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STRUCTURE, CHIRALITY, AND FIELD TESTING OF A MALE-PRODUCED AGGREGATION PHEROMONE OF ASIAN PALM WEEVIL *Rhynchophorus bilineatus* (Montr.) (Coleoptera: Curculionidae)

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Abstract—4-Methyl-5-nonanol is a male-produced aggregation pheromone of the Asian palm weevil, *Rhynchophorus bilineatus* (Montr.). The pheromone was identified by coupled gas chromatographic-electroantennographic detection (GC-EAD) and coupled GC-mass spectrometric (MS) analyses of male- and female-produced volatiles. Analyses by GC-EAD and GC-MS of weevil-produced and stereoselectively synthesized isomers of 4-methyl-5-nonanol on a Cyclodex B column, which separated isomers with baseline resolution, revealed that only (4*S*,5*S*)-4-methyl-5-nonanol is EAD active and produced by the males. In field experiments in Papua New Guinea, (4*S*,5*S*)-4-methyl-5-nonanol and a racemic mixture of disatereoisomers of it enhanced attraction of male and female weevils to sugarcane-baited traps. (4*S*,5*S*)-4-Methyl-5-nonanol is also an aggregation pheromone of two other Asian palm weevils, *R. ferrugineus* (Oliv.) and *R. vulneratus* (Panz.). The stereoisomeric mixture of 4-methyl-5-nonanol is currently used to manage populations of *R. bilineatus* in Papua New Guinea.

Key Words—Coleoptera, Curculionidae, *Rhynchophorus bilineatus*, aggregation pheromone, pheromone chirality, (4*S*,5*S*)-4-methyl-5-nonanol, coconut palm.

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INTRODUCTION

In Papua New Guinea the palm weevil *Rhynchophorus bilineatus* (Montr.) causes mortality of coconut palms used as shade trees in cocoa plantations. While the weevil may directly attack the palms, they are frequently attracted to and ovipost in feeding tunnels of the New Guinea rhinoceros beetle, *Scopanes australis* (Boisd.). Feeding by weevil larvae often destroys the terminal bud, contributing to the death of the palm (Wattanapongsiri, 1966).

Male-produced aggregation pheromones have been reported for most economically important species of palm weevils (Rochat et al., 1991, 1993a,b; Oehlschlager et al., 1992a; Gries et al., 1993; Hallett et al., 1993a,b; Zagatti et al., 1993; Weissling et al., 1994). This paper reports the structural elucidation, stereochemistry, antennal response to, and behavioral activity of a male-produced aggregation pheromone for *R. bilineatus*.

METHODS AND MATERIALS

Laboratory Analysis. Male and female *R. bilineatus* of mixed age and sex were collected in cocoa plantations with interspersed coconut palms near Rabaul, East New Britain Province, Papua New Guinea. Collections were made in modified 19-liter plastic buckets containing 10–20 pieces of cut sugarcane (10–15 cm). Because weevils may mate within a few days of emergence (Wattanapongsiri, 1966) prior to and after capture in the buckets, it is likely that mainly mated weevils were used in aeration experiments. Twenty-five male and 20 female *R. bilineatus* were aerated separately for 6 to 7 days in a modified Nalgene desiccator containing 0- to 4-day-old sectioned apples. A water aspirator was used to draw charcoal-filtered air through the chamber and insect- and sugarcane-produced volatiles were trapped on Porapak Q. Volatiles were eluted from Porapak Q with pentane, concentrated by distillation (Oehlschlager et al., 1988), and subjected to gas chromatographic analysis (Figure 1). Gas chromatographic–electroantennographic detection (GC-EAD, Figure 2) (Hewlett Packard 5890A) (Arn et al. 1975) and GC-mass spectrometry (MS) (Hewlett Packard 5985B) in both electron impact (EI) and chemical ionization (CI) mode, employing a 30-m × 0.25-mm-ID fused silica column coated with SP-1000 (Supelco Inc., Belafonte, Pennsylvania), revealed 4-methyl-5-nonanol as an antennally active male-produced volatile. For GC-EAD recordings, a weevil antenna was removed from the rostrum and suspended between two glass capillary electrodes with the antennal base inserted into one and the olfactory club impaled by the other electrode. GC-MS-EI in selected ion monitoring mode (SIM) of natural and synthetic chiral isomers of 4-methyl-5-nonanol were further conducted on a fused silica Cyclodex-B-coated column (30 m × 0.25 mm ID, J & W Sci-

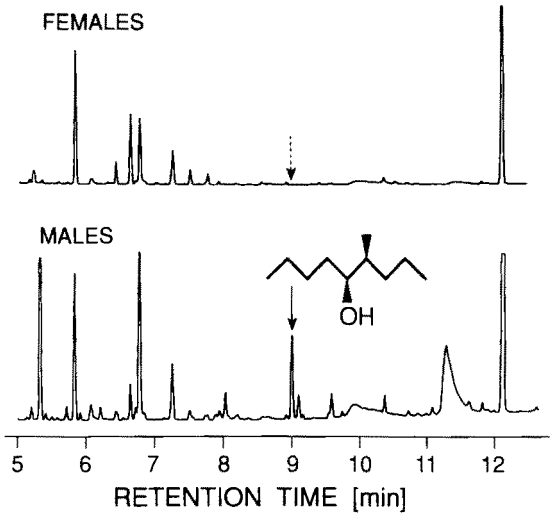

FIG. 1. Gas chromatograms of volatiles obtained from female and male *R. bilineatus* feeding on apples. Chromatography: SP-1000 fused silica column; temperature program: 1 min at 50°C, 10°C/min to 180°C.

entific, Folsom, California; isothermal 100°C) to deduce which stereoisomer was weevil-produced (Figure 3). For GC-MS-EI-SIM, a full-scan mass spectrum of synthetic 4-methyl-5-nonanol, [(±)-**1**], was obtained to select diagnostic ions [*m/z* = 69, (M^+-C_5H_{11}) 87 and (M^+-C_4H_9) 101]. Synthetic (±)-**1**, hexane, and concentrated weevil-produced compound were then injected in split mode and analyzed by scanning for the diagnostic ions. GC-EAD of synthetic chiral isomers of 4-methyl-5-nonanol was also conducted on this column to determine which stereoisomer was antennally active (Figure 4).

Synthesis. Synthetic (±)-**1** was prepared by slow addition of freshly distilled 2-methyl-1-pentanal in ether to butyl lithium in hexane cooled in an ice-water bath. After the usual work-up distillation of the crude product under reduced pressure yielded 67% of (±)-**1** in >95% purity (1.5:1 *syn*:*anti*). The *syn* and *anti* diastereoisomers of (±)-**1** separated on an SP-1000 column. In analogy to previous work (Perez et al., 1994a), *R. bilineatus*-produced **1** was hypothesized to be the *syn* diastereoisomer, which was confirmed by stereoselective synthesis of all isomers of (±)-**1**. Preparation of (4*R*,5*R*)-**1** (97% ee) and (4*S*,5*S*)-**1** (98% ee) was according to methodology previously reported (Perez, et al. 1994a) for the preparation of chiral isomers of 3-methyl-4-octanol and 5-methyl-4-octanol. Details of the preparation of (4*R*,5*R*)-**1** and (4*S*,5*S*)-**1** is described in another paper (Perez et al. 1994b).

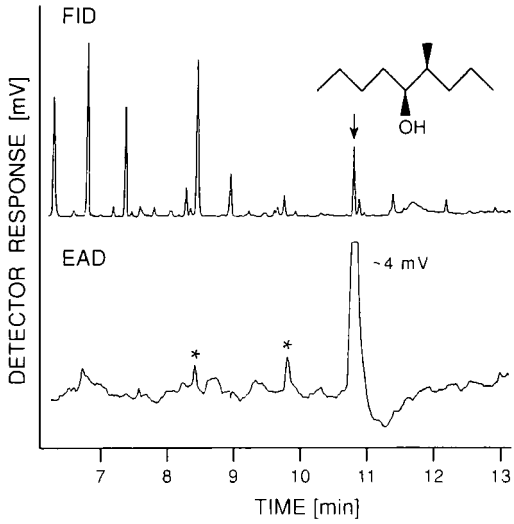

FIG. 2. Flame-ionization (FID) and electroantennographic detector (EAD: female *R. bilineatus* antenna) responses to volatiles obtained from male *R. bilineatus* feeding on apples (*apple-derived components). FID trace: 0.8 weevil hours of an extract containing 4200 weevil hours of volatile production. Column as in Figure 1.

Field Experiments. Field experiments were conducted in coconut-shaded cocoa plantations near Kervera and Vunabang, Papua New Guinea. Modified white 19-liter plastic bucket traps were attached to palms at chest height in randomized blocks with traps at 27-m intervals and blocks 80 m apart (Oehlschlager et al., 1993b). Detergent-laced (0.3%) water (2–3 cm) in the bottom of each trap retained captured weevils. A wire mesh above the water held halved sugarcane stalks. Every three to four days sugarcane was changed and weevils counted.

The first three-treatment experiment tested the attractiveness of (±)-**1** (3 mg/day), sugarcane stalk, and both combined (Figure 5). The second treatment experiment tested attraction of male and female weevils to sugarcane stalk alone and the same combined with (±)-**1** (0.3 mg/day, Figure 6). The third four-treatment experiment tested sugarcane alone and in combination with (±)-**1** released at 0.3, 3.0, or 30 mg/day (Figure 7). The fourth four-treatment experiment tested sugarcane alone and in combination with (±)-**1** (0.4 mg/day), 4*S*,5*S*-**1**, or 4*R*,5*R*-**1** (each stereoisomer released at 0.1 mg/day, Figure 8). Release rates were determined by weight loss of release devices maintained at 25°C. For the low release rate, (±)-**1** was evaporated from three glass capillary tubes (1 mm ID), cut 1 cm above the liquid meniscus and each placed inside

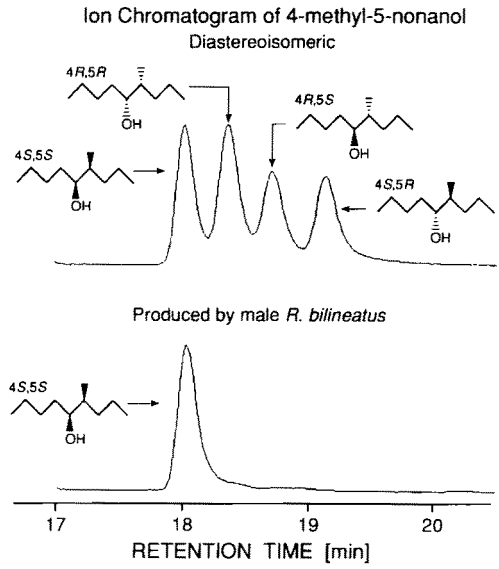

FIG. 3. Selected ion m/z = 69, 87, and 101 chromatogram (Hewlett Packard 5985B GC-MS) of stereoisomeric and weevil-produced **1**. m/z was the parent ion of the full scan EI mass spectrum of $(M^{+}\text{-}C_4H_9)$ 101 or $(M^{+}\text{-}C_5H_{11})$ 87. Analysis on a Cyclodex B column, isothermal 100°C, linear flow velocity of carrier gas 35 cm/sec, split injection and injector temperature 220°C.

capped 300-μl plastic centrifuge tubes with two 2-mm holes near the top. For the medium and high release rates, (±)-**1** was evaporated from 1 or 10 dispensers (heat-sealed plastic bags providing constant release rates under isothermal conditions; ChemTica Internacional, S. A., Apdo. 159-2150, San Jose, Costa Rica).

Statistical Analysis. Assumptions of data normality and homogeneity of variance were tested by graphical assessment of log (variance) versus log (mean), and Bartlett's test, respectively (SAS, 1990). Data were transformed by $(X + 0.5)^{0.5}$ to eliminate heteroscedasicity (Zar, 1984) and were subjected to ANOVA using PROC GLM (SAS) with means compared by Bonferroni's t-test (SAS, 1990).

RESULTS AND DISCUSSION

Analyses of volatiles from males and females revealed several male specific compounds (Figure 1). GC-EAD revealed only one male-specific compound that elicited strong antennal response from male and female weevils (Figure 2).

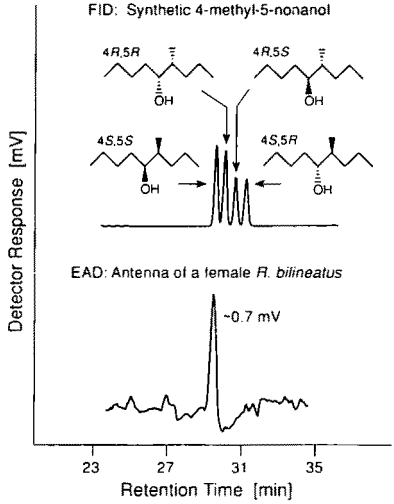

FIG. 4. Flame-ionization (FID) and electroantennographic detector (EAD: female *R. bilineatus* antenna) responses to isomers of 4-methyl-5-nonanol. Column as in Figure 3.

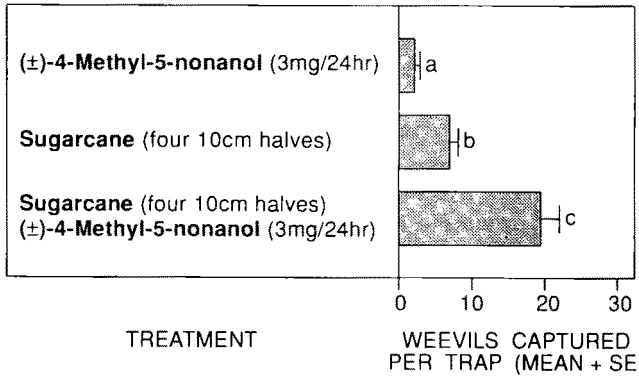

FIG. 5. Mean (+ standard error) captures of *R. bilineatus* in bucket traps baited with sugarcane, (±)-**1**, or both. Experiment ($N = 10$) was conducted in Kervera plantation, November 2–December 4, 1992. Data transformed to approximate homogeneity are presented untransformed. ANOVA, $F = 29.18$; $df = 2,15$; $P < 0.001$. Means followed by the same letter are not significantly different (Bonferonni's t test, $P < 0.05$).

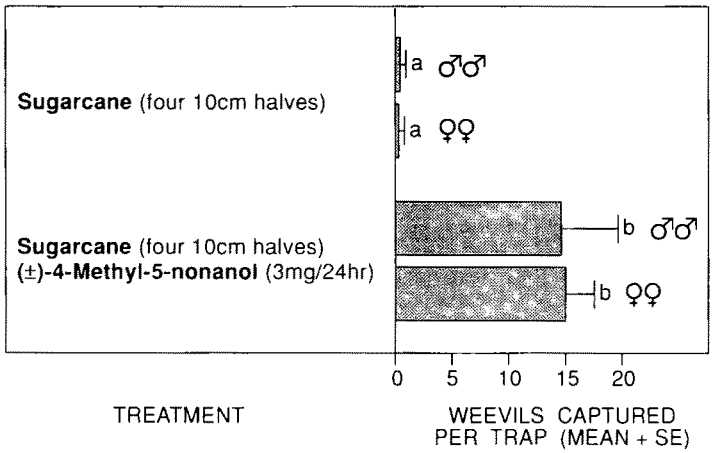

FIG. 6. Mean (+ standard errors) captures of *R. bilineatus* in bucket traps baited with sugarcane or (±)-**1**. Experiment ($N = 4$) was conducted in Vunabang plantation, May 4–14, 1994. Analysis determined there was not a date effect. ANOVA on pooled trap catches for each date, for males $F = 8.84$; $df = 1,6$; $P < 0.02$; for females $F = 35.84$; $df = 1,6$; $P < 0.001$. Means followed by the same letter are not significantly different (Bonferonni's t test, $P < 0.05$).

Mass spectral and retention characteristics of the EAD-active compound were consistent with 4-methyl-5-nonanol [(±)-**1**], the recently identified aggregation pheromone of *R. ferrugineus* and *R. vulneratus* (Hallett et al., 1993a,b; Perez et al., 1993). This nonanol is also a male-specific volatile produced by *R. palmarum* (Hallett et al., 1993a). Analysis by GC-MS of synthetic chiral isomers of **1** and of **1** produced by male *R. bilineatus* on a Cyclodex B column revealed that 4*S*,5*S*-**1** was produced by male *R. bilineatus* (Figure 3). Analysis of synthetic chiral isomers of **1** by GC-EAD revealed that only 4*S*,5*S*-**1** was antennally active (Figure 4).

The first field experiment revealed that (±)-**1** or sugarcane alone was not very attractive, but in combination they caught many weevils (Figure 5). Equal attraction of males and females to traps containing (±)-**1** and sugarcane demonstrated that **1** constitutes an aggregation pheromone (Figure 6). Synergistic attraction of *R. bilineatus* to traps containing both aggregation pheromone and food is consistent with findings in five other species of the Rhynchophorinae (Chinchilla and Oehlschlager, 1992, Oehlschlager et al., 1992a, 1993a,b; Gries et al., 1993, 1994; Hallett et al., 1993a,b; Perez et al., 1993; Weissling et al., 1994).

A third experiment tested sugarcane alone and in combination with (±)-**1**

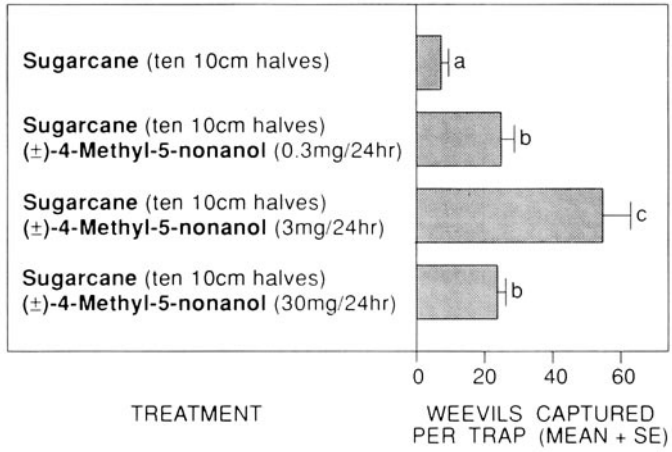

FIG. 7. Mean (+ standard mean error) captures of *R. bilineatus* in bucket traps baited with sugarcane alone or combined with (±)-**1** at three release rates. Experiment (N = 15) conducted at Vanabang plantation, November 25, 1992–January 5, 1993. Data transformed to approximate homogeneity are presented untransformed. ANOVA, $F = 24.67$; $df = 12,67$; $P < 0.0001$. Means followed by the same letter are not significantly different (Bonferonni's t test, $P < 0.05$).

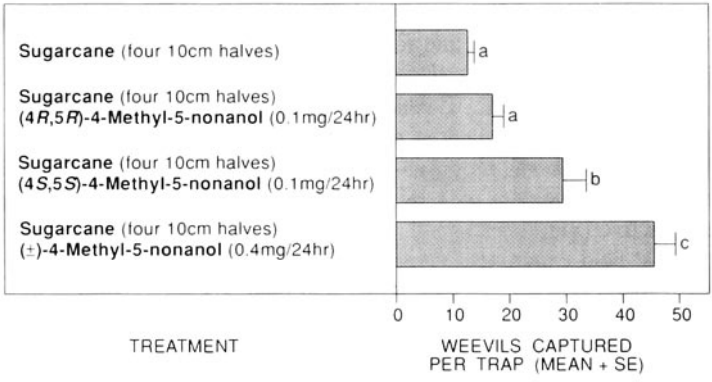

FIG. 8. Mean (+ standard error) captures of *R. bilineatus* in bucket traps baited with sugarcane alone or in combination with (±)-**1**, (4*S*,5*S*)-**1**, or (4*R*,5*R*)-**1**. Experiment (N = 10) at Vimy plantation, January 21–February 22, 1994. Analysis determined there was not a date effect. ANOVA on pooled trap captures for each date gave $F = 22.22$; $df = 3,36$; $P < 0.0005$. Means followed by the same letter are not significantly different (Bonferonni's t test, $P < 0.05$).

released at 0.3, 3.0, or 30 mg/day. Attraction was optimal to those traps from which 3 mg/day of (±)-**1** was released (Figure 7). *R. palmarum* responds equally well to the same three release rates of its pheromone when combined with food, but without food the beetles prefer a 30 mg/day dose (Oehlschlager et al., 1992a, 1993b). In the presence of palm tissue, *R. ferrugineus* is more strongly attracted to traps from which 3 mg/day rather than 0.3 mg/day of (±)-**1** are released (Hallett et al., 1993a,b).

In further field experiments (±)-**1** or 4*S*,5*S*-**1** but not 4*R*,5*R*-**1** enhanced attraction to sugarcane baited traps (Figure 8). The better attractiveness of (±)-**1** than 4*S*,5*S*-**1** is not understood because only the latter is detected in male volatiles (Figure 3) and elicits antennal responses (Figure 4). Interestingly, 4*S*,5*S*-**1** is an aggregation pheromone of *R. ferrugineus* and *R. vulneratus* (Mori, 1993b; Perez et al., 1993). The same stereoisomer of **1** is also produced by male *R. palmarum* (Perez et al., 1993). In Central America, (±)-**1** alone does not enhance attraction of *R. palmarum* to taps containing sugarcane (A.C. Oehlschlager et al., unpublished data).

Production of and antennal response to only one stereoisomer of the aggregation pheromone in *R. bilineatus*, *R. ferrugineus*, and *R. vulneratus* (Perez et al., 1993) is consistent with findings in other Rhynchophorinae. *R. phoenicis* and *R. cruentatus* produce and respond only to (3*S*,4*S*)-3-methyl-4-octanol (Mori et al., 1993a; Perez et al., 1993, 1994a) and (4*S*,5*S*)-5-methyl-4-octanol, respectively (Perez et al., 1994a). Similarly, *R. palmarum* produces and responds to (4*S*,2*E*)-5-methylhepten-4-ol with the antipode being inactive (Oehlschlager et al., 1992a).

The occurrence of 4*S*,5*S*-**1** in the American and three Asian palm weevils supports the hypothesis that the American palm weevil speciated from Asian weevils. This would be consistent with the prevailing theory that palm trees evolved in Southeast Asia, spread through Africa and eventually arrived in the Americas (Harries, 1991). The production of and response solely to 4*S*,5*S*-**1** by *R. ferrugineus*, *R. vulneratus*, and *R. bilineatus* suggest that other mechanisms contribute to reproductive isolation of these weevils. Geographic isolation may allow *R. bilineatus* to use 4*S*,5*S*-**1** as its major pheromone without competition from *R. ferrugineus* or *R. vulneratus*. Sympatric *R. ferrugineus* and *R. vulneratus* are cross-attracted, and males produce the same unique volatiles, suggesting similar communication systems (Hallett et al., 1993a,b). This has raised doubts as to their being separate species (Hallett et al., 1993a,b). Pheromone communication in *R. ferrugineus* and *R. vulneratus* may be different than that of *R. bilineatus* due to the ketone corresponding to **1** that is produced by both the former species (Hallett et al., 1993a) but not by male *R. bilineatus*. The ketone is EAD active in both producing species (Hallett et al., 1993a).

Traps containing (±)-**1**, sugarcane, and detergent-laced water are presently used in Papua New Guinea for management of *R. bilineatus* (R.N.B. Prior and

S. Laup, personal communication). Pheromone-based trapping over one year has proven effective in reducing populations of *R. palmarum* by >80% in commercial oil palm and is used operationally in Central and South America to manage red ring disease vectored by this insect (Oehlschlager et al., 1992b, Chinchilla et al., 1993).

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