Evaluation of a Single-Matrix Food Attractant Tephritid Fruit Fly Bait Dispenser for Use in Federal Trap Detection Programs

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Abstract. The use of synthetic food attractant lures for Tephritid fruit fly trapping is presently being incorporated into U.S. state and federal detection programs. These lures consist of ammonium acetate, trimethylamine hydrochloride and putrescine contained in individual packages that are attached to the inside (top) of plastic McPhail-type traps. Two chemical packets are placed in the traps for *Anastrepha spp.,* where as three are attached for *Ceratitis capitata*. This report presents data on trap captures of the above species comparing the current (individually packaged) baits with a novel dispenser containing either two or three components into a single matrix. Tests were conducted in Florida and Hawaii using hand release of sterile Caribbean fruit fly (Caribfly), *Anastrepha suspensa* and the Mediterranean fruit fly, *Ceratitis capitata* (medfly)/ aerially released medfly/ and wild caribfly populations (Florida) and wild medfly (Hawaii). Observations in the Florida study indicated that minor formulation adjustment should increase the efficacy of the *Anastrepha* attractant, whereas less of an adjustment may be required to capture *Ceratitis capitata* . Results in open field tests in Hawaii indicated that the three-component synthetic food attractant in a single cone unit was just as effective in capturing wild male and female Medflies as the same food attractants in individual packets. The single matrix has some advantages in handling and ease-of-use, especially with the Multilure trap.

Key words: female food attractants; detection tools; *Anastrepha* spp.; *Ceratitis capitata*

Introduction

Synthetic food attractants for detection and delimitation of *Ceratitis capitata* (Mediterranean fruit fly) and *Anastrepha* spp. (Caribbean/ Mexican fruit fly) have recently been developed/ field tested and are currently being utilized in State and Federal survey programs (Heath et al., 1997, Thomas et al., 2001 and FDACS, 2001). These attractant baits replace the aqueous slurry of torula yeast, which have long been the industry standard for tephritid fruit fly surveillance programs using food type baits (Burditt, 1982 and Cunningham, 1989). In addition to the tephritid flies, the protein-based liquids are attractive to a broad range of non-target insects, but this is less of a problem with the newer synthetic lures (Aluja, 1999, Heath et al., 1995 and Katsoyannos et al., 1999). The synthetic baits are packaged in adhesive-backed packets that can be either attached to the top inside surface of a two-piece plastic McPhail-type trap (Thomas et.al. 2001) or clipped from the center top of the trap. Two of the attractants (putrescine and ammonium acetate) are used for attracting *Anastrepha* spp. and three attractants (putrescine, ammonium acetate and trimethylamine) are used to

attract *Ceratitis capitata* (medfly) males and females. For direct attachment to the cover, the cover of the adhesive strip is usually removed from each bait packet, before it is "taped" to the trap, individually or together. Additionally, regardless of the method of presentation, a protective cover must be removed from the packet surface after removal of the packet from the package before lure "vaporization" is initiated. The attractants are active for at least 6 weeks under normal field conditions according to the manufacturer's instructions.

The latter procedure is somewhat time consuming, in that care must be taken to avoid damage to the membrane of the bait packet. If the trap attachment process is not correctly performed, one or more of the baits, when used in "wet-type traps" (i.e. McPhail-type with either propylene glycol or water/borax/surfactant preservative solution) could drop into the solution rendering the baits useless. This is not a problem when non-aqueous methods for capturing flies are used (i.e. vaportape (DDVP) or sticky panels). Care must also be taken not to confuse bait packages, as two of the three medfly baits are identical in appearance. Alternative dispenser technology, which employs a solid matrix, is currently being used for dispensing of Trimedlure, the male attractant used in state and federal detection programs. We recently evaluated this dispenser technology in which two or three of the components identified above were combined into a single matrix, which alleviated some of the handling problems (above) that had been identified. The new dispenser requires only the removal from the foil packages for access to the (2 in 1 or 3 in 1) bait that is then placed directly into a plastic "basket" incorporated into a recess at the top of the Multilure trap. Our hypothesis was that a single (multi-component) matrix could be developed that was as effective as the multi-unit packets currently available for detection of these fruit fly species. The purpose of this study was to directly evaluate the current synthetic food baits using two (*Anastrepha* sp.) and three (*Ceratitis capitata*) individual packets (Suterra product) with the single-matrix unit (Scentry product). The overall objective is to provide to federal government fruit fly eradication project/ Preventative Release Program managers, a more time (and handling) efficient, less costly, and equally (or more) effective bait dispenser system for use in detection programs against these pests.

Materials and Methods

Tests were initially conducted in (1.6 hectares or less) citrus groves in Florida using ground releases of sterile Caribbean or Mediterranean fruit flies previously emerged and nourished in modified Rubbermaid Palletote screened cages. Later, tests were run within a sterile fly Protective Release Program (PRP), with the flies being released aerially and/or in areas where native populations of caribfly existed, generally in urban host plants. Tests were also carried out in commercial coffee plantations on the island of Kauai, Hawaii where established medfly populations exist. Both the glass McPhail and a plastic "McPhail trap" (Multilure®, Better World Inc, Fresno, CA.) were used in this study. The standard glass trap containing torula yeast/ borax tablets dissolved in water was in all cases but one, used as the control, with the Multilure trap containing either the synthetic bait packets (Suterra Inc., Bend, OR.) or the candidate baits combined into a "cone" matrix (Scentry Biologicals Inc., Billings, MT.). A Multilure trap with torula yeast/ borax was used once as a control in lieu of glass McPhail in one of the Florida tests. Trap/ bait fly captures and longevity for the cone baits, (ammonium acetate/ putrescine for the caribfly) (ammonium acetate, putrescine plus trimethylamine hydrochloride for the medfly), were measured against the standard packet configuration.

Preliminary development of the single cone unit, (2 components-in-one (PA) or 3 components-in-one (PTA), evolved from initial tests of three or four initial configurations which were rejected as being cumbersome, oversized, poorly shaped or not effective. This work

was conducted initially in South Africa and to a lesser extent, in Florida (TCH unpublished). However, these initial trapping results were encouraging enough to continue to pursue the further development of a combined cone unit. In 2001, a cone-shaped polymeric matrix appeared to show promise. The three components for medfly and the two components only, were layered singly into a polymeric "cone", in identical volumetric measurements for each component respectively that were effective in attracting male and female flies into wet and dry traps (unpublished data).

Insects. Sterile male and female caribfly were obtained from the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL. Flies were held upon emergence in modified Rubbermaid Palletote (23.5"L x 16.5"W x 12.5"H) screened cages and fed a mixture of sugar and water, presented in an agar block on the top of the cage. Sterile male and female medfly were obtained from the U.S., Guatemalan, and Mexico Moscamed Program Rearing Facility at El Pino, Guatemala. Pupae were enclosed in the screened cages as detailed above for caribfly. Male only medflies (temperature sensitive genetic sexing strain designated as Vienna-7 TSL) were utilized in the September 12 to October 31, 2002 tests. These flies were also obtained from the Moscamed mass-rearing facility in Guatemala.

Florida Test I. Tests were conducted in a citrus grove managed by the University of Florida, Gainesville on July 8 through September 13, 2002. The purpose of these tests was to compare trap captures of caribfly and medfly for the individual Suterra (PA) or (PTA) with the Scentry (PTA) and the standard TYB pellets. The four treatments were: (1) Scentry cone containing putrescine, ammonium acetate and trimethylamine (PTA) in a Multilure trap placed in the well of the trap top with a 10% solution of propylene glycol (PG) (Prestone Low Tox® antifreeze); (2) Suterra packets (3 individual packets) (BioLure®) containing PTA in a Multilure trap with 10% PG; (3) Suterra packet (2 individual components containing putrescine and ammonium acetate (PA) in a Multilure trap with 10% PG; and (4) Four torula yeast/ borax tablets (TYB) dissolved in water in the standard glass McPhail trap. Ten traps for each of the treatments were randomly placed in the citrus grove and inspected at seven-day intervals for a period of ten weeks. Traps were alternated between trees and rows with traps at a distance of 25 feet from one another. Both male and female, sterile caribfly (7–10 d) and medfly (5–7 d) were released (by hand) from 30 cm x 30 cm x 30 cm screened cages at four centrally equidistant points within the grove.

Florida Test II. Tests were conducted again within the Sarasota PRP sterile male medfly release area during November 14, 2002 through January 30, 2003. In these tests we compared the Suterra (PA) or (PTA) with the Scentry (PA) or (PTA) and TYB pellets. Wild densities of caribfly populations were utilized despite fly populations not at their highest during this time period. The three treatments were as follows: (1) Scentry (PTA); (2) Suterra (PTA); and (3) TYB in water. All treatments utilized the Multilure trap with PG. There was however, a difference in the formulation of the putrescine in this test from those conducted previously with the Scentry cone. In this study a liquid form of putrescine was used rather than putrescine salt, which was used exclusively in both the initial testing and the above trials. Ten traps for each of the treatments were inspected on seven-day intervals for a period of 12 weeks. The field test design was a one by six city block area, one trap of each treatment per block.

Florida Test III. Tests were conducted under the Bradenton PRP sterile male medfly release area during March 18, 2005 through April 20, 2005. Suterra (PTA) was compared to Scentry (PTA) in multilure traps with a 10% solution of propylene glycol (PG). There were 43-45 traps of each treatment. Traps were placed in eight different sites; five of each treatment type per nine trappers, for a total six- week test period.

Hawaii Test I. Field tests were conducted in wild populations of Mediterranean fruit flies

in Kauai Coffee Plantation, located on the island of Kauai during the period November 5, 2002 through February 6, 2003. These tests compared the Suterra (PA) or (PTA) with the Scentry (PA) or (PTA) as well as improved Scentry cones that did not stick to the recessed basket in the Multilure trap. Six treatments were tested: (1) Scentry (PTA); (2) Scentry (PA); (3) an improved three-component Scentry cone made of trimethylamine, ammonium acetate and liquid putrescine (PTA-II); (4) Suterra (PTA); (5) Suterra (PA); and (6) a (blank) control. Treatments were tested in Multilure traps with a vaportape strip (Hercon Industries) (containing10% dichlorvos) as a toxicant in each trap. Cone formulations were placed in the recessed well, built in the cover of the Multilure trap. Individual Suterra (BioLure®) packets were adhered to the inner cover of the trap. The vaportape was suspended from the insert holder on the underside of the Multilure trap cover. The first trap in a row was placed 10 trees 5 m in from the end of the row and each trap within the row was spaced 20 trees 15 m apart. Treatment rows were spaced 5 rows 15m apart. Traps were serviced for a total 8-week test period. Flies were collected and male and female captures were counted and recorded.

Hawaii Test II. A second test of the cones were carried out during April-May 2005, in commercial coffee fields on the island of Kauai, Hawaii. Fifty Multilure traps each containing either the Scentry cone II (PTA) or Suterra (PTA) were deployed in the fields in a similar configuration as the first test. Ten blank control traps were also included. However in these tests the traps contained 10% solution of propylene glycol (PG) as in the most recent Florida tests.

Data analysis. ***Florida studies.*** All data presented in the Florida studies I and II were analyzed using Statistix for Windows (ver. 2.2) computer software. Both an ANOVA and Tukeys comparison of means were applied in the evaluation of significance ($P < 0.05$). The data in test III was analyzed using SAS version 8.2 (SAS Institute, Cary, North Carolina). An analysis of variance (PROC GLM) was performed on the data followed by a Tukey's test for mean separation.

Hawaii studies. Data are presented as mean fly capture per trap per day. Male and female trap captures were analyzed separately. Data was transformed to square root (x + .5). An analysis of variance (PROC GLM) was performed on the data followed by a Tukey's test for mean separation. Significant differences were determined at the $P < 0.05$ level. The data was analyzed using SAS version 8.2 (SAS Institute, Cary, North Carolina).

Results

Florida Test I. July 8 through September 13, 2002. Caribfly: Both the 2 component (PA) and the 3 component (PTA) Suterra products captured significantly more flies than the 3 component (PTA) cone from Scentry ($F = 7.45$, $P = 0.0001$). However neither the Suterra (PTA) nor the Scentry (PTA) were significantly better at trapping flies than the Torula yeast (TYB) in this study. The Suterra (PA) appeared to capture the most caribflies (Table 1). With medfly there was no significant difference in trap capture between the Scentry cone (PTA) or Suterra (PA) and PTA. Both Suterra products were significantly better than TYB ($F= 6.54$, $P= 0.0003$). The Scentry (PTA) was not significantly different than TYB in this study (Table 1).

Florida Test II. November 14, 2002 through January 30, 2003. In this test, there were no significant differences in fly response for either the caribfly or medfly between Multilure traps baited with Scentry (PTA), Suterra (PTA) or TYB pellets ($F = 0.18$, $P = 0.8354$, $F = 2.59$, $P = 0.0761$) (Table 2). Trap captures were lower overall in this residential setting than in the previous tests, which occurred in an orange grove.

Florida Test III. March 18, 2005 through April 20, 2005. In this test there were no

Table 1. Evaluation of Scentry 3-component cone. Release recovery of sterile Caribbean fruit fly and Mediterranean fruit fly at the University of Florida, Citrus Grove, Gainesville, July 8–September 13, 2002.

Treatment	N	Mean no. of males and females ± s.e	
		Caribfly	Medfly
Scentry PTA	100	8 ± 1.7 c	15 ± 2.2 ab
Suterra PTA	110	23 ± 4.3 ab	22 ± 3.2 a
Suterra PA	90	32 ± 7 a	18 ± 2.9 a
Torula yeast	100	19 ± 3.2 bc	9.7 ± 1.3 b

Numbers followed by the same letter are not significantly different.

Table 2. Evaluation of Scentry 3-component cone. Response of wild Caribbean fruit flies and sterile Mediterranean fruit flies in PRP area of residential Sarasota, November 14–January 30, 2003.

Treatment	N	Mean no. of males and females ± s.e	
		Caribfly	Medfly
Scentry PTA	120	0.48 ± 0.17 a	1.40 ± 0.22 a
Suterra PTA	120	0.70 ± 0.48 a	1.50 ± 0.55 a
Torula yeast	120	0.56 ± 0.20 a	0.95 ± 0.20 a

Numbers followed by the same letter are not significantly different.

Table 3. Response of sterile released Medflies to Scentry 3-component cones and Suterra 3-component packets in Bradenton, Florida, March 21–April 22, 2005.

Treatment	N	Weeks 1–6, mean flies/trap/day ± s.e.
Suterra PTA	118	11.3 ± 1.2a
Scentry cone II PTA	133	10.1 ± 1.1a

Data analyzed by PROC GLM; Means followed by the same letter in a column are not significantly different ($p > 0.05$) by Tukey's test

significant differences between Suterra (PTA) compared to Scentry (PTA); (F=0.92, P= 0.4912) (Table 3).

Hawaii Test I. November 5, 2002 through February 6, 2003. The results from field tests conducted in Hawaii showed that there were no significant differences in female trap captures of medflies between the Scentry (PTA), Suterra (PTA), the improved Scentry (PTA-II) and Suterra PA but all of the above treatments captured significantly more female

medflies than the control. Scentry (PTA), Suterra (PTA), and the improved Scentry (PTA-II) were significantly different from Scentry (PA). Suterra (PA) and Scentry (PA) were not significantly different in female trap captures but significantly different compared to control ($F = 24.05$, $p < 0.0001$). The data was also subjected to a repeated measures analysis. In assessing longevity of the treatments, time was a large source of variation ($F = 157.84$, $p < 0.0001$), due to the depletion of the treatment over time. There was a weak time and treatment interaction. Results showed a significant effect of treatment ($F = 6.25$, $p < 0.0013$).

In the eight weeks of testing there were no significant differences in male medfly trap captures between the Scentry (PTA), Suterra (PTA), Scentry (PTA-II) and the Suterra (PA) but, captured significantly more male medflies than the control ($F = 23.04$, $p < 0.0001$). Suterra (PA) was not significantly different from Scentry (PA). Scentry (PA) was not significantly different from control (Table 4). Treatment effects were determined for the six- week manufacturer's recommended use period.

Hawaii Test II. In a second study comparing Suterra (PTA) and Scentry cone II (PTA), there were no significant differences in male and female Medfly trap captures for weeks 1–8. Both treatments were significantly different compared to control ($F = 41.41$, $F = 78.65$, $p < 0.0001$) (Table 5).

Discussion

Federal and state detection programs for exotic fruit fly pests are a critical first line of defense against the establishment of these quarantine pests. Improving the components of any detection program aimed at these pests continues to be a high priority of state and federal action agencies.

The Florida studies showed that both products tested were capable of attracting both caribfly and medfly in either the 2 (caribfly) or 3-component (medfly) configuration and in either the individual packets (Suterra) or the combined matrix (Scentry). Additionally, both products continued to capture flies for 6–10 weeks. Thus the main advantage of the Scentry cone is ease in handling of the product by reducing the need to open three individual packets and the resulting time needed to properly secure the packets to the sides of the trap (personal communication).

Despite the fact that the Scentry cone did not always out capture larger number of flies than the Suterra PA/ PTA, it appears that the present formulation, (i.e. ratio and amounts of product placed in the cone) is comparable to the current commercial product. Perhaps as for the PA, and as observed with caribfly, the ratio of the components could be adjusted. This may be required for use at low fly densities or in eradication efforts were the sensitivity of the bait is critical.

Overall test results in Hawaii showed that for the recommended time of maximum effectiveness (6 weeks), of these synthetic food attractants, the single three component cone unit was just as effective in capturing wild male and female medflies compared to the individual 3 component packets. Initially the 2 component formulations were also tested for attractancy of oriental and melon fruit flies. There were some flies detected in the traps but due to low populations, there were no significant trap captures.

Based on our results, we believe that the new all-in-one three-component dispenser will perform as well as the single-packet ensemble currently available commercially from Suterra. Further improvements in both the composition and formulation of fruit fly food-baits should reduce the overall costs of state and federal fruit fly action programs and continue to improve our ability to ensure that these pests do not become established in the continental U.S.

Table 4. Response of wild female and male Mediterranean fruit flies to Scentry 3-component and 2-component cones vs, 3-component and 2- component packets in Kauai Coffee Plantation, November 5, 2002 to February 6, 2003.

		Weeks 1–8, mean flies/trap/day ± s.e.	
Treatment	N	Male	Female
Scentry cone PTA	48	37 ± 7.5 a	83 ± 10 a
Scentry cone II PTA	48	36 ± 7.6 a	81 ± 10 a
Suterra packets PTA	48	39 ± 8.3 a	93 ± 11 a
Suterra packets PA	48	25 ± 5 ab	59 ± 6.4 ab
Scentry cone PA	48	15 ± 3.1 bc	39 ± 4.2 b
Control	48	0.19 ± .05 c	0.44 ± .09 c

Data analyzed by PROC GLM; Means followed by the same letter in a column are not significantly different ($p > 0.05$) by Tukey's test

Table 5. Response of wild Medflies to Scentry 3 component cones and Suterra 3 component packets in Kauai Coffee Plantation. March 16,2005- April 11, 2005

		Weeks 1–8, mean flies/trap/day ± s.e.	
Treatment	N	Male	Female
Suterra PTA	376	1.7 ± .12 a	5 ± .24 a
Scentry cone II PTA	392	1.8 ± .15 a	4.9 ± .27 a
Control	80	0.05 ± .01 b	0.21 ± .03 b

Data analyzed by PROC GLM; Means followed by the same letter in a column are not significantly different ($p > 0.05$) by Tukey's test

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