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SEX-SPECIFIC PRODUCTION OF IPSDIENOL AND MYRCENOL BY *Dendroctonus ponderosae* (COLEOPTERA: SCOLYTIDAE) EXPOSED TO MYRCENE VAPORS[1]

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Abstract—Male mountain pine beetles, *Dendroctonus ponderosae* Hopkins, produced ipsdienol [97.0% ± 0.3 *S*-(+)] and myrcenol (90.3% ± 4.0 *E*) when exposed to myrcene vapors. Females which were exposed to myrcene vapors did not produce any ipsdienol, but did produce low levels of myrcenol (98.0% ± 0.7 *E*). Neither sex produced detectable levels of ipsdienol or myrcenol when fed for 24 hr on lodgepole pine, *Pinus contorta* var. *latifolia* Engelmann. The sex-specific conversion of myrcene to ipsdienol and myrcenol suggests that these compounds may have behavioral significance within the species. In addition, the *S*-(+)-ipsdienol produced by male *D. ponderosae* probably functions as a repellent allomone against *Ips pini* (Say).

Key Words—*Dendroctonus ponderosae*, bark beetle, Coleoptera, Scolytidae, myrcene, aggregation pheromones, ipsdienol, myrcenol.

INTRODUCTION

Although males of *Ips* spp. produce the terpene alcohol pheromones ipsdienol [2-methyl-6-methylene-2,7-octadien-4-ol] and/or ipsenol [2-methyl-6-methylene-7-octen-4-ol] when exposed to the host tree (*Pinus* spp.) monoterpene, myrcene (Hughes, 1974), these compounds have been found only rarely in *Den-*

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droctonus spp. Hughes (1973) reported that males of *D. brevicomis* LeConte, *D. valens* LeConte, *D. ponderosae* Hopkins, and *D. pseudotsugae* Hopkins produced ipsdienol when exposed to myrcene vapors, but females were apparently not exposed to myrcene, and the behavioral significance of ipsdienol was not investigated. Renwick et al. (1976) and Byers (1982) found that only male *D. brevicomis* produce ipsdienol when exposed to myrcene vapors. Byers (1982) also discovered that this ipsdienol was predominantly the *S*-(+) enantiomer, and that, in the field, racemic ipsdienol significantly reduced the response by *D. brevicomis* of both sexes to a mixture of myrcene, *exo*-brevicomin, and frontalin.

The terpene alcohol myrcenol (2-methyl-6-methylene-octa-2,7-dien-1-ol] was also found in *Dendroctonus* spp. after beetles were exposed to myrcene. *D. brevicomis* of both sexes contained significant amounts of myrcenol after exposure to myrcene vapors (Renwick et al., 1976), and female *D. ponderosae* produced small quantities of myrcenol when fed on pine logs (Conn, 1981); neither study reported whether the compound was *E*- or *Z*-myrcenol or a mixture of both isomers. Conn (1981) found myrcenol to be attractive to *D. ponderosae* of both sexes in laboratory bioassays, but in the field, response to other attractants was slightly inhibited by the presence of *E*-myrcenol (Conn et al., 1983).

Our objectives were to confirm that *D. ponderosae* can produce ipsdienol and myrcenol, to determine which sex(es) produce the compounds, and to identify which geometric isomers of myrcenol or enantiomers of ipsdienol are produced.

METHODS AND MATERIALS

Logs of lodgepole pine, *Pinus contorta* var. *latifolia* Engelmann, infested with the mountain pine beetle were obtained near Princeton in the southwestern interior of British Columbia. The cut ends of the logs were sealed with hot paraffin wax, and the logs were stored at 4°C. To obtain adult *D. ponderosae*, logs containing brood were placed in cages at 27°C. Emergent beetles were collected daily and were stored for a maximum of two weeks on moistened paper toweling at 2–4°C in loosely capped, screw-top jars. When beetles were needed for experiments, the jars were rewarmed to room temperature, and active individuals were selected and their sex determined using the method of Lyon (1958).

D. ponderosae of both sexes were exposed to myrcene by allowing the beetles to bore in fresh lodgepole pine logs for 24 hr or by exposing them to the vapors from a 2-ml vial containing 24 μl of myrcene (>99% pure) in a 500-ml jar for 24 hr. Abdomens from individual insects were excised and extracted in twice distilled pentane. The amounts of ipsdienol and myrcenol pre-

sent in these abdominal extracts were analyzed by gas–liquid chromatography (Hewlett Packard HP5880 A) in the direct injection mode on a glass capillary column (30 m × 0.50 mm ID) coated with SP-1000 (Supelco, Inc., Bellafonte, Pennsylvania). Racemic ipsdienol was obtained from Borregaard, A.S., Sarpsborg, Norway. *E*-Myrcenol was synthesized by the selenium dioxide oxidation of myrcene (Büchi and Wüest, 1967). Analysis of the distilled product (Hewlett Packard 5985B GC-MS) revealed an *E/Z* ratio of 97:3.

The chirality of the ipsdienol produced by individual beetles was determined by the technique developed by Slessor et al. (1986). To a pentane solution (10–30 μl), containing both racemic 3-octanol (4.1 ng/μl) as an internal standard and the insect extract containing >40 ng of ipsdienol, was added a solution of pyridine (50 mg/ml) in ether (15 μl), followed by 25 μl of acetyl-*S*-lactyl chloride reagent (25 mg/ml) in methylene chloride (30 μl) prepared from chirally pure *S*-(+)-lactic acid. The components were mixed, cooled to −20°C, and sealed in a glass ampoule. Ampoules were kept at room temperature overnight. The samples were then diluted with hexane (50 μl), washed by agitation with water (50 μl), and the aqueous phase removed. The organic phase was further washed with aqueous 5% sodium bicarbonate (3 × 50 μl) and once more with water (50 μl). The samples were analyzed by splitless capillary gas chromatography on a Hewlett Packard HP5890 using a 30-m × 0.2-mm ID methyl silicone DB-1 (J&W Scientific, Inc., Rancho Cordova, California) programmed at 60°C for 2 min, 7°C/min to 130°C, 2°C/min to 240°C. Helium carrier gas was used at a flow rate of 1 ml/min, with an injector temperature of 200°C and detector temperature of 250°C. Retention times for the free alcohols, 3-octanol and ipsdienol, were 5.80 and 8.92 min, respectively. The acetyl *S*-(+)-lactic derivatives exhibited retention times of 16.21 and 16.39 min for *R*-(−)- and *S*-(+)-3-octanol, and 19.23 and 19.45 min for *S*-(+)- and *R*-(−)-ipsdienol.

RESULTS

Only male *D. ponderosae* contained detectable levels of ipsdienol (>10 ng/beetle) after exposure to myrcene vapors (Table 1). This ipsdienol was found to be predominantly the *S*-(+) enantiomer (Table 1), with relatively little variation between individuals (Figure 1). However, there was considerable variability in the amount of ipsdienol produced by individual beetles; some produced none at all, while one male contained over 7 μg of the compound (Figure 2). Neither male nor female beetles contained detectable levels of ipsdienol after feeding on *P. contorta*, either alone or with an individual of the other sex. Ipsenol was not produced at any time by either sex.

D. ponderosae of both sexes contained myrcenol, predominantly the *E*-

TABLE 1. QUANTITIES AND ISOMERIC COMPOSITION OF IPSDIENOL AND MYRCENOL PRODUCED BY MALE AND FEMALE *D. ponderosae* WHEN EXPOSED TO MYRCENE THROUGH FEEDING OR VAPORS

Method of exposure to myrcene	Sex	Ipsdienol			Myrcenol		
		No. exposed	ng/beetle $\bar{X} \pm$ SE	% (+) $\bar{X} \pm$ SE	No. exposed	ng/beetle $\bar{X} \pm$ SE	% E $\bar{X} \pm$ SE
Vapors, 24 hr	Male	20	1946 ± 497	97.0 ± 0.3	9	1497 ± 215[a]	90.3 ± 4.0
	Female	17	< 10	—	9	427 ± 119[a]	98.0 ± 0.7
Fed alone for 24 hr on lodgepole pine logs	Male	12	< 10	—	12	< 10	—
	Female	12	< 10	—	12	< 10	—
Fed for 24 hr with an individual of the other sex	Male	12	< 10	—	12	< 10	—
	Female	12	< 10	—	12	< 10	—
No exposure; held for 24 hr at room temperature	Male	12	< 10	—	12	< 10	—
	Female	12	< 10	—	12	< 10	—

[a]Myrcenol content significantly different between sexes, Mann-Whitney U test, $P < 0.01$.

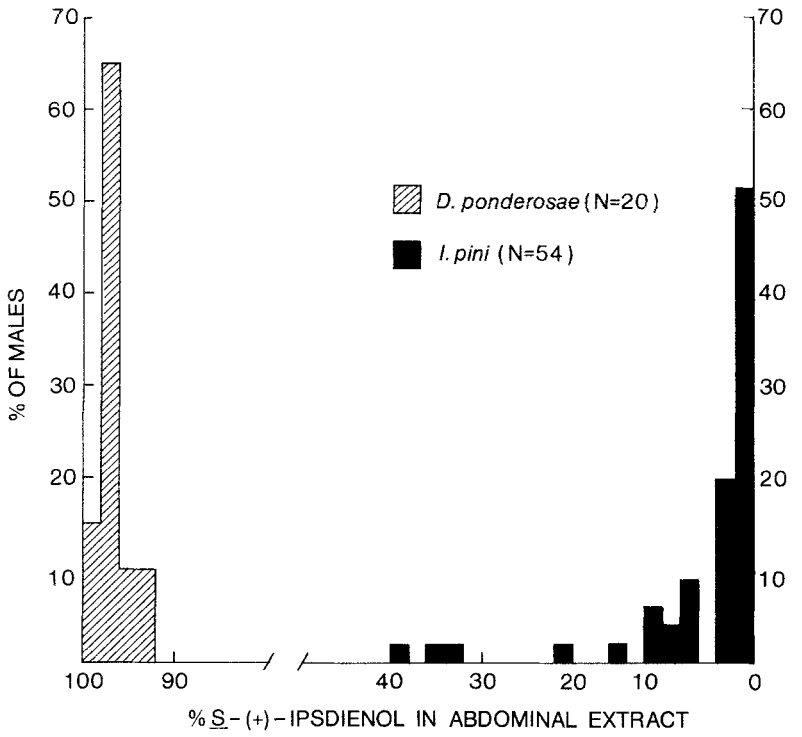

FIG. 1. Chirality of ipsdienol produced by individual male *D. ponderosae* exposed to myrcene vapors and *I. pini* fed on *Pinus ponderosa*. *I. pini* data adapted from Slessor et al. (1986).

isomer, after exposure to myrcene vapors, although males contained significantly more than females (Table 1). Neither males nor females contained detectable levels of myrcenol after feeding on *P. contorta*.

DISCUSSION

In most bark beetles, myrcene is converted to ipsdienol more efficiently if the exposure is through feeding, while α-pinene is converted to *cis*- and *trans*-verbenol more efficiently through exposure to vapors. As a result, ipsdienol has been termed a "frass" pheromone, while *cis*- and *trans*-verbenol are termed "contact" pheromones (Vité et al., 1972). Our data, as well as those presented by Byers (1982) and Hughes (1974), indicate that in *Dendroctonus* spp. myrcene is actually oxidized much more efficiently with vapor exposure than with feeding. Apparently this oxidation is not performed in the same way as in *Ips* spp. This conclusion is in agreement with the hypothesis presented by Vité et

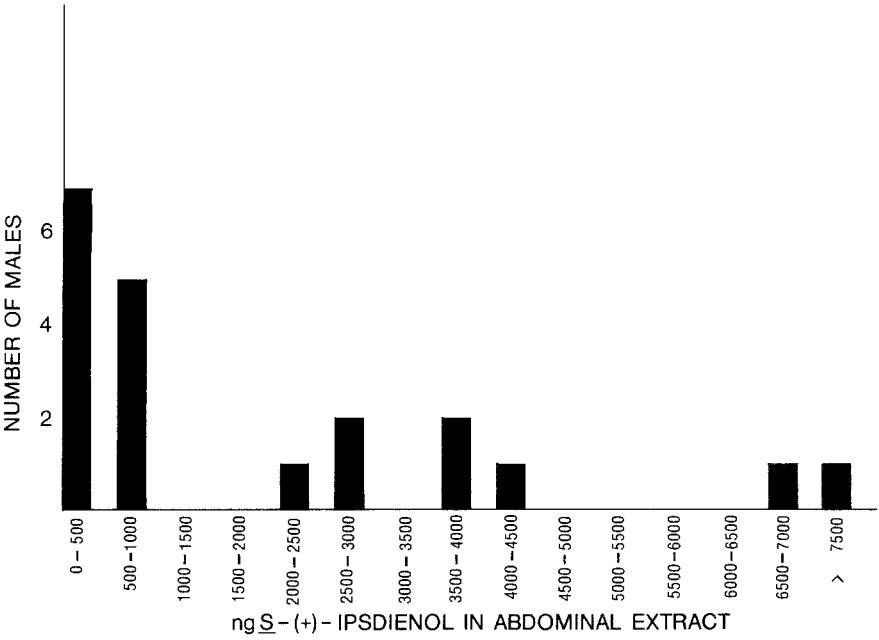

FIG. 2. Variation in *S*-(+)-ipsdienol production by 20 individual male *D. ponderosae*.

al. (1972), suggesting that aggressive bark beetles, such as many *Dendroctonus* spp., begin oxidizing monoterpenes upon initial contact with a new host, while less aggressive species, such as many *Ips* spp., depend on feeding for the conversion of monoterpenes.

The variation in chirality of *S*-(+)-ipsdienol produced by individual male *D. ponderosae* is similar to but not as extensive as the variation in the enantiomeric composition of *R*-(−)-ipsdienol in *Ips pini* (Say) from southeastern British Columbia (Figure 1) (Slessor et al., 1986). Therefore, if ipsdienol proves to be of behavioral significance to *D. ponderosae*, there seems to be less potential than in *I. pini* for natural or artificial selection pressures, such as pheromone-based trapping programs, to select for individuals that utilize different enantiomeric mixtures. The high degree of variation in total ipsdienol content (Figure 2) is in agreement with data on *trans*-verbenol in *D. ponderosae* (Borden et al., 1985), ipsdienol in *I. pini* (Borden et al., 1985) and *cis*-verbenol in *Ips typographus* Linnaeus (Birgersson et al., 1984). Borden et al. (1985) speculated that the few beetles with a capacity for producing very large quantities of terpene alcohol pheromones may be those that are successful pioneer beetles.

There is no overlap in the range of chirality of ipsdienol produced by individual *D. ponderosae* and *I. pini* (Figure 1). Birch et al. (1980) have shown

that *I. pini* from California are attracted to *R*-(−)-ipsdienol, the naturally predominating enantiomer, while *S*-(+)-ipsdienol interrupted the response of *I. pini* to an attractive source in field tests. Therefore, as in the hypothesized interaction between *D. brevicomis* and *I. pini* (Byers, 1982), the *S*-(+)-ipsdienol produced by *D. ponderosae* may function as a repellent allomone, inhibiting the orientation of *I. pini* and thus reducing interspecific competition for the same host. Moreover, the inhibitory effect of racemic ipsdienol on pheromone-positive orientation by *D. brevicomis* (Byers, 1982) suggests that the *R*-(−)-ipsdienol produced by *I. pini* may function reciprocally as a repellent allomone for *D. brevicomis*.

The sex-specific conversion of myrcene vapors to ipsdienol and myrcenol by *D. ponderosae* suggests that either or both products may have behavioral significance within the species. However, with the exception of the work by Byers (1982) on *D. brevicomis*, and Conn (1981) and Conn et al. (1983) on *D. ponderosae*, the oxidation of myrcene by *Dendroctonus* spp., and its possible biological roles, have not been studied. This omission is likely due to the fact that in most pheromone isolation studies beetles are induced to produce pheromones by allowing them to bore in host phloem tissue in cut logs that no longer produce copious amounts of monoterpene-rich resin. Thus, it is not surprising that oxidation products of monoterpene vapors have been largely overlooked. In addition, female *Dendroctonus* spp., which have been studied more intensively than males because they initiate attacks on new host trees, do not appear to oxidize myrcene to the same extent as males. As a result the possible behavioral significance of ipsdienol and myrcenol for *Dendroctonus* spp. has been largely ignored. This topic is presently under study.

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