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Evaluation of mass trapping and bait stations to control *Anastrepha* (Diptera: Tephritidae) fruit flies in mango orchards of Chiapas, Mexico

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Abstract

The use of insecticide baits is one of the most common and efficient methods worldwide to control fruit flies (Diptera: Tephritidae). However, this strategy has commonly been associated with environmental contamination and undesirable effects on non-target organisms. The use of lure-and-kill devices (bait stations) or mass trapping could be alternative control methods to overcome these ecological concerns. In this study, we evaluated several mass trapping devices and attractants in comparison with ground-applied insecticide bait sprays for the control of *Anastrepha obliqua* (Macquart) and *Anastrepha ludens* (Loew) (Diptera: Tephritidae) in mango orchards in Chiapas, Mexico. Among the bait stations evaluated, we found that a wide-mouth 2 L plastic bottle baited with Cera Trap®, an enzymatic hydrolyzed protein, and used at the density of 25 traps per ha was most efficient at reducing the fruit fly populations. Our results showed that bait stations to control fruit flies, in addition to using less insecticide, were as effective as ground sprays and were superior under rainy conditions. These properties represent a great advantage considering the tropical conditions in several mango production zones around the world.

Key Words: *Anastrepha ludens*; *Anastrepha obliqua*; spinosad; sterile insect; insecticide application

Resumen

El uso de cebos con insecticidas es uno de los métodos más comunes y eficaces en diversas regiones del mundo para el control de moscas de la fruta (Diptera: Tephritidae). Sin embargo, esta estrategia ha sido cuestionada debido a la contaminación ambiental y los efectos no deseados en organismos no blanco. El uso de dispositivos de atracción y muerte llamados estaciones cebo (BS) o el trampeo masivo pueden ser métodos de control alternativos para superar estos impactos ecológicos. En este estudio se evaluaron diferentes dispositivos usados para el trampeo masivo y atrayentes en comparación con aspersiones terrestres de insecticidas cebo para el control de *Anastrepha obliqua* (Macquart) y *Anastrepha ludens* (Loew) (Diptera: Tephritidae) en huertos de mango en Chiapas, México. Se encontró que el contenedor de plástico de 2 litros cebado con Cera Trap®, proteína hidrolizada enzimáticamente, a una densidad de 25 trampas por hectárea fue el más eficaz en la reducción de las poblaciones de mosca de la fruta. Nuestros resultados indican que el uso de estaciones cebo puede ser tan eficaz como las aspersiones terrestres de cebo tóxico, pero superior bajo condiciones de lluvia. Lo anterior resulta de gran ventaja teniendo en cuenta las condiciones climáticas tropicales de varias zonas de producción de mango en el mundo.

Palabras Clave: *Anastrepha ludens*; *Anastrepha obliqua*; spinosad; insect estéril; aplicación terrestre de insecticidas

Integrated fruit fly management includes strategies such as the sterile insect technique and the application of insecticide bait sprays, both independently and in complementary strategies, in pest eradication or suppression actions (Mangan & Moreno 2007). Currently, spinosad plus food attractants is formulated as GF-120® NF 0.02 Naturalyte® CB (Dow AgroSciences LLC, Indianapolis, Indiana) and applied worldwide as a toxic bait by aerial sprays or by ground application against *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (McQuate et al. 2005), *Anastrepha ludens* (Loew) (Diptera: Tephritidae) (Prokopy et al. 2000), and *Bactrocera* species (Diptera: Tephritidae) (Piñero et al. 2009). However, these toxic bait sprays have low effectiveness during the rainy season because rain can wash off active compounds (Piñero et al. 2010). To overcome this situation, bait stations (lure-and-kill devices) that use specific attractants and selective insecticides represent an alternative for fruit fly control and reduce both environmental impact and damage to non-target organisms (Vayssières et al. 2007), including effects on natural enemies and pollinators (Desneux et al. 2007; Blacquière et al. 2012). Bait stations can be highly effective in controlling small, low-density, isolated populations and have the potential to add value to long-term pest management (El-Sayed et al. 2009). Additionally, bait stations can be used in a mass trapping strategy to retain and kill the target populations of the pest.

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Lasa et al. (2013, 2014a,b) indicated that affordable traps and the prolonged efficacy of attractants can favor the development of mass trapping programs; however, more studies using different trap densities and weather conditions are needed to confirm the efficacies of these technologies against fruit flies.

Bait stations and mass trapping also are alternative methods for population suppression in areas such as organic and backyard orchards, where it is not possible or convenient to spray toxic bait. However, the development of these pest control devices needs to consider their cost effectiveness and the need for long-lasting attractants and killing agents, and it should target female fruit flies. Jemâa et al. (2010) showed that mass trapping using a female-targeted lure (Tri-pack®, Kenogard SA, Barcelona, Spain) for the control of *C. capitata* was not only as effective as malathion used at the dose of 500 mL/ha every 10 d but also did not leave pesticide residues on the fruit. Leza et al. (2008) concluded that mass trapping with 50 traps per ha, supplemented when necessary with insecticide bait formulations, was significantly more effective in reducing *C. capitata* female populations in citrus orchards compared with insecticide bait application alone.

In Mexico, several handcrafted models of bait stations made of various materials, such as corn cobs, sawdust bags (jute bags filled with sawdust and impregnated with toxic bait), and plastic bottles with holes in the sides and baited with hydrolyzed protein and malathion or spinosad, have shown positive results when tested (Flores & Montoya 2010). An ideal bait station should have low cost and low environmental impact and should be easy to use, selective, long lasting, safe, and easy to install (FAO/IAEA 2009).

Mexico is the largest mango exporter in the world (Galán-Saúco 2009), and *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae) and *A. ludens* are two of the most economically important pests in mango orchards in several parts of the country. Therefore, in this study we evaluated various devices and attractants used as bait stations to control *A. obliqua* and *A. ludens* populations in mango orchards in the state of Chiapas, Mexico.

Materials and Methods

This study was conducted in 3 trials from Apr 2009 to Jul 2012, including (in some trials) tests during the dry and raining seasons. The different devices evaluated are shown in Fig. 1.

RELEASE OF STERILE FRUIT FLIES

To ensure the presence of flies during the evaluation of all trials described below, sterile adults of *A. ludens* and *A. obliqua* were released in the plots (Arredondo et al. 2014). The insects were obtained as irradiated pupae (at 85 Gy in a gamma irradiator model GB-127, Nordion International Inc., Ottawa, Ontario, Canada) and dyed with fluorescent DayGlo® A-11 Aurora Pink pigment (DayGlo Color, Cleveland, Ohio) from the Moscafrut facility in Metapa, Chiapas. The pupae were packed in paper bags with a 1:24 mixture of hydrolyzed protein (ICN Biomedicals, Inc., Aurora, Ohio) and sugar as food (Liedo et al. 2013) and kept at 25 °C, 70 to 75% RH, and a photoperiod of 14:10 h L:D under laboratory conditions for adult emergence. The adult flies were released when approximately 5 d old, at specific densities described for each trial. Sterile and wild flies were monitored by installing 1 Multilure® (Better World Manufacturing, Inc., Fresno, California) trap per ha, baited with hydrolyzed protein and serviced and inspected each week (FAO/IAEA 2013). For each released species, captured fruit flies were sorted, separated using a stereomicroscope, and counted by sex to record the total number of flies per trap by treatment type for each replication. Sterile flies were identified by the fluorescent pink pigment on the ptilinal suture by observation of specimens with an epifluorescent stereomicroscope SMZ1500 (Nikon, Japan).

COMPARISON OF BAIT STATIONS AND GROUND APPLICATION OF TOXIC BAIT

Trial 1: Evaluation of 600 mL Polyethylene Terephthalate Bottles

The trial was conducted from Apr to Jun 2009 in the 'Ataulfo' mango orchard "El Cebollero" (14.78400°N, 92.20110°W) located in Frontera Hidalgo Municipality, Chiapas, Mexico. This study was carried out over two 6 wk periods. One period occurred during the dry season (Nov to Apr) and the other during the rainy season (May to Oct). The orchard was divided into 9 plots of 4 ha each, and we used the central ha inside each 4 ha plot for sampling in order to diminish the border effect. We carried out 3 replicates randomly distributed of the following treatments: 1, untreated control; 2, GF-120 Naturalyte ground spray (see next paragraph); and 3, polyethylene terephthalate (PET) bottle with holes.

No pest control activity was implemented in the plot used as the control. Ground treatment with 40% GF-120® NF 0.02 Naturalyte® CB (spinosad) (Dow AgroSciences LLC, Indianapolis, Indiana) (hereafter: GF-120 Naturalyte) was performed once per wk by using a 20 L Jacto® backpack sprayer (Máquinas Agrícolas Jacto SA, Säo Paulo, Brazil), at a rate of 5 L of GF-120 Naturalyte solution per ha on the underside of foliage in alternate rows (50% of the trees). Ground bait sprays were applied 1 d after the release of 1,500 sterile flies per ha of both *A. ludens* and *A. obliqua*. We baited 600 mL PET bottles (recycled soft drink bottles) containing three 1-cm-diameter holes in the upper third of each bottle with 50 mL of the hydrolyzed protein Captor 300® (Promotora Agropecuaria Universal S.A. de C. V., Mexico City, Mexico) bait. The PET bottles were installed at a density of 25 bottles per ha (SAGARPA-SENASICA 2012), and the attractants were changed every 3 wk.

Trial 2: Evaluation of PET Bottle vs. MS® Trap vs. Wax Matrix Bait Station

This trial was conducted in 3 mango orchards of Tapachula in Chiapas: "Las Carmelas" (14.7975778°N, 92.3371611°W), "Las Delicias" (14.74183°N, 92.27490°W), and "El Progreso" (14.75485°N, 92.26964°W), from Jan to Apr 2011. Each orchard represented a replicate and was divided into five 4 ha plots, and we used the central ha inside each 4 ha plot for sampling in order to diminish the border effect. The treatments evaluated were: 1, untreated control; 2, GF-120 Naturalyte ground spray; 3, PET bottle with windows; 4, MS® mass trapping device; and 5, wax matrix bait station.

Control and GF-120 Naturalyte ground spray treatments were performed as described for Trial 1. For Trial 2, a 600 mL PET bottle with three 3 × 3 cm square openings at the top (in the upper middle area) was baited with 150 mL of a solution consisting of GF-120 Naturalyte and water at a 1:1.5 ratio. The MS® trap (Proveedora Fitozoosanitaria S. A. de C. V., Texcoco, Mexico) (Fig. 1) was a commercial 2-piece, bottle-shaped device with 1 transparent upper piece (with three 3 x 3 cm windows, hinged at the top in the middle region of the bottle) and 1 yellow bottom piece baited with Atrayente® (Proveedora Fitozoosanitaria S. A. de C. V., Texcoco, Mexico) (mixture of 30% hydrolyzed protein, 10% propylene glycol, 5% malathion, and adjuvants). The wax matrix bait station (Epsky et al. 2012) was a waxed green box

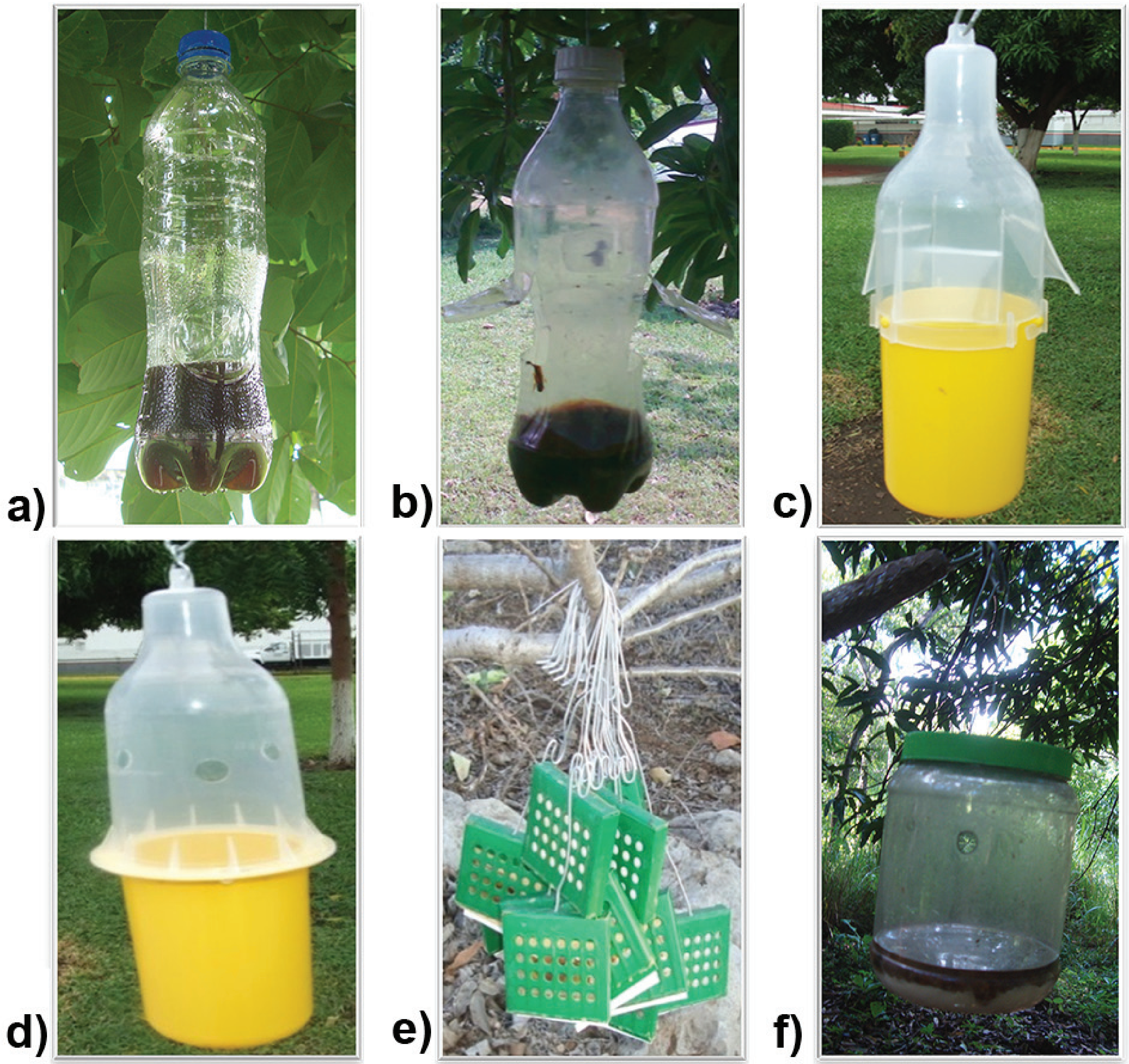

Fig. 1. Devices used as treatments during field evaluations: a) 600 mL polyethylene terephthalate (PET) bottle with three 1 × 1 cm holes in the upper third, baited with 50 mL of hydrolyzed protein; b) 600 mL PET bottle with two 3 × 3 cm windows in the upper middle, baited with 150 mL of GF-120 Naturalyte (80 ppm); c) MS® is a commercial bait station consisting of a 2-piece, bottle-shaped device, with a transparent upper piece with three 3 × 3 cm windows in the middle part and a yellow bottom, baited with Atrayente® (mix of 30% hydrolyzed protein, 10% propylene glycol, 5% malathion, and adjuvants; d) MS2® is the same MS® device as in 'c' but with the upper piece containing three 1-cm-diameter holes in the middle part, baited with 250 mL of Cera Trap®; e) wax matrix bait station is a waxed green box with slots baited with BioLure® synthetic attractant (ammonium acetate and putrescine); f) INIFAP trap is a 2 L, 14-cm-diameter, and 14 cm high cylindrical container, with three 1-cm-diameter holes at mid-height, baited with 250 mL of Cera Trap®.

with slots baited with a 2-component (ammonium acetate and putrescine) BioLure® synthetic attractant (Suterra® LLC, Bend, Oregon). The 3 treatment devices were placed at a density of 25 devices per ha and remained in the field for 3 mo without service. Sterile insects of *A. ludens* and *A. obliqua* were released at a density of 3,000 adults per ha of each species. We increased the fly release rate in this trial to obtain better recapture rates of sterile flies.

Trial 3: Evaluation of MS2® Trap vs. INIFAP Trap

Two mass trapping devices, the INIFAP trap (National Institute of Phytosanitary, Agricultural and Fisheries Research, Montemorelos, Mexico, http://www.inifapcirne.gob.mx/Biblioteca/Publicaciones/660.pdf) and the MS2® trap (Proveedora Fitozoosanitaria S. A. de C. V., Texcoco, Mexico), were evaluated from Mar to Jul 2012 in the mango orchard "Las Delicias." Because the principal objective of this trial was to compare the performance of the mass trapping devices, in this trial we used the GF-120 Naturalyte ground spray as a relative control. The orchard was divided into nine 4 ha plots, with 3 replicates of each treatment distributed randomly. We used the central ha inside each 4 ha plot for sampling in order to diminish the border effect. The treatments were: 1, GF-120 Naturalyte ground spray; 2, MS2® trap; and 3, INIFAP trap.

The GF-120 Naturalyte ground spray areas were treated as described for Trial 1. The MS2® trap is a 2-component plastic bottle with a yellow base covered by a transparent lid perforated with 3 small (1 cm in diameter) holes 5 cm apart and baited with 250 mL of Cera Trap® hydrolyzed enzymatic protein (Bioibérica, Barcelona, Spain). The INIFAP trap is a 2 L cylindrical container (12 cm in diameter, 16 cm high) with 6 openings of 1 cm diameter at mid-height and baited with 250 mL of Cera Trap®. In each mass trapping plot, 25 devices per ha were placed and maintained during the entire period of the trial. Sterile insects of *A. ludens* and *A. obliqua* were released at a density of 3,000 adults per ha of each species. For mass trapping treatments, in each replicate we recorded weekly the number of flies of each species captured in 5 MS2® traps and in 5 INIFAP traps.

DATA ANALYSES

To compare the numbers of captured flies in the traps for each treatment, the number of adults per trap was divided by the number of days per sampling interval, giving flies per trap per d (FTD). The numbers of flies per trap per d were calculated as percentage of recapture per release date, and later were arcsine transformed prior to 1-way analysis of variance. The Tukey multiple range test ($\alpha = 0.05$) was used for comparisons of mean trap captures ($\alpha = 0.05$). Sex ratio among treatments was compared using a logistic regression. All analyses were performed using JMP® software version 7 (JMP 2007).

Results

EVALUATION OF MASS TRAPPING DEVICES

TRIAL 1: EVALUATION OF PET BOTTLE

The comparison between PET bottles and ground sprays of GF-120 Naturalyte in the rainy and dry seasons is shown in Fig. 2. In the rainy season, ground spraying was ineffective in reducing the captures of both *A. ludens* and *A. obliqua* sterile flies. For sterile *A. ludens*, the percentages of fly recapture in Multilure® traps were significantly different among treatments ($F_{2,33} = 33.5$; $P < 0.001$). Similarly, for sterile *A. obliqua*, the captures were significantly different among treatments ($F_{2,33} = 27.8$; $P < 0.001$). Means comparisons with the Tukey test indicated that fly captures were greater in the control and ground spray treatments than in the plots with PET bottles for both *A. ludens* ($\alpha = 0.05$) and *A. obliqua* ($\alpha = 0.05$). The numbers of captured wild flies were not large enough to be analyzed statistically.

In the dry season, the captures were significantly different among treatments for sterile *A. ludens* ($F_{2,33} = 70.3$; $P = 0.001$) and for sterile *A. obliqua* ($F_{2,33} = 66.0$; $P = 0.001$). Significantly greater percentages of flies were recaptured in the untreated control plots than in the treated plots for both *A. ludens* ($\alpha = 0.05$) and *A. obliqua* ($\alpha = 0.05$), but no significant differences were observed between the plots ground sprayed with GF-120 Naturalyte and the plots with baited PET bottles.

The percentage of females (± SE) of *A. ludens* captured was 50.2 ± 6.9% female, and the sex ratios did not differ among treatments ($\chi^2_2 = 0.82$; $P = 0.661$) or between seasons ($\chi^2_1 = 0.56$; $P = 0.452$). For *A. obliqua*, the percentage of females captured was 74.2 ± 4.0%, and the sex ratios did not differ among treatments ($\chi^2_2 = 0.12$; $P = 0.939$) or between seasons ($\chi^2_1 = 1.68$; $P = 0.195$).

Trial 2: Evaluation of PET Bottle vs. MS® Trap vs. Wax Matrix Bait Station

In this trial, the analysis indicated significant differences of sterile insect capture in Multilure® traps among treatments for *A. ludens* ($F_{4,55} = 8.6$; $P < 0.001$) and *A. obliqua* ($F_{4,55} = 4.4$; $P = 0.003$). Recapture percentages in plots with both MS® traps and GF-120 Naturalyte ground treatments were significantly lower than captures in control plots for both *A. ludens* and *A. obliqua* (Fig. 3).

The percentage of captured females (± SE) was not different among treatments for *A. ludens* ($\chi^2_4 = 6.3$; $P = 0.175$) or *A. obliqua* ($\chi^2_4 = 4.5$; $P = 0.344$), with an average of 46.8 ± 3.0% and 57.9 ± 5.0% females, respectively.

Trial 3: Evaluation of MS2® Trap vs. INIFAP Trap

In Multilure® traps, the recapture percentages of sterile *A. ludens* did not differ among treatments ($F_{2,33} = 1.1$; $P = 0.327$), but we found significant differences among treatments for *A. obliqua* (Fig. 4). For sterile *A. obliqua*, the catches were significantly lower in the treatment with INIFAP traps than in the GF-120 Naturalyte plots ($\alpha = 0.05$). The sex ratio was not different among treatments for the 2 species ($\chi^2_2 > 4.6$; $P = 0.098$).

Between the mass trapping devices, the number of sterile adults captured was greater in the INIFAP trap than in the MS2® trap, and this difference was significant for both *A. ludens* ($F_{1,22} = 88.3$; $P = 0.001$) and *A. obliqua* ($F_{1,22} = 46.2$; $P = 0.005$) (Fig. 5). The percentages (± SE) of sterile females in MS2® traps and INIFAP traps were 53.3 ± 2.4 and 50.3 ± 1.7%, respectively, for *A. ludens* ($\chi^2_1 = 4.0$; $P = 0.045$), and 61.7 ± 2.7% and 56.9 ± 2.0%, respectively, for *A. obliqua* ($\chi^2_1 = 7.6$; $P = 0.006$); significant differences between traps for each species were obtained.

Discussion

Mass trapping with bait stations can be used to reduce or eliminate the use of insecticide bait sprays in various integrated pest management programs against fruit flies (Navarro-Llopis et al. 2011; Lasa et al. 2013, 2014 a,b; Yokoyama 2014). Our study supports the use of mass trapping devices to reduce the densities of *Anastrepha* fruit flies in mango orchards because the tested devices are less expensive than toxic bait sprays, and their performance is not affected by the rainy season.

The INIFAP trap, PET bottle, MS® trap, and MS2® trap reduced the numbers of sterile adult captures similarly to ground spraying with GF-120 Naturalyte. When PET bottles were baited exclusively with hydrolyzed protein (without malathion), results were not different from those in the control, possibly due to escape of the flies. Our results are consistent with the data published by Piñero et al. (2009), which suggested that installing bait stations can be as efficient as ground spraying to control *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) populations. In our opinion, these devices represent a control option in the rainy season (Piñero et al. 2010) or where aerial or ground spraying have little effect or little acceptance, depending on specific agro-ecological conditions or social concerns about the harmful effect of insecticides. In the rainy season, the ground sprays can be ineffective as a result of the dilution or removal of the product (Peck & McQuate 2000). This is especially true in the case of GF-120 Naturalyte because flies need to consume a specific dosage of the active ingredient to be killed (Flores et al. 2011). The use of

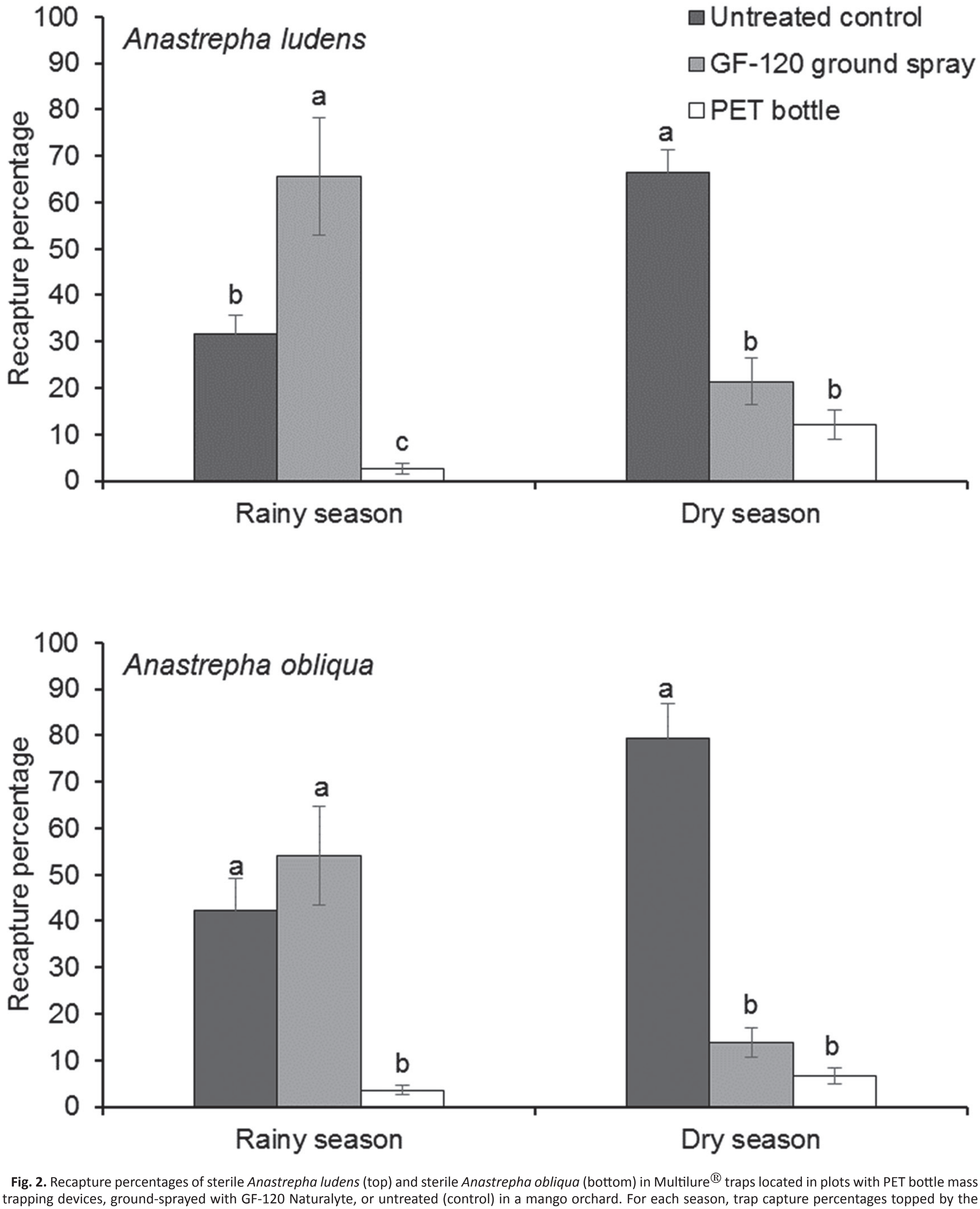

Fig. 2. Recapture percentages of sterile *Anastrepha ludens* (top) and sterile *Anastrepha obliqua* (bottom) in Multilure® traps located in plots with PET bottle mass trapping devices, ground-sprayed with GF-120 Naturalyte, or untreated (control) in a mango orchard. For each season, trap capture percentages topped by the same letter are not significantly different (α = 0.05).

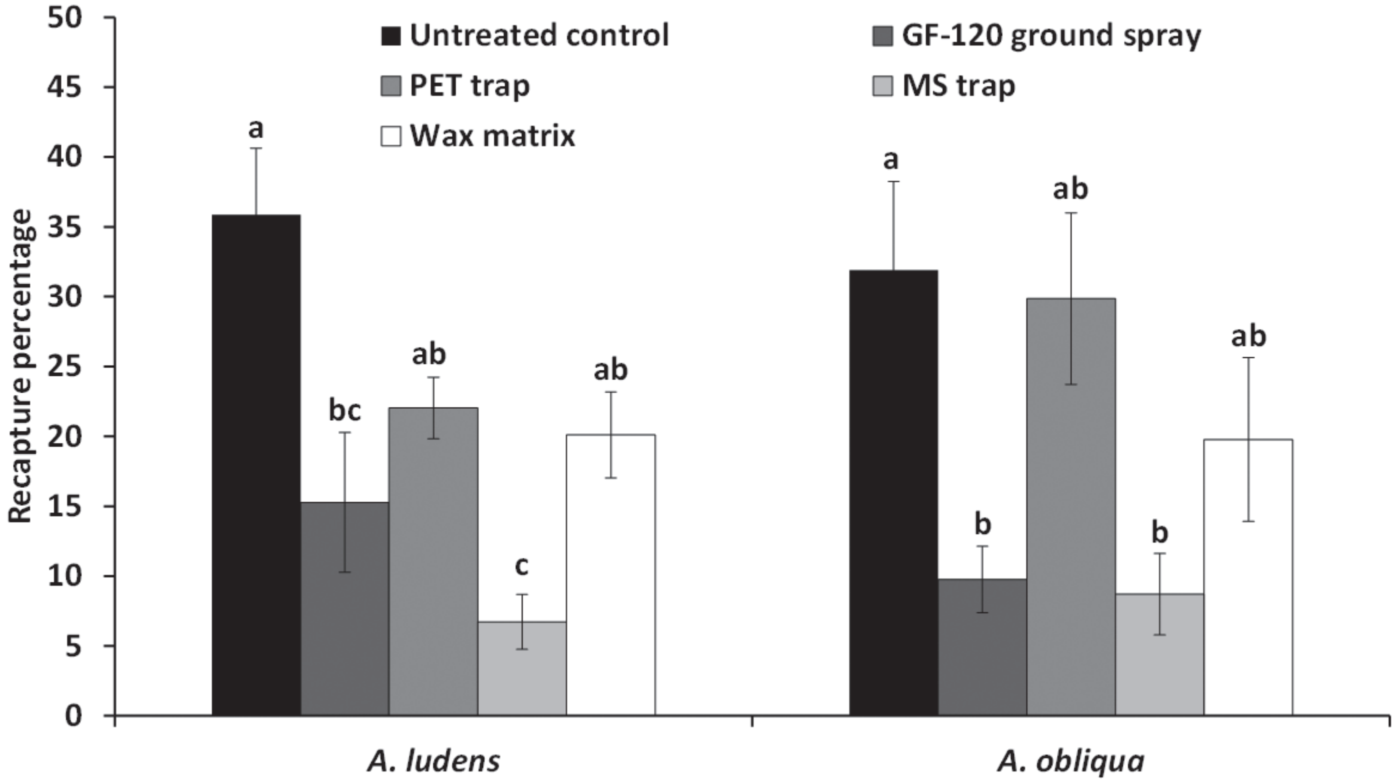

Fig. 3. Recapture percentages of sterile *Anastrepha ludens* and *Anastrepha obliqua* flies in Multilure® traps in plots with different bait station devices. For each species, trap capture percentages topped by the same letter are not significantly different (α = 0.05).

bait devices during the rainy season is an effective alternative to ground spraying. These devices can also protect photosensitive bait from degradation by ultraviolet light. Also, a visual stimulus, such as an attractive color, can be added to the devices to improve their efficacy (Piñero et al. 2010). Our data confirm this advantage of using protective devices such as PET bottles during the rainy season.

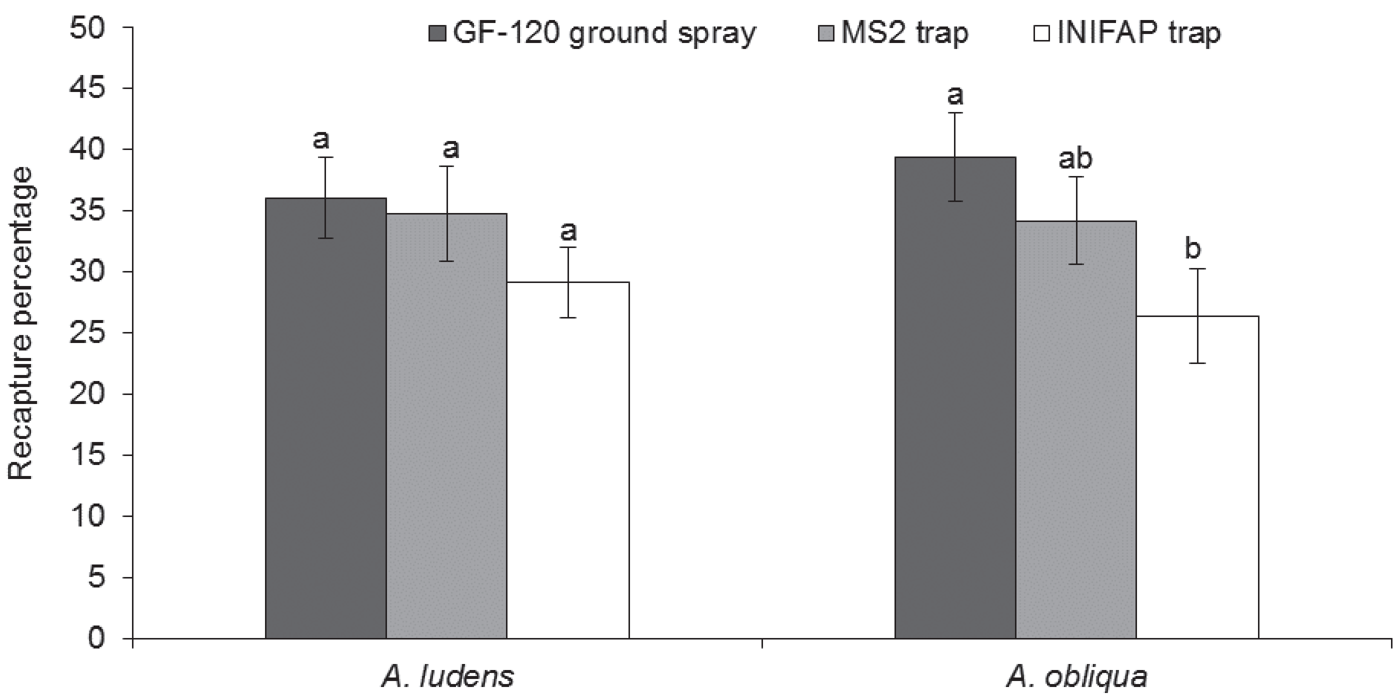

Fig. 4. Recapture of sterile *Anastrepha ludens* and *Anastrepha obliqua* in Multilure® traps in different treatments. For each species, trap capture percentages topped by the same letter are not significantly different (α = 0.05).

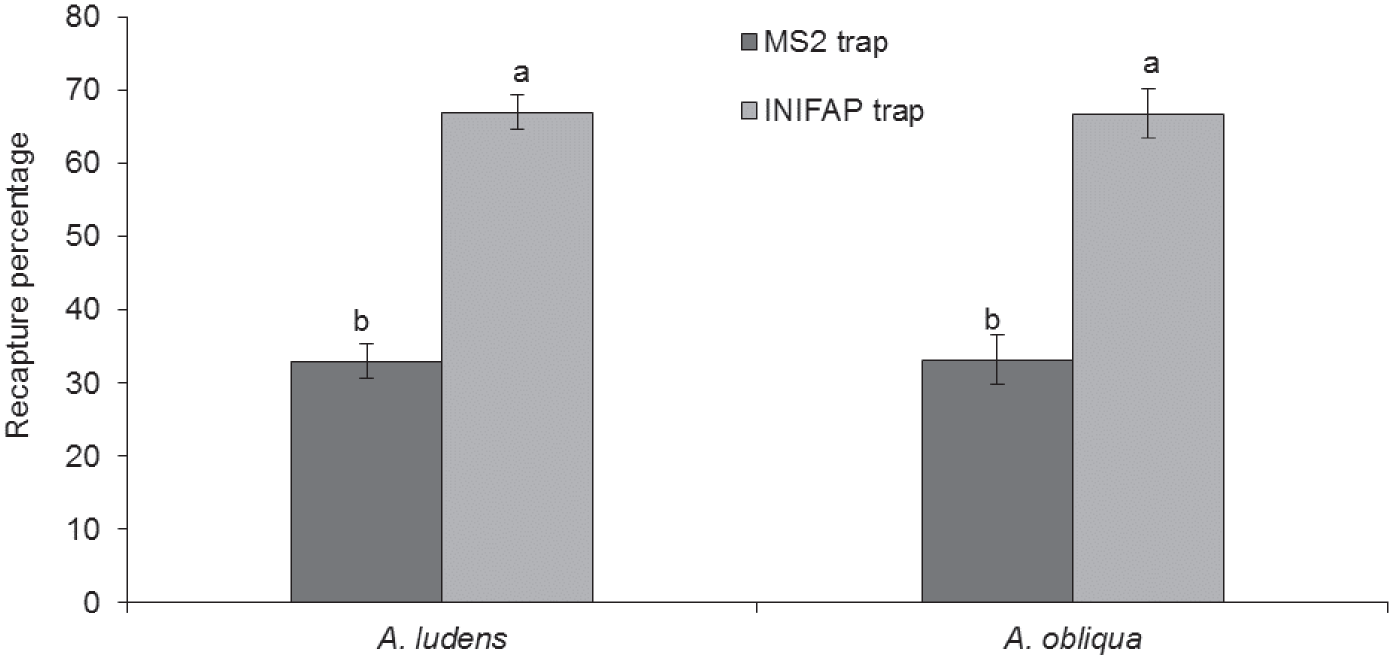

Fig. 5. Recapture percentages of sterile and wild *Anastrepha ludens* and *Anastrepha obliqua* in 2 types of mass trapping devices. For each species and strain, trap capture percentages topped by the same letter are not significantly different (α = 0.05).

Lasa et al. (2013, 2014b) evaluated the capture of *A. ludens* with different mass trapping devices with several attractants. They found differences among the devices but concluded that PET bottles with an enzymatic hydrolyzed protein (Cera Trap®) represented a cost-effective and highly efficient method for *A. ludens* control in citrus (Lasa et al. 2015). Manrakhan & Kotze (2011) and Piñero et al. (2011) mentioned that the type of attractant is critical in the performance of bait stations. Some attractants can be improved when combined with substances such as oils (McQuate & Peck 2001) or antifreeze (Thomas 2008) that increase the efficiency and long-term stability in the field (Lasa et al. 2015). The use of a more effective attractant such as Cera Trap® in the INIFAP trap may represent an improvement as a bait station, because the larger surface, larger volume, and longer period of effectiveness in the field, compared with 600 mL PET bottles, permit this trap to hold a greater number of captured flies before saturation. We found that 1 INIFAP device baited with Cera Trap® may remain effective for 10 wk without servicing under rainy conditions, and 4 to 6 wk during the dry season. Importantly, this means that these devices require servicing only 1 or 2 times during the production and harvest seasons to protect the fruit. However, a limitation in the case of *Anastrepha* species is the current lack of an attractant that is effective in dry traps (as in the case of *C. capitata*), although some research has produced promising results (Robacker & Czokajlo 2005). Thus, liquid attractants continue to be used in traps for capture of fruit flies, resulting in the need for bait replacement during the fruit harvest season.

We found that 25 bait stations per ha, containing about 70 mango trees, resulted in the same level of control as ground application of insecticides, and that any device baited with Cera Trap® resulted in a higher percentage of captured females. The mass trapping devices are not totally efficient in retaining flies inside the trap because some flies escape (Lasa et al. 2014a,b), although flies that ingest the bait and escape from the trap have their reproductive traits negatively affected (Perea-Castellanos et al. 2015).

We observed that *A. obliqua* was always captured more frequently (as sterile insects released or as wild flies), irrespective of the species released. This was observed previously by Arredondo et al. (2014), possibly because this species is more attracted to protein-based lures than *A. ludens*. However, because *A. ludens* is the most important pest for commercial mango production in Chiapas, Mexico, research on the use of bait stations should continue.

The use of bait stations to control fruit flies offers the possibility to save labor and insecticide costs relative to toxic bait sprays (Piñero et al. 2010; Lasa et al. 2015) in addition to reducing the non-target impacts associated with insecticide treatment. Our results indicate that trapping devices such as the INIFAP trap are at least as effective as ground sprays and superior under rainy conditions, which is an important finding considering the tropical climate conditions in several fruit production zones around the world.

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