El Colegio de la Frontera Sur

Efecto de los volátiles del hospedero (*Macadamia integrifolia*) en la atracción de *Gymnandrosoma aurantianum* (Lepidoptera: Tortricidae) a su feromona sexual

TESIS
presentada como requisito parcial para optar al grado de
Maestro en Ciencias en Recursos Naturales y Desarrollo Rural
Con orientación en Biotecnología Ambiental

Por

Alvaro Jose Campuzano Granados

2022

ECOSUR

El Colegio de la Frontera Sur

Tapachula, Chiapas; 01 de febrero de 2021.

Las personas abajo firmantes, integrantes del jurado examinador de:

Alvaro Jose Campuzano Granados

hacemos constar que hemos revisado y aprobado la tesis titulada:
Efecto de los volátiles del hospedero (*Macadamia integrifolia*) en la atracción de *Gymnandrosoma aurantianum* (Lepidoptera: Tortricidae) a su feromona sexual

para obtener el grado de **Maestro en Ciencias en Recursos Naturales y Desarrollo Rural.**

Nombre Firma

Director: Dr. Leopoldo Caridad Cruz López

Asesor: Dr. Edi Álvaro Malo Rivera

Asesor: Dr. Guillermo López Guillén

Sinodal adicional: Dra. Rebeca González Gómez

Sinodal adicional: Dr. Samuel Cruz Esteban

Sinodal suplente: M.C. Juan Cisneros Hernández

DEDICATORIA

A:

Dios, por darme la oportunidad de vivir, por haberme permitido llegar hasta este punto y haberme dado salud para lograr mis objetivos, además de haber puesto en mi camino a aquellas personas que han sido mi soporte y compañía durante todo este tiempo.

Mi madre Angélica Granados, por darme la vida, quererme mucho, creer en mí y porque siempre me apoyaste y estuviste ahí en cada momento, por tus consejos, por la motivación constante que me ha permitido ser una persona de bien, pero más que nada, por tu amor incondicional que me has brindado.

Mi padre Juan Campuzano, por los ejemplos de perseverancia y constancia que me ha infundado siempre, por el valor mostrado para salir adelante y por mostrarme el camino que hay que seguir.

Mi hermano Emmanuel, por estar conmigo en cada momento, por el apoyo constante que me has brindado a lo largo de mi vida y por ser el ejemplo de un hermano mayor.

Todo este trabajo ha sido posible gracias a ellos.

AGRADECIMIENTOS

Al Consejo Nacional de Ciencia y Tecnología, por la beca otorgada para realizar este posgrado, porque sin ella hubiera sido imposible dedicarme de forma exclusiva al proyecto.

A El Colegio de la Frontera Sur (ECOSUR), por las facilidades otorgadas para el uso del equipo y materiales dentro de los laboratorios del proyecto de Ecología Química en el Grupo de Ecología de Artrópodos y Manejo de Plagas.

A mi director de tesis, Dr. Leopoldo Caridad Cruz López por brindarme la oportunidad de ser parte de su grupo, por su esfuerzo y dedicación, quien, con sus conocimientos, su experiencia, su paciencia y motivación me ha dado el impulso necesario para concluir con éxito este trabajo.

Expreso mi más sincero reconocimiento y agradecimiento al grupo de Doctores que integraron mi consejo tutelar: Dr. Edi A. Malo Rivera y Dr. Guillermo López Guillén y a mis revisores externos Dr. Samuel Cruz esteba, Dra. Rebeca Gonzáles Gómez y M.C. Juan Cisneros Hernández, quienes con su atenta lectura y atinadas sugerencias han ayudado en el enriquecimiento del artículo y la tesis.

Al M.C Antonio Santiesteban y al Técnico Armando Virgen por el apoyo brindado en la realización del proyecto, así también por brindarme el conocimiento necesario para realizar las diferentes actividades en los laboratorios.

A mis compañeros y amigos del grupo de Ecología Química (Samuel, Fabián, David, Fernando, Lupita Meneses, Wilmar), por ser parte de mi formación profesional y la amistad brindada.

Y A todas las personas involucradas directa e indirectamente que aportaron un granito de arena en construir mi formación académica muchas gracias.

ÍNDICE

RESUMEN

La palomilla de la nuez de macadamia, *Gymnandrosoma aurantianum* Lima (Lepidoptera: Tortricidae), es considerada la plaga de mayor importancia del cultivo de *Macadamia integrifolia* en Centroamérica. Particularmente en Guatemala, este insecto plaga se controla por medio de insecticidas sintéticos, los cuales tienen efectos colaterales en el ambiente y la salud humana, por lo que es importante buscar nuevas alternativas para el control de esta especie. Los volátiles de la planta hospedera pueden modificar la atracción de los insectos al percibir compuestos específicos, los cuales al integrarse con los componentes de las feromonas sexuales liberadas por las hembras pueden producir una sinergia o inhibir la respuesta de los machos. Se investigaron algunos aspectos sobre la olfacción de la palomilla de la nuez de macadamia (*G. aurantianum*) a los volátiles de la planta hospedera (*Macadamia integrifolia*) y su feromona sexual (*E*-8-dodencenial acetato). Se evaluó la respuesta comportamental y electrofisiológica de machos y hembras de *G. aurantianum* a los volátiles de hojas, frutos y flores de *M. integrifolia*. Adicionalmente, por medio de cromatografía de gases acoplado a un electroantenodetector (GC-EAD), se identificaron los compuestos que presentaron respuesta antenal. Finalmente, se llevaron a cabo pruebas de campo para conocer si los volátiles de *M. integrifolia* sinergizan la respuesta de los machos con la feromona sexual. Los resultados mostraron que ambos sexos de *G. aurantianum* respondieron a los volátiles de su planta hospedera. La mezcla de ocimeno, uno de los volátiles de la planta hospedera con la feromona sexual, no tuvo efecto sinérgico en la captura de machos en campo, pero encontramos captura de hembras. Por lo que la captura de machos y hembras en la misma trampa proporciona una ventaja para el monitoreo y control de esta especie.

Palabras Clave: Electroantenográfica, volátiles de planta hospedera, barrenador de la nuez, macadamia, (E)-8-dodecenil acetato.

CAPÍTULO I

INTRODUCCIÓN

1.1. Olfacción en Lepidópteros

En lepidópteros, como en la mayoría de los insectos, las antenas son el órgano principal para detectar moléculas odoríferas en su entorno y perciben constantemente estas moléculas presentes en el medio ambiente (Cunningham et al. 2013), sin embargo, sólo algunas moléculas son detectadas y presentan relevancia comportamental. Los machos son altamente sensibles a los compuestos feromonales de la hembra y en algunos casos suelen ser detectados a grandes distancias y pueden percibir volátiles de sus plantas hospederas donde es posible encontrar a hembras vírgenes para copular. Mientras que las hembras son capaces de percibir y seleccionar plantas de buena calidad y libres de competidores conespecíficos o heteroespecíficos para ovipositar (Martin y Hildebrand 2010). Así también, en algunos lepidópteros, los volátiles del hospedero pueden modificar o estimular la producción y liberación de la feromona sexual e incluso pueden inhibir la respuesta de los machos a estas (Light et al. 1993; Landolt y Phillips, 1997 y Fang et al. 2018).

1.2. Modelos de interacción feromona sexual-volátiles de planta hospedera

Se han reportado diferentes modos de interacción entre los componentes de la feromona sexual y volátiles de la planta huésped, los cuales pueden generar una respuesta sinérgica por la integración de compuestos específicos, pero también pueden ocasionar la inhibición en la percepción olfativa de la feromona sexual en machos de algunas especies. Por ejemplo, Ochieng y colaboradores (2002) reportaron que el componente principal (*Z*)-11-hexadecenal de la feromona sexual emitida por las hembras de *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), no fue afectado por la exposición a los volátiles de la planta huésped, esto mediante pruebas realizadas por Registro de Sensilla Única (RSU). Sin embargo, los estímulos con mezclas binarias del componente de feromona y dosis crecientes de linalol o (*Z*)-3-hexenol sinergizaron significativamente la activación de la neurona específica de feromona en comparación con las respuestas al componente principal de la feromona en machos de esta especie. Esto sugiere que la respuesta a la mezcla de feromonas más los volátiles de la planta huésped, es mayor cuando se compara con la respuesta a la feromona sola. Así también, Fang et al. (2018) evaluaron los efectos de compuestos volátiles de la planta huésped con la integración de la feromona sexual de *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) por medio de pruebas electroantenográficas (EAG), en laboratorio y campo. Estos autores reportaron que el benzaldehído, fenilacetaldehído y el alcohol bencílico incrementaron significativamente la respuesta antenal en machos, mientras que el longifoleno, (*E*)-β-cariofileno y (*E*)-β-ocimeno no tuvieron un efecto significativo sobre la atracción de la feromona sexual. Sin embargo, el (±)-linalol disminuyó significativamente la atracción de machos de *S. litura* con la integración de la feromona sexual a lo largo de la vía olfativa. Xiang, et al. (2019), reportaron un incremento en la respuesta de *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) tanto en pruebas de laboratorio como en campo, cuando se integró (*E*)-β-ocimeno o (*E*)-β farneseno con la feromona sexual de esta especie, mejorando la eficiencia en las capturas en comparación con la feromona sola.

También se han reportado algunas pruebas de campo, donde los machos de palomillas son atraídos en mayor número a las trampas cebadas con mezclas de feromona más volátiles de plantas, en comparación con las trampas cebadas únicamente con feromonas (Light et al. 1993; Landolt y Phillips, 1997; Deng et al. 2004). Además, se ha observado un incremento en el número de vuelos orientados hacia mezclas constituidas por feromona más volátiles de la planta huésped en condiciones de laboratorio (Schmidt et al. 2009; Varela et al. 2011; Trona et al. 2013).

1.3. Modelo de estudio

1.3.1. Palomilla de la nuez de macadamia

La palomilla de la nuez de macadamia *Gymnandrosoma aurantianum* Lima (Lepidoptera: Tortricidae) fue descrita por Lima (1927) en Brasil como una plaga de naranjos. Los adultos suelen ser pequeños con una longitud alar delantera de 6.2 a 10.5 mm aproximadamente, con un ala delantera de color marrón oscuro con marcas indistintas de color marrón rojizo y negro. El ala anterior es ampliamente triangular con el tegmen (margen exterior) débilmente convexo. Las hembras son ligeramente más grandes que los machos (Adamski y Brown, 2001). *G. aurantianum* es la principal plaga de la nuez de macadamia en la región de Centro y Sudamérica, en países como Costa Rica, Hondura y Guatemala teniendo como principal hospedero los cultivos de la nuez de macadamia (*Macadamia integrifolia*). Las hembras ponen huevos en los frutos y las larvas recién nacidas penetran y se alimentan de estos. El ciclo de vida de *G. aurantianum* (de huevo a adulto) dura casi 36 días, y las especies pueden producir hasta 10 generaciones por año, dependiendo de las condiciones climáticas y la disponibilidad de alimentos. El daño ocasionado por esta especie puede reducir de un 40 - 60% la producción de nuez.

1.3.2. Hospederos

En Brasil principalmente, se ha convertido en una de las plagas de mayor importancia económica de los cítricos, ocasionando considerables pérdidas al sector agrícola de esta región. Las áreas infestadas han experimentado pérdidas sobre la producción del 5 - 50% aproximadamente (Carvalho, 2003). Además, se han reportado pérdidas importantes ocasionada por daños de esta especie en otros países y en otros cultivos. Se sabe que *G. aurantianum* ataca *M. integrifolia* (nuez de macadamia) (Blanco-Metzler, 1994), *Theobroma cacao* (cacao) (Pest, 2018) y *Plukenetia volubilis* (nuez inca) (Leandro, 2013), dañando la fruta y reduciendo el rendimiento de estos cultivos.

1.3.3. Biología

En la nuez de macadamia, las palomillas (*G. aurantianum*) ovipositan de forma individual en nueces inmaduras que tienen un diámetro de 8 – 20 mm, generalmente dentro de los 3 m del suelo, donde la producción de nueces es más alta (Blanco-Metzler, 1994). Por lo general, pone un sólo huevo, pero a veces de dos a cuatro huevos por nuez. Los huevos eclosionan en 5 – 6 días. Durante su vida, las hembras ponen un promedio de 37 huevos. Una vez que la larva emerge del huevo deambula por la nuez hasta encontrar un sitio adecuado para iniciar la perforación. Una vez que la larva logra penetrar la cascara de la nuez esta tiende a barrenar hacia los extremos debido a que el grosor de esta es mayor. Conforme la larva va alcanzando su madurez, el orificio de entrada y salida se hace cada vez más visible, logrando detectar el daño ocasionado a la nuez, así mismo ésta va dejando un rastro de los desechos acumulados en la entrada. En la mayoría de las larvas éstas prefieren alimentarse del meso y endocarpo de la nuez, sin embargo, si la cubierta de la nuez no se ha endurecido pude continuar alimentándose hasta llegar a la almendra. Generalmente se ha encontrado una larva por nuez dañada, aunque en algunos casos cuando la población es elevada se pueden encontrar más de una larva por nuez. Cuando existen más de una larva por fruto estas se encuentran regularmente en diferentes estadios, lo que demuestra que, la hembra es atraídas a ovipositar en nueces que ya tienen daño. Conforme se acercan a la etapa de pupa, las larvas dejan de alimentarse y elaboran un capullo con los desechos y una tela de seda que ellas producen, conforme transcurren los días el capullo se va endureciendo hasta tornar en un color café oscuro, la duración del estado de pupa es de 7 -15 días aproximadamente. Las pupas de las hembras suelen ser ligeramente más grande que la de los machos. Este proceso de pupación se lleva a cabo principalmente en las nueces, aunque también se ha observado en el musgo que crece en el árbol, ramas laterales, en el peciolo del racimo de los frutos y entre las nueces. Los adultos emergen generalmente durante el día, sin embargo, algunos lo hacen durante la noche o a tempranas horas de la mañana (Blanco-Metzler, 1994).

Aunque aún no se tiene claro la conducta del apareamiento para esta especie (*G. aurantianum*) se estima que ocurre cerca del anochecer, cuando la actividad de las hembras se incrementa, estas mueven constantemente sus alas, levantando el abdomen para exponer la glándula feromonal al exterior, mientras que los machos realizan una serie de vuelos cortos a traves de la pluma de feromonas hasta dar con la hembra para aparearse (Blanco-Metzler, 1994).

1.3.4. Feromona sexual

Los componentes de la feromona sexual de *G. aurantianum*, han sido identificados como: acetato de dodecilo (12: Ac), acetato de (*E*)-8-dodecenilo (*E*8-12: Ac), (*E*) -8-dodecen-1-ol (*E*8-12: OH) y acetato de (*Z*)-8-dodecenilo (*Z*8-12: Ac) (Chamberlain et al., 2003). De acuerdo con pruebas de laboratorio y campo, el compuesto mayoritario (*E*8-12: Ac) ha mostrado mayor atracción en machos de *G. aurantianum* a dosis de 1 mg.

CAPÍTULO II

MACADAMIA VOLATILES AFFECT THE ATTRACTION OF THE MOTH *Gymnandrosoma aurantianum* TO (*E*)-8-DODECENYL ACETATE THE MAIN COMPONENT OF ITS SEX PHEROMONE

Manuscrito enviado a: Journal of Chemical Ecology

MACADAMIA VOLATILES AFFECT THE ATTRACTION OF THE MOTH *Gymnandrosoma aurantianum* TO (*E*)-8-DODECENYL ACETATE THE MAIN COMPONENT OF ITS SEX PHEROMONE

ALVARO J. CAMPUZANO-GRANADOS[1], EDI A. MALO[1], JAIME GOMEZ-RUIZ[1], GUILLERMO LÓPEZ-GUILLÉN[2] AND LEOPOLDO CRUZ-LÓPEZ[1]*

*Corresponding Author: Leopoldo Cruz-López, lcruz@ecosur.mx

[1]*Grupo de Ecología de Artrópodos y Manejo de Plagas, Departamento de Agricultura, Sociedad y Ambiente, El Colegio de la Frontera Sur, Carretera Antiguo Aeropuerto Km 2.5, 30700 Tapachula, Chiapas, Mexico.*

[2]*Campo Experimental Rosario Izapa, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Tuxtla Chico, Chiapas, C. P. 30780, Mexico.*

Abstract-The macadamia nut borer moth *Gymnandrosoma aurantianum*, is the main pest of macadamia (*Macadamia integrifolia*) in Central America. This study investigates the effect of the host (*M. integrifolia*) on attraction of *G. aurantianum* to its sex pheromone. Y-Tube bioassays showed that females *G. aurantianum* were attracted to volatiles from *M. integrifolia* leaves and flowers, while males responded to volatiles from flowers. Both sexes had significantly different electroantennographic responses (EAG) to the extracts of volatiles from flowers, fruits and leaves and (*E*)-8-dodecenyl acetate (main component of the sex pheromone of *G. aurantianum*). Females *G. aurantianum* exhibited electroantennographic responses by CG-EAD to phenylacetaldehyde, (1*Z*)-3-methylbutanal oxime and (*E*)-β-ocimene, while the males showed antennal activity in response to phenylacetaldehyde, (1*E*)-3-methylbutanal oxime, (1*Z*)-3-methylbutanal oxime, present in the extracts of *M. integrifolia*. The EAG dose-response with ocimene (mix of isomers) showed that female antennal activity increases as the dose increases, while with males, the highest dose elicited a response that was significantly different from the control. In field tests, the mixture (ocimene/(*E*)-8-dodecenyl acetate) with the proportion of 10:1

was the treatment that captured the highest number of males and females. Also, we observed that the lowest number of male captures was obtained with the proportion of 1:1, compared to the traps baited with only (*E*)-8-dodecenyl acetate. These results suggest that the binary mixture of ocimene plus (*E*)-8-dodecenyl acetate in a proportion of 10:1 could be an option for monitoring this pest because obtained captures of both sexes.

Key Words-Sex pheromones, host plant volatiles, *Gymnandrosoma aurantianum*, macadamia, (*E*)-8-dodecenyl acetate.

INTRODUCTION

Most phytophagous insects use plant volatiles to localize their hosts, which provide food, habitat, and oviposition sites (Bruce et al. 2005). For some lepidopterans, host volatiles can modify or stimulate the production and release of sex pheromone, or they can inhibit male response to the sex pheromone produced by females (Light et al. 1993; Landolt and Phillips 1997; Fang et al. 2018). Moreover, it is known that host plant volatiles can produce a synergic effect on response to the sex pheromone in several lepidopteran species. For example, Ochieng et al. (2002) described the synergy between linalool and the main component of the sex pheromone (*Z*)-11-hexadecenal of the moth *Helicoverpa zea* (Lepidoptera: Noctuidae), based on the hypothesis that the interaction between the sex pheromone and host plant volatiles can increase male attraction. Also, a study conducted by Fang et al. (2018) indicated that the benzaldehyde, phenylacetaldehyde, and benzyl alcohol significantly increased attraction of *Spodoptera litura* (Lepidoptera: Noctuidae). In contrast, longifolene, (*E*)-β-caryophyllene and (*E*)-β-ocimene did not have significant effect on attraction, while (±)-linalol significantly decreased attraction of the male *S. litura* when it was mixed with the sex pheromone. According to the hypothesis, host plant volatiles provide valuable information about insects because they act as habitat signals that facilitate the location of mates. In some field tests, male moths have been attracted in larger numbers to traps baited with mixtures of pheromone and host plant volatiles than to traps baited with only sex pheromones (Light et al. 1993; Landolt and Phillips 1997; Deng et al. 2004). It has also been observed that the number of flights toward the mixtures of pheromone and host plant volatiles increased in the laboratory (Schmidt et al. 2009; Varela et al. 2011; Trona et al. 2013).

The macadamia nut borer moth *Gymnandrosoma aurantianum* (Lepidoptera: Tortricidae), synonymously known also as *Ecdytolopha torticornis* (Lepidoptera: Tortricidae) (López-Guillén et al. 2021), is the principal pest of macadamia (*Macadamia integrifolia* Maiden and Betche; Proteaceae) in Central America (Masís and Soto-Manitiu, 1992; López-Guillén et al. 2021). The components of the sex pheromone of this moth species have been reported as: dodecyl acetate (12: Ac), (*E*)-8-dodecenyl acetate (*E*8-12: Ac), (*E*)-8-dodecen-1-ol (*E*8-12: OH) and (*Z*)-8-dodecenyl acetate (*Z*8-12: Ac) (Chamberlain et al. 2003). In laboratory and field tests, the main component (*E*8-12: Ac) showed to be attractive to *G. aurantianum* males (Chamberlain et al. 2003). Recently, Navas-Franco (2019) also showed that this compounds attracts males of *G. aurantianum.* However, it is not known whether there is a synergic effect of host plant volatiles (*M. integrifolia*) with the main component of *G. aurantianum* sex pheromone (*E*8-12: Ac). For this reason, we studied the behavioral and electrophysiological response of *G. aurantianum* males and females to volatiles from *M. integrifolia* leaves, fruits, and flowers. The compounds that elicited antennal response were then identified by gas chromatography-mass spectrometry. Finally, field tests were done to determine whether one of the identified compounds synergize the response of *G. aurantianum* males to *E*8-12: Ac the main component of the sex pheromone of *G. aurantianum*.

METHODS AND MATERIALS

Insects. We collected *G. aurantianum* larvae on the Buena Vista Farm, located in the Republic of Guatemala (14° 57’ 51’’ N and 91° 59’ 49 W) and kept them in controlled conditions (23 ± 2 °C, 75 ± 5 % relative humidity and a photoperiod of 12:12 h (L:D) until the adult stage. The larvae were fed an artificial diet (García and Parra, 1999), and after emergence, the adults were fed a 10% sucrose solution. For all the tests, we used 2 – 5 day-old virgin specimens.

Plant material. Plant material was collected from *M. integrifolia* cultivar Kau which is considered highly susceptible to the nut borer. Two sizes of macadamia fruits were collected: small (2 cm diameter) and large (3 cm), to determine whether females have some preference in terms of fruit size. In addition, flowers and leaves were collected in the same site as mentioned above.

Collection of volatiles. Volatiles were collected using the method of dynamic headspace. The samples (fruits, flowers, and leaves) were placed inside of a glass aeration chamber (30 x 60 cm). Volatiles were collected by airflow passage at 0.5 L/min, (previously purified by an activated carbon filter) over the plant material. Volatiles were collected in a glass column contained 200 mg Porapak Q 50–80 (Waters Corporation, Milford, MA) for a period of 24 h, at a temperature of 26 ± 2 °C, RH 70 ± 5 %, and a photoperiod of 12:12 h (L:D). Later, the volatiles were eluted from the column with 400 μL dichloromethane which were concentrated to 100 μL under a nitrogen flow and stored in a 1mL glass vial at -20 °C until use.

Bioassays. The response of *G. aurantianum* adults to volatiles collected from fruits, flowers and leaves were evaluated in a glass Y-tube olfactometer (trunk of the Y-tube 15.52 cm long and the two arms 12 cm long, at 45°, internal diameter 2.5 cm). The plant volatile extracts and the dichloromethane (control) were placed at the ends of each arm (1 μL), and later, humidified air filtered with activated carbon was made to pass through each of the arms, while the insect was introduced at the base of the trunk of the olfactometer. A positive response was recorded when the insect crossed the "line of election" (7 cm after passing the intersection of the olfactometer arms) and remained at the site for 10 seconds. Each bioassay had a duration of 5 min, and those insects that did not select after 5 min were excluded from the analysis. A total of 30 replications were performed per treatment. The bioassays were conducted in a dark room at a temperature of 25 ± 1 °C between 18:00 and 22:00 h. The room was illuminated by a red light at a distance of 120 cm (8-10 Lux). During all the tests, the sample and the control were interspersed to avoid biases in the responses. After five replications, the olfactometer was washed with water, soap and acetone and dried in an oven at 90 °C for one hour.

Electroantennography. The antennal response of *G. aurantianum* females and males to *M. integrifolia* volatiles, to *E*8-12: Ac and ocimene were determined by electroantennography (EAG). The head of the insect was carefully cut, and the reference electrode was inserted into its base, using a glass capillary, which was filled with a physiological saline solution. A segment of the antenna was cut, and the distal end of the antenna was inserted at the end of the glass capillary placed inside the recording electrode. The signals generated by the antenna passed through a high impedance amplifier (NL 1200; Syntech, Hilversum, The Netherlands) and were visualized in a monitor using Syntech software to process the EAG signals. A regulator of stimulus flows (CS-05; Syntech) was used to generate stimuli at intervals of 1 min. A constant current of pure humidified air (0.7 L/min) was directed over the antenna through a glass tube 10 mm in diameter into which the sample was placed for its analysis. A standard aliquot (1 μl) of each plant extract was loaded in a piece of filter paper (0.5 x 3.0 cm, Whatman, No. 1; Whatman

International, Maidstone, United Kingdom) exposed to air for 30 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 antenna replicate. A cartridge with a piece of filter paper loaded with 1 µL of dichloromethane was used as control. A current of humidified pure air (0.7 L/min) was constantly directed onto the antenna through a 10 mm diameter glass tube. 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. A stimulus flow controller (CS-05; Syntech) was used to generate a stimulus at 1 min intervals. The duration of the stimulus was 1 s. The continuous flow of clean air through the airflow tube and over the preparation ensured that odours were removed immediately from the vicinity. The plant extract was presented in random order and the test doses for ocimene were presented sequentially from the lowest to the highest concentration (0.01, 0.1, 1, 10 y 100 µg/µL). Control (dichloromethane) stimuli were presented at the beginning and end of each EAG analysis. One replicate was made with one antenna. Each plant extract and ocimene were tested on 2 – 5 day-old 10 males and 10 females of *G. aurantianum*.

Gas chromatography coupled to an electroantenodetector (CG-EAD). To determine the antennal active compounds in the extracts, a gas chromatograph (Shimadzu GC-2010 Plus, Tokyo, Japan) coupled with an electroantenodetector (EAD, Syntech, Hilversum, The Netherlands), equipped with a non-polar capillary column Factor Four VF-5ms (30 m long with 0.25 mm internal diameter) and a flame ionization detector (FID), as well as a split/splitless injector was used. The samples were injected in splitless mode, using hydrogen as the carrier gas at a flow of 2.0 ml/min. The effluent of the capillary column was divided into two parts (1:1) using a transfer line (glass capillary with no phase) that was connected to the FID and the other to the EAD. The samples were analyzed using a temperature schedule for the chromatograph oven: initial temperature 50 °C for 2 min, increasing 5 °C/min up to 250 °C, with an isotherm for 10 min. The injector temperature was 200 °C and that of the detector was 250 °C. The signals generated by the antenna and by the FID detector passed through a high incidence amplifier (NL 1200, Syntech, Hilversum, The Netherlands) and were visualized with a monitor using the software Syntech version 2.6 (1993-2003) to process the GC-EAD signals. The antennae, previously cut at their base, were inserted into two glass capillaries (for the base and the end of the antennal flagellum) provided with a saline solution. A total of 10 replications of each sample (fruits, flowers and leaves) were analyzed, using an antenna from a different specimen in each replication. Compounds were considered antennally active if they showed an incidence rate of ≥ 60% in each sample analyzed.

Identification of volatiles. The compounds that were antennally active by GC-EAD were identified in a Varian Star 3400 CX gas chromatograph coupled to a Varian selective mass detector, model Saturn 4D (Palo Alto, CA, USA). The compounds were separated using a column of methyl silicone (DB5-MS) 30 m long by 0.25 mm interior diameter. The samples were injected in splitless mode. A program of 50 °C initial temperature (2 min) to a final temperature of 280 °C (2 min) with increments of 15 °C per min. Helium was used as the carrier gas. Volatiles were analyzed using Saturn GC/MS Workstation software. Compounds were identified by comparing retention times and mass spectra of the available synthetic standards. Other compounds were identified tentatively based on comparison with the spectra library of the National Institute of Standards and Technology 2.0 (NIST) and retention indexes with those reported in the literature.

Chemicals. (*E*)-8-dodecenyl acetate (Alfa Chemistry, NY, USA), (*E*)-β-ocimene (Cayman Chemicals, Michigan, USA), ocimene (mix of isomers) (Aldrich, Toluca, Mexico).

Field tests. This assay was conducted on the Buena Vista Farm, municipality of San Pablo, department of San Marcos, Guatemala (Cordenadas). White delta-type sticky traps (12 x 18 cm) were used to capture *G. aurantianum*. These traps were baited with rubber septa that contained ocimene (10 mg), (*E*)-8-dodecenyl acetate (1 mg) and a mixture of the two in different proportions (1:1, 5:1 and 10:1 mg/mg); entomological glue was placed on the bottom of the traps. The experimental design was a completely randomized block design replicated in five blocks, each block contained all treatments evaluated. The blocks were set out in parallel lines (transects) with a separation of 50 m between blocks. The traps were placed at a height of 3 m above the ground with a separation of 50 m between traps. The traps were examined every week, and the moths captured per treatment were collected and recorded. After each examination, the traps were rotated within the blocks, while the baits were renewed every 14 days. Sexing was performed using the taxonomic keys employed by Adamski and Brown (2001).

Statistical analysis. The data obtained in the olfactometry tests in “Y” tubes were analyzed using the *G-test* with Williams’ correction. The field capture data and the EAG responses were analyzed using a one-way ANOVA. Field capture data was transformed with Log (x + 0.5) to comply with the assumptions of normality and homoscedasticity. The comparison of means was performed using the *HSD Tukey* test ($\alpha = 0.5$). Data were analyzed using the program R (v. 4.0.5).

RESULTS

Attraction of Gymnandrosoma aurantianum adults to M. integrifolia volatiles. *G. aurantianum* females were significantly more attracted to *M. integrifolia* leaf ($\chi^2 = 4.2$; df = 1; $P < 0.05$) and flower ($\chi^2 = 3.89$; df = 1; $P < 0.05$) extracts compared to the control (dichloromethane), but they were not attracted to volatile extracts from fruits (large and small) (Fig. 1). While males were attracted only to flower volatiles ($\chi^2 = 5.36$; df = 1; $P < 0.05$) (Fig. 2).

Electrophysiological response (EAG) of Gymnandrosoma aurantianum to Macadamia integrifolia volatiles and sex pheromone. The EAG responses of *G. aurantianum* females to plant volatile extracts of *M. integrifolia* and to (*E*)-8-dodecenyl acetate showed significant differences ($F = 13.54$; df = 5, 54; $P < 0.0001$). The EAG response elicited by leaves volatiles was significantly different than those obtained the control, but the response elicited by fruit (large and small) volatiles was not, while the responses EAG elicited by flowers volatiles and the main component of the sex pheromone were statistically like the responses EAG elicited by the fruit volatiles but different to EAG produced by control (Fig 3). The EAG of *G. aurantianum* males to plant volatile extracts and to (*E*)-8-dodecenyl acetate showed significant differences ($F = 28.53$; df =5, 54; $P < 0.0001$). It was observed that the EAG responses elicited by flowers and fruits volatiles were similar but significantly different to the EAG responses produced by control, while the EAG responses elicited by leaves volatiles and (*E*)-8-dodecenyl acetate showed significantly larger responses than control (Fig. 4).

Response detected by gas chromatography coupled to an electroantenodetector (GC-EAD). The GC-EAD analysis of macadamia flowers volatiles showed that *G. aurantianum* females consistently exhibited antennal responses to three compounds: (1*Z*) -3-methylbutanal oxime (peak 1), (*E*)-β-ocimene (peak 2) and phenylacetaldehyde (peak 3) (Fig. 5a). (*E*)-β-ocimene was the only compound of the *M. integrifolia* leaf volatile extract that produced an EAG response to *G. aurantianum* female (Fig. 5b). Females showed no EAG response to volatiles from fruits (small and large). Flower volatiles: (1*Z*)-3-methylbutanal oxime (peak1), (1*E*)-3-methylbutanal oxime (peak 2) and phenylacetaldehyde (peak 3), elicited EAG responses only in males (Fig. 6).

The phenylacetaldehyde (major compound in flowers extract [43.7%, Table 1]) elicited the larger EAG response (0.44 mV), followed the EAG response elicited by (1*Z*)-3-methybutanal oxime (0.37 mV) in *G. aurantianum* females. The (*E*)-β-ocimene (major compound in leaves extract [100%, Table 1]) elicited the larger EAG response (0.39 mV), followed the EAG response elicited by (1*Z*)-3-metilbutanal oxime (0.36 mV), and phenylacetaldehyde (0.33 mV) (both present in flower extract) in *G. aurantianum* males. (*E*)-β-ocimene is found in a larger proportion in leaf extract than in flower extract (Table 1).

Evaluation of response dose by EAG. The analysis of the EAG responses elicited by mixture ocimene (mix of isomers) of different doses evaluated in *G. aurantianum* females showed significant differences (*F* = 261.1; df =5, 54; *P* < 0.0001), observing an increase in EAG response as the doses increased; the highest concentration evaluated was 100 μg. The comparison of means showed that all the doses evaluated elicited EAG response different the control, obtaining a greater response at the highest dose (Fig. 7). The doses evaluated in males showed that the dose of 1 μg and the highest dose (100 μg) were those that elicited larger EAG response (*F* = 13.29; df =5, 54; *P* < 0.0001).

Field experiment. Females were captured in the traps baited with ocimene (mix of isomers). The concentration of 10 mg of ocimene captured significantly more females (4.48 ± 0.74 moths/seek) when it was mixed with (*E*)-8-dodecenyl acetate (sex pheromone), compared with traps baited with only ocimene (2.6 ± 0.04 moths/week) (*F* = 10.18; df =3, 96; *P* < 0.0001) (Fig. 8). In contrast, when 1 mg ocimene was mixed with 1 mg of (*E*)-8-dodecenyl acetate, the capture of males was less (2 ± 0.30 moths/week) than with (*E*)-8-dodecenyl acetate alone (4.84 ± 0.74 moths/week) ($F = 9.96$; df =3, 96; $P < 0.0001$). The traps baited with a proportion of 10:1 (10 mg ocimene/1mg (*E*)-8-dodecenyl acetate) did not show significant differences in captures (5.12 ± 0.74 moths/week), when compared with pheromone alone (Fig. 8).

DISCUSSION

In this study, we investigated the behavioral and electrophysiological response of *G. aurantianum* males and females to volatiles from *M. integrifolia* leaves, fruits, and flowers. We found that females were attracted to volatiles from leaves and flowers, while males responded to flower volatiles, but neither sex responded to fruits in the bioassays. Moreover, in field tests, we found that the addition of one of the host plant volatiles (ocimene) to (*E*)-8-dodecenyl acetate (the main component of the sex pheromone) had no synergic effect in the capture of males, but females were captured in these traps. The result of the capture of *G. aurantianum* males and females suggests that it can be used to monitor and control *G. aurantianum*. However, evaluation of the synergy of the other identified compounds is required.

These results suggest that volatiles from flowers may be involved in long-distance attraction of females and males of *G. aurantianum*, since it is known that some moths are attracted by the chemical signals of the host plant (Bäckman et al. 2001). Furthermore, these compounds can stimulate females to release their sex pheromone and mate with their conspecifics (Castrovillo and Cardé, 1980; Curkovic and Brunner 2007). Males can detect these signals from the pheromone and host plant volatiles acting synergically to detect females more easily (Yang et al. 2004; Xian et al. 2009).

When we analyzed the EAG responses of *G. aurantianum* to the plant volatiles by GC-EAD, we observed differences in responses between males and females. (*E*)-β-Ocimene was the compound that more consistently (100 %). In various studies, β-ocimene is reported as a common compound, which is released in significant quantities in the leaves and flowers of many plant species, which can perform various biological functions within plants by potentially affecting visitors and by mediating defensive responses to herbivory (Farré-Armengol 2017). The fact that males and females of *G. aurantianum* respond differently to the macadamia volatiles might be related to their olfactory system, as it happens in some lepidopteran species, for example, Röstelien et al. (2000), found *Heliothis virescens* possess specific receptor neurons for (*E*)-β-ocimene. In addition, several studies have reported the attraction of some moths using (*E*)-β-ocimene in behavioral tests (Rajapakse et al. 2006; Sun et al. 2012; Borrero et al. 2015). Phenylacetaldehyde has also been informed as a compound that synergizes the response to the sex pheromone of males *Spodoptera litura* (Fang et al. 2018).

Neither female nor male *G. aurantianum* showed attraction to fruit volatiles (small and large) in the laboratory bioassays, possibly because the volatiles emitted by these fruits are found in small quantities, as in the case of *Helicoverpa armiguera* female moths, the majority of which were attracted to volatiles of whole plants over fruits alone and preferred to oviposit when they were stimulated with whole plants (Jallow et al. 1999). Although the mechanism of host fruit localization of this species (*G. aurantianum*) is still not clear because it is a nocturnal species, orientation by host volatiles seems to be the most likely mechanism for locating fruits. It is considered that this species could be using visual or physical mechanisms to locate fruits, as is the case of some lepidopteran species (Fenemore 1988; Renwick and Chew 1994). Another apparent reason that the females were not attracted to fruits in the bioassays could be that the selected fruits were not in a suitable physiological state since, according to a study by Blanco et al. (1993), ripe fruits do not favor oviposition in nut borer females (*Ecdytolopha torticornis* Lepidoptera: Tortricidae). For this reason, in future work, we recommend broadening fruit selection.

In field test, ocimene plus (*E*)-8-dodecenyl acetate in different proportions captured males and females. However, no synergic effect was observed in the capture of males. The mixture (ocimene/(*E*)-8-dodecenyl acetate) with the proportion of 10:1 was the treatment that captured the highest number of males and females. In contrast, it was found that the binary mixture in a 1:1 proportion could be inhibiting the response to (*E*)-8-dodecenyl acetate in the field tests. Thus, the effect of the dose may have influenced *G. aurantianum* behavior in the field tests. In this sense, Yang et al. (2004) reported that the volatile compounds from plants can synergize attraction of the codling moth *Cydia pomonella* to the sex pheromone (*E*, *E*)-8,10-dodecadien-1-ol (codlemone); the mixture of pheromone and host plant volatiles in a proportion of 1:100 attracted more males than the mixtures of 1:1 or 1:10,000 in wind tunnel laboratory tests.

In contrast with our results, generally, in Lepidoptera, a synergist attractiveness effect of plant volatiles to sex pheromone occurs. For example, Xiang et al. (2019) reported an increase in the response of *Grapholita molesta* (Lepidoptera: Tortricidae) in both laboratory and field tests when (*E*)-β-ocimene or (*E*)-β-farnesene were integrated with the sex pheromone of this species, improving capture efficiency when compared with the pheromone alone. Moreover, a study conducted by Fang et al. (2018) indicated that compounds, such as benzaldehyde, phenylacetaldehyde and benzylic alcohol, significantly increased attraction of *Spodoptera litura*.

In contrast, longifolene, (*E*)-β-caryophyllene and (*E*)-β-ocimene had no significant effect on attraction, while (±)-linalol significantly decreased attraction of the *S. litura* male, supporting the hypothesis that host plant volatiles act as habitat signals that can facilitate the location of a mate.

In lepidopterans, there is little information on female captures when host volatiles are mixed with sex pheromone. Using the mixture of ocimene and (*E*)-8-dodecenyl acetate has the advantage of capturing both sexes, which can be useful in monitoring and controlling *G. aurantianum*.

In conclusion, in this study we found that both sexes of *G. aurantianum* responded behaviorally and electrophysiologically, to host plant volatiles. Mixing one of the host plant volatiles (ocimene) with (*E*)-8-dodecenyl acetate did not have a synergic effect in attracting males, but the capture of females in the field is reported. The mixture (ocimene/(*E*)-8-dodecenyl acetate) with the proportion of 10:1 was the treatment that captured the highest number of males and females. The capture of males and females in the same trap is advantageous for monitoring or a mass trapping system for the management of *G. aurantianum*. However, it is necessary to evaluate the synergy of the other identified compounds.

REFERENCES

Adamski D, Brown J (2001) Systematic revision of the Ecdytolopha group of genera (Lepidoptera: Tortricidae: Grapholitini) in the New World. Entomol Scand Suppl 58:1-86.

Bäckman A, Bengtsson M, Borg-Karlsson A, Liblikas I, Witzgall P (2001) Volatiles from apple (*Malus domestica*) eliciting antennal responses in female codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae): effect of plant injury and sampling technique. Z Naturforsch, C J Biosci 56(3-4):262-268. https://doi.org/10.1515/znc-2001-3-415

Blanco-Metzler H, Watt A, Cosens D (1993) Ciclo de vida y comportamiento de oviposición de *Ecdytolopha torticornis* (Lepidoptera: Tortricidae) barrenador de la nuez de macadamia. Manejo Integr Plagas 29:36-39.

Borrero F, Becher P, Birgersson G, Bengtsson, M, Witzgall P, Saveer A (2015) Flight attraction of *Spodoptera littoralis* (Lepidoptera, Noctuidae) to cotton headspace and synthetic volatile blends. Front Ecol Evol 3:1-56. https://doi.org/10.3389/fevo.2015.00056

Bruce T, Wadhams L, Woodcock C (2005) Insect host location: a volatile situation. Trends Plant Sci 10(6):269-274. https://doi.org/10.1016/j.tplants.2005.04.003

Castrovillo P, Cardé R (1980) Male codling moth (*Laspeyresia pomonella*) orientation to visual cues in the presence of pheromones and sequences of courtship behaviors. Ann Entomol Soc Am 73:100-105. https://doi.org/10.1093/aesa/73.1.100

Chamberlain D, Beevor P, Alan C, David R (2003) (*E*)-8-Dodecenyl acetate: Major component of the female sex pheromone of a Macadamia nut borer, *Ecdytolopha torticornis*. Entomol Exp Appl 4:1-3. https://doi.org/10.1046/j.1570-7458.2003.00035.x

Curkovic T, Brunner J (2007) Short communication. Pheromone inhibitors for *Pandemis pyrusana* males (Lepidoptera: Tortricidae). Span J Agric Res 5:385-388. https://doi.org/10.5424/sjar/2007053-264

Deng J, Wei H, Huang Y, Du J (2004) Enhancement of attraction to sex pheromones of *Spodoptera exigua* by volatile compounds produced by host plants. J Chem Ecol 30:2037-2045. https://doi.org/10.1023/b:joec.0000045593.62422.73

Fang Y, Zeng R, Lu S, Dai L, Wan X (2018) The synergistic attractiveness effect of plant volatiles to sex pheromones in a moth. J Asia Pac Entomol 21:380-387. https://doi.org/10.1016/j.aspen.2018.01.009

Fenemore P (1988) Host-plant location and selection by adult potato moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae): a review. J Insect Physiol 34(3):175-177.

Farré-Armengol G, Filella I, Llusiá J, Peñuelas J (2017) β-Ocimene, a key floral and foliar volatile involved in multiple interactions between plants and other organisms. Molecules (Basel, Switzerland) 22(7):1148. https://doi.org/10.3390/molecules22071148

García M, Parra J (1999) Comparação de dietas artificiais, com fontes protéicas variáveis, para criação de *Ecdytolopha aurantiana* (Lima) (Lepidoptera: Tortricidae). An Soc Entomol 28(2):219-232. https://doi.org/10.1590/S0301-80591999000200004

Jallow M, Zalucki M, Fitt G (1999) Role of chemical cues from cotton in mediating host selection and oviposition behaviour in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Aust J Entomol 38:359-366. https://doi.org/10.1046/j.1440-6055.1999.00131.x

Landolt P, Phillips T (1997) Host plant influences on sex pheromone behavior of phytophagous insects. Annu Rev Entomol 42:371-391. https://doi.org/10.1146/annurev.ento.42.1.371

Light D, Flath R, Buttery R, et al. (1993) Host-plant green-leaf volatiles synergize the synthetic sex pheromones of the corn earworm and codling moth (Lepidoptera). Chemoecology 4:145-152. https://doi.org/10.1007/BF01256549

López-Guillén G, Gómez Ruiz J, Brown J, Cruz-López L, Metz M, Solís M (2021) First records of lepidoptera damaging *Macadamia integrifolia* (Proteaceae) fruits in Guatemala. Proc Entomol Soc Wash 123(2):341-349. https://doi.org/10.4289/0013-8797.123.2.341

Masís C, Soto-Manitiu J (1992) Insectos asociados a *Macadamia integrifolia* en Costa Rica. Agron. Costarricense 16(1):137-138.

Ochieng S, Park K, Baker T (2002) Host plant volatiles synergize responses of sex pheromone-specific olfactory receptor neurons in male *Helicoverpa zea*. J Comp Physiol A 188:325-333. https://doi.org/10.1007/s00359-002-0308-8

Rajapakse C, Walter G, Moore C, Hull C, Cribb B (2006) Host recognition by a polyphagous lepidopteran (*Helicoverpa armigera*): primary host plants, host produced volatiles and neurosensory stimulation. Physiol Entomol 31(3):270-277. https://doi.org/10.1111/j.1365-3032.2006.00517.x

Renwick J, Chew F (1994) Oviposition behavior in Lepidoptera. Annu Rev Entomol 39(1):377-400. https://doi.org/10.1146/annurev.en.39.010194.002113

Röstelien T, Borg-Karlson A, Mustaparta H (2000) Selective receptor neurone responses to (E)-beta-ocimene, beta-myrcene, E,E-alpha-farnesene and homo-farnesene in the moth *Heliothis virescens*, identified by gas chromatography linked to electrophysiology. J Comp Physiol A 186(9):833-847. https://doi.org/10.1007/s003590000136

Schmidt D, Von M, Guerin P (2009) Host plant volatiles serve to increase the response of male European grape berry moths, *Eupoecilia ambiguella*, to their sex pheromone. J Comp Physiol A 195:853-864. https://doi.org/10.1007/s00359-009-0464-1

Sun J, Huang L, Wang C (2012) Electrophysiological and behavioral responses of *Helicoverpa assulta* (Lepidoptera: Noctuidae) to tobacco volatiles. Arthropod Plant Interact 6:375-384. https://doi.org/10.1007/s11829-012-9190-7

Trona F, Anfora G, Balkenius A, Bengtsson M, Tasin M, Knight A (2013) Neural coding merges sex and habitat chemosensory signals in an insect herbivore. Proc R Soc Lond B Biol Sci 280:2-67. https://doi.org/10.1098/rspb.2013.0267

Varela N, Avilla J, Anton S, Gemeno C (2011) Synergism of pheromone and host plant volatile blends in the attraction of *Grapholita molesta* males. Entomol Exp Appl 141:114-122. https://doi.org/10.1111/j.1570-7458.2011.01171.x

Xiang, H, Chen Z, Li X, Guo Y, li X, Ma R (2019) Two terpenoids activates close mating behavior and enhances trap efficiency of sex pheromone of *Grapholita molesta*. J Asia-Pac Entomol 22(4):1109-1114. https://doi.org/10.1016/j.aspen.2019.08.003

Yang Z, Bengtsson M, Witzgall P (2004) Host plant volatiles synergize response to sex pheromone in codling moth, *Cydia pomonella*. J Chem Ecol 30:619-629. https://doi.org/10.1023/B:JOEC.0000018633.94002.af

Declarations

Funding: *The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.*

Conflicts of interest/ competing interests: none

Author Contributions: Conception and design of the project (LCL, AJC); collection of data (AJC); performed analyses (LCL, AJC, EAM); wrote manuscript (LCL, AJC, EAM, GLG)

Table 1 Antennal response (mV) of *G. aurantianum* to *M. integrifolia* volatiles (^{F} = Flowers and ^{L} = Leaves)

Compounds	Antennal response (mV) ± EE		Relative abundance (%) ± EE	
	Females	Males	Flowers ^{F}	Leaves ^{L}
(1*Z*)-3-methybutanal oxime	0.37 ± 0.01	0.36 ± 0.02	19.37 ± 1.03	
(1*E*)-3-methybutanal oxime		0.28 ± 0.02	10.51 ± 1.03	
(*E*)-β-ocimene	0.33 ± 0.01 ^{F} 0.39 ± 0.01 ^{L}		9.49 ± 0.75	100
Phenylacetaldehyde	0.44 ± 0.03	0.33 ± 0.02	43.70 ± 1.88	

Figure legends

Fig. 1 Attraction of *Gymnandrosoma aurantianum* females to *Macadamia integrifolia* volatiles using a Y-tube olfactometer. NR = Number of non-responding females. The asterisks indicate significant values in each comparison (* $P < 0.05$). N = 30

Fig. 2 Attraction of *Gymnandrosoma aurantianum* males to *Macadamia integrifolia* volatiles using a Y-tube olfactometer. NR = Number of non-responding males. The asterisks indicate significant values in each comparison (* $P < 0.05$). N = 30

Fig. 3 EAG responses (means ± SE) of *Gymnandrosoma aurantianum* females to *Macadamia integrifolia* volatiles and sex pheromone (E8-12: Ac). Significantly different means are indicated with different letters (*HSD Tukey*, $P < 0.05$).

Fig. 4 EAG responses (means ± SE) of *Gymnandrosoma aurantianum* males to *Macadamia integrifolia* volatiles and sex pheromone (E8-12: Ac). Significantly different means are indicated with different letters (*HSD Tukey*, $P < 0.05$).

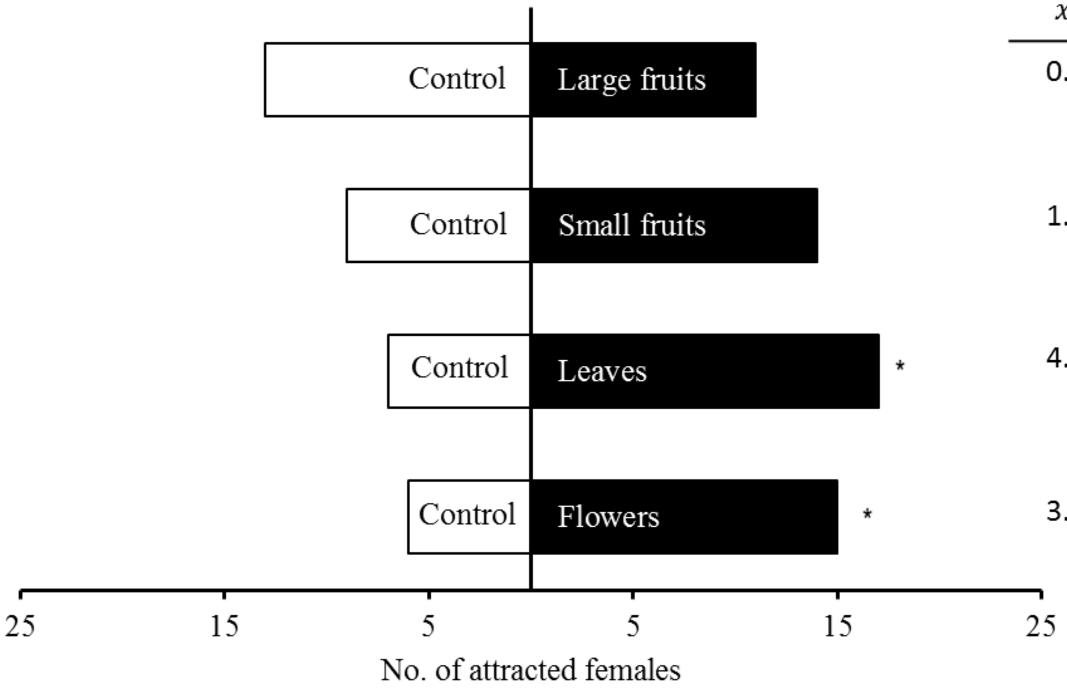
Fig. 5 Simultaneous responses of *Gymnandrosoma aurantianum* females to *Macadamia integrifolia* volatiles using the flame ionization detector (FID) and electroantennographic detection (EAD). a) flower volatiles; peak 1(1Z) -3-methylbutanal oxime), peak 2 (E)-β-ocimene), peak 3(Phenylacetaldehyde). b) leaf volatiles; peak 1((E)-β-ocimene).

Fig. 6 Simultaneous responses of *Gymnandrosoma aurantianum* males to *Macadamia integrifolia* flower volatiles using the flame ionization detector (FID) and electroantennographic detection (EAD). Peak 1((1Z)-3-methylbutanal oxime), peak 2 ((1E)-3-methybutanal oxime) and peak 3 (Phenylacetaldehyde).

Fig. 7 EAG responses (means ± SE) to different doses of ocimene of *Gymnandrosoma aurantianum* females (white bars) and males (black bars) with increasing doses of ocimene. Means followed by different letters (uppercase letters for males and lowercase for females) are significantly different (*HSD Tukey*, $P < 0.05$).

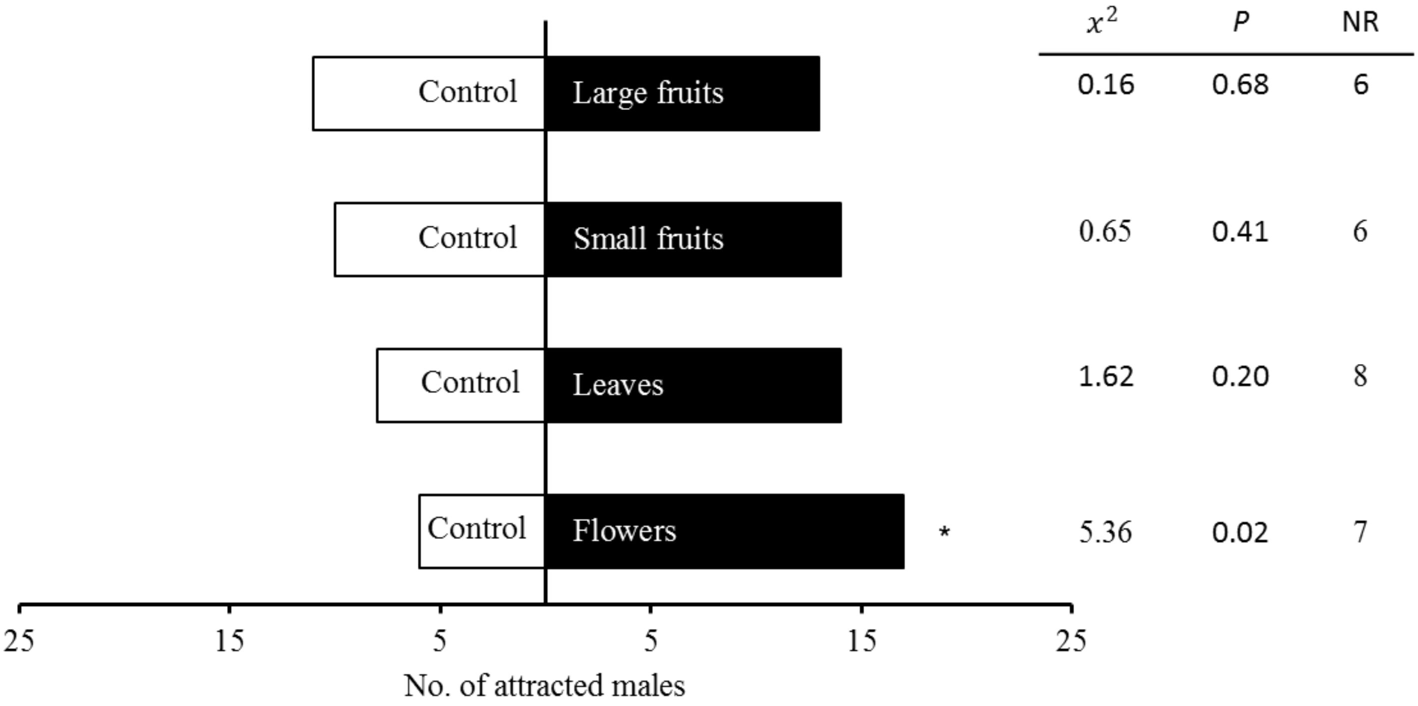
Fig. 8 Capture (means ± SE) of *Gymnandrosoma aurantianum* females (white bars) and males (black bars) in traps baited with ocimene (10 mg), pheromone (1 mg) and binary mixtures. The proportions used in the mixtures were 1mg ocimene/1mg pheromone (1:1), 5 mg ocimene/1 mg pheromone (5:1), 10 mg ocimene/1mg pheromone (10:1). Means followed by different letters (uppercase letters for males and lowercase for females) are significantly different (*HSD Tukey*, $P < 0.05$).

Fig. 1



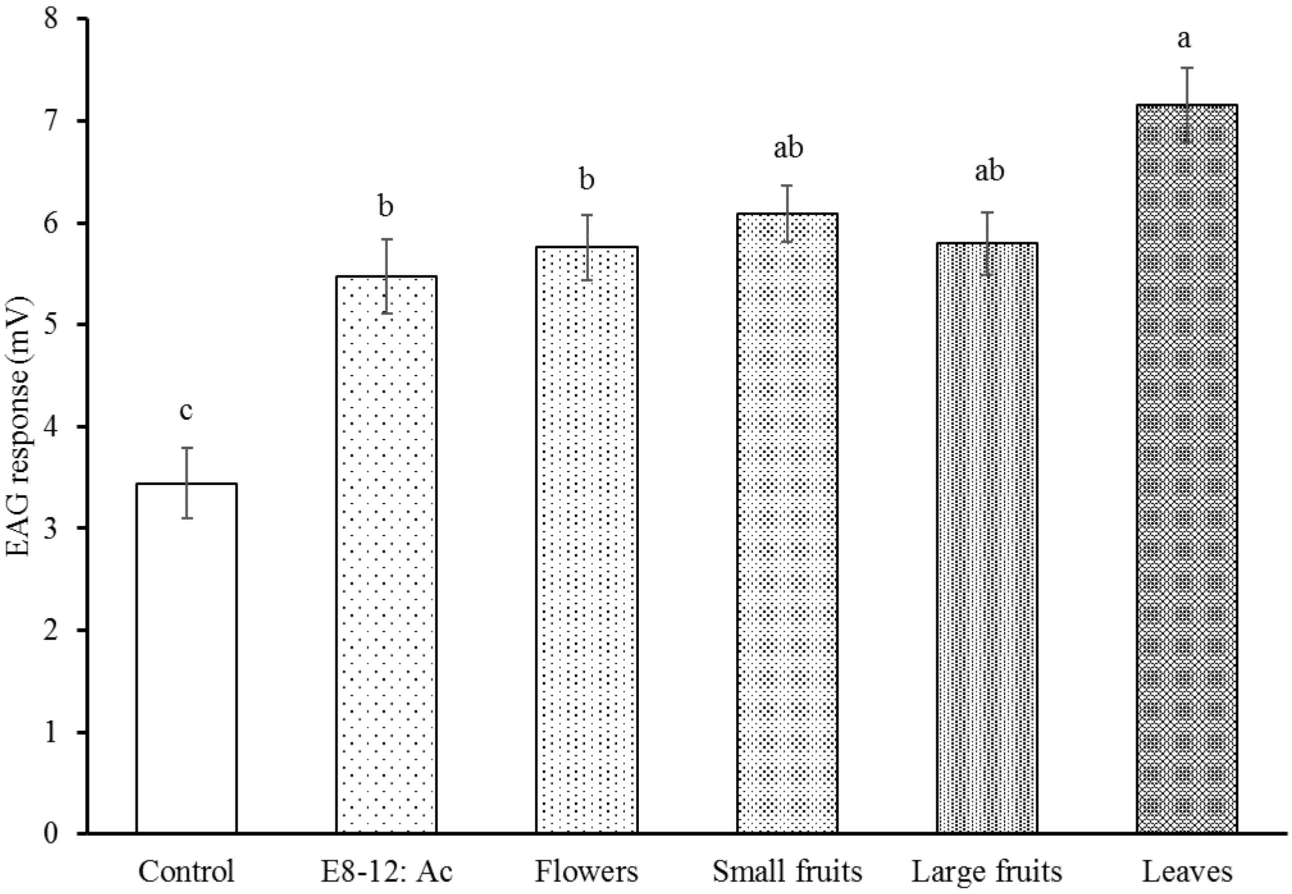
x^2	P	NR
0.16	0.68	6
1.07	0.30	7
4.20	0.04	6
3.89	0.04	9

Fig. 2



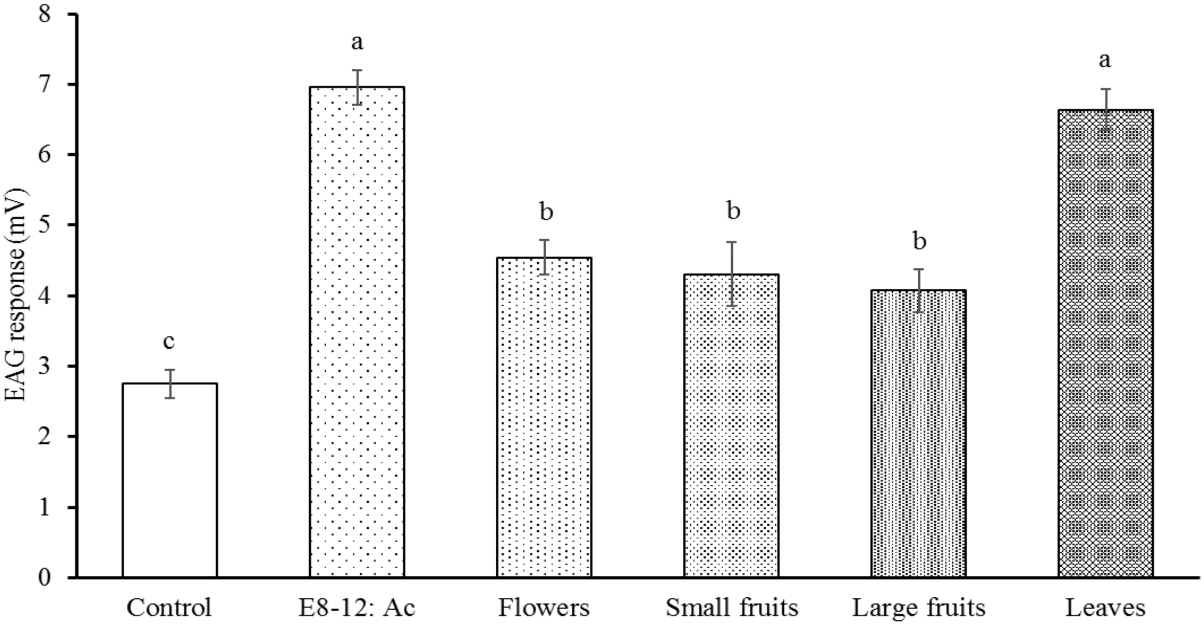
x^2
P
NR
Control
Large fruits
0.16
0.68
6
Control
Small fruits
0.65
0.41
6
Control
Leaves
1.62
0.20
8
Control
Flowers
*
5.36
0.02
7
25
15
5
5
15
25
No. of attracted males

Fig. 3



EAG response (mV)
0
1
2
3
4
5
6
7
8
c
b
b
ab
ab
a
Control
E8-12: Ac
Flowers
Small fruits
Large fruits
Leaves

Fig. 4



EAG response (mV)
0
1
2
3
4
5
6
7
8
c
a
b
b
b
a
Control
E8-12: Ac
Flowers
Small fruits
Large fruits
Leaves

Fig. 5

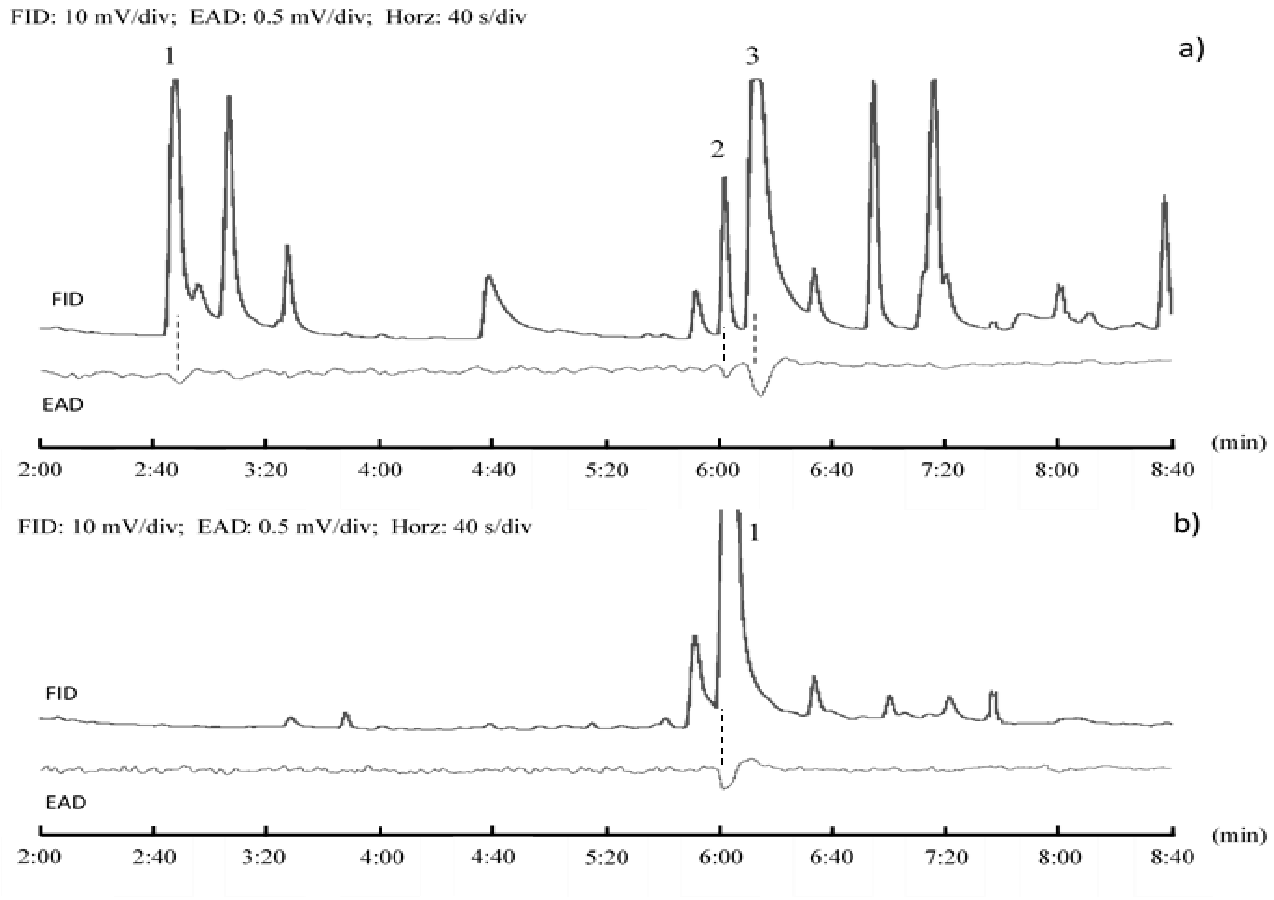

FID: 10 mV/div; EAD: 0.5 mV/div; Horz: 40 s/div
a)
1
2
3
FID
EAD
2:00
2:40
3:20
4:00
4:40
5:20
6:00
6:40
7:20
8:00
8:40
(min)
FID: 10 mV/div; EAD: 0.5 mV/div; Horz: 40 s/div
b)
1
FID
EAD
2:00
2:40
3:20
4:00
4:40
5:20
6:00
6:40
7:20
8:00
8:40
(min)

Fig. 6

FID: 10 mV/div; EAD: 0.5 mV/div; Horz: 40 s/div

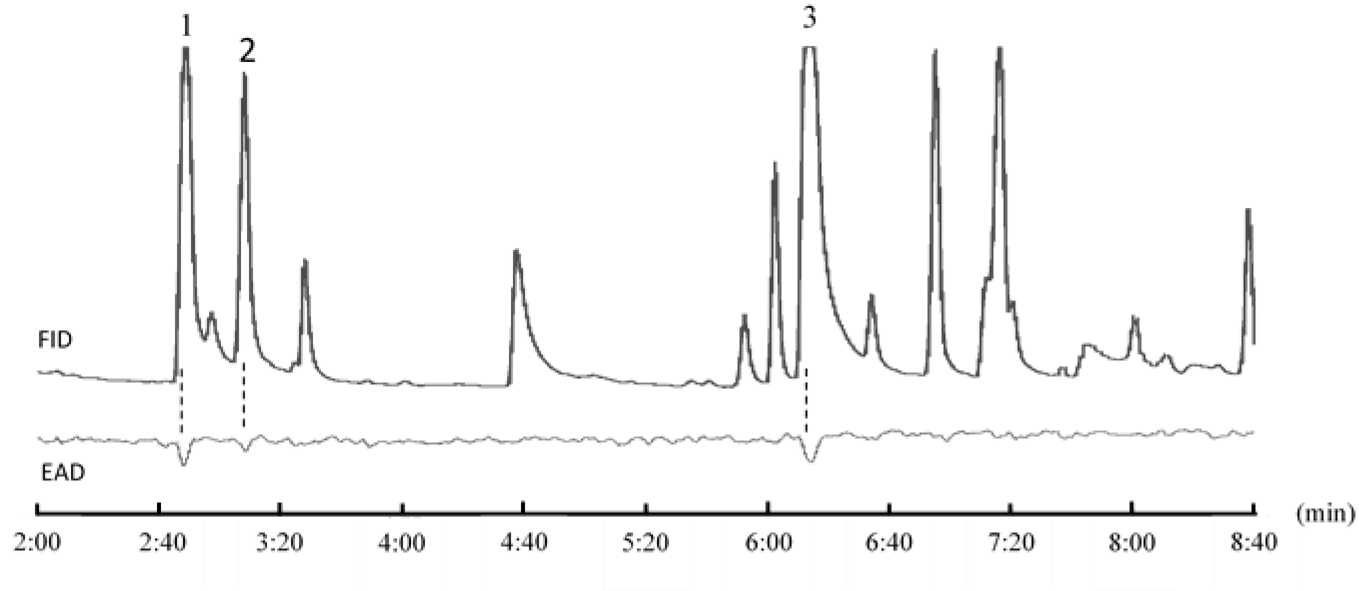


Fig. 7

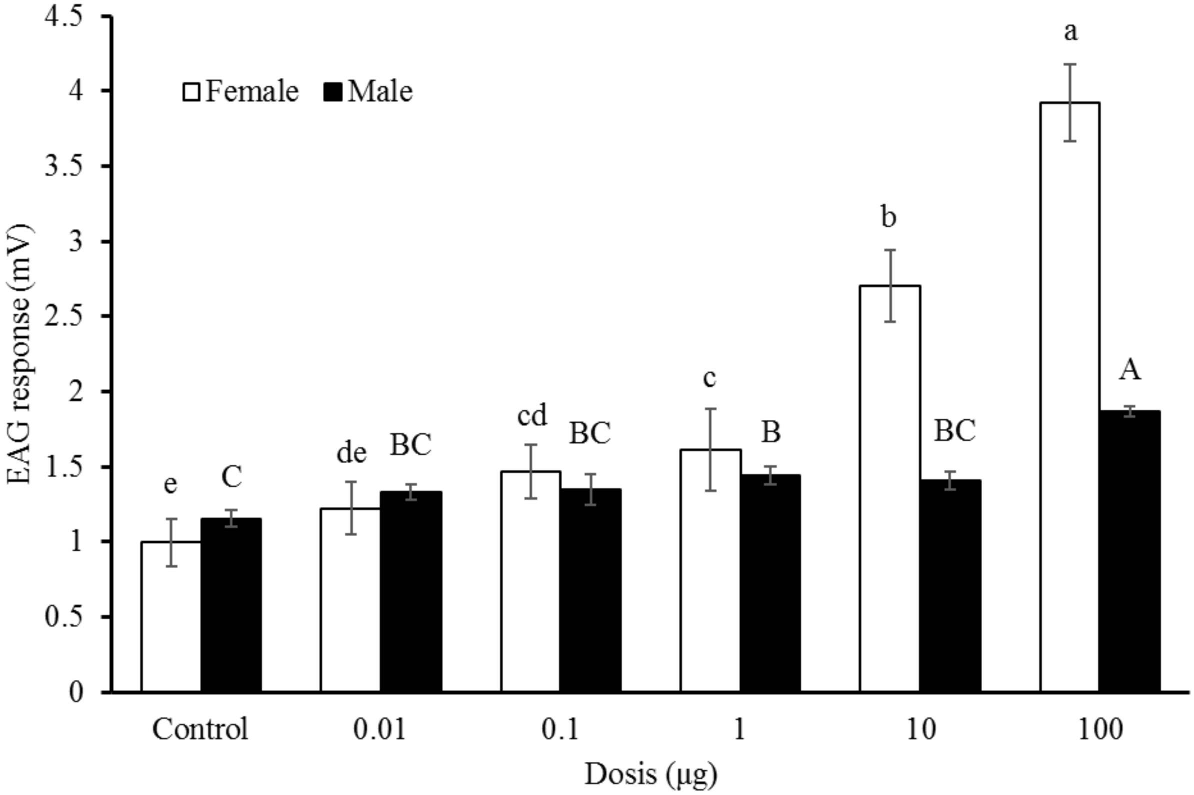
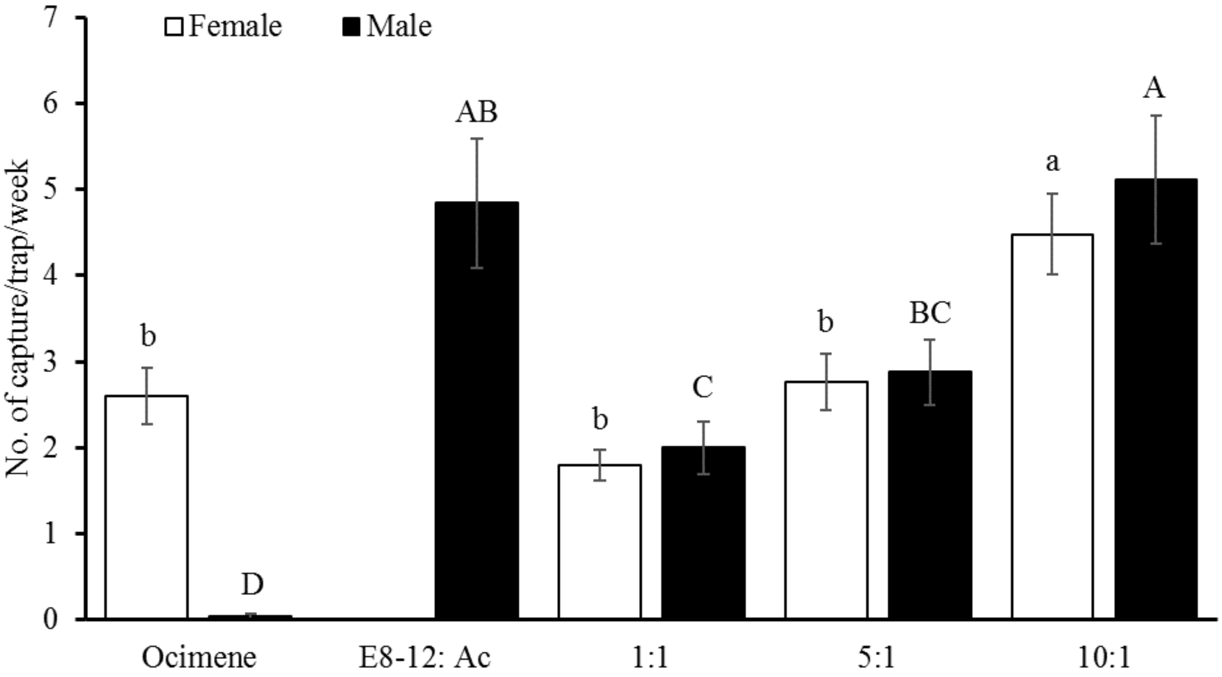

4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
EAG response (mV)
□Female ■Male
e
C
de
BC
cd
BC
c
B
b
BC
a
A
Control
0.01
0.1
1
10
100
Dosis (µg)

Fig. 8



No. of capture/trap/week
0
1
2
3
4
5
6
7
□Female
■Male
b
D
AB
b
C
b
BC
a
A
Ocimene
E8-12: Ac
1:1
5:1
10:1

CAPÍTULO III

CONCLUSIONES

En este trabajo se encontró que ambos sexos de *G. aurantianum* respondieron tanto comportamental como electrofisiológicamente a los volátiles de su planta hospedera (*M. integrifolia*).

En los bioensayos se observó que las hembras de *G. aurantianum* fueron atraídas a los volátiles de flores y hojas, mientras que los machos respondieron a los volátiles de las flores únicamente, ambos sexos no mostraron respuesta a los volátiles de frutos.

En las pruebas realizadas por Electroantenográfia (EAG), tanto machos como hembras, el extracto de hojas de macadamia presento la mayor respuesta (mV), en comparación con los demás tratamientos (flores y frutos), así mismo los machos respondieron significativamente a la feromona sexual.

En las pruebas por CG-EAD con extracto de flores, se encontraron tres compuestos antenalmente activos para machos, los cuales se identificaron como: (1*Z*)-3-metilbutanal oxima, (1*E*)-3-metilbutanal oxima y fenilacetaldehído. Los demás extractos no provocaron respuesta antenal. Por su parte, las hembras mostraron respuesta antenal al extracto de flores, cuyos compuestos se identificaron como: (1*Z*)-3-metilbutanal oxima, (*E*)-β-ocimeno, fenilacetaldehído, mientras que en el extracto de hojas se observó respuesta antenal al (*E*)-β-ocimeno.

En la evaluación de dosis respuesta por EAG con la mezcla de isómeros de ocimeno, se observó una relación positiva (directamente proporcional) en las respuesta antenales de las hembras con las dosis evaluadas, por lo que la dosis más alta (100 µg) presentó la mayor respuesta antenal. Mientras que, en machos las respuestas antenales más altas fueron provocadas con las dosis de 1 y 100 µg.

La mezcla de ocimeno con la feromona sexual, no tuvo efecto sinérgico en la atracción de machos, pero se registraron capturas de hembras en campo con este compuesto. El uso de la mezcla ocimeno más feromona sexual tiene la ventaja de capturar ambos sexos de *G. aurantianum* en cultivos de macadamia.

LITERATURA CITADA

Adamski D, Brown J. 2001 Systematic revision of the Ecdytolopha group of genera (Lepidoptera: Tortricidae: Grapholitini) in the New World. Entomol. Scand. Suppl. 58:1-86.

Blanco-Metzler H, Watt AD, Cosens D. 2013. The effect of quality of food source on macadamia nut damage by *Gymnandrosoma aurantianum* (Lepidoptera: Tortricidae). Agronomía Costarricense 37(2): 83-90.

Blanco-Metzler H. 1994. Biology and ecology of the macadamia nutborer *Ecdytolopha torticornis* in Costa Rica. [Tesis de Doctorado] University of Edinburgh, 158 p.

Carvalho DR. 2003. Comparação de métodos de monitoramento e controle do bicho-furão, *Ecdytolopha aurantiana* (Lima, 1927) (Lepidoptera: Tortricidae), em citros. [Tesis de Maestria] University of São Paulo, 38 pp.

Chamberlain D, Beevor P, Alan C, David R (2003) (E)-8-Dodecenyl acetate: Major component of the female sex pheromone of a Macadamia nut borer, Ecdytolopha torticornis. Entomol Exp Appl 4:1-3. https://doi.org/10.1046/j.1570-7458.2003.00035.x

Cunningham JP, West S, Zalucki M. 2013. Host selection in phytophagous insects: a new explanation for learning in adults. Oikos 95: 537-543.

Deng J, Wei H, Huang Y, Du J. 2004. Enhancement of attraction to sex pheromones of *Spodoptera exigua* by volatile compounds produced by host plants. J. Chem. Ecol. 30: 2037-2045 https://doi.org/10.1023/b:joec.0000045593.62422.73.

Fang Y, Zeng R, Lu S, Dai L, Wan X. 2018. The synergistic attractiveness effect of plant volatiles to sex pheromones in a moth. J. Asia. Pac. Entomol. 21: 380-387 https://doi.org/10.1016/j.aspen.2018.01.009.

Landolt P y Phillips T (1997) Host plant influences on sex pheromone behavior of phytophagous insects. Annu. Rev. Entomol. 42: 371-391 https://doi.org/10.1146/annurev.ento.42.1.371.

Leandro H. 2013. Identificación y estudio de la biología de *Gymnandrosoma aurantianum* Costa Lima Perforador del fruto sacha inchi (*Plukenetia volubilis* L.) en Tingo María. [Tesis de Licenciatura] Universidad Nacional Agraria de la Selva.

Light D, Robert A, Ronald G, Frank G, Richard E, Joseph C, Eric B. 1993. Host-plant green-leaf volatiles synergize the synthetic sex pheromones of the corn earworm and codling moth (Lepidoptera). Chemoecology: 4: 145-152 https://doi.org/10.1007/BF01256549.

Martin JP,Hildebrand JG. 2010. Innate recognition of pheromone and food odors in moths: a common mechanism in the antennal lobe? Front. Behav. Neurosci 4: 1-8.

Ochieng S, Park K, Baker T. 2002. Host plant volatiles synergize responses of sex pheromone-specific olfactory receptor neurons in male *Helicoverpa zea*. J. Comp. Physiol. A. 188: 325-333 https://doi.org/10.1007/s00359-002-0308-8.

Pest ID. 2018. Ad Hoc 309 Report - *Gymnandrosoma aurantianum*. United States Department of Agriculture, Animal and Plant Health Inspection Service. https://aqas.aphis.usda.gov/pestid.

Schmidt D, Von M, Guerin P. 2009. Host plant volatiles serve to increase the response of male European grape berry moths, *Eupoecilia ambiguella*, to their sex pheromone. J. Comp. Physiol. A. 195: 853-864 https://doi.org/10.1007/s00359-009-0464-1.

Trona F, Anfora G, Balkenius A, Bengtsson M, Tasin M, Knight A. 2013 Neural coding merges sex and habitat chemosensory signals in an insect herbivore. Proc. R. Soc. Lond. B. Biol. Sci. 280: 2-67 https://doi.org/10.1098/rspb.2013.0267.

Varela N, Avilla J, Anton S, Gemeno C. 2011 Synergism of pheromone and host plant volatile blends in the attraction of *Grapholita molesta* males. Entomol. Exp. Appl. 141: 114-122 https://doi.org/10.1111/j.1570-7458.2011.01171.x.

Xiang H, Chen Z, Li X, Guo Y, li X, Ma R. 2019 Two terpenoids activates close mating behavior and enhances trap efficiency of sex pheromone of *Grapholita molesta*. Journal of Asia-Pacific Entomology 22(4): 1109-1114 https://doi.org/10.1016/j.aspen.2019.08.003.