Responses of *Ips pini* (Say), *Pityogenes knechteli* Swaine and Associated Beetles (Coleoptera) to Host Monoterpenes in Stands of Lodgepole Pine[1]

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J. Entomol. Sci. 38(4): 602–611 (October 2003)

Abstract We conducted seven experiments in stands of mature lodgepole pine in southern British Columbia to elucidate the role of host volatiles in the semiochemical ecology of the pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae), with particular reference to the behavioral responses of predators and competing species of bark beetles. Our results demonstrated that the attraction of *Ips pini* and the bark beetle predators *Lasconotus complex* LeConte (Colydiidae), *Thanasimus undatulus* (Say) (Cleridae) and a *Corticeus* sp. (Tenebrionidae) were increased by 3-carene. In contrast, attraction of the bark beetle *Pityogenes knechteli* Swaine (Scolytidae) to ipsdienol was interrupted by 3-carene and α-pinene. Attraction of *L. complex* to ipsdienol was increased by γ-terpinene, a compound attractive to the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Scolytidae). Terpinolene interrupted the attraction of *I. pini* to ipsdienol.

Key Words *Ips pini, Pityogenes knechteli, Dendroctonus ponderosae,* 3-carene, γ-terpinene, β-pinene, α-pinene, terpinolene, ipsdienol

The pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae), is an occasional forest pest of pine stands in western North America (Furniss and Carolin 1980). In British Columbia, adult *I. pini* aggregate on suitable hosts and construct brood galleries under the bark of pines, particularly lodgepole, *Pinus contorta* var. *latifolia* Engelm., and ponderosa pine, *P. ponderosa* Laws. (Bright 1976). The formation of these aggregations is facilitated by semiochemicals such as the pheromones, ipsdienol and lanierone (Miller et al. 1997), and various host kairomones (Miller and Borden 1990, 2000).

Monoterpenes are abundant in the phloem and xylem tissues of pines throughout North America (Mirov 1961, Smith 2000), serving as host kairomones for numerous species of bark beetles (Borden 1982, Byers 1989). In stands of lodgepole pine, *I. pini* were attracted to traps baited with the host compound, β-phellandrene, with and without the pheromone, ipsdienol (Miller and Borden 1990). Attraction of *I. pini* to ipsdienol-baited traps was increased by the monoterpenes β-phellandrene, 3-carene and β-pinene but interrupted by myrcene and terpinolene, all in a dose-dependent fashion (Miller and Borden 2000).

[1]Received 20 February 2003; accepted for publication 25 May 2003.

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However, 3-carene and β-pinene failed to increase catches of *I. pini* to traps baited with ipsdienol when one of the other treatments was β-phellandrene with ipsdienol (Miller and Borden 1990). Of seven monoterpenes tested by Miller and Borden (2000), only β-phellandrene has been tested by itself (Miller and Borden 1990). Therefore, our objective was to determine the behavioral responses of *I. pini* to five monoterpenes commonly found in the sapwood of lodgepole pine (Shrimpton 1973, Smith 2000), alone and in combination with the pheromone, ipsdienol.

Our secondary objective was to determine the response of predators and associated bark beetles to the same treatments. More than 50 species of bark and ambrosia beetles breed in pines in British Columbia (Bright 1976), yet pheromones and kairomones have been identified for only a few species (Lindgren and Borden 1989). Smith (2000) found considerable variation of xylem monoterpene compositions within and between populations of lodgepole and ponderosa pine in western North America, suggesting significant opportunities for selection pressures within and between populations based simply on host chemistry. Yet, the effect of host physiology on reproductive success of bark beetles has been limited to studies with *Dendroctonus ponderosae* Hopkins (Scolytidae) and *I. pini* with most studies of competition in stands of lodgepole and ponderosa pine involving only three species: *D. ponderosae, I. pini* and *Pityogenes knechteli* Swaine. There are many more species interactions that need to be identified to better understand the evolution and maintenance of pine bark beetle semiochemical ecology.

Materials and Methods

Chemicals and release devices. Δ-(+)-3-Carene, (–)-α-pinene, (–)-β-pinene and γ-terpinene (chemical purities >95%) were obtained from Aldrich Chemical Co. (Milwaukee, WI); whereas, terpinolene (chemical purity 94%) was obtained from D. Vanderwel (Department of Chemistry, Simon Fraser University). Phero Tech Inc. (Delta, BC) supplied bubblecap devices that release (±)-ipsdienol (chemical purity 98%) at a rate of 0.2 to 0.3 mg/d at 24°C (determined by collection of volatiles on Porapak-Q and analysis by capillary gas chromatography). The monoterpenes were released separately from sealed polyethylene transfer pipettes (3.5 mL) (Saint-Amand Mfg. Co., San Fernando, CA) at rates of about 184, 143, 121, 294, and 343 mg/d at 27 to 30°C for 3-carene, α-pinene, β-pinene, γ-terpinene and terpinolene, respectively (determined by weight loss).

Experimental design. In 1989, five concurrent experiments were conducted in stands of mature lodgepole pine near Princeton, BC. In each experiment, 8 to 10 replicates of four 12-unit, multiple-funnel traps (Lindgren 1983) (PheroTech Inc.) were set in grids of 2 × 2. Replicate grids were spaced >200 m apart, and traps were spaced 10 to 15 m apart within each replicate. Each trap, measuring approximately 1 m in height, was suspended between trees by rope such that the top funnel of each trap was 1.3 to 1.5 m above ground. No trap was within 2 m of any tree.

Experiment 1 ran from 20 August to 22 September with the following treatments, randomly assigned within each replicate: (1) blank; (2) 3-carene alone; (3) ipsdienol alone; and (4) 3-carene and ipsdienol. Experiments 2 to 5 were similar to Experiment 1 but with 3-carene replaced by α-pinene, β-pinene, γ-terpinene and terpinolene, respectively, and set from 24 August to 22 September, 30 August to 26 September, 20 July to 2 September and 10 to 22 September, respectively.

Catches were tallied for total numbers of *I. pini* and the bark beetles *Pityogenes*

knechteli, Dendroctonus ponderosae, Hylurgops porosus (LeConte), as well as *Lasconotus complex* LeConte (Colydiidae), *Thanasimus undatulus* (Say) (Cleridae) and *Corticeus* Piller & Mitterpacher sp. (Tenebrionidae). *Thanasimus undatulus* is a common bark beetle predator with adults feeding on bark beetle adults and larvae feeding on bark beetle larvae (Dahlsten 1982). The larvae of various species of *Lasconotus* and *Corticeus* are considered to be predaceous on bark beetle larvae (Furniss and Carolin 1980, Bowers and Borden 1992). Sexes of *I. pini* in random subsamples (n = 30 to 50 beetles) for each trap catch were determined using declivital characters (Lanier and Cameron 1969). Voucher specimens were deposited at the Entomology Museum, Simon Fraser University.

Statistical analyses. Trap catch data were analyzed with the SYSTAT (1998) statistical package. Data were transformed by $\log_{10}(y + 1)$ to remove heteroscedasticity and subjected to full-factoral two-way analysis of variance (ANOVA) using monoterpene, ipsdienol and the interaction between monoterpene and ipsdienol as model factors. Synergistic effects of individual monoterpenes on attraction of beetles to ipsdienol were determined as in Miller et al. (1997), with a significant interaction term between a monoterpene and ipsdienol in the ANOVA indicating that the proportional increases due to both factors were not additive. Sex ratio data for *I. pini* were analyzed by *t* test.

Results

Ipsdienol and some monoterpenes had significant effects on catches of *I. pini* in all five experiments (Table 1, Figs. 1A-E). 3-Carene significantly enhanced catches of *I. pini* in funnel traps ($F = 5.46$; df = 1, 36; $P = 0.025$), although not in a synergistic fashion with ipsdienol ($F = 0.02$; df = 1, 36; $P = 0.883$). Catches of *I. pini* were not affected by β-pinene ($F = 0.62$; df = 1, 29; $P = 0.439$), γ-terpinene ($F = 0.16$; df = 1, 28; $P = 0.690$) or α-pinene ($F = 0.09$; df = 1, 36; $P = 0.766$). Terpinolene significantly reduced trap catches of *I. pini* ($F = 6.25$; df = 1, 36; $P = 0.017$).

Attraction of *P. knechteli* was strongly affected by ipsdienol, as in Savoie et al. (1998), as well as several monoterpenes (Table 2, Figs. 1F-J). Attraction of *P. knechteli* to ipsdienol-baited traps was interrupted by 3-carene ($F = 7.96$; df = 1, 20; $P = 0.011$) and α-pinene ($F = 6.13$; df = 1, 365; $P = 0.018$); whereas, β-pinene significantly increased catches of beetles ($F = 0.07$; df = 1, 11; $P = 0.035$). Catches of *P. knechteli*

Table 1. Significance levels for analyses of variance (ANOVAs) on total trap catches of *Ips pini* in multiple-funnel traps baited with ipsdienol and/or one of five monoterpenes

Monoterpene	Monoterpene (M)	Ipsdienol (Id)	M * Id
3-Carene	0.025	<0.001	0.883
β-Pinene	0.439	<0.001	0.976
γ-Terpinene	0.690	<0.001	0.181
α-Pinene	0.766	<0.001	0.836
Terpinolene	0.017	<0.001	0.160

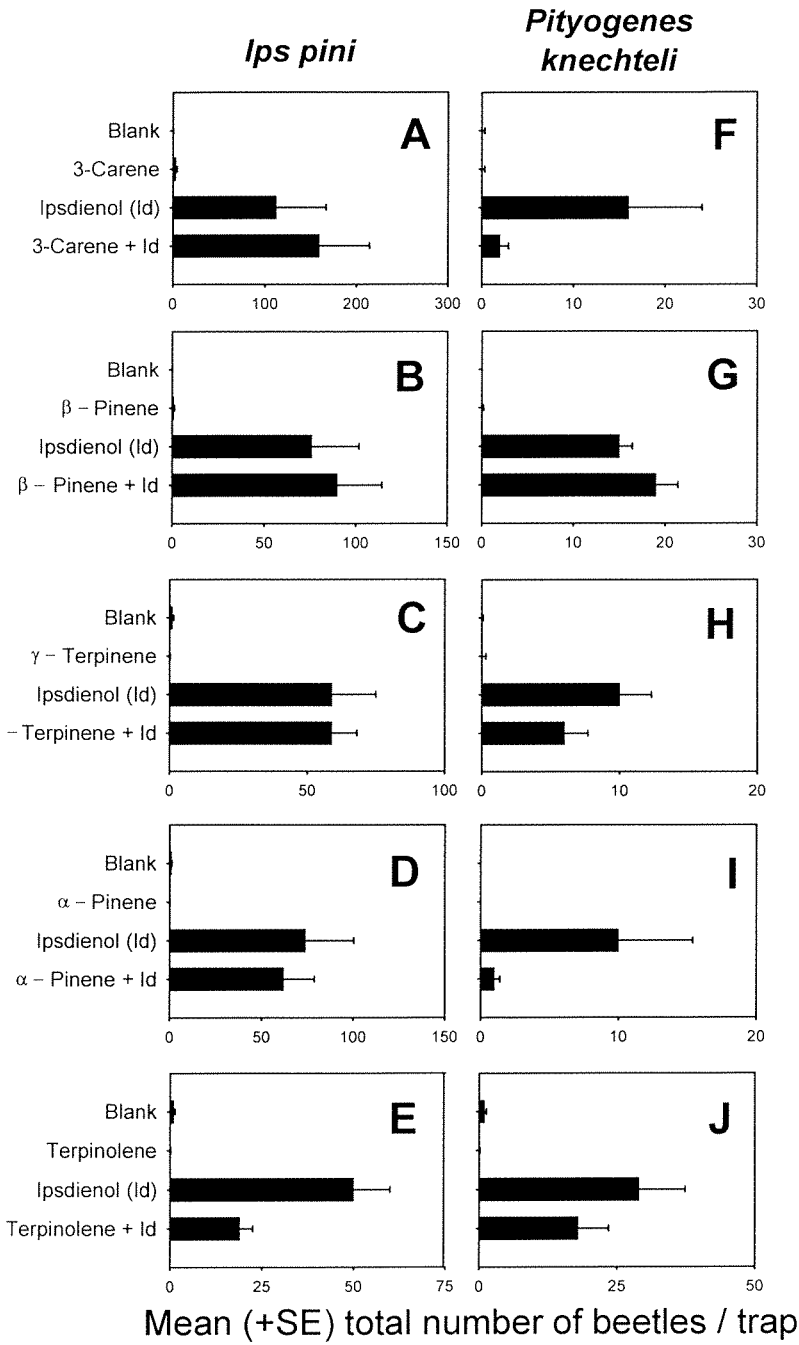

Fig. 1. The effect of various monoterpenes, with or without ipsdienol, on the total mean catches of *Ips pini* (A-E) and *Pityogenes knechteli* (F-J) in multiple-

Table 2. Significance levels for analyses of variance (ANOVAs) on total trap catches of *Pityogenes knechteli, Dendroctonus ponderosae* and *Hylurgops porosus* in multiple-funnel traps baited with ipsdienol and/or one of five monoterpenes

Monoterpene	Monoterpene (M)	Ipsdienol (Id)	M * Id
Pityogenes knechteli			
3-Carene	0.011	<0.001	0.011
β-Pinene	0.035	<0.001	0.775
γ-Terpinene	0.372	<0.001	0.267
α-Pinene	0.018	<0.001	0.018
Terpinolene	0.203	<0.001	0.636
Dendroctonus ponderosae			
γ-Terpinene	0.006	0.917	0.287
Hylurgops porosus			
γ-Terpinene	0.180	0.002	0.321

were not affected by γ-terpinene (F = 0.825; df = 1, 28; P = 0.372) or terpinolene (F = 1.68; df = 1, 36; P = 0.203).

In Experiment 4, γ-terpinene increased catches of *D. ponderosae* to funnel traps (F = 8.81; df = 1, 28; P = 0.006); whereas, ipsdienol had no effect (F = 0.01; df = 1, 28; P = 0.917) (Table 2, Fig. 2A). In contrast, catches of *H. porosus* were unaffected by γ-terpinene (F = 1.93; df = 1, 20; P = 0.180) but strongly affected by ipsdienol (F = 12.77; df = 1, 20; P = 0.002 (Table 2, Fig. 2B).

The cylindrical bark beetle, *L. complex,* was significantly attracted to funnel traps

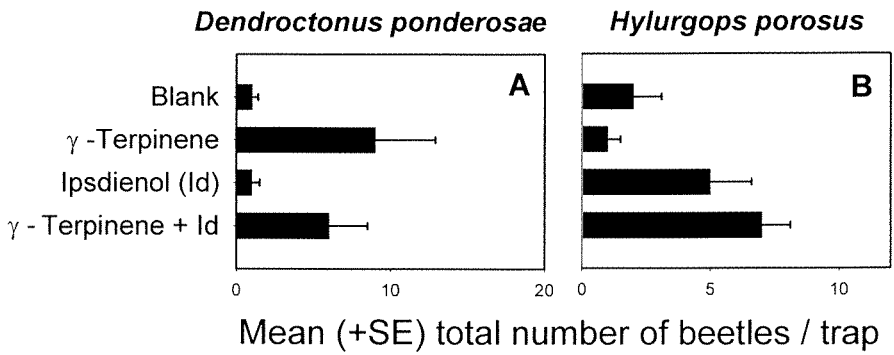

Fig. 2. The effect of γ-terpinene, with or without ipsdienol, on the total mean catches of *Dendroctonus ponderosae* (A) and *Hylurgops porosus* (B) in multiple-funnel traps near Princeton, BC, in 1989.

by ipsdienol in all five experiments as well as several monoterpenes (Table 3, Figs. 3A-E). Catches of *L. complex* were significantly increased by 3-carene ($F = 71.03$; df = 1, 36; $P < 0.001$) in an additive fashion ($F = 0.64$; df = 1, 36; $P = 0.431$). In contrast, catches of *L. complex* were unaffected by γ-terpinene alone ($F = 2.41$; df = 1, 28; $P = 0.132$) but significantly increased by γ-terpinene in a synergistic fashion with ipsdienol ($F = 6.34$; df = 1, 28; $P = 0.018$). *Lasconotus complex* was not affected by α-pinene ($F = 0.87$; df = 1, 36; $P = 0.358$), β-pinene ($F = 0.05$; df = 1, 18; $P = 0.827$) or terpinolene ($F = 0.10$; df = 1, 28; $P = 0.450$).

The checkered beetle, *T. undatulus,* and a darkling beetle, *Corticeus* sp., were significantly attracted to ipsdienol ($F = 41.13$; df = 1, 24; $P < 0.001$ and $F = 69.20$; df = 1, 32; $P < 0.001$, respectively) (Table 3, Figs. 3F-G). Catches to ipsdienol-baited traps were significantly synergised by 3-carene for both *T. undatulus* ($F = 6.73$; df = 1, 24; $P = 0.016$) and *Corticeus* sp. ($F = 27.56$; df = 1, 32; $P < 0.001$).

Discussion

Specificity in responses to monoterpenes was evident with both *I. pini* and *P. knechteli* (Fig. 1). 3-Carene increased attraction of *I. pini* but interrupted attraction of *P. knechteli; P. knechteli* was attracted to β-pinene which had no effect on *I. pini. Pityogenes knechteli* typically breeds in lodgepole pine material of smaller diameter than that used by *I. pini,* although galleries can readily intermingle (Poland and Borden 1994). Attraction of *P. knechteli* to its pheromone, ipsdienol (Savoie et al. 1998), was interrupted by 3-carene and α-pinene (Figs. 1F and 1I). 3-Carene and α-pinene are two dominant monoterpenes in ponderosa pine and western white pine, *P. monticola* Dougl *ex* D. Don respectively (Mirov 1961, Smith 2000), which are utilized by *Pityogenes carinulatus* (LeConte) and *P. fossifrons* (LeConte), respectively. β-Pinene

Table 3. Significance levels for analyses of variance (ANOVAs) on total trap catches of *Lasconotus complex, Thanasimus undatulus* and *Corticeus* sp in multiple-funnel traps baited with ipsdienol and/or one of five monoterpenes

Monoterpene	Monoterpene (M)	Ipsdienol (Id)	M * Id
Lasconotus complex			
3-Carene	<0.001	<0.001	0.431
β-Pinene	0.827	<0.001	0.562
γ-Terpinene	0.132	<0.001	0.018
α-Pinene	0.358	<0.001	0.547
Terpinolene	0.450	<0.001	0.450
Thanasimus undatulus			
3-Carene	0.016	<0.001	0.016
Corticeus sp			
3-Carene	<0.001	<0.001	<0.001

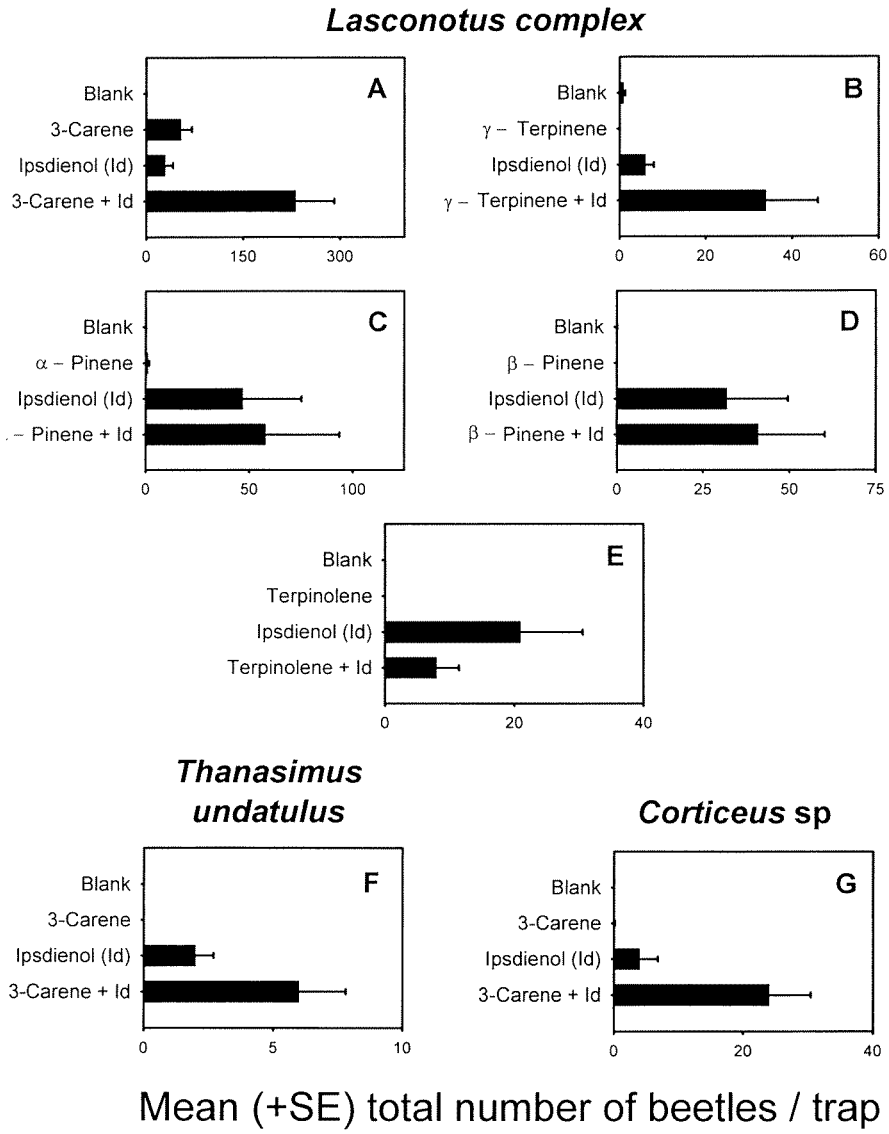

Fig. 3. The effect of various monoterpenes, with or without ipsdienol, on the total mean catches of *Lasconotus complex* (A-E), *Thanasimus undatulus* (F) and a *Corticeus* sp. (G) in multiple-funnel traps near Princeton, BC, in 1989.

is second only to β-phellandrene in abundance in lodgepole pine (Smith 2000). *Ips pini,* on the other hand, breeds freely in both lodgepole and ponderosa pines (Bright 1976). *Ips montanus* (Eichhoff) is the dominant ips species in western white pine (Bright 1976). Monoterpene specificity may help to maintain separation of these spe-

cies, even on the same host. Shrimpton (1973) found substantial variation in quantities of 3-carene, β-pinene and terpinolene between resinous and non-resinous lodgepole pine.

The monoterpene, γ-terpinene had little, if any effect, on *I. pini* (Fig. 1C), *P. knechteli* (Fig. 1H) or *H. porosus* (Fig. 2B) but was significantly attractive to *D. ponderosae* (Fig. 2A). Miller and Borden (2000) found that γ-terpinene released at rates comparable to those used in this study significantly increased attraction of *D. ponderosae* to its pheromones, *exo*-brevicomin, and *trans*- and *cis*-verbenol. γ-Terpinene may be an important kairomone for *D. ponderosae,* along with other monoterpenes such as myrcene, 3-carene, β-phellandrene, and α- and β-pinene (Lindgren and Borden 1989). However, only γ-terpinene has been found to be attractive to *D. ponderosae* alone (Fig. 2A) and may serve a role in initial selection of suitable host trees, particularly at endemic population levels. γ-Terpinene has not been previously tested against *D. ponderosae.*

Our results are not consistent with those of Miller and Borden (2000) in demonstrating that terpinolene interrupts attraction of *I. pini* to ipsdienol (Fig. 1E). As in Miller and Borden (2000), but not Miller and Borden (1990), 3-carene increased catches of beetles in ipsdienol-baited traps (Fig. 1A). β-Pinene increased catches of *I. pini* to ipsdienol in Miller and Borden (2000) but not in this study (Fig. 1B) or Miller and Borden (1990).

The variation in responses of *I. pini* to 3-carene between the three studies may relate to variation in the range of suitable host species for *I. pini.* Lodgepole and ponderosa pines are the two most common hosts for *I. pini* in British Columbia (Furniss and Carolin 1980) with the range of lodgepole pine far exceeding that of ponderosa pine (Farrar 1995). 3-Carene is the most abundant monoterpene in ponderosa pine, accounting for 51 to 64% of total monoterpenes on average in the northwest (Mirov 1961, Smith 2000). The principal monoterpene in lodgepole pine is β-phellandrene with 3-carene accounting for only 7 to 15% of total monoterpenes (Shrimpton 1973, Smith 2000). The present study and that of Miller and Borden (1990) were conducted in southern British Columbia (within the range of ponderosa pine); whereas, the third study (Miller and Borden 2000) was conducted in central British Columbia (well outside the range of ponderosa pine).

Variation in responses may also relate to differences in release rates of devices used in the three studies. In Miller and Borden (1990), the release rates for β-pinene, 3-carene and terpinolene were about 9.3, 22.9 and 29.5 mg/day, respectively, at 27°C. These rates were considerably lower than the rates used in the present study (121, 181, and 343 mg/day, respectively, at 27°C). In contrast, Miller and Borden (2000) used devices with release rates as high as 1199, 1217, and 2065 mg/day at 27-30° for β-pinene, 3-carene and terpinolene, respectively. The threshold for responses of *I. pini* may not be the same for all monoterpenes.

Our results and those of Miller and Borden (1990, 2000) provide some of the best evidence of tri-trophic level interactions in the semiochemical ecology of northern pine bark beetles, with both bark beetles and their predators using host odors emanating from pine trees. In Texas, Dixon and Payne (1980) found that attraction of several species of predators of the southern pine bark beetle, *Dendroctonus frontalis* Zimm., to the pheromone frontalin were increased by turpentine volatiles. In Wisconsin, Ergilbin and Raffa (2001) demonstrated that attraction of the bark beetle predators *Thanasimus dubius* (F.) (Cleridae), *Platysoma cylindrica* (Paykull) (Coleoptera: Histeridae) and *Corticeus paralellus* Melsh (Tenebrionidae) to the pheromones ipsenol,

ipsdienol and lanierone were all increased by α-pinene; 3-carene interrupted the response of *C. paralellus* to the pheromones ipsdienol and lanierone. Other studies, such as Bowers and Borden (1992), have documented the attraction of predators to pheromones produced by bark beetles but not to host pine odors.

Further advances in semiochemical ecology of bark beetles will require studies on tri-trophic level associations for all bark beetles, pine hosts, and their predators and associates. We need more studies on the association of host volatiles with preferred host association and the effects of variation in host monoterpene composition on reproductive success, particularly for endemic populations of bark beetles.

Acknowledgments

We thank T. M. Poland, P. Spaine, K. O. Britton and two anonymous reviewers for comments on the manuscript. L. J. Chong, J. Gandy, C. Matteau, and L. Wheeler provided field and laboratory assistance. This research was supported by the H.R. Macmillan Family Fellowship Fund, the Science Council of British Columbia and the Natural Sciences and Engineering Research Council of Canada.

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