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STEREOSPECIFIC ANTENNAL RESPONSE BY RED TURPENTINE BEETLE, *Dendroctonus valens* TO CHIRAL MONOTERPENES FROM PONDEROSA PINE RESIN

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Abstract—The antennal response of *Dendroctonus valens* to host monoterpenes from the resin of ponderosa pine was studied using the electroantennogram (EAG) technique. Male and female beetles were given a single dose of each of 11 different monoterpenes. Response amplitude to the different compounds did not vary between sexes and was generally well correlated with results from field attraction studies. Response to (*S*)-(−)-β-pinene was greatest. The relative amplitude of the responses to the (*R*)-(+) and (*S*)-(−) enantiomers of α-pinene, however, were reversed from their relative attractiveness in the field. A dose-response study was conducted for the (*R*)-(+) and (*S*)-(−) enantiomers of α-pinene, plus a reciprocal differential saturation test with successive doses of first one enantiomer of α-pinene and then the other. Comparison of EAG traces suggests different receptors for the two stereoisomers of α-pinene. Differential saturation curves suggest that while one set of receptors may respond to both enantiomers, some receptors respond only to the (*S*)-(−) enantiomer.

Key Words—Coleoptera, Scolytidae, Dendroctonus valens, EAG, electroantennogram, enantiomer, kairomone, host attraction, bark beetle, α-pinene, β-pinene

INTRODUCTION

Dendroctonus valens (Leconte) is attracted to diseased and wounded conifers principally in the genus *Pinus*. Unlike its congeners and more well-known bark

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beetles, *D. valens* does not typically mass attack its host and is not generally an aggressive tree-killing species (Eaton and Lara, 1967). Instead of a powerful aggregation pheromone, *D. valens* may rely more on host odors or kairomones to locate and select its host. *D. valens* is attracted to the resin of its host ponderosa pine, *Pinus ponderosa* Lawson (Vité and Gara, 1962; Owen, 1985; Hobson, 1992). The principal attractants in the resin are the monoterpenes (*S*)-(−)-β-pinene, (*S*)-(+)-Δ-3-carene and (*R*)-(+)-α-pinene. The (*S*)-(−) enantiomer of α-pinene interrupts attraction of *D. valens* to its optical isomer, (*R*)-(+)-α-pinene (Hobson et al., 1993).

In the foothills of California's central Sierra Nevada *D. valens* feeds on two principal hosts, *P. ponderosa* and sugar pine, *Pinus lambertiana* Dougl. The resin of *P. ponderosa* is composed principally of (*S*)-(−)-β-pinene, (*S*)-(+)-Δ-3-carene and (*S*)-(−)-α-pinene with smaller amounts of myrcene and (*S*)-(−)-limonene (Smith, 1977; Hobson et al., 1993). The resin of *P. lambertiana* is composed principally of (*R*)-(+)-α-pinene, (*S*)-(−)-α-pinene, (*S*)-(−)-β-pinene, (*S*)-(+)-Δ-3-carene, and myrcene in decreasing order of percent composition (Hobson et al., 1993).

This study was conducted in tandem with the identification and field testing of the attractants for *D. valens* from ponderosa pine resin. The aim was to investigate the sensitivity and specificity of the antennal response of *D. valens* to a range of monoterpenes, for correlation with field test results, and thus to help further our understanding of host tree location mechanisms.

METHODS AND MATERIALS

D. valens adults were collected at the University of California's Blodgett Forest Research Station (El Dorado County, California) in the foothills of the central Sierra Nevada in August 1988. Adults were captured in Lindgren flight traps (Lindgren, 1983) baited with commercial gum turpentine (T&R Chemical, Clinton, Texas) and sexed by male stridulation (Pajares and Lanier, 1990).

The antennal response of *D. valens* to individual compounds was determined using the EAG technique of Schneider (1957), with methods described previously by White and Birch (1987). Responses were recorded from isolated heads of *D. valens*, mounted ventral surface uppermost on a glass stage using double-sided adhesive tape. The recording electrode (a glass micropipet filled with insect saline) was inserted into a hole punched in the terminal club segment using an etched tungsten needle, while the indifferent electrode was inserted into the back of the head. The electrodes were connected via chloridized silver wires to a Grass P-16 preamplifier, and EAG responses were displayed on a Tektronix 502 oscilloscope. A permanent record of EAG responses was made

using a Gould Brush 220 pen recorder, from which measurements of EAG amplitudes were made.

Odor stimuli were delivered from cartridges as 1.0-sec pulses into a continuous airstream (1000 ml/min) directed onto the antenna, as described previously by White and Birch (1987). Each material (10 μl) was applied to a filter paper strip and the solvent allowed to evaporate before use. A blank cartridge (solvent only) was presented before each test material, and the resulting EAG response subtracted from the subsequent test response before analysis of the results, to control for the effects of the solvent and mechanical disturbance of the airflow in the first experiment. Eleven compounds, obtained from Aldrich Chemical Company with chemical purities shown in Table 1, were tested. Nine were previously identified as the major monoterpenes from resin extracts of ponderosa pines at Blodgett Forest: (*R*)-(+)-α-pinene, (*S*)-(−)-α-pinene, (*S*)-(−)-β-pinene, β-phellandrene, (*S*)-(+)-Δ-3-carene, myrcene, (*S*)-(−)-limonene, (*R*)-(+)-limonene, and terpinolene (Hobson et al., 1993); two (longifolene, a sesquiterpene and estragole, a resin and foliage aromatic ether) were included to test the response of the antennae to terpenes from sympatric conifers and foliage of ponderosa pine. Each was tested at a single (10-μg) dose applied to a filter paper strip in 10 μl of dichloromethane, which was allowed to evaporate before the paper was inserted into the cartridge. The order of presentation of compounds was randomized between beetles. A total of 10 individuals (five male, five female) were tested.

The results from testing the 11 compounds were analyzed using a two-layered analysis of variance to investigate the effect of beetle sex, and the different chemicals on the EAG response followed by Bonferroni pairwise tests ($\alpha = 0.05$) (Crunch Interactive Software, San Francisco, California).

TABLE 1. CHEMICAL PURITIES OF TESTED COMPOUNDS

(*R*)-(+)-α-pinene	98% (optical purity 96%)[a]
(*S*)-(−)-α-pinene	98% (optical purity 91%)[a]
(*S*)-(−)-β-pinene	99%
β-Phellandrene	[b]
(*S*)-(+)-Δ-3-Carene	95%
Myrcene	85%
(*S*)-(−)-Limonene	97% (optical purity 91%)
(*R*)-(+)-Limonene	97% (optical purity 91%)
Longifolene	98%
Terpinolene	[b]
Estragole	98%

[a] Determined by chiral GC column (Hobson et al., 1993).
[b] Not recorded.

In a separate experiment, a log dilution series in dichloromethane was made up for each of the two stereoisomers of α-pinene. Each series was presented to six male *D. valens*, again randomizing the order of presentation.

To determine the specificity of the antennal receptors to the two enantiomers of α-pinene, a differential saturation test was carried out (Payne and Dickens, 1976) using methods previously described White (1987). This involved recording the EAG response (as described above), while the antennal receptors were presented first with a continuous saturating dose (1 mg) of one enantiomer, then, after a 1.5-sec delay, with a simultaneous pulse (1.0-sec duration) of the other enantiomer (1 mg). The odors were presented using a three-inlet delivery tube, keeping the total airflow over the antennae at 1000 ml/min throughout the presentation. The procedure was repeated for three male individuals. In each case the antenna was presented with first one enantiomer of α-pinene as the saturating dose and then the other. To ensure that the first presentation did saturate the EAG response, each antenna was also presented with a saturating dose of each enantiomer, followed by a test dose of the same enantiomer.

RESULTS AND DISCUSSION

EAG Responses to Individual Components. Analysis of variance showed no difference in EAG responses between the sexes ($F = 0.07$, $P = 0.8$), therefore, the data were pooled for presentation. There were clear differences, however, between the responses to the components tested ($F = 12.5$, $P < 0.001$), with (*S*)-(−)-β-pinene giving the largest EAG responses and longifolene the smallest (Table 2). Where optically active components were tested, there was evidence of chiral specificity in the response of *D. valens* antennal receptors. There was a clear difference between the responses to (*R*)-(+)-α-pinene and (*S*)-(−)-α-pinene ($P < 0.01$, Bonferroni test), although there was no specificity shown between (*R*)-(+)-limonene and (*S*)-(−)-limonene. The lack of differences in response between the sexes suggests that both male and female *D. valens* antennae possess similar numbers and types of receptor sites (Payne, 1975).

The lack of sexual differences in EAG responses agrees with the overall even ratio of sexes caught in the traps (Hobson et al., 1993). The closely related *D. terebrans* also showed very little sexual difference in EAG response to α-pinene, β-pinene, turpentine, and six scolytid pheromones (Delorme and Payne, 1990). *Dendroctonus frontalis* (Zimmermann) males and females did not differ in their EAG response to four different enantiomeric blends of frontalin (Payne et al., 1982). *Dendroctonus ponderosae* (Hopkins) males and females did not differ in their response to three terpenoid pheromones (*trans*-verbenol, *cis*-verbenol, verbenone) and differed in their response to three bicyclic ketal pheromones (*exo*-brevicomin, frontalin, *endo*-brevicomin) only at the highest

TABLE 2. RESPONSE OF *D. valens* TO HOST MONOTERPENES[a]

Compound	EAG (μV)[2b]	*D. valens* catch[3c]	*D. valens* catch[4d]
(*S*)-(−)-β-Pinene	467 ± 165[a]	27.8[a]	61.1[a]
(*S*)-(+)-Δ-3-Carene	193 ± 115[c]	2.6[b]	11.2[b]
(*R*)-(+)-α-Pinene	162 ± 92[c]	2.2[b]	0.7[d]
(*S*)-(−)α-Pinene	452 ± 155[a]	0.1[c]	
Myrcene	385 ± 262[ab]		2.5[c]
(*R*)-(+)-Limonene	146 ± 71[c]		0.8[d]
(*S*)-(−)-Limonene	208 ± 154[bc]		
β-Phellandrene	162 ± 162[bc]		
Terpinolene	125 ± 125[bc]		
Estragole	421 ± 173[ab]		
Longifolene	21 ± 28[c]		

[a] Means followed by the same superscript are not significantly different ($\alpha = 0.05$).
[b] Mean and SD for 10 beetles.
[c] Mean *D. valens* caught in test 5 (Hobson et al., 1993).
[d] Mean *D. valens* caught in test 2; α-pinene and limonene tested as natural enantiomeric mix in *P. ponderosa* resin (Hobson et al., 1993).

two concentrations (10 and 100 μg) tested (Whitehead et al., 1989). In contrast, female *D. pseudotsugae* had a higher EAG response to limonene and more receptor cells that responded to either α-pinene or limonene (unspecified enantiomers) than did males (Dickens et al., 1983, 1984). *D. pseudotsugae* females were also more attracted to host odors than were males (Rudinsky, 1966).

EAG responses to individual components of the resin correlated to some extent with their attractiveness in field tests, although not all compounds tested on the antenna were tested in the field. (*S*)-(−)-β-pinene gave the largest EAG responses at the dose tested and was the most effective in attracting beetles to traps, although (*S*)-(−)-α-pinene and myrcene gave EAG responses that were not significantly different from (*S*)-(−)-β-pinene, yet produced much lower trap catches. A notable exception to the correlation between EAG response and attraction in the field was α-pinene, where (*R*)-(+)-α-pinene produced a lower EAG response than (*S*)-(−)-α-pinene but was significantly more attractive in the field (Table 2).

This stereospecificity was confirmed in the second experiment, where the dose–response curve of the two stereoisomers of α-pinene showed a significantly greater EAG response to the (*R*)-(+) enantiomer at all doses >1 μg (Figure 1).

The presence of a large EAG response suggests the presence of many receptor sites for a given compound, although it cannot determine whether such compounds will act as attractants, repellents, or in some other way. Ecologically

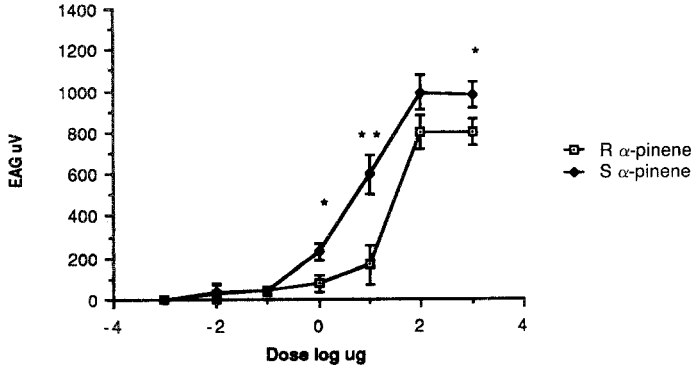

FIG. 1. Dose–response curve of EAG response (mean ± SE) of six male *D. valens* to dilutions of (*R*)-(+)- and (*S*)-(−) α-pinene. *$P < 0.05$; ** $P < 0.01$.

relevant materials with high EAG activity are likely, however, to be of behavioral significance to the insect (see Masson and Mustaparta, 1990, for a review). The greater EAG response to the *S* enantiomer of α-pinene, relative to the *R* enantiomer, suggests that the *S* enantiomer may have a role to play in the host location mechanism. Trap catch results have shown that (*S*)-α-pinene acts as an interruptant and reduces the trap catch caused by (*R*)-(+)-α-pinene (Hobson et al., 1993). This, together with the abundance of (*S*)-(−)-α-pinene in sympatric nonhost conifers, suggests that (*S*)-(−)-α-pinene may be a cue whereby *D. valens* can discriminate nonhosts (Hobson et al., 1993).

The reversal of the relative amplitudes of the antennal EAG response from the preference exhibited in the field prompted a closer examination of the shape of the EAG curves for the two enantiomers of α-pinene (Figure 2). Measurements of the time taken for the EAG response to recover to half of the maximum response amplitude showed that recovery following stimulation by (*S*)-(−)-α-pinene takes significantly longer than recovery following stimulation by (*R*)-(+)-α-pinene ($t = 5.48$; 5 *df*, $P < 0.01$ for 100-μg doses). This suggests that different receptor populations may be involved. The differential saturation curves support this conclusion (Figure 3). When 1 mg of (*R*)-(+)-α-pinene was delivered, followed by 1 mg of (*S*)-(−)-α-pinene there was an initial strong antennal response and a subsequent additional response, indicating that a separate set of (*S*)-(−) receptors were capable of responding after a saturation dose of (*R*)-(+). Saturation of the antennal receptors was demonstrated at this dose level by presenting two successive doses of 1 mg of (*R*)-(+) and obtaining only one response (Figure 3b). In contrast, a 1-mg dose of (*S*)-(−) followed by the same dose of (*R*)-(+) produced only one response (Figure 3c). Again, saturation at the 1-mg level of (*S*)-(−) was demonstrated by two successive 1-mg pulses of (*S*)-(−), which produced only one response.

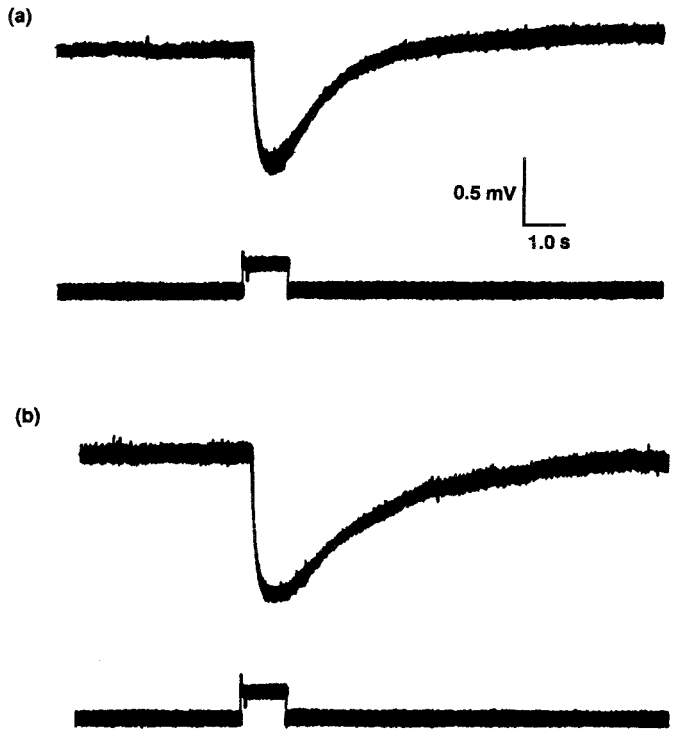

FIG. 2. EAG trace of *D. valens* to 100 μg of (*R*)-(+)-α-pinene (a) or (*S*)-(−)-α-pinene (b) Lower trace shows pulse duration of odor stimulus.

Delorme and Payne (1990) suggest from saturation curves that *D. terebrans* receptors for α-pinene and β-pinene may be triggered by other monoterpenes, but *D. terebrans* antennal receptors continued to respond to doses of α- and β-pinene beyond the saturation dose of turpentine. However, the relative proportions of (*R*)-(+) and (*S*)-(−) enantiomers were not known for the α-pinene or the turpentine.

The responses of *D. valens* antennae suggest at least two sets of receptors of α-pinene, those that may respond to either (*R*)-(+) or (*S*)-(−), (at least at high concentrations) and those that respond to (*S*)-(−) only. These results do not rule out the existence of receptors specific for the *R* enantiomer. This technique tests extremely high concentrations of the odors; specificity for the *R* enantiomer may be shown by some receptors at lower, physiologically and ecologically more relevant concentrations. Single-unit recordings would be required to confirm this. At the high concentrations presented here, such discrimination may break down and the *R* receptors may be saturated by the *S*

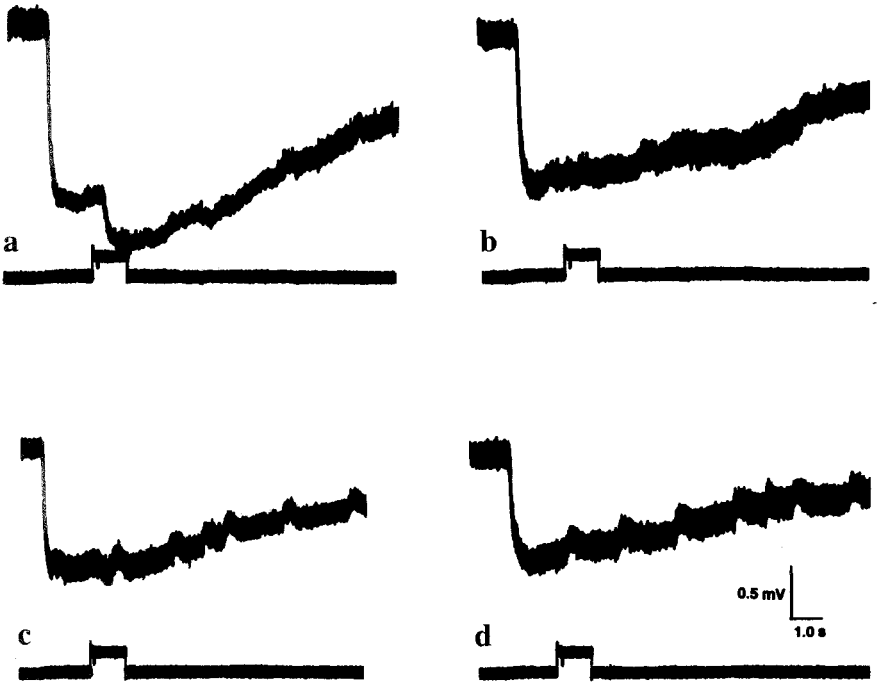

FIG. 3. Differential saturation EAG trace of *D. valens* to 1-mg doses of α-pinene enantiomers: (*R*)-(+) then (*S*)-(−) (a), (*R*)-(+) then (*R*)-(+) (b), (*S*)-(−) then (*R*)-(+) (c), and (*S*)-(−) then (*S*)-(−) (d). EAG (top trace) shows onset of first (saturating) odor stimulus, which was kept on for 5 sec. Lower trace shows pulse duration of second (test) odor stimulus.

enantiomer. These results do demonstrate, however, that there are at least two distinct populations of receptors for α-pinene and that one population responds specifically to the *S* enantiomer.

The specificity of the putative (*S*)-(−) receptors is indicated by the additional response obtained after 100 μg of (*R*)-(+) failed to exhaust the antennae's capacity to respond to (*S*)-(−). Similarly *I. pini* antennal receptors sensitive to ipsdienol maintained their specificity when tested with a 500-μg dose of other odors (Mustaparta et al., 1979). In contrast, the most specific antennal receptors of *D. terebrans* (sensitive to 3,2-MCHone and 3,2-MCHol) responded to a 1-μg dose of other odors such as α-pinene (Dickens et al., 1984). The specificity of the putative (*S*)-(−) receptor in *D. valens* antennae suggests that the ability to distinguish the two enantiomers of α-pinene that are widespread and abundant in host and nonhost conifers is important in host selection. Separate receptors that discriminate different enantiomers of pheromones are known for *Ips para-*

confusus Lanier, *Ips pini* (Say), and *Scolytus scolytus* (F.) (Mustaparta et al., 1979, Wadhams et al., 1982) although *D. pseudotsugae* and *D. frontalis* received both (+)- and (−)-frontalin on a single receptor (Dickens et al., 1985; Payne et al., 1982). Prior to the current study, however, few comparative EAG or single cell electrophysiological tests of enantiomers of chiral host monoterpenes have been done. Both enantiomers of limonene and α-pinene elicited significant EAG responses from *Anthonomus grandis* Boh., but there was no evidence of chiral specificity (Dickens, 1984).

Additional EAG or single-cell dose–response studies with other host monoterpenes that are attractive to *D. valens* in field tests (e.g., β-pinene and Δ-3-carene) are likely to further our understanding of *D. valens*' host selection. Ultimately we may answer the question of how *D. valens* can, by olfaction, identify its host species and individual hosts in a forest of mixed species and variable individuals with broadly overlapping host odor components.

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REFERENCES

DELORME, J.L. and PAYNE, T.L. 1990. Antennal olfactory responses of black turpentine beetle, *Dendroctonus terebrans* (Olivier), to bark beetle pheromones and host terpenes. *J. Chem. Ecol.* 16:1321–1329.

DICKENS, J.C. 1984. Olfaction in the boll weevil *Anthonomus grandis* Boh. (Coleoptera: Curculionidae): Electroantennogram studies. *J. Chem. Ecol.* 10:1759–1785.

DICKENS, J.C., GUTMANN, A., PAYNE, T.L., RYKER, L.C., and RUDINSKY, J.A. 1983. Antennal olfactory responsiveness of Douglas-fir beetle *Dendroctonus pseudotsugae* Hopkins (Coleoptera: Scolytidae) to pheromones and host odors. *J. Chem. Ecol.* 9:1383–1395.

DICKENS, J.C., PAYNE, T.L., RYKER, L.C., and RUDINSKY, J.A. 1984. Single cell responses of the Douglas-fir beetle *Dendroctonus pseudotsugae* Hopkins (Coleoptera: Scolytidae) to pheromones and host odors. *J. Chem. Ecol.* 10:583–600.

DICKENS, J.C., PAYNE, T.L., RYKER, L.C., and RUDINSKY, J.A. 1985. Multiple acceptors for pheromonal enantiomers on single olfactory cells in the Douglas-fir beetle *Dendroctonus pseudotsugae* Hopk. (Coleoptera: Scolytidae). *J. Chem. Ecol.* 11:1359–1370.

EATON, C.B., and LARA, R.R. 1967. Red turpentine beetle *Dendroctonus valens* LeConte, *in* A.G. Davidson and R.M. Prentice (eds.). Important Forest Insects of Mutual Concern to Canada, the United States and Mexico. Canada Department of Forestry and Rural Development Pub. 1180. Ottawa, 248 pp.

HOBSON, K.R. 1992. Studies on host selection and biology of *Dendroctonus valens* and fungal host interactions of *Dendroctonus brevicomis*. PhD dissertation. University of California, Berkeley, 65 pp.

HOBSON, K.R., WOOD, D.L., COOL, L.G., WHITE, P.R., OHTSUKA, T., KUBO, I., and ZAVARIN, E. 1993. Chiral specificity in responses by the bark beetle *Dendroctonus valens* to host kairomones. *J. Chem. Ecol.* In press.

LINDGREN, B.S. 1983. A multiple funnel trap for scolytid beetles (Coleoptera). *Can. Entomol.* 115:299–302.

MASSON, C., and MUSTAPARTA, H. 1990. Chemical information processing in the olfactory system of insects. *Physiol. Rev.* 70:199–245.

MUSTAPARTA, H., ANGST, M.E., and LANIER, G.N. 1979. Specialization of olfactory cells to insect- and host-produced volatiles in the beetle *Ips pini* (Say). *J. Chem. Ecol.* 5:109–123.

OWEN, D.R. 1985. The role of *Dendroctonus valens* and its vectored fungi in the mortality of ponderosa pine. PhD dissertation. University of California, Berkeley, 64 pp.

PAJARES, J.A. and LANIER, G.N. 1990. Biosystematics of the turpentine beetles *Dendroctonus terebrans* and *D. valens* (Coleoptera: Scolytidae). *Ann. Entomol. Soc. Am.* 83:171–188.

PAYNE, T.L. 1975. Bark beetle olfaction. III antennal olfactory responsiveness of *Dendroctonus frontalis* Zimmerman and *D. brevicomis* LeConte (Coleoptera: Scolytidae) to aggregation pheromones and host tree terpene hydrocarbons. *J. Chem. Ecol.* 1:233–242.

PAYNE, T.L., and DICKENS, J.C. 1976. Adaptation to determine receptor system specificity in insect olfactory communication. *J. Insect Physiol.* 22:1569–1572.

PAYNE, T.L., RICHERSON, J.V., DICKENS, J.C., WEST, J.R., MORI, K., BERISFORD, C.W., HEDDEN, R.L., VITÉ, J.P., and BLUM, M.S. 1982. Southern pine beetle: Olfactory receptor and behavior discrimination of enantiomers of the attractant pheromone frontalin. *J. Chem. Ecol.* 8:873–881.

RUDINSKY, J.A. 1966. Host selection and invasion by the Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins, in coastal Douglas-fir forests. *Can. Entomol.* 98:98–111.

SCHNEIDER, D. 1957. Elektrophysiologische Untersuchungen von Chemo- und Mechanorezeptoren der Antennae des Seidenspinners. *Bombyx mori* L. *Z. Vergl. Physiol.* 40:8–41.

SMITH, R.H. 1977. Monoterpenes of ponderosa pine xylem resin in western United States. USDA Tech. Bull. 1532. Washington, D.C., 48 pp.

VITÉ, J.P., and GARA, R.I. 1962. Volatile attractants from ponderosa pine attacked by bark beetles (Coleoptera: Scolytidae). *Contrib. Boyce Thompson Inst.* 21:251–274.

WADHAMS, L.J., ANGST, M.E., and BLIGHT, M.M. 1982. Responses of the olfactory receptors of *Scolytus scolytus* (F.) (Coleoptera: Scolytidae) to the stereoisomers of 4-methyl-3-heptanol. *J. Chem. Ecol.* 8:477–492.

WHITE, P.R. 1987. Responses of the saw-toothed grain beetle, *Oryzaephilus surinamensis*, to food odours and pheromones. D Phil thesis. University of Oxford, Oxford, U.K.

WHITE, P.R., and BIRCH, M.C. 1987. Female sex pheromone of the common furniture beetle *Anobium punctatum* (Coleoptera: Anobiidae): extraction, identification and bioassays. *J. Chem. Ecol.* 13:1695–1706.

WHITEHEAD, A.T., SCOTT, D.T., SCHMITZ, R.F., and MORI, K. 1989. Electroantennograms by mountain pine beetle *Dendroctonus ponderosae* Hopkins, exposed to selected chiral semiochemicals. *J. Chem. Ecol.* 17:2089–2099.