BEHAVIORAL ECOLOGY

Can chemical communication be cryptic? Adaptations by herbivores to natural enemies exploiting prey semiochemistry

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Abstract Predators and parasites commonly use chemical cues associated with herbivore feeding and reproduction to locate prey. However, we currently know little about mechanisms by which herbivores may avoid such natural enemies. Pheromones are crucial to many aspects of herbivore life history, so radical alterations of these compounds could be disadvantageous despite their exploitation by predators. Instead, minor modifications in pheromone chemistry may facilitate partial escape while maintaining intraspecific functionality. We tested this hypothesis using *Ips pini*, an endophytic beetle that develops in the phloem tissue of pine trees. Its predominant predators in the Great Lakes region of North America are *Thanasimus dubius* and *Platysoma cylindrica*, both of which are highly attracted to *I. pini's* pheromones. However, there are significant disparities between prey and predator behaviors that relate to nuances of pheromone chemistry. *Thanasimus dubius* is most attracted to the (+) stereoisomer of ipsdienol, and *P. cylindrica* is most attracted to the (−) form; *Ips pini* prefers racemic mixtures intermediate between each predator's preferences. Further, a component that is inactive by itself, lanierone, greatly synergizes the attraction of *I. pini* to ipsdienol, but has a weak or no effect on its predators. A temporal component adds to this behavioral disparity: lanierone is most important in the communication of *I. pini* during periods when its predators are most abundant. The difficulties involved in tracking prey are further compounded by spatial and temporal variation in prey signaling on a local scale. For example, the preferences of *I. pini* vary significantly among sites only 50 km apart. This chemical crypsis is analogous to morphological forms of camouflage, such as color and mimicry, that are widely recognized as evasive adaptations against visually searching predators. Presumably these relationships are dynamic, with predators and prey shifting responses in microevolutionary time. However, several factors may delay predator counter adaptations. The most important appears to be the availability of alternate prey, specifically *I. grandicollis*, whose pheromone ipsenol is highly attractive to the above predators but not cross-attractive with *I. pini*. Consistent with this view, the specialist parasitoid, *Tomicobia tibialis*, has behavioral preferences for pheromone components that closely correspond with those of *I. pini*. These results are discussed in terms of population dynamics and coevolutionary theory.

Keywords Predator–prey · Coevolution · Kairomones · Bark beetles · Pheromones

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Introduction

In recent years there has been a tremendous increase in our understanding of how parasites and predators exploit chemical signals associated with herbivore feeding and reproduction to locate prey (Turlings et al. 1995; Zuk and Kolluru 1998; Haynes and Yeargan 1999; Thaler 1999; De Boer and Dicke 2004). By responding to cues indicative of herbivore metabolism, communication, and feeding, natural enemies can rapidly orient to suitable habitats and hosts. Chemically mediated attraction is especially important to natural enemies seeking cryptic prey such as endophytic insects. Insect herbivores are known to possess a variety of adaptations that inhibit their capture, such as morphological camouflage, chemical defense, behavioral resistance, and evasion (Stamp and Bowers 1993; Rowellrahier et al. 1995; Karban and English-Loeb 1997; Hunter 2000). However, the extent and mechanisms by which prey might avoid detection by predators exploiting kairomonal signals are poorly understood (Mathis et al. 1995; Hare and Morgan 2000; Peckarsky et al. 2002; De Moraes and Mescher 2004). Likewise, it is uncertain whether semiochemical adaptations would confer costs to prey, similar to those incurred by physical avoidance and suboptimal host utilization patterns (Ball and Baker 1996; Bjorkman et al. 1997; Losey and Denno 1998; Lill and Marquis 2001; Stamp 2001).

Bark beetles (Curculionidae: Scolytinae) provide a useful system for exploring ecological and evolutionary implications of semiochemical interactions among predators and prey. They spend all but a brief portion of their life cycle within the subcortical tissues of host plants, and rely on chemical signaling for a variety of life history and ecological processes, including mating, host plant procurement, and niche partitioning (Wood 1982). Bark beetle pheromones are typically complex, multicomponent, and relatively species-specific signals (Seybold et al. 1995). These beetles are attacked by a relatively narrow group of predators that can be highly effective at exploiting beetle pheromones. The predator guilds of most bark beetles are typically dominated by just a few species (Amman 1984; Schroeder and Weslien 1994; Reeve 1997; Dahlsten et al. 2004).

Most bark beetles are limited to severely stressed trees, but several can also colonize healthy hosts. These can be major agents of tree mortality, and can have important ecological roles in shaping canopy structure, gap formation, succession, and fire cycles (Romme et al. 1986). Adult beetles emerge from brood trees, locate susceptible hosts, and bore through the outer bark. As beetles tunnel through the bark they produce pheromones that attract mates. They mate under the bark and oviposit in galleries that extend from the mating chamber. The progeny complete development

within the phloem and inner bark. Beetles typically develop within a host for only one generation, so the emerging brood adults must repeat the process. Because bark beetles develop within conductive tissue, colonization of the main stem is usually lethal to the host. This has selected for complex constitutive and inducible plant defenses against bark beetle attack (Franceschi et al. 2005; Raffa 1991). In general, solitary beetles cannot avoid, tolerate or overcome the concentrations of toxins produced by healthy trees. Rather, their pheromones rapidly concentrate high densities of both sexes, thereby exhausting the tree's defensive capacity within a few days (Wood 1982). These pheromones are closely linked to tree physiology, as they are converted from and or synergized by host monoterpenes (Miller and Borden 2000; Seybold et al. 2006). Where several sympatric species exploit the same host, the specificity of pheromones can serve as a barrier against exploitation by competitors and help structure niche partitioning (Schlyter and Anderbrandt 1993; Ayres et al. 2001).

Predators can strongly influence the survival and population dynamics of bark beetles. Turchin et al. (1999) observed evidence for delayed density-dependent regulation of southern pine beetle, *Dendroctonus frontalis* Zimm., and concluded that these patterns were most consistent with higher trophic level responses. Others have reported high mortality by natural enemies under field conditions (Amman 1984), and increased proportionate mortality at high prey densities (Reeve 1997). Field exclusion and laboratory amending experiments provide additional evidence of high natural enemy impacts on within-tree brood production (Linit and Stephen 1983; Schroeder and Weslien 1994; Aukema and Raffa 2002), and density-dependent numerical and functional responses have been described in the field (Reeve 1997). The life histories of parasites of bark beetles are fairly well known, but there is less evidence they affect reproductive success or population dynamics (Linit and Stephen 1983; Sullivan and Berisford 2004).

Numerous studies have demonstrated that predators are highly attracted to bark beetles' aggregation pheromones (Wood 1982; Herms et al. 1991). In some cases their attraction may be even stronger than by the bark beetles themselves (Aukema et al. 2000). However, it is not clear what possible counter-options are available to avoid such detection. The complex and multifunctional nature of bark beetle pheromones (e.g., mate procurement, host procurement, and resource partitioning) may pose severe restrictions on the potential for major semiochemical alterations.

We posited that minor alterations in pheromone stereochemistry and secondary components may provide viable options for partially avoiding predators that exploit chemical cues. Previous experiments provide some support for this view (Raffa and Klepzig 1989; Herms et al. 1991; Aukema et al. 2000), but these studies were either short-term, focused

on a single predator, focused on a single feature of phero-mone chemistry, or conducted over a small geographic scale. In the current study we evaluated the responses of all natural enemies and bark beetles responding to mixtures of stereoisomeric ratios of pheromone and synergistic components over three years at five widely separated sites. We asked:

(a) Do the preferences of the predominant predators for specific combinations of pheromone components coincide with or deviate from those of their prey?

(b) To what extent is herbivore preference for pheromone components uniform, and to what extent does it vary with space and time within a region?

(c) To what extent do semiochemical alterations represent an avenue for escape from generalist versus specialist natural enemies?

Methods

Description of model system

The pine engraver, *Ips pini* (Say), is the most abundant tree-killing bark beetle in the Great Lakes region (Erbilgin et al. 2002). Males initiate attacks and each is joined by several females. The pine engraver's principal pheromone component is ipsdienol, 2-methyl-6-methylene-2,7-octadien-4-ol. Geographically isolated populations vary markedly in their stereochemistry. For example, California and Idaho populations are attracted to *R*-(−)-ipsdienol, while New York populations are most strongly attracted to a 50% (−):50% (+) blend (Seybold et al. 1995). Eastern populations also produce lanierone (2-hydroxy-4,4,6-trimethyl-2,5-cyclo-hexadien-1-one), which synergizes attraction to ipsdienol but is not attractive by itself (Teale et al. 1991). Western populations do not produce lanierone in substantial quantities. Despite these differences, laboratory hybridization experiments and molecular analyses support the single species status of *I. pini* (Seybold et al. 1995; Cognato et al. 1997).

The predominant predators of *I. pini* in Wisconsin are *Thanasimus dubius* (F.) (Cleridae) and two closely related histerids *Platysoma cylindrica* (Paykull) and *P. parallelum* Say (Raffa and Klepzig 1989; Herms et al. 1991; Aukema et al. 2000; Erbilgin et al. 2002). These predators feed on both adult bark beetles, as adults during the prey's colonization phase, and on bark beetle eggs, larvae, and pupae, as larvae after the responding adults oviposit under the bark. Each of these predators is highly attracted to pine engravers boring into host tissue and to synthetic ipsdienol (Aukema et al. 2000). Analyses of field populations indicate strong density-dependent relationships (Erbilgin et al. 2002), and

laboratory assays indicate strong effects on beetle survival and reproduction (Aukema and Raffa 2002). Further, each predator species exerts equivalent effects on beetle reproduction, and there are no nonadditive interference or augmentation effects (Aukema et al. 2004). These predators can be considered habitat generalists, in that they develop exclusively within trees killed by bark beetles, but feed on several species of primary colonizers and secondary opportunists in such trees (Erbilgin et al. 2002). In Wisconsin, their main alternate prey is *I. grandicollis* Eichoff, which produces ipsenol 2-methyl-6-methylene-7-octen-4-ol) (50% (+)/50% (−)), and to a lesser extent *I. perroti* Swaine which produces a blend of ipsdienol plus ipsenol (Ayres et al. 1999).

Bioassays: responses of bark beetles and associates to pheromone components of bark beetles

We tested the response of *I. pini* and its associates to six combinations of ipsdienol and lanierone, ipsenol, and a blank control over a three-year period (1995–1997) at five 30–50-year-old red pine *Pinus resinosa* Ait. sites in southern and central Wisconsin. In 1995, the treatments were three ratios of (+)/(−) ipsdienol, each with either high or trace amounts of lanierone, to simulate the possible range of signals arising from a population of variable individuals (Miller et al. 1989). In 1996 and 1997, we included two additional treatments, ipsenol and blank controls. We deployed pheromone baits in "bubble cap" slow release devices from PheroTech Inc. (Delta, BC, Canada). Release rates (25 °C) for lanierone were 0.2 mg/day (high) or 0.0002 mg/day (trace). Release rates of ipsdienol and ipsenol were 0.135 and 0.240 mg/day.

Insects were sampled using 12-funnel flight traps. Treatments were deployed in a hierarchically nested design, with 16 blocks nested among five sites each year. Blocks within a site were each greater than 200 m apart. Each block contained a full array of lures. Traps within a block were arranged in a circle with roughly 4–5 m between each trap. Sites ranged from 13 to 108 km from each other, with a mean intersite distance of 66 km. Traps were sampled at roughly weekly intervals throughout the flight period of *I. pini* (approximately late April to late September with some variation by year). We re-randomized treatments within each block after each collection of insects. Our experimental unit was the individual trap collection on a particular date. Insects were identified to species, and *I. pini* were identified to sex.

Statistical analyses

The number of insects captured in each sample period and each trap were analyzed using mixed effects ANOVA models.

Pheromone treatments, site, season (spring vs. summer), and year were included as fixed effects, while random effects included samples nested within blocks. Assumptions of normality and homogeneity of variances were checked with residual plots. Statistical analysis of pheromone trap catch data can be impeded by large numbers of zeroes within control samples, increasing heteroscedasticity. Data transformations (square root or logarithmic) were performed where necessary to reduce heteroscedasticity. Further, we follow the recommendation of Reeve and Strom (2004), and first test whether each insect was attracted to ipsdienol relative to blanks, and subsequently proceed to analyses containing only the six ipsdienol combinations. Means comparisons among pheromone treatments were performed using protected t tests ($P = 0.05$) and Bonferronni corrections. We limit these analyses to species caught in adequate numbers, i.e., at least an average across all treatments of 1.5 insects per trap per sampling period. Because of trends in seasonal abundance (see below), spring and summer samples were analyzed separately. All data analysis was performed in R (Ihaka and Gentleman 1996; R Development Core Team 2006).

Results

Bark beetles, predators, and parasites responding to semiochemicals

The most abundant herbivore was *I. pini*, of which over 160,000 were obtained (Table 1). This insect was widely distributed across all sites and years. Approximately 6,000 *I. grandicollis* were also obtained, and like *I. pini*, were widely distributed in space and time. *Ips perroti* was obtained less commonly, with the highest populations in two of the northern sites, Lake Delton and Marquette.

The most abundant predators were the clerid beetle *T. dubius*, of which nearly 9,000 were obtained, and two closely related histerid beetles, *P. cylindrica* and *P. parallelum*, with a combined total of over 12,000 (Table 1). Each of these predators was widely distributed across all sites and years. Three additional clerid beetles were obtained less commonly, representing less than 6% of all predators. Approximately a third of *Thanasimus undulatus* (Say) were obtained at one site, Marquette, 87% of *Enoclerus nigripes* (Say) were obtained at the three northern sites, and *Enoclerus nigrifrons* (Say) were more uniformly distributed. The only moderately abundant parasitoid obtained was the specialist wasp, *Tomicobia tibialis* Ashmead (Pteromalidae), which attacks adults. Its abundance was substantially below that of the major predators (Table 1). Its highest populations were at Lake Delton and Marquette.

Attraction relative to blank controls, and absolute trap catch values, are shown in Table 2. *Ips pini* was significantly

Table 1 Summary of insects sampled in multiple-funnel traps in five sites over three years in Wisconsin

Insect		Total
Herbivores		
Curculionidae: Scolytinae		
Ips pini	160,933	
Male	70,311	
Female	85,952	
Not sexed	4,670	
Ips grandicollis	5,953	
Ips perroti	2,804	
Total *Ips spp.*		169,690
Predators		
Cleridae		
Thanasimus dubius	8,775	
Thanasimus undulatus	342	
Enoclerus nigripes	403	
Enoclerus nigrifrons	534	
Histeridae		
Platysoma cylindrical	6,754	
Platysoma parallelum	5,454	
Total Cleridae and Histeridae		10,054
Parasitoids		
Pteromalidae		
Tomicobia tibialis	682	682

attracted to all combinations of ipsdienol, except 70% (−) with trace lanierone. Even the latter treatment approached significance, with $P < 0.036$ prior to correction for multiple comparisons, and an overall mean $>10\times$ that in controls. The ratio of *I. pini* per trap with ipsdienol (among all six treatments) to control trap was 79. *Ips grandicollis* was not attracted to any form of ipsdienol, with or without lanierone. *Ips perroti* was predominantly attracted to 70% (−) ipsdienol, being significant with high lanierone and approaching significance (e.g., $26\times$ that of controls in 1996) with low lanierone. The most abundant predator, *T. dubius*, was highly attracted to ipsdienol, including all combinations of chirality and lanierone content. The ratio of *T. dubius* per trap with ipsdienol (among all six treatments) to controls was 23. Similarly, the predators *P. cylindrica* and *P. parallelum* were highly attracted to ipsdienol, and to most combinations of chirality and lanierone content. The ratio of *Platysoma spp.* per trap with ipsdienol to controls was 38.

Behavioral preferences of *Ips pini* and major predators with respect to enantiomeric ratios of ipsdienol and lanierone

The most pronounced overall pattern was that even though each major predator was attracted to the pheromones of

Table 2 Effect of ipsdienol lanierone content and chirality on trap catches of bark beetles and predators; in each case the mean (SEM) is reported along with the associated t values and P values in comparison to the blank controls beneath

Insect	Blank Control	High lanierone (0.2 mg per day)			Trace lanierone (0.0002 mg per day)		
		10% (−) ipsd	40% (−) ipsd	70% (−) ipsd	10% (−) ipsd	40% (−) ipsd	70% (−) ipsd
Spring							
Ips pini	0.165 (0.42)	29.999 (0.42)	51.156 (0.42)	38.997 (0.42)	3.656 (0.42)	5.185 (0.52)	2.323 (0.42)
t		6.59***	7.63***	7.10***	2.78*	3.35**	2.10 (NS)
Ips grandicollis	0.118 (0.08)	0.071 (0.07)	0.087 (0.07)	0.052 (0.07)	0.071 (0.07)	0.107 (0.07)	0.115 (0.07)
t		0.44 (NS)	0.29 (NS)	0.61 (NS)	0.43 (NS)	0.10 (NS)	0.02 (NS)
Ips perroti	0.004 (0.1)	0.176 (0.09)	0.268 (0.09)	0.560 (0.09)	0.058 (0.09)	0.073 (0.09)	0.199 (0.09)
t		1.24 (NS)	1.83 (NS)	3.47**	0.41 (NS)	0.52 (NS)	1.40 (NS)
Thanasimus dubius	0.071 (0.142)	3.508 (0.13)	2.533 (0.13)	1.925 (0.13)	2.678 (0.13)	2.332 (0.13)	1.505 (0.13)
t		7.89***	6.55***	5.51***	6.77***	6.23***	4.67***
Platysoma cylindrica	0.000 (0.27)	0.554 (0.27)	1.863 (0.27)	2.679 (0.27)	0.490 (0.27)	1.708 (0.27)	2.494 (0.27)
t		1.32 (NS)	3.12*	3.86***	1.20 (NS)	2.96*	3.71**
Platysoma parallelum	0.040 (0.130)	0.980 (0.122)	1.766 (0.122)	2.220 (0.122)	0.892 (0.122)	1.550 (0.122)	2.166 (0.122)
t		3.84***	5.82***	6.73***	3.57**	5.34***	6.63***
Summer							
Ips pini	0.357 (0.46)	41.991 (0.45)	86.308 (0.45)	80.193 (0.45)	11.696 (0.45)	17.123 (0.45)	9.531 (0.46)
t		6.53***	7.87***	7.73***	4.23***	4.90***	3.87***
Ips grandicollis	0.031 (0.04)	0.020 (0.04)	0.019 (0.04)	0.021 (0.042)	0.031 (0.04)	0.049 (0.04)	0.052 (0.04)
t		0.18 (NS)	0.22 (NS)	0.16 (NS)	0.004 (NS)	0.28 (NS)	0.33 (NS)
Ips perroti	0.051 (0.99)	0.257 (0.10)	0.342 (0.10)	0.615 (0.10)	0.150 (0.10)	0.202 (0.10)	0.286 (0.10)
t		1.35 (NS)	1.85 (NS)	3.25**	0.68 (NS)	1.02 (NS)	0.98 (NS)
Thanasimus dubius	0.036 (0.09)	1.004 (0.09)	0.813 (0.09)	0.655 (0.09)	0.895 (0.09)	0.824 (0.09)	0.663 (0.09)
t (p)		5.20 (<0.0001)	4.41 (<0.0001)	3.69 (0.01)	4.76 (<0.0001)	4.46 (<0.0001)	3.73 (0.01)
Platysoma cylindrica	0.000 (0.11)	0.136 (0.11)	0.422 (0.11)	0.643 (0.11)	0.156 (0.11)	0.482 (0.11)	0.659 (0.11)
t		0.86 (NS)	2.36 (NS)	3.23**	0.97 (NS)	2.64 (NS)	3.39**
Platysoma parallelum	0.037 (0.06)	0.126 (0.06)		0.299 (0.06)	0.139 (0.06)	0.213 (0.06)	0.317 (0.06)
t		1.00 (NS)	2.12 (NS)	0.235 (0.06) 2.74*	1.14 (NS)	1.905 (NS)	2.91*

Data corrected by Bonferroni method. Insects with low trap numbers (<1.5 per trap per sample) excluded

Spring $df = 1{,}296$ except $1{,}292$ for *I. pini* and $1{,}293$ for *I. grandicollis* and *I. perroti*. Summer $df = 1{,}201$ except $1{,}196$ for *I. pini*, *I. grandicollis*, and *I. perroti*

P values indicated as follows: NS = $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

I. pini, specific features of their attraction differed markedly from those of their prey (Fig. 1). *Ips pini* preferred 40% (−):60% (+) ipsdienol over the more (−) or (+) blends, and its attraction was strongly synergized by lanierone. The extent of this synergism was highest at the optimal chiral ratio, by a factor of approximately 7.2. Like *I. pini*, the predominant predators, *P. cylindrica*, *P. parallelum*, and *T. dubius* were highly attracted to ipsdienol (Table 2). However, their preferences differed from *I. pini* and from each other. The two *Platysoma* species (individually and pooled) preferred highly (−) ipsdienol, whereas *T. dubius* preferred the more (+) ipsdienol (Fig. 1). In addition, all of these predators were either unresponsive or weakly responsive to lanierone. *Platysoma cylindrica* and *P. parallelum* were not affected by lanierone content, and *T. dubius*

showed a statistically significant but biologically weak response, with an overall synergism ratio (total trap catch at high/trace lanierone composition at all three enantiomeric ratios) of only 1.2. Only 17% of *T. dubius*, 18.5% of *P. cylindrica*, and 18.3% of *P. parallelum* were attracted to the most favored pheromone composition of *I. pini*. In contrast to the more generalist predators, attraction by the specialist parasitoid *T. tibialis* closely mirrored that of its host. These wasps showed strong preferences for 40% (−):60% (+) ipsdienol, high lanierone, and their interaction, as did *I. pini*. Overall 38.3% of the *T. tibialis* and 37.3% of the *I. pini* were attracted to this preferred combination. This overall pattern among herbivores, predators, and parasites was consistent across years (data not shown to conserve space, but available upon request). There was some exception in 1997,
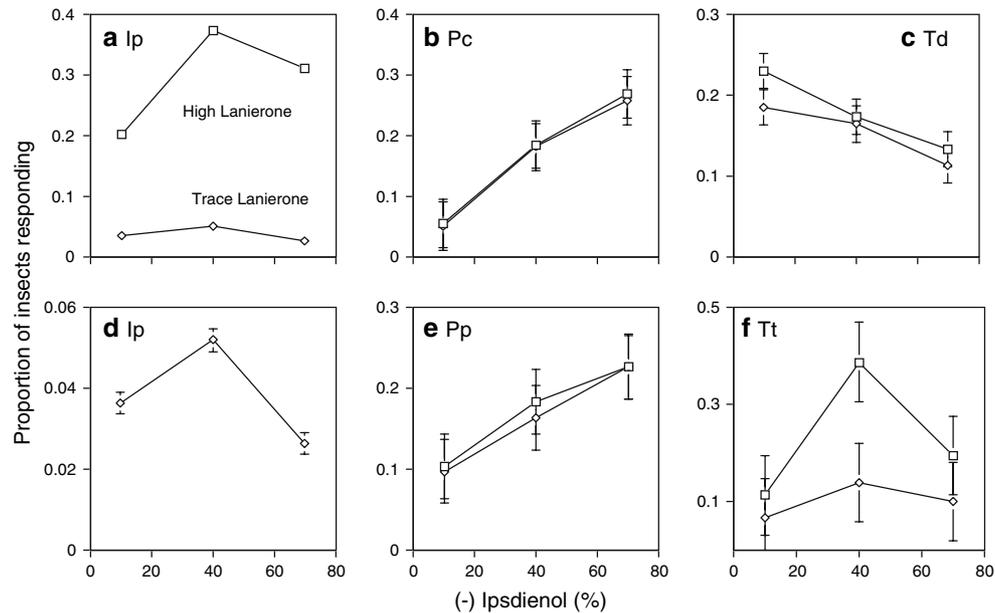
Fig. 1a–f Effects of pheromone stereochemistry and synergistic components [high lanierone (*squares*) and trace lanierone (*diamonds*)] on attraction by (**a**, **d**) the herbivore *Ips pini* (Ip) and its predominant natural enemies to ipsdienol [mean (−) enantiomer ± 1 SEM, data pooled over three years]. The natural enemies include three predacious beetles, **b** *Platysoma cylindrica* (Pc), **c** *Thanasimus dubius* (Td),

e *Platysoma parallelum* (Pp), and **f** one specialist parasitic wasp *Tomicobia tibialis* (Tt). **d** A magnification of the response of *I. pini* to (−) ipsdienol with only trace lanierone is shown to better illustrate the effect of sterochemistry. *Error bars* are present for all *I. pini*, but are not always visible due to the large sample sizes (see Table 1)

but those results should be used with caution, as they include the lowest numbers: 6% of *I. pini*, 9% of *T. dubius*, 4% of *P. cylindrica* and 16% of *P. parallelum*, and only a total of 3 *T. tibialis*.

Full statistical analyses are shown in Appendix 1. All of the predominant species displayed strong preferences for particular stereoisomeric ratios of ipsdienol, as indicated by significant ipsdienol chirality effects (Appendix 1). There was a lanierone effect for *I. pini*, *T. dubius*, *T. tibialis*, *Ips pini*, and *T. tibialis*, none of the predators showed an ipsdienol × lanierone interaction. Additional interactions are discussed below.

The two other herbivores differed from each other, and from *I. pini* (Table 2). *Ips perroti* varied due to ipsdienol chirality, lanierone content, and their interaction. *Ips grandicollis* varied with season and lanierone. Mean numbers of *I. grandicollis* at a particular ipsdienol chirality were always lower with high lanierone present, with an overall synergism ratio of 0.69, suggesting inhibition. Mean numbers of *I. perroti* at a particular enantiomeric ratio of ipsdienol were always higher with high lanierone present, with an overall synergism ratio of 2.5.

Male and female *I. pini* showed similar responses to the various pheromone components. The rank orders of preference, 40, 70, and 10% (−) ipsdienol with high lanierone, followed by 40, 10, and 70% (−) with trace lanierone, were the same for males and females. Comparisons between treatments yielded similar results for males and females; of

30 combinations between treatments (analyzed separately for spring and summer), there were only three instances where the significance between them was not the same for males as for females.

Variation of behavior, abundance, and their interactions in space and time

Despite the consistency of the overall pattern, there was substantial quantitative variation in behavioral preferences across space and time. In particular, there were several strong temporal patterns associated with portions of the flight period within years. The synergism of the attraction of *I. pini* to ipsdienol by lanierone differed markedly between spring and summer, as manifested by a strong lanierone by season interaction (Appendix 1). Overall, ipsdienol synergism by lanierone was 7.7 times as high in spring as in summer (Fig. 2a). This change occurred in all sites and in all years. High synergism occurs when the major predators are most abundant, spring (Fig. 2b; Table 1). In contrast to these univoltine species, *I. pini* is more evenly distributed, with the majority present in the summer as numbers build over successive generations. Among the other natural enemies, most *T. undatulus* (72%), *E. nigripes* (71%), and *T. tibialis* (61%) were trapped in spring, with the only exception being *E. nigrifrons*, which was most active in summer (97%).
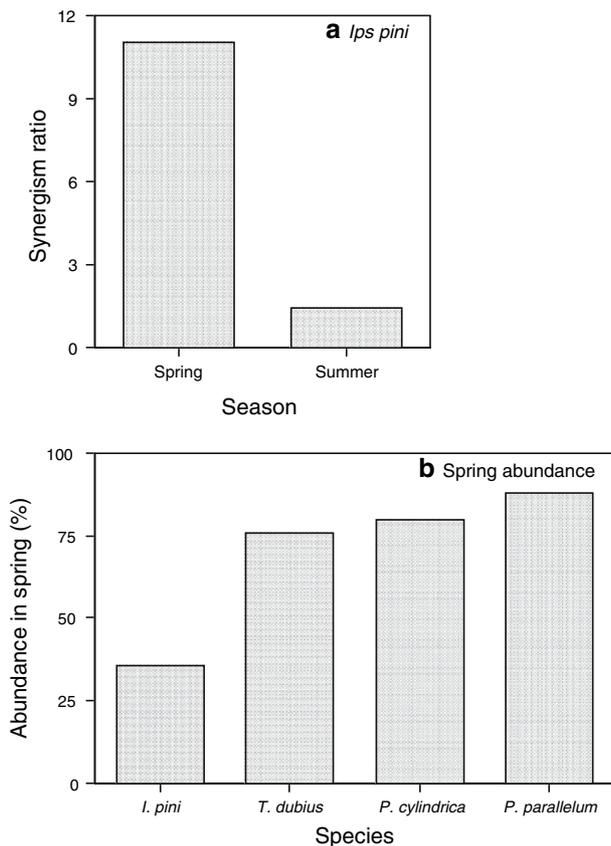
Fig. 2a–b a Seasonal variation in synergism of the attraction of the herbivore *I. pini* to ipsdienol by lanierone. The synergism ratio is the ratio of ipsdienol treatments with high lanierone to ipsdienol treatments with trace lanierone. Predators show no or little response to lanierone (see Fig. 1). **b** Seasonal abundance of *I. pini* and its three predominant predators in spring, 1995–1997, where abundance of spring plus summer for each insect sums to 100%

Behavioral responses to the various stereoisomers of ipsdienol also varied seasonally (Appendix 1). However, these were less pronounced. Mean chiral preference by *I. pini*, expressed as $\{[(\text{no. beetles} \times 0.10) + (\text{no. beetles} \times 0.40) + (\text{no. beetles} \times 0.70)]/3\}$, increased 10% from spring to summer.

There was also site-to-site variation in the behavior of *I. pini*. Due to the complex relationships identified in Appendix 1, we focus on one year for the purposes of presentation, using 1996, when the highest numbers were trapped. Again, the responses by *I. pini* were complex (Appendix 2). There were site effects, interactions of pheromone chemistry with site and season, and a site × ipsdienol × lanierone interaction. The result is a mosaic of signaling in space and time (Table 3; Appendix 2, 3). For example, synergism in Marquette was 30% higher than in Necedah, only 64 km away. Likewise, the decline in synergism ratio from spring to summer was nearly 50% higher in Spring Green and Arena than in Lake Delton and Marquette, 76 km away. The increase in mean chiral preference that

occurred from spring to summer varied from 3% in Lake Delton to 18% in Necedah, a distance of only 50 km.

The major predators also showed some season by chemical interactions (Appendix 1). However, these must be treated with caution owing to large differences in absolute numbers between spring and summer, and the relatively weak biological differences in their responses between these periods (Table 3). There was significant site-to-site variation in absolute numbers of predators, but there were relatively few instances of site by chemical preference interaction (Appendix 1). The synergism ratio (spring) by *T. dubius* varied among sites, being 50% higher in Lake Delton than in Necedah, but again this must be viewed within the context that all synergized attraction by this insect is weak. There was no relationship between the synergism ratio (spring or summer) of *T. dubius* and *I. pini* across sites. Responses by the major predators to various enantiomers of ipsdienol showed significant variation (Appendix 1), but they were relatively weak (Table 3).

Ips pini showed significant year to year variation in their degree of preference for various stereoisomeric ratios of ipsdienol, and in the extent of synergism by lanierone (Appendix 1). These were mostly subtle differences in the magnitude of the preference, as the overall directions remained relatively constant. Likewise, the major predators showed some statistically significant year by chemistry interactions (Appendix 1), but these were largely in degree not direction (Fig. 1).

Responses by bark beetles and associates to alternate chemical signals

Ips grandicollis was highly attracted to ipsenol, with a ratio of 163 beetles caught per treatment to control trap (Table 4). There was no cross attraction by either herbivore, *I. pini* or *I. perroti*. The predominant predators all showed strong attraction to ipsenol, at least when they were abundant in the spring. During this period, the ratio of insects caught in ipsenol-baited to control traps was 23.1 for *T. dubius*, 50.9 for *P. cylindrica*, and 28.5 for *P. parallelum*. The parasitoid *T. tibialis* was not attracted to ipsenol.

Discussion

Across a large spatial scale and over three years, *I. pini* consistently preferred different blends of its pheromone components than did its predominant predators. This is consistent with the hypothesis that herbivores can gain partial escape from natural enemies that exploit kairomonal signals by modifying subtle features of their pheromone chemistry. Specifically, at least six species of predators, including three that are very abundant, are highly attracted

Table 3 Spatial and seasonal variation in responses of *I. pini* and its predominant predators to components of *I. pini* pheromone

Site	*Ips pini*				*Thanasimus dubius*				*Platysoma cylindrica*				*Platysoma parallelum*			
	Ipsdienol (−)				Ipsdienol (−)				Ipsdienol (−)				Ipsdienol (−)			
	Lan syn	10%	40%	70%	Lan syn	10%	40%	70%	Lan syn	10%	40%	70%	Lan syn	10%	40%	70%
Spring																
Spring Green	14.41	0.28	0.44	0.28	1.33	0.47	0.29	0.24	0.97	0.12	0.43	0.45	1.31	0.14	0.31	0.55
Arena	12.00	0.27	0.42	0.31	1.38	0.44	0.36	0.21	0.94	0.11	0.38	0.51	1.05	0.18	0.34	0.48
Lake Delton	13.47	0.38	0.34	0.29	1.87	0.47	0.24	0.30	1.34	0.08	0.28	0.64	1.44	0.15	0.39	0.46
Marquette	18.38	0.29	0.39	0.32	1.41	0.47	0.33	0.21	1.43	0.12	0.37	0.51	1.23	0.17	0.33	0.50
Necedah	14.08	0.33	0.44	0.23	1.24	0.35	0.35	0.29	0.96	0.12	0.37	0.51	1.23	0.23	0.38	0.38
Summer																
Spring Green	5.77	0.21	0.39	0.41	0.91	0.37	0.35	0.28	0.60	0.15	0.40	0.45	1.53	0.39	0.29	0.32
Arena	4.72	0.23	0.45	0.33	0.83	0.39	0.35	0.26	0.97	0.11	0.39	0.50	2.06	0.24	0.30	0.46
Lake Delton	7.82	0.23	0.45	0.33	0.96	0.30	0.51	0.19	0.99	0.10	0.36	0.54	2.59	0.00	0.23	0.77
Marquette	10.00	0.23	0.39	0.38	1.11	0.37	0.37	0.26	0.97	0.14	0.32	0.54	1.09	0.18	0.42	0.40
Necedah	6.38	0.24	0.40	0.36	1.39	0.41	0.31	0.28	1.48	0.08	0.37	0.55	0.87	0.18	0.37	0.45

Lanierone synergism (Lan syn) is the ratio of trap catches in treatments containing high versus trace lanierone

Values under ipsdienol are proportions of insects caught in treatments containing 10, 40, or 70% (−) ipsdienol, with lanierone treatments pooled

to pheromones of this herbivore. However, these predators prefer different stereoisomers of the herbivore's primary pheromone component than do the prey (Fig. 1). The positioning of the preferences of *I. pini* midway between the preferences of the two major predators may arise from equal and opposing pressures they exert, i.e., they occur in approximately equal numbers (Table 1), show equivalent numerical relationships with *I. pini* populations in nature (Erbilgin et al. 2002), and have equivalent and noninteracting per-predator effects on *I. pini* reproduction within hosts (Aukema et al. 2004). Incorporating a synergist into their pheromone plume gives *I. pini* further opportunity to communicate with only moderate or with no additional eavesdropping by predators. Despite this complexity, chemical communication between male and female *I. pini* appears to be completely congruous (as evidenced by their similar rank orders and between-treatment comparisons), so there is no loss of intraspecific functioning. Together, these results suggest that widely recognized adaptations by which insects avoid visual detection by predators, such as camouflage and mimicry, have a chemical counterpart.

The variability in chemical signaling by *I. pini* is consistent with localized adaptation and counter-adaptation in microevolutionary time. The prey's preferences for both lanierone content and stereoisomeric ratios of ipsdienol vary in degree spatially, seasonally within years, among years, and in all combinations thereof. Further, the modification of the communication system of *I. pini* by lanierone is strongest when predators are most abundant, and declines when predator numbers diminish in an annually predictable fashion (Fig. 2). Similarly, preferences by *I. pini* for specific

chiralities appear more pronounced when predators are most abundant, but this effect was less than with lanierone (Appendix 1). Such variability adds a spatiotemporal dimension to complex semiochemical interactions, confronting predators with a moving target that is complex, dynamic, and partly unpredictable. These results support the views of Hunter (2003) and Cronin and Reeve (2005) that studying semiochemical interactions under natural conditions and at a landscape scale can improve our overall understanding of their ecological roles.

Disparities between the preferences of *I. pini* and those of its predominant predators are not unique to Wisconsin. We observed a similar pattern in California, where *I. pini* produce (−) ipsdienol, and the major predators prefer blends with a higher (+) component (Dahlsten et al. 2004). Intriguingly, the attraction of these predators to ipsdienol is strongly synergized by lanierone, even though *I. pini* there do not utilize this component or do so to only a minor extent (Seybold et al. 1992; Miller et al. 1997; Dahlsten et al. 2004). Further evidence for chemical shifts in microevolutionary time comes from patterns of inter-regional variation. Local predators of *I. pini* in both California and Wisconsin show stronger attraction to *I. pini* from the corresponding distant than the local population (Raffa and Dahlsten 1995). Thus, behavioral disparities among predators and *I. pini* have been demonstrated at three levels of scale, within forest stands in two different regions (Raffa and Klepzig 1989; Dahlsten et al. 2004), stands within regions (Herms et al. 1991; Aukema et al. 2000), and between geographic regions (Raffa and Dahlsten 1995; Miller et al. 1997). Consistent with the underlying assumptions of our

Table 4 Effect of ipsenol on trap catches of bark beetles and associates

Insect	Ipsenol mean	SE	Control mean	SE	t	P
Spring						
Ips pini	0.26	0.43	0.17	0.43	0.15	NS
Ips grandicollis	12.16	0.08	0.12	0.08	23.44	<0.0001
Ips perroti	0.18	0.10	0.004	0.10	1.21	NS
Thanasimus dubius	2.37	0.14	0.07	0.14	6.05	<0.0001
Platysoma cylindrica	2.42	0.27	0	0	3.61	0.01
Platysoma parallelum	2.42	0.13	0.04	0.13	6.85	<0.0001
Tomicobia tibialis	0.03	0.04	0.04	0.04	0.08	NS
Summer						
Ips pini	0.34	0.46	0.36	0.45	0.02	NS
Ips grandicollis	3.20	0.04	0.03	0.04	23.11	<0.0001
Ips perroti	0.13	0.10	0.05	0.10	0.55	NS
Thanasimus dubius	0.33	0.10	0.04	0.09	1.93	NS
Platysoma cylindrica	0.31	0.11	0	0	1.80	NS
Platysoma parallelum	0.27	0.06	0.04	0.060	2.39	NS

t values and *P* values in comparison to blank controls, corrected by Bonferroni method; NS; *P* value > 0.05; *df* as in Table 2

model, the pheromone components of *I. pini* are under strong genetic control and reflect assortative mating (Hager and Teale 1996; Cognato et al. 1997; Domingue et al. 2006).

Modification of chemical signals does not function alone, but rather in conjunction with other temporal and spatial processes. For example, *I. pini* gains some measure of temporal escape through additional generations over *T. dubius* and *P. cylindrica* (Fig. 2) (Aukema et al. 2005). Similarly, Erbilgin and Raffa (2002) found that predator populations were often highest in sites where populations of *I. pini* were relatively low. A solely temporally based escape strategy for the herbivore is constrained, however, by the decline in nutritional quality of phloem that begins by mid-to-late summer (Redmer et al. 2001). Likewise, the purely spatial component is limited by the superior dispersal ability of predators relative to bark beetles, as has been observed with *D. frontalis* (Cronin et al. 2000) and *I. pini* (Costa et al., unpublished data).

Alterations in chemical signals to escape predators must be compatible with other functions such as avoiding competitors. For example, where sympatric *Ips* species produce the same compound, differing ratios of their stereoisomers can contribute to resource partitioning (Wood 1982). However, in our region the only other abundant colonizer of pine stems is *I. grandicollis*. These two species utilize different chemicals between which there is no cross-attraction. Further, lanierone inhibits the arrival of *I. grandicollis* at traps baited with ipsdienol, even as it synergizes the response of *I. pini*. There appears to be some overlap in signaling between *I. pini* and *I. perroti*. Interestingly, their preferred stereoisomeric ratios differ. Further, numbers of *I. perroti* were very low and highly localized in this and previous studies in southern Wisconsin (Aukema et al.

2000; Erbilgin et al. 2002), as this beetle occurs mostly at higher latitudes (Ayres et al. 1999, 2001). The relative importance of competition and predation in influencing signaling likely varies with local selection forces. For example, in California at least four congeneric pine-colonizing *Ips* produce ipsdienol (Wood 1982), so competition probably plays a more active role there.

Predators likely adjust their behaviors to prey alterations through generations. For example, *T. dubius* in Wisconsin show some attraction to lanierone. The most important factor delaying counter-adaptation by predators is likely the availability of alternate prey. All of the predominant predators are attracted to ipsenol, the pheromone of *I. grandicollis*. Spatial complexity (Table 3) likely further complicates predator counter-adaptations. For example, Ryall and Fahrig (2005) observed that habitat fragmentation reduces the ratio of *T. dubius* to *I. pini*. Most *T. dubius* disperse less than 2 km (maximum approximately 8 km) (Cronin et al. 2000), which suggests sites with the separation studied here can be partially isolated. The basis for differences between *Thanasimus* and *Platysoma* sp. (Fig. 1) is unknown, but may represent a degree of resource partitioning between these predators. For example, they have differing responses to semiochemicals associated with various *Dendroctonus* bark beetles with which they overlap (Haberkern and Raffa 2003).

In contrast to predators, the specialist wasp *T. tibialis* showed nearly perfect correspondence with the chemical preferences of *I. pini*. Likewise, in the inter-regional reciprocal exchange experiment described above (Raffa and Dahlsten 1995), this specialist preferred *I. pini* of local origin. This close correspondence likely reflects the strong selective pressures on *T. tibialis* to closely track its host. However, the low numbers of this parasitoid found in this

(Table 1) and previous (Senger and Roitberg 1992) studies suggests that *T. tibialis* would exert low selective pressures on *I. pini*. Thus, our model of semiochemical escape seems more applicable to generalists than specialists, at least when specialists occur in low numbers.

Although the patterns we observed are consistent with adaptive responses for evading predators, a number of questions remain. First, the extent and pattern by which pheromone biosynthesis, as opposed to the behavioral responses we measured here, varies among individuals in natural populations is poorly understood. In particular, we do not know how the emission of pheromone components and quantities varies at the level of individual trees. Whether plumes emanating from single trees are comprised of relatively homogeneous or highly heterogeneous signals depends on the assemblages of beetles that arrive, and could strongly affect benefits at the individual colonizer level. Second, we do not know the extent to which predators incorporate and integrate visual and chemical cues once they have landed on trees, which could likewise affect the benefits to beetles of producing various signals. Similarly, the extent to which these predators learn from experience has not been investigated and could likewise be important, particularly at the scale of forest stands. Third, these predators incorporate plant monoterpenes into their search behavior, and their attraction to *Ips* pheromones is modulated by the chirality of these compounds (Erbilgin and Raffa 2001). The extent to which host compounds either allow natural enemies to compensate for pheromonal modifications by the prey, or provide the prey with additional opportunities for evasion (De Moraes and Mescher 2004), is unknown.

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- The following material is contained in the electronic appendices -

App. 1: Sources of variation in responses by *I. pini* and associates to 6 combinations of ipsdienol stereoisomeric ratios (ipsd) and lanierone

content (lan) during spring and summer in 1995-1997 (n=2), year of the study (n=3) and their interactions

a. Predominant members of each guild: herbivores, predators, parasitoids

Source of Variation	ndf	*Ips pini* ddf	F	p	*Thanasimus dubius* ddf	F	p	*Platysoma cylindrica* ddf	F	p	*Platysoma parallelum* ddf	F	p	*Tomicobia tibialis* ddf	F	p
(Intercept)	1	1942	534.7	<.0001	1950	173.7	<.0001	1950	174.3	<.0001	1950	28.1	<.0001	1950	15.1	0.0001
ipsd	2	1942	73.9	<.0001	1950	48.3	<.0001	1950	354.0	<.0001	1950	111.1	<.0001	1950	18.9	<.0001
lan	1	1942	3177.0	<.0001	1950	11.2	0.0008	1950	0.8	0.3602	1950	1.6	0.2083	1950	30.9	<.0001
season	1	389	120.1	<.0001	389	79.3	<.0001	389	104.2	<.0001	389	161.3	<.0001	389	0.1	0.8147
year	2	389	65.4	<.0001	389	12.6	<.0001	389	28.0	<.0001	389	2.9	0.0565	389	5.1	0.0067
ipsd:lan	2	1942	34.9	<.0001	1950	1.6	0.2039	1950	0.0	0.9627	1950	0.6	0.5313	1950	6.3	0.0019
ipsd:season	2	1942	5.5	0.0041	1950	8.5	0.0002	1950	54.2	<.0001	1950	30.8	<.0001	1950	2.0	0.1388
lan:season	1	1942	65.4	<.0001	1950	6.0	0.0141	1950	3.4	0.0662	1950	1.6	0.2104	1950	3.1	0.0786
ipsd:year	4	1942	10.0	<.0001	1950	1.8	0.1185	1950	25.0	<.0001	1950	12.6	<.0001	1950	2.6	0.0325
lan:year	2	1942	183.6	<.0001	1950	6.2	0.0021	1950	0.0	0.9901	1950	1.2	0.2963	1950	5.5	0.004
season:year	2	389	14.2	<.0001	389	2.6	0.0786	389	6.0	0.0028	389	3.3	0.0381	389	5.2	0.0059
ipsd:lan:season	2	1942	1.0	0.3842	1950	0.4	0.6725	1950	0.1	0.9446	1950	0.1	0.9285	1950	0.2	0.7791
ipsd:lan:year	4	1942	1.5	0.1937	1950	1.2	0.2899	1950	0.5	0.7653	1950	1.4	0.2249	1950	1.1	0.3719
ipsd:season:year	4	1942	2.0	0.0913	1950	3.0	0.0176	1950	3.9	0.0035	1950	0.8	0.5279	1950	0.7	0.5775
lan:season:year	2	1942	8.3	0.0002	1950	4.0	0.0182	1950	0.5	0.6056	1950	2.0	0.1325	1950	3.4	0.0347
ipsd:lan:season:year	4	1942	1.5	0.1864	1950	0.2	0.9525	1950	0.6	0.68	1950	1.9	0.0999	1950	0.2	0.9242

App 2: Within-season sources of variation in responses by *I. pini* and associates to 6 combinations of ipsdienol stereoisomeric ratios and lanierone content across five sites in Wisconsin: 1996

Source of Variation	*ndf*	*ddf*	*Ips pini* F	*p*	*Thanasimus dubius* ddf	F	*p*	*Platysoma cylindrica* ddf	F	*p*	*Platysoma parallelum* ddf	F	*p*	*Tomicobia tibialis* ddf	F	*p*
(Intercept)	1	918	1130.2	<.0001	920	355.5	<.0001	920	68.1	<.0001	920	124.9	<.0001	920	30.9	<.0001
ipsd	2	918	40.5	<.0001	920	21.2	<.0001	920	175.7	<.0001	920	43.6	<.0001	920	8.6	0.0002
lan	1	918	2098.0	<.0001	920	7.9	0.005	920	0.1	0.7846	920	2.6	0.1053	920	24.1	<.0001
site	4	918	21.3	<.0001	920	17.6	<.0001	920	7.4	<.0001	920	1.7	0.152	920	20.0	<.0001
season	1	117	13.8	0.0003	117	41.5	<.0001	117	42.8	<.0001	117	122.6	<.0001	117	2.2	0.1379
ipsd:lan	2	918	22.2	<.0001	920	0.5	0.5842	920	0.3	0.7203	920	1.9	0.1554	920	3.3	0.0372
ipsd:site	8	918	1.7	0.0948	920	0.7	0.6686	920	1.3	0.2221	920	0.8	0.6207	920	2.0	0.0487
lan:site	4	918	7.3	<.0001	920	0.3	0.8508	920	0.8	0.501	920	3.1	0.0148	920	5.0	0.0005
ipsd:season	2	918	5.2	0.0056	920	5.6	0.0038	920	34.3	<.0001	920	30.3	<.0001	920	0.5	0.6007
lan:season	1	918	44.6	<.0001	920	11.4	0.0007	920	1.4	0.2367	920	0.0	0.8521	920	3.9	0.0486
site:season	4	918	7.2	<.0001	920	20.5	<.0001	920	2.2	0.0661	920	0.6	0.6513	920	2.1	0.076
ipsd:lan:site	8	918	2.7	0.0057	920	0.7	0.6645	920	0.8	0.6029	920	0.4	0.92	920	0.8	0.5598
ipsd:lan:season	2	918	0.2	0.8388	920	0.3	0.7345	920	0.3	0.7411	920	2.5	0.0826	920	0.0	0.9663
ipsd:site:season	8	918	0.5	0.8836	920	2.0	0.0427	920	1.1	0.3615	920	1.2	0.3034	920	1.2	0.322
lan:site:season	4	918	0.8	0.5306	920	1.2	0.3185	920	1.3	0.2819	920	3.2	0.0124	920	0.5	0.7229
ipsd:lan:site:season	8	918	1.2	0.2866	920	0.6	0.7771	920	0.2	0.9833	920	0.8	0.5728	920	0.3	0.9736

App 3: Variation in space and time of responses by *I. pini* to its pheromone components. For each of five sites, ranging from 13 to 108 km from each other, the lanierone synergism ratio and chiral preference are shown for spring (circles) and summer (triangles). The synergism ratio is the ratio of ipsdienol treatments with high lanierone to ipsdienol treatments with trace lanierone. Chiral preference is the mean response by beetles across three stereoisomeric ratios: 10, 40, and 70 % of (-) ipsdienol

Necedah

Lanierone Synergism Ratio vs Chiral Preference (-) Ipsdienol (%)

Marquette

Lanierone Synergism Ratio vs Chiral Preference (-) Ipsdienol (%)

Spring Green

Lanierone Synergism Ratio vs Chiral Preference (-) Ipsdienol (%)

Arena

Lanierone Synergism Ratio vs Chiral Preference (-) Ipsdienol (%)

Lake Delton

Lanierone Synergism Ratio vs Chiral Preference (-) Ipsdienol (%)

65 km