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ANTENNAL OLFACTORY RESPONSES OF BLACK TURPENTINE BEETLE, *Dendroctonus terebrans* (OLIVIER), TO BARK BEETLE PHEROMONES AND HOST TERPENES[1]

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Abstract—Electroantennograms (EAGs) were recorded from male and female black turpentine beetles, *Dendroctonus terebrans* (Olivier), exposed to bark beetle pheromones and host terpenes. The dose–response curves indicated similarities in the receptor mechanisms for both sexes for each compound. Antennal sensitivity was greatest to *endo*-brevicomin, which correlates with the importance of the compound in the behavior of the beetles. At above-threshold concentrations, EAGs were greatest to *endo*-brevicomin and frontalin, suggesting a large population of antennal receptors for these compounds. A large population of receptors would be expected for compounds that play such a significant role in this beetle's behavior. Beetles were also shown to have receptors that respond to the *Ips* pheromones, ipsenol, and ipsdienol.

Key Words—Electroantennogram, electrophysiology, black turpentine beetle, *Dendroctonus terebrans*, Coleoptera, Scolytidae, host odors, pheromones.

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INTRODUCTION

Payne et al. (1987) showed that male and female black turpentine beetles, *Dendroctonus terebrans* (Olivier), possess antennal olfactory receptors that respond to host terpenes and pheromones of the sympatric southern pine beetle, *D. frontalis*. They also showed that *D. terebrans* produce and respond behaviorally to some of the same pheromones as *D. frontalis* and proposed a relationship in which the pheromones produced by one species may function as kairomones for the other species. In order to further explore the ability of *D. terebrans* to detect pheromones of sympatric species, we conducted electrophysiological investigations using host odors, *D. frontalis* pheromones, and racemic mixtures of the sympatric *Ips* species pheromones ipsenol and ipsdienol (Silverstein et al., 1966; Renwick and Vité, 1972; Vité et al., 1976a, 1978).

METHODS AND MATERIALS

Beetles. Adult beetles of unknown age were collected from stumps or the bases of pine trees in the east Texas pine forest. Beetles were sexed (Godbee and Franklin, 1978), isolated in Petri dishes with moistened filter paper, and stored for 1–40 days at 6°C in a domestic refrigerator without photoperiodic control, prior to use in experiments.

Compounds. Nine beetle- and host tree-produced compounds were used in the study. The source and purity of each compound is listed in Table 1. Racemic mixtures of chiral compounds were used, even though chirality is important to

TABLE 1. SOURCE AND PURITY OF PHEROMONES AND HOST TERPENES USED IN STUDY

Compound	Source of supply	Purity (%)
endo-Brevicomin	Chem. Samp. Co.	99
Frontalin	BASF	99
Ipsenol	Borregard	81
Ipsdienol	Borregard	89
α-Pinene	Aldrich Chemical Co.	97
β-Pinene	Aldrich Chemical Co.	97
Verbenone	Chem. Samp. Co.	98
trans-Verbenol	Chem. Samp. Co.	99
Turpentine	Short path distillation of loblolly pine oleoresin	99

the response of sympatric bark beetles (Vité et al., 1976a,b, 1978). All compounds were diluted in nanograde pentane.

Electroantennograms. Electroantennogram (EAG) techniques (Dickens and Payne, 1977) were modified from earlier techniques (Schneider, 1957; Payne, 1970, 1975). Glass capillary Ag–AgCl electrodes filled with insect Ringer's solution (Barbosa, 1974) were used. Following prepuncture with a sharpened tungsten needle, the indifferent electrode was inserted into the head capsule; the recording electrode was inserted in the distal end of the antennal club. EAGs were displayed on a Tektronix 5223 digitizing oscilloscope and recorded on a Soltec x–y plotter.

Stimuli were delivered at 5-min intervals in increasing concentrations as 5 μl aliquots on filter paper (20 × 7 mm) placed into glass cartridges (75 mm long, 5 mm ID) and oriented toward the antennal preparation from ca. 1 cm. Stimulus duration was 2 sec in a 1 liter/min airflow filtered through activated charcoal. The initial depolarization upon stimulation was recorded as response to a given stimulus.

Serial dilutions of each compound were presented from the lowest to the highest concentration. The pentane solvent (5 μl on filter paper) was used as a control. The *D. frontalis* aggregation pheromone frontalin, at 5 μg, was used as the standard. Response to the standard was for males, $\bar{X} = 1.42$ mV $\pm$ SE $= 0.7$ mV, $N = 40$, and for females, $\bar{X} = 1.80$ mV $\pm$ SE $= 0.33$ mV, $N = 40$. Response to the control was for males, $\bar{X} = 0.31$ mV $\pm$ SE $= 0.03$ mV, $N = 40$, and for females, $\bar{X} = 0.37$ mV $\pm$ SE $= 0.05$ mV, $N = 40$. Response to the control was subtracted from the response to each stimulus. Response to the standard was recorded after the control and again after every two stimulations in a dilution series. Response to each dilution in the series was calculated as a percent of the mean of the two closest responses to the standard. This was done to normalize the data to control for variation between preparations and within preparations over time (Payne, 1975). Four beetles of each sex were tested for each compound. A single beetle may have been stimulated by more than one compound if the EAG elicited by the standard was greater than or equal to 1 mV.

All statistical analyses were computed using the Statistical Analysis System (SAS Institute Inc., Cary, North Carolina). The threshold of response to each compound was the minimum stimulus concentration at which, relative to the standard, the percent EAG to the stimulus was significantly greater ($P \geq 0.05$) than the percent EAG to the corresponding pentane control, as determined by the t test. Analysis of variance was used to compare percent response values between compounds for each sex at and above threshold. Means were separated using the least significant difference test. The t test was used to compare percent response values between sexes for each compound at each dose.

RESULTS AND DISCUSSION

All compounds used elicited EAG responses within the range of concentrations tested (Figure 1A–I). The similarity in shapes of the dose–response curves for male and female beetles to each compound suggest similar receptor mechanisms for both sexes. Significant differences in percent response values between the sexes did occur for turpentine at 5 μg, for α-pinene at 50 μg, and for *trans*-verbenol and verbenone at 0.5 μg on filter paper. These differences

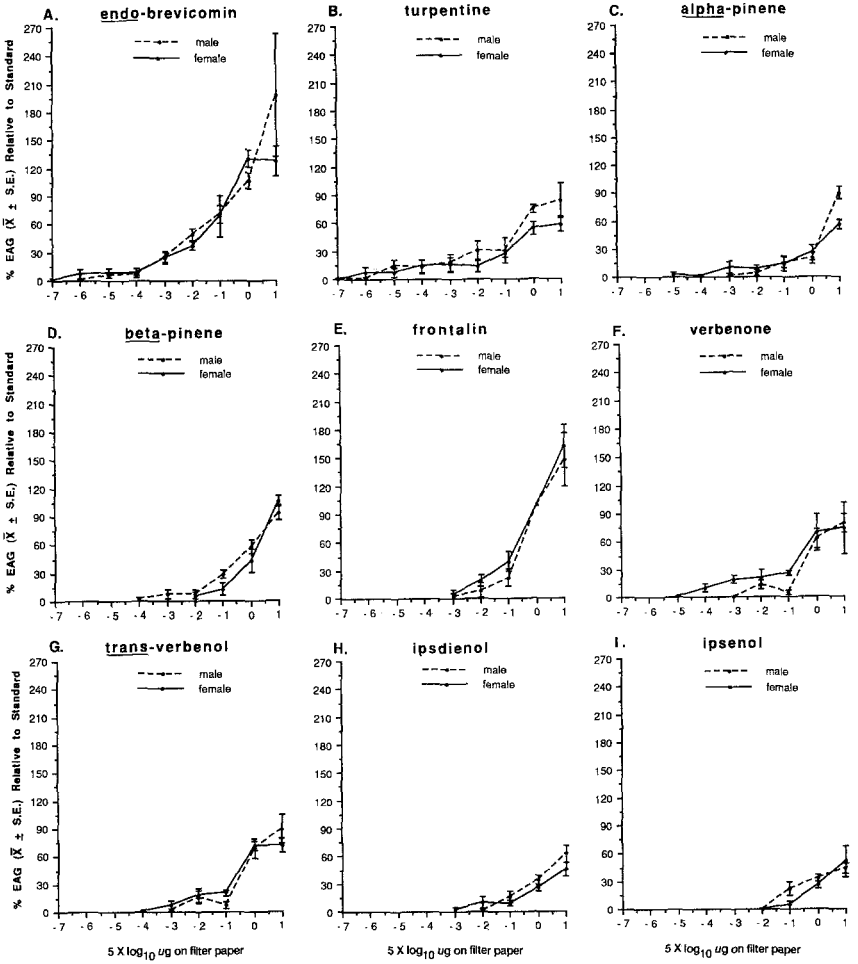

FIG. 1. Mean percent EAGs ± SE from *Dendroctonus terebrans* to pheromones and host terpenes, relative to the standard frontalin.

were not consistent over all concentrations and were likely the result of variability among the different preparations.

Response thresholds were lowest for *endo*-brevicomin (Table 2). The low threshold and wide concentration range to receptor saturation suggests that this compound may function in long-range orientation for *D. terebrans* (Dickens, 1981). Payne et al. (1987) found that *endo-brevicomin* was highly attractive to *D. terebrans* females in a pedestrian bioassay and when released with large quantities of turpentine in the field.

The higher thresholds for the other compounds suggest that they are more likely to play a role in intermediate- or short-range host orientation or synergism (Dickens, 1981).

At threshold doses and above (5 μg and 50 μg on filter paper), EAGs were greater for *endo*-brevicomin and frontalin than for the other compounds (Table 3), suggesting a larger population of receptors for these compounds than for the other compounds tested. This difference was significant ($P \leq 0.05$) at 5 μg, but not at 50 μg on filter paper. *endo*-Brevicomin is a male-produced *D. frontalis* pheromone that inhibits arrestment of conspecifics on mass-attacked trees (Vité and Renwick, 1971; Rudinsky et al., 1974; Payne et al., 1978; Richerson and Payne, 1979). Although *endo*-brevicomin is produced in only trace amounts by male *D. terebrans*, it is highly attractive to females of this species (Payne et al., 1987). The degree of antennal sensitivity and magnitude of response at higher concentrations, coupled with the strong female behavioral response, indicates that the *D. frontalis*-produced *endo*-brevicomin may function as a kairomone that benefits host-seeking *D. terebrans* females by directing them to weakened hosts.

Payne et al. (1987) reported that frontalin was highly attractive to male *D. terebrans* both in a pedestrian bioassay and in the field when released with large

TABLE 2. EAG THRESHOLDS OF *Dendroctonus terebrans* TO PHEROMONES AND HOST TERPENES

Compound	Female threshold	Male threshold
α-Pinene	10^{0}	10^{0}
β-Pinene	10^{0}	10^{-1}
endo-Brevicomin	10^{-3}	10^{-3}
Frontalin	10^{-1}	10^{-1}
Ipsdienol	10^{0}	10^{0}
Ipsenol	10^{0}	10^{0}
trans-Verbenol	10^{-2}	10^{0}
Turpentine	10^{-1}	10^{-2}
Verbenone	10^{-1}	10^{0}

TABLE 3. MEAN PERCENT EAG RESPONSE RELATIVE TO STANDARD[a] OF *D. terebrans* TO BARK BEETLE PHEROMONES AND HOST TERPENES

Concentration (μg)	Chemical	$\bar{X}$ ♀ Response ± SE	$\bar{X}$ ♂ Response ± SE
5	*endo*-Brevicomin	131.1 ± 9.0 a	107.2 ± 8.8 a
	Frontalin	100.0 ± 0.0 b	100.0 ± 0.0 a
	trans-Verbenol	71.5 ± 4.7 c	68.6 ± 10.3 b
	Verbenone	69.1 ± 19.7 cd	62.5 ± 10.3 b
	Turpentine	54.0 ± 6.7 cd	75.0 ± 4.4 b
	β-Pinene	43.0 ± 12.7 de	58.1 ± 7.0 b
	Ipsdienol	25.9 ± 3.9 e	33.9 ± 5.6 c
	Ipsenol	25.9 ± 3.4 e	33.5 ± 3.2 c
	α-Pinene	25.5 ± 7.9 e	20.0 ± 5.8 c
50	*endo*-Brevicomin	128.2 ± 16.3 ab	198.5 ± 64.0 a
	Frontalin	162.2 ± 22.4 a	147.7 ± 28.2 ab
	β-Pinene	106.3 ± 5.6 bc	94.3 ± 8.2 bc
	Verbenone	74.8 ± 7.8 cd	79.0 ± 9.9 bc
	trans-Verbenol	71.4 ± 7.8 cd	89.2 ± 15.7 bc
	Turpentine	58.3 ± 8.2 d	84.2 ± 18.4 bc
	α-Pinene	55.5 ± 5.2 d	89.7 ± 6.7 bc
	Ipsenol	51.2 ± 16.8 d	44.1 ± 5.4 c
	Ipsdienol	45.2 ± 7.2 d	62.7 ± 9.4 c

[a] 5 μg frontalin on filter paper.
[b] Means in the same column followed by the same letter are not significantly different at the 5% probability level according to the least significant difference test.

quantities of turpentine. Analogous results were reported by Phillips et al. (1989). Both studies found that this compound is produced and released by *D. terebrans* females. Female production, the magnitude of response at the higher concentrations, and the strong behavorial response by males implicate frontalin as a sex pheromone for *D. terebrans*.

The attraction of *D. terebrans* to turpentine is important in host finding. Turpentine-baited traps attract large numbers of *D. terebrans* (Fatzinger, 1985; Fatzinger et al., 1987). Thresholds for the monoterpenes α- and β-pinene were much higher than for turpentine. Turpentine consists of more than one volatile component (Renwick and Vité, 1970), and the lower threshold may result from the summation of receptor potentials from the simultaneous firing of receptors for the different components. Delorme (unpublished) found that *D. terebrans* males responded significantly to β-pinene in a pedestrian bioassay. The monoterpenes may play a role in close-range orientation for *D. terebrans*, as sug-

gested for *D. frontalis* (Payne, 1980). Receptor saturation for turpentine was reached at 5 μg on filter paper for both sexes, whereas response to α- and β-pinene was still increasing at 50 μg on filter paper. This suggests that receptors for α- and β-pinene also might be responsive to other monoterpenes in turpentine.

Verbenone, produced by male and female *D. frontalis*, inhibits arrestment of conspecifics on mass-attacked trees (Renwick and Vité, 1970; Payne et al., 1978). Phillips et al. (1989) reported that both sexes of *D. terebrans* produce verbenone during gallery construction, but did not discover a behavioral function for the compound. The high threshold and narrow range to saturation suggests that verbenone may function in close-range orientation or synergism (Dickens, 1981).

The dose–response curve for *trans*-verbenol has similar characteristics to that of verbenone, indicating that *trans*-verbenol also functions in close-range orientation or synergism. Payne et al. (1987) and Phillips et al. (1989) reported the production of *trans*-verbenol by both sexes of *D. terebrans*. Fatzinger et al. (1987) reported that *trans*-verbenol by itself was not attractive to *D. terebrans* in the field but had a synergistic effect when released with turpentine and ethanol. In contrast, Phillips et al. (1989) did not find any effect of *trans*-verbenol on the attraction of turpentine and frontalin to *D. terebrans*. Payne et al. (1987) reported that *D. terebrans* males were significantly attracted to *trans*-verbenol in a pedestrian bioassay.

Although ipsenol and ipsdienol elicited EAGs, the high thresholds and low responses at high concentrations (Figure 1H and I) indicate a relatively small population of receptors for these compounds. Billings (unpublished) found a significant decrease in field response of male *D. terebrans* to large quantities of turpentine when a mixture of 2% each of ipsenol, ipsdienol, and *cis*-verbenol was released with the host material. Delorme (unpublished) found that female *D. terebrans* response to ipsdienol was lower than to a pentane control in a pedestrian bioassay. It is known that olfactory interactions play an important role in resource partitioning among sympatric *Dendroctonus* and *Ips* species (Birch et al., 1980; Byers and Wood, 1980; Svihra et al., 1980; Flamm et al., 1987). The *Ips* pheromones may function to regulate host colonization by *D. terebrans* and sympatric *Ips*, especially *Ips calligraphus*, which can have overlapping ranges of distribution within the bole (Smith, 1957; Birch and Svihra, 1979; Flamm et al., 1988). The interaction of *D. terebrans* with *Ips* species warrants further investigation in the field.

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