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Identification of sulcatol, a potential pheromone of the ambrosia beetle *Gnathotrichus materiarius* (Col., Scolytidae)

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Abstract: We report the identification of a potential pheromone for *Gnathotrichus materiarius* (Fitch) (Col., Scolytidae). The population sex ratio is close to 1 : 1, and males initiate attacks on host trees. Headspace and hindgut samples from single males showed the presence of the putative pheromone 6-methyl-5-hepten-2-ol, sulcatol. Unmated males released sulcatol for at least 12 days, and ceased producing the pheromone after 20 days. The peak sulcatol release occurred after 2 days. Males cease production of sulcatol 24 h after being paired with females. Single females were unable to initiate galleries, and no sulcatol was detected from their headspace and hindgut samples. The chiral ratio of the pheromone, observed from headspace samples, was 31% (S)-(+)- and 69% (R)-(−)-sulcatol.

Key words: enantiomeric ratio, headspace analysis, hindgut analysis, SPME technique, sex ratio

1 Introduction

Bark and ambrosia beetles are the two main groups within the family Scolytidae, which includes many of the most important forest insect pests (Wood, 1982). The majority of the economic impact attributed to these insects is inflicted by bark beetles (Drooz, 1985; Paine et al., 1997). Comparatively, losses caused by ambrosia beetle attacks are smaller (Samaniego and Gara, 1970). These insects attack mainly felled and/or weakened trees (Beaver, 1988), where their galleries and staining of wood cause damage of lumber. The resulting pinholes lead to a degradation of the lumber, reducing its market value, increasing the slabbing and manufacturing time, and rejection of exported wood material (Graham and Boyes, 1950; Borden and McLean, 1980). In coastal British Columbia ambrosia beetle damage may exceed $60 million per year (McLean, 1985).

Several ambrosia beetle species are known to respond to primary attractants, responding to odours emanating from the stressed, dying or dead host trees (Klimetzek et al., 1986; Phillips et al., 1988; Kelsey, 1994; Flechtmann et al., 1995). However, secondary attraction has been intensively studied only in a few ambrosia beetles. Sulcatol was determined to be the pheromone of *Gnathotrichus sulcatus* (LeConte), at the chiral ratio of 65% (S)-(+)- and 35% (R)-(−)-enantiomers (Byrne et al., 1974) while in *Gnathotrichus retusus* (LeConte) (Borden and McLean, 1979) it is composed of 99% of the (S)-(+)- enantiomer (Borden et al., 1980). The pheromone produced by *Trypodendron lineatum* (Olivier) was identified as lineatin (Macconnel et al., 1977). These species are considered to be the main pests in British Columbia (Borden and McLean, 1980; McLean, 1985).

No pheromone has been previously reported for *Gnathotrichus materiarius* (Fitch), although its production has been suggested (Dixon and Payne, 1979). This species is widespread in the eastern US from Florida to Canada (Atkinson et al., 1991), and it breeds in the lower portions of trunks of dead and dying pines, spruce, balsam fir and other conifers (Drooz, 1985).

Gnathotrichus materiarius appears to be of more economic concern in Europe than in its original native range. It was accidentally introduced from the US in infested logs into Europe (Ménier, 1972), being first recorded in France in 1934 (Hoffmann, 1936). It has now been established in other countries, including Germany (Postner, 1974), the Netherlands (Doom, 1967), Switzerland (Hirschheydt, 1992) and most recently Finland (Valkama et al., 1998). In Europe this species also attacks dying trees, felled trees, stumps and trees attacked by bark beetles (Postner, 1974; Dengler, 1990), where they degrade lumber and render infested boles useless for cellulose-producing industries because of the staining (Doom, 1967; Dengler, 1990).

The objectives of our research were to verify production and identification of the putative pheromones produced by *G. materiarius,* and to learn how they vary over time.

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2 Materials and methods

2.1 Source of *Gnathotrichus materiarius*

Loblolly pine (*Pinus taeda* L.) billets (length 0.70 cm, diameter 15–20 cm) were exposed to *G. materiarius* attack at the US Department of Energy Savannah River Site (Barnwell County, SC) in a longleaf pine (*Pinus palustris* Miller) forest in the summer of 1997. After initiation of the attacks, about 10 billets were brought to the lab and placed in an emergence box at weekly intervals (Browne, 1972). Emerged beetles were collected daily, sexed (Wood, 1982) and males and females were placed in separate Petri dishes containing filter paper moistened with distilled water and kept under refrigeration (4°C) for further analysis (Borden and McLean, 1979).

2.2 Bole infestation and volatile collection

Loblolly pine billets (1 m length, 20 cm diameter) were artificially infested in the laboratory (room temperature 22°C) with 100 male and 50 female beetles. Individual males were caged under a gelatin capsule over a hole punctured to a depth of approx. 1 mm to facilitate initial boring. One female was added to each half of the gelatin capsules containing males on the second day, immediately after volatile collection. Volatiles were collected from individual gallery entrances every 24 h for the first 7 days, then again on the 12th and on the 20th day after the initial attack.

Volatiles were collected from five each of unpaired males and females, pairs of males and females and uninfested entrance holes. For the male and pair treatments, only entrance holes with abundant frass were selected for volatile collections. An entrance artificially drilled with a 1/16-inch drill to a depth of approx. 4 mm each day, to simulate beetle boring was used as control.

For the volatile collection, the gel capsule was removed, and a pasteur pipette (approx. 2.9 ml volume) was placed over the gallery entrance including the extruded frass, creating a small headspace. The tip of the pipette was covered with aluminium foil and left in place for 30 min for the volatiles to reach equilibrium in the headspace, before volatile collection. The volatiles were collected using a solid phase microextraction (SPME) technique (Arthur and Pawliszyn, 1990), with a fibre coated with 100 μm of polydimethylsiloxane as the stationary phase (Supelco, Bellefonte, PA, USA). The fibre was exposed for 1 min inside the headspace, after which it was withdrawn into the needle, and the tip was sealed with a rubber septum. The fibres were kept on ice until their transfer to a freezer, set at −82°C. Volatiles were analysed on the day of collection. After each volatile collection, the gallery entrance was cleaned of frass, and the gel capsule was replaced to prevent beetle escape.

We used an external calibration (Yang and Peppard, 1994), where the fibres were exposed to the headspace of undecane and ethyl caprate standard (1 μl of 250 μg/μl) in a 500-ml mason jar. We did not attempt to quantify the volatiles because such a method is not suitable for quantification in SPME techniques (Yang and Peppard, 1994). The abundance of volatiles was expressed as the relative abundance based on undecane abundance.

2.3 Enantiomeric determination

A loblolly pine billet was infested with male *G. materiarius* and volatiles of 23 actively boring males were collected after 24 h, by the methodology previously described.

A response factor curve was calculated, based on exposing fibres to the headspace of 1 μl of the two enantiomers of the pheromone and of the standards undecane and ethyl caprate at 1, 10 and 100 μg/μl inside a 500 ml mason jar.

2.4 Hindgut extractions

Loblolly pine billets were infested with male and female *G. materiarius* in separate holes. After 24 h, 20 frass-producing males and 20 live, active females were removed from the logs and immediately placed on dry ice. On the same day, females were added to the remaining males and 24 h later the pairs were excised from the logs and placed on dry ice. All beetles were transferred to a −82°C freezer and kept there until hindgut extraction. The hindguts of unpaired males, unpaired females and pairs of males and females were excised with fine tip forceps and transferred in groups of five to a vial containing 100 μl of 95% pentane : 5% ether (Gries et al., 1990). Samples from the extracts were analysed using the chiral column.

2.5 Instrumental methods

A Hewlett-Packard GCD G1800A gas chromatograph-mass spectrum equipped with a HP-FFAP fused-silica capillary column (46.8 m × 0.2 mm inside diameter; 0.33 mm film thickness) (Hewlett-Packard Corp., Palo Alto, CA, USA) was used for the identification of chemicals. The temperature was programmed as follows: 40°C for 1 min, 16°C/min to 80°C, 7°C/min to 202°C, then 40°C/min to 230°C for 6 min. The carrier gas was helium and the flow rate was 0.7 ml/min. The injector was at 220°C and the mass spectral detector at 230°C.

A HP-BETA DEX fused-silica capillary column (29.9 m × 0.25 mm ID; 0.25 mm film thickness) (Hewlett-Packard Corp.) was used for the enantiomeric determination of the sulcatol identified on the HP-FFAP column. The temperature programme was the same as described above. The carrier gas was helium and the flow rate was 1.0 ml/min.

For both columns, injections were carried out manually (Bartelt, 1997), using a specialized SPME holder (Supelco). The injector was equipped with a 0.75-mm ID liner, and runs were splitless for 1 min (Czerwiński et al., 1996). The enantiomers were identified based on their mass spectra and retention times matching known standards. Enantiomers of the putative pheromone identified were generously donated by Phero Tech, Delta, BC, Canada; (+)-sulcatol was 99% pure, while (−)-sulcatol was 77% pure.

2.6 Experimental design and data analysis

Differences between numbers of males and females emerging in the emergence box were compared by a *t*-test (proc TTEST; SAS, 1990b). Pheromone production by males and females were analysed by proc ANOVA with treatment mean separated by Tukey test (SAS, 1990a).

3 Results

A total of 3166 *G. materiarius* were collected from the emergence box, giving a sex ratio of 1.00 ♂ : 1.09 ♀, which was not statistically different from 1 : 1 ($t = 0.2145$, d.f. = 36, P = 0.8314).

Of the initial 50 females used to infest the logs, all failed to bore into the sapwood, and only a few of them

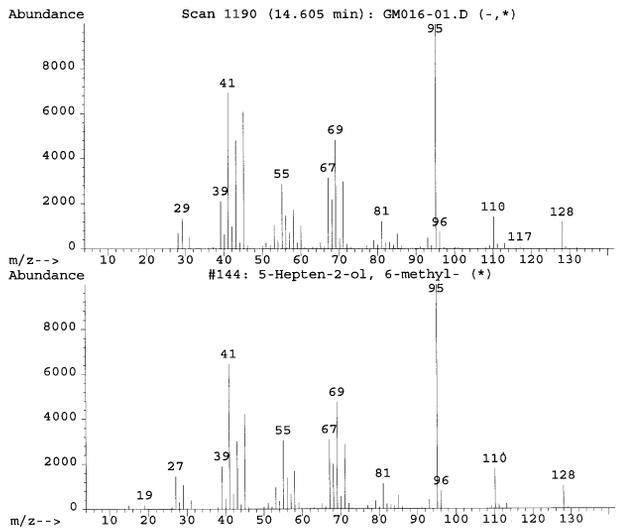

Fig. 1. *Comparison of mass spectra from hindgut extract of a male* Gnathotrichus materiarius *with library reference spectra for sulcatol, with 96% match quality (NIST standard reference database, 1994)*

bored slightly into the bark. After 8 days all but one female had died. Conversely, about 90% of the males bored into the sapwood 24 h after being placed on the logs.

From the headspace samples of males, we identified the component 6-methyl-5-hepten-2-ol, which has the common name sulcatol (fig. 1). No evidence of this chemical was found in the female headspaces. Unpaired males had two peaks of sulcatol production during the first 7 days, occurring on the second and sixth days (fig. 2).

When females were introduced into male galleries on the second day, sulcatol in the headspace dropped sharply, and was not detected after the fourth day,

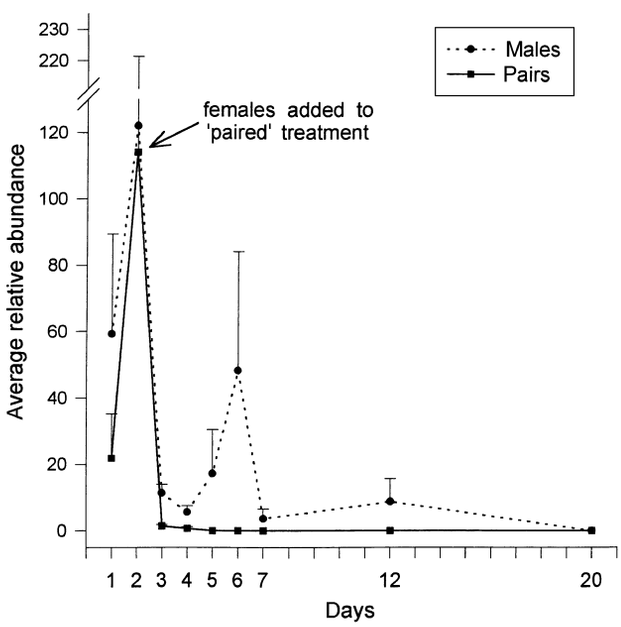

Fig. 2. *Mean + SE of relative abundance of sulcatol release over time by males and pairs of* Gnathotrichus materiarius *in artificially infested* Pinus taeda *logs*

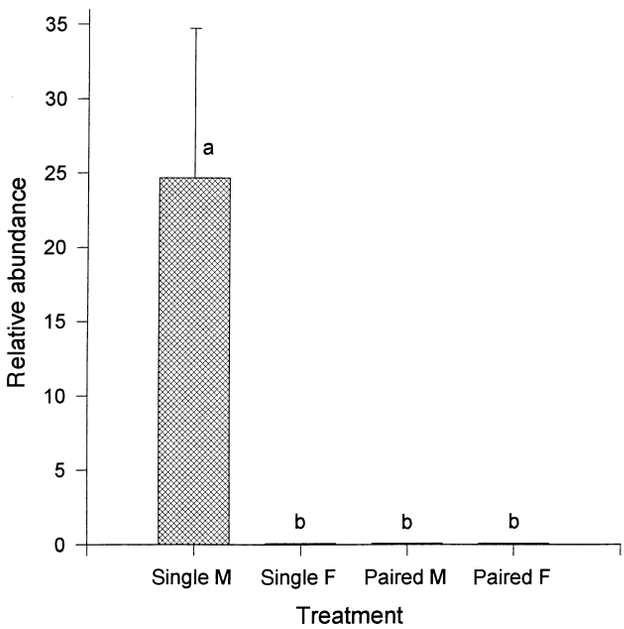

Fig. 3. *Mean + SE of relative abundance of sulcatol in hindgut samples of unmated males (single M), unmated females (single F), mated males (paired M) and mated females (paired F) of* Gnathotrichus materiarius

while in unpaired males there was an initial steep decline followed by a significant increase. Sulcatol was detected in single males only up to day 12 (fig. 2), although many were still alive and actively producing frass until day 20. A comparison of the sulcatol production by males that remained unpaired and those to which females were added after the second day, prior to female introduction (days 1 and 2), revealed that they were not statistically different ($F = 0.13$; d.f. = 1, 18; $P = 0.7197$). After the introduction the female, sulcatol production in single males was significantly higher than that of coupled males for the remainder of the sampling period ($F = 5.39$, d.f. = 1, 68; $P = 0.0233$).

Chiral analysis of sulcatol indicated that it was present in the headspace at a ratio of 30.73 ± 0.74% of (S)-(+)-sulcatol and 69.27 ± 0.74% of (R)-(−)-sulcatol, which corresponded respectively to 4.31 ± 0.25 and 9.49 ± 0.18 ng released by each individual male beetle.

The results of hindgut extractions showed that single males contained sulcatol, while hindguts of paired males did not contain any sulcatol 24 h after female introduction. No potential pheromone was detected in either single or paired females (fig. 3). Only the (+)-enantiomer of sulcatol was found, in very low amounts (less than 1 ng/5 ♂) in single males. Even when samples equivalent to a total of 20 ♂♂ were combined, (−)-sulcatol was found in quantities too low for certain identification.

4 Discussion

The sex ratio of *G. materiarius* found in this study was similar to that found for this species in the Netherlands

(Doom, 1967). The male is the pioneer beetle, as it is for *G. sulcatus* and *G. retusus* (Farris, 1963; Liu and McLean, 1989). Only males bored through the bark into the sapwood in our experiment. Dixon and Payne (1979) reported that males predominate in the initial arrivals on host trees in the field.

The putative pheromone of *G. materiarius* appears to be composed of a single component, sulcatol. Coleoptera pheromones are usually composed of more than one compound (Tumlinson and Teal, 1982), which makes *T. lineatum, G. sulcatus, G. retusus* and now potentially *G. materiarius,* all single-component pheromone producers, more the exception than the rule (Silverstein, 1974). However, considering that sulcatol in this case is a chiral compound, where each enantiomer may elicit a distinct response in the beetle (Borden et al., 1980), it is questionable if this compound could be considered as a putative single component pheromone (Silverstein and Young, 1976).

Sulcatol was detected only in unmated males, both in headspace and hindgut samples (figs 2 and 3), which is consistent with results obtained for the other two species of this genus (Borden and Stokkink, 1973; Borden and McLean, 1979). Males to which females were added had no detectable sulcatol in their hindguts after 24 h (fig. 3). The presence of detectable sulcatol in headspace samples of paired males up to 48 h after the females were added (fig. 2) was most likely residual pheromone on the walls of the galleries (Borden and Stokkink, 1973). Similar results were observed for male *Ips typographus* (L.), where the amounts of some alcohols of potential pheromonal activity dropped sharply after they were joined by females (Birgersson et al., 1984). Males apparently cease to produce sulcatol in the presence of females; it was observed in a few cases that when a female died or was rejected after a few days, males resumed pheromone release.

Sulcatol released by unmated male beetles was identified as being a mixture of approx. 31% (S)-(+)- and 69% (R)-(−)-sulcatol. This ratio differs from the values reported for *G. sulcatus,* which has 65% of (+) and 35% of the (−) enantiomer (Byrne et al., 1974), and *G. retusus,* whose pheromone is composed of 99% of (−)-sulcatol (Borden et al., 1980). *Gnathotrichus materiarius* overlaps in its geographical distribution range with *G. sulcatus* westbound (Wood, 1982). Considering the different enantiomer ratios of sulcatol for these two species, this would still enable the pheromone to be species-specific, leading to a reproductive isolation (Borden et al., 1980).

The release of sulcatol was highly variable among individuals (fig. 2), which is apparently common in pheromone-producing scolytids (Grosman et al., 1997; Birgersson et al., 1988). The highest peak was observed 2 days after males bored into the wood (fig. 2), similar to results obtained for *G. sulcatus,* whose pheromone peak was observed after 3 days (Borden and Stokkink, 1973). We found males still producing sulcatol after 12 days, although they were still alive after 20 days (fig. 2). If the males of this species behave similarly to those of *G. sulcatus,* which do not feed until joined by females (Byrne et al., 1974), it may be reasonable to assume that sulcatol is a beetle-produced compound.

Although both enantiomers of sulcatol were detected in the headspace, only the (+)-enantiomer was consistently detected in the hindgut. The analysis of body tissues does not necessarily accurately represent the behaviourally active compounds, which are better characterized from headspace volatile collections (Peacock, 1975; Silk et al., 1980, 1982; Francke, 1988; Birgersson and Bergström, 1989). Sulcatol is produced by various microorganisms, many of which are associated with scolytid beetles (Birkinshaw and Morgan, 1950; Collins, 1976; Brand and Barras, 1977; Francke et al., 1995; Tidswell et al., 1997). As it appears that little ambrosia fungus growth occurs until males are joined by females (McLean and Borden, 1975), and that sulcatol production ceases when females are present, it seems unlikely that microorganisms could explain the differences observed in headspace and hindguts. Two possible hypotheses are (i) the (−)-enantiomer is released by the scolytid so fast that it would not be stored in the beetle (Gries et al., 1988) or (ii) it is being produced or concentrated somewhere else other than the hindgut (Birgersson et al., 1990).

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