FIELD RESPONSE OF *RHYNCHOPHORUS CRUENTATUS* (COLEOPTERA: CURCULIONIDAE) TO ITS AGGREGATION PHEROMONE AND FERMENTING PLANT VOLATILES

ROBIN M. GIBLIN-DAVIS,[1] THOMAS J. WEISSLING,[1,2] A. C. OEHLSCHLAGER,[3] AND LILLIANA M. GONZALEZ[3]

[1]Fort Lauderdale Research and Education Center,
University of Florida, Institute of Food and Agricultural Sciences
3205 College Avenue, Fort Lauderdale, FL 33314

[2]Present Address: USDA-ARS, 3706 W. Nob Hill Blvd., Yakima, WA 98902

[3]Department of Chemistry, Simon Fraser University,
Burnaby, British Columbia, V5A 1S6, CANADA

ABSTRACT

Semiochemicals from 2.5 kg of chopped stem tissue from cabbage palmetto, *Sabal palmetto* (Walter), frozen or fresh stem tissue from sugarcane, *Saccharum officinarum* L., or syncarp tissue from pineapple, *Anana comosus* (L.), were equally suitable for field attraction of *Rhynchophorus cruentatus* (F.) when used with 0.4 mg/d of its aggregation pheromone, 5-methyl-4-octanol (cruentol). Twenty-eight different chemicals known to be fermentation products from palm sap were screened with 0.4 mg/d cruentol for field attraction of *R. cruentatus* adults. Good chemically-mediated field trapping of *R. cruentatus* was achieved with cruentol plus ethyl acetate (852 mg/d) and to a lesser degree with each of the following: (*S*)-(-)-ethyl lactate (release rate not determined; ND), ethyl isobutyrate (40 mg/d), ethyl butyrate (255 mg/d), or ethanol (51 mg/d). However, none of the test chemicals with cruentol were as effective as 1.5 kg of fermenting sugarcane or *S. palmetto* tissue plus cruentol. Also, none of these chemicals were attractive by themselves at the rates tested. A combination of individually released ethanol (48 mg/d), ethyl acetate (131 mg/d), ethyl butyrate (34 mg/d), ethyl isobutyrate (40 mg/d), and (*S*)-(-)-ethyl lactate (ND) with cruentol was as effective for the capture of *R. cruentatus* as cruentol plus any of the individual components at the rates tested. Several trap designs were evaluated for future research and implementation of semiochemically-mediated monitoring and management of *R. cruentatus*.

Key Words: Chemical ecology, palmetto weevil, semiochemicals, field trapping.

RESUMEN

Semioquímicos de 2.5 kg de tejido picado del tallo del palmetto de col, *Sabal palmetto* (Walter) tejido de caña de azucar, *Saccharum officinarum* L. o congelado o fresco, y tejido de sincarpo de piña, *Anana comosus* (L). fueron igualmente satisfactorios para atracción en el campo de *Rhynchophorus cruentatus* (F.) cuando fué usado con 0.4 mg/d de su feromona de agregación, 5-metilo-4-octanol (cruentol). Veintiocho diferentes químicos conocidos como productos de la fermentación de la savia de las palmeras fueron examinados con 0.4 mg/d cruentol para atracción en el campo de los adultos de *R. cruentatus*. Se logró buen atrapamiento de campo de *R. cruentatus* con cruentol más acetato de etilo (852 mg/d), y a un nivel menos con cada uno de las combinaciones siguientes: (S)-(-) lactato de etilo (razon de liberar no determinado; ND), isobutirato de etilo (40 mg/d), butirato de etilo (255 mg/d), etanol (51 mg/d). Sin embargo, nignuno de los químicos con cruentol fueron tan efectivo como 1.5 kg de caña en fermentación o tejido de *S. palmetto* más cruentol. Además, ninguno de estos químicos fueron atractivos

por si mismo a las concentraciones examinadas. Una combinación de etanol (48 mg/d), acetato de etilo (131 mg/d), butirato de etilo (34 mg/d), isobutirato de etilo (40 mg/d) y (S)-(-)-lactato de etilo (ND) liberados individualmente con cruentol fue efectivo para la captura de *R. cruentatus* como cruentol más cualquiera de los componentes individuales por las razones examinadas. Se evaluaron varios diseños de trampas para investigaciones futuras y para implemetación de monitoreo y control de *R. cruentatus* basado en semioquímicos.

Rhynchophorus cruentatus (F.) is a pest of stressed palms in the southeastern United States (Giblin-Davis & Howard 1989). Feeding damage by the larvae of this weevil is difficult to detect before the regenerative apical meristem (bud) of the palm has been destroyed (Giblin-Davis & Howard 1988). The congeneric palm weevil, *R. palmarum* (L.) is the major vector of the red ring nematode, *Bursaphelenchus cocophilus* (Cobb), which causes the lethal and economically important red ring disease of coconut (*Cocos nucifera* L.) and African oil palm (*Elaeis guineensis* Jacq.) in the southern Caribbean, Mexico, and Central and South America (Giblin-Davis 1993). Other species of *Rhynchophorus* may be capable vectors of the red ring nematode as well (Giblin-Davis 1993).

Recently, male-produced aggregation pheromones have been identified for six species of *Rhynchophorus* (Gries et al. 1993, Hallet et al. 1993, Oehlschlager et al. 1992, 1993b, Rochat et al. 1991, 1993, Weissling et al. 1994). The aggregation pheromone for *R. cruentatus* has been identified as 5-methyl-4- octanol (cruentol) (Weissling et al. 1994). Semiochemicals from fermenting host tissue and male-produced aggregation pheromones interact synergistically to provide enhanced trapping of *Rhynchophorus* spp. (Gries et al. 1993, Hallet et al. 1993, Oehlschlager et al. 1992, 1993a,b, Weissling et al. 1994). In nature, *Rhynchophorus* spp. are normally attracted to wounded, stressed, or dying palms (Wattanapongsiri 1966) and male-produced aggregation pheromones apparently aid in recruitment of weevils of both sexes to patchily distributed resources.

Identification of host-derived semiochemicals for *Rhynchophorus* spp. would allow for the development and production of longer lasting and more easily implemented traps when used with their specific aggregation pheromones. Chemically-mediated traps would have applications in monitoring and management attempts for red ring nematode-infested *R. palmarum* or other species of *Rhynchophorus*.

The cabbage palmetto, *Sabal palmetto* (Walter), is the most frequently reported host of *R. cruentatus* (Giblin-Davis & Howard 1989). Freshly chopped *S. palmetto* crown and stem tissue is a reliable bait for trapping *R. cruentatus* and is most attractive 24-72 h after harvest (Weissling et al. 1992). However, *S. palmetto* is labor and time consuming to procure for short duration field attractancy studies. A more convenient alternative plant tissue with similar attractancy when released with cruentol is needed for future work. In addition, we wanted to see if there was something unique about fermenting *S. palmetto* tissue or if ubiquitous fermentation products from a variety of plant tissues would be attractive with cruentol for *R. cruentatus* adults.

The purpose of this study was to field-evaluate the attraction of different fermenting plant tissues with cruentol to *R. cruentatus* adults. We also took a pragmatic approach to field-evaluating the attractancy of a number of volatile fermentation products when used alone or released with cruentol for the capture of *R. cruentatus* adults. Lastly, several trap designs were evaluated for future experiments with semiochemically-mediated trapping of *R. cruentatus*.

MATERIALS AND METHODS

Field Response of Weevils to Fermenting Plant Tissues and Cruentol

A trap for capturing and retaining live *R. cruentatus* adults (Weissling et al. 1992) was used for comparisons of the attractancy of different fermenting plant tissues with cruentol. Briefly, each trap consisted of a 19-liter black, high density polyethylene (HDPE) bucket (United States Plastic Corp., Lima, OH) with a 32-cm diam composite PVC pipe lid (2.4 cm ID for each individual PVC tube). Each trap had two 5-mm diam holes drilled into the bottom for drainage and was suspended in a *S. palmetto* tree with nylon cord so that the bottom of the bucket was about 0.5 m from the ground.

Experiment No. 1 was conducted 11-18 May 1992 and the treatments were 2.5 kg of chopped tissue from the following fresh sources; 1) *S. palmetto* stem and crown, 2) sugarcane, *Saccharum officinarum* L., stem, 3) pineapple, *Anana comosus* (L.), syncarp, and 4) saw palmetto, *Serrenoa repens* (Bartram), stem (Table 1).

Racemic 5-methyl-4-octanol (cruentol) was synthesized (> 95% purity) and released from four 40 µl capillary tubes with 10 µl each of pheromone. Each capillary tube was cut 1 cm above the meniscus and placed into a 300 µl microcentrifuge tube with two 3-mm holes drilled 5 mm below the sealed top (release rate 0.4 mg/d @ 27-34° C) as described by Weissling et al. (1994) for all experiments in this paper.

Experiment No. 2 was conducted 5-12 June 1992 and the treatments were the same as for experiment No. 1 except that chopped frozen sugarcane stem replaced the saw palmetto stem (Table 1).

Traps for both experiments were arranged in a randomized complete block design in a 300 ha pasture interspersed with *S. palmetto* and *S. repens* at the A. Duda & Sons, Inc. citrus unit, 12 km south of La Belle, Hendry Co., FL. Traps were spaced about 20 m apart within blocks and blocks were separated by ≥ 300 m. There were four blocks for experiment No. 1 and five for experiment No. 2. Total numbers of adult male and female *R. cruentatus* collected per trap for each 7-day trapping period were used for analysis. Data were transformed $(x + 0.5)^{0.5}$ and a Statistical Analysis System general linear models procedure (SAS Institute 1985) for unbalanced analysis of variance (ANOVA) was used. Untransformed means are presented. A Waller-Duncan k-ratio t-test ($k = 100$, $P \leq 0.05$) was used for mean separations.

Weevil Attraction to Fermentation Products and Cruentol

Chemicals were chosen for preliminary evaluations, with or without cruentol, for attractancy to *R. cruentatus* from a list of empirically-tested chemicals that (1) elicited *R. palmarum* electroantennogram (EAG) activity greater or equal to the response to 72 h fermented sap from African oil palm (Rochat 1987), and/or (2) from a list of characterized volatiles from fermented African oil palm sap (palm wine) (Nagnan et al. 1992), and/or (3) from a list of major volatiles from Sri Lankan arrack (coconut palm wine distillate)(Samarajeewa et al. 1981). The chemicals were obtained from Aldrich Chemical Co. (ACC), Eastman Kodak Co. (EKC), Fisher Scientific (FS), Matheson, Coleman & Bell Manufacturing Chemists (MCB), or Sigma Chemical Co. (SCC) and were ≥ 85% chemically pure.

Preliminary studies included Y-tube screening of certain chemicals versus air (zero grade) for *R. cruentatus* attraction (N = 10 or 20 weevils of each sex) (see Weissling et al. 1993 for Y-tube methods). The following chemicals were tested in the Y-tube olfactometer: *cis*-2-hexen-1-ol (92%; ACC), citronellal (93%; EKC), 95% ethanol, ethyl butyrate (99%; ACC), ethyl isobutyrate (99%; ACC), ethyl (*S*)-(-)-lactate (98%; ACC), 1-hexanol (98%; EKC), isoamyl acetate (99%; SCC), and 2-methyl-1-propanol (99.9%; FS).

TABLE 1. ATTRACTION OF *RHYNCHOPHORUS CRUENTATUS* ADULTS TO FIELD TRAPS DESIGNED FOR LIVE INSECT CAPTURE AND BAITED WITH DIFFERENT PLANT TISSUES (2.5 KG) AND SYNTHETIC 5-METHYL-4-OCTANOL (RACEMIC MIXTURE OF CRUENTOL RELEASED AT 0.4 MG/DAY) IN HENDRY CO., FLORIDA FOR 7-DAY SAMPLING PERIODS.

Treatment	No. of Replicates	No. Weevils Per Trap (Mean + S.E.)[1] Females	Males
Experiment no. 1 (11-18 May 1992)			
Chopped *Sabal palmetto* stem	4	3.8 ± 2.3ab	2.8 ± 1.3a
Chopped fresh sugarcane stem	4	5.8 ± 1.3a	2.5 ± 2.3a
Fresh pineapple (quartered)	4	2.3 ± 0.6ab	3.5 ± 0.9a
Chopped *Serrenoa repens* stem	4	0.0 ± 0.0b	0.0 ± 0.0b
Experiment no. 2 (5-12 June 1992)			
Chopped *Sabal palmetto* stem	4	15.4 ± 2.7a	6.9 ± 2.1a
Chopped fresh sugarcane stem	5	15.2 ± 6.1a	7.2 ± 4.0a
Fresh pineapple (quartered)	5	10.4 ± 2.5a	4.2 ± 1.2a
Chopped frozen sugarcane stem	4	7.0 ± 1.1a	3.8 ± 1.8a

[1]Means followed by the same letter are not significantly different according to a Waller-Duncan k-ratio t-test on $(x + 0.5)^{0.5}$ transformed data ($k = 100$, $P \leq 0.05$). Untransformed means are presented.

All chemicals except citronellal and 1-hexanol were screened for *R. cruentatus* attractancy in combination with cruentol (0.4 mg/day) in single replicate field response trials during September 1992. In addition, the following chemicals were screened for attractancy with cruentol; acetaldehyde (99%; FS), 1-butanol (99.9%; FS), *n*-butyl acetate (99%; ACC), ethyl acetate (99%; MCB), ethyl caprate (99%; ACC), ethyl caproate (99%; ACC), ethyl caprylate (99%; ACC), glacial acetic acid (99.7%; FS), hexanoic acid (99%; ACC), isobutyric acid (99%; ACC), isopropyl acetate (99.9%; EKC), isovaleric acid (99%; ACC), lactic acid (85%; FS), methanol (99%; FS), 3-methyl-1-butanol (99%; FS), 1-pentanol (99%; FS), octanoic acid (99.5%; ACC), 1-propanol (99%; FS), and α-terpineol (98%; ACC). One ml of each test chemical was placed in a 2-ml Gold Brand® ampule (designated AMP) with a 4 mm diam opening (Wheaton, Millville, NJ). The AMP was put into a 25.9 ml polystyrene vial with wires for hanging it in the trap.

The lethal trap described by Weissling et al. (1993) was used for these preliminary studies and modified for the subsequent replicated field studies. The trap consisted of a 19-liter black HDPE bucket with a composite PVC pipe lid. In the preliminary screenings, a 4.8-liter, tapered, beige, two-part flower pot (opening covered with a galvanized hardware mesh cone with 6 mm openings) was used to house the test stimulus. Tissue controls or test chemicals were placed inside the inverted flower pot and it was suspended 3 cm from the composite lid by two wires. The buckets were positioned in the field, filled with about 2 liters of tap water and 50-60 g of Alconox detergent (Alconox, Inc. New York, NY), and anchored with two metal bars through the composite lid into the ground. Traps were arranged in a completely randomized design in the pasture at A. Duda & Sons, Inc. and spaced ≥ 20 m apart.

Chemicals that elicited significant attraction from *R. cruentatus* in the Y-tube olfactometer and/or lured ≥ 6 weevils per trap after 7 days in the field during September 1992 were selected for a replicated field evaluation. These were ethanol, ethyl acetate,

ethyl butyrate, ethyl caproate, ethyl caprylate, ethyl isobutyrate, ethyl (*S*)-(-)-lactate, isopropyl acetate, lactic acid, *n*-butyl acetate, and octanoic acid. Each of these chemicals was tested using the AMP release device with or without 0.4 mg/d of cruentol.

Three other chemical release devices were used besides the AMP design to modulate release rates for selected chemicals: 1) 5 ml of test chemical in a 14.8 ml glass vial (OD x H = 21 x 70 mm) with a screw top with a 4 mm diam hole (designated 4DH), 2) 5 ml of test chemical in a 14.8 ml glass vial without a top (11.4 mm diam opening) (designated 4NT), and 3) 25 ml of test chemical in a 40.7 ml glass vial (OD x H = 28 x 108 mm) without a top (17.8 mm diam opening) (designated 11NT). Release rates were quantified gravimetrically at the end of each 7-day trapping period. Transportation of chemicals to and from the field was done with lids tightly sealed onto vials or with plastalina modeling clay (Van Aken International, Rancho Cucamonga, CA) which was used to plug ampules. Chemicals were placed in a 120-ml HDPE specimen container (Fisher Scientific Co., Pittsburgh, PA) (4DH, 4NT, and 11NT designs) or a 25.9 ml polystyrene vial (AMP design) which was suspended from the inside of the test stimulus container by wires.

Chemicals with relatively high weevil counts in the preliminary study were tested using at least one additional release device to the AMP device with and without cruentol. These chemicals and the release devices used were: ethanol (4DH, 11NT), ethyl acetate (4DH, 11NT), ethyl butyrate (4NT, 11NT), ethyl isobutyrate (4NT), and ethyl (*S*)-(-)-lactate (4NT).

The previously described lethal trap design was modified slightly for this experiment. The stimulus container was changed from the flower pot to a translucent white, 946 ml capacity, HDPE container (Fisher Scientific Co., Pittsburgh, PA). An 8.5-cm diam hole was cut in the lid of the container and a 9.5-cm diam piece of aluminum screening (1.5 mm square openings) was glued to the inside circumference of the lid. These lids were used to retain 0.5 kg of chopped *S. palmetto* stem and crown tissue within the inverted container. The controls were 0.5 kg of *S. palmetto* tissue with or without cruentol and an empty trap with or without cruentol. Traps of the old design with an inverted flower pot stimulus container with 1.5 kg of chopped *S. palmetto* tissue with or without cruentol were also included for comparison with previous studies of *R. cruentatus* attraction (Weissling et al. 1993, 1994).

Traps were spaced 20 m apart in a completely randomized design and treatments were replicated weekly for ten consecutive 7-day sampling periods starting 6 October 1992 and running through 15 December 1992 at the pasture at A. Duda & Sons, Inc. Total trap counts of *R. cruentatus* adults were transformed and analyzed as previously described.

The lethal trap was modified again for a field experiment to test the efficacy of cruentol and ethanol (AMP), ethyl acetate (4DH), ethyl butyrate (4NT), ethyl isobutyrate (AMP), ethyl (*S*)-(-)-lactate (4NT), and a combination of each of these chemicals released separately from 2 ml ampules in a 120 ml HDPE specimen holder. The stimulus container was changed to a translucent white, 4.9 liter capacity, HDPE container (Fisher Scientific Co., Pittsburgh, PA). A 13.0 cm diam hole was cut from the lid of the container and a 17.5 cm diam piece of galvanized hardware cloth (6 mm square openings) was glued to the inside circumference of the lid. These lids were used to retain 1.5 kg of chopped fresh sugarcane stem tissue within the inverted container which was suspended 2 cm from the opening of the bucket with a PVC crossbar capped with PVC cap and tee. A metal post was driven through the PVC tee into the ground to help prevent damage by raccoons and cattle. A bungee chord was used to secure the bottom of the trap to the post. The previously used PVC composite lid was not used (see Fig. 1C for approximate trap design). Sugarcane tissue with or without cruentol and an empty trap with or without cruentol served as controls. Traps were arranged in a randomized complete

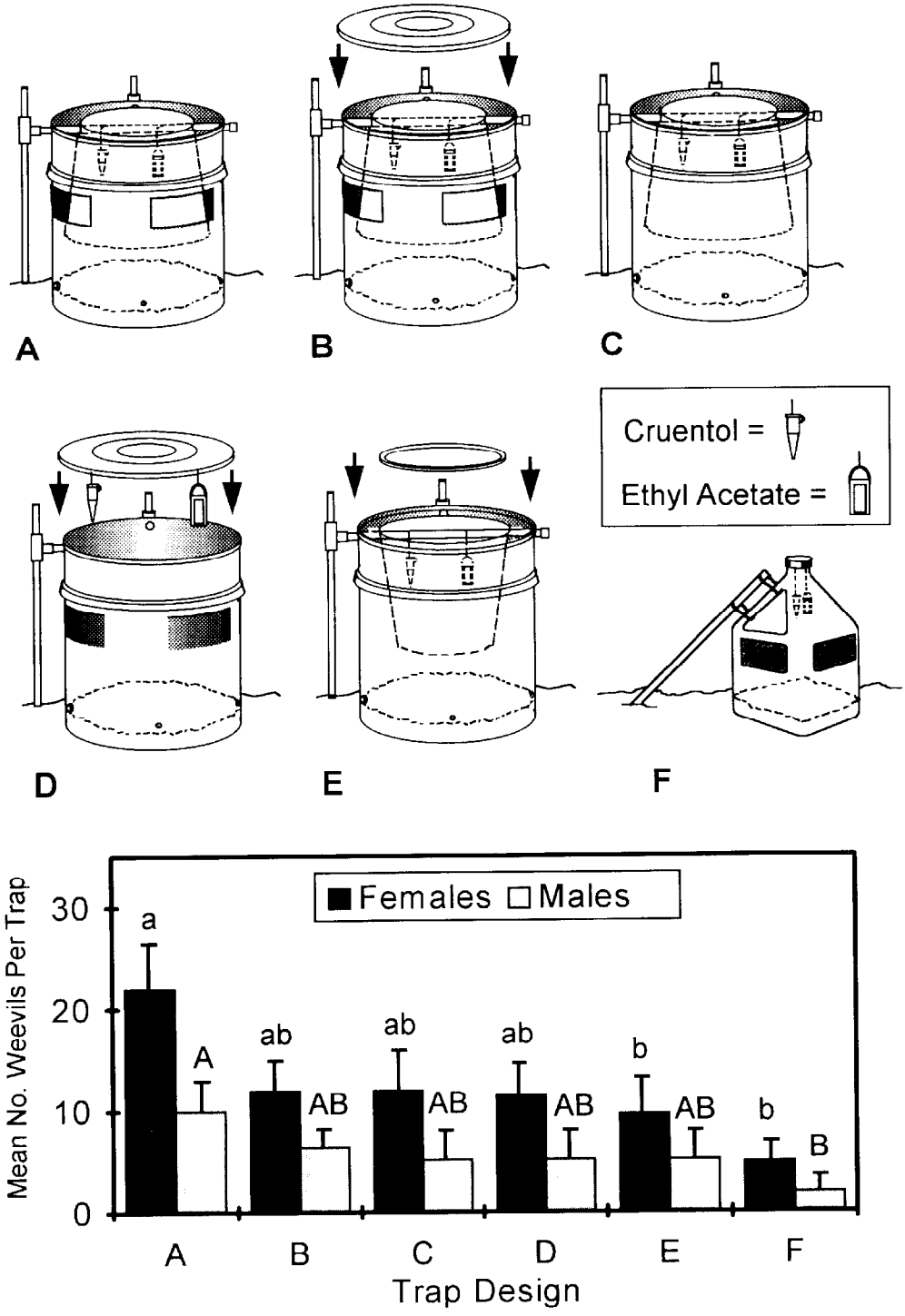

Fig. 1. Comparisons of mean 7-day trap counts of adult female and male *Rhynchophorus cruentatus* from six different semiochemically-mediated lethal traps, each baited with 0.4 mg/d 5-methyl-4-octanol (cruentol) and 742.3 mg/d ethyl acetate (n =7). Means followed by the same letter are not significantly different according to a Waller-Duncan k-ratio t-test on $(x + 0.5)^{0.5}$ transformed data ($k = 100$, $P \leq 0.05$). Untransformed means are presented. Error bars indicate standard error of the mean.

block design in the pasture at A. Duda & Sons, Inc. and spaced ≥ 20 m apart within each block and blocks were separated by ≥ 50 m. Studies were conducted 29 June-6 July 1993 and 6-13 July 1993. Treatments within each block were rerandomized between sample periods. Adult male and female counts of *R. cruentatus* were transformed and analyzed as previously described.

Modifications of Semiochemically-Mediated Traps

Several modifications of the lethal trap were field-tested with cruentol and ethyl acetate (4DH release device with 8 ml of ethyl acetate) as the semiochemical bait to determine the best trap design for future research (Figs. 1A-F). The basic trap design involved the 19-liter black HDPE plastic bucket with drainage holes and contained detergent solution as previously described (Figs. 1A-E). In three designs, three 4 x 13 cm openings were cut and spaced 10 cm apart and 21 cm from the bottom around the circumference of the 19-liter bucket (Figs. 1A, B, D). The black lids for these 19-liter buckets were used to prevent excessive rainfall and tampering in two designs (Figs. 1B, D). In the design pictured in Fig. 1D, two eye bolts were attached through the lid for hanging baits. A translucent white, 4.9-liter, HDPE stimulus container was inverted and suspended 2 cm from the opening of the bucket with a PVC crossbar capped with a PVC cap and tee in three designs (Figs. 1A-C) and not inverted in one design (Fig. 1E). In this design (Fig. 1E), the bottom of the stimulus container was removed and the lid was used to keep rain and animals away from the semiochemical baits while allowing easier access for changing semiochemicals. PVC tees were attached through each bucket to a small PVC piece and capped at 90° from the crossbar. No crossbar was needed in the design pictured in Fig. 1D which had 2 PVC tees attached through the bucket at 90° from each other. Metal posts were driven through the PVC tees into the ground to anchor the traps and prevent tampering (Figs. 1A-E). One lethal trap design (Fig. 1F) consisted of a clean 3.78-liter HDPE milk container with three 4 x 10 cm openings cut 12 cm from the bottom. The trap was attached to a metal rod by the handle with duct tape. This design had an eye bolt through the top for suspending semiochemicals and was filled with about 600 ml of tap water and 20 g of Alconox detergent (Alconox, Inc. New York, NY).

Traps were arranged in a randomized complete block design in the pasture at A. Duda & Sons, Inc. and spaced ≥ 20 m apart within each block and blocks were separated by ≥ 50 m. The experiment was conducted 25 August to 1 September 1993 with 7 replicates. Adult male and female counts of *R. cruentatus* were transformed and analyzed as previously described.

Results and Discussion

Field Response of Weevils to Fermenting Plant Tissues and Cruentol

In experiment No. 1, fermenting *S. palmetto* plus cruentol, sugarcane plus cruentol, and pineapple plus cruentol were equally attractive for *R. cruentatus* females, but sugarcane plus cruentol was more attractive than *S. repens* plus cruentol ($F = 4.43$; $df = 3$; $P = 0.0358$) (Table 1). Fermenting *S. palmetto* plus cruentol, sugarcane plus cruentol, and pineapple plus cruentol were equally attractive for *R. cruentatus* males, but more attractive than *S. repens* plus cruentol ($F = 4.44$; $df = 3$; $P = 0.0355$) (Table 1). The lack of attractancy of *S. repens* plus cruentol is probably indicative of a lack of moisture in this tissue which may have affected the quality of fermentation and the quantity of attractive volatiles released. *Serrenoa repens* is a host of *R. cruentatus* (R.M.G-D. unpublished data, Giblin-Davis & Howard 1989) and under different condi-

tions from this experiment may release attractive volatiles. Sugarcane stem and fermenting pineapple syncarp are suitable larval food sources for *R. cruentatus*, although these plants are usually not observed as natural hosts (Giblin-Davis et al. 1989).

In experiment No. 2, cruentol plus fermenting *S. palmetto*, fresh and frozen sugarcane, and pineapple were equally attractive for *R. cruentatus* females ($F = 1.19$; df = 3; $P = 0.3537$) and males ($F = 0.45$; df = 3; $P = 0.7190$) (Table 1). This study shows that semiochemicals from fermentation (≤ 7 days) of a variety of plant tissues are equally suitable for attraction of *R. cruentatus* when cruentol is present. Our results are similar to those reported by Diegado & Moreno (1986) with *R. palmarum*. They found that tissue from fruits of papaya, banana, pineapple, and orange, and stem tissue from African oil and coconut palms were attractive to adults of *R. palmarum* (Diegado & Moreno 1986).

Weevil Attraction to Fermentation Products and Cruentol

An average of eight times more *R. cruentatus* adults were captured in lethal traps baited with *S. palmetto* tissue (0.5 kg) and cruentol than with tissue alone (0.5 kg) or cruentol alone; no weevils were captured in unbaited traps (Table 2). These results corroborate previous studies (Weissling et al. 1994). *Sabal palmetto* tissue (1.5 kg) with cruentol was nearly three times more effective for trap capture of *R. cruentatus* than 0.5 kg of tissue plus cruentol (Table 2). The palm tissue pieces for the 0.5 kg treatment were tightly packed into the 946 ml containers and retained with a fine screen which reduced the efficiency of volatile release. Fermenting *S. palmetto* tissue (0.5 and 1.5 kg) plus cruentol are equally attractive when used in the flower pot stimulus holder or in a 4.9-liter HDPE container with a coarse screen and greater surface area for volatile release (R.M.G-D., unpublished data).

None of the chemicals with cruentol were as effective as the 1.5 kg of *S. palmetto* tissue plus cruentol (Table 2). Also, none of the chemicals tested were attractive alone, although ethyl acetate (1971 mg/d) captured about 1 weevil per trap (Table 2). Adults of *R. cruentatus* responded to cruentol combined with either ethyl acetate (1843 and 482 mg/d) or ethyl (*S*)-(-)-lactate (4NT) equally as well as to *S. palmetto* (0.5 kg) plus cruentol (Table 2).

Ethyl (*S*)-(-)-lactate with cruentol was significantly more attractive to *R. cruentatus* adults when released from the 4NT device than from the AMP device (Table 2). Antennae from males of *R. palmarum* gave the strongest EAG response to ethyl lactate compared with 72 h fermented African oil palm sap (Rochat 1987). Isopropyl acetate elicited an EAG response from *R. palmarum* similar to African oil palm sap (72 h) (Rochat 1987). However, it was not attractive when released to *R. cruentatus* at 67 mg/d with cruentol compared with cruentol alone (Table 2). Ethanol elicited a small relative EAG response from *R. palmarum* male antennae (Rochat 1987) but was attractive at 34 and 564 mg/d with cruentol to *R. cruentatus* (Table 2).

Ethyl isobutyrate (30 mg/d) was attractive with cruentol, but not attractive when released at about 276 mg/d compared with cruentol alone (Table 2). Ethyl butyrate at 229 mg/d with cruentol was equally as attractive as ethyl isobutyrate at 30 mg/d with cruentol and slightly more attractive than ethyl butyrate at 17 or 449 mg/d with cruentol (Table 2). Ethyl butyrate was detected in the distilled extract of fermented African oil palm sap but not in methylene chloride extracted sap (Nagnan et al. 1992). Octanoic acid and its ethyl ester (ethyl caprylate), ethyl caproate, *n*-butyl acetate, lactic acid, and isopropyl acetate, which appeared attractive with cruentol in our preliminary studies, were not as attractive as cruental alone at the rates used (Table 2).

Good chemically-mediated trapping of *R. cruentatus* was achieved with cruentol plus ethyl acetate (852 mg/d) and to a lesser degree with cruentol plus (*S*)-(-)-ethyl lactate (4NT), ethyl isobutyrate (40 mg/d), ethyl butyrate (255 mg/d), and ethanol (51 mg/d)

TABLE 2. ATTRACTION OF *RHYNCHOPHORUS CRUENTATUS* ADULTS TO LETHAL FIELD TRAPS IN HENDRY CO., FLORIDA BAITED WITH SELECTED CHEMICALS, CHOPPED *SABAL PALMETTO* STEM TISSUE, AND/OR SYNTHETIC 5-METHYL-4-OCTANOL (RACEMIC MIXTURE OF CRUENTOL RELEASED AT 0.4 MG/DAY) FOR 7-DAY SAMPLING PERIODS (6 OCTOBER THROUGH 15 DECEMBER 1992).

Treatments [Release Device][1]	Cruentol (+ or -)	No. of Traps	Total No. Weevils/Trap (Mean ± S.E.)[2]
Chopped *Sabal palmetto* stem (1.5 kg)	+	10	32.0 ± 5.1a
Chopped *Sabal palmetto* stem (0.5 kg)	+	10	11.7 ± 3.7b
Ethyl acetate [11NT] (1843.1 ± 124.4 mg/day)	+	8	9.9 ± 3.5bc
Ethyl (*S*)-(-)-lactate [4NT]* (hygroscopic; release rate not quantified)	+	4	9.5 ± 4.0bc
Ethyl acetate [4DH] (482.4 ± 21.9 mg/day)	+	7	8.6 ± 2.0bc
Ethyl isobutyrate [AMP]* (30.3 ± 2.5 mg/day)	+	8	7.1 ± 2.1cd
Ethyl butyrate [4NT]* (229.4 ± 35.9 mg/day)	+	4	6.8 ± 4.1cd
Ethyl acetate [AMP]* (107.8 ± 7.4 mg/day)	+	8	6.1 ± 2.2cde
Ethanol [AMP] (34.0 ± 2.4 mg/day)	+	8	5.8 ± 1.4cdef
Ethanol [11NT]* (564.1 ± 50.3 mg/day)	+	4	5.8 ± 1.7cd
Ethyl butyrate [AMP] (17.1 ± 1.0 mg/day)	+	8	4.4 ± 1.3defg
Chopped *Sabal palmetto* stem (1.5 kg)	-	10	4.3 ± 1.3defg
Ethyl butyrate [11NT]* (448.6 ± 87.8 mg/day)	+	4	3.5 ± 1.3defg
Ethyl caprylate [AMP]* (0.9 ± 0.5 mg/day)	+	5	3.0 ± 0.8defgh
Octanoic acid [AMP]* (hygroscopic; release rate not quantified)	+	5	2.8 ± 1.3fghi
Ethyl (*S*)-(-)-lactate [AMP]* (hygroscopic; release rate not quantified)	+	8	2.6 ± 0.8efghi

TABLE 2. (Continued)

Treatments [Release Device][1]	Cruentol (+ or -)	No. of Traps	Total No. Weevils/Trap (Mean ± S.E.)[2]
Isopropyl acetate [AMP]* (67.0 ± 1.9 mg/day)	+	5	2.4 ± 0.8ghij
Ethanol [4DH]* (169.9 ± 23.5 mg/day)	+	4	2.3 ± 1.0ghijk
Ethyl caproate [AMP]* (2.5 ± 0.1 mg/day)	+	5	1.6 ± 0.4ghijkl
Chopped *Sabal palmetto* stem (0.5 kg)	-	10	1.4 ± 0.7hijklm
No treatment	+	9	1.2 ± 0.7hijklm
Ethyl isobutyrate [4NT]* (275.6 ± 26.3 mg/day)	+	4	1.0 ± 1.0ijklm
Ethyl acetate [11NT] (1971.4 ± 156.5 mg/day)	-	8	0.8 ± 0.5ijklm
N-butyl acetate [AMP]* (14.6 ± 0.7 mg/day)	+	5	0.4 ± 0.4klm
Lactic acid [AMP]* (hygroscopic; release rate not quantified)	+	5	0.4 ± 0.2jklm
Ethyl acetate [4DH] (509.9 ± 38.3 mg/day)	-	7	0.1 ± 0.1lm
Ethanol [AMP] (33.6 ± 2.0 mg/day)	-	8	0.1 ± 0.1lm
Ethyl butyrate [AMP] (17.8 ± 1.0 mg/day)	-	8	0.1 ± 1.0lm
No treatment	-	10	0.0 ± 0.0m

[1]Chemicals were released from one or more of the following different kinds of containers to modulate release rates; (AMP) = 1 ml of test chemical in a 2 ml Gold Brand ampule with a 4 mm diam opening (Wheaton, Millville, NJ), (4DH) = 5 ml of test chemical in a 14.8 ml glass vial (OD x H = 21 x 70 mm) with a screw top with a 4 mm diam hole, (4NT) = 5 ml of test chemical in a 14.8 ml glass vial without a top (11.4 mm diam opening), and (11NT) = 25 ml of test chemical in an 40.7 ml glass vial (OD x H = 28 x 108 mm) without a top (17.8 mm diam opening). Release rates were quantified gravimetrically.
Treatments ranked according to total weevils captured per trap.

[2]Means followed by the same letter are not significantly different according to a Waller-Duncan k-ratio t-test on $(x + 0.5)^{0.5}$ transformed data ($k = 100$, $P \leq 0.05$). Untransformed means are presented.

*No male or female weevils were captured using this same treatment but without cruentol. Data for the treatment without cruentol is not shown but is equal to the no treatment without cruentol.

(Table 3). Release rates from the same release devices were higher in this experiment (Table 3) than in the previous experiment (Table 2) because of seasonally higher ambient temperatures. The ethyl acetate release device (4DH) ran dry at about 6 days under these summer conditions and data in Table 3 probably underestimate the 7-day weevil counts. None of the rest of the chemicals tested ran dry during the experiment. Sex ratios were skewed towards females when cruentol was present, but not when either *S. palmetto* or sugarcane was used without pheromone (Table 2 data not shown, Table 3).

TABLE 3. ATTRACTION OF *RHYNCHOPHORUS CRUENTATUS* ADULTS TO LETHAL FIELD TRAPS IN HENDRY CO., FLORIDA BAITED WITH SELECTED CHEMICALS, CHOPPED SUGARCANE STEM TISSUE, AND/OR SYNTHETIC 5-METHYL-4-OCTANOL (RACEMIC MIXTURE OF CRUENTOL RELEASED AT 0.4 MG/DAY) FOR 7-DAY SAMPLING PERIODS (29 JUNE-6 JULY 1993 AND 6-13 JULY 1993).

Treatments [Release Device][1]	Cruentol (+ or -)	No. of Replicates	No. Weevils Per Trap (Mean ± S.E.)[2] Females	Males
Chopped sugarcane (1.5 kg)	+	10	37.0 ± 6.4a	20.3 ± 3.9a
Ethyl acetate [4DH] (852.4 ± 34.9 mg/day)	+	10	26.7 ± 3.2b	9.7 ± 1.8b
Combination*	+	9	21.7 ± 3.1bc	7.7 ± 2.0bc
Ethyl (*S*)-(-)-lactate [4NT] (hygroscopic; release rate not quantified)	+	10	16.0 ± 2.0cd	6.5 ± 1.1bc
Ethyl isobutyrate [AMP] (40.2 ± 1.3 mg/day)	+	10	13.1 ± 2.4de	5.8 ± 1.5c
Ethyl butyrate [4NT] (254.5 ± 13.6 mg/day)	+	10	13.1 ± 1.6de	4.4 ± 1.1c
Ethanol [AMP] (51.0 ± 2.0 mg/day)	+	10	10.3 ± 1.8ef	5.1 ± 0.9c
Chopped sugarcane (1.5 kg)	-	10	5.3 ± 1.7g	4.9 ± 1.1c
No treatment	+	10	5.6 ± 0.6fg	1.8 ± 0.5d
No treatment	-	10	0.1 ± 0.1h	0.0 ± 0.0e

[1]See legend of Table 2 for description of release devices. Treatments ranked according to total weevils captured per trap.

[2]Means followed by the same letter are not significantly different according to a Waller-Duncan *k*-ratio *t*-test on $(x + 0.5)^{0.5}$ transformed data ($k = 100$, $P \leq 0.05$). Untransformed means are presented.

*Combination = ethanol (48.4 ± 4.9 mg/d), ethyl acetate (131.2 ± 1.9 mg/d), ethyl butyrate (34.0 ± 7.7 mg/d), ethyl isobutyrate (39.6 ± 1.0 mg/d), and ethyl lactate (release rate unknown) each released separately from an AMP device.

Ethyl acetate was one of the most attractive chemicals that we tested with cruentol (Tables 2,3). Trap counts appeared to be dose related for ethyl acetate and cruentol. However, there were no significant differences among the 108, 482, and 1843 mg/d release rates for ethyl acetate with cruentol (Table 2). Ethyl acetate, ethyl lactate, and ethanol were among the list of major volatiles identified by Samarajeewa et al. (1981) from coconut palm wine distillates and arrack and performed well in our study when released with cruentol (Tables 2, 3). The other major components, acetaldehyde, amyl-alcohol, methanol, 2-methyl-1- propanol, and 1-propanol were not attractive with cruentol to *R. cruentatus* at the rates tested in our study (unpublished data). Samarajeewa et al. (1981) found ten-fold increases in the concentrations of ethyl acetate and ethyl lactate

in naturally-fermented samples of coconut sap compared with controlled fermentations with commercial strains of *Saccharomyces* yeasts. The role of mixed microflora in the qualitative and quantitative profiles of host-plant fermentation products for attraction of *Rhynchophorus* spp. has not been studied. It could prove interesting. Also, the role of host-plant volatiles in host apparency to *Rhynchophorus* spp. has not been studied. It could be important in understanding management practices that encourage red ring nematode transmission in African oil palm and coconut palm plantations by *R. palmarum* or establishment of other species of *Rhynchophorus* in host palms.

The combination treatment [ethanol + ethyl acetate + ethyl butyrate + ethyl isobutyrate + (*S*)-(-)-ethyl lactate] with cruentol was as effective for the capture of *R. cruentatus* as any of the individual components with cruentol at the rates tested (Table 3). This suggests that an important class of semiochemicals may be missing or that further optimization of release rates and ratios is needed. Jaffé et al. (1993) have recently shown that a mixture of 68% ethanol, 27% ethyl acetate, and 5% pentane was as attractive to *R. palmarum* in a laboratory movement assay as odors from red ring-diseased coconut palm stem. Unfortunately, 2-methyl-5(E)-hepten-4-ol (male-produced aggregation pheromone of *R. palmarum*) plus ethanol, ethyl acetate, isoamyl acetate, 3-methyl-1-butanol, hexanal, or pentane alone (release rates undetermined) and as mixtures were not effective in the field.

Modifications of Semiochemically-Mediated Traps

There were no significant differences in the release rates of ethyl acetate among the six different trap designs ($F = 0.49$; df = 5; $P = 0.7792$) and the pooled release rate was 742.3 ± 7.9 mg/d (mean ± S.E.). Trap counts of females and males of *R. cruentatus* were equal for all but one of the five 19-liter black bucket designs (Fig. 1). The design pictured in Fig. 1E did not catch as many males and the 3.78-liter milk container design (Fig. 1F) did not catch as many females or males as the 19-liter black bucket design pictured in Fig. 1A. Future research with semiochemically-mediated trapping of *R. cruentatus* should utilize the designs pictured in Figs. 1B,D because these traps are the simplest to make and use. The trap designated in Fig. 1B could be used when a trap baited with tissue is to be compared with a trap (Fig. 1D) baited with only chemicals.

Currently, we are identifying and quantifying EAG active semiochemicals for *R. cruentatus* from perfusions of fermenting *S. palmetto*, sugarcane, and pineapple. Work is in progress on the optimization of ratios of plant-derived semiochemicals to be used with cruentol in a chemically-mediated trap for *R. cruentatus* that is equal to, or better than, fermenting sugarcane or *S. palmetto* tissue plus cruentol.

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