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Atracción de *Anastrepha obliqua* (Macquart) a los compuestos volátiles de frutos de guayaba

TESIS

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Atracción de *Anastrepha obliqua* (Macquart) a los compuestos volátiles de frutos de guayaba

para obtener el grado de **Maestro (a) en Ciencias en Recursos Naturales y Desarrollo Rural**

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CONTENIDO

I. RESUMEN

En los insectos, la búsqueda de hospederos es una parte fundamental ya que de esto depende su supervivencia y la de su progenie. Estudios previos han demostrado que *Anastrepha obliqua* (Macquart) es atraída por esteres, terpenos y alcoholes, que han sido identificados en compuestos de tres frutos hospederos diferentes. El objetivo de este estudio fue identificar los compuestos volátiles que median la atracción de *A. obliqua* a dos variedades y tres estados de madurez de frutos de guayaba. Los volátiles emitidos por los frutos fueron capturados con adsorbente Super Q. Los extractos fueron evaluados en jaulas de campo y analizados por cromatografía de gases acoplada a electroantenografía (CG-EAD). Los compuestos que resultaron antenalmente activos se identificaron por cromatografía de gases acoplada a espectrometría de masas (CG-EM). En los extractos de las dos variedades de guayaba madura se encontraron seis compuestos antenalmente activos, los cuales fueron identificados como: 1) butirato de etilo, 2) cis-3-hexen-1-ol, 3) hexanoato de etilo, 4) acetato de cis-3-hexenilo, 5) benzoato de etilo y 6) octanoato de etilo. Con los compuestos identificados se preparó una mezcla sintética a una concentración similar a la encontrada en cada variedad y fue evaluada en jaulas de campo, encontrándose que no hay diferencias entre los extractos y las mezclas de cada variedad. También se observó que *A. obliqua* discriminó entre los tres estados de madurez de guayaba, teniendo mayor preferencia por frutos sazones y maduros.

Palabras claves: CG-EAD, CG-EM, hospedero, olfacción, semioquímicos.

II. INTRODUCCION

Aproximadamente más de un millón de especies de insectos se alimentan de plantas (Camarena 2009), y la supervivencia de estos insectos y la de su progenie está relacionada con su capacidad de localizar sitios adecuados con plantas hospederas en donde puedan satisfacer sus requerimientos nutricionales y de refugio, así como los de su progenie (Renwick 1989; Honda 1995; Castrejón 2006; Schoonhoven et al. 2006). Durante el proceso de búsqueda de hospederos, un insecto puede hacer uso de señales visuales, olfativas, gustativas y táctiles (Visser 1998; Bruce et al. 2005; Schoonhoven et al. 2006). Los árboles frutales, al igual que otras plantas emiten compuestos volátiles que pueden ser percibidos por los insectos frugívoros como señales útiles para localizar a un fruto hospedero (Schoonhoven et al. 2006). Se ha documentado que los compuestos volátiles pueden funcionar como atrayente o repelentes para los insectos herbívoros. A pesar de que algunos insectos usan compuestos específicos para localizar a su planta hospedera, la mayoría de las especies estudiadas usan mezclas de compuestos ubicuos (Bruce et al. 2005).

La búsqueda de hospedero es un proceso difícil para los insectos herbívoros, ya que los volátiles liberados por las plantas consisten en diversas estructuras químicas que son emitidos a través de diferentes órganos con diferentes propósitos biológicos (Dudareva et al. 2004; Conchou et al. 2019). Por ejemplo, las flores emiten compuestos volátiles que sirven como atrayentes de polinizadores (Reinhard et al. 2004) o en respuesta al daño por herbivoría (Farré-Armengol et al. 2015). Bajo condiciones naturales las plantas son expuestas a diversos factores que alteran o modifican la emisión de sus compuestos volátiles, lo cual puede perturbar las interacciones mediadas por los volátiles. Las emisiones de volátiles varían incluso entre plantas de la misma especie, esto debido a que cada planta responde de forma diferente a los factores abióticos y bióticos (Conchou et al. 2019). Los volátiles emitidos son controlados por factores abióticos como luz, temperatura, concentración de dióxido de carbono atmosférico y tipo de suelo (nutrientes, suelo seco, entre otros) (Loreto y Schnitzler 2010). Por ejemplo, Loreto et al. (1996)

encontraron que la emisión de α-pineno por *Quercus ilex* es dependiente de la luz, además el aumento de temperatura aumentó la emisión de α-pineno. Entre los factores bióticos se encuentra la edad de la planta, que contribuye a la variación en la emisión de volátiles (Dudareva et al. 2004). Una muestra de lo anterior ocurre con los frutos de guayaba que a medida que van madurando el perfil de volátiles va cambiando (Soares 2007). La herbívora es otro factor que modifica la liberación de volátiles. En este sentido, Farré-Armengol et al. (2015) encontraron que, en respuesta a la florivoría y folivoría, la rabaniza blanca (*Diplotaxis erucoides)* aumentó las emisiones de compuestos como el metanol, acetato de etilo y 3-butenonitrilo. Por otro lado, Peñuelas et al. (2014) encontraron que la supresión de la microbiota floral cambió la composición y proporción de terpenos en plantas de sauco (*Sambucus nigra)*.

Anastrepha obliqua (MacQuart) es una especie de mosca de la fruta con distribución en regiones de México, Centroamérica y Sudamérica (Fu et al. 2014). Es una especie generalista ya que se alimenta de una gran diversidad de frutas tropicales, se han observado infestaciones en campo por esta especie en frutos de las familias Anacardiaceae, Annonaceae, Ebenaceae, Euphorbiaceae, Fabaceae, Bignoniaceae, Malpighiaceae, Moraceae, Myrtaceae, Oxalidaceae, Passifloraceae, Rosaceae, Rutaceae, Salicaceae y Sapotaceae (Norrbom y Kim 1988). Sin embargo, algunos de los registros pueden ser erróneos debido a la mala identificación de la especie, particularmente debido a la confusión con *A. fraterculus*, o la no confiable identificación del hospedero (Birke et al. 2013). Las plantas preferidas por *A*. *obliqua* pertenecen a los géneros de las familias Anacardiaceae (*Spondias* spp., *Mangifera indica* L.), de la familia Myrtaceae (*Psidium guajava*, *Syzygium jambos*) y Oxalidaceae (*Averrhoa carambola*) (Birke et al. 2013). Por ser considerada una especie polífaga en sus hábitos alimenticios se hipotetiza que debería ser atraída por compuestos ubicuos no específicos presentes en diferentes especies o variedades de frutos. En estudios previos sobre la ecología química de *A. obliqua* se han identificado compuestos químicos, como terpenos y esteres, de tres especies de frutos hospederos maduros (jobo, mango y carambola) que son atractivos para machos y hembras de esta especie (Cruz-López et al. 2006;

Espinoza-Roblero 2017; Toledo et al. 2009; Malo et al. 2012). Esto pareciera indicar que *A. obliqua* es atraída por una amplia gama de compuestos volátiles presentes en diferentes hospederos. Sin embargo, estudios con otras especies, variedades y estados de madurez y de frutos, es indispensable confirmar esta hipótesis. Por lo tanto, en este trabajo se seleccionó como modelo de estudio la guayaba (*Psidium guajava*). La guayaba es una especie de fruta de origen tropical de la familia Myrtaceae, nativo de América Central, desde México al norte de Sudamérica (GISD c1998-2000). Investigaciones previas sobre la composición química de frutos de guayaba muestran que hay una variación tanto cualitativa como cuantitativa entre variedades y estados de madurez (Paniandy et al. 2000; Pino et al. 2002; Chen et al. 2006; Quijano y Pino 2007; Thuaytong y Anprung 2011; Pino y Bent 2013; Elizalde-González et al. 2018). Sin embargo, se desconoce cuáles son los compuestos que están involucrados en la atracción de *A*. *obliqua* a los frutos de esta especie. Por tanto, esta investigación se llevó a cabo para conocer cuáles son los compuestos atrayentes de la guayaba para *A*. *obliqua*. En primer lugar, en jaulas de campo se evaluó la atracción de *A. obliqua* a frutos de dos variedades y tres estados de madurez de guayaba. Posteriormente, se identificaron los compuestos antenalmente activos obtenidos a partir de los extractos de guayaba, y se identificó químicamente cada compuesto. Finalmente, la actividad biológica de los compuestos identificados fue evaluada con mezclas sintéticas en pruebas realizadas en jaulas de campo. La información obtenida da los elementos para ampliar nuestro conocimiento sobre la olfacción y la búsqueda de hospedera de esta mosca de la fruta.

III.

The ripeness stage but not the variety affects the attraction of *Anastrepha obliqua* to guava fruits

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The ripeness stage but not the variety affects the attraction of *Anastrepha obliqua* to guava fruits

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Abstract The West Indian fruit fly, *Anastrepha obliqua* (Macquart), feeds on a wide diversity of tropical fruits. Previous studies suggest that *A. obliqua* is attracted to volatile compounds common in different hosts. However, all studies have used ripe fruits for the identification of attractive compounds. In this study, firstly, we investigated the behavioral responses of *A. obliqua* females and males to two varieties and three ripening stages of guava (*Psidium guajava*). Secondly, we identified the attractive compounds to *A. obliqua* by combined gas chromatography-electroantennographic detector (GC-EAD) and gas chromatography-mass spectrometry (GC-MS). Thirdly, we evaluated the biological activity of the identified compounds in field-cage tests. We found that both sexes of *A. obliqua* showed no preference for either of the two varieties of guava evaluated. In contrast, flies were more attracted to ripe and half-ripe fruits than to unripe ones. GC-EAD analysis of extracts of ripe Creole or Thai varieties showed that six compounds elicited antennal responses by *A. obliqua* females and males. The compounds were identified by GC-MS as ethyl butyrate, cis-3-hexen-1-ol, ethyl hexanoate, cis-3-hexenyl acetate, ethyl benzoate, and ethyl octanoate. Half-ripe guavas emit ethyl butyrate, cis-3-hexen-1-ol, ethyl hexanoate, and cis-3-hexenyl acetate, while unripe guavas only release cis-3-hexenyl-acetate in traces. Field cage tests with synthetics confirmed that the compounds identified are responsible for the attraction of *A. obliqua* flies to guava fruits.

Keywords Fruit volatiles, Host searching behavior, Olfaction, *Psidium guajava*, Tephritid fruit flies

Introduction

Plants may emit hundreds of volatile organic compounds (VOCs) through their roots, leaves, flowers or fruits (Dudareva et al. 2004). The vast majority of the emitted compounds are ubiquitous, while some are species-specific, which provide the characteristic flavor or aroma of a particular plant (Schoonhoven et al. 2005). The VOCs mediate a number of ecological interactions among plants and insects (Visser 1986; Bruce et al. 2005). For instance, COVs may affect the host searching behavior of phytophagous insects (Bernays and Chapman 1994; Conchou et al. 2017). Most insect species use blends of ubiquitous compounds for host searching, but in some cases the preference of the phytophagous insect, particularly in specialists, is influenced by species-specific compounds (Bruce 2005; Ghaninia and Amooghli-Tabari 2016). Phytophagous insects are able to recognize hosts and non-hosts based on the identity, the ratio, and concentration of the VOCs emitted by plants (Bruce et al. 2005; Najar-Rodriguez et al. 2010; Cha et al. 2011). However, the ratio and concentration of VOCs in the blends can be affected by biotic or abiotic factors (Conchou et al. 2019), thus altering the host searching behavior of phytophagous insects. For instance, a frugivorous insect may encounter different odor landscapes during host searching because of the surrounding vegetation of the orchard or because in the orchard there may be fruits with a different stage of ripeness (Vallat and Dorn 2005).

The West Indian fruit fly, *Anastrepha obliqua* (Macquart), has a distribution spanned from northern Mexico to southern Brazil (Ruiz-Arce et al. 2012), but it has a high potential to settle in the tropics and subtropics of other continents (Fu et al. 2014). This fruit fly feeds on a wide diversity of tropical fruits, therefore is considered a generalist species. The plants preferred by *A. obliqua* are *Spondias* spp. (Anacardiaceae), followed by

Mangifera indica L. (Anacardiaceae), *Syzygium jambos* L. (Alston) (Myrtaceae), *Averrhoa carambola* L. (Oxalidaceae) and *Psidium guajava* L. (Myrtaceae) (Birke et al. 2013). Previous studies on the chemical ecology of *A. obliqua* have identified volatiles compounds, such as terpenes and esters, of three species of host fruits (*Spondias mombin* L., *M. indica*, and *A. carambola*) that are attractive to males and females (Cruz-López et al. 2006; Malo et al. 2012; Espinoza-Roblero 2017). This suggests that *A. obliqua* is attracted to volatile compounds common in different hosts. However, all studies have used ripe fruits for the identification of attractive compounds.

In this study, first we investigated the behavioral responses of *A. obliqua* females and males to two varieties and three ripening stages of guava. After, we identified the attractive compounds to *A. obliqua* by combined gas chromatography-electroantennographic detector (GC-EAD) and gas chromatography-mass spectrometry (GC-MS). Finally, we evaluated the biological activity of the identified compounds in field-cage tests.

Materials and methods

Insects

A. obliqua pupae were obtained from the Moscafrut facility located in Metapa de Domínguez, Chiapas. The insects were fed with an artificial diet (Artiaga-López 2004; Orozco-Dávila et al. 2017). The pupae were placed in glass boxes (30 cm x 30 cm x 40 cm) and kept in controlled conditions of temperature (25±1 °C), relative humidity (60-70%) and a photoperiod of 12:12 h (L: D). Upon emergence, adults were kept inside the boxes to allow mating. Flies were fed *at libitum* with a mixture of enzymatic yeast (MP Biomedical, Irvine, CA) and sucrose (1: 3 ratio). Water was provided through a tube covered with cotton wool. Females and males, presumably mated at 8-13 days old, were used in the experiments.

Fruits

Guava fruits used in the experiments were selected from backyard trees in houses located in the surroundings of Tapachula city, in the State of Chiapas, Mexico (14° 54′ 0″ North, 92° 16′ 0″ West). Unripe fruits were covered with mosquito net fabric to protect them from any possible insect damage. When the fruits were unripe, half-ripe or ripe they were cut and transported to the laboratory for bioassays and sampling of VOCs. The sugar content was measured in the guava fruits with a refractometer. The effect of the variety type on the response of *A. obliqua* was investigated using ripe (12-14° Brix) guava fruits of two varieties (Thai and Creole). The effect of ripeness on the attraction of *A. obliqua* was evaluated using unripe (5-7 °Brix), half-ripe (8-10 °Brix), and ripe (12-14 °Brix) guava fruits of the Creole variety.

Bioassays

The attraction of the flies to guava fruits was evaluated in two-choice (effect of variety) or three-choice (effect of maturity) tests in cylindrical nylon-screen field cages (2.80 m diameter x 2.20 m high). One fruit of each treatment was placed inside a Multilure trap (Better World Manufacturing, Fresno, CA). In each trap, 250 ml of propylene glycol was also added to retain the attracted flies. This compound does not interfere with the behavioral responses of *A. obliqua* to fruit volatiles (J.C.R., unpublished data). Traps were spaced equally and randomly hung 10 cm from the cage roof. In total, 50 flies of one sex were released into the cages at 08:00 h, and the number of flies captured was recorded 24 h later. After emptying the traps, fruits were replaced and traps were rotated for avoiding a positional bias. In total, 18 and 10 replicates per sex were performed for evaluating the effect of variety and ripeness, respectively.

Volatile sampling

The sampling of fruit volatiles of the two varieties and three stages of maturity was performed by dynamic headspace technique as described elsewhere (Cruz-López et al. 2006). Briefly, about 450 g of guava fruits were placed into glass aeration containers. A charcoal-filtered air stream (1 L/min) was passed through the glass aeration containers for 24 h. Guava volatiles were trapped into collection traps containing Super Q adsorbent (Sigma Scientific LLC, Micanopy, Fl). At the end of each air entrapment, the volatiles were eluted from the adsorbent with 400 µL of dichloromethane (Baker, HPLC grade, Sigma-Aldrich, Toluca, Mexico) and stored in vials at -20 °C for later use.

Gas chromatographic-electroantennodetection analysis (GC-EAD)

The extracts of the two varieties were analyzed by GC-EAD using a system described elsewhere (Diaz-Santiz et al. 2016) using a Shimadzu GC-2010 plus gas chromatograph equipped with a VF-5MS Factor Four (Supelco, Toluca, Mexico) capillary column (30 m x 0.25 mm). The GC was coupled to an electroantennogram (Syntech, Kirchzarten, Germany). The initial GC temperature was 40 ^{o}C, held for 1 min, and then ramped to 280 ^{o}C at 8 ^{o}C per min and held at 280 ^{o}C for 10 min. A split/splitless injector was used with a constant temperature of 250 °C. The injector was operated in splitless mode. Hydrogen was used as a carrier gas. A fly´s head was carefully removed and the reference glass capillary electrode was inserted into its base, whereas the recording electrode was inserted into the distal end of the antenna. The signal was amplified and analyzed with GC-EAD software (Syntech, Kirchzarten, Germany). A current of humidified pure air (0.7 l/min) was constantly passed onto the antenna through a 10-mm diameter glass tube as a stimulus flow controller (Syntech). One microliter of each extract was tested on one different antenna. In total, 10 extracts per sex per treatment were analyzed.

Chemical analysis

The GC-EAD active peaks in extracts of each guava variety and ripeness stage were identified by gas chromatography-mass spectrometry (GC-MS) using a Varian CP-3800 GC coupled with a Varian 220 mass spectrometer, with a nonpolar capillary column (Factor Four VF-5 ms, 30 m x 0.25 mm i.d., Supelco, Toluca, Mexico). One microliter of each extract was injected into the GC in splitless mode; the injector temperature was held at 250 ^{o}C. The temperature program was the same as that described for the GC-EAD analyses. The chromatographic profiles were similar to those found in GC-EAD analyses, therefore it was possible to match the peaks. The carrier gas was helium at constant flow rate of 1 ml/min. Ionization was by electron impact at 70 eV with a scan mass range of 40-350 m/z. Compounds were identified by comparison of retention times and mass spectral matches with available standards. The standards were acquired from Sigma-Aldrich (Toluca, Mexico) and were 97-99% pure according to the supplier. The amounts of compounds in the extracts were quantified using a calibration standard of each compound identified (1-100 ng).

Attraction of *A. obliqua* to synthetic blends

The attractiveness of the synthetic blends was compared with the fruit extracts in two-choice tests using multilure traps placed in field cages as described above. The synthetic mixture of each variety was prepared according to the concentrations in which each compound was found in the natural extracts of varieties (Table 1) or ripeness stages (Table 2). In all experiments, 100 µl of extract or synthetic blend were loaded into rubber septa, unless otherwise specified. In the first experiment, we evaluated the attractiveness of the synthetic blends of the Creole variety or Thai variety against its respective extracts. In the second experiment, we compared the attractiveness of the synthetic blend of Creole guava versus the synthetic blend of Thai guava. In the third experiment, we evaluated the attraction of *A. obliqua* to the synthetic blends derived from the ripeness stage extracts. Unripe guava fruits only released cis-3-hexenyl-acetate in traces, therefore we arbitrarily used 20 ng of this compound for the unripe guava lure. The trials were performed under similar conditions as mentioned above. Approximately 50 females or 50 males were released by cage. The flies were used only once. Eight replicates were performed for the evaluation of the synthetic blend of Thai guava against its respective extract. Twelve replications were used for the rest of the evaluations. Each cage was considered as a replicate.

Statistical analysis

Data were analyzed using software R version 3.6.0. Field cage data were analyzed using a linear mixed-effects model. Data were chi-square transformed to satisfy the assumptions of homogeneity of variances and normality. The comparison of means was performed by a Tukey test with a level of significance of $\alpha < 0.05$.

Results

Effect of guava variety on the attraction of *A. obliqua*

The difference in preference between varieties was not significant for females (F = 0.78; df = 17; P> 0.05), nor for males (F = 1.30; df = 17; P> 0.05) (Fig. 1).

Effect of guava ripeness on the attraction of *A. obliqua*

The attraction of females (F = 8.54; df = 22; P <0.01) and males (F = 5.95; df = 22; P <0.01) was affected by the ripeness stage of guava fruits. Both sexes were more attracted to ripe than unripe guava fruits. The attraction of flies to half-ripe fruits was intermediate and not significantly different from that shown to ripe fruits and unripe guava fruits (Fig. 2).

GC-EAD and GC-MS analyses of fruit extracts

The analysis by GC-EAD of extracts of Creole or Thai guava mature showed that six compounds elicited antennal responses by *A. obliqua* females and males (Fig. 3, Table 1). The same compounds were found in both varieties, although their concentrations varied between varieties (Table 1). The compounds were identified by GC-MS as ethyl butyrate, cis-3-hexen-1-ol, ethyl hexanoate, cis-3-hexenyl acetate, ethyl benzoate, and ethyl octanoate. The major compound was cis-3-hexenyl acetate. Four of them were identified from half-ripe of Creole guava extracts: ethyl butyrate, cis-3-hexen-1-ol, ethyl hexanoate, and cis-3-hexenyl acetate. The other two compounds were found in traces. In the headspace of non-ripe guavas only cis-3-hexenyl-acetate was found in traces, while the other five compounds were absent (Table 2).

Attraction of *A. obliqua* to synthetic blends

The results of the first experiment showed that the number of females ($F = 0.28$; $df = 11$; $P > 0.05$) and males ($F = 0.01$; $df = 11$; $P > 0.05$) caught in the traps baited with the of six-component blend formulated from the Creole guava extracts was not different from those caught in the traps baited with Creole guava natural extracts (Table 2). Similarly, the traps baited with the six-component blend derived from the Thai guava extracts showed no significant difference in the catches of females ($F = 2.28$; $df = 7$; $P > 0.05$) and males ($F = 0.92$; $df = 7$; $P > 0.05$) compared to the traps baited with Thai guava natural extracts (Table 2). The results of the second experiment showed that there were no differences in the catches of females ($F = 1.58$; $df = 11$; $P > 0.05$) and males ($F = 0.97$; $df = 11$; $P > 0.05$) by traps baited with synthetic blend of Creole guava and those baited with the synthetic blend of Thai guava (Table 2).

The results of the third experiment showed that the ripening stage affected the catches of females ($F = 7.38$; $df = 22$; $P < 0.05$) and males ($F = 16.41$; $df = 22$; $P < 0.001$). Traps baited with ripe and half-ripe Creole guava blends captured more females and males than those baited with unripe Creole guava blend (Fig. 4).

Discussion

The chemical composition of guava varies qualitatively and quantitatively between varieties and ripening stages (Pino et al. 2002; Chen et al. 2006; Thuaytong and Anprung 2011; Pino and Bent 2013; Elizalde-Gonzalez et al. 2018). However, so far it was unknown what compounds were responsible for the attraction of *A. obliqua* to this fruit species.

In this study, we found that both sexes of *A. obliqua* showed no preference for either of the two guava varieties. In contrast, females and males could discriminate among fruits of the different ripeness stages. Additionally, we identified the compounds emitted by both varieties and the different ripening stages of the Creole variety. Field cage tests with synthetic compounds confirmed that the compounds identified were responsible for the attraction of *A. obliqua* flies to guava fruits.

The fact that *A. obliqua* was not able to discriminate between the two varieties, contrasts with two previous studies with this fruit fly species. Malo et al. (2012) found that both sexes of *A. obliqua* showed greater preference for mango fruits of the Amate variety compared to that exhibited for the Coche and Ataulfo varieties. More recently, Lopez-Ley et al. (2016) reported that *A. obliqua* was more attracted and oviposited more frequently on carambola fruits of the Maha variety than over Golden Star variety fruits. The differences between the present study and the previous ones may be due to several factors. It is possible that the volatile profile did not vary qualitatively among the guava varieties but it did in the mango and carambola varieties. GC-MS analyses showed that 24, 22, and 19 volatile compounds were identified for Amate, Ataulfo, and Coche mango varieties, respectively (Malo et al. 2012). They also found that there were quantitative differences in the common compounds among the three varieties (Malo et al. 2012). Thus, the difference in the volatile profiles of the mango varieties may explain the preference of *A. obliqua*. In the present study, we found only one (ethyl hexanoate) of the six EAD-active compounds varied significantly in both guava varieties. It is generally known that most herbivorous insects are attracted to ubiquitous compounds and that they are able to discriminate between different plant species or plants of different quality using the different ratios or concentration in which the volatile compounds are emitted by host plants (Visser 1986; Bruce et al. 2005).

In contrast, with the lack of preference of *A. obliqua* for the guava varieties, flies were able to discriminate between the different fruit ripeness stages. Both sexes preferred ripe and half-ripe guava fruits over unripe ones. This result confirms what was previously found with *A. obliqua* and other tephritid species. For instance, both sexes of *A. obliqua* were more attracted and females preferred to oviposit on ripe and half-ripe carambola fruits than on unripe ones (López-Ley et al. 2016). *Bactrocera dorsalis* (Hendel) is more often attracted to ripe mango, guava and papaya fruits than to unripe fruits (Jang and Light 1991; Cornelius et al. 2000). *Bactrocera tryoni* (Froggatt) females were more attracted to overripe guavas compared to ripe and unripe fruits (Cunningham et al. 2016). In contrast, García-Ramirez et al. (2004) found that both sexes of *Anastrepha ludens* (Loew) were more attracted to volatiles from unripe mango and orange fruits compared to ripe ones. However, few studies have investigated the volatile compounds that mediate the preference of tephritid fruit flies for ripe fruits over unripe ones. Our study shows that unripe guava fruits only release traces of cis-3-hexenyl acetate, while ripe and half-ripe emit six and four EAD-active compounds, respectively. The lack of emission of volatiles, except traces of cis-3-hexenyl acetate, by unripe guava fruits seems to explain their low attractiveness. This was confirmed when the synthetic mixtures derived from the three ripeness stages were evaluated in field-cage tests. The response of flies to traps baited with unripe fruits can be also explained by the color of the trap. Lopez-Guillen et al. (2009) found that *A. obliqua* was more attracted to lemon-green spheres baited with fruit volatiles than unbaited lemon-green spheres and transparent spheres.

Our results suggest that *A. obliqua* shows behavioral plasticity in response to volatiles emitted by its host fruits. It is possible that *A. obliqua* needs to have the plasticity to recognize certain key compounds or variation in the ratio or concentration of fruit volatile compounds because in the field it would be expected that the identity as well as ratio/concentration of the compounds will vary as their host fruits are subjected to different physiological or ecological factors. In this way, we found that *A. obliqua* flies showed similar responses to ripe and half-ripe fruits even though the former fruits emitted two more EAD-active compounds compared to half-ripe fruits. This suggests that some of the EAD-active compounds are behaviorally redundant or are not crucial in mediating fly attraction to host fruits. Previously, Cruz-Lopez et al. (2006) reported that both sexes of *A. obliqua* respond antennally to nine compounds emitted by *S. mombin* fruits. However, field tests showed that the blend of the two major compounds (ethyl butyrate and ethyl hexanoate) were as attractive as the nine-components blend or the blend of the seven minor components (Toledo et al. 2009). It seems that ethyl butyrate is the crucial compound in the attraction of *A. obliqua* to fruits because it is as active as the binary blend of the two major compounds. In other tephritid fruit flies a similar situation have been reported. For instance, *Rhagoletis pomonella* (Walsh) is attracted to a six-component blend constituted by ethyl acetate, 3-methylbutan-1-ol, isoamyl acetate, nonatriene, butyl hexanoate and ionone (Nojima et al. 2003). The subtraction of ethyl acetate and isoamyl acetate from the six-component blend did not affect the fly attraction. Cunningham et al. (2016) identified 11 compounds from overripe guavas that were attracted to *B. tryoni*. However, they found that a blend consisted of ethyl acetate, ethyl propianate and ethyl butyrate was as attractive as the 11-component blend, which lost its attractiveness when these three compounds were subtracted (Cunningham et al. 2016). We also found that the Creole variety emitted seven times more ethyl hexanoate then the Thai variety, but despite this variation, the fly attraction to guava varieties was not affected. This situation has been exemplified with *Grapholita molesta* (Busck) females, where the level of one component (benzonitrile) in a peach-derived blend can vary by two orders of magnitude without affecting the attraction to moths (Najar-Rodriguez et al. 2010). In contrast, other herbivorous insects are affected when the ratio or the concentration of any of the components of the blends is altered (Cha et al. 2011, 2017). For instance, the specialist grape berry moth *Paralobesia viteana* (Clemens) is attracted to seven-component blend derived from its host plant. When doubling any compound, except one, of the seven-component blend the attraction of females is reduced in comparison to the original blend (Cha et al. 2011).

Cunningham et al. (2016) have hypothesized that fruit ripening volatiles are probable indicators of a suitable host resource, rather than fruit species. In this way, the compounds identified here are used by *A. obliqua* as cues for host habitat location. In contrast to host cues, habitat cues are emitted in larger amounts, consequently are more detectable over extended distances, and generally these are not specific. Thus, habitat cues may give an effective way for insects to augment their likelihood of subsequently finding specific host cues (Webster and Cardé 2016). Because fruit ripening volatiles are share for a number of fruits, the question is how a fruit fly is able to discriminate between both host and non-host fruits or host of different quality. The sequential cues hypothesis proposes that a herbivorous insect will perceive the common volatile compounds released from different host plants species (Silva and Clarke 2019). In absence of other cues, the common volatiles are enough on their own to lead host habitat and acceptance. However, if within the host habitat the insect perceives more host specific cues, it may show preference for different hosts. A number of studies have reported that generalist herbivores insects detect general host cues as well as host specific cues (Silva and Clarke 2019). However, depending the identity, the concentration or ratio, general host cues may be used by insects for discriminate between hosts and non-hosts or between host of different quality. For instance, the addition of isopentyl butyrate or ethyl octanoate inhibits the attraction of *A. obliqua* females to ethyl butyrate. Interestingly, the presence of these compounds as a part of the nine-component blend does not affect the attraction of *A. obliqua* females (J.C.R., unpublished data). Thus, if under some circumstances, the fruit does not release the whole blend, the female get away from this fruit. It also needs to be considered that non-host plants emit volatiles or have visual cues that may inhibit the attraction of fruit flies to non-hosts. Alternatively, once within host habitat guided by ripening volatiles, females may land indiscriminately on fruits, and after evaluating the physical or chemical characteristics of fruits, insects may accept the fruits or leave for still searching for suitable fruits. The last situation would be similar as predicted by the appropriate/inappropriate landing´s theory (Finch and Collier 2000). For example, *B. dorsalis* females oviposit more often on ripe and fully-ripe mangoes than on unripe ones (Rattanapun et al. 2009). Pericarp toughness seems to explain the preference of *B. dorsalis* females for ripe and fully-ripe fruits, unripe fruits have harder pericarp compared to ripe and fully-ripe fruits (Rattanapun et al. 2009). Unripe fruits of a number species contain latex or resin ducts that can affect the oviposition behavior or harm the tethritid larvae (Joel 1978; Seo et al. 1983; Rattanapun et al. 2009; Guillén et al. 2017).

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Author contribution

Fernando Cortés-Martinez and Julio C. Rojas conceived the study and designed the experiments. Fernando Cortés-Martinez performed the experiments, analysed the data, and wrote the first draft of the manuscript. Julio C. Rojas supervised the project and assisted in the manuscript development. Leopoldo Cruz-López supported in the chemical analysis. Pablo Liedo provided critical feedback to the manuscript. All authors read and approved the final manuscript.

Competing interest

The authors declare that they no competing interest

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Tables

Table 1. Mean (±SE) concentration of EAD-active compounds in the extracts of both guava mature varieties obtained from a calibration curve by gas chromatography (n = 6).

No.	Compound	RI	Creole (ng/µl)	Thai (ng/µl)
1	Ethyl butyrate	814	4.04 ± 0.48	3.52 ± 0.55
2	Cis-3-hexen-1-ol	864	2.12 ± 0.45	2.47 ± 0.56
3	Ethyl hexanoate	1003	396.26 ± 62.76	55.33 ± 2.73
4	Cis-3-hexenyl acetate	1009	864.14 ± 39.04	820.08 ± 35.55
5	Ethyl benzoate	1186	56.43 ± 6.29	40.09 ± 4.39
6	Ethyl octanoate	1198	42.08 ± 0.66	32.38 ± 0.79

RI: Retention index

Table 2. Mean (±SE) number of *A. obliqua* captured by multilure traps baited with Creole extract vs Creole synthetic blend (n = 12), Thai extract vs Thai synthetic blend (n = 8), or Creole synthetic blend vs Thai synthetic blend (n = 12).

Sex	Treatment	Mean (±SE)	P-value
Female	Creole extract	6.16 ± 1.57	0.62
	Creole synthetic blend	4.66 ± 0.85	
	Thai extract	6 ± 1.47	0.13
	Thai synthetic blend	4 ± 1.13	
	Creole synthetic blend	8.83 ± 5.89	0.19
	Thai synthetic blend	6.25 ± 2.89	
Male	Creole extract	5.58 ± 1.21	0.94
	Creole synthetic blend	5.83 ± 1.23	
	Thai extract	3.75 ± 0.86	0.48
	Thai synthetic blend	4.87 ± 1.23	
	Creole synthetic blend	7.75 ± 1.10	0.87
	Thai synthetic blend	7.66 ± 1.29	

Table 3. Mean (±SE) concentration (ng/ul) of compounds in guava fruits extracts of different ripening stage obtained from a calibration curve by gas chromatography (*n* = 6).

Compound	RI	Ripe	Half-ripe	Unripe
Ethyl butyrate	814	4.04 ± 0.48	27.13 ± 7.98	ND
Cis-3-hexen-1-ol	864	2.12 ± 0.45	7.74 ± 2.39	ND
Ethyl hexanoate	1003	396.26 ± 62.76	33.03 ± 14.48	ND
Cis-3-hexenyl acetate	1009	864.14 ± 39.04	196.59 ± 31.24	T
Ethyl benzoate	1186	56.43 ± 6.29	T	ND
Ethyl octanoate	1198	42.08 ± 0.66	T	ND

T: Traces; ND: Compound no detected; RI: Retention index

Figure legends

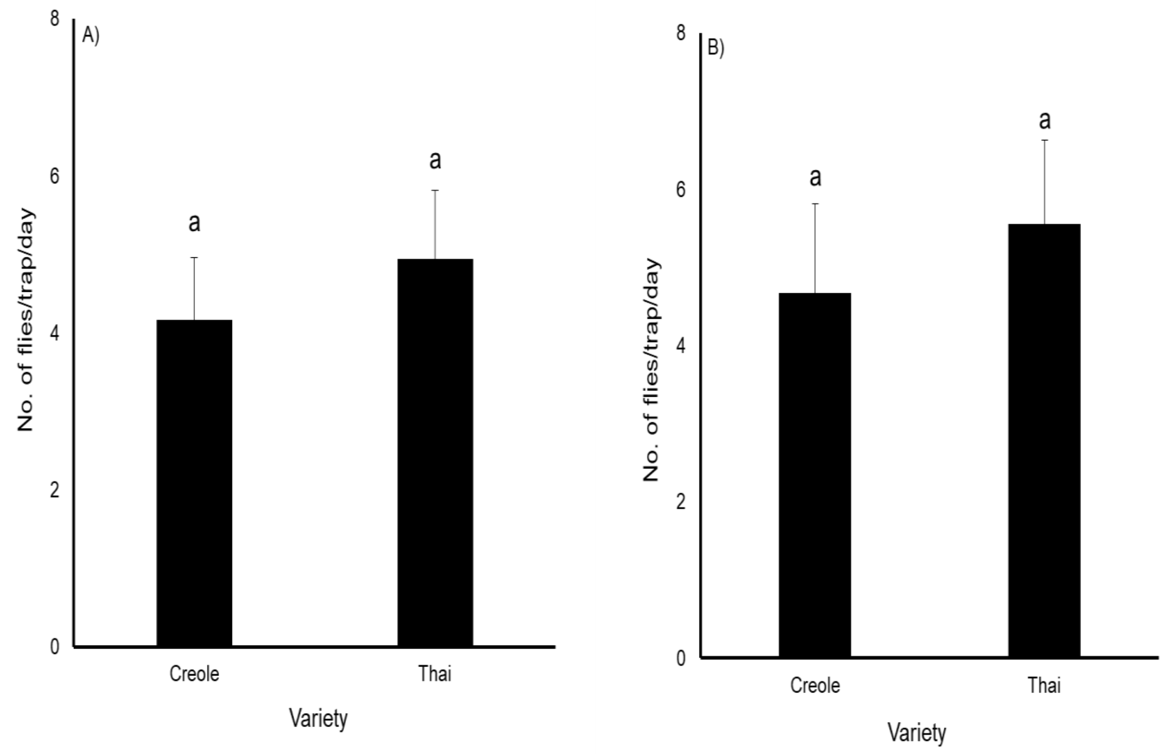
Fig. 1. Attraction of *A. obliqua* females (A), and males (B) to two guava mature varieties in field-cage tests. One trap was baited with one Creole guava fruit, while another was baited with Thai guava fruit. Bars with the same letter are not significantly different according Tukey test at P > 0.05.

Fig. 2. Attraction of *A. obliqua* females (A), and males (B) to three ripening stages of Creole guava in field-cage tests. One trap was baited with one ripe fruit, another was baited with half-ripe fruit, and the other was baited with unripe fruit. Bars with the same letter are not significantly different according to Tukey test at P > 0.05.

Fig. 3. Simultaneous responses of flame ionization detector (FID) and electroantennographic detection (EAD) using the antennae of mated *A. obliqua* females to headspace collected from (A) Creole and (B) Thai guava fruits on a nonpolar column. Numbered peaks indicate EAD-active compounds (see also Table 1).

Fig. 4. Attraction of *A. obliqua* females (A) and males (B) to synthetic compounds of three Creole guava ripening stages in field-cage tests. One trap was baited with a septum loaded with 100 µl of a six-component mixture (ripe), another was baited with a septum loaded with 100 µl of a four-component mixture (half-ripe), and the other was baited with a septum loaded with 20 µl of cis-3-hexenyl acetate (unripe). Bars with the same letter are not significantly different according to Tukey test at P > 0.05.

Fig.1



A)
8
6
4
2
0
No. of flies/trap/day
a
a
Creole
Thai
Variety
B)
8
6
4
2
0
No. of flies/trap/day
a
a
Creole
Thai
Variety

Fig.2

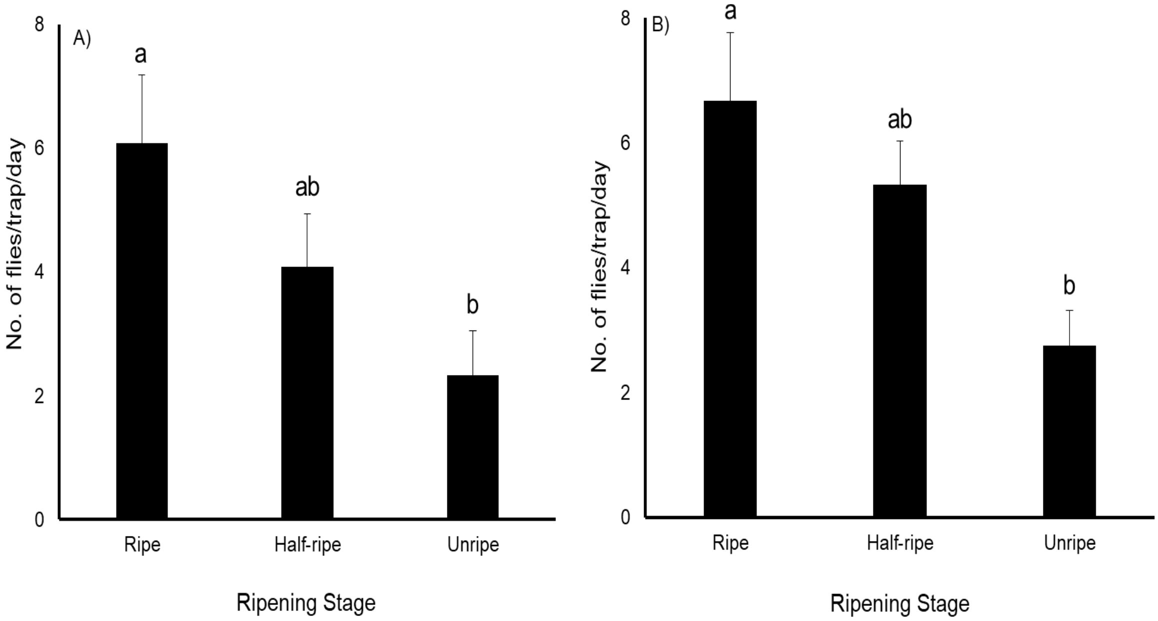

A)
No. of flies/trap/day
0
2
4
6
8
a
ab
b
Ripe
Half-ripe
Unripe
Ripening Stage
B)
No. of flies/trap/day
0
2
4
6
8
a
ab
b
Ripe
Half-ripe
Unripe
Ripening Stage

Fig. 3

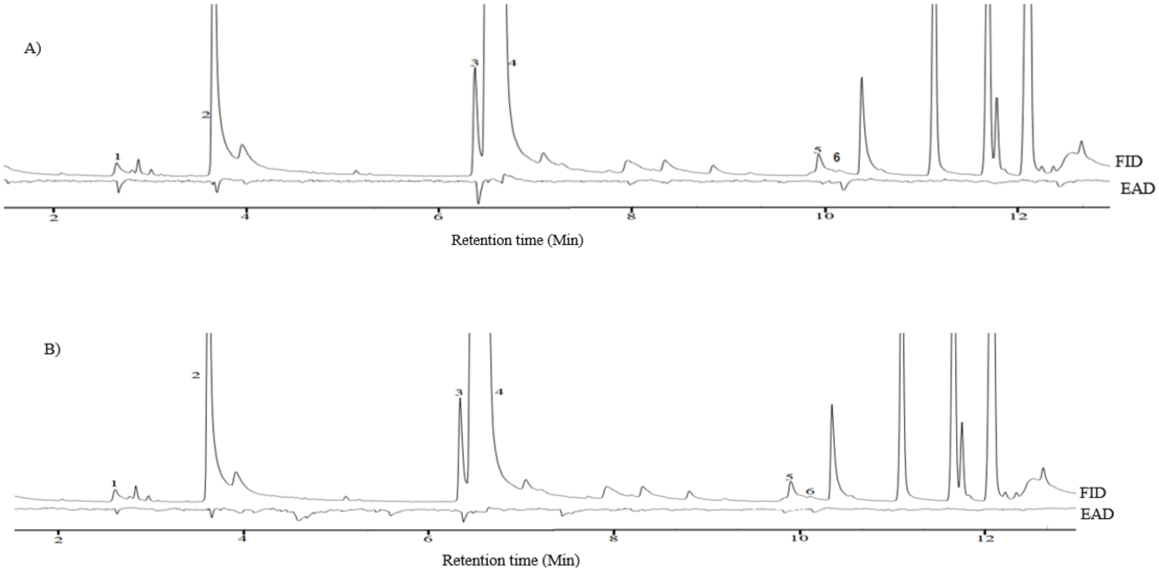
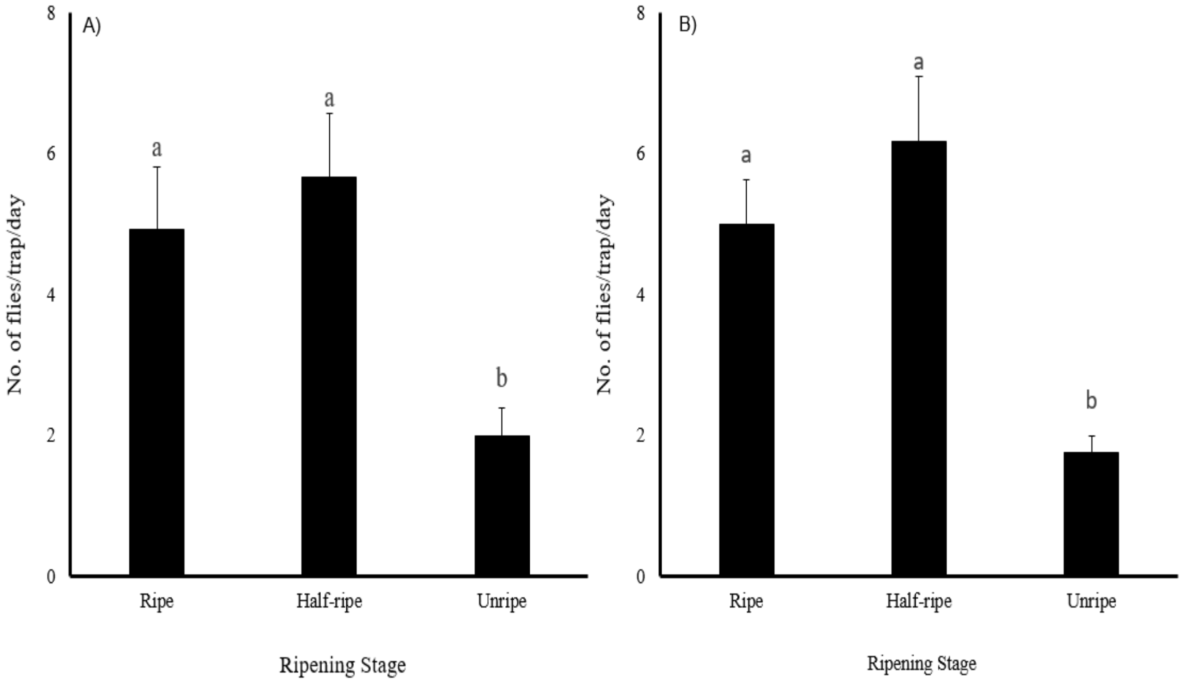

A)
1
2
3
4
5
6
FID
EAD
2
4
6
8
10
12
Retention time (Min)
B)
1
2
3
4
5
6
FID
EAD
2
4
6
8
10
12
Retention time (Min)

Fig. 4


A)
B)
No. of flies/trap/day
0
2
4
6
8
a
a
b
Ripe
Half-ripe
Unripe
Ripening Stage

VI. CONCLUSIONES

En este trabajo se encontró que hembras y machos de *A. obliqua* son atraídos de igual forma por frutos de guayaba criolla y guayaba tailandesa.

Los análisis de los extractos de frutos de guayaba realizados por cromatografía de gases acoplado a un electroantenodetector mostraron que esta especie de mosca de la fruta responden de forma consistente a seis compuestos presentes en frutos de las dos variedades estudiadas.

Los compuestos que resultaron antenalmente activos fueron identificados como: 1) butirato de etilo, 2) cis-3-hexen-1-ol, 3) hexanoato de etilo, 4) acetato de cis-3-hexenilo, 5) benzoato de etilo y 6) octanoato de etilo.

Las mezclas sintéticas preparadas con los seis compuestos, que fueron formuladas a una concentración similar a la natural y colocadas en septos de caucho y evaluadas en jaulas de campo con pruebas de doble elección, resultaron tan atractivas como los extractos de las dos variedades de guayaba.

Hembras y machos de *A. obliqua* discriminaron entre los estados de madurez del fruto, teniendo mayor preferencia por frutos sazones y maduros que por los frutos verdes.

Los análisis químicos mostraron que los frutos verdes solo liberan trazas del acetato de cis-3-hexenilo, mientras que las frutas maduras y sazonas liberan seis y cuatro de los compuestos antenalmente activos, respectivamente.

Las mezclas sintéticas preparadas con los compuestos antenalmente activos, butirato de etilo, cis-3-hexen-1-ol, hexanoato de etilo, acetato de cis-3-hexenilo, benzoato de etilo y octanoato de etilo, para frutos maduros; butirato de etilo, cis-3-hexen-1-ol, hexanoato de etilo, acetato de cis-3-hexenilo, para frutos sazones y acetato de cis-3-hexenilo para frutos verdes, formuladas a una concentración similar a la natural, confirmaron la mayor atracción de los frutos maduros y sazones sobre los frutos verdes.

Con esta investigación se contribuye a ampliar el conocimiento de los compuestos volátiles responsables de la atracción, encontrándose que son compuestos reportados en otros hospederos. Este hecho indicó que *A. obliqua* no detecta un amplio rango de compuestos.

V. LITERATURA CITADA

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