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AGGREGATION PHEROMONE FOR THE PEPPER WEEVIL, *Anthonomus eugenii* CANO (COLEOPTERA: CURCULIONIDAE): IDENTIFICATION AND FIELD ACTIVITY[1]

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Abstract—This study describes the identification of an aggregation pheromone for the pepper weevil, *Anthonomus eugenii* and field trials of a synthetic

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pheromone blend. Volatile collections and gas chromatography revealed the presence of six male-specific compounds. These compounds were identified using chromatographic and spectral techniques as: (*Z*)-2-(3,3-dimethylcyclohexylidene)ethanol, (*E*)-2-(3,3-dimethylcyclohexylidene)ethanol, (*Z*)-(3,3-dimethylcyclohexylidene)acetaldehyde, (*E*)-(3,3-dimethylcyclohexylidene)acetaldehyde, (*E*)-3,7-dimethyl-2,6-octadienoic acid (geranic acid), and (*E*)-3,7-dimethyl-2,6-octadien-1-ol (geraniol). The emission rates of these compounds from feeding males were determined to be about: 7.2, 4.8, 0.45, 0.30, 2.0, and 0.30 μg/male/day, respectively. Sticky traps baited with a synthetic blend of these compounds captured more pepper weevils (both sexes) than did unbaited control traps or pheromone-baited boll weevil traps. Commercial and laboratory formulations of the synthetic pheromone were both attractive. However, the commercial formulation did not release geranic acid properly, and geranic acid is necessary for full activity. The pheromones of the pepper weevil and the boll weevil are compared. Improvements for increasing trap efficiency and possible uses for the pepper weevil pheromone are discussed. A convenient method for purifying geranic acid is also described.

Key Words—Attractant, alcohol, aldehyde, geranic acid, monitoring, aggregation pheromone, *Anthonomus eugenii*, pepper weevil, Coleoptera, Curculionidae.

INTRODUCTION

The pepper weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae), is an important pest of both sweet and hot peppers (*Capsicum* spp.) in the southern United States, Mexico, and Central America (Elmore et al., 1934; Goff and Wilson, 1937). The most important damage is yield reduction resulting from premature abscission of infested fruit. Infested fruit not aborted may contain frass and decaying plant tissue, making them unmarketable. Additionally, the pepper weevil has been implicated in the transmission of internal mold of peppers (Bruton et al., 1989). Because the pepper weevil larvae and pupae are protected within the environment of the pepper pod, insecticide treatments must be directed against the emerged adults. Effective chemical control of adult pepper weevils is hindered by problems associated with detecting adults prior to economic injury (Genung and Ozaki, 1972). Predictive models for pepper weevil adult emergence are unavailable, and decisions regarding adulticide treatments and timing are generally based on classical calendar spraying regimes (Riley, 1990). Although a damage-based threshold has recently been described (Cartwright et al., 1990), visual counts of adults on terminal buds is the most widely accepted sampling method for adult pepper weevils (Andrews et al., 1986; Riley, 1990). Action thresholds for the pepper weevil are low: 5% terminal bud damage (Cartwright et al., 1990) and between 1 adult/400 terminals (Riley et al., 1992) and 1 adult/100 terminals (Andrews et al., 1986). Because both

sampling methods are tedious, time-consuming, and may only detect weevils after they have passed economic levels, a better monitoring system is needed for this pest.

There is previous evidence for a male aggregation pheromone in pepper weevils. Male pepper weevils have been shown to attract females and males in the field (Patrock, 1986; Patrock et al., 1992). In addition, males and dichloromethane extracts of males are reported to attract females and males in a laboratory olfactometer (Coudriet and Kishaba, 1988). A synthetic pepper weevil pheromone, if available, could provide a reliable and economic sampling method for detecting adult pepper weevil presence and determining density for making management decisions.

The objectives of this study were to isolate and identify the male aggregation pheromone and to field test a synthetic pepper weevil pheromone.

METHODS AND MATERIALS

Insects. A laboratory culture of pepper weevils was established from insects collected in Florida and Texas. Pepper weevils were reared according to methods described by Patrock (1986). Fresh jalapeño peppers were purchased locally, grown in a greenhouse, or grown in an outdoor garden. Emerging adult pepper weevils were held individually in 30-ml diet cups and fed sliced fresh jalapeño pepper, black nightshade (*Solanum nigrum*) berries, or a piece of artificial diet (Toba et al., 1969). Adult pepper weevils were sexed using CO_2 anesthetization and characters as described for sexing boll weevils (Agee, 1964). In addition, males can be identified by the presence of a metatibial mucro (Eller, 1994).

Collection of Volatiles. Initially, volatiles were collected using a 50-ml filtering flask and a Tenax porous polymer trap system as described by Bartelt et al. (1990). Later volatile collections were made using a volatile collection system consisting of a 20-cm × 2.2-cm-ID Pyrex glass tube sealed on each end with a No. 11 cork stopper. One cork held a prefilter (7-cm × 4-mm-ID glass tube) with ca. 6 mm of Super Q porous polymer (80/100 mesh; Alltech Associates, Inc., Deerfield, Illinois) held between a stainless steel screen (325 mesh; F.P. Smith Wire Cloth Co., Franklin Park, Illinois) and a glass wool plug. The other cork held a similar filter with ca. 4 mm of Super Q to collect volatiles. Air was drawn through the tube with either the house vacuum system or a vacuum pump at a flow of ca. 130 ml/min.

Volatiles were collected from both male and female pepper weevils to identify male-specific compounds (i.e., the putative aggregation pheromone components). Typically, volatiles were collected from individual pepper weevils on small (i.e., ca. 5-cm or shorter) jalapeño fruit, although occasionally volatiles were collected from groups of weevils, and the plant material was pepper buds

or nightshade berries. Volatiles were also collected from formulations of synthetic pheromones to determine their release rates and component ratios. Collections were made for periods of one to five days, and collected volatiles were extracted using 240-μl hexane for Tenax filters and methylene chloride or hexane for Super Q filters. Ten microliters of a 250 ng/μl solution (i.e., 2500 ng) of α-terpineol was added to each filter extract as an internal standard to quantify collected volatiles and calculate pheromone release rates.

Gas Chromatography. Gas chromatography was performed using a Hewlett-Packard 5890 Series II gas chromatograph (GC) with a Spectra-Physics SP4400 integrator and a Varian model 3700 GC with a Hewlett-Packard 3396A integrator. The columns used were a fused silica Hewlett-Packard HP-5 (0.17-μm film thickness, 25 m $\times$ 0.32 mm ID) (Hewlett Packard Co., Avondale, Pennsylvania) and a fused silica Durabond DB-1 (1.0-μm film thickness, 15 m $\times$ 0.25 mm ID) (J & W Scientific, Folsom, California), respectively. The temperature programs were: 50°C for 3 min then 10°C/min to 220°C and 70°C to 200°C at 10°C/min, respectively. For both gas chromatographs, the injector and detector temperatures were 170°C and 250°C, respectively, and each was equipped with a flame ionization detector with helium as the carrier gas. Injections of 1–2 μl were made in the splitless mode and changed to the split mode after 0.60 min. Retention indices (RI) were calculated relative to *n*-alkene standards according to Poole and Schuette (1984).

GC–Mass Spectrometry (GC-MS). Electron-impact mass spectra (EI-MS) were obtained on a Hewlett-Packard 5970 Mass Selective Detector. An ionizing potential of 70 eV was used for EI spectra. Sample introduction was through a Hewlett-Packard 5890 GC fitted with a DB-1 (0.25-μm film thickness, 15 m $\times$ 0.25 mm ID) capillary column. Chemical-ionization mass spectra (CI-MS) were obtained on a Finnigan 4535 quadrapole mass spectrometer. The reagent gas was isobutane. Sample introduction was through a GC fitted with a DB-1 (0.25-μm film thickness, 15 m $\times$ 0.25 mm ID) capillary column.

Infrared Spectroscopy. Vapor-phase infrared spectra were obtained using a Mattson Instruments Galaxy Series 6020 FT-IR spectrometer with light pipe accessory. Samples were introduced through the Hewlett-Packard GC and column described earlier using the same temperature program. Absorbances are reported in reciprocal centimeters (cm^{-1}).

High-Performance Liquid Chromatography. Prior to proton nuclear magnetic resonance spectroscopy, compounds **1, 2,** and **5** (Figure 1) were purified to ca. 99% pure by high-performance liquid chromatography (HPLC). HPLC separations were performed using a Spectra Physics SP8700 solvent delivery system and Spectra Physics SP8750 pump and Waters R401 refractive index detector. Compounds **1** and **2** were separated using a silica column (5 μm, 4.6 mm diam. $\times$ 250 mm long) and the mobile phase was 25% ether in hexane at a flow rate of 1 ml/min. Their retention volumes were ca. 11.5 and 12.2 ml,

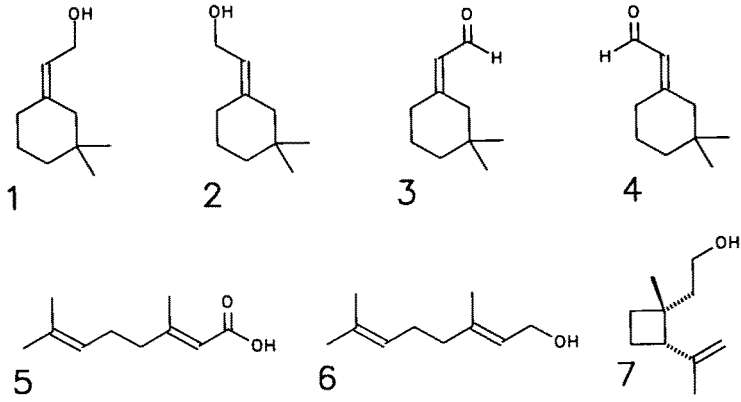


FIG. 1. Structures of compounds discussed in the text and assigned structure numbers.

respectively. Compound **5** was purified using a C-18 reversed-phase column (5 μm, 4.6 mm diam. $\times$ 250 mm long), and the mobile phase was 75:25 methanol–water at a flow rate of 1 ml/min. Compound **5** had an elution volume of ca. 7.0 ml.

Proton Nuclear Magnetic Resonance Spectroscopy (^1H *NMR*). Nuclear magnetic resonance (NMR) proton spectra were obtained on a Bruker 300 MHz instrument using deuterochloroform as the solvent. Shifts are reported in parts per million (δ) relative to tetramethylsilane.

Synthetic Derivatives. The methyl ester derivative of **5** was prepared using diazomethane in ether (Fales et al., 1973).

Synthetic Chemicals. Synthetic **1** was purchased from Frank Enterprises, Inc. (Columbus, Ohio) (95% pure by GC) or Bedoukian Research, Inc. (Danbury, Connecticut) (98% pure by GC). Synthetic **2** (98% pure by GC) and a mixture of **3** and **4** (95% pure by GC) were purchased from Bedoukian Research, Inc. A technical grade of synthetic **5** was purchased from ICN Biomedicals, Inc. (Cleveland, Ohio) (59% pure by GC; major impurity was nerolic acid) and was used without purification in experiments 1 and 2. For experiment 3, synthetic **5** was purified (99% pure by GC) by repeated (ca. 10$\times$) recrystallizations from acetone (70% geranic acid, 30% acetone, by volume) at ca. $-70°C$. Synthetic **6** was purchased from (Aldrich Chemical Co., Milwaukee, Wisconsin) (98% pure by GC).

NCAUR Pheromone Formulation. The synthetic pheromone was formulated using Miraspers (pregelatinized corn starch, pass 100 mesh; A.E. Staley, Decatur, Illinois). The general procedure consisted of adding 0.1% (by weight) 2,6-di-*tert*-butyl-4-methylphenol (BHT) (Aldrich Chemical Co.) as an antioxidant to the pheromone blend (compounds **1, 2, 3, 4,** and **6** in a ratio of

56:38:2:2:2, respectively). This pheromone/BHT blend was subsequently combined with the starch to obtain a mixture containing ca. 8% (by weight) pheromone. Approximately 0.5 g of the pheromone–starch mixture was placed inside a piece of glass tube (ca. 2.5 cm long × 0.5 cm diam.), and the tube was subsequently sealed inside a polypropylene (4 mil) bag (ca. 3.8 cm × 4.4 cm). Compound **5** (geranic acid) was formulated separately and was mixed with an equal amount of mineral oil (Fischer Scientific, Fairlawn, New Jersey) to slow the release of this compound, otherwise the same procedure was used. The pheromone was formulated to release the six components in a ratio of ca. 48:32:2:2:14:2 for compounds **1, 2, 3, 4, 5,** and **6,** respectively, at a total release rate of ca. 13.5 μg/hr.

Field Assays. Field tests of the synthetic pheromone were set up in three separate experiments, reflecting progressive improvements in the pheromone/trap combination. The first experiment was designed to compare commercial boll weevil traps (Great Lakes IPM, Vestaburg, Michigan) with sticky traps (6 in. × 12 in. yellow strips, Olson Products, Medina, Ohio). Previous research has shown that boll weevil traps baited with live male pepper weevils captured pepper weevils; however, pepper weevils were observed to move in and out of the inspection dome (Patrock et al., 1992). In addition, during preliminary tests of synthetic pepper weevil pheromone using boll weevil traps, it was noted on several occasions that, when traps were visually checked without removing captured insects and later rechecked, some pepper weevils had escaped between the two checks. The apparent inefficiency of boll weevil traps at capturing pepper weevils prompted the testing of the sticky traps in experiment 1. The NCAUR pheromone formulation was used (six-component blend with an approximate total release rate of 13.5 μg/hr). Four treatments were compared: unbaited (control) boll weevil traps, boll weevil traps baited with the six-component blend, unbaited (control) sticky traps, and sticky traps baited with the six-component blend. Traps were placed on bamboo stakes just above the tops of the pepper plants and were separated by ca. 10 m. Both baited and unbaited boll weevil traps contained a small piece (ca. 1 cm^3) of Pest Strip (Loveland Industries, Inc., Greely, Colorado) to kill captured insects. Pheromone baits were placed inside the inspection dome of the boll weevil traps and were attached to the sticky traps using a pin and cork. The test was conducted between November and March in Florida and Texas near the cities indicated (Table 2 below). Treatments were set out in random order and the traps were checked at two- to five-day intervals.

A second experiment was set up to compare the attractiveness of the NCAUR formulation to a commercial formulation containing the same components, using yellow sticky traps. The commercial formulation was similar to the Hercon boll weevil lure (Hercon Environmental, Emigsville, Pennsylvania). Ten milligrams per lure of the six-component blend were loaded in ratio of ca.

45:35:3:3:12:2. The NCAUR formulation was the same as described in the first experiment. The test was conducted between March and May in Florida and Texas near the cities indicated (Table 3 below). Treatments were set out in random order and the traps were checked at two- to five-day intervals.

Because the commercial formulation did not release compound **5** as desired, a third experiment was set up to determine whether this compound was a necessary component of the pheromone blend or whether **5** could simply be omitted for practical purposes. A comparison was made of the attractiveness of Hercon lures containing only compounds **1, 2, 3, 4,** and **6,** with and without compound **5** (using the NCAUR formulation). Ten milligrams per lure of components **1–4** and **6** were loaded in ratio of ca. 50:41:3.5:3.5:2. Compound **5** was formulated alone in the NCAUR formulation as described earlier; for this study, highly purified rather than technical grade **5** was used so that any effects of impurities would not confound the experiment. The pheromone treatments were compared using yellow sticky traps as described earlier. The test was conducted during May and June in Florida and Texas and June through October in New Mexico near the cities indicated (Table 4 below). Treatments were set out in random order and the traps were checked at two- to five-day intervals.

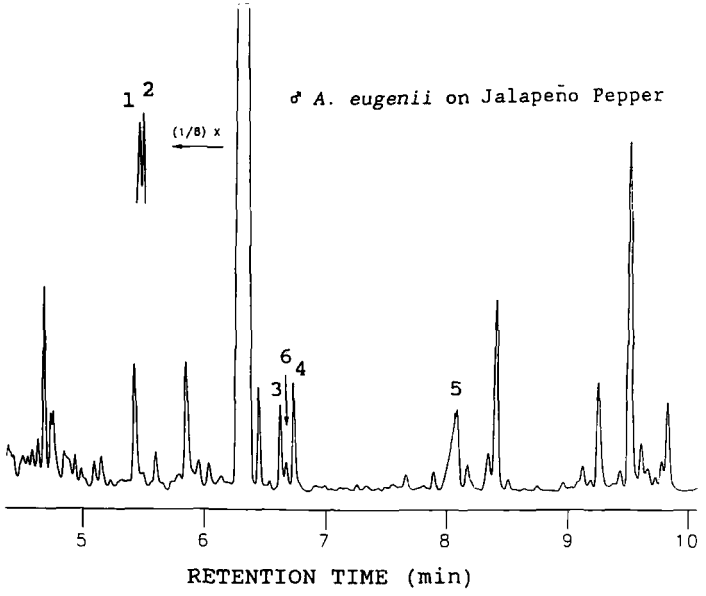
Captured insects were examined using a dissecting microscope to separate pepper weevils from other species of weevils and to determine the sex of the captured pepper weevils.

Statistical Analyses. Trap capture data were analyzed using Statistix 4.0 (Analytical Software, Saint Paul, Minnesota). The analyzed variable was the total trap catch (over test period) after $\log(X + 1)$ transformation for each treatment, location, and replication. Significance levels were 0.05 for all tests.

RESULTS

Male-Specific Compounds. A comparison of the GC profiles of volatile collections of males and females revealed the presence of six male-specific compounds designated 1–6 (Figure 2). Peaks 1–6 were found to correspond to the structures shown in Figure 1 with the same numbers. The GC retention data for these compounds and approximate release rates as determined from Super Q volatile collections are given in Table 1. Analytical results supporting these identifications are summarized in the following paragraphs.

EI-MS spectra for GC peaks 1 and 2 were very similar and had molecular ions at *m/z* 154. A search of the mass spectral library (NBS) gave an essentially perfect match with (*Z*)-2-(3,3-dimethylcyclohexylidene)ethanol **(1),** which is the second most abundant component of the boll weevil pheromone (Tumlinson et al., 1969). At this point, GC peaks 1 and 2 were tentatively identified as (*Z*)-2-(3,3-dimethylcyclohexylidene)ethanol and (*E*)-2-(3,3-dimethylcyclohexyli-



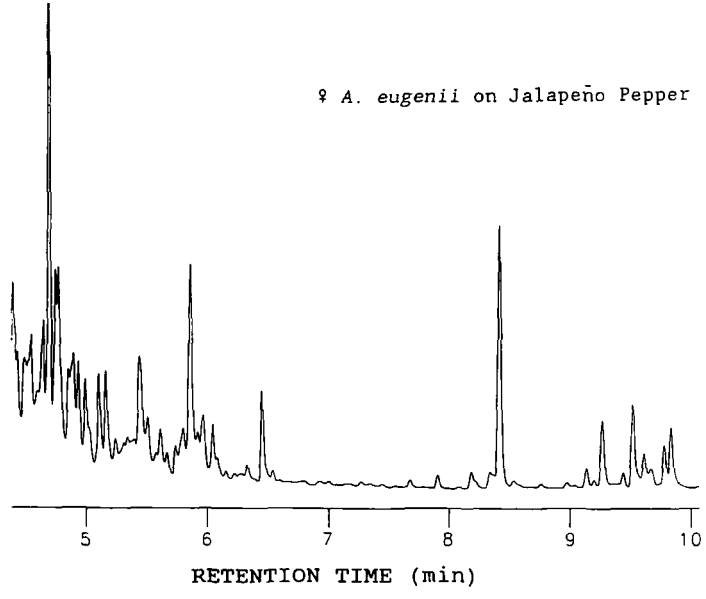


FIG. 2. Gas chromatograms (HP-5 column) of volatile collections of male (top) and female (bottom) pepper weevils feeding on jalapeño peppers.

TABLE 1. RELEASE RATES AND RELATIVE ABUNDANCE OF COMPONENTS OF PEPPER WEEVIL PHEROMONE IN VOLATILE COLLECTIONS, COMPARISON TO BOLL WEEVIL PHEROMONE, AND GAS CHROMATOGRAPHIC RETENTION INDICES (RI) OF THESE COMPOUNDS

	Pepper weevil		Boll weevil	RI	
Compound	Amount emitted (μg/male/day)	Relative abundance	Relative abundance[a]	DB-1	HP-5
1	7.20	48	35	12.14	12.24
2	4.80	32		12.16	12.27
3	0.45	3	14	12.33	12.50
4	0.30	2	15	12.40	12.59
5	1.95	13		13.33	13.62
6	0.30	2		12.35	12.52
7			36	11.90	12.12

[a]Relative abundances as reported by Chang et al. (1989).

dene)ethanol, respectively (compounds **1** and **2,** Figure 2). The GC retention times of the synthetic standards matched those of the pepper weevil-derived compounds on both GC columns. Mass spectral and NMR analyses both gave identical spectra for the weevil-derived and the synthetic compounds **1** and **2,** and the spectra were consistent with those reported earlier (Tumlinson et al., 1971).

GC peaks 3 and 4 both produced mass spectra with molecular ions at *m/z* 152, two units less than alcohols **1** and **2.** By analogy to the boll weevil system, these GC peaks were compared with synthetic standards of the aldehydic boll weevil compounds, (*Z*)-(3,3-dimethylcyclohexylidene)acetaldehyde and (*E*)-(3,3-dimethylcyclohexylidene)acetaldehyde (compounds **3** and **4,** respectively, Figure 2). The GC retention times of the synthetic standards matched those of the pepper weevil-derived compounds on both GC columns. Mass spectral analyses gave identical spectra for the weevil-derived and the synthetic compounds **3** and **4,** and the spectra were consistent with those reported earlier (Tumlinson et al., 1971). The small quantities of natural aldehydes precluded NMR analysis.

EI-MS analysis of GC peak 5 did not reveal an obvious molecular ion; however, CI-MS analysis gave a base peak at *m/z* 169, which was presumably the M+1 ion. FTIR analysis and a search of the EPA vapor-phase library suggested that this compound was an unsaturated carboxylic acid, specifically because of absorptions at 3585, 1752, and 1652 (cm^{-1}). The compound represented by GC peak 5 reacted with diazomethane to give the corresponding methyl ester. The methyl ester gave a much sharper GC peak than did the

original compound, and subsequent EI-MS analysis gave a presumptive molecular ion at *m/z* 182. A search of the mass spectral library (NBS) gave an essentially identical match for this derivative with methyl (*E*)-3,7-dimethyl-2,6-octadienoate (geranic acid methyl ester). Therefore, GC peak 5 was tentatively identified as (*E*)-3,7-dimethyl-2,6-octadienoic acid (geranic acid) **(5).** The GC retention time of the synthetic standard matched that of the pepper weevil-derived compound on both GC columns. Mass spectral and NMR analyses both gave identical spectra for the weevil-derived and synthetic compound **5,** and the mass spectrum was consistent with that reported earlier (Renhold et al., 1974).

EI-MS analysis of GC peak 6 revealed a molecular ion at *m/z* 154, and a subsequent search of the mass spectral library (NBS) gave an essentially perfect match with (*E*)-3,7-dimethyl-2,6-octadien-1-ol (geraniol). The GC retention time of synthetic geraniol matched that of the pepper weevil-derived compound on both GC columns. Mass spectral analyses of pepper weevil-derived compound **6** and synthetic geraniol gave identical spectra. The small quantity of natural material precluded NMR analysis.

Field Assays. The results of experiment 1 and statistical analysis are shown in Table 2. Because the treatment × location interaction was significant, treatments are only compared within a given location. Both the baited and unbaited boll weevil traps captured very few pepper weevils. Overall, pheromone-baited sticky traps caught the most pepper weevils, significantly more than pheromone-baited boll weevil traps at all seven locations, and significantly more than unbaited sticky traps at five of the seven locations. Considering all treatment–location combinations, females accounted for 50–67% of the captured pepper weevils, with an overall average of 60% females.

The results of experiment 2 and statistical analysis are shown in Table 3. Sticky traps baited with either the NCAUR or Hercon formulation captured significantly more pepper weevils than did unbaited sticky traps at five of the six locations. Overall, the two formulations (i.e., NCAUR and Hercon) captured about equal numbers of pepper weevils. The two formulations were statistically equal at three locations, the NCAUR caught significantly more weevils at two locations, and the Hercon caught significantly more weevils at one location. Considering all location–treatment combinations, females accounted for 50–100% of the captured pepper weevils, with an overall average of 84% females.

The results of experiment 3 and statistical analysis are shown in Table 4. The geranic acid (i.e., compound **5**) treatment caught the smallest number of pepper weevils and was statistically equivalent to controls at all seven locations. The Hercon lure alone was statistically equivalent to the controls at five locations but caught significantly more weevils than controls at two locations. The Hercon lure plus geranic acid captured the most pepper weevils and caught significantly more weevils than did the Hercon lure alone or controls at five locations and

TABLE 2. FALL 1992 AND WINTER 1993 PHEROMONE EXPERIMENT COMPARING OLSON STICKY TRAPS AND BOLL WEEVIL TRAPS WITH AND WITHOUT PEPPER WEEVIL PHEROMONE (NCAUR FORMULATION) (EXPERIMENT 1)

Location (test period)	Mean trap catch (range)[a]				Replications (*N*)
	Sticky, pheromone	Sticky, control	Boll weevil, pheromone	Boll weevil, control	
Weslaco, Texas (11/18–12/2)	8.2 a (1–14)	0.4 b (0–1)	0.0 b	0.0 b	5
Loxahatchee, Florida (1/18–2/10)	6.7 a (1–14)	0.0 b	0.0 b	0.0 b	3
Boyton Beach, Florida (1/19–1/29)	5.6 a (1–10)	8.0 a (1–15)	0.0 c	1.6 b (0–3)	5
Immokalee, Florida (1/27–3/4)	2.8 a (0–4)	1.8 b (0–5)	0.4 b (0–2)	0.0 b	5
Bradenton, Florida (1/29–2/19)	4.7 a (1–10)	2.3 a (1–7)	0.3 b (0–1)	0.0 b	3
Delray Beach, Florida (2/9–3/1)	1.2 a (0–3)	0.0 b	0.0 b	0.0 b	5
Immokalee, Florida (2/18–3/29)	17.8 a (2–59)	6.2 b (0–14)	0.4 c (0–2)	0.2 c (0–1)	5
Overall number captured	212	89	5	9	31

[a] In each line, treatments without letters in common differ significantly (LSD, 0.05 level). Overall differences among treatments: $F_{3,96} = 42.92$, $P \ll 0.0001$. Treatment × location interaction: $F_{18,96} = 2.25$, $P = 0.006$.

the geranic acid alone at six locations. Considering all location–treatment combinations, females accounted for 50–100% of the captured pepper weevils, with an overall average of 72% females.

DISCUSSION

Comparison of Pepper Weevil and Boll Weevil Pheromones. The total amount of pheromone released by male pepper weevils was ca. 15 μg/male/day and was higher than that reported for the boll weevil (ca. 4.2 μg/male/day) (Chang et al., 1989). The boll weevil (Tumlinson et al., 1969) and the pepper weevil have three pheromone components in common (i.e., **1**, **3**, and **4**). These three compounds are designated II, III, and IV, respectively in the boll weevil literature (Tumlinson et al., 1969). The relative percentages of the boll weevil pheromone as determined by collection of volatiles from boll weevils feeding on cotton (Chang et al., 1989) are shown in Table 1 for comparison. Compound **1** is the most abundant component of the pepper weevil pheromone and is the

TABLE 3. SPRING 1993 PHEROMONE EXPERIMENT COMPARING NCAUR FORMULATION AND COMMERCIAL HERCON FORMULATION USING OLSON STICKY TRAPS (EXPERIMENT 2)

Location (test period)	Mean trap catch (range)[a]			Replications (*N*)
	Pheromone (NCAUR)	Pheromone (Hercon)	Control	
Immokalee, Florida (3/12–5/14)	6.6 a (0–4)	11.0 a (0–8)	0.4 b (0–1)	5
Jupiter, Florida (3/17–5/17)	7.8 a (7–10)	9.0 a (5–12)	0.6 b (0–3)	5
Loxahatchee, Florida (3/22–4/4)	5.0 b (5–5)	17.5 a (16–19)	0.0 c	2
Bradenton, Florida (3/24–4/17)	3.5 b (0–7)	17.5 a (5–30)	19.0 a (7–31)	2
Immokalee, Florida (4/1–5/17)	20.4 a (6–37)	20.0 a (7–28)	8.0 b (5–16)	5
Weslaco, Texas (4/21–5/7)	17.8 a (4–45)	5.4 b (0–12)	0.4 c (0–1)	5
Overall number captured	280	297	85	24

[a] In each line, treatments without letters in common differ significantly (LSD, 0.05 level). Overall differences among treatments: $F_{2,57} = 28.02$, $P \ll 0.0001$. Treatment × location interaction: $F_{10,57} = 3.29$, $P = 0.002$.

second most abundant component of the boll weevil pheromone. In addition, compound **1** has been isolated from female pecan weevils, *Curculio caryae* (Horn) (Coleoptera: Curculionidae) (Hedin et al., 1979). Pepper weevils and boll weevils also have the aldehyde components (i.e., **3** and **4**) in common, although their relative percentages of the blend differ widely. Compounds **2, 5,** and **6** isolated from male pepper weevils have not been reported from the boll weevil and are apparently not part of its aggregation pheromone. Compound **5,** however, is reported to be part of the Nasonov pheromone of the honeybee, *Apis mellifera* (Pickett et al., 1980) and is attractive to foraging bees (Williams et al., 1981). A somewhat similar compound, (*E*)-3,7-dimethyl-2-octen-1,8-dioic acid (callosobrusic acid) has been isolated from female azuki bean weevils, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) (Mori et al., 1983). Compound **6** (geraniol) is not reported to be produced by boll weevils, although Tumlinson et al. (1970) suggested that geraniol was a possible precursor to the boll weevil pheromone components. Their reasoning can be applied to the production of all of the pepper weevil compounds, including compound **5** (i.e., geranic acid), which is the corresponding acid of geraniol. The most abundant

TABLE 4. SUMMER 1993 PHEROMONE EXPERIMENT COMPARING COMMERCIAL HERCON LURE WITH AND WITHOUT GERANIC ACID (NCAUR FORMULATION) USING OLSON STICKY TRAPS (EXPERIMENT 3)

Location (test period)	Mean trap catch (range)[a]				Replications (*N*)
	Hercon + Geranic acid	Hercon alone	Geranic acid alone	Control	
Immokalee, Florida (5/15–5/26)	3.8 a (1–6)	1.2 b (0–3)	0.8 b (0–2)	0.4 b (0–1)	5
Immokalee, Florida (5/17–5/25)	15.5 a (6–25)	15.0 a (6–24)	1.5 b (1–2)	0.5 b (0–1)	2
Bradenton, Florida (5/17–6/7)	12.8 a (11–16)	4.0 b (2–9)	5.5 b (0–10)	8.0 ab (3–18)	4
Weslaco, Texas (5/19–6/17)	26.0 a (12–39)	7.6 b (1–16)	4.6 b (2–7)	3.4 b (1–6)	5
Lantana, Florida (5/19–6/17)	27.2 a (21–34)	24.4 a (17–28)	22.2 a (19–26)	24.6 a (18–29)	5
Loxahatchee, Florida (5/25–6/2)	17.6 a (11–24)	4.8 b (1–7)	1.0 c (0–3)	0.8 c (0–2)	5
Las Cruces, New Mexico (6/28–10/14)	199.7 a (0–2828)	43.6 b (0–615)	26.4 b (0–357)	19.7 b (0–267)	15
Overall number captured	3440	890	437	474	41

[a] In each line, treatments without letters in common differ significantly (LSD, 0.05 level). Overall differences among treatments: $F_{3,134} = 36.28$, $P \ll 0.0001$. Treatment × cooperator interaction: $F_{12,134} = 4.40$, $P < 0.0001$.

component of the boll weevil pheromone (i.e., **7**) is apparently not produced by male pepper weevils.

Field Studies with Pepper Weevil Pheromone. Pheromone-baited sticky traps generally captured at least twice as many pepper weevils as did control traps, with several cases of over 20 times as many. This indicates that the synthetic pheromone is attractive to pepper weevils. Patrock et al. (1992) reported that male-baited boll weevil traps caught ca. three times as many pepper weevils as did control boll weevil traps. The three experiments reflect progressive improvement of pheromone baits. The ratio of overall trap catch by the best treatment to that by the sticky-trap control increased from 2.4:1 to 3.4:1 to 6.3:1 for experiments 1, 2, and 3, respectively. The trap captures were generally low, but it should be noted that most experiments were conducted in commercial fields where growers were applying insecticides to suppress pepper weevil populations.

The results of experiment 2 clearly indicate that the pheromone-baited

yellow sticky traps were more effective at capturing pepper weevils than either baited boll weevil traps or unbaited sticky traps. In all field experiments, unbaited control traps (especially yellow sticky cards) captured pepper weevils. This is probably a result of an attraction to the color of the traps. Previous research has shown that unbaited sticky traps (especially yellow and white) are attractive to pepper weevils (Segarra-Carmona and Pantoja, 1988; Riley, 1990). The yellow sticky cards and boll weevil traps used in these tests were found to have peak reflectances at 563 and 542 nm, respectively (unpublished data), and were very similar to the reflectance pattern of the yellow sticky cards used by Segarra-Carmona and Pantoja (1988). Segarra-Carmona and Pantoja (1988) reported that unbaited sticky traps were an effective monitoring technique for the pepper weevil and were superior to other sampling methods, including direct counting. The addition of the synthetic pheromone and resulting increased trap captures should make the pheromone–sticky card combination an even more effective monitoring technique.

Although both the NCAUR and Hercon formulations used in experiment 2 were attractive, the NCAUR formulation is made by hand and is impractical for large-scale production. The Hercon lure, on the other hand, is mass-produced. However, the Hercon lures used in experiment 2 released much less of compound **5** (geranic acid) than do male pepper weevils. In addition, the synthetic compound **5** used in experiments 1 and 2 contained a large amount of impurities with unknown effects. Although the lures used in experiments 1 and 2 were attractive, it is believed that a synthetic blend more closely approximating that released by males would be even more attractive. This difficulty in formulating compound **5** prompted the testing of whether or not it was an essential component of the synthetic blend (i.e., experiment 3). The blend of the synthetic boll weevil pheromone has a significant effect on its attractiveness. Tumlinson et al. (1969) reported that the individual components of the boll weevil pheromone were nearly inactive and that the two alcohols (i.e., **7** and **1**) were both required with at least one of the aldehydes (i.e., **3** or **4**) but response was highest to the complete blend. Our results of experiment 3 indicate that the pheromone blend is important for the maximum attractancy of the pepper weevil as well. Although geranic acid is inactive by itself, the six-component blend containing geranic acid is much more attractive than the five-component blend without geranic acid. Therefore either a new commercial lure that can properly release all six components must be developed or two separate formulations must be used to achieve this. Other preliminary data suggest the individual alcohols **1** and **2** are inactive by themselves and the two aldehydes together (i.e., **3** and **4**) are also inactive (unpublished). The effects of the individual components and blend optimization need to be investigated further.

In all three experiments, the treatment × location interaction was significant. We believe that much of this interaction was due to differences between

the locations in pepper phenologies and pepper weevil densities, which subsequently affected the response of weevils to the pheromone. The biologies of the pepper weevil and the boll weevil are very similar (Burke, 1976), and factors affecting one species are likely to affect the other species as well. The efficiency of traps baited with male boll weevils or synthetic pheromone is affected by both cotton phenology and boll weevil population dynamics (Hardee et al., 1970a; Ridgway et al., 1971). Traps baited with live male boll weevils only capture more boll weevils than control traps when cotton was in the pre-squaring stage. Midseason (i.e., after fruiting), control traps capture as many boll weevils as do male-baited traps, which elicit little or no response even though the field population is increasing. In addition, traps baited with male boll weevils capture the highest numbers at the end of the season. Several factors have been suggested to account for these observations. Early in the season, there is little competition from native males and a low availability of food/oviposition sites. Midseason, however, the attractiveness of the boll weevil pheromone decreases with the increased availability of food/oviposition sites, increased competition from native males, an increase in the percentage of mated females (which are no longer attracted to the pheromone) (Hardee et al., 1970b), and decreased movement from field to field. At the end of the season, the pheromone becomes attractive again as the boll weevils migrate out and as the cotton becomes unsuitable (i.e., a lack of oviposition sites).

Our data may reflect a similar situation for the pepper weevil. Early in the season (i.e., before fruiting) or when pepper weevil populations are low, the pheromone traps are more attractive than control traps, although relatively low numbers of weevils are captured. This was generally the case at most locations and all three experiments. As the peppers mature, competition from native males increases, the proportion of mated females increases, and the effect of the pheromone becomes less evident. This appeared to be the case at Lantana, Florida, where the pepper field had been abandoned and the pepper weevil population was very high (Table 4). At one location, (i.e., Bradenton, Florida), the pheromone-baited sticky traps never captured statistically more weevils than did control sticky traps (Tables 2–4). This location is a relatively small plot on an experiment station farm, and the results may be due to a population of pepper weevils on nightshade in the area adjacent to this plot. At the end of the season, when the pepper fields are destroyed by plowing or killed by frost, pheromone traps are again more attractive than control traps. After a pepper field was disked in Mexico, pheromone trap captures of over 200 pepper weevils per trap per day were recorded, compared to four per trap per day for control traps (Laborde, personal communication). In addition, after a frost killed the pepper plants in New Mexico, individual pheromone traps captured over 800 pepper weevils (virtually covering the sticky surface) over a three-day period, compared with ca. 20 on control traps.

The fact that male and female pepper weevils were captured in baited traps in both these experiments and in those of Patrock et al. (1992) suggests that the pepper weevil pheromone acts as an aggregation pheromone similar to that described for the boll weevil (McKibben et al., 1971). Although it has not been tested, the pepper weevil pheromone may act as an aggregation pheromone early in the season and more as a true sex pheromone (attracting primarily females) later in the season, as is true for the boll weevil pheromone (Tumlinson, 1985). Although the captured weevils from all locations were not available for examination, we were able to analyze for sex-by-treatment interactions for eight data sets (experiment–location combinations) (chi-square tests). Of these, four of the cases had significant interactions, and there was a tendency for traps with the complete pheromone to catch more females than control traps or traps baited with only the geranic acid. However, these latter traps captured relatively few insects and conclusions based on these results are tenuous.

Other Considerations. It may be possible to enhance the attractiveness of the pepper weevil pheromone by the addition of pepper compounds in a manner analogous to the enhanced attractiveness of grandlure by the addition of water extracts of cotton squares (Hardee et al., 1971), cotton essential oils (Dickens, 1986), or green leaf volatiles (Dickens, 1989). The effects of pepper compounds on the activity of the synthetic pepper weevil pheromone will be investigated in future studies.

The commercial availability of compounds **1–6** should expedite their use as attractants for pepper weevils. Three of these are available as boll weevil pheromone components (i.e., **1, 3,** and **4**), and **2** is also available as a "by-product" of the synthesis of **1.** Synthetic **5** (i.e., geranic acid) is also available commercially. Although the purity of compound **5** from commercial sources is low (ca. 60%), it can be purified relatively easily by recrystallization from acetone (70:30 mixture by volume, respectively) at −70°C. Compound **6** (i.e., geraniol) can be purchased in high purity.

We feel that the pepper weevil pheromone will be of greatest utility for the early detection of pepper weevil adults. In several instances during the course of this study, pepper weevils were captured on pheromone-baited traps in commercial pepper fields before they were detected by visual scouting (T.F.M., B.J., J.H.D.). If a strong correlation between pheromone trap captures and pepper weevil density or damage can be established, action thresholds based on pheromone trap captures may be more effective than those based on visual counts of adults (Andrews et al., 1986) or counts of damaged buds (Cartwright et al., 1990). In addition, it may be possible to control pepper weevils by using a system analogous to the bait-stick developed for control of the boll weevil (McKibben et al., 1991). The pepper weevil pheromone has great potential as part of a management program for the pepper weevil, just as the boll weevil

pheromone, grandlure, is an effective tool for the management of the boll weevil (Hardee et al., 1974).

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