EL COLEGIO DE LA FRONTERA SUR

Efecto del metopreno incorporado al alimento sobre el apareamiento y emisión de volátiles de machos de *Anastrepha obliqua* (Diptera: Tephritidae)

TESIS

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Siempre me has sustentado.

A todos mis seres amados, como símbolo de agradecimiento y amor.

A mis amados hijos. La buena voluntad,

la perseverancia y el amor, dan frutos de felicidad.

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Índice

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1. Introducción.

Las moscas de la fruta del género *Anastrepha* son consideradas de las plagas más importantes a nivel mundial por su impacto económico en la producción de frutas y las estrictas restricciones cuarentenarias (Aluja y Mangan, 2008). Pertenecen al orden Díptera, clasificadas dentro de la familia Tephritidae. Esta familia agrupa alrededor de 4,223 especies. En el continente Americano se encuentran cerca de 977 especies, de las cuales el 73% han sido registradas para la región Neo-tropical (desde México hasta Chile y Argentina) (Norrbom, et al., 1998). La mosca de las Indias Occidentales, *Anastrepha obliqua* (Macquart) es considerada la segunda plaga de importancia económica del genero *Anastrepha* en México, atacando frutos de mango (*Mangifera indica* L.), constituyendo un problema serio para la fruticultura del país (Aluja, 1994; Aluja, et al., 1996). Como una medida de control, el Gobierno Mexicano implementó la Campaña Nacional contra Moscas de la Fruta, teniendo como estrategia fundamental el uso de la Técnica del Insecto Estéril (TIE) y el Control Biológico Aumentativo (Reyes, et al., 2000).

La TIE es un procedimiento usado como parte del manejo integrado en área extensas para la erradicación o supresión de plagas como moscas de la fruta (Enkerlin, 2005). En México, ha sido ampliamente usada para la erradicación de la mosca del Mediterráneo, *Ceraitis capitata* (Wiedeman), *Anastrepha ludes* (Loew) *y A. obliqua*, estableciendo áreas libres en el país (Rull, et al., 1996; Reyes, et al., 2000). La TIE consiste en la producción masiva de la especie a controlar y liberar machos estériles sexualmente maduros en zonas infestadas con poblaciones silvestres para que se apareen con las hembras silvestres. Las cópulas de machos estériles sexualmente maduros producen

huevos infértiles, reduciendo así la fertilidad de las poblaciones silvestres (Knipling, 1970; Klassen y Curtis, 2005).

Aproximadamente 150 millones de individuos de *A. ludens* y 40 millones de *A. obliqua* son liberados semanalmente a través de la Campaña Nacional contra las Moscas de la Fruta (Gutiérrez, 2010). La fase final de la TIE consiste en la liberación de moscas estériles en el campo, siendo éstas empacadas como pupa en cajas PARC (Plastic Adult Release Container) o en torres de emergencia y finalmente ser liberadas a través del sistema de adulto frío (Dowell, et al., 2005; Shelly, et al., 2006, 2009; Domínguez, et al., 2010; Hernández, et al., 2010).

El período entre la emergencia del adulto y la liberación de machos de *A. obliqua* representa un problema para la aplicación de la TIE en esta especie. Los insectos deben alimentarse en salas acondicionadas por un período de 4-5 días, antes de alcanzar la madurez sexual y ser liberados. Para incrementar la eficiencia de la TIE se han planteado reducir el tiempo para alcanzar la madurez sexual de los machos en las salas de emergencia a través del uso de análogos sintéticos de la hormona juvenil (metopreno). Algunos trabajos realizados con *A. ludens* y *A. suspensa* (Loew) han arrojado resultados alentadores, ya que el uso de metopreno aceleró la maduración sexual (Pereira, 2005; Gómez, et al., 2013).

El metopreno es un análogo de la hormona juvenil (JHA) que actúa en el sistema endocrino de los insectos, interfiriendo en procesos esenciales de la vida de estos, tales como la metamorfosis y la reproducción (Vogel, et al., 1979). La hormona juvenil (JH) pertenece a un grupo de sesquiterpenoides aciclicos que regulan la metamorfosis y el

desarrollo de señales sexuales con maduración de gametos en muchas especies de insectos (Cusson, et al., 1994). Existen tres tipos de hormonas juveniles identificadas: JH I y JH II, ambas sesquiterpenoides epóxido métil esteres, encontrados en lepidópteros; mientras la JH III es un homologo trimetil, detectado en todos los órdenes de insectos (Cusson, et al., 1994).

Los análogos de la hormona juvenil (AHJ) son compuestos sintéticos cuyos grupos funcionales han sido reemplazados. Uno de los análogos de la hormona juvenil, es el metopreno, un ester llamado isopropyl (2E-4E)-11-metoxy-3,7,11-trimethyl-2-4 dodecadienato (Crosby y Minyard, 1991). El metopreno aplicado en la etapa pre-pupal impide que la larva se transforme en pupa. Así, el metopreno puede ser usado como un insecticida contra mosquitos (Norland y DeWitt, 1975) y otros dípteros (Glare y O'Callaghan, 1999).

En moscas de la fruta (Tephritidae) se han realizado investigaciones relacionados al uso del análogo de la hormona juvenil para acelerar la madurez sexual. Por ejemplo, cuando es suministrado en el alimento para adultos de *A. suspensa*, reduce el tiempo de maduración sexual (Teal, et al., 2000). Estos autores demostraron que los extractos de hemolinfa de machos con experiencia sexual contenían tres veces más hormona juvenil que machos no experimentados, y mediante espectrometría de masas identificaron en la hemolinfa de estos insectos a la hormona juvenil III bisepóxido, existiendo una diferencia en proporción de 2.5:1 en machos experimentados y no experimentados. Una forma para incrementar la hormona juvenil es la aplicación por vía tópica de metropeno o fenoxicarb, lo que permite que los machos jóvenes precoces liberen más feromona por unidad de tiempo que los machos no tratados, concluyendo

que la hormona juvenil media un factor que garantiza que machos que se apareen a una edad temprana podrían desplazar a los vírgenes de la misma edad por las oportunidades de apareamiento (Teal, et al., 2002). Adicionalmente se conoce que la efectividad del metopreno requiere de la presencia de alimentos ricos en proteína (Pereira, et al., 2009; Haq, et al., 2010 a,b).

En ese sentido, Teal, et al. (2007), sugirieron la posibilidad de incorporar HJ (metopreno y fenoxicarb) en el alimento (proteína y azúcar) de los adultos de *A. ludens* en cría masiva; y recientemente, Gómez, et al. (2013) reportaron una reducción del índice de aislamiento de machos estériles después del tratamiento con metopreno y un aumento del porcentaje de apareamiento entre machos estériles de laboratorio y hembras silvestres en jaulas de campo. Concluyendo que la incorporación del metopreno en el alimento del adulto mejora el rendimiento sexual de machos de esta especie.

Haq, et al. (2010a), en estudios con machos de *Bactrocera cucurbitae* (Coquillet) de seis días de edad tratados con: proteína y metopreno, sólo proteína o sólo metopreno, observaron que en el inicio de formación de leks, llamados, apareamiento, interacción macho-macho e índice de aceptación de la hembra fue superior tratados con proteína y metropeno que no tratados, a pesar de que los no tratados tenían mayor edad y eran sexualmente maduros. Estos autores llegaron a la conclusión de que el metopreno más la proteína tiene un efecto sinérgico que mejora el rendimiento sexual de los machos.

En *A. obliqua*, trabajando con poblaciones silvestres, provenientes de larvas colectadas directamente de frutos hospederos (*Spondias* sp.), y posteriormente los adultos alimentados *ad libitum* con una dieta artificial a base de azúcar y proteína en una

proporción 3:1 más metopreno, se reportó que el metopreno no aceleró la madurez sexual de los insectos tratados en comparación con los no tratados (Aluja, et al., 2009). En contraste, Teal, et al. (2007) y Chacón, et al. (2013) encontraron una mayor proporción de cópulas y emisión de volátiles cuando machos de *A. obliqua* fueron tratados de manera tópica con metopreno comparados con machos no tratados.

Sin embargo, la aplicación tópica es impráctica a nivel masivo en la aplicación de la TIE. En este trabajo, propusimos determinar el efecto de diferentes concentraciones de metopreno incorporado al alimento para adultos a diferentes proporciones de azúcar:proteína (A:P) sobre la emisión de volátiles y propensión a la cópula de machos de *A. obliqua*. Consideramos que una anticipada maduración de los machos de esta especie bajo una metodología práctica a nivel masivo puede conducir a la reducción de costos en el manejo en las instalaciones de pre-liberación debido a un ahorro de espacio e insumos, además de la liberación de machos maduros sexualmente, aumentado la eficiencia de la TIE en esta especie.

2. Capítulo de artículo enviado.

Journal of Economic Entomology: Ecology and Behavior section Muñoz-Barrios et al. Effect of methoprene on sexual-related behavior *A. obliqua*	Dr. Edi A. Malo El Colegio de la Frontera Sur (ECOSUR). Grupo de Ecología y Manejo de Artrópodos, Carretera Antiguo Aeropuerto, Km. 2.5 30700, Tapachula, Chiapas, México Tel: Mex (52) 9626289800 Fax: Mex (52) 962 62 89806 Email: emr@ecosur.mx

Influence of methoprene incorporated into adult food on age-related mating propensity and volatile emission of *Anastrepha obliqua* (Diptera: Tephritidae) males

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ABSTRACT. It has been demonstrated that the application of juvenile hormone analog (JHA), methoprene, reduces the time required for sexual maturation and enhances mating success in several species of tephritid fruit flies. This study examined the effect of different concentrations of methoprene incorporated into the diet of adult flies, consisting of distinct proportions of sugar: protein (S:P), on age-related mating propensity and pheromone emission of *Anastrepha obliqua* males. Our results demonstrated that a concentration of 0.02% and 0.05% methoprene combined with a S:P ratio of 3:1 and 24:1, enhanced the sexual maturation of treated males compared to untreated males. In subsequent assays, the enhancement of male volatile emissions and sexual maturation by the incorporation of 0.02% methoprene into a 24:1 (S: P) diet, was confirmed. Among the volatiles released by males, (*Z*)-3- nonenol and (*Z*,*Z*)-3,6-nonadienol were emitted at higher quantities by flies treated with methoprene than untreated ones. Our results show that methoprene accelerates sexual maturation of mass-reared *A. obliqua* flies and increases their mating propensity. This would reduce the time required to attain sexual maturation by sterile flies, thus reducing fly maintenance costs and improving the efficacy of the sterile insect technique.

KEY WORDS West Indian fruit fly, Juvenile hormone, Mating, Sexual maturation, Volatiles

Fruit flies belonging to the *Anastrepha* (Diptera: Tephritidae) genus are one of the most important pests worldwide, due to their economic impact on fruit production and strict quarantine restrictions (Aluja and Mangan 2008). *Anastrepha* fruit flies are native to the American continent (Norrbom et al. 1998). The West Indian fruit fly, *Anastrepha obliqua* (Macquart) is considered the second most economically important *Anastrepha* fruit fly species and presents a serious challenge for fruticulture in Mexico, attacking mainly mango fruits (*Mangifera indica* L.) (Aluja 1994, Aluja et al. 1996). As a control measure, the Mexican government implemented the National Fruit Fly Program, including the use of the Sterile Insect Technique (SIT) and augmentative biological control (Reyes et al. 2000).

The SIT is a component of area-wide integrated pest management (AW-IPM) of tephritid fruit flies (Enkerlin 2005). In Mexico, SIT has been applied with the aim of eradicating the Medfly, *Ceratitis capitata* (Wiedemann), *Anastrepha ludens* (Loew) and *A. obliqua* from northwest Mexico, succeeding in the establishment of pest free areas (Rull et al. 1996, Reyes et al. 2000). SIT involves the mass production of the target species and then the release of sterile males in areas infested with wild flies to increase the incidence of mating between sterile males and wild females. The mating of sterile insects yields infertile eggs, thus reducing the fertility of the field population of the target species (Klassen 2005, Shelly et al. 2009, Dominguez et al. 2010). Approximately 150 million sterile *A. ludens* and 40 million sterile *A. obliqua* are released on a weekly basis (Hernández et al. 2010). The period between adult emergence and sexual maturity of *A. obliqua* males presents a problem for SIT programs, as males must be held for a lengthy period before attaining sexual maturity prior to release. To improve the efficacy of SIT programs, a reduction in the time required for male flies to reach sexual maturity was proposed by incorporating a juvenile hormone analog (methoprene) into the food of adult flies. Furthermore,

this treatment could result in an increase in both the emission of male volatiles and mating propensity (Gómez et al. 2013).

Methoprene is a juvenile hormone (JH) analog that acts on the endocrine system of insects, interfering with essential life process such as metamorphosis and reproduction (Vogel et al. 1979). Several studies have reported that the topical application of methoprene and incorporated into diet to *A. ludens* and *A. suspensa* (Loew) males results in an acceleration of sexual maturity (Gómez et al. 2013, Pereira 2005). However, Aluja et al. (2009), using wild *A. obliqua,* males (obtained from larvae collected directly from infested host plant fruits) fed *ad libitum* on an artificial diet consisting of a 3:1 sugar: protein proportion (S:P) plus methoprene, found that the juvenile hormone analog did not accelerate sexual maturation. In contrast, Teal et al. (2007) and Chacon et al. (2013) found that *A. obliqua*, males treated topically with methoprene, demonstrated a highly significant number of copulations and increased volatile emissions when compared with untreated controls. However, the topical application of methoprene in SIT programs is impractical. Haq (2010 a,b) reported that adding methoprene and protein to the diet produced synergic effects on the mating behavior of *Bactrocera cucurbitae* (Coquillett) males. This suggests that in addition to methoprene, the amount of protein may influence sexual maturity in other species of fruit fly. The aim of this study was to determine the effect of different methoprene concentrations and sugar: protein (S:P) ratios, on volatile emission and mating propensity of *A. obliqua* males. We considered that a rapid maturation of *A. obliqua* males would result in significant cost reductions at fly handling facilities, due to the use of less space and supplies, as well as more efficient SIT programs whereby released sexually mature males demonstrate an increase in mating propensity.

Materials and methods

Insects. Pupae of *A. obliqua* that had been irradiated 48 h pre-emergence with 80 Gy were used in these experiments. These pupae were obtained from the Moscafrut (DGSV-SENASICA) facility, located in Metapa, in the state of Chiapas, Mexico. Mass-rearing procedures and conditions are described by Artiaga-Lopez et al. (2004). In all experiments, we used 100 recently-emerged male flies per treatment; these insects were placed in separate acrylic cages (30 x 30 x 30 cm). Recently-emerged female flies were arranged in groups of 500 and placed in acrylic cages (30 x 30 x 40 cm). Males and females were kept at 26 ± 1°C, 70-80% RH, and a photoperiod of 12:12 (L:D). The photophase began at 07:00 hours and ended at 19:00 hours.

Methoprene. The commercial product used was Precor, which contains 1.2% methoprene (Wellmark International, Schaumburg, IL.). The experiments which investigated the effect of different concentrations of methoprene were performed using 0.01, 0.02, 0.5, 0.1 and 0.2% methoprene diluted in water.

Food preparation. The food was prepared by mixing the protein and sugar in powder form and then incorporating the methoprene solution (Gómez et al. 2013). Treated adult male flies were fed with a mixture of sucrose and protein (hydrolyzed yeast) (S:P) at different ratios plus methoprene. Control males were fed with the same food but without methoprene. Food was prepared one day prior to fly emergence, allowing it to dry before being consumed. Females used in all experiments were fed with 3:1 (S:P) without methoprene. Male and female flies were provided with water in vials covered with cotton wicks and feeding was *ad libitum* during the experimental period.

Effects of methoprene concentration and S:P ratio on the mating propensity of *A. obliqua* males.

Recently-emerged males were separated into groups of 100 individuals; each group was placed in 30 x 30 x 30 cm acrylic cages and provided with food according to a given treatment. For each treatment, 10 flies of a given age were separately placed into 30 x 30 x 30 acrylic cages. Twelve sexually mature virgin females (8 to 12 d- old) were immediately added to each cage. The mating behavior of male flies was observed from 7:00 to 11:00 h, corresponding to the period of peak sexual activity (Aluja et al. 2000). During the mating evaluation period, male and female flies were provided with water in glass tubes covered with cotton wicks; however, no food was available. The number of matings was recorded for each treatment and age. Flies (females and males) that copulated were removed from the cages.

This experiment consisted of 18 treatments, three food ratios: 3:1, 9:1, 24:1 (S:P) and six methoprene concentrations: 0, 0.01, 0.02, 0.05, 0.1 and 0.2% (Table 1). All 18 combinations were tested each day, with 3 to 11 day old male flies. The number of matings achieved by males for each treatment were recorded and transformed to the proportion of mating per treatment.

Male mating behavior. Male treated was fed on a diet consisting of 0.02% methoprene and a sugar: protein proportion of 24:1. The control consisted of flies fed with the same 24:1 (S:P) diet but without methoprene. This experiment was conducted in order to corroborate the effect of the methoprene and 24:1 S:P diet on the mating propensity and emission of volatiles by *A. obliqua* males.

One hundred recently-emerged male flies were placed in a 30 x30 x 30 cm acrylic cage and provided with food according to treatment. Ten males of a given age and treatment were placed in 30 x 30 x 30 acrylic cages. Twelve sexually mature virgin females (8 to 12 d- old) were immediately introduced into each cage. Males aged 3 to 11d old were evaluated. Male mating behavior was observed from 7:00 to 11:00 h. In all treatments, male and female flies were provided with water in vials covered with cotton wicks. No food was provided during the experimental periods. The number of matings for each age was recorded. Mated male and female flies were discarded and not used again. Eleven replicates were performed.

Volatile collection. Only male flies were used for volatile collection. The experiment consisted of two treatments, males treated with 0.02% methoprene and a 24:1 S:P ratio and the control that consisted of the same 24:1 S:P ratio but without methoprene. Food was provided to groups of 100 insects that were placed inside 30 x 30 x 30 cm acrylic cages according to treatment. Ten males were separated from the original group and placed inside an Erlenmeyer 150 ml clean-oven matrass, which was then stoppered with foil aluminum. Volatile collection was carried out using the Solid Phase Microextraction technique (SPME). A previously-conditioned SPME syringe with a solid phase of Polydimethylsiloxane (Supelco Inc., Bellefont, PA) was introduced at the top of each Erlenmeyer matrass. Four, six, eight and ten day-old males were used in this experiment. Volatiles were collected between 07:00 and 09:00 a.m. Males were only used once and then discarded. The area under each peak was the analyzed variable. Eleven replicates for each age and treatment were performed.

144 **Volatile analysis.** The chemical analysis of the volatiles captured by using SPME fibers was
145 performed in a Varian CP-3800 Gas Chromatograph coupled to a Varian Saturn 4D 2200 (GC-
146 MS) Mass Spectrometer. A non-polar fused silica capillary column DB5-MS (30 m x 0.25 mm
147 i.d.) (Agilent Tech. Santa Clara, CA) was used. The analysis was performed at an initial
148 temperature of 50 °C (for 2 min) increasing 15 °C/min to 280 °C (for 10 min). Helium was used
149 as the carrier gas. Injector temperature was 200 °C. Ionization was by electronic impact at 70 eV.
150 The identification of the peaks was based on comparison of our data with those of synthetic
151 standards ((*Z*)-3-nonenol, (Z,Z)-3,6-nonadienol, farnesene racemic (including (*Z*,*E*)-α-farnesene
152 and (*E*,*E*)-α-farnesene) supplied by Sigma-Aldrich (Toluca, Mexico).

153

154 **Statistical analysis.** Statistical analyses of all data were carried out using R software (R version
155 3.1.1). The first and the second experiments were analyzed using a logistic regression model with
156 binomial response. In the first experiment, methoprene dose was the first factor; the S:P ratio and
157 male fly age were the second and third factors respectively. The second experiment consisted of
158 males of different ages treated with methoprene compared against the control. The analysis of the
159 identified volatiles was conducted using an analysis of variance (ANOVA), obtained by applying
160 a generalized linear model for each compound, analyzing the treatment, control and the
161 interactions between treatment and age.

162

163 **Results**

164 **Effects of methoprene concentrations and S:P ratios on the mating propensity of *A. obliqua***
165 **males.** Methoprene concentrations (χ^2= 151.03; df= 5; P < 0.001), male age (χ^2= 714.19; df= 8; P

166 < 0.001) and the S:P ratios (χ^2= 7.84; df= 2; P < 0.05) had a significant effect on the number of
167 matings by *A. obliqua* males. The interactions methoprene concentrations x male age (χ^2=
168 133.77; df= 40; P < 0.001), methoprene concentrations x S:P ratios (χ^2= 22.86; df= 10; P <
169 0.011), and S:P ratios x male age (χ^2= 34.79; df= 16; P < 0.004) were all significant. The
170 interaction methoprene concentrations x male age x S:P ratios (χ^2= 62.95; df= 80; P > 0.05) was
171 not significant.

172 Considering the three S:P ratios, the mating propensity of young *A. obliqua* males treated with
173 methoprene was low, reaching a maximum at 5 to 6 d-old and then decreasing between 7 and
174 11-d-old (Fig. 1). The mating propensity of untreated males peaked at 8 d-old, followed by a
175 continual decrease with age. Six-day old *A. obliqua* males presented the highest mating
176 propensity when treated with 0.02% methoprene, followed by 0.05% and 0.01%. Males displayed
177 the lowest mating propensity when treated with the highest methoprene concentrations (0.1% and
178 0.2%). Three and 4 d-old males treated with methoprene showed greater mating propensity than
179 non-treated males of the same age (Fig. 1).

180 When analyzing the effect of increasing methoprene concentration and S:P ratios on male mating
181 propensity for all age groups, diets consisting of 0.02% and 0.05% methoprene and 3:1 and 24:1
182 S:P resulted in an increase in male mating propensity; however, treatments with 0.1 and 0.2%
183 methoprene resulted in a decrease in mating frequency (Fig. 2). Surprisingly, male mating
184 propensity revealed a different profile when flies were fed with the 9:1 S:P diet (Fig. 2). Males
185 fed with the 9:1 S:P diet demonstrated the lowest mating propensity. Control males and those
186 treated with methoprene doses of 0.01%, 0.1% showed similar results, independent of diet (Fig.
187 2). Males treated with 0.2% methoprene presented a decrease in mating propensity (Fig. 2).
188 Untreated males demonstrated similar mating propensities, independent of the S:P ratio.

189 Therefore, males treated with a diet containing 0.05% methoprene and 3:1 S:P, and those treated
190 with 0.02% methoprene and 3:1 and 24:1 S:P ratios, exhibited a highest number of matings (Fig.
191 2).

192 Without considering methoprene, the mating propensity of 3 to 5-d-old males was similar for all
193 three S:P ratios. Six-d-old males fed with a 3:1 S:P diet presented the highest mating propensity.
194 Seven and 8-d-old males fed on a 24:1 S:P ratio diet also exhibited high mating propensity (Fig.
195 3).

196

197 **Male mating behavior.** When only considering the 0.02% methoprene / 24:1 S:P ratio diet and
198 the control , significant differences between treatments (χ^2= 42.4; df= 1; P < 0.001), male age
199 (χ^2= 312.8; df= 8; P < 0.001) and the interaction between treatment and male age (χ^2= 35.04; df=
200 8; P <0.001) were revealed. Specifically, differences between treated and untreated males at 4
201 (χ^2= 8.2, df= 1, P > 0.01), 5 (χ^2= 19.5, df= 1, P < 0.001), 6 (χ^2= 8.9, df= 1, P > 0.01) and 7-d-old
202 (χ^2= 20.36, df= 1, P < 0.001) were significant (Fig. 4). Regarding 8 to 11 d-old males, no
203 significant differences (P > 0.05) were observed among treatments and control (Fig. 4). The
204 highest mating propensity was observed in treated 7 d-old males. Among untreated males, the
205 highest mating propensity was observed at 9 days-old. Mating propensity in untreated males was
206 always lower than in treated males, although differences were not significant between 8 and 11
207 days old (Fig. 4).

208

209 **Volatile analysis.** Six compounds were identified: (Z)-3-nonenol, (Z,Z)-3,6-nonadienol, (Z,E)-α-
210 farnesene, (E,E)-α-farnesene, and a farnesene isomer. Statistical analysis of revealed that

methoprene treatments ($F= 40.97$; $df=1,71$; $P < 0.001$), and male age ($F= 25.25$; $df=3,64$; $P < 0.001$) had a significant effect increasing the amount of (Z)-3-nonenol. The treatment x age interaction was not significant ($F= 0.77$; $df=3,64$; $P < 0.51$) (Fig. 5A). The (Z,Z)-3,6-Nonadienol demonstrated significant differences in the amount of the alcohol between the treatments and control ($F= 40.54$; $df=1,71$; $P < 0.001$) and male age ($F= 9.11$; $df=3,64$; $P < 0.001$); the treatment x male age interaction was significant ($F= 4.16$; $df=3,64$; $P < 0.01$) (Fig. 5B). (Z,E)-α-Farnesene amounts demonstrated significant differences with respect to male age ($F= 35.87$; $df= 3,64$; $P < 0.0001$), but not treatments ($F= 2.64$; $df=1,64$; $P > 0.05$) or treatment x male age interactions ($F= 1.56$; $df=3,64$; $P > 0.05$). Amounts of (E,E)-α-Farnesene were influenced by male age ($F= 37.1$; $df=3,64$; $P < 0.001$), but not treatment ($F= 2.59$; $df=1,71$; $P > 0.05$); the treatment x male age interaction was not significant ($F= 1.36$; $df=3,64$; $P > 0.1$). The levels of the farnesene isomer presented significant differences among male age ($F= 40.67$; $df=3,64$; $P < 0.0001$); however, no differences were evident with respect to treatments ($F= 1.14$; $df=1,64$; $P > 0.05$) and treatment x male age interactions ($F= 2.22$; $df=3,64$; $P > 0.05$).

Discussion

In this study, we found that methoprene incorporated into adult fly food accelerated the sexual maturation of *A. obliqua* males. Treated males presented higher mating percentages and an increased release of volatiles when compared with control males. The GC-MS analysis of volatiles released by treated and untreated *A. obliqua* males consistently revealed the same compounds as those previously reported for this species, with the exception of (Z,Z)-3,6-nonadienol (López-Guillén et al. 2008, Chacon et al. 2013). Two alcohols ((Z)-3-nonenol and (Z,Z)-3,6-nonadienol) were released in higher quantities by male flies treated with methoprene than control males. However, the farnesenes compounds were not affected by the methoprene.

235 Furthermore, males treated with methoprene began releasing alcohols earlier than control males.
236 López-Guillen et al. (2008) found that (Z)-3-nonenol was antennal and behavioral active to *A.*
237 *obliqua* females. Chacon et al. (2013) investigated the effect of topical methoprene application on
238 *A. obliqua* males, finding a higher amount of farnesenes and (Z)-3-nonenol. The present study
239 reports for the first time the presence of the (Z,Z)-3,6-nonadienol compound in *A. obliqua*.
240 However, in other studies, (Z,Z)-3,6-nonadienol was released by calling males of *A. ludens* and
241 *A. suspensa* (Nation 1983). Surprisingly, we observed that mating propensity is proportional to
242 the quantity of (Z,Z)-3,6-nonadienol emitted by treated males.
243
244 Mating behavior in males treated with methoprene peaked at 6 to 7-d-old, whereas mating
245 frequency in control males peaked at 8 to 9-d-old. This indicates that the JH analogue reduced the
246 time required by *A. obliqua* males to achieve sexual maturation by two days. Similar results were
247 reported by Chacon-Benavente et al. (2013), where topical application of methoprene to males of
248 *A. obliqua* also reduced the time required to achieve sexual maturation by two days. In this study,
249 we found that methoprene incorporated into the adult diet resulted in a higher proportion of
250 matings when compared with the control. In contrast, Aluja et al. (2009) found that wild *A.*
251 *obliqua* males, collected as larvae infesting *Spondias pupurea* L., did not present any significant
252 differences among males treated with methoprene and those used as control when fed with a 3:1
253 S:P diet. This result may have been due to only one concentration of methoprene being tested.
254 Our results demonstrated that high concentrations of methoprene (0.2%) reduce the number of
255 matings by treated males when compared with the control, indicating that the effect of
256 methoprene was dose-dependent. An additional factor that could have played a significant role in
257 the lack of an association between methoprene levels and mating percentages is the accumulation
258 of nutrients during larval development, depending on the origin of the population. Aluja et al.

(2009), tested two populations of *A. ludens*, one collected as larvae from *Casimiroa greggii* (S.Watson) and the second from *Citrus paradise* (Macf). These authors revealed significant differences in achieved matings between *C. greggii* males treated with methoprene and control males. In contrast, treated males of *C. paradisi* showed similar mating percentages to control males. In this study, we used *A. obliqua* mass-reared flies that as larvae were fed on a diet rich in carbohydrates and protein.

We found that 4 to 7-d-old *A. obliqua* males fed on a methoprene diet showed a higher proportion of mating than males used as control. In contrast, we did not find any differences between treated 8 to 11-d-old adult flies and control males. Similar results were reported for *A. ludens* by Pereira et al. (2011) who found that the use of methoprene increased the number of matings in 4 to 7-d-old treated males compared with non-treated males; however, 8 to 10-d-old males treated with methoprene and non-treated males presented a similar number of matings. In *A. suspensa*, males treated with juvenoids engaged in sexual signals, pheromone release, and mated at earlier ages than control males (Teal et al. 2000). The topical application of methoprene and the addition of protein to the diet of *A. suspensa* males resulted in earlier sexual development compared with the control flies (Teal et al. 2002, Pereira et al. 2009, 2010 and 2011).

With respect to sugar: protein ratios, in our first experiment, a higher number of matings was attained by males fed on a 3:1 S:P diet, followed by 24:1 and 9:1. Liedo et al. (2013) tested different S: P proportions (without methoprene) in the diet of *A. obliqua* and *A. ludens*; males and female flies fed with 24:1 (S:P) achieved similar mating percentages but improved survival and longevity compared with 3:1, 9:1 (S:P) and sugar only. The authors suggest a S:P proportion of 24:1 when preparing the food of both species. In addition, preparing a 24:1 S:P diet is more cost-

283 effective than implementing 3:1 and 9:1 proportions, or other types of diet (Orozco-Davila et al.
284 2015). Taking this and the results of the first experiment into consideration, we tested males
285 treated with methoprene at a 0.02% dose and a 24:1 S:P ratio. We corroborate that 5, 6 and 7 d-
286 old treated males presented higher mating percentages than the control. Similar results were
287 reported with *A. ludens* males when treated with methoprene and fed on a diet rich in protein
288 (Teal et al. 2007, Gómez 2013, Pereira et al. 2011). Segura et al. (2009, 2013), confirmed that
289 methoprene incorporated into the diet of *A. fraterculus* (Wiedemann) showed a significant effect
290 on the sexual development of males of this species. In contrast, methoprene had no effect on the
291 sexual maturity of *C. capitata* (Faria et al. 2008, Shelly et al. 2009). The authors mention that the
292 absence of a methoprene effect on sexual maturity may be due to the short pre-mating period (2-4
293 days) of this species, reducing the opportunity to enhance sexual maturity by adding methoprene.
294 Haq et al. (2010 a,b) reported that adding methoprene and protein to the diet produced synergic
295 effects on the mating behavior of *Bactrocera cucurbitae* males. Furthermore, other reports
296 document that methoprene and protein contributed significantly to the longevity and survival of
297 male *Bactrocera cucurbitae* flies, which improved after three days feeding (Haq et al. 2013 a,b).
298
299 In summary, for *A. obliqua* flies, we recommend the incorporation of 0.02% methoprene to a
300 24:1 sugar: protein diet. This treatment reduces the time required for sexual maturation by two
301 days, and enhances the mating propensity of treated males.
302
303
304
305
306

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Footnotes

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Table 1. Treatments used for evaluating the effect of methoprene and diet on the age-related mating propensity of *A. obliqua* males.

Methoprene concentration (%)	S:P ratios *	Replicates
0 (control)	3:1	8
	9:1	8
	24:1	8
0.01	3:1	8
	9:1	8
	24:1	8
0.02	3:1	8
	9:1	8
	24:1	8
0.05	3:1	8
	9:1	8
	24:1	8
0.1	3:1	6
	9:1	7
	24:1	7
0.2	3:1	6
	9:1	7
	24:1	7

*S:P, sugar: protein

Figure legends

Fig.1. Mean proportion of matings by *Anastrepha obliqua* males in relation to methoprene concentration (0.01, 0.02, 0.05, 0.1, 0.2% and control) in the diet. The data analysis was performed using the global results for the three ratio diets.

Fig.2. Mean (± S. E.) proportion of matings by *Anastrepha obliqua* males fed on different sugar: protein ratios (3:1, 9:1, 24:1), and methoprene concentrations (0, 0.01, 0.02, 0.05, 0.1, 0.2%) diets. The data analysis was performed using the global results considering all male age groups.

Fig.3. Mean (± S. E.) proportion of matings by *Anastrepha obliqua* males of different ages, fed with distinct sugar: protein ratios (3:1, 9:1, 24:1). The data analysis was performed with the global results considering matings with and without methoprene.

Fig.4. Age-specific mean (± S. E.) proportion of mating by *Anastrepha obliqua* males treated with 0.02 % methoprene and 24:1 (S:P) ratio diets.

Fig.5. Mean (± S. E.) of areas (Mcounts) of pheromone volatiles emitted by 10 males of *Anastrepha obliqua*. A) (*Z*)-3-Nonenol. B) (*Z*,*Z*)-3,6-Nonadienol. Males were treated with 0.02% methoprene and fed with 24:1 (S:P); control flies were not treated with methoprene.

Fig. 1.

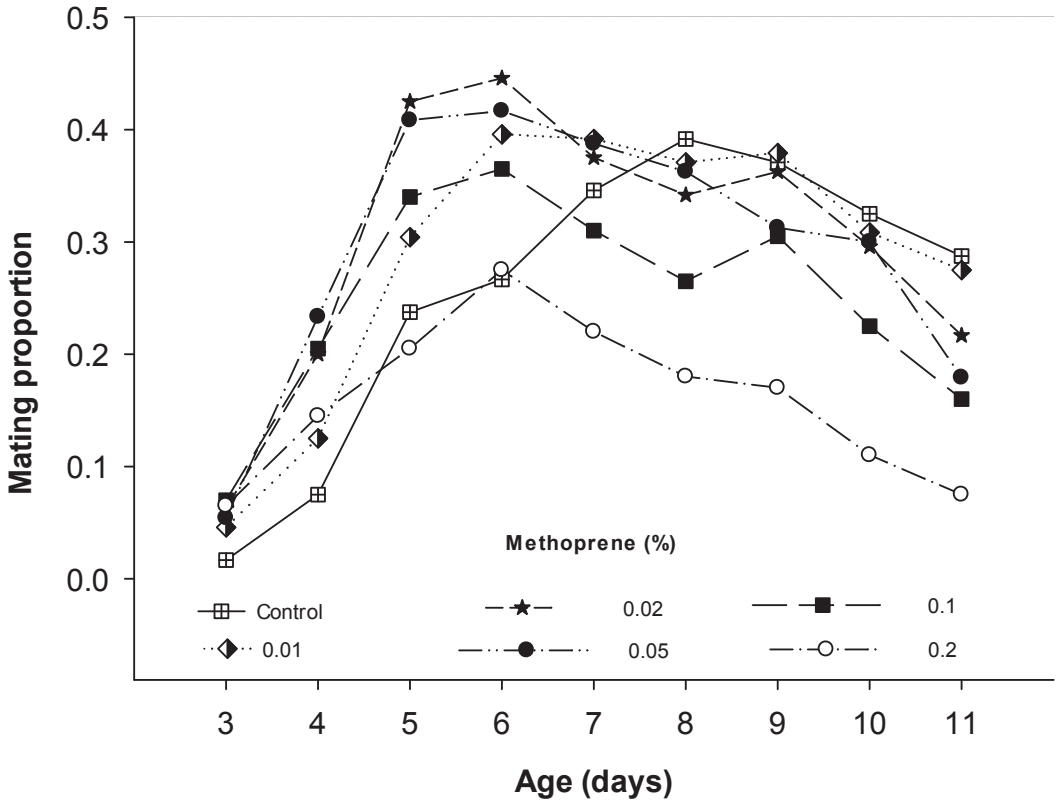

Mating proportion
0.5
0.4
0.3
0.2
0.1
0.0
Methoprene (%)
Control
0.01
0.02
0.05
0.1
0.2
3
4
5
6
7
8
9
10
11
Age (days)

Fig. 2.

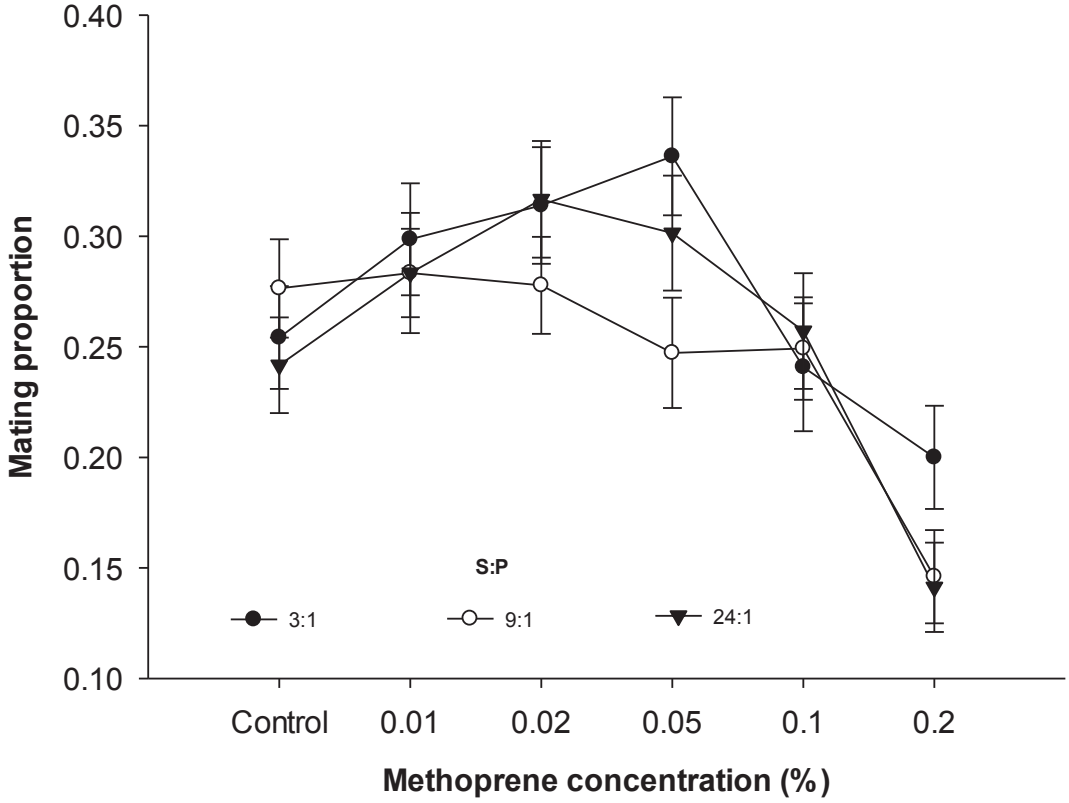

0.40
0.35
0.30
0.25
0.20
0.15
0.10
Mating proportion
S:P
3:1
9:1
24:1
Control
0.01
0.02
0.05
0.1
0.2
Methoprene concentration (%)

Fig. 3.

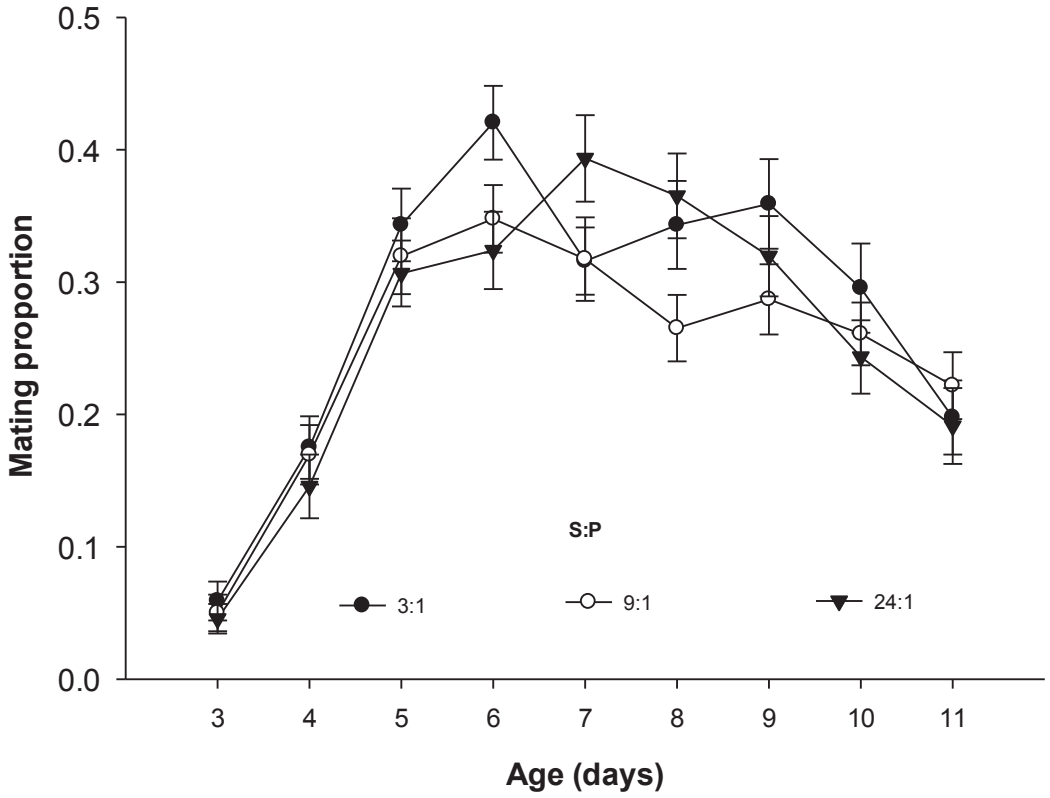
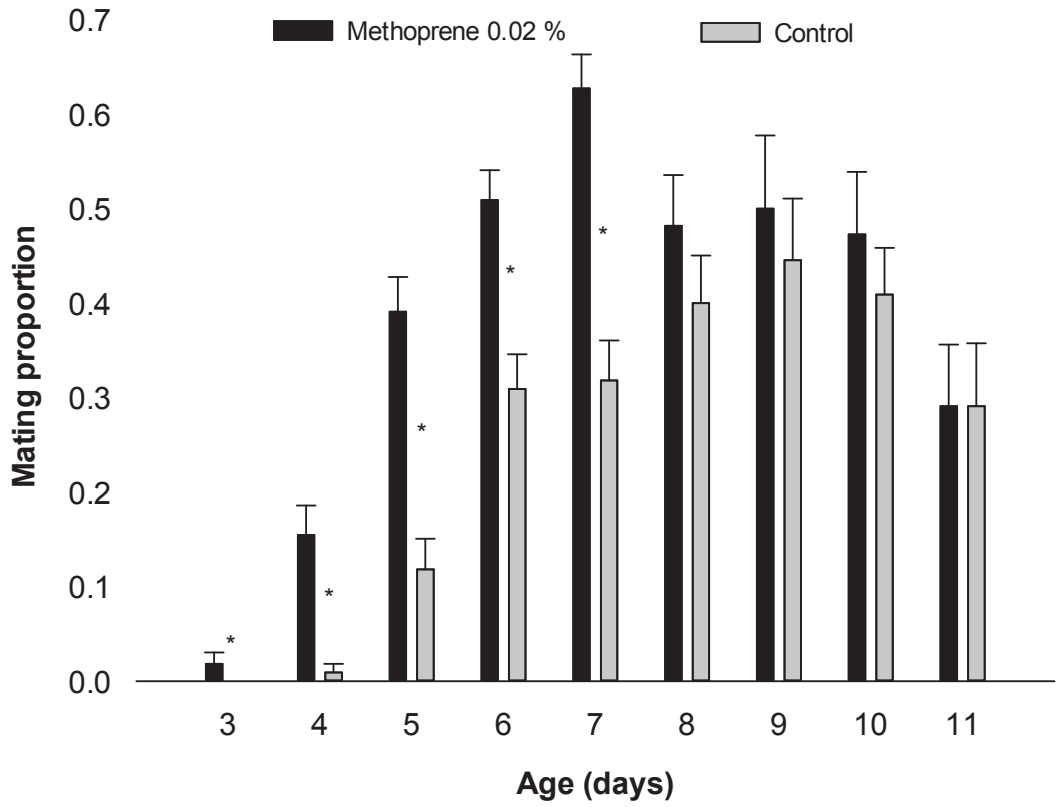
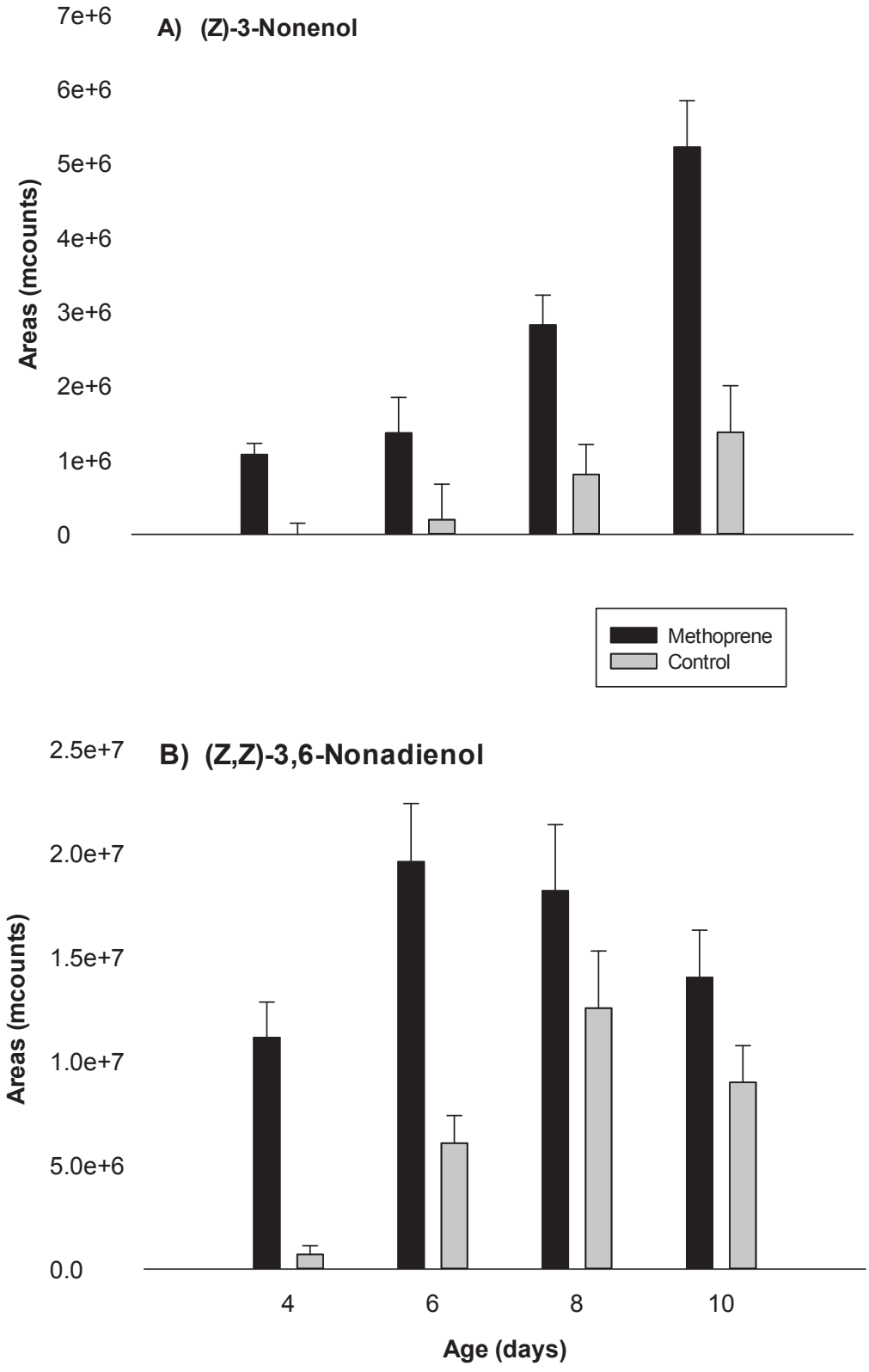

0.5
0.4
0.3
0.2
0.1
0.0
Mating proportion
S:P
3:1
9:1
24:1
3
4
5
6
7
8
9
10
11
Age (days)

Fig. 4.


Methoprene 0.02 %
Control
Mating proportion
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
3
4
5
6
7
8
9
10
11
Age (days)
*

649 Fig. 5.


A) (Z)-3-Nonenol
7e+6
6e+6
5e+6
4e+6
3e+6
2e+6
1e+6
0
Areas (mcounts)
Methoprene
Control
B) (Z,Z)-3,6-Nonadienol
2.5e+7
2.0e+7
1.5e+7
1.0e+7
5.0e+6
0.0
Areas (mcounts)
4
6
8
10
Age (days)

650

3. Conclusiones.

1. El metopreno (análogo de la hormona juvenil) incorporado en el alimento para machos de *A. obliqua*, aceleró la madurez sexual en esta especie, alcanzando los machos tratados su pico máximo de cópulas dos días antes que los machos no tratados.

2. La proporción máxima de cópulas alcanzada por los machos tratados con metopreno fue superior al pico máximo logrado por los machos control.

3. Los volátiles (*Z*)-3-nonenol y (*Z*,*Z*)-3,6-nonadienol fueron liberados en mayor proporción y anticipadamente en los machos tratados con metopreno que en los machos control.

4. Los volatiles (*Z*,*E*)-α-farnesene, (*E*,*E*)-α-farnesene y un isomero del farneseno, no fueron afectados por el uso del análogo de la hormona juvenil.

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