FOREST ENTOMOLOGY

Trap Type, Chirality of a-Pinene, and Geographic Region Affect Sampling Efficiency of Root and Lower Stem Insects in Pine

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ABSTRACT Root and lower stem insects cause significant damage to conifers, vector phytopathogenic fungi, and can predispose trees to bark beetle attacks. The development of effective sampling techniques is an important component in managing these cryptic insects. We tested the effects of trap type and stereochemistry of a-pinene, in combination with ethanol, on catches of the root colonizing weevils (Coleoptera: Curculionidae) *Hylobius* spp. [mostly *Hylobius pales* (Herbst)], and *Pachylobius picivorus* (Germar), the root colonizing bark beetle (Coleoptera: Scolytidae) *Hylastes porculus* Erickson, and the lower stem colonizing bark beetle *Dendroctonus valens* (LeConte). We tested for inter-regional differences by conducting similar field assays in the northern (Wisconsin) and southern (Louisiana) United States. The more effective trap type varied with region. Root weevils were caught primarily in pitfall traps in Wisconsin, whereas they were caught mostly in lower stem flight traps in Louisiana. In Wisconsin, root colonizing bark beetles were also caught primarily in pitfall traps, but lower stem colonizing bark beetles were caught primarily in lower stem flight traps. The root feeding weevils preferred (−) over (+)-a-pinene in both regions. Some exceptions relating to trap type or gender occurred in southern populations. The two root and lower stem colonizing bark beetles in Wisconsin showed no preference between (+) and (−)-α-pinene in combination with ethanol. No bark beetles were caught in the south. Our results suggest that modifying trap type and enantiomeric ratios of monoterpenes for different insect groups and in different regions can improve sampling efficiency for these important pests.

KEY WORDS *Pinus, Hylobius, Hylastes,* Dendroctonus, root insects, chemical ecology

INSECTS THAT COLONIZE conifer roots and lower stems have increased in importance due to intensive forest management practices in Europe (Nordlander et al. 1986, 1997), and North America (Nord et al. 1982, Lynch 1984, Schowalter 1985, Witcosky et al. 1986a, Klepzig et al. 1991). The primary pest species are weevils (Curculionidae) and bark beetles (Scolytidae), which injure trees either directly by adult (Nord et al. 1982, Nordlander et al. 1997) and larval feeding (Wilson and Millers 1983), or indirectly by vectoring phytopathogenic fungi (Witcosky et al. 1986b; Owen et al. 1987; Klepzig et al. 1991; Nevill and Alexander 1992a, 1992b) and predisposing trees to subsequent attack by bark beetles (Owen 1985; Klepzig et al. 1991, 1995).

Detection and quantitative sampling of root insects are particularly difficult, because the larvae feed underground, and the adults are nocturnal and hide in the litter or soil. Several techniques have been developed to sample root insects attacking conifers. Bait logs can attract large numbers of weevils (Lynch 1984) but have inherent disadvantages: arriving insects are not trapped (unless pesticides are included), emissions from logs vary among trees and through time, and deploying logs is labor-intensive and expensive. Pitfall traps baited with host volatiles have been used to sample root weevils in Sweden (Tilles et al. 1986a, 1986b; Nordlander 1987) and the Great Lakes region of the United States (Hunt and R&a 1989; Rieske and Raffa 1991, 1993a). These traps have the advantages of being standardized, easily deployed, and able to capture arriving beetles. However, the effectiveness of baited pitfall traps varies regionally for reasons that are not understood. For example, large numbers of *Hylobius abietis* L. are caught using pitfall traps throughout Scandinavia, but their performance appears less effective in Great Britain (Wilson and Day 1995). Likewise, this method has been relatively less effective in Virginia than in Wisconsin, USA (Fettig and Salom 1998). Various forms of flight traps have been reported to capture root and lower stem insects in the southern United States (Fatzinger et al. 1987) and to a lesser extent in the northern United States (Klepzig et al. 1991, Rieske and Raffa 1993a), but no direct comparisons of trap efficiency have been conducted.

Host volatiles, particularly monoterpenes and ethanol, have been used to monitor populations of conifer root and stem feeding insects (Tilles et al. 1986b; Fatzinger et al. 1987; Nordlander 1987, 1989; Chénier and Philogbne 1989; Hunt and Raffa 1989; Nordenhem and Eidmann 1991; Rieske and Raffa 1991; Lindelöw et

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al. 1993). For example, ethanol and α-pinene interact synergistically to attract *abietis* adults in Europe (Nordenhem and Nordlander 1994). Similarly, ethanol and components of turpentine, particularly α-pinene, interact synergistically for four species of pine root feeding weevils in the Great Lakes region (Hunt and Raffa 1989, Hoffman et al. 1997, Rieske and Raffa 1999). α-Pinene is the most abundant monoterpene in the three major pine species, red, *P. resinosa* (Aitman), white, *Pinus strobus* L., and jack, *Pinus banksiana* Lamb, in the Great Lakes region (Bridgen et al. 1979; Klepzig et al. 1995, 1996; Raffa and Smalley 1995; Wallin and Raffa 1999), and in loblolly pine, *Pinus taeda* L. in the south (Hodges and Lorio 1975, Hodges et al. 1979). Ethanol is likewise emitted from pines, and show strong seasonal patterns (Crawford and Baines 1977, Kimmerer and Kozlowski 1982) that coincide with peak behavioral attraction by ***Hylobius*** spp. (Hoffman et al. 1997). Currently little is known about the role of stereochemistry in the behavior of North American species. In addition, the importance of various ratios of specific stereoisomers to ethanol, and possible sources of geographic variations, have not been tested.

Chirality has been shown to be important in the chemical ecology of many Curculionoidea. For example, Hobson et al. (1993) found that (+) -α-pinene, but not (−) -a-pinene, attracted ***Dendroctonus valens*** (LeConte) in California, and that (−) -α-pinene inhibited attraction to (+) -α-pinene. Likewise, the stereochemistry of host monoterpenes can affect the synthesis of and response to bark beetle pheromones (Renwick et al. 1976, Seybold et al. 1995, Erbilgin and Raffa 2000), and pheromone stereochemistry can greatly affect activity (Wood 1982). Strong geographic variation can occur in the responses of phloeophagous insects to semiochemicals. For example, most western populations of *Ips pini* (Say) are attracted primarily to R- (−) -ipsdienol, whereas eastern and midwestern populations are more strongly attracted to racemic ipsdienol (Lanier et al. 1972, Miller et al. 1989, Raffa and Klepzig 1989, Seybold et al. 1995).

A complex of curculionids and scolytids colonize the root and lower stems of pine trees (Drooz 1985). In the Great Lakes region, the pales weevil, ***Hylobius pales*** (Herbst), the pine root collar weevil, *Hylobius radicis* Buchanan, and the pitch eating weevil, *Pachylobius picivorus* (Germar), colonize the root collars and large portions of lateral roots, whereas the pine root tip weevil, ***Hylobius assimilis*** Boheman (=*Hylobius rhizophagus* Millers, Benjamins and Warner) colonizes the small lateral roots. ***Hylobius radicis*** and ***H. assimilis*** (primary species) breed in healthy hosts, whereas ***H. pales*** and ***P. picivorus*** (secondary species) breed in dying or newly killed trees or stumps (Drooz 1985). Two scolytids, ***D. valens*** and *Hylastes porculus* Erickson, are also associated with declining pines, occurring in the lower stem and root collar, and lateral roots, respectively. ***Hylobius pales*** and *P.* picivorus also colonize the roots of southern pines. Feeding by adults on pine seedlings in the south typically causes more extensive damage than that seen in the north (Lynch 1984), and larval development in the southern United States is faster (Drooz ***1985). Hylastes porculus, Hylastes salebrosus*** Eichhoff and ***Dendroctonus terebt-ans*** (Olivier) colonize the lateral roots and lower stems, respectively, of southern pines, can be damaging by themselves, and are suspected of contributing to complex stand declines (Higley and Tattar 1985, Rane and Tattar 1987) in a manner similar to that of *D. valens* and *H. porculus* in Wisconsin (Klepzig et al. 1991). All of these species are attracted to combinations of monoterpenes and ethanol (Fatzinger et al. 1987, Fettig and Salom 1998), but the optimal ratio can vary (Rieske and Raffa 1991, 1999).

The three objectives of this study were as follows: (1) determine effects of trap type on sampling efficiency of the complex of beetles attacking pine roots and lower stems, (2) determine effects of stereochemistry of α-pinene, and the interaction of stereoisomers of α-pinene with relative amounts of ethanol, on these responses, and (3) compare responses of co-occurring and related species in northern and southern regions of the central United States.

Materials and Methods

Traps and Chemicals. We conducted experiments using baited pitfall and lower stem flight traps in 40- to 50-yr-old ***P.*** *resinosa* plantations in south central Wisconsin in 1996, and ***P. taeda*** plantations in central Louisiana in 1998 and 1999. The pitfall traps are described by Tilles et al. (1986a) as modified by Hunt and Raffa (1989), and capture adult insects as they walk on the soil surface. Twenty centimeters high × 10 cm diameter PVC plastic drain pipe sections containing eight equally spaced holes (0.6 cm diameter) around the circumference of the upper section of the pipe. The pipes were capped with removable plastic lids at both ends, and placed in the soil, with the holes at ground level. A thin layer of Fluon (DuPont, Wilmington, DE) was applied to the inner walls of the trap to prevent the escape of insects that entered through the holes. Two holes (0.28 cm diameter) were drilled in the bottom lid for water drainage, and the top lids were removable to allow collection of captured insects. Vials containing lures were suspended from thin aluminum wire passing through two holes (0.2 cm diameter) in the walls of the trap at ground level. Lower stem flight traps described by Klepzig et al. (1991) were used to capture adult insects in flight. This trap was fashioned from a 3.78-liter plastic (10-gallon) milk jug by removing three of its sides and retaining two vertical strips as supporting columns. The jug was inverted, and the striking surface (remaining fourth side) was attached with a thin aluminum wire to the stem, without wounding the tree, ≈25 cm above ground. A plastic jar screwed over the mouth of the jug served as the collection vessel. Vials were suspended from the aluminum wire.

We used (−) -α-pinene (Chemical Purity (CP): >99%; Enantiomeric Ratios: (ER): 96.6%(−)/ 3.4% (+)) , (+) -a-pinene (CP: > 99%; ER: 95% (+) / 5%(-)) (Aldrich, Milwaukee, WI), and 95% ethanol

Table 1. Statistical analyses of effects of trap type and stereochemistry of α-pinene, in combination with ethanol, on responses of root- and lower stem-insects in Wisconsin and Louisiana

	df (N, D)	*F*	*P*	df (N, D)	*F*	*P*	df (N, D)	*F*	*P*	df (N, D)	*F*	*P*
All traps[b]		*Hylobius* spp			*P. picivorus*			*H. porculus*			*D. valens*	
					Wisconsin”							
Trap	1, 10.1	27.0	0.0004	1,244	11.1	0.001	1, 10	18.2	0.0016	1, 9.9	12.5	0.0055
Treatment	4,235	11.9	0.0001	4,244	6.8	0.0001	4,235	4.64	0.0013	4,235	4.7	0.0012
Trt X Trap	4,235	8.4	0.0001	4,244	2.9	0.0218	4, 235	2.5	0.0442	4,235	2.6	0.0403
Paired contrasts												
(+)-α vs. (-)-a	1,234	9.1	0.0030	1,244	5.4	0.021	1,234	0.07	0.80	1,234	0.04	0.84
1:5 vs. 1:1 ratio	1,234	4.9	0.0281	1,244	4.4	0.037	1,234	0.01	0.925	1, 234	2.5	0.115
Stereo x Ratio	1,234	7.9	0.0054	1,244	1.0	0.318	1,234	6.70	0.0102	1,234	5.2	0.0237
Within trap types												
Flight traps												
Treatment	4,121	2.9	0.026	4,121	1.9	0.107	4,121	3.1	0.018	4,121	4.5	0.0022
Paired contrasts												
(+)-α vs. (-)-a	1,121	0.4	0.72	1,121	0.1	0.84	1,121	0.2	0.67	1,121	0.6	0.43
1:5 vs. 1:1 ratio	1,121	5.1	0.026	1,121	0.0	0.99	1,121	0.6	0.43	1,121	2.6	0.11
Stereo x Ratio	1,121	1.8	0.181	1,121	3.2	0.08	1,121	7.1	0.009	1,121	10.5	0.0015
Pitfall traps												
Treatment	4,123	11.8	0.0001	4,123	8.0	0.0001	4,123	7.4	0.0001	4,123	2.4	0.6
Paired contrasts												
(+)-α vs. (-)-a	1,123	5.7	0.0183	1,123	6.5	0.0122	1,123	0.8	0.37	1,123	0.1	0.82
1:1 vs 1:5 ratio	1, 123	1.9	0.1732	1,123	2.5	0.12	1,123	1.2	0.27	1,123	2	0.16
Stereo X Ratio	1,123	7.1	0.009	1,123	1.6	0.21	1,123	0.6	0.43	1.123	0.1	0.73
					Louisiana”							
		H. pales (6)			*H. pales* (♀)			If. *pales* (Total)				
All traps												
Trap	1, 1260	0.0	0.99	1, 1260	13.8	0.0002	1.1254	3.6	0.06			
Treatment	2, 1260	29.2	0.0001	2.1260	23.2	0.0001	2, 4.01	20.1	0.008			
Trt X Trap	2, 1260	4.01	0.0183	2, 1260	6.1	0.0024	2, 1254	4.0	0.018			
Within trap types												
Flight traps												
Treatment	2,630	17.	0.0001	2,630	17.0	0.0001	2, 6.1	12.6	0.007			
Pitfall traps												
Treatment	2,680	17.	0.0001	2,380	7.1	0.001	3,678	20.4	0.0001			
		P. picivorus (♂)			*P. picivorus* (♀)			*P. picivorus* (Total)				
All traps												
Trap	1, 12	loo.3	0.0001	1, 12	97.4	0.0001	1, 2	126.1	0.008			
Treatment	2, 12	54.6	0.0001	2, 12	44.7	0.0001	2, 7.93	104.7	0.0001			
Trt x Trap	2, 12	28.3	0.0001	2, 12	23.7	0.0001	2.7.93	44.7	0.0001			
Within trap types												
Flight traps												
Treatment	2, 4	44.3	0.002	2,628	177.5	0.0001	2,628	247.6	0.0001			
Pitfall traps												
Treatment	2,680	22.8	0.0001	2,680	18.8	0.0001	3,680	38.4	0.0001			

[a] *P. resinosa* plantations, 1996.

[b] Data were analyzed by repeated measure analysis in PROC mixed. Fisher's Protected LSD test ($P < 0.05$) was used for multiple comparisons of means of transformed data [$\sqrt{x}$]. Data were pooled for paired comparisons between (+) versus (−)-α-pinene, or 1:1 versus 1:5 ratios of α-pinene: ethanol in Wisconsin.

[c] *P. taeda* plantations, 1999.

(5% water). Volatiles were released from separate 40-ml glass vials (3.5 cm high X 1.2 cm diameter). Volatilization rates at 23°C were 200 mg/24 h for ethanol and 40 mg/24 h for a-pinene. For the test involving 5-I ratios, compounds were released using 20 ml and 4 ml glass vials, respectively.

Trap Deployment and Sampling. Wisconsin. We deployed pitfall and lower stem flight traps in six 40- to 50-yr-old *P. resinosa* plantations in Sauk, Juneau, and Necedah counties in south central Wisconsin. We placed five flight and five pitfall traps at each site. Traps were separated by approximately 5 m, and trap types were alternated. We randomly deployed the following treatments to both trap types in each site: (1) 1:5 (+)-α-pinene:ethanol, (2) 1:1 (+)-α-pinene: ethanol:, (3) 1:5 (-)-a-pinene:ethanol, (4) 1:1 (−)-α-pinene:ethanol, and (5) unbaited control. We sampled traps every 2 wk from the beginning of May through the end of August. We caught no root -and lower stem- insects after the end of July, so we omitted these periods, yielding five sample periods from the data analysis. We rerandomized and replenished traps at each collection period. We identified bark beetles and *P. picivorus* to species, and *Hylobius* spp. to genus. Our rationale was to emphasize practical sampling methodology of infested stands for forest managers. However, based on related work, we can estimate the *Hylobius* spp. captured as being mostly *H. pales:* In similar stands in Wisconsin the next year, 67% of 5,886 *Hylobius* in baited pitfall traps were *H. pales, 24%* were

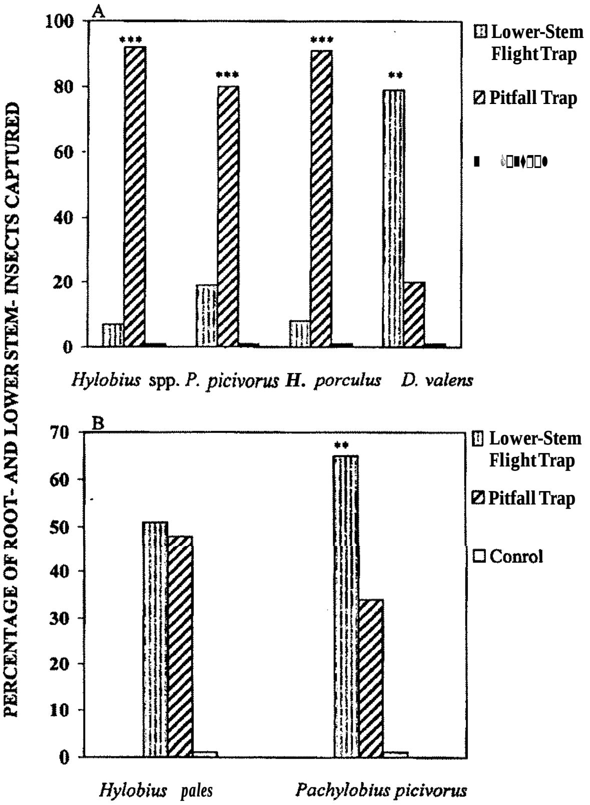

Fig. 1. Percentage of root- and lower stem- insects captured in pitfall traps and lower-stem flight traps in (A) Wisconsin, and (B) Louisiana. Comparisons between trap types were analyzed by one-way ANOVA: repeated measure analysis (PROC Mixed) with *, $P < 0.05$, **, $P \le 0.01$; ***, $P \le 0.001$. Statistics are presented in Table 2. Wisconsin and Louisiana studies were conducted in *P. resinosa* plantations in 1996 and *P. taeda* plantations in 1999, respectively.

radicis, and 9% were *H. assimilis* (Erbilgin and Raffa 2001).

Louisiana. We deployed identical pitfall traps in three *P. taeda* plantations in the Kisatchie National Forest (Rapides and Grant Parishes, LA) in 1998. Each site contained 15 pitfall traps, with one of three treatments randomly assigned to each trap. In 1999, we also included 15 pitfall traps and 15 lower-stem flight traps in each of three sites in forested land within Camp Beauregard (Rapides Parish, LA). Traps were separated by approximately 5 m. One of the following treatments was assigned to each trap: (1) 1:1 (−)-α-pinene:ethanol, (2) 1:1 (+)-α-pinene:ethanol, and (3) blank control. We identified and sexed weevils. Because we caught very low numbers of insects in pitfall traps in 1998, we only used data from 1999 for statistical analysis. We checked traps weekly throughout the season. Of 18 collection periods, three yielded no weevils in pitfall traps and six yielded no weevils in flight traps, so we only used data from 15 collection periods for pitfall traps and 12 collection periods for flight traps.

Monoterpene Composition of Host Pines. We determined monoterpene compositions for the native pine hosts in Wisconsin by gas chromatography, using previously described methods (Wallin and Raffa 1999). Approximately 2.5 g of phloem samples from fifteen trees each of *P.* resinosa, *P. banksiana,* and *P. strobus* pines were finely chopped with a razor, weighed, and extracted in 10 ml hexane for 24 h at 25°C. We separated extracts by vacuum filtration, and dried them over calcium chloride for 1 h. Analyses were performed using a Shimadzu gas liquid chromatography (GLC)-17 A (Shimadzu Scientific Instruments, Columbia, MD) on 30 m X 0.25-mm-i.d. Cyclodex B chiral column (J&W Scientific, Folsom, CA), with helium carrier gas at 30 cm/s, temperature 60°C for 10 min, increased to 200°C by + 10°C per minute.

Data Analysis. We analyzed the data using Analysis of Variance (ANOVA). We conducted a graphical analysis of residuals of raw data (Neter et al. 1983), which indicated that response variables had non-normal distributions and error terms, and that transformation was required. Because the data contained large numbers of zeros for some treatments, especially controls, and some sampling periods had low overall catches, we conducted a sensitivity analysis by applying several different transformations, e.g., log $(x + 1)$, $\sqrt{x}$, $x/(1+x)$ (Zar 1996). The statistical inferences were consistent throughout these transformations, in that the values of F and P did not vary. We selected $\sqrt{x}$, because the insect counts approximated the Poisson distribution, for which this transformation is most suitable (Steel and Torrie 1980, Snedecor and Cochran 1989). We analyzed each insect using the Repeated Measures Analysis in PROC Mixed (SAS Institute 1996). The model was $Y_{ijk} = \mu + \text{Treatment}_i + \text{Time}_j + \text{Trap}_k + (\text{Treatment X Time})_{ij} + (\text{Treatment X Trap})_{ik} + (\text{Time X Trap})_{jk} + (\text{Treatment X Time X Trap})_{ijk} + \delta_{ijk}$, where μ corresponds to population mean; Treatment_i corresponds to treatment effect; Time_j corresponds to time effect; Trap_k corresponds to trap effect; Treatment X Time_{ij} corresponds to trt by time interaction; Treatment X Trap_{ik} corresponds to treatment by trap interaction; Time X Trap_{jk} corresponds to time by trap interaction; Treatment X Time X Trap_{ijk} corresponds to treatment, time, by trap interaction; δ_{ijk} corresponds to plot error. If the covariance parameter for interactions between a random and fixed factor (e.g., site X treatment) was not significant, we eliminated that term from the random statement in the model. This test was performed by a comparison of the '-2 residual log likelihood' values of the model with and without that term. This results in a chi-square analysis (df = 1) at the $P < 0.05$ level. If chi-square analysis revealed significance, then we included the term in the random statement (SAS Institute 1996).

Comparisons between enantiomers of α-pinene, between ratios, and the interactions between chirality of α-pinene and ratios within and between trap types, were analyzed by Contrasts in PROC-Mixed (SAS Institute 1996). For pairwise comparisons between (+) and (-) enantiomers of a-pinene and between 1:1 and 1:5 ratios of a-pinene: ethanol, data were pooled accordingly, and analyzed using Fisher pro-

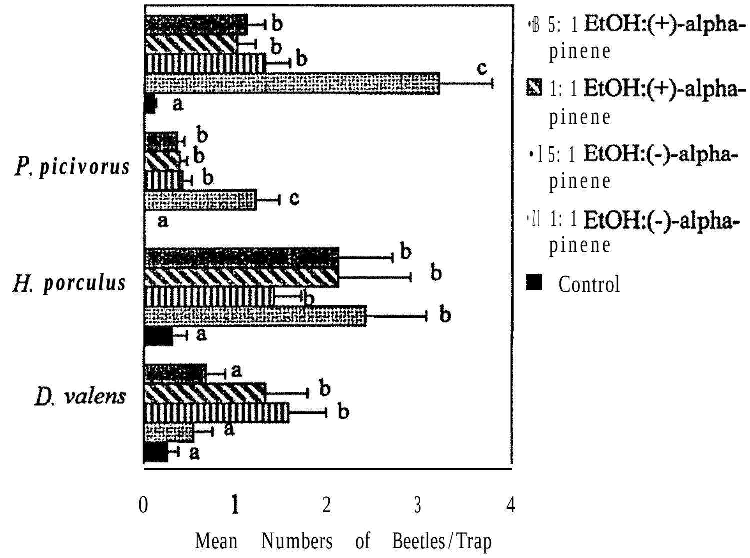

Fig. 2. Responses of root- and lower stem- insects to stereoisomers of cr-pinene, in combination with ethanol in Wisconsin. Data collected in *P. resinosa* plantations during **1996.** Multiple comparisons among treatments for ***Hylobius*** spp., ***P. picivorus,*** and *H. porculus* were conducted within pitfall traps, and those for D. *valens* were conducted within lower-stem flight traps. Means followed by the same letter within a species are not significantly different ($P < 0.05$, repeated measures analysis in PROC Mixed). Fisher protected LSD test ($P < 0.05$) was used for multiple comparisons of means of transformed data [$\sqrt{x}$]. Untransformed means are reported. Statistical parameters are presented in Table 1.

tected least significant difference (LSD) test ($P < 0.05$).

Where there were significant trap effects, comparisons among treatments were performed only within a trap type, and we used the trap type, which caught the most of each insect (see *Results*).

Results

We caught a total of 3,765 beetles representing five genera. In Wisconsin, 315 were ***H. porculus,*** **255** were ***Hylobius*** spp., 191 were ***D. valens,*** **100** were *P. picivorus,* and 36 were *Pissodes* spp. In Louisiana, 2,624 were ***P. picivorus,*** and 244 were ***H. pales.*** The proportions of female ***H. pales*** and ***P.*** picivorus in Louisiana were 51.21 and 55.5%, respectively.

There were significant trap and trap by treatment interaction effects on the numbers of all species in Wisconsin (Table 1) and *P.* picivorus in Louisiana (Table 1), and significant trap by treatment interactions on ***H. pales*** in Louisiana. Based on these results, we conducted multiple comparisons within pitfall traps for ***Hylobius, P. picivorus,*** and *H. porculus,* and within lower stem flight traps for ***D. valens*** in Wisconsin. Similarly, we conducted multiple comparisons for *P. picivorus* in Louisiana within lower stem flight traps only. Because the total ***H. pales*** in Louisiana were caught equally in both trap types, we conducted comparisons for both pitfall and lower stem flight traps.

Curculionidae. Pitfall traps captured 14 times more ***Hylobius*** spp. than did lower stem flight traps in Wisconsin (Fig. 1A). By contrast, no significant differences were observed in attraction of total ***H. palm*** to lower stem flight or pitfall traps in Louisiana (Fig. 1B). Female ***H. pales*** were caught in significantly higher numbers in lower-stem flight traps than pitfall traps, whereas male ***H. pales*** numbers showed no difference among trap types (Table 1).

Traps baited with all combinations of α-pinene plus ethanol caught significantly more ***Hylobius*** spp. than did unbaited traps, in Wisconsin (Fig. 2). Three times as many ***Hylobius*** spp. were caught in traps baited with 1:1 ethanol: (-) -α-pinene ratio as with any other treatment. Contrast analysis in Wisconsin showed that the catch of ***Hylobius*** spp. was significantly higher with (−) -α-pinene than (+) -a-pinene, plus ethanol (Fig. 3A). ***Hylobius*** spp. did not respond differently to different ratios of a-pinene to ethanol (Fig. 3B).

In Louisiana, attraction of ***H. pales*** to a-pinene plus ethanol was likewise greater than to unbaited controls (Fig. 4A), in both lower stem flight traps and pitfall traps (Fig. 4B). In lower stem flight traps, the highest number of ***H. pales*** responded to (-)-a-pinene plus ethanol (Fig. 4A), but in pitfall traps, the number of ***H. pales*** did not vary by chirality (Fig. 4B).

The effect of trap type on ***P. picivorus*** varied between regions (Table 1). Pitfall traps caught over 4 times more ***P. picivorus*** than lower stem flight traps in Wisconsin (Fig. 1A), whereas lower stem flight traps caught 2 times more *P. picivorus* than pitfall traps in Louisiana (Fig. 1B). Both male and female ***P. picivorus*** showed stronger attraction to lower stem flight traps than to pitfall traps in Louisiana.

In Wisconsin, all combinations of α-pinene plus ethanol attracted more ***P. picivorus*** than did unbaited controls (Fig. 2). Significantly more ***P. picivorus*** were caught in traps baited with 1:1 ratio of ethanol:(−)-cr-pinene than the other treatments. Overall, treatments with (-) -a-pinene attracted 2.3 times more *P.* picivorus than (+)-a-pinene (Fig. 3A). Attraction of

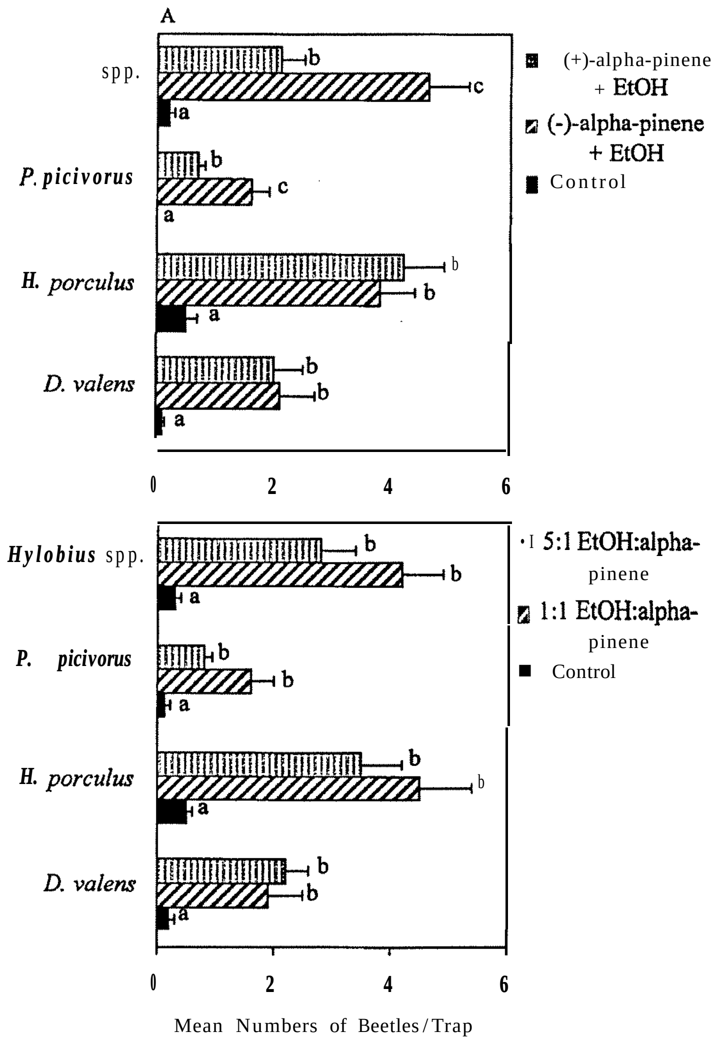

Fig. 3. Responses of root- and lower stem- insects to (A) stereochemistry of α-pinene in combination with ethanol, and (B) different ratios of a-pinene to ethanol, in Wisconsin. Data collected in *P. resinosa* plantations during 1996. Data for similar enantiomers of a-pinene [(+)-a-pinene versus (-)-a-pinene] and similar ratios (5:1 ratio versus 1:1 ratio) were pooled. Means followed by the same letter within a species are not significantly different ($P < 0.05$, Repeated Measures Analysis in PROC Mixed). Fisher protected LSD test ($P < 0.05$) was used for multiple comparisons of means of transformed data [$\sqrt{x}$] . Untransformed means are reported. Statistical parameters are presented in Table 1.

P. *picivorus* to pitfall traps did not vary with different ratios of ethanol and a-pinene when (+)- and (−)-cr-pinene are pooled (Fig. 3B).

In Louisiana, cr-pinene plus ethanol likewise attracted more ***P. picivorus*** than unbaited controls (Fig. 4C). More *P.* ***picivorus*** were caught in lower stem flight traps containing (−) -α-pinene plus ethanol than (+) - α-pinene plus ethanol. Catches of female *P.* ***picivorus*** were significantly higher in traps baited with (−)-α-pinene plus ethanol than in traps baited with (+)-a-pinene plus ethanol.

Scolytidae. Trap type, chemical treatment, and their interactions significantly affected catches of *H. porculus* (Table 1). Pitfall traps were more efficient than lower-stem flight traps, by a factor of nearly 12 times (Fig. 1A). All combinations of α-pinene plus ethanol were more attractive to ***H. porculus*** than unbaited controls (Fig. 2). However, attraction did not vary among baited treatments.

Attraction of *D. valens* varied with trap type and ratio of ethanol:a-pinene (Table 1). The lower stem flight traps captured nearly five times as many as ***D. valens*** as did pitfall traps (Fig. 1A). All combinations of α-pinene plus ethanol, with the exceptions of 5:1 ratio of ethanol: (+)-α-pinene and 1:1 ratio of ethanol: (-) -α-pinene, attracted more ***D. valens*** than did unbaited controls (Fig. ***2). Dendroctonus valens*** did not show any overall preference between the enantiomers of a-pinene or between the two ratios (Fig. 3 A and B) .

Monoterpene Composition of Host Pines. The principal monoterpenes, and their stereoisomers, in the cortical resin of ***P. resinosa, P. banksiana,*** and ***P. strobus*** are shown in Table 2. cx-Pinene was the predominant monoterpene and β-pinene was the second most

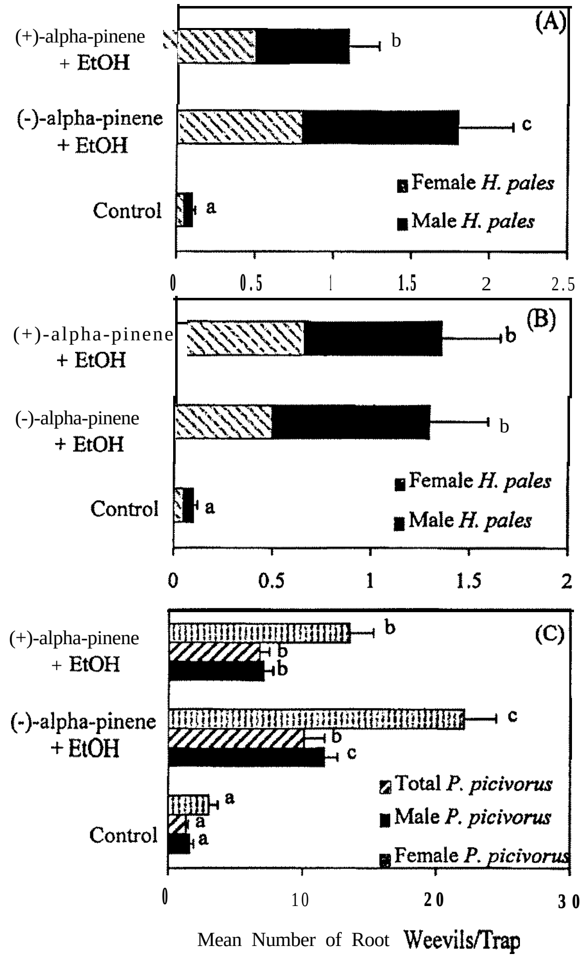

Fig. 4. Responses of root insects to stereochemistry of α-pinene, in combination with ethanol in Louisiana. Data collected in *P. taeda* plantations during 1999. (A) H. pales in lower-stem flight traps, (B) *H. pales* in pitfall traps, (C) *P. picivorus* in lower-stem flight traps. Means followed by the same letter within a species are not significantly different ($P < 0.05$, repeated measures analysis in PROC Mixed). Fisher protected LSD test ($P < 0.05$) was used for multiple comparisons of means of transformed data $[\sqrt{x}]$. Untransformed means are reported. Statistical parameters are presented in Table 1.

abundant monoterpene in all three species. The proportion of α-pinene occurring as the (−) stereoisomer ranged from 13.7% in resinosa to 23.4% in *P. strobus*. β-Pinene and limonene occurred almost entirely as the (−) enantiomer in all three species.

Discussion

The relative efficiency of different trap types that can be used to sample pine root insects varies with region. The superior performance of pitfall traps in Wisconsin, and of lower stem flight traps in Louisiana, suggests that geographically based behavioral differences may partially explain the observation by Fettig and Salom (1998) that pitfall traps in Virginia were less efficient than reported in the upper Midwest (e.g., Hunt and Raffa 1989; Hunt et al. 1993; Rieske and Raffa 1991, 1993b). These differences could reflect relatively more flight during host searching periods by southern pine root weevils. Mark-recapture studies conducted during early June (Rieske and Raffa 1999) and direct observations (Wilson and Millers 1983) suggest that most *H. pales* and *P. picivorus* in the Great Lakes region do not disperse very far, and appear to do so mostly by walking, at least during the periods examined. Although no direct comparisons of flight behavior have been conducted, high catches in wading-pool flight traps suggest that airborne dispersal is more important to root weevils in southern regions (Fatzinger 1985, Fatzinger et al. 1987). Perhaps the shorter development time, more rapid population buildup, more rapid exploitation of the resource, and warmer nocturnal temperatures in the southern United States favor more flight.

The observation that these root weevils prefer specific stereoisomers of host monoterpenes agrees with results with other root and stem colonizing insects of conifers (e.g., Nordenhem and Nordlander 1994, Hobson et al. 1993). However, in this system, we found no evidence of inter regional variation in preference for different enantiomers.

In some cases, our assays yielded unexpected results. For example, attraction of *D. valens* did not vary with the stereochemistry of α-pinene (Fig. 3A), even though (−)-α-pinene attracted more *D. valens* in funnel traps in Wisconsin (Erbilgin and Raffa 2000). However, the current study included ethanol in the treatments, whereas the previous study did not. Likewise, increasing the ratio of ethanol to (+)-α-pinene did not increase catch of *Hylobius* and *Pachylobius* in Wisconsin (Fig. 3 A and B), even though higher ratios of ethanol to mixtures of monoterpenes (i.e., turpentine) resulted in higher trap catch (Rieske and Raffa 1991). These results add further support to the view that specific compounds may elicit different behaviors, depending on whether they are emitted individually or in various combinations (Wood 1982).

Table 2. Composition of monoterpenes in phloem of *P. resinosa*, *P. banksiana*, and *P. strobus* in Wisconsin

Species	α-pinene	β-pinene	limonene	sabinene	3-carene	δ-terpinene	terpinolene	α-terpinol	myrcene	α-terpinene
P. resinosa	78.1 (13.7)	9.2 (96.3)	1.8 (97)	0.8 (0)	0.9 (0)	2.7	2.4	2.4	1	1
P. banksiana	75.2 (18.4)	12 (90.6)	1.2 (100)	0.7 (0)	0.0 (0)	1.8	1.7	3.7	3.1	0.7
P. strobus	70 (23.4)	23.2 (96)	1.0 (100)	1.2 (0)	0.0 (0)	1.2	1.3	0.9	1	0.4

Data given as the relative amount (%) of each constituent of the monoterpene fraction. The relative amount of the (−)-enantiomer (%) is included within brackets for those monoterpenes, which display optical activity.

Based on these results, we can make specific recommendations for monitoring complexes of root- and lower stem- insects in pest management programs. In the Great Lakes region, pitfall traps baited with (−)-α-pinene plus ethanol are more effective for root insects, and lower stem flight traps baited with (+)-α-pinene plus ethanol are more effective for lower stem insects. Although both trap types are effective for catching root weevils in Louisiana, lower stem flight traps appear more effective, especially for attracting females. In general, (-)-a-pinene plus ethanol seems the best option for attracting both sexes.

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