

Effect of varying monoterpene concentrations on the response of *Ips pini* (Coleoptera: Scolytidae) to its aggregation pheromone: implications for pest management and ecology of bark beetles

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- Abstract**
- 1 Host plant terpenes can influence attraction of conifer bark beetles to their aggregation pheromones: both synergistic and inhibitory compounds have been reported. However, we know little about how varying concentrations of individual monoterpenes affect responses.
 - 2 We tested a gradient of ratios of α -pinene, the predominant monoterpene in host pines in the Great Lakes region of North America, to *Ips pini*'s pheromone, racemic ipsdienol plus lanierone.
 - 3 *Ips pini* demonstrated a parabolic response, in which low concentrations of α -pinene had no effect on attraction to its pheromone, intermediate concentrations were synergistic and high concentrations were inhibitory. These results suggest optimal release rates for population monitoring and suppression programmes.
 - 4 Inhibition of bark beetle attraction to pheromones may be an important component of conifer defences. At terpene to pheromone ratios emulating emissions from trees actively responding to a first attack, arrival of flying beetles was low. This may constitute an additional defensive role of terpenes, which are also toxic to bark beetles at high concentrations.
 - 5 Reduced attraction to a low ratio of α -pinene to pheromone, as occurs when colonization densities become high and the tree's resin is largely depleted, might reflect a mechanism for preventing excessive crowding.
 - 6 *Thanasimus dubius*, the predominant predator of *I. pini*, was also attracted to ipsdienol plus lanierone, but its response differed from that of its prey. Attraction increased across all concentrations of α -pinene. This indicates that separate lures are needed to sample both predators and bark beetles effectively. It also provides an opportunity for maximizing pest removal while reducing adverse effects on beneficial species. This disparity further illustrates the complexity confronting natural enemies that track chemical signals to locate herbivores.

Keywords α -pinene, bark beetles, *Ips*, pheromones, predators, solid phase microextraction, terpenes, *Thanasimus*.

Introduction

Pheromones play important roles in the ecology of conifer bark beetles (Coleoptera: Scolytidae), and are therefore exploited widely for pest management (Wood, 1982;

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Borden, 1989). Attractive and repellent compounds have been identified for many of the most damaging species, and synthetic analogues have been deployed for population monitoring and suppression. Monitoring regimes use estimates of both bark beetles and their predators to forecast outbreaks (Billings, 1988; Grégoire *et al.*, 1992a, b), and pheromones therefore provide the most efficient means for detecting non-indigenous species (Hoebeke, 1994). Population suppression tactics include direct trap-out, disruption of dispersal and interference with host colonization (Borden, 1989).

Despite the identification and bioassay of numerous pheromone components, substantial variation occurs in field trials and implementation. This variation arises in part from complex ratios among pheromone mixtures, stereoisomers, synergists and host plant volatiles (Wood, 1982; Seybold *et al.*, 1995). A number of studies have demonstrated that host plant compounds, particularly monoterpenes, can either synergize or inhibit the attraction of bark beetles to their aggregation pheromones (Billings *et al.*, 1976; Miller & Borden, 1990, 2000; Erbilgin & Raffa, 2000). However, we currently know little about how various release rates of these semiochemicals affect beetle response (Miller & Borden, 1990, 2000). Relationships between host monoterpenes and host selection behaviour, for example, can be strongly dose dependent. Low concentrations can incite tunnelling, whereas high concentrations can be repellent or toxic (Coyne & Lott, 1961; Raffa & Smalley, 1995; Wallin & Raffa, 2000).

The pine engraver, *Ips pini* (Say), is distributed across North America. It preferentially colonizes weakened or recently killed trees, but can sometimes reach damaging levels on healthy trees (Schenk & Benjamin, 1969; Miller & Borden, 1990). In the Great Lakes region, *I. pini* colonizes native red pine, *Pinus resinosa* (Aitman), jack pine, *Pinus banksiana* Lamb, and white pine, *Pinus strobus* L. Each of these trees contains α -pinene as its predominant monoterpene: 78.1%, 75.2% and 70%, respectively (Erbilgin *et al.*, 2001). Monoterpenes have complex and dose-dependent functions in conifer-bark beetle-fungal interactions. For example, the concentrations present in induced reaction tissue can be toxic to adult beetles and their brood, and inhibitory to their associated fungi (Delorme & Lieutier, 1990; Raffa & Smalley, 1995). Monoterpene concentrations often decline in response to severe drought (Lorio *et al.*, 1995), root infection (Klepzig *et al.*, 1995), physical damage (Byers, 1989) or defoliation (Wallin & Raffa, 1999).

During attacks, adult males in this region produce racemic ipsdienol (2-methyl-6-methylene-2,7-octadien-4-ol) and lanierone (2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one) (Wood, 1982; Teale *et al.*, 1991; Seybold *et al.*, 1995), which attract flying beetles. Ipsdienol is attractive by itself, whereas lanierone is not attractive but synergizes response to ipsdienol in some geographical races. α -Pinene by itself is not attractive to *I. pini* (Erbilgin & Raffa, 2000). The mechanism by which aggregation is terminated is unknown. The most abundant predators associated with Midwestern *I. pini* are *Thanasimus dubius* (F.) (Coleoptera: Cleridae) and *Platysoma cylindrica* (Paykull) (Coleoptera: Histeridae). These insects are also attracted to *Ips* pheromones (Raffa, 1991; Aukema *et al.*, 2000).

The purpose of this study was to test how varying ratios of the host allelochemical, α -pinene, to the bark beetle pheromone racemic ipsdienol plus lanierone affect the arrival of flying *I. pini* and its predominant predators.

Materials and methods

Field sites and experimental design

Field experiments were conducted in 45–55-year-old *Pinus resinosa* plantations in Sauk County, Wisconsin, between

10 and 30 July 2001. We tested four ratios of α -pinene to ipsdienol plus lanierone, ipsdienol plus lanierone and a blank control. Each treatment was assigned to a multiple funnel trap (Lindgren, 1983) within a randomized block design. There were seven blocks, and each block contained six traps. The distance between treatments within each block was 10–15 m, and the distance between blocks was 200 m. No-pest strips (Pest STRIP, Loveland Industries, Inc., Greeley, CO) were placed in the collection jars to kill arriving insects and prevent predation. Arriving insects were sampled every 4 days, identified to species and tabulated. Treatments were re-randomized at each collection date to guard against spurious effects due to trap positions. There were five collection periods.

Release rates of monoterpenes

Pheromones and α -pinene release devices were obtained from Phero Tech, Inc. (Delta, British Columbia, Canada). The pheromones ipsdienol (chemical purity >98%, enantiomeric ratios: 50%(-):50%(+)) and lanierone (chemical purity >98%) were released from membrane release devices with bubble cap reservoirs at a rate of 110 and 100 $\mu\text{g}/\text{day}$, respectively, at 25 °C (Delta). α -Pinene (chemical purity >99%, enantiomeric ratios: 50%(-):50%(+)) was released from 1.8-mL or 15-mL polyethylene release devices at a rate of 1500 and 100 000 $\mu\text{g}/\text{day}$, respectively (Phero Tech, Delta). Because the available α -pinene release devices did not give us every ratio we desired, the release rates of some 15-mL release devices were adjusted by changing the exposed surface area. The desired ratios of α -pinene to pheromone were then achieved by deploying different numbers and combinations of 1.8-mL, unmodified 15-mL and modified 15-mL α -pinene release devices (Table 1). The release rates of some 15-mL α -pinene release devices were adjusted by covering 46% of the surface area of the release device with aluminium foil fastened with duct tape.

Release rates of α -pinene from unmodified and modified 15-mL release devices were confirmed using Solid Phase MicroExtraction (SPME; Supelco, Inc., Bellefonte, PA), followed by gas chromatography (GC) (Czerwinski *et al.*, 1996; Supelco, 1998). An α -pinene release device was placed in a glass jar (12 cm diameter \times 20 cm high) and sealed airtight with a lid containing a silicon rubber septum. After 5 min of equilibration, the α -pinene released into the headspace of the glass jar was sampled by adsorption onto the SPME fibre (fused silica fibre with a 100- μm polydimethylsiloxane coating) for 5 s. The α -pinene was desorbed by exposing the SPME fibre for 30 s in the heated injection port of the GC. The amount of α -pinene sampled was quantified by comparing these peak areas with standard equations obtained with known quantities of α -pinene (Powell & Raffa, 1999). Peak integrations were performed using Class-VP software (Shimadzu Scientific Instruments, Inc., Columbia, MD). The amount of α -pinene recovered from modified release devices was 50% of the unmodified devices. From these results, a release rate of 50 000 $\mu\text{g}/\text{day}$ from the modified devices was inferred.

Table 1 Release ratios of α -pinene to ipsdienol plus lanierone assayed for responses by *Ips pini* and predators

Treatment	Desired ratio (α -pin : ipsd)	No. of bottles ^a			Total α -pinene (μ g/day)	Ratio achieved ^c
		1.8 mL	15 mL	Covered ^b 15 mL		
1	Blank control	0	0	0	0	0
2	Ipsdienol alone	0	0	0	0	0 : 1
3	50 : 1	4	0	0	6000	54.6 : 1
4	500 : 1	4	0	1	56 000	509.1 : 1
5	1000 : 1	7	1	0	110 500	1004.6 : 1
6	5000 : 1	0	5	1	550 000	5000 : 1

^aRelease rates of ipsdienol and α -pinene from release devices given in the text.

^bTotal surface area = 40.27 cm²; bottom (4.16 cm²) and 40% (14.45 cm²) of vertical surface area covered.

^cDetermined by gas chromatography.

All analyses were performed on a Shimadzu GLC 17A fitted with an AOC 20i autosampler (Shimadzu Scientific Instruments), using a 25-m \times 0.25-mm bonded fused silica open tubular polyethylene glycol column (Alltech Assoc., Deerfield, IL), as described by Powell & Raffa (1999). The oven temperature was 60 °C for 10 min and increased to 160 °C at 10 °C/min increments. Helium was used as the carrier gas at 30 cm/s.

Statistical analyses

Data were evaluated by analysis of variance. Each variable was tested to satisfy assumptions of normality and homogeneity of variance (Zar, 1996) by graphical analysis of residuals (Neter *et al.*, 1983). We used a $1/\sqrt{x}$ transformation because the insect counts approximated the Poisson distribution, for which this transformation is most suitable (Steel & Torrie, 1980; Snedecor & Cochran, 1989), and resultant distributions satisfied assumptions in all cases. Dependent variables were analysed as a randomized block design, treating sites as blocks (Proc Mix, SAS Institute, 1996). Collection time was included as an additional blocking factor for all experiments. Block and trap within block were treated as random factors. A Protected LSD test was used for multiple comparisons of means. Data from the first sample were excluded because unfavourable weather reduced trap catches to just a few beetles.

Results

The phloeophagous and predacious insects obtained are shown in Table 2. These were dominated by *I. pini* and *T. dubius*, of which there were 1357 and 127, respectively. Abundances of other species were deemed too low for statistical analysis.

There were no significant time by treatment interactions for total *I. pini* ($F_{15,107} = 1.09$, $P = 0.37$), male *I. pini* ($F_{15,107} = 1.17$, $P = 0.31$), female *I. pini* ($F_{15,107} = 1.33$, $P = 0.19$) or *T. dubius* ($F_{15,107} = 0.49$, $P = 0.94$). Likewise, there were no sex by treatment interaction effects on the numbers of *I. pini* caught ($F_{6,107} = 1.12$, $P = 0.3336$), indicating male and female *I. pini* responded similarly to the same

treatments. The overall male to female ratio of *I. pini* was 53 : 47.

The ratio of α -pinene to ipsdienol plus lanierone had a significant effect on trap catches of both sexes of *I. pini* (Fig. 1) ($F_{5,107} = 47.15$, $P < 0.0001$ for male; $F_{5,107} = 39.36$, $P < 0.0001$ for female). All baited traps attracted significantly more *I. pini* than blank controls. The interaction with ipsdienol plus lanierone varied with the ratio of α -pinene to pheromones. Responses by *I. pini* to ipsdienol plus lanierone were synergized by the 510 : 1 ratio. By contrast, α -pinene released at 5001 : 1 reduced the attraction of male and female *I. pini* to ipsdienol plus lanierone by 2.89 and 3.44 times, respectively. The overall relationship was fit by a polynomial model ($y_1 = 275 + 0.0654x - 0.00002x^2$;

Table 2 Total numbers and species of insects caught in treatments containing different release ratios of α -pinene to pheromone of *Ips pini*, ipsdienol plus lanierone

Insect species	Total numbers of insects
Phoeophagous	
Scolytidae	
<i>Ips pini</i> (Say)	1357
<i>Ips grandicollis</i> (Eichhoff)	38
<i>Hylastes porculus</i> Erichson	20
<i>Gnathotrichus materiarius</i> Fitch	6
<i>Dendroctonus valens</i> LeConte	3
<i>Orthotomicus caelatus</i> Eichhoff	2
Cerambycidae	
<i>Xylotrechus sagittatus</i> (Germar)	20
<i>Monochamus carolinensis</i> (Olivier)	10
Predacious	
Cleridae	
<i>Thanasimus dubius</i> (F.)	127
<i>Enoclerus nigrifrons</i> (Say)	12
<i>Thanasimus undatulus</i> (Say)	3
<i>Zenodosus sanguineus</i> Say	1
Tenebrionidae	
<i>Corticeus parallelus</i> (Melsheimer)	43
<i>Tenebroides collaris</i> (Sturm)	33
Histeridae	
<i>Platysoma cylindrica</i> (Paykull)	5
<i>Platysoma parallelum</i> Say	3

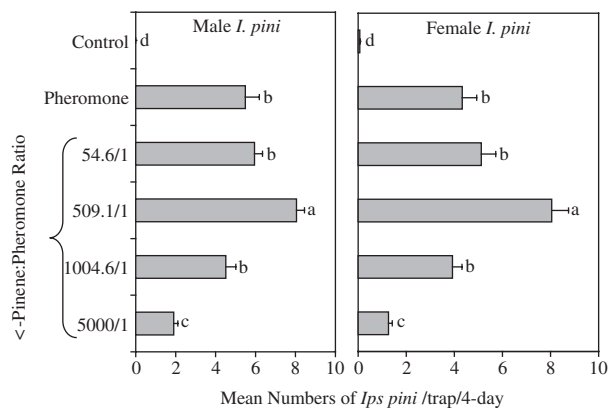


Figure 1 Responses of male and female *Ips pini* to various release ratios of racemic α -pinene and ipsdienol plus lanierone in red pine plantations in Wisconsin.

$R^2 = 0.57$), where y_1 is the total number of *I. pini* attracted and x is the ratio of monoterpene to pheromone.

Responses by *T. dubius* also varied with the ratio of host plant to insect-produced compounds ($F_{5,107} = 13.5$, $P < 0.0001$), but in a pattern different from *I. pini*. The number of *T. dubius* generally increased with increasing release rates of α -pinene, and was highest at traps baited with the highest release ratio of α -pinene to pheromone (Fig. 2). The overall relationship was fit by $y_2 = 5 + 0.0305x - 0.000004x^2$; $R^2 = 0.93$, where y_2 is the total number of *T. dubius* attracted and x is the ratio of monoterpene to pheromone.

Discussion

These results demonstrate that individual monoterpenes can both synergize and inhibit responses of bark beetles to their aggregation pheromones, depending on concentration. The optimal ratio tested for monitoring *I. pini* in the Great Lakes region was approximately 500:1 (Fig. 1). A ratio of 5000:1 was most effective for sampling predators (Fig. 2). For purposes of pheromonally based trap-out, 500:1 was optimal among tested treatments for removing *I. pini* while minimizing removal of predators. Presumably optimal ratios could vary with absolute amounts of pheromone, which can also affect attraction (Aukema & Raffa, 2000).

These results agree closely with previous work in Wisconsin in which an α -pinene to ipsdienol plus lanierone ratio of 2572 reduced attraction by approximately 44% (Erbilgin & Raffa, 2000). However, they differ from results in British Columbia, Canada, in which there was no relationship between α -pinene concentration and *I. pini* attraction to ipsdienol (without lanierone) (Miller & Borden, 2000). The latter study reported strong synergism by α -phellandrene, the predominant monoterpene in *I. pini*'s primary western host, *Pinus contorta* var. *latifolia* Engelm. Thus there appear to be regional, host species and/or signal-specific differences in these relationships.

Predators are known to exploit both bark beetle pheromones and host terpenes in their foraging behaviour (Mizell *et al.*, 1984; Billings, 1985; Miller & Borden, 1990;

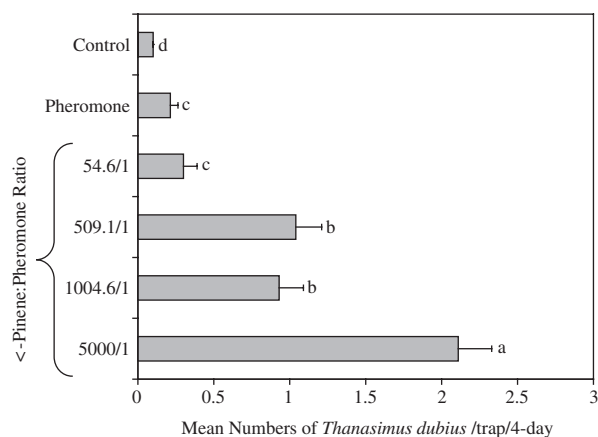


Figure 2 Responses by the predator, *Thanasisimus dubius*, to various release ratios of racemic α -pinene and ipsdienol plus lanierone in red pine plantations in Wisconsin.

Grégoire *et al.*, 1992a, b; Erbilgin & Raffa, 2001). Beetles appear to have adapted to these selection pressures by evolving modifications in their chemical signals, such as altered ratios of stereoisomers and synergists (Raffa & Klepzig, 1989; Herms *et al.*, 1991; Raffa, 1991; Raffa & Dahlsten, 1995; Aukema & Raffa, 2000). Our results suggest that differential responses to various mixtures of plant and insect volatiles add to the complexity of chemical signals that predators must track to exploit a continually coevolving prey (Figs 1, 2). However, we clearly need additional information on the chemical ecology of *T. dubius*, as its attraction to signals associated with low *I. pini* success seems paradoxical.

Field observations indicate that beetle entry into trees does not always elicit aggregation. The success of pioneer beetles can be as low as 50%, even during outbreaks (Raffa & Berryman, 1983). By contrast, beetles that initiate attacks as part of 'switching' from neighbouring trees undergoing the late stages of attack are almost always successful (Geiszler *et al.*, 1980; Raffa & Berryman, 1983). This suggests trees may be able to inhibit communication while the number of entering beetles is still very low (Raffa, 2001). The underlying mechanisms could include physical obstruction of volatiles by gummy resins, an absence of attractive kairomones, the presence of compounds that inhibit attraction to pheromones such as those emitted by non-host tree species (Dickens *et al.*, 1992; Borden *et al.*, 1998; Byers *et al.*, 1998) or high ratios of host monoterpenes to beetle pheromones. Our best estimate of the ratio of monoterpenes to pheromones emitted from the first attacking beetle is approximately 2581, based on various reports in the literature (Ikeda *et al.*, 1980; Byers *et al.*, 1985; Raffa & Smalley, 1995; Erbilgin & Raffa, 2000). Based on Fig. 1, this mixture is moderately attractive relative to blank controls, but less attractive than pheromone alone. If additional males arrive rapidly, their total emissions would increase and successful aggregation could occur. However, if induced reactions have time to proceed, such as when a heavy flow of constitutive resin delays beetle progress, ratios of monoterpene

to pheromone can rise rapidly. For example, the 10 times increase within 3 days in *P. resinosa* (Raffa & Smalley, 1995) would raise the monoterpene to pheromone ratio to over 25 000, which is nearly completely inhibitory based on the equation derived from Fig. 1. Thus, inhibition of attraction to aggregation pheromones may be an important component of integrated host defences against bark beetles. The resulting selective pressures could explain in part why beetles avoid trees with high monoterpene concentrations (Wallin & Raffa, 2000), and why simultaneous 'switching' to neighbouring trees is advantageous (Geiszler *et al.*, 1980; Raffa & Berryman, 1983).

A dose-dependent distinction between the allomonal and kairomonal roles of monoterpenes is consistent with observations that high resin flow is associated with both a decreased likelihood and delayed onset of aggregation, and also increased arrival rates if and when aggregation can be elicited (Raffa & Berryman, 1983). Tree interference with beetle communication also has implications for the experimental design of host resistance studies. For example, artificial elicitation of mass attack is a useful tool for attracting enough beetles to challenge resultant tree responses, but may have the limitation of circumventing an important component of integrated conifer defences.

Variable responses to rapidly changing monoterpene to pheromone ratios during colonization may also prevent overcrowding (Anderbrandt *et al.*, 1985; Robins & Reid, 1997). *Ips pini* is not known to employ an anti-aggregation pheromone, so reduced attraction to very low monoterpene to pheromone ratios (Fig. 1), such as would occur once tree defences are exhausted, may contribute to the termination of aggregation.

The context-specific role of individual monoterpenes, in which they function as allomones inhibiting attraction to pheromones at high concentrations, but are amenable to exploitation as kairomones at low concentrations, probably prevails among other bark beetle species. We hypothesize that various species display unique response profiles, and that these relate to the physiological condition of trees they typically colonize. For example, those species typically associated with healthy trees may demonstrate synergism at monoterpene to pheromone ratios high enough to inhibit attraction to pheromones in species associated with stressed trees. Exploring such relationships could contribute to a more mechanistic understanding of what is generally termed 'aggressiveness'. Contextual roles of host compounds may also provide a basis for intraspecific plasticity associated with population phase, as proposed by Birgersson *et al.* (1988). For example endemic-phase beetles may show response profiles similar to those of less 'aggressive' species, but eruptive populations may have shifted response profiles as a mechanism for exploiting more well-defended hosts. We are currently testing these predictions.

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