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ELECTROPHYSIOLOGICALLY AND BEHAVIORALLY ACTIVE VOLATILES OF BUFFALO GOURD ROOT POWDER FOR CORN ROOTWORM BEETLES[1]

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Abstract—The dried, powdered roots of buffalo gourd, *Cucurbita foetidissima*, were tested in a cornfield and shown to attract adult northern and southern corn rootworm beetles. Coupled gas chromatography–electroantennography (GC-EAG) analyses of headspace samples of the root powder showed several GC-EAG-active compounds on the antennae of female northern, southern, and western corn rootworms. Among other techniques, solid-phase microextraction and GC-mass spectrometry identified the following GC-EAG-active compounds: hexanol, nonanal, 1-octen-3-ol, benzaldehyde, benzyl alcohol, (*E*)-3-octen-2-one, (*E,E*)-3,5-octadien-2-one, and (*E,Z*)-3,5-octadien-2-one. EAG dose–response studies of several of the identified root powder volatiles also were performed and compared with results from known attractants. Field tests of synthetic root powder volatiles in commercial cornfields showed that northern corn rootworm adults were attracted to (*E,E*)-3,5-octadien-2-one. The antennae of the *Diabrotica* species and the field tests showed specificity for different geometrical isomers of 3,5-octadien-2-one, with a behavioral preference for (*E,E*)-3,5-octadien-2-one. In addition, we have shown that the efficacy of buffalo gourd root powder as a feeding stimulant and arrestant can be enhanced for northern and western corn rootworm adults by augmenting buffalo gourd root powder with additional (*E,E*)-3,5-octadien-2-one.

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[1]The Editors apologize to Drs. Cossé and Baker. This paper should have appeared in the June 1998 issue (Volume 24, Number 6). By an error of filing, the paper was "lost" in the editorial office for some months.

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Key Words—Coleoptera, Chrysomelidae, buffalo gourd root powder, *Cucurbita foetidissima, Diabrotica virgifera virgifera, D. undecimpunctata howardi, D. barberi,* attractants, solid-phase microextraction, gas chromatography–electroantennography.

INTRODUCTION

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte; the southern corn rootworm, *D. undecimpunctata howardi* Barber; and the northern corn rootworm, *D. barberi* Smith and Lawrence (Coleoptera: Chrysomelidae) are among the more economically important damaging insect pests of corn, *Zea mays* L., in the United States (Metcalf, 1986). It is estimated that control costs and crop losses due to *Diabrotica* spp. approach $1 billion annually (Metcalf, 1986). Applications of soil insecticides to reduce the larval densities in the root zone are the primary management practice for *Diabrotica* spp. in continuous corn (Mayo and Peters, 1978; Mayo, 1986). Baits containing a combination of cucurbitacins, volatile attractants, and small quantities of insecticide have shown promise as an alternative management tool that targets adult beetles (Metcalf et al., 1987; Lance and Sutter, 1990, 1991, 1992; Weissling and Meinke, 1991a,b). The nonvolatile, oxygenated, tetracyclic triterpenoid cucurbitacins found in many species of Cucurbitaceae evoke compulsive feeding and locomotory arrestment in diabroticite beetles (Metcalf et al., 1980, 1982). Adding a volatile attractant, such as a mixture of 1,2,4-trimethoxybenzene, indole and (*E*)-cinnamaldehyde (compounds emitted by the flowers of Cucurbitaceae) to a formulation of the powdered roots of the buffalo gourd, *Cucurbita foetidissima* H. B. K., enhances the efficacy of the bait (Metcalf et al., 1987; Lance, 1988a,b; Weissling and Meinke, 1991a,b).

This study presents the results of experiments designed to determine whether the dried, powdered roots of the buffalo gourd contain volatiles that are electrophysiologically active and to see whether these naturally occurring compounds can attract *Diabrotica* beetles, thereby potentially increasing the effectiveness of the current toxicant-feeding baits for *Diabrotica* species.

METHODS AND MATERIALS

Insects. Male and female western and southern corn rootworm adults were obtained from a rearing facility operated by French Agricultural Research, Inc., Lamberton, Minnesota. In addition, western, southern, and northern corn rootworm adults were collected from cornfields in and around the Iowa State University campus. Male and female beetles were placed in separate cages and kept at 25°C under a 14L:10D photoperiod. Adults had access to water and slices of squash.

Collection of Buffalo Gourd Root Powder Volatiles. Buffalo gourd root powder was obtained from MicroFlo Company (Lakeland, Florida). Headspace collections of volatiles from the powder were obtained by three different methods. In the first method, which does not require the use of solvents, buffalo gourd root powder volatiles were collected directly from 2-dram (7.4-ml) sample vials by using solid-phase microextraction (SPME) (Supelco, Inc., Bellefonte, Pennsylvania). The SPME unit consisted of a fiber coated with 100 μm of polydimethylsiloxane housed within a syringe. The fiber was extruded from the syringe and inserted into the headspace of a 2-dram vial containing 1.5 g of buffalo gourd root powder at room temperature (ca. 25–28°C). After 20 min it was retracted and immediately inserted into the injector port (250°C) of a HP 5890 (Hewlett-Packard Comp., Palo Alto, California) gas chromatograph (GC), and thermally desorbed.

The second method (no solvents needed), involved placing 1 g of buffalo gourd root powder in a 25-ml fritted sparger that was connected to a Tekmar 2000 purge and trap concentrator fitted with a turbocool unit (Tekmar, Cincinnati, Ohio). The conditions for collecting volatiles on a cooled (−20°C) Tenax trap, housed in the Tekmar 2000 instrument, were as follows: 2 min prepurge, sample at 50°C, 2 min preheat, 10 min purge (He, 20 ml/min), preheat desorb at 195°C, and 4 min desorb at 200°C. The Tekmar 2000 was directly interfaced to the coupled gas chromatograph–electroantennograph (GC-EAG) setup (see below).

For the third method, 35 g buffalo gourd root powder samples were placed in a two-necked 250-ml flask. Charcoal-filtered air was blown over the powder at a rate of 10 ml/sec for 9 hr at room temperature. Volatiles were collected in a glass tube (10 cm long × 4 mm ID) containing 350 mg of precleaned Tenax TA (Alltech Associates, Inc., Deerfield, Illinois) placed between two glass wool plugs. The Tenax TA trap was desorbed with 3 ml of redistilled HPLC-grade hexane. Samples were stored at −20°C for later analyses by GC-EAG and coupled GC-mass spectrometry (GC-MS).

Chemical Analyses. Collected volatiles resulting from all three methods were injected in splitless mode on a 30-m × 0.25-mm-ID fused silica capillary gas chromatographic column (DB-1, DB-5, or DB-225 model, J&W Scientific, Folsom, California) for analysis by GC-EAG and GC-MS. Column conditions were as follows: He carrier gas flow of 1.5 ml/min, injector temperature 250°C, 1 min delay on inlet purge, and 4 min at 35° C then 25°C/min to 320°C (DB-1) or 250°C (DB-5) or 10°C/min to 220°C (DB-225). The SPME samples were analyzed by using the above-mentioned conditions with a 4-min delay on inlet purge. GC-MS analyses were performed by using the HP 5890 gas GC with a direct interface to an Hewlett-Packard 5972 mass selective detector (electron impact, 70 eV).

Electroantennogram Responses. Coupled gas chromatography–electroantennography and EAG analyses were performed according to standard methods (e.g., Cossé et al., 1995; Cossé and Baker, 1996) by using an HP 5890 GC. EAG recordings were made by inserting a glass pipet Ag–AgCl saline electrode

(World Precision Instruments, Sarasota, Florida) into the back of an excised beetle head. A second saline recording electrode was placed in contact with the distal end of one antenna. Both pipets were filled with Beadle-Ephrussi saline (Ephrussi and Beadle, 1936). To examine antennal sensitivity to the identified buffalo gourd root powder volatiles, we recorded EAGs that were not coupled to the GC from female western and southern corn rootworm beetle antennae in response to a dose–response series of commercial compounds. In addition, EAGs were obtained from male and female antennae of northern and western corn rootworm beetles to a 100-μg dose of selected synthetic compounds. Serial dilutions of the tested compounds were made in HPLC-grade methylene chloride such that the tested compounds were applied to filter paper strips (0.5 cm × 3.0 cm, Whatman No. 1) in 10 μl of solvent. The filter paper strips were placed inside glass Pasteur pipets (15 cm long). The antenna was continuously flushed with a charcoal-filtered, humidified airstream. The air, flowing at a rate of 20 ml/sec, was delivered through a glass tube (8 mm ID) ending 10 mm in front of the preparation. Two milliliters of volatiles from the stimulus pipet were puffed into the constant airstream by a mechanical puffing device (Syntech, Hilversum, The Netherlands), delivering puffs of 0.1-sec duration. The test compound was injected into the airstream 15 cm upstream from the antenna. Solvent blank puffs (filter paper plus solvent) were presented to each EAG preparation before and after each dose level of test compound. The EAG amplitudes in response to the synthetic compounds were corrected for mechanoreceptive response by subtracting the mean amplitudes of the EAGs generated from the solvent blank puffs. Within a particular series of test compounds, presentation order of the test compounds was randomized within doses, beginning with the 0.01-μg odor cartridges and working upward to the 10-μg odor cartridges. The resulting EAG data were subjected to analysis of variance (ANOVA) and mean responses were compared by the LSD method (Sokal and Rohlf 1981).

Chemicals. Indole, benzyl alcohol, benzaldehyde, and hexanol were obtained from Sigma Chemical Co. (St. Louis, Missouri). The compounds 1-octen-3-ol and nonanal were purchased from Bedoukian (Danbury, Connecticut) and (*E*)-cinnamaldehyde and 1,2,4-trimethoxybenzene were obtained from Aldrich Chemical Co. (Milwaukee, Wisconsin). Samples of (*E*)-3-octen-2-one and (*E,E*)-3,5-octadien-2-one, in quantities sufficient for physiological and behavioral tests, were synthesized by W. L. Roelofs, Department of Entomology, New York State Agricultural Experiment Station, Cornell University, Geneva, New York. The (*E,E*)-3,5-octadien-2-one came in several batches, containing different proportions of the 3,5-octadien-2-one isomers (see Figure 1 for additional details). Initial samples of authentic 3,5-octadien-2-one for identification were kindly supplied as a gift by Joerg D. Hardege (Gatty Marine Laboratory, St. Andrews, Fife, Scotland).

Field Tests. In August 1994, prior to the chemical and physiological anal-

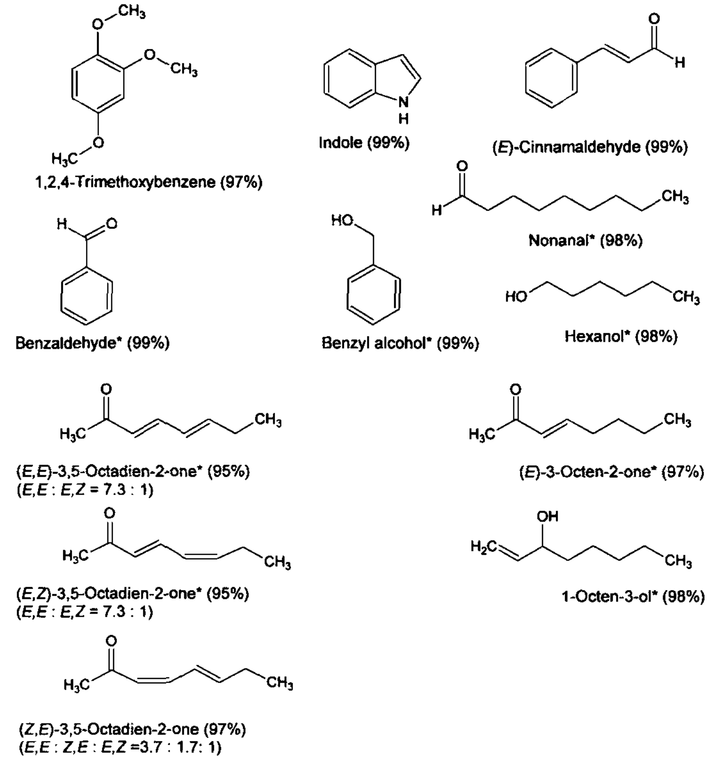

FIG. 1. Structure and purity (in parentheses) of test compounds. Asterisks indicate compounds identified in the headspace of buffalo gourd root powder.

yses, buffalo gourd root powder was tested for attracting corn rootworm beetles on a small experimental corn plot (40 × 60 m) located on the Iowa State University campus. Five grams of compacted buffalo gourd root powder were placed in sachets made of several layers of cheesecloth and affixed to the glue of yellow Pherocon A. M. sticky traps (Trécé, Inc., Salinas, California). A control trap received no treatment. Traps were affixed to corn plants at about ear height, 20 m apart, around the edges of the experimental plot. Five replicate pairs were left in the field for 24 hr, after which time they were replaced by new traps and fresh baits. The 24-hr trapping period was repeated five times (August 5–9) with corn at late milk to early dough stage. Trap catch data was transformed [log $(x + 1)$] to normalize variances and subjected to two-way ANOVA with replication.

In August 1996, two different sets of field tests were performed. In the first test, several identified EAG-active compounds were tested in a commercial cornfield located in Story County, Iowa. Traps were baited with candidate attractants (dissolved in acetone) that were applied to cotton dental rolls (3.8 cm long × 1 cm diam., Peerless Int., Inc., Massachusetts) as a 0.8-ml mixture of acetone and mineral oil (1 : 1). Control traps were baited with dental rolls that were only treated with the acetone–mineral oil mixture. Trap catches in response to EAG-active compounds were compared with similarly prepared known attractant mixtures containing 1,2,4-trimethoxybenzene, indole, and (*E*)-cinnamaldehyde (1 : 1 : 1). Traps were deployed at ear height, 30 m apart, in a randomized complete block design with six replicates that were placed along the edges of the cornfield. Traps were left in the field for 72 hr during August 20–22 with corn at late milk to early dough stage.

In the second test, we compared the numbers of corn rootworm beetles arriving on corn leaves that were sprinkled with buffalo gourd root powder and buffalo gourd root powder augmented with a wick containing synthetic volatile attractant. A salt shaker was used to sprinkle four corn leaves, two below and two above the corn ear with 200 mg of buffalo gourd root powder. Dental rolls containing 30 mg of 1,2,4-trimethoxybenzene, indole, and (*E*)-cinnamaldehyde (1 : 1 : 1) applied as a 0.5-ml mixture of acetone and mineral oil (1 : 1) were affixed to some of these corn plants at ear height. Other plants receive dental rolls that were only treated with the acetone–mineral oil mixture. Treated plants were laid out in a randomized complete block design with five replicates with blocks and plants 15 m apart, starting on the fourth row from the edge of the field. Beetles were counted on the four treated and the four control leaves 5 hr after the start of the experiment. Corn was at milk stage during this test (August 21). The same experiment was repeated a year later with the exception that the synthetic attractant was replaced by 100 mg of (*E*,*E*)-3,5-octadien-2-one (*E*,*E*:*E*,*Z* = 7.3 : 1). Corn was at silking stage during this test (July 28, 1997). Data were transformed [log $(x + 1)$] to normalize variances and subjected to ANOVA, means were compared using the Student-Newman-Keuls test. Untransformed data are presented in the tables.

RESULTS

Field Trapping with Buffalo Gourd Root Powder. Analysis of variance of the mean numbers of beetles that were trapped per day in the small-plot field test indicated significantly higher numbers of southern corn rootworm beetles ($F = 35.30$; $df = 4, 40$; $P \ll 0.001$) and northern corn rootworm beetles ($F = 8.59$; $df = 4, 40$; $P \ll 0.001$) in buffalo gourd root powder-baited traps than in control traps. The daily averages (±SD) were the highest for southern corn rootworm beetles (7.12 ± 4.84) followed by northern corn rootworm beetles (1.92 ± 1.68), and western corn rootworm beetles (1.48 ± 0.87), respectively. The daily control averages (±SD) for southern, northern, and western corn rootworm beetles were 0.76 ± 0.88, 0.92 ± 0.91, and 0.28 ± 0.29, respectively. Because the beetles did not contact the root powder itself, these results indicated that behaviorally active volatiles were being emitted from the buffalo gourd root powder.

Analyses of Buffalo Gourd Root Powder Volatiles. The GC profiles obtained by the three methods used to collect buffalo gourd root powder volatiles did not reveal any prominent qualitative variation. However, each method had its own advantages. With the SPME technique, headspace samples could be analyzed quickly (20 min) by both GC-MS and GC-EAG without the presence of solvent. Interfacing the purge and trap method with the GC-EAG allowed for higher amounts of trapped volatiles to be transferred to the GC column and subsequently to the antenna of the beetle. However, sample analysis by this method was limited to the GC-EAG setup and took a relatively long time (1 hr). The adsorption of buffalo gourd root powder volatiles on Tenax TA allowed for relatively large quantities to be collected and stored for later analysis by both GC-EAG and GC-MS. However, solvent used to desorb collected volatiles could possibly interfere with GC peaks of interest, and this setup also required testing the trap for breakthrough and precleaning the adsorbent.

Thirty-six GC-EAG analyses of volatiles, equally distributed over the three collection methods and the three GC columns, collected from buffalo gourd root powder were obtained from 18 female southern corn rootworm and 18 female western corn rootworm antennae. Only those GC peaks that consistently elicited simultaneous EAG activity were targeted for further analysis. These GC-EAG analyses consistently revealed 14 GC peaks on all three GC columns having corresponding EAG activity on both female southern corn rootworm and western corn rootworm antennae (Figure 2; Table 1). The retention times and GC-MS spectra of eight synthetic compounds presented in Table 1 were identical to those of peaks 2, 5, 8, 9, 10, 12, 13, and 14 detected in the headspace of buffalo gourd root powder on all three columns. Results from an additional four GC-EAG analyses from four different female northern corn rootworm antennae by using the SPME collection method on DB-1 and DB-225 columns showed GC-EAG activity identical to that obtained with southern corn rootworm and western corn rootworm antennae. Using three different isomers of 3,5-octadien-2-one, GC-EAG and GC-MS analyses revealed that only the *E,E* and *E,Z* isomers, and not the *Z,E* isomer, evoked EAG responses regardless of which of the three corn rootworm species' antennae were used.

Electroantennogram Responses. The EAG responses of female western and southern corn rootworm antennae to puffs of three GC-EAG-active compounds plus three known adult corn rootworm attractants were recorded at four dosages (Figure 3). The EAG amplitudes increased as the dosages in the cartridges

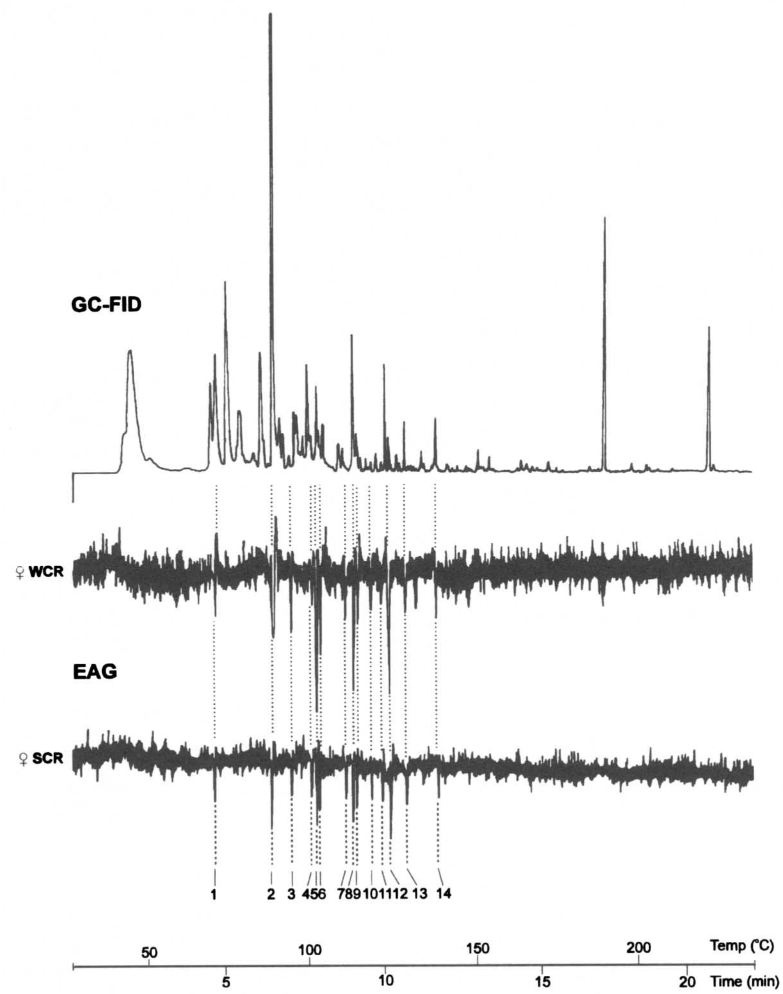

FIG. 2. Simultaneously recorded gas chromatogram [flame ionization detector (FID)] of buffalo gourd root powder volatiles collected by solid-phase microextraction and electroantennograms (EAG) from female western (WCR) and southern corn rootworm (SCR). Numbered peaks indicate EAG-active compounds (see also Table 1).

increased from 0.01 to 10 μg for all compounds tested, except when female southern corn rootworm antennae were exposed to 1,2,4-trimethoxybenzene. Statistical analyses of the dose–response data showed that both female western and southern corn rootworm antennae gave significantly higher responses to benzyl alcohol and indole than to the other four compounds tested.

TABLE 1. IDENTIFIED BUFFALO GOURD ROOT POWDER VOLATILES WITH ELECTROPHYSIOLOGICAL ACTIVITY ON FEMALE WESTERN, SOUTHERN, AND NORTHERN CORN ROOTWORM ANTENNAE

Peak no.[a]	Compound	Identification[b]
1	unknown	—
2	hexanol	GC-MS
3	cyclohexanol	MS
4	cyclooctanone	MS
5	1-octen-3-ol	GC-MS
6	aromatic hydrocarbon	MS
7	aromatic hydrocarbon	MS
8	benzaldehyde	GC-MS
9	(*E*)-3-octen-2-one	GC-MS
10	nonanal	GC-MS
11	aromatic hydrocarbon	MS
12	(*E*,*Z*)-3,5-octadien-2-one	GC-MS
13	(*E*,*E*)-3,5-octadien-2-one	GC-MS
14	benzyl alcohol	GC-MS

[a]Numbers correspond to labeled peaks in Figure 2.
[b]Identification is based on a mass spectrum library (Wiley 138K) search (MS) and/or comparison of mass spectra and retention times of the natural materials with those of the identified synthetic compounds (GC-MS).

Comparison of EAG responses between female western and southern corn rootworm antennae indicated significantly higher responses of female western corn rootworm antennae to benzaldehyde (F = 48.8; df = 1, 32; $P \ll 0.001$) benzyl alcohol (F = 22.2; df = 1, 32; $P \ll 0.001$), and indole (F = 27.6; df= 1, 32; $P \ll 0.001$), than were obtained from southern corn rootworm antennae. Significantly higher EAG responses were evoked in response to (*E*)-cinnamaldehyde from southern corn rootworm antennae (F = 121.43; df= 1, 32; $P \ll 0.001$) than from western corn rootworm antennae.

Comparisons of the EAG responses of male and female antennae of northern and western corn rootworms to single dosages of 1-octen-3-ol, (*E*,*E*)-3,5-octadien-2-one, and indole showed no significant sex differences, and male and female responses were therefore pooled for further analysis (Figure 4). The EAG responses of northern corn rootworm antennae were significantly higher to 1-octen-3-ol and (*E*,*E*)-3,5-octadien-2-one than to indole (F = 54.8; df = 2, 57; $P \ll 0.001$). Similar results were obtained with western corn rootworm antennae (F = 30.4; df = 2, 57; $P \ll 0.001$). Comparison between the EAG responses of northern and western corn rootworm antennae to 1-octen-3-ol, (*E*,*E*)-3,5-octadien-2-one, and indole revealed no significant differences (F = 1.3; df = 1, 114; P = 0.26).

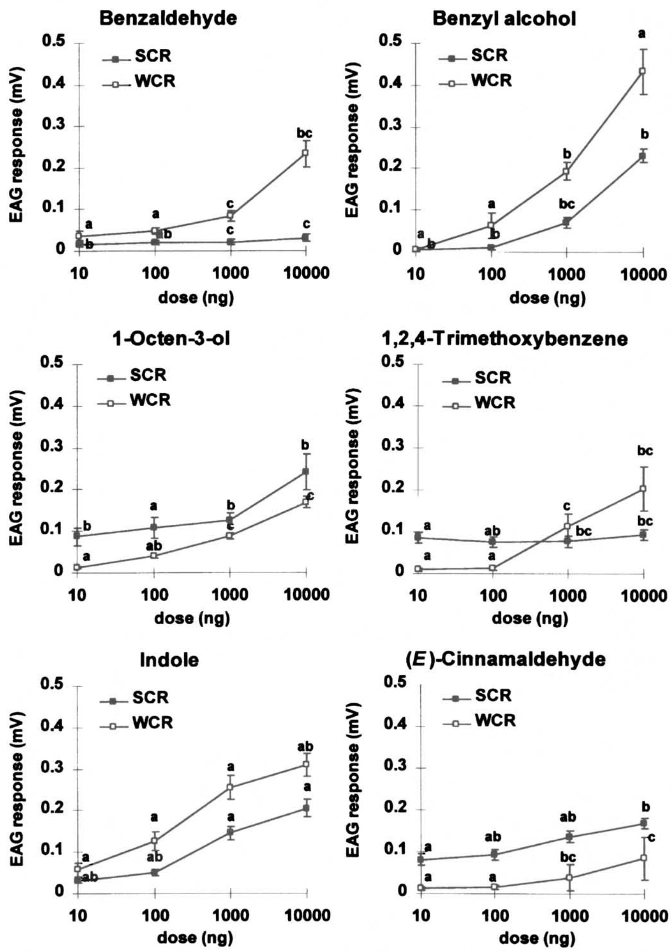

FIG. 3. Electroantennogram (EAG) dose–response of female western (WCR) and southern corn rootworm (SCR) antennae to selected volatile compounds emitted from buffalo gourd root powder. Stimuli were synthetic standards. Standard deviations of mean responses are presented as error bars ($N = 5$). The EAG responses of WCR and SCR antennae for each dosage were compared by ANOVA, and if significant at $P < 0.05$, the differences among means were tested for significance according to the LSD method.

Field Trapping. The mean number of corn rootworm beetles captured on traps baited with selected GC-EAG active buffalo gourd root powder volatiles is presented in Table 2. As expected, a mixture of 1,2,4-trimethoxybenzene, indole, and (*E*)-cinnamaldehyde (1 : 1 : 1) attracted significantly more north-

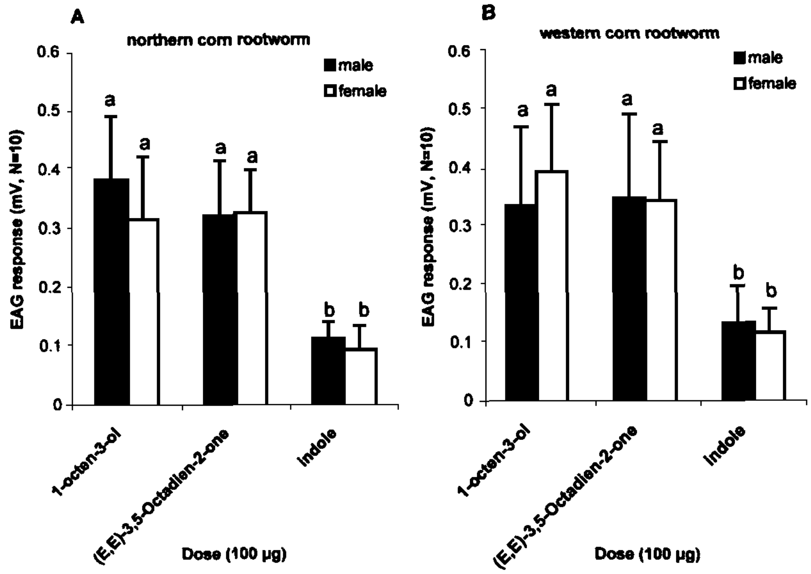

FIG. 4. Electroantennogram (EAG) responses of male and female western (WCR) and northern corn rootworm (NCR) antennae to a single dose (100 μg) of selected volatile compounds emitted from buffalo gourd root powder. Stimuli were synthetic standards. Standard deviations of mean responses are presented as error bars. The EAG responses were compared by ANOVA, and if significant at $P < 0.05$, the differences among means were tested for significance according to the LSD method.

ern, western, and southern corn rootworm beetles than did an unbaited control. When beetle captures on traps baited with isomeric mixtures of 3,5-octadien-2-one as well as with a 1 : 1 mixture of (*E*)-3-octen-2-one and 3,5-octadien-2-one were compared with captures on control traps, only one isomeric mixture of 3,5-octadien-2-one attracted significantly more beetles than did the control and attraction was limited to northern corn rootworms. Although there was no significant difference in the number of northern corn rootworms attracted to the two isomer mixtures of 3,5-octadien-2-one, only the 7.3 : 1 (*E*,*E*:*E*,*Z*) mixture attracted significantly more beetles than did the control. Traps baited with (*E*)-3-octen-2-one or a 1 : 1 mixture of (*E*)-3-octen-2-one and 3,5-octadien-2-one (3.7 : 1, *E*,*E*:*E*,*Z*) captured northern corn rootworm beetles, but the numbers were not significantly different from those of the control traps. No western or southern corn rootworms were captured on the yellow-sticky control traps, whereas, on average, 3 (±1.7 SD) northern corn rootworms were captured on the controls. The number per plant (±SD) of northern, western, and southern corn rootworm beetles on four leaves of 10 corn plants

TABLE 2. MEAN NUMBERS OF NORTHERN (NCR), WESTERN (WCR), AND SOUTHERN CORN ROOTWORM (SCR) ADULTS CAPTURED ON YELLOW PHEROCON A. M. STICKY TRAPS BAITED WITH CHEMICALS DERIVED FROM BUFFALO GOURD ROOT POWDER, STORY COUNTY, IOWA, AUGUST 20–22, 1996[a]

	Capture/trap (mean ± SD)		
Treatment (100 mg/compound)[b]	NCR	WCR	SCR
1,2,4,-Trimethoxybenzene + indole + (*E*)-cinnamaldehyde (1 : 1 : 1)	70.2 ± 30.7a	5.5 ± 4.5a	2.3 ± 1.0a
(*E,E*)-3,5-Octadien-2-one (3*E*,5*E* : 3*E*,5*Z* = 7.3 : 1)	11.8 ± 3.3b	0.2 ± 0.4b	0.2 ± 0.4b
(*E,E*)-3,5-Octadien-2-one (3*E*,5*E* : 3*E*,5*Z* = 3.7 : 1)	8.0 ± 5.6bc	0.2 ± 0.4b	0.2 ± 0.4b
(*E*)-3-Octen-2-one	5.8 ± 3.8c	0.0 ± 0.0b	0.0 ± 0.0b
(*E*)-3-Octen-2-one + (*E,E*)-3,5-Octadien-2-one (3*E*,5*E* : 3*E*,5*Z* = 3.7 : 1)	5.8 ± 2.6c	0.0 ± 0.0b	0.0 ± 0.0b
Control	3.0 ± 1.7c	0.0 ± 0.0b	0.0 ± 0.0b
F statistics	25.3[c]	24.9[c]	26.0[c]

[a]Corn stage; late milk-early dough (R3-R4). Means within columns followed by the same letter do not differ by Student-Newman-Keuls test, $P > 0.05$.
[b]Compounds were applied on dental wicks as an acetone-mineral oil mixture (0.8 ml, 1 : 1).
[c]Statistical significance at $P < 0.001$ ($df = 5, 25$).

just prior to the start of the experiment was 0.8 (±0.9 SD), 0.3 (±0.8 SD), and 0.1 (±0.6 SD), respectively. An overall lower capture of western and southern compared with northern corn rootworm (Table 2) might be due to the somewhat higher population density of northern corn rootworm indicated in the beetle-per-plant counts.

The mean numbers of corn rootworm beetles observed on corn leaves treated with buffalo gourd root powder with or without the presence of a wick containing synthetic attractant are presented in Table 3. As expected, leaves treated with buffalo gourd root powder had significantly more western and northern corn rootworm beetles than did the untreated control leaves. The addition of a point-source wick of the synthetic attractants significantly increased the numbers of these beetles per corn plant compared with those found on plants only treated with the buffalo gourd root powder. With either the mixture of 1,2,4-trimethoxybenzene, indole, and (*E*)-cinnamaldehyde or the newly identified (*E,E*)-3,5-octadien-2-one, the mean number of western corn rootworms per four leaves increased by a factor of 2.8. Similar significant trends were observed for the northern corn rootworm. However, overall beetle counts were lower than those of the western corn rootworm. For the southern corn rootworms, no significant differences were observed.

TABLE 3. MEAN NUMBERS OF WESTERN (WCR), NORTHERN (NCR), AND SOUTHERN CORN ROOTWORM (SCR) ADULTS COUNTED ON CORN LEAVES AFTER APPLICATION OF BUFFALO GOURD ROOT POWDER (BGRP) EITHER WITH OR WITHOUT DIFFERENT CHEMICAL ATTRACTANTS APPLIED TO A COTTON WICK AFFIXED TO THE PLANT

	Counts/plant (mean ± SD)[a]		
Treatment[b]	WCR	NCR	SCR
August 21, 1996			
BGRP + TIC	30.0 ± 16.6a	2.0 ± 1.2a	0.2 ± 1.2a
BGRP	7.6 ± 3.4b	1.4 ± 1.1ab	0.0 ± 0.0a
Control	1.6 ± 1.9c	0.0 ± 0.0b	0.0 ± 0.0a
F statistics[c]	19.5**	15.2*	NS
July 28, 1997			
BGRP + (*E*,*E*)-3,5-octadien-2-one	21.4± 11.5a	1.4 ± 1.1a	0.4 ± 0.5a
BGRP	7.6 ± 4.2b	0.4 ± 0.5b	0.4 ± 0.5a
Control	3.0± 1.9c	0.0 ± 0.0b	0.2 ± 0.4a
F statistics[c]	17.2**	5.2*	NS

[a]Beetle counts were made on four corn leaves, two above and two below the corn ears, 5 hr after application. Corn stage August 21, 1996: milk (R3); corn stage July 28, 1997: early silking (R1).
[b]BGRP = 200 mg applied over four leaves; TIC = 1,2,4-trimethoxybenzene, indole, and (*E*)-cinnamaldehyde, 30 mg (1 : 1 : 1); (*E*,*E*)-3,5-octadien-2 one = 100 mg (3*E*,5*E* : 3*E*,5*Z* = 7.3 : 1). Chemicals were applied on dental wicks as acetone-mineral oil mixtures (0.5 ml, 1 : 1) and affixed to the corn stalk at ear height.
[c]Asterisks denote statistical significance at $P < 0.05$ (*) and $P < 0.001$ (**), NS denotes $P > 0.05$ ($df = 2, 8$). Means within dates and columns followed by the same letter do not differ by Student-Newman-Keuls test, $P > 0.05$.

DISCUSSION

Our results demonstrate that volatiles from the dry, powdered roots of the buffalo gourd can attract corn rootworm adults in the absence of an additional synthetic attractant. Furthermore, our approach of combined GC-EAG analyses demonstrated that several volatile constituents of the buffalo gourd root powder are EAG-active on the antennae of western, northern, and southern corn rootworm beetles and that at least one of these volatiles, (*E*,*E*)-3,5-octadien-2-one, is capable of attracting northern corn rootworms in significant numbers in field trapping experiments. In addition, we have shown that the arrival and retention of northern and western corn rootworm beetles on leaves sprinkled with buffalo gourd root powder can be enhanced with additional (*E*,*E*)-3,5-octadien-2-one presented on a cotton wick.

Adult corn rootworms are attracted to volatile compounds from corn plants and to volatiles isolated or derived from the blossoms of *Cucurbita* species (Hammack, 1996, 1997; Prystupa et al., 1988, Metcalf et al., 1995; Metcalf and

Metcalf, 1992, and references therein), and several studies have shown that contact of the beetles with the nonvolatile cucurbitacins present in buffalo gourd root powder can be increased with the addition of synthetic attractants identified from flowers (Metcalf et al., 1987; Lance and Sutter, 1990, 1991, 1992; Weissling and Meinke, 1991a,b).

Several of the volatile EAG-active compounds identified in our headspace samples of the buffalo gourd root powder previously have been identified from *Cucurbita* flowers and tested as attractants for *Diabrotica* species in the field. For example, benzaldehyde and benzyl alcohol, identified in this study from root powder, are attractive to several *Diabrotica* species, but only at relatively high dosages (>200 mg) (Lampman et al., 1987; Metcalf and Lampman, 1989; Metcalf and Metcalf, 1992). A compound previously identified from corn, 1-octen-3-ol, also identified from root powder in this study, was not attractive to northern or western corn rootworms (Hammack, 1997). However, the identified EAG-active volatiles, (*E,E*)-3,5-octadien-2-one, (*E,Z*)-3,5-octadien-2-one, and (*E*)-octen-2-one, had not been previously identified or tested for attractancy. Our EAG results showed specificity for all three beetle species for two of the three tested geometrical isomers of 3,5-octadien-2-one. Significant EAG activity occurred only in resposne to the *E,E* and *E,Z* isomers, and not to the *Z,E* isomers. This specificity also was expressed behaviorally where mixtures with different ratios of *E,E* and *E,Z* isomers attracted northern corn rootworms in the field, but only traps baited with mixtures higher in *E,E* content attracted significantly more beetles than the control traps. (*E*)-Octen-2-one did not attract significant numbers of corn rootworm beetles, nor were any beetles attracted when (*E*)-octen-2-one was tested in combination with one of the isomer mixtures of 3,5-octadien-2-one.

That buffalo gourd root powder alone is capable of attracting *Diabrotica* species could be important information when synthetic attractants in combination with formulations of buffalo gourd root powder are evaluated as baits for controlling adult corn rootworms. Without the proper controls, the presence of buffalo gourd root powder could unduly increase the effectiveness of the tested attractant.

Corn silk has been shown to attract western and northern corn rootworms in wind-tunnel tests (Prystupa et al., 1988), and several studies on field attractancy of *Diabrotica* species to volatile compounds have noted significantly decreased trap catch during the silking stage of the corn (Hammack, 1996; Lampman et al., 1987; Andersen and Metcalf, 1986). This decrease in trap catch could be due to the presence of (*E,E*)-3,5-octadien-2-one. This compound was recently identified together with two other volatile compounds from senescing silk that protrudes from the tips of the corn ears and was shown to be EAG-active on western corn rootworm antennae (Hibbard et al., 1997a). Our beetle counts on corn leaves, dusted with buffalo gourd root powder and augmented with the presence of additional (*E,E*)-3,5-octadien-2-one, were obtained during the silking period of the corn. However, we observed beetles feeding on the silks as well as on the buffalo gourd root powder particles of corn plants that received the buffalo gourd root powder–attractant combination.

After submission of this paper it came to our attention that (*E,E*)-3,5-octadien-2-one had been identified from extracts of buffalo gourd root powder and was shown to be EAG-active on western corn rootworm antennae (Hibbard et al., 1997b). These results corroborate our EAG findings. However, additional research is needed to study the novelty of (*E,E*)-3,5-octadien-2-one as an attractant for corn rootworm beetles as well as the effects of blends of all identified attractants in the headspace of buffalo gourd root powder. Studies to examine whether these compounds alone or in combination can increase the feeding stimulant effect of buffalo gourd root powder in commercial cornfields at different crop stages could contribute to the efficacy of the toxicant-feeding stimulant formulations for controlling adult *Diabrotica* beetles.

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