PHEROMONE-BASED AGGREGATION IN *ORTHOTOMICUS CAELATUS* (EICHHOFF) (COLEOPTERA: SCOLYTIDAE)

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Abstract

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Two field experiments were conducted to determine if *Orthotomicus caelatus* (Eichhoff) was attracted to pine bolts infested with conspecifics and to assess the roles of the sexes in attraction. Traps baited with pine bolts artificially infested with males attracted both males and females, but traps baited with uninfested bolts or bolts with females caught very low numbers of beetles. The addition of females to bolts with males reduced the attraction produced by males in a second experiment. Bolts with males and females did not reduce the attraction produced by other males in adjacent bolts, suggesting that females do not produce a masking pheromone. The pheromone system of *O. caelatus* is similar to those known for other species in the Ipini in which males initiate gallery construction, produce an attractant, and attract females and opportunistic males. Preliminary gas chromatographic analyses of extracts of hindguts and frass from males boring on pine bolts suggested the presence of ipsdienol and ipsenol, two commonly occurring pheromones in other species of the Ipini. The pheromone system of *O. caelatus* is discussed with regard to the complexity of the pine bark beetle guild in the southeastern United States.

Résumé

On a effectué deux expériences de terrain afin de savoir si *Orthotomicus caelatus* (Eichhoff) est attiré par des bûches de pin infestées d'individus conspécifiques et de comprendre le rôle des sexes dans le phénomène d'attraction. Des pièges appâtés de bûches de pin infestées artificiellement de mâles ont attiré des mâles et des femelles, alors que des pièges appâtés de bûches non infestées ou infestées de femelles seulement ont attiré peu de charançons. Dans une deuxième expérience, l'ajout de femelles à des bûches portant déjà des mâles a réduit l'effet d'attraction obtenu avec des mâles. Des bûches porteuses de mâles et de femelles n'ont pas réduit l'effet attirant de bûches adjacentes porteuses de mâles, indiquant que la femelle ne produit pas de phéromone masquante. Le système phéromonal de *O. caelatus* est similaire à ceux d'autres Ipini dont c'est le mâle qui initie la galerie, produit un attractant et attire des femelles et des mâles opportunistes. L'analyse chromatographique préliminaire d'extraits d'intestin et de fèces de mâles en train de forer des bûches de pin indique la présence d'ipsdiénol et d'ipsénol, deux phéromones communément rapportées chez d'atures Ipini. On discute du système phéromonal de *O. caelatus* en rapport avec la complexité de la guilde des charançons des pins du sud des USA.

Introduction

Orthotomicus caelatus (Eichhoff) is a widely distributed and common species of bark beetle that infests coniferous trees of the Pinaceae throughout North America (Wood 1982). *Orthotomicus caelatus* breeds in the phloem of logging slash and the boles, branches, and root collar regions of host trees that are moribund or severely stressed. Because the species is not aggressive, it is considered to have little or no economic significance (Baker 1972; Furniss and Carolin 1977; Drooz 1985). The ecological role of *O. caelatus* is that of a secondary bark beetle (Stark 1965) that generally infests host material already being colonized by other phloeophagus insects. Because *O. caelatus* is a common associate in different groups of coniferous bark beetle species throughout North America (e.g. Beal and Massey 1945; Reid 1955), its role in interspecific interactions and its mechanism of host colonization are of interest. Very little is known about the biology and host selection behavior of this common insect (Wood 1982).

While conducting a field experiment on pheromones of the eastern sixspined engraver, *Ips calligraphus* (Germar) (unpublished data), we discovered that *O. caelatus* might use

an attractant pheromone. Small slash pine bolts, *Pinus elliottii* Engelm. var. *elliottii*, artificially infested with 10 males of *I. calligraphus* and wrapped with aluminum screening, were deployed with bark beetle traps to assess the responses of feral *I. calligraphus*. No *I. calligraphus* were trapped at those bolts, but many individuals of *O. caelatus* were attracted and caught in traps. At first we suspected that *O. caelatus* was attracted to *I. calligraphus* pheromones because of the taxonomic similarity of the two species (Wood 1982) and the fact that related species of bark beetles are sometimes cross-attractive (e.g. Lanier and Wood 1975; Birch *et al.* 1980). However, upon inspection of the pine bolts we found that *O. caelatus* had passed through the mesh of the aluminum screening and were colonizing the bolts. We therefore investigated the possibility of a beetle-produced attractant in *O. caelatus* with controlled experiments, the results of which are reported here.

Methods and Materials

Two field experiments were conducted in a 16-year-old slash pine plantation located east of Gainesville, Alachua Co., FL. The first experiment, conducted 25 September to 9 October 1987, was designed to determine if either males or females produced an attractant while feeding on host material. Slash pine bolts, approximately 15 by 25 cm, were cut from the upper boles of standing trees. Adult *O. caelatus* used as sources of attractant were trapped alive while responding to attractive bolts in our *I. calligraphus* experiment described above. Beetles were sexed according to differences in morphology of the elytral declivity (Wood 1982). The three treatments in the first experiment were as follows: (1) a bolt only as a control; (2) a bolt artificially infested with six females; and (3) a bolt artificially infested with six males. Beetles were confined with pieces of saran screening in 2-mm-diameter holes drilled through the phloem and evenly spaced on the bark of the bolts. Bolts were completely covered with multiple wraps of 30-mesh saran screening and 18-mesh aluminum screening to prevent escape of introduced beetles and volunteer attacks by other insects. Treatment bolts were hung adjacent to the mid-point of 16-unit multiple funnel traps (Lindgren 1983). Traps were hung from PVC pipe standards so that the collecting jars were within 30 cm of the ground. The three treatments were deployed in five completely randomized blocks. Traps within an experimental block were spaced 20 m apart in a row, and blocks were spaced at least 30 m apart.

Because the first experiment showed that males were attractive (see below), the second field experiment, conducted 3–25 March 1988, examined the effect of females on the attraction produced by males. Beetles used in this experiment emerged in the laboratory from slash pine logs; bolts for the experiment, 15 by 25 cm, were cut from 16-year-old slash pines. The three treatments in the second experiment were as follows: (1) a slash pine bolt artificially infested with eight males; (2) a male-infested bolt to which two females were added to the galleries with each of the eight males (total of 16 females); and (3) a bolt with eight males deployed beside another bolt containing eight males with 16 females, as in the second treatment. Males were introduced onto all bolts and, for bolts in the second and third treatments, females were added 1 day later. Bolts were screened and deployed with funnel traps as in the first experiment; four completely randomized blocks were established. The second treatment was designed to determine if the presence of females in bolts with males reduced the male-produced attraction, and the third treatment tested the possibility that volatiles from bolts with paired males and females might interrupt the response of beetles to the attractant from bolts with males only.

Data from both field experiments, recorded as the numbers of male and female *O. caelatus* trapped at each treatment in each block for the duration of the study were transformed by calculating $y = \sqrt{x + 0.5}$. Transformed data for the responses of males and females separately were subjected to analysis of variance followed by the Student-Newman-Keuls test for means comparisons.

Gas chromatographic (GC) analyses were conducted on various extracts of *O. caelatus* volatiles to determine the presence of candidate pheromones. Field-collected males were introduced in pre-drilled holes on slash pine bolts in the laboratory, and frass (feces and boring chips) was collected in one-half of a 00 gelatin capsule affixed to each hole. Individual females were also infested on bolts and treated in the same manner. Frass was collected daily, pooled among individuals by sex, and placed in 1.0 mL of chilled pentane. After 6 days of feeding the beetles were removed from the bolts and their abdomens dissected and extracted in pentane. Abdomens from 10–20 beetles were pooled for each sex, placed in a 1.0-mL glass vial with chilled pentane (about 5.0 μL per abdomen), macerated with a glass probe, sonicated for 15 s for further extraction, and stored at −60°C until analysis. Qualitative GC analysis was performed primarily on a Varian 3700 instrument equipped with a flame ionization detector (FID) and a Hewlett-Packard 3390A recording integrator. The column was a 30 m by 0.25 mm ID fused silica capillary with a DB-1 phase (J & W Scientific, Inc.). Injection of 1–2 μL of pentane extract was done in splitless mode. Injector temperature was 180°C, the detector was 250°C, and the oven was held at 50°C for 12 min, then heated at 2°C per minute to 75°C and held for 25 min; He gas flow was 25.1 cm per second at 100°C. Identity of GC peaks was tentatively assigned based on congruence of retention times with those of known standard compounds. A second GC system, using a polar column, was then used to corroborate certain peak identities by retention time comparisons with standards. A Varian 2400 GC with an FID and the Hewlett-Packard 3390A integrator was equipped with an 8 m by 0.53 mm ID Superox column (Alltech Assoc.). The column oven was held at 100°C isothermally while the injector was 170°C, the detector was 240°C, and He flow was 34.0 cm per second; injection was directly on-column.

Results and Discussion

Male *O. caelatus* feeding in pine bolts produced a conspecific attraction for both males and females (Fig. 1). About twice as many females as males were attracted to male-infested bolts. Traps baited with females in bolts caught low numbers of both sexes, and these were not statistically different from the numbers responding to uninfested bolts. The occurrence of a male-produced sex pheromone in *O. caelatus* is consistent with what is known about the life history of this species and with the chemical ecology of related species in the scolytid tribe Ipini. All species in this tribe display a harem polygynous mating system (see Kirkendall 1983) in which males initiate attack on a host and are joined by two or more females (Wood 1982). Beetles in the genus *Ips* are well known for male-produced pheromones (e.g. Anderson 1948; Silverstein *et al.* 1966; Lanier and Wood 1975), and cases of male-produced pheromones are also known from *Pityogenes* (Franke *et al.* 1977) and *Pityokteines* (Harring and Vité 1975). A male-produced pheromone has been identified for the European *O. erosus* (Giesen *et al.* 1984).

Male *O. caelatus* joined by females in pine bolts attracted significantly lower numbers of flying females compared with the attraction to unjoined males in the second experiment; response of males did not differ between the two treatments (Fig. 2). Bolts with males and females apparently retained some attractiveness, but this could not be fully assessed because this experiment lacked an uninfested bolt as a control. The third treatment, a male-infested bolt adjacent to a bolt containing males and females, attracted significantly more *O. caelatus* than the first treatment with just a male-infested bolt. The presumed residual attraction from bolts with males and females may have augmented the attraction produced by the adjacent bolts containing males only in the third treatment. Alternatively, females joining males may produce their own pheromones that augment attractive pheromones produced by males alone on adjacent bolts, although we found no evidence of an attractant from females only in the first experiment. It is obvious that the bolts with males and females had no inhibitory or interrupting effect on the attractiveness of bolts with just males.

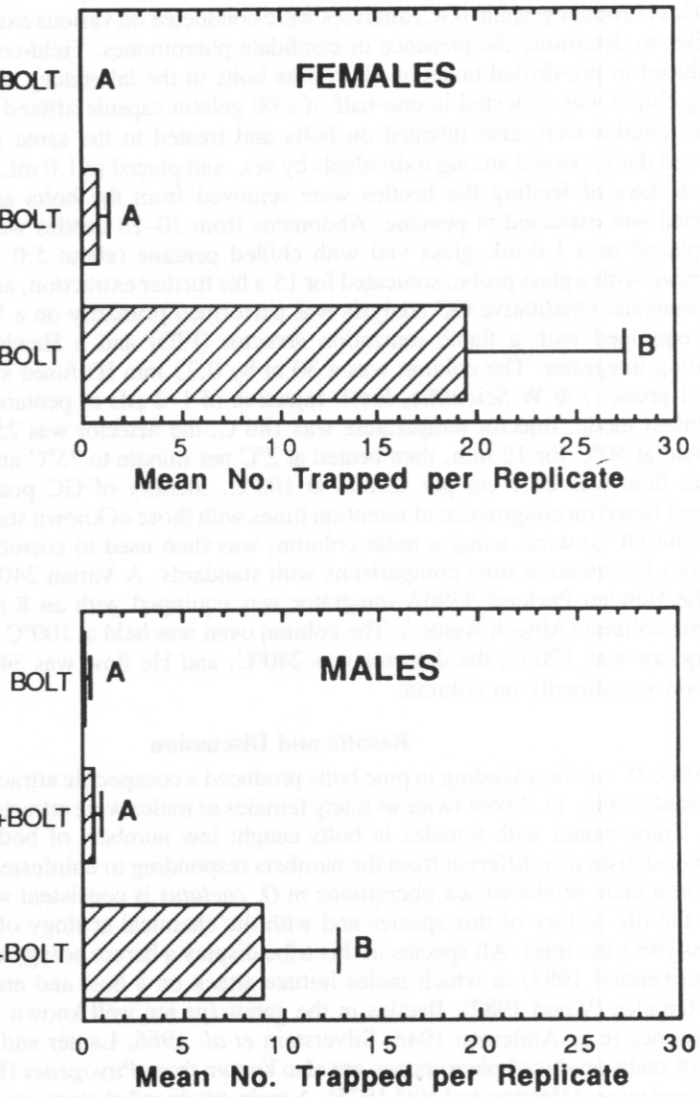

FIG. 1. Responses of *O. caelatus* females and males to pine bolts artificially infested with either six females or six males in the first experiment. Histograms represent mean numbers caught in traps and bars are the standard errors of the means ($N = 5$). Means followed by the same letter are not significantly different ($P < 0.05$, Student-Newman-Keuls test).

There are two possible explanations for the reduction of attraction to bolts containing *O. caelatus* males and females (Fig. 2, second treatment). Males may simply produce less attractant as a consequence of being joined by females, or joining females may produce a pheromone that masks the effect of male-produced attractant and thus interrupt the response of other beetles (''antiaggregation pheromones'' of some authors). In all the Ipini for which pheromones have been investigated, females join colonizing males that are producing pheromones (Borden 1982). These females are not known to produce attractants themselves. Frass from male *I. paraconfusus* Lanier (=*I. confusus* LeConte) loses its attractiveness after females enter the nuptial chamber (Borden 1967). Byers (1981) showed

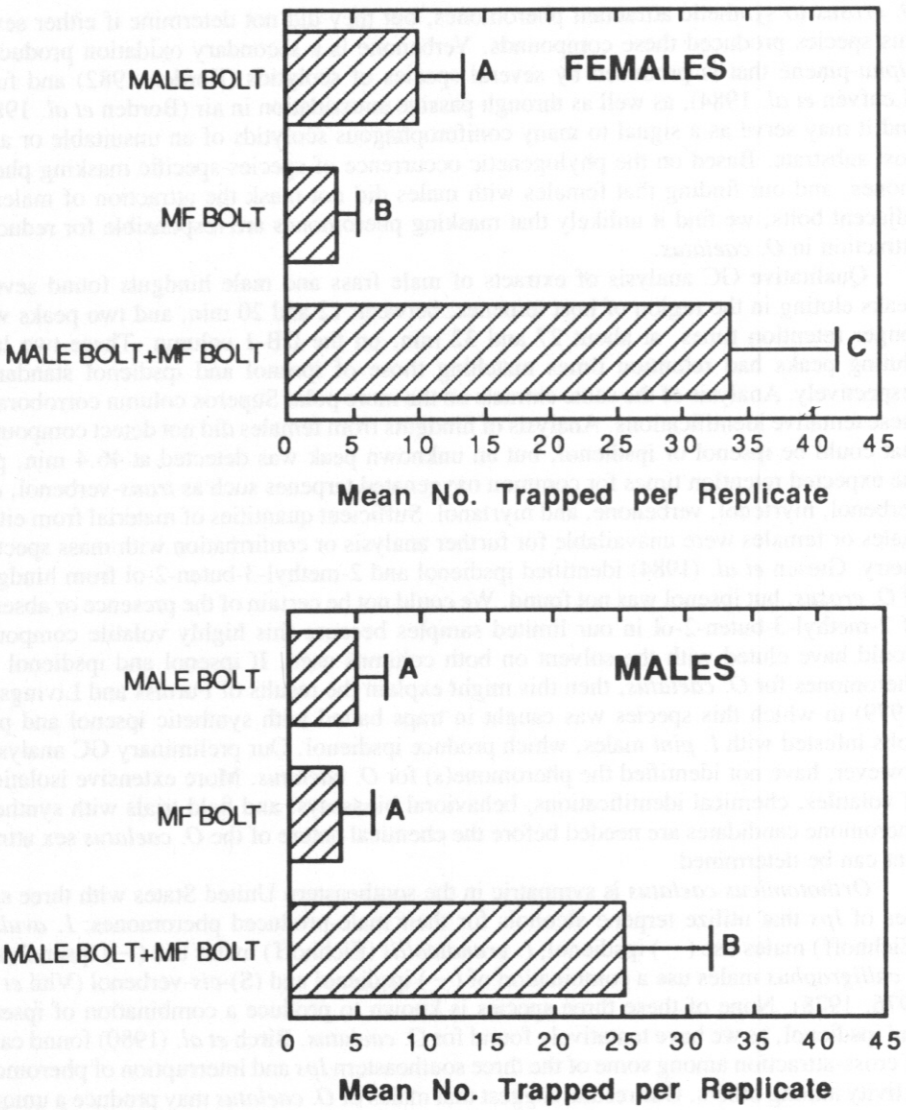

FIG. 2. Responses of *O. caelatus* females and males to traps baited with pine bolts, either singly or in combination, that were artificially infested with either males (MALE BOLT), or males each joined by two females (MF BOLT), in the second experiment. Histograms represent mean numbers caught in traps and bars are the standard errors of the means ($N=4$). Means followed by the same letter are not significantly different ($P<0.05$, Student-Newman-Keuls test).

that this reduced attraction by male *I. paraconfusus* was due to a reduction in the amounts of pheromones produced as females entered the gallery. Female-produced masking pheromones are not known in the Ipini, although masking pheromones do occur in several *Dendroctonus* species, in which they are produced by males that join colonizing females (e.g. Alcock 1982; Phillips *et al.* 1989; reviewed in Borden 1982). If a female-produced masking pheromone is operating in *O. caelatus*, then our results (Fig. 2) suggest it could only influence the attractant produced by males in the same piece of host material. Paiva *et al.* (1988) recently reported that *cis*-verbenol and verbenone reduced the response of

pines in northern peninsular Florida (Atkinson *et al.* 1988; unpublished data), including *O. caelatus*. Additionally, there is a rich fauna of ambrosia beetles (Scolytidae and Pla-

O. erosus to synthetic attractant pheromones, but they did not determine if either sex of this species produced these compounds. Verbenone is a secondary oxidation product of *alpha*-pinene that is produced by several species of scolytids (Borden 1982) and fungi (Leufvén *et al.* 1984), as well as through passive autoxidation in air (Borden *et al.* 1986), and it may serve as a signal to many coniferophagous scolytids of an unsuitable or aged host substrate. Based on the phylogenetic occurrence of species-specific masking pheromones, and our finding that females with males did not mask the attraction of males in adjacent bolts, we find it unlikely that masking pheromones are responsible for reducing attraction in *O. caelatus*.

Qualitative GC analysis of extracts of male frass and male hindguts found several peaks eluting in the region of host terpenes, between 12 and 20 min, and two peaks with longer retention times, at about 27 and 32 min, on the DB-1 column. These two late-eluting peaks had retention times matching those of ipsenol and ipsdienol standards, respectively. Analysis of the same extracts on the more polar Superox column corroborated these tentative identifications. Analysis of hindguts from females did not detect compounds that could be ipsenol or ipsdienol, but an unknown peak was detected at 46.4 min, past the expected retention times for common oxygenated terpenes such as *trans*-verbenol, *cis*-verbenol, myrtenol, verbenone, and myrtanol. Sufficient quantities of material from either males or females were unavailable for further analysis or confirmation with mass spectrometry. Giesen *et al.* (1984) identified ipsdienol and 2-methyl-3-buten-2-ol from hindguts of *O. erosus*, but ipsenol was not found. We could not be certain of the presence or absence of 2-methyl-3-buten-2-ol in our limited samples because this highly volatile compound would have eluted with the solvent on both columns used. If ipsenol and ipsdienol are pheromones for *O. caelatus*, then this might explain the results of Furniss and Livingston (1979) in which this species was caught in traps baited with synthetic ipsenol and pine bolts infested with *I. pini* males, which produce ipsdienol. Our preliminary GC analyses, however, have not identified the pheromone(s) for *O. caelatus*. More extensive isolations of volatiles, chemical identifications, behavioral bioassays, and field trials with synthetic pheromone candidates are needed before the chemical nature of the *O. caelatus* sex attractant can be determined.

Orthotomicus caelatus is sympatric in the southeastern United States with three species of *Ips* that utilize terpene alcohols for their male-produced pheromones: *I. avulsus* (Eichhoff) males use (−) ipsdienol; *I. grandicollis* (Eichhoff) males use (−) ipsenol; and *I. calligraphus* males use a combination of (−) ipsdienol and (S)-*cis*-verbenol (Vité *et al.* 1976, 1978). None of these three species is known to produce a combination of ipsenol and ipsdienol, as we have tentatively found for *O. caelatus*. Birch *et al.* (1980) found cases of cross-attraction among some of the three southeastern *Ips* and interruption of pheromone activity among others. Our results suggest that males of *O. caelatus* may produce a unique, species-specific combination of terpene alcohols among the conifer-infesting Ipini of the Southeast. However, a total of 61 *I. grandicollis* and 51 *Pityophthorous pulicarius* (Zimmermann) were attracted to bolts infested with male *O. caelatus* in our two field tests, but only five *I. calligraphus* and no *I. avulsus* were attracted. It is also possible that the pheromones produced by male *O. caelatus* interrupt responses by other species to their pheromones. When we first discovered *O. caelatus* flying to experimental pine bolts containing male *I. calligraphus*, and then determined that the bolts were contaminated with *O. caelatus* (described above), no *I. calligraphus* were attracted to those bolts.

Recent literature purports that the pine bark beetle guild of the southeastern United States is composed of just five scolytid species, three species of *Ips* and two of *Dendroctonus* (e.g. Birch *et al.* 1980; Coulson *et al.* 1986; Smith *et al.* 1988). This is an oversimplification that disregards the complexity of competitive interactions among phloem-feeding species in trees and the degree by which chemical signals are exploited between species for host location. There are 14 species of phloem-feeding scolytids that infest slash

pines in northern peninsular Florida (Atkinson *et al.* 1988; unpublished data), including *O. caelatus*. Additionally, there is a rich fauna of ambrosia beetles (Scolytidae and Platypodidae), Curculionidae, Buprestidae, and Cerambycidae found in slash pine that adds further complexity to the system. The total number of species in the pine beetle guild colonizing southern pines is probably over 40 (Atkinson *et al.* 1988). Many of these species are known to locate hosts via attraction to constitutive host odors (e.g. terpenes), volatiles resulting from degradation of host tissues or as by-products of host metabolism (e.g. ethanol), beetle-produced semiochemicals, or mixtures of these (Phillips *et al.* 1988). The chemical ecology of most of these species is unstudied. It is apparent that *O. caelatus* uses an attractant for mating and host colonization, and that its presence at host trees may affect the colonization and behavior of other members of the pine bark beetle guild.

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References

Alcock, J.A. 1982. Natural selection and communication among bark beetles. *Fla. Ent.* **65**: 17–32.

Anderson, R.F. 1948. Host selection by the pine engraver. *J. econ. Ent.* **41**: 596–602.

Atkinson, T.H., J.L. Foltz, and M.D. Conner. 1988. Flight patterns of phloem- and wood-boring Coleoptera (Scolytidae, Platypodidae, Curculionidae, Buprestidae, Cerambycidae) in a north Florida slash pine plantation. *Environ. Ent.* **17**: 259–265.

Baker, W.L. 1972. Eastern Forest Insects. *U.S.D.A. Forest Serv. Misc. Publ.* 1175. 642 pp.

Beal, J.A., and C.L. Massey. 1945. Bark beetles and ambrosia beetles (Coleoptera: Scolytidae): with special reference to species occurring in North Carolina. *Duke Univ., School of Forest., Bull.* 10. 178 pp., illus.

Birch, M.C., P. Svihra, T.D. Paine, and J.C. Miller. 1980. Influence of chemically mediated behavior on host tree colonization by four cohabiting species of bark beetles. *J. Chem. Ecol.* **6**: 395–414.

Borden, J.H. 1967. Factors influencing the response of *Ips confusus* (Coleoptera: Scolytidae) to male attractant. *Can. Ent.* **99**: 1164–1193.

———1982. Aggregation pheromones. pp. 74–139 *in* Mitton, J.B., and K.B. Sturgeon (Eds.), Bark Beetles in North American Conifers. University of Texas Press, Austin. 527 pp.

Borden, J.H., D.W.A. Hunt, D.R. Miller, and K.N. Slessor. 1986. Orientation in forest Coleoptera: an uncertain outcome to responses by individual beetles to variable stimuli. pp. 97–109 *in* Payne, T.L., M.C. Birch, and C.E.J. Kennedy (Eds.), Mechanisms in Insect Olfaction. Oxford University Press, Oxford. 364 pp.

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

Coulson, R.N., R.O. Flamm, P.E. Pulley, T.L. Payne, E.J. Rykiel, and T.L. Wagner. 1986. Response of the southern pine bark beetle guild (Coleoptera: Scolytidae) to host disturbance. *Environ. Ent.* **15**: 850–858.

Drooz, A.T. 1985. Insects of Eastern Forests. *U.S.D.A. Forest Serv. Misc. Publ.* 1426. 608 pp.

Franke, W., V. Heeman, B. Gerken, J.A.A. Renwick, and J.P. Vité. 1977. 2-Ethyl-1,6-dioxaspiro [4.4]nonane, principal aggregation pheromone of *Pityogenes calcographus* (L.). *Naturwissenschaften* **64**: 590–591.

Furniss, M.M., and R.L. Livingston. 1979. Inhibition by ipsenol of pine engraver attraction in northern Idaho. *Environ. Ent.* **8**: 369–372.

Furniss, R.L., and V.M. Carolin. 1977. Western Forest Insects. *U.S.D.A. Forest Serv. Misc. Publ.* 1339. 654 pp.

Giesen, H., U. Kohnle, J.P. Vité, M.-L. Pan, and W. Francke. 1984. Das Aggregationspheromon des mediterranen Kiefernborkenkäfers *Ips* (*Orthotomicus*) *erosus*. *Z. ang. Ent.* **98**: 95–97.

Harring, C.M., and J.P. Vité. 1975. "Ipsenol," der Populations lockstroff des krummzahnigen Tannenborkenkäfers. *Naturwissenschaften* **62**: 488.

Kirkendall, L.R. 1983. The evolution of mating systems in bark and ambrosia beetles (Coleoptera: Scolytidae and Platypodidae). *Zool. J. Soc.* **77**: 293–352.

Lanier, G.N., and D.L. Wood. 1975. Specificity of response to pheromones in the genus *Ips* (Coleoptera: Scolytidae). *J. Chem. Ecol.* **1**: 9–23.

Leufvén, A., G. Bergström, and E. Falsen. 1984. Interconversion of verbenols and verbenone by identified yeasts isolated from the spruce bark beetle *Ips typographus*. *J. Chem. Ecol.* **10**: 1349–1361.

Lindgren, B.S. 1983. A multiple funnel trap for scolytid beetles (Coleoptera). *Can. Ent.* **115**: 299–302.

Paiva, M.R., M.F. Pessoa, and J.P. Vité. 1988. Reduction in the pheromone attractant response of *Orthotomicus erosus* (.) and *Ips sexdentatus* Boern. (Col., Scloytidae). *J. Appl. Ent.* **106**: 198–200.

Phillips, T.W., J.L. Nation, R.C. Wilkinson, and J.L. Foltz. 1989. Secondary attraction and field activity of beetle-produced volatiles in the black turpentine beetle, *Dendroctonus terebrans*. *J. Chem. Ecol.* **15**: 1513–1533.

Phillips, T.W., A.J. Wilkening, T.H. Atkinson, J.L. Nation, R.C. Wilkinson, and J.L. Foltz. 1988. Synergism of turpentine and ethanol as attractants for certain pine-infesting beetles. *Environ. Ent.* **17**: 456–462.

Reid, R.W. 1955. The bark beetle complex associated with lodgepole pine slash in Alberta. *Can. Ent.* **87**: 311–323.

Silverstein, R.M., J.O. Rodin, and D.L. Wood. 1966. Sex attractants in frass produced by male *Ips confusus* in ponderosa pine. *Science* **154**: 509–510.

Smith, M.T., G.R. Busch, T.L. Payne, and J.C. Dickens. 1988. Antennal olfactory responsiveness of three sympatric *Ips* species [*Ips avulsus* (Eichhoff), *Ips calligraphus* (Germar), *Ips grandicollis* (Eichhoff)], to intra- and interspecific behavioral chemicals. *J. Chem. Ecol.* **14**: 1289–1304.

Stark, R.W. 1965. Recent trends in forest entomology. *A. Rev. Ent.* **10**: 303–324.

Vité, J.P., R. Hedden, and K. Mori. 1976. *Ips grandicollis*: field response; to optically pure pheromone. *Naturwissenschaften* **63**: 43–44.

Vité, J.P., G. Ohloff, and R.F. Billings. 1978. Pheromone chirality and integrity of aggregation response in southern species of the bark beetle *Ips* sp. *Nature* **272**: 817–818.

Wood, S.L. 1982. The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), a taxonomic monograph. *Great Basin Naturalist Mem.* 6. 1359 pp.

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