BEHAVIORAL AND ELECTROPHYSIOLOGICAL RESPONSES OF THE MEXICAN FRUIT FLY (DIPTERA: TEPHRITIDAE) TO GUAVA VOLATILES

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ABSTRACT

The behavioral and electrophysiological responses of males and females of the Mexican fruit fly *Anastrepha ludens* (Loew) to guava (*Psidium guajava* L.) volatiles were investigated in laboratory tests. Males and females were significantly more attracted and landed more often on guava fruits than yellow spheres used as control in the wind tunnel. Also, both sexes were more attracted to Porapak Q extracts of guava than to solvent controls. Gas chromatography-electroantennographic detection (GC-EAD) analysis of the behaviorally active extracts showed that consistently eight and seven compounds elicited antennal response from male and female, respectively. The compounds were identified by gas chromatography-mass spectrometry (GC-MS) as ethyl butyrate, (*E*)-3-hexenol, (*Z*)-3-hexenol, hexanol, ethyl hexanoate, hexyl acetate, (*Z*)-3-hexenyl butyrate and ethyl octanoate. The electrophysiological activity of the identified compounds at three different doses was evaluated with electroantennography (EAG). An analysis of covariance of the EAG amplitude revealed that synthetic chemicals, sex, dose, and the synthetic chemical × dose interaction significantly influence the antennal response of *A. ludens*. Males and females were significantly more attracted to septa loaded with the eight-component synthetic blend compared to solvent controls in the wind tunnel.

Key Words: *Anastrepha ludens*, guava volatiles, attractants, GC-EAD, GC-MS

RESUMEN

Las respuestas conductuales y electrofisiológicas de machos y hembras de la mosca Mexicana de la fruta *Anastrepha ludens* (Loew) a volátiles de guayaba (*Psidium guajava* L.) fueron investigadas en experimentos de laboratorio. Machos y hembras fueron significativamente mas atraídos y aterrizaron mas frecuentemente en fruta de guayaba que en la esfera amarilla usada como control en el túnel de vuelo. Así también, ambos sexos fueron más atraídos al extracto de frutos de guayaba que al disolvente usado como control. El análisis por cromatografía de gases-electroantenografia de los extractos comportalmente activos mostró que consistentemente ocho y siete compuestos fueron antenalmente activos a machos y hembras, respectivamente. Los compuestos fueron identificados por cromatografía de gases-espectrometría de masas como butirato de etilo, (*E*)-3-hexenol, (*Z*)-3-hexenol, hexanol, hexanoato de etilo, acetato de hexilo, butirato de (*Z*)-3-hexenilo y octanoato de etilo. Los resultados del análisis de la amplitud del pico EAG a estímulos de compuestos sintéticos identificados a tres diferentes dosis estudiadas, mostraron que la respuesta antenal de *A. ludens* es significativamente afectada por los compuestos químicos, sexo, dosis y la interacción compuestos químicos × dosis. Los machos y las hembras fueron significativamente mas atraídos a septos de hule cargadas con la mezcla de los ocho compuestos, comparadas contra el disolvente en el túnel de viento.

Translation provided by the authors.

The Mexican fruit fly, *Anastrepha ludens* (Loew), is one of the most important species attacking more than 50 tropical fruits from eight families, including grapefruit, orange, mango, and common guava (Eskafi & Cunningham 1987; Norrbom & King 1988; Norrbom et al. 2000). In Mexico losses of citrus, mango, and guava due to *A. ludens* are estimated at 25% (Enkerlin et al. 1989). This species was originally native to Mexico although currently it is found in Central and South America, and southern Texas, USA (Hernández-Ortiz & Aluja 1993).

McPhail traps baited with fermenting sugars, yeast and hydrolyzed protein have been used for many years to monitor *A. ludens* and other species of fruit flies. Nevertheless, low capture efficiency, catch of non-target insects, difficulties in managing the liquid-baited McPhail traps in the field, and high cost have led to searches for more effective attractants and traps (Epsky et al. 1993; Heath et al. 1996). For instance, promising attractants have been found in bacteria, avian feces, and human urine (Robacker et al. 1998, 2000; Piñero et al. 2003). Several studies have also doc-

umented that both sexes of *A. ludens* are attracted to fruit volatiles (Robacker & Fraser 2002, 2003), although generally chemical identity of the compounds responsible for attraction remains unknown (but see Robacker et al. 1990, 1992). The isolation and identification of behaviorally active compounds may be a laborious task because a ripening fruit is a complex mixture of frequently over a hundred(s) detectable volatile compounds, possessing various functional groups and ranging from simple to complex structures (e.g., Buttery 1981; Maarse 1991). Nevertheless, analytical tools such as gas chromatographic-electroantennographic detection (GC-EAD) (Arn et al. 1975) may facilitate rapid identification of active compounds present in complex blends of fruit volatiles, eliminate from consideration compounds without biological activity, and suggest candidates for behavioral and field studies (Cossé et al. 1995; Zhang et al. 1999; Nojima et al. 2003).

In this study, firstly, we evaluated the attractiveness of guava fruits and their volatiles to male and female *A. ludens* in a wind tunnel; secondly, we located electrophysiologically-active compounds from guava extracts by using GC-EAD; thirdly we identified the EAD-active compounds by GC-mass spectrometry (GC-MS); and finally we evaluated the electrophysiological and behavioral activity of the identified compounds with an electroantennogram (EAG) bioassay in a wind tunnel, respectively.

Materials and Methods

Insects

The pupae were obtained from the Moscafrut (SAGARPA-IICA) mass rearing facility located in Metapa de Domínguez, Chiapas, Mexico. They were reared on an artificial diet previously described by Domínguez et al. (2000). The adults were maintained at 25°C ± 1°C, 70 ± 5% RH, and a photoperiod of 12:12 (L:D) h. Adults were fed *ad libitum* with a mixture of enzymatic yeast hydrolystate (ICN Biomedicals, Costa Mesa, CA) and sucrose (1:3) (unless otherwise specified). Water was provided in test tubes covered with cotton plugs. Adult flies of 8-20 d old were used for behavioral bioassays and GC-EAD and EAG analysis.

Chemicals

Synthetic chemicals were purchased from Sigma/Aldrich (Toluca, Mexico) and Bedoukian Research (Danbury, CT), and the purities were >95% based on the results with capillary gas chromatography.

Fruits

Ripe fruits were collected from a guava orchard (*Psidium guajava* L., native type) near Tapachula, Chiapas. The fruits were collected when they were of a yellow color and placed in plastic bags and immediately carried to the laboratory for bioassays and volatile collection. Because the color of guava fruits is not a good indicator of ripeness, we included the content of sugar determined as Brix degree. This degree is used to measure liquid density, especially sugar concentration in fruit and vegetable juices (Hogness & Jones 1984). We measured the Brix degree from a random sample of 10 yellow guava fruits with a refractometer (Iroscope, Mexico City), and the values ranged from 9.8 to 13.5 Brix degree.

Collection of Volatiles

Ripe healthy guava fruits (1.6 kg) were placed in a cylindrical glass aeration chamber (58 cm long × 18.5 cm i.d.). A charcoal-filtered airstream (1 l/min) was maintained through the glass aeration chamber for 16 h. Fruit volatiles were collected on 350 mg of Porapak Q (50-80 mesh, Water Associates, Inc., Milford, MA) packed between plugs of silanized glass wool in a Pasteur pipette. Porapak Q was cleaned previously by heating it in a nitrogen stream at 150°C for 5 h and then washed with diethyl ether. Fruit volatiles were eluted from the Porapak Q with 2 ml of diethyl ether (HPCL grade) and concentrated to 600 µl by slow evaporation under a gentle stream of nitrogen. The extract was stored at a -20°C until bioassays and analysis.

Wind Tunnel Bioassays

The observations were carried out in a flight wind tunnel, 120 cm long and 30 cm high and wide. A fan was used to pull air through the tunnel with a velocity of 0.4 m/s. Activated charcoal was used to filter intake air. Illumination was provided by four fluorescent bulbs (39 W daylight GE, Mexico City) mounted 60 cm above the wind tunnel giving a light intensity of 2,380 lux. The insects were evaluated in groups of 25 individuals (males or females), which were placed in a plastic container (6 cm high × 8 cm diameter, release cylinder) with screen top 18 h before testing. Water, but not food, was provided with the cylinders. They were allowed to acclimatize in the wind tunnel room conditions (25 ± 1°C, 60 ± 5% relative humidity) for at least one h before being assayed. In three different trials, the responses of flies to guava fruits, Porapak Q volatile extracts, and the synthetic blend were evaluated in non-choice tests. Polystyrene spheres (5 cm diameter) painted with vinyl acrylic water-based paints mixed to match (as detected by the human eye) the yellow of guava fruit were used for dispensing either the guava extracts (2nd trial) or the synthetic blend (3rd trial), and as control (in all trials). One g equivalent of guava fruit extract or 1 mg of

the synthetic blend prepared according to the relative proportions of each compound in the natural extracts was loaded on a rubber septum (Agrisense, England). The rubber septum was inserted into a yellow painted sphere. A rubber septum loaded with 10 µl of diethyl ether and placed in a yellow painted sphere was used as control. The target stimulus (e.g., fruit, unscented sphere, or scented sphere) was hung in the center of the wind tunnel, 16 cm from the upwind end. Each observation was started by placing the release cylinder on a 12 cm high platform at the downwind end of the tunnel and insects were released and observed for 10 min. The insects were recorded for upwind flight and for landing on odor source or control. Upwind movement was recorded if insects passed a point two-thirds of the distance from the release cylinder to the odor source or control (Robacker and Fraser 2002). Landing behavior was scored if the flies touched and stayed at least two minutes on the odor source. The flies were only used once during the bioassays. All bioassays were conducted between 8:00 and 13:00 h.

EAG Analysis

Antennal receptivity of adult males and females of *A. ludens* to synthetic compounds was determined by EAG. The insect head was cut off carefully, and a reference electrode was inserted into its base with a glass capillary filled with physiological saline solution (Malo et al. 2004). The distal end of the antenna was inserted into the tip of the recording glass capillary electrode. One replicate was made with one fly antenna. The signals generated by the antenna were passed through a high-impedance amplifier (NL 1200; Syntech, Hilversum, The Netherlands) and displayed on a monitor by Synthech software for processing EAG signals. A stimulus flow controller (CS-05; Syntech) was used to generate a stimulus at 1-min intervals. A current of humidified pure air (0.7 l/min) was constantly directed onto the antenna through a 10-mm-diameter glass tube. At least 12 individuals of each sex were used in these tests.

Serial dilutions of the synthetic compounds were prepared in HPLC-grade hexane to make 1, 10, and 100 µg/µl solutions. A standard aliquot (1 µl) of each test dilution was pipetted onto a piece of filter paper (0.5 × 3.0 cm, Whatman, No. 1) exposed to air 20 s to allow the solvent to evaporate, then inserted into a glass Pasteur pipette or sample cartridge, and left for 40 s before applying. A new cartridge was prepared for each insect. To present a stimulus, the pipette tip containing the test compound was inserted through a side hole located at the midpoint of a glass tube through which humidified pure air flowed at 0.5 l/min. The duration of stimulus was 1 s. The continuous flow of clean air through the airflow tube and over the preparation ensured that odors were removed immediately from the vicinity. The synthetic compounds were presented in random order and the test doses for each compound were presented sequentially from the lowest to highest concentration. Control stimuli (hexane) were presented at the beginning and end of each EAG analysis, and in the analysis the value was included as the mean of two measures.

GC-EAD Analysis

GC-EAD analysis (Arn et al. 1975) was carried out to locate the antennally active components in Porapak Q extracts. The system consisted of a gas chromatograph (Varian 3600, Palo Alto, CA) coupled to an electroantennogram apparatus (Syntech, Hilversum, The Netherlands). The GC was equipped with a capillary column (DB-5MS, 30 m × 0.25 mm i.d., film thickness 0.5 µm; J & W Scientific, Folsom, CA), a flame ionization detector (FID) and a split/splitless injector. Temperature oven was programmed at 50°C for 2 min, then 3°C/min to 280°C, and held for 10 min. Injector and detector temperatures were 250°C and 300°C, respectively. The injector was operated in splitless mode. Helium was used as carrier gas at 2 ml/min and nitrogen as make-up gas. At the end of the capillary column a fixed outlet splitter (VSOS, Scientific Instruments Services, Ringoes, NJ) distributed the effluent from the column to FID and to a transfer line towards the EAD preparation. Both connection tubings were made of deactivated fused silica of the same length and diameter such that the column effluent was split approximately 1:1. The EAG set up was reported above. Before injection of a sample, the antenna was stimulated with linalool to check sensitivity. If the antennae elicited a clear response different to that of signal-noise, then the guava fruit extract or synthetic compounds was injected. A minimum of 9 different antennae per sex were used, and for each test an antenna was used only once.

Chemical Analysis

The GC-MS analysis was conducted with a Varian Star 3400 CX chromatograph linked to a Varian Saturn 4D mass spectrometer. The samples were analyzed in a fused silica column (DB5-MS, 30 m × 0.25 mm, film thickness 0.5 µm) that was programmed at 50°C for 2 min, then 3°C/min until 280°C, and held for 10 min. The carrier gas was helium (l ml/min). The injector port temperature was held at 250°C. Ionization was by electron impact at 70 eV. Identifications were based on retention time, mass spectral analysis of the natural compounds, and comparison with synthetic standards.

Statistical Analysis

Data were analyzed with the Statistica Software Package version 6.0 (StatSoft, Inc., 2003). Data from behavioral and EAG experiments were analyzed for homogeneity of variances and normality. When necessary, data were transformed with log (x + 1) to stabilize the variance and normality. Results of the wind tunnel were subjected to *t*-test. The values of EAG depolarization amplitude after exposure to synthetic chemicals in the dose-response studies were analyzed by three-way ANCOVA in a block design, where the hexane response was the covariate (the EAG amplitude response to hexane was a typical mechanical response produced by the air because the solvent was evaporated), and significant ANCOVAs were followed by a posthoc Tukey's test for multiple comparison of means ($P < 0.05$). The treatments tested were synthetic products, sex, and dose. The insects represent replicates.

Results

Response to Guava Fruits and Extracts

Males ($t = 5.2$; $df = 28$; $P < 0.001$), and females ($t = 2.4$; $df = 28$; $P = 0.02$) were more attracted to guava fruits than to yellow sphere (Table 1). Both males ($t = 5.0$; $df = 28$; $P < 0.001$) and females ($t = 5.7$; $df = 28$; $P < 0.001$) landed more often on guava fruits than on yellow spheres (Table 1). Also, flies of both sexes were more attracted to guava fruit extracts (males: $t = -4.5$; $df = 18$; $P < 0.001$; females: $t = 6.7$; df 18; $P < 0.001$), but few females and no males landed on yellow spheres dispensing fruit volatiles (Table 1).

GC-EAD Analysis

GC-EAD analysis of guava extracts eluted from Porapak Q revealed eight and seven compounds that elicited consistent antennal responses from male and female *A. ludens*, respectively (Table 2). The corresponding EAD active compounds were identified as ethyl butyrate, (*E*)-3-hexenol, (*Z*)-3-hexenol, hexanol, ethyl hexanoate, hexyl acetate, (*Z*)-3-hexenyl butyrate and ethyl octanoate, respectively, by comparison of mass spectra and GC-MS retention times with synthetic standards. Ethyl butyrate, ethyl hexanoate, (*Z*)-3-hexenol, (*E*)-3-hexenol, and ethyl octanoate elicited a great antennal response from female antennae (Table 2). The response of male antennae was lower than the females, but again ethyl butyrate, ethyl hexanoate, and ethyl octanoate evoked the strongest antennal responses (Table 2). Antennal activity of the eight natural compounds was confirmed by antennal responses elicited by 1 mg of their respective synthetic compounds. The ratio of the different compounds in headspaces samples, estimated by GC-MS, were: ethyl butyrate, (*E*)-3-hexenol, (*Z*)-3-hexenol, hexanol, ethyl hexanoate, hexyl acetate, (*Z*)-3-hexenyl butyrate, and ethyl octanoate (14:1:28:10:18:120:6: 4), respectively.

EAG Analysis

The ANCOVA analysis of the amplitude of the EAG revealed that synthetic chemicals, sex, dose. and the synthetic chemicals × dose interaction significantly influenced the antennal response of *A. ludens* (Table 3). The interaction between synthetic chemicals × dose is shown in Fig. 1. Multiple comparisons revealed that, at the dose of 1 µg, ethyl hexanoate and ethyl octanoate evoked significantly larger EAG responses compared with those elicited by hexanol and hexyl acetate. The antennal response evoked by (*Z*)-3-hexenyl butyrate, (*Z*)-3-hexenol, (*E*)-3-hexenol, and ethyl butyrate were intermediate between, and not significantly different from, those elicited by ethyl hexanoate and ethyl octanoate, and hexanol and hexyl acetate. At a dose of 10 µg, ethyl hexanoate evoked significantly larger antennal response compared with those elicited by hexanol and hexyl acetate. The EAG response evoked by (*Z*)-3-hexenyl butyrate, (*Z*)-3-hexenol, (*E*)-3-hexenol, ethyl octanoate, and ethyl butyrate were intermediate between and not significantly different from

Table 1. Mean percentages (± SE) of *A. ludens* that exhibited attraction to and landed on guava fruits and extract in a wind tunnel.

	Attraction		Landing	
Treatment	Female	Male	Female	Male
Guava fruit	27.7 ± 3.2 a	23.7 ± 2.4 a	7.2 ± 1.1 a	8.8 ± 1.3 a
Yellow sphere	15.5 ± 4.1 b	9.1 ± 1.9 b	0.8 ± 0.5 b	1.1 ± 0.5 b
Guava extract	25.6 ± 1.9 a	12.8 ± 0.9 a	2.4 ± 1.1 a	0.0 a
Yellow sphere + solvent	9.6 ± 1.3 b	6.8 ± 0.8 b	0.0 a	0.0 a

Guava fruit was compared to a yellow sphere ($n = 15$ replicates per treatment per sex), and guava volatiles extract compared with ethyl ether as control ($n = 10$ replicates per treatment per sex). Means within columns followed by the same letter are not significantly different (*t*-test, $P > 0.05$).

TABLE 2. VOLATILES ELICITING ELECTROPHYSIOLOGICAL ACTIVITY DURING GC-EAD RECORDING FROM FEMALE AND MALE *A. LUDENS* ANTENNAE EXPOSED TO HEADSPACE COLLECTIONS OF GUAVA FRUITS.

	Male antennae		Female antennae	
Compound[a]	EAG responses in 9 runs[b]	EAG intensity (mV) (Mean ± S. E.)	EAG responses in 10 runs[c]	EAG intensity (mV) (Mean ± S. E.)
Ethyl butyrate	9	0.35 ± 0.07	10	0.85 ± 0.18
(*E*)-3-Hexenol	6	0.17 ± 0.07	8	0.63 ± 0.18
(*Z*)-3-Hexenol	7	0.18 ± 0.08	10	0.60 ± 0.17
Hexanol	6	0.08 ± 0.05	6	0.33 ± 0.07
Ethyl hexanoate	9	0.60 ± 0.08	10	1.16 ± 0.19
Hexyl acetate	7	0.15 ± 0.07	2	—
(*E*)-3-hexenyl butyrate	8	0.18 ± 0.08	5	0.62 ± 0.22
Ethyl octanoate	9	0.46 ± 0.07	7	0.77 ± 0.12

[a]Identification is based on comparison of mass spectra and retention times of the natural materials with those of authentic synthetic standards.
[b]Based on four different headspaces samples and 9 different insects.
[c]Based on four different headspaces samples and 10 different insects.

those elicited by ethyl hexanoate, and hexanol and hexyl acetate. At the higher dose tested (100 µg), the EAG response to (*E*)-3-hexenol was significantly higher in comparison to those evoked by hexanol, hexyl acetate, ethyl butyrate, ethyl hexanoate, ethyl octanoate, and (*Z*)-3-hexenyl butyrate. There was no significant difference in the EAG response by (*E*)-3-hexenol and (*Z*)-3-hexenol. Hexanol and ethyl octanoate evoked the lowest EAG responses.

Behavioral Response to the Blend of the EAD-Active Compounds

Results of the behavioral response to the blend of the EAD-active compounds are shown in Fig. 2. Both males (t = 3.52, df = 18, P = 0.002) and females (t = 3.27, df = 18, P = 0.004) were more attracted to septa loaded with the eight-component guava blend compared to a solvent control. However, few insects landed on spheres dispensing the guava blend, with no differences between the number of flies landing on the blend source and the control spheres (male: t = 0.45, df = 18, P = 0.65; females: t = 0.64, df = 18, P = 0.52).

DISCUSSION

This study showed that both males and females of *A. ludens* were attracted to and landed on guava fruits more often than on yellow spheres in a wind tunnel. The weak responses of *A. ludens* to guava fruits and their volatiles in the wind tunnel are similar to those obtained with other fruit species (e.g., Robacker et al. 1990; Robacker & Fraser 2002). The fact that Porapak Q extracts evoked few landings on the source compared with those observed with fruits could indicate that the compounds eliciting this particular behavior are lacking in these extracts or they are not in the appropriate concentrations to elicit landings in the wind tunnel. This idea seems to be supported by

TABLE 3. TEST OF SIGNIFICANCE OF THE FACTORS INVOLVED IN THE ANCOVA ANALYSIS OF THE EAG RESPONSE OF MALES AND FEMALES OF *A. LUDENS* TO SYNTHETIC VOLATILES AT DIFFERENT DOSES.

Source of Variation	SS	*df*	MS	*F*	*P*
Covariate (Hexane)	30.60	1	30.600	1117.80	<0.0001
Sex	0.54	1	0.540	19.70	<0.0001
Synthetic chemicals	4.90	7	0.690	25.50	<0.0001
Dose	8.30	2	4.150	151.60	<0.0001
Sex-Synthetic products	0.22	7	0.030	1.20	0.31
Sex-Dose	0.02	2	0.010	0.44	0.64
Synthetic products-Dose	6.70	14	0.480	17.50	<0.0001
Sex-Synthetic products-Dose	0.13	14	0.009	0.34	0.98
Block	2.90	11	0.260	9.60	<0.0001
Error	14.10	516	0.020		
Total	82.60	575			

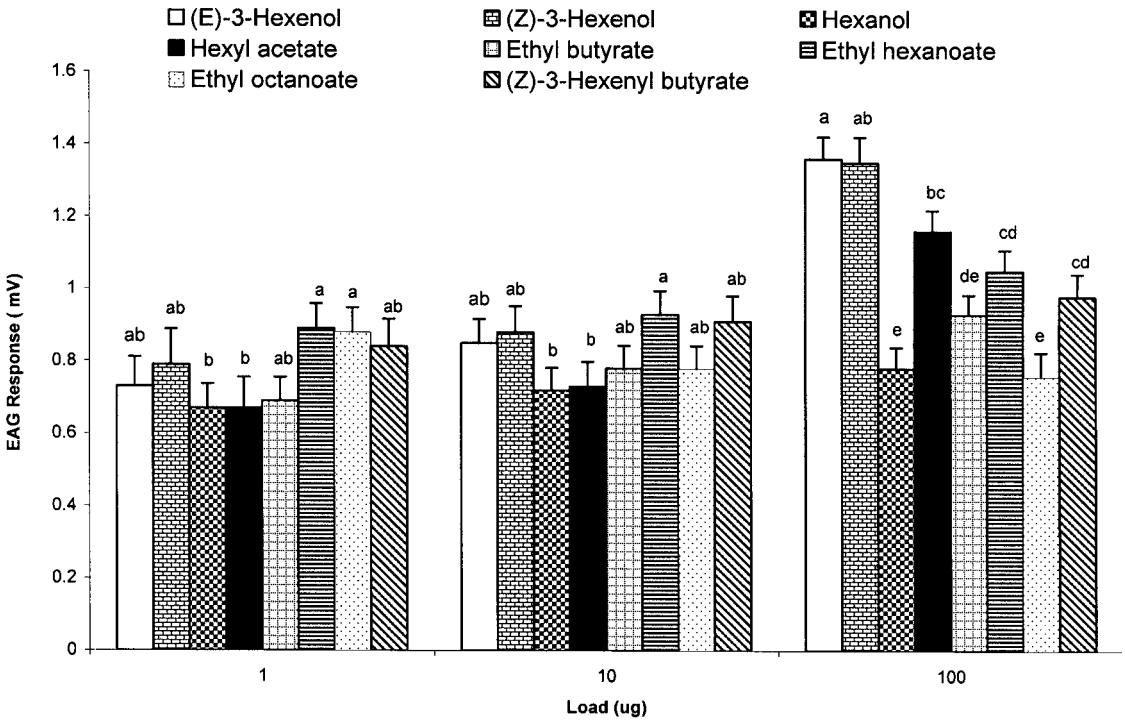

Fig. 1. Mean EAG amplitude response (± SEM) of *A. ludens* to synthetic chemicals isolated from guava fruits. Data from both sexes per compound were combined for this figure (n = 24 EAG recordings for each compound). Significant differences within-dose comparisons are indicated by different letters (Tukey test, $P < 0.05$).

the results obtained during the evaluation of synthetic compounds identified from guava extracts in which few flies landed on yellow spheres baited with the guava blend.

In the present study we showed that GC-EAD technique can be a useful tool for identifying EAG-active compounds and so avoiding the evaluation of compounds from host fruit without biological activity. This electrophysiology approach has been previously used for the identification of attractants for two fruit flies species (Cossé et al. 1995; Zhang et al. 1999; Nojima et al. 2003). For example, Zhang et al. (1999) using solid-phase microextraction and GC-EAD were able to identify a new blend of volatiles from apples as attractants for apple-origin *Rhagoletis pomonella* (Walsh) flies. The new five-component blend identified contained three compounds in common with the previous seven-component blend (Fein et al. 1982), plus two new components. In wind tunnel bioassays, sticky red spheres baited with the new five-component blend caught more flies than the previous seven-component blend or butyl hexanoate, a compound used commercially to monitor *R. pomonella*. In field experiments, red spheres baited with the five-component blend also captured more flies than butyl hexanoate (Zhang et al. 1999).The behavioral evaluation of all compounds identified in host volatiles and their possible mixtures may be a time demanding task. For instance, Robacker et al. (1990) identified over 70 compounds from fermented chapote. When the identified compounds were tested individually, only 16 of the chemicals were slightly attractive to *A. ludens*. In bioassays, the number of compounds in an attractive mixture was reduced to three by elimination of unnecessary compounds. The three compounds identified were 1, 8-cineole, ethyl hexanoate, and hexanol and the three-component blend (CEH) was 1.8 times more attractive than aqueous solutions of torula dried yeast and borax to *A. ludens*. In a subsequent study, 16 compounds previously identified in volatiles from chapote fruit, but not evaluated in earlier work, were tested for individual attractiveness and for their capacity to enhance the attractiveness of CEH when combined with it (Robacker et al. 1992). Of all compounds evaluated, ethyl octanoate was found to increase the attractiveness of CEH and this four-component blend (CEHO) was more attractive than torula yeast in McPhail traps during flight-chamber tests.

Some of the compounds identified in the present study have been reported to influence the behavior of other fruit flies. For mated female *Ceratitis capitata* (Wied.), the kairomonal activity

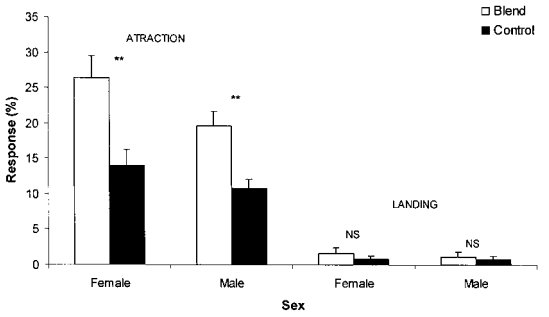

Fig. 2. Behavioral response of male and female *A. ludens* to synthetic blend compounds in the wind tunnel. Double asterisks (**) mean significant difference in the response of the synthetic blend compared to control; NS means not significant (*t*-test, $P > 0.05$).

of the odor of nectarine, a highly-preferred host fruit, was synergistically enhanced by a blend of ethyl hexanoate, and methyl and ethyl octanoate (Light & Jang 1996). Hexenyl acetate individually was attractive to *Bactrocera dorsalis* (Hendel) (Hwang et al. 2002), and blended with another six esters, was attractive to *R. pomonella* (Fein et al. 1982).

In conclusion, this study shows that both sexes of *A. ludens* are attracted to guava fruits and guava extracts in a wind tunnel. The compounds responsible for this attraction were identified by GC-EAD and GC-MS techniques. The behavioral evaluation of the identified compounds showed that they are attractive to male and female *A. ludens* in a wind tunnel. Finally, this study showed that an approach similar to that used here could be useful in the searching of potential host fruit attractants for *A. ludens* and other *Anastrepha* fruit flies. The behavioral activity of the compounds identified here will be evaluated in field conditions in future studies.

ACKNOWLEDGMENTS

We are grateful for the technical assistance of Gustavo Rodas, Azucena Oropeza, and Milton A. Rasgado, and the statistical advice of Javier Valle-Mora (ECOSUR). We also thank Julio Domínguez, Moscafrut (National Fruit Fly Campaign-SAGARPA-IICA) mass rearing facility director, for providing the flies used in this study. This project was supported by a grant from CONACyT (project 36490-B).

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