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INEXPENSIVE TRAPS FOR USE IN MASS TRAPPING *ANASTREPHA LUDENS* (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Mass trapping is being developed to control the fruit fly *Anastrepha ludens* (Loew) (Diptera: Tephritidae) in some citrus-growing areas of Mexico using a trap-lure pack composed of CeraTrap® lure and a specific bait station (MS2®). The technique is based on placing a sufficient density of baited traps so that enough flies are captured to account for an important reduction in fruit infestation in the orchard. Trap-lure efficacy, trap density and cost ratio are crucial for broad implementation of this technique by growers. The use of handmade traps, constructed from 500 mL plastic bottles, with 3 10 mm holes at $\frac{2}{3}$ up from the base, had similar attraction but captured more flies under caged conditions than 2 commercial traps sold in Mexico for this purpose. Under field conditions, the capture of *A. ludens* with plastic bottle traps was statistically superior to that of MS2® traps. When placed at a density of 40 traps per ha in an orange (*Citrus sinensis* [L.]; Rutaceae) orchard, bottle traps had a significantly higher capture rate than a similar density of MS2 traps and provided satisfactory crop protection, with 0.3- 2.3% of fallen fruits infested at harvest. The low cost of this trap, (~US\$ 0.18), has the potential to greatly reduce mass trapping costs and could favor broad implementation of this strategy in Latin American countries that produce fruit crops affected by *A. ludens*.

Key Words: *Anastrepha ludens*, inexpensive traps, CeraTrap, mass trapping

RESUMEN

Las estrategias de trapeo masivo para el control de *Anastrepha ludens* (Loew) (Diptera: Tephritidae) están siendo desarrolladas en algunas zonas cítricas de México mediante el uso de la estación cebo MS2® con el cebo líquido CeraTrap®. La técnica se basa en la colocación de una densidad suficiente de trampas que capturen un elevado número de moscas que reduzca la cantidad de fruta infestada en el huerto. La eficacia de la combinación trampa/cebo, la densidad de trampas y el coste son cruciales para una amplia implementación de esta técnica por parte de los productores. Las trampas fabricadas manualmente con botellas de plástico de 500 mL, con tres agujeros de 10 mm a $\frac{2}{3}$ de la altura desde la base, tuvieron la misma atracción pero capturaron más moscas en condiciones de jaula que dos trampas comerciales de México. En condiciones de campo, la captura de *A. ludens* con trampas construidas con botellas de plástico fue significativamente superior a la de la trampa MS2®. Cuando se colocaron trampas a una densidad de 40 por ha en un huerto de naranja (*Citrus sinensis* [L.]; Rutaceae), las construidas con botellas de plástico tuvieron un nivel de capturas significativamente más alto que las trampas MS2® a la misma densidad, y proporcionan una protección satisfactoria del cultivo con 0.3-2.3% de frutos caídos infestados en el momento de la recolección. El bajo coste de esta trampa (~US\$ 0.18) tiene el potencial de mejorar los costes de trapeo masivo y podría favorecer un amplio uso de esta estrategia en países de Latinoamérica que tienen cultivos frutales atacados por *A. ludens*.

Palabras Clave: *Anastrepha ludens*, trampas económicas, CeraTrap, trapeo masivo

The Mexican fruit fly, *Anastrepha ludens* (Loew) (Diptera: Tephritidae), is one of the most important pest species of fruits grown in Mexico, and its detection triggers quarantine restrictions for export of commercial fruit of citrus (Sapindales: Rutaceae) crops to importing countries (Enkerlin et al. 1989; Aluja 1993). Control relies heavily on applications of chemical pesticides such as malathion and more recently the biorational product spinosad (GF120, Dow Agro-Sciences, Indianapolis, Indianapolis). Up to 8 applications of GF120 are applied per fruiting season, at weekly intervals in some regions of Mexico, leading to the possibility of the development of resistance in a few years. Moreover, some of these treatments are applied near the harvest, making it difficult to adhere to the preharvest safety period in the case of insecticides with residual toxicity, such as organophosphates.

Countries that import fresh or processed grapefruit (*Citrus × paradise* Macfad.) and orange (*Citrus × sinensis* (L.) Osbeck) products apply strict control procedures for detection of pesticide residues due to consumer health concerns. As a result of these requirements, the continued use of insecticides is increasingly limited near harvest, making it necessary to evaluate other control strategies for inclusion in integrated pest management programs to reduce Mexican fruit fly populations.

The wide host range of *A. ludens* favors the maintenance of substantial populations during most of the year, making it necessary to monitor and control this pest for at least 2 months before the fruit harvest. Mass trapping strategies have been developed for control the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and have attracted interest due to their efficacy, specificity and low environmental impact (Navarro-Llopis et al. 2008; Martínez-Ferrer et al. 2010). Mass trapping consists of the use of traps and baits that release specific volatile substances that attract insects to the trap, in which fruit flies are captured and killed (El Sayed et al. 2006). The efficiency of the traps and the attractiveness of baits must be high enough to reduce pest infestation levels in the crop. At the same time, the use of effective lures and inexpensive traps is essential to obtain a good cost/benefit ratio.

In Mexico, previous trials developed with the bait station MS2® (Fitozoosanitaria S.A. de C.V., Texcoco, Mexico), revealed promising results when used with the liquid lure CeraTrap® (De los Santos et al. 2012). CeraTrap® is a protein-based lure, derived from enzymatic hydrolysis, which has proven to be very attractive to female Mexican fruit flies (Lasa et al. 2013; Lasa et al. 2014). The present study evaluated the efficacy of 2 inexpensive handmade traps fashioned from polyethylene (PET) bottles, and compared their performance to 2 commercial traps currently sold

in Mexico. Trap performance was evaluated in field cages and field conditions. A mass trapping trial was then performed to evaluate the traps' effectiveness in reducing crop infestations.

MATERIAL AND METHODS

Insect Source, Trap Types, and the Lure

Anastrepha ludens adults obtained from a colony maintained at the Instituto de Ecología A. C., Xalapa, Mexico, were used for the cage and laboratory tests. Newly emerged adults were placed in acrylic cages (20 × 20 × 20 cm) covered with organdy, and flies were fed ad libitum with a mixture of sugar and hydrolyzed protein (3:1) until they were 3-5 days old. Water was supplied via wet cotton. Flies and pupae were reared under laboratory conditions at 25 ± 1.5 °C and 55 ± 10% (RH).

The following 4 trap models (Fig. 1) were evaluated: i) MS2® (Fitozoosanitaria S.A. de C.V., Texcoco, Mexico), a bottle-shaped trap constructed with a transparent top that has three 11 mm diam circular holes spaced 5 cm apart and a yellow base. The trap holds 250 mL of CeraTrap lure; ii) A & C Trap® (Mubarqui, Tamaulipas, Mexico), a yellow cylindrical cup-shaped trap with 4 translucent lateral access conical tubes (8 mm diam outside and 6 mm inside), affixed to an oval translucent top and with a total capacity of 350 mL of liquid lure; iii) 500 mL blue polyethylene (PET) bottles (Tecní Plastic Containers S.A. de C. V., Martínez de la Torre, Mexico) that were modified by drilling three 10 mm diam holes, 5 cm apart, ⅓ above the base, with a capacity of 250 mL of liquid lure; and iv) transparent colorless 500 mL polyethylene (PET) bottles (Tecní Plastic Containers S.A. de C.V., Martínez de la Torre, Mexico), that were perforated in an identical manner to the blue PET bottles described above.

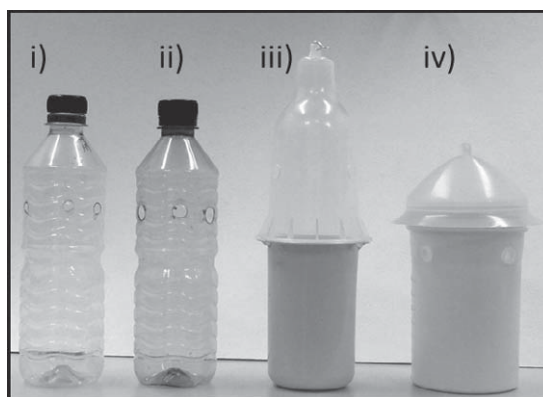


Fig. 1. Trap models evaluated: i) transparent colorless PET bottle; ii) blue PET bottle; iii) MS2 trap; and iv) A & C trap.

The lure “CeraTrap®” (Bioibérica, Barcelona, Spain) was used in all traps for all experiments. This product is supplied as a soluble concentrate derived from enzymatic hydrolysis of proteins, whose evaporation causes the emission of volatile compounds, primarily amines and organic acids that are highly attractive to adult tephritids, especially females (Marín 2010).

Trap Efficacy and Physical Retention of Flies under Field Conditions

Cage tests were conducted at the experimental orchard of the company Cítricos Ex S.A. de C.V. in Martínez de la Torre, Veracruz. Five small (1.2-1.4 m) potted grapefruit (var. ‘Rio red’), *Citrus paradisi* (Macfad), plants were placed in the cage (one in the center and one at each corner); each cage was 1 × 1 × 1.8 m high constructed from screen mesh (1 mm aperture). One each of the 4 types of traps, each baited with 250 mL of CeraTrap®, was hung from the cage roof at points 0.55 m equidistant from the center of the cage giving a total of 4 traps per cage. Traps were initially positioned at random and subsequently rotated clockwise in position for each new replicate. On the day of a new replicate, 30 flies (15 females and 15 males) starved overnight were released inside the cage at 10:00 am. Trap attraction was evaluated from 12:00-13:00 h and again from 15:00-16:00 h. During these periods, the number of flies that landed on each trap was noted and their sex recorded. Twenty-four hours after the flies were released, the number of flies of each sex captured by each trap was counted. After that, the remaining flies inside the cage were removed. A total of 16 replicates was performed over a 3-week period in Oct 2013.

The physical retention of flies for each trap model was evaluated according to the methodology described previously (Lasa et al. 2014). For this, each trap was hung inside a 30 × 30 × 30 cm fly rearing cage. Twelve laboratory flies (6 males and 6 females) were released inside the trap using an entomological aspirator and observed for a period of 30 min. Flies that managed to escape from the trap during 30 min were counted and classified by sex. All traps were evaluated without any attractant and in the absence of any insecticide or other retention system. Experiments were performed under laboratory conditions at 27 ± 1 °C and 55 ± 10% RH. All traps were evaluated simultaneously and evaluations were performed a total of 16 times.

Trap Efficacy under Field Conditions

An orange orchard (*Citrus sinensis* L., var. ‘Valencia’), located in the municipality of San José Acateno, (N 20° 6' 0.73" W 97° 15' 1.55") in Puebla

State, Mexico, was selected for this experiment. The orchard was infested by the Mexican fruit fly. Twenty four traps, baited with 250 mL of CeraTrap®, were randomly distributed in 6 blocks over a 3 ha area. Traps were placed one per tree, 3-4 m above the ground with a distance of 12-15 m between adjacent traps. The distribution of treatments in each block was randomized initially. Traps were checked at 7-day intervals for a period of 8 weeks between Nov 2012 and Jan 2013. Trapped insects were collected in vials with 70% (v/v) alcohol for future counting and sex identification in the laboratory. The position of traps was rotated sequentially each week during the 8-week evaluation. Only captures of *A. ludens* were analyzed statistically.

Mass Trapping Assay

A 12-ha orchard of orange (*Citrus sinensis* L., var. ‘Valencia’) adjacent to the previously described orchard was selected for a mass trapping experiment. This orchard was divided into 12 plots of 1 ha each. Four blocks per treatment were selected randomly and contained one of 3 trap models: i) transparent PET bottle, ii) MS2, and iii) A & C trap. Traps were hung 1 per tree, 3-4 m above the ground on the inside canopy, and at a density of 40 traps per ha. Traps were evenly distributed throughout the plot in mid-Nov 2012 and were maintained in the field until mid-Feb 2013 without being re-baited. During the experiment, the number of fruit flies trapped by 10 traps per plot, randomly selected and marked, was counted directly in the field every 14 days. At the same time, 30 fallen fruits per plot (120 fruits per treatment) were randomly chosen and evaluated individually for the presence or absence of fruit fly larvae. To detect larvae, fruit were cut into small slices (~1 mm thick) starting from the bottom of the fruit. One week before fruit harvest (14-16 Feb), all traps were taken down from trees and the total number of fruit flies captured per trap was counted. The amount of lure still remaining was also measured in all traps. Several PET and MS2 traps were discarded for this analysis of remaining lure because more than 250 mL was observed, suggesting that rainwater had entered the traps. The temperature, relative humidity, and rainfall in the orchard were recorded hourly throughout the experiment with a weather station model WS-2811 SAL-IT La Crosse Technology (La Crosse, Wisconsin, USA) placed in the same orchard.

Statistical Analysis

In cage bioassays, landings and captures were normally distributed; data were $\sqrt{(x + 0.5)}$ transformed to stabilize the variance and subjected to

2-way analysis of variance (ANOVA), with sex and trap design as factors. Percentages of flies that escaped the trap in 30 min were arcsine square root transformed for stabilization of the variance. As sex had no statistically significant effect on physical retention, traps were subsequently compared by one way ANOVA on the total number of flies that escaped the trap. Means separation was achieved by HDS Tukey's test. Under field conditions, numbers of flies captured were rank transformed and subjected to 2-way ANOVA. In the mass trapping assay, total flies captured per trap were rank transformed and subjected to 2-way ANOVA. Average percentage of females per trap was calculated with traps that captured at least 1 fruit fly. Percentage of females values were not normally distributed and were subjected to a Kruskal Wallis nonparametric test. Remaining lure inside traps was normally distributed and analyzed by one-way ANOVA. The percentage of infested fallen fruits per treatment was subjected to one way ANOVA. All analyses were performed using SPSS v.19 software (SPSS Inc., Chicago, Illinois).

RESULTS

Trap Efficacy and Physical Retention under Cage Conditions

Of the 480 flies released in the cage (16 replicates \times 32 flies), only 166 (34.6%) were recaptured. Both sex ($F = 12.24$; $df = 1,120$; $P < 0.01$) and trap type ($F = 16.95$; $df = 3,120$; $P < 0.01$) significantly affected the capture rate. However, the interaction of the 2 factors was not significant. Traps captured significantly more females (109; 65.7%) than males (57; 34.3%). PET bottles showed significantly higher captures of flies than the commercial traps MS2 and A & C trap (Table 1).

During the 32 h of observation (2 hours \times 16 reps), a total of 216 flies were observed landing on traps. No significant differences were observed in the number of visits to the 4 trap models ($F = 0.823$; $df = 3,120$; $P = 0.484$). However, signifi-

cantly more females than males visited traps ($F = 17.11$; $df = 1,120$; $P < 0.01$), with 66.2% of visits by females (143 flies) and 33.8% by males (73 flies).

As for the physical retention of the traps, the number of flies that escaped differed significantly among the 4 trap models ($F = 13.13$; $df = 3,60$; $P < 0.01$); in the absence of lure or insecticide, the A & C Trap was significantly more efficient in retaining flies than the other traps (Table 1).

Trap Efficacy under Field Conditions

During the 8 weeks when 24 traps were exposed for fly capture, a total of 394 fruit flies were trapped of which 324 (82.2%) were *A. ludens*, 57 (14.5%) were *Anastrepha obliqua* (Macquart), 10 (2.5%) were *Anastrepha striata* (Schiner) and 3 (0.8%) were *Anastrepha serpentina* (Wiedemann). Analysis was only performed on the number of *A. ludens* captured. Significant differences were observed between sexes ($F = 32.71$; $df = 1,376$; $P < 0.01$) and among trap types ($F = 37.64$; $df = 3,376$; $P < 0.01$). The interaction of these 2 factors was not statistically significant. The number of captured females (69.1%; 224 individuals) was significantly higher than that of males (30.9%; 100 individuals). Although the number of trapped flies in the transparent PET trap was numerically higher, no significant differences were observed between the colorless and the blue PET bottles. However, significantly fewer captures were observed in the MS2 and the A & C traps compared to PET bottles (Fig. 2).

Mass Trapping Assay

A total of 452 traps were recovered in the mass trapping assay. Twenty eight traps could not be evaluated because they had fallen to the ground or disappeared. Of the total of 4,749 fruit flies trapped, 3,672 (77.3%) were *A. ludens*, 978 (20.6%) were *A. obliqua*, 61 (1.3%) were *A. striata* and a few specimens (< 1%) were *A. serpentina*, *Anastrepha fraterculus* (Wiedemann) and *Anastrepha cordata* (Aldrich). Only *A. ludens* were considered for analysis. Significant differences

TABLE 1. AVERAGE (\pm SE) OF NUMBER OF FLIES THAT WERE CAPTURED OR THAT LANDED ON TRAPS DURING TWO PERIODS OF OBSERVATION AND PERCENTAGE OF FLIES THAT ESCAPED FROM TRAPS IN 30 MIN AFTER BEING RELEASED INSIDE.

Trap model	Trapped flies	Flies that landed	% Escaped flies
	Flies/Trap and Rep. \pm S.E.	Flies/Trap and Rep. \pm S.E.	% Flies/Trap and Rep. \pm S.E.
Transparent PET bottle	3.88 \pm 0.60 a	2.63 \pm 0.48 a	33.85 \pm 3.61 a
Blue PET bottle	4.13 \pm 0.68 a	3.94 \pm 0.80 a	38.02 \pm 3.88 a
MS2	1.69 \pm 0.35 b	3.81 \pm 0.76 a	42.71 \pm 4.80 a
A & C Trap	0.69 \pm 0.34 b	3.13 \pm 0.54 a	13.54 \pm 2.83 b

Averages followed by the same letter within a column are not significantly different ($P = 0.05$), Tukey's mean separation test.

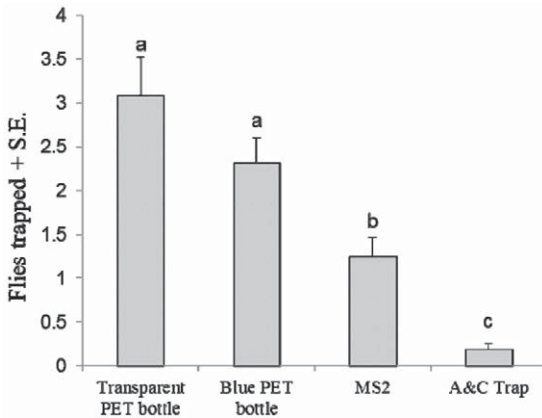


Fig. 2. Mean (\pm S.E.) number of flies per trap and repetition captured by 4 different trap models in a blocks design under field conditions. Bars labeled with identical letters were not significantly different after comparisons among treatments (ANOVA, HDS $\alpha = 0.05$, Tukey's test).

in captures were observed between sexes ($F = 168.19$; $df = 1,897$; $P < 0.001$), among trap types ($F = 229.18$; $df = 2,897$; $P < 0.001$) and in the interaction of these factors ($F = 11.99$; $df = 2,897$; $P < 0.001$). Overall, colorless transparent PET bottles

captured 1.5 and 6.4 times more flies per day than the MS2 and A & C traps, respectively (Table 2). Moreover, higher numbers of flies per trap and day were captured by the PET bottles during the entire period of the study (Fig. 3). No significant differences between traps were observed in the percentage of females caught (Kruskal Wallis, $P = 0.332$). The average percentage of infested fallen fruits by *A. ludens* in plots protected with the A & C trap was 3.4 times higher than with the colorless PET bottle, although no significant differences were observed in relation to the crop protection with the use of the different traps ($F = 2.22$; $df = 2,15$; $P = 0.151$) (Table 2). Lure evaporation also differed significantly between traps ($F = 20.85$; $df = 2,422$; $P < 0.01$). Significantly more lure evaporated in the PET bottles and MS2 traps compared to the A & C trap (Table 2).

DISCUSSION

The results of this study indicate that the yellow color of the commercial traps did not significantly increase the attractiveness of the traps when they were baited with CeraTrap®. Color has been mentioned by several authors as an important component of the visual attraction of traps (Prokopy 1968; Cytrynowicz et al. 1982; Economopoulos 1989; Sivinski 1990; Robacker

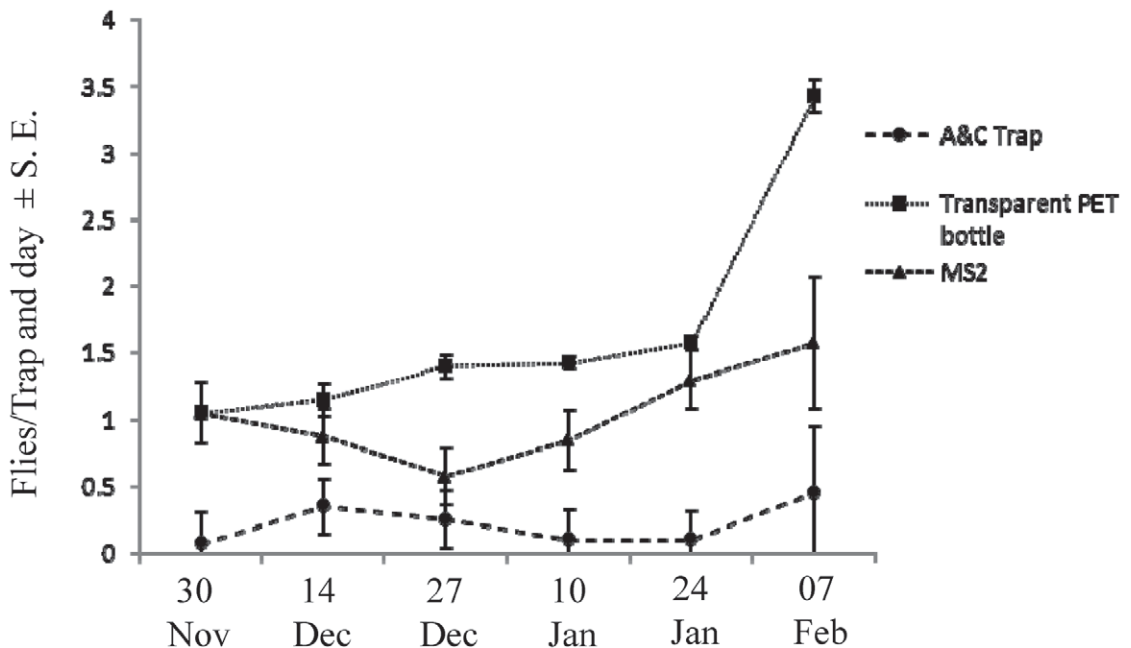


Fig. 3. Mean (\pm S.E.) number of flies per trap and day captured in forty traps of each model, at 2-week intervals during the course of the mass trapping experiment.

TABLE 2. NUMBER OF TRAPS RECOVERED PER TREATMENT, MEAN TOTAL *A. LUDENS* CAPTURED PER TRAP, AVERAGE PERCENTAGE OF FEMALES TRAPPED, PERCENTAGE OF FRUITS DAMAGED BY THE MEXICAN FRUIT FLY IN FRUITS FALLEN TO THE GROUND DURING THE COURSE OF THE EXPERIMENT AND THE VOLUMES OF LURE REMAINING INSIDE THE TRAPS AT THE END OF THE MASS TRAPPING EXPERIMENT.

Trap Model	No. traps recovered	Mean Flies/Trap	% females	% infested fruits (n = 600)	Vol. (mL)
Transparent PET bottle	142	13.76 ± 0.88 a	74.35 ± 1.62 a	0.33 ± 0.2 a	146.8 ± 0.44 a
MS2	153	9.02 ± 0.65 b	73.82 ± 1.77 a	1.17 ± 0.2 a	143.2 ± 0.31 a
A & C Trap	157	2.15 ± 0.31 c	71.34 ± 3.60 a	2.33 ± 1.1 a	166.6 ± 0.25 b

Mean values are indicated ± S.E.

Means followed by same letter within a column were not significantly different ($P = 0.05$, Tukey's mean separation test).

et al. 1990; Robacker 1992; López-Guillén et al. 2009). Shape, size and color influence fruit fly attraction, especially when traps are built to mimic host fruits (Cytrynowicz et al. 1982; Sivinski 1990). However, other authors observed that the contrast of the trap against the background seems to be a very important factor in fly attraction (Nakagawa et al. 1978; Drummond et al. 1984). Attraction to our colorless transparent trap model could be explained by the contrast of the trap with the background, and could possibly be enhanced by the white-cream color of the trap when baited with the CeraTrap lure. In the A & C trap, the access through narrow conical holes managed to retain significantly more flies, but this feature often hinders the ability of flies to gain entry to the trap (personal observation).

Trap efficacy for Mexican fruit fly control will clearly depend on the trap and bait combination used (Lasa et al. 2014). For mass trapping, besides the trap and lure used, the density of traps per ha and their distribution within the orchard will be crucial in determining the efficacy of this technique as a pest control measure (El Sayed et al. 2006). In the region of Martínez de la Torre, *A. ludens* population levels in citrus crops are significantly higher in fruits harvested in May than in those harvested between late Sep and Nov (Aluja et al. 2012). As a result, the density of 40 traps per ha used for mass trapping in seasonal grapefruit or orange could be insufficient to provide a good level of control in grapefruit for the May harvest due to an increase of fruit fly populations during this fruiting period. Consequently, the number of traps per ha will need to be evaluated and adjusted to the level of pest pressure and crop susceptibility at a given time (Martínez-Ferrer et al. 2010). In Spain, more than 50,000 ha of citrus groves are currently protected against the Mediterranean fruit fly, *C. capitata* (Wiedemann) by mass trapping strategies; and densities of ~50 traps per ha hung from trees 3 months before harvest provide good levels of pest control in this region (Navarro-Llopis et al. 2008).

CeraTrap® lure is highly attractive to *A. ludens* females (Lasa et al. 2013; Lasa et al. 2014). While CeraTrap® lure is more expensive than other

protein-derived lures, its durability and chemical stability makes it suitable for long-term trapping programs. Less than 50% of the lure evaporated during the fruit ripening period, when fruit were susceptible to fly infestation (3 months), and attractancy was maintained right until the end of the trial period (Fig. 3). However, lure evaporation will in part depend on the diam of the holes and the climatic conditions during the ripening period. Low evaporation of the lure in our experiment could be explained by relatively low prevailing temperatures (~20.0 °C), high RH (~82 %) and high rainfall (133 mm cumulative precipitation) during the trial. Under warmer conditions, such as those in mango crops, more than 50% of the lure can evaporate in just 2 months (personal observation). In our study, the cost of the control program was reduced because of no need to re-bait traps.

Field results showed higher levels of captured females (69-74%) than males (26-31%), and this greater attraction for females brings with it an important benefit in reducing crop infestations. Similar results were observed by our group using CeraTrap® in other tests (Lasa et al. 2013; Lasa et al. 2014). Females tend to be more active in seeking sources of protein because of the critical requirement for this nutrient for the maturation of eggs (Hendrichs et al. 1991; Katsoyannos et al. 1991), as was widely observed with other protein baits (Houston 1981; Aluja et al. 1989; Piñero et al. 2002; Conway & Forrester 2007; Martínez et al. 2007). The levels of infestation of fallen fruits observed throughout the 12 ha of the orchard is considered very low in relationship to the number of fly captures (> 3,700 *A. ludens* flies captured during the trial), corroborating the efficacy of the mass trapping strategy. Only 0.4% of sampled fruit was infested in blocks that used the colorless transparent PET traps although no significant differences in crop protection were observed among the different treatments. This study demonstrates the high efficiency of simple traps that are easily constructed from a transparent PET bottle, which cost only ~US\$0.18, including the labor for drilling holes and attaching the hanging wire. This cost is just 13% of that of commercial traps pro-

duced in Mexico, which sell for ~US\$2.3-2.8 per unit. At a density of 40 traps/ha, this represents a difference of US\$7.20 (PET) against ~US\$100 (MS2) per ha in trap costs alone. Thus, the use of PET bottles could represent significant savings for growers using mass trapping control strategy. Moreover, although durability of traps was not evaluated, PET traps have been re-used during 2 consecutive years and remain in good enough condition for use during a third year. In contrast, MS2 traps are continuously discarded because the plastic structure connecting the base and the lid, breaks from use.

Although the use of these kinds of affordable traps and the prolonged efficacy of CeraTrap can favor the development of mass trapping programs, more studies with different trap densities, varieties of citrus and weather conditions are needed to elucidate and confirm the efficacies of these innovative technologies against *A. ludens*.

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