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PRODUCTION OF PHEROMONE COMPONENTS, CHALCOGRAN AND METHYL (*E*, *Z*)-2,4-DECADIENOATE, IN THE SPRUCE ENGRAVER *PITYOGENES CHALCOGRAPHUS*

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Abstract—Capillary gas chromatography and mass spectrometry were used to quantify the amounts of *E*- and *Z*- chalcogran and methyl (*E*,*Z*)-2,4-decadienoate (*E*,*Z*-MD), pheromone synergists of the bark beetle *Pitygenes chalcographus* (Coleoptera: Scolytidae). Males were exposed or not to vapours of host (Norway spruce, *Picea abies*) oleoresin or allowed to feed in host logs prior to extraction of body parts and hindguts for pheromone synergists. *E*,*Z*-MD and chalcogran were produced sex-specifically in males, and only after feeding on host-plant tissue. The pheromone synergists were not produced during exposure to oleoresin vapours. Several oxygenated monoterpenes (including *trans*-verbenol, myrtenol, and *trans*-myrtanol) were found in feeding males. The amounts of the pheromone synergists in unmated feeding males remained relatively constant over a 3.5 day period. In contrast to many other pheromone components of bark beetles, including chalcogran, *E*,*Z*-MD was found primarily in the male's body (head and thorax) with less in the hindgut (abdomen). The probable acetogenic origins of both pheromone components indicate that the species has evolved control over production and is thus not dependent on host precursors as expected in many other bark beetles.

Key Word Index: Pheromone; *Pityogenes chalcographus*; Coleoptera; Scolytidae; methyl (*E*,*Z*)-2,4-decadienoate; chalcogran; *Picea abies*; synergist; sex-specific

INTRODUCTION

In Europe, *Pityogenes chalcographus* ("Kupferstecher") is a serious pest of Norway spruce, *Picea abies* (L.) Karst, killing primarily the smaller diameter and younger trees. These tiny bark beetles (2-mm long) aggregate on certain host trees in response to a male-produced pheromone which consists of two synergistic components, chalcogran (Francke *et al.*, 1977) and methyl (*E*,*Z*)-2,4-decadienoate (*E*,*Z*-MD, Byers *et al.*, 1988; Byers *et al.*, 1990). *P. chalcographus* males produce both the *E*- and *Z*-isomers of chalcogran (Francke *et al.*, 1977). The *E*-isomer is produced only in the 2*S*,5*R*-form of two possible enantiomers: 2*S*,5*R*- and 2*R*,5*S*-chalcogran, while the *Z*-isomer also is produced only in the 2*S*,5*S*-form of two possible enantiomers: 2*S*,5*S*- and 2*R*,5*R*- (Schurig and Weber, 1984). Byers *et al.* (1990) found that of the two naturally produced enantiomers, the *E*- (2*S*,5*R*-) is synergistically active with *E*,*Z*-MD in eliciting attraction while the *Z*-chalcogran (2*S*,5*S*-) is essentially inactive.

The objective of the study was to observe the distribution of *E*- and *Z*-chalcogran and *E*,*Z*-MD within the body of male *P. chalcographus* while feeding in host logs or exposed to host oleoresin or not. Differences in the relative distribution of isomers and components as well as oxygenated monoterpenes may indicate differences in the sites of biosynthesis. We also wanted to measure amounts of the pheromone components in a cohort of males at several times over a several-day period of colonization in host logs.

MATERIALS AND METHODS

P. chalcographus beetles were reared in a laboratory culture originating from Lardal, Norway, on freshly cut Norway spruce logs at 27°C. At emergence, beetles were automatically collected and maintained at 4°C for several days until used in experiments (Anderbrant *et al.*, 1991).

The amounts of *E* and *Z* isomers of chalcogran, *E*,*Z*-MD, 1-hexanol, 2-phenylethanol, and various oxygenated monoterpenes were quantified by analytical gas chromatography and mass spectrometry (GC-MS) using a Finnigan model 4021. A fused silica column (25 m × 0.15 mm i.d.) coated with Superox® RA (Alltech Associates, d.f. = 0.3 μm) was used with a temperature program of 50°C for 4 min, 8°C/min to 200°C, then isothermal for 10 min. Helium at 20 cm/s was used as mobile phase. The identities of the reported semiochemicals were confirmed by matching their GC retention time and mass spectra with authentic standards using GC–MS. Quantification was done by GC–MS using total ion chromatograms (TIC) and extracted ion current profiles (EICP; Chen, 1979; Garland and Powell, 1981). A quantification standard of 1 ng heptyl

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Table 1. Relative amounts of *E* and *Z*-chalcogran and methyl (*E*,*Z*)-2,4-decadienoate (*E*,*Z*-MD) in mid/hindguts and gutted bodies of *P. chalcographus* exposed to Norway spruce oleoresin or not for 10 h, or fed in host logs for 45–72 h (14 April 1985)

Extract	Amounts relative to chalcogran: Chalcogran *E*:*Z*	*E*,*Z*-MD
15 males; oleoresin; "guts"	*	*
18 males; no oleoresin; "guts"	*	*
30 males; fed 45 h; "guts"	51:49	7
31 males; fed 45 h; "bodies"	50:50	58
28 females; fed 48 h; "guts"	*	*
18 males; fed 72 h; "guts"	52:48	2†

*Amounts were below the limit of quantification (0.01 ng/GC injection, EICP).
†Limit of quantification.

acetate per μl diethyl ether extraction solvent was used in the analyses.

In the first experiment (15 April 1985), various numbers of males and females were fed in host logs for 45 or 72 h and then mid/hindguts were removed and extracted with 200–300 μl diethyl ether. Males were each exposed to a "saturated" atmosphere of host oleoresin (100 μl on filter paper) or clean air by placing 15–18 in a screen cage within 100 ml bottles for 10 h until dissection and extraction with diethyl ether. In a second experiment (29 April, 1985), hindguts were removed from 81 males after they had fed in host logs for 67 h. The hindguts were extracted and analysed on GC–MS in order to quantify their content of pheromone components and 1-hexanol as described above. In the third experiment (beginning 30 April, 1985), feeding males were taken out of host logs after varioius periods and extracted in groups of 10–15 for the pheromone components. In order to increase the quantification sensitivity and reduce the amounts of sesquiterpenes and oxygenated monoterpenes on the beetle's cuticle, 66 males were surface washed in ethanol (7 May 1985) before dividing them into head + thorax and abdomen parts for extraction and quantification of the chemical constituents of interest. In the fifth experiment (14 May 1985), males were allowed to feed in two host logs, separated into body parts, extracted in groups of 10–14, and then analysed for pheromone components.

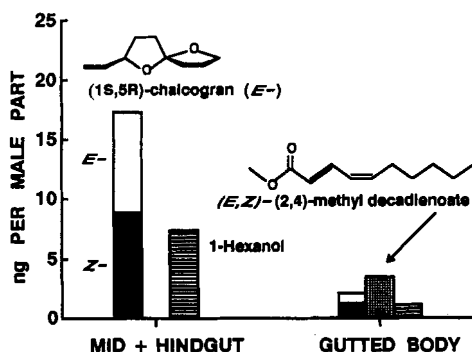

Fig. 1. Amounts of *E*- and *Z*-chalcogran, methyl (*E*,*Z*)-2,4-decadienoate (*E*,*Z*-MD), and 1-hexanol in mid/hindguts and corresponding gutted bodies of 81 male *P. chalcographus* fed in a Norway spruce log for 67 h (29 April 1985). *E*,*Z*-MD was detected in the hindguts but was below our limit of quantification (<0.1 ng/gut).

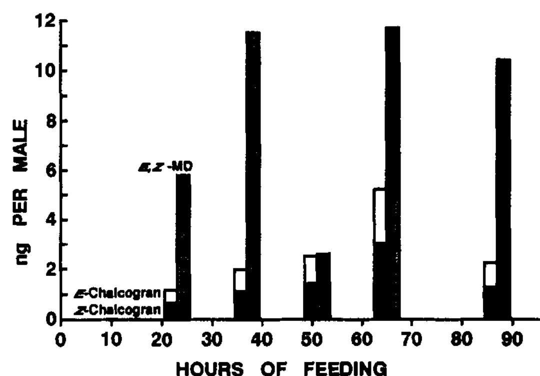

Fig. 2. Amounts of the pheromone components, chalcogran and methyl (*E*,*Z*)-2,4-decadienoate (*E*,*Z*-MD), in male *P. chalcographus* after various periods of feeding in a Norway spruce log (30 April 1985).

RESULTS

Neither of the pheromone components, *E*,*Z*-MD nor chalcogran, were found in hindguts of males exposed to oleoresin vapours or to air alone in the first experiment (Table 1). Neither were *E*,*Z*-MD nor chalcogran found in feeding females (Table 1). In the second experiment, the mid/hindguts contained more 1-hexanol than the gutted body (Fig. 1). In feeding males, *E*,*Z*-MD was found predominately in the male's gutted body, with little differences in the ratios of *E*:*Z* chalcogran (Table 1 and Fig. 1). More specifically, there was more *E*,*Z*-MD in the head + thorax of the male (11.2 ± 4.6 ng, $\pm$SD) compared to the abdomen (2.3 ± 0.9 ng). In contrast, only 2.6 ± 1.4 ng chalcogran (46% *E*:54% *Z*) was found in the male's head + thorax compared to 10.4 ± 2.4 ng in the abdomen in experiments 4 and 5 (Tables 2 and 3). The ratios of *E*- to *Z*-chalcogran were rather constant at about 46:54 regardless of the body part extracted (Tables 1–3; Figs 1 and 2).

The amount of *E*,*Z*-MD and chalcogran in hindguts of males appeared rather stable during several days of feeding in host logs (Fig. 2). Various monoterpene alcohols (*trans*-verbenol, myrtenol, and *trans*-myrtanol), and 2-phenylethanol were also found in higher proportions in the abdomen than in the head + thorax (Table 2).

Table 2. Amounts of pheromone components, chalcogran and methyl (*E*,*Z*)-2,4-decadienoate (*E*,*Z*-MD), some oxygenated monoterpenes, and 2-phenylethanol in 66 male *P. chalcographus* fed in a Norway spruce log for 67 h

Compound	ng per male: Head + thorax	Abdomen
E-chalcogran	1.03	5.48
Z-chalcogran	1.22	6.36
E,*Z*,-MD	3.03	0.79
cis-Verbenol	*	*
trans-Verbenol	0.37	4.59
Myrtenol	0.28	0.73
trans-Myrtanol	0.09	0.95
2-Phenylethanol	0.99	2.35

*Amount less than limit of identification (<0.01 ng/GC injection, EICP).
After their removal from the log, the males were washed in ethanol to remove any possible host resin contaminants before separation into head + thorax and abdomen (containing the mid/hindgut), 7 May 1985.

Table 3. Amounts of the pheromone synergists, chalcogran (*E*- and *Z*-isomers) and methyl (*E,Z*)-2,4-decadienoate (*E,Z*-MD), in male *P. chalcographus* fed in Norway spruce logs for 48 h (14 May 1985)

	ng per Male				
	Chalcogran				
Analysis group	*E*-isomer	*Z*-isomer	*E* + *Z* total	*E,Z*-MD	Total/*E,Z*-MD ratio
Log No. 1					
10 abdomens	6.08	7.72	13.80	1.79	7.72
10 heads + thoraxes	0.71	0.86	1.57	7.48	0.21
14 abdomens	4.56	5.48	10.07	2.94	3.41
14 heads + thoraxes	2.12	2.50	4.63	13.43	0.35
Log No. 2					
10 abdomens	3.83	5.86	9.69	1.34	7.24
10 heads + thoraxes	0.63	0.92	1.56	7.18	0.22
13 abdomens	3.40	4.72	8.08	3.13	2.59
13 heads + thoraxes	1.08	1.68	2.76	16.54	0.17
Average (abdomens)	4.41	5.83	10.23	2.41	4.24
Average (heads + thoraxes)	1.21	1.59	2.81	11.69	0.24

DISCUSSION

Sex-specificity of pheromone component production generally resides in the male sex in the polygynous bark beetle mating systems exemplified by the important pest genera *Ips* and *Pityogenes*. The male initiates the gallery or nuptial chamber and "controls" the access of females to the food/habitat resource. In *Ips paraconfusus*, only the male can produce the pheromone components ipsenol and ipsdienol (Hughes, 1974; Byers *et al.*, 1979) but females can produce about 36% as much as males of the third pheromone component, *cis*-verbenol (Byers, 1981a). Males of *I. typographus* specifically produce the pheromone component 2-methyl-3-buten-2-ol while both sexes produce the second component *cis*-verbenol (Birgersson *et al.*, 1984).

In *P. chalcographus*, in addition to the *E*- and *Z*-chalcogran produced by males, Francke *et al.* (1977) found another sex-specific compound, 1-hexanol. However, they stated that it had no apparent synergistic effect although they cautioned that "it is also possible that the enantiomeric composition of the isomers (of chalcogran with or without 1-hexanol?) is critical for maximal beetle response". Subsequently, 1-hexanol has not been shown to be synergistically active since its subtraction from the natural pheromone component blend did not affect attraction (Byers *et al.*, 1990). We found that the mid/hindguts contained more 1-hexanol than the gutted body (Fig. 1).

Another sex-specific compound in *P. chalcographus*, 2-phenylethanol, has been found previously only in males in several species and genera of bark beetles (*Ips* and *Dendroctonus*), regardless of the mating system (Renwick *et al.*, 1966, 1976b; Vité *et al.*, 1976; Birgersson *et al.*, 1984). In *I. paraconfusus*, the male produces 2-phenylethanol during feeding in logs (Renwick *et al.*, 1966, 1976b) or after synthetic juvenile hormone is applied (Hughes and Renwick, 1977). It has been reported that 2-phenylethanol synergizes the attraction to naturally-infested host logs of ponderosa pine (Renwick *et al.*, 1976b), although the statistical evidence is weak and the finding could not be confirmed (Byers, unpublished). *I. typographus* also produce 2-phenylethanol male-specifically (Birgersson *et al.*, 1984) but the compound has no apparent effect on attraction to a blend of compounds containing the essential pheromone components 2-methyl-3-butene-2-ol and *cis*-verbenol (Schlyter *et al.*, 1987). The behavioural function of 2-phenylethanol, if any, remains to be elucidated.

Sex-specificity of production is also shown for *E,Z*-MD in *P. chalcographus*, but the distribution within the body appears significantly different from that of the other compounds observed. The predominance of *E,Z*-MD in the head + thorax is unusual compared to most bark beetles studied, where pheromone components and host-derived monoterpene alcohols are concentrated mostly in the hindgut within the abdomen (Pitman *et al.*, 1965; Renwick *et al.*, 1966; Zenther-Møller and Rudinsky, 1967; Kinzer *et al.*, 1969; Vité *et al.*, 1972; Coster and Vité, 1972; Hughes, 1973, 1974; Borden, 1974; Renwick *et al.*, 1976a; Francke *et al.*, 1977; Klimetzek and Francke, 1980; Hughes and Renwick, 1977; Byers, 1981a, b, 1983a, b, 1989; Byers *et al.*, 1979, 1984; Birgersson *et al.*, 1984; Lanne *et al.*, 1987). It may be that the biosynthetic site of *E,Z*-MD is located in a different area of the insect compared to the sites of biosynthesis for chalcogran as well as other constituents (Table 3), but all compounds are probably released with the faecal pellets.

Many of the above studies have assumed that only the hindgut contained significant amounts of pheromone. However, some evidence has been presented, in addition to ours, which contradicts the prevailing view that bark beetle pheromones are found essentially in the hindgut. Hughes (1973) noted that after *D. valens* and *D. ponderosae* were exposed to α-pinene vapours both species contained relatively more *cis*-3-pinen-2-ol than *trans*-verbenol in the haemolymph than in the hindgut. This suggests either different sites of biosynthesis or differential rates of diffusion/transport. Gore *et al.* (1977) found that α-multistriatin is concentrated in an accessory gland near the anus while the other pheromone component, 4-methyl-3-heptanol, was found in the abdomen but neither component was within the hindgut. Recently, Madden *et al.* (1988) found that *cis*-verbenol and verbenone were distributed about equally between the anterior (head + prothorax) and posterior (meso-metathorax + abdomen) parts of *D. pseudotsugae* while *trans*-verbenol was found in much larger amounts in the posterior region. In *I. paraconfusus*, the biosynthetic system converting α-pinene to the

cis- and *trans*-verbenol and the system converting myrcene to the ipsdienol and ipsenol were suggested to reside in different areas (Byers, 1983a). This hypothesis was based on (1) differences in pheromone component production rates between beetles exposed to monoterpene (pheromone precursor) vapours compared with host feeding (Byers *et al.*, 1979; Byers, 1981a, 1983a) and on (2) differential sensitivity of the biosynthetic systems to antibiotic (Byers and Wood, 1981) and to synthetic juvenile hormone (Hughes and Renwick, 1977).

The distribution of *trans*-verbenol, myrtenol and *trans*-myrtanol between the head + thorax and the abdomen in *P. chalcographus* is consistent with that found in *I. paraconfusus* and *D. brevicomis* (Byers, 1983a). However, the absence of *cis*-verbenol relative to amounts of *trans*-verbenol, that are comparable to the pheromone components (Table 2), is not characteristic of either *I. parconfusus* (Renwick *et al.*, 1976a; Byers, 1981a, 1983a) or *I. typographus* (Klimetzek and Francke, 1980; Birgersson *et al.*, 1984) but more similar to that found in *D. brevicomis* (Byers, 1983b). One reason for the absence of *cis*-verbenol may be that *P. chalcographus* does not use this compound as a pheromone, as many *Ips* species do, but detoxifies and solubilizes the host tree α-pinene by conversion to trans-verbenol and myrtenol, as well as β-pinene to *trans*-myrtanol.

It is unusual that the two pheromone components of *P. chalcographus* appear to be biosynthetically derived from acetogenic and not terpenic pathways (W. Francke, personal communication) as are most other beetle pheromones (Vanderwel and Oehlschlager, 1986; Byers, 1989). Esters such as (*E*,*Z*)-MD have not been discovered in Scolytidae other than *P. chalcographus*. (*E*,*Z*)-MD is a representative of a new class of semiochemical esters. Williams *et al.* (1981) and Cogburn *et al.* (1984) have shown that the lesser grain borer (Coleoptera: Bostrichidae) uses (*S*)-1-methylbutyl (*E*)-2-methyl-2-pentenoate and (*S*)-1-methylbutyl (*E*)-2,4-dimethyl-2-pentenoate esters as aggregation pheromone components.

Schurig and Weber (1984) found that *P. chalcographus* contains only two isomers of chalcogran [(2*S*,5*R*)- and (2*S*,5*S*)-chalcogran] of four possible configurations. It appears that the beetle produces one or both of these isomers and that in the acidic gut they epimerize to the 46:54 ratio of (2*S*,5*R*):(2*S*,5*S*) isomers. The (2*S*,5*R*)-isomer is the most active synergist with *E*,*Z*-MD while (2*S*,5*S*)- is the least active (Byers *et al.*, 1989b). The other two isomers, (2*R*,5*S*)- and (2*R*,5*R*)- are not present in the beetle and are of intermediate behavioural activity. The presence of the inactive natural enantiomer and the two unnatural enantiomers of chalcogran does not interfere with the synergistic activity of 2*S*,5*R*-chalcogran with *E*,*Z*-MD (Byers *et al.*, 1989b).

The amounts of chalcogran and *E*,*Z*-MD in males were relatively constant throughout the 3.5 day period of colonization. The amounts probably would have decreased if females had been allowed to join the males since pheromone component amounts decline in other bark beetle species after mating such as in the genera *Dendroctonus* (Hughes, 1973; Coster and Vité, 1972; Byers *et al.*, 1984), *Scolytus* (Peacock *et al.*, 1971; Gore *et al.*, 1977), *Ips* (Byers, 1981b; Birgersson *et al.*, 1984), and *Tomicus* (Lanne *et al.*, 1987; Byers *et al.*, 1989a).

Investigations into the biosynthetic systems for chalcogran and *E*,*Z*-MD in *P. chalcographus* may prove to be more intractable, since they appear to be produced *de novo* in an acetogenic pathway, compared with systems in other bark beetles in which host monoterpenes can serve as pheromone precursors (Byers, 1989). Thus, during evolution *P. chalcographus* seems to have gained full control over biosynthesis of its pheromone components and would not be affected by variations in precursor supply from host trees as might be expected in other bark beetles.

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REFERENCES

Anderbrant O., Byers J. A., Jirle E., Jönsson J., Löfqvist J. and Schlyter F. (1991) Continuous rearing and automatic collection of some European spruce bark beetles. *J. Appl. Entomol.* Submitted for publication.

Birgersson G., Schlyter F., Löfqvist J. and Bergström G. (1984) Quantitative variation of pheromone components in the spruce bark beetle *Ips typographus* from different attack phases. *J. chem. Ecol.* **10**, 1029–1055.

Borden J. H. (1974) Pheromone mask produced by male *Trypodendron lineatum* (Coleoptera: Scolytidae) to male attractant. *Can. Entomol.* **99**, 1164–1193.

Byers J. A. (1981a) Pheromone biosynthesis in the bark beetle, *Ips paraconfusus*, during feeding or exposure to vapours of host plant precursors. *Insect Biochem.* **11**, 563–569.

Byers J. A. (1981b) Effect of mating on terminating aggregation during host colonization in the bark beetle, *Ips paraconfusus*. *J. chem. Ecol.* **7**, 1135–1147.

Byers J. A. (1983a) Influence of sex, maturity and host substances on pheromones in the guts of the bark beetles, *Dendroctonus brevicomis* and *Ips paraconfusus*. *J. Insect Physiol.* **29**, 5–13.

Byers J. A. (1983b) Bark beetle conversion of a plant compound to a sex-specific inhibitor of pheromone attraction. *Science* **220**, 624–626.

Byers J. A. (1989) Chemical ecology of bark beetles. *Experientia* **45**, 71–283.

Byers J. A. and Wood D. L. (1981) Antibiotic-induced inhibition of pheromone synthesis in a bark beetle. *Science* **213**, 763–764.

Byers J. A., Lanne B. S. and Löfqvist J. (1989a) Host tree unsuitability recognized by pine shoot beetles in flight. *Experientia* **45**, 489–492.

Byers J. A., Birgersson G., Löfqvist J. and Bergström G. (1988) Synergistic pheromones and monoterpenes enable aggregation and host recognition by a bark beetle. *Naturwissenschaften* **75**, 153–155.

Byers J. A., Wood D. L., Craig J. and Hendry L. B. (1984) Attractive and inhibitory pheromones produced in the bark beetle, *Dendroctonus brevicomis*, during host colonization: regulation of inter- and intraspecific competition. *J. chem. Ecol.* **10**, 861–877.

Byers J. A., Birgersson G., Löfqvist J., Appelgren M. and Bergström G. (1990) Isolation of pheromone synergists of bark beetle, *Pityogenes chalcographus*, from complex insect-plant odors by fractionation and subtractive-combination bioassay. *J. chem. Ecol.* **16,** 861–876.

Byers J. A., Högberg H. E., Unelius R., Birgersson G. and Löfqvist J. (1989b) Structure-activity studies on aggregation pheromone components of *Pityogenes chalcographus* (Coleoptera: Scolytidae): all stereoisomers of chalcogran and methyl 2,4-decadienoate. *J. chem. Ecol.* **15,** 685–695.

Byers J. A., Wood D. L., Browne L. E., Fish R. H., Piatek B. and Hendry L. B. (1979) Relationship between a host plant compound, myrcene and pheromone production in the bark beetle *Ips paraconfusus*. *J. Insect Physiol.* **25,** 477–482.

Chen E. C. M. (1979) Selective ion monitoring. In B. S. Middleditch (ed.) *Practical Mass Spectrometry—A Contemporary Introduction* (Edited by Middleditch B. S.). Plenum Press, New York.

Cogburn R. R., Burkholder W. E. and Williams H. J. (1984) Field tests with the aggregation pheromone of the lesser grain borer (Coleoptera: Bostrichidae). *Environ. Entomol.* **13,** 162–166.

Coster J. E. and Vité J. P. (1972) Effects of feeding and mating on pheromone release in the southern pine beetle. *A. Ent. Soc. Am.* **65,** 263–266.

Garland W. A. and Powell M. L. (1981) Quantitative selected ion monitoring (QSIM) of drugs and/or metabolites in biological matrices. *J. Chromat. Sci.* **19,** 392–434.

Gore W. E., Pearce G. T., Lanier G. N., Simeone J. B., Silverstein R. M., Peacock J. W. and Cuthbert R. A. (1977) Aggregation attractant of the European elm bark beetle, *Scolytus multistriatus*: production and individual components and related aggregation behavior. *J. chem. Ecol.* **3,** 429–446.

Francke W., Heemann V., Gerken B., Renwick J. A. A. and Vité J. P. (1977) 2-ethyl-1-6-dioxaspiro[4,4]nonane, principal aggregation pheromone of *Pitogenes chalcographus* (L.). *Naturwissenschaften* **64,** 590–591.

Hughes P. R. (1973) *Dendroctonus*: production of pheromones and related compounds in response to host monoterpenes. *Z. angew. Entomol.* **73,** 294–312.

Hughes P. R. (1974) Myrcene: a precursor of pheromones in *Ips* beetles. *J. Insect Physiol.* **20,** 1271–1275.

Hughes P. R. and Renwick J. A. A. (1977) Neural and hormonal control of pheromone biosynthesis in the bark beetle, *Ips paraconfusus*. *Physiol. Entomol.* **2,** 117–123.

Kinzer G. W., Fentiman A. F. Jr, Page T. F. Jr, Foltz R. L., Vité J. P. and Pitman G. B. (1969) Bark beetle attractants: identification, synthesis and field bioassay of a new compound isolated from *Dendroctonus*. *Nature* **221,** 477–478.

Klimetzek D. and Francke W. (1980) Relationship between the enantiomeric composition of α-pinene in host trees and the production of verbenols in *Ips* species. *Experientia* **36,** 1343–1345.

Lanne B. S., Schlyter F., Byers J. A., Löfqvist J., Leufvén A., Bergström G., Van Der Pers J. N. C., Unelius R., Baeckström P. and Norin T. (1987) Differences in attraction to semiochemicals present in sympatric pine shoot beetles, *Tomicus minor* and *T. piniperda*. *J. chem. Ecol.* **13,** 1045–1067.

Madden J. L., Pierce H. D. Jr, Borden J. H. and Butterfield A. (1988) Sites of production and occurrence of volatiles in Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins. *J. chem. Ecol.* **14,** 1305–1317.

Peacock J. W., Lincoln A. C., Simeone J. B. and Silverstein R. M. (1971) Attraction of *Scolytus multistriatus* (Coleoptera: Scolytidae) to a virgin-female-produced pheromone in the field. *A. Ent. Soc. Am.* **64,** 1143–1149.

Pitman G. B., Kliefoth R. A. and Vité J. P. (1965) Studies on the pheromone of *Ips paraconfusus* (LeConte). II. Further observations on the site of production. *Contrib. Boyce Thompson Inst.* **223,** 13–17.

Renwick J. A. A., Hughes P. R. and Krull I. S. (1976a) Selective production of *cis*- and *trans*-verbenol from (−) and (+) alpha-pinene by a bark beetle. *Science* **191,** 199–201.

Renwick J. A. A., Pitman G. B. and Vité J. P. (1976b) 2-Phenylethanol isolated from bark beetles. *Naturwissenschaften* **63,** 198.

Renwick J. A. A., Pitman G. B. and Vité J. P. (1966) Detection of a volatile compound in hindguts of male *Ips confusus* (LeConte) (Coleoptera: Scolytidae). *Naturwissenschaften* **53,** 83–84.

Schlyter F., Birgersson G., Byers J. A., Löfqvist J. and Bergström G. (1987) Field response of spruce bark beetle, *Ips typographus*, to aggregation pheromone candidates. *J. chem. Ecol.* **13,** 701–716.

Schurig V. and Weber R. (1984) Use of glass and fused-silica open tubular columns for the separation of structural, configurational and optical isomers by selective complexation gas chromatography. *J. Chromat.* **289,** 321–332.

Vanderwel D. and Oehlschlager A. C. (1987) Biosynthesis of pheromones and endocrine regulation of pheromone production in Coleoptera. In *Pheromone Biochemistry* (Edited by Prestwich G. D. and Blomquist G. J.), pp. 175–215. Academic Press, New York.

Vité J. P., Bakke A. and Renwick J. A. A. (1972) Pheromones in *Ips* (Coleoptera; Scolytidae): Occurrence and production. *Can. Entomol.* **104,** 1967–1975.

Vité J. P., Lühl R., Hughes P. R. and Renwick J. A. A. (1976) Pine beetles of the genus Dendroctonus: pest populations in Central America. *FAO Plant Protec. Bull.* **24,** 178–184.

Williams H. J., Silverstein R. M., Burkholder W. E. and Khorramshahi A. (1981) Dominicalure 1 and 2: Components of aggregation pheromone from male lesser grain borer *Rhyzopertha dominica* (F.). *J. chem. Ecol.* **7,** 759–780.

Zenther-Møller O. and Rudinsky J. A. (1967) Studies on the site of pheromone production in *Dendroctonus pseudotsugae* (Coleoptera: Scolytidae). *A. Ent. Soc. Am.* **60,** 575–582.