80	58	68	71	75
Emerged insects	301	255	351	320	135	128	86	79

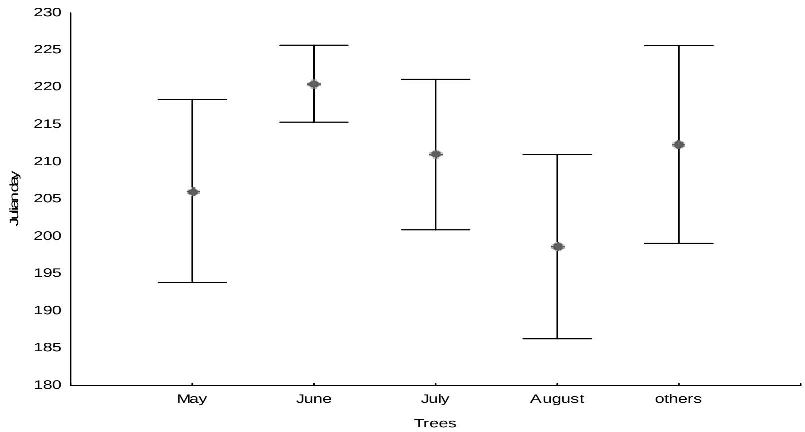

Figure 2 - Emergence on the year 2002 (dates in Julian days, mean ± SD) of *M. galloprovincialis* from tree-traps created in 2001 and from other non-specified dead pines ("others")

Females were significantly bigger than males (F= 26.3; df= 1; P≤0.005), although the weight did not differ between the two sexes (F= 1.89; df= 1; P=0.170). Adult beetles had different sizes according to the region of the tree from which they emerged (F= 3.52; df= 3; P= 0.015), with larger insects from the trunk bolts while smaller individuals emerged from the upper crown branches. The thinnest branch with emergence had a 2.2 cm diameter while the largest trunk segment had a diameter of 20.6 cm.

When dissecting the pine bolts after emergence to investigate larval mortality (Study 5.2), an additional 64 *M. galloprovincialis* larvae which had not emerged were found alive in their galleries inside the wood, although they died a few weeks later after being removed from the wood and kept at 23ºC under laboratory conditions.

Study 2 - Flight period of *M. galloprovincialis* at Setúbal peninsula

The earliest *M. galloprovincialis* were captured in mid-May while the latest beetles appeared in the end of October, with a total of 1886 insects caught over the three years. The flight activity of the beetles' generally extended for a consecutive period of over four months, with the most abundant captures occurring in June and July. The pattern of beetle captures differed between the years (Kruskal-Wallis test: χ^2 = 164.72, d.f. = 2, P ≤0.005), with mean captures occurring earlier in 2003 than on 2001 and 2002 (Figure 3).

Study 3 - Seasonal Development in relation to oviposition time

The logs contained a mean of 4.1±0.4 eggs each, which hatched in less than two weeks under the ambient temperatures. The young larvae developed very rapidly and actively fed under the bark for about one month before tunnelling into the wood, and although a few precocious larvae begun their xylem tunnels just two or three weeks after hatching, they continued to feed on the phloem for a few more weeks (Table 2).

None of the eggs or larvae died during the experiment, and after one year a total of nineteen adult insects (five males and 14 females) emerged from the logs. Emergencies occurred in July and August 2002 for the five logs independently of the oviposition dates. When dissecting the pine logs after emergence, a single live *M. galloprovincialis* larva was found in a gallery from the September 15th log, although it died a few weeks after its removal from the wood.

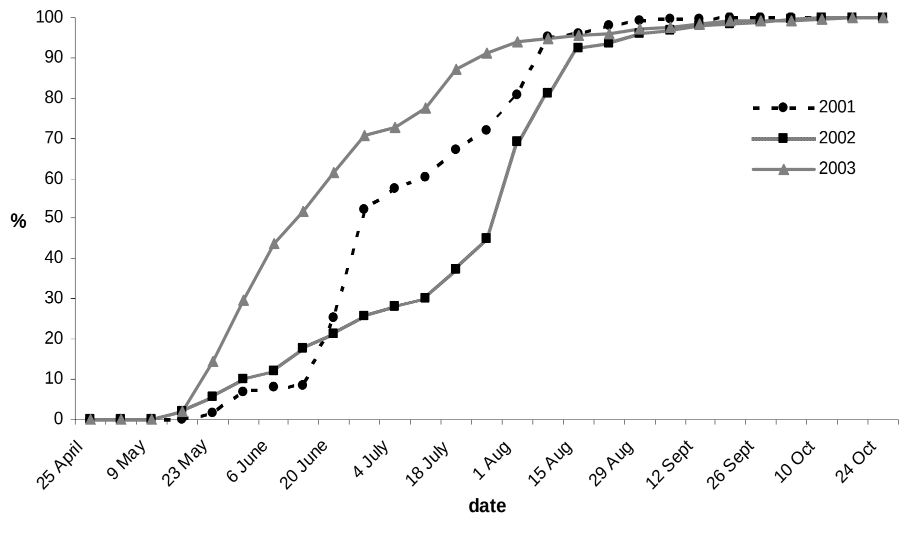

Figure 3 - Cumulated captures (as %) of adult *M. galloprovincialis* on Setúbal peninsula between 2001 and 2003

Table 2 - Developmental instars of *M. galloprovincialis* on pine logs kept at ambient temperature and periodically sampled from June 2001 to April 2002. *scl* – larvae in galleries beneath the bark (sub-cortical); *xl* – larvae in galleries inside the wood (xylem)

Sampling Month	**Oviposition Date**				
	25 May	**20 June**	**15 July**	**15 August**	**15 Sept.**
June	eggs/scl	eggs			
July	scl	scl	eggs		
August	scl/xl	scl/xl	scl	eggs	
September	xl	scl/xl	scl	scl	eggs/scl
October	xl	scl/xl	scl/xl	scl/xl	scl
November	xl	xl	xl	scl/xl	scl
December	xl	xl	xl	xl	scl
January		xl	xl	xl	scl/xl
February			xl	xl	scl/xl
March				xl	xl
April					xl

Study 4 - Determination of the number of larval instars

Head capsule measurements were made on 993 larvae and plotted as a frequency distribution which can be visualized on figure 4. The distribution pattern is generally irregular and presents overlapping peaks that impede the determination of the exact number of larval instars. Only the first peak (corresponding to the first instar larvae) was discrete and could be defined, with a mean head capsule width of 0.937±0.008mm and extreme values of 0.640mm and 1.120mm. The remaining peaks are continuous due to overlap of head capsule widths and thus do not allow for clear distinction, although overall between four and six peaks are apparent in the distribution.

Study 5 - Mortality of the immature stages

Study 5.1 - Mortality of eggs and sub-cortical larvae

The pine logs contained a mean of 6±0.8 *M. galloprovincialis* eggs, some of which laid in the terrain by wild pine sawyer females and also incorporated in the study (additional seven eggs and 14 larvae). During the two-month study 16% of the 44 *M. galloprovincialis* eggs did not hatch due to desiccation, resinosis and unknown causes. The mortality of the recently-hatched larvae was also very low, affecting 11% of the 80 individuals and caused by resinosis, the fungi *Beauveria bassiana* (Bals.) and other non-identified agents (empty galleries without larvae). After one month on the field the larvae gradually begun tunnelling into the wood, and through the following months none of the 28 individuals found on the xylem galleries were found dead.

Overall, due to the very low number of dead larvae detected (only nine) no relation could be established between the larvae's mortality and possible inter-specific competition with other insects present in the logs or with adverse climatic conditions. The pine logs were colonised by four other insect species, namely the small scolytidae *Orthotomicus erosus* (Wollaston) (by far the most abundant coloniser) along with the bark beetle *Hylurgus ligniperda* (Fabricius), the cerambycid *Arhopalus syriacus* (Reitter) and an unidentified small Buprestid. No predators or parasitoids were observed in the logs or associated with the larvae. The weather conditions were normal for the season with a daily mean temperature of 23ºC during the 70-day period and absolute maximum values of 38ºC for a few days of August and September. Rainfall was scarce (totalling 16.2 mm) during five non--consecutive days.

Study 5.2 - Mortality of mature larvae, pupae and pre-emerging adults

As the mortality did not differ between years (F= 3.36; df= 1; P= 0.067) the results of both years were analysed jointly. The initial density of larvae tunnelling into the wood (per m^2 of wood) was different between tree sections (F= 4.61; df= 4; P= 0.001), being higher in the upper trunk and lower in the lower crown (Table 3). Nevertheless, larval mortality also differed among tree segments (F= 11.31; df= 4; P≤0.001) and affected mainly the upper crown branches, thus resulting in more insects emerging per m^2 of wood on larger trunk bolts while the lowest emergence values were found on the smaller upper crown branches (F= 6.36; df= 4; P≤0.001).

Overall, mortality affected 26% of the mature larvae inside the wood. The dissection of 579 larval galleries without emergence found that more than half of the mortality was due to unknown causes (when dead larvae were found without any apparent mortality factor present) and/or missing individuals (galleries inside the wood with no larvae or larval remains) (Figure 5).

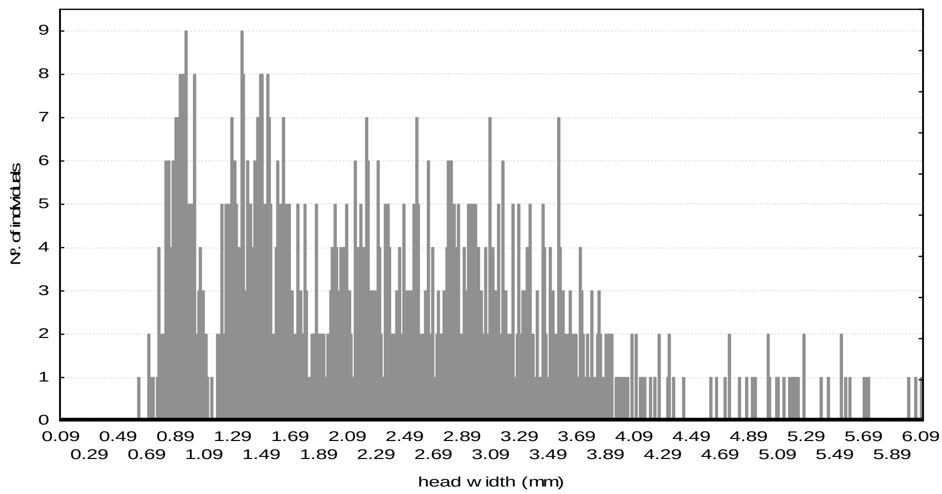

Figure 4 - Frequency distribution of head capsule measurements of 993 *M. galloprovincialis* larvae

Table 3 - Comparison of tree sections (in parenthesis number of bolts analysed) on bolt diameter (in mm), number of larvae penetrating into the wood (per m^2), adult emergencies (per m^2) and larval mortality inside the wood (%)

Tree Section		Diameter	Larvae in Wood	Emergencies	Mortality
Crown	Upper (75)	34 ± 1.4 a	52 ± 3.2 ab	24 ± 3.3 a	44 ± 5.1 a
	Mid (158)	52 ± 1.3 b	45 ± 2.7 a	29 ± 2.8 ab	27 ± 3.5 b
	Lower (150)	60 ± 1.8 c	43 ± 2.4 a	25 ± 2.3 a	26 ± 3.7 b
Trunk	Upper (93)	70 ± 1.9 d	60 ± 4.2 b	39 ± 4.0 bc	22 ± 4.3 b
	Lower (83)	120 ± 2.6 e	49 ± 3.0 ab	42 ± 3.0 c	11 ± 2.6 c

[1] Means within each column followed by the same letter are not significantly different, Tukey HSD test P≤0.05

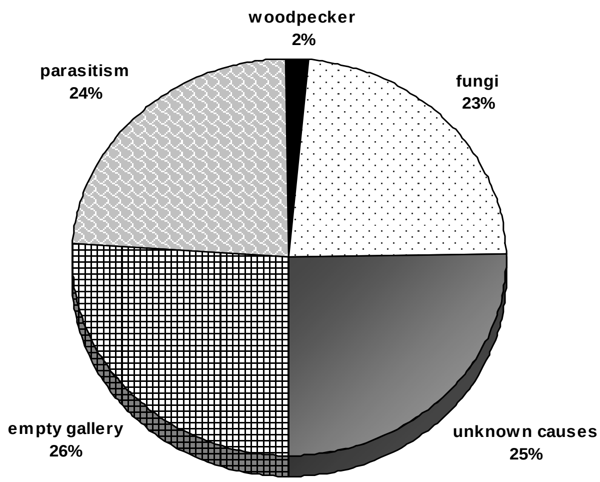

Figure 5 - Mortality (in %) of *M. galloprovincialis* larvae from galleries inside the wood

Parasitism accounted for almost one-quarter of the mortality, being *Cyanopterus flavator* Fabricius (Hymenoptera; Braconidae) the only species found. Fungi were found on the dead larvae, namely *Beauveria bassiana* and, less frequently, unidentified species from the Order Mucorales. Predation by woodpeckers was residual and affected only 2% of the galleries. No correlation was found between the frequency of any of the mortality agents and the location of the pine bolts on the tree or bolt diameter. Only 0.8% of the pupae died inside the pupal chambers while 2% of the adults died in the course of boring the exit holes to the exterior.

Discussion

Despite being the vector of the pathogenic pine wood nematode in

Portugal, the seasonal development and life history of *M. galloprovincialis* had not been thoroughly studied until now. Our studies revealed that adult emergence was extremely regular during the four years and consistently peaked during July, extending for up to three consecutive months. The yearly fluctuations of the emergence appear to be associated with variations of annual temperatures, and in the hotter years of 2003 and 2004 (PIRES *et al*., 2004; SANTO *et al*., 2005) the larval development was accelerated resulting in earlier emergences. The pattern of emergence from Tróia (Study 1) corroborated well with the overall flight curve obtained for the Setúbal peninsula (Study 2), which was also influenced by annual temperature variations.

The earlier emergence of males in advance of females is a common trend for the Cerambycidae in general (LINSLEY, 1959) and has also been reported for other species of the *Monochamus* genus (e.g., TOGASHI and MAGIRA, 1981; SCHOEMAN *et al*., 1998; SHIBATA, 1998), being a strategy for the males to find the usually scarce oviposition sites which they defend until the posterior arrival of females. The *M. galloprovincialis*' sex ratio of 0.48 reported in Study 1 is similar to the value of 0.50 described by FRANCARDI and PENNACCHIO (1996) for a smaller number of insects.

The size of the emerging *Monochamus* varied according to their location in the tree (Study 1), and as the dimension of the adult beetles is mostly determined by the availability and quality of the food during the larval growth (DILLON and DILLON, 1941; AKBULUT and LINIT, 1999b), the larger size of the insects from the trunk could derive from a higher nutritional value of the local phloem and/or higher availability of food due to lower intra-specific competition.

Trap trees from May to August were colonised by breeding insects, consistently with the emergence and flight pattern previously discussed. The absence of eggs on September and October might result from the gradually diminishing population of reproducing females, along with a higher availability of suitable breeding hosts during the late summer/early autumn months when in the field appear an increasingly growing number of pines wilting and dying due to *B. xylophilus* infection.

Eggs developed in one or two weeks during the summer and the newly-hatched larvae had an extremely fast development during the warm months, and therefore practically all the insects over-wintered as last-instar larvae in galleries inside the wood, except for the eggs laid from September onwards.

The number of larval instars could not be determined because the capsule width frequency distribution did not adequately define discrete the number of larval instars for this species. Other methods like direct observation of larval moults is difficult to conduct on field-collected populations due to the species' cryptic feeding habits and the within-wood development, while rearing larvae on artificial diets can originate variable instars or differential growth (*e.g.*, PERSHING and LINIT, 1989; ALOO and KATAGIRI, 1994).

Mortality of the larvae varied according to their location on the trees (Study 5.2), with the thicker bark of the main trunk providing additional protection against natural enemies and adverse environmental conditions, compared to the thinner bark of the upper branches. Nevertheless, mortality was generally low for all developmental instars, varying from a maximum of 26% for the mature larvae inside the wood to a minimum of 0.8% for the pupae. TOGASHI (1990) also found that *M. alternatus* pupae had the lowest mortality among all develop-mental stages, although he reported a 6% rate. The mortality of the eggs and sub-cortical larvae described on Study 5.1 might have underestimated the situation on the terrain because the experiment was conducted with laboratory-reared logs with relatively low larval density, and density-dependent death due to intra-specific competition and cannibalism has been found to be an important mortality factor of several *Monochamus* species (ROSE, 1957; KOBAYASHI *et al*., 1984; TOGASHI, 1986; SHIBATA 1987; TOGASHI, 1990; VICTORSSON and WIKARS, 1996; DODDS *et al*., 2001).

Mortality by inter-specific competition was not observed as the pine sawyer larvae are always bigger and more aggressive than neighbouring bark-beetle and buprestid larvae, while the similarly-sized and equally aggressive *A. syriacus* is usually found on larger-diameter trunk sections (SOUSA *et al*., 2000). Further-more, the young *M. galloprovincialis* larvae appear to withstand high temperatures quite well, as individuals submitted to peak ambient temperatures of 38ºC (which might have been higher on the surface of the sun-exposed logs) were apparently unaffected (Study 5.1).

Overall, the most important mortality agents were fungi and a parasitic wasp. The fungus *B. bassiana* is one of the most important pathogens of *M. alternatus* in Japan (e.g., KISHI, 1995; OKITSU *et al*., 2005) and China (e.g., WANG *et al*., 2003). The parasitic wasp *C. flavator* had already been reported with *M. galloprovincialis* in a previous study (NAVES *et al*., 2005), although with lower parasitism rates. Mortality by woodpecker predation was residual and our results support the hypothesis that bird predation is density dependent, with an effective impact at low and intermediate prey densities but not at high densities (HOLMES, 1990). Similar observations were made in Japan for *M. alternatus*, which is efficiently controlled by woodpeckers only at low population densities (KOBAYASHI *et al*., 1984; KISHI, 1995).

More than half of the larval mortality was due to unidentified agents, either non-surveyed pathogenic micro--organisms like yeasts, bacteria and nematodes, intra-specific cannibalism or unidentified predator(s). Combining the mortality of the various instars (Studies 5.1 and 5.2) we obtain a within log generation survivorship from egg to adult of 53%, higher than for other *Monochamus* species (ROSE, 1957; HELLRIGL, 1971; LINIT, 1985; TOGASHI, 1990; AKBULUT and LINIT, 1999a, 1999b; DODDS and STEPHEN, 2000; AKBULUT *et al*., 2004), although similar values have also been reported for *M. alternatus* in Japan (TOGASHI, 1989; KISHI, 1995). Due to the low within-log generation mortality a mean value of 31 emerging insects per m^2 of wood was recorded.

The *M. galloprovincialis* population studied was found to have a univoltine life cycle as the insects required ten to thirteen months to emerge from both the trees of Study 1 and the pine logs of Study 3. A minor proportion of the insects (4% on Study 1 and 5% on Study 3) did not emerge after one year and would eventually develop through a two-year-long life cycle, this being a normal pattern for populations of central and northern Europe. In fact, in southern Finland the seasonal development is practically the inverse of Portugal as 90% of the individuals need two years to emerge and only 10% have a one-year generation (TOMMINEN, 1993).

Overall, our observations suggest the existence of a larval winter dormancy which lengthens and regulates the insect's life cycle. A genetically-induced and obligatory period of dormancy would explain the fixed one-year life cycle regardless of temperature variations, the fairly synchronised emergence of different-aged insects and the interruption of larval development even at favourable temperatures. Similar winter larval dormancies have been described for other species of the genus, namely the Asian *M. alternatus* and *M. saltuarius* (TOGASHI, 1991; TOGASHI *et al.*, 1994), and have previously been suggested to occur on *M. galloprovincialis* (NAVES *et al.*, 2007).

With the insect's prolonged emergence any control action (like the use of traps baited with attractives) must be carried out for a period of no less than six consecutive months. Our results suggest that the limited efficacy of such methods and the low natural control observed in the terrain do not limit efficiently the pine sawyer populations, which in part explain the high pine mortality observed in the regions where *B. xylophilus* and its vector jointly occur.

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