Response of the Sapote Fruit Fly, Anastrepha serpentina (Diptera: Tephritidae), to Commercial Lures and Trap Designs in Sapodilla Orchards

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Response of the sapote fruit fly, *Anastrepha serpentina* (Diptera: Tephritidae), to commercial lures and trap designs in sapodilla orchards

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Abstract

Anastrepha serpentina (Wiedemann) (Diptera: Tephritidae) is one of the least studied of the pestiferous Neotropical tephritid flies despite its propensity to attack several commercial fruit crops, mainly in the Sapotaceae (Ericales). Few studies have been performed to improve monitoring traps and lures specifically targeted at this species. Management currently is achieved by using the hydrolyzed protein lure (Captor® with borax) and a Multilure® trap in combination with chemical control measures. Here, we aimed to evaluate the efficacy of other commercial lures and traps for monitoring purposes in sapodilla orchards. The efficacies of 3 commercial lures, namely, Captor (chemically hydrolyzed protein) + borax, CeraTrap® (enzymatically hydrolyzed protein), and Biolure® (dry lure based on ammonium acetate and putrescine), were compared in 2 independent experiments. In a 1st experiment, CeraTrap caught twice as many *A. serpentina* flies per trap per day as Captor + borax. In a 2nd experiment, trapping efficacy of CeraTrap and Biolure was similar, and both lures caught more *A. serpentina* flies per day per trap than Captor + borax. No significant differences in the capture of *A. serpentina* were observed among a Multilure trap, a Tephi Trap®, or a simple polyethylene bottle trap, when baited with CeraTrap. This study contributes with additional information on the response of *A. serpentina* to commercial lures, showing that CeraTrap could represent an effective alternative to monitor this pest using simple and cheap polyethylene bottle traps.

Key Words: CeraTrap; Biolure; Multilure; bottle trap

Resumen

Anastrepha serpentina (Wiedemann) (Diptera: Tephritidae) es una de las especies de tefrítidos Neotropicales menos estudiada a pesar de que tiende a atacar varios frutos comerciales, principalmente de las Zapotáceas (Ericales). Pocos estudios han sido realizados para mejorar trampas y cebos en el monitoreo de esta especie. El manejo actual se realiza utilizando la proteína hidrolizada (Captor® con bórax) y una trampa Multilure® en combinación con controles químicos. Nuestro objetivo fue evaluar la eficacia de otros cebos comerciales y trampas con miras al monitoreo en huertos de Chico Zapote. La eficacia de tres cebos comerciales, Captor (proteína de hidrólisis química) + bórax, CeraTrap® (proteína de hidrólisis enzimática), y Biolure® (cebo seco a base de acetato de amonio y putrescina) fue comparada en dos experimentos diferentes. En un primer experimento CeraTrap capturó dos veces más moscas de *A. serpentina* por trampa y día que Captor + bórax. En un segundo experimento, la eficacia de captura de CeraTrap y Biolure fue similar, y ambos cebos capturaron más moscas de *A. serpentina* por trampa y día que Captor + bórax. No hubo diferencias significativas de captura de *A. serpentina* entre una trampa Multilure, una Tephi Trap® o una trampa simple hecha con una botella de polietileno. Este estudio provee información adicional sobre la respuesta de *A. serpentina* a cebos comerciales en donde CeraTrap y una simple trampa hecha con una botella de plástico puede representar una alternativa eficaz para el monitoreo de esta.

Palabras Clave: CeraTrap; Biolure; Multilure; trampa de botella

The sapote fruit fly, *Anastrepha serpentina* (Wiedemann) (Diptera: Tephritidae), is one of the least studied species of pestiferous Neotropical tephritid fruit flies. This species has a wide distribution from northern Mexico to northern Argentina (Hernández-Ortiz & Aluja 1993) and sporadic intrusions into the southern United States (Mangan et al. 2011). It has been recorded from 45 species of fruit in 17 families (Norrbom 2002), but it is considered an important pest of fruit only in the family Sapotaceae (Ericales). In Mexico, the commercial crops mostly affected are sapodilla, *Manilkara zapota* (L.) P. Royen (known as chico zapote in Mexico), and mamey sapote, *Pouteria sapota* (Jacq.) H. E. Moore & Stearn (known as zapote mamey in Mexico) (Hernández-Ortiz & Aluja 1993). Developing larvae grow easily in the pulp of mature fruit of both species. Losses due to pest infestation can reach up to 80% of mature fruit in sapodilla crops (Hernández-Ortiz 1992). This fact, and the growing market value of sapodilla and mamey crops, has prompted inclusion of *A. serpentina* in the Mexican federal government's National Fruit Fly Eradication Program (Reyes et al. 2000). Several studies have been performed on the biology and behavior of *A. serpentina,* yet few studies have specifically addressed issues around monitoring or control of this pest (reviewed by Aluja & Norrbom 2000).

In the central region of Veracruz State, Mexico, sapote fruit production occurs throughout almost the entire year with a major peak in production between May and Aug. The presence of unharvested mature fruit during winter favors maintenance of populations of the pest during this period, triggering rapid population rises when maturing fruits become abundant during the main production period. In this period,

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A. serpentina populations are so large that growers are obligated to harvest green fruit as a measure to avoid infestation (personal observation). High captures of *A. serpentina* have been recorded in studies performed to monitor populations of the West Indian fruit fly, *Anastrepha obliqua* (Macquart) in mango *Mangifera indica* (L.) (Sapindales: Anacardiaceae) trees during the May–Jun period in the same region (Lasa & Cruz 2014). Although collecting green fruit and ripening it artificially can reduce economic losses, there is interest among growers in the development of an efficient and cheap system to monitor populations of this fly. Like other *Anastrepha* species in Mexico, this pest currently is monitored using a Multilure® trap and a liquid lure based on hydrolyzed protein, Captor® + borax, under guidelines established by Mexico's phytosanitary authority (NOM-023-1995) for the Mexican National Fruit Fly Campaign (Anonymous 1999). In a previous trial targeted at monitoring *A. obliqua* in mango orchards, an enzymatic hydrolyzed animal protein product, CeraTrap® (Sierras et al. 2006), was reported to capture more *A. serpentina* flies than the Captor + borax lure. However, 2 different trap models were used in that trial, making direct comparisons of lure efficacy impossible.

The objective of the present study was to examine the efficacy of the CeraTrap lure for monitoring *A. serpentina* and compare it with 2 lures widely used to monitor other pestiferous species of *Anastrepha*. In order to further reduce costs and produce accessible management tools for resource-poor small-scale growers, we also evaluated the efficacy of a simple polyethylene (PET) bottle trap, which was found to be effective for monitoring *Anastrepha ludens* (Loew) in citrus crops (Lasa et al. 2015), and compared it with the widely used Multilure trap and a Tephri Trap®, which proved effective for *A. obliqua* monitoring in previous tests (Lasa & Cruz 2014).

Materials and Methods

LURES AND TRAPS

Three different commercial odor lures, commonly used against *Anastrepha* fruit flies, were compared in the field: i) Captor (Promotora Agropecuaria Universal, Mexico City, Mexico), a chemically hydrolyzed protein prepared following the Mexican phytosanitary authority's standard using 10 mL hydrolyzed protein Captor 300, 5 g borax (J. T. Baker, Mexico City, Mexico), and 235 mL water (Anonymous 1999); ii) CeraTrap (Bioibérica, Barcelona, Spain), a liquid hydrolyzed animal protein that releases a number of volatile compounds, mostly amines and organic acids, that are highly attractive to *Anastrepha* species (Lasa et al. 2013); and iii) Biolure® (Suterra LLC, Bend, Oregon, USA), a dry lure in individual sachets that releases ammonium acetate and putrescine (Heath et al. 1997).

Several trap models were used in the experiments: i) glass McPhail traps (McPhail 1937); ii) Multilure traps (Better World Manufacturing Inc, Fresno, California, USA) (Martínez et al. 2007), which are invaginated plastic traps with 2 components that are recommended by the Mexican phytosanitary authority to monitor *Anastrepha* species (Anonymous 1999); iii) Tephri Trap (Sorygar, Madrid, Spain), which is a yellow cylindrical trap with a funnel base and four 22 mm non-invaginated lateral holes; and iv) a transparent colorless 500 mL PET bottle (Tecni Plastic Containers S.A. de C.V., Martinez de la Torre, Mexico) that was modified by drilling three 10 mm diameter holes (previously described by Lasa et al. 2014b).

EVALUATION OF LURES

Experiment 1. Captor + borax and CeraTrap lures were compared in an area of sapodilla orchards in the locality of Apazapan (19°19ˈ35.04" N, 96°43ˈ22.75"W) in the state of Veracruz, Mexico, during Apr–May 2014. Four independent blocks of ~1 ha each, located within a sapodilla orchard (10 ha) surrounded by mango trees. Each block received 2 glass McPhail traps, 1 baited with Captor + borax and 1 baited with CeraTrap. Both traps were baited with 250 mL of liquid lures. Hydrolyzed protein was replaced every 7 d, whereas CeraTrap was not replaced during the entire course of the experiment, and only 15 to 50 mL of lure was added every week in order to maintain the correct fluid volume (250 mL). Traps were placed on sapodilla trees at a height of 4.0 to 4.5 m, within the canopy, and spaced at a distance of 20 to 30 m between traps. Placement of traps within each block was initially randomized, and the position of traps was rotated sequentially when checked weekly during the 6 wk experiment (3 times per position). Captured insects were collected and placed in vials with 70% ethanol, counted, and identified to species and sex.

Experiment 2. An additional experiment was performed during June–Jul 2014 in an orchard located in central Veracruz State (19°20ˈ16.49"N, 96°43ˈ53.52"W) to compare the attraction of Captor + borax with that of CeraTrap and Biolure. A sapodilla orchard (~2 ha) was divided in five blocks of 0.4 ha each. Each block contained Multilure traps baited with 3 different lures: i) Multilure trap with Captor + borax, ii) Multilure trap with CeraTrap, iii) Multilure trap with Biolure. All traps were baited with 250 mL of lure or 10% polyethylene glycol in water as a retention method in the case of the dry lure, Biolure. Traps were placed randomly 1 to a tree, at a height of 3.5 to 4.0 m and with a distance of 20 to 22 m between adjacent traps within a block. Blocks were located 25 to 30 m apart. Traps were monitored every 7 d, and captured insects were counted and identified to species and sex. The location of traps was rotated sequentially in each block every week during the 6 wk evaluation period (2 times per position). Captor + borax was re-baited every week, whereas CeraTrap and 10% polyethylene glycol were renewed after 3 wk of exposure. Dry Biolure was not replaced during the experiment. During the experiment, CeraTrap and polyethylene glycol were maintained at a 250 mL volume by adding 15 to 50 mL of product every week if necessary. In this experiment, numbers of *A. obliqua* flies also were recorded due to high capture levels of this species, given the proximity of mango orchards.

Experiment 3. A 3rd experiment was performed to determine the relative efficacy of a simple PET bottle trap and to compare it with the standard Multilure trap and the Tephri Trap. A block design with 4 blocks and 3 traps per block was set up between Jan and Feb 2015 in a sapodilla orchard in Apazapan (19°19'2.80"N, 96°43'23.87"W), Veracruz, Mexico. All traps were baited with 250 mL of CeraTrap due to the greater efficacy of this lure in the previous experiments. Traps were placed at a height of 3.5 to 4.0 m on trees and spaced 15 m apart. Four replicate blocks, at least 21 m apart, were established within a 2 ha test plot. Traps were randomized initially and rotated clockwise every 7 d during the 6 w experimental period (2 times per position). Insects captured by each trap were placed in 70% alcohol and taken to the laboratory, where the numbers of males and females of *Anastrepha* species captured per trap were counted. CeraTrap was not replaced during the experiment, but volumes of 15 to 50 mL of lure lost through evaporation or during fly recovery were replaced at sampling.

STATISTICAL ANAYLYSES

For the evaluation of lures and traps, the numbers of captured *A. serpentina* flies were transformed to flies per trap per day (FTD). In all cases, the percentage of females was calculated for traps that captured at least one fly. FTD was square root transformed and subjected to a *t*-test in experiment 1 and 1-way analysis of variance (ANOVA) in experiments 2 and 3. The percentages of females were arcsine root

Table 1. Mean (± SE) number of *A. serpentina* adult flies captured per trap per day (FTD), percentage of females captured, and number of traps that did not capture any flies in field experiments with McPhail traps baited with Captor + borax or CeraTrap. Field experiments were conducted in a sapodilla orchard in Veracruz, Mexico.

Trap with lure	FTD	Females (%)	Zero captures[a] (*n* = 24)
McPhail with Captor + borax	5.3 ± 1.3a	86.9 ± 3.1a	0
McPhail with CeraTrap	12.1 ± 2.5b	85.8 ± 3.2a	0

Means in columns followed by the same letter were not significantly different (Turkey test on square root FTD or arcsine square root transformed percentage of females, *P* = 0.05; non-transformed means shown).

[a]Number of traps that did not capture any *A. serpentina* during the experiment.

transformed to stabilize variance and subjected to a *t*-test in experiment 1 and 1-way ANOVA in experiments 2 and 3. Mean separation in experiments 2 and 3 was performed by Tukey test. The numbers of *A. obliqua* captured in experiment 2 were transformed and analyzed as described for *A. serpentina*. The proportion of traps with zero captures was compared by *Z*-test for both experiments. All statistical analyses were performed using SPSS v.19 software (SPSS Inc., Chicago, Illinois, USA).

Results

EVALUATION OF LURES

Experiment 1. In total, 3,043 tephritid flies were captured during the 6 wk period of trap exposure. Of these, 2,882 (94.7%) were *A. serpentina*, 136 (4.5%) were *A. obliqua*, and 25 (<1%) were *A. ludens*. In total, 2,171 individuals and 872 individuals were captured in traps baited with CeraTrap and Captor + borax, respectively. Values of flies captured per trap per day (FTD) differed significantly between lures, with CeraTrap being more attractive to *A. serpentina* than Captor + borax ($t = 2.67$; df = 46; $P = 0.011$) (Table 1). There was no effect of lure on the percentage of *A. serpentina* females in the total capture ($t = 0.245$; df = 46; $P = 0.807$), and all traps of both lures captured at least 1 *A. serpentina* individual during the experiment (Table 1).

Experiment 2. In total, 11,754 tephritid flies were captured during the 6 wk trap exposure period, of which 10,804 (91.9%) were *A. serpentina*, 911 (7.8%) were *A. obliqua*, and 39 (<1%) were *A. ludens*. In total, 5,397 flies, 4,410 flies, and 1,947 flies were found in traps baited with CeraTrap, Biolure, and Captor + borax, respectively. Treatment affected capture of both *A. serpentina* ($F = 16.66$; df = 2, 87; $P < 0.001$) and *A. obliqua* ($F = 11.69$; df = 2, 87; $P < 0.001$) (Table 2). More *A. serpentina* flies were captured in traps baited with Biolure or CeraTrap than in traps baited with Captor + borax. In contrast, more *A. obliqua* flies were captured in traps baited with CeraTrap than in traps with Biolure or Captor + borax. There were no differences in percentage of females among lures for *A. serpentina* ($F = 1.04$; df = 2, 87; $P = 0.358$) or *A. obliqua* ($F = 1.91$; df = 2, 81; $P = 0.155$) (Table 2). All traps of all lures captured at least 1 *A. serpentina* individual during the experiment, but for *A. obliqua,* although CeraTrap showed a greater sensitivity with a lower frequency of zero captures, there was no difference among treatments ($Z = 1.24$; df = 2; $P = 0.558$).

EFFICACY OF TRAPS

Experiment 3. In total, 749 *Anastrepha* individuals were captured during the 6 wk trap period, of which 650 (86.8%) were *A. serpentina*, 66 (8.8%) were *A. obliqua*, 60 (8.0%) were *A. ludens*, 13 (1.7%) were *Anastrepha striata* (Schiner), and 5 (<1%) were *Anastrepha bicolor* (Stone). In total, 214 flies, 239 flies, and 296 flies were trapped in the PET bottle, Multilure trap, and Tephri Trap, respectively. For *A. serpentina*, FTD values did not differ among traps ($F = 1.84$; df = 2, 69; $P = 0.166$) (Table 3). Significant differences were not observed among trap models in the percentage of females in the total capture ($F = 1.84$; df = 2, 63; $P = 0.166$) (Table 3). No significant differences were observed in the trap sensitivity (number of zero captures) for *A. serpentina* capture among traps ($Z = 1.07$; df = 2; $P = 0.585$).

Discussion

The enzymatically hydrolyzed protein CeraTrap outperformed Captor + borax in the sapodilla orchards used in the first 2 experiments. In the 2nd experiment, the attraction of CeraTrap was superior to that of Captor + borax but similar to that of the dry lure Biolure when trapping *A. serpentina* in the same orchard. When trapping *A. obliqua* in sapodilla (experiment 2), CeraTrap outperformed both Biolure and Captor + borax in line with previous observations aimed at monitoring the pest in mango orchards of this region (Lasa & Cruz 2014). About 2.3 to 2.7 times more *A. serpentina* individuals were captured with CeraTrap than with the standard lure Captor + borax, across the first 2 trails. However, all traps captured at least 1 *A. serpentina* fly which indicated no difference in the sensitivity of lures at very low population levels. All lures tended to capture more females than males as expected because females are more attracted to proteinaceous sources as these substances are required for egg maturation (Hendrichs et al. 1991). This tendency has also been observed in other tephritid species trapped using food-based lures (Díaz-Fleischer et al. 2014) including other *Anastrepha* species (Aluja et al. 1989; Conway & Forrester 2007; Lasa et al. 2014a; Martínez et al. 2007).

Table 2. Mean (± SE) number of *Anastrepha serpentina* and *A. obliqua* adult flies per trap per day (FTD) and percentage of females captured with traps baited with Captor + borax, CeraTrap, or Biolure in sapodilla trees.

Fruit fly species	Trap with lure	FTD	Females (%)	Zero captures[a] (*n* = 24)
A. serpentina	Multilure with Captor + borax	8.4 ± 1.3a	68.8 ± 2.7a	0
	Multilure with CeraTrap	23.1 ± 2.8b	70.3 ± 1.9a	0
	Multilure with Biolure	20.0 ± 2.2b	74.0 ± 1.8a	0
A. obliqua	Multilure with Captor + borax	0.9 ± 0.2a	62.1 ± 4.8a	3a
	Multilure with CeraTrap	2.5 ± 0.4b	72.1 ± 3.0a	1a
	Multilure with Biolure	1.0 ± 0.1a	74.0 ± 5.0a	3a

Means in columns followed by the same letter were not significantly different (Turkey test on square root transformed FTD or arcsine square root transformed percentage of females, *P* = 0.05, or *Z*-test in the case of zero captures; non-transformed means shown).

[a]Number of traps that did not capture any *A. serpentina* and *A. obliqua* during the experiment.

Table 3. Mean (± SE) number of *Anastrepha serpentina* adult flies per trap per day (FTD) and percentage of females captured in PET bottles, Multilure, and Tephri Traps baited with CeraTrap.

Trap with lure	FTD	Females (%)	Zero captures[a] (*n* = 24)
PET bottle with CeraTrap	0.96 ± 0.15a	76.8 ± 3.5a	2a
Multilure with CeraTrap	1.14 ± 0.31a	78.8 ± 5.1a	3a
Tephri Trap with CeraTrap	1.50 ± 0.23a	74.0 ± 1.8a	1a

Means in columns followed by the same letter were not significantly different (Turkey test on square root transformed FTD or arcsine square root transformed percentage of females, *P* = 0.05, and *Z*-test in the case of zero captures; non-transformed means shown).
[a]Number of traps that did not capture any *A. serpentina* during the experiment.

The response of *A. serpentina* to CeraTrap over the 6 wk period in the 1st experiment clearly indicated the stability and durability of this lure, as previously reported in this region (Lasa et al. 2015). Due to its stability, this liquid lure has also been effectively used for mass trapping *A. ludens* in citrus crops and was found to remain effective during 10 consecutive weeks (Lasa et al. 2014b).

While evaluating options available to resource-poor mango producers under field conditions, Piñero et al. (2003) reported that human urine and chicken feces were less attractive to *A. serpentina* than Captor (without borax) or torula yeast with borax. In a subsequent test, Aluja & Piñero (2004) reported greater attraction of *A. serpentina* to diluted human urine compared with Captor in sapodilla orchards, whereas Captor outperformed dilute human urine as a lure for trapping *A. serpentina* and *A. obliqua* in mango orchards. Martínez et al. (2007) reported significantly greater capture of *A. serpentina* flies in yellow or green Multilure traps baited with Biolure than in glass McPhail traps baited with torula yeast. Nevertheless, more detailed studies, specifically addressing *A. serpentina* responses to lures and traps, are still needed to develop cost-effective sustainable management practices in sapodilla orchards.

The present study also revealed that a simple and cheap colorless PET bottle trap baited with CeraTrap was as effective for capture of *A. serpentina* as a Multilure or a Tephri Trap, each of which costs 4 to 6 US$. Similar results were observed with the same colorless PET bottle trap when compared with commercial traps, including the Multilure trap, for capture of *A. ludens* using CeraTrap lure in a grapefruit orchard in central Veracruz State (Lasa et al. 2015). Long-distance attraction of foraging flies to host plants and odor-baited traps is mediated by chemical volatiles, whereas at short distances, when a fly has landed on a tree, host finding and trap capture is driven mainly by visual cues (Aluja & Prokopy 1993; Finch & Collier 2000). It has widely been studied that several trap features can favor or affect trap capture of tephritid pests (Prokopy 1968; Nakagawa et al. 1978; Cytrynowicz et al. 1982; Economopoulos 1989; Sivinski 1990; Robacker 1992; Epsky et al. 1995; López-Guillen et al. 2009; Lunau 2014).

Given the magnitude of the local market, and the rising international demand for non-traditional exotic fruit, developing management tools for *A. serpentina*, a key pest of Neotropical Sapotaceae, could be considered as being of strategic importance. This study provides novel information on the response of *A. serpentina* to an enzymatically hydrolyzed protein product, CeraTrap. The CeraTrap lure is less expensive than alternatives such as Biolure and can be employed in the field during several weeks without the need for re-baiting. Moreover, this bait can be deployed effectively in a simple perforated PET bottle that is easy to manufacture using empty drink bottles at an extremely low cost. Developing effective and inexpensive monitoring tools constitutes a necessary initial step towards rational pest management in developing countries. Further studies on the response of flies to visual and odor cues may aid in designing efficient traps for use in mass trapping programs and reduce growers' reliance on insecticide applications for tephritid fly control, an alternative that still eludes pest species in the genus *Anastrepha*.

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