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## COMPARISON OF SYNTHETIC FOOD-BASED LURES AND LIQUID PROTEIN BAITS FOR CAPTURE OF *ANASTREPHA SUSPENS*A (DIPTERA: TEPHRITIDAE) ADULTS

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### ABSTRACT

Field tests were conducted in south Florida to compare capture of the Caribbean fruit fly, *Anastrepha suspensa* (Loew), in Multilure traps baited with either of the liquid protein baits torula yeast/borax or Nulure/borax, or with food-based synthetic lures including two-component Biolure (ammonium acetate, putrescine) and three-component Biolure (ammonium acetate, putrescine, trimethylamine). The highest relative proportion of females captured was in traps baited with the two-component Biolure (44-61%), intermediate capture was in traps baited with the three-component Biolure (14-24%) or torula yeast/borax (8-25%), and the lowest capture tended to be in traps baited with Nulure/borax (0-19%). Similar results were obtained for capture of males. Tests of the unipak two-component Biolure, which has a reduced ammonium acetate release rate and is a single package with both ammonium acetate and putrescine sections, captured similar numbers of both females and males as Biolure formulated in 2 individual packages. Traps baited with unipak Biolure combined with the addition of a trimethylamine lure captured fewer females than the unipak alone, but this was greater than capture in traps baited with torula yeast/borax. Our studies confirmed that the best lure for *A. suspensa* is ammonium acetate and putrescine. However, *C. capitata*-targeted traps baited with three-component Biolure should be as effective for *A. suspensa* detection and monitoring as traps baited with torula yeast/borax. The unipak two-component Biolure will provide the improved handling that has been requested by users.

**Key Words:** Caribbean fruit fly, Biolure, ammonium acetate, unipak, torula yeast

### RESUMEN

En pruebas de campo realizadas en el sur de Florida, se compararon resultados de captura de la mosca de la fruta del Caribe *Anastrepha suspensa* (Loew), capturadas en trampas Multilure que habian sido cebadas con cebos de proteina liquida de levadura torula/borax o con Nulure/borax, o con atrayentes alimenticios sinteticos los cuales incluian Biolure con dos componentes (acetato de amonnia, putrescina) y Biolure con 3 componentes (acetato de amonnia, putrescina, trimethylamina). El mas alto porcentaje de captura de hembras ocurrio en trampas cebadas con Biolure de dos componentes (44-61%), un nivel de captura intermedio ocurrio en trampas cebadas con Biolure de 3 componentes (14-24%) o con los cebos de levadura torula /borax (8-25%), mientras que la captura mas baja fue en aquellas trampas cebadas con Nulure/borax (0-19%). Se obtuvieron resultados similares en la captura de machos. Pruebas unipak del Biolure de dos componentes, el cual da una tasa de liberacion reducida de acetato de amonio y el cual es un atrayente individual con secciones de acetato de amonio y putrescina, no mostro diferencias en captura de machos o hembras entre el Biolure de dos componentes formulado como atrayentes individuales o como unipak. La captura intermedia de las hembras fue obtenida en trampas cebadas con unipak Biolure combinado con un atrayente individual de trimethylamina, pero esta captura fue mayor que la obtenida cuando se usaron trampas cebadas con levadura torula /borax. Nuestros estudios confirmaron que el mejor atrayente para *A. suspensa* es el acetato de amonio y putrescina. Sin embargo, trampas destinadas a atrapar *C. capitata* y cebadas con acetato de amonio, putrescina y trimethylamina deben ser tan efectivas como aquellas trampas cebadas con levadura torula /borax para la deteccion y monitoreo de *A. suspensa*. El unipak con Biolure de dos componentes es igualmente efectivo y dara una mejor facilidad para manipulacion, la cual ha sido pedida por los usuarios.

Translation provided by the authors.

Tephritid fruit flies are among the most important pests of fruits and vegetables in the world, and use of traps and lures are important components of fruit fly pest management programs. Standard trapping protocols have been developed for fruit fly detection and monitoring and, depending on target species, different female-targeted baits may be used (IAEA 2003). Selection of trap and lure depends on the purpose of trapping, availability of materials and cost. Although use of a single trap type that would capture the highest number of females and males of multiple species would have many advantages, species-specific traps that use lures specific to the target fruit fly and environmental conditions are the best systems available currently (Díaz-Fleischer et al. 2009). Among the numerous liquid protein baits, agencies primarily use torula yeast/borax solutions for detecting and monitoring *Anastrepha* spp. and NuLure/borax solutions for the Mediterranean fruit fly, *Ceratitis capitata* Weidemann (Epsky et al. 1993; Heath et al. 1993, 1994).

Food-based synthetic attractants have been developed based on volatile chemicals released from liquid protein baits. A two-component Biolure attractant comprised of ammonium acetate and putrescine is used in traps that target *Anastrepha* spp. (Heath et al. 1995; Epsky et al. 1995, Thomas et al. 2001). Addition of trimethylamine is used in traps that target *C. capitata* (Heath et al. 1997), and McPhail-type traps baited with the Biolure three-component attractant are equal to or better than liquid protein-baited traps for capture of *C. capitata* females (Epsky et al. 1999). Capture of *Anastrepha* spp. flies by traps baited with either the two-component or three-component attractant, however, is more variable (IAEA 2007). Initial studies found no difference in capture of the Mexican fruit fly, *Anastrepha ludens* (Loew), in traps baited with Biolure ammonium acetate and putrescine with or without the third Biolure component, trimethylamine (Heath et al. 1997). However, Holler et al. (2006) found that addition of trimethylamine reduced capture of the Caribbean fruit fly, *Anastrepha suspensa* (Loew).

Regulatory agencies deploy McPhail-type traps baited with the three-component Biolure (ammonium acetate, putrescine, and trimethylamine; Suterra LLC, Bend, OR), which is commercially available, primarily to monitor for new infestations of *C. capitata* in areas currently fly-free, and questions remain on the effectiveness of this trapping system for detecting and/or monitoring *A. suspensa*. Due to problems with the deployment of the synthetic attractants as individual lures, several versions of the components combined in a single lure have been developed and tested (Jang et al. 2007; Navarro-Llopis et al. 2008) including two-component and three-component unipak versions of Biolure (Holler et al. 2009). We report results of field tests that were

conducted in south Florida to compare capture of *A. suspensa* in traps baited with either of the liquid protein baits or with the Biolure two- and three-component food-based synthetic attractant (both individual lures and unipak lure) to determine relative effectiveness of these standard trapping systems.

## MATERIALS AND METHODS

### Traps and Lures

MultiLure traps (Better World Manufacturing Inc., Fresno, CA, USA) were used in all experiments. Liquid protein baits included aqueous solutions of torula yeast/borax (three 5-g pellets, 2.25:2.75 yeast:borax, in 300 mL water) (ERA International, Freeport, NY) and NuLure (Miller Chemical & Fertilizer Co., Hanover, PA) as a 300 mL aqueous solution of 9% NuLure (vol:vol) and 3% borax (wt:vol; sodium tetraborate decahydrate). Synthetic attractants included individual component Biolure formulations of ammonium acetate, putrescine, and trimethylamine, and the unipak two-component Biolure formulation of ammonium acetate and putrescine (Suterra LLC, Bend, OR). The membrane-release area of the ammonium acetate lure in the two-component unipak has been reduced from ~35 mm diam. on individual component lure to ~23 mm diam. on unipak lure. This lowers the release rate of ammonium acetate, which improves capture of the Mexican fruit fly, *Anastrepha ludens* (Loew) but has no effect on capture of *A. suspensa* (Thomas et al. 2008). Traps baited with synthetic lures contained 300 mL 10% polypropylene glycol (vol:vol; LowTox, Prestone, Danbury, CT, USA) aqueous solution to retain captured flies.

### Field Tests

Field tests were conducted at the Tropical Research and Education Center (TREC), University of Florida, Homestead, FL. Experiment 1 compared capture of flies in traps baited with (1) NuLure/borax, (2) torula yeast/borax, (3) two-component Biolure (individual ammonium acetate and putrescine lures), and (4) three-component Biolure (individual ammonium acetate, putrescine, and trimethylamine lures). Experiment 2 compared capture of flies in traps baited with (1) torula yeast/borax, (2) two-component Biolure, (3) unipak two-component Biolure, and (4) unipak two-component Biolure plus trimethylamine individual Biolure. Tests were conducted in two hosts, Surinam cherry, *Eugenia uniflora* L., and guava, *Psidium guajava* L., for experiment 1; and in one host, guava, for experiment 2. For the tests in Surinam cherry, all 4 treatments were placed around the periphery of a large tree in fruit. There were 3 blocks (replicates) of traps, with 2 m

between traps within a block and 10 m between blocks. For the tests in guava, there was 1 trap per tree with the traps placed in 3 rows (blocks/replicates) of trees. There were at least 10 m between rows and 10 m between traps within a row. For both experiments, traps were sampled every 7 d, and numbers of male and female flies were recorded. Traps were sampled for 4 wk per sampling period, for a total of 4 sampling periods in experiment 1 (sample periods 1-2 in Surinam cherry, sample periods 3-4 in guava), and 1 sampling period in experiment 2. The protein bait solutions were replaced every 7 d, but the synthetic lures were not replaced during a sampling period. A complete randomized design was used for trap placement at the start of each sampling period. Traps were rotated sequentially to the next position within a block at time of sampling, so that all treatments were in all positions within each sampling period. Trees were in fruit throughout both experiments, but the tests in Surinam cherry were conducted toward the end of the fruiting season (18 Jun to 13 Aug, 2008) and the experiments in guava were conducted in the beginning of the fruiting season (experiment 1 - Jun 25 to Aug 20, 2008; experiment 2 - Aug 27 to Sep 17, 2009). Fruiting in guava trees began earlier in 2008 than in 2009 because the trees were trimmed in spring 2009.

#### Statistical Analysis

Effect of treatment and either sample period (experiment 1) or sample week (experiment 2) were analyzed by two-way ANOVA in a factorial model with interaction (Proc GLM, SAS Institute 2000) followed by LSD mean separation ( $P = 0.05$ ) for significant factors. When necessary, data were transformed prior to analysis to satisfy conditions of equal variance (Box et al. 1978). Numbers of total flies per trap per day and percentage females for flies captured in traps baited with the Biolure two-component attractant were analyzed as assessments of population level. Summary statistics are presented as average  $\pm$  standard deviation.

## RESULTS

### Experiment 1

Number of total *A. suspensa* captured per trap per day in traps baited with the two-component Biolure was affected by sampling period ( $P = 9.08$ ,  $df = 3, 44$ ;  $P < 0.0001$ ;  $\log(x + 1)$  transformed data). Numbers of *A. suspensa* in traps in Surinam cherry decreased from  $4.0 \pm 4.4$  flies per trap per day in sampling period 1 to  $2.0 \pm 2.3$  flies per trap per day in sampling period 2. Numbers in traps in guava increased from  $0.4 \pm 0.2$  in sampling period 3 to  $5.8 \pm 5.0$  in sample period 4. Per-

centage of females captured in these traps was also affected by sampling period ( $P = 3.27$ ,  $df = 3, 48$ ;  $P = 0.0321$ ; square-root ( $x + 0.5$ ) transformed data). All captures were female biased, and percentage of females captured during sampling periods 1, 2, 3 and 4 were  $86.6 \pm 10.6$ ,  $80.8 \pm 14.5$ ,  $60.8 \pm 32.0$ , and  $76.1 \pm 16.7$ , respectively.

Numbers of female and male flies per trap per block were converted to relative trapping efficiency to facilitate comparisons among the range of population levels tested during the different sampling periods (Epsky et al. 1999). There was a significant interaction between sampling period and treatment for female ( $F = 3.17$ ;  $df = 9, 32$ ;  $P = 0.0075$ ) but not for male ( $F = 0.97$ ;  $df = 9, 32$ ;  $P = 0.4825$ ) relative trapping efficiencies. Therefore, one-way analyses were used to test effect of treatment within each sampling period for females and over all sampling periods for males. Traps baited with the two-component Biolure had the highest relative trapping efficiency for females for all sampling periods (Table 1). The next highest relative trapping efficiencies were for traps baited with the three-component Biolure and with torula yeast/borax solution. Relative trapping efficiency in traps baited with NuLure/borax was significantly less than capture in traps with the three-component attractant for the sampling periods in which the population was the lowest (sample periods 2 and 3). Treatment also had an effect on capture of males ( $F = 24.24$ ;  $df = 3, 44$ ;  $P < 0.0001$ ). The highest relative trapping efficiency of males was  $64.5 \pm 20.2\%$  in traps baited with the two-component Biolure, and this was higher than traps baited with the three-component Biolure, NuLure/borax or torula yeast/borax ( $12.4 \pm 10.9$ ,  $12.2 \pm 12.7$ , and  $10.9 \pm 10.5$ , respectively).

### Experiment 2

Number of total *A. suspensa* captured per trap per day in traps baited with the two-component Biolure was affected by sample week ( $P = 23.83$ ,  $df = 3, 8$ ;  $P = 0.0003$ ;  $\log(x + 1)$  transformed data). Numbers increased from  $6.5 \pm 0.6$  in week 1 to  $29.0 \pm 6.2$  in week 4. Percentage of females captured in these traps was not affected by sampling period ( $P = 1.62$ ,  $df = 3, 8$ ;  $P = 0.2591$ ; square-root ( $x + 0.5$ ) transformed data). Overall, captures were female biased, but the percentage of females captured decreased slightly from  $74.7 \pm 8.9$  in week 1 to  $65.9 \pm 5.1$  in week 4.

As in experiment 1, numbers of female and male flies per trap per block were converted to relative trapping efficiency for subsequent analysis. There was no interaction between treatment and sample week, so data from all sample weeks were pooled and effect of treatment was analyzed with one-way ANOVA. Both individual and unipak two-component Biolure formulations captured more females than traps baited with torula yeast/

TABLE 1. RELATIVE TRAPPING EFFICIENCY (%) FOR CAPTURE OF FEMALE *ANASTREPHA SUSPENS*A IN FIELD TESTS CONDUCTED IN HOMESTEAD, FL. MULTILURE TRAPS WERE USED FOR ALL BAITS AND EACH SAMPLE PERIOD WAS 4 WEEKS. SURINAM CHERRY WAS AT THE END OF THE FRUITING SEASON IN SAMPLE PERIOD 2 AND GUAVA WAS AT THE BEGINNING OF THE FRUITING SEASON IN SAMPLE PERIOD 3.

Bait*	Surinam cherry**		Guava	
	Sample period 1	Sample period 2	Sample period 3	Sample period 4
Two-component BioLure	44.1 ± 4.5 a	60.2 ± 10.2 a	60.9 ± 12.8 a	60.9 ± 20.5 a
Three-component BioLure	19.4 ± 8.5 b	24.2 ± 13.9 b	14.3 ± 9.4 b	23.7 ± 15.8 b
Torula yeast/borax	17.3 ± 7.2 b	10.1 ± 4.3 bc	24.8 ± 12.6 b	7.5 ± 2.2 b
NuLure/borax	19.2 ± 12.8 b	5.5 ± 2.9 c	0 c	8.0 ± 4.4 b
F	4.22	21.56	27.17	11.06
df	3,8	3,8	3,8	3,8
P	0.0459	0.0003	0.0002	0.0032

\*Ammonium acetate and putrescine alone (two-component Biolure) or with trimethylamine (three-component Biolure) in traps with 300 mL 10% propylene glycol solution, 3 torula yeast/borax pellets in 300 mL water, 9% NuLure and 3% borax in 300 mL water.

\*\*Means within a column followed by the same letter are not significantly different (LSD mean separation test on square root ( $x + 0.5$ )-transformed data, non-transformed mean ± standard deviation presented).

borax (Fig. 1 solid bars;  $F = 7.73$ ;  $df = 3, 44$ ;  $P = 0.0003$ ), with intermediate capture with unipak two-component Biolure plus trimethylamine. There was no difference in number of males captured in traps baited with two-component Biolure or unipak two-component Biolure (Fig. 1 shaded bars;  $F = 3.37$ ;  $df = 3, 44$ ;  $P = 0.0268$ ), and both treatments captured more males than traps baited with either with unipak Biolure plus trimethylamine or torula yeast/borax.

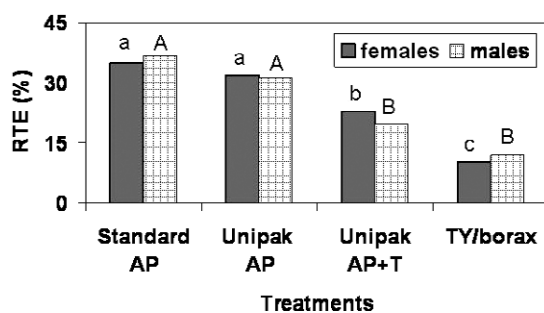


Fig. 1. Relative trapping efficiency (%) for capture of female (solid bars) and male (shaded bars) *Anastrepha suspensa* in field tests conducted in Homestead, FL. Multilure traps were used for all baits and traps were sampled for 4 weeks. Treatments included two-component Biolure as individual ammonium acetate and putrescine lures (Standard AP), unipak two-component Biolure (Unipak AP), unipak two-component Biolure plus trimethylamine individual Biolure (Unipak AP + T) in traps with 300 mL 10% propylene glycol solution, and 3 torula yeast/borax pellets (TY/borax) in 300 mL water. Bars headed by the same lowercase (females) or uppercase (males) letter are not significantly different (LSD mean separation test on square root ( $x + 0.5$ )-transformed data, non-transformed means presented).

## DISCUSSION

Glass McPhail traps baited with torula yeast/borax solution have been the standard trapping system for *Anastrepha* spp. since early studies found that torula yeast performed better than a number of other yeast formulations (Lopez et al. 1971), and most studies have evaluated liquid protein baits in these traps. Torula yeast/borax captured more *A. suspensa* than NuLure/borax in tests with glass McPhail traps (Epsky et al. 1993). Plastic McPhail traps baited with two-component Biolure captured more flies than glass McPhail traps baited with either torula yeast/borax or two-component Biolure in tests in Florida in Surinam cherry, loquat (*Eriobotrya japonica* [Thunb.] Lindl.), and guava (Thomas et al. 2001; Hall et al. 2005). Tests in grapefruit, *Citrus paradisi* Macfady, in Florida found that captures in Multilure traps baited with either two-component Biolure or torula yeast/borax were higher than in traps baited with NuLure/borax (Thomas et al. 2008). Tests in sapodilla, *Manilkara zapota* Van Royen, and mamey sapote, *Pouteria sapota* (Jacq.), in Puerto Rico, however, found more *A. suspensa* were captured in Multilure traps baited with torula yeast/borax than with two-component Biolure (Pingel et al. 2006). In the only previous test of three-component Biolure, Holler et al. (2006) found that the highest capture was in plastic McPhail traps baited with two-component Biolure, and that there was no difference between capture in plastic McPhail traps baited with three-component Biolure or glass McPhail traps baited with torula yeast/borax. Results from that study, in which traps were placed in scattered wild guava trees, were the same as the results from our study.

Unipak versions of two-component and three-component Biolure have been shown to be equal

to the same components formulated in single lure formulations for capture of sterile *C. capitata* released in Florida and for capture of *A. suspensa* in traps placed in backyard plantings of host fruit trees in Sarasota/Bradenton and in Ft. Pierce (Holler et al. 2009). We recorded similar results for *A. suspensa* in our tests in south Florida. Additionally, we found that the unipak two-component in combination with trimethylamine may be better than torula yeast/borax for *A. suspensa* monitoring. This may be due to the lower release rate of ammonia from the unipak two-component formulation. The greater amount of ammonia from the individual lure formulation used in experiment 1, when combined with the amines from the trimethylamine lure, may have been repellant to *A. suspensa*.

Our studies confirmed that the best lure for *A. suspensa* is two-component Biolure ammonium acetate and putrescine, however, *C. capitata*-targeted traps baited with three-component Biolure ammonium acetate, putrescine and trimethylamine should be as effective as traps baited with torula yeast/borax for *A. suspensa* detection and monitoring. The unipak two-component Biolure is equally effective and will provide the improved handling that has been requested by users.

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