El Colegio de la Frontera Sur

Infección de adultos de *Anastrepha ludens* y *A. obliqua* con diseminadores de conidios de *Beauveria bassiana* en campo

TESIS

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Sergio Eduardo Campos Carbajal

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

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INDICE

I. Introducción

La mosca Mexicana de la fruta, *Anastrepha ludens* (Loew) y la mosca de las Indias Occidentales, *Anastrepha obliqua* (Macquart) son consideradas las plagas de mayor importancia económica en mango en México, debido al daño que ocasionan al infestar frutos de las variedades comerciales, lo que implica restricciones cuarentenarias para su exportación (Aluja 1993). El control de estas plagas se realiza bajo un enfoque de manejo integrado de plagas (MIP) que incluye trampeo, muestreo de frutos, control químico a base de cebo orgánico (i.e. GF-120), control mecánico mediante la destrucción de frutos, control biológico con liberación de parasitoides y aplicación de la técnica del insecto estéril (TIE) (Gutiérrez 2010).

Para algunos fruticultores, los insecticidas químicos son la herramienta convencional en el control de moscas de la fruta y otros insectos plaga. Sin embargo, los reportes de su impacto negativo sobre la entomofauna benéfica, su residualidad, el desarrollo de resistencia en algunas plagas, y la repercusión en la salud humana por su uso desmedido, han obligado a evaluar otras alternativas con menor impacto al ambiente (Shahid et al. 2012). Entre estas se incluye el uso de hongos entomopatógenos, dentro de los cuales destacan *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikoff) Sorokin y *Paecilomyces fumosoroseus* (Wize) Brown & Smith, que son reconocidos por provocar infecciones en dípteros (Castillo et al. 2000).

Estos patógenos se han utilizado como una estrategia de control que complementa a la técnica del insecto estéril (TIE), que consiste en inocular con conidios de hongos a machos estériles de moscas de la fruta (Flores et al. 2013, San Andrés et al. 2014, Toledo et al. 2017), o empleando dispositivos que atraen e infectan insectos y facilitan su infección (Dimbi et al. 2013, Ekesi et al. 2016). Una vez que son infectados los machos estériles o silvestres actúan como vectores al diseminar el inóculo y transmitirlo a individuos silvestres no infectados por medio del contacto a través de cópulas, intentos de cópulas y formación de leks.

El potencial que ofrecen los diseminadores es una estrategia conocida como “atracción e infección”, por lo que deben ser cebados con atrayentes eficaces como

paraferomonas o cebos alimenticos (Navarro-Llopis et al. 2015, Toledo et al. 2017). Con estos dispositivos se reduce la cantidad de conidios a utilizar, ya que solo actúan sobre los individuos que son atraídos y además el inóculo se puede proteger de factores ambientales adversos como la luz solar, lluvia, etc., lo que incrementa su viabilidad en condiciones naturales (Ekesi et al. 2007, Baverstock et al. 2010). Los diseminadores de conidios se han evaluado bajo condiciones de campo cebados con Trimedlure contra adultos de *Ceratitis capitata* (Wiedemann) con excelentes resultados al obtenerse hasta ~48% de infección (Quesada-Moraga et al. 2008, Toledo et al. 2017).

El objetivo de esta investigación fue determinar la infección de adultos de *Anastrepha ludens* y *A. obliqua* con diseminadores de *B. bassiana* en campo utiizando diseminadores de *B. bassiana* cebados con conidios. El estudio se realizó en tres fases: 1) Se comparó la efectividad de dos tipos de diseminadores de conidios en jaulas de campo; 2) Evaluación de diferentes densidades de diseminadores en condiciones de campo; y 3) se comparó la eficacia de dispositivos diseminadores contra el trampeo masivo en el control de *A. ludens* y *A. obliqua*.

II.

Infección de adultos de *Anastrepha ludens* y *A. obliqua* con diseminadores de conidios de *Beauveria bassiana* en campo

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Running Head:

Campos et al.: Infection of fruit flies with *B. bassiana*

Infection of Adults of *Anastrepha ludens* and *A. obliqua* with *Beauveria bassiana* disseminator devices in Mango orchard.

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Abstract

The efficacy of dissemination devices was evaluated in terms of the transmission of *Beauveria bassiana* conidia to sterile and wild adults of *Anastrepha ludens* and *A. obliqua*. In field cages were compared the infection as result of the use of cylindrical and panel type devices. In mango orchards we determined the relationship between cylindrical device density and percentage of fly infection, and compare the efficacy of such devices as control method regarding the use of mass trapping. In the field cages, the cylindrical device caused greater infection in *A. ludens* than the panel type device. Under field conditions, the level of infection of sterile flies increased directly with device density, although these differences were not significant. Infection caused by the dissemination devices was 30.7 and 31.5% in sterile *A. ludens* and *A. obliqua*, respectively. No significant difference was found between the cylindrical devices and mass trapping as control strategy. The viability of the conidia in the devices under field conditions varied from 93.0 to 70.1% after 28 days. Our results suggest that the use of ten dissemination devices per hectare, with conidia of the *B. bassiana*, represents a strategy that could be integrated in a management program of fruit flies of the genus *Anastrepha*.

Key words: autoinoculators, fruit flies, viability, entomopathogenic fungi, mango.

Introduction

Fruit flies of the genus *Anastrepha* (Diptera: Tephritidae) are distributed in tropical and subtropical regions of America, with approximately 200 species (Norrbom et al. 1999). Among the most important of these flies are the Mexican fruit fly, *Anastrepha ludens* (Loew), which attacks orange [*Citrus sinensis* (L.) Osbeck] and mango (*Mangifera indica* L.), and the West Indian fruit fly, *A. obliqua* (Macquart), which infests the yellow mombin (*Spondias mombin* L.), purple mombin (*Spondias purpurea* L.), Guava (*Psidium guajava* L), and mango (Aluja et al. 2014). Both fruit fly species cause economic losses in these crops (Aluja and Liedo, 1986).

Control strategies against fruit flies include application of the sterile insect technique (SIT), biological control through the release of parasitoids and the use of toxic baits (e.g. GF-120), complemented with bait stations to reduce wild populations (Flores et al. 2011). Another biological control option are some strains of *Beauveria bassiana* Bals. (Vuill.) and *Metarhizium anisopliae* Mestch. (Sorokin) that have showed potential for the control of species of Diptera, Coleoptera, Lepidoptera and Orthoptera (Maniania 1991). The entomopathogenic fungi represent an option for the control of pests in both conventional and organic agricultural systems (Lomer et al. 2001, Meyling and Eilenberg 2007, Flores et al. 2013). The viability and transmission capacity of the pathogen determines the range of propagation in the host, based on the application method (Vega et al. 2000, Toledo et al. 2007, Quesada-Moraga et al. 2008). This new approach can be conducted with dissemination devices or with sterile insects inoculated in order to act as vectors (Vega et al. 2000, Maniania 2002, Toledo et al. 2017). Sterile males of *Ceratitis capitata* (Wied.) inoculated with *B. bassiana* conidia were released over large areas in Guatemala, showed high potential for horizontal transmission towards wild flies (Flores et

al. 2013).

Conidia dissemination devices have been developed in order to attract and infest pest insects so they can subsequently contaminate other conspecific individuals (Vega et al. 2000, Dimbi et al. 2003). Dispersion of fungal conidia by disseminators devices and sterile insects as vectors, are other alternatives proposed for the control of fruit flies (Toledo et al. 2007, 2017).

The aims of this study were: (1) to evaluate transmission of *B. bassiana* conidia in *A. ludens* and *A. obliqua* with disseminator devices, (2) to evaluate the efficiency of different device densities per hectare, in terms of fruit fly control, and (3) to compare their effectiveness against a mass trapping system baited with Ceratrap® in mango orchards.

Materials and methods

Biological material. The *B. bassiana* strain Bb-Et was obtained from the Laboratory of Beneficial organisms located in Tuxtla Chico, in Chiapas, Mexico. It was formulated with Celite 400 dust at a concentration of 2.9×10^9 conidia/g and an initial viability of ≥90%. The Moscafrut Plant, located in Metapa de Domínguez, Chiapas, Mexico, provided *A. obliqua* and *A. ludens* pupae dyed with Aurora Pink (DayGlo, Color Corp., Cleveland, OH, USA) colorant and irradiated at 80 Gy with a Cobalt 60 source in a gamma beam irradiator (model GB-127 Nordion International Inc., Ottawa, Ontario, Canada), from which emerged sterile fruit fly adults.

Infection of adults with disseminators of conidia in field cages. In order to estimate the transmission of fungal conidia to fruit flies, tests were conducted in field cages (3 m in diameter X 2 m in height) with two mango trees (1.25 m in height) inside each cage.

Recently emerged *A. obliqua* and *A. ludens* adults were separated by sex in glass cages (30 X 30 X 30 cm) and provided with water and a 1:3 mixture of hydrolyzed protein (ICN Biomedicals, Costa Mesa, CA) and sugar as food. These cages were maintained for seven days at 25 ± 2 °C at a photoperiod of 12:12 hours light: darkness prior to release of the flies into field cages.

The conidia disseminators were developed by Toledo et al. (2017) for *C. capitata* and adapted for evaluation purposes as described below:

a) Panel type device: This consisted of a piece of waxed cardboard (23 X 14 cm), covered with a yellow felt cloth over which two grams of the fungal formulate was distributed. A 3 cm diameter perforation was made in the center of the cardboard in order to place a small plastic basket with cotton wicks impregnated with 10 ml of the enzymatic hydrolyzed protein Ceratrap® (Bioibérica, Barcelona). To protect the device from the sun and rain, a 20 cm diameter yellow colored plate was fixed to the upper part by inserting the wire of the hook through the plate.

b) Cylinder type device: This consisted of a plastic cylindrical receptacle of capacity 500 ml with two 3.3 cm diameter holes on the mid part, covered with yellow felt cloth over which two grams of the fungal formulate were deposited. Inside the receptacle, 90 ml of Ceratrap were placed. The devices were also protected from the sun and rain with a plastic plate through which the hook wire was inserted.

The disseminator devices were exposed to field conditions (28.2 - 31.3 °C and precipitation of 376.6 mm) below the mango tree canopy. In each field cage we hanged one type of disseminator and 25 pairs of flies of each species were released. In order to differentiate between species, the adults were marked by supplying them with three grams of solid food (hydrolyzed protein + sugar) mixed with 30 µl of red vegetal colorant

127 McCormick® (McCormick & Company, Mexico) for *A. obliqua* and green colorant for *A.*
128 *ludens* (Novelo-Rincón et al. 2009). The released insects remained in the field cages
129 exposed to the devices for five hours, and were subsequently collected in Plexiglass cages
130 (30 x 30 x 30 cm) with water and food. The cages containing the flies were kept under
131 laboratory and mortality recorded over ten consecutive days. Dead flies were removed from
132 the cages and placed in moist chambers (Petri dishes with absorbent paper saturated with
133 distilled water) in order to facilitate growth of the fungus. Six days later, the presence of the
134 fungus was verified through observations made under a stereoscopic microscope. For each
135 type of disseminator, six repetitions were realized.
136 **Evaluation of different densities of disseminator devices under field conditions.** Three
137 densities of cylindrical devices were evaluated, since the highest fly infection was obtained
138 with this device in the previous experiment. Over six weeks (from January to March 2016),
139 treatment densities of 5, 10 and 20 devices per hectare were evaluated, as control we used
140 10 devices without conidia. Each treatment consisted of three replicates, giving a total of 12
141 experimental units. Each experimental unit consisted of a 1 ha plot within the mango cv.
142 Ataulfo orchards "Los Ángeles" (15 ha, 14° 46' 39" N and 92° 21' 25" W) and "El Tesoro
143 II" (130 ha, 14° 43' 27" N and 92° 20' 9" W), in the municipality of Tapachula, Chiapas
144 México.
145 Every week, 400 pupae of *A. obliqua* and 400 pupae of *A. ludens*, from which
146 emerged approximately 375 flying adults, were placed in Kraft No. 20 paper bags
147 (Morysan®). Within each bag was placed a strip (0.3 x 0.3 m) of Marlin paper impregnated
148 with the food Mubarqui® (FAO 2007) and another wrinkled strip (0.3 X 1.0 m) of Marlin
149 paper as a rest area. The bags remained for seven days at 25 ± 2 °C and RH of 80 ± 5% so
150 that the adult flies could reach sexual maturity. Each week, 1500 flying adults (750 pairs)

per species, distributed in four bags, were released at 20 meters from the central point of the plot, one bag towards each of the four cardinal points. In order to monitor the flies, the day after release, two Multilure® (Better World Manufacturing) traps baited with Ceratrap protein were installed at opposite positions15 m from center of each plot. The traps were inspected on the fourth and seventh day, replacing the attractant at each inspection in order to reduce the risk of contamination between captured individuals. The captured flies were recorded per species and sex, then washed with sterile water and placed in a humid chamber (a Petri dish with wet filter paper) to facilitate development of *B. bassiana* and corroborate infection by presence of hyphae and spores on the flies using a stereoscopic microscope..

Comparison between the use of devices and mass trapping under field conditions.

A density of 10 cylindrical devices per ha (density selected from the previous experiment) was compared with mass trapping, which consisted in ten devices per hectare distributed uniformly; each device consisted of a recycled 600 ml polyethylene terephthalate (PET) bottle with three 1 cm diameter holes cut into the upper third and baited with 200 ml of Ceratrap protein in each plot. The experiment was conducted from April to June 2016 in the mango cv. Ataulfo orchard "Galicia" (80 ha, 14° 43' 47" N and 92° 17' 33" W), where nine 1 ha plots were delimited. The treatments were as follows: 1) dissemination devices, 2) mass trapping 3) Control (without treatment). In the treatment with disseminators, 10 cylindrical devices were prepared as described in previous experiments and installed. For each treatment, three replicates (three 1 ha plots) were conducted. The disseminators and PET bottles were re-baited every 21 days. The release of sterile *A. ludens* and *A. obliqua* was conducted as described in the previous experiment. The day after release, two Multilure traps were installed per ha, baited with Ceratrap protein and inspected and re-

baited every third day. The collected material was transported to the laboratory for identification and quantification. Specimens were washed with sterile water and then placed in a moist chamber to promote fungal growth and corroborate *B. bassiana* infection.

Viability of conidia

In parallel to the evaluation of devices in the field, the conidia viability was determined. Once installed in each plot, three devices were chosen at random number and sampled at 0, 7, 14, 21 and 28 days of exposure, by scraping part of the felt cloth of the devices with a sterile bacteriological inoculation loop. Each conidia sample was placed in an Eppendorf tube with 3 ml of sterile distilled water and transported to the laboratory. The samples were agitated for two minutes at 250 RPM in a Vortex (Genie 2 Scientific Industries USA) and two drops of the resulting fungal suspension were placed on the microculture with the culture medium Saboraud Dextrose Agar, and maintained for 24 h at 25 ± 2 °C. Germination was quantified 14 h after conducting the microculture, with three counts taken using a Neubauer chamber (Hemacytometer) (Jiménez 1989, Wright et al., 2007). A conidium was considered to have germinated when the length of the germinative tube reached at least twice the diameter of the conidium (Inglis et al. 2012).

Data analysis

The normalized percentages of infection and percentages of captured were arcsine$\sqrt{X}$ transformed and subjected to an analysis of variance (ANOVA) of one factor. Subsequent comparison of means was conducted with the Tukey method at a 95% level of significance ($\alpha = 0.05$). Conidia viability, expressed as a percentage, was subjected to a correlation analysis considering viability as the dependent variable and time of exposure as the independent variable. All analyses were conducted using the program JMP 7.0 (SAS

198 Institute 2007).

199 **Results**

200 **Infection of adults with conidia disseminators in field cages.** The percentage of infection
201 of *A. obliqua* with the panel and cylindrical disseminators was 22.34 ± 2.97% and 24.09 ±
202 2.34%, respectively, with no significant difference between values (F = 0.04; d.f. = 1, 36; P
203 = 0.832). For *A. ludens*, with the panel type device, a percentage of infection of 8.0 ± 1.2%
204 was found, but with the cylindrical device this value was 14.4 ± 2.6%. The difference
205 among these values was significant (F = 4.56; d.f. = 1, 36; P = 0.039) (Fig. 1).

206 **Evaluation of different disseminator densities under field conditions.** The capture of
207 sterile adults, expressed in percentages, presented a significant difference among treatments
208 for *A. ludens* (F = 4.83; d.f. = 3, 80; P = 0.004) and *A. obliqua* (F = 8.32; d.f. = 3, 80; P <
209 0.001). In both species, there was greater capture in the control compared to the three
210 device densities while no significant difference was found among densities (Fig. 2).
211 Infection of sterile *A. ludens* adults was 29.3%, 32.1% and 49.7%, with 5, 10 and 20
212 devices per hectare, respectively. However, the differences among these values were not
213 significant (F = 2.98; d.f. = 2, 60; P = 0.058) (Fig. 3). In the case of *A. obliqua*, infection of
214 adults was 19.5%, 24.31% and 28.4% for 5, 10 and 20 devices, respectively. While
215 percentage of infection increased as a function of density, the values obtained did not differ
216 significantly (F = 0.30; d.f. = 2, 60; P = 0.744) (Fig. 3). Sporulation was not recorded in the
217 captured flies in control plots.

218 **Comparison between disseminators and massive trapping under field conditions.** The
219 percentage capture of sterile *A. ludens* was greater in the control than in the areas with
220 disseminators or massive trapping. The differences were significant between treatments (F
221 = 19.9; d.f. = 2, 15; P < 0.001). For sterile *A. obliqua*, no significant difference was found

between percentages of capture in the different treatments (F = 3.33; d.f. = 2, 15; P = 0.064) (Fig. 4).

The percentage of infection of sterile adults of *A. ludens* with disseminators was 30.7 % (354 infected from 1151 adults captured), while for *A. obliqua* this value was 31.5 % (323 infected from 1026 adults captured). The difference between these values was not significant (F = 0.003; d.f. = 1,10; P = 0.956).

Conidia viability

Conidia viability reduced significantly over time in disseminators, from 93.0 to 70.1% after 28 days of exposure under field conditions. Differences between these values were significant (Fig. 5) (F = 443.1; d.f. = 4, 40; P < 0.001).

Discussion

Our results show that under field conditions, the disseminators evaluated (particularly the cylindrical type) were effective at attracting and infecting adults of *A. ludens* and *A. obliqua*, and that the conidia on the surface of the devices can remain viable for up to three weeks and can cause infection in 25 to 30% of adults in the population. Equally, the use of ten cylindrical devices per hectare was found to be as effective as massive trapping for the control of both fruit fly species in mango orchards.

Of the two device designs evaluated, the cylindrical type produced greater infection in adults of both fly species (statistically significant difference in *A. ludens* only), which is considered a consequence of the quantity of attractant used (90 ml in the cylindrical *vs.* 10 ml in the panel type device), although it has been reported that *A. obliqua* is more attracted to cylinders or spherical forms than to flat surfaces (López-Guillén et al. 2009). When different devices treated with conidia of *M. anisopliae* in field cages were evaluated, up to 90 % infection was recorded in both females and males of *C. capitata*

(Quesada-Moraga et al. 2008). For their part, Dimbi et al. (2003) evaluated a plastic cylindrical tube covered with velvet cloth impregnated with the conidia of *B. bassiana* y/o *M. anisopliae* under laboratory conditions and observed ranges of mortality from 7 to 100%, 11 to 100% and 72.78% for *C. capitata*, *C. fasciventris* (Karsh) and *C. cosyra* (Walker), respectively. In addition, in field cages with devices containing *M. anisopliae* conidia on maize cob, gauze or Petri dishes, these authors obtained ranges of mortality of 70 to 93% in *C. capitata* and *C. fasciventris*.

Regardless of design, device efficacy depends to a large extent on the fly attractant utilized (Baverstock et al. 2010), although the combination of device type and attractant used is essential to the efficacy of the strategy (López-Guillén et al. 2009; Lasa et al. 2014). For other species, such as *C. capitata*, *Bactrocera dorsalis* (Hendel), the parapheromones that have been formulated are highly efficient for attracting males and are of long duration in autoinoculators in the field (Vargas et al. 2012). For species of the genus *Anastrepha*, there are currently only attractants of the food type, which are less efficient. For this reason, it is a priority to evaluate more effective compounds that last longer in the field (Lasa et al. 2014, Gonzalez et al. 2016). Development of this strategy will enable an improvement to this approach of conidia application and should help to reduce management costs.

The direct relationship between device density and levels of infection in adults of *A. ludens* and *A. obliqua* is based on the distance that exists between the points of attraction (disseminator devices), which determines the efficacy of the baits (Suckling et al. 2014). Similar results were obtained with the detection systems, where effectiveness is directly related to trap density (Lance and Gates 1994). Ekesi et al. (2007) reported 80% infection in adults of *Bactrocera invadens* Drew, Tsuruta & White, when using an autoinoculator of

M. anisopliae conidia baited with liquid protein and installed in trees at a density of 40 devices per hectare. Likewise, 24 autoinoculators of *M. anisopliae* conidia baited with trimedlure per hectare produced effective control in wild populations of *C. capitata* (Navarro-Llopis et al. 2015). In mango orchards, the use of devices with a commercial formulate of *M. anisopliae* (Met 69) at 30 devices per hectare produced a reduction in the population of *B. dorsalis* of between 79.1 and 94.6%, and fruit infestation of between 6.4 and 11.6% compared to that of between 61.7 and 62.2% recorded in non-treated orchards (Ekesi et al. 2016).

A similar reduction in the capture of *A. ludens* and *A. obliqua* was achieved using the mass trapping strategy, indicating that both strategies are equally effective in the control of these pests. The efficacy of both strategies is based on the initial attraction of the flies, but in mass trapping, the devices attract the flies, which are immediately eliminated (Heath et al. 2009, Navarro-Llopis et al. 2013). In contrast, with the autoinoculators, the insects that are attracted become infected but, before dying, disseminate the fungus to other individuals of the same species thus increasing the degree of infection and occasionally generating epizooty among adults of the pest (Maniania 2002, Toledo et al. 2007, Quesada-Moraga et al. 2008, Baverstock et al. 2010, Lacey et al. 2015).

In our study, conidia viability was 70% following four weeks of exposure in field conditions. This supports that reported for *M. anisopliae* (Ekesi et al. 2007), which presented 68% viability in an autodisseminator after five weeks of exposure, while Maniania (1998) reported that, in infection chambers of *Glossina*, conidia viability was 80% after 21 days (20% loss of viability). In general, it is assumed that solar radiation is the main factor behind conidia viability loss in the devices (Ekesi et al. 2007), although Maniania **(**2002) exposed disseminator devices to the sun and shade for 31 days and found

294 that viability reduced from 86% to 62% and 86% to 43%, respectively, which was
295 attributed mainly to high humidity that condensed on the surface and could induce
296 germination of the conidia. Loss of conidia viability was evaluated by Navarro-Llopis et al.
297 (2015), who reported that *M. anisopliae* conidia placed on a semi-solid carboxymethyl
298 cellulose gel including an attractant (TML) had infected 30% of *C. capitata* males after
299 three months.

300 To increase and prolong the useful life of the device and maintain the conidia
301 viability using some form of protection such as the semi-solid carboxymethyl cellulose gel
302 or coconut oil in order to reduce viability loss (Fernandes et al. 2015, Navarro-Llopis et al.
303 2015).

304 In conclusion, this study demonstrated that the use of disseminator devices can be a reliable
305 strategy to control fruit fly populations, being the cylindrical devices the most effective for
306 attracting and infecting fruit flies of the genus *Anastrepha*. We also determine that a density
307 of 10 devices per hectare is as effective as the use of massive trapping to control fruit fly
308 pets in mango orchards.

309

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Figure captions

Figure 1. Percentage of infection of adults (average ±SE) of *Anastrepha ludens* and *Anastrepha obliqua* exposed to disseminator devices in field cages. For each species, bars with different letters indicate statistical differences ($\alpha = 0.05$).

Figure 2. Capture of sterile adults (average ±SE) of *Anastrepha ludens* and *Anastrepha obliqua* using three densities of conidia disseminator devices (5, 10, 20 devices per ha) and a control under field conditions. For each species, bars with different letters indicate statistical differences ($\alpha = 0.05$).

Figure 3. Percentage of infection of adults (average ±SE) of *Anastrepha ludens* and *Anastrepha obliqua* exposed to three densities of disseminator devices (5, 10, 20 devices per ha) in the field. For each species, bars that share letters do not present significant differences on comparison between the two devices ($\alpha = 0.05$).

Figure 4. Capture of sterile adults (average ±SE) of *Anastrepha ludens* and *Anastrepha obliqua* in areas treated with disseminator devices, bait stations and a control under field conditions. For each species, bars with different letters indicate statistical differences ($\alpha = 0.05$).

Figure 5. Conidia viability of *B. bassiana* in cylindrical devices installed in mango orchards at 0, 7, 14, 21 and 28 days of exposure.

Figure 1

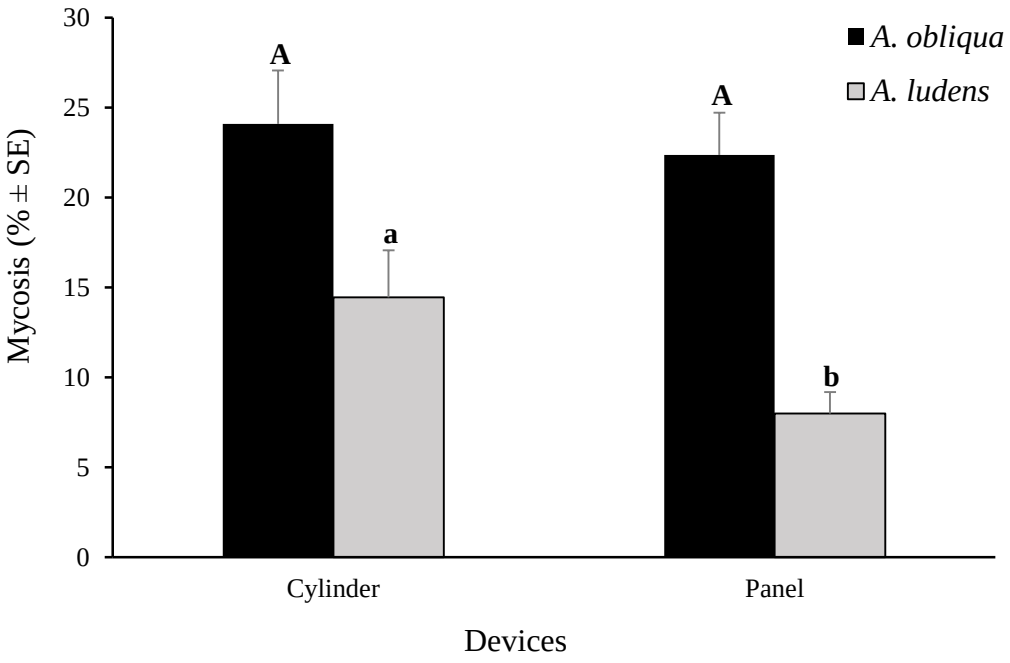

30
25
20
15
10
5
0
Mycosis (% ± SE)
A
a
A
b
A. obliqua
A. ludens
Cylinder
Panel
Devices

Figure 2

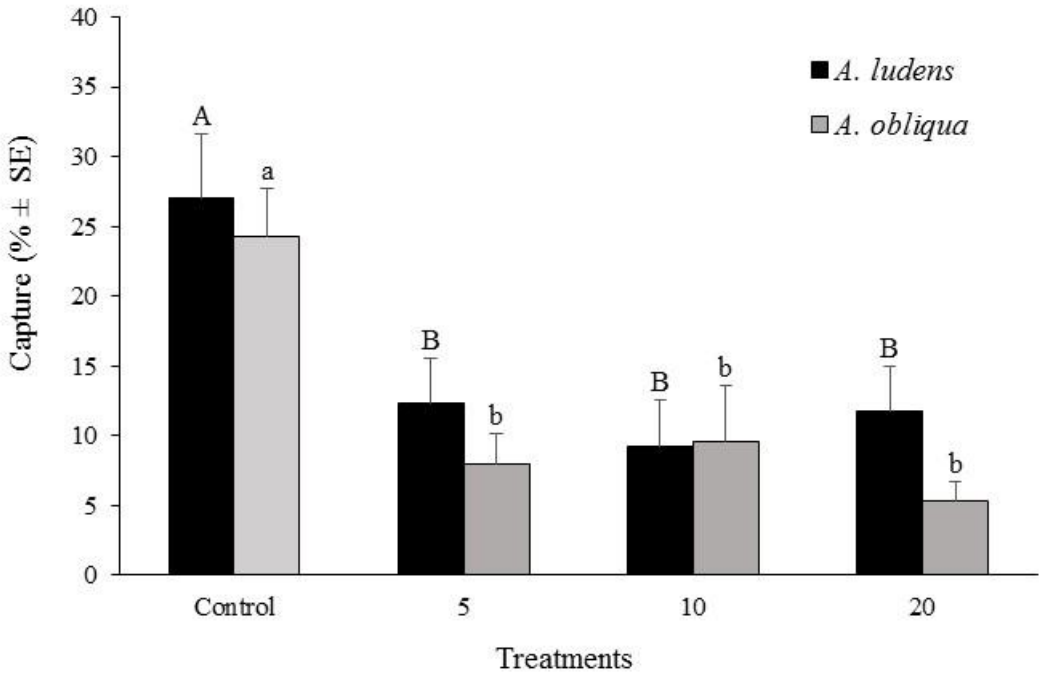

40
35
30
25
20
15
10
5
0
Capture (% ± SE)
A
a
B
b
B
b
B
b
A. ludens
A. obliqua
Control
5
10
20
Treatments

Figure 3

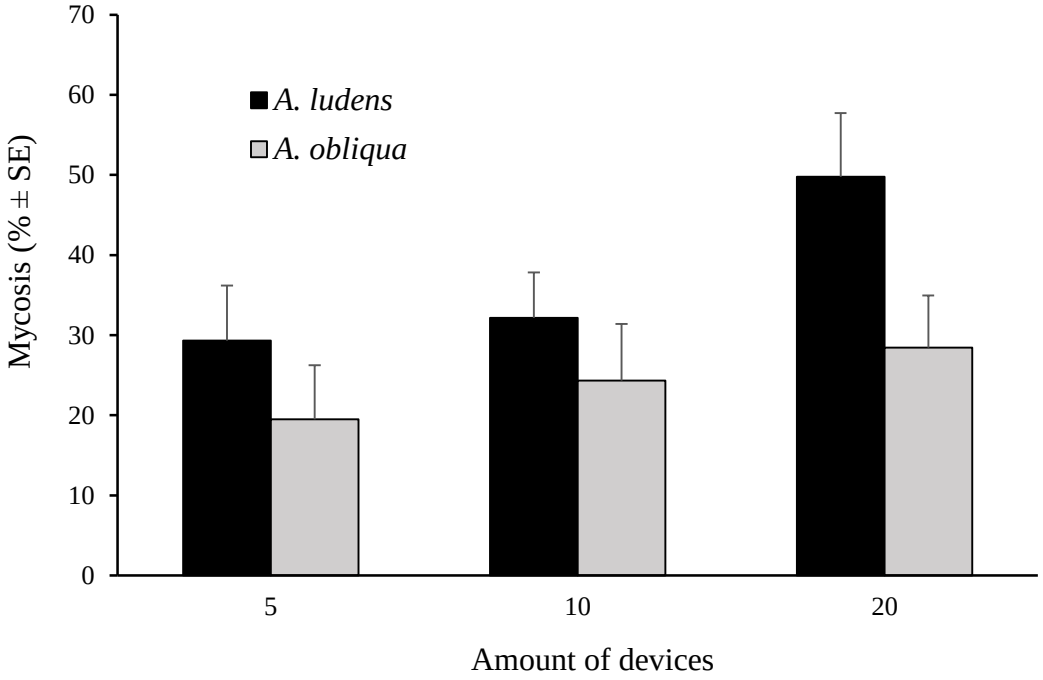

70
60
50
40
30
20
10
0
Mycosis (% ± SE)
A. ludens
A. obliqua
5
10
20
Amount of devices

Figure 4

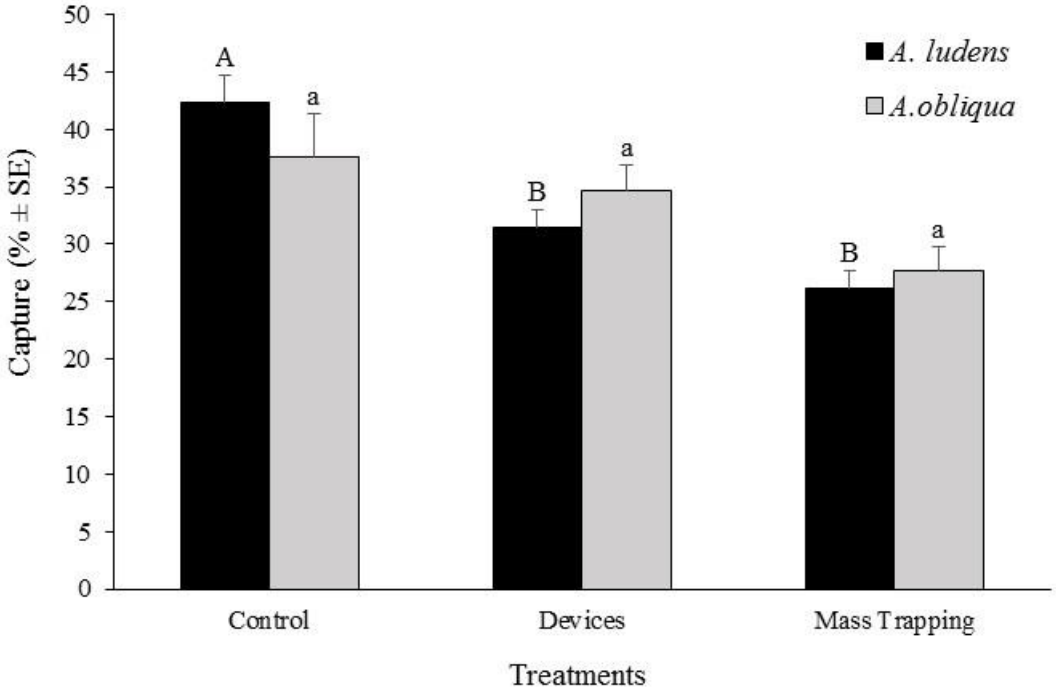
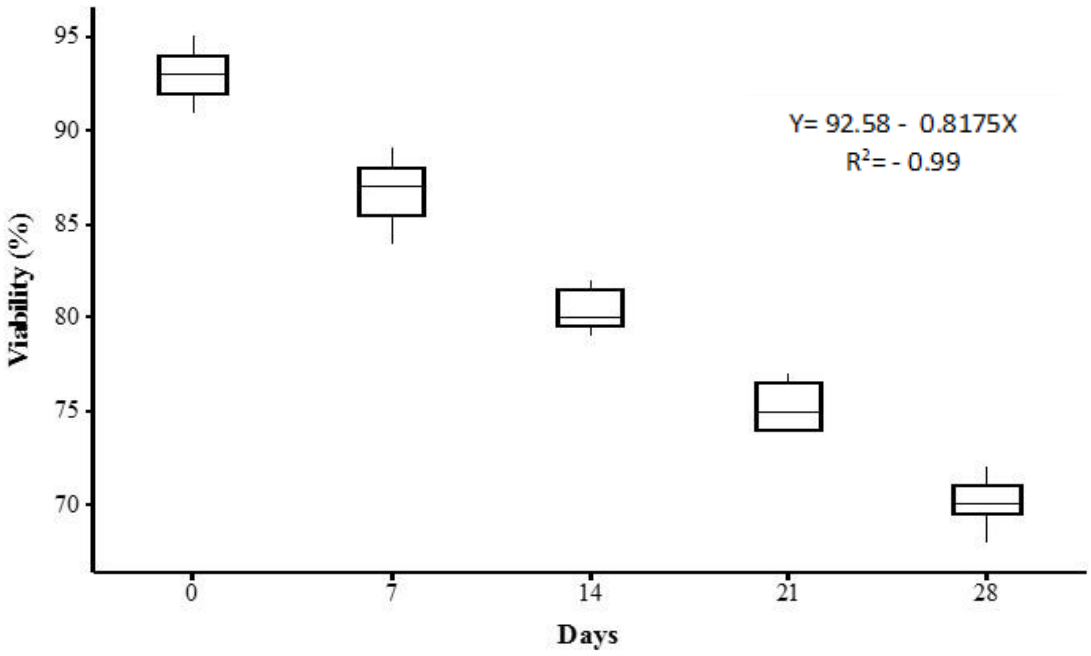

50
45
40
35
30
25
20
15
10
5
0
Capture (% ± SE)
A
a
B
a
B
a
A. ludens
A.obliqua
Control
Devices
Mass Trapping
Treatments

Figure 5



III. Discusión

La Técnica del Insecto Estéril (TIE) es un método de control de plagas considerado amigable por ser especifico. Para su aplicación se requiere de la producción masiva y liberación de millones de insectos estériles para reducir gradualmente la población silvestre de la plaga. Su éxito depende de la competitividad de los machos estériles liberados (Lance et al. 2000, Orozco et al. 2002) y una alta relación estéril: silvestre para favorecer los apareamientos entre machos estériles y hembras silvestres (Hendrichs et al. 2005). Sin embargo, con frecuencia se ha observado que ocurren intentos de apareamientos no exitosos. (Novelo-Rincón 2009). En el caso de los dispositivos diseminadores de *B. bassiana* son eficaces para la atracción de *A. ludens* y *A. obliqua* lo que depende en gran medida del tipo de atrayente (Lasa et al. 2014). En las pruebas de campo se presenta reducción en capturas y se presenta infección de *B. bassiana* en las tres densidades evaluadas, siendo 10 dispositivos una densidad adecuada para inducir infección en moscas estériles obteniéndose 32.1% de infección en *A. ludens* y 24.3% para *A. obliqua*. (Ekesi et al. 2007) reportaron transmisión de conidios utilizando 40 dispositivos por hectárea, mientras que Navarro-LLopis et al. (2015) reportaron que con 24 dispositivos se controla en forma eficaz las poblaciones de *C. capitata.*

Al comparar el trampeo masivo con los diseminadores no se encontraron en capturas diferencias entre ambos, con los dispositivos la infección en adultos estériles recapturados fue de 30.7% para *A. ludens* y 31.4% para *A. obliqua*. La pérdida de viabilidad en este estudio fue de 22.9% durante 28 días de exposición en condiciones de campo. Ekesi et al (2007) reportan una disminución en la viabilidad de 22% en los dispositivos después de cinco semanas de exposición.

Uno de los efectos adicionales de los hongos entomopatógenos es la disminución de la fecundidad de las hembras infectadas. Castillo et al. (2000) obtuvieron 40 - 50% de reducción en *C. capitata* con *M. anisopliae*. Sookar et al. (2014) reportaron que contra *B. cucurbitae* se redujo la producción de huevos, pero la fertilidad (eclosión de huevos) no fue afectada. Toledo et al. (2007) reportaron que la fecundidad de *A. ludens* fue menor (36.5 h/d) en las moscas tratadas con *B. bassiana* mientras que las del control

tuvieron una mayor fecundidad (52.6 h/d), y la fertilidad no fue afectada. Ouna (2011) reportó que la fecundidad de *Bactrocera zonata* fue menor en moscas tratadas (6.8 huevos/día) comparadas con las moscas del control (28.4 huevos/día), también hubo una reducción en la fertilidad de las moscas tratadas. Por lo tanto, el uso de machos estériles como vectores de conidios o el uso de diseminadores de conidios de hongos (*B. bassiana* o *M. anisopliae*) representa una opción viable y eficaz (Ekesi et al. 2007). El beneficio de esta estrategia está basado en la incorporación de un entomopatógeno como un factor de mortalidad de la plaga (Toledo et al. 2017). Ambas estrategias de control evaluadas contra *C. capitata* utilizando insectos vectores o con dispositivos diseminadores se observó un efecto en la trasmisión de conidios hacia la población silvestre. (Flores et al. 2013, Toledo et al. 2017).

El potencial de este enfoque se ilustra con el modelo teórico de Knipling (1955) incluyendo la TIE y el uso de hongos entomopatógenos (Tabla 1). La población natural sin control aumenta en tamaño exponencialmente. En el caso del control con *Beauveria bassiana* puede observarse una reducción de la población gradual y si se integra con la TIE el nivel de control podría ser mayor a 90%, mientras que si utilizamos la TIE con *B. bassiana* se observa el efecto de ambos métodos de control. Considerando que los dispositivos diseminadores ocasionan 40% de infección sobre adultos de la población, después de 4 generaciones se ocasionará una supresión del 87% de la población. Integrando ambas estrategias se estima una supresión de 99.99%. Al combinar las estrategias los adultos infectados pueden diseminar los conidios en hojas, refugios, y otros lugares de agregación de las moscas. En estos sitios es difícil hacer aplicaciones de insecticidas o llevar a cabo otras acciones de control.

Tabla 1. Modelo teórico de Knipling del efecto de los diseminadores de *B. bassiana* más la TIE

	Control (100%)	**Bb (60%)**		**TIE (10%)**		**TIE+ Bb (6%)**	
Generación	**N**	**N**	% Supresión	**N**	% Supresión	**N**	% Supresión
P	1,000	1,000	0	1,000	0	1,000	0
F1	5,000	3,000	40	500	90	300	94
F2	25,000	9,000	64	250	99	90	99.6
F3	125,000	27,000	78	125	99.9	27	99.9
F4	625,000	81,000	87	62.5	99.99	8	99.9

IV. Conclusiones

1.- Los dispositivos diseminadores fueron atractivos a ambas especies de moscas de la fruta, con el dispositivo cilíndrico se logró la mayor transmisión de *Beauveria bassiana* de 14.4 y 24.0% para *Anastrepha ludens* y *Anastrepha obliqua* en condiciones de jaulas de campo.

2.- Bajo condiciones de campo, las densidades de 5, 10, y 20 dispositivos por ha redujeron las capturas de *A. ludens* y *A. obliqua,* estériles, en comparación con el Control. La densidad de 10 dispositivos resulto ser eficiente.

3. Conforme se aumentó la densidad de dispositivos, se incrementó la trasmisión de *Beauveria bassiana,* aunque las diferencias no fueron significativas.

4.- Al comparar los dispositivos diseminadores con el trampeo masivo (EC) no hubo diferencia significativa, lo que indicó que ambas estrategias son eficaces para el control de moscas de la fruta.

5. En las parcelas donde se instalaron diseminadores de *Beauveria bassiana* se registró una infección de 30.7% y 31.5% en adultos estériles de *A. ludens* y *A. obliqua*, respectivamente.

6. La viabilidad del hongo sufrió una disminución gradual con relación al tiempo de exposición. Después de 28 días bajo condiciones de campo tuvo 22.9% de pérdida de viabilidad.

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