El Colegio de la Frontera Sur

Comparación de las respuestas de *Anastrepha ludens* y *Anastrepha obliqua* (Díptera: Tephritidae*)* al atrayente sintético "BioLure"

TESIS
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Contenido

I. Introducción

El género *Anastrepha* (Schiner) es considerado el grupo más diverso de especies de la familia Tephritidae en América (Hernández-Ortiz 2003). En México se reportan 37 especies de *Anastrepha,* de las cuales únicamente cuatro son consideradas de importancia económica: *Anastrepha ludens* (Loew) que ataca a cítricos (*Citrus* spp. L.) y mango *(Mangifera indica* L.); *A. obliqua* (Mcquart) que infesta mango y diversas especies de jobos o ciruelas (*Spondias* spp); *A. serpentina* (Wiedemann) que ataca a las sapotáceas como el mamey (*Calocarpum mammosum* L.) y chicozapote (*Manilkara zapota* L.), y *A. striata* (Schiner) principal plaga de la guayaba (*Psidium guajava* L.) (Hernández-Ortiz y Aluja 1993).

Los climas tropicales y subtropicales favorecen la existencia de una gran cantidad de especies vegetales que son hospederas de las moscas de la fruta. La naturaleza polífaga de estas plagas permite que tengan una amplia distribución en el territorio nacional, representando un alto riesgo para el sector frutícola. Esto ha generado el desarrollo de programas fitosanitarios para su control (IAEA 2003).

En 1992, el gobierno federal implementó el Programa Nacional contra Moscas de la Fruta, con el objetivo de controlar, y erradicar donde fuera factible, a estas cuatro especies de importancia económica (Reyes et al. 2000).

La tecnología de control está sustentada en un sistema de Manejo Integrado de Plagas (MIP) que comprende acciones de detección (trampeo de adultos y muestreo de frutos), de control mediante aspersiones de cebo tóxico, saneamiento a través de labores culturales, liberación de enemigos naturales y de moscas estériles (Montoya et al. 2010). La aplicación coordinada de estas actividades está encaminada a lograr el establecimiento de zonas libres y de baja

prevalencia de la plaga, que permita a los fruticultores producir fruta de óptima calidad fitosanitaria para el mercado nacional y de exportación (Gutiérrez 2010).

Como parte de las acciones de protección, se han establecido redes de trampeo, labor importante en las actividades de campo que permite conocer la presencia o ausencia de la plaga, sus densidades relativas y su distribución (Montoya et al. 2010). El conocimiento de la densidad de las poblaciones y la distribución de las plantas hospederas en el campo, son factores determinantes para establecer las estrategias de manejo.

Cuando se establece un programa de monitoreo, se debe considerar que habrá una fuerte interacción con una gran variedad de estímulos presentes en el ambiente, por lo que los atrayentes utilizados deberán competir exitosamente contra dichos estímulos, de manera que los índices de captura puedan referir a la poblaciones existentes de manera confiable (Montoya et al. 2002).

Los insectos frugívoros como las especies del género *Anastrepha* necesitan de la ingesta de proteína para promover el desarrollo ovárico y la ovogénesis en las hembras (Aluja 1994, Aluja et al. 2001). La identificación de fuentes de nutrientes ha permitido el desarrollo de atrayentes (Díaz-Fleischer y Castrejón 2012).

Los primeros sistemas de trampeo de moscas de la fruta se basaron en cebos hechos de proteína y azúcar fermentada (Gurney 1925 en Díaz-Fleischer y Castrejón 2012). Para las especies de *Anastrepha*, el principal atrayente utilizado ha sido un fagoestimulante, la proteína hidrolizada (Heath and Epsky 1993), aunque en los últimos años han surgido los atrayentes alimenticios sintéticos (Epsky et al. 1995, Heath et al. 1995). Estos ofrecen ventajas como una concentración definida de sus compuestos y una tasa de liberación controlada y de larga duración en campo (Robacker y Czokajlo 2005). El uso de acetato de

amonio más putrescina en forma sintética ofrece una opción para capturar adultos en comparación con las proteínas hidrolizadas en estado liquidas o "pellets" de levadura de torula (*Candida utilis*) que se utilizan tradicionalmente. Se ha comprobado que trampas cebadas con acetato de amonio y putrescina permiten monitorear eficientemente adultos de *A. ludens* (Heath et al. 2004). El primer representante en el mercado de este tipo de atrayentes lo constituye el BioLure® (Suterra LLC, Inc., Bend, OR) (Heath et al. 1995, 1997), el cual está enfocado principalmente hacia la detección y monitoreo de hembras de tefrítidos en varios países (Epsky et al. 1998). Estudios recientes indican que no hay diferencia significativa en las capturas de *A. ludens* y de *A. obliqua,* utilizando el atrayente BioLure, así como la predominancia de hembras en las capturas (Arredondo et al. 2014).

En la región del Soconusco, Chiapas se ha observado que las trampas cebadas con proteína hidrolizada, registran mayores capturas de *A. obliqua* son mayores que las de *A. ludens* (Aluja et al. 1996). Así mismo los programas de trampeo implementados en la región del Soconusco para el monitoreo de moscas de la fruta, utilizando el BioLure como atrayente, han detectado consistentemente que las capturas de *A. obliqua* son mayores que las capturas de *A. ludens* (P. L. datos no publicados, ver anexo). Por otro lado, en las empacadoras de mango se ha registrado que *A.ludens* es la especie que más invade el mango Ataulfo (>90%), mientras que la infestación por *A. obliqua* es rara (CESAVE, Chiapas, ver anexo). ¿Cómo puede explicarse esta aparente contradicción de que *A. obliqua,* la especie que más se captura, no es la que más infesta el mango Ataulfo? ¿Será que su mayor captura obedece a que es más atraída por el cebo de las trampas que *A. ludens*?

Por lo anterior, el objetivo del presente estudio fue comparar las respuestas de *A. ludens* y *A. obliqua* al atrayente sintético BioLure. Para ello se utilizaron tres enfoques metodológicos:

1) Evaluación de la respuesta electrofisiológica a diferentes dosis de los volátiles del BioLure, utilizando la técnica de electroantenografía (EAG); 2) Pruebas comportamentales utilizando trampas MultiLure (Better World MFG Inc., Fresno, CA) cebadas con BioLure en condiciones de jaulas de campo; y 3) Pruebas de liberación y recaptura de moscas estériles en condiciones naturales de campo.

A continuación se anexa el manuscrito que fue sometido a publicación al Journal of Economic Entomology en donde se discuten los resultados obtenidos.

II.

Comparación de las respuestas de *Anastrepha ludens* y *Anastrepha obliqua* (Díptera: Tephritidae) al atrayente sintético "BioLure"

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Comparative responses of *Anastrepha ludens* and *Anastrepha obliqua* (Diptera: Tephritidae) to the synthetic attractant BioLure*

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Abstract. The responses of wild and sterile *Anastrepha ludens* (Loew) and *Anastrepha obliqua* (Mcquart) fruit flies to the synthetic attractant BioLure were determined by electroantennography (EAG), in field cage tests using MultiLure traps, and by release-recapture field experiments using sterile flies. In test EAG bioassays, responses from *A. obliqua* females to BioLure were higher than to those from *A. ludens* females. Non-significant differences were observed between males of both species, and no significant difference was found between wild and sterile flies. In field cage tests, the number of flies captured was influenced by species, sex and age. More *A. ludens* than *A. obliqua* individuals were captured. In *A. ludens,* there was not significant difference between the number of females and males captured, whereas in *A. obliqua* more females than males were caught. Age showed a bimodal response in both species and both sexes, with peaks at 4 and 14 d old. In the release-recapture experiments, there were significant differences between species, sexes, and orchards and among the days after release. More individuals of *A. ludens* than *A. obliqua* were recaptured. Only in *A. obliqua* the difference between the sexes was significant, with a 3.60:1 female: male ratio. Orchard conditions affected the recapture rate, but in both orchards the largest number of flies recaptured occurred during the first day after release (46 and 88% in each orchard). Our results show that the response to this synthetic lure is species-specific and contribute to better interpret trapping data.

Key words: Mexican fruit fly, West Indies fruit fly, food attractant, electroantennography, trapping, release-recapture.

53 The most important fruit fly pests in Mexico are *Anastrepha ludens* (Loew) that
54 infests some citrus species (*Citrus* spp. L) and mango *(Mangifera indica* L.), and *A. obliqua*
55 (Mcquart) that attacks mango and tropical plums (*Spondias* spp.), mainly (Hernández-Ortiz
56 and Aluja 1993). In 1992, the Fruit Fly National Program was implemented to control, and
57 eradicate where feasible, the species of economic importance (Reyes et al. 2000).
58 With an area-wide integrated pest management approach, this program considers the
59 application of monitoring and control actions, including trapping nets, bait spraying, and
60 release of parasitoids and sterile flies (Montoya et al. 2010). Its aim was to establish free and
61 low pest prevalence areas, which allow growers to produce quality fruit for the domestic and
62 export markets (Gutiérrez 2010).
63 Trapping nets serve to determine the presence of the pest, its relative density and
64 distribution. The lures used for this purpose most compete with host plants odors and other
65 environmental stimuli to produce reliable capture figures that can be related to population
66 densities (Montoya et al. 2010).
67 *Anastrepha* fruit flies need to ingest proteins to fully develop their ovaries and
68 produce eggs (Aluja 1994, Aluja et al. 2001). The identification of food sources and their
69 volatiles has permitted the development of lures (Díaz-Fleischer and Castrejón 2012). The
70 most common attractant used for *Anastrepha* flies has been the hydrolyzed protein (Heath
71 and Epsky 1993). Efforts to develop a synthetic food attractant and an adequate trap design
72 were undertaken (Epsky et al. 1995, Heath et al. 1995). The first food based synthetic
73 attractant in the market was BioLure® (Suterra LLC, Inc., Bend, OR), which is a combination
74 of ammonium acetate and putrescine (Epsky et al. 1998). Its formulation allows a defined
75 concentration and a long lasting controlled release rate (Robacker and Czokajlo 2005).

Currently, as part of an area-wide integrated fruit fly management program for mango production in the Soconusco region in Chiapas Mexico, BioLure baited traps are used to detect wild flies. It has been found consistently, that wild *A. obliqua* are captured in higher numbers than *A. ludens* (PL unpublished data, Supp. Table S1). However, when mango fruits of the Ataulfo cultivar, the most common variety, come into the packing houses and are sampled, the most frequent species found has been *A. ludens* (>90% according to CESAVE, Chiapas, Supp. Table S2). How this contradictory information between numbers of flies captured in traps, and fruit infestation can be explained? Could be because a greater response of *A. obliqua* to BioLure than *A. ludens*?

Our aim in this study was to compare the response of *A. ludens* and *A. obliqua* to BioLure. Three methodological approaches were used: 1) electroantennogram responses (EAG) of both species to different doses of volatiles from BioLure; 2) behavioral tests using Multilure traps (Better World MFG Inc., Fresno, CA) baited with BioLure under field cage conditions; and 3) sterile flies release-recapture experiments under natural field conditions.

Materials and Methods

Study Insects. Wild and mass-reared sterile *A. ludens* and *A. obliqua* flies were used. Sterile flies were obtained from the MOSCAFRUT (SAGARPA-IICA) mass-rearing facility located in Metapa, Chiapas, Mexico. Flies were reared following the standard procedures described by Domínguez et al. (2010). Wild *A. ludens* flies were obtained as larvae from infested sour oranges (*Citrus aurantium* L.*)* collected in Tuxtla Chico, Chiapas, Mexico (14º 56" N, 92º 10" W). Wild *A. obliqua* flies were obtained as larvae from infested creole mangos (*Mangifera indica* L.) collected in Pumpuapa, Tapachula, Chiapas, Mexico (14° 55´50.35" N, 92°21´39.27" W). When larvae were mature, approximately one week after field

collection, were removed from the fruits and placed in containers with moistened vermiculite to promote pupation. Pupae were monitored daily until adult emergence. One hundred adult females and 100 males of each species and condition were placed in 30 X 30 X 30 cm glass cages and maintained under laboratory conditions. For the field cage and release and recapture experiments, 10,000 to 15,000 sterile flies were placed in four 70 X 50 X 70 cm metal frame cages covered with mesh fabric. Since adult emergence, sugar and water were provided *ad libitum* to all flies before the experiments. No source of protein was provided. Laboratory conditions were 25±2 °C temperature, 65±5% relative humidity and a 12:12 h L:D photoperiod.

EAG Assays. The antennal responses of wild and sterile *A. ludens* and *A. obliqua* flies to volatiles of BioLure were determined by EAG technique. We used virgin males and females that were sorted out just after emergence and maintained as described in the previous section. The technique consisted in cut off carefully the head of flies with dissection scissors, and inserted into its base with a glass capillary filled with physiological saline solution (Malo et al. 2004) to constitute the reference electrode. The distal end of the antenna was inserted into the tip of the recording glass capillary electrode. The signals generated by the antenna were passed through a high-impedance amplifier (NL 1200, Syntech, Hilversum, The Netherlands) and displayed on a monitor using Syntech version 2.6 software for processing signals. A current of humidified pure air (0.7 l/min) was constantly directed onto the antenna through a 10-mm-diameter glass tube by using a stimulus flow controller (CS-05, Syntech). The stimuli to test was prepared 24 h previously by placing two patches of BioLure inside a 200 ml Erlenmeyer clean-oven matrass, which was then stoppered with adherent plastic in a laboratory room with temperature of 26 ± 2°C to collect the volatiles. The volatiles were

123 collected using a new plastic no-reusable syringe (Terumo Medical Corporation, Japan). The
124 doses tested were 1, 2, 3, 4 and 5 ml, which were injected directly to the clean air flux to the
125 insect´s antennae. After used once the syringes were discarded. The duration of the stimulus
126 was 1 s. The continuous flow of clean air through the air flow tube and over the preparation
127 ensured that odors were removed immediately from the vicinity. The EAG value when the
128 antennae was stimulated correspond to the EAG amplitude peak and they were expressed in
129 mV. Each replicate consisted of one fly antenna. Six females and six males of 13-18 d old
130 wild *A. ludens* and the same number of sterile *A. ludens* but 7-12 d old were tested. For wild
131 *A. obliqua,* six males and six females of 10-15 d old and the same number of sterile flies of
132 8-13 d old, were tested. We used clean air as control. The EAG tests were performed between
133 8:00-14:00 h, at 24 ± 2°C temperature and 65 ± 5% relative humidity.
134
135 **Field Cage Experiment**. Field cage tests were carried out at a mango orchard during the
136 fruiting season (July of 2015). Four 3 m in diameter by 2 m height field cages were used.
137 Distance between cages was 7 to 10 m. In the center of each cage a small potted mango tree
138 without fruit was placed. At the top central part of each cage, a Multiure trap was hanged.
139 This trap was baited with BioLure, and 200 ml of propylene-glycol (IMC, Chemicals Corp.,
140 Perrysburg, USA) were added to retain the flies entering the trap. The BioLure membranes
141 were not replaced during the 2 wk of the experiment. Trap were setup between 0830 and
142 0900 hours and 15 min later, 50 sterile males and 50 sterile females of each species were
143 released in each cage. After 24 h, the flies captured in the traps were placed in vials with 50
144 ml alcohol at 70% and taken to the laboratory for species and sex identification and recording.
145 Those flies that were not captured were removed out from the cage. To assess the effect of
146 age, flies were tested from 1 to 10 d, 12 and 14 old. For each age, four replicates were done.

147 **Release and Recapture Experiment**. Four releases were made in two different Ataulfo
148 mango orchards. The first two releases were done during March and May, which was fruiting
149 season, in the orchard "El Refugio" (14°55´29.8"N 92°16´48.7"W), at an altitude of 231 m
150 above sea level. Recorded temperatures ranged from 22 to 30 °C. The third and fourth
151 releases were done in August, out of fruiting season, in the orchard "El Paraiso" (14°46´49.7"
152 N 92°22´36.2" W), at an altitude of 23 m above sea level. Recorded temperatures ranged
153 from 24 to 37 °C. Fifty Multilure traps baited with BioLure were placed in concentric circles
154 (Fig. 1). In each trap, 200 ml of propylene glycol plus water solution were included to retain
155 the attracted flies. One day before traps setup, 30,000 sterile flies (males and females) of each
156 species were released. Flies were 6 d old and were marked as pupae with fluorescent dye
157 (Aurora Pink, Day-Glo Color Corp., Cleveland, OH,) to distinguish them from wild flies.
158 Traps were hanged at about 3 m height and distance between trees ranged from 15 to 60 m.
159 Traps were inspected daily for 7 consecutive days. Captured flies were placed in vials with
160 50 ml of alcohol at 70%. This distinction of sterile from wild flies was based on the color
161 marking observed at an epifluorescent microscope (Carl Zeiss model SMZ1500, Germany).
162 Species identification was based on thorax and wings taxonomic characters (López et al.
163 2010).

164 **Statistical analysis**. EAG data (mV) were analyzed by a mixed model where antennae
165 response was the random effects and the different doses of the attractant were the fixed
166 effects. Comparison of group means was carried out by orthogonal contrasts. The data from
167 the field cage experiment were analyzed by a Generalized Lineal Model (GLM) of repeated
168 means with binomial response, with three factors: species, sex and age. Each field cage was
169 considered as a replicate. The data from the release-recapture experiment were adjusted to a

GLM with binomial negative response. Four factors were considered: species, sex, orchards and days after release. Each of the four releases was considered as a replicate. Multiple comparisons were made using Tukey test. When $P<0.05$ the difference was considered significant. All analyses were made using R software version 3.2.1 (R Development Core Team 2014).

Results

EAG Assays. The mean (± SE) responses of wild and sterile *A. obliqua* females to control (air) was 0.59 ± 0.03 mV, and 0.53 ± 0.07 mV, respectively. In *A. obliqua* males it was 0.48 ± 0.07 mV for wild, and 0.55 ± 0.06 mV for sterile flies. In *A. ludens,* it was 0.35 ± 0.06 mV, and 0.34 ± 0.07 mV for wild and sterile females, respectively, and 0.40 ± 0.07 mV and 0.48 ± 0.08 mV for wild and sterile males, respectively. The antennal response to BioLure was significantly affected by dose, dose X condition interaction, species X sex, and dose X species X condition interactions (Table 1). However, the antennal response was not affected by species, condition (sterile or wild) or sex (Table 1). The difference between the dose and the random effects suggested the use of orthogonal contrasts (Supp. Table S3). These kind of analyses showed marginal or no significant differences between females and males of *A. ludens* ($t = 1.36$; df = 42; $P = 0.07$), both wild and sterile. Neither between *A. obliqua* males and females ($t = 1.4$; df = 42; $P = 0.16$) on both conditions. However, the comparison between females of both species, wild and sterile (Fig. 2A), showed significant differences ($t = 2.75$, df = 42; $P= 0.008$), a higher response biased towards the sterile condition. In males, differences between species were not significant ($t = 0.48$; df = 42; $P = 0.62$) (Fig. 2B).

192 **Field Cage Experiment**. The effect of the three factors considered: species, sex and age
193 were highly significant, as were the age X species, age X sex, species X sex and age X species
194 X sex interactions (Table 2). *A. ludens* was the species with the greatest number of individuals
195 captured in this experiment. Out of 2,143 flies, 1,108 were females and 1,035 were males
196 (1.07:1 female: male ratio). For *A. obliqua,* out of 1,679 flies, 1,007 were females and only
197 672 were males (1.50:1 female: male ratio).

198 The difference between sexes in *A. ludens* was not significant, whereas in *A. obliqua*
199 significantly more females than males were captured. Age showed a bimodal effect on both
200 species and both sexes (Supp. Table S4). Peak captures were observed when flies were 4 and
201 14 d old, and the minimum capture occurred when flies were 9 d old (Fig. 3).
202
203 **Release and Recapture Experiment**. In the first two trials in orchard "El Refugio" 4,607
204 flies of both species were recaptured, which represented 3.83% of the flies released. In the
205 last two trials at "El Paraiso" orchard, 5,178 flies were recaptured of both species,
206 representing 4.31% of the flies released.

207 We found significant differences between species, sexes, orchards and among the
208 days after release (Table 3). The average (± SE) total catch in the four releases for *A. ludens*
209 was 120.25 ± 42.11, while for *A. obliqua* it was 54.48 ± 20.83 flies. The species X sex
210 interaction was significant in *A. obliqua,* with a 3.60:1 female: male ratio. In *A. ludens* no
211 significant difference was observed between the number of females and males recaptured
212 (Fig. 4), with a 0.97:1 female: male ratio (Supp. Table S5).

213 In both orchards, a high fraction of the flies recaptured occurred during the first days
214 after release (Fig. 5). In the orchard "El Refugio", 46 and 26% of captures occurred in the

first and second day after release, respectively. In "El Paraiso" orchard, 88% of the flies recaptured occurred in the first day after release and 8% in the second one.

Discussion

Our results show that the response to the synthetic attractant BioLure is species specific. The differences between the two species were observed in the EAG test, in the capture of flies in Multilure traps in field cages, and in the release-recapture experiment. Differences in the antennal responses of both sexes were significant in the case of *A. obliqua*, but not in *A. ludens.* The response to the attractant was also affected by the age of the flies. In the EAG assays, *A. obliqua* females showed the greatest response to BioLure in comparison to *A. ludens* females. No species differences were observed in males.

Similar studies have been carried out previously with other species of *Anastrepha* (Kendra et al. 2005, 2009, Epsky et al. 2006, Jenkins et al. 2012), these authors evaluated the antennal responses of *Anastrepha suspensa* (Loew) and *A. obliqua* to ammonium bicarbonate (instead of ammonium acetate) and putrescine. The general pattern in all these studies confirmed that *A. obliqua* showed greater responses than *A. suspensa*, as we found when we compared the responses of the former species with *A. ludens*. Regarding sexual, Kendra et al. (2009) found a lower response by *A. suspensa* males to putrescine and greater response to ammonium bicarbonate than in females. Similarly, Jenkins et al. (2012) found that *A. obliqua* females showed higher responses than males, as we found in this study. In *A. suspensa,* these authors found no sexual differences, similar to our results with *A. ludens*. It should be noted that the difference between these previous studies and our study was that we used both sterile

237 (irradiated) and fertile (non-irradiated) flies, while the other studies used only fertile flies.
238 However, we found no differences between EAG responses from sterile and fertile flies.

239 In the field cage tests, fly captures were significantly affected by species, sex and age.
240 The pattern of age-specific response for both species and both sexes were similar. Captures
241 increased rapidly during the first days, with a peak when flies were 4 d old. Then, the number
242 of insects captured decreased with minimum catch when flies were 9 d old, and increased
243 again until flies were 14 d old, when the test ended. This bimodal pattern could be partly
244 explained by the dynamics on the accumulation and use of reserves. Nestel et al. (2005)
245 observed a cyclic pattern in lipids and protein reserves in adult *Ceratitis capitata*
246 (Wiedemann), particularly during the first days of adult life.

247 Regarding differences between the sexes, the greater response of females in *A.*
248 *obliqua* compared to males was expected. However, the no difference between females and
249 males in *A. ludens* was unexpected. Generally, it has been known that females have a greater
250 need to ingest protein sources than males because of egg production (Aluja et al. 2001).
251 Therefore, they are more attracted to volatiles associated to proteins (Epsky et al. 1998).
252 However, recent studies have shown the important effect of protein consumption in males
253 mating and reproductive success (Liedo et al. 2013, Pereira et al. 2013), which could explain
254 the high response of *A. ludens* males to BioLure.

255 The greater capture of *A. ludens* flies was not associated to a greater EAG response.
256 The lack of concordance between electrophysiological and behavioral tests have been
257 documented in a number of studies. For instance, Epsky et al. (2006) found that *A. suspensa*
258 females showed a greater antennal response to a Nulure/borax solution (Miller Chemical and
259 Fertilizer Corp., Hanover, PA) than to a TYB solution (ERA Intl., Baldwin, NY). However,

in wind tunnel essays, more flies responded to the TYB solution than to the Nulure/borax solution. Kendra et al. (2005) found that 1 to 5 d old (sexually immature) *A. suspensa* females showed more antennal sensitivity to an ammonium compound than 9 to 14 d old (sexually mature) females, but in wind tunnel essays immature flies showed a lower response than sexually mature females. Also, our results contrast to those obtained by Díaz-Fleischer et al. (2009) in field cage tests. These authors found that *A. obliqua* was more attracted to BioLure baited traps than *A. ludens*. The disagreement between both studies could be attributed to that both studies used different batch of attractants, insects' physiological state and the diet used to feed the insects before the test (Arredondo et al. 2014).

Our results from the release-recapture experiment showed that both sexes of *A. ludens* were similarly attracted to BioLure baited traps, whereas *A. obliqua* females were more attracted compared to the conspecific males. Several studies have shown that fruit fly females than males are more attracted to food based lures than males (Arredondo et al. 2014, Dutra et al. 2009, Díaz-Fleischer et al. 2009, Lasa et al. 2013, Martínez et al. 2007, Piñero et al. 2002), which agree with our results with *A. obliqua*. However, in the case of *A. ludens* no significant difference was observed between females and males. Robacker and Thomas (2007), working with feral *A. ludens* in Texas and Northeastern Mexico, reported no sexual differences in the captures. Also, in Guatemala, Heath et al. (2004), testing combinations of three compounds (ammonium acetate, putrescine, and trimethylamine), found no difference in the number of females and males of *A. ludens* captured. In the case of *A. suspensa*, Epsky et al. (2006) found no significant difference between the number of females and males captured by traps baited with ammonium acetate and putrescine. In another study in Hawaii, using traps baited with ammonium acetate, putrescine and trimethylamine to attract *C. capitata*, no differences were found between sexes, although the number of females

284 decreased when putrescine was absent (Leblanc et al. 2010). Considering previous reported
285 results and our findings, we can see that there is consistency in the species-specific sex ratio
286 response. Whereas in *A. obliqua* more females than males respond to BioLure, in other
287 species such as *A. ludens* and *A. supensa* there is not difference between the sexes. The
288 reasons for these species differences are unknown and deserve further research.

289 Regarding the comparison between species, Arredondo et al. (2014) found no
290 significant difference between the number of *A. ludens* and *A. obliqua* flies captured in
291 BioLure baited traps. This disagreement with our results could be explained by variations in
292 environmental conditions (see below) interacting with the release density and the presence
293 of other stimuli competing in the attraction of flies.

294 There were significant differences in the number of flies captured of each species in
295 the two orchards. However, the patterns were similar, in both orchards, more *A. ludens* than
296 *A. obliqua* flies were captured, more females than males were recorded for *A. obliqua*, but
297 not for *A. ludens*, and most of the captures occurred during the first two days after release.
298 Release–recapture tests in guava (*Psidium guajava* L.) orchards with wild and sterile *A.*
299 *suspensa* females, resulted with the largest fraction captured the first day after release
300 (Kendra et al. 2010). The difference between orchards in the number of flies captured could
301 be attributed to the presence of host fruits and abiotic conditions (Aluja 1993). When the
302 tests were done in "El Paraíso" orchard, it was out of the fruiting season, temperatures were
303 higher, relative humidity and the availability of water was lower than in "El Refugio"
304 orchard. These conditions could make the traps more attractive because there were no other
305 competing stimuli, but flies survival could be lower. Robacker and Czokajlo (2005) pointed
306 out that when temperatures are high and water availability is scarce, flies are more attracted
307 to traps baited with liquid solutions. In "El Refugio" orchard, temperatures were lower and

308 there was more water available than in “El Paraiso” orchard because a stream pass near the
309 orchard. This probably reduced fruit flies stress and favored their survival, but the
310 availability of fruits, both those in the trees and those in decomposition on the soil were
311 stimuli competing with traps attractiveness. Host fruit availability is an important factor in
312 determining wild fruit fly populations (Aluja and Prokopy 1992, Ledesma et al. 2013,
313 Rodriguez et al. 2015), and therefore it affects trapping effectiveness. Here, by using a known
314 populations of sterile flies, we were able to assess the effect of this competing stimuli in
315 trapping.

316 In conclusion, *A. obliqua* females showed higher responses in EAG in comparison to
317 conespecific males and both sexes of *A. ludens*. We found no difference in the response of
318 wild and mass-reared sterile flies, suggesting that sterile flies could serve as model organisms
319 for this type of physiological tests. There were differences in the responses of *A. ludens* and
320 *A. obliqua* to Biolure, founding that *A. ludens* flies were captured in greater numbers in
321 comparison to *A. obliqua*. Thus, the greater number of *A. obliqua* flies captured in the
322 trapping net of the area-wide management program could not be explained as a greater
323 response of this species to BioLure. This species-specific difference was also apparent in the
324 sex ratio, where *A. obliqua* females showed a greater response than conspecific males,
325 whereas no sexual differences were observed in *A. ludens*. Fly age and environmental
326 conditions affected the response to BioLure. This knowledge contributes to better interpret
327 field data obtained from trapping nets.

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480 **Table 1.** ANOVA results from the mixed model comparing the EAG response from wild
481 and sterile condition *A. ludens* and *A. obliqua* flies according to different doses of the
482 BioLure volatiles.

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	numdf	Dendf	*F*	*P*
Dose	4	656	151.79	**<.0001**
Species	1	42	2.57	0.11
Condition	1	42	1.96	0.16
Sex	1	42	0.09	0.76
Dose X Species	4	656	1.60	0.17
Dose X Condition	4	656	5.47	**0.0002**
Species X Condition	1	42	0.02	0.86
Species X Sex	1	42	5.26	**0.0268**
Dose X Species X Condition	4	652	3.76	**0.0049**

484 Values in bold are significant.

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Table 2. Results from the Generalized Lineal Model of repeated means with bimodal response and three factors (species, sex and age) using data obtained from trap captures of *A. ludens* y *A. obliqua* in field cages.

	χ^2	df	*P*
Age	579.985	11	**< 2.2e-16**
Species	98.325	1	**< 2.2e-16**
Sex	78.393	1	**< 2.2e-16**
Age X Species	78.228	11	**3.244e-12**
Age X Sex	70.769	11	**8.725e-11**
Species X Sex	42.019	1	**9.042e-11**
Age X Species X Sex	26.079	11	**0.006316**

Values in bold are significant.

515 **Table 3.** Results from release–recapture experiments with four factors analyzed: species,
516 sex, orchard and days after release (the latest was adjusted by means of a polynomial of
517 second order).
518

	χ^2	Df	*P*
Days (Day 2)	327.85	2	**< 2.2e-16**
Orchard	63.67	1	**1.470e-15**
Sex	16.43	1	**5.057e-05**
Species	23.90	1	**1.017e-06**
Days (Day 2) X Orchard	37.17	2	**8.495e-09**
Sex X Species	15.63	1	**7.704e-05**

519 Values in bold are significant.
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Figure legends

Figure 1. Maps showing the location of traps in the orchards used for the release–recapture experiment.

Figure 2. Mean (+SE) of the antennal responses of females (A) and males (B) *A. ludens* and *A. obliqua* Wild (W) and sterile (S) flies to different doses of BioLure volatiles. Females showed significant difference. Different letters indicate significant difference.

Figure 3. Probability of recapture according to the estimated recapture model with respect to age under field cage conditions.

Figure 4. Flies captured (±SE) of the number of sterile females and males of *A. ludens* and *A. obliqua* recaptured in Multilure traps baited with BioLure in the release-recapture experiment (differences were significant, Tukey test P=1^e-05).

Figure 5. Mean number of sterile females and males *A. ludens* and *A. obliqua* captured per day after release in the release–recapture experiment at the orchards "El Refugio" (A) and "El Paraíso" (B).

554 **Figure 1.**

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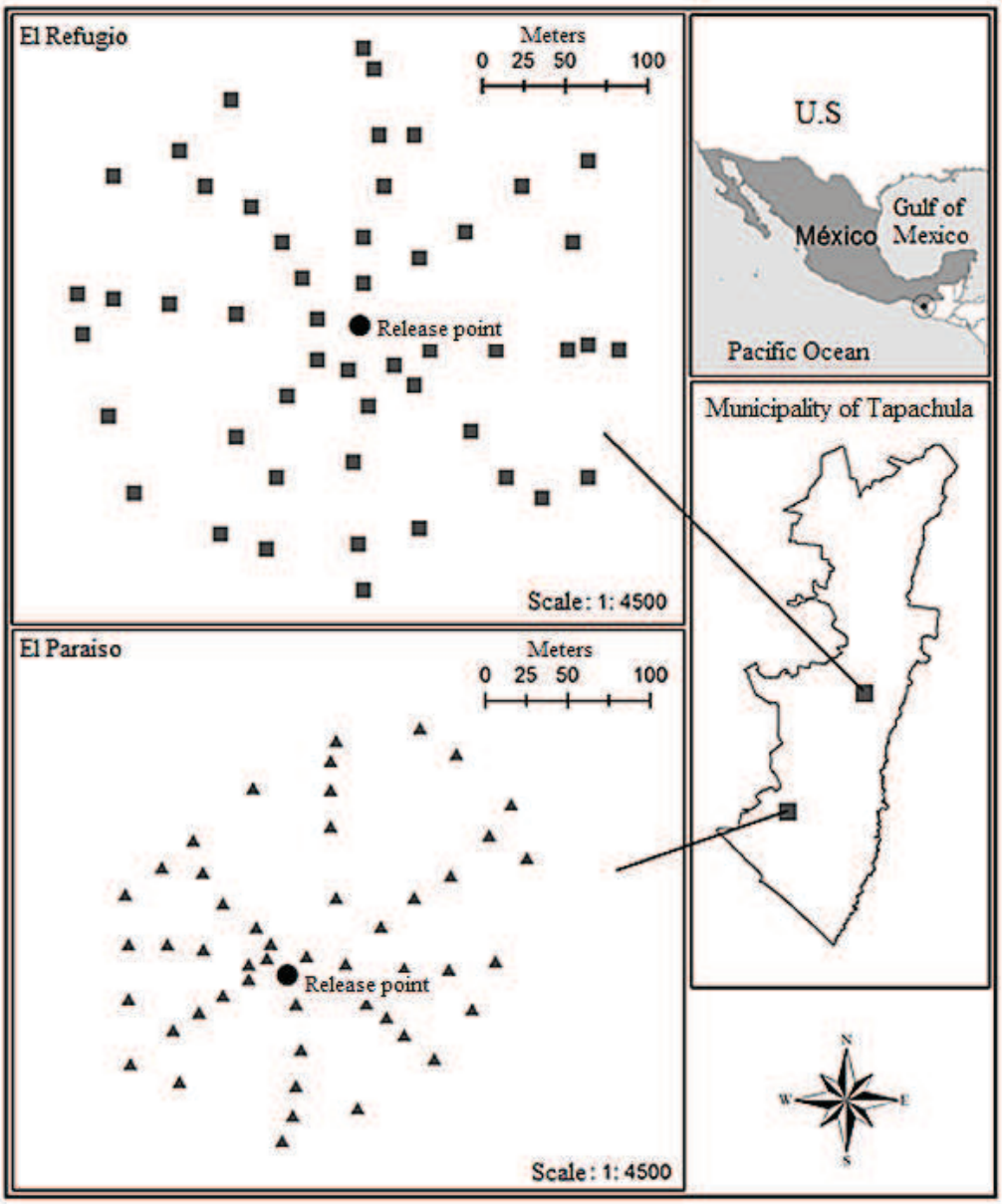

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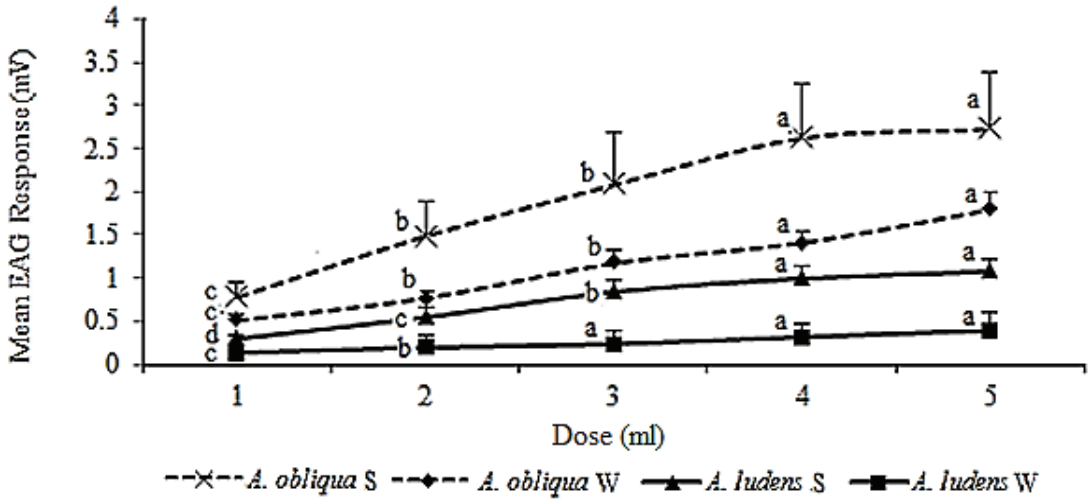
Figure 2.

A


Mean EAG Response (mV)
Dose (ml)
A. obliqua S
A. obliqua W
A. ludens S
A. ludens W

B

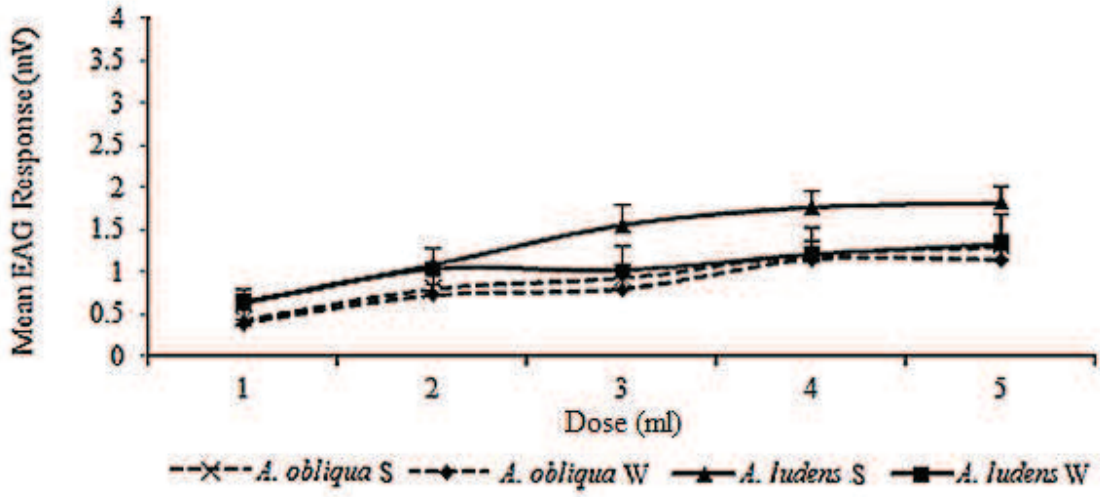
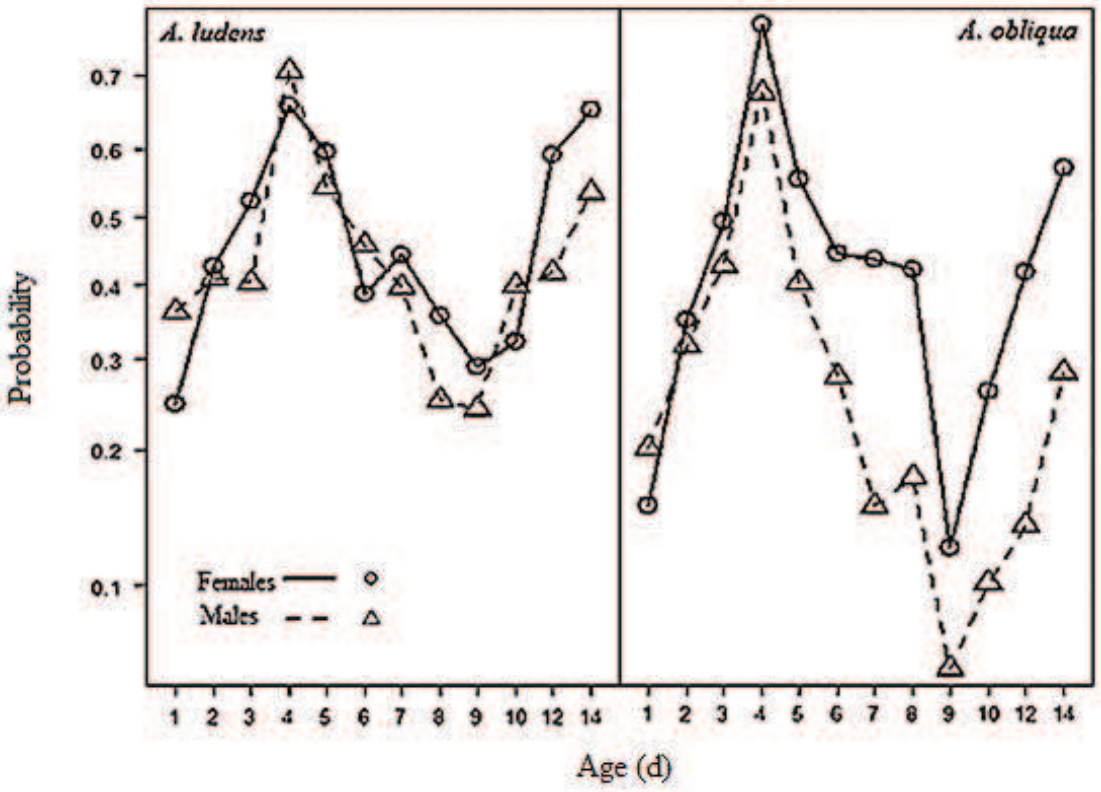

Mean EAG Response (mV)
Dose (ml)
A. obliqua S
A. obliqua W
A. ludens S
A. ludens W

Figure 3.


A. ludens
A. obliqua
Probability
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Females
Males
1 2 3 4 5 6 7 8 9 10 12 14
1 2 3 4 5 6 7 8 9 10 12 14
Age (d)

586 **Figure 4.**

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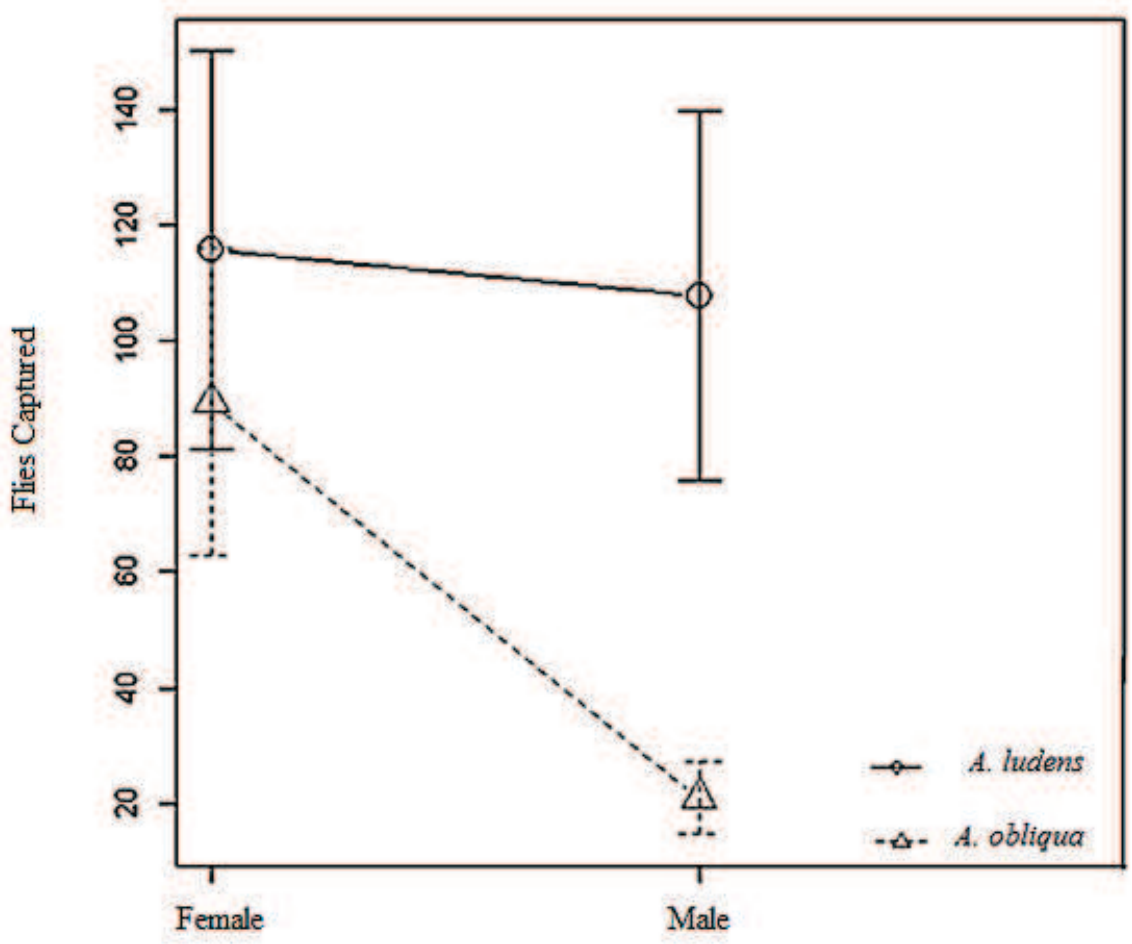

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599 **Figure 5.**

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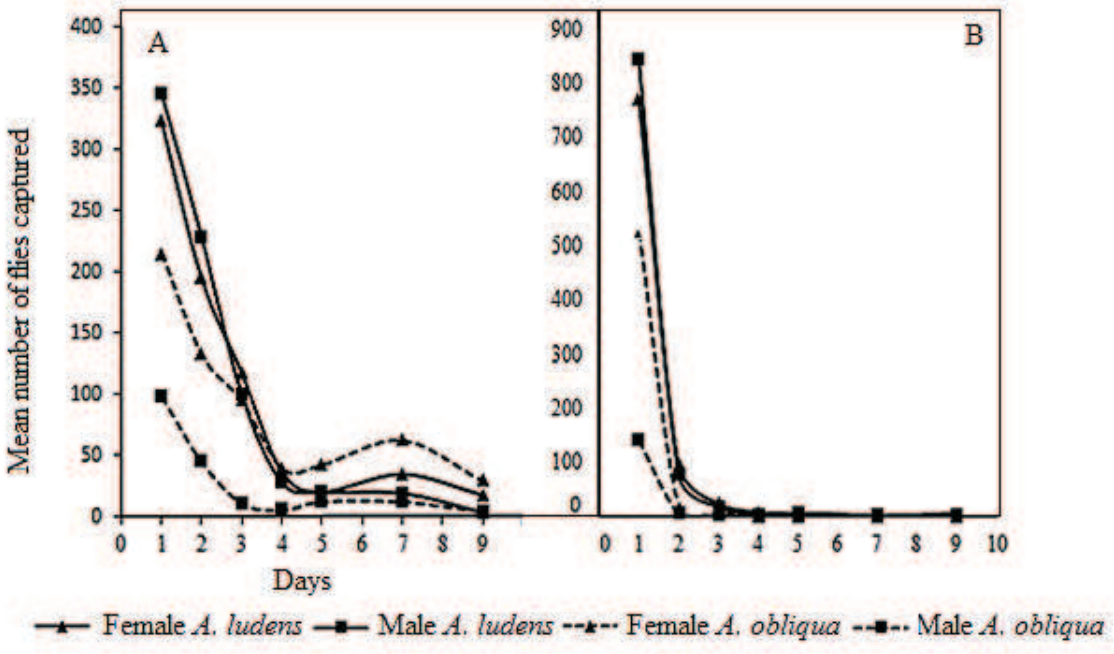

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614 **Supplemental Material**

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616 **Table S1. Total number of flies captured per year in 79 traps baited with BioLure**
617 **located in the Soconusco region, Chiapas, Mexico.**

	Female *A. obliqua*	Female *A. ludens*	Male *A. obliqua*	Male *A. ludens*
2012	3,859	783	1,799	381
2013	9,940	1,629	4,728	849
2014	11,737	553	4,552	265
2015	774	298	356	189

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Table S2. Number of larval infested shipments and number of individuals per species per year recorded at the packing houses in the Soconusco region, Chiapas, Mexico.

	Shipments	*A. obliqua*	*A. ludens*
2012	154	21	390
2013	151	9	401
2014	143	6	236
2015	143	4	253

Source: Comité Estatal de Sanidad Vegetal de Chiapas

662 **Table S3. Comparison of the responses of *A. ludens* and *A. obliqua* fruit flies to BioLure**
663 **volatiles in EAG essays by orthogonal contrasts of the interactions dose, species and**
664 **condition (sterile or wild).**

665

Contrast	EAG intensity (mV) (Mean ± S. E.)	df	Contrast	EAG intensity (mV) (Mean ± S. E.)	df
5ml X *A. ludens* X Sterile	1.448±0.02 a	656	5ml X *A. ludens* X Wild	1.061±0.02 a	656
4ml X *A. ludens* X Sterile	1.381±0.02 a	656	4ml X *A. ludens* X Wild	0.932±0.02 a	656
3ml X *A. ludens* X Sterile	1.200±0.02 b	656	3ml X *A. ludens* X Wild	0.812±0.02 a	656
2ml X *A. ludens* X Sterile	0.816±0.02 c	656	2ml X *A. ludens* X Wild	0.788±0.02 b	656
1ml X *A. ludens* X Sterile	0.469±0.02 d	656	1m X *A. ludens* X Wild	0.494±0.02 c	656
5ml X *A. obliqua* X Sterile	1.448±0.02 a	656	5ml X *A. obliqua* X Wild	1.476±0.02 a	656
4ml X *A. obliqua* X Sterile	1.384±0.02 a	656	4ml X *A. obliqua* X Wild	1.285±0.02 a	656
3ml X *A. obliqua* X Sterile	0.989 ±0.02 b	656	3ml X *A. obliqua* X Wild	0.991±0.02 b	656
2ml X *A. obliqua* X Sterile	0.825±0.02 b	656	2ml X *A. obliqua* X Wild	0.751±0.02 b	656
1ml X *A. obliqua* X Sterile	0.481±0.02 c	656	1ml X *A. obliqua* X Wild	0.457±0.02 c	656

666 Means in each column followed by the same letter are not significantly different.

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Table S4. Values of estimated probabilities of the number of flies recaptured by age of female and male *A. ludens* and *A. obliqua.*

		95% confidence interval		Estimated Probabilities	
Age		*A. ludens*	*A. obliqua*	*A. ludens*	*A. obliqua*
1	Female	0.138 - 0.402	0.078 - 0.275	0.247	0.152
	Male	0.220 - 0.535	0.110 - 0.344	0.363	0.202
2	Female	0.269 - 0.604	0.210 - 0.526	0.428	0.352
	Male	0.255 - 0.586	0.185 - 0.488	0.411	0.318
3	Female	0.355 - 0.690	0.327 - 0.663	0.526	0.495
	Male	0.251 - 0.577	0.271 - 0.602	0.403	0.429
4	Female	0.488 - 0.799	0.605 - 0.867	0.660	0.759
	Male	0.539 - 0.831	0.507 - 0.811	0.705	0.677
5	Female	0.423 - 0.749	0.383 - 0.716	0.596	0.556
	Male	0.373 - 0.707	0.251 - 0.577	0.546	0.403
6	Female	0.237 - 0.563	0.284 - 0.621	0.387	0.446
	Male	0.294 - 0.633	0.159 - 0.442	0.458	0.279
7	Female	0.284 - 0.617	0.280 - 0.612	0.444	0.439
	Male	0.247 - 0.572	0.078 - 0.275	0.398	0.152
8	Female	0.214 - 0.532	0.264 - 0.598	0.358	0.422
	Male	0.171 - 0.409	0.092 - 0.307	0.252	0.175
9	Female	0.169 - 0.457	0.061 - 0.231	0.292	0.123
	Male	0.135 - 0.396	0.028 - 0.137	0.242	0.063
10	Female	0.189 - 0.494	0.148 - 0.422	0.323	0.262
	Male	0.246 - 0.575	0.050 - 0.196	0.399	0.101
12	Female	0.418 - 0.744	0.263 - 0.592	0.591	0.419
	Male	0.263 - 0.592	0.070 - 0.253	0.419	0.137
14	Female	0.481 - 0.794	0.397 - 0.731	0.654	0.572
	Macho	0.363 - 0.702	0.162 - 0.449	0.536	0.284

Table S5. Results of the Tukey test for the release –recapture experiment.

	Estimate	SE	Z value	*P*
Male *A. ludens* X Female *A. ludens*	-0.07238	0.23688	-0.306	0.990
Female *A. obliqua* X Female *A. ludens*	-0.25798	0.23788	-1.085	0.699
Male *A. obliqua* X Female *A. ludens*	-1.70679	0.25151	-6.786	<1e-05
Female *A. obliqua* X Male *A. ludens*	-0.18560	0.23823	-0.779	0.864
Male *A. obliqua* X Male *A. ludens*	-1.63441	0.25178	-6.491	<1e-05
Male *A. obliqua* X Female *A. obliqua*	-1.44881	0.25256	-5.736	<1e-05

III. Conclusiones

Los resultados del presente trabajo, partiendo de nuestro objetivo de comparar las respuestas experimentales de *A. obliqua* y *A. ludens* bajo enfoques electrofisiológicos y comportamentales, determinaron que la mayor captura de *A. obliqua* en el Soconusco no se puede atribuir a que esta especie tenga mayor atracción al BioLure que la especie de *A. ludens*. En las pruebas electrofisiológicas, los resultados mostraron que *A. obliqua* en las dos condiciones evaluadas (silvestres y estériles), respondieron mayormente a los volátiles del cebo que *A. ludens*, también en las dos condiciones. Sin embargo en pruebas comportamentales en jaula de campo y en las de liberación y recaptura, las respuestas fueron iguales o menores en *A. obliqua* comparada con *A. ludens.*

Nuestros resultados de las pruebas comportamentales sustentan una marcada diferencia entre las dos especies y en la respuesta por sexo. En el caso de *A. ludens* hay similitud entre hembras y machos, lo que fue una respuesta inesperada dada la evidencia de diferentes estudios similares donde generalmente las hembras son mayormente atraídas (Arredondo et al. 2014, Díaz-Fleischer et al. 2009, Lasa et al. 2013). En el caso de *A. obliqua,* esto sí se cumplió*,* ya que hubo un marcado sesgo hacia una mayor respuesta por las hembras.

En los ensayos en jaula de campo, a pesar de la diferencia entre sexos, se encontró una similitud en la respuesta con relación a la edad. En nuestros ensayos, los primeros días de edad se mostró una mayor atracción al cebo, con un valor máximo a los 4 días de edad para ambas especies y ambos sexos. Este nivel de respuesta descendió gradualmente hasta su valor mínimo a los 9 días de edad, para posteriormente volver ascender en las últimas edades evaluadas (14 días). En la prueba de liberación y recaptura no se observó este repunte, ya que

las capturas posteriores a los dos días de edad siempre fueron menores. Esto es posible a que hay una disminución de la población en campo ocasionada por la dispersión o la muerte de las moscas liberadas, y no necesariamente a una menor respuesta a esas edades.

Se necesitarán realizar investigaciones futuras para abordar preguntas que surgieron a partir de nuestros resultados como: ¿Por qué no hay relación directa positiva entre EAG y las respuestas observadas en los experimentos en jaula de campo y de liberación y recaptura?, ¿Por qué la diferencia en la respuesta de hembras y machos por especie?, ¿Cómo se explica la consistente respuesta de hembras y machos de ambas especies con relación a la edad considerando que sus tiempos de maduración son diferentes? y ¿Por qué en campo abierto no se observó el repunte que se observó en jaula de campo con relación a la edad?

A partir de los resultados obtenidos se sugiere que probablemente la mayor captura de *A. obliqua* en la región, se deba a que sus poblaciones son mayores que las de *A. ludens* y se descarta que esto pueda obedecer a que *A. obliqua* tenga una mayor respuesta al BioLure. Sin embargo, *A. ludens* es la especie que mayormente infesta al mango Ataulfo. ¿Como se explica esta aparente contradicción? Estudios realizados por Aluja et al. (2014) indicaron que hay diferencias en la preferencia por los diferentes cultivares de mango de cada especie. Una posible línea de investigación sería analizar si los diferentes cultivares de mango tienen algún efecto sobre las tasas de desarrollo y crecimiento de las poblaciones, como fue demostrado para el caso de la mosca del Mediterráneo y sus diferentes especies hospederas (Krainacker et al. 1987). En la medida que se avance el conocimiento sobre el comportamiento de respuesta a los atrayentes, el efecto de los factores fisiológicos y ambientales en esa respuesta, y sus mecanismos, será posible diseñar sistemas de detección más eficientes e interpretar mejor los resultados que se obtienen de estos sistemas.

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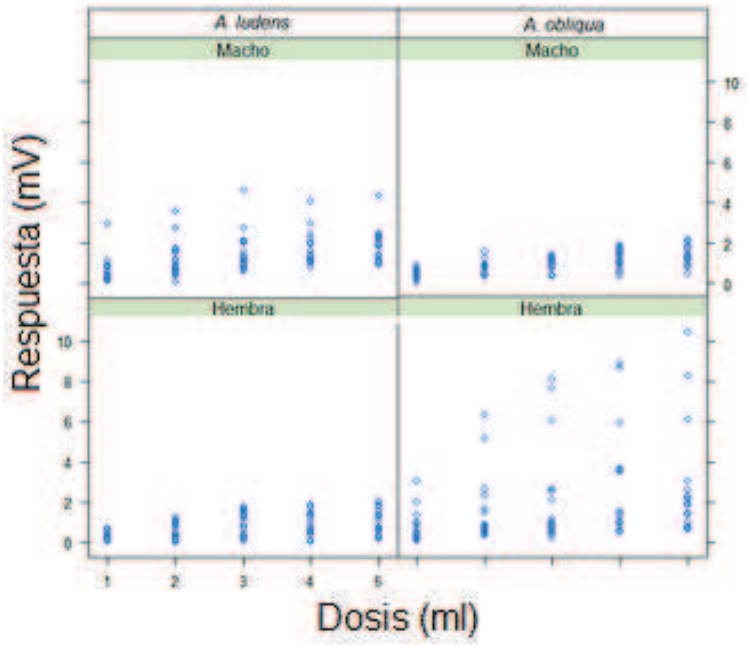
ANEXOS

Tabla 1. Resultados del análisis de devianza (significativos) del modelo con error binomial negativo con cuatro factores, especie, sexo, tipo de huerto y días de trampeo.

	Estimate	SE	z value	*P*
(Intercept)	4.37087	0.18543	23.572	< 2e-16
Día _(Día, 2)	-9.07471	1.22141	-7.430	1.09e-13
Día (Día, 2)_2	2.32861	1.22033	1.908	0.05637
Huerto_(huerto2)	-1.60369	0.17609	-9.107	< 2e-16
Sexo_(Macho)	-0.07238	0.23688	-0.306	0.75994
Especie_(*A.obliqua*)	-0.25798	0.23788	-1.085	0.27814
Día(Día, 2)1_ Huerto (h2)	-9.31206	1.85430	-5.022	5.12e-07
Poly(Dia, 2)2_ Huerto (h2)	5.71492	1.83607	3.113	0.00185
Sexo_(Macho)	-1.37643	0.34623	-3.976 7	7.02e-05
Especie_(*A.obliqua*)				

A



B

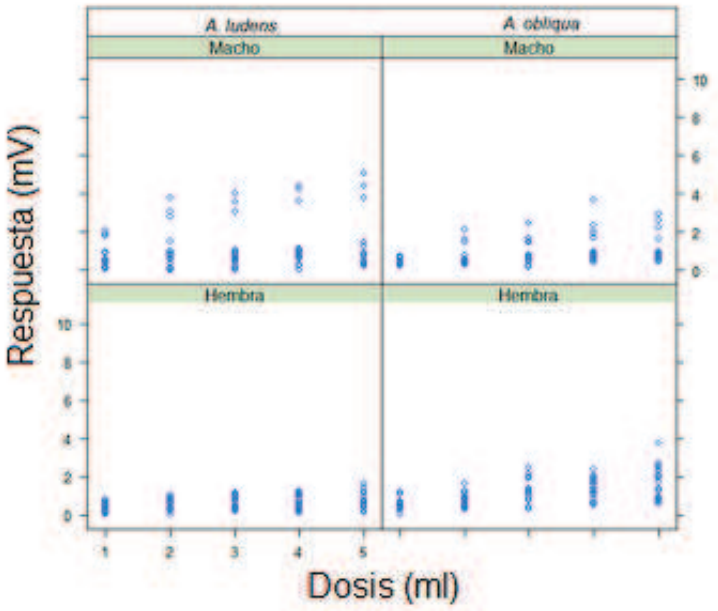


Figura 1. Graficas de puntos de la respuesta de EAG de hembras y machos de *A. ludens* y *A. obliqua* estériles (A) y silvestres (B) con respecto a diferentes dosis.

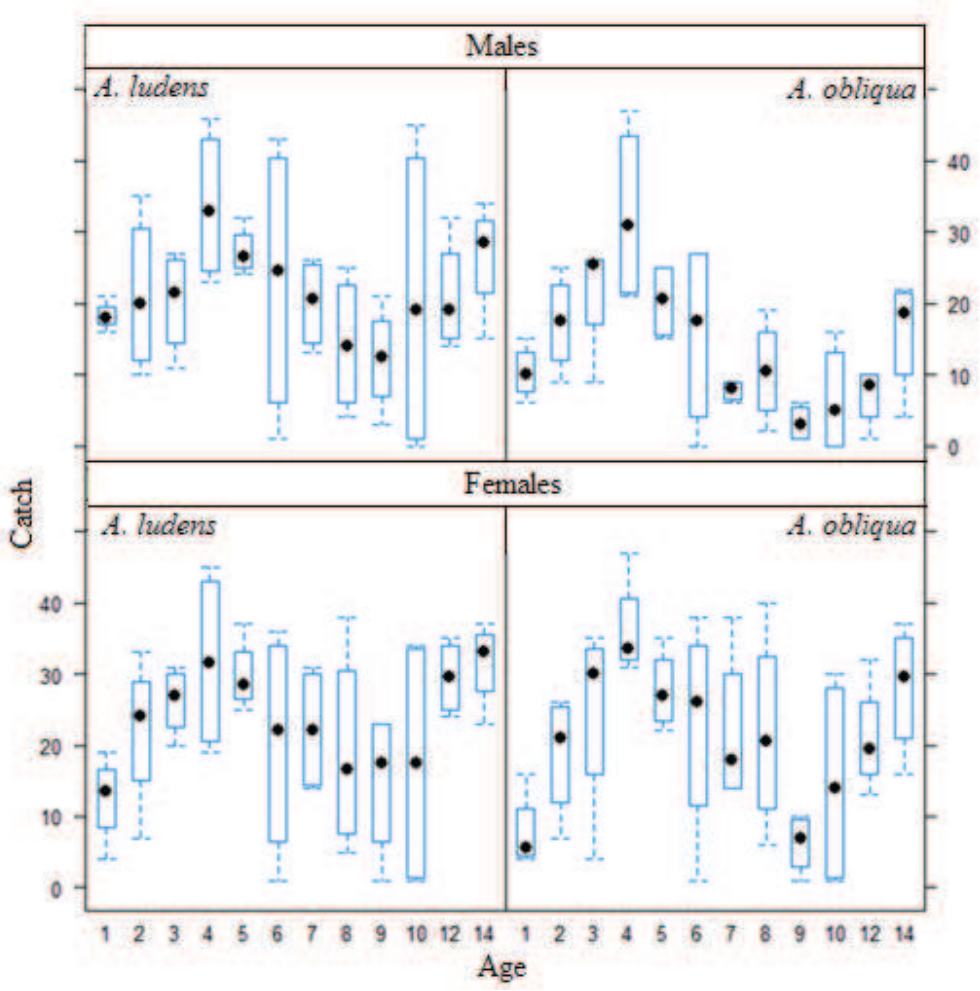


Figura 2. Numero de Capturas ±(ES) de hembras y machos de *A. ludens* y *A. obliqua* con respecto a edad, bajo condiciones de jaula de campo.

Tapachula Chiapas
Enero 2015

Nayeli Déctor Pacheco
Estudiante de Maestría de ECOSUR.
Presente

Atendiendo a su solicitud, atentamente me dirijo a usted para enviarle información sobre el número de lotes de mango Ataulfo que resultaron larvados en las inspecciones realizadas en de las empacadoras en los últimos cuatro años, indicando las especies identificadas:

Año	*Lotes*	*A. obliqua*	*A. ludens*
2012	154	21	390
2013	151	9	401
2014	143	6	236
2015	143	4	253

Esperando que le sirva de referencia y sin otro particular, reciban un cordial saludo.

Atentamente,

Ing. Fredy Orlando Gálvez Cárdenas,
Comité Estatal de Sanidad Vegetal de Chiapas.