JOURNAL OF APPLIED ENTOMOLOGY

J. Appl. Entomol.

ORIGINAL CONTRIBUTION

# Field response of the six-toothed pine bark beetle, *Ips sexdentatus* (Col.: Curculionidae, Scolytinae), to pheromonal blend candidates

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**Keywords**

bark beetle predators, 2-methyl-3-buten-2-ol, cis-verbenol, ipsdienol, ipsenol, myrtenol

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Received: June 19, 2011; accepted: September 22, 2011.

doi: 10.1111/j.1439-0418.2011.01682.x

## Abstract

The identification and description of the chemical signals involved in the aggregation of bark beetles may lead to the development of integrated pest management strategies using synthetic pheromones. *Ips sexdentatus* Boern. (Col.: Scolytinae) colonizes trees of the *Pinus* genus across Europe, causing severe damage in occasions. The effect of *cis*-verbenol, ipsenol, 2-methyl-3-buten-2-ol (MB) and myrtenol in relation to the major pheromonal compound ipsdienol on the aggregation behaviour of *I. sexdentatus* was studied on four field bioassays. The ternary blend of racemic ipsdienol, *cis*-verbenol and racemic ipsenol consistently caught the highest number of bark beetles, resulting in large standardized mean differences ($d_{\text{unbiased}} > 0.8$). The binary blends between ipsdienol and ipsenol, and ipsdienol and *cis*-verbenol also improved the performance of ipsdienol, although only ipsenol did it significantly. On the other hand, catches were reduced ($d_{\text{unbiased}} = -0.96$) when MB was released along ipsdienol, although the effect was found to be non-significant. On a third bioassay, the relative release rates between ipsdienol, ipsenol and *cis*-verbenol were studied. Although no differences were found between the ternary blends, a ratio of 1 : 0.25 : 0.5 for ipsdienol, ipsenol and *cis*-verbenol, respectively, scored the strongest effect size ($d_{\text{unbiased}} = 1.17$). A fourth bioassay studied the behavioural effects of myrtenol and found no significant modifications to previously established findings. Myrtenol on its own attracted almost no individuals of *I. sexdentatus*. High numbers of bark beetle predators *Thanasimus formicarius* L. and *Temnochila caerulea* Olivier were trapped during the trials. The binary blend between ipsdienol and ipsdienol was shown to catch the highest significant amount of *T. formicarius*, whereas numbers of *T. caerulea* caught were highest on the binary blend between *cis*-verbenol and ipsdienol. Presented results establish the ternary blend between ipsdienol, ipsenol and *cis*-verbenol as a reference functional aggregative lure ready to be used on the management of *I. sexdentatus*.

## Introduction

The influence of bark and ambrosia beetles (Curculionidae: Scolytinae) on forest ecosystems is regarded as unique and very significant, as they can cause major effects on landscape and great economic losses (Lieutier et al. 2004). Well more than 500 of 6000 bark beetle species described are estimated to be endophytic parasites of trees of the genus *Pinus* Linnaeus. Interspecific competition may be minimized by niche partitioning as many species might colonize the same host, but exploit different parts of

the tree, and segregate temporally between recently dead, weakened or even live hosts (Amezaga and Rodríguez 1998; Seybold et al. 2006). While boring into their hosts, they can inoculate pathogenic and saprophytic fungi, playing a key role during wood decay and recycling of nutrients (Paine et al. 1997).

The six-toothed pine bark beetle (*Ips sexdentatus* Börner) occurs in *Pinus* forests throughout the Eurasian continent (Gil and Pajares 1986) where it follows a secondary life pattern. Severe damages by *I. sexdentatus* have been reported after forest fires in Spain (Sánchez et al. 2008) or wind storms in France (Jactel et al. 2001), but it is probably the outbreak that followed the massive felling of *Pinus pinaster* Aït. by storm Klaus in January 2009 in south-western France, one of the most remarkable. The wind affected 43.1 million cubic metres (14% of the standing volume), and by the end of September 2010, 40% of the remaining trees were found to be affected by bark beetles. About 3.9 million cubic metres have been lost because of their activity (EFI, 2010).

During host colonization, several primary and secondary species release aggregation pheromones that attract both mates and additional colonizers, which help overcoming tree defences inducing mass attack (Wood 1982). The successful isolation and identification of three terpene alcohols from *Ips paraconfusus* Lanier (Silverstein et al. 1966) and their re-isolation in related species (Vité et al. 1972) set the initial milestone for the study of semiochemicals in relation to the intraspecific and interspecific communication among bark beetles and associated saproxylic insects, particularly natural enemies (Seybold et al. 2006). The identification and description of the signals involved in the chemical communication has also allowed the development of integrated pest management strategies using these synthetic semiochemicals (Vité and Baader 1990; Kohnle 1991).

Regarding *I. sexdentatus*, most of the research performed during the last 30 years has been directed to the characterization and formulation of its aggregation pheromone blend. Ipsdienol was established as the main pheromonal component during pioneering studies within genus *Ips* (Vité et al. 1972, 1974) and was isolated later again from beetle hindguts (Meyer 1993). The attractive effect of ipsdienol was proven in field experiments (Klimetzek and Vité 1986). Racemic ipsenol was detected in hindgut and frass extracts in ratio of ipsdienol to ipsenol ranging from 1 : 0.5 to 1 : 0.025 (Vité et al. 1972; Francke et al. 1986; Kohnle 1991), and its combined emission with ipsdienol seemed to reduce the number of beetles captured when the release rates were high and the ipsdienol-to-ipsenol ratio was 1 : 2 (Vité et al. 1974). However, if the ratio was brought to more natural figures (i.e. 1 : 0.05), attraction was apparently unaffected or slightly improved (Kohnle et al. 1992).

Exposition of *I. sexdentatus* to (−) − α-pinene vapours resulted in the stereoselective oxidation products *trans*-verbenol (8.2%), *cis*-verbenol (15.8%) and myrtenol (72%; Kohnle 1991). On the other hand, verbenols have not been detected in hindgut extracts of male *I. sexdentatus* (Francke et al. 1986; Kohnle 1991; Meyer 1993), although *trans*-verbenol was detected for females (Francke et al. 1986). *Cis*-verbenol is a common semiochemical among bark beetles, e.g. it is one of the major compounds of *Ips typographus* (L.) aggregation pheromone (Bakke et al. 1977). Its effect on the aggregation of *I. sexdentatus* has either been negative when released at high rates (Paiva et al. 1988), reported to increase catches (Bakke, personal communication in Schonherr et al. 1983), or not significant at low rates (Kohnle et al. 1992). A similar response has been observed for *trans*-verbenol (Kohnle et al. 1992). Myrtenol, along ipsdienone and ipsdienol, represents a major product in the hindguts of successfully boring *I. sexdentatus* (Francke et al. 1995), but its behavioural effect has not been tested, as it has for other *Ips* (Zhang et al. 2007).

The isoprene derivative 2-methyl-3-buten-ol (MB) has been shown to be part of the aggregation pheromone of *I. typographus* (Bakke et al. 1977; Schlyter et al. 1987a) and *Orthotomicus* (*Ips*) *erosus* (Wollaston; Giesen et al. 1984) but has never been isolated from *I. sexdentatus* extracts. Even so, the compound has been used in field trials on several occasions as solvent and long-distance signal (Klimetzek and Vité 1986; Paiva et al. 1988; Kohnle 1991; Kohnle et al. 1992; Lozzia 1995), although only two studies tested its effect, which turned to be either not significant or inhibiting (Kohnle 1991; Kohnle et al. 1992).

Thus, besides the general acceptance that ipsdienol is the main component in its aggregation signal, there is not a reference pheromone blend for *I. sexdentatus* yet, in spite of numerous attempts. Some commercial blends that include host compounds that may act as kairomones are available in the market, but lack information on the response to different compounds they are composed of. The present work reports the work performed testing some of the mentioned compounds with the aim of developing an effective aggregative lure for *I. sexdentatus* that would eventually help within the frame of integrated control programs. Besides, we report the response of some of the associated saproxylic beetles that are trapped along with *I. sexdentatus*, particularly

 

of some recognized natural enemies of bark beetles, and the implication of these catches on pest management is discussed.

## Material and Methods

The influence of candidate semiochemicals improving the aggregation pheromone mixture used as lure for *I. sexdentatus* was tested through four different trapping bioassays during 2006, 2007 and 2008 seasons (table 1). The first two experiments were carried out during spring and summer of 2006, at a site near Otero de Bodas (Zamora, Castile and Leon, Spain), enclosed within UTM 29T 7350 4640 coordinates and with an altitude ranging from 900 to 1090 m AMSL. *Ips sexdentatus* population was considered to be at endemic level. The area is mainly reforested by pure ca. 40-year-old *P. pinaster*, with younger ca. 30-year-old *Pinus sylvestris* L. stands covering north faced hillsides. Climatic data for the experimental periods were retrieved from a nearby weather station at Villardeciervos (Zamora, Castile and Leon, Spain). The experiments in 2007 and 2008 were carried out at a site enclosed within UTM 29T 7390 4729 coordinates (1050–1130 m AMSL) in Quintana del Castillo municipality (Leon, Castile and Leon, Spain). Although a few ca. 50-year-old *P. pinaster* stands are present, reforested stands of about 30-year-old *Pinus nigra salzmannii* J. F. Arnold cover the southern area of the experimental site. A large forest fire had occurred in the area 2 years before the onset of experiment, and breeding material was still available for *I. sexdentatus*; population levels had become high enough to be considered epidemic. In fact, during the first season of experiments in this site, a few small infestation foci were located in the sampling area. By the spring 2008, there was roughly no breeding material left from the forest fire. Climatic data for the experimental periods were retrieved from the nearby weather station at Leon Airport (Leon, Castile and Leon, Spain).

At each experiment, treatments were evaluated in terms of beetle catches in 12-unit multiple funnel traps (Former Phero Tech Inc., now Contech Enterprises Inc., British Columbia, Canada; Lindgren 1983). Traps were suspended two metres above ground from metal poles and spaced >75 m apart, outside of the surrounding stands. A total of seven blocks of treatments were defined along firebreaks and dirt roads that held uniform conditions across the experimental sites. For the site used in the experiments in 2007 and 2008, one of the blocks was taken a mean of 300 m outside of the main forest stand, uphill into the burnt area with the aim of evaluating the effectiveness on catches when traps were located outside the main stand. Traps were checked on a weekly basis when catches were collected and kept in 70% ethanol until identification and quantification. Besides the target species, other related saproxylic beetles were taken into consideration, especially two of the main *I. sexdentatus* predators, *Thanasimus formicarius* L. (Col.: Cleridae) and *Temnochila caerulea* Olivier (Col.: Trogossitidae) (Pajares et al. 2008). Numbers of other beetles such as *Orthotomicus* (*Ips*) *erosus* Wollaston (although present, not considered during 2006) or *Hylurgus ligniperda* Fabricius (Col.: Scolytinae), or the facultative zoophages *Ampedus* sp. and *Lacon punctatus* Herbst (Col.: Elateridae) or *Rhagium inquisitor* L. (Col.: Cerambycidae), or the smaller clerid zoophage *Allonyx quadrimaculatus* Schaller were also registered (Gil and Pajares 1986; Dajoz 2000; Lieutier et al. 2004).

### Trapping bioassay 1

From 27 April to 10 August 2006, the effect of the addition of *cis*-verbenol, 2-methyl-3-buten-2-ol (MB) and ipsenol to the major known aggregation pheromonal compound, ipsdienol, was tested. Release rates and compound ratios were as described in table 1. A total of five treatments were thus evaluated within a complete randomized block design. Besides the initial random position assignment, re-randomization of treatment allocation to experimental units within experimental blocks was undertaken on 31 May and 10 August (Fettig et al. 2006). Baits were replaced after 6 weeks, well before to the expected duration (SEDQ LLC, Barcelona, Spain). In addition, a set of five sachets per each treatment was hanged in an extra trap in the vicinity of the study area. These dispensers were collected after 0, 10, 20, 30, 60 and 90 days and sent to SEDQ for analysis to estimate compound release rates. Content of the devices was hexane extracted at room temperature during 20 h, and the extract was subsequently quantified using a HP-5 non-polar GC column, using n-nonyl acetate as an internal standard. This procedure allowed estimating the remaining amounts of pheromonal compounds and thus quantifies their release in the field.

### Trapping bioassay 2

As a continuation of the previous experiment, a new treatment was included in the previous set-up at the same locality from 10 August 2006 to 27 September 2006. Preliminary results of the first bioassay had

 

**Table 1** Semiochemicals, release rates, release ratios and dispensers used in field trapping experiments. All semiochemical purities ≥95%. Experimental periods, localities, and mean average (Avg), minimum (Min) and maximum (Max) air temperatures are specified for each bioassay. In bioassay 3 (v) and (Ph) notations on treatment labels, refer to the PE vial and Phero Tech bubble cap dispensers, respectively. A single dispenser was used per trap and treatment, unless otherwise specified. Release rates also specify the number of dispensers used for their calculation

| Treatment label | Semiochemicals | Source | Dispenser | Release rates (mg/24 h) | Ratio |
|---|---|---|---|---|---|
| Bioassay 1 (n = 7) 27/04–10/08, 2006 | Sierra de la Culebra (Zamora, SP). Mean period T°C: Avg 17.54°C Min 7.89°C Max 24.48°C | | | | |
| Id | (+/–) Ipsdienol | SEDQ | Aluminium Pouch | 1.53 ± 0.26, n = 6 | |
| Id + cV | (+/–) Ipsdienol | SEDQ | Aluminium Pouch | 1.58 ± 0.3, n = 6 | 1 : 0.25 |
| | *cis*-verbenol | | | 0.39 ± 0.05, n = 6 | |
| Id + Ie | (+/–) Ipsdienol | SEDQ | Aluminium Pouch | 1.53 ± 0.26, n = 6 | 1 : 0.1 |
| | (+/–) Ipsenol | Phero Tech | Bubble Cap | 0.2 at 20°C | |
| Id + MB | (+/–) Ipsdienol | SEDQ | Aluminium Pouch | 1.69 ± 0.49, n = 6 | 1 : 1 |
| | 2-methyl-3-buten-2-ol | | | 1.31 ± 0.70, n = 6 | |
| Ie | (+/–) Ipsenol | Phero Tech | Bubble Cap | 0.2 at 20°C | |
| Bioassay 2 (n = 7) 10/08–27/09, 2006 | Sierra de la Culebra (Zamora, SP). Mean period T°C: Avg 19.76°C Min 8.96°C Max 28.4°C | | | | |
| Id | (+/–) Ipsdienol | SEDQ | Aluminium Pouch | 1.53 ± 0.26, n = 6 | |
| Id + cV | (+/–) Ipsdienol | SEDQ | Aluminium Pouch | 1.58 ± 0.3, n = 6 | 1 : 0.25 |
| | *cis*-verbenol | | | 0.39 ± 0.05, n = 6 | |
| Id + Ie | (+/–) Ipsdienol | SEDQ | Aluminium Pouch | 1.53 ± 0.26, n = 6 | 1 : 0.1 |
| | (+/–) Ipsenol | Phero Tech | Bubble Cap | 0.2 at 20°C | |
| Id + Ie + cV | (+/–) Ipsdienol | SEDQ | Aluminium Pouch | 1.58 ± 0.3, n = 6 | 1 : 0.25 : 0.1 |
| | *cis*-verbenol | | | 0.39 ± 0.05, n = 6 | |
| | (+/–) Ipsenol | Phero Tech | Bubble Cap | 0.2 at 20°C | |
| Id + MB | (+/–) Ipsdienol | SEDQ | Aluminium Pouch | 1.69 ± 0.49, n = 6 | 1 : 1 |
| | 2-methyl-3-buten-2-ol | | | 1.31 ± 0.70, n = 6 | |
| Ie | (+/–) Ipsenol | Phero Tech | Bubble Cap | 0.2 at 20°C | |
| Bioassay 3 (n = 7) 16/04–16/07, 2007 | Palaciosmil (Leon, SP). Mean period T°C: Avg 14.4°C Min 8.05°C Max 20.7°C | | | | |
| Id + cV | (+/–) Ipsdienol | SEDQ | Aluminium Pouch | 1.11 ± 0.17, n = 6 | 1 : 0.25 |
| | *cis*-verbenol | | | 0.32 ± 0.11, n = 5 | |
| Id + 0.25 cV + 0.1 Ie | (+/–) Ipsdienol | SEDQ | Aluminium Pouch | 1.21 ± 0.61, n = 6 | 1 : 0.25 : 0.1 |
| | *cis*-verbenol | | | 0.29 ± 0.14, n = 6 | |
| | (+/–) Ipsenol | | | 0.13 ± 0.03, n = 6 | |
| Id + cV + Ie(v) | (+/–) Ipsdienol | SEDQ | Aluminium Pouch | 1.11 ± 0.17, n = 6 | 1 : 0.25 : 0.2 |
| | *cis*-verbenol | | | 0.32 ± 0.11, n = 6 | |
| | (+/–) Ipsenol | SEDQ | Closed 250$\mu$l PE vial | 0.21 ± 0.12, n = 6 | |
| Id + cV + Ie(Ph) | (+/–) Ipsdienol | SEDQ | Aluminium Pouch | 1.11 ± 0.17, n = 6 | 1 : 0.25 : 0.2 |
| | *cis*-verbenol | | | 0.32 ± 0.11, n = 5 | |
| | (+/–) Ipsenol | Phero Tech | Bubble Cap | 0.2 at 20°C | |
| Id + 0.25 cV + 0.5 Ie | (+/–) Ipsdienol | SEDQ | Aluminium Pouch | 0.68 ± 0.17, n = 6 | 1 : 0.25 : 0.5 |
| | *cis*-verbenol | | | 0.13 ± 0.03, n = 6 | |
| | (+/–) Ipsenol | | | 0.38 ± 0.09, n = 6 | |
| Id + 0.25 cV + Ie | (+/–) Ipsdienol | SEDQ | Aluminium Pouch | 0.64 ± 0.24, n = 6 | 1 : 0.25 : 1 |
| | *cis*-verbenol | | | 0.11 ± 0.05, n = 6 | |
| | (+/–) Ipsenol | | | 0.61 ± 0.20, n = 6 | |
| Bioassay 4 (n = 6) 16/04–11/06, 2008 | Palaciosmil (Leon, SP). Mean period T°C: Avg 11.90°C Min 6.96°C Max 16.84°C | | | | |
| Id + cV | (+/–) Ipsdienol | SEDQ | Aluminium Pouch | 1.2 at 24°C | 1 : 0.25 |
| | *cis*-verbenol | | | 0.3 at 24°C | |
| Id + Ie | (+/–) Ipsdienol | SEDQ | Aluminium Pouch | 1.2 at 24°C | 1 : 0.1 |
| | (+/–) Ipsenol | | | 0.13 at 24°C | |
| Id + My | (+/–) Ipsdienol | SEDQ | Aluminium Pouch | 1.2 at 24°C | 1 : 0.1 |
| | (–) Myrtenol | | | 0.14 at 24°C | |

**Table 1**(Continued)

| Treatment label | Semiochemicals | Source | Dispenser | Release rates (mg/24 h) | Ratio |
|---|---|---|---|---|---|
| Id + Ie + My | (+/–) Ipsdienol | SEDQ | Aluminium Pouch | 1.2 at 24°C | 1 : 0.1 : 0.1 |
| | (+/–) Ipsenol | | | 0.13 at 24°C | |
| | (–) Myrtenol | | | 0.14 at 24°C | |
| Id + Ie + cV | (+/–) Ipsdienol | SEDQ | Aluminium Pouch | 1.2 at 24°C | 1 : 0.25 : 0.1 |
| | (+/–) Ipsenol | | | 0.13 at 24°C | |
| | *cis*-verbenol | | | 0.3 at 24°C | |
| Id + cV + Ie + My | (+/–) Ipsdienol | SEDQ | Aluminium Pouch | 1.2 at 24°C | 1 : 0.25 : 0.1 : 0.1 |
| | (+/–) Ipsenol | | | 0.13 at 24°C | |
| | *cis*-verbenol | | | 0.3 at 24°C | |
| | (–) Myrtenol | | | 0.14 at 24°C | |
| My | (–) Myrtenol | SEDQ | Aluminium Pouch | 0.14 at 24°C | |

shown an improvement in *I. sexdentatus* catches when *cis*-verbenol or ipsenol was released together with ipsdienol. The new treatment tested the combined emission of all three compounds (table 1).

### Trapping bioassay 3

After the two first initial trials, the additive effect of the combination of ipsenol, *cis*-verbenol and ipsdienol on *I. sexdentatus* catches was further studied from 16 April to 16 July 2007. Ipsdienol plus *cis*-verbenol (1 : 0.25) and the triple ipsdienol, *cis*-verbenol and ipsenol mixtures were set as positive controls, and four additional treatments evaluated (i) the possibility of having an all-in-one release device in comparison with the release of ipsenol from a closed microcentrifuge tube or Phero Tech's bubble-cup and (ii) three ratios of ipsdienol to ipsenol to screen for a release level where ipsenol could eventually turn repellent, as was initially reported by Vité and collaborators (1974). As the observed natural ipsdienol-to-ipsenol ratios are very low, e.g. 1 : 0.15 during initial days of *I. sexdentatus* males boring into *P. pinaster* logs or 1 : 0.06 in *P. sylvestris* (Kohnle 1991), and the inhibitory effect was reported at a 1 : 2 ratio, it was decided to set a minimum ratio of 1 : 0.1, emulating the natural ratios during maximum beetle aggregation, and a maximum ratio at 1 : 1, the highest reported ratio during host colonization. A third intermediate ratio was set to 1 : 0.5. Although individual release rates per compound changed between treatments, the accumulated semiochemical release per treatment was held constant (table 1). As for the previous season, sets of release devices were analysed for emission rates by SEDQ following the procedure described earlier. Lure sachets were renewed after 6 weeks in the field. Re-randomizations of treatment allocation to experimental units were undertaken on 14 May and on 18 June.

### Trapping bioassay 4

The effect of myrtenol on the improvement of aggregation power of the lure developed so far was evaluated in a fourth trial at the same locality as in 2007. During the experiment, from 16 April to 11 June 2008, double, triple and quadruple combinations of ipsenol, *cis*-verbenol or myrtenol with ipsdienol were released from aluminium poaches developed in cooperation with SEDQ (table 1). Myrtenol was found to be present in the hindguts of *I. sexdentatus* boring into *P. sylvestris* (Meyer 1993), as well as in *P. pinaster* (Kohnle 1991), but tests on its behavioural effects had not been reported before. During this season, analyses of emission rates were not performed in the field and were estimated at SEDQs laboratory. As in the previous years, lure sachets were renewed after 6 weeks. Re-randomizations of treatment allocation to experimental units were carried out on 30 April, 14 and 28 May.

### Statistical analyses

The total number of collected insects over the experimental period per block and treatment was used as the appropriate value for the replicate of response variable, avoiding temporal pseudoreplication (Hurlbert 1984; Fettig et al. 2006). Disturbed traps, i.e. traps that were blown over by the wind, suffered climatic bias or felled, were deleted from the data set. Taking into consideration the count data nature of the response variable, it was fitted against treatment and block factors and to a Poisson error distribution in a generalized linear model (GLM) with a log-link

 

function, (Crawley 2007). Treatments with variances close to 0 were removed from the analysis, thus keeping error rates near their specified values (Reeve and Strom 2004). If significant treatment effects were detected, then mean values were separated using Tukey's HSD test, applying the Bonferroni correction to the value of $\alpha$ for the confidence intervals (Reeve and Strom 2004). The conservative Hedges's unbiased standardized effect sizes ($d_{\text{unbiased}}$), along with their approximated 95% confidence interval (CI) widths, were also calculated (Nakagawa and Cuthill 2007). The effect size scales the difference between treatments and allows the comparison of the effect of different lures across different experiments and species. Calculations and statistical analyses were carried out under the R statistical environment and language (The R Development Core Team, 2010).

## Results

### Trapping bioassay 1

The set of sachets analysed by SEDQ for the study of release rates revealed that there was no 2-methyl-3-buten-2-ol (MB) left once the dispenser had been in the field over 21 days. Thus, only data collected within those initial 3 week days were used for the analysis, with release rates estimated to be those presented in table 1. As lure sachets were renewed after 6 weeks of the onset of experiment, catches during the following 3 weeks from renewal were added to the first period. Ipsenol on its own caught almost no *I. sexdentatus* (fig. 1) and was left out of treatment analysis (Reeve and Strom 2004). A significant treatment effect was found among remaining treatments [$F_{3,18}$ 10.79, P(> F) < 0.001], and Tukey's HSD test revealed that there was a significant increase in *I. sexdentatus* catches when ipsenol was released along ipsdienol. According to the outcome of this test, no significant differences in the mean accumulated catches of *I. sexdentatus* could be found when *cis*-verbenol or MB was released along Id. If resulting accumulated mean catches are used for the estimation of conservative Hedges's $d_{\text{unbiased}}$ (table 2), a similar picture arises. Calculated effect sizes indicate that the Id + cV combination may have a medium positive effect on trap catches ($d_{\text{unbiased}} = 0.39$) and the Id + MB combination may have a large negative effect ($d_{\text{unbiased}} = -0.95$). But in either case, as the approximate confidence intervals overlapped zero, none of both results could be considered confident. On the other hand, the Id + Ie combination had a

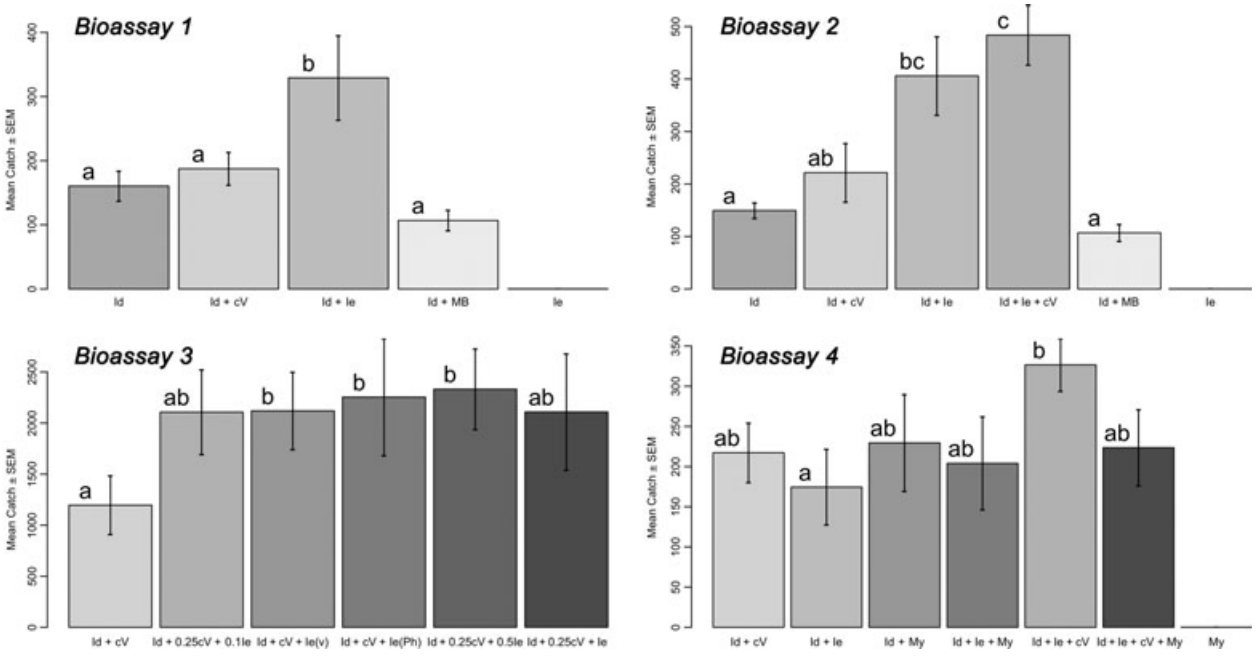


**Fig. 1** Bar plots representing mean accumulated *Ips sexdentatus* catches ± SEM per treatment and bioassay on multiple funnel traps. Trapping bioassays 1 and 2 placed in Otero de Bodas (Zamora, Castile and Leon, Spain) during the 2006 season (n = 7). Trapping bioassays 3 (n = 7) and 4 (n = 6) placed in Quintana del Castillo (Leon, Castile and Leon Spain) during 2007 and 2008 seasons. cV, *cis*verbenol; Id, ipsdienol; Ie, ipsenol; MB, methyl butenol; My, myrtenol. Further information and release rates in table 1. Bars sharing the same letter are not significantly different (Tukey's HSD, Bonferroni's adjustment, P < 0.05).

**Table 2** Standardized mean differences (Hedges's $d_{unbiased}$) and their 95% CI, between shown treatments and Id in bioassays 1 and 2 and Id + cV in bioassays 3 and 4 in mean accumulated catches of *Ips sexdentatus*. Treatment labels as in table 1. Approximate width of confidence interval for effect sizes calculated after equation 15 in Nakagawa and Cuthill (2007)

| Bioassay<br>Treatment | | Hedges's $d_{unbiased}$<br>and 95% CI |
|---|---|---|
| Bioassay 1 (n = 7) | | |
| Id | | |
| Id + cV | 0.39 | −0.76 to 1.54 |
| Id + Ie | 1.21 | −0.03 to 2.44 |
| Id + MB | −0.95 | −2.16 to 0.25 |
| Ie | **−3.43** | **−5.22 to −1.64** |
| Bioassay 2 (n = 7) | | |
| Id | | |
| Id + cV | 0.63 | −0.54 to 1.79 |
| Id + Ie | **1.68** | **0.36 to 3.01** |
| Id + Ie + cV | **2.85** | **1.23 to 4.47** |
| Id + MB | −0.96 | −2.16 to 0.24 |
| Ie | **−4.96** | **−7.26 to −2.66** |
| Bioassay 3 (n = 7) | | |
| Id + cV | | |
| Id+0.25cV+0.1Ie | 0.90 | −0.29 to 2.10 |
| Id + cV + Ie(v) | 0.97 | −0.23 to 2.18 |
| Id + cV + Ie(Ph) | 0.83 | −0.36 to 2.01 |
| Id + 0.25 cV + 0.5 Ie | 1.17 | −0.07 to 2.40 |
| Id + 0.25 cV + Ie | 0.72 | −0.46 to 1.89 |
| Bioassay 4 (n = 6) | | |
| Id + cV | | |
| Id + Ie | −0.39 | −1.65 to 0.88 |
| Id + My | 0.09 | −1.16 to 1.35 |
| Id + Ie + My | −0.10 | −1.36 to 1.15 |
| Id + Ie + cV | 1.20 | −0.16 to 2.55 |
| Id + Ie + cV + My | 0.06 | −1.19 to 1.31 |
| My | **−3.17** | **−5.04 to −1.29** |

Bold values highlight effect sizes with CIs not overlapping zero.

quite clear and very large effect on *I. sexdentatus* catches ($d_{unbiased}$ = 1.21), whereas ipsenol alone could be considered unattractive ($d_{unbiased}$ = −3.43).

Treatment effect was found to significantly affect catch levels of *A. quadrimaculatus* ($F_{4,24}$ = 3.30, P = 0.027), *H. ligniperda* ($F_{4,24}$ = 2.98, P = 0.039), *T. caerulea* ($F_{4,24}$ = 7.65, P < 0.001) and *T. formicarius* ($F_{4,24}$ = 58.47, P < 0.001). The presence of ipsenol clearly influenced the response of the tallied clerids (table 3). Calculated effect sizes showed a significant, very large increase for *T. formicarius* when ipsenol was the only semiochemical released ($d_{unbiased}$ = 1.31). Although *T. caerulea* was apparently not affected by ipsenol, its catches were significantly increased when *cis*-verbenol was released along the major compound ipsdienol, reaching numbers of the same order of magnitude of its prey *I. sexdentatus*. Accordingly, the estimated effect size reflected a very large mean difference between ipsdienol and Id-cV ($d_{unbiased}$ = 1.39; table 3).

### Trapping bioassay 2

As the estimated duration of the MB dispenser was 3 weeks, only catches for the first 3 weeks of the experiment were used for the analysis. Once treatment ipsenol was removed from the analysis, a highly significant treatment effect was detected on *I. sexdentatus* catches ($F_{4,24}$ = 14.98, P < 0.001). The triple blend, Id + Ie + cV, yielded a highly significant improvement in comparison with the reference treatment (ipsdienol), although not significantly better than Id + Ie (fig. 1). Treatment Id + cV pooled in between ipsdienol and Id + Ie, whereas Id + MB caught less beetles but not significantly. The standardized mean differences pointed to similar results; both Id + Ie and Id + Ie + cV treatments had a large effect in contrast to ipsdienol ($d_{unbiased}$ = 1.68 and 2.85, respectively, table 2), whereas Id + cV held a medium effect as in bioassay 1 ($d_{unbiased}$ = 0.63). The standardized mean difference between Id + cV and Id + Ie + cV, on the other hand, was estimated at $d_{unbiased}$ = 1.40. The evaluated Id + MB showed again a large negative effect ($d_{unbiased}$ = −0.96), although as in bioassay 1, the width of the CI overlapped zero (table 2).

As the flight period of many of the tallied beetles was over by the onset of bioassay 2, only a few *H. ligniperda* and *T. caerulea* were caught during the experiment (table 3).

### Trapping bioassay 3

During the 2007 season, the significant increase in response to *I. sexdentatus* for the triple Id + Ie + cV blend detected during the previous year was re-tested through a set of treatments that evaluated different compound ratios and release methods. The chemical analysis for the estimation of lure release rates showed that overall values were a bit lower than during previous season, thus resulting in slightly different semiochemical ratios, and the Id / Ie ratio in the Id + cV + Ie(Ph) treatment changed from 1 : 0.1 to 1 : 0.2 (table 1). Estimated mean average and maximum air temperatures had been three to four degrees celsius lower in this locality than in the previous experimental site and season (table 1) and could have influenced evaporation rates of tested compounds.

**Table 3** Mean accumulated catches (±SEM) and standardized mean differences (Hedges's $d_{unbiased}$) of associated saproxylic beetles of *Ips sexdentatus* after trapping bioassays. Treatment labels as in table 1. Treatments sharing the same letter are not significantly different (Tukey's HSD, Bonferroni's adjustment, $P < 0.05$). Hedges's $d_{unbiased}$ estimated using treatment Id as control in bioassays 1 and 2 and Id + cV in bioassays 3 and 4

| | Catches per treatment (mean ± SM) and standardized mean differences (Hedges's $d_{unbiased}$) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | *Allonyx quadrimaculatus* | | *Ampedus* sp. | | *Hylurgus ligniperda* | | *Lacon punctatus* | | *Orthotomicus erosus* | | *Rhagium inquisitor* | | *Temnochila caerulea* | | *Thanasimus formicarius* | |
| Bioassay 1 (n = 7) | | | | | | | | | | | | | | | | |
| Id | 2.0 ± 0.7ab | | 2.9 ± 0.9a | | 85.6 ± 19.4a | | 10.0 ± 2.8a | | – | | 2.4 ± 1.1a | | 41.0 ± 8.4a | | 10.1 ± 1.8a | |
| Id + cV | 1.6 ± 0. ab | −0.30 | 1.9 ± 0.8a | −0.41 | 86.1 ± 20.3a | 0.01 | 10.3 ± 3.4a | 0.03 | – | | 2.1 ± 0.5a | −0.12 | 117.0 ± 26.0b | **1.39** | 11.6 ± 2.8a | 0.21 |
| Id + Ie | 2.1 ± 0.8ab | 0.07 | 2.3 ± 0.4a | −0.28 | 79.9 ± 13.3a | −0.12 | 7.9 ± 2.8a | −0.27 | – | | 0.1 ± 0.1a | −1.00 | 47.7 ± 6.7a | 0.31 | 75.1 ± 7.6b | **4.15** |
| Id + MB | 1.0 ± 0.4a | −0.64 | 2.6 ± 0.8a | −0.12 | 87.7 ± 17.1a | 0.04 | 11.9 ± 3.7a | 0.20 | – | | 2.4 ± 1.2a | 0.00 | 42.6 ± 12.9a | 0.05 | 5.3 ± 1.4a | −1.04 |
| Ie | 5.0 ± 1.8b | 0.77 | 1.1 ± 0.3a | −0.86 | 32.1 ± 6.7a | **−1.30** | 8.3 ± 2.2a | −0.24 | – | | 2.0 ± 0.7a | −0.16 | 27.0 ± 9.6a | −0.55 | 18.1 ± 2.4a | **1.31** |
| Bioassay 2 (n = 7) | | | | | | | | | | | | | | | | |
| Id | 0 | | 0 | | 2.4 ± 0.7a | 0.27 | 0.3 ± 0.3 | | – | | 0 | | 5.4 ± 1.5a | | 0 | |
| Id + cV | 0 | | 0 | | 3.0 ± 0.8a | 0.83 | 0 | −0.50 | – | | 0 | | 13.6 ± 6.9a | 0.58 | 0 | |
| Id + Ie | 0 | | 0 | | 4.6 ± 1.1a | 0.28 | 0 | −0.50 | – | | 0 | | 6.9 ± 2.5a | 0.24 | 0 | |
| Id + Ie + cV | 0 | | 0 | | 3.0 ± 0.7a | 0.31 | 0 | −0.50 | – | | 0 | | 8.6 ± 3.0a | 0.47 | 0 | |
| Id + MB | 0 | | 0 | | 3.3 ± 1.2a | −0.58 | 0 | −0.50 | – | | 0 | | 2.7 ± 0.8a | −0.79 | 0 | |
| Ie | 0 | | 0 | | 1.4 ± 0.5a | 0.27 | 0 | −0.50 | – | | 0 | | 2.1 ± 0.9a | −0.94 | 0 | |
| Bioassay 3 (n = 7) | | | | | | | | | | | | | | | | |
| Id + cV | 1.3 ± 0.4a | | 1.6 ± 0.8a | | 40.0 ± 11.0a | | 1.1 ± 0.6a | | 272.3 ± 109.1a | | 0.3 ± 0.3a | | 18.4 ± 6.0ab | | 3.0 ± 1.4a | |
| Id + 0.25 cV+ 0.1 Ie | 1.7 ± 0.6a | 0.28 | 2.1 ± 0.6a | 0.27 | 37.3 ± 17.6a | −0.07 | 1.1 ± 0.5a | 0.00 | 164.0 ± 38.6a | −0.47 | 1.6 ± 0.7a | 0.87 | 14.6 ± 4.7b | −0.25 | 8.6 ± 1.0ab | **1.65** |
| Id + cV + Ie(v) | 3.0 ± 0.8ab | 0.91 | 3.1 ± 1.5a | 0.46 | 45.9 ± 11.8a | 0.18 | 0.3 ± 0.3a | −0.69 | 255.4 ± 51.1a | −0.07 | 1.1 ± 0.7a | 0.56 | 27.1 ± 7.8ab | 0.45 | 14.9 ± 2.7ab | **1.97** |
| Id + cV + Ie(Ph) | 3.4 ± 0.8ab | 1.25 | 2.6 ± 0.8a | 0.44 | 57.3 ± 25.1a | 0.32 | 1.1 ± 0.6a | 0.00 | 223.3 ± 56.9a | −0.20 | 1.9 ± 1.0a | 0.78 | 26.6 ± 4.9ab | 0.53 | 13.0 ± 2.4ab | **1.79** |
| Id + 0.25 cV + 0.5 Ie | 4.3 ± 1.2b | 1.17 | 1.7 ± 0.7a | 0.06 | 50.1 ± 12.8a | 0.30 | 0.7 ± 0.4a | −0.33 | 248.6 ± 61.5a | −0.09 | 1.4 ± 0.4a | 1.11 | 37.4 ± 9.6a | 0.84 | 19.4 ± 5.0b | **1.58** |
| Id + 0.25 cV + Ie | 2.0 ± 0.5ab | 0.55 | 2.0 ± 0.3a | 0.24 | 55.0 ± 18.5a | 0.35 | 1.6 ± 0.7a | 0.24 | 231.4 ± 103.9a | −0.14 | 0.9 ± 0.6a | 0.46 | 28.7 ± 8.8ab | 0.48 | 20.6 ± 4.6b | **1.82** |
| Bioassay 4 (n = 6) | | | | | | | | | | | | | | | | |
| Id + cV | 0.3 ± 0.2a | | 0.7 ± 0.3a | | 46.2 ± 15.4ab | | 0.7 ± 0.4 | | 44.2 ± 10.8ab | | 1.2 ± 0.5a | | 1.5 ± 1.1a | | 0.2 ± 0.2a | |
| Id + Ie | 0.7 ± 0.2a | 0.60 | 1.0 ± 1.0a | 0.17 | 19.0 ± 4.7ab | −0.91 | 0 | 0.00 | 40.2 ± 7.8ab | −0.16 | 0.3 ± 0.3a | −0.77 | 0.5 ± 0.3a | −0.46 | 3.0 ± 1.4a | 1.09 |
| Id + My | 0.3 ± 0.2a | 0.00 | 2.8 ± 2.4a | 0.48 | 32.0 ± 9.5ab | −0.42 | 0 | −0.69 | 46.7 ± 11.0a | 0.09 | 0.7 ± 0.3a | −0.46 | 2.2 ± 2.0a | 0.16 | 0.3 ± 0.2a | 0.34 |
| Id + Ie + My | 0.7 ± 0.3a | 0.46 | 3.3 ± 1.3a | 1.05 | 22.5 ± 4.4ab | −0.80 | 0.3 ± 0.2 | 0.24 | 63.8 ± 15.0a | 0.58 | 0.3 ± 0.3a | −0.77 | 2.2 ± 1.8a | 0.17 | 3.5 ± 0.9a | **2.00** |
| Id + Ie + cV | 1.3 ± 0.6a | 0.83 | 4.8 ± 1.9a | 1.14 | 59.8 ± 13.5a | 0.36 | 0.3 ± 0.3 | −0.33 | 64.7 ± 16.4a | 0.56 | 1.3 ± 0.3a | 0.15 | 2.2 ± 0.9a | 0.25 | 3.5 ± 1.9a | 0.92 |
| Id + Ie + cV + My | 0.3 ± 0.2a | 0.00 | 1.5 ± 0.5a | 0.75 | 26.7 ± 11.1ab | −0.56 | 0.1 ± 0.1 | 0.00 | 48.7 ± 12.2a | 0.15 | 0.7 ± 0.3a | −0.46 | 1.2 ± 0.5a | −0.14 | 2.0 ± 0.7a | 1.32 |
| My | 0.0 ± 0.0 | −0.85 | 2.3 ± 1.6a | 0.55 | 1.5 ± 0.6b | **−1.57** | 0.4 ± 0.3 | 0.00 | 1.8 ± 0.8b | **−2.12** | 0.0 ± 0.0 | −1.32 | 0.3 ± 0.2a | −0.55 | 0.0 ± 0.0 | −0.54 |

Bold values highlight effect sizes with CIs not overlapping zero.

Total *I. sexdentatus* catch levels registered about a 10-fold increase from bioassay 1 and tallied between 1196.3 ± 286.1 and 2332.6 ± 395.3 SEM (n = 7) individuals per treatment. Even if the effective experimental period was longer, the detected increase was probably caused by the high local population of *I. sexdentatus*. A significant treatment effect ($F_{5,30}$ = 4.11, P = 0.006) was detected, mainly caused by higher trap catches on treatments releasing ipsenol (fig. 1). Although the highest catch was recorded for treatment Id + 0.25 cV + 0.5 Ie, no significant differences among those treatments with ipsenol in their blend could be detected (fig. 1). In fact, the more conservative effect size approach revealed large effects for all evaluated treatments compared to Id + cV, but all estimates had their lower CI end overlapping zero, treatment Id + 0.25 cV + 0.5 Ie being the only one with the lower tail close to 0 (table 2).

Only catches of predatory species showed a significant treatment effect among associated saproxylic beetles (table 3). In case of *T. caerulea*, although the effect was found to be significant ($F_{5,30}$ = 2.73, P = 0.038), only the treatments Id + 0.25 cV + 0.5 Ie and Id + 0.25 cV + 0.1 Ie were found to differ. The clerids showed a clear preference for treatments with highest ipsenol presence, causing a significant treatment effect in the analysis of both *A. quadrimaculatus* ($F_{5,30}$ = 4.10, P = 0.006) and *T. formicarius* ($F_{5,30}$ = 6.09, P < 0.001). The large effect of ipsenol on *T. formicarius* catches was also apparent in the conservative standardized mean differences (table 3). Elaterids were less abundant than on bioassay 1 and, as before, showed no preference among treatments (table 3). Finally, no significant treatment effects were detected for trapped *H. ligniperda* and *O. erosus* ($F_{5,30}$ = 0.51, P = 0.766 and $F_{5,30}$ = 0.86, P = 0.519, respectively).

### Trapping bioassay 4

The effect on *I. sexdentatus* catches and associated beetles of the release of myrtenol alone and combined with previously tested compounds was evaluated (table 1). Temperature records revealed that the experimental period had been very cold. In fact, only during 3 of the 8 sampling weeks was the mean maximum week temperature above the minimum flight threshold (18°C, Bakke 1968), and consequently, the mean of the maximum daily temperature during the trial was below that threshold (table 1). Even if *I. sexdentatus* catches were severely reduced in comparison with the previous season, the response was judged to be sufficient to carry out the analysis. However, outliers were detected on an experimental block with some trap positions exposing north and had to be removed from the analysis.

Myrtenol on its own caught just a single *I. sexdentatus* individual, whereas catches in the other treatments ranged between 174.3 ± 47 and 326.5 ± 32.7 SEM (n = 6) insects. A significant treatment effect was detected among these treatments ($F_{5,25}$ = 2.67, P = 0.045), but significant mean differences were only found between treatments Id + Ie and Id + Ie + cV (fig. 1). Lures containing myrtenol in their blend did not significantly differ from treatment Id + cV or Id + Ie + cV, which could be considered the reference treatments upon the previous results. Large effect sizes were only detected for treatments Id + Ie + cV ($d_{\text{unbiased}}$ = 1.20) and myrtenol ($d_{\text{unbiased}}$ = −3.17), although only for the second could the standardized mean difference be tagged as different to 0 (table 2).

Significant treatment effects were only found for the scolytids *H. ligniperda* and *O. erosus* among associated beetles ($F_{6,30}$ = 3.73, P = 0.007 and $F_{6,30}$ = 5.37, P < 0.001, respectively), mainly because of the low catches on the My treatment (table 3). None of the catches of other tallied saproxylic beetles made it to the tens and were often clumped in few experimental blocks. Medium-to-large negative effect sizes on *H. ligniperda* catches were detected for treatments releasing myrtenol, although only treatment My deviated from 0. *Orthotomicus erosus* showed a similar pattern to *I. sexdentatus*, effect sizes only confirming the negative large effect for treatment My (table 3).

## Discussion

The release of ipsenol along with the major pheromonal compound, ipsdienol, consistently reinforced the attractive power of the latter when tested in binary combinations, catching significantly more *I. sexdentatus* than ipsdienol alone (fig. 1). Ipsenol on its own was clearly not attractive at all, and thus, its combination with ipsdienol could be classed as synergic. Used ipsdienol-to-ipsenol ratio was 1 : 0.1 (table 1), much lower than in previously reported trials. Vité et al. (1974) reported inhibition by ipsenol at a ratio of 1 : 2. Besides, conclusions were drawn after an experiment with just two replicates and compounds being released at very high rates, 192 and 384 mg/24 h for ipsdienol and ipsenol, respectively. Pheromone compounds that might be very attractive at low release rates have been found interruptive or repellent at high

 

rates (Borden 1997). In any case, dose–response curves have not been studied for *I. sexdentatus* in relation to any of its pheromonal compounds besides ipsdienol, in one study that used MB as solvent (Klimetzek and Vité 1986) and in another in which it was released along increasing amounts of E-myrcenol (Schlyter et al. 2001). On another trial, the effect of ipsenol on *I. sexdentatus* catches was found not significant (Kohnle et al. 1992) but hinted in the direction that using natural ratios among these terpene alcohols could improve the attraction power of the pheromonal blend, as results showed low negative ($d_{\text{unbiased}} = -0.04$) or medium positive effect sizes ($d_{\text{unbiased}} = 0.55$). However, these results could have been masked by the effect of α-pinene, 2-methyl-3-butin-2-ol and 2-phenylethanol, which were released along (Kohnle et al. 1992). Results presented here clearly point a very large and consistent effect ($d_{\text{unbiased}} = 1.21$ and 1.68) for the binary combinations on bioassays 1 and 2 (table 2).

Anyway, the most significant outcome of bioassay 2 was the highly significant effect that *cis*-verbenol and ipsenol had on the aggregative power of ipsdienol, when released at a ratio of 1 : 0.25 : 0.1 (fig. 1). Catches of *I. sexdentatus* were found to be increased threefold, with an estimated effect size of $d_{\text{unbiased}} = 2.85$ (table 3). This result encouraged further trials studying the relationship between ipsdienol and ipsenol on a third field bioassay. The binary *cis*-verbenol and ipsdienol combination was kept as the blend to improve, and as in the previous trial, results confirmed the performance of the ternary blend. In any case, while on bioassay 2 treatment Id + Ie + cV had a standardized mean difference to Id + cV of $d_{\text{unbiased}} = 1.40$, registered effects on bioassay 3 were weaker and overlapping zero (table 2). The lack of differences between ternary blends with different release ratios put a stop to a dose modelling analysis for the response of *I. sexdentatus* to the presence of ipsenol in the blend. More extreme ratios could help reveal optimum blends. Improving the relative release rates of pheromone components should increase the aggregation power of the lures as has been suggested for *I. paraconfusus* (Seybold et al. 2006). Meanwhile, the Id + cV + Is blend might be kept as a reference for a functional aggregation lure, with the strongest effect sizes registered for compound ratios between 1 : 0.25 : 0.5 and 1 : 0.25 : 0.1.

*Ips sexdentatus* can colonize several pine species, sharing hosts with other *Ips* beetles, for example, it competes with *Ips acuminatus* in *P. sylvestris* and with *O. erosus* in *P. nigra*, *Picea orientalis* (L.) and *P. pinaster* (Gil and Pajares 1986; Kohnle et al. 1988). Although niche overlap in bark beetles is avoided to a certain degree by differences in the preference for bark thickness (Bakke 1968), there is an obvious mediation of semiochemicals on the interspecific competition, by their chirality and/or by the combination of their terpenoid aggregation pheromones (Kohnle et al. 1988). Besides, the ratios at which these compounds are released could also play a key role (Kohnle et al. 1992).

The behavioural effect of 2-methyl-3-buten-2-ol (MB) on *I. sexdentatus* has rarely been studied (Kohnle et al. 1992), but it has been used on various trials as solvent and/or assuming that it is involved in long-distance signalling (Serez and Schonherr 1985; Klimetzek and Vité 1986; Paiva et al. 1988; Lozzia 1995). On *I. typographus,* MB plays a key role on the pheromonal blend (Bakke et al. 1977; Schlyter et al. 1987a) and might be a key cue to induce landing of beetles (Bakke et al. 1983). This hemiterpenoid is also a major compound for the aggregation pheromone of *O. erosus* (Giesen et al. 1984; Seybold et al. 2006). Although significant differences were not found in our study (fig. 1), large negative standardized mean differences ($d_{\text{unbiased}} = -0.95$ and $-0.96$, table 2) are comparable to those reported earlier ($d_{\text{unbiased}} = -0.67$) by Kohnle et al. (1992) and confirm that MB may inhibit the response of *I. sexdentatus* in a similar fashion in which it does to the aggregation of *I. acuminatus* (Bakke 1978; Kohnle et al. 1992). Thus, MB might be signalling the presence of the competitor *O. erosus* and repel *I. sexdentatus*. Even if bark thickness has been pointed as a segregation factor for these species, niche overlap is frequent, as *I. sexdentatus* is able to colonize areas of the tree where the bark is thinner than its own mean body depth (Amezaga and Rodríguez 1998). Both species share ipsdienol as the major compound of their pheromone blend, even at the optical isomer level (Francke et al. 1995; Seybold et al. 2006), signalling host suitability in the long distance, so it is likely that inhibition of *I. sexdentatus* might occur when landing, aiding the beetles localize trees suitable for colonization and compartmentalizing themselves along the tree using chemical signals. The importance of aggregation pheromones in the colonization pattern of *I. sexdentatus* has been suggested earlier (Bouhot et al. 1988).

A similar situation may occur when *I. acuminatus* and *I. sexdentatus* colonize *P. sylvestris*. Whereas *I. acuminatus* utilizes the thin bark area of the upper stem, *I. sexdentatus* inhabits the thick bark parts of the stem

 

(Bakke 1968, 1978). 95% (+)/5% (−) ipsdienol, 5% (+)/95% (−) ipsenol and *cis*-verbenol are recognized as the major compounds within the pheromonal blend of *I. acuminatus* (Bakke 1978; Francke et al. 1986). On the other hand, ipsdienol was found to be present at a constant 20% (+)/ 80% (−) enantiomeric composition in hindguts of *I. sexdentatus* (Meyer 1993), although a previous experiment failed to show any preference between enantiomers and the racemic mixture (Francke et al. 1986). Thus, the optical isomer of ipsdienol might play a key role in distinguishing the aggregation signal for both bark beetles. Furthermore, cross-attraction to trees under attack to both species might occur as both species respond strongly to racemic ipsdienol (fig. 1; Bakke 1978; Vité et al. 1974), which, in turn, would benefit both species. Results published so far and those presented here raise the possibility that *cis*-verbenol may also play a key role. Whereas *cis*-verbenol has not been detected in male *I. sexdentatus* hindguts (Francke et al. 1986; Kohnle 1991; Meyer 1993), it does respond to it (Schonherr et al. 1983; Paiva et al. 1988; Kohnle et al. 1992), suggesting its role as an allelochemical that may act as a kairomone when released at low rates, in a ratio of ipsdienol to *cis*-verbenol of 1 : 0.25 (fig. 1), and as allomone in a ratio of 1 : 100 (Paiva et al. 1988). Accordingly, blend ratios of 1 : 0.5 yielded no significant differences to the reference ipsdienol treatment (Kohnle et al. 1992). If effect sizes are considered, $d_{\text{unbiased}}$ increases as the ratio favours ipsdienol in the mentioned studies, −0.89 at Paiva et al. (1988), −0.24 at Kohnle et al. (1992) and 0.39 and 0.63 for our bioassays 1 and 2, respectively. Besides, it is to be considered that a ratio of 1 : 3.33 of ipsdienol to *cis*-verbenol together with MB has proven attractive to *O. erosus* (Klimetzek and Vité 1986). All in all, the moderate increase in catches detected after the combined release of *cis*-verbenol and ipsdienol has been taken into consideration on the development of lure for *I. sexdentatus* (SEDQ, personal communication).

The outcome of bioassay 4 was severely affected by the low temperatures registered during the field trial (table 1). Although the ternary blend described earlier yielded highest catches and a very large positive effect size ($d_{\text{unbiased}}$ = 1.20) comparable to those in the previous trials, the good results of treatment Id + Ie could not be confirmed. Although one of the experimental blocks was removed because of obvious internal exposure heterogeneity, there was a high chance that randomization procedures did not minimize positional effects, as experimental units were not equally represented (Fettig et al. 2006). Myrtenol is present in high amounts in *I. sexdentatus* hindgut extracts (Francke et al. 1995), but its behavioural effect had not been tested on this bark beetle. It had been identified and tested on *Ips subelongatus* Motsch., which, although it could detect it, did not change response when the compound was withdrawn from the full pheromonal blend (Zhang et al. 2007). Similar results have been reported for *I. typographus* (Schlyter et al. 1987a), as myrtenol did not alter the response to the major pheromonal compounds MB and *cis*-verbenol. Bark beetles may avoid the oxidation of α-pinene to bioactive products such as *cis*-verbenol and *trans*-verbenol, by producing myrtenol (Kohnle 1991). The results presented above support the previous findings. Myrtenol on its own caught almost no *I. sexdentatus*, and treatments that released it showed no significant differences with remaining treatments. Thus, it can be concluded that myrtenol exerted no behavioural changes on the aggregation of *I. sexdentatus*.

Many bark beetle associated saproxylic beetles, including predators, are known to locate grazing grounds using host, competitor or prey chemical cues (Wood 1982; Raffa 2001; Lieutier et al. 2004). In our experiments, at least seven other species were found to some extent in the traps. As mentioned earlier, *O. erosus* uses ipsdienol and MB as major pheromonal components (Seybold et al. 2006), and consequently, variation of ipsenol ratios in bioassay 3 did not affect catches. The same response to ipsenol has been reported earlier (Klimetzek and Vité 1986). Opposing results for *cis*-verbenol are found in the literature; Klimetzek and Vité (1986) reported a positive influence on catches, whereas Paiva et al. (1988) showed inhibitory effects. Our results are inconclusive, as no differences were found in bioassays 3 or 4, except for myrtenol alone which caught almost no beetles (table 3). As for *H. ligniperda* Fabricius, this secondary beetle is generally attracted to host semiochemicals but has also been shown to be attracted to those of bark beetles (Reay and Walsh 2002; Etxebeste and Pajares 2011).

Bark beetle predators, on the other hand, show clear preference for certain semiochemicals. *Temnochila caerulea* was captured in high numbers during bioassay 1, and the binary blend of ipsdienol and *cis*-verbenol caught significantly more than any other treatment, pointing to *cis*-verbenol as a strong kairomone (table 3). North American *Temnochila* species have been shown to be attracted to verbenols (Billings and Cameron 1984), and *T. caerulea* has been trapped earlier with bark beetle kairomones (Pajares et al. 2004), though not in the same amounts.

 

Similarly, numbers of *T. formicarius* caught in treatment Id + Ie were significantly higher on bioassay 1, revealing a synergistic effect between these two compounds. Similar responses have been described earlier (Bakke and Kvamme 1981; Schlyter et al. 1987b). Owing to the relevant predation that *T. caerulea* and *T. formicarius* exert on *I. sexdentatus* (Pajares et al. 2008), their bycatch in such high numbers should be seriously considered within the context of integrated pest management programs.

Taken together, the results on the present work help establish a reference functional aggregation lure for *I. sexdentatus*, improving our knowledge on the behavioural effects of some of the most common semiochemicals for *Ips* bark beetles. Such blend could help improve the operational monitoring and control of this important beetle. The development of an effective aggregation lure can also be integrated in other management strategies in combination with repellents, such as 'push-and-pull' (Cook et al. 2007), as inhibition of *I. sexdentatus* response has been reported for known repellents, such as verbenone or non-host volatiles (Jactel et al. 2001; Etxebeste and Pajares 2011). Future work should focus on refining the aggregation blend by determining pheromone compound ratios and optical isomers and testing and incorporating host signals to further increase the effectiveness of the lure, not just by total numbers, but also by reducing captured non-targeted species.

## Acknowledgements

We are to acknowledge the aid provided by a number of people, specially Ana B. Martín, and Luis Miguel from the Regional Forest Health Centre at Calabazanos (Palencia, Castile and Leon) and Dionisio Pozo and José Manuel Heras from the Department of Environment of the Regional Castile and Leon Government. This work has been financed by the Spanish Science and Education Ministry, within the AGL 2004-07507-C04-04 and AGL 2007-61152 research projects. The first author was supported by a scholarship within a fellowship between the University of Valladolid and the Department of Environment of the Castile and Leon Autonomous Government.

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