

# An operative kairomonal lure for managing pine sawyer beetle *Monochamus galloprovincialis* (Coleoptera: Cerymbycidae)

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**Abstract:** *Monochamus galloprovincialis* Olivier (Col., Cerambycidae) is a vector of the pine wood nematode, *Bursaphelenchus xylophilus*, causing the destructive pine wilt disease. An effective lure for monitoring and/or mass-trapping would be of great interest in the management of this pine sawyer. Males and females of this species show an attractive kairomonal response to blends composed of four pheromone compounds used by *Ips* spp. bark beetles and two host volatiles from pines. This six-component lure is highly attractive but may be too complex and costly for practical use as each component is released from a separate lure. The role of each component, ipsdienol, ipsenol, *cis*-verbenol, methyl butenol,  $\alpha$ -pinene and ethanol as attractants for *M. galloprovincialis* was field tested in Spain to obtain a simpler but equally effective bait. Ipsenol was confirmed as the strongest kairomonal signal to *M. galloprovincialis* synergizing response to  $\alpha$ -pinene by 95 times. The addition of methyl butenol to this blend doubled the number of males and females trapped. On the other hand, neither ipsdienol, *cis*-verbenol nor ethanol improved the results when incorporated into the above three-component blend. A lure consisting of ipsenol, methyl butenol and  $\alpha$ -pinene may be very cost-efficient in operational monitoring or mass trapping of *M. galloprovincialis*. Three potentially repellent candidates, (–)verbenone, methyl cyclohexenone and *trans*-conophthorin, were also tested against the attractive three-component bait. *trans*-Conophthorin significantly reduced male catches of *M. galloprovincialis*; methyl cyclohexenone had no effect. Verbenone significantly enhanced the response of females to the attractive combination of  $\alpha$ -pinene, ipsenol and methyl butenol.

**Key words:** Ipsenol,  $\alpha$ -pinene, methyl-butenol, verbenone, *trans*-conophthorin, attractive

## 1 Introduction

The genus *Monochamus* Dejean comprises about 130 species of longhorn beetles with a broad distribution worldwide. Five of these species are endemic to Europe and two of them occur in Spain, *Monochamus galloprovincialis* (Olivier) and *Monochamus sutor* (Linnaeus) (Vives 2000). Most of the cerambycid species are usually considered secondary forest pests (Hanks 1999), but some species are able to kill healthy trees and act as primary forest pests (Hanks et al. 1995; Hanks 1999; Smith and Humble 2000; Macleod et al. 2002). All the European *Monochamus* form a group of wood-boring species usually colonizing woody plants that are dead, dying or severely stressed by fire, drought or by other organisms.

In some areas, as in North America, economic damage produced by longhorned beetles is due to timber degradation caused by larvae boring into the sapwood and heartwood (McIntosh et al. 2001; Evans et al. 2004). However, wood-borers of the genus *Monochamus* have particular relevance to forests because, in addition to causing significant wood damage, they are vectors of the pine wood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner et Buhner)

Nickle, the causal agent of pine wilt disease. The PWN is endemic to North America and causes a fatal wilting disease in susceptible species of pines (Winfield et al. 1982). Healthy trees are inoculated with nematodes when maturing adult beetles feed on the pine shoots (Mamiya and Enda 1972) and nematode transmission also occurs when females oviposit into susceptible dying or dead trees. Pine forest in Japan experienced widespread damage and heavy losses after the introduction of PWN into Japan around 1905. PWN has spread into China, Korea and Taiwan during the past 35 years and is thought to have reached these locations from Japan (Ikeda 1984; Bajoun and Qouli 1989). On several occasions, interception of PWN in wood imported from Canada and United States in European countries (Schroeder and Magnusson 1992; Tomminen and Nuorteva 1992) raised concern, but recent discovery of the PWN causing death of *Pinus pinaster* trees in Portugal (Mota et al. 1999) has set the alarm in Europe and currently it is declared a quarantine organism in the European Union. *Monochamus galloprovincialis* has been confirmed as the vector of PWN in Portugal (Sousa et al. 2001).

The introduction of the PWN poses a great risk to many European pine forests as most of the native *Pinus* species are very susceptible to the disease and warm summer conditions required for disease development (Rutherford and Webster 1987; Rutherford et al. 1990) are prevalent in several areas, particularly in the Mediterranean countries. Effective methods for management of the native vector *M. galloprovincialis* are thus required before the disease spreads further through Europe.

Woodborers colonizing dying conifers are attracted by host odours (Phillips et al. 1988; Chénier and Philogène 1989; Morewood et al. 2002; Miller 2006), so most commercial baits recommended for capturing these beetles are based on host monoterpenes, usually  $\alpha$ -pinene or turpentine, and ethanol, simulating the bouquet of stressed or dying trees. Billings and Cameron (1984) and Billings (1985) demonstrated a kairomonal response in *Monochamus titillator* to a blend of *Ips* spp. bark beetle pheromones (ipsdienol, ipsenol and *cis*-verbenol), synergized by the addition of *Pinus taeda* turpentine. Allison et al. (2001) later reported a kairomonal response by four *Monochamus* species in Canada (*M. clamator*, *M. scutellatus*, *M. notatus* and *M. obtusus*) to a blend of bark beetle pheromones consisting of ipsenol, ipsdienol, 3-methyl-2-cyclohexenone and frontalin. Further studies on the attractive response by *Monochamus clamator* and *Monochamus scutellatus* confirmed that the pheromonal compounds of *Ips* spp., ipsenol and ipsdienol, increased the response to  $\alpha$ -pinene plus ethanol lures (Allison et al. 2003). In the same manner, De Groot and Nott (2004) found that traps baited with ipsenol caught more *M. s. scutellatus* than unbaited traps, and its blend with  $\alpha$ -pinene was found to be more attractive than the monoterpene alone. On the contrary, none of that happened with ipsdienol, alone or combined with lanierone, *cis*-verbenol or frontalin. For *Monochamus mutator*, the addition of ipsdienol or frontalin to  $\alpha$ -pinene did not increase the attractive response, whereas the blend of ipsenol and  $\alpha$ -pinene did not lead to the trapping of more *M. notatus* than each compound separately. More recently, Miller and Asaro (2005), testing the response of *M. titillator* to bark beetle pheromones in the south-eastern United States, found that ipsenol, ipsdienol or a combination of both led to higher catches of *M. titillator* than unbaited traps.

In Europe, responses by *M. galloprovincialis* have been recently studied by Pajares et al. (2004). They observed that multiple funnel traps baited with host volatiles  $\alpha$ -pinene or turpentine plus ethanol were scarcely attractive for this species, but the addition of *Ips* spp. pheromonal compounds significantly increased trap catches of both sexes. They found that ipsenol alone was more attractive than host volatiles ( $\alpha$ -pinene plus ethanol) but it was greatly synergized by them, confirming previous reports of this semiochemical as a strong kairomonal signal for some North American *Monochamus* (Allison et al. 2003). Besides, it was also observed that catches obtained by the triple combination of  $\alpha$ -pinene, ethanol and ipsenol were not increased by the addition of ipsdienol, but they were highly increased if not only ipsdienol but *cis*-verbenol and methyl butenol

were also incorporated into the triple mixture. This six-component lure is very effective in attracting *M. galloprovincialis* but may be too complex and costly for operational use in management programmes.

Response to two bark beetle antiaggregation pheromones, verbenone and methyl cyclohexenone (MCH), has also been tested in North America. Both components are antennally active to both sexes of *M. scutellatus*, *M. clamator* and *M. obtusus* and have been field tested in blends with other *Ips* or *Dendroctonus* pheromonal compounds and host volatiles (Allison et al. 2001). Blends containing verbenone were as attractive as unbaited traps or as traps baited with host volatiles and reduced response to the attractive blend when both were released together. Neither attractive nor repellent effect was observed when MCH was released together with the host volatiles  $\alpha$ -pinene and ethanol (Allison et al. 2003). Morewood et al. (2003) tested several non-host volatiles and showed that *trans*-conophthorin was behaviourally active, disrupting the attraction of *M. scutellatus* and *M. clamator* to traps baited with the host volatiles  $\alpha$ -pinene and ethanol and the bark beetle pheromones ipsenol plus ipsdienol.

In this article, we report results of field experiments aimed to further explore the attractive role of each compound in the mentioned six-component blend in trapping *M. galloprovincialis*. The current six-component blend is costly and complex as it requires separate release devices for each component. Our goal is to develop a simpler cost-effective lure combination for trapping *M. galloprovincialis*. In addition, three potentially repellent compounds, (-)-verbenone, MCH and *trans*-conophthorin, were also tested against attractive baits.

## 2 Materials and Methods

Four experiments were conducted on the responses of *M. galloprovincialis* to several combinations of semiochemicals. All experiments were set up in Sierra Espuña (Murcia) natural park, south-east Spain. The site was a *Pinus halepensis* mature forest planted approximately 100 years ago. Experiments 1, 2 and 3 were conducted from 23 June to 5 August, from 5 August to 29 September 2004 and from 4 July to 14 August 2005 respectively. In these experiments, we compared several combinations of semiochemicals in a subtractive or additive manner to the highly attractive six-component lure. Thus, several five-, four-, three- and two-component blends of  $\alpha$ -pinene, ethanol, ipsenol, ipsdienol, *cis*-verbenol and methyl butenol were tested for response by *M. galloprovincialis*.

Experiment 4, conducted from 14 August to 22 September 2005, was devised to test the repellent effect of three potential candidates, verbenone, MCH and *trans*-conophthorin. Tables 1 and 2 show treatments and compounds tested in the four experiments, respectively. Bark beetle semiochemicals were released at rates instructed by commercially available dispensers and host compounds were emitted at rates used in operational trapping of woodborers, similarly to other studies on *Monochamus* (Allison et al. 2001, 2003).

In all the experiments, 12-unit multiple funnel traps (Phero Tech Inc., Delta, BC, Canada) were suspended from ropes between trees, with the top funnel 1.8 m above ground. All experiments were deployed in seven randomized complete blocks. Each block consisted of different groups of traps.

**Table 1.** Treatments for all experiment testing potential baits for *Monochamus galloprovincialis*

Experiment	Treatments	
	Host compounds	<i>Ips</i> compounds
1	$\alpha$ P + et	+ Is + Id + cV + Mb
	$\alpha$ P + et	+ Is + Id + cV
	$\alpha$ P + et	+ Is + Id + Mb
	$\alpha$ P + et	+ Is + cV + Mb
	$\alpha$ P + et	+ Is + Mb
	$\alpha$ P + et	+ Is + cV
2	$\alpha$ P	–
	$\alpha$ P	+ Is
	$\alpha$ P	+ Id
	$\alpha$ P	+ Mb
	$\alpha$ P	+ Is + Mb
	$\alpha$ P	+ Id + Mb
	$\alpha$ P + et	+ Is + Mb
	$\alpha$ P + et	+ Id + Mb
3	–	Is
	et	+ Is
	$\alpha$ P	+ Is
	$\alpha$ P + et	+ Is
	–	Is + Mb
	$\alpha$ P	+ Is + Mb
	$\alpha$ P + et	+ Is + Mb
	$\alpha$ P	+ Is + Id
	$\alpha$ P + et	+ Is + Id
	Attractive bait	Repellent
4	$\alpha$ P + Is + Mb	–
	$\alpha$ P + Is + Mb	+ Vb
	$\alpha$ P + Is + Mb	+ MCH
	$\alpha$ P + Is + Mb	+ tC

$\alpha$ P,  $\alpha$ -pinene; et, ethanol; Id, ipsdienol; Is, ipsenol; cV, *cis*-verbenol; Mb, 2-methyl-3-butenol; Vb, verbenone; MCH, methyl-cyclohexenone; tC, *trans*-conophthorin.

Distance between traps was at least 100 m and nearest blocks were 700 m apart. Collecting cups were provided with a small amount of dimethyl 2,2-dichlorovinyl phosphate (DDVP) insecticide (Econex S. L., Murcia, Spain) to prevent escape of beetles from traps. Captured *M. galloprovincialis* were collected every 10–15 days and stored in the freezer until identified and sexed (Vives 2000).

Data were transformed by  $\log_{10}(x + 1)$  to meet assumptions of normality and homoscedasticity, and subjected to

ANOVA (GLM) for randomized complete blocks with the SAS System software (SAS Institute Inc. 1999–2000). Mean catches were compared by least significant difference (LSD) Fisher's test at  $\alpha = 0.05$ .

### 3 Results

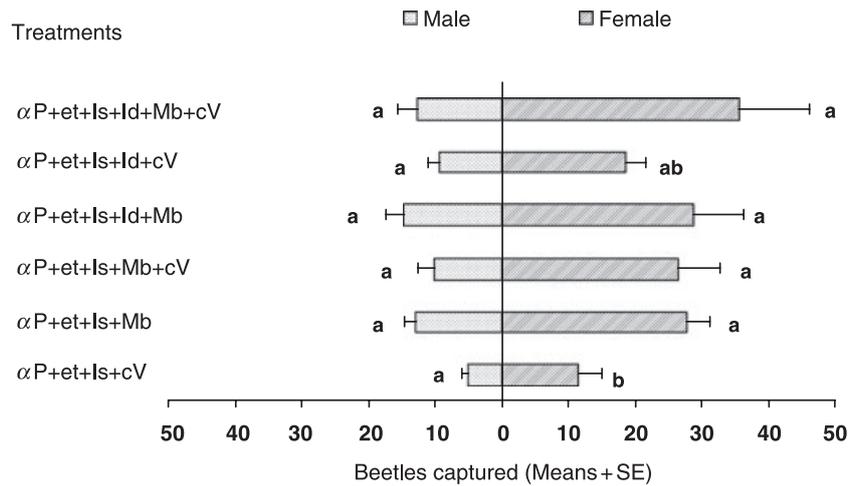
In experiment 1, we subtracted one or two of the components in the triple blend that strongly synergized the combination of  $\alpha$ -pinene, ethanol and ipsenol (Pajares et al. 2004), to find out the real contribution of ipsdienol, *cis*-verbenol and methyl butenol separately. Results showed that female catches were quite reduced, although not significantly enough, when methyl butenol was excluded in a five-component lure, but it did not happen if ipsdienol or *cis*-verbenol was removed (fig. 1). Moreover, if only methyl butenol was kept in a four-component blend, there was no difference in the number of trapped *M. galloprovincialis* females compared with the six-component reference bait. On the contrary, if only *cis*-verbenol was incorporated as the synergist, female catches were significantly reduced. Ipsdienol was not tested here as a single synergist of the  $\alpha$ -pinene, ethanol and ipsenol blend as it was shown to have no effect in an earlier experiment (Pajares et al. 2004; though turpentine was used instead of  $\alpha$ -pinene). In all treatments, catches of females were two to three times higher than those of males. No significant differences were found in trapped males among the treatments.

In experiment 2, we aimed to find out first, the net synergistic effect to  $\alpha$ -pinene of ipsenol, ipsdienol and methyl-butenol in two- or three-component blends and second, if ethanol was a really attractive contributor to the combination of  $\alpha$ -pinene and bark beetle semiochemicals. Poor performance in experiment 1 precluded testing of *cis*-verbenol. Host volatile  $\alpha$ -pinene alone, at the doses tested, was found to be poorly attractive, but addition of ipsenol increased *M. galloprovincialis* catches by 95 times, confirming the high synergistic effect observed before (Pajares

**Table 2.** Compounds tested as attractants for *Monochamus galloprovincialis* in field experiments

Compound	Chemical purity (%)	Enantiomeric ratio (+ : –)	Source*	Release device <sup>†</sup>	Release rate (mg/24 h) <sup>‡</sup>
Ipsenol	99	50 : 50	Phero Tech	Bubble cap	0.40
Ipsdienol	95	50 : 50	Phero Tech	Bubble cap	0.20
<i>cis</i> -Verbenol	90 (5% <i>trans</i> )	20 : 80	Phero Tech	Bubble cap	0.25
2-Methyl-but(3)en(1)ol	99	–	Phero Tech	Bubble cap	11
Verbenone	97	11 : 89	Phero Tech	Bubble cap	70
3-Methyl-cyclohex(2)en(1)one	99	–	Phero Tech	Bubble cap	3.5
<i>trans</i> -Conophthorin	97	50 : 50	Phero Tech	Polyethylene vial	– <sup>§</sup>
$\alpha$ -Pinene	95	5–10 : 90–95	Esencias Catalá	Polyethylene bag	2859
Ethanol	96	–	Panreac	Plastic vial	1179

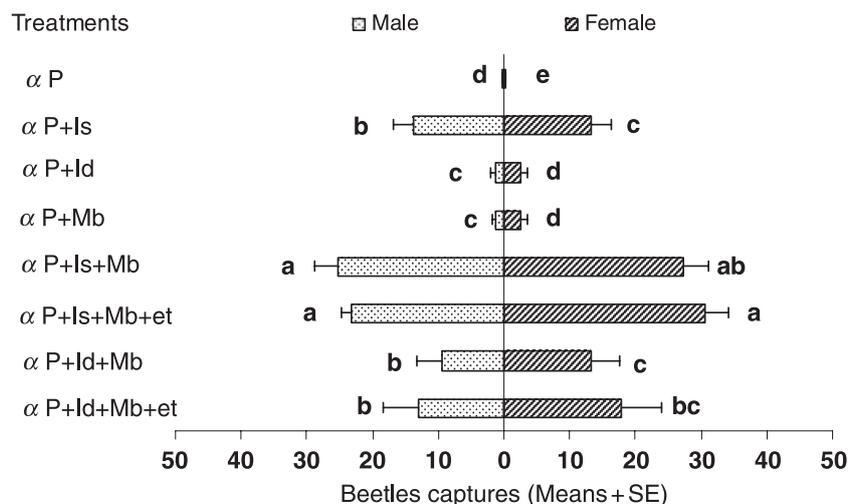
\*Phero Tech Inc. Delta, British Columbia, Canada; Esencias Catalá S.L., Gandía, Valencia, España; Panreac Química S.A., Montcada i Reixac, Barcelona, España; Unión Resinera Española S. A., Madrid, Spain.  
<sup>†</sup>Release devices for ipsdienol, ipsenol, *cis*-verbenol 2-methyl-but(3)en(1)ol were from Phero Tech Inc. All compounds were released separately.  
<sup>‡</sup>Release rates for ipsdienol, ipsenol, *cis*-verbenol, 2-methyl-but(3)en(1)ol, verbenone and 3-methyl-cyclohex(2)en(1)one were determined at 25°C by Phero Tech Inc.; release rates for  $\alpha$ -pinene and ethanol were gravimetrically calculated under field conditions in Sierra Espuña (Murcia, Spain).  
<sup>§</sup>53 mg in 250  $\mu$ l polyethylene microcentrifuge tube; release rate not provided.



**Fig. 1.** Catches of *Monochamus galloprovincialis* in Sierra Espuña, Murcia, Spain, from 23 June to 5 August 2004 to multiple-funnel traps baited with host volatiles plus *Ips* spp. semiochemicals. Compounds are:  $\alpha P$  =  $\alpha$ -pinene; *et* = ethanol; *Is* = ipsenol; *Id* = ipsdienol; *cV* = *cis*-verbenol, *Mb* = 2-methyl-3-butenol. For each sex, bars followed by the same letter are not significantly different. LSD test,  $P > 0.05$ . ANOVA statistic are: male:  $F = 1,643$ ,  $d.f. = 11$ ,  $P = 0.140$ ,  $n = 7$ ; female  $F = 2,366$ ,  $d.f. = 11$ ,  $P = 0.032$ ,  $n = 7$

et al. 2004) (fig. 2). Similarly, ipsdienol or of methyl butenol significantly increased attraction to  $\alpha$ -pinene, but their synergistic effect was significantly less stronger than by ipsenol (13 times increase in each of both compounds). However, when methyl butenol was released together with  $\alpha$ -pinene and ipsenol, trap catches of both sexes were noticeably increased compared with only the two later components (two times increase, and 183 times respect to  $\alpha$ -pinene alone). Combining both methyl butenol and ipsdienol with  $\alpha$ -pinene also led to a significant increment of trapped beetles compared with the release of only each of them plus the monoterpene, but results of such a three-component lure was not different from those of the simpler blend of  $\alpha$ -pinene plus ipsenol. Finally, addition of ethanol to each of both three-component blends did not increase catches in any of both sexes.

Considering the results obtained in experiments 1 and 2, in experiment 3 we tried to test how host volatiles  $\alpha$ -pinene and ethanol, and bark beetle semiochemicals ipsdienol and methyl butenol, synergized the highly attractive ipsenol in two-, three- and four-component combinations. As suggested in experiment 2, ethanol did not synergize ipsenol in capturing *M. galloprovincialis* males and slightly increased catches of females, but a strong synergism in attracting both sexes was obtained by  $\alpha$ -pinene (fig. 3). Again, addition of ethanol to this attractive mixture did not result in any increase of catches. Methyl butenol was found to be a better synergist of ipsenol, than  $\alpha$ -pinene; however, the differences were not significant. The combination of ipsenol,  $\alpha$ -pinene and methyl butenol, resulted in the highest male and female catches of all treatments. When methyl butenol was replaced by



**Fig. 2.** Catches of *Monochamus galloprovincialis* in Sierra Espuña, Murcia, Spain, from 5 August to 29 September 2004 to multiple-funnel traps baited with host volatiles alone or with *Ips* spp. semiochemicals. Compounds are:  $\alpha P$  =  $\alpha$ -pinene; *et* = ethanol; *Is* = ipsenol; *Id* = ipsdienol; *Mb* = 2-methyl-3-butenol. For each sex, bars followed by the same letter are not significantly different. LSD test,  $P > 0.05$ . ANOVA statistic are: male:  $F = 24,9$ ,  $d.f. = 13$ ,  $P < 0.0001$ ,  $n = 7$ ; female:  $F = 21,6$ ,  $d.f. = 13$ ,  $P < 0.0001$ ,  $n = 7$

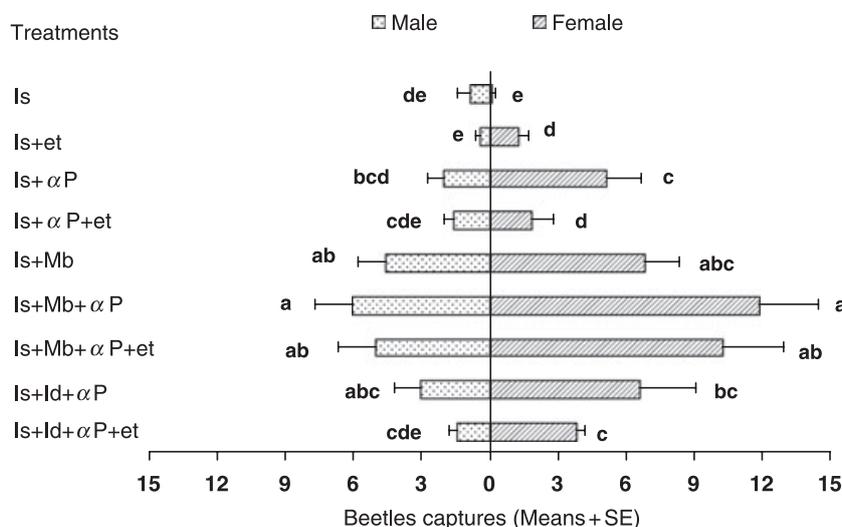
ipsdienol in the triple blend, both sexes of *M. galloprovincialis*, significantly females, were found to be less attracted. Finally, the treatments incorporating ethanol into both triple lures did not lead to the trapping of more beetles than the corresponding baits without the alcohol (mean values were even lower).

Testing of the three potentially repellent compounds in experiment 4 offered interesting results. Contrary to expectations, verbenone reinforced the attractive combination of  $\alpha$ -pinene, ipsenol and methyl butenol, significantly trapping 5.2 times more females and, non-significantly, 2.5 times more males (fig. 4). On the other hand, release of *trans*-conophthorin significantly reduced male catches of *M. galloprovincialis* but had no effect on the response of females. None of the sexes resulted less trapped when methyl-cyclohexenone was added.

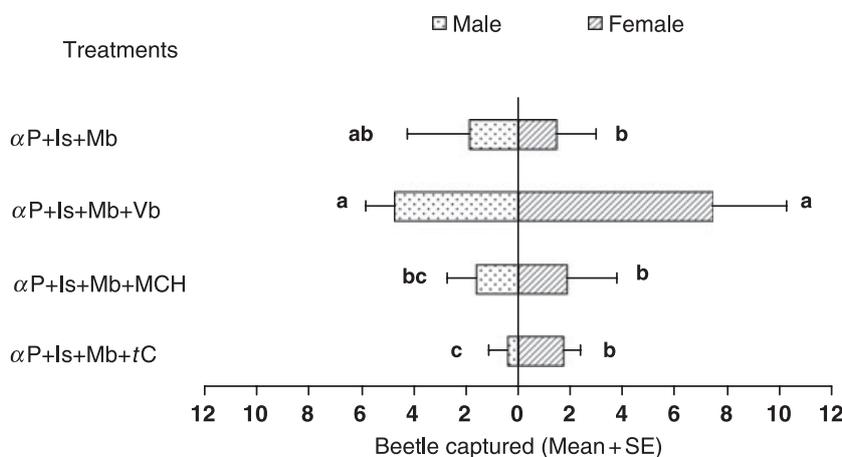
### 4 Discussion

Ipsenol was confirmed as a strong kairomone to *M. galloprovincialis*, particularly when it was released together with the host monoterpene  $\alpha$ -pinene or with methyl butenol (experiments 2 and 3, figs 2 and 3), and it must be included in any attractive lure intended for practical monitoring or mass trapping of *M. galloprovincialis*. Such a clear and higher response to ipsenol has also been reported in North America for several *Monochamus* spp. pine sawyers (Allison et al. 2003; De Groot and Nott 2004; Miller and Asaro 2005).

The subtractive method applied in experiment 1 (fig. 1) showed that when removing methyl butenol from the complete six-component lure, female catches were reduced by half (35.6 vs. 18.6 females per trap,  $P = 0.06$ ), and when two compounds were excluded



**Fig. 3.** Catches of *Monochamus galloprovincialis* in Sierra Espuña, Murcia, Spain, from 4 July to 14 August 2005 to multiple-funnel traps baited with host volatiles plus *Ips* spp. semiochemicals. Compounds are:  $\alpha$ P =  $\alpha$ -pinene; et = ethanol; Is = ipsenol; Id = ipsdienol; Mb = 2-methyl-3-butenol For each sex, bars followed by the same letter are not significantly different. LSD test,  $P > 0.05$ . ANOVA statistic are: male:  $F = 4,097$ ,  $d.f. = 14$ ,  $P < 0.0001$ ,  $n = 7$ ; female:  $F = 10,068$ ,  $d.f. = 14$ ,  $P < 0.0001$ ,  $n = 7$



**Fig. 4.** Catches of *Monochamus galloprovincialis* in Sierra Espuña, Murcia, Spain, from 14 August to 22 September 2005 to multiple-funnel traps baited with host volatiles and *Ips* spp. semiochemicals and bark beetles repellent. Compounds are:  $\alpha$ P =  $\alpha$ -pinene; Is = ipsenol; Mb = 2-methyl-3-butenol, Vb = verbenone; MCH = Methyl-cyclohexenone; tC = *trans*-conophthorin. For each sex, bars followed by the same letter are not significantly different. LSD test,  $P > 0.05$ . ANOVA statistic are: male:  $F = 8,928$ ,  $d.f. = 9$ ,  $P < 0.0001$ ,  $n = 7$ ; female:  $F = 9,124$ ,  $d.f. = 9$ ,  $P < 0.0001$ ,  $n = 7$

from the combination, only keeping methyl butenol in the lure resulted in similar catches than the complete combination. These results suggesting that methyl butenol is a key component to synergize *M. galloprovincialis* attraction to a blend of host volatiles and ipsenol, were further confirmed in other experiments (figs 2 and 3). Pajares et al. (2004) had tested methyl butenol within multicomponent blends, but it is the first time here that this bark beetle pheromone component has been thoroughly studied for kairomonal response by pine sawyers, so it is unknown whether its synergistic enhancement of ipsenol could also be extended to other North American *Monochamus* species.

In earlier studies, *cis*-verbenol was found to be non-attractive to several North American *Monochamus* species in blends with either host volatiles plus *Dendroctonus* spp. pheromones (Allison et al. 2001), host volatiles (Allison et al. 2003) or ipsdienol (De Groot and Nott 2004). As with methyl butenol, *cis*-verbenol had previously been tested for response to European *M. galloprovincialis* only as part of five- or six-component lures and its role in attraction remained uncertain (Pajares et al. 2004). Our first experiment showed that *cis*-verbenol scarcely contributed to the attractability of the six-component lure, as *M. galloprovincialis* response did not decrease when this component was removed from the blend. Furthermore, the four-component bait releasing *cis*-verbenol resulted in lower female catches of all treatments (experiment 1, fig. 1). These results led to *cis*-verbenol being discarded from the following experiments.

Kairomonal response by *Monochamus* spp. to ipsdienol has proved diverse among species and sites in North America. Studies have found that ipsdienol was attractive to *M. titillator* (Miller and Asaro 2005) and enhanced attraction of *M. clamator* and *M. scutellatus* to host volatiles (Allison et al. 2003). On the contrary, De Groot and Nott (2004) found that ipsdienol alone or combined with lanierone or *cis*-verbenol was unattractive and did not reinforce response to  $\alpha$ -pinene either by *M. s. scutellatus* or by *M. mutator*. In Europe, ipsdienol was part of multicomponent host plus bark beetle blends that were attractive to *M. galloprovincialis*, it did not enhance response by *M. galloprovincialis* to baits releasing ipsenol plus host volatiles (Pajares et al. 2004). In our experiments, ipsdienol significantly increased attraction to host  $\alpha$ -pinene, in a manner similar to that of methyl-butenol, though to a much lesser extent when compared with the strong synergism showed by ipsenol (experiment 2, fig. 2). However, when ipsdienol was subtractively excluded in experiment 1, combinations excluding it resulted in catches similar to the all-component lure (fig. 1). Similarly, ipsdienol could not effectively replace methyl butenol as a synergist of  $\alpha$ -pinene plus ipsenol (experiment 3, fig. 3). These findings clearly point out that, even though *M. galloprovincialis* seems to kairomonally respond to ipsdienol, this pheromone would not improve any bait that is already releasing ipsenol, which is much more attractive.

It is known that many coniferophagous woodborers colonizing dying or severely stressed trees positively respond to host volatiles, particularly monoterpenes

( $\alpha$ -pinene) and ethanol. Within *Monochamus*, several species have been shown to be attracted to  $\alpha$ -pinene alone (Chénier and Philogène 1989; De Groot and Nott 2004; Miller 2006) or within a blend of monoterpenes (Ikeda et al. 1980, 1981). Contrary to these and other studies, response by *M. galloprovincialis* to host volatiles, whether turpentine or  $\alpha$ -pinene, both combined with ethanol, were consistently very low, even though release rates were high (approx. 2200 mg/day) and comparable to commercial baits and other tests (Pajares et al. 2004). Similar poor results were obtained by De Groot and Nott (2004) in three North American *Monochamus* species, though release rate of  $\alpha$ -pinene was 14 times lower. Our results again showed that *M. galloprovincialis* is almost not captured in traps baited only with  $\alpha$ -pinene (0.3 beetle/trap compared with 54 beetles/trap in the most attractive combination; experiment 2, fig. 2), though no unbaited controls were included. Poorly attractive as it might be by itself,  $\alpha$ -pinene proved to be a potent synergist to the kairomonal response by *M. galloprovincialis* to bark beetle pheromones particularly to ipsenol (Pajares et al. 2004; experiment 2, fig. 2). Thus, there is no doubt on the convenience of including host monoterpenes ( $\alpha$ -pinene) in the lures for this species.

Contrarily to the active role of  $\alpha$ -pinene, the real contribution of ethanol remained unclear. Previous studies have reported a synergistic effect of ethanol on the response to monoterpenes by some North American *Monochamus* (Fatzinger 1985; Phillips et al. 1988; Miller 2006). Pajares et al. (2004) did not test them separately on *M. galloprovincialis* thus, some treatments of our experiments 2 and 3 were specifically designed to know the effect of ethanol. In none of four combinations of  $\alpha$ -pinene with bark beetle compounds, addition of ethanol did increase the number of beetles trapped (experiments 2 and 3, figs 2 and 3), thus it would be advisable to eliminate it from operative baits devised for *M. galloprovincialis* without losing effectivity.

Experiment 4 with antiaggregative or repellent semiochemicals provided a somewhat unexpected result, as the response of *M. galloprovincialis* females was significantly increased when verbenone was added to the attractive bait. Catches of males were more than doubled, but not significantly. MCH, the other bark beetle antiaggregative pheromone tested, had no effect on trap catches. Allison et al. (2001) suggested a potential repellent effect of verbenone to *M. scutellatus* that was not confirmed in further experiments (Allison et al. 2003). However, in a manner similar to that with aggregation pheromones, positive orientation towards antiaggregative signals could be also adaptative for *Monochamus* spp., as pointed out by Allison et al. (2001), as they may indicate a suitable host. Kairomonal response to aggregative pheromones could guide *M. galloprovincialis* adults to fallen, dying or fire-damaged trees that represent a suitable breeding material for bark beetle species. Our studies were carried out in forest stands where secondary *Ips* (*Orthotomicus*) *erosus* is the main sympatric bark beetle colonizing hosts suitable for breeding of *M. galloprovincialis* during the summer. Semiochemical aggregants released by this scolytid are ipsdienol and

methyl-butenol (Giesen et al. 1984) and other known ipsenol-releasing *Ips* species (Kohnle et al. 1988) are not present in the area, so the high response to ipsenol detected could be ancestral to *Monochamus* pine sawyers and thus general and not related to specific adaptations to current bark beetle species or populations. On the other hand, verbenone is released when the host is fully occupied and no longer appropriate for new bark beetle attacks, but it could still be suitable for *M. galloprovincialis* breeding if facultative intraguild predation of *M. galloprovincialis* larvae on bark beetle larvae occurs as observed in *Monochamus carolinensis* (Dodds et al. 2001).

*Trans*-Conophthorin is a compound produced by several bark beetles, both in angiosperm and in conifer twig and cone species (Birgersson et al. 1995; Miller et al. 2000; Zhang et al. 2002). It is also a major volatile in the bark of many broadleaves (Francke et al. 1995; Byers et al. 1998; Huber et al. 1999) and several studies have documented a repellent effect of this chemical on several conifer bark beetles (Huber et al. 2000, 2001; Zhang et al. 2000, 2001; Huber and Borden 2001; Jactel et al. 2002). Recently, Morewood et al. (2003) found that *trans*-conophthorin significantly decreased the captures of *M. scutellatus* and *M. clamator* when combined with baits composed of host volatiles and bark beetle pheromones, but it had no effect when it was tested only with host volatiles. In our experiment, *trans*-conophthorin seemed to reduce the numbers of male *M. galloprovincialis* trapped but not of females, compared with the standard attractive bait, thus partially confirming the above results. Results of experiment 4 on verbenone and *trans*-conophthorin must be confirmed in further experiments, as the number of insects that responded to the attractive bait in the same site and period were 10 times lower compared with the previous year. This might be due to the extremely dry conditions that could have provided a surplus of breeding material likely colonized by bark beetles and thus competing with the traps. The other reason for the sharp decrease in trapped *M. galloprovincialis* is the removal of 3800 individuals during the experiments carried out in 2003 and 2004 (Pajares et al. 2004). This figure might be a good example of the effectivity of trapping that could be achieved by these kairomonal lures in a mass-trapping programme.

Our results confirm earlier findings on the kairomonal response by the pine sawyer *M. galloprovincialis* to host and bark beetle semiochemicals (Pajares et al. 2004). The high attractive response by *M. galloprovincialis* to a six-component lure releasing two host volatiles,  $\alpha$ -pinene and ethanol, and four bark beetle pheromones, ipsenol, ipsdienol, *cis*-verbenol and methyl-butenol, found before was corroborated in our field assays. More importantly, doubts about the practical use of such complex and costly lure have been cleared, as our results demonstrated that a simpler but similarly attractive lure can be achieved. Here we have shown that *M. galloprovincialis* responds strongly to the blend of kairomones  $\alpha$ -pinene, ipsenol and methyl-butenol, and that such a lure would be very costly/efficient for the operational management of this pine sawyer.

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