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MALE-SPECIFIC CONVERSION OF THE HOST PLANT COMPOUND, MYRCENE, TO THE PHEROMONE, (+)-IPSDIENOL, IN THE BARK BEETLE, *Dendroctonus brevicomis*[1]

JOHN A. BYERS[2,3]

[2]*Department of Entomological Sciences, University of California Berkeley, California 94720*

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Abstract—When both sexes of the bark beetle, *Dendroctonus brevicomis* LeConte, were exposed to vapors of myrcene from ponderosa pine, only the male produced (+)-ipsdienol. In the field, racemic ipsdienol significantly reduced the attraction of both sexes in flight to a mixture of myrcene and the aggregation pheromones, *exo*-brevicomin and frontalin. This suggests that ipsdienol may be involved in regulating colonization density of *D. brevicomis*. The implications of the biosynthesis of various enantiomers of ipsdienol by *D. brevicomis* and the cohabitating bark beetles, *Ips paraconfusus* and *I. pini*, in relation to their behavioral responses are discussed in regard to reducing interspecific competition.

Key Words—*Dendroctonus brevicomis*, Coleoptera, Scolytidae, *Pinus ponderosa*, pheromone biosynthesis, bark beetle, myrcene, ipsdienol, *exo*-brevicomin, frontalin, attractants, pheromones, competition.

INTRODUCTION

The process of aggregation of the western pine beetle, *D. brevicomis* LeConte (Coleoptera: Scolytidae), on a ponderosa pine, *Pinus ponderosa* Laws., begins when a female initiates the entrance hole and begins excavating a nuptial chamber and tunnel in the phloem tissue. One component of the aggregation pheromone, *exo*-brevicomin, is synthesized only in the female during boring and feeding in the tree, is released by defecation, and causes a low-level attraction of females and males (Silverstein et al., 1968). A male

[1]Coleoptera: Scolytidae.
[3]Present address: Department of Animal Ecology, University of Lund, S-22362 Lund, Sweden.

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joins the female in the nuptial chamber and immediately releases frontalin which is synergistically attractive with *exo*-brevicomin (Kinzer et al., 1969; Pitman et al., 1969; Bedard et al., 1970; Wood et al., 1976). Myrcene, a host monoterpene, presumably is released primarily at the entrance hole from exuding oleoresin and beetle frass, and it enhances the attraction of *D. brevicomis* to the pheromones (Bedard et al., 1969, 1970).

Hughes (1973) reported that males of *D. brevicomis* produce a "large quantity" of ipsdienol when exposed to myrcene vapors (concentration undetermined). The production of ipsdienol is probably the result of a simple hydroxylation of myrcene at carbon 4. However, Hughes did not mention whether females had been exposed to myrcene. Furthermore, he indicated it was not certain that myrcene (96%) was converted to ipsdienol since it was "not possible to determine whether closely related monoterpenes cause the formation of different quantities of the same products or if the small amounts of other terpenes present as impurities are the cause." Since ipsdienol was not known to have a behavioral effect on *D. brevicomis* and sex-specificity in production was not reported, the finding until now has not been regarded as significant.

The present study was undertaken to confirm the report of Hughes and to determine whether females could synthesize ipsdienol. The quantities and chirality of the ipsdienol produced in beetles after exposure to known vapor concentrations of purified myrcene were determined. In the field, the effect of ipsdienol on the response of *D. brevicomis* in flight to a mixture of its aggregation pheromones and myrcene was tested.

METHODS AND MATERIALS

D. brevicomis adults were reared from bark removed from naturally infested ponderosa pine trees in the Sierra National Forest, California, at about 1000 m elevation (Browne, 1972). Both sexes of *D. brevicomis* were exposed to various concentrations of myrcene vapor (GLC purified >99.8%) or no vapor in bottles for 18 ± 0.5 hr at 21 ± 1.7° C (Byers et al., 1979) on several occasions under natural photoperiods (Table 1). The myrcene concentrations were determined near the end of the exposure periods by withdrawing headspace air from each bottle with a gas-tight syringe for quantification by GLC analysis on a 1.8-m × 2-mm ID glass column of 3% Apiezon L on 100/120 Gas Chrom Q at 100° C and N_2 flow of 12 ml/min. The mid- and hindguts of beetles from each bottle (Table 1) were excised and extracted with 0.2 ml diethyl ether. The amounts of ipsdienol present in these gut extracts were analyzed by GLC (3.6-m × 2-mm ID glass column of Ultrabond II on 100/120 mesh at 100° C and N_2 flow of 30 ml/min; and the Apiezon L column described above). Authentic ipsdienol (GLC purified,

TABLE 1. QUANTITIES OF IPSDIENOL IN MALE AND FEMALE *D. brevicomis* AFTER AN 18-HOUR EXPOSURE TO MYRCENE VAPOR OR NO VAPOR.

Date	Sex	No. exposed	Myrcene concentration (10^{-6} g/ml)[a]	Ipsdienol per beetle ($\times 10^{-8}$ g)
Oct. 13, 1977	♂	20	4.4 ± 0.3	310
	♂	20	None	16
	♀	20	4.4 ± 0.3	<1[d]
	♀	20	None	<1[d]
Nov. 16, 1977	♂	20	3.9 ± 0.6	210
	♀	20	3.9 ± 0.6	<1[d]
May 3, 1980	♂	30[b]	2.0 ± 0.4	152 ± 5
	♂	30[b]	None	12 ± 3
	♀	30[b]	2.0 ± 0.4	<0.5[d]
	♀	30[b]	None	<0.5[d]
May 10, 1980	♂	800[c]	2.1 ± 0.1	169 ± 9

[a]Values represent average ±SEM for all groups exposed on the same date.
[b]Groups of 15 per bottle.
[c]Groups of 100 per bottle.
[d]None detected at GLC sensitivity employed.

>99.5%, Chem Sample Co.) was compared to gut extracts by GLC for retention time and peak area to quantify the production in the beetles.

Eight groups of 100 males were exposed to myrcene (Table 1) as described above and the guts of each group extracted with 0.4 ml diethyl ether so that sufficient amounts of ipsdienol could be obtained for determination of its optical rotation. The ipsdienol produced by these males was isolated by injecting about 100 μl of gut extract for each GLC run and collecting the compound from the 50:1 stream-split effluent from a 2-m × 4.5-mm ID Teflon column of 3% Apiezon L on 100/120 Gas Chrom Q at 100° C and N_2 flow of 100 ml/min. A glass U tube, 150-mm × 4-mm ID, filled 1 cm deep with Glasperline glass beads (0.45–0.5 mm diam) and immersed in liquid N_2 was used to condense the ipsdienol from the effluent gas. After collection of ipsdienol, the U tube was washed with ethanol and the sample rechromatographed, collected, and washed from the U tube with a total of 300 μl of ethanol. This sample was analyzed by GLC to determine the final concentration of the ipsdienol collected (5.02×10^{-7} g/μl). The rotations of the ipsdienol produced by male *D. brevicomis* and the authentic ipsdienol used in the field tests were determined with an electro balancing polarimeter (Autopol III). The ipsdienol from males and the authentic ipsdienol were further analyzed by GCMS on a SCOT 40-m capillary column of OV-101 at 115° C. Mass spectra (EI) were obtained with a V. G. Micromass 7070F mass spectrometer with computerized data system.

Six trap pairs in the Sierra National Forest at about 1000 m elevation were used to determine if ipsdienol (GLC purified >99.5%) released from one trap of each pair had any effect on the attraction of *D. brevicomis* to *exo*-brevicomin, frontalin, and myrcene released at approximately equal rates from both traps. The trap consisted of a 6-mm mesh metal-screen cylinder (19 cm diam × 30.5 cm high) coated with Stickem Special® (Bedard and Browne, 1969) placed 1.2 m above the ground. The traps of each pair were spaced 9–10 m apart and at least 100 m away from other pairs. Treatment and check were assigned at random within each pair each day. *exo*-Brevicomin, frontalin, and myrcene were each released at about 2 mg/day from glass tubes inside a glass salt shaker (Byers and Wood, 1980). Ipsdienol was released at about 0.6 mg/day from another salt shaker containing two 4- × 65-mm glass tubes each filled with 30 mg ipsdienol (GLC >99.5%). The trap catches on July 1–6, 1980, of *Enoclerus lecontei* (Wolcott) (Coleoptera: Cleridae), *Temnochila chlorodia* (Mannerheim) (Coleoptera: Trogositidae), and both sexes of *D. brevicomis* on each trap pair were compared with Wilcoxon signed rank tests (Lehmann, 1975). Sex ratio comparisons of *D. brevicomis* for the treatment and check were performed by chi-square tests.

RESULTS

Female *D. brevicomis* did not contain detectable quantities of ipsdienol ($<1 \times 10^{-8}$ g/female) after exposure to myrcene vapors while males contained at least 1.5×10^{-6} g ipsdienol per male after similar treatment (Table 1). Females unexposed to myrcene also did not contain ipsdienol; however, unexposed males appeared to have as much as 10% of the amount of ipsdienol that myrcene-exposed males contained (verified by GLC only). Ipsenol was not detected in either sex. The rotation of the ipsdienol isolated by GLC from males exposed to myrcene had a specific rotation of $[\alpha]_D^{21} = +12 \pm 4°$ (ethanol) which is similar to $[\alpha]_D^{20} = +10 \pm 0.9°$ reported by Silverstein et al. (1967) for ipsdienol isolated from *Ips paraconfusus* Lanier (Coleoptera: Scolytidae) [95% (+) enantiomer, Plummer et al., 1976].

The GC-MS fragmentation pattern of the purified ipsdienol obtained from vapor-exposed males matched the mass spectra of authentic ipsdienol. The masses of the following major fragments are listed with their percentages of the base peak because they differ quantitatively from those published earlier (Silverstein et al., 1967): 32:4.1, 39:3.0, 41:8.1, 53:2.8, 55:4.9, 65:2.8, 67:7.7, 68:4.9, 69:2.4, 79:5.0, 85:100.0 (base peak), 91:8.2, 109:4.6, 119:6.5, 134:5.1, 152:2.7. The quantitative differences in the spectra might be the result of some thermal decomposition of ipsdienol on the GLC column used by Silverstein et al.

The number of *D. brevicomis* caught on traps releasing aggregation

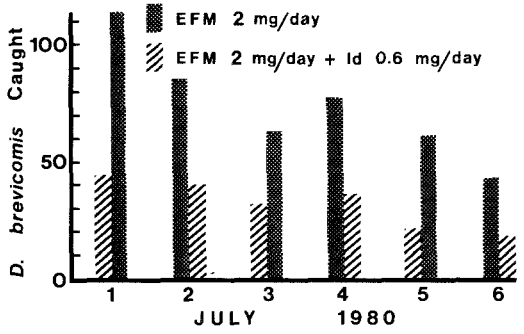

FIG. 1. The reduction in the attraction of *D. brevicomis* in flight to *exo*-brevicomin (E), frontalin (F), and myrcene (M) by ipsdienol (Id) as shown by the comparison of daily catch totals on 6 pairs of traps from July 1 to 6, 1980, in the Sierra National Forest, California. The catch totals for treatment and check are shown alternating each day to indicate random placement of ipsdienol.

components and ipsdienol (191, 93 male : 98 female) was significantly less than the number attracted to traps with aggregation components alone (443, 198 male : 245 female) (Figure 1). The response of both sexes to the aggregation pheromones appears to be inhibited (significantly lowered catch) by ipsdienol ($P < 0.001$ in each case), and there was no significant difference between the responses of the sexes as indicated by the ratio of catch by sex on the treatment and check traps ($P > 0.1$). The attraction of *T. chlorodia*, a predator, to the aggregation components was not effected by release of ipsdienol (75 vs. 67 on ipsdienol, $P > 0.1$). However, the catch of another predator, *E. lecontei*, was significantly increased at traps with ipsdienol (31 vs. 5, $P < 0.001$) which is consistent with their response to ipsdienol alone (Wood et al., 1966). The rotation of the ipsdienol used in the field tests was $[\alpha]_D^{21} = -0.35°$ (4% in ethanol) or racemic [52% (−) : 48% (+)].

DISCUSSION

Myrcene appears to play a role in several important ecological functions for *D. brevicomis*. The release rate of myrcene from the tree is increased by the boring activity of beetles which use the compound in combination with aggregation pheromones to locate its host and breeding sites. In the present study, myrcene was converted to (*S*)-(+)-ipsdienol only in the male beetle, and racemic ipsdienol, released at rates comparable to the aggregation pheromones, inhibited the attraction of both sexes in the field. This suggests that ipsdienol may function in regulating the density of attack and/or terminating the attack in an area depending on the compound's release rate in relation to

release rates of other behavioral chemicals during the colonization period. The precise function of ipsdienol remains to be elucidated. Another role that myrcene may play in the chemical ecology of *D. brevicomis* was shown by Byers and Wood (1981a) in which at least one of the myrcene-derived pheromones of a cohabitating bark beetle, *I. paraconfusus*, ipsenol and ipsdienol (Hughes, 1974; Byers et al., 1979; Hendry et al., 1980), synergized with another pheromonal component, *cis*-verbenol, to inhibit the response of *D. brevicomis* to its pheromones in the laboratory. The response of *D. brevicomis* to pheromone from naturally infested logs was inhibited by the presence of logs infested with *I. paraconfusus* males (Byers and Wood, 1980). These results indicated that ipsdienol/ipsenol plus *cis*-verbenol from *I. paraconfusus* may function to reduce interspecific competition for food and space.

In California, *I. pini* (Say), *I. paraconfusus*, and *D. brevicomis* may all compete for food and space on the same host tree. *I. pini* produces (−)-ipsdienol as its primary pheromone and this inhibits the response of *I. paraconfusus* to its pheromones (Light and Birch, 1979). On the other hand, *I. paraconfusus* produces (+)-ipsdienol (Plummer et al., 1976), and this inhibits the response of *I. pini* to its pheromone (Light and Birch, 1979). *D. brevicomis* probably produces (+)-ipsdienol under natural conditions because it feeds on phloem containing myrcene (Byers, 1981). Therefore, it remains to be established if a sufficient quantity of (+)-ipsdienol is released by *D. brevicomis* to inhibit *I. pini* response and thus could function to reduce interspecific competition. Release of (+)-ipsdienol from *D. brevicomis* would not inhibit *I. paraconfusus* since this beetle uses the enantiomer as one component of its aggregation pheromone. However, Byers and Wood (1981a) showed that verbenone from male *D. brevicomis* was very effective in inhibiting the response of *I. paraconfusus* to its pheromones.

It appears that only specific host monoterpenes can be converted to specific pheromones in most bark beetles studied. For example, myrcene is not converted to *cis*-verbenol in *I. paraconfusus* (Byers et al., 1979), and another major monoterpene found in ponderosa pine, α-pinene, is not converted to ipsdienol or ipsenol in the same insect (Renwick et al., 1976; Byers, 1981). α-Pinene is converted to the pheromones *cis*-verbenol in *I. paraconfusus* (Brand et al., 1975; Renwick et al., 1976; Byers, 1981) and *trans*-verbenol in *D. ponderosae* (Pitman, 1971; Hughes, 1973). Exposure of *I. paraconfusus* to Δ-3-carene, β-pinene, and limonene did not result in synthesis of any of the above pheromones, but other specific compounds (unidentified) were produced (Byers, unpublished). Similar exposure of *D. frontalis* to host monoterpenes resulted in the production of compounds specific to "a particular terpene" (Hughes, 1973).

One reason that these bark beetle species, as well as *D. brevicomis* (and

possibly *I. pini*), have evolved to use myrcene and α-pinene as precursors for certain of their pheromones may be due to the variation of monoterpenes in ponderosa pine. For example, Smith (1964) reported that the minimum percentages of myrcene and α-pinene in the oleoresin of 64 ponderosa pines in the central Sierra Nevada of California were higher (Myrcene the highest) than the sometimes trace amounts of Δ-3-carene, β-pinene, and limonene. In a similar study, 369 trees located at 27 sites from southern to northern California had much more narrow ranges of percentages of myrcene and α-pinene than the other three major monoterpenes (Smith et al., 1969). Thus, there would appear to be a selective advantage for those insects that utilize host monoterpenes which are less variable in their distribution for the conversion to pheromones so crucial for bark beetle survival.

Byers and Wood (1981b) found that the antibiotic, streptomycin, inhibited the conversion of myrcene to ipsdienol and ipsenol in male *I. paraconfusus*, which suggests a symbiotic microorganism within the intestine of the beetle. The biosynthetic system in *D. brevicomis* appears to be different in that only ipsdienol is produced, and it is not reduced to ipsenol. Further work is needed to determine if microorganisms play a role in *D. brevicomis*.

Unequivocal proof that ipsdienol is a pheromone of *D. brevicomis* can only be presented when quantitative comparisons are made between the release of the chemical from naturally infested trees and the amounts needed to effect behavior. It appears that ipsdienol could be useful in protecting pines from *D. brevicomis* attack or in mass release applications to "confuse" the beetle. Release of ipsdienol and verbenone (Byers and Wood, 1980) would have the advantage of inhibiting the responses of *D. brevicomis*, *I. paraconfusus*, and *I. pini* to any pine substrate infested by any one of these species in order to suppress movement of the infestation to surrounding pines or reduce the attack rate sufficiently for the tree to overcome the beetles with resin. Further work is needed to determine if these inhibitors of attractive response might even repel beetles away from a place where susceptible trees are growing.

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