PHEROMONE PRODUCTION AND CONTROL MECHANISMS IN *DENDROCTONUS PONDEROSAE* HOPKINS

by

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ABSTRACT

Several combinations of potentially attractive volatiles for the mountain pine beetle, Dendroctonus ponderosae Hopkins, were tested in laboratory and field bioassays. Volatiles tested were: trans-verbenol, a known aggregation pheromone; 3-caren-10-ol, acetophenone, and myrcenol, all produced by females boring in host pine logs; and 2 host monoterpenes, α-pinene and 3-carene. In the laboratory, 3-caren-10-ol alone was attractive to beetles of both sexes. When added to 3-carene plus trans-verbenol in field trapping experiments, 3-caren-10-ol caused a switch in sex ratio in favour of males. Seven out of 8 trap trees baited with the ternary mixture of 3-carene, trans-verbenol, plus 3-caren-10-ol were attacked, compared to 3 baited with the binary mixture of 3-carene plus trans-verbenol, and none baited with α-pinene plus trans-verbenol, the components of a U.S.-patented product, Pondelure. Myrcenol and acetophenone appeared to be attractive in the laboratory. However, in field tests, the addition of myrcenol to a mixture of 3-carene, trans-verbenol, and 3-caren-10-ol caused a decrease in total beetle catch, while the addition of acetophenone had no effect. Emerged males contained large amounts of exo-brevicomin, a pheromone of other Dendroctonus spp. Within 24 h of being paired with females in a log, the males contained no exo-brevicomin. However, the females retained their complement of volatiles. Topical application of 100 µg of synthetic juvenile hormone (JH III) to emerged females induced them to produce trans-verbenol and acetophenone. Exposure of females for 24 h to 120 $\mu g/cm^2$ of precocene 2 on a glass surface resulted in inhibition of trans-verbenol production after 24 h in a fresh host log, and inability to produce brood during 12 days paired with males in a host log.

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INTRODUCTION

Bark and ambrosia beetles in the family Scolytidae are selective in host colonization. It was originally proposed for the western pine beetle, Dendroctonus brevicomis LeConte, that primary attractants emanated from weakened ponderosa pine, Pinus ponderosa Lawson, in the form of volatile oils (Person 1931). By this hypothesis, beetles attracted to these trees subsequently mass attacked them, overcoming the trees' natural resistance. Host odours as primary attractants have been demonstrated for several scolytid beetles: Dendroctonus pseudotsugae Hopkins and Dryocoetes autographus (Ratzeburg) (Chapman 1966); Ips typographus L. (Rudinsky et al. 1971); Scolytus multistriatus (Marsham) (Peacock et al. 1971); Gnathotrichus sulcatus (LeConte) and G. retusus (LeConte) (Chapman 1966); and Trypodendron lineatum (Olivier) (Chapman 1962, 1966). However, pioneer D. brevicomis, like some other aggressive species, land at random (Moeck et al. 1981).

Regardless of the role of primary attractants, almost all scolytids depend on secondary attraction (Borden et al. 1975), a combination of host and beetle-produced volatiles, which are released from a host after a "pioneer beetle" of the first-attacking sex has created an attraction centre (Borden 1974). Anderson (1948) first demonstrated that logs attacked by Ips pini (Say) attracted beetles of both sexes in the field. Wood et al. (1966) isolated and Silverstein et al. (1966) identified and synthesized the first bark beetle pheromones from male Ips paraconfusus (Lanier) and subsequently demonstrated their attractiveness in laboratory bioassays and field tests. Since then, many scolytid pheromones have been isolated and identified (Borden 1981).

In I. paraconfusus (Wood et al. 1968) and some other Ips spp. (Bakke 1976, 1978; Renwick and Vité 1972; Vité et al. 1976b; Birch et al. 1977a), pheromones are attractive alone in the field and host volatiles either do not enhance attractiveness or have not yet been tested rigorously. Some pheromones of Dendroctonus spp. (Bedard et al. 1969; Renwick and Vité 1969; Pitman and Vité 1970; Dyer 1973, 1975) as well as pheromones of G. sulcatus (Byrne et al. 1974), G. retusus (Borden et al. 1980a), and Scolytus scolytus (Blight et al. 1979) are attractive alone in laboratory and field tests, but for many of these species, the activity of pheromones is enhanced by the addition of one or more host volatiles (Bedard et al. 1969; Renwick and Vité 1969; Pitman and Vité 1970; Pearce et al. 1975; Lanier et al. 1977; Blight et al. 1978 ; Borden et al. 1980b).

Bark beetles have evolved a mechanism for detoxifying host-produced monoterpenes, which are toxic to them (Smith 1961, 1965; Hughes, 1973a, 1973b, 1974). demonstrated that exposing beetles to individual monoterpenes could induce the production of the corresponding terpene alcohols. These metabolic "waste-products" either with or without monoterpenes as synergists, have been utilized secondarily as pheromones to create the centre of attraction necessary to overcome the resistance of host trees (Smith 1966) or to utilize isolated host resources fully (Atkins 1966).

Trans-verbenol, isolated from the hindguts of female mountain pine beetles, Dendroctonus ponderosae Hopkins, was identified by Pitman et al. (1968). In the field trans-verbenol alone did not attract beetles, but when sprayed onto logs of western white pine, Pinus monticola Douglas, infested with females it increased attractiveness compared to female-infested logs alone. A communication system consisting of host- plus

beetle-produced volatiles was proposed for 3 species of Dendroctonus by Renwick and Vité (1970), in which the proposed system for D. ponderosae was less complex than for either the southern pine beetle, D. frontalis Zimmerman or D. brevicomis. It was proposed that the response of D. ponderosae depends on the presence of fresh resin or α-pinene to synergize trans-verbenol and that exo- and endo-brevicomin identified in hindguts of males by Pitman et al. (1969) had no apparent function.

When trans-verbenol was tested with several monoterpenes, α-pinene was superior to other monoterpenes in white pine forests (Pitman 1971). However, Billings et al. (1976) found that both myrcene and terpinolene were better synergists than α-pinene in ponderosa pine forests. β-Phellandrene, the major monoterpene in lodgepole pine (Mirov 1961; Shrimpton 1973; von Rudloff 1975), has not been tested. Since the combination of trans-verbenol and α-pinene is not effective in lodgepole pine forests (Pitman et al. 1978), I hypothesized that either additional pheromones exist in D. ponderosae infesting lodgepole pine, or that different host volatiles combined with these additional pheromones and/or trans-verbenol would successfully attract beetles.

Juvenile Hormone (JH) regulates reproduction and metamorphosis in insects (Wigglesworth 1934, 1936). Borden et al. (1969) induced the production of aggregation pheromones in male I. paraconfusus by topical application of a synthetic JH (10,11-epoxyfarnesenic acid methyl ester), and hypothesized that pheromone production might be controlled by JH. Hughes and Renwick (1977a,b) proposed 2 different control mechanisms for pheromone biosynthesis, one for I. paraconfusus and one for D. brevicomis. In I. paraconfusus the proposed sequence is: ingestion of host material

stretching the gut which removes neural inhibition of JH released by the corpora allata (CA), stimulation by JH of brain hormone (BH) release from the corpora cardiaca (CC), and promotion by BH of the conversion of the monoterpene, myrcene, to ipsdienol which is then reduced to ipsenol. For D. brevicomis Hughes and Renwick (1977b) propose that a host compound acts as a stimulant for JH release, i.e. gut distension has no effect, and BH apparently has no role in pheromone production.

However, exposure to volatile monoterpenes induces pheromone production by several species of Dendroctonus (Hughes 1973a, 1973b) and Ips (Hughes 1974) without a further host stimulus or gut stretching by ingestion of food. Therefore, there are at least 2 means by which pheromone production can be stimulated in bark beetles.

Precocene, a chromene isolated from the bedding plant, Ageratum houstoninum L., was found to have antiallatal properties including the induction of precocious metamorphosis and inhibition of egg production by Oncopeltus fasciatus (Dall) (Bowers et al. 1976). Precocene causes degeneration of the CA in Oncopeltus fasciatus (Unnithan et al. 1977) or inhibits its development (Bowers and Martinez-Pardo 1977), thereby preventing JH secretion. A topical application of JH reverses the effect of precocene in O. fasciatus (Bowers et al. 1976). The effects of precocene have not been extensively researched in the Coleoptera, although Sahota and McMullen (1979) showed that topical application of precocene 2 to female Pissodes strobi Peck reduced the number of oviposition punctures and progeny per female. No experiments with precocene have been done on scolytid beetles. I hypothesized that precocene would inhibit yolk deposition and pheromone production by female D. ponderosae by preventing JH secretion and

that this effect would be reversible by a topical application of JH.

My objectives were:

1) to identify new volatiles produced by female D. ponderosae and to test their attractiveness in laboratory bioassays and field tests in lodgepole pine forests,

2) to utilize gas liquid chromatography, and JH treatments to elucidate mechanisms of pheromone production in D. ponderosae, and

3) to examine the effect of precocene on pheromone production and reproductive capacity of female D. ponderosae.

MATERIALS AND METHODS

Collection and Maintenance of Beetles

Adult beetles were collected as they emerged from lodgepole or ponderosa pine logs in cages at room temperature. All logs came from attacked trees in an infestation on Osprey Lake Road northeast of Princeton, British Columbia.

Beetles were kept at 2-4 C in 450 ml screw-top jars with moistened cotton dental wicks and paper tissue to prevent dehydration and to minimize contact between individuals.

In all cases only those beetles which passed a simple walking test were used for experimental purposes. This test consisted of warming beetles to room temperature and placing them on a paper towel. Beetles that walked normally and had no missing appendages were used.

Bioassay Procedures

Bioassays were run in a well-ventilated darkened room in an open arena olfactometer similar to that described by Borden et al. (1968). Beetles were tested in groups of 10 and released from a point 3 cm to the left and 4 cm downwind of the outlet of an airstream with a flow rate of 1400 ml/min. A rectangle 8 x 10 cm in area, traced onto an 18.5 cm diameter disposable filter paper served as the response arena. A microscope light was placed to the right of the arena to draw the photopositive beetles into the airstream. The filter paper was changed each time a new stimulus was tested.

Each stimulus consisted of 20 µl of pentane extract of test material dispensed on a rolled up filter paper inside a glass tube [1 cm internal diameter (ID x 7 cm long]. This tube was then placed to allow the air

strean to flow through it into the centre of the response arena. The stimulus was changed for each new group of beetles and at least 40 beetles were bioassayed for each stimulus.

After release, beetles were given 2 min. to respond. To be classed as a positive responder a beetle had to walk into the airstream, turn upwind toward the test material, and come to within 1 cm of the stimulus source. Non-responders walked off the arena, usually toward the light source, or remained in the arena longer than 2 min. without responding.

Between bioassays the groups of beetles were kept in a refrigerator at 2-4 C, but were brought out about 2 min. prior to use so they would be active at room temperature. At least 45 min. passed before any group of beetles was reused. This procedure resulted in no difference in response between beetles tested repeatedly in this or in studies with other scolytids (Borden et al. 1968; Stock 1981).

When monoterpene host odours were used, approximately 1 ml of the test compound was put in a 6 ml vial with a 4 mm hole in the lid. The vial was placed upright in an Erlenmeyer flask through which the air stream passed. The monoterpene was released from the vial at approximately 5 µg/min. When the monoterpene was changed or no further host odour was desired, all the glassware beyond and including the flask was washed, rinsed with distilled water, and then acetone before the next stimulus was tested.

A standard reference stimulus used in bioassays was a pentane extract of frass (excrement plus boring dust) produced by female beetles boring in ponderosa or lodgepole pine. This extract was tested at a concentration of 0.02 g equiv., which is equivalent to the amount of frass produced by one female in 83.5 min. At this concentration, the standard stimulus

induced consistent responses by beetles of both sexes. This standard was used in all bioassays to verify that the test beetles were responsive prior to testing with new stimuli. If the response was 50% or greater for males or 37.5% or greater for females, tests were conducted. Response was poor on days with low barometric pressure. Tests were not run on these days.

Dissection and Extraction Technique

To induce pheromone production, female beetles were put into preformed entrance holes in fresh pine logs and allowed to bore in the inner bark for 24 h. They were then chipped out of the bark and held over ice until a sufficient number were accumulated for dissection. Abdomens were removed and immediately immersed in twice distilled pentane in a 2 ml glass vial in dry ice. Extracted abdomens for pheromone isolation were at a concentration of 1 abdomen/10 µl of pentane. For analysis by gas liquid chromatography (GLC), extracts were made of individual abdomens in 100 µl of pentane. Each vial had a teflon liner in the lid and was stored at -44 C.

Gas Liquid Chromatographic Analyses

Internal standards for GLC analysis were 2- and 3-octanol. Each standard was made up separately at a concentration of 1.22 µl (1 mg) in 5 ml of pentane.

Single beetle abdomens in pentane which had been kept at -44 C were warmed to room temperature and 2.5 µl of the 3-octanol standard solution was added to each. The samples were frozen over dry ice and the beetle abdomens were crushed with a small metal spatula. After the samples were rewarmed to room temperature, 2.5 µl of the 2-octanol standard solution was added to each.

The amounts of the 2 octanols were compared to check for possible loss of other volatiles during processing of the sample. Samples were then transferred to clean 2 ml vials along with 2 pentane rinses of approximately 25 µl each. Samples not analysed during the day they were prepared were kept at -44 C until use. Five µl of each sample was injected into the GLC; all samples from one treatment were run on the same day.

Analyses by GLC were conducted on a Hewlett-Packard 5830 gas chromotograph equipped with a 18835B capillary inlet system and a flame ionization detector. The injection port and detector temperatures were 260 C and 275 C, respectively.

Single beetle extracts were analysed in the direct injection mode on a glass capillary column (30 m x 0.66 m ID) coated with SP-1000 (Supelco, Inc., Bellafonte, PA).

Each day a standard sample made up of authentic acetophenone and the monoterpene oxidation products, trans-verbenol, 3-caren-10-ol, verbenone, geraniol, and perilla alcohol (for comparison with female volatiles), or exo-brevicomin (for male beetle comparison), was put through the GLC under identical conditions as the beetle extract samples. In addition, the standards were added to one sample and that mixture was also analysed to ensure that compounds identified in samples were identical to authentic ones.

In calculating the actual amount of volatiles per insect, the area under the 3-octanol peak was used as a reference for all samples.

Pheromone Isolation and Identification

To isolate the attractive pheromones from female D. ponderosae, several hundred beetles were placed on ponderosa pine logs for 24 h,

chipped out of the bark, and their abdomens extracted in pentane. The steam-distilled volatiles were analysed by combined GC/MS, and individual compounds were identified by comparison of their mass spectra to those of authentic specimens. This collaborative research with Drs. A. C. Oehlschlager,[1] H. D. Pierce, Jr.,[1] and J. H. Borden[2] resulted in the identification of the following volatiles in the female abdominal extracts (Fig. 4): acetophenone, estragole (tentative), trans-verbenol, verbenone, 1-methyl-5-(α-hydroxyisopropyl)-cyclohexa-1,3-diene, 1-methyl-4-(α-hydroxyisopropyl)-cyclohexa-1,5-diene, 3-caren-10-al, geranyl acetate, myrtenol, α-methylbenzyl alcohol, geraniol, p-mentha-2-en-7-ol, myrcenol, 3-caren-5-one, 3-caren-10-ol, p-mentha-1,3-dien-7-ol, perilla alcohol, p-mentha-1,4(8)-dien-9-ol, p-mentha-1,4(8)-dien-10-ol, p-mentha-1,4-dien-7-ol, p-mentha-1,4(8)-dien-7-ol (tentative) and cuminyl alcohol.

Three compounds found most attractive in laboratory bioassays were considered as candidate aggregation pheromones. They were 3-caren-10-ol, acetophenone, and myrcenol. These 3, as well as trans-verbenol, a known pheromone of D. ponderosae (Pitman et al. 1968), were bioassayed in several combinations with D. ponderosae of both sexes. In all bioassays and field tests (+)-trans-verbenol and (+)-3-caren-10-ol were used. The monoterpenes, α-pinene and 3-carene, were tested for synergism in several combinations with the beetle-produced compounds.

To compare activity levels, candidate pheromone mixtures were bioassayed with male D. ponderosae at graded concentrations. The most attractive mixture, trans-verbenol, 3-caren-10-ol, acetophenone and myrcenol, was then tested with the major monoterpenes from lodgepole pine (Shrimpton 1973) at the

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following levels of purity: α-pinene, 99%; β-pinene, 100%; myrcene, 90%; 3-carene, 95%; β-phellandrene, 94%; and terpinolene, 91%.

Field Experiments with Attractive Volatiles

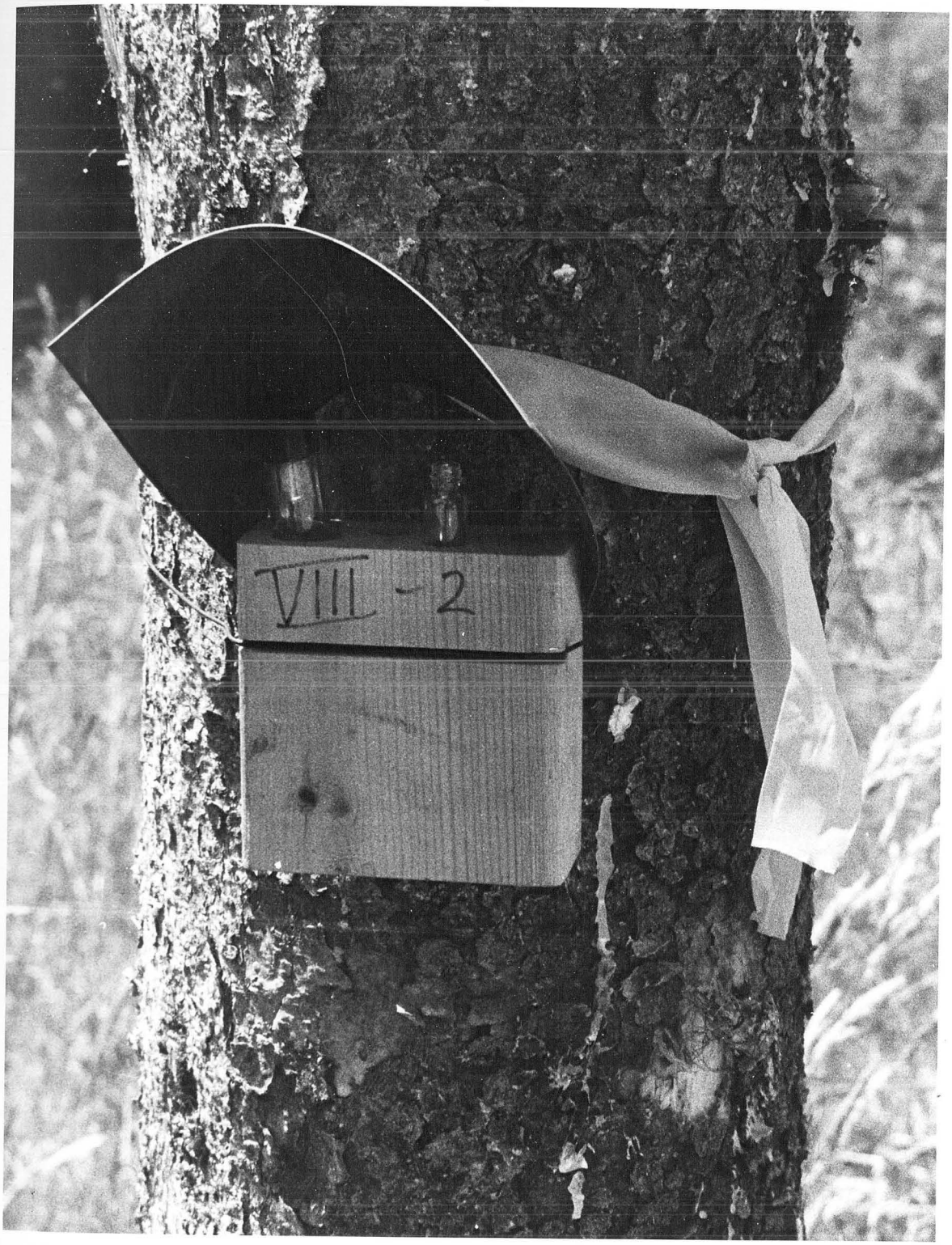
Chemicals used in field experiments were released in 2 ways. Trans-verbenol was released from 1.7 mm ID glass pipettes which were sealed and kept at -44 C until use. Acetophenone was released from 1.0 mm ID pipettes. Approximate release rates in the laboratory at 20 C were 200 and 100 µg/day for trans-verbenol and acetophenone, respectively. As both myrcenol and 3-caren-10-ol polymerized in glass pipettes, open 2 ml vials with 4 mm apertures were used for their release. These gave release rates in the laboratory at 20 C of 200 and 100 µg/day for 3-caren-10-ol and myrcenol, respectively. These release rates reflected the relative amounts and ratios of the compounds in GLC traces of abdominal extracts of female beetles. Monoterpenes were released from 6 ml vials with 3 mm diameter holes in the lids, at a rate in the laboratory of approximately 7 mg/day.

The pipettes and vials were put into recessed holes in small wooden blocks (Fig. 1), which were suspended inside traps or fitted with a plastic rain shield and wired to trees at breast height.

Two study areas were used, one for trapping experiments and one for trap-tree experiments. Both were located near Osprey Lake Road, northeast of Princeton, British Columbia. The trap-tree site extended from approximately 34 to 38 km from Princeton in an area of primarily unattacked lodgepole pine with only 2-3 small groups of brood-containing trees in 1980. In this area, the experiment was not in danger of being obliterated by a natural mass infestation.

Trees chosen for trap-trees were mature lodgepole pines of medium

Fig. 1. Host monoterpene and beetle volatile release system used in trap-tree experiment for D. ponderosae. Wooden block with recessed holes contains a 6 ml vial for monoterpene release, a 2 ml vial for 3-caren-10-ol release, and a 1.7 mm ID glass pipette for trans-verbenol release.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diameter at breast height (18-38 cm) about 50 m apart with no obvious deformaties such as "witches' brooms" caused by dwarf mistletoe infections. Normally, trees in this area suffer from moisture stress as they inhabit the dry interior zone (Lyons 1952). The summer of 1980, however, was exceptionally cold and rainy so it is unlikely that they were as susceptible to beetle attack as during previous summers. Trees were chosen as much as possible with the next nearest lodgepole pine at a distance of 3 m or more so that beetles drawn into the area would concentrate on the baited trees, and not be diverted by a prominent, nearby silhouette.

The trap-tree experiment was laid out June 10-13, 1980, with 8 replicates in a randomized block design with the following bait treatments: trans-verbenol + 3-caren-10-ol; 3-carene + trans-verbenol; α-pinene + trans-verbenol; 3-carene + 3-caren-10-ol; trans-verbenol; 3-caren-10-ol; 3-carene + trans-verbenol + 3-caren-10-ol; unbaited control. On October 10-12, 1980, all the trees in the experiment were inspected and the numbers of pitch tubes were counted in the lower 2 m of the bole of attacked trees.

The trapping site was located approximately 45 km from Princeton, in a stand that had sustained an epidemic but relatively static D. ponderosae population for several years (Andrews and Unger 1979). Numerous lodgepole and some ponderosa pines in the area contained vigorous brood from the 1979 attack.

Two infested lodgepole pines were cut down in the area in June. Bolts from these trees were put into field cages so that emergent beetles would serve as an indication of when flight would begin. Norwegian drainpipe traps (Borregaard A. S., Sarpsborg, Norway; 1979 model; 13 cm ID x 135 cm long) were set at a minimum of 25 m apart in 7 trap lines. This trap lay out was used for 2 experiments, the first from June 18 to July 20, 1980,

and the second from July 21 to August 5, 1980.

The first experiment was set up in a Latin square design and included the following baits chosen for their attractiveness in laboratory bioassays: pentane extract of frass from females boring in ponderosa pine (0.02 g equiv.); α-pinene + trans-verbenol; 3-carene + trans-verbenol + 3-caren-10-ol; 3-carene + trans-verbenol + 3-caren-10-ol + myrcenol; 3-carene + trans-verbenol + 3-caren-10-ol + acetophenone; 3-carene + trans-verbenol + 3-caren-10-ol + myrcenol + acetophenone; and unbaited control.

In the second experiment, 3 compounds, 3-carene, trans-verbenol, and 3-caren-10-ol, were tested alone and in all possible combinations along with an unbaited control in a 6 replicate randomized block design.

Captured beetles were removed from the collecting jars in the traps and held in 95% ethanol until they were counted and their sex determined.

Pheromone Biogenesis

To compare the attractiveness of mated and unmated female D. ponderosae, females were allowed to bore into ponderosa pine logs for 24 h. At this time, they were either excised from the bark and their abdomens extracted, or they were paired (mated) with males. After a further 24 h, the paired males and females were chipped out of the bark and their abdomens were extracted in separate 2 ml vials. Abdominal extracts of D. ponderosae males were dissected within 24 h of their emergence from lodgepole or ponderosa pine logs. Extracts were bioassayed with beetles of both sexes, and were also analysed by GLC.

Juvenile Hormone Experiments

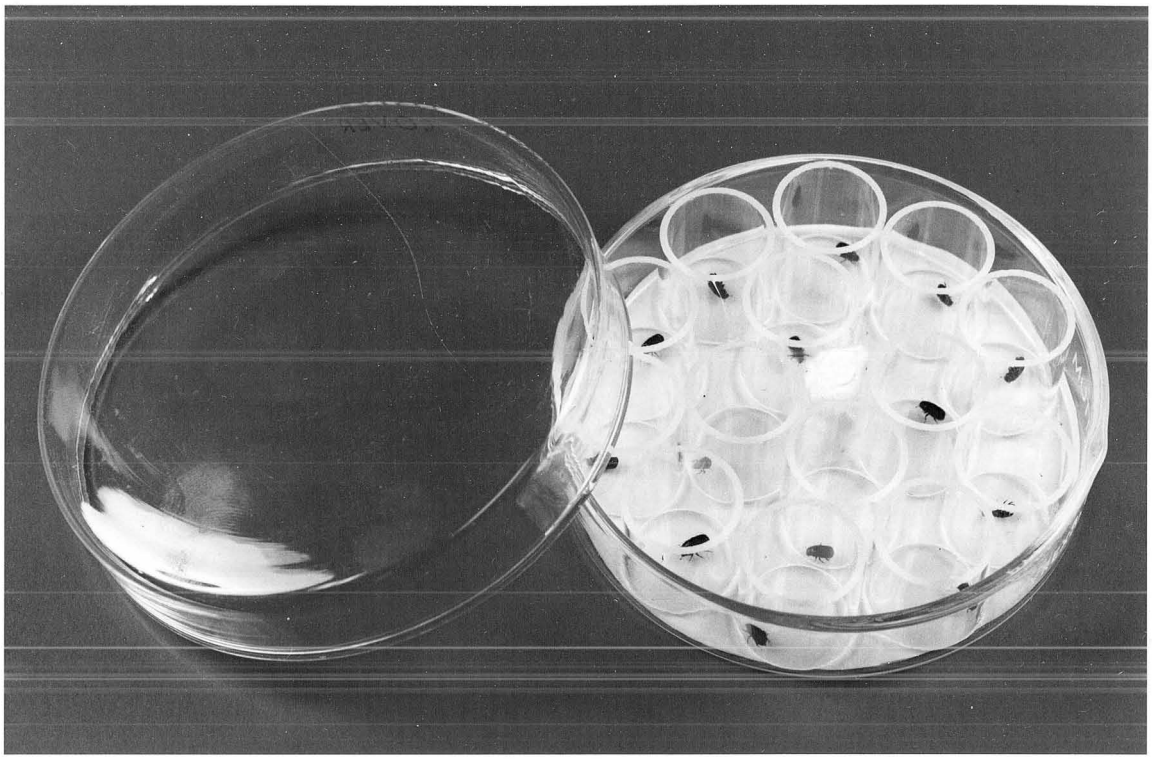
JH III (Calbiochem-Behring Corp. La Jolla, Ca., 10 mg in hexane) was

diluted in methanol or acetone and stored at -44 C in 2 ml vials with teflon-lined lids. Using a 25 µl Hamilton syringe (no. 702) attached to a Hamilton repeating dispenser (no. PB-600-1), 1 µl of solvent or JH III in solvent was applied topically to the abdominal venter of beetles and allowed to air dry. Treated and control beetles were either held for 24 h in individual glass cylinders (Fig. 2) in the dark at 20 C or allowed to bore into a log for 24 h. Abdominal extracts for GLC analysis were then made from these beetles. A preliminary GLC analysis for trans-verbenol production utilized pooled abdominal extracts of 20 fed or unfed female D. ponderosae treated with acetone or 25 µg JH III. Abdominal extracts of fed or unfed, single, female D. ponderosae treated with acetone or 100 µg of JH III were analysed for production of the known pheromone, trans-verbenol, and 3 other candidate pheromones, 3-caren-10-ol, acetophenone, and myrcenol.

Precocene Experiments

Earlier experiments with Ips paraconfusus Lanier showed high mortality after beetles were exposed to precocene 2 for 24 h followed by a 1 µl treatment of acetone (J. E. Conn, unpublished results). Therefore, 28 female D. ponderosae were exposed for 24 h in glass cylinders (Fig. 2) to a glass surface treated with one of the following solvents to be tested as a precocene carrier: acetone, ethanol, dimethylsulphoxide (DMSO), or methanol. Survivors were treated topically with 1 µl of the same solvent and then placed in gelatin capsules on ponderosa pine logs for 24 h. Beetles boring successfully were used as indicators of low toxicity. DMSO caused high mortality and was rejected as a potential solvent. Ethanol was no less toxic than acetone but was rejected because it is a synergist with pheromones of ambrosia beetles (Vité and Bakke 1979; Borden et al. 1980b) and

Fig. 2. Apparatus for exposure of beetles to precocene. One beetle placed in each of 14 glass cylinders (1.4 cm ID) for 24 h exposure to glass surface of 9 cm petri dish coated with precocene. Moistened dental cotton in centre prevents desiccation.



D. pseudotsugae (Pitman et al. 1975). As methanol was numerically (although not significantly) superior to acetone (23/28 as compared to 18/28 successfully boring beetles), it was used as the solvent for subsequent experiments.

Walking beetles were exposed to precocene 2 (6,7-dimethoxy-2,2-dimethylchromene) 99%, Aldrich Chemical Company, Milwaukee, Wisconsin, on the glass surface of a petri dish. Precocene was dissolved in methanol to make a stock solution of 50 mg/ml. The amount of this solution to be put on a 9 cm diameter petri dish was determined by area. For example, for coverage of 15 $\mu g/cm^2$, 21.25 µl of precocene solution was used. To ensure complete coverage, 3 ml of methanol was first pipetted into the petri dish followed by the desired amount of precocene solution and two, 100 µl methanol rinses of the syringe. The dish was slowly rotated so the bottom of the dish was evenly covered by the precocene. Control dishes received methanol only.

After the methanol evaporated, beetles were placed individually in the dish in hollow glass cylinders 1.4 cm ID, 14/dish (Fig. 2). Separation of the beetles in this manner prevented mutual injury, and ensured that each beetle would be exposed to the same amount of precocene. A piece of moistened dental cotton was placed in the centre of each dish to prevent desiccation of the beetles. Dishes were then kept in the dark at 20 C for 24 h. To test for pheromone production, treated and control beetles were then confined with gelatin capsules in preformed holes in ponderosa pine logs; 24 or 48 h later they were removed from the logs and their abdomens were excised and extracted in pentane.

Female D. ponderosae were treated with increasing concentration of precocene 2 to determine if any dose was effective in inhibiting or reducing

pheromone production. Abdominal extracts made from females after 24 h exposure to precocene 2 and 24 h on ponderosa pine logs were analysed by GLC to determine the amounts of trans-verbenol and 3-caren-10-ol produced. Solvent control beetles were exposed to methanol-treated petri dishes for 24 h followed by 24 h on logs.

Exogenous JH III was applied topically to assess whether it could reverse the effect of precocene 2 application. Female D. ponderosae were exposed to precocene 2 at 120 µg/cm^2 for 24 h, treated topically with 100 µg JH III in 1 µl of methanol, and then were allowed to bore in a ponderosa pine log for 24 h. Some beetles did not bore into the logs and thus were exposed in the preformed entrance holes to host odours (principally monoterpenes) for 24 h. Control beetles were exposed to methanol alone in the petri dishes (precocene control) or treated topically with methanol (JH III control) prior to being placed on host logs. Abdominal extracts were made of all beetles and analysed by GLC for trans-verbenol and 3-caren-10-ol production.

To assess the effect of precocene 2 on the reproductive capacity of female D. ponderosae, females were exposed to 120 µg/cm^2 prococene 2 in petri dishes then confined in gelatin capsules in preformed holes in lodgepole pine logs for 12 days. Controls were exposed to methanol and then put on logs. Males, after exposure for 24 h to 120 µg/cm^2 precocene 2 or to methanol (as a control) in petri dishes, were paired (mated) with the females in logs. Twelve days after females were placed on logs, the bark was stripped off the logs; the lengths of the females' galleries were measured, and the number of larvae/female was counted.

RESULTS AND DISCUSSION

Laboratory Bioassay of Female-Produced Volatiles and Host Monoterpenes

D. ponderosae of both sexes responded to host and beetle-produced volatiles alone and in various combinations (Tables 1-4). For simplicity, the results in Tables 1-4 are segregated into responses to single compounds (Table 1), binary mixtures (Table 2), ternary mixtures (Table 3) and greater than ternary mixtures (Table 4). The responses to pentane and standard frass extracts are common to all 4 tables.

Of the single compounds bioassayed only 3-caren-10-ol elicited responses by beetles of both sexes as high as to the female frass standard (Table 1). The responses to *trans*-verbenol, a female-produced pheromone (Pitman et al. 1968), were no better than to the pentane controls. The responses by males to myrcenol and to 2 host monoterpenes, α-pinene and 3-carene, were significantly higher than to pentane but not as high as to 3-caren-10-ol.

The binary combination of 3-carene plus *trans*-verbenol was the only stimulus which induced a significantly higher response by males than the female frass standard (Table 2). The combination of α-pinene plus *trans*-verbenol, the constituents of Pondelure (Pitman 1971, Billings et al. 1976) evoked a significantly lower response by males than 3-carene plus *trans*-verbenol. Responses by males to stimuli containing 3-caren-10-ol were generally high, while those to stimuli containing myrcenol and acetophenone were lower, but still suggestive that these compounds have pheromonal activity (Table 2).

Response by females to binary stimulus combinations was lower than that by males. Four binary stimuli elicited a response by females which was no different from that to the female frass standard: 3-caren-10-ol +

Table 1. Ranked responses of *D. ponderosae* to monoterpenes and female-produced volatiles tested singly in a laboratory olfactometer.

Sex	Stimulus	Concentration	No. Beetles Tested	Percent Response[a]
Males	pentane	20 µl	239	13.5 a
	acetophenone	200 ng	40	13.8 a
	trans-verbenol	500 ng	40	20.0 ab
	α-pinene	5 µg/min.	120	28.3 bc
	myrcenol	200 ng	40	30.0 bc
	3-carene	5 µg/min.	80	31.3 bc
	3-caren-10-ol	500 ng	40	42.5 cd
	standard female frass extract	0.02 g equiv.	248	58.0 d
Females	pentane	20 µl	80	17.5 a
	3-carene	5 µg/min.	40	20.0 a
	acetophenone	200 ng	40	22.5 a
	trans-verbenol	500 ng	40	25.0 ab
	α-pinene	5 µg/min.	40	27.5 ab
	myrcenol	200 ng	40	27.5 ab
	3-caren-10-ol	500 ng	40	47.5 bc
	standard female frass extract	0.02 g equiv.	80	51.3 c

[a]Values for each sex followed by same letter are not significantly different, χ^2 test, $P<0.05$.

Table 2. Ranked responses of *D. ponderosae* to monoterpenes and female-produced volatiles tested in selected binary combinations in a laboratory olfactometer.

Sex	Stimulus	Concentration	No. Beetles Tested	Percent Response[a]
Males	pentane	20 µl	239	13.5 a
	trans-verbenol + myrcenol	500 + 200 ng	80	21.0 ab
	trans-verbenol + acetophenone	500 + 200 ng	40	27.5 ab
	α-pinene + acetophenone	5 µg/min. + 200 ng	50	32.0 bc
	3-caren-10-ol + acetophenone	500 + 200 ng	40	32.5 bc
	α-pinene + myrcenol	5 µg/min. + 200 ng	40	35.0 bcd
	3-carene + acetophenone	5 µg/min. + 200 ng	40	42.5 cde
	3-carene + α-pinene	5 µg/min. + 5 µg/min.	40	42.5 cde
	α-pinene + *trans*-verbenol	5 µg/min. + 500 ng	80	45.0 cde
	acetophenone + myrcenol	200 + 200 ng	40	45.0 cde
	3-caren-10-ol + myrcenol	500 + 200 ng	40	50.0 cde
	3-carene + myrcenol	5 µg/min. + 200 ng	40	50.0 cde
	3-carene + 3-caren-10-ol	5 µg/min. + 500 ng	39	53.8 cde
	trans-verbenol + 3-caren-10-ol	500 + 500 ng	40	55.0 de
	standard female frass extract	0.02 g equiv.	248	58.0 e
	3-carene + *trans*-verbenol	5 µg/min. + 500 ng	40	85.0 f

Table 2. (cont'd)

Sex	Stimulus	Concentration	No. Beetles Tested	Percent Response[a]
Females	trans-verbenol + acetophenone	500 + 200 ng	40	10.0 a
	pentane	20 µl	80	17.5 ab
	α-pinene + myrcenol	5 µg/min. + 200 ng	40	20.0 ab
	3-carene + trans-verbenol	5 µg/min. + 500 ng	40	22.5 abc
	3-caren-10-ol + myrcenol	500 + 200 ng	40	25.0 abc
	3-carene + acetophenone	5 µg/min. + 200 ng	40	27.5 abc
	trans-verbenol + myrcenol	500 + 200 ng	40	27.5 abc
	α-pinene + trans-verbenol	5 µg/min. + 500 ng	40	27.5 abc
	3-carene + myrcenol	5 µg/min. + 200 ng	40	30.0 bcd
	acetophenone + myrcenol	200 + 200 ng	40	30.0 bcd
	α-pinene + acetophenone	5 µg/min. + 200 ng	40	20.0 bcd
	3-caren-10-ol + acetophenone	500 + 200 ng	40	40.0 cde
	3-carene + α-pinene	5 µg/min. + 5 µg/min.	40	40.0 cde
	standard female frass extract	0.02 g equiv.	80	51.3 de
	3-caren-10-ol + trans-verbenol	500 + 500 ng	40	52.5 e
	3-carene + 3-caren-10-ol	5 µg/min. + 500 ng	40	52.5 e

[a] Values for each sex followed by same letter are not significantly different, χ^2 test, $P<0.05$.

Table 3. Ranked responses of D. ponderosae to monoterpenes and female-produced volatiles tested in selected ternary combinations in a laboratory olfactometer.

Sex	Stimulus	Concentration	No. Beetles Tested	Percent Response[a]	
Males	pentane	20 µl	239	13.5	a
	3-carene + α-pinene + myrcenol	5 µg/min. + 5 µg/min. + 200 ng	40	27.5	b
	3-carene + 3-caren-10-ol + myrcenol	5 µg/min. + 500 ng + 200 ng	40	30.0	b
	3-carene + α-pinene + trans-verbenol	5 µg/min. + 5 µg/min. + 500 ng	40	32.5	b
	3-carene + α-pinene + acetophenone	5 µg/min. + 5 µg/min. + 200 ng	40	32.5	b
	trans-verbenol + acetophenone + myrcenol	500 ng + 200 ng + 200 ng	40	35.0	bc
	3-caren-10-ol + acetophenone + myrcenol	500 ng + 200 ng + 200 ng	40	40.0	bc
	3-carene + acetophenone + myrcenol	5 µg/min. + 200 ng + 200 ng	40	45.0	bcd
	3-carene + 3-caren-10-ol + acetophenone	5 µg/min. + 500 ng + 200 ng	40	45.0	bcd
	α-pinene + trans-verbenol + myrcenol	5 µg/min. + 500 ng + 200 ng	40	50.0	bcd
	α-pinene + trans-verbenol + acetophenone	5 µg/min. + 500 ng + 200 ng	40	57.5	cde
	standard female frass extract	0.02 g equiv.	248	58.0	cde
	trans-verbenol + 3-caren-10-ol + myrcenol	500 ng + 500 ng + 200 ng	20	60.0	cdef
	trans-verbenol + 3-caren-10-ol + acetophenone	500 ng + 500 ng + 200 ng	40	67.5	def
	3-carene + trans-verbenol + acetophenone	5 µg/min. + 500 ng + 200 ng	40	80.0	ef
	3-carene + trans-verbenol + myrcenol	5 µg/min. + 500 ng + 200 ng	40	82.5	f
	3-carene + trans-verbenol + 3-caren-10-ol	5 µg/min. + 500 ng + 500 ng	40	85.0	f

Table 3 (cont'd)

Sex	Stimulus	Concentration	No. Beetles Tested	Percent Response[a]
Females	3-carene + α-pinene + acetophenone	5 µg/min. + 5 µg/min. + 200 ng	40	15.0 a
	3-carene + trans-verbenol + acetophenone	5 µg/min. + 500 ng + 200 ng	40	15.0 a
	pentane	20 µl	80	17.5 a
	3-caren-10-ol + acetophenone + myrcenol	500 ng + 200 ng + 200 ng	40	20.0 ab
	3-carene + 3-caren-10-ol + myrcenol	5 µg/min. + 500 ng + 200 ng	40	20.0 ab
	α-pinene + trans-verbenol + acetophenone	5 µg/min. + 500 ng + 200 ng	40	22.5 ab
	3-carene + trans-verbenol + myrcenol	5 µg/min. + 500 ng + 200 ng	40	22.5 ab
	3-carene + acetophenone + myrcenol	5 µg/min. + 200 ng + 200 ng	40	25.0 abc
	α-pinene + trans-verbenol + myrcenol	5 µg/min. + 500 ng + 200 ng	40	30.0 abc
	3-carene + α-pinene + trans-verbenol	5 µg/min. + 5 µg/min. + 500 ng	40	32.5 abc
	3-carene + α-pinene + myrcenol	5 µg/min. + 5 µg/min. + 200 ng	40	35.0 abc
	3-carene + 3-caren-10-ol + acetophenone	5 µg/min. + 500 ng + 200 ng	40	35.0 abc
	trans-verbenol + 3-caren-10-ol acetophenone	500 ng + 500 ng + 200 ng	40	37.5 bcd
	trans-verbenol + acetophenone + myrcenol	500 ng + 200 ng + 200 ng	40	42.5 bcd
	trans-verbenol + 3-caren-10-ol + myrcenol	500 ng + 500 ng + 200 ng	20	50.0 cd
	standard female frass extract	0.02 g equiv.	80	51.3 d
	3-carene + trans-verbenol + 3-caren-10-ol	5 µg/min. + 500 ng + 500 ng	40	82.5 e

[a]Values for each sex followed by same letter are not significantly different, χ^2 test, $P<0.05$.

Table 4. Ranked responses of D. ponderosae to monoterpenes and female-produced volatiles in selected four and five way combinations in a laboratory olfactometer.

Sex	Stimulus	Concentration	No. Beetles Tested	Percent Response[a]
Males	pentane	20 µl	239	13.5 a
	3-carene + 3-caren-10-ol acetophenone + myrcenol	5 µg/min. + 500 ng + 200 ng + 200 ng	40	32.5 b
	trans-verbenol + 3-caren-10-ol + acetophenone + myrcenol	500 ng + 500 ng + 200 ng + 200 ng	80	36.2 b
	3-carene + α-pinene + acetophenone + myrcenol	5 µg/min. + 5 µg/min. + 200 ng + 200 ng	40	37.5 b
	α-pinene + trans-verbenol acetophenone + myrcenol	5 µg/min. + 500 ng + 200 ng + 200 ng	40	52.5 bc
	standard female frass extract	0.02 g equiv.	248	58.0 c
	3-carene + α-pinene + trans-verbenol + acetophenone	5 µg/min. + 5 µg/min. + 500 ng + 200 ng	80	73.8 cd
	3-carene + α-pinene + trans-verbenol + myrcenol	5 µg/min. + 5 µg/min. + 500 ng + 200 ng	80	78.8 d
	3-carene + trans-verbenol + acetophenone + myrcenol	5 µg/min. + 500 ng + 200 ng + 200 ng	40	82.5 d
	3-carene + trans-verbenol + 3-caren-10-ol + myrcenol	5 µg/min. + 500 ng + 500 ng + 200 ng	80	87.5 d
	3-carene + trans-verbenol + 3-caren-10-ol + acetophenone	5 µg/min. + 500 ng + 500 ng + 200 ng	40	87.5 d

Table 4. (cont'd)

Sex	Stimulus	Concentration	No. Beetles Tested	Percent Response[a]
Males (cont'd)	pentane	20 µl	239	13.5 a
	3-carene + α-pinene + trans-verbenol + acetophenone + myrcenol	5 µg/min. + 5 µg/min. + 500 ng + 200 ng + 200 ng	80	57.5 b
	standard female frass extract	0.02 g equiv.	248	58.0 b
	3-carene + trans-verbenol + 3-caren-10-ol + acetophenone myrcenol	5 µg/min. + 500 ng + 500 ng + 300 ng + 200 ng	40	62.5 b
Females	pentane	20 µl	80	17.5 a
	α-pinene + trans-verbenol acetophenone + myrcenol	5 µg/min. + 500 ng + 200 ng + 200 ng	40	30.0 ab
	3-carene + α-pinene + trans-verbenol + acetophenone	5 µg/min. + 5 µg/min. + 500 ng + 200 ng	40	30.0 ab
	3-carene + trans-verbenol acetophenone + myrcenol	5 µg/min. + 500 ng + 200 ng + 200 ng	40	32.5 ab
	trans-verbenol + 3-caren-10-ol + acetophenone + myrcenol	500 ng + 500 ng + 200 ng + 200 ng	40	32.5 ab
	3-carene + α-pinene + acetophenone + myrcenol	5 µg/min. + 5 µg/min. + 200 ng + 200 ng	40	37.5 bc
	3-carene + α-pinene + trans-verbenol + myrcenol	5 µg/min. + 5 µg/min. + 500 ng + 200 ng	80	40.0 bc

Table 4. (cont'd)

Sex	Stimulus	Concentration	No. Beetles Tested	Percent Response[a]
Females (cont'd)	3-carene + trans-verbenol + 3-caren-10-ol + acetophenone	5 µg/min. + 500 ng + 500 ng + 200 ng	40	42.5 bc
	3-carene + 3-caren-10-ol + acetophenone + myrcenol	5 µg/min. + 500 ng + 200 ng + 200 ng	40	45.0 bc
	standard female frass extract	0.02 g equiv.	80	51.3 cd
	3-carene + trans-verbenol + 3-caren-10-ol + myrcenol	5 µg/min. + 500 ng + 500 ng + 200 ng	40	66.3 d
	pentane	20 µl	80	17.5 a
	3-carene + trans-verbenol + 3-caren-10-ol + acetophenone + myrcenol	5 µg/min. + 500 ng + 500 ng + 200 ng + 200 ng	40	30.0 a
	3-carene + α-pinene + trans-verbenol + acetophenone + myrcenol	5 µg/min. + 5 µg/min. + 500 ng + 200 ng + 200 ng	40	32.5 a
	standard female frass extract	0.02 g equiv.	80	51.3 b

[a] Values for each sex followed by same letter are not significantly different, χ^2 test, $P<0.05$.

acetophenone; 3-carene + α-pinene; trans-verbenol + 3-caren-10-ol; and 3-carene + 3-caren-10-ol (Table 2). As for males, the data suggest that 3-caren-10-ol, acetophenone, and myrcenol are attractive pheromones for females.

There was no obvious synergism between a beetle volatile and a host terpene for female responders but there was for males. Responses by males to trans-verbenol alone and 3-carene alone were 20% and 31.5%, respectively (Table 1), but the response to the combination was 85% (Table 2).

Four ternary stimuli evoked a response by male D. ponderosae that was as good as to the female frass standard, and 2 stimuli, 3-carene + trans-verbenol + myrcenol and 3-carene + trans-verbenol + 3-caren-10-ol evoked a significantly higher response (Table 3). The most attractive binary stimulus, 3-carene + trans-verbenol (Table 2), was found in the 2 most attractive ternary stimuli. Three ternary stimuli evoked as high a response by female D. ponderosae as the female frass standard (Table 3). The superior response by females to 3-carene + trans-verbenol + 3-caren-10-ol compared to the insignificant response to the binary stimulus of 3-carene + trans-verbenol alone (Table 2) indicates that 3-caren-10-ol may be an essential part of secondary attraction.

Response by males to 4 quarternary mixes was significantly higher than to the female frass standard (Table 4). All 4 contained the most attractive binary mix, 3-carene + trans-verbenol, and the 2 highest also contained 3-caren-10-ol. Response by females to one quarternary mix, 3-carene + trans-verbenol + 3-caren-10-ol + myrcenol was as high as to the standard (Table 4). The responses by both males and females to 5 component stimuli were inexplicably lower than to the quarternary stimuli (Table 4).

When graded concentrations of binary, ternary and quarternary stimuli were tested, male D. ponderosae responded to increasingly lower concentrations as components were added to the stimulus (Table 5). At the lowest concentration only the quarternary stimulus of trans-verbenol + 3-caren-10-ol + acetophenone + myrcenol evoked as high a response as did the female frass standard, suggesting that each of these volatiles contributes in some way to secondary attraction.

The addition of 3-carene or β-phellandrene to the quarternary mixture of female-produced volatiles induced responses significantly higher for males and females, respectively, than to the female-produced volatiles alone (Table 6). Alpha-pinene appeared to be a moderately good synergist for males, but was ineffective for females. As 3-carene was a good synergist for males, and apparently also good for females (Table 6) and had produced good results in other bioassays (Tables 1-5), it was chosen as a standard synergist for field experiments. There was insufficient time during the summer of 1980 to field test all six monoterpenes. However, myrcene and terpinolene were superior synergists to 3-carene in one field test (Billings et al., 1976) and β-phellandrene has never been field tested.

The results of laboratory bioassays yielded the following information which served as a basis for field experiments with D. ponderosae: confirmation that trans-verbenol acts as a pheromone; numerous data suggesting that acetophenone, myrcenol and particularly 3-caren-10-ol are aggregation pheromones; and corroboration that monoterpenes, especially 3-carene, act as synergists in secondary attraction; and data suggesting that 3-carene is a good candidate for operational field trials in an attractive bait mixture.

Table 5. Ranked responses of male *D. ponderosae* to graded concentrations of monoterpenes and beetle-produced volatiles in various combinations in a laboratory olfactometer.

Stimulus[a]	Respective Concentration (ng) Of Each Component In Test Stimuli	No. Beetles Tested	Percent Response[b]
trans-verbenol + 3-caren-10-ol	32 + 32	40	20.0 a
pentane (20 µl)	-	80	27.5 a
trans-verbenol + 3-caren-10-ol	125 + 125	40	32.5 a
trans-verbenol + 3-caren-10-ol	500 + 500	40	42.5 ab
standard female frass extract (0.02 g equiv.)	-	40	62.5 b
pentane (20 µl)	-	80	27.5 a
trans-verbenol + 3-caren-10-ol + myrcenol	32 + 32 + 12	40	37.5 ab
trans-verbenol + 3-caren-10-ol + myrcenol	125 + 125 + 50	40	47.5 bc
trans-verbenol + 3-caren-10-ol + myrcenol	500 + 500 + 200	40	55.0 bc
standard female frass extract (0.02 g equiv.)	-	40	62.5 c
pentane (20 µl)	-	80	27.5 a
trans-verbenol + 3-caren-10-ol + acetophenone	32 + 32 + 32	40	35.0 a
trans-verbenol + 3-caren-10-ol + acetophenone	125 + 125 + 50	40	42.5 ab
trans-verbenol + 3-caren-10-ol + acetophenone	500 + 500 + 200	40	62.5 b
standard female frass extract (0.02 g equiv.)	-	40	62.5 b
pentane (20 µl)	-	80	27.5 a
trans-verbenol + 3-caren-10-ol + acetophenone + myrcenol	32 + 32 + 12 + 12	40	52.5 b
trans-verbenol + 3-caren-10-ol + acetophenone + myrcenol	125 + 125 + 50 + 50	40	60.0 b
standard female frass extract (0.02 g equiv.)	-	40	62.5 b
trans-verbenol + 3-caren-10-ol + acetophenone + myrcenol	500 + 500 + 200 + 200	40	67.5 b

[a] Responses to pentane and standard female frass extract common to each stimulus combination

[b] Values for each data group followed by same letter are not significantly different, χ^2 test, $P<0.05$.

Table 6. Ranked responses by D. ponderosae to a quaternary mixture of female-produced volatiles tested at a low concentration in a laboratory olfactometer alone and with lodgepole pine monoterpenes.

Sex	Stimulus[a]	No. Beetles Tested	Percent Response[b]
Males	pentane (20 µl)	80	23.7 a
	female volatile mix	40	52.5 b
	standard female frass extract (0.02 g equiv.)	80	53.8 bc
	female volatile mix + β-pinene	40	57.5 bc
	female volatile mix + myrcene	40	60.0 bc
	female volatile mix + α-pinene	40	62.5 bc
	female volatile mix + β-phellandrene	40	67.5 bc
	female volatile mix + terpinolene	40	72.5 bc
	female volatile mix + 3-carene	40	80.0 c
Females	pentane (20 µl)	40	15.0 a
	female volatile mix + α-pinene	40	20.0 ab
	female volatile mix	40	32.5 ab
	standard female frass extract (0.02 g equiv.)	40	37.5 b
	female volatile mix + β-pinene	40	40.0 b
	female volatile mix + terpinolene	40	43.5 b
	female volatile mix + myrcene	40	47.5 bc
	female volatile mix + 3-carene	40	55.0 bc
	female volatile mix + β-phellandrene	40	67.5 c

[a]Components of female volatile mix and concentration/stimulus as follows: trans-verbenol, 32 ng; 3-caren-10-ol, 32 ng; acetophenone, 12 ng; myrcenol, 12 ng. Monoterpenes introduced into airstream at 5 µg/min.

[b]Values for each set followed by same letter are not significantly different, χ^2 test, $P < 0.05$.

Field Experiments with Attractive Volatiles

The drainpipe traps used in field trapping experiments caught low numbers of D. ponderosae (Table 7). They had been chosen for convenience to avoid problems commonly encountered with traps covered with sticky substances, because they had apparently been effective traps in preliminary field studies (Moeck 1980), and because they offered a prominent vertical silhouette. A dark vertical silhouette was found to be attractive to D. ponderosae in laboratory studies (Shepherd 1966), and vertical cylindrical traps were more effective than horizontal ones in the field (Billings et al. 1976), although another study yielded the opposite result (Pitman and Vite 1969). Moreover, D. ponderosae is an aggressive beetle which primarily attacks standing trees. Possible reasons for the low catches are that the beetles do not readily enter the holes in the trap and that the bait placement at the top of the trap was suboptimal. Traps with baits at the middle or bottom are more effective at catching ambrosia beetles, apparently because convection currents inside the trap carry the volatiles upward (J. H. Borden[2] and L. Friskie[2] pers. comm.).

In the first trapping experiment, both males and females responded best to the ternary mixture of 3-carene + trans-verbenol + 3-caren-10-ol (Table 7). The constituents of Pondelure, α-pinene + trans-verbenol, were ineffective as attractants. However, Pondelure offers a mixture of nine parts trans-verbenol to one part α-pinene, whereas the inverse ratio was used in my experiments. The addition of acetophenone to the attractive ternary stimulus made no significant difference in beetles caught, but the addition of myrcenol caused a significant decrease in the total catch. When both acetophenone and myrcenol were present, the numbers of beetles caught neither

Table 7. Ranked responses of *D. ponderosae* in 2 experiments to drainpipe traps baited with host monoterpenes and beetle-produced volatiles.

Experiment No. and no. replicates	Stimulus[a]	Numbers Captured[b] Males	Females	Total	Percent Female
1	blank control	0 a	0 a	0 a	-
7 reps	pentane extract of female frass (0.02 g equiv.)	0 a	0 a	0 a	-
	α-pinene + *trans*-verbenol	0 a	1 a	1 a	-
	3-carene + *trans*-verbenol + 3-caren-10-ol + myrcenol	10 ab	9 ab	19 a	47.4
	3-carene + *trans*-verbenol + 3-caren-10-ol + acetophenone + myrcenol	24 ab	15 ab	39 ab	38.5
	3-carene + *trans*-verbenol + 3-caren-10-ol + acetophenone	25 ab	19 ab	44 ab	43.2
	3-carene + *trans*-verbenol + 3-caren-10-ol	32 b	35 b	67 b	52.2
2	*trans*-verbenol	0 a	0 a	0 a	-
6 reps	blank control	0 a	1 a	1 a	-
	3-carene	0 a	1 a	1 a	-
	3-caren-10-ol	1 a	1 a	2 a	-
	3-carene + 3-caren-10-ol	1 a	4 a	5 a	-
	trans-verbenol + 3-caren-10-ol	7 a	2 a	9 a	22.2
	3-carene + *trans*-verbenol	50 b	29 b	77 b	37.7[c]
	3-carene + *trans*-verbenol + 3-caren-10-ol	71 c	12 b	83 b	14.4

[a]Release rates for volatiles determined in the laboratory as follows: 3-carene and α-pinene, 7 mg/day each; *trans*-verbenol and 3-caren-10-ol, 200 µg/day each; acetophenone and myrcenol, 100 µg/day each.

[b]Values for each experiment followed by same letter are not significantly different, Newman-Keul's test, $P < 0.05$.

[c]Mean sex ratios of beetles caught to 3-carene + *trans*-verbenol and 3-carene + *trans*-verbenol + 3-caren-10-ol are significantly different, t-test, $P<0.05$.

increased nor decreased significantly. On the basis of these results, acetophenone and myrcenol appear to lack potential as effective attractants in the field. The results fail to confirm whether or not 3-caren-10-ol is an effective attractant, as the binary combination of 3-carene + trans-verbenol was not tested for comparison.

The next experiment rectified this error as the three components of this bait were tested alone and in all possible combinations (Table 7). Only 3-carene + trans-verbenol and 3-carene + trans-verbenol + 3-caren-10-ol attracted significant numbers of beetles. The addition of 3-caren-10-ol to 3-carene + trans-verbenol caused a significant shift in sex ratio in favour of males. 3-Carene was a necessary and effective synergist in combination with trans-verbenol or trans-verbenol + 3-caren-10-ol, but not with 3-caren-10-ol.

The trap-tree experiment disclosed further evidence of the activity of 3-caren-10-ol (Table 8). 3-Carene + trans-verbenol + 3-caren-10-ol was clearly the most effective bait. The fact that 3-caren-10-ol appears to attract more males than females (Table 7) suggests that attracted males might have contributed to the effectiveness of this ternary bait.

The least effective bait was the combination of a-pinene + trans-verbenol. Based on the results of laboratory bioassays (Tables 2-4, 6), and field experiments (Tables 7, 8) and the results of other researchers (Alexander et al. 1976; Billings et al. 1976; Pitman et al. 1978) I conclude that Pondelure should not be recommended for use in lodgepole pine forests.

Pheromone Biogenesis and Effects of Mating

Responses to abdominal extract stimuli disclosed attraction in both

Table 8. Ranked attack by *D. ponderosae* on lodgepole pines baited with host monoterpenes and beetle-produced volatiles. N = 8 replicates.

Bait Treatment	Number of Trees	
	Attacked (1-10 pitch tubes)	Mass Attacked (>10 pitch tubes from ground level to 2 m high)
blank control	0	0
α-pinene + *trans*-verbenol	0	0
3-caren-10-ol	1	0
3-carene + 3-caren-10-ol	1	0
trans-verbenol + 3-caren-10-ol	1	1
trans-verbenol	2	1
3-carene + *trans*-verbenol	3	2
3-carene + *trans*-verbenol + 3-caren-10-ol	7	5

Release rates for volatiles determined in the laboratory as follows: α-pinene and 3-carene, 7 mg/day each; trans-verbenol and 3 caren-10-ol, 200 µg/day each.

unmated and mated females as well as in mated males (Table 9). The response to mated males was probably due to the presence of both monoterpenes and terpene alcohols (small but detectable amounts) in male extracts, since males do feed following mating.

Female D. ponderosae contained only trace amounts of active volatiles on emergence (Fig. 3), but produced them after 24 h boring in a host log (Fig. 4). All GlC analyses disclosed considerable variation in amounts of volatiles per beetle. Of 4 female-produced volatiles, 3 were not significantly different in amounts in unmated or mated females (Table 10). Only acetophenone had decreased significantly in amount in mated beetles 24 h after females had been paired with males. This result suggests that mated females retain their ability to attract other beetles for longer than 24 h after mating. There is no compelling evidence for an antiaggregation pheromone in mated females, either from biological studies (Edson 1978) (Table 9) or gas chromatographic analyses (Table 10). Like female S. multistriatus (Elliot et al. 1975) female D. ponderosae probably gradually loose their attractiveness following mating. However, it is also possible that the insignificant increase in myrcenol content in mated females may prove to be real with further study. Myrcenol was partially inhibitory when combined with other volatiles in drainpipe traps (Table 7). Moreover, the amounts of verbenone, a known antiaggregation pheromone in D. brevicomis (Renwick and Vite 1970; Rudinsky et al. 1974) and D. frontalis (Rudinsky 1973), rose from 0.09 to 0.18 µg/female following mating (data not significantly different, Mann-Whitney U test, $P < 0.05$). Therefore, these compounds may be responsible in part for termination of aggregation on trees mass attacked by D. ponderosae.

Exo-brevicomin was prominent in emerged males but was absent from mated

Table 9. Ranked responses of D. ponderosae to abdominal extracts of emerged and mated males and unmated females excised from ponderosa pine logs. Forty beetles tested to each stimulus in a laboratory olfactometer.

Males		Females	
Source of Extract Stimulus	Percent[a] Response	Source of Extract Stimulus	Percent[a] Response
pentane control	15.0 a	pentane control	10.0 a
emerged males	20.0 ab	emerged males	25.0 a
females in log 24 h, then paired with males 24 h	37.5 b	females in log 24 h, then paired with males 24 h	42.5 b
males paired in log with females 24 h	45.0 bc	males paired in log with females 24 h	42.5 b
females in log 24 h	45.0 bc	females in log 24 h	45.0 b
standard female frass extract (0.02 g equiv.)	65.0 c	standard female frass extract (0.02 g equiv.)	52.5 c

[a]Values for each sex follows by same letter are not significantly different, χ^2 test, $P<0.05$.

Fig. 3. Gas chromatogram of abdominal extract volatiles from a single female D. ponderosae emerged from lodgepole pine. Peaks identified by letters are as follows: solvent (S1), 2-octanol (2oc), 3-octanol (3oc), trans-verbenol (tv), and verbenone (v).

Fig. 4. Gas chromatogram of abdominal extract volatiles from a single female D. ponderosae after boring 24 h in lodgepole pine log. Peaks identified by letters are as follows: solvent (S1), 2-octanol (2oc), 3-octanol (3oc), acetophenone (a), trans-verbenol (tv), verbenone (v), 1-methyl-5-(α-hydroxyisopropyl)-cyclohexa-1,3-diene (M5), 3-caren-10-al (C1), geranyl acetate (ga), unidentified sesquiterpene (S2), myrtenol (mt), geraniol (g), p-menth-2-en-7-ol (P1), myrcenol (m), 3-caren-10-ol (C2), p-mentha-1,3-dien-7-ol (P2), perilla alcohol (pa), and cuminyl alcohol (ca).

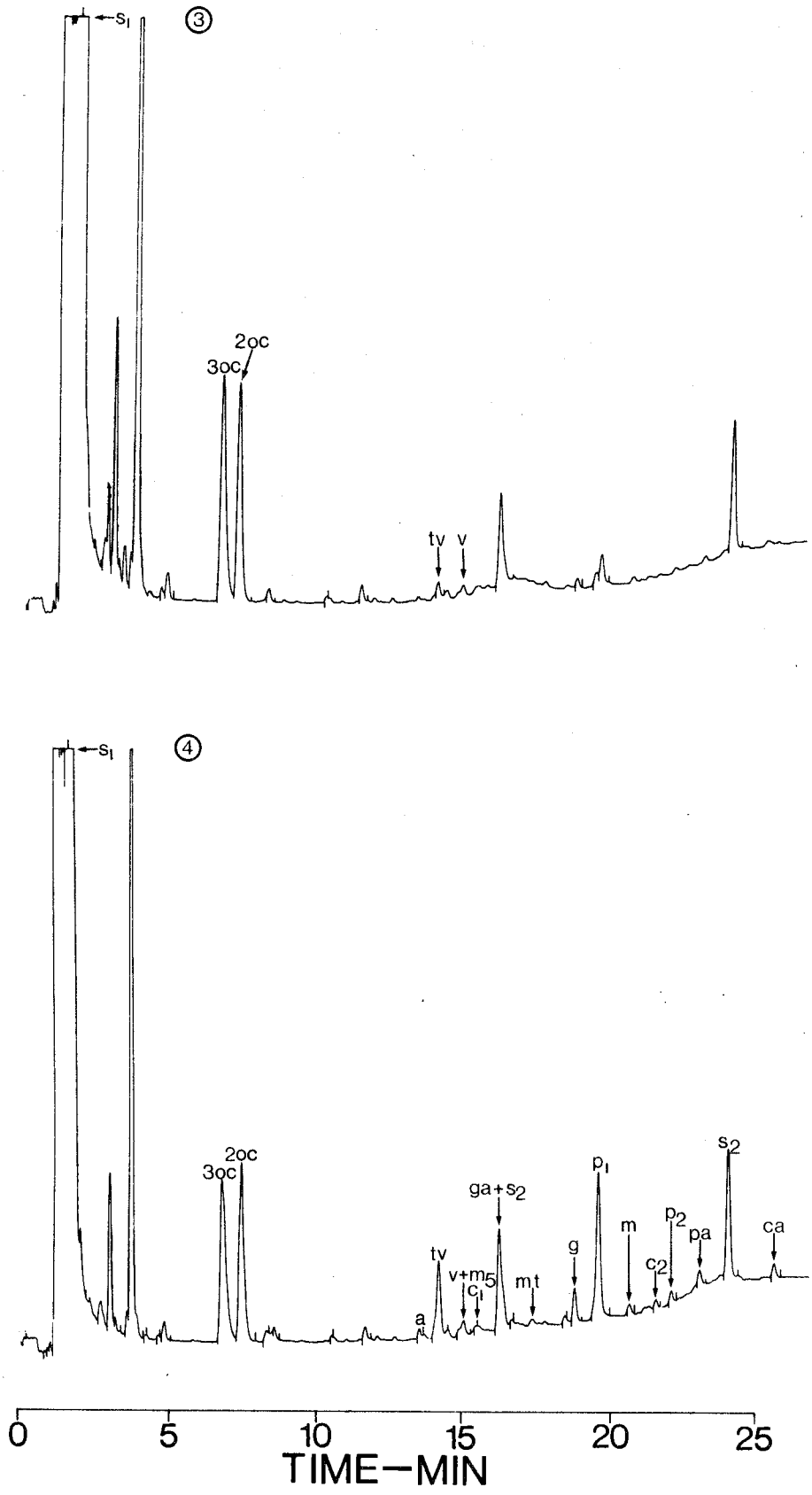

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Table 10. Amounts of proven or candidate aggregation pheromones in abdominal extracts of emerged and mated males and mated and unmated females excised from ponderosa pine. GLC analysis done on 8 beetles of each sex.

Sex	Treatment	Compound	ng/beetle[a]
Males	emerged	exo-brevicomin	82 a
	paired in log with female 24 h		0 b
Females	in log 24 h	trans-verbenol	76 a
	in log 24 h, then paired with males 24 h		96 a
	in log 24 h	3-caren-10-ol	10 a
	in log 24 h, then paired with males 24 h		12 a
	in log 24 h	acetophenone	36 a
	in log 24 h, then paired with males 24 h		8 b
	in log 24 h	myrcenol	10 a
	in log 24 h, then paired with males 24 h		26 a

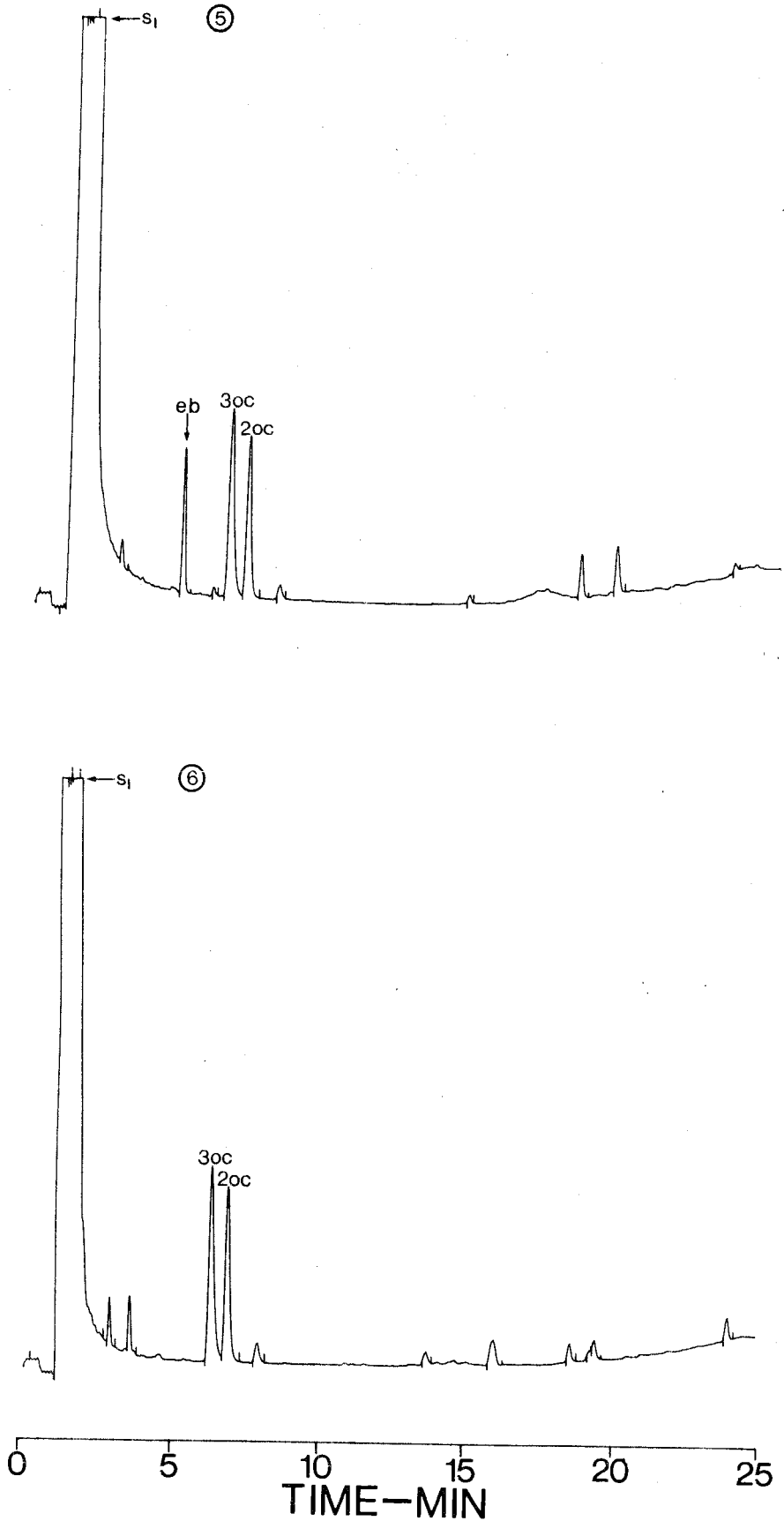
[a]Values for each pair of treatments followed by same letter are not significantly different, Mann-Whitney U test, $P<0.05$.

males (Figs. 5, 6; Table 10). This result does not agree with other studies in which it was reportedly detected in feeding males and females (Pitman et al. 1969), although it was most prominent in emerged males (Rudinsky et al. 1974). A very low concentration of exo-brevicomin added to the attractive mixture of trans-verbenol, α-pinene and ponderosa pine resin enhanced attraction of males in laboratory and field bioassays; higher concentrations were apparently inhibitory but the results were not supported by statistical analyses (Rudinsky et al. 1974). A stimulus containing a low concentration of exo-brevicomin added to Pondelure prevented mass attack in a white pine forest, but not in a lodgepole pine forest (McKnight 1979).

I hypothesize that exo-brevicomin acts as an aggregation pheromone at low concentrations for lodgepole pine-infesting D. ponderosae. Early bioassays with frass (L. Chong[2] and J. H. Borden[2], unpublished data) disclosed an increased response by females to mixed male and female frass compared with frass from either sex alone. In bioassays with male abdominal extracts (Table 9) there was no difference between female response to emerged male extracts and pentane. However, females probably require the odour of host monoterpenes with exo-brevicomin, since in nature they would respond to a host tree after pioneer female D. ponderosae have begun boring successfully (releasing host odours) and have been joined by males containing exo-brevicomin. At the height of a mass attack, high concentrations of exo-brevicomin might have an antiaggregation effect. This effect would be consistent with Rudinsky's (1974) results and might be the basis for attack switching to nearby unattacked trees (Geiszler and Gara 1978).

Fig. 5. Gas chromatogram of abdominal extract volatiles from a single male D. ponderosae emerged from lodgepole pine. Peaks identified by letters are as follows: solvent (S1), 2-octanol (2oc), 3-octanol (3oc), exo-brevicomin (eb).

Fig. 6. Gas chromatogram of abdominal extract volatiles from a single male D. ponderosae paired (mated) with a female 24 h in a ponderosa pine log. Peaks identified by letters are as follows: solvent (S1), 2-octanol (2oc), 3-octanol (3oc).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Effects of Juvenile Hormone on Pheromone Production

In both pooled and individual beetle samples, female D. ponderosae treated 24 h earlier with JH III contained more trans-verbenol than the solvent-treated control beetles, even though they were denied access to host logs (Figs. 7, 8; Table 11). Therefore, female D. ponderosae must sequester a precursor of trans-verbenol, possibly α-pinene in a conjugate form as suggested by Hughes (1975) for D. frontalis and D. terebrans (Oliv.). Production and release of trans-verbenol would begin after JH release occurred in attacking females. In D. brevicomis, this release is stimulated by an ethanol-soluble component of host phloem tissue (Hughes and Renwick 1977b). Further trans-verbenol production would occur as the attacking beetles converted α-pinene from host resin to trans-verbenol (Hughes 1973b).

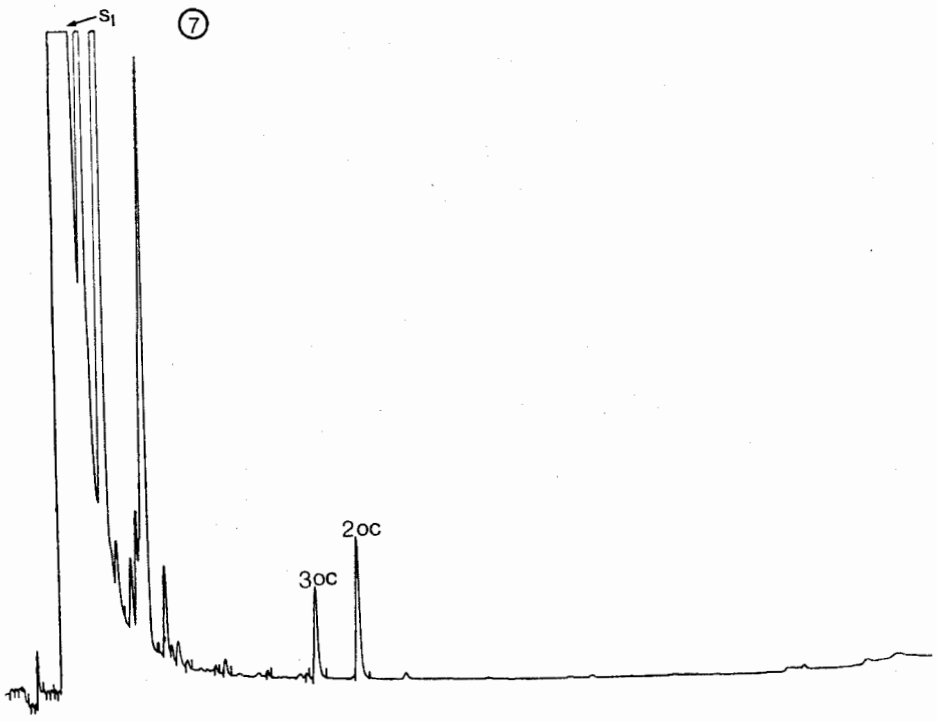
Acetophenone also increased significantly in unfed females following JH treatment (Table 11), suggesting that it should be re-evaluated as a potential pheromone. However, JH treatment failed to induce a significant production of myrcenol or 3-caren-10-ol in unfed females. These compounds are probably produced only after the beetles encounter a host tree. The production of such compounds is consistent with Hughes' (1973a, 1973b) hypothesis that the beetles detoxify inhaled or ingested host monoterpenes to terpene alcohols, and secondarily use some of the products as pheromones.

The fact that JH-treated, unfed females contained so much trans-verbenol may be partly because it accumulated in the hindgut and was not released with the fecal pellets, since the beetles were not allowed to feed.

Beetles treated with 100 µg of JH III contained less trans-verbenol and 3-caren-10-ol after 24 h in ponderosa pine than acetone-treated, fed

Fig. 7. Gas chromotogram of abdominal extract volatiles from a single female D. ponderosae emerged from lodgepole pine and 24 h following topical treatment with 1 µl acetone. Peaks identified by letters are as follows: solvent (Sl), 2-octanol (2oc), 3-octanol (3oc).

Fig. 8. Gas chromatogram of abdominal extract volatiles from a single female D. ponderosae emerged from lodgepole pine and 24 h following topical treatment with 100 µg JH III. Peaks identified by letters are as follows: solvent (Sl), 2-octanol (2oc), 3-octanol (3oc), acetophenone (a), trans-verbenol (tv).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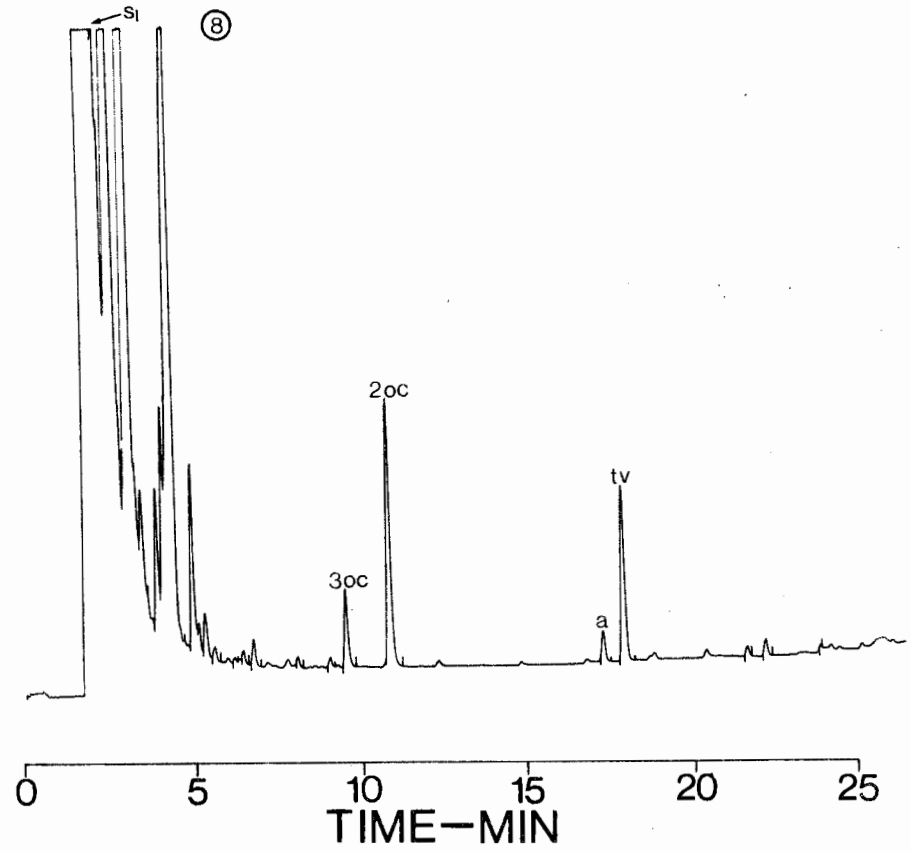
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Table 11. Effects of treatment with JH III on amounts of proven or candidate aggregation pheromones in abdominal extracts of fed or unfed female *D. ponderosae*.

Sampling Method	Treatment	No. Beetles	Compound	ng/beetle[a]
pooled sample	acetone (1µl), 24 h at 20 C	20	trans-verbenol	26
	JH III (25 µg), 24 h at 20 C	20		80
	acetone (1µl), 24 h in ponderosa pine	20		18
	JH III (25 µg), 24 h in ponderosa pine	20		20
individual beetle samples	acetone (1µl), 24 h at 20 C	10	trans-verbenol	0 a
	JH III (100 µg), 24 h at 20 C	8		114 c
	acetone (1µl), 24 h in ponderosa pine	10		60 c
	JH III (100 µg), 24 h in ponderosa pine	9		20 b
	acetone (1µl), 24 h at 20 C	10	3-caren-10-ol	0 a
	JH III (100 µg), 24 h at 20 C	8		2 a
	acetone (1µl), 24 h in ponderosa pine	10		32 b
	JH III (100 µg), 24 h in ponderosa pine	9		4 a
	acetone (1µl), 24 h at 20 C	10	acetophenone	0 a
	JH III (100 µg), 24 h at 20 C	8		34 b
	acetone (1µl), 24 h in ponderosa pine	10		26 b
	JH III (100 µg), 24 h in ponderosa pine	9		28 b
	acetone (1µl), 24 h at 20 C	10	myrcenol	8 a
	JH III (100 µg), 24 h at 20 C	8		4 a
	acetone (1µl), 24 h in ponderosa pine	10		24 a
	JH III (100 µg), 24 h in ponderosa pine	9		10 a

[a]Mean values of individual beetle samples for each compound followed by same letter are not significantly different, Mann-Whitney U test, $P<0.05$.

controls (Table 11). A similar result was obtained by Bridges (1981) for D. frontalis. The excess of exogenous JH (4x that in the pooled sample) may stimulate more rapid release of pheromones by feeding females, or may induce a negative feedback reaction resulting in decreased pheromone production. However, this result was not repeated in another experiment (Table 13).

Effects of Precocene 2 on Pheromone Production and Reproductive Capacity

Exposure to a surface coated with 120 μg/cm^2 of precocene 2 significantly reduced the abiltiy of treated females to produce trans-verbenol (Fig. 9; Table 12). The significant decrease in production of trans-verbenol, but not 3-caren-10-ol, provides additional evidence for JH control of trans-verbenol synthesis. Treatment with precocene 2 did not affect the amounts of acetophenone or myrcenol in female beetles. Treatment with JH III following exposure to precocene 2 for 24 h restored the ability of female D. ponderosae to synthesize trans-verbenol when they fed for 24 h on ponderosa pine (Fig. 10; Table 13). When insects did not feed but were exposed for 24 h to ponderosa pine volatiles, α-pinene was apparently converted to trans-verbenol (Table 13). Neither precocene nor JH had any effect on this detoxification mechanism, suggesting that it is not under neural or hormonal control. However, these beetles were not feeding, or therefore defecating, so trans-verbenol probably accumulated in them.

The decrease in amount of 3-caren-10-ol following precocene treatment in the dose response experiment was not significant (Table 12). In the precocene/JH experiment, the content of 3-caren-10-ol decreased significantly following exposure to precocene, and increased following subsequent treatment with 100 μg of JH III (Table 13). Very little 3-caren-10-ol was

Fig. 9. Gas chromotogram of abdominal extract volatiles from a single female D. ponderosae emerged from lodgepole pine and after 24 h exposure to 120 µg/cm^2 precocene 2 on a glass surface, and a further 24 h boring in ponderosa pine. Peaks identified by letters are as follows: solvent (Sl), 2-octanol (2oc), 3-octanol (3oc), acetophenone (a), trans-verbenol (tv), verbenone (v), 3-caren-10-ol (C2).

Fig. 10. Gas chromatogram of abdominal extract volatiles from a single female D. ponderosae emerged from lodgepole pine and after 24 h exposure to 120 µg/cm^2 precocene, then a topical treatment of 100 µg JH III and a further 24 h boring in ponderosa pine. Peaks identified by letters are as follows: solvent (Sl), 2-octanol (2oc), 3-octanol (3oc), acetophenone (a), trans-verbenol (tv), myrcenol (m), 3-caren-10-ol (C2).

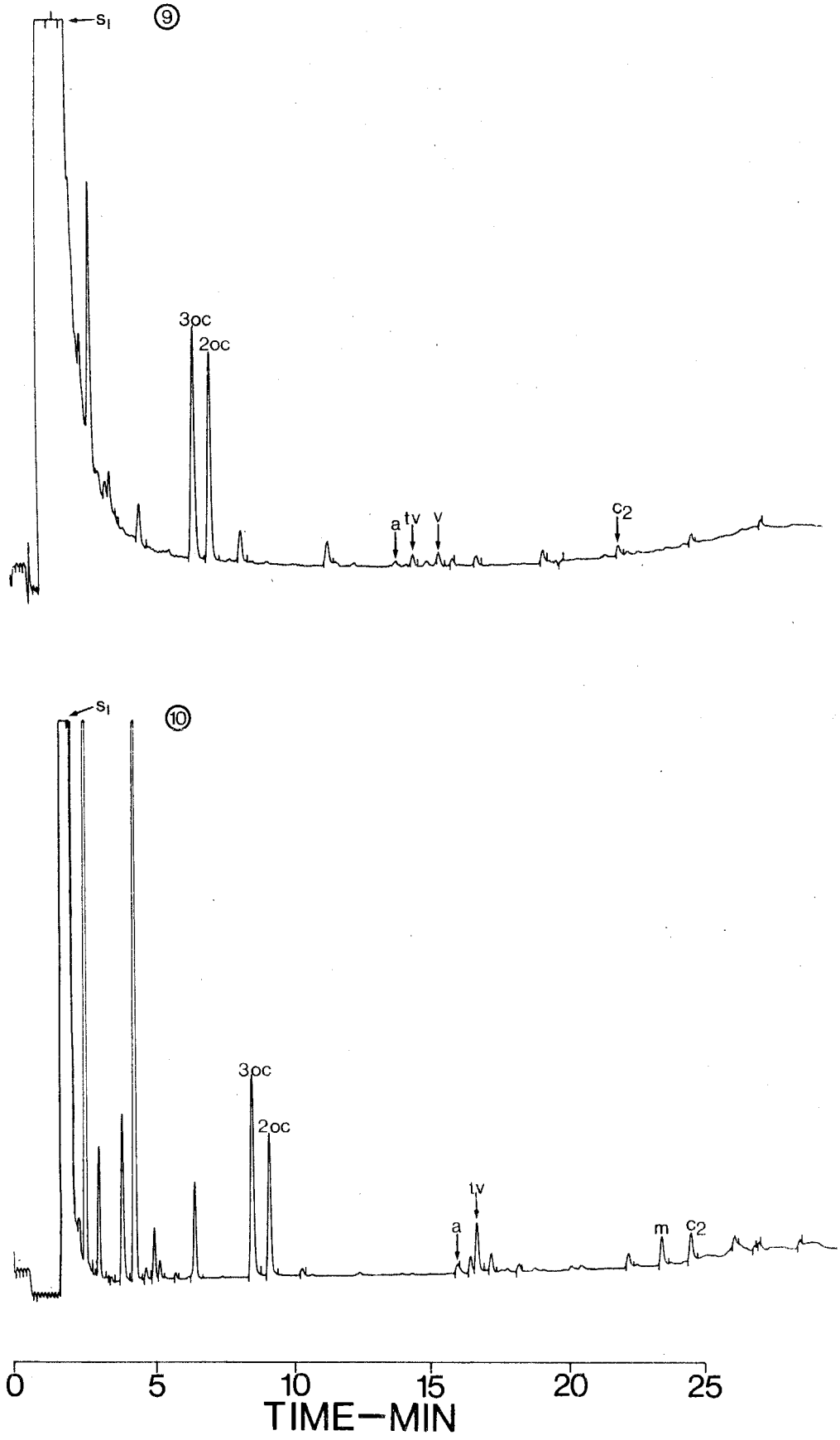
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Table 12. Effect of graded doses of precocene 2 on production of trans-verbenol and 3-caren-10-ol by female D. ponderosae. N=6 beetles/treatment.

Treatment of Beetles	Dose	ng/beetle[a]	
		trans-verbenol	3-caren-10-ol
24 h in petri dish treated with methanol, 24 h in ponderosa pine log	46.6 µl/cm^2	66 a	10 a
24 h in petri dish treated with precocene 2, 24 h in ponderosa pine log	30 µg/cm^2	58 ab	8 a
	60 µg/cm^2	96 a	2 a
	120 µg/cm^2	12 b	4 a

[a]Mean values for each compound followed by same letter are not significantly different, Mann-Whitney U test, $P<0.05$.

Table 13. Effect of treatment with precocene 2 followed by treatment with 100 µg JH III on production of *trans*-verbenol and 3-caren-10-ol by female *D. ponderosae*. N=7 beetles/treatment.

Treatment		ng/beetle[a]	
0-24 h, in petri dish treated with methanol (46.6 µl/cm^2) or precocene (120 µg/cm^2)	24-48 h, topical treatment with methanol (1 µl) or 100 µg JH III, then fed in ponderosa pine log	*trans*-verbenol	3-caren-10-ol
methanol	methanol	114 a	88 a
methanol	JH III	170 a	44 ab
precocene 2	methanol	20 b	4 b
precocene 2	JH III	66 a	30 ab
precocene 2	methanol (did not feed on log)	54 a	8 b
precocene 2	JH III (did not feed on log)	60 a	2 b

[a]Mean values for each compound followed by same letter are not significantly different, Mann-Whitney U test, $P<0.05$.

produced following exposure to host volatiles. Therefore, a feeding stimulus may be required to initiate 3-caren-10-ol production. The ability to respond to such a stimulus may be maintained by a high titre of JH.

Precocene has been shown to act on O. fasciatus by causing degeneration of the CA (Unnithan et al. 1977). A similar effect probably occurs in Coleoptera. Female P. strobi (Curculionidae) treated with precocene 2 made fewer oviposition punctures and laid fewer eggs than untreated females (Sahota and McMullen 1979). The evidence is more compelling for the Scolytidae. JH can stimulate pheromone production in many scolytids (Borden et al. 1969; Hughes and Renwick 1977a,b; Bridges 1981), including D. ponderosae (Tables 11, 13). Normal pheromone production by D. ponderosae can be inhibited by treatment with precocene 2 and reversed in part by treatment with JH III (Table 13), strongly implicating the CA in control of pheromone production and release. The suggestion by Borden et al. (1969) that topically-applied JH may be a pheromone precursor is probably wrong. JH also controls reproductive maturation in female scolytids including T. lineatum (Fockler and Borden 1973) and D. pseudotsugae (Sahota et al. 1970). The lack of reproductive success by precocene-treated female D. ponderosae but not males (Table 14) further implicated the CA as organs which control reproductive functions in the Scolytidae.

Table 14. Effect of treatment with precocene 2 on reproductive success of *D. ponderosae*. Beetles allowed to bore in lodgepole pine log for 12 days after treatment.

Treatment (24 h in petri dish treated with methanol (46.6 µl/cm²) or precocene 2 (120 µg/cm²))		No. Beetle Pairs	Length of Egg Gallery (cm)[a]	Larvae/ Female[a]
Males	Females			
methanol	methanol	8	45.9 a	40.0 a
precocene 2	methanol	7	41.1 a	36.1 a
methanol	precocene 2	7	13.9 b	0 b
precocene 2	precocene 2	8	13.2 b	0 b

[a]Mean values within a column followed by same letter are not significantly different, Mann-Whitney U test, $P<0.05$.

CONCLUSIONS

To date the role of semiochemicals in host selection by many species of bark beetles has been well researched (Borden 1981). Such chemicals have been used in increasingly successful programs aimed at managing scolytid pests, e.g. S. multistriatus (O'Callaghan et al. 1980; Peacock et al. 1981), D. frontalis (Billings 1981), D. brevicomis (Bedard and Wood 1981), and I. typographus (Lie and Bakke 1981).

The control of pheromone biogenesis has been well researched for many scolytids, and JH has been accorded a critical role in control of pheromone production (Borden 1981). Although some research on Coleoptera has been done on the effects of antiallatal agents, e.g., precocene (Sahota and McMullen 1979), no research has been done on scolytids. My results contribute to knowledge in both of the above areas.

There is strong evidence that 3-caren-10-ol is an aggregation pheromone for D. ponderosae infesting lodgepole pine. It was attractive alone and in combination with other attractants in laboratory bioassays (Tables 1-6) and shifted the attraction in favour of males when combined with a monoterpene and the known aggregation pheromone, trans-verbenol in field traps (Table 7). The same combination was far more effective in inducing attack on baited lodgepole pine than any binary combination of the bait chemicals (Table 8). While Pondelure is apparently effective for practical use against D. ponderosae infesting western white pine, Pinus monticola (Pitman et al. 1978), other chemicals are probably necessary for optimal use against populations infesting lodgepole pine. Therefore, I recommend that 3-caren-10-ol be rigorously evaluated with other active chemicals as a population management tool for such populations.

Other compounds probably act as pheromones for D. ponderosae in lodgepole pine. Exo-brevicomin has strong potential as part of an attractant complex made up of host monoterpenes and pheromones. It disappears in males following mating (Table 10) which suggests that it may attract females. A low concentration increased attractiveness in the field (Rudinsky et al. 1974) and prevented a mass attack in a white pine forest but not in a lodgepole pine forest (McKnight 1979). Since Rudinsky et al. (1974) demonstrated that exo-brevicomin at high concentrations had antiaggregative activity, I suggest that exo-brevicomin should be field-tested at both high and low concentrations with other attractants in lodgepole pine forests to determine the precise nature of its contribution to an attractive monoterpene-pheromone complex.

Acetophenone and myrcenol had not been tested as potential attractants for D. ponderosae before this study. While neither added significantly to the attractive mix of 3-carene + trans-verbenol + 3-caren-10-ol in the field (Table 7), they were both part of the female volatile mix that attracted male D. ponderosae at the lowest concentration in the laboratory (Table 5) and in synergist tests with host monoterpenes (Table 6). In addition, acetophenone increased significantly following JH treatment (Table 11) and decreased significantly following mating (Table 10) strongly suggesting that its production is controlled by the CA. Myrcenol increased following mating (although not significantly) (Table 10) and reduced attractiveness in the field (Table 7). Both should be re-evaluated in the field: acetophenone as a potential attractant, possibly at close range, and myrcenol as a potential inhibitor.

My data indicate that, as in other scolytids (Borden 1981), pheromone production in D. ponderosae is in part controlled by JH. Trans-verbenol

was synthesized immediately following JH treatment but 3-caren-10-ol was not. The production of both compounds was inhibited by exposure to precocene. This inhibition was reversed by JH application, implicating the CA. These results suggest 2 modes of pheromone production: 1) trans-verbenol or its precursor is sequestered and immediately synthesized or released in exposed beetles; and 2) trans-verbenol may also be produced by the conversion of α-pinene during attack on a new host. 3-Caren-10-ol is not sequestered but is the result of the conversion of host material. This 2 component system is similar to that in Ips cembrae, in which JH stimulates the production of methylbutenol, but not ipsenol and ipsdienol which are produced when the males attack a new host or are exposed to myrcene vapours (Renwick and Dickens 1979).

There was no transfer of the effect of precocene by treated males (Table 14). Therefore direct contact of precocene by females is necessary to reduce their reproductive capacity. In practical situations this requires that the insects contact precocene, which may prove difficult with cryptic scolytids. It may be some time before methods are developed for the use of precocene and other antiallatal agents against bark or ambrosia beetles. There may be some promise in exploiting natural sources of antiallotropins, e.g. white spruce trees which cause reversal of yolk deposition in D. rufipennis (Sahota and Ibaraki 1979).

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