Journal of Chemical Ecology, Vol. 20, No. 3, 1994

AGGREGATION PHEROMONE OF PALMETTO WEEVIL, *Rhynchophorus cruentatus* (F.) (COLEOPTERA: CURCULIONIDAE)

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(Received May 26, 1993; accepted October 15, 1993)

Abstract—5-Methyl-4-octanol is the major aggregation pheromone of the palmetto weevil, *Rhynchophorus cruentatus* (F.). The pheromone (cruentol) was identified by coupled gas chromatographic–electroantennographic (GC-EAD) analysis of male-produced volatiles, coupled GC-mass spectrometry (MS) in electron impact and chemical ionization mode, and coupled GC-high resolution MS. In laboratory and field assays, a diastereomeric mixture of synthetic cruentol greatly enhanced attraction of weevils to cabbage palmetto, *Sabal palmetto* (Walter), stem tissue, indicating that cruentol and host volatiles are synergistically attractive. An attractive lure in combination with efficient traps should facilitate development of semiochemical-based management for *R. cruentatus*.

Key Words—Coleoptera, Curculionidae, *Rhynchophorus cruentatus*, palmetto weevil, *S. palmetto*, aggregation pheromone, 5-methyl-4-octanol, cruentol.

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INTRODUCTION

Rhynchophorus cruentatus (F.), the palmetto weevil, is the only species of palm weevils found in the continental United States (Wattanapongsiri, 1966). This large (24- to 33-mm-long) beetle ranges from the Florida Keys through the coastal regions of South Carolina and Texas (Wattanapongsiri, 1966). Unlike several of its congeners, *R. cruentatus* is not considered a major pest of palms. However, this species will attack transplanted or otherwise stressed ornamental palms (Giblin-Davis and Howard, 1988, 1989). In Florida, *R. cruentatus* is sympatric with the native cabbage palmetto, *Sabal palmetto* (Walter) (Woodruff, 1967), a palm often used as mature specimens in landscaping due to its low cost, natural abundance, and high transplanting survivorship.

Volatiles emanating from moribund palms are attractive to *R. cruentatus* males and females (Chittenden, 1902; Wattanapongsiri, 1966; Weissling et al., 1992). Females lay eggs in leaf bases or directly in the wounds of dying host palms, and immature stages develop in the crown and stem region. Recent studies suggest that *R. cruentatus* males produce an aggregation pheromone that plays a substantial role in weevil colonization of susceptible trees (Weissling et al., 1993). This paper addresses the identification and the electrophysiological and behavioral activity of the aggregation pheromone of *R. cruentatus*.

METHODS AND MATERIALS

Volatile Collections. Weevils were field-collected (Weissling, et al., 1992) or laboratory-reared (Giblin-Davis et al., 1989) and maintained on *S. palmetto* tissue until three days before volatile collections. Groups of 30–40 weevils, separated by sex, were placed in modified 9-liter Nalgene desiccators with or without sugarcane (500 g). A charcoal-filtered airstream (1.5 cm^3/min) was maintained through the desiccators for seven days, collecting insect and plant volatiles on 10 g of Porapak Q packed in Pyrex glass tubing (Oehlschlager et al., 1988, 1992). Volatiles were eluted from the Porapak Q with distilled pentane and concentrated by distillation.

Instrumental Methods. Porapak Q extracts were analyzed by gas chromatographic–electroantennographic detection (GC-EAD) (Arn et al., 1975), using a Hewlett Packard (HP) 5885B equipped with a DB-5-coated, fused silica column (30 m × 0.25 mm ID) (J&W Scientific, Folsom, California) (linear flow velocity: 35 cm/sec; injector and detector temperature: 220°C; temperature programming: 70°C (1 min), 10°C/min to 240°C). A coupled HP 5985B GC-mass spectrometer (MS) equipped with a SP-1000-coated, fused silica column (30 m × 0.25 mm ID) (Supelco Inc., Bellefonte, Pennsylvania) was used for GC-MS in both electron impact (EI) (70 eV) and chemical ionization (CI) mode. A Kratos MS80RFA fitted with a DB-5-coated, fused silica column (30 m ×

0.25 mm ID) (J&W Scientific) was used for coupled GC-high resolution MS in EI mode.

Pheromone Synthesis. Racemic 5-methyl-4-octanol (Pinazzi et al., 1976, 1977) was synthesized by reacting butanal at 0°C in diethyl ether with the Grignard reagent of 2-bromopentane to give the corresponding alcohol as a diastereoisomeric mixture. Analytical data were as follows: MS (EI), *m/z* (percent relative abundance): 101 (13), 83 (28), 73 (100), 55 (83), 43 (34); ^{1}H NMR (relative to TMS): δ 0.95 (9H, m); δ 1.23 (4H, m); δ 1.45 (4H, m); δ 1.5 (1H, br s, D_2O exchangeable); δ 3.48 (1H, m). ^{13}C NMR [diaspereoisomeric] ($CDCl_3$, ppm); 75.91, 75.04, 38.70, 38.07, 36.81, 35.73, 34.26, 20.54, 20.51, 19.51, 19.37, 15.33, 14.23, 14.38, 14.21, 13.65; analytical calculation for $C_9H_{20}O$: C, 74.92; H, 13.98; found: C, 74.71; H, 13.96. The alcohol was purified by flash chromatography (SiO_2, 60% hexanes/diethylether) to afford 5-methyl-4-octanol (90% yield) as a colorless liquid (98% purity).

Laboratory Assay. Weevil response to synthetic pheromone and host-palm volatiles was tested in a binary choice, Y-tube olfactometer (Weissling et al., 1993) with a humidified airstream at 250 ml/min (27°C). For each experiment, 60–100 weevils of each sex were individually tested for 5 min. A positive response was recorded if a weevil walked at least 3 cm into one arm of the Y-tube. Experiments 1–3 tested pheromone released at three rates versus humidified clean air. For the medium (120 $\pm$ 10.6 μg/hr) and high (1300 $\pm$ 194 μg/hr) release rates, one and four 40-μl hematocrit tubes (Fisher Scientific, Pittsburgh, Pennsylvania), respectively, loaded with 10 μl of pheromone were placed in 300-μl plastic centrifuge tubes with two 3-mm-diam. holes drilled 5 mm below the sealed top. For the low release rate (0.18 $\pm$0.02 μg/hr), one 5-μl microcap tube loaded with 5 μl of pheromone was placed in a 300-μl centrifuge tube. Release rates for the high and medium treatments were determined gravimetrically, whereas release of the low rate treatment was determined volumetrically ($N = 6$). Experiments 4 and 5 tested pheromone (1300 μg/hr) alone and pheromone combined with *S. palmetto* tissue (50 g, 24–72 hr old) versus palm tissue. A final experiment tested pheromone (1300 μg/hr) combined with palm versus pheromone alone.

Field Experiments. Four field experiments were conducted in a 300-ha pasture interspersed with *S. palmetto* and saw palmetto, *Serenoa repens* (Bartr.) 12 km south of La Belle, Florida, using complete randomized blocks with traps at 20-m intervals and blocks 300 m apart. Two types of traps were used. "Live traps" were constructed from 19-liter black plastic buckets covered with tops made from polyvinyl chloride tubes (5 cm long, 2.4 cm inside diameter) glued together longitudinally (Weissling et al., 1992). These traps allow weevils in but prevent escape and provide harborage for sustenance. "Lethal traps" (Weissling et al., 1993), similar in exterior appearance to live traps, were also used. However, weevils entering these traps cannot escape and drown in soapy

water. In experiments 1 and 2, live traps were suspended 1.5 m above the ground from *S. palmetto* trees, whereas in experiments 3 and 4, lethal traps were placed on the ground. Ten microliters of synthetic pheromone loaded into 40-μl capillary tubes were placed four per 300-μl microcentrifuge tube as described above. Unless otherwise indicated, this served as the standard release device for all tests. Centrifuge tubes were suspended with a copper wire inside traps 10 cm from the top. Release rates of pheromone were determined gravimetrically ($N = 4$) at 24-hr intervals for seven days.

The first four-treatment, four-replicate experiment tested an unbaited trap (control), the pheromone alone, fresh *S. palmetto* bud and distal stem tissue (2.5 kg) alone, and pheromone and palm combined. All traps without palm tissue in this and subsequent experiments contained four water-moistened sponges. Tests were conducted from March 24 to April 21, 1992. Captured weevils were removed from traps weekly, and clean traps were baited and rerandomized within each block. To determine if the weevil population in the study site was too low for extended testing (indicated by high recapture of the same insects), captured weevils were marked by scratching a number on the metathoracic sternal plate with a dissecting probe and were released in the middle of the study site.

The second experiment compared pheromone released from the standard device versus pheromone released at 10 times this rate. For the high release rate, 80 μl of pheromone was added to 300 μl microcentrifuge tubes packed with cotton. Each release rate of pheromone was tested alone and combined with 2.5 kg *S. palmetto* tissue. Traps were placed in the field as described above (five blocks) from April 20 to 27, 1992.

In the third, five-replicate experiment, the influence of semiochemicals released by trapped weevils on other weevils was removed by using lethal traps. Treatments were identical to those in the first experiment. Tests were conducted from June 5 to 12, and 12 to 19, 1992. Traps were cleaned, rebaited, and rerandomized between test periods.

In the fourth, four-replicate experiment, lethal traps were baited with live *R. cruentatus* males to determine if male-produced volatiles enhanced attraction to synthetic pheromone. Treatments included traps baited with 1.5 kg of palm tissue plus 10 *R. cruentatus* males, 1.5 kg of palm tissue plus pheromone, and 1.5 kg palm tissue plus both 10 *R. cruentatus* males and pheromone. Tests were conducted from July 2 to 9, and 9 to 16, 1992.

Statistical Analyses: Data from laboratory assays were converted to percent attractancy [(number of insects entering a sample arm/total number responding) $\times$ 100], and analyzed by the Kruskal-Wallis test (SAS Institute, 1985). Data from field tests were square root transformed ($x + 0.01$) and analyzed by analysis of variance using PROC ANOVA (SAS Institute, 1985). Treatment effects were estimated both over time and within time periods. Least significant differ-

ence tests (SAS Institute, 1985) were used for mean separation where significant ($P < 0.05$) statistical differences occurred.

RESULTS

Volatile Collections. Analysis (GC-EAD) of volatiles from fed and unfed male weevils revealed a strongly EAD-active compound (Figure 1). Mass spectra of the candidate pheromone (Figure 2) suggested a methyl-branched, secondary alcohol with a molecular weight of 144 (M − 1 in the CI spectrum of Figure 2). The high-resolution mass spectrum revealed that fragment *m/z* 73 contained one oxygen, indicating that the hydroxyl group was bonded to either C-2, C-3, or C-4. Based on mass spectrometric data and retention index cal-

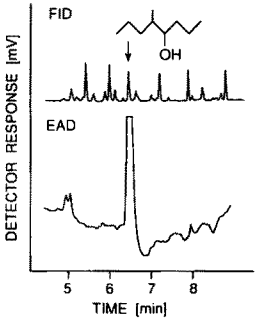

FIG. 1. Flame ionization detector (FID) and electroantennographic detector (EAD) responses to volatiles collected for seven days from unfed *R. cruentatus* males. The antennal recording (EAD) was carried out with a *R. cruentatus* male antenna. Gas chromatographic conditions: linear flow velocity: 35 cm/sec, injector and detector temperatures: 220°C, temperature programming: 70°C (1 min), 10°C/min to 240°C; DB-5 column (30 m × 0.25 mm ID).

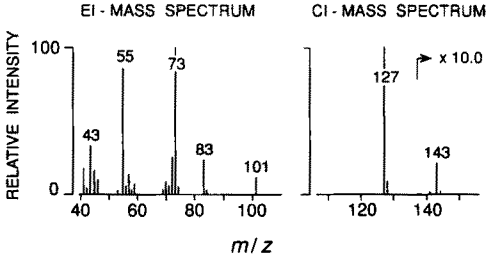

FIG. 2. Mass spectra of 5-methyl-4-octanol.

culations with authentic methyl-branched secondary octanols and nonanols, we hypothesized that the candidate pheromone was 5-methyl-4-octanol. Identical mass spectra, identical Kovats retention indices on two columns with different retention characteristics (DB-5: 1060, DC-23: 1388) and similar GC-EAD responses to equivalent amounts of synthetic 5-methyl-4-octanol and the male-produced compound confirmed the structural assignment.

Laboratory Assay. More male and female *R. cruentatus* were attracted to synthetic 5-methyl-4-octanol (all rates) than to the clean air control (Figure 3, experiments 1–3). Attraction of males to pheromone (1300 μg/hr) exceeded that to palm tissue (Figure 3, experiment 4). The combination of pheromone (1300 μg/hr) and palm tissue was more attractive to males and females than palm alone and more attractive to females than pheromone alone (Figure 3, experiments 5 and 6).

Field Experiments. Racemic 5-methyl-4-octanol was released from the standard device at the rate of 0.41 ± 0.03 mg/day (range: 0.2–0.9 mg/day). Marked weevils comprised 1.5% of total capture (N = 852 weevils) in live traps from March 24 to April 27, 1992, indicating a large population in the study site area.

Treatment effects in experiment 1 were estimated separately for each collection date because of a significant treatment × date interaction (male: F = 5.8; df = 9, 25; $P < 0.01$; females: F = 3.6; df = 9, 25; $P < 0.01$). 5-Methyl-4-octanol was effective at increasing capture of *R. cruentatus* males and females in live traps baited with host-palm tissue (Figure 4). More weevils were caught

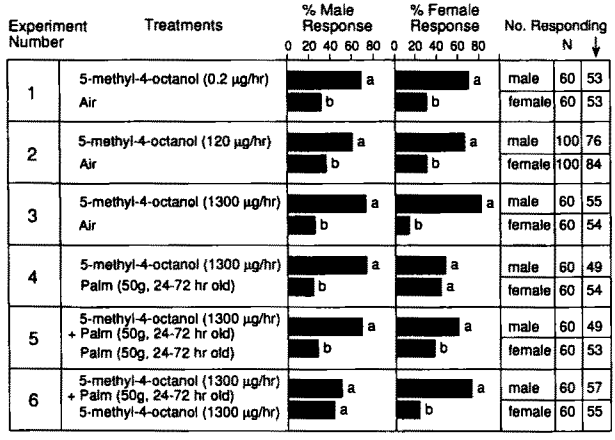

FIG. 3. Response of individual male and female *R. cruentatus* to various Y-tube olfactometer treatments. Weevil responses within a gender, followed by the same letter are not significantly different ($P < 0.05$; Kruskal-Wallis test).

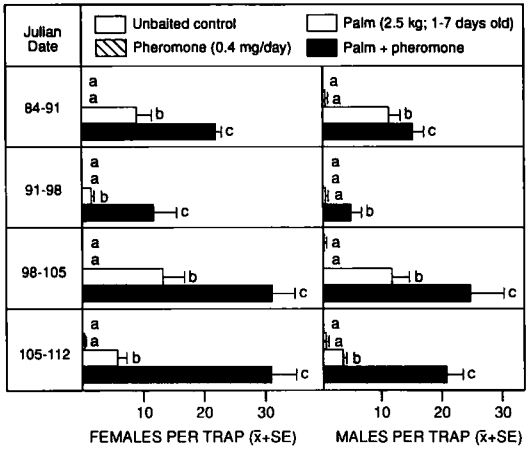

FIG. 4. Mean (+SEM) capture of *R. cruentatus* in live traps baited with *S. palmetto* tissue, (±)-5-methyl-4-octanol, or both combined, La Belle, Florida, 1992. Means within a sex and julian date grouping followed by the same letter are not significantly different ($P < 0.05$; least significant difference).

in traps baited with palm and pheromone than with any other treatment each week. In addition, more weevils were caught in traps baited with palm tissue than in unbaited traps or in traps baited with pheromone during weeks 1, 3, and 4 (Figure 4).

Low (0.4 mg/day) and high (4 mg/day) release of pheromone equally enhanced attraction of weevils to palm tissue (means ± SEM, males; low rate pheromone: 0 ± 0, high rate pheromone: 0 ± 0; palm plus low rate pheromone: 23.0 ± 8.3, palm plus high rate pheromone: 13.0 ± 1.0; females, low rate pheromone: 0.3 ± 0.3, females, high rate pheromone: 0.3 ± 0.3; palm plus low rate pheromone: 33.5 ± 11.8; palm plus high rate pheromone: 22.0 ± 4.0). Significantly more weevils were caught in traps baited with palm tissue and pheromone than in traps baited with pheromone alone (males: $F = 29.7$; $df = 3, 9$; $P < 0.01$, females: $F = 26.6$; $df = 3, 9$; $P < 0.01$).

Weevils captured in lethal traps were killed before contributing to trap attraction. Ten to 15 times more weevils were captured in traps baited with palm plus pheromone than with any of the other treatments (Figure 5). In addition, more females were caught in traps baited with pheromone than in unbaited control traps (Figure 5).

The addition of live conspecific males to lethal traps baited with palm tissue and pheromone did not enhance weevil capture over traps baited only with palm plus pheromone. More males and females were caught in traps baited with palm

tissue plus both 10 conspecific males and pheromone than in traps baited with palm plus 10 males (Figure 6). Additionally, more weevils were caught in traps baited with palm plus pheromone than in traps baited with palm plus 10 males (Figure 6).

DISCUSSION

Following the demonstration of a male-produced aggregation pheromone in *R. cruentatus* (Weissling et al., 1993), we have identified the compound as 5-methyl-4-octanol, and propose the trivial name "cruentol." Cruentol and palm

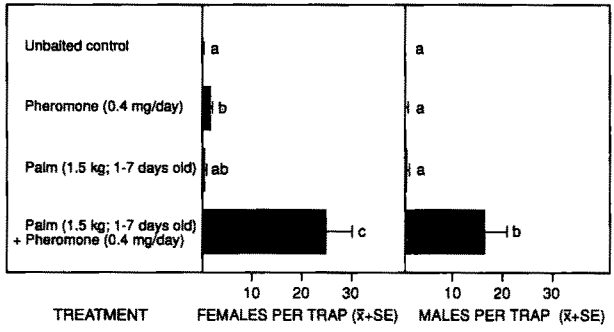

FIG. 5. Mean (+SEM) capture of *R. cruentatus* in lethal traps baited with *S. palmetto* tissue, (±)-5-methyl-4-octanol, or both combined, La Belle, Florida, June 5–19, 1992. Means within a sex followed by the same letter are not significantly different ($P < 0.05$; least significant difference), treatment effects; males: $F = 25.9$; $df = 3, 12$; $P < 0.01$; females: $F = 38.0$; $df = 3, 12$; $P < 0.01$.

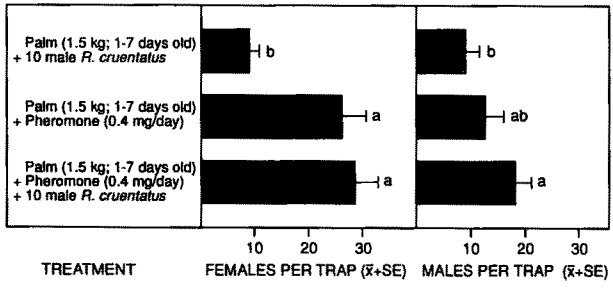

FIG. 6. Mean (+SEM) capture of *R. cruentatus* in lethal traps baited with *S. palmetto* tissue in combination with either (±)-5-methyl-4-octanol, 10 *R. cruentatus* males, or both, La Belle, Florida, July 2–16, 1992. Means within a sex followed by the same letter are not significantly different ($P < 0.05$; least significant difference), treatment effects; males: $F = 6.0$; $df = 2, 6$; $P < 0.04$; females: $F = 6.9$; $df = 2, 6$; $P < 0.03$.

tissue together were as attractive as cruentol, palm, and 10 conspecific males together (Figure 6), suggesting that cruentol is the only pheromone essential for optimal attraction.

Male-produced pheromones have been identified from the curculionid genera *Pissodes* (Booth et al., 1983; Phillips et al., 1984), *Sitophilus* (Phillips et al., 1985; Schmuff et al., 1984), *Sitona* (Blight et al., 1984), *Metamasius* (Perez et al., 1994), and *Rhynchophorus*. In the Rhynchophorinae, aggregation pheromones recently have been identified from the American palm weevil, *R. palmarum* (L.) (Rochat et al., 1991; Oehlschlager et al., 1992), the African palm weevil, *R. phoenicis* (F.) (Gries et al., 1993), and the Asian palm weevils, *R. vulneratus* (Panz.), *R. ferrugineus* (Oliv.) (Hallett et al., 1993), and *R. bilineatus* (Mont.) (Oehlschlager et al., 1994). Unlike their Asian congeners, which produce methyl-branched, secondary nonanols, and unlike *R. palmarum*, which produces a methyl-branched heptenol, *R. cruentatus* and *R. phoenicis* produce methyl-branched, secondary octanols as aggregation pheromones.

In olfactometer tests, cruentol at all release rates was more attractive to walking weevils than the clean air control. It was also more attractive to males than palm tissue (Figure 3). In field tests, however, very few weevils were caught in traps baited only with cruentol, even when cruentol was released at 4 mg/day (experiment 2). We have demonstrated that neither palm tissue or cruentol are efficient trap baits when used alone in lethal traps and that the palm tissue and cruentol grouping is very synergistic (Figure 5). As cruentol by itself did not attract weevils into traps but into the general study area (T.J.W. and R.M.G.-D., personal observation), we hypothesize that the pheromone functions mainly as a long-range attractant, whereas host-plant volatiles are required for weevils to orient to and enter traps. Synergistic combinations of plant- and weevil-produced volatiles have also been demonstrated in *Pissodes nemorensis* Germar (= *P. approximateus*, synonymized by Phillips et al., 1987) (Booth et al., 1983), *Sitona lineatus* L. (Blight and Wadhams, 1987), *R. palmarum* (Oehlschlager et al., 1992), *R. phoenicis* (Gries et al., 1993), *R. vulneratus* and *R. ferrugineous* (Hallett et al., 1993), and *R. bilineatus* (Oehlschlager et al., 1994).

The identification of cruentol as a male-produced aggregation pheromone in *R. cruentatus* is an important step towards understanding the biology and chemical ecology of this insect and furthers the development of management programs. While a stereoisomeric mixture of synthetic cruentol combined with palm tissue attracted large numbers of weevils, optimal attraction may require only one stereoisomer in combination with synergistic host volatiles. In addition, several early fermentation products of palm sap found to be attractive with cruentol (Giblin-Davis et al., 1994) can be used in place of palm tissue to further simplify trapping.

Acknowledgments—We thank J. Cangiamila, B. Center, and M. Stanaland for technical assistance, and A. Duda and Sons for providing *S. palmetto* trees and research sites. We are also grateful to R. Scheffrahn and B. Howard for critically reviewing an earlier version of this manuscript. This research was supported in part by a USDA Special Grant in Tropical and Subtropical Agriculture CRSR-90-34135-5233 to R.M.G.-D., R.H. Scheffrahn, and J.P. Toth. This manuscript is Florida Agricultural Research Station Journal Series R-03197.

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