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Seasonal pheromone response by *Ips pini* in northern Arizona and western Montana, U.S.A.

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Abstract 1 Populations of *Ips pini* (Say) in northern Arizona and western Montana, U.S.A., were studied to determine regional pheromone response and to evaluate seasonal shifts in that response. A range of enantiomeric blends of the attractant ipsdienol, alone and in the presence of the synergist lanierone, were tested during spring and summer seasons over several years.

2 Both populations were most attracted to high levels of (*R*)-(–)-ipsdienol, and lanierone was highly synergistic.

3 A significant seasonal shift in pheromone response between spring and summer seasons was found in both regions in both years. Shifts resulted in a more specific preference for the pheromone treatment of 97% (*R*)-(–)-ipsdienol with lanierone.

4 Several coleopteran insect associates of *I. pini* also displayed responses to the ipsdienol and lanierone treatments. *Temnochila chlorodia* (Mannerheim) (Trogositidae), *Enoclerus sphegeus* (F.) (Cleridae) and, to a limited extent, *Lasconotus laqueatus* (LeConte) (Colydiidae) were attracted to higher proportions of (*R*)-(–)-ipsdienol with no apparent reaction to the presence of lanierone. *Orthotomicus latidens* (LeConte) (Curculionidae: Scolytinae) was strongly attracted to (*S*)-(+)-ipsdienol with *Enoclerus lecontei* (Wolcott) (Cleridae), *Pityogenes carinulatus* (LeConte) (Curculionidae: Scolytinae) and *Hylurgops porosus* (LeConte) (Curculionidae: Scolytinae) demonstrating some preferences for the (*S*)-(+)-enantiomer. However, lanierone was synergistic for *E. lecontei* and *P. carinulatus*, inhibitory for *O. latidens*, and produced no significant reaction for *H. porosus*. *Elacatis* sp. (Salpingidae, previously Othniidae) was attracted to the presence of ipsdienol but displayed no preference to the enantiomeric ratios of ipsdienol or the presence of lanierone.

Keywords Bark beetle, competitor, enantio-specificity, pheromone response, pine engraver, predator, seasonal abundance, seasonal behavior.

Introduction

Bark beetles are an important disturbance agent in forest ecosystems, with many species causing widespread tree mortality (Rudinsky, 1962; Furniss & Carolin, 1977). One of the most common and widely-distributed species of bark beetle is the pine engraver *Ips pini* (Say) (Wood, 1982; Kegley *et al.*, 1997). *Ips pini* is considered to be moderately aggressive, generally attacking recently downed coniferous host material. However, this species is capable of killing large numbers of live pines when their abundance is high and forest stands are stressed (Kennedy, 1969; Parker, 1991; Kegley *et al.*, 1997). Usually, sapling or pole-sized trees are killed, although tops of larger trees may be colonized when the lower bole has been attacked by more aggressive bark beetle species, or after the tree has been infected by a pathogen (e.g. dwarf mistletoe or a root disease) or damaged by abiotic factors such as wind, snow, lightning or fire (Livingston, 1979; Klepzig *et al.*, 1991; Parker, 1991; Kegley *et al.*, 1997). Coniferous hosts include most species of *Pinus* and, in rare cases, species of *Picea*, as well as *Larix laricina* (Du Roi) K. Koch (Furniss & Carolin, 1977; Wood, 1982; Gandhi & Seybold, 2002).

Male *I. pini* initiate attack on host material. As they feed, they produce the attractant pheromone ipsdienol (2-methyl-6-methylene-2,7-octadien-4-ol), which occurs as

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two enantiomers (*R*)-(–) and (*S*)-(+) (Birch *et al.*, 1980; Seybold *et al.*, 1995), and the synergistic compound lanierone (2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one), which is achiral (Teale *et al.*, 1991). Other insect species may also use these allelochemicals to avoid interspecific competition or to locate prey (Bakke & Kvamme, 1981). Thus, semiochemicals play an important role in inter- and intra-specific interactions of *I. pini* within the forest ecosystem (Seybold, 1993; Birch, 1978; Light *et al.*, 1983; Savoie *et al.*, 1998; Raffa, 2001).

Pheromone response by *I. pini* has been found to vary geographically. Three pheromonal populations have been identified; the 'New York' population prefers 32–56% (*R*)-(–)-ipsdienol, the 'California' population prefers 94–98% (*R*)-(–)-ipsdienol and the 'Idaho' population, generally considered a hybrid of the New York and California populations, prefers 91–95% (*R*)-(–)-ipsdienol (Lanier *et al.*, 1972, 1980; Miller *et al.*, 1989; Seybold *et al.*, 1995). The New York pheromonal population is described as ranging from southeastern Appalachia, along the Atlantic coast, through the Great Lakes region and southern British Columbia, Canada (BC), with the California pheromonal population ranging from Washington to Arizona and into New Mexico, and the Idaho pheromonal population ranging over southeastern BC, Idaho and Montana (Seybold *et al.*, 1995; Miller *et al.*, 1996). Local variability within these larger geographically defined pheromone types has also been noted (Miller *et al.*, 1989; Herms *et al.*, 1991; Miller *et al.*, 1996). In addition, the strength of lanierone as a synergist varies geographically, with it being strongly synergistic in New York and Wisconsin, weakly synergistic in Montana and BC, and minimally synergistic in California (Teale *et al.*, 1991; Seybold *et al.*, 1992; Miller *et al.*, 1997; Dahlsten *et al.*, 2003). Genetic evidence supports these pheromone-based population delineations (Cognato *et al.*, 1999; Domingue *et al.*, 2006).

In addition to geographical differences in pheromone response, *I. pini* may also undergo a seasonal change in pheromone response (Birch, 1974; Teale & Lanier, 1991; Teale *et al.*, 1991; Aukema *et al.*, 2000; Ayres *et al.*, 2001; Dahlsten *et al.*, 2003). This shift may involve a change in the preferred enantiomeric ratio of the attractant ipsdienol, as well as the selection for other semiochemical compounds, such as lanierone (Teale & Lanier, 1991; Seybold *et al.*, 1992; Miller *et al.*, 1997; Aukema *et al.*, 2000; Ayres *et al.*, 2001; Dahlsten *et al.*, 2003).

Pheromone response by bark beetles may be influenced by a number of factors, including reinforcement of reproductive isolation (Lanier & Wood, 1975; Birch *et al.*, 1980; Miller & Borden, 1992), avoidance of interspecific competition (Birch & Wood, 1975; Birch *et al.*, 1980; Light *et al.*, 1983) or escape from predation (Raffa & Klepzig, 1989; Raffa & Dahlsten, 1995; Aukema & Raffa, 2000). Beetle condition (e.g. reproductive stage, physiology, age, symbionts; Atkins, 1966; Bennett & Borden, 1971; Hagen & Atkins, 1975; Hunt & Borden, 1990; Gast *et al.*, 1993; Seybold *et al.*, 2000), population density (Teale & Lanier, 1991; Teale *et al.*, 1991; Wallin & Raffa, 2002) or host material condition (Renwick *et al.*, 1976; Klimetzek & Francke, 1980; Seybold *et al.*, 1995; Seybold *et al.*, 2000; Wallin & Raffa, 2002) may also be important. Spatial or temporal changes in one or more of these factors may affect changes in pheromone production with a concomitant change in pheromone response.

We chose the two regions of Flagstaff, Arizona and Missoula, Montana, U.S.A., for our research due to their location in ponderosa pine (*Pinus ponderosa* P&C Lawson) dominated forests in the interior west of the U.S.A., similarities in climate, and similar *I. pini* life cycles. However, Missoula was located within the 'Idaho' pheromonal population and Flagstaff population within the 'California' pheromonal population, allowing for potential contrasts between the two groups. The objectives of the present study were to: (i) characterize pheromone response by *I. pini* and their associates in two geographic locations not previously tested, (ii) determine whether male and female *I. pini* differ in pheromone response, (iii) determine whether pheromone response by *I. pini* in these locations shift seasonally, and (iv) explore some of the possible mechanisms that influence seasonal pheromone response by *I. pini*.

Materials and methods

Study sites

We conducted this study in the ponderosa pine forests of northern Arizona, within 32 km of Flagstaff, and in western Montana, within 48 km of Missoula. At an elevation of 2133 m a.s.l. and a latitude of 35.10°N, Flagstaff experiences four distinct seasons, as does Missoula much further to the north at an elevation of 975 m and a latitude of 46.55°N. With mean annual temperatures of 6.6°C and 7.6°C, and a mean annual precipitation of 34 and 58 cm, respectively, Missoula and Flagstaff fall into Holdrige's cool temperate steppe/moist forest life-zone class (Smith, 1986; NOAA, 2008a, b). In both regions, *I. pini* populations are typically bivoltine with spring beetle flights (FL1) consisting of overwintering adults beginning in early April and mid-April, in Arizona and Montana, respectively, and summer flights (FL2) of the first new generation of beetles beginning in mid- to late June in both regions (Parker, 1991; Villa-Castillo, 1994; Gibson & Weber, 2004).

Treatments

Five ratios of ipsdienol (ID) enantiomers [given as the percent of the (*R*)-(–) enantiomer: 3% (–)-ID, 25% (–)-ID, 50% (–)-ID, 75% (–)-ID, 97% (–)-ID] were tested. These five ratios were deployed with and without the synergistic compound lanierone (L). Two control traps were also used, one trap with no semiochemicals (C) and one trap with lanierone only (C+L), for a total of 12 pheromone treatments. Bubble caps were replaced every 28 days to ensure a continual release of pheromone throughout the experiment. Elution rates of ipsdienol and lanierone dispensers (Pherotech International, British Columbia, Canada) were 110 and 10 μg/day, respectively, at 25 °C. We did not correct for differences in elution rates of the two ipsdienol enantiomers [e.g. 97% (–)-ID eluted approximately 106.7 μg/day of (*R*)-(–)-enantiomer at 25 °C whereas 3% (–)-ID eluted only 3.3 μg/day].

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Plot layout and experimental design

In all trials (2000–2002), bubble caps were hung in 8-unit Lindgren funnel traps (Lindgren, 1983), except in Montana during 2000 when 12-unit traps were used. Traps were hung so that the tops of traps were 1.5–2.0 m above ground and more than 1 m from the nearest tree (host or nonhost). All traps were spaced 18–25 m apart to minimize possible neighbour-trap effects. Dichlorvos-impregnated wax bar pieces (3 × 3 cm) (No Pest Strip, Loveland Industries, Greeley, Colorado) were added to collection cups (dry cup method) in 2000 and 2001 to prevent escape and minimize predation of samples. In 2002, collection cups were modified to use propylene glycol to preserve catches better (wet cup method). Each week, trap catches were collected and traps were re-randomized within the site to minimize possible location or neighbour-trap effect.

Trapping was conducted in 2000 during the summer *I. pini* flight (FL2) in both Arizona and Montana. In each region, four replicates of the 12 treatments were placed in a single stand along a 6 × 8 grid. Trap catches were collected weekly for 5 weeks in Arizona and 4 weeks in Montana. In 2001, we placed one replicate of each of the 12 pheromone treatments along a 3 × 4 grid at four different sites in each region. All trapping sites were at least 16 km apart. In Arizona, we trapped from the beginning of spring flight through summer flight for a total of 20 weeks. In Montana, we trapped for 4 weeks during the spring flight (FL1) and summer flight (FL2) for a total of 8 weeks. In 2002, we chose three new trapping sites in each region and again deployed one replicate of each of the 12 treatments along a 3 × 4 grid. Collections in 2002 were made for approximately 4 weeks during spring (FL1) and summer (FL2) flights in both regions.

In 2002, we conducted additional trapping to monitor seasonal abundance of *I. pini* and their associates. These traps used a subset of the 12 treatments used to test pheromone response and were deployed at/near the pheromone response sites. In Arizona, we used four pheromone treatments [50% (–)-ID, 50% (–)-ID+L, 97% (–)-ID, 97% (–)-ID+L] placed at the far corners of the 3 × 4 trap grids. These four monitoring treatments were deployed between and after the 12-treatmement pheromone response trials. Thus, pheromone response and monitoring traps collectively sampled populations from 21 February to 12 November. In Montana, we used five pheromone treatments for monitoring [50% (–)-ID, 50% (–)-ID+L, 75% (–)-ID+L, 97% (–)-ID, 97% (–)-ID+L] with traps placed near the 12-treatment pheromone response trials. These monitoring traps were deployed from 29 March to 13 September, and used dry collection cups. Additional information on each trapping session is summarized in Table 1.

Trap catches were sorted, identified, and counted. Sex determination of *I. pini* was conducted using known differences in the declivital spines (Wood, 1982). Insect associates of *I. pini* captured in sufficient numbers were identified and evaluated for seasonal abundance and pheromone response. Identifications of associated insect to genus and species were conducted by comparison with voucher specimens and/or professional verification.

Statistical analysis

Seasonal abundances of *I. pini* and its principal associates were determined as the sum of individuals caught in all traps at all sites during each 1-week period. We assumed that beetles chose one of the available traps regardless of the total number deployed at the site. In Arizona and Montana in 2001, the 12 traps at all four sites were summed whereas, in 2002, all available traps, pheromone response and/or monitoring, were summed over the three sites. Weekly insect counts were used only in analysing seasonal abundance. In all other analyses, the count data for each trap were summed over the time period of interest. For example, descriptions of regional pheromone response by *I. pini* were calculated by summing catches for each trap over the 2000 trapping period (5 weeks in Arizona and 4 weeks in Montana). For evaluation of gender difference in pheromone response, the proportions of males to females were used.

Trap catches in 2001 and 2002 were summed over the 3–5-week-long spring (FL1) and summer (FL2) flight periods (Table 1). However, insect abundance differed across sites, regions and years, requiring that data be standardized to allow comparison of treatments. Standardization was accomplished by dividing the total number of individuals caught in each trap during a given time period by the total number of individuals caught at that site for the same time period. This resulting 'proportion-of-total-catch' for each trap was used in analyses of seasonal pheromone response by *I. pini* (× 100 for 'percent-of-total-catch' used in figures). In an additional analyses of seasonal pheromone shift by *I. pini*, 'proportion-of-total-catch' was recalculated using only the three preferred treatments [50% (–)-ID+L, 75% (–)-ID+L, and 97% (–)-ID+L] where five consecutive month periods of trapping data were available (Arizona 2001 and Montana 2002-monitoring, Julian dates 68–208 and 116–256, respectively).

The allomonal/kairomonal responses to ipsdienol and lanierone by eight coleopteran species associated with *I. pini* were calculated using trapping periods during which all 12 treatments were deployed in 2001 and 2002 [12 weeks in AZ 2001, 4 weeks in MT 2001 (FL2), and 8 weeks in both AZ 2002 and MT 2002 (FL1 + FL2) for a maximum of seven replicates per state]. If fewer than ten individuals (of the species) were captured at a site during the year, data for that site were not used in further calculations for that species. Potential seasonal shifts in pheromone response of associates were not evaluated due to the univoltine life cycles of most species and/or the limited number of individuals caught. The 'proportion-of-total-catch' for each trap used in analyses was calculated as described for *I. pini*.

In the few cases where trap data were lost for a given week, we estimated the number of missing *I. pini* by determining the value that best maintained the 'proportion-of-total-catch' for that particular 'treatment × week' and 'treatment × site'. These *I. pini* estimates were not used in analysis of seasonal abundance, however, and associates in missing traps were not estimated.

Due to the summary of weekly catch data over longer time periods, only four observations (replicates) of each treatment were available for analyses of *I. pini* in each region in 2000.

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Table 1 Summary of trapping experiments for *Ips pini* (IP) and associated insects (AS) in Arizona (AZ) and Montana (MT), 2000–2002

Year	Location	Trap	Cup	Experiment	Dates (and flight No.)	Julian date	Design	Deviations	Tests
2000	AZ	8-unit	Dry	Pheromone response	1 June to 6 July (FL2)	152–187	Four replicates of 12 treatments at one site (48 traps) (completely randomized design)		Pheromone response (IP), Sex ratio response (IP)
	MT	12-unit	Dry	Pheromone response	23 June to 25 July (FL2)	174–206	Four replicates of 12 treatments at one site (48 traps) (completely randomized design)		Pheromone response (IP), Sex ratio response (IP)
2001	AZ	8-unit	Dry	Pheromone response	9 March to 27 July (13 April to 18 May = FL1) (15 June to 13 July = FL2)	68–208 103–138 166–194	Twelve treatments at four sites (48 traps) (completely randomized block design)	Treatment 25%(−)-ID and 25%(−)-ID+L not available until 4 May	Seasonal abundance (IP, AS), Seasonal pheromone response (IP), Five months of response (IP), Pheromone response (AS)
	MT	8-unit	Dry	Pheromone response	15 April to 10 May (FL1) 28 June to 26 July (FL2)	105–130 179–207	Twelve treatments at four sites (48 traps) (completely randomized block design)	Treatment 25% (−)-ID and 25%(−)-ID + L not available in FL1	Seasonal abundance (IP, AS), Seasonal pheromone response (IP), Pheromone response (AS)
2002	AZ	8-unit	Wet	Pheromone response	29 March to 6 May (FL1) 7 June to 5 July (FL2)	88–126 158–186	Twelve treatments at three sites (36 traps) (completely randomized block design)	Two of three sites terminated 29 June	Seasonal abundance (IP, AS), Seasonal pheromone response (IP), Pheromone response (AS)
	MT	8-unit	Wet	Pheromone response	9 May to 7 June (FL1) 21 June to 19 July (FL2)	129–158 172–200	Twelve treatments at three sites (36 traps) (completely randomized block design)	One of three sites terminated 27 June	Seasonal abundance (IP, AS), Seasonal pheromone response (IP), Pheromone response (AS)
2002	AZ	8-unit	Wet	Monitoring	21 February to 28 March 7 May to 6 June 6 July to 12 November	52–87 127–157 187–316	Four treatments at three sites (12 traps) (completely randomized block design)		Seasonal abundance (IP, AS)
	MT	8-unit	Dry	Monitoring	20 March to 13 September	79–256	Five treatments at three sites (15 traps) (completely randomized block design)	One of three sites without data from 28 June to 18 July	Seasonal abundance (IP, AS), Five months of response (IP)

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In 2001 and 2002, maxima of four and three observations were available, respectively, during each flight season in each region for analysis of the *I. pini* response. Similarly, only four and three observations in 2001 and 2002, respectively, were available for each treatment at each location for analysis of *I. pini* associates.

Several statistical tests were used to analyze our data. Analyses of variance (ANOVA) (PROCMIXED command in SAS; SAS Institute, 2002) were conducted on 2001–2002 *I. pini* 'proportions-of-total-catch' [transformed using $\ln(Y+1)$] to assess the importance of various test factors. However, use of ANOVA for other analyses was limited because assumptions of normality and homogeneity of variance could not be met. Instead, multiple response permutation procedures for one-factor designs (MRPP) and multiple response permutation procedure for unreplicated block designs (MRBP) were used due to their insensitivity to data distribution and variance structure (Petrondas & Gabriel, 1983; Zimmerman *et al.*, 1985; Mielke & Berry, 2001). Their only test assumptions are: (i) the data are a representative sample of the desired target population; (ii) each observation belongs to only one group; and (iii) all possible permutations of observations among groups have equal probability of occurrence (for MRPP) or equal probability within each block (for MRBP) (Mielke & Berry, 2001). However, analyses are limited to only one variable. If MRPP/MRBP tests suggested treatments were not similar, we conducted simultaneous multiple comparisons ($\alpha \leq 0.05$) using the Peritz closure method (Petrondas & Gabriel, 1983). In some cases, insufficient data were available for these multiple comparisons (e.g. for three and four replicates, the smallest exact *P*-values for the two-group multiple comparisons are only 0.25 and 0.125, respectively).

Regression analyses were also used to determine pheromone response profiles of *I. pini* (2000) and associated insects (2001–2002) (PROC LNIN command in SAS; SAS Institute, 2002). With the exception of *Elacatis* sp., *Enoclerus lecontei* and *Hylurgops porosus*, regression analyses were conducted using the transformed response variable $[\ln(Y+1)]$ to meet assumptions of normality and homogeneity of variance although figures are shown untransformed. Due to the curvilinear nature of most responses, a two-parameter power model $[Y = b_0 \times X^{b1}$ where X is the % (–)-ID] was used to describe the response profiles. Separate models were created for treatments with lanierone and without lanierone when the two models resulted in significantly less residual error than the single, pooled model ($P < 0.05$) (Bates & Watts, 1988). Potential effects of region were also tested where appropriate to see if separate models should be created for Arizona and Montana. Control treatments were not included.

Results

Pheromone response by *I. pini*

Regression analysis of beetles caught in the year 2000 indicated that, in both regions, attraction of *I. pini* beetles significantly increased with increased percentages of (*R*)-(–)-ipsdienol although ipsdienol treatments in Arizona without lanierone had an R^2 of only 0.02 (Fig. 1). Overall catches were greatly increased by the addition of lanierone. In Arizona, analyses using MRPP showed no significant difference between ipsdienol treatments without lanierone and empty control ($P = 1.0$, $n = 24$), confirming that ipsdienol was not attractive without lanierone present. Thus, the most attractive treatment in both regions was 97% (–)-ID+L. The numbers of beetles caught in ipsdienol treatments for Arizona and Montana with and without lanierone in 2000 are given in Table 2.

When gender (sex ratio) was tested as a factor affecting *I. pini* response to treatments (using treatments with ≥ 10 beetles per site), MRPP tests suggested that sex ratios were not significantly different in Arizona ($P = 0.1461$, $n = 12$), but were dissimilar in Montana ($P = 0.0010$, $n = 32$). However, simultaneous multiple comparisons of the eight treatments in Montana [only treatments with ≥ 25% (–)-ID had ≥ 10 beetles per site] failed to detect significant differences between pairs ($\alpha > 0.05$). The data suggest that males in Montana may be more strongly attracted to lanierone than females are, and that females may be more strongly attracted to ipsdienol treatments with the highest ratios of the (*R*)-(–) enantiomer (Fig. 2).

Initial tests of the importance of flight period on pheromone response were conducted using 2001 and 2002 data [transformed using $\ln(Y+1)$], but were limited to ipsdienol treatments with lanierone because individual treatments lacking lanierone often had fewer than ten beetles (for catch numbers, see Table 2). The ANOVA results on 'proportion-of-total-catch' are given in Table 3. Not only do test results confirm the importance of ipsdienol enantiomeric composition in pheromone response, but also they indicate that flight period significantly interacts with this response. Figure 3 shows the mean response by *I. pini* to each of the 12 treatments during spring (FL1) and summer (FL2) flight periods. The data suggest there is an increase in the proportion of beetles being caught with 97% (–)-ID+L with corresponding decreases in several of the other treatments. In addition, the proportion of beetles caught with pheromone blends lacking lanierone tends to decrease in the summer. Analyses of the proportion of male *I. pini* caught by each treatment in spring and summer (% males in trap/% males at site) using MRBP showed no significant seasonal difference in male response to treatments ($P = 0.24$, $n = 122$), suggesting that gender is not a factor in a shift in seasonal pheromone preference.

When the three preferred treatments were evaluated over five consecutive months, it appeared that a shift in pheromone response occurred over time (Fig. 4). Response to the three treatments was not significantly different in the first month(s) ($P = 0.66$, $n = 12$ and $P = 0.67$, $n = 9$, for AZ and MT, respectively) but became significant as time progressed to the fifth month ($P = 0.004$, $n = 12$ and $P = 0.03$, $n = 9$, respectively). Looking at changes in individual treatments over time, the data suggest that the response shifted from an initial broad attraction to these three treatments to a much more specific response to the highest proportions of (*R*)-(–)-ipsdienol.

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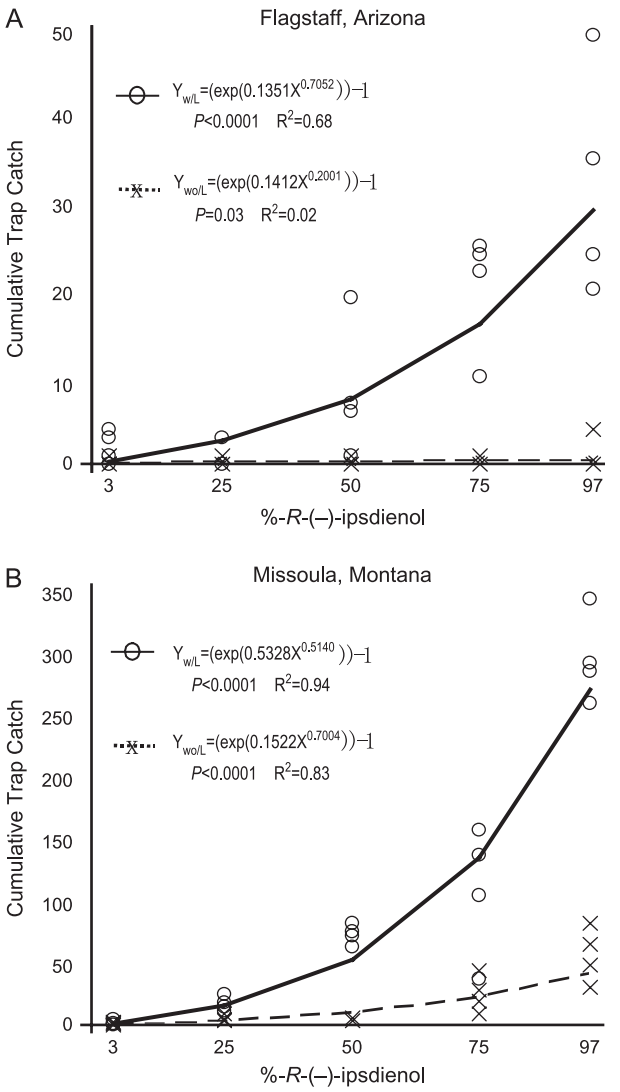

Figure 1 Pheromone response profiles for *Ips pini* in Flagstaff, Arizona (A) and Missoula, Montana (B), 2000. Points represent the total number of beetles caught by each of the four replicates (traps) of each pheromone treatment. Treatments with lanierone (w/L) and without lanierone (wo/L) are represented by (o) and (x), respectively. Regression analyses indicated that in both regions separate equations for ipsdienol treatments with lanierone and without lanierone should be used ($P<0.0001$). Analyses were conducted on the transformed response [$\ln(Y+1)$] but the final graphics and model equations are provided as untransformed numbers.

To test density as a potential factor in seasonal shift, we compared spring and summer 'average weekly site catch' and 'maximum weekly site catch' using a paired two-sample *t*-test with a two-tailed distribution for pairs where both spring and summer total catches were ≥10. Spring and summer values were not significantly different for either 'average weekly' or 'maximum weekly' site catch ($P=0.66$, d.f. $=8$, *t*-critical $=2.3$ and $P=0.78$, d.f. $=8$, *t*-critical $=2.3$, respectively).

Table 2 Number of *Ips pini* captured during the spring flights (FL1) and summer flights (FL2) in Arizona (AZ) and Montana (MT) (with lanierone, without lanierone)

	AZ		MT	
2000				
FL2	269	(259, 10)	2330	(1962, 368)
2001				
FL1	270	(245, 25)	1597	(1319, 278)
FL2	115	(113, 2)	948	(818, 130)
2002				
FL1	256	(157, 99)	65	(44, 21)
FL2	187	(156, 31)	1178	(982, 196)

Seasonal abundance of *I. pini* and associated insects

Data on seasonal abundance in 2001 and 2002 indicated that, in Arizona, *I. pini* spring peaks (FL1) began around 13 April with a second peak (FL2) beginning around 22 June (Julian dates 103 and 173, respectively). In 2002, additional peaks began around 20 July and 20 September (Julian dates 221 and 263, respectively), representing additional progeny flights or pre-winter feeding flights (Fig. 5A, B). In Montana in 2001, we are unable to determine exact timing of peak flights, although a large number of beetles were caught during both the April/May and July trapping sessions (Fig. 6A, B). In 2002, unusually cold and wet spring weather delayed the first peak catch until 6 June (high populations lasting through 19 July) with another peak at 9 August (Julian dates 157 and 221, respectively).

Many other insect species were captured during trapping trials. Based on abundance and probable relationship with *I. pini*, eight species of Coleoptera of interest were identified. In Arizona, these species included *Elacatis* sp.

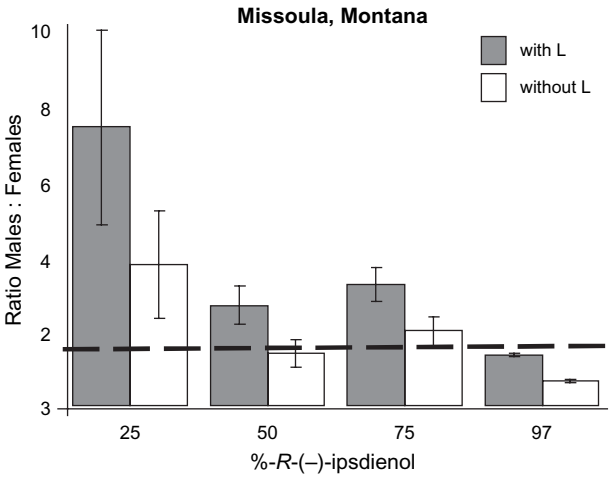

Figure 2 Ratio of Ips pini males to females for Missoula, Montana, 2000. Means ± SE for each treatment with and without lanierone (L) are shown. The dashed line indicates the overall male : female ratio of 1.6 found at the trap site. Values above 1.6 indicate a higher ratio of males than expected. Values below 1.6 indicate a higher ratio of females than expected.

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Table 3 Results of analysis of variance on *Ips pini* trap catches for ipsdienol treatments with lanierone (2001 and 2002 pooled)

Effect	d.f.	*F*	*P*
Location	1, 112	0.16	0.6938
Ipsdienol	4, 112	35.37	< 0.0001*
Flight	1, 112	0.20	0.6539
Location × Ipsdienol	4, 112	0.96	0.4315
Location × Flight	1, 112	0.13	0.7202
Ipsdienol × Flight	4, 112	15.65	< 0.0001*
Location × Ipsdienol × Flight	4, 112	1.29	0.2764

*Significant effects ($P < 0.05$).

[Salpingidae, previously Othniidae, (Borror *et al.*, 1992; Arnett *et al.*, 2002)], *Enoclerus sphegeus* (F.) [Cleridae], *E. lecontei* (Wolcott) [Cleridae], *Orthotomicus latidens* (LeConte) [previously *Ips latidens* (LeConte), (Cognato & Vogler, 2001)] [Curculionidae: Scolytinae], *Pityogenes carinulatus* (LeConte) [Curculionidae: Scolytinae], and *Temnochila chlorodia* (Mannerheim) [Trogositidae], although too few *E. sphegeus* were captured to graph (Fig. 5C–M). Many of the same species were found in Montana, including *Elacatis* sp., *E. sphegeus*, *E. lecontei*, *P. carinulatus*, and *T. chlorodia*, although too few *P. carinulatus* were captured to graph. *Hylurgops porosus* (LeConte) [Curculionidae: Scolytinae] and *Lasconotus laqueatus* (LeConte) [Colydiidae] were also abundant in our Montana traps (Fig. 6C–P).

Allelochemic responses by *I. pini* associates

Response profiles to ipsdienol and lanierone treatments suggest that for some species, response may to be more strongly associated with the (*R*)-(–)-enantiomer of ipsdienol whereas others appear more responsive to the (*S*)-(+)-enantiomer, and lanierone may or may not have an effect on response. Both *T. chlorodia* and *E. sphegeus* were attracted to (*R*)-(–)-ipsdienol with no apparent reaction to the presence of lanierone (ten and five replicates, respectively; Fig. 7A, B). *Enoclerus lecontei* and *P. carinulatus* were slightly more attracted to the (*S*)-(+)-enantiomer of ipsdienol with attraction increased by the presence of lanierone (ten replicates each; Fig. 7E, G). *Orthotomicus latidens* strongly preferred (*S*)-(+)-ipsdienol, and lanierone acted as an attraction inhibitor (seven replicates; Fig. 7F). *Elacatis* sp., *L. laqueatus*, and *H. porosus* were slightly attracted to ipsdienol but without apparent sensitivity to enantiomeric composition or presence of lanierone (seven, six and three replicates, respectively; Fig. 7D, C, H).

Discussion

Geographic variation in pheromone response by *I. pini*

Our research indicated that the pheromone response profile for the *I. pini* population in Missoula, Montana was similar to

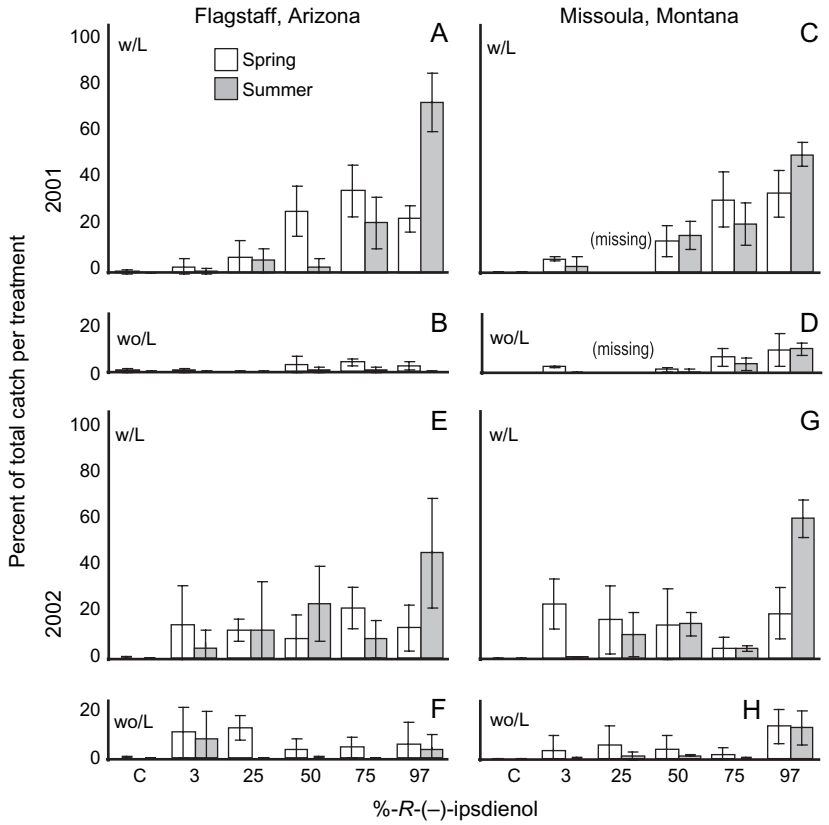

Figure 3 The average percentage of *Ips pini* caught by the 12 pheromone treatments during spring (white) and summer (grey) flights for Flagstaff, Arizona in 2001 (A, B) and 2002 (C, D), and for Missoula, Montana in 2001 (E, F) and 2002 (G, H). Error bars indicate the 95% confidence intervals.

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that previously described for the 'Idaho' pheromonal population, with greatest positive response to high proportions of (*R*)-(–)-ID (Miller *et al.*, 1989; Seybold *et al.*, 1995) with lanierone (Miller *et al.*, 1997). *Ips pini* in Flagstaff, Arizona, considered to be part of the 'California' pheromonal population (Seybold *et al.*, 1995; Cognato *et al.*, 1999), demonstrated a very strong preference for (*R*)-(–)-ipsdienol similar to that found for beetles in California (Birch *et al.*, 1980). In Arizona, however, lanierone was necessary for significant attraction to ipsdienol, contrary to descriptions of the 'California' pheromonal population, which indicate that lanierone is not an important component of an attractant blend (Seybold *et al.*, 1992; Miller *et al.*, 1997). We suspect that the response to lanierone may be either highly variable within the 'California' pheromone population or that *I. pini* in Arizona are more closely related to the 'Idaho' pheromonal population, which they more closely resemble in pheromone preference. A study of mitochondial DNA suggests that Arizona beetles, similar to beetles in the 'Idaho' pheromonal population, have a higher variety of haplotypes (including haplotypes found in beetles from the 'New York' pheromonal population) than do beetles in California (Cognato *et al.*, 1999). This could explain the high response to lanierone that is more similar to the 'New York' and 'Idaho' pheromone populations than the 'California' populations. Further discussion of forces affecting geographical variation in the production of and attraction by ipsdienol and lanierone is provided in Seybold *et al.* (1992, 1995), Cognato *et al.* (1999) and Domingue *et al.* (2006).

Although we did not find significant differences in the response of males and females to various pheromone blends in Arizona, significant differences were found in Montana. In particular, attraction of male beetles in Montana appeared to be more strongly synergized by lanierone than were female beetles. Females also tended to be more strongly attracted by 97% (–)-ID, especially in the absence of lanierone. Past research provides similarly varied results for differences in pheromone response by gender. In New York and California, selection of ipsdienol enantiomeric blends was similar for both sexes (Teale *et al.*, 1994; Dahlsten *et al.*, 2003). However in BC, ipsdienol enantiomeric ratios significantly affected sex ratios with gender shifts similar to those demonstrated at our Montana site (Miller *et al.*, 1996, 1997). The addition of lanierone to traps baited with ipsdienol tended to increase male bias in BC (southeast and southwest), New York and Wisconsin (Miller *et al.*, 1997). In California, Miller *et al.* (1997) also found a significant increase in male representation, yet Seybold *et al.* (1992) found that neither the presence, nor dosage of lanierone had a significant effect on sex ratios. In contrast to our findings, studies by Miller *et al.* (1997) in Montana found that the presence of lanierone did not have a significant effect on sex ratios.

Interestingly, Miller *et al.* (2005) have shown that the proportion of males (in BC) is inversely proportional to elution rate of racemic ipsdienol. Because the higher ratios of (*R*)-(–)-ipsdienol also have higher elution rates of the attractive (*R*)-(–)-enantiomer [and decreased elution rates of the disruptive (*S*)-(+)-enantiomer], it is unclear if male bias against 97% (–)-ID is due to the ratio of enantiomers or the elution rates of specific enantiomers (D. Huber, personal communication).

Allelochemic responses and seasonality of *I. pini* associates

Of the eight insect associates of *I. pini*, *E. sphegeus*, *E. lecontei* and *T. chlorodia* are known bark beetle predators (Furniss & Carolin, 1977; Amman, 1984; DeMars *et al.*, 1986). Some species within the genera *Elacatis* and *Lasconotus* have also been found to prey on bark beetle larvae or adults (Hackwell, 1973; Rohlfs & Hyche, 1984; Cibrian-Tovar, 1987; Bowers *et al.*, 1996), although little is known about the species in the present study. Similar to *I. pini*, *O. latidens*, *P. carinulatus* and *H. porosus* are often found in recently downed coniferous host material (Furniss & Carolin, 1977), although the interactions among these three species are not clearly understood.

Treatment response by these associates is largely similar to that found in previous studies, although variation in response is also apparent. For example, both *T. chlorodia* and *E. sphegeus* in California exhibited greater attraction to ipsdienol than to empty traps, with no added attraction by lanierone (Seybold *et al.*, 1992; Miller *et al.*, 1997). *T. chloradia* in California also demonstrated preference for the (R)-(–)enantiomer of ipsdienol (Dahlsten *et al*., 2003). *E. sphegeus* exhibited attraction to ipsdienol in south/central BC (Miller & Borden, 1990) but failed to respond to either ipsdienol or lanierone in south eastern BC during a later study (Miller *et al*., 1997). *Enoclerus lecontei* exhibited greater attraction to the presence of ipsdienol over blank traps in Montana and southeastern BC, with attraction to ipsdienol significantly increased in southeastern BC by the addition of lanierone (Miller *et al.*, 1997). In California, results have been mixed with significant attraction by ipsdienol alone or only in the presence of lanierone (Miller & Borden, 1990; Seybold *et al.*, 1992; Miller *et al.*, 1997), with increased attraction to ipsdienol with the addition of lanierone supported by Dahlsten *et al.* (2003). A review of Dahlsten *et al.* (2003) suggests that enantiomeric ratios of ipsdienol are not important in attraction.

A study near Flagstaff, Arizona, conducted in 2002 and 2003, captured the same *Elacatis* sp. in attraction found in the present study by using traps baited with racemic ipsdienol [*I. pini* lure of 50% (*R*)-(–)-ID], or the three-terpene blend of α-pinene, β-pinene, and 3-carene (*Dendroctonus valens* LeConte lure) (Gaylord *et al.*, 2006). Thus, it appears that ipsdienol is one of several compounds attractive to *Elacatis* sp. but that neither the ipsdienol enantiomeric composition, nor the presence of lanierone greatly affects attraction. No additional information has been found on *Elacatis*. in the U.S.A. Several studies have captured *Lasconotus* spp., most often *Lasconotus subcostulatus* Kraus; however, numbers were often too low for analyses of pheromone response or no response was found (Seybold *et al.*, 1992; Dahlsten *et al.*, 2003). In California, Dahlsten *et al.* (2003) found no pattern of response to three different enantiomeric ratios of ipsdienol (97%, 50% and 25% (–)-ID) or the presence of lanierone, nor were there response differences due to time of year. Similar to *Elacatis* sp., this suggests that ipsdienol may be attractive but that neither the ipsdienol enantiomeric composition,

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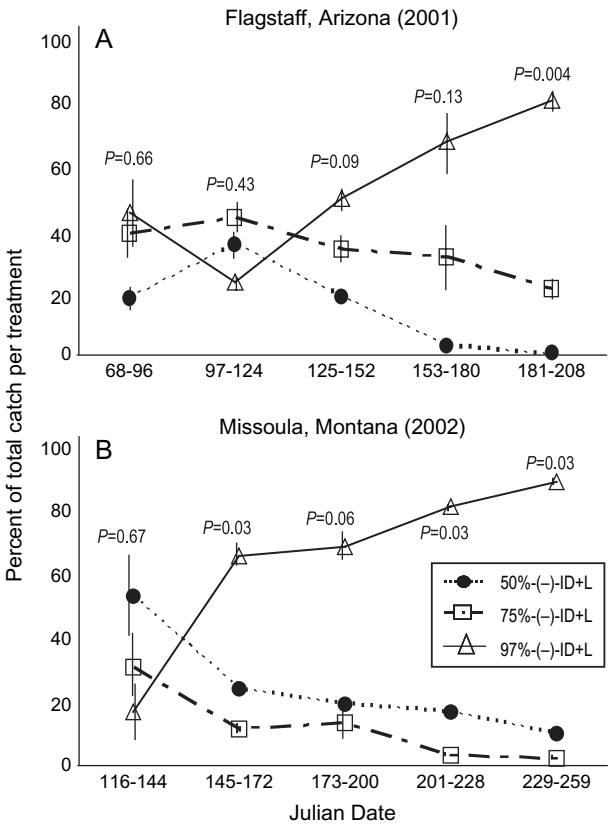

Figure 4 Pheromone response by *Ips pini* at five consecutive 1-month periods for Flagstaff, Arizona in 2001 (A) and Missoula, Montana in 2002 (B) using the three pheromone treatments of 50%, 75% and 97% (R)-(–)-ipsdienol (ID) plus lanierone (L). The multiple response permutation procedure for unreplicated block design tests did not reject similarity of treatments during the first month(s) ($P > 0.05$) but did reject their similarity in the final month(s) ($P < 0.05$). Multiple comparisons between individual treatments were not conducted due to the small sample size (four and three replicates in Arizona and Montana, respectively). Data are the mean ± SE.

nor the presence of lanierone has a significant effect on response.

The principle attractant pheromone for *O. latidens* has been identified as ipsenol (Miller *et al.*, 1991). Ipsdienol (racemic) with lanierone was also found to be attractive at least in northern Arizona (Gaylord *et al.*, 2006). However, in south-central BC. Miller *et al.* (1991) found that (*S*)-(+)-ipsdienol inhibited attraction of *O. latidens* to ipsenol while (*R*)-(–)-ipsdienol had no effect. No previously published information was found on the effect of lanierone on *O. latidens*. *Pityogenes carinulatus* in eastern Oregon was attracted to ipsdienol, with attraction significantly increased by addition of lanierone (Zhou *et al.*, 2001). In California, few individuals were captured but 38 of 40 were found in ipsdienol treatments with lanierone (Seybold *et al.*, 1992). Few studies report on *H. porosus*. However, in BC, Miller and Borden (2003) found this species was significantly more attracted to their only ipsdienol treatment (racemic) than to the blank trap.

Seasonal variation in pheromone response by *I. pini*

Our findings of seasonal shifts in response to pheromone blends are similar to results reported for New York (Teale & Lanier, 1991; Teale *et al.*, 1991), Wisconsin (Aukema *et al.*, 2000; Ayres *et al.*, 2001), and California (Dahlsten *et al.*, 2003) suggesting that *I. pini* in most geographic regions of the U.S.A. experiences a seasonal shift in pheromone response. In both Arizona and Montana, significant changes in response were due to increased attraction of beetles to the (*R*)-(–)-enantiomer of ipsdienol and the presence of lanierone. This shift occurred gradually over time, although it was unclear whether the gradual change was due to an ever increasing proportion of new (summer/FL2) generation of beetles and/or to an incremental change in the response of individual overwintering beetles. Furthermore, we found that relative proportions of either sex were not affected by season (spring versus summer flight), suggesting that seasonal shifts in pheromone response occurred similarly for both male and female beetles. We evaluated changes in response between the first two peak flights: spring and summer. Peak flights later in the season may have shown additional changes in response. For example, Ayres *et al.* (2001), trapping *I. pini* with racemic ipsdienol in Wisconsin, found that lanierone had a strong synergistic effect in spring and early summer, but that this effect was significantly reduced in late summer.

Factors affecting seasonal pheromone response in *I. pini*

Although genetics appears to be the principal mechanism explaining geographic variation in pheromone response and in gender differences within the geographic groups (Seybold, 1993; Seybold *et al.*, 1992, 1995; Cognato *et al.*, 1999; Domingue, *et al.*, 2006), forces creating seasonal shifts are less clearly understood. Shifts in seasonal pheromone response have been attributed to changes in intraspecific population density (Teale & Lanier, 1991) as well as changes in predation pressure (Aukema *et al.*, 2000).

In the present study, we did not find significant differences between spring (FL1) and summer (FL2) population densities of *I. pini*. We also noted that the principal predators in both regions selected for greater proportions of (*R*)-(–)-ipsdienol (e.g. *T. chlorodia*, *E. sphegeus*, *L. laqueatus*) or were synergized by the presence of lanierone (e.g. *E. lecontei*). Nevertheless, *I. pini* in both regions shifted their selection to the predator-preferred 97% (–)-ID+L treatment. Thus, seasonal shifts in our two study areas were toward the pheromone blends preferred by predators, which would not be expected if avoidance of predation was the principle force causing seasonal shift.

Shift in pheromone response by *I. pini* does appear, however, to be away from the pheromone blends preferred by the bark beetle species *O. latidens*, *P. carinulatus* and *H. porosus*. These three species have been found inhabiting logs of similar size and condition as those selected as host material by *I. pini* (B. Steed, personal observation).

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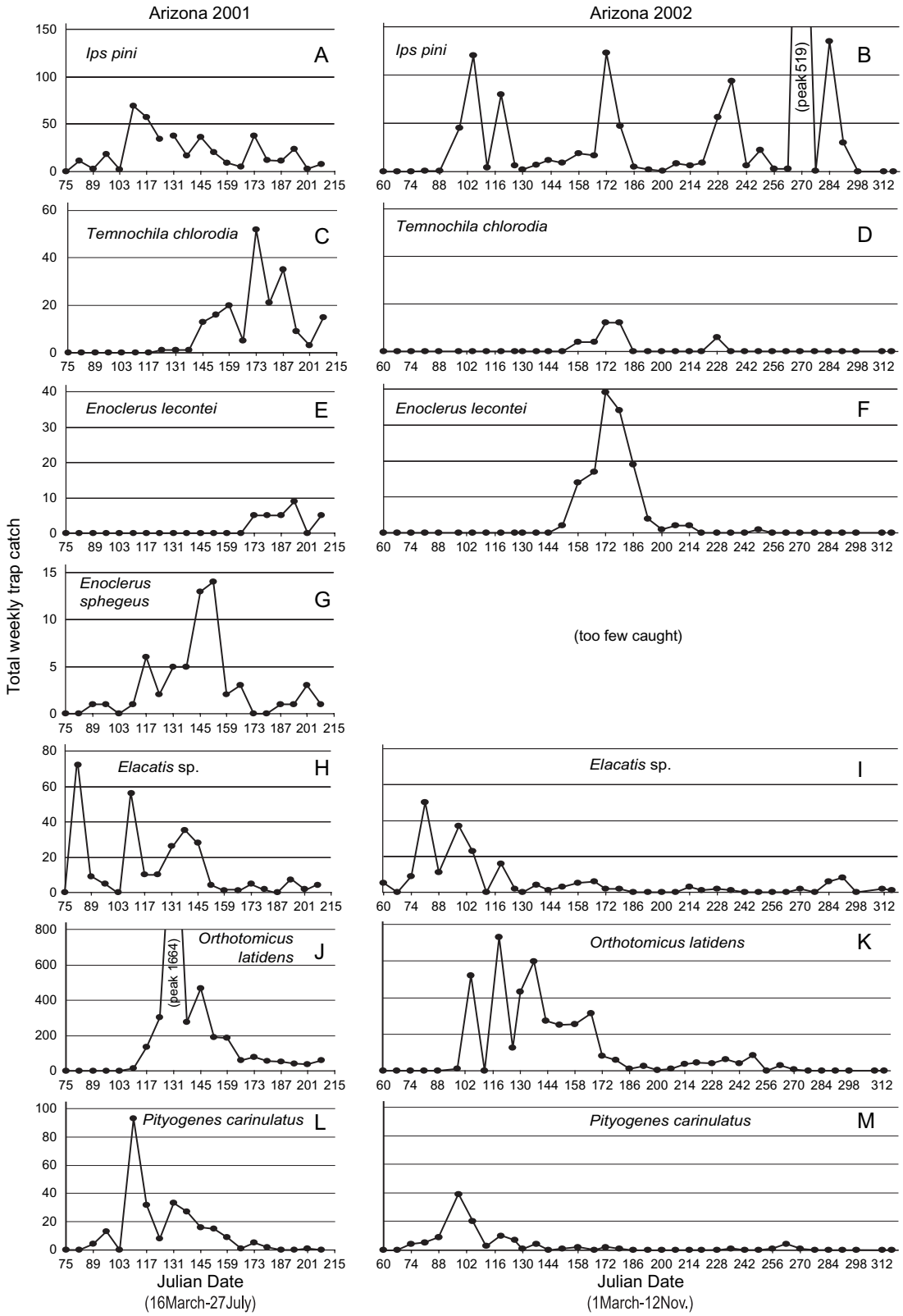

Figure 5 Seasonal abundance of *Ips pini* and its most common associates during 2001 (A, C, E, G, H, J, L) and 2002 (B, D, F, I, K, M) in Flagstaff, Arizona. Values for 2001 are based on the sum of 48 traps (four sites with 12 treatments) for each 1-week period. Values for 2002 are based on the sum of all available traps, ranging from 12 traps (three sites with four monitoring treatments) to 36 traps (three sites with 12 treatments). Catches at Julian dates 187–207, 2002; are from only one site so are lower than expected.

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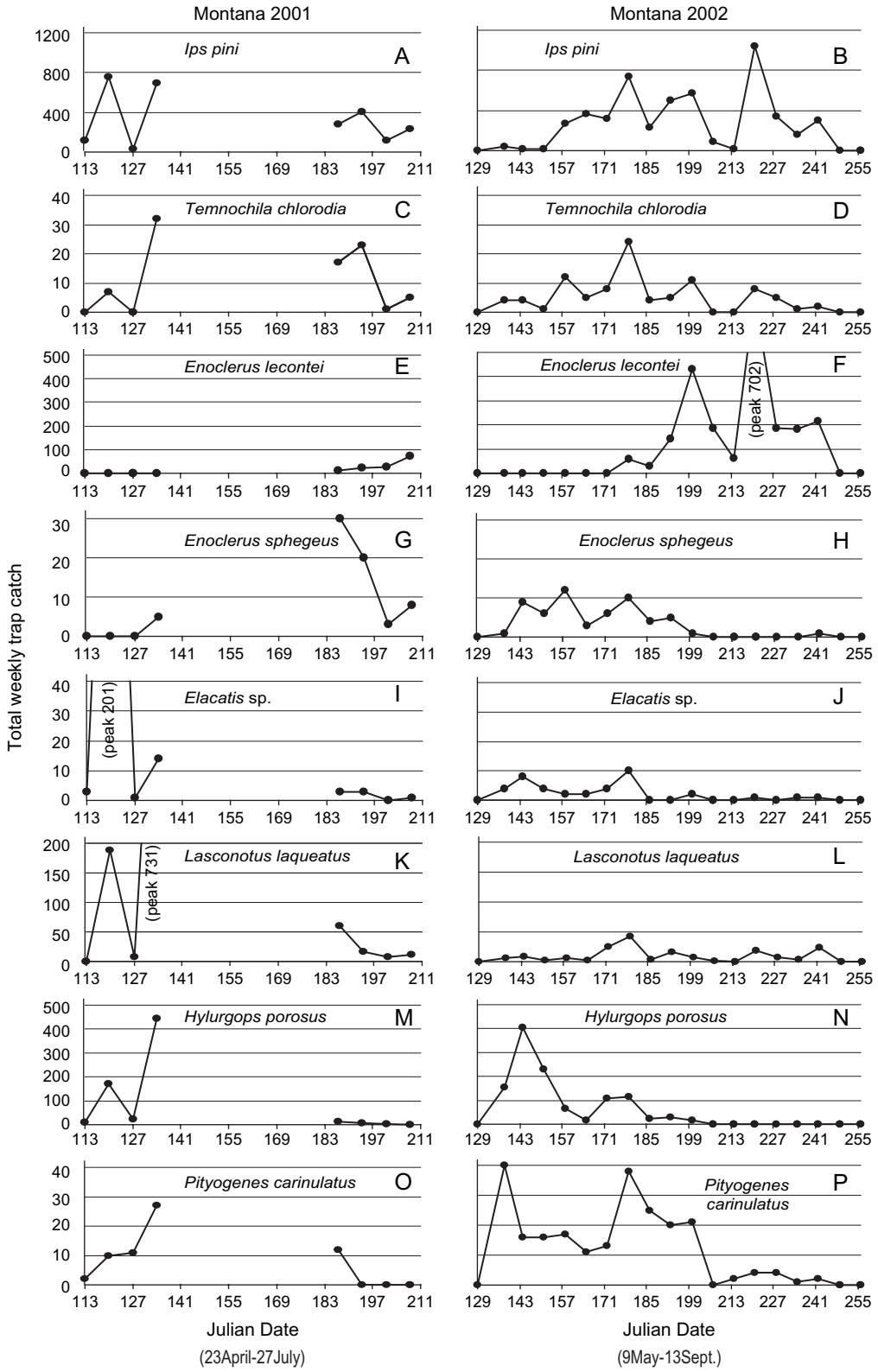

Figure 6 Seasonal abundance of *Ips pini* and its most common associates during 2001 (A, C, E, G, I, K, M, O) and 2002 (B, D, F, H, J, L, N, P) in Missoula, Montana. Values for 2001 are based on the sum of 48 traps (four sites with 12 treatments) for each 1-week period. Values for 2002 are based on the sum of all available traps (five monitoring traps plus 12 response traps, when deployed).

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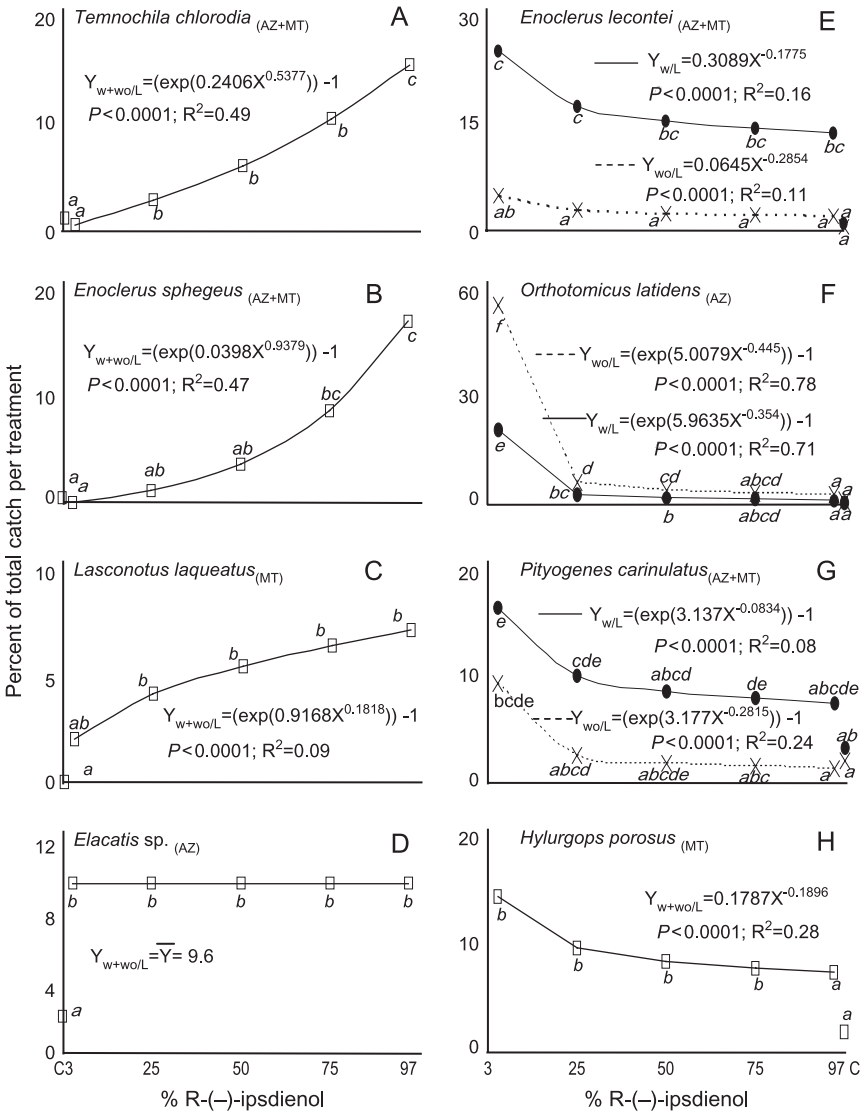

Figure 7 Response profiles for eight coleopteran associates of *Ips pini* in Flagstaff, Arizona (A, B, D, E, F, G) and Missoula, Montana (A, B, C, E, G, H). Although all regression equations were significant ($P < 0.05$), the low R^2 of 0.0013 for *Elacatis* sp. indicated that the best description of response was the average for all treatments ($\bar{Y}$). Additional analyses of treatment separations, including control treatments, were conducted on the untransformed data using multiple response permutation procedures for one-factor designs with simultaneous multiple comparisons. Similar letters (lower case) indicate treatments that were not significantly different ($\alpha \leq 0.05$).

In Arizona, we noted that *O. latidens* and *I. pini* avoided infesting the same log, but that *P. carinulatus* would co-infest logs with either *O. latidens* or *I. pini* (unpublished data from tests described in Steed & Wagner, 2004). Thus, the relationship of *I. pini* with *O. latidens* is probably competitive via resource exclusion, whereas relationships with *P. carinulatus* and *H. porosus* are less definite (see Light *et al.*, 1983; Miller & Borden, 1992; Poland & Borden, 1998a, b; Savoie *et al.,* 1998). These results support previous suggestions that maintenance of reproductive isolation and competitive interspecific interactions for space and food can be the most critical factors in evolution of pheromone use, including the formation of seasonal pheromone response (Birch, 1978; Birch *et al.*, 1980; Seybold *et al.*, 1995; Poland & Borden, 1998a, b).

We found that peak flights of several bark beetle species overlapped considerably with peak flights of *I. pini*, indicating that competitive interspecific interactions can not be fully avoided by temporal variation in seasonal flights. In addition, each species' flights appeared to vary greatly with temperature and precipitation (B. Steed, personal observation), making flight overlaps more unpredictable. Similar overlap of flights among *Ips* spp. (including *O. latidens*) was also noted by Ayres *et al.* (2001) and Gaylord *et al.* (2006), indicating that interspecific competition is as likely to be active during spring season as summer season. This suggests that perhaps it is more important to consider why the increased enantiomeric specificity observed during summer is not also found in spring.

Previous research has found that response to pheromones in field-collected bark beetles decreases markedly during

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adverse seasons when beetles are not normally active (Birch, 1978; Teale & Lanier, 1991). Birch (1974) demonstrated that not only did female *I. pini* response to pheromone (male-produced frass) decrease during winter diapause, but also that male production of pheromone (in the frass) was simultaneously reduced. Teale and Lanier (1991) suggested that decreased pheromone use in bark beetles may be related to a decrease in reproductive activity. Studies on mechanisms of pheromone production have found that juvenile hormone (JH) plays an important role in stimulating and regulating pheromone production (Borden *et al*., 1969; Vanderwel, 1994; Tillman *et al.*, 1998, 1999; Seybold *et al.*, 2000). Changes in JH titre of bark beetles may be triggered by changes in beetle physiology (e.g. distended abdomen from feeding, expelled air bubble, or exercise) (Atkins, 1966; Bennett & Borden, 1971; Hagen & Atkins, 1975; Gast *et al.*, 1993; Wallin & Raffa, 2002), in the host material (e.g. presence/absence, monoterpene content) (Renwick *et al.*, 1976; Klimetzek & Francke, 1980; Wallin & Raffa, 2002), in intraspecific density (Teale & Lanier, 1991; Teale *et al.*, 1991; Wallin & Raffa, 2002), in interspecific interactions with predators (Raffa & Klepzig, 1989; Raffa & Dahlsten, 1995; Aukema & Raffa, 2000; Aukema *et al.*, 2000) or competitors (Birch & Wood, 1975; Birch *et al.*, 1980; Light *et al.*, 1983), in changes in type or quantity of symbionts (Hunt & Borden, 1990), or possibly in the abiotic environment (e.g. temperature, photoperiod). Further discussion of these factors is provided in Vanderwel (1994) and Seybold *et al.* (2000). Thus, overwintering (diapausing) populations may experience a lag period in their development of pheromone sensitivity after a period of insensitivity (Birch, 1974), resulting in less specific pheromone preference in the spring flight. Similarly, a decrease in pheromone specificity would be expected as beetles begin to move back into the overwintering stage (Teale and Lanier, 1991; Ayres *et al.*, 2001).

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