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OLFACTORY RESPONSES OF *ANASTREPHA OBLIQUA* (DIPTERA: TEPHRITIDAE) TO VOLATILES EMITTED BY CALLING MALES

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ABSTRACT

The West Indian fruit fly, *Anastrepha obliqua* (Macquart), is considered one of the most important pests of mango (*Mangifera indica* L.) and hog plums (*Spondias* spp.) in Latin America. A better understanding of the role of chemical compounds during the sexual behavior of *A. obliqua* may be useful to improve the monitoring of this tephritid fruit fly pest. The objectives of this study were: (1) to evaluate the attraction of females and males to live calling males and to Super Q extracts of calling males; (2) to measure the female and males antennal responses to extracts from live calling males; (3) and to identify the compounds emitted by *A. obliqua* males during calling by gas chromatography-mass spectrometry (GC-MS). Both sexes were attracted to live males and to male extracts. Extracts from males elicited significant antennal responses from both sexes compared to those evoked by the control. GC-MS analyses of the volatile extracts showed that calling *A. obliqua* males consistently emitted 9 compounds, 6 of which are reported for the first time for this fruit fly species. Preliminary bioassays showed that females and males were attracted to (*Z*)-3-nonenol and (*Z*)-3-nonenol + β-farnesene. Further identification of the unknown compounds and their synthesis remain to be performed in order to evaluate their biological activity.

Key Words: West Indian fruit fly, sexual behavior, male volatiles, sexual/aggregation attraction

RESUMEN

La mosca de la fruta de las Indias Occidentales, *Anastrepha obliqua* (Macquart), se considera una de las plagas más importantes del mango (*Mangifera indica* L.) y jobo o jocote (*Spondias* spp.) en América Latina. Un mejor conocimiento del papel que juegan los compuestos químicos durante el comportamiento sexual de *A. obliqua* puede ayudar a mejorar los sistemas de trampeo. Los objetivos de esta investigación fueron evaluar la atracción de hembras y machos a volátiles emitidos por machos vivos y extractos colectados de machos vivos con filtro Super Q; evaluar la respuesta antenal (EAG) de hembras y machos a extractos colectados de machos vivos con filtro Super Q; e identificar los compuestos volátiles liberados por los machos. Las hembras y los machos fueron atraídos por los machos vivos y a extractos colectados de machos vivos con filtro Super Q. La respuesta medida con EAG mostró que ambos sexos tuvieron actividad antenal a extractos colectados de machos vivos con filtro Super Q. El análisis de los volátiles de machos mostró la presencia de nueve compuestos, de los cuales seis son nuevos para esta mosca de la fruta. Los bioensayos prelimares con los compuestos sintéticos disponibles permitieron demostrar que las hembras y los machos fueron atraídos al compuesto (*Z*)-3-nonenol y a la mezcla binaria constituida por (*Z*)-3-nonenol y β-farneseno. La identificación total y síntesis química de los compuestos desconocidos y conocidos que faltan debe ser realizada para evaluar su actividad biológica.

The West Indian fruit fly, *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae), is considered a pest of several economically important fruit crops such as mango (*Mangifera indica* L.) and hog plums (*Spondias* spp.) (Enkerlin et al. 1989). It has been recorded from the USA (Florida and Texas) to South America, including the Caribbean Islands (Hernández-Ortiz & Aluja 1993).

The sexual behavior of *Anastrepha* spp. includes a calling display by the males whereby they inflate their pleural pouches and fan their wings to produce sounds and disseminate volatile compounds that are released through the mouth, anus and sometimes directly from the cuticle by evaporation to attract females (Nation 1989). During calling behavior, males of *Anastrepha ludens* Loew, *Anastrepha suspensa* (Loew), *Anastrepha fraterculus* (Wiedemann), and *Anastrepha serpentina* (Wiedemann) released numerous volatiles (Sivinski & Calkins 1986; Landolt & Averill

1999; Heath et al. 2000). The compounds released by these species have been identified as lactones, monoterpenes, sesquiterpenes, alcohols, esters, pyrazines, and aldehydes (Landolt & Averill 1999; Heath et al. 2000; De Lima et al. 2001; Robacker et al. 2009). Some of the identified compounds are thought to be sexual pheromone components of these species but only in a few cases have their biological activity been evaluated (e.g. De Lima et al. 2001; Robacker & Hart 1985; Robacker et al. 2009). Additionally, preliminary studies have shown that the volatile compounds released from male salivary glands were attractive to females in laboratory bioassays (De Lima et al. 2001).

In this study the aims were: (1) to evaluate the attraction of females and males to live calling males and to Super Q extracts from calling males, which are volatiles releases by males; (2) to measure the female and males antennal responses to extracts from live calling males; and (3) to identify the compounds emitted by *A. obliqua* males during calling. This work resulted is the first report of biological activity of *A. obliqua* females and males in response to volatiles emitted by calling males.

Materials and Methods

Insects

Non-irradiated *A. obliqua* flies used in the experiments were obtained from MOSCAFRUT facility, located in Metapa de Domínguez, Chiapas, Mexico, with periodic introductions of wild material; the last introduction occurred in 2002. Flies were reared under the standard operational protocol (Artiaga-López et al. 2004) for about 115 generations.

For our experiments, insects were placed in wooden cages covered with mesh on the sides (65 × 65 × 45-cm) and kept at 27 ± 2 °C, at 60 - 70% RH, and a photoperiod of 12:12 hr L:D. The photophase began at 0700 h and ended at 1900 h. Flies were separated by sex 2 days after eclosion to keep the flies virgin. Flies were fed *ad libitum* with a mixture of enzymatic yeast hydrolysate (MP Biomedical, Irvine, California), sucrose (1:3, wt:wt) and water and maintained at a density of 100 flies in a cage with 30 × 30 × 30-cm covered on one side by a 2-mm tulle mesh. No food or water was given to males during the collection of volatiles.

Collection of Volatiles

Ten 8-10 d old *A. obliqua* males were confined in a 100 mL glass entrapment container (4.8 cm internal diam × 12.5 cm high). Volatiles were drawn from the container using purified air, previously passed through an activated charcoal trap, into a glass volatile collection trap (4 mm ID × 40 mm long) containing 50 mg Super Q adsorbent (Alltech Associates Inc. Deerfield, Illinois). Air was drawn through the trap at a rate of 1 L/min. At the conclusion of each air entrapment, the volatiles were eluted from the adsorbent with 200 µL of methylene chloride (Baker, HPLC grade).

Volatiles emitted by *A. obliqua* males were collected in 2 periods: from 0700 to 1800 h and during peak calling (0700 to 1000 h). The volatiles collected during the peak calling period were used for electrophysiological tests and for chemical analyses, while volatiles collected from 0700 to 1800 h were used in field cage tests. A longer time was used for collecting volatiles for bioassays in field cages, because these males had been used as baits from 0700 to 1800 h; and therefore the extracts contained amounts of volatiles similar to those emitted by live males. *A. obliqua* males released more volatiles in the morning, less around midday, followed by an increase in the afternoon (López-Guillén et al. 2008).

All volatile collections were performed in a room without windows at 25 ± 2 °C and 50-60% RH, and 1190 lux (illumination was provided by four 39-watt fluorescent lamps placed 3 m above volatile collection devices).

Behavioral Responses of *Anastrepha obliqua* to Live Conspecifics and Male Extracts

The response of *A. obliqua* males and females to live conspecifics was evaluated in seminatural conditions using cylindrical clear nylon screen field cages (2.85 m diam × 2-m high). In the center of each cage, 3 potted mango trees (1.20 m tall) were placed to provide resting and calling sites for fruit flies. The cages were placed under the shade of Primavera trees (*Tabebuia donnell-smithii* Rose). Fifty virgin females or 50 virgin males (8 to 10 d old) were placed into different field cages at least 1 h before testing (0600 h). Then, 10 males, 10 females and 10 males + 10 females, respectively were each placed as a lure in the container of a Mutilure trap (Better World Manufacturing Inc., Fresno, California, USA), which was lined with mesh to prevent the escape of live flies. The 3 traps containing live flies and a trap without flies (control) were hung 10 cm from the top of cage, and equidistantly from each other, and 35 cm from the perimeter of the cage. Traps were maintained with 250 mL of water and Tween 80 (ICI, Wilmington, Delaware) (2 mL of Tween 80/liter of water) to break the surface tension and to prevent trapped flies from escaping. Traps were placed daily at 0700 h, and the number of flies caught by the traps was recorded at 1700 h (11 h after insect introduction), and uncaught flies were removed from the field cages and never used again. After each replication, the traps were rotated counter-clockwise to reduce

positional effects. The temperature ranged from 25 to 32 °C and RH from 60 to 95% during the experiments. A total of 16 replications per field cage were performed.

The response of *A. obliqua* flies to Super Q extracts from live males was evaluated in a similar bioassay as described above. Fifty virgin females or 50 virgin males (8 to 10 d old) of *A. obliqua* were placed into field cages. Flies were introduced into the field cages at least 1 h before testing. Flies were provided with water, which was withdrawn before beginning the bioassay. One hundred µL of volatile extract of 10 males or 100 µL of hexane—used as a control—were loaded into rubber septa (Sigma-Aldrich, Toluca, Mexico). The extract was collected during 11 h using the dynamic headspace technique as described above. The rubber septa were placed into the containers of Multilure traps. As a positive control, 10 live males were placed into the container of another Multilure trap. The number of flies captured by each trap was registered daily. A total of 16 replications per field cage were performed.

Chemical Analyses of Extracts

Chemical analyses of the extracts were conducted in a Varian CP-3800 gas chromatograph coupled with a Varian Saturn 2200 (GC-MS) mass spectrometer (Varian, Palo Alto, California). The extracts were analyzed using a non-polar (VF-5MS) capillary column (30 m × 0.25 mm) or polar (CP-Wax 57 CB) capillary column (25 m × 0.25 mm) (Agilent Technologies, Santa Clara, California, USA). The analyses were performed using an initial temperature of 50 °C (for 2 min) increasing 15 °C/min to 280 °C (for 10 min). Helium was used as the carrier gas. Temperature of the injector was 200 °C. Ionization was by electronic impact at 70 eV. The compounds were identified by comparing Kovat's index and mass spectra of the synthetic standards. The mass spectrum of (*Z,E*)-α-farnesene was compared with that in the Wiley/NBS spectral data base (McLafferty & Stauffer 1989). Other compounds were tentatively identified based by comparison with spectra from the NIST/EPA/NIH Mass Spectral library (version 2.0, 2002).

Electroantennography (EAG)

Antennal receptivity of the *A. obliqua* females and males to the Super Q extracts was determined by EAG. A female or male's head was cut off carefully and the reference glass capillary electrode inserted into its base. The tip of the recording glass capillary electrode was inserted into the distal end of the antenna. The capillaries were filled with saline solution (Cruz-López et al. 2006). The signals generated by the antenna were displayed on a monitor using Syntech software for processing EAG signals (Syntech NL 1200, Hilversum, The Netherlands). Extracts collected from 5, 10, 20 or 50 calling males were prepared in methylene chloride (Baker, HPLC grade). For each extract 1µL was applied to a filter paper (0.5 by 3.0-cm, Whatman no. 1), left for 20 s to allow the solvent to evaporate and then inserted into a glass Pasteur pipette or sample cartridge for 40 s before testing. New cartridges were prepared for every insect tested. A stimulus controller (CS-05, Syntech) was used to generate stimuli 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 to ensure that odors were immediately removed from the antennal preparation. To present a stimulus, the pipette tip containing the test extract was inserted through a side hole located at the midpoint of the glass tube through which humidified pure air flowed at 0.5 L/min. The duration of the stimulus was 1 s. Control stimuli (hexane) were presented at the beginning, followed by extracts in random order, and finally, a control stimulus was applied again. The amplitude value in mV was used for analyzing the EAG recordings. One fly antenna for each series of the extracts was tested.

Behavioral Response of *Anastrepha obliqua* Females to Selected Synthetic Compounds

The response of virgin *A. obliqua* flies to synthetic compounds available commercially was evaluated in glass chambers (30 × 30 × 30 cm) as previously described (Robacker & Hart 1984). Twenty five females or males were placed inside the chambers at least 1 h before testing. Solutions of the synthetic standards were prepared to 1 µg/µL dissolved in hexane and its combinations to a load of 20 µg. Twenty µL of hexane was used as control. The treatments were deposited onto 2 filter papers with test chemical and 2 filter papers with hexane (2.5 × 2.5-cm, Whatman no. 4) positioned with thread on the corner of top of the chamber. One paper with a treatment and another, a control, were positioned at diagonally opposite each corners. The farnesene mixture β-farnesene and (*Z*)-3-nonenol were obtained from Aldrich (Toluca, Mexico). The number of flies beneath each paper was counted every 2 min for 30 min. A total of 5 repetitions were performed. The number of males or females beneath each treatment was counted every 2 min for 30 min. A total of 5 repetitions per sex were performed.

Statistical Analysis

All analyses were conducted using the computer program, Statistica (Statsoft 2003). Data were first checked for normality and transformed when necessary. The number of females or males captured by each trap was analyzed using a main

effects analysis of variance (ANOVA). EAG data also were analyzed by a one-way ANOVA. Significant differences were followed by Tukey test for multiple comparisons of means with the significance of $\alpha = 0.05$. Data from the response of virgin females and males to synthetic compounds were analyzed by using a paired Student's *t*-test.

RESULTS

Behavioral Responses of *Anastrepha obliqua* to Live Conspecifics and Male Extracts

The responses of *A. obliqua* females to live conspecifics were affected by treatment ($F = 3.63$; df = 3, 63; $P = 0.019$). In contrast, the responses of *A. obliqua* males to live conspecifics were not affect by treatment ($F = 2.41$; df = 3, 63; $P = 0.070$). Traps baited with live males caught more females than those captured by unbaited traps. The numbers of females captured by traps baited with live females and live males + females were not significantly different to that caught by unbaited traps (Fig. 1).

The responses of the virgin *A. obliqua* females ($F = 14.97$; df = 2, 47; $P = 0.000$) and males ($F = 8.62$; df = 2, 47; $P = 0.001$) were affected by treatment. Traps baited either with the Super Q extracts of live calling males (LME), or and live calling males (LM) caught significantly more females and males than those captured by unbaited traps (Fig. 2).

Chemical Analyses of Extracts

GC-MS analyses of the volatile extracts showed that calling males of *A. obliqua* consistently released 9 compounds: (1) (*Z*)-3-nonenol, (2) nonadienol, (3) unknown sesquiterpene, (4) β-farnesene, (5) (*E,E*)-α-farnesene, (6) (*Z,E*)-α-farnesene, (7), (8) and (9) sesquiterpenes (Table 1).

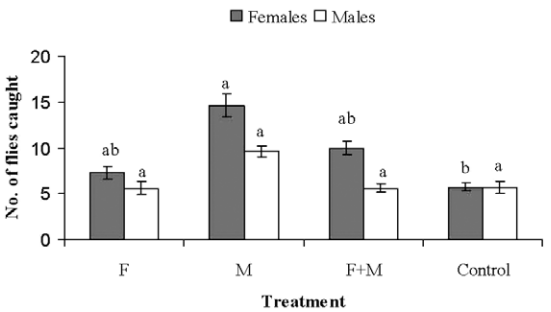

Fig. 1. Mean (±SE) of *Anastrepha obliqua* males and females caught by traps baited with conspecific live females (F), live males (M), live females + males (F + M), or without flies (control). Bars with different letters are significantly different (Tukey test, $P < 0.05$).

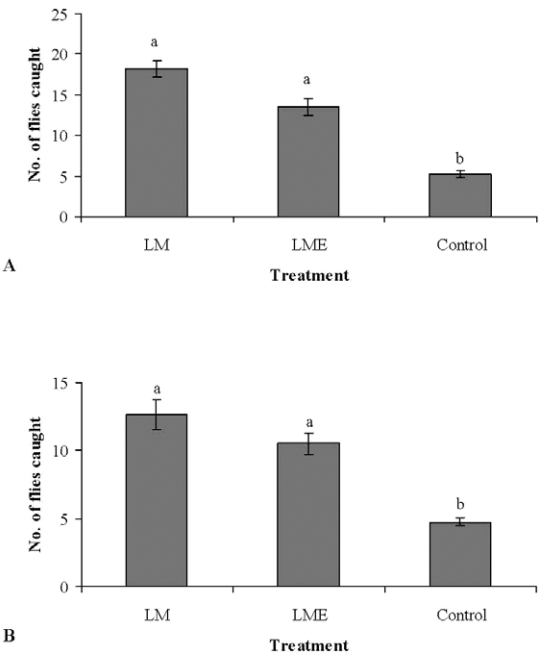

Fig. 2. Mean (±SE) of *Anastrepha obliqua* females (A) and males (B) caught in traps baited with live males (M), Porapak Q extracts of live males (ME), or hexane (control). Bars with different letters are significantly different (Tukey test, $P < 0.05$).

Compounds (2), (3), (4), (7), (8) and (9) are being reported for the first time for *A. obliqua*. Mass spectral data of (2) indicated that this compound was a nonadienol which readily dehydrated to give an ion at m/e 122 and other prominent ions at m/e 67, 93, 79, 55 and 41; mass spectrum of (3) resembles a sesquiterpene with molecular ion of m/z 204, with characteristic ions at 49 (base peak), 84, 105, 133, 147, 161, 175 and 189; the mass spectrum of peak (7) showed characteristic ions at 49 (base peak), 93, 107, 119, 135, 161, 189 and molecular ion m/z 204; peak (8) displayed a molecular ion of m/z 204, with characteristic ions at 41, 91, 197, 135 (bases peak); and peak (9) had a molecular ion of m/z 204, with characteristic ions at 49 (base peak), 84, 105, 133, 147, 161, 175 and 189. Unidentified compounds 7, 8 and 9 were considered as farnesene isomers since mass spectra and retention times match those found in the farnesene mixture obtained from Aldrich (Toluca, Mexico).

Electroantennography

The extracts from live calling males affected the antennal responses of *A. obliqua* females ($F = 22.90$; df = 4, 54; $P < 0.001$). Multiple comparisons revealed that extracts of 20 and 50 calling males

TABLE 1. RELATIVE AMOUNT (%) OF VOLATILE COMPOUNDS RELEASED BY CALLING *ANASTREPHA OBLIQUA* MALES.

Peak	Compound	Kovat's Index[1]	(Mean ± SE)
1	(*Z*)-3-nonenol[3]	1138	8.01 ± 6.10
2	Nonadienol[2,3]	1138	t
3	Sesquiterpene[2]	1410	0.58 ± 0.45
4	*β*-farnesene	1393	1.01 ± 0.74
5	(*Z,E*)-α-farnesene	1481	56.22 ± 41.08
6	(*E,E*)-α-farnesene	1493	13.17 ± 9.64
7	Farnesene isomer[2]	1500	0.33 ± 0.25
8	Farnesene isomer[2]	1580	20.47 ± 14.96
9	Farnesene isomer[2]	1604	0.21 ± 0.16

[1]Kovat´s index calculated from retention times data on a SPB-1 capillary column.
[2]Unknown chemicals.
[3](Z)-3-nonenol and the nonadienol do not separate from each other on the nonpolar column used in this investigation.
t = traces.

elicited EAG responses that were significantly larger than those evoked by the extract of 5 calling males and control (Fig. 3A).

The extracts from live calling males affected the antennal responses of *A. obliqua* males (F = 14.05; df = 4, 55; $P < 0.001$). Multiple comparisons revealed that extracts of calling males elicited EAG responses that were significantly higher than control, but there were no differences among extracts (Fig. 3B).

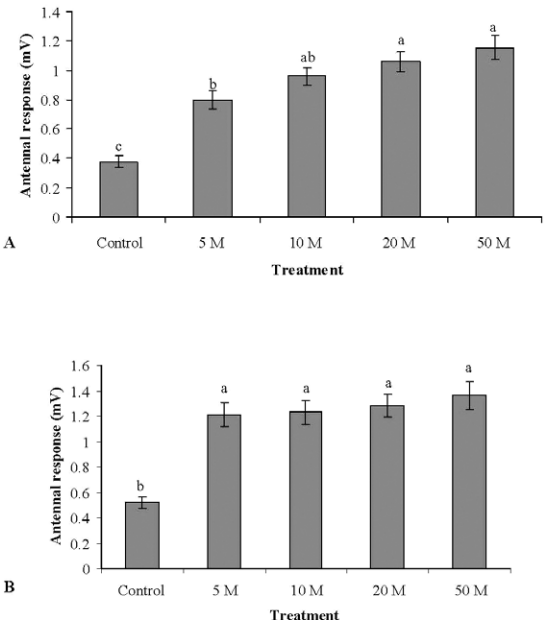

Fig. 3. Mean (±SE) antennal responses of *Anastrepha obliqua* females (A) and males (B) to Super Q extracts equivalent to different numbers of calling males (M), and to a control (hexane). Bars with different letters are significantly different (Tukey test, $P < 0.05$).

Behavioral Responses of *A. obliqua* Females and Males to Selected Synthetic Compounds

Virgin females were significantly more attracted to (*Z*)-3-nonenol (t = 3.05, df = 8, P = 0.03), and the mixture of (*Z*)-3-nonenol + *β*-farnesene (t = 3.09, df = 8, P = 0.03) than to the control. Females were not significantly more attracted to racemic farnesene alone (t = 0.70, df = 8, P = 0.95), *β*-farnesene alone (t = 2.28, df = 8, P = 0.08), or the blend of (*Z*)-3-nonenol + racemic farnesene (t = 1.22, df = 8, P = 0.29) when compared with control (Fig. 4A).

Virgin males were significantly more attracted to (*Z*)-3-nonenol (t = 9.08, df = 8, P = 0.001), and the mixture of (*Z*)-3-nonenol + *β*-farnesene (t = 4.82, df = 8, P = 0.01) than to control. Males were not significantly more attracted to racemic farnesene alone (t = 2.24, df = 8, P = 0.09), *β*-farnesene alone (t = 1.44, df = 8, P = 0.22), or the blend of (*Z*)-3-nonenol + racemic farnesene (t = 0.80, df = 8, P = 0.47) when compared with control (Fig. 4B).

DISCUSSION

This work represents a further step to identify and evaluate the volatiles released by males of *A. obliqua* attractive to conspecific females and males. Our results showed that *A. obliqua* females were attracted to live males in field cage tests. Similar results were reported in *A. suspensa* and *A. serpentina* where females responded to live males used as lures in laboratory and field studies (Webb et al. 1983; Robacker et al. 2009). We also found that this attraction was mediated, at least in part, by compounds emitted by males because *A. obliqua* virgin females and males were attracted to Super Q extract of 10 live males collected from 0700 to 1800 h. Similarly, Nation (1975) reported that *A. suspensa* females were attracted to male extracts, while *A. ludens* females also responded to abdominal male extracts (Ro-

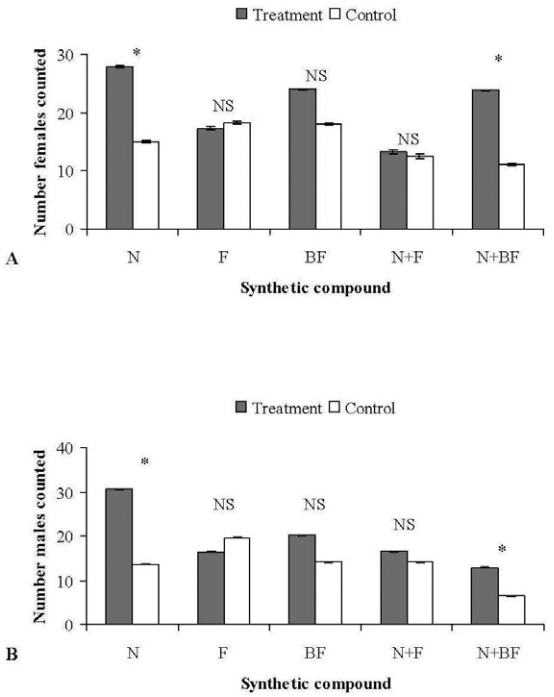

Fig. 4. Mean (±SE) number of females (A) and males (B) attracted to different treatments of synthetic compounds and their blends: N = (*Z*)-3-nonenol, F = racemic farnesene, BF = β-farnesene, and C = control (hexane). Bars marked with NS indicate non-significant differences ($P > 0.05$); bars marked with * indicate significant differences (paired *t*-test, $P < 0.05$).

backer & Hart 1984). Our findings also are agree with Castrejón-Gómez (2006), who reported that *A. serpentina* females were attracted to Super Q extracts of live calling males in wind-tunnel bioassays. The males of *A. obliqua* used as lures often inflated their pleural pouches and fanned their wings, behaviors commonly observed in other *Anastrepha* (Nation 1989). These behaviors have been associated with the periodicity of release of volatiles by males.

In general, we found that *A. obliqua* males were attracted to live conspecific males, which was confirmed when Super Q extracts of calling males were used. Thus this male attraction to conspecific males possibly evidences an aggregation attraction, such as have been reported previously in *A. suspensa* and *A. ludens* males (Perdomo et al. 1976; Burk 1984; Robacker & Hart 1986). In contrast, it seems probable that the response of *A. obliqua* females to conspecific males is evidence of a sexual attraction, such as have been suggested by others researchers (Nation 1972; Robacker & Hart 1986; Robacker et al. 2009). In addition to the chemical cues, fruit flies may be attracted to other short-range cues such as visual and acoustic displays (Nation 1972; Landolt & Averill 1999). Thus it appeared that both sexes of *A. obliqua* were also attracted to physical cues emitted by calling conspecific males. The effect of the interaction of odors, sounds, and visual cues on the attraction of *A. obliqua* flies should be investigated in future experiments.

Some of the compounds found in this study have previously been reported in mass-reared *A. obliqua* flies originally collected from field-collected in mangos of Haiti (Heath et al. 2000), and have also been found in the volatiles of other tephritid fruit fly species. For example, (*Z*)-3-nonenol, (*E,E*)-α-farnesene and (*Z,E*)-α-farnesene were reported as volatiles emitted by *A. obliqua* males by Heath et al. (2000). Also (*Z*)-3-nonenol has been reported as a component of male volatiles of *A. suspensa*, and *A. ludens* (Nation 1983b). The (*E,E*)-α-farnesene has been previously found among volatiles of *A. suspensa*, *A. ludens* and *C. capitata* (Baker et al. 1985; Rocca et al. 1992), while the (*Z,E*)-α-farnesene has been reported in *A. fraterculus* and *A. suspensa* (De Lima et al. 2001; Lu & Teal 2001). The compound (*Z,Z*)-3,6-nonadienol, released by calling males of *A. ludens* and *A. suspensa* (Nation 1983a), probably is the same nonadienol found in the volatiles *A. obliqua* in the present study. In addition, in this work we reported the presence of 4 unknown sesquiterpene compounds and β-farnesene, which have not been previously found in the volatiles of any *Anastrepha* species. The similarity between the compounds released by *A. obliqua*, *A. ludens*, *A. suspensa, A. striata* and *A. fraterculus* seems to be related to their phylogeny, because all belong to the *fraterculus* group. DNA mitochondrial analyses provided evidence that *A. suspensa*, *A. fraterculus*, *A. ludens* and *A. obliqua* are closely related evolutionarily, and also to *A. striata*, which does not belong to the same group (McPheron et al. 2000). Additional information from laboratory and field tests of *A. obliqua* could provide a more reasonable interpretation of the evolutionary and chemotaxonomical significance of the volatiles reported here.

The EAG responses of *A. obliqua* flies to extracts from 5 to 50 live calling males showed that antennal receptors of females and males responded more intensely when the extracts contained more male emissions, suggesting that extracts contain a higher amount of volatile compounds. A possible explanation to the different antennal responses is that there is a minimum threshold response to male volatiles. In fact, the *Anastrepha* females are attracted to males when these form a lek of at least 3 males calling simultaneously (Aluja & Birke 1993). Similarly, in field test with *A. suspensa*, 40 males were more attractive to females than either 10 or 20 males (Perdomo 1974).

In bioassays with synthetic compounds identified in volatiles *A. obliqua* males, we found that

females and males were attracted by (*Z*)-3-nonenol alone, and by its binary blend with *β*-farnesene. For the bioassays results, we can consider that (*Z*)-3-nonenol plays a crucial role in attraction. For example when combined with racemic farnesene, this binary mixture was not attractive. However when (*Z*)-3-nonenol was combined with *β*-farnesene, the mixture was attractive in bioassays but no more so than (*Z*)-3-nonenol alone. This apparently indicates that *β*-farnesene does not have a synergistic, additive or inhibitory effect when was added to (*Z*)-3-nonenol. Similarly *A. suspensa* females also were attracted to (*Z*)-3-nonenol alone or in blend with others 3 compounds (Nation 1975). Robacker & Hart (1985) also reported that *A. ludens* were attracted to (*Z*)-3-nonenol, when this alcohol was combined with (*S*,*S*)-(-)-epianastrephin. Preliminary EAG tests showed that (*Z*)-3-nonenol elicited significant electrophysiological responses in the antennae of *A. obliqua* females (López-Guillén, unpublished data). Similar results were found for *A. suspensa*, and *A. ludens* females (Robacker et al. 1986; Robacker & Hart 1987). The major compounds released by *A. obliqua* males identified by us do not exist commercially, and their synthesis is difficult due to the presence of isomers for which we did not control in these studies.

In conclusion, *A. obliqua* females and males were attracted to live males during periods when the latter exhibited calling behavior. These responses are indicative of sexual/aggregation attraction. The Super Q extracts collected from live males elicited antennal and behavioral responses from both females and males. We also report 6 additional components emitted by calling *A. obliqua* males. Moreover, we found preliminarily that females and males were attracted to (*Z*)-3-nonenol, as well as to (*Z*)-3-nonenol + *β*-farnesene. The complete identification and synthesis of the unknown compounds remain to be accomplished, and this is a critical need with respect to evaluating their biological activity.

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