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PHEROMONE PRODUCTION BY THE BARK BEETLE, *Ips paraconfusus*,[1] IN THE NONHOST, WHITE FIR[2,3]

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Abstract—Male *I. paraconfusus* confined to artificial entrance tunnels in white fir logs produced the pheromone compounds ipsenol and ipsdienol in their hindguts. The hindguts were attractive to females in a laboratory olfactometer and the male infested logs were attractive in field bioassay. The amount of pheromones produced and the amount of feeding and boring activity is much less in white fir than in ponderosa pine. There were no pheromones detected in the hindguts of recently emerged, unfed males.

Key Words—*Ips paraconfusus*, pheromones, nonhost, *Abies concolor*, *Pinus ponderosa*, ipsenol, ipsdienol, bark beetle.

INTRODUCTION

The host range of *Ips paraconfusus* Lanier consists of the species of pine that grow in California and southern Oregon. Several previous studies have shown that *I. paraconfusus* males can produce attractants when boring in nonhost trees. Wood et al. (1966) showed that *I. paraconfusus* confined to artificial entrance holes in the non-hosts Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] and white fir (*Abies concolor* Lindl.) will produce odors attractive to female *I. paraconfusus* in a laboratory olfactometer. Vité et al. (1963) state

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that an attractant is produced in a Douglas fir log, but they do not present evidence. Lanier and Wood (1975) found that *I. paraconfusus* produced an attractant in sitka spruce [*Picea sitchensis* (Bong.) Carr.] in laboratory experiments. Production of attractants in nonhost trees by other bark beetle species in field experiments has been demonstrated by Jantz and Rudinsky (1965) and Lanier and Wood (1975). There are, however, no published data proving that *I. paraconfusus* can produce attraction to the nonhost in the field. Nor has anyone shown that the attractants produced are the same pheromone compounds produced in the host. The purpose of this study was to determine if pheromones are produced by *I. paraconfusus* males boring in white fir, one of the most common nonhosts in the range of this insect.

METHODS AND MATERIALS

Fifty male *I. paraconfusus* were placed individually in artificial entrance holes drilled through the outer bark of ponderosa pine (*Pinus ponderosa* Laws.) or white fir logs (Bedard and Browne, 1969; Wood et al., 1968). A strip of aluminum screen was stapled over each hole to prevent the beetles from escaping. Each log was then wrapped in screen to prevent access by any additional beetles that might be attracted to the logs. The logs, 28 cm long, were cut from living trees about 15–20 cm in diam. They were then transported to Berkeley and stored at 4° C for no more than 4 weeks prior to the experiments. Both the logs and beetles in this study came from Blodgett Experimental Forest, El Dorado County, California or from near Bass Lake, Madera County, California.

The field test consisted of a ponderosa pine and white fir log each infested with 50 male *I. paraconfusus* and uninfested logs of pine and fir as controls. Control logs were prepared exactly as treatment logs except that no beetles were introduced into the drilled holes. Each log was placed upright in a hardware cloth, "sticky basket" trap mounted on a 1.5 m pipe driven into the ground (Bedard and Browne, 1969). The logs were positioned in a line 16 m apart.

Every morning the beetles caught during the previous 24 hr on the sticky trap were removed and counted. In order to reduce the possible position effects within the line, each log was moved each morning to the adjacent position. Thus, at the end of 4 days, each log had occupied each position once. Three trials of this experiment were conducted simultaneously at different locations at an altitude of 1520 m near Bass Lake, California, in October 1976. Each trial ran for 4–5 days. At the end of the experiment the bark was removed from the logs to determine the extent of boring activity by measuring the lengths of galleries constructed.

In laboratory experiments the pine and fir logs containing male *I.*

paraconfusus and prepared as in the field experiments were placed on a laboratory bench beside paper cartons containing recently emerged, unfed males on moist paper towels. At the end of 48 hr the screen and bark were removed from the logs. The males from the logs and the unfed males were dissected and their hindguts were stored in approximately 0.3 ml diethyl ether in vials cooled by dry ice. The presence or absence of ingested material in the hindguts was noted.

The presence of attractants in the diethyl ether solutions containing the dissected hindguts was assayed by counting the number of recently emerged female *I. paraconfusus* walking upwind in an open arena olfactometer (Wood et al., 1966). Beetles were released in groups of 10 and given two opportunities to traverse the 20 cm upwind to the source. The five treatments in this assay were gut extracts from: (1) 56 males from white fir; (2) 18 males from ponderosa pine; (3) 56 unfed males; (4) a pheromone standard consisting of racemic ipsenol, ipsdienol, and *cis*-verbenol (Chemical Samples Co., >97% purity) mixed in equal proportions in ether solution at 1×10^{-8} g/μl; and (5) the ether solvent by itself. The gut extract solutions, the pheromone standard, and the blank solvent were eluted with a power-driven syringe (L.E. Browne, unpublished) at a rate of 1 μl/min or approximately 0.1 guts/min. The numbers tested, proportions responding, and associated confidence intervals are given in Table 2. The ratio of compounds and the release rate of the pheromone standard was one that produced a strong attraction in previous laboratory assays (Byers et al., 1979).

Gut extracts from 211 males boring in white fir, 38 in ponderosa pine, and 282 unfed males were analyzed by gas chromatography in conjunction with electron-impact mass spectroscopy (Finnigan 1015B). One tenth of the insect extracts were chromatographed isothermally on 3% Carbowax, 20 M, at 115° C. Quantification of the pheromone compounds ipsenol and ipsdienol was achieved by comparing the peak areas of selected ions to those of known amounts of the compounds. We did not examine the hindgut extracts for *cis*-verbenol, a third pheromone compound isolated from the frass of male *I. paraconfusus* (Silverstein et al., 1966a, b) because this compound is not found in the hindguts of males feeding in host material (Byers, 1978).

RESULTS

Field Test. The daily trap catches (Table 1) showed that a total of 88 beetles were caught at the male-infested white fir log in the first trial. This number was significantly different from the 755 beetles caught at the male-infested ponderosa pine log (Wilcoxon signed rank test, $\alpha = 0.05$) and from the one beetle caught at each control. In 2 other trials the catch at white fir was not significantly different from the controls. The catch at ponderosa pine in

TABLE 1. DAILY CATCH OF *I. paraconfusus* AT TRAPS BAITED WITH WHITE FIR AND PONDEROSA PINE LOGS EACH INFESTED WITH 50 MALES, AND WITH PINE AND FIR CONTROL LOGS WITHOUT MALES (OCTOBER 9–13, 1976, BASS LAKE, CALIFORNIA)[a]

	Treatment				Control (without males)			
	Ponderosa pine		White fir		Ponderosa pine		White fir	
Day	No.	♂♂/♀♀	No.	♂♂/♀♀	No.	♂♂/♀♀	No.	♂♂/♀♀
Trial 1								
1	86	23/63	13	2/11				
2	179	18/161	45	18/27	0	0/0	0	0/0
3	124	11/113	20	7/13	1	1/0	1	1/0
4	113	21/92	7	4/3	0	0/0	0	0/0
5	253	42/211	3	1/2	0	0/0	0	0/0
Total	755	115/640	88	32/56	1	1/0	1	1/0
Trial 2								
1	1	0/1	0	0/0	1	0/1	0	0/0
2	15	6/9	1	0/1	0	0/0	0	0/0
3	15	0/15	1	0/1	1	1/0	0	0/0
4	48	11/37	3	2/1	1	1/0	0	0/0
Total	79	17/62	5	2/3	3	2/1	0	0/0
Trial 3								
1	9	1/8	0	0/0	0	0/0	0	0/0
2	56	3/53	1	1/0	4	2/2	0	0/0
3	86	10/76	3	2/1	2	0/2	0	0/0
4	45	9/36	1	0/1	2	2/0	0	0/0
Total	196	23/173	5	3/2	8	4/4	0	0/0

[a]There was a difference in the number of beetles caught at white fir and ponderosa pine treatments, white fir treatment and the white fir control, and ponderosa pine treatment and ponderosa pine control (Wilcoxon signed rank test). These tests were significant at a simultaneous error rate of $\alpha \leqslant 0.05$ (Bonferroni inequality).

these trials was much lower than in the first trials, but it was significantly different from the controls. There was a significant difference in the ratio of males to females (Pearson's χ^2 test, $\alpha = 0.01$) caught at the ponderosa pine treatment (1:5.56) compared to the white fir treatment (1:1.75) in the first trial. The same difference in sex ratio was apparent in the second and third trials, although the numbers caught at the fir treatment were very low.

Dissection of the logs at the end of the field test revealed that significantly more boring was done in the ponderosa pine logs than in the white fir logs (Wilcoxon rank sum test, $\alpha = 0.05$). The mean gallery length in ponderosa pine was 38.5 mm (SD = 19.5), and in white fir 7.5 mm (SD = 4.35).

Laboratory Assay. The response of females to gut extracts from beetles feeding in white fir (Table 2) was significantly different from the response to the ether blank, to the gut extracts from unfed beetles, and also to gut extracts from beetles feeding in ponderosa pine and the synthetic pheromones. There was clearly more ingested material in the hindguts of beetles from ponderosa pine than from white fir (Table 3) and no ingested material in the hindguts of unfed beetles.

Identification of Pheromone Compounds. GC-MS analyses (Table 4) showed that ipsenol and ipsdienol were produced in the hindguts of males boring in white fir and ponderosa pine. Ipsenol and ipsdienol had retention times of 2.0 and 3.8 min, respectively. There were no detectable amounts of these compounds in the hindguts of the unfed males. The males boring in ponderosa pine produced 72 times as much ipsenol and 85 times as much ipsdienol per beetle as those boring in white fir.

TABLE 2. WALKING RESPONSE OF FEMALE *I. paraconfusus* TO MALE GUT EXTRACTS IN LABORATORY OLFACTOMETER (BERKELEY, CALIFORNIA, OCTOBER 1977)

Treatment	No. females tested	Elution rate per min	Proportion responding	Confidence interval[a]
Pheromone standard[b]	40	1×10^{-8} g	0.70	0.54–0.83 a[c]
Gut extracts, fed in ponderosa pine	60	0.06 gut equivalents	0.65	0.52–0.77 a
Gut extracts, fed in white fir	110	0.18 gut equivalents	0.40	0.32–0.50 b
Gut extracts, unfed	60	0.18 gut equivalents	0.15	0.07–0.26 c
Ether blank	40	1.0 μl	0.12	0.04–0.27 c

[a]Confidence intervals for binomial proportions cover simultaneously with a probability of 0.95.
[b]Ipsdienol, ipsenol, and *cis*-verbenol each at a 1×10^{-8} g/μl.
[c]Any treatments sharing the same lowercase letter are not significantly different by pairwise comparisons with a simultaneous error rate of $\alpha = 0.05$. (Miller, 1966; Chap. 6, Sect. 2.2).

TABLE 3. PERCENT OF MALE *I. paraconfusus* WITH VARIOUS AMOUNTS OF MATERIAL IN THEIR HINDGUTS AFTER 48 HR EXPOSURE TO DIFFERENT TREATMENTS: UNFED AND BORING IN PONDEROSA PINE AND WHITE FIR[a]

Treatment	Trial no.	No. of males examined	Percent of males			
			Empty	Scattered fragments	1/2 Full	Full
Ponderosa pine	1	16	0	0	25	75
	2	39	0	3	30	67
	Total	55	0	2	29	69
White fir	1	53	28	26	36	9
	2	59	25	57	15	2
	Total	112	27	43	25	5
Unfed	1	56	100	0	0	0
	2	149	100	0	0	0
	Total	205	100	0	0	0

[a]Chi-square analysis compared the distribution of males over the four gut content categories. There was a significant difference between males from ponderosa pine and white fir, ponderosa pine and unfed, and white fir and unfed, with a simultaneous error rate of $\alpha \leq 0.05$ (Bonferroni inequality).

TABLE 4. AMOUNT OF IPSENOL AND IPSDIENOL PER BEETLE FROM HINDGUTS OF MALE *I. paraconfusus* BORING IN PONDEROSA PINE AND WHITE FIR BY GC-MS.

	Ipsenol (ngs)/beetle						
Fragment ions (m/e)	68	69	85	93	121	136	$\bar{X}$
38 males in ponderosa pine	189.2	197.1	190.1	226.3	213.5	154.5	195.1
211 males in white fir	3.8	3.1	3.1	2.0	1.5	—	2.7

	Ipsdienol (ngs)/beetle				
Fragment ions (m/e)	85	109	119	134	$\bar{X}$
38 males in ponderosa pine	41.1	60.2	54.5	48.6	51.1
211 males in white fir	0.4	0.5	0.3	1.1	0.6

Ratio ipsenol/ipsdienol		Ratio ponderosa/white fir	
Ponderosa	3.8:1	Ipsenol	72.3:1
White fir	4.5:1	Ipsdienol	85.2:1

DISCUSSION

The field and laboratory experiments showed that *I. paraconfusus* males can produce attractants when boring in the nonhost white fir. GC-MS analyses confirmed that two pheromone compounds, ipsenol and ipsdienol, were present in the hindguts of males boring in white fir. No pheromones were found in recently emerged, unfed males. However, the amount of attraction and pheromone compounds produced by males boring in white fir was much less than that produced in ponderosa pine. There are two possible explanations for this result. First, because the beetles bored and fed much less in white fir than in ponderosa pine, they may have produced less pheromones. In this study, there was less boring and feeding in white fir than in ponderosa pine. This alone might account for the lowered pheromone production. Second, the beetles may have been less able to synthesize pheromones from white fir. Both of these explanations may be true.

The ability of *I. paraconfusus* males to produce attractants in the white fir is consistent with studies of the pheromone biosynthesis of this insect. Hughes (1974) found ipsenol and ipsdienol in the hindguts of male *I. paraconfusus* following aeration with myrcene. Byers et al. (1979) have subsequently confirmed these findings. Myrcene is one of the terpene hydrocarbons found in the xylem oleoresin of ponderosa pine (Mirov, 1961) and also in the cortical blister resin of white fir (Zavarin, 1968). The amount of xylem resin extractives is much less in the firs than the pines (Zavarin and Snajberk, 1965) and there are no xylem resin canals in the firs (Zavarin, personal communication). This fact may account for the reduced attraction produced by males boring in white fir.

Even if the beetles had not fed at all in white fir, they might have produced pheromones by absorption of myrcene vapors in the entrance tunnels. However, Byers (1978) has shown that mere exposure to the myrcene vapors in the bark of ponderosa pine is not sufficient for pheromone production probably because the concentration of these vapors is not high enough. The beetles must feed in order to produce sufficient pheromones to elicit an upwind walking response in the laboratory (Wood et al., 1966).

The reason for the failure of significant attraction to white fir in two of the three trials of the field test is unknown. The white fir logs used in these two trials came from a different tree than in the first trial. The amount of myrcene precursor in white fir or the stimuli that elicit feeding and boring behavior may vary from one tree to another. The numbers caught at the ponderosa pine were also much reduced compared to the first trial, indicating lowered flight activity. Even so, the relative attractiveness of fir to pine is not as great in these two trials. Whatever the explanation for the absence of attraction to white fir in the second and third trials, the results of the first trial are unequivocal. We know that under some conditions *I. paraconfusus* males boring in white fir

can produce attractants in the field. These results corroborate previous reports of attractants produced by *I. paraconfusus* in nonhost trees (Wood et al., 1966; Lanier and Wood, 1975; Vité et al., 1963).

The ratio of males to females (1 : 5.56) at the ponderosa pine treatment in the first trial of the field experiment is consistent with previously reported sex ratios captured at ponderosa pine logs baited with male *I. paraconfusus* (Wood et al., 1968; Lanier and Wood, 1975) and at traps baited with synthetic pheromones (Wood et al., 1968). Byers (unpublished) has recently shown that the response to pheromones of male and female *I. paraconfusus* differ depending on the pheromone concentrations. In this study, the different male/female ratio caught at white fir compared to ponderosa pine may be due to the lower concentration of pheromones produced. However qualitative differences in the pheromones may also help explain this phenomenon.

The results of this study have implications for host selection. Elkinton and Wood (1980) suggest a possible, although unlikely, mechanism of host selection based on the failure of pheromone production in the nonhost. The males might bore and feed readily in the nonhost, but not produce pheromones; thus the mass attack and colonization would never occur on nonhost trees. The results of this study do not support this hypothesis. Since *I. paraconfusus* can produce attractants in white fir, nonhost status must result from the failure to bore and feed in this tree species. One might still argue that the greatly reduced production of pheromones in white fir compared to ponderosa pine might mean that insufficient beetles would ever be attracted to colonize white fir. However, there is strong evidence from an earlier study (Elkinton and Wood, 1980) that stimuli in the white fir deterred or failed to elicit sustained boring and feeding behavior.

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