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INFLUENCE OF SEX, MATURITY AND HOST SUBSTANCES ON PHEROMONES IN THE GUTS OF THE BARK BEETLES, *IPS PARACONFUSUS* AND *DENDROCTONUS BREVICOMIS*

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Abstract—Pheromones and metabolites of host (ponderosa pine) compounds were found in association with the hindgut of both naturally fed and of non-fed, host vapour-exposed bark beetles, *Ips paraconfusus* and *Dendroctonus brevicomis*. Much smaller amounts were found in the corresponding heads and mid guts. Sex-specific differences in content of pheromones were observed as in earlier studies. Exposure of *I. paraconfusus* to vapours of a pheromone component, ipsenol and other monoterpene alcohols resulted in their accumulation in the hindgut but relatively very low amounts in the head. The possible sites of pheromone biosynthesis are discussed. Exposure of male *I. paraconfusus* to vapours of host compounds, myrcene and α-pinene, revealed that immature adults do not produce the pheromone components, ipsenol and ipsdienol, as mature adults do while both immature and mature sexes produced another pheromone component, *cis*-verbenol, as well as *trans*-verbenol and myrtenol. Immature *D. brevicomis* adults did not contain pheromones until their exposure to vapours of (−)-α-pinene which caused production of *trans*-verbenol but only about 10% that of mature adults treated similarly. Verbenone, a male-produced inhibitory pheromone of *D. brevicomis*, apparently was not synthesized from (−)-α-pinene in females nor was its synthesis in males enhanced by exposure to this host compound.

Key Word Index: Ips paraconfusus, Dendroctonus brevicomis, Coleoptera, Scolytidae, *Pinus ponderosa*, pheromone biosynthesis, bark beetle, myrcene, α-pinene, monoterpenes, ipsenol, ipsdienol, *cis*-verbenol, *trans*-verbenol, myrtenol, *exo*-brevicomin, frontalin, verbenone, pheromone, digestive tract

INTRODUCTION

PHEROMONES of bark beetles (Coleoptera: Scolytidae) have been shown to be associated with the posterior portion of the gut in most cases where they have been studied (WOOD, 1982). However, no study has attempted a more precise localization. PITMAN *et al.* (1965) excised portions of the digestive tract from male *Ips paraconfusus* (Lanier) fed on ponderosa pine (*Pinus ponderosa*, Laws.) and showed that extracts from hindgut parts were the most attractive to walking females. At that time the three pheromone components of the beetle had not yet been identified so it was not possible to determine if certain of these compounds were distributed in other gut portions and to what extent. *Dendroctonus brevicomis* LeConte also feeds on ponderosa pine and its attractive pheromone components, *exo*-brevicomin and frontalin (SILVERSTEIN *et al.*, 1968; PITMAN *et al.*, 1969), have been found in the hindgut of the appropriate sex upon emergence and during host colonization (HUGHES, 1973; BYERS and WOOD, 1980). Other pheromone components which inhibit the attractive response, verbenone and *trans*-verbenol (RENWICK and VITÉ, 1970; BEDARD *et al.*, 1980; BYERS, 1983), also have been found in the hindgut of *D. brevicomis* (RENWICK, 1967; VITÉ and RENWICK, 1970; BYERS and WOOD, 1980).

The aggregative pheromone of *I. paraconfusus* was isolated and identified from male boring frass as a mixture of three components: ipsenol, *cis*-verbenol and ipsdienol (SILVERSTEIN *et al.*, 1966; WOOD *et al.*, 1966). All three compounds are necessary for maximal attraction in the field (WOOD *et al.*, 1968). Only ipsenol alone shows some activity in the laboratory while the addition of either *cis*-verbenol or ipsdienol increases the attraction response (WOOD *et al.*, 1967; WOOD, 1970). Males and females produce *cis*-verbenol in their hindguts after exposure to vapours of the (−)-enantiomer of α-pinene, a major host monoterpene, which indicates they would produce *cis*-verbenol under natural conditions (RENWICK *et al.*, 1976; BYERS, 1981a). HUGHES (1974) reported that after males of *I. paraconfusus* were exposed to vapours of another host terpene, myrcene, they contained ipsenol and ipsdienol in the hindgut. BYERS *et al.* (1979) determined the quantitative relationship between vapour concentrations of myrcene and the subsequent conversions to ipsenol and ipsdienol and they showed that the synthesis of these compounds was male specific.

The systems for biosynthesis of pheromones in *D. brevicomis* are less well characterized since precursors have not been found for the attractive components. However, recently *trans*-verbenol was shown to be an inhibitor of female attraction (BYERS, 1983), and since it is produced in the largest amounts at the initiation of colonization (BYERS and WOOD, 1980)

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this indicates a function in attack density regulation and intraspecific competition. Earlier, HUGHES (1973) had showed that female *D. brevicomis* produced *trans*-verbenol in linear proportion to the length of exposure to α-pinene vapour. Therefore, further biosynthetic work needs to be done with this host precursor/pheromone system.

In bark beetles it appears that only *I. paraconfusus* has been studied regarding the ability of immature adults to produce pheromones. Callow adults dissected from beneath the bark and then forced to colonize host material did not attract females for at least six days compared to only a one-day lag period for mature males (BORDEN, 1967). HUGHES and RENWICK (1977a) reported that callow males and females did not produce ipsenol and ipsdienol after exposure to myrcene vapours, although their vapour concentrations and level of sensitivity for pheromone detection were not presented. Therefore, the first objective of this study was to more fully investigate the ability of both sexes of immature and mature *I. paraconfusus* and *D. brevicomis* to biosynthesize pheromones from the host-plant precursors, myrcene and α-pinene. The second objective was to determine the amounts of pheromones in different parts of the digestive tracts of both species and sex during host feeding or exposure to vapours. Differences in the distribution of compounds between feeding (movement of compounds?) and non-feeding might reveal the sites of production.

MATERIALS AND METHODS

Distribution of pheromones in the digestive tracts of male and female bark beetles after exposure to plant compound vapours or after feeding in the host

I. paraconfusus adults were reared from naturally infested ponderosa pine logs (logging debris) and *D. brevicomis* adults from bark of naturally infested trees collected in the Sierra National Forest, California, at about 1000 m elevation. The adults were stored on moistened paper at 4°C from 2 to 15 days before use. Each sex of *I. paraconfusus* was exposed separately to a mixture of vapours from 5 μl of myrcene (GLC purified >99.8%, Chemical Samples Co.) and 5 μl of (−)-α-pinene (GLC purified >99.8%, $[\alpha]_D^{22} = -41.6°$C, Aldrich) in 70 ml jars (BYERS and WOOD, 1981) for about 18 hr at 21°C. From 15 to 20 beetles of each sex were exposed on 19 November 1976, and two similar groups (one control) on 27 April 1980. Samples of headspace air (60 μl) were withdrawn from the jars with a gas-tight syringe during the exposure period to determine the vapour concentrations by GLC analysis (Varian 2700 f.i.d.) on a 1.8 m × 2 mm i.d. glass column of 10% FFAP on 80/100 Gas Chrom Q at 50°C and N_2 flow of 30 ml/min (1976) or on a similar glass column of 3% Apiezon L on 100/120 Gas Chrom Q at 100°C and N_2 flow of 12 ml/min (1980).

After the exposure period, the posterior portion of the beetles' guts, including the mid and hindguts, were removed, divided into three sections and separately extracted in diethyl ether, about 150 μl/10 guts (BYERS *et al.*, 1979). Each mid-hindgut was laid on a glass slide which had been previously sprayed and covered with a fine mist of water. Unused portions of razor blades were used to slice each gut into sections. The posterior section consisted of the anus and rectum and a portion of the hindgut to about midway between the anus and the pyloris (see Fig. 1). The mid section contained the pyloris, and often a few short broken strands of Malpighian tubules, and ended just posterior to the posterior venticulus which has the parallel rows of short gastric papillae. The anterior section consisted of the posterior ventriculus and an indefinite but short portion anterior to the posterior ventriculus. For comparison, extractions were made of the heads plus some attached cervical muscles from the beetles whose guts were sectioned. The whole mid and hindguts of male and female control groups were extracted to determine the loss of pheromones during the sectioning procedure. The fresh and dry weight of 10 male heads were compared to the corresponding weights of 10 male mid-hindguts by reweighing after heating at 77°C for 1 hr. The gut extracts were analysed in 1976 on the FFAP column at 100°C and Apiezon L column or in 1980 on the Apiezon L column and a 3.7 m × 2 mm i.d. glass column of Ultrabond II (monolayer Carbowax 20 M, 100/120 mesh) at 100°C and N_2 flow of 30 ml/min. The quantities of ipsenol, ipsdienol, *cis*-verbenol, myrtenol ($[\alpha]_D^{22} = -47.5°$, >99%, Aldrich) and compound 'B' were determined in the extracts by comparison of GLC peak areas with known amounts of these compounds as external standards, compound B quantified in ipsenol equivalents (chemicals from Chemical Samples Co. and GLC purified >99%, except *cis*-verbenol >95%).

The distribution of pheromones in the posterior digestive tract of *D. brevicomis* was determined in the same general way as above with the same numbers and three groups (one control). However, the groups of each sex were exposed only to vapours from 20 μl of (−)-α-pinene in 0.45 l bottles, 5 May 1980 (BYERS *et al.*, 1979). Headspace analyses were performed on the Apiezon L column. The gut extracts were analysed on the Apiezon L and Ultrabond II columns. The quantities of myrtenol, *trans*-verbenol and verbenone were determined by comparison to known amounts of these compounds (chemicals GLC purified >99%, Aldrich, Glidden Organics and Chemical Samples Co., respectively).

The distributions of pheromones in the posterior digestive tract of both bark beetle species when feeding in ponderosa pine were investigated using the same procedures as above. Pine logs 14–17 cm dia. × 35 cm long were cut from trees in the Sierra National Forest and stored at 4°C for less than 1.5 months before use. Two groups of 50 *I. paraconfusus* males were introduced head first into holes drilled in two logs and allowed to excavate nuptial chambers for 62 hr until their removal on 27 April 1980. Females were introduced to the second male-infested log and allowed to feed for 48 hr. The procedures for sectioning of the guts from males feeding alone and the females and solvent extraction were as described above (see Fig. 1). GLC analyses were performed on the Apiezon L and Ultrabond II columns. Similarly, 50 female *D. brevicomis* were introduced into drilled holes in a pine log (5 May 1980) and allowed to construct a gallery for 74 hr while males were added to each entrance for the last 26 hr before both sexes were removed and analysed as above for fed *I. paracon-*

Table 1. Accumulation of ipsenol, *trans*-verbenol and (−)-myrtenol in hindguts of mature *I. paraconfusus* during exposure to these vapours for 18 hr

Vapour exposure: Body part extracted	Vapour concentration ($\times 10^{-8}$ g/ml)	Quantity ($\times 10^{-8}$ g) per body part Ipsenol	*trans*-verbenol	(−)-myrtenol
Ipsenol:				
Male hindgut	24.9	362.7	0*	0
Male head	24.9	1.0	0	0
Trans-verbenol:				
Male hindgut	6.2	0	394.2	0
Male head	6.2	0	1.2	0
(−)-Myrtenol:				
Male hindgut	4.4	0	0	157.3
Male body, no gut	4.4	0	0	38.0
Female hindgut	3.8	0	0	146.4
Female body, no gut	3.8	0	0	21.6

* All zeros indicate that none was detected by GLC ($< 1 \times 10^{-8}$ g/body part).

fusus. In addition to *trans*-verbenol and verbenone mentioned before, the quantities of frontalin and *exo*-brevicomin were determined in gut extracts by comparison of peak areas to those of known amounts (both >95%, Chemical Samples Co.).

Accumulation of ipsenol, trans*-verbenol and myrtenol in the hindgut of* I. paraconfusus *after exposure of adults to these vapours*

Male and female *I. paraconfusus* were exposed for 18 hr to the vapour of *trans*-verbenol (23 January 1976), ipsenol, or (−)-myrtenol (11 November 1976) in the 70 ml jar (see Table 1). The mid and hindguts were extracted and analysed by FFAP (*trans*-verbenol) or by Apiezon L as described above. Headspace analyses were conducted as described before on these respective columns. The heads and degutted bodies were extracted (300–400 μl diethyl ether) and analysed similarly.

Pheromones produced in immature and mature bark beetles exposed to plant monoterpene vapours

Callow adults of *I. paraconfusus* were removed from beneath the bark of infested ponderosa pine. Only the yellow coloured or unsclerotized beetles were immediately placed in the 70 ml jars and exposed or not to vapours of myrcene or myrcene plus α-pinene (1:1 mixture of (−), rotation as above, and (+), $[\alpha]_D^{22} = +45.8°$) for 18 hr (see Table 2). Mature adults that had recently emerged were treated similarly. Gut extracts were made as described above and analysed by GLC on the FFAP and Apiezon L columns. The guts also were tested to determine if pheromones were present by the attraction of walking females in the laboratory olfactometer (Byers *et al.*, 1979). Pupae and callow adults of *D. brevicomis* were removed from the bark of pine, sex was determined, and these beetles plus emerged adults were exposed or not to (−)-α-pinene vapour for 18 hr in the 0.45 l bottles (see Table 3). GLC analyses of the gut extracts were made on the Apiezon L and Ultrabond II columns.

RESULTS

Distribution of pheromones in the digestive tracts of male and female bark beetles after exposure to plant compound vapours or after feeding in the host

The metabolites of myrcene and (−)-α-pinene were found in the largest amounts in the posterior half of the hindgut of both male and female *I. paraconfusus* (Fig. 1). Females, as expected, did not contain ipsenol and ipsdienol (Byers *et al.*, 1979) while males had a ratio of 5.9 (ipsenol/ipsdienol) in agreement with that in Byers (1981a) (not 5:9 as misprinted). Both sexes contained relatively large amounts of an unidentified metabolite of myrcene, compound 'B' (Byers and Wood, 1981), which did not enhance the attraction of beetles to various mixtures of *cis*-verbenol plus ipsdienol or to ipsenol (Table 2 in Byers *et al.*, 1979) indicating that compound B cannot substitute for any of the three components. Quantities of the pheromone components and metabolites decreased progressively toward the posterior midgut section. The male and female control groups (whole gut) contained slightly larger quantities of about 130 and 120%, respectively, than that in the gut-sectioned beetles. Host fed females did not contain detectable amounts ($< 0.3 \times 10^{-8}$ g) of the chemicals shown in Fig. 1. Fed males contained large amounts of ipsenol and ipsdienol again predominately in the posterior hindgut with levels decreasing anteriorly. The fed male control contained approximately 96% as much of these two components as gut-sectioned beetles. The male head and cervical muscles have about twice as much fresh weight (4.9×10^{-4} g) as the mid and hindgut (2.3×10^{-4}g). This indicates that on a per tissue weight basis the heads contain about half that shown in relation to amounts in the hindgut (Fig. 1).

In *D. brevicomis* the distributions of (−)-α-pinene metabolites, *trans*-verbenol and myrtenol, in the digestive tract were somewhat different than that in *I. paraconfusus* since about equal amounts of each compound were found in the anterior and posterior sections of the hindgut (Fig. 2). Verbenone was not produced in females, but in males its levels were high-

Table 2. Pheromones produced in callow and mature males and females of *I. paraconfusus* exposed to vapours of mycene and α-pinene or no vapours and the attractive response of females to gut extracts from these vapour-exposed beetles

Maturity: sex	Vapour exposure	% Responding to gut extracts* (95% B.C.L.)	Quantity ($\times 10^{-8}$ g) per gut Ipsenol	*cis*-Verbenol	Ipsdienol	*trans*-Verbenol
Callow ♂†	No terpenes	7 (0–22)	0‡	0	0	0
Callow ♀†	No terpenes	0 (0–12)	0	0	0	0
Callow ♂†	Myrcene/α-pinene§	7 (0–22)	0	120.6	—‖	162.5
Callow ♂¶	Myrcene/α-pinene§	2 (0–9)	0	48.2	—	77.9
Callow ♀¶	Myrcene/α-pinene§	5 (1–14)	0	29.0	—	43.4
Callow ♂**	Myrcene††	3 (0–12)	0	0	0	0
Callow ♀**	Myrcene††	4 (0–12)	0	0	0	0
Mature ♂¶	No terpenes	3 (1–12)	0	0	0	0
Mature ♀¶	No terpenes	2 (0–9)	0	0	0	0
Mature ♂†	Myrcene/α-pinene§	43 (25–62)	16.0	217.5	—	280.4
Mature ♂¶	Myrcene/α-pinene§	48 (35–61)	7.6‡‡	182.0	—	253.7
Mature ♀¶	Myrcene/α-pinene§	8 (2–18)	0	111.9	—	190.7
Mature ♂**	Myrcene††	58 (45–70)	35.7‡‡	0	7.2‡‡	0
Mature ♀**	Myrcene††	4 (0–12)	0	0	0	0

* The release rate was estimated to be about 0.09 gut equivalents in diethyl ether per min; B.C.L. = binomial confidence limits.

† 27 February 1976, for each row 14–20 insects extracted × 1 replicate; and $N = 30$ tested for response.

‡ All zeros indicate that no compound was detected by GLC ($<0.3 \times 10^{-8}$ g/gut).

§ Beetles exposed for 18 hr in a jar to vapours in equilibrium with 150 μl myrcene and 100 μl (−) and 100 μl (+) α-pinene.

‖ Ipsdienol quantities could not be determined as it elutes with *trans*-verbenol (from α-pinene exposure), however, ipsdienol has been shown consistently to be about 1/6 that of ipsenol (BYERS, 1981a).

¶ 3 March 1976, for each row 10–12 insects extracted × 2 replicates; and $N = 60$ tested for response.

** 29 March 1976, for each row 20 insects extracted × 2 replicates; and $N = 60$ tested for response.

†† Beetles exposed for 18 hr in a jar to vapours of GLC-purified myrcene (24.8×10^{-7} g myrcene/ml, S.E.M. = 1.8×10^{-7} g/ml, $N = 8$).

‡‡ Indicates that the amount of compound was significantly greater than other values in the same column on each date ($\alpha = 0.05$, *t*-test).

Table 3. Pheromone components produced in pupae, callow and mature males and females of *D. brevicomis* exposed to vapours of (−)-α-pinene ($7.8 \pm 0.8 \times 10^{-8}$ g/ml, ± S.E.M., $N = 12$) for 18 hr (23 April–3 May 1980)

Maturity: sex	(Number) × Groups exposed to:	Quantity ($\times 10^{-8}$ g) per gut ± S.E.M. F	E	tV	V	M
	(10–15) × 1					
Pupae ♂	No terpenes	0*	0	0	0	0
Pupae ♀	No terpenes	0	0	0	0	0
Callow ♂	No terpenes	0	0	0	0	0
Callow ♀	No terpenes	0	0	0	0	0
	(20) × 6					
Mature ♂	No terpenes	24	0	30 ± 5†	279 ± 36†	8 ± 3
Mature ♀	No terpenes	0	0	66 ± 13†	0	6 ± 1
	(10–15) × 2					
Pupae ♂ + ♀	α-Pinene vapour	0	0	4 ± 2	0	1 ± 0.5
Callow ♂	α-Pinene vapour	0	0	226 ± 30	0	34 ± 8
Callow ♀	α-Pinene vapour	0	0	148 ± 51	0	23 ± 10
	(20) × 6					
Mature ♂	α-Pinene vapour	—‡	—	2181 ± 206†	229 ± 51†	286 ± 4†
Mature ♀	α-Pinene vapour	—	—	2088 ± 289†	0	376 ± 55†

F corresponds to frontalin, E to *exo*-brevicomin, tV to *trans*-verbenol, V to verbenone, and M to myrtenol.

* Zeros indicate that no compound was detected by GLC ($<0.3 \times 10^{-8}$ g/gut).

† Indicates that the amounts were significantly different from the corresponding treatment of the callow sex (*t*-test, $\alpha = 0.05$).

‡ Quantities not determined.

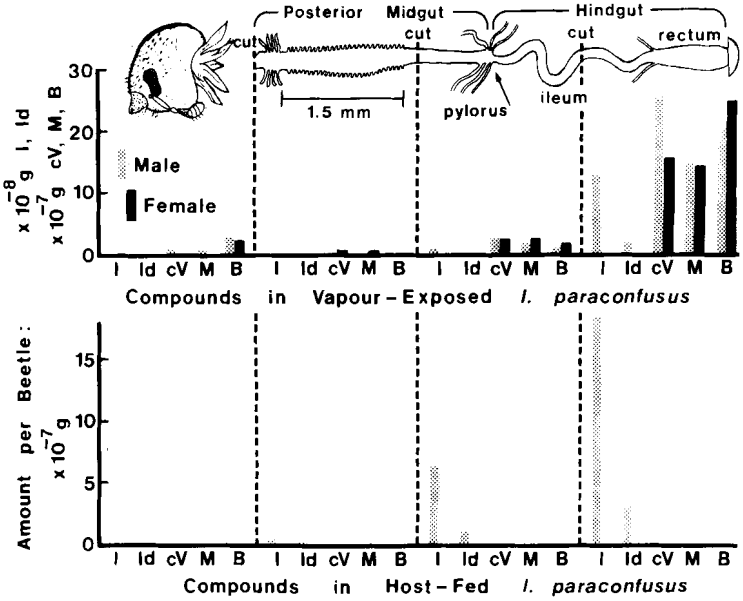

Fig. 1. Distribution of pheromonal compounds and volatiles in various sections of the digestive tract of male and female *I. paraconfusus* exposed to vapours of myrcene and (−)-α-pinene (18 hr) or allowed to feed in pine logs for 62 hr (the males alone, the females with males). Ipsenol = I, ipsdienol = Id, *cis*-verbenol = cV, myrtenol = M and compound B = B. The absence of a compound indicates that none was detected by GLC ($< 0.3 \times 10^{-8}$ g/beetle).

est in the posterior hindgut. Very small amounts of *trans*-verbenol were found in the head and even smaller if based on a fresh weight basis. Control groups of males and females contained about 69 and 72% as much of the compounds as gut-sectioned beetles. Fed males contained increasing amounts of frontalin and verbenone toward the posterior end of the gut. However, *trans*-verbenol was highest in the anterior hindgut, although a difference has not been shown statistically. Fed females showed the same general distribution pattern for *exo*-brevicomin and *trans*-verbenol as that for the male's frontalin, but females did not contain either frontalin or verbenone (Fig. 2). Fed control groups of males and females had about 83 and 74% as much pheromone components as gut-sectioned beetles. The morphology of male and

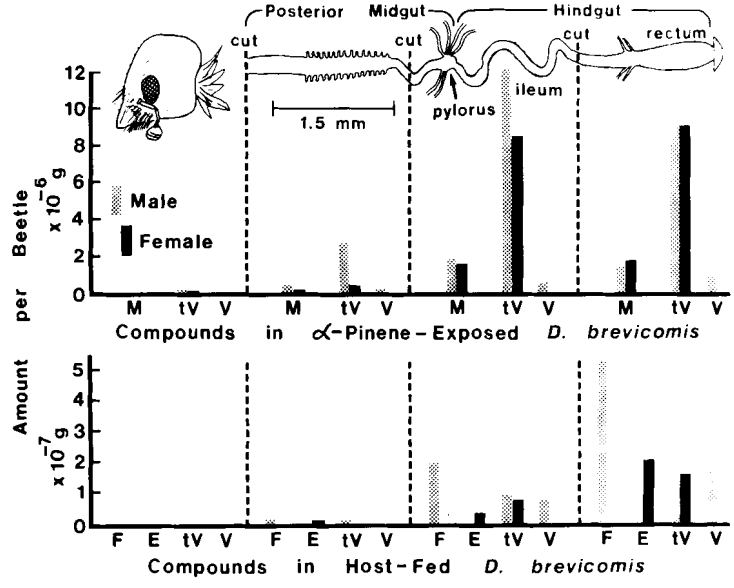

Fig. 2. Distribution of pheromonal compounds and volatiles in various sections of the digestive tract of male and female *D. brevicomis* exposed to vapour of (−)-α-pinene (18 hr) or allowed to feed together in pine logs (females for 74 hr, males added last 26 hr). Myrtenol = M, *trans*-verbenol = tV, verbenone = V, frontalin = F and *exo*-brevicomin = E. The absence of a compound indicates that none was detected by GLC ($< 0.3 \times 10^{-8}$ g/beetle).

female digestive tracts of *D. brevicomis* appeared identical with the light microscope (× 100) as did the guts of male and female *I. paraconfusus*.

Accumulation of ipsenol, trans*-verbenol and myrtenol in the hindgut of* I. paraconfusus *after exposure of adults to these vapours*

In beetles exposed to vapours of ipsenol, *trans*-verbenol and (−)-myrtenol, large amounts of these compounds were absorbed or accumulated in the mid and hindgut as compared to the head (Table 1). However, some (−)-myrtenol was found in/on degutted males and females exposed to vapours of this compound but less than that contained in the gut.

Pheromones produced in immature and mature bark beetles exposed to plant monoterpene vapours

Monoterpene alcohols were not found in either callow or adult *I. paraconfusus* which had not been exposed to host-plant vapours (Table 2). Both callow males and females produced *cis*- and *trans*-verbenol during exposure to vapours of α-pinene enantiomers, although somewhat less than mature adults (Table 2). Neither callow males or females nor mature females could synthesize ipsenol and ipsdienol from myrcene while these pheromone components were made in mature males. The walking response of females was significantly higher only to those gut extracts shown by GLC to contain ipsenol and ipsdienol and in some also *cis*-verbenol (Table 2).

Pupae and callow adults of both sexes of *D. brevicomis* did not contain detectable quantities of pheromonal compounds when they were not exposed to host-plant vapours (Table 3). Mature adults of both sexes contained *trans*-verbenol and myrtenol, but frontalin and verbenone were found only in males, and no detectable levels of *exo*-brevicomin were found in females. Both callow males and females produced *trans*-verbenol and myrtenol during exposure to high concentrations of (−)-α-pinene while pupae had only traces of these compounds under the same conditions. Frontalin, *exo*-brevicomin and verbenone were not found in these immature beetles exposed to (−)-α-pinene vapour. Exposure of mature males and females to (−)-α-pinene vapours resulted in large quantities of *trans*-verbenol and myrtenol, but did not appear to increase the amount of verbenone already present in emerged males and no verbenone was detected in females (Table 3).

DISCUSSION

Some of the first studies which indicated that the digestive tract of bark beetles contain pheromones were reported by WOOD and BUSHING (1963) and VITÉ *et al.* (1963) who found pheromone activity in faecal pellets. Later reports showed pheromone activity associated with hindgut extracts (PITMAN *et al.*, 1965; ZETHNER-MØLLER and RUDINSKY, 1967) and volatile compounds suspected of being pheromonal were found in hindguts by RENWICK *et al.* (1966), PITMAN *et al.* (1966) and RENWICK and VITÉ (1972). Identified pheromones have been isolated from the hindguts of many bark beetles by PITMAN *et al.* (1969), VITÉ *et al.* (1972), HUGHES (1973, 1975), RENWICK *et al.* (1976), COSTER and VITÉ (1972), BORDEN (1974), BAKKE *et al.* (1977), and BYERS and WOOD (1980). However, bark beetle pheromones have been found in two cases elsewhere than the digestive tract. HUGHES (1973) identified *trans*-verbenol from haemolymph withdrawn with a syringe from *D. valens* and *D. ponderosae*. He proposed that this indicated its synthesis occurred in the haemocoel and was then transported via the Malpighian tubules to the hindgut to be concentrated by reabsorption of water. However, the possibility that the *trans*-verbenol had diffused from the hindgut or had been reabsorped by the cryptonephridial Malpighian tubules cannot be excluded. In the other case, GORE *et al.* (1977) believe that α-multistriatin is located in an accessory gland near the anus of *Scolytus multistriatus*. Another pheromone component, 4-methyl-3-heptanol, was found in the abdomen but neither compound was detected in the hindgut.

In the present study when *I. paraconfusus* was exposed to plant monoterpene vapours, there were no apparent differences within the gut in the distributions of the metabolites of myrcene, ipsenol and ipsdienol (ipsdienol system), and in the distributions of the metabolites of α-pinene, *cis*-verbenol and myrtenol (*cis*-verbenol system). However, as shown previously (BYERS *et al.*, 1979) females do not have the ipsdienol system. On the other hand, feeding on the host caused production of large amounts of ipsenol and ipsdienol while *cis*-verbenol could not be detected. Thus feeding appears to increase the synthetic rate of the ipsdienol system (HUGHES and RENWICK, 1977a) and/or increases the rate of myrcene introduction over vapour exposure alone (BYERS, 1981a). Feeding also appears to inhibit the synthetic rate of the *cis*-verbenol system or does not provide sufficient α-pinene compared to vapour exposure (BYERS, 1981a).

It would seem to be more economical for bark beetles to have the biosynthetic sites of pheromones near their release point, the anus, such as in the rectum, so that losses by diffusion into the body would be minimized. Also, it would be more efficient to have the sites of biosynthesis in or near the digestive tract and accessible to host precursors diffusing from the food material. If the insect manufactures pheromone in certain cells or glands in the haemocoel then additional mechanisms are needed to concentrate and excrete the volatiles to the outside via the anus. Furthermore, symbiotic bacteria which have been implicated in the *cis*-verbenol system (*Bacillus cereus*, BRAND *et al.*, 1975) and ipsdienol system (BYERS and WOOD, 1981) in *I. paraconfusus* would most likely reside in the gut (as most other bacteria do) rather than in the haemocoel.

While it may be reasonable to suspect that the site of pheromone biosynthesis corresponds to the site of accumulation in the hindgut, there is some evidence that synthesis of *cis*-verbenol may occur in the haemocoel or anterior portion of the gut. The synthesis of *cis*- and *trans*-verbenol (from α-pinene) and compound B (from myrcene) may not occur in the gut since they were not found in host-fed males while large amounts of ipsenol and ipsdienol were. The quantities of α-pinene and myrcene in pine tissue are comparable (BYERS, 1981a) so adequate precursors for both the *cis*-verbenol and ipsdienol systems should have been available in the gut during feeding. How-

ever, another explanation which might account for the relative differences between fed males and vapour-exposed males is that synthesis of ipsenol and ipsdienol may reside in the gut epithelium, so that they accumulate, while synthesis of *cis*-verbenol and other metabolites occur in the gut lumen, and are thus rapidly purged during feeding. This hypothesis would predict large amounts of *cis*-verbenol relative to ipsenol/ipsdienol in the faecal pellets. However, SILVERSTEIN *et al.* (1967) found a ratio of 1:267 of *cis*-verbenol:ipsenol in the frass (boring particles and faecal pellets) which is in agreement with the ratio found in guts of fed males (undetectable *cis*-verbenol) but opposite to the ratio of 10–20:1 in vapour-exposed males found here and in BYERS (1981a). Furthermore, the *cis*-verbenol system was not seemingly affected by streptomycin while the ipsdienol system was inhibited (BYERS and WOOD, 1981), and this antibiotic, a trisaccharide, is not known to transverse the gut epithelium except in trace amounts, at least in mammals (FRANKLIN and SNOW, 1971). Therefore, this evidence suggests that the ipsdienol system is located in a different place than the *cis*-verbenol system and that the ipsdienol system is probably in the gut lumen or epithelial cells.

There were no apparent differences in gut morphology between the sexes of each bark beetle species. However, PITMAN *et al.* (1965) reported that the Malpighian tubules of male *I. paraconfusus* lengthen about 9 mm as they feed and an earlier conclusion (PITMAN and VITÉ, 1963) that hindgut epithelium of males is thicker than females was retracted. If morphological differences are found between the sexes this may not necessarily be associated with sex-specific pheromone biosynthesis.

In *D. brevicomis* the distribution of pheromonal compounds in the gut of vapour-exposed beetles was similar to *I. paraconfusus*. However, it appears that the anterior hindgut of *D. brevicomis* contained higher amounts relative to the posterior hindgut than in *I. paraconfusus*. The distributions of compounds in feeding beetles of both species were even more similar. These similarities may either mean that no matter where the pheromones are synthesized they eventually accumulate in the hindgut or that these bark beetles synthesize the compounds in the same general area, the hindgut. An attempt to test these competing hypotheses was done by exposing *I. paraconfusus* to vapours of certain monoterpene alcohols. If these compounds then had been evenly distributed throughout the insect this would have indicated that the excretory system was not able to concentrate the compounds in the hindgut, and thus it would support the idea that the biosynthetic sites are in the hindgut. However, since the monoterpene alcohols were found in the hindgut, one cannot determine whether this is the result of enzyme/cofactor affinity for the products (monoterpene alcohols) in the hindgut or the result of normal excretory processes. The isolation of various tissues from the beetle and their exposure to pheromone precursors *in vitro* may provide an answer.

The *trans*-verbenol/myrtenol production from α-pinene in callow *D. brevicomis* was only about 10% that of mature adults indicating an effect of maturation. However, no pheromones or volatiles were detected in the gut of callow sexes dissected from the bark. Significantly, callow females and males as well as mature females did not make verbenone when exposed to (−)-α-pinene while mature males contained verbenone, but the amounts were not apparently different from unexposed males. It is surprising that α-pinene apparently was not converted to verbenone, at least at this time, as verbenone is structurally related to *trans*-verbenol (keto vs alcohol at C_4) and no other major host monoterpenes are as closely related as α-pinene is to verbenone. The (+) enantiomer of α-pinene also appears not to be converted to verbenone (BYERS, 1983).

HUGHES (1975) reported that *D. frontalis* males exposed to α-pinene contained about twice as much verbenone as unexposed males (GLC peak height of 80.7 vs 44.3 cm). However, in another group of adults just emerging the difference apparently was not significant (80.7 vs 73.1 cm). HUGHES (1975) further showed pupae of *D. frontalis* and *D. terebrans* did not contain significantly more *trans*-verbenol after exposure to α-pinene, but that adults reared from these pupae contained several times more *trans*-verbenol than adults reared from unexposed pupae. HUGHES (1975) suggested that pupae may conjugate some form of α-pinene with an unknown compound(s) and this conjugate would be later metabolized by the young adult to yield *trans*-verbenol and verbenone. This hypothesis may explain some of the results of the present study and BYERS (1983) in which *trans*-verbenol and verbenone quantities increased in adult *D. brevicomis* held at room temperature for 18 hr in the absence of α-pinene vapours. It is also possible that α-pinene is not detected in sufficient amounts in the hindgut to account for the production of pheromone components because it is stored in some other part of the body or it is diffused throughout. In this latter hypothesis, pupal exposure may enhance *trans*-verbenol and verbenone synthesis in adults of *Dendroctonus* by induction of synthetic enzymes and/or by increasing the precursor reservoir. The changes in pheromone production during maturation are probably further affected by feeding, as female *D. brevicomis* at this time produce *exo*-brevicomin (Fig. 2; HUGHES, 1973) and juvenile hormone application alone caused an even higher production of this component (HUGHES and RENWICK, 1977b).

The present study supports the statement of HUGHES and RENWICK (1977a) that neither callow male nor female *I. paraconfusus* have a functioning ipsdienol system. Callow adults of both sexes did not have pheromone even though food material was found in almost all beetles. The *cis*-verbenol system was found to operate in both callow sexes, apparently at a slightly reduced rate compared to mature adults. This could mean that the same oxidative enzymes of the beetle (detoxification?, HUGHES, 1973) or bacteria are present in immature and mature adults. Again, juvenile hormone may be involved with pheromone synthesis (HUGHES and RENWICK, 1977a) by regulating the enzyme system of the beetle or bacteria. Other possibilities are that symbiotic bacteria may be eaten before emergence and/or allowed to flourish during development or feeding. The systems for biosynthesis of pheromones in *D. brevicomis* and *I. paraconfusus* appear to be complex and may be affected by sex, developmental stage, bacteria, antibiotic, juvenile hor-

mone, host plant compounds, feeding, mating and ageing (HUGHES, 1973, 1975; BRAND *et al.*, 1975; RENWICK *et al.*, 1976; HUGHES and RENWICK, 1977a,b; BYERS *et al.*, 1979; BYERS and WOOD, 1981; BYERS, 1981a,b, 1982, 1983).

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