Limonene: Attractant Kairomone for White Pine Cone Beetles (Coleoptera: Scolytidae) in an Eastern White Pine Seed Orchard in Western North Carolina

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ABSTRACT I report on the attraction of the white pine cone beetle, *Conophthorus coniperda* (Schwarz) (Coleoptera: Scolytidae), to traps baited with the host monoterpene limonene in western North Carolina. Both (+)- and (−)-limonene attracted male and female cone beetles to Japanese beetle traps in an eastern white pine, *Pinus strobus* L., seed orchard near Murphy, NC. Catches of cone beetles were directly proportional to the release rate of (−)-limonene; (+)-limonene was not tested for dose response. Attraction of cone beetles to the pheromone (±)-*trans*-pityol was increased significantly by both enantiomers of limonene. In all experiments, catches of *C. coniperda* were strongly male biased with no treatment effect on sex ratio. (−)-Limonene had no effect on trap catches of the predator *Enoclerus nigripes* (Say) to pityol, whereas (+)-limonene interrupted the attraction of *E. nigripes* to traps baited with pityol. Of six monoterpenes commonly found in white pine cones, only (−)-α-pinene elicited attraction of *E. nigripes* to Japanese beetle traps.

KEY WORDS *Conophthorus coniperda*, Scolytidae, kairomone, Cleridae, *Enoclerus nigripes*

Some species of insects pose significant threats to the production of valuable seed crops in conifer seed orchards throughout North America (Hedlin et al. 1981). In eastern white pine, *Pinus strobus* L., the white pine cone beetle, *Conophthorus coniperda* (Schwarz) (Coleoptera: Scolytidae), can cause seed losses of up to 100% within seed orchards (Graber 1964, Odera 1968, Morgan and Mailu 1976, DeBarr et al. 1982). Such losses can be particularly acute as seed orchards are generally limited to only a handful per region. The loss of cones and seed also can impact natural regeneration of eastern white pine after forest fires (Godwin and ODell 1965). Prescribed fire can be used to reduce populations of cone beetles within seed orchards (Wade et al. 1989). However, such efforts are constrained by environmental conditions, susceptibility of trees to mortality from scorch and costs of application (Wade et al. 1989). Control tactics using semiochemicals could provide cost-effective protection of seed resources with minimal environmental costs (Rappaport et al. 2000, Trudel et al. 2004).

Typically, white pine cone beetles emerge from fallen cones of eastern white pine in the spring and attack fresh cones in the tree canopy with attacks initiated by female beetles (Godwin and ODell 1965). Females produce the pheromone, (+)-*trans*-pityol, which attracts predominantly male beetles; (−)-*trans*-pityol has no effect on the response of *C. coniperda* (Birgersson et al. 1995). Male beetles produce conophthorin, which interrupts attraction of conspecific males (Birgersson et al. 1995, de Groot et al. 1998). The same is true for the red pine cone beetle, *Conophthorus resinosae* Hopkins, in seed orchards of red pine, *Pinus resinosa* Ait., and the ponderosa pine cone beetle, *Conophthorus ponderosae* Hopkins, in stands of ponderosa pine, *Pinus ponderosa* Douglas ex P. & C. Laws., and western white pine, *Pinus monticola* Dougl. ex D. Don (Pierce et al. 1995, de Groot and DeBarr 2000, Miller et al. 2000, Rappaport et al. 2000).

Host odors play an important role in the chemical ecology of cone beetles. The common pine monoterpene, (−)-α-pinene, synergizes attraction of male and female *C. coniperda* and *C. ponderosae* to pityol-baited traps (de Groot et al. 1998; de Groot and DeBarr 2000; Miller et al. 2000, 2003) but not *C. resinosae* (de Groot et al. 2002). Moreover, de Groot et al. (2002) found that attraction of *C. resinosae* to pityol was interrupted by (+)-α-pinene.

To date, catches of *C. coniperda* in traps with attractants have been largely male biased with no strong attractant yet identified primarily for females. de Groot et al. (1991) found that female *C. coniperda* were attracted to volatiles emitted by eastern white pine cones, *Pinus strobus* L., in both laboratory walking bioassays and field trapping studies. Miller et al. (2003) found that the monoterpene blends released by un-

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attacked cones of several eastern white pines in western North Carolina in March differed in composition from blends released by cones from the same trees in May. Specifically, the relative proportion of limonene was high in volatiles from March cones but low in volatiles from May cones in the same year. Overwintering adult cone beetles typically start to emerge from fallen cones in March in western North Carolina (D.R.M., unpublished data).

My objective was to test both enantiomers of the host monoterpene, limonene, as attractant kairomones for *C. coniperda* in an eastern white pine seed orchard in western North Carolina. The ultimate goal is to develop a lure that is significantly attractive to female beetles, thereby providing an opportunity for a mass-trapping program in white pine seed orchards (Rappaport et al. 2000, Trudel et al. 2004). The effects of (+)- and (−)-limonene on the responses of the beetle predator *Enoclerus nigripes* (Say) (Coleoptera: Cleridae) also were determined. Previously, Miller et al. (2003) found that attraction of *E. nigripes* (misreported as *Thanasimus dubius* L.) was strongly influenced by (−)-α-pinene.

Materials and Methods

Chemicals and Release Devices. Pherotech International Inc. (Delta, British Columbia, Canada) supplied polyethylene bubblecap lures containing 40 mg of (±)-*trans*-pityol (hereafter pityol) (chemical purity >98%), released at ≈0.14 mg/d at 23°C, and tested previously for efficacy (de Groot and DeBarr 1998). Sigma-Aldrich Co. (Milwaukee, WI) supplied (−)-α-pinene, (−)-β-pinene, Δ-(+)-3-carene, myrcene, (−)-limonene, and (+)-limonene. The chemical purity of myrcene was 90%, whereas the chemical purities of the remaining monoterpenes were >95% (enantiomeric purities >97%). In experiments 1–4, (−)-α-pinene, (−)-β-pinene, Δ-(+)-3-carene, myrcene, (−)-limonene, and (+)-limonene were released separately from closed 15-ml low-density polyethylene (LDPE) Boston-round screw-cap bottles (O. Berk Co., Union, NJ) (device 1) at rates of ≈105, 100, 235, 170, 295, and 295 mg/d (at 23–25°C in still air), respectively. In experiment 5, several additional devices were used to release (−)-limonene at various rates: closed 1.8-ml polyethylene micro-centrifuge tubes (Evergreen Scientific, Los Angeles, CA) (device 2), closed 8-ml Nalgene wide-mouth screw-cap bottle (Cole-Palmer Instrument Co., Vernon Hills, IL) (device 3), and closed 15-ml LDPE screw-cap bottle (Pherotech International Inc.) (device 4). The release rates of limonene from these devices were ≈10, 150, and 610 mg/d (at 23–25°C in still air), respectively.

Experimental Design. Five experiments were conducted in orchards of eastern white pine at the Beech Creek Seed Orchard (USDA Forest Service, Nantahala National Forest) near Murphy, NC, over a 3-yr period (2002–2004). The trees were ≈40 yr of age and spaced at 10 by 10 m. The mean ± SE height and diameter (at breast height) of trees were 19.5 ± 0.39 m and 50.0 ± 1.06 cm, respectively. Almost all previous work on the chemical ecology of white pine cone beetles in western North Carolina has been conducted at the Beech Creek Seed Orchard (de Groot et al. 1991, 1998, Birgersson et al. 1995, de Groot and DeBarr 1998, 2000; Rappaport et al. 2000; Miller et al. 2003).

In each experiment, yellow Japanese beetle traps (Trécé Inc., Salinas, CA), as recommended by de Groot and DeBarr (1998), were suspended by ropes and pulleys in the crowns of mature eastern white pines (15–25 m in height), adjacent to cones, with only one trap per tree. The distance between trees varied from 10 to 30 m. Beetles were collected in 500-ml plastic Mason jars, filled with ≈200 ml of pink propylene glycol solution (Peak RV and Marine Antifreeze, Old World Industries Inc., Northbrook, IL) as a killing and preservation agent. Trap catches were collected at 2–3-wk intervals with the glycol solution replaced on each occasion. Sexes of captured cone beetles in subsamples ($n = 20–30$) were determined by examination of the seventh and eighth abdominal tergites (Kinzer and Ridgill 1972, Santiago-Blay and Young 1995). Voucher specimens of *C. coniperda* and *E. nigripes* were deposited in the Entomology Collection, Museum of Natural History, University of Georgia (Athens, GA). Identifications of *C. coniperda* and *E. nigripes* were confirmed by comparison to specimens in the Entomology Collection.

Experiment 1. This experiment was a broad survey on the attractiveness of six monoterpenes typically released from cones of eastern white pine (Miller et al. 2003). The trapping period was 28 March–30 May 2002. Each of 75 traps was randomly assigned one of the following treatments: 1) unbaited control; 2) (−)-α-pinene; 3) (−)-β-pinene; 4) (+)-3-carene; 5) myrcene; 6) (−)-limonene; and 7) (+)-limonene, resulting in 10, 12, 10, 11, 12, 11, and 9 replicates of each treatment, respectively. Except for control traps, each trap was baited with one closed LDPE bottle (device 1) filled with an appropriate monoterpene. In spite of differences in release rates among the various monoterpenes (105–295 mg/d at 23–25°C), the LDPE bottle was chosen as the standard device, because devices releasing α-pinene at higher rates than 105 mg/d (at 23–25°C) resulted in a decrease in attraction of *C. coniperda* to pityol-baited traps (Miller et al. 2003). Cone beetle flight at the Beech Creek Seed Orchard generally starts in mid-March and ends in late May with only one generation per year (D.R.M., unpublished data).

Experiment 2. This experiment examined the interaction between (−)- and (+)-limonene on the attraction of cone beetles. Thirty-two traps were set in eight replicate blocks of four traps per block. The trapping period was 1 March–30 May 2003. The following treatments were randomly assigned to traps within each block: 1) unbaited control; 2) (−)-limonene; 3) (+)-limonene; and 4) (−)-limonene and (+)-limonene. For treatment 4, traps were baited with two release devices; one device for each enantiomer of limonene.

Experiments 3 and 4. These experiments examined the interaction of (+)- and (−)-limonene, respectively, with α-pinene and pityol in attracting cone beetles to traps. In both experiments, traps were set in replicate blocks of eight traps per block with five blocks per experiment. The trapping period for experiment 3 was 1 March–30 May 2003, whereas the trapping period for experiment 4 was reduced to 1 March–30 April 2003 due to vandalism and loss of seven traps in early May. In experiment 3, the following treatments were randomly assigned to traps within each block: 1) unbaited control; 2) (+)-limonene; 3) (−)-α-pinene; 4) pityol; 5) (+)-limonene and (−)-α-pinene; 6) (+)-limonene and pityol; 7) (−)-α-pinene and pityol; and 8) (+)-limonene, (−)-α-pinene, and pityol. In experiment 4, the following treatments were randomly assigned to traps within each block: 1) unbaited control; 2) (−)-limonene; 3) (−)-α-pinene; 4) pityol; 5) (−)-limonene and (−)-α-pinene; 6) (−)-limonene and pityol; 7) (−)-α-pinene and pityol; and 8) (−)-limonene, (−)-α-pinene, and pityol.

Experiment 5. This experiment determined the effect of the release rate of (−)-limonene in attracting cone beetles to traps. Fifty traps were set in 10 replicate blocks of five traps per block. The trapping period was 27 March–10 May 2004. All traps were baited with devices releasing (−)-limonene with treatments differing by release rate. The following release rate treatments were randomly assigned to traps within each block: 50, 150, 295, 610, and 1,830 mg/d (at 23–25°C in still air). The highest release rate was obtained with three of device 2 and the lowest release rate was obtained with five of device 4. The three intermediate release rates were obtained with a single device 3, 1 and 4, respectively.

Statistical Analyses. The data were analyzed with the SYSTAT version 11.00.01 and the SigmaStat version 3.1 statistical packages (Systat Software, Inc., Point Richmond, CA). All analyses were conducted on total trap catches over the trapping periods. Trap catch data were transformed by $\ln(y + 1)$ or $\ln(y)$, as required, to remove heteroscedasticity (Pepper et al. 1997). Data in experiments 1–4 were subjected to one-way analysis of variance (ANOVA) followed by the Holm–Sidak multiple comparison test when $P \leq 0.05$. Because of significant differences in release rates for the different monoterpenes, treatment means in experiment 1 were compared only with those of controls, whereas all pairwise comparisons were examined in experiments 2–4. In addition, data in experiment 2 were subjected to full factoral two-way ANOVA by using replicate, (+)-limonene, (−)-limonene, and the interaction between (+)- and (−)-limonene as model factors, whereas those in experiments 3 and 4 were subjected to full factoral three-way ANOVA by using replicate, (−)-α-pinene, pityol, limonene, and the interactions between each pair as well as the three-way interaction as model factors. Data from experiment 5 was subjected to regression analysis. Sex ratio data (using proportion of males in catches as the test variable) for treatments with sufficient numbers (>10 beetles) were subjected to one-way ANOVA.

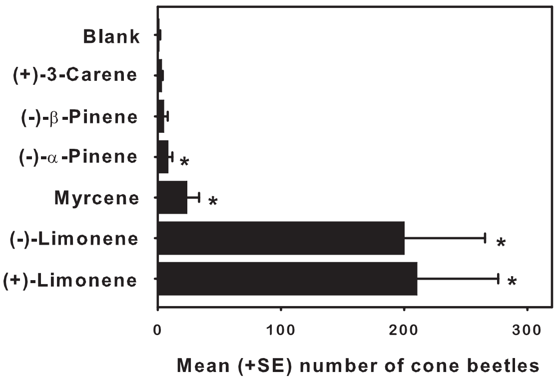

Fig. 1. Effects of six common pine monoterpenes on mean + SE total catches of *C. coniperda* in Japanese beetle traps from 28 March to 30 May 2002 in experiment 1 (n = 9–12). Treatment means followed by an asterisk are significantly different from the control mean + SE trap catch of 0.40 ± 0.16 at $\alpha = 0.05$ (Holm–Sidak multiple comparison test).

Results

Experiment 1. The total number of white pine cone beetles caught in experiment 1 was 4,565 with 90% of beetles captured in traps baited with either (+)- or (−)-limonene. There was a significant treatment effect in the experiment ($F = 26.652$; df = 6, 68; $P < 0.001$). Catches in traps baited with (−)-α-pinene, myrcene, (−)-limonene, and (+)-limonene were greater than those in blank controls (Fig. 1). There was no significant effect of these four treatments on sex ratio of captured beetles ($F = 0.122$; df = 3, 22; $P = 0.946$). Fourteen samples were not included in the sex ratio analysis due to low trap catches (<10 beetles). The mean ± SE proportions of males in catches for traps baited with (−)-α-pinene, myrcene, and (−)- and (+)-limonene were 0.83 ± 0.01 (n = 4), 0.83 ± 0.07 (n = 3), 0.85 ± 0.04 (n = 10), and 0.83 ± 0.04 (n = 9), respectively.

Experiment 2. The traps caught 727 white pine cone beetles in total in experiment 2 with catches significantly affected by both (+)-limonene ($F = 4.247$; df = 1, 21; $P = 0.052$) and (−)-limonene ($F = 16.218$; df = 1, 21; $P = 0.001$). Catches of beetles in traps baited with either enantiomer of limonene were significantly higher than those in blank control traps (Fig. 2). However, the effect of the two enantiomers was not additive ($F = 8.556$; df = 1, 21; $P = 0.008$). Catches in traps baited with both enantiomers of limonene were not significantly different from those baited with either enantiomer alone (Fig. 2). There was no effect of the three limonene treatments on the sex ratio of cone beetles in trap catches ($F = 0.185$; df = 2, 14; $P = 0.833$). Seven samples were not included in the sex ratio analysis due to low trap catches (<10 beetles). The mean ± SE proportions of males in catches for traps baited with (−)-limonene, (+)-limonene, and both (+)- and (−)-limonene were 0.96 ± 0.03 (n = 4), 0.93 ± 0.05 (n = 7), and 0.92 ± 0.03 (n = 6), respectively.

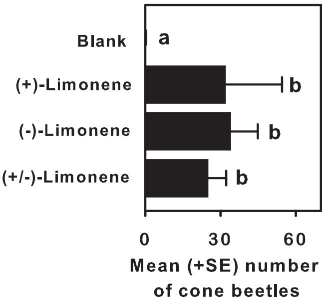

Fig. 2. Effects of (−)- and (+)-limonene on mean + SE total catches of *C. coniperda* in Japanese beetle traps in experiment 2 ($n = 8$). Means followed by the same letter are not significantly different at $\alpha = 0.05$ (Holm–Sidak multiple comparison test).

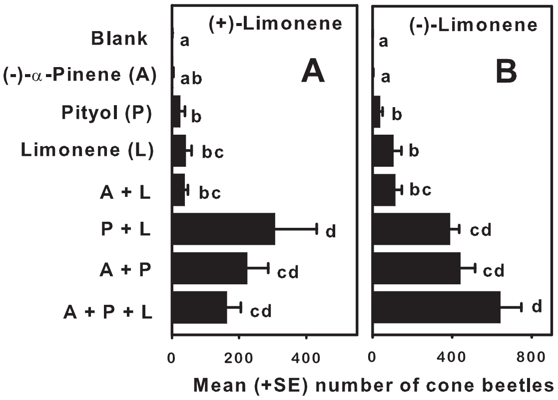

Fig. 3. Effects of interactions between (−)-α-pinene and pityol with (+)-limonene (A) and (−)-limonene (B), separately, on mean + SE total catches of *C. coniperda* in Japanese beetle traps in experiments 3 and 4, respectively ($n = 5$). Means followed by the same letter are not significantly different at $\alpha = 0.05$ (Holm–Sidak multiple comparison test).

Experiments 3 and 4. Traps captured 4,007 and 8,657 white pine cone beetles in experiments 3 and 4, respectively. The responses of cone beetles in the two experiments were significantly affected by pityol, (−)-α-pinene, and limonene (Table 1). Catches in traps baited with either limonene or pityol were significantly greater than those in blank control traps (Fig. 3A and B). Traps baited with limonene or the combination of limonene and (−)-α-pinene were as attractive as those baited solely with pityol in both experiments. Catches in traps baited with pityol and the addition of limonene, (−)-α-pinene, or both limonene and (−)-α-pinene were significantly greater than those in traps baited with only pityol.

In experiments 3 and 4, the interaction between pityol and (−)-α-pinene was additive ($P = 0.576$ and $P = 0.055$, respectively) (Table 1). In both experiments, trap catches in traps baited with both compounds were greater than those in traps baited with each compound separately (Fig. 3A and B). The interaction between (−)-α-pinene and limonene was not additive in the two experiments ($P = 0.029$ and $P = 0.042$, respectively). The interaction between pityol and limonene was additive for (+)-limonene in experiment 3 ($P = 0.062$), but not for (−)-limonene in experiment 4 ($P = 0.001$) (Table 1). In both experiments, catches of cone beetles in traps baited with pityol and limonene were significantly greater than those in traps baited with either compound alone (Fig. 3A and B). The only significant difference between the two experiments was that catches in traps baited with the three-component lure were greater than those in traps baited solely with limonene or the combination of limonene with (−)-α-pinene in experiment 4 (Fig. 3B) but not in experiment 3 (Fig. 3A).

Table 1. Analyses of variance of effects of pityol and (−)-α-pinene with (+)- and (−)-limonene on catches of *C. coniperda*

Source	df	(+)-Limonene (exp. 3)		(−)-Limonene (exp. 4)	
		F	*P*	*F*	*P*
Replicate	4	0.044	0.996	0.559	0.694
(−)-α-Pinene (A)	1	4.980	0.034	15.176	0.001
Limonene (L)	1	34.343	<0.001	86.747	<0.001
Pityol (P)	1	57.345	<0.001	111.544	<0.001
A × L	1	5.311	0.029	4.560	0.042
A × P	1	0.322	0.576	3.996	0.055
L × P	1	3.789	0.062	14.306	0.001
A × L × P	1	2.960	0.097	4.174	0.051
Error	28				

I found no evidence of female-bias in any of the limonene treatments in experiments 3 and 4 ($F = 0.572$; df = 5, 16; $P = 0.687$, $F = 1.873$; df = 5, 21; $P = 0.142$, respectively). Data for control traps and traps baited with (−)-α-pinene alone were not used in the analyses due to insufficient numbers of beetles (<10) for determining sex ratios. The mean ± SE proportions of males in trap catches for the experiments 3 and 4 were 0.95 ± 0.01 ($n = 22$) and 0.90 ± 0.01 ($n = 27$), respectively.

Experiment 5. Trap catches of white pine cone beetles were directly proportional to the release rate of (−)-limonene, although the regression equation explained only 12% of the variation in trap catches (Fig. 4). Trap catches of cone beetles were significantly lower in 2004 (total catch of 2,526 *C. coniperda*) than in 2002–2003 (experiments 1, 3, and 4). It is likely that populations of cone beetles at the Beech Creek Seed Orchard were reduced significantly before the establishment of experiment 5. Unlike the previous 2 yr, a prescribed burn was conducted throughout the orchard in early March 2004 in an attempt to reduce cone beetle populations by direct consumption of beetles while still in fallen cones. Prescribed fire is an effective tool in controlling cone beetles (Wade et al. 1989). There was no effect of limonene release rate on the sex ratio of captured cone beetles ($F = 0.100$; df = 1, 24; $P = 0.754$) with a mean ± SE proportion of males in trap catches of 0.97 ± 0.01 ($n = 26$).

Responses of *E. nigripes*. The total catch of *E. nigripes* in experiment 1 was 81 beetles, which was sufficient to discern a treatment effect ($F = 2.386$; df = 6, 68; $P = 0.038$). Catches of beetles in traps baited with

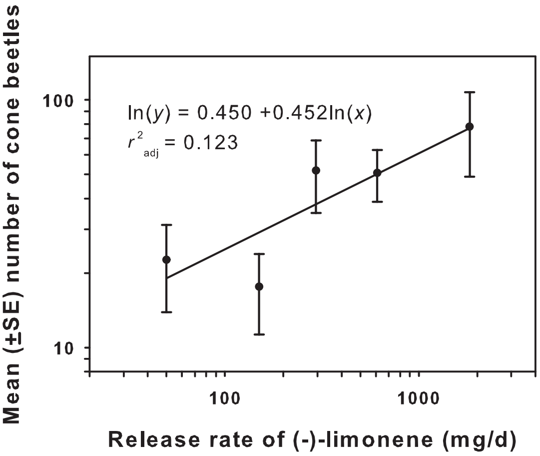

Fig. 4. Effect of release rate of (−)-limonene on mean ± SE total catches of *C. coniperda* in Japanese beetle traps in experiment 5. Slope of regression line is significantly different from zero ($P = 0.007$).

(−)-α-pinene were significantly greater than those in control traps (Fig. 5). Because only 24 *E. nigripes* were caught in experiment 2, the data could not be analyzed with sufficient statistical power. In experiment 5 (total catch of 147 beetles), catches of *E. nigripes* were unaffected by the release rate of (−)-limonene ($F = 0.650$; df = 1, 48; $P = 0.424$).

In experiments 3 and 4, trap catches of *E. nigripes* were significantly affected by pityol and both enantiomers of limonene (Table 2). In experiment 3, catches of beetles in traps baited with pityol alone, or in combination with (−)-α-pinene, were greater than those in traps with most of the other treatments (Fig. 6A). In experiment 4, catches of beetles in traps baited with pityol and (−)-α-pinene were greater than those in traps with five of the remaining treatments (Fig. 6B). There was no evidence of an increase in attraction of *E. nigripes* by limonene in either experiment (Fig. 6A and B). The principal effect of (+)-limonene in experiment 3 seemed to be an interruption of the attraction of beetles to pityol-baited traps (Fig. 6A). Catches of beetles in traps baited with pityol alone were significantly greater than those in traps baited with pityol and (+)-limonene. Similarly, catches of beetles in traps baited with pityol and (−)-α-pinene were significantly greater than those baited with all three compounds.

Table 2. Analyses of variance of effects of pityol and (−)-α-pinene with (+)- and (−)-limonene on catches of *E. nigripes*

Source	(+)-Limonene (exp. 3)			(−)-Limonene (exp. 4)		
	df	*F*	*P*	df	*F*	*P*
Replicate	4	0.184	0.945	5	0.831	0.066
(−)-α-Pinene (A)	1	2.808	0.105	1	1.103	0.302
Limonene (L)	1	21.575	<0.001	1	6.548	0.016
Pityol (P)	1	40.184	<0.001	1	28.349	<0.001
A × L	1	1.409	0.245	1	0.707	0.407
A × P	1	0.943	0.340	1	4.322	0.046
L × P	1	10.249	0.003	1	3.608	0.067
A × L × P	1	0.066	0.800	1	0.035	0.852
Error	28			30		

Discussion

Limonene is an attractive, host-produced kairomone for the white pine cone beetle, *C. coniperda*, in western North Carolina. Limonene is abundant in cones of eastern white pines in March when beetles are attacking cones and less abundant in cones in May when beetles cease attacks (Miller et al. 2003). White pine cone beetles were attracted in large numbers to traps baited simply with limonene, irrespective of enantiomeric composition (Figs. 1 and 2). The response of *C. coniperda* was dependent on the release rate of (−)-limonene (Fig. 4). Catches in pityol-baited traps were significantly increased by either enantiomer of limonene (Fig. 3A and B). I found no evidence of

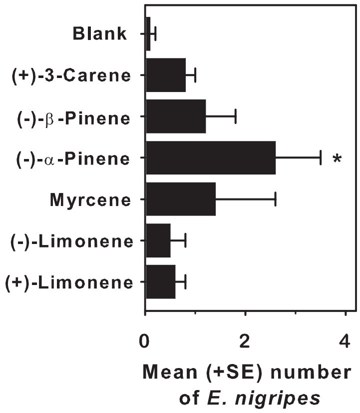

Fig. 5. Effects of six common pine monoterpenes on mean + SE total catches of *E. nigripes* in Japanese beetle traps in experiment 1 ($n = 9–12$). Treatment means followed by an asterisk are significantly different from the control at $\alpha = 0.05$ (Holm–Sidak multiple comparison test).

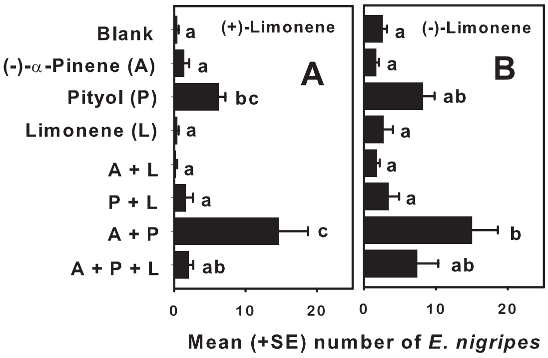

Fig. 6. Effects of interactions between (−)-α-pinene and pityol with (+)-limonene (A) and (−)-limonene (B), separately, on mean (+SE) total catches of *E. nigripes* in Japanese beetle traps in experiments 3 and 4, respectively ($n = 5, 6$). Means followed by the same letter are not significantly different at $\alpha = 0.05$ (Holm–Sidak multiple comparison test).

synergy between limonene and (−)-α-pinene in attraction of *C. coniperda* (Fig. 3A and B).

Catches of both male and female *C. coniperda* were enhanced by limonene, although catches of cone beetles were male biased in all experiments. The mean ± SE proportion of males in trap catches of *C. coniperda* ranged from 0.839 ± 0.109 in experiment 1 to 0.968 ± 0.005 in experiment 5. There was no significant effect of treatment on sex ratio of captured beetles in any of the five experiments. It is possible that the right compounds (or blend of compounds) to attract female *C. coniperda* in high numbers have yet to be tested. In laboratory and field assays, female *C. coniperda* were attracted to volatiles extracted from uninfested and infested eastern white pine cones, with some preference for those containing male beetles (de Groot et al. 1991).

Alternatively, the low proportion of females in trap catches of *C. coniperda* may reflect a lower probability of capture. It is possible that female cone beetles spend less time than males in choosing cones. Females must first initiate attacks on uninfested cones before males can choose among cones attacked by females. Although production of pityol by female cone beetles likely helps males to find female-infested cones, males likely fly between trees and branches before choosing the right cones. In addition, females may be choosy in accepting males, resulting in an increase in searching time by males as they fly off to other cones when rejected. Once mated, females need to spend time in cones laying eggs, whereas males may be free to leave cones to find other female-attacked cones. Godwin and ODell (1965) found that the sex ratio of *C. coniperda* in infested cones of eastern white pine was female biased at a sex ratio of ≈2:1, whereas the sex ratio of overwintering *C. coniperda* in cones on the ground was 1:1. Considerable research is still needed to understand the basic behavioral ecology of cone beetles.

Geographic variation in the use of semiochemicals is an important issue with many species of bark beetles (Byers 1989), and it may be an important consideration for *C. coniperda* with respect to limonene as all of my studies were conducted in western North Carolina. The range of *C. coniperda* mirrors that of eastern white pine, ranging from eastern Canada down to the Southern Appalachians (Wood 1982). In studies conducted in 2004, Brauner and de Groot (2006) found that limonene, irrespective of enantiomeric composition, significantly increased attraction of *C. coniperda* to pityol-baited traps in two eastern white pine seed orchards in southern Ontario. Moreover, catches of both male and female cone beetles in traps baited with pityol and (−)-limonene were directly proportional to the release rate of (−)-limonene. As in this study, Brauner and de Groot (2006) did not find any evidence of synergy between limonene and (−)-α-pinene in attracting cone beetles. The addition of (−)-α-pinene interrupted attraction of female *C. coniperda*, but not male *C. coniperda*, to traps baited with pityol and (−)-limonene. Unlike the present results (Figs. 1 and 2), the data of Brauner and de Groot (2006) did not indicate significant attraction of beetles to traps baited simply with limonene. My results and those of Brauner and de Groot (2006) provide solid evidence that limonene is a kairomone for *C. coniperda* in the southern and northern parts of its range. More information on the responses of white pine cone beetles in the middle of its range should be obtained to verify the general responses to limonene.

The complete role of host volatiles in the chemical ecology of white pine cone beetles is still unclear, although limonene and (−)-α-pinene are evidently important. Numerous other monoterpenes are present in cones of eastern white pine (Miller et al. 2003). In the current study, myrcene released at a rate of ≈170 mg/d (at 23–25°C) was attractive to cone beetles (Fig. 1). The lack of response to the other compounds may be due to differences in release rates. Attraction of cone beetles to (−)-α-pinene is dose dependent with attraction increasing as release rates increase and then decreasing when rates get high (Miller et al. 2003). At present, there is little, if any, information regarding in situ release rates of host volatiles from cones and branches of eastern white pine. Previous studies on the chemical ecology of *C. coniperda* focused on composition of volatiles rather than rates of emission (Birgersson et al. 1995, Miller et al. 2003, Brauner and de Groot 2006). There is a distinct need to evaluate the composition and rates of host volatile emissions by cones on eastern white pines to better understand the role of monoterpenes in the chemical ecology of *C. coniperda.*

Variation in chemical composition between cones, conelets, and shoot tips also may be important in understanding the chemical ecology of cone beetles. Cone beetle biology has been generalized as follows: beetles overwinter in cones, emerge, and attack second-year cones that then fall to the ground. The cycle repeats the following year (ODell and Godwin 1964). However, attacks on twigs and first-year conelets have been noted for several species of cone beetles, including *Conophthorus lambertianae* Hopkins (=*C. ponderosae* Wood) and *C. resinosae* and *C. coniperda* (Struble 1947, Lyons 1956, Godwin and ODell 1965). Mattson (1980) found significant damage to cones, conelets, and shoots by *C. resinosae* in red pine stands over a period of 11 yr. In stands of jack pine, *P. banksiana* Lamb. an ecovariant of *C. resinosae* (=*C. banksianae*) feeds primarily on shoots, overwintering in downed shoot tips on the forest floor, and it rarely attacks cones (McPherson et al. 1970, de Groot and Borden 1991). Attacks by *C. resinosae* on jack pine shoots may be classified as feeding, breeding, ovipositioning, or overwintering attacks (Mattson 1989).

Presumably, white pine cone beetles attack shoot tips and conelets during periods of low abundance of second-year cones or when population levels of beetles are high (Godwin and ODell 1965). Godwin and ODell (1965) note that, whereas most *C. coniperda* overwinter in cones on the ground, a considerable number of beetles emerge in late summer and early fall and attack first-year conelets with males initiating as many attacks as females. Some beetles leave cones on the ground and move to other damaged cones on the ground. Shoots of red pine are often attacked in the fall by *C. resinosae,* possibly improving overwintering survivorship of the beetles (Lyons 1956).

Godwin and ODell (1965) found that white pine cone beetles formed small aggregations before attacking conelets in the fall with as many as eight beetles clustered around the conelet–petiole junction. Mattson (1980) generalized that cone beetles aggregate in small feeding groups before attacking cones for oviposition in the spring as well. Morgan and Mailu (1976) found aggregative feeding attacks by overwintered *C. coniperda* on both white pine twigs and cones in the spring with up to 12 beetles per site. Thigmotropic aggregative behaviors in *C. coniperda* are related to temperature and humidity, possibly serving a role in ensuring overwintering survivorship and maximal cone attack success in the spring (Henson 1961, 1964).

My results demonstrate that limonene interrupts the attraction of *E. nigripes* to the cone beetle pheromone pityol (Fig. 6A), even though limonene increases the attraction of *C. coniperda* to traps baited with pityol. Similarly, Miller et al. (2003) found that *E. nigripes* (misreported as *T. dubius*) preferred pityol-baited traps with lures releasing (−)-α-pinene at the highest rate tested (579 mg/d at 23°C), whereas *C. coniperda* preferred traps with lures releasing (−)-α-pinene at lower rates (107 mg/d at 23°C). In eastern North America, *E. nigripes* preys on numerous species of bark and wood boring beetles (USDA Forest Service 1985). It is possible that generalist predator species, such as *E. nigripes,* that feed on a broad array of prey use a broad olfactory search image as opposed to a narrow search image associated with only one prey species. A similar relationship was apparent in California where the pine engraver, *Ips pini* (Say), preferred ipsdienol-baited traps with lures releasing the pheromone lanierone at lower rates (0.1–100 μg/d under field conditions) than those preferred by the generalist bark beetle predator *Enoclerus lecontei* (Wolcott) (2 mg/d under field conditions) (Seybold et al. 1992).

Alternatively, the responses of *E. nigripes* in my experiments may relate to maximizing survivorship of larvae rather than adults. Generally, adult clerids feed on adult scolytid beetles, whereas larval clerids feed on larval scolytids (USDA Forest Service 1985). Cones attacked by cone beetles in late April and early May are apt to be larger than those attacked in March. It is possible that larger cones can sustain more cone beetle larvae than smaller cones. Adult clerids may be selecting attacked cones on the basis of suitability for survivorship of larval clerids. Because *E. nigripes* are attracted to pityol, especially when combined with α-pinene (de Groot and DeBarr 2000, Miller et al. 2003), attraction to traps releasing high rates of α-pinene and low rates of limonene may be indicative of responses to larger cones.

Because most studies on the chemical ecology of white pine cone beetles have been conducted in seed orchards, further studies should consider the unique nature of eastern white pine seed orchards in contrast to natural stands of eastern white pine. Trees within an orchard are typically widely spaced to encourage open crowns and greater cone production. The understory vegetation in seed orchards is generally controlled by mowing, herbicides or prescribed burns. It is possible that the patterns seen in orchards may differ substantially from those in natural stands. Moreover, the patterns in the use of semiochemicals observed in orchards may be derived from selection pressures occurring within natural stands but not within seed orchards.

My work and that of Brauner and de Groot (2006) with limonene suggest that host volatiles other than α-pinene may play a significant role in the chemical ecology of both male and female white pine cone beetles in eastern white pine seed orchards. Studies on the volatiles associated with feeding and oviposition attacks on cones, conelets, and shoots (in spring and fall) may provide insights into potential attractants for female cone beetles. Such work should be tied to analyses of volatiles produced by cones relative to genetic composition and across various spatial and temporal scales.

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