

Trapping *Monochamus galloprovincialis* (Coleoptera: Cerambycidae), Vector of the Pine Wood Nematode, with Pine Allelochemicals, in Portugal

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Abstract. Seven different trap models or variations and ten different chemical lures or combinations thereof were compared as to their efficiency to attract and capture the pine sawyer *Monochamus galloprovincialis*, vector of the Pine Wood Nematode (PWN) (*Bursaphelenchus xylophilus*) in Portugal, between 2001 and 2004. Traps were made at the INRB laboratories (former EFN), with and without a visual silhouette, with chemical lures (ethanol, α -pinene and/or turpentine) from the maritime pine (*Pinus pinaster*), the only host of the PWN and its vector in Portugal. They were tested in four successive assays on pine stands; the less efficient were eliminated and the best were accepted for subsequent tests. The best combination was a transparent cross-vane interception trap lured with ethanol and turpentine in separate vials. The implications of the results for control of *M. galloprovincialis* are discussed.

Key words: Pine wilts disease; maritime pine; pine sawyer; traps; lures

Desenvolvimento de Métodos para a Captura de *Monochamus galloprovincialis* (Coleoptera: Cerambycidae), Vetor do Nemátodo da Madeira do Pinheiro, com Compostos Aleloquímicos do Pinheiro Bravo, em Portugal

Sumário. Diferentes armadilhas e variações (sete), iscadas com atraentes químicos, foram testadas em Portugal entre 2001 e 2004. O objetivo era verificar a eficiência na atração e captura do longicórnio do pinheiro, *Monochamus galloprovincialis*. Este é o inseto vetor do nemátodo da madeira do pinheiro (NMP) (*Bursaphelenchus xylophilus*). Todas as armadilhas testadas foram construídas no laboratório do INRB (ex - Estação Florestal Nacional), sendo algumas de interceção enquanto outras apresentavam silhueta visual. Em todas foram colocados atraentes químicos (etanol, aguarrás e α -pineno) originários do hospedeiro do NMP, o pinheiro-bravo. Foram testadas em quatro ensaios realizados de modo sucessivo, em que as piores combinações foram eliminadas e as melhores admitidas no ensaio seguinte. A melhor combinação era constituída por uma armadilha de painéis cruzados transparentes com um conjunto atrativo constituído por etanol e aguarrás, apresentados em recipientes separados. Finalmente são discutidas as implicações dos resultados obtidos no controlo das populações de *M. galloprovincialis*.

Palavras-chave: *Bursaphelenchus xylophilus*; armadilhas; atraentes químicos; pinheiro-bravo

Développement de Méthodes pour la Capture de *Monochamus galloprovincialis* (Coleoptera: Cerambycidae), Vecteur du Nématode du Pin, avec des Composants Alelochimiques du Pin Maritime au Portugal

Résumé. Différentes pièges et variations (sept), avec des attractives chimiques, ont été testés entre 2001 et 2004 pour leur efficacité à attirer et capturer *Monochamus galloprovincialis*, insecte vecteur du nématode du pin (PWN) (*Bursaphelenchus xylophilus*) au Portugal. Toutes les pièges ont été fabriquées dans le laboratoire de l'INRB (anciennement Station Forestière Nationale), quelques-unes d'interception tandis que d'autres avaient silhouette pour contact visuel de l'insecte. Toutes avaient des produits chimiques attractifs (éthanol, essence de térébenthine et α -pinène) provenant de l'hôte du nématode de pin, le pin maritime, et ont été testées dans quatre essais réalisés en succession, dans laquelle la pire des combinaisons a été éliminée et la meilleure admise à l'essai suivante. La meilleure combinaison finale était composée d'un piège à panneaux transparents plié avec des attractants constitué d'éthanol et essence de térébenthine dans des conteneurs individuels.

Mots clés: Maladie du nématode du pin; longicorne du pin; pin maritime; piège; attractante chimique

Introduction

The pine wood nematode (PWN) *Bursaphelenchus xylophilus* (Steiner & Bühner) Nickle, the causal agent of pine wilt disease (MOTA *et al.*, 1999), was discovered in Portugal in 1999. Soon after, the main study allowed identification of its unique vector, the native secondary forest insect *Monochamus galloprovincialis* (Olivier) (SOUSA *et al.*, 2001).

Since the detection of the first infected pines, enormous efforts have been made to control the disease, namely by cutting and destroying the infested trees before the exit of the insect vector and by capturing the insect vector during its flight period.

For this last purpose, early assays used traps, made at the INRB Laboratory based on descriptions from publications, with kairomone lures from the maritime pine tree host (ethanol, turpentine and α -pinene), as tested in other countries where PWN causes mortality: Asia (Japan) and exotic pines in the USA.

Many traps were developed to catch conifer bark and wood-boring beetles.

They can be divided into four different types: transparent flight interception (i.e. cross-vane traps) (BILLINGS and CAMERON, 1984), silhouette or barrier traps (i.e. multi-funnel traps) (LINDGREN, 1983; FATZINGER, 1985), sticky traps (IKEDA *et al.*, 1980) and vertical stove-pipe traps (CHÉNIER and PHILOGÈNE, 1989b).

Different traps and lures to catch the PWN *Monochamus* vector have already been studied, mainly in USA and Japan. As is the case with most cerambycids, communication in the *Monochamus* genus is based on chemical stimuli, flying in the host volatiles' plume (TUNSET *et al.*, 1988; ALLISON *et al.*, 2004). These are namely terpenes (CHÉNIER and PHILOGÈNE, 1989a; DYER and SEABROOK, 1978; ZHANG *et al.*, 1993) and ethanol (CHÉNIER and PHILOGÈNE, 1989b). Sometimes they also need visual clues (SEYBERT and GARA, 1970; VASECHKO, 1978; NIEMEYER, 1985) and mechanical search after landing (DICKE and GROSTAL, 2001).

Cerambycids are sensitive to volatiles produced by the host, using the chemical clues for feeding, mating and egg-laying.

Host selection might initially be based on chemical odors and its acceptance regulated by tasting, all depending on the volatile terpene content of the wood (HANKS, 1999; FACCOLI *et al.*, 2005; GINZEL and HANKS, 2003).

The objectives of this paper are to study the response of *M. galloprovincialis* to the host semiochemical odors, to turpentine and ethanol and to test several trap designs (LINDGREN, 1983; FATZINGER, 1985).

Materials and methods

Traps and lures developed to catch *M. galloprovincialis* were tested in the initially affected area of Setúbal Península, at Tróia (2001 and 2002) and Comporta (2004). It was necessary to displace the assays further south from Comporta because the insect population levels at Tróia decreased due to the success of eradication procedures, with an impact on trap captures.

All assays occurred from July to September, in order to overlap with the flight period of *M. galloprovincialis* adults (NAVES *et al.*, 2008). Traps were visited weekly to collect captured bark beetles and wood borers and lures were renewed when vials were found to be almost empty.

Traps and/or lures with the best results in one assay were used again in the following assay against new sets. Table 1 presents the list of materials used in the different assays. All traps used in the work described in this paper were built at the INRB Laboratory. In the field, traps were hung high in the lower canopy of adult pines. This procedure was followed in order to improve trapping efficiency by placing them at

the height of *M. galloprovincialis* flight and trunk colonization.

The multi-funnel trap was based on Lindgren's trap (1983) using nine green plastic funnels (16cm high and 20cm wide) with a narrow end cut. The bottom funnel was glued to a 50cl plastic cup containing a DDVP insecticide strip (Figure 1a).

The stove pipe trap was based on the Fatzinger trap (1985), using a black PVC pipe 80cm high and 15cm wide, attached on the bottom to a black, round 50cm-wide trash bin cover. The whole setup was covered with the entomologic glue *Temocid* (Quelmer, Lisboa, Portugal). Attractants were hung inside the tube and to allow dispersal of odors, two rings of holes were drilled into the tube at 25cm from each end. To prevent the capture of small birds or bats in the glue a 5cm-wide metallic net was attached to the border of the trash bin cover (Figure 1b).

The transparent cross vane trap was based on traps described by WILKENING *et al.* (1981) and a Japanese commercial trap for *M. alternatus* (NAKAMURA *et al.*, 1999). The trap consisted of two acrylic plates, 30cm wide and 40cm high, cross-connected in the center through 15cm slits. A 30cm funnel was hung under the vanes to lead the insect that crashed into them to a 50cl plastic vial glued at the funnel end and containing a DDVP insecticide strip (Figure 1c).

The first variation of the cross vane trap was completely black, as described by McIntosh *et al.* (2001) (Figure 1d), and the second had a 10cm vertical black band at the middle, where the vanes cross (GROOT and NOTT, 2001) (Figure 1e).

Table 1 – Traps and lures used in assays for the capture of *Monochamus galloprovincialis*

Date/ Lure	Traps				
	Multi-funnel	Stove Pipe	Transparent Cross-Vane	Black Cross-Vane	Profile Cross-Vane
2001 (8 June-13 July)					
Turpentine ⁽¹⁾	4	4			
Ethanol ⁽³⁾	4	4			
2002 (20 June-10 Sept)					
Ethanol ⁽³⁾	3	3	3		
α -pinene ⁽⁴⁾	3	3	3		
α -pinene ⁽⁴⁾ +ethanol ⁽³⁾	3	3	3		
Turpentine ⁽²⁾	3	3	3		
Turpentine ⁽²⁾ +ethanol ⁽³⁾	3	3	3		
Oleoresin ⁽⁵⁾	3	3	3		
Oleoresin ⁽⁵⁾ +ethanol ⁽³⁾	3	3	3		
Control	3	3	3		
2004a (25 June-8 Oct)					
Turpentine ⁽²⁾ +ethanol ⁽²⁾	4		4	4	4
2004b (9 July-3 Sept)					
Turpentine ⁽⁴⁾ +ethanol ⁽²⁾			16		

⁽¹⁾ 78% α -pinene, 17% β -pinene, 5% limonene (Resipez Lda., Leiria, Portugal), in 20ml open vials.

⁽²⁾ 82% α -pinene, 15% β -pinene, 1% limonene, 1% camphene (Prorresina, Alvares, Portugal), in two open plastic tubes, each with 10ml (2002); in two open plastic vials, each with 100ml (after 2002).

⁽³⁾ Ethanol 99% (Panreac, Barcelona, Spain), in two plastic tubes with 10ml, closed and with six holes close to the cover.

⁽⁴⁾ α -pinene 99,9% (Fluka, Chemika), in two plastic tubes with 10ml, closed and with six holes close to the cover.

⁽⁵⁾ Oleoresin collected during the previous 24 hours from pines near the plots.

Experimental design

The assay done in 2002, in Tróia pine stands, compared three different traps (multi-funnel, stove pipe and transparent cross vane), seven allelochemical combinations (oleoresin, turpentine, α -pinene) and a control (no lure).

Eight groups were established with 100m between them, with one trap of each type having the same attractant, placed 50m apart. Every week attractants were replaced by new ones of a different kind following a clockwise order (Figure 2).

This way, after eight weeks all lures had been used in all traps and the assay ended. This design was repeated three times and as a result this assay used 72

traps simultaneously.

In all the other experiments the design used was the Latin square, with traps placed 100m from each other and, in each line or column of traps, all tested variables were represented without repetition.

In 2001 multi-funnel and stove pipe traps were tested, lured with turpentine+ethanol and pinene+ethanol. In a first assay in 2004 the three variations of the cross-vane and multi-funnel traps were compared, all lured with turpentine+ethanol. Finally, in a second 2004 assay, 16 transparent cross vane traps were used to test four combinations of turpentine and ethanol, mixed at different ratios (1:1, 1:2 and 2:1) as well as in separate vials.



Figure 1 – Traps used in assays to capture *Monochamus galloprovincialis* at Tróia and Comporta. a- multi-funnel; b- stove pipe; c to e – cross vane traps

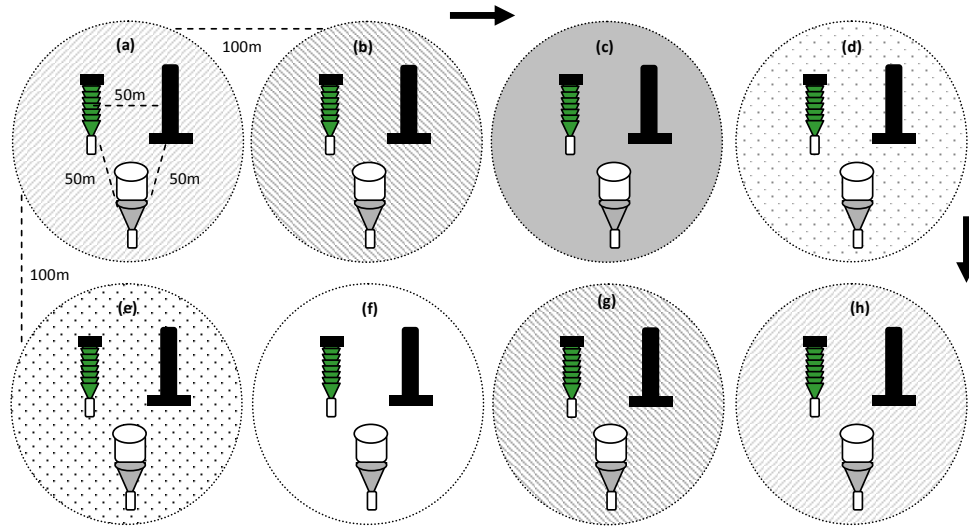
Statistical analysis

In all the assays' results one-way ANOVA was applied to assess the effect of each variable: chemical lure, trap and time. Afterward, variables were tested together using multiple ANOVA, where interaction between fixed (trap and lure) and random (time) factors was evaluated for its effectiveness in attracting and capturing *M. galloprovincialis* adults.

Since frequently less than 10 beetles were captured and some traps did not

even capture a single insect, results were transformed by $\sqrt{(x + 0,5)}$, also used to assure data normality and homocedasticity (ROELOFS and CARDE, 1977; ZAR, 1984).

When statistically significant differences were obtained a post-hoc Fisher *Least Square Difference* was adjusted, because it is considered to be more robust than other available tests such as *Tukey HSD*, *Newman-Keuls*, *Bonferroni*, *Scheffé* and *Duncan* (ZAR, 1984).



	23 Jul.	30 Jul.	6 Aug.	13 Aug.	20 Aug.	27 Aug.	3 Sept.	10 Sept.
a	Turpentine + ethanol	Turpentine	Pinene+ ethanol	Control	Oleoresin	Oleoresin+ ethanol	Ethanol	Pinene
b	Pinene	Turpentine + ethanol	Turpentine	Pinene+ ethanol	Control	Oleoresin	Oleoresin+ ethanol	Ethanol
c	Ethanol	Pinene	Turpentine + ethanol	Turpentine	Pinene+ ethanol	Control	Oleoresin	Oleoresin+ ethanol
d	Oleoresin+ ethanol	Ethanol	Pinene	Turpentine + ethanol	Turpentine	Pinene+ ethanol	Control	Oleoresin
e	Oleoresin	Oleoresin+ ethanol	Ethanol	Pinene	Turpentine + ethanol	Turpentine	Pinene+ ethanol	Control
f	Control	Oleoresin	Oleoresin+ ethanol	Ethanol	Pinene	Turpentine + ethanol	Turpentine	Pinene+ ethanol
g	Pinene+ ethanol	Control	Oleoresin	Oleoresin+ ethanol	Ethanol	Pinene	Turpentine+ ethanol	Turpentine
h	Turpentine	Pinene+ ethanol	Control	Oleoresin	Oleoresin+ ethanol	Ethanol	Pinene	Turpentine+ ethanol

Figure 2 - Experimental design used to test traps and chemical lures to capture *Monochamus galloprovincialis*, with 3 simultaneous replications on the field. Arrows indicate the direction of clockwise rotation of the lures between groups of traps, according to the time sequence described in the table

Results

The time pattern of *M. galloprovincialis* captures was studied for the 2002 assay and a concentration was observed in the first two weeks, with 18 and 17 beetles respectively (79,5% of 44 insects caught during the whole test). Variance analysis revealed significant concentration during

these weeks and a weak contribution during the third and eighth weeks (Figure 3). The reason for the sudden decrease during the third week was due to unstable weather observed (rain and strong winds) that surely conditioned the beetles' activity. Disregarding this week the pattern showed a slow decrease towards the end of the assay, in late

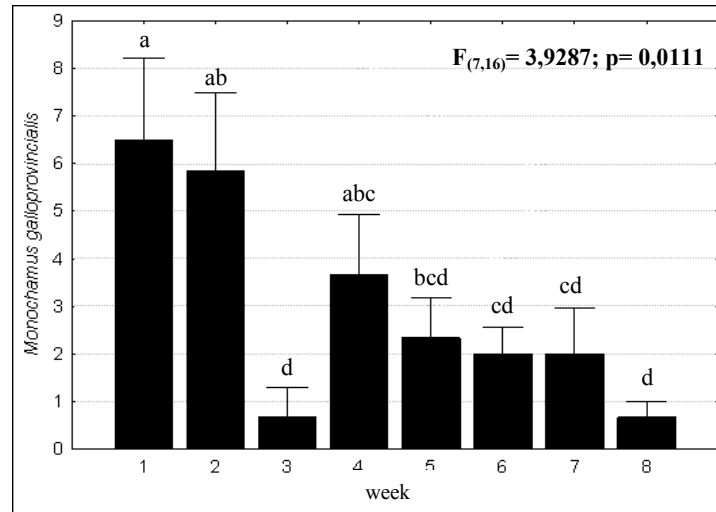


Figure 3 – Weekly pattern of total *Monochamus galloprovincialis* captures observed in the 2002 assay. Lines are standard errors associated to means (in black bars) and letters are Homogeneous LSD groups

September, when the emergence period of *M. galloprovincialis* ends.

Traps

The first assay to study the effect of trap design was conducted in 2001, when 38 adult *M. galloprovincialis* were captured by two kinds of traps, mainly the stove-pipe trap (27 specimens against 11 in the multi-funnel trap) but no significant statistical difference was found ($F_{(6,1)} = 0,3139$; $p = 0,5956$) (Figure 4).

In the 2002 assay, concerning the performance of different traps, the multi-funnel traps did not catch any *M. galloprovincialis*, therefore the ANOVA analysis showed significant differences in relation to the other two trap types, that showed similar capabilities ($F_{(2,69)} = 11,2129$, $p < 0,0001$) (Figure 5).

In the trap assay done in 2004 the introduction of a visual effect in cross-

vane traps was tested. Once again the multi-funnel trap gave the worst results (though having captured four beetles during the 16 weeks of the test period).

Transparent cross-vane traps captured 24 *M. galloprovincialis* beetles, followed by the completely black traps with 23 beetles; profile traps caught 26 beetles (although almost half of them were caught during the first week in a single trap). Transparent cross-vane traps showed more constant captures distributed between the four traps used and along the weeks of the assay.

One-way ANOVA analysis reported statistically significant differences in total captures ($F_{(3,12)} = 2,7594$; $p = 0,0882$) and of males ($F_{(3,12)} = 3,6371$; $p = 0,0449$), with negative displacement of multi-funnel in relation to cross-vane traps (Figure 6). Females were captured in lesser numbers ($n = 31$) and equally in all kinds of traps ($F_{(3,12)} = 1,4412$; $p = 0,2796$).

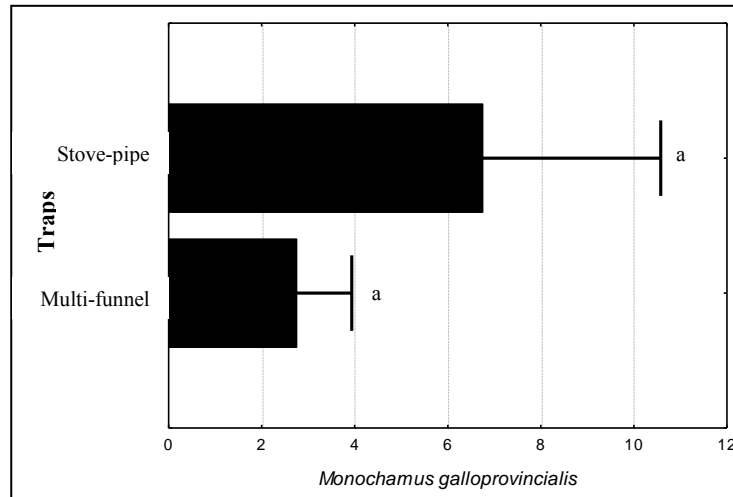


Figure 4 – Total captures of *Monochamus galloprovincialis* adults obtained per trap in two different traps tested in 2001. Lines are standard errors associated to means (in black bars) and letters are Homogeneous LSD groups

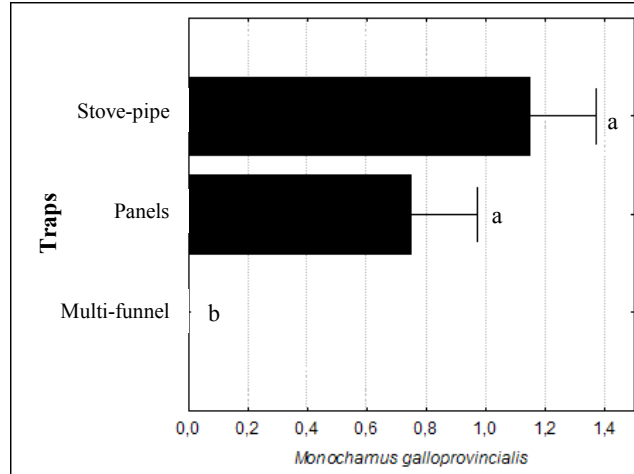


Figure 5 – Total numbers of *Monochamus galloprovincialis* caught per trap and per week in assay of 2002. Lines are standard errors associated to means (in black bars) and letters are Homogeneous LSD groups

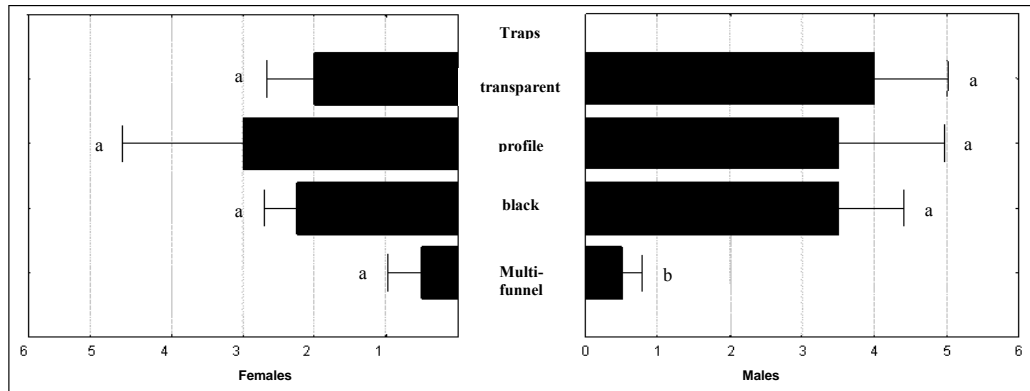


Figure 6 – Total numbers of *Monochamus galloprovincialis* caught per trap and per week in assay of 2004a. Lines are standard errors associated to means (in black bars) and letters are Homogeneous LSD groups

Lures

During the 2001 assay, *M. galloprovincialis* were only caught in traps lured with ethanol and pinene; none were caught in traps with turpentine.

The most consistent result obtained in the 2002 assay was the synergistic effect of ethanol when combined with other compounds; the *M. galloprovincialis* captures obtained were multiplied by 1,7 with α -pinene, by 2,3 with oleoresin and by 7,7 when combined with turpentine, giving a strong contribution to the statistical differences observed with ANOVA analysis ($F_{(7,64)} = 2,9065$, $p = 0,0106$). Ethanol alone proved to be more attractive than turpentine or oleoresin placed in isolation (Figure 7).

The combined effects of traps and lures decreased previous observed differences ($F_{(14,48)} = 1,3116$; $p = 0,2359$). However, the best results of all traps were always obtained when used with ethanol and turpentine (a total of eight beetles in transparent panels and seven beetles in stove-pipe).

In the other assay done in 2004 with

the purpose of clarifying the best way to present ethanol and turpentine, when these attractants were placed separately, more than half of the total *M. galloprovincialis* beetles were caught (54 of 103 beetles).

Statistical differences were only found with trapped males ($F_{(3,12)} = 3,3813$; $p = 0,0543$) because there was a concentration of females in one trap with separated chemicals (17 out of 25 total females caught), resulting in a big standard error, either in relation to female trapping ($F_{(3,12)} = 1,3619$; $p = 0,3013$) or total trapping ($F_{(3,12)} = 2,5865$; $p = 0,1016$).

Different mixtures of ethanol and turpentine gave similar results (Figure 8).

Considering other wood-boring species, in the 2002 assay a total of 240 cerambicids, 47 buprestids and 20 curculionids were caught. Besides the 44 *M. galloprovincialis* specimens, the other cerambicids caught were 95 *Ahropalus syriacus*, 7 *A. ferus*, 32 *S. buprestoides*, 50 *P. perroudi*, 11 *A. griseus* and 1 *Trichoferus* sp..

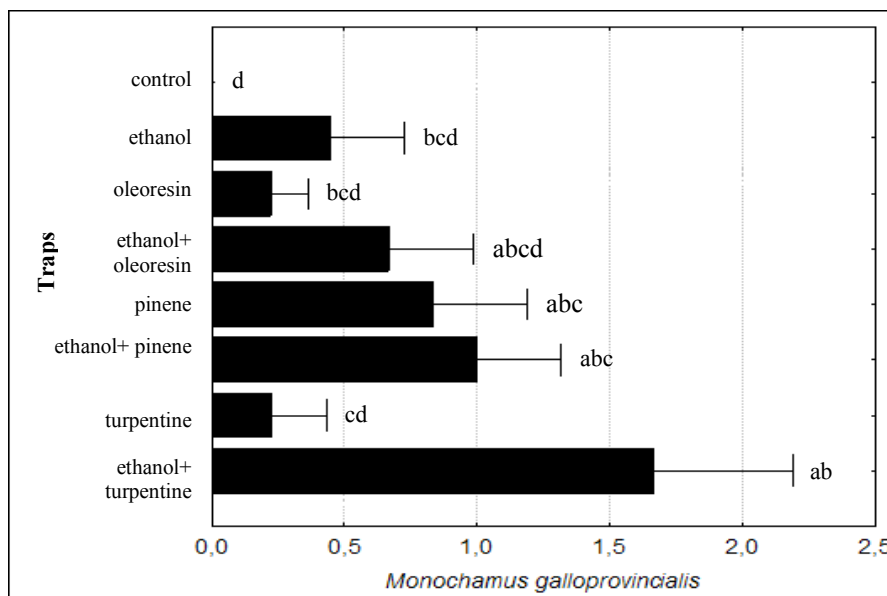


Figure 7 - Total numbers of *Monochamus galloprovincialis* caught per trap and per week in the 2002 assay. Lines are standard errors associated to means (in black bars) and letters are Homogeneous LSD groups

The captures of *Ahropalus* genus followed the same pattern as *M. galloprovincialis* in terms of either the worst performance of multi-funnel traps ($F_{(2,69)} = 7,5053$; $p = 0,0011$) or the best results when lured with ethanol+turpentine ($F_{(7,64)} = 2,2656$; $p = 0,0400$).

It was not possible to identify curculionid species and only two *C. solieri* and three from the genus *Anthaxia* were identified from the captured buprestids.

Discussion

In our assays most of the *M. galloprovincialis* beetles were captured in transparent cross-vane traps, so a visual stimulus is not important for catching *M. galloprovincialis*; odors therefore play the major role, as referred by PAJARES *et al.*

(2004).

Silhouette and stove-pipe traps have a strong visual effect which is important to many species that need visual stimuli to stop their flight and land. Flight interception traps only rely on the attractive ability of the lure and have proven to be efficient in catching large forest insects (YOUNAN and HAIN, 1982). Most North American *Monochamus* species such as *M. titillator*, *M. carolinensis* (FATZINGER, 1985) and *M. notatus* (CHENIÉR and PHILOGÈNE, 1989b) were caught in higher numbers in traps with a black vertical silhouette than in interception or multi-funnel traps, although in some situations no statistical differences were found (CHÉNIER and PHILOGÈNE, 1989a; GROOT and NOTT, 2001; 2003; MOREWOOD *et al.*, 2002). On the other hand, GROOT and NOTT (2001;

2003) and McINTOSH *et al.* (2001) found differences between *Monochamus* species, where visual stimuli is important for *M. scutellatus* but not for *M. mutator*.

In our assays, multi-funnel traps always showed the worst results when compared with the others. Multi-funnel traps are useful for monitoring but not efficient for pine sawyer population limitation (NAKAMURA, 2003; LOYD *et al.*, 2004), as the Japanese trap for *M. alternatus* (SHIBATA *et al.*, 1996; SAKAKIBARA *et al.*, 1998; NAKAMURA *et al.*, 1999; NAKAMURA and SONÉ, 2004). The use of sticky traps, such as the stove-pipe, although they had proven to be efficient in catching *M. galloprovincialis*, is not practical for large-scale trapping and it is impossible to extract nematodes, needed for infestation assessment within the affected area.

Since in many of the assays no

statistical differences were found between the traps used, the attractive effect of chemical compounds is apparently the most important variable for catching *M. galloprovincialis*.

The first two assays used different turpentines and the 2001 turpentine was produced from a mixture of oleoresin from *P. pinaster* and *P. pinea* with higher limonene content. This difference must have had a strong repellent effect on *M. galloprovincialis*. Several studies report that limonene has an opposite effect to the attraction produced by α -pinene on *H. abietis* (LINDGREN *et al.*, 1996; KLEPZIG and SCHLYTER, 1999), inhibits egg-laying of pine processionary moth (TIBERI *et al.*, 1999; ZHANG *et al.*, 2003), is toxic to many bark beetles (BYERS, 1995) and is a feeding deterrent for the larvae of *M. alternatus* (FAN and SUN, 2006).

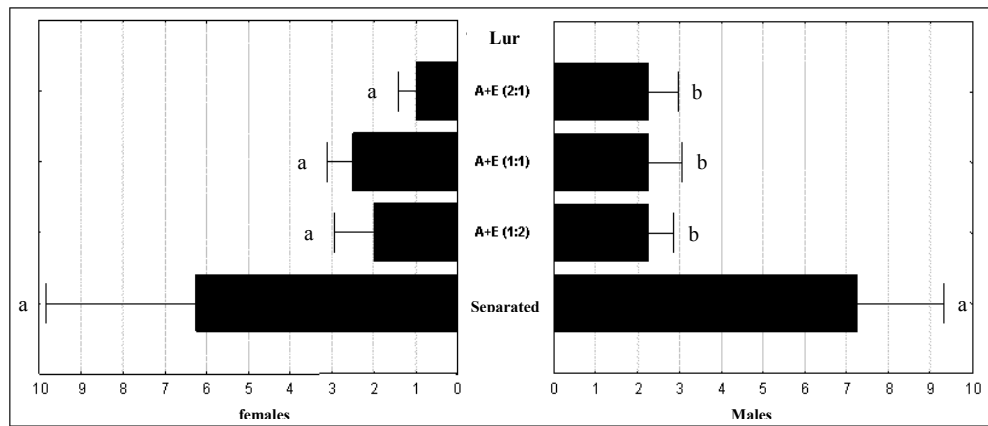


Figure 8 – Total numbers of *Monochamus galloprovincialis* caught per trap and per week in the 2004b assay. Turpentine (A) and Ethanol (E) were placed separately and in mixtures at different proportions (2:1; 1:2 and 1:1). Lines are standard errors associated to means (in black bars) and letters are Homogeneous LSD groups

The strong synergistic effect of ethanol when combined with oleoresin compounds found in our results was also reported by other authors and may be related to the fact that ethanol is a sub-product of microbial activity inside tree hosts and is interpreted by secondary insects as a sign of a weakened host (IKEDA and ODA, 1980; BYERS, 1989a; b; SATO and MAETO, 2006).

The most abundant terpene of the pines' oleoresin is α -pinene (around 80% in Portuguese *P. pinaster*) (PESTANA, 1993); when combined with ethanol, it has proven to be very attractive to bark and wood-boring beetles (BORDEN *et al.*, 1982; SCHROEDER and LINDELOW, 1989; BYERS, 1992).

This time pattern observed in our assays, with a strong effect in the first two weeks, was also reported for *M. alternatus* adults and related to high initial sensitivity to α -pinene, becoming less effective as the insect matures sexually (SAKAI *et al.*, 1992).

However, we obtained better results with turpentine than with α -pinene alone, as in other studies with wood-boring beetles (FATZINGER, 1985; IKEDA *et al.*, 1986; PHILLIPS *et al.*, 1988; CHÉNIER and PHILOGÈNE, 1989b; ALLISON *et al.*, 2001) and pine processionary moth (ZHANG *et al.*, 2003). This reveals that *M. galloprovincialis* may be sensitive to a minor compound of oleoresin, still to be isolated.

The trapping of *M. galloprovincialis* with lures based on allelochemicals produced by the pine tree host, inside the area affected by the pine wood nematode, has a limited effect because all year round many diseased trees can be found highly attractive to beetles.

To improve the efficiency of traps in capturing *M. galloprovincialis* in order to

serve as an important tool for pine sawyer population regulation and therefore reduce contamination by the pine wood nematode *B. xylophilus*, it is essential to find non-tree host odors that would easily be detected by the beetles, even in a high-mortality pine stand.

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