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Response of *Anastrepha suspensa* to Liquid Protein Baits and Synthetic Lure Formulations

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ABSTRACT: The host list for the Caribbean fruit fly, Anastrepha suspensa (Loew), includes nearly 100 fruit trees including citrus. In south Florida, it is primarily a pest of dooryard fruit trees including Surinam cherry, Eugenia uniflora; loquat, Eriobotrya japonica; and tropical almond, Terminalia catappa; and it is a production pest of common guava, Psidium guajava. In areas of Florida with commercial citrus production, fly-free zones are designated areas that are certified as A. suspensa controlled areas for citrus export. In addition to Florida, A. suspensa is found in Cuba, Jamaica, Hispaniola and Puerto Rico. Highly effective traps for this species and for other Anastrepha spp are needed for suppression of fruit flies in areas in which they occur and for early detection in areas currently free of these pests. McPhail-type traps baited with a two component food-based synthetic lure containing ammonium acetate and putrescine outcapture McPhail-type traps baited with the liquid protein baits Nulure/borax or torula yeast/borax in some field trials, but results may be highly variable among different host plants or different environmental conditions. We report on the results of laboratory and field tests conducted to further evaluate the effects of ammonia dose and formulation on A. suspensa response, as well as comparative studies with other liquid protein bait types and formulations.

Key Words: Caribbean fruit fly, trapping, synthetic lures, aqueous baits, host tree

INTRODUCTION

Improved attractants are needed for *Anastrepha* spp. fruit flies, including the Caribbean fruit fly, *A. suspensa* (Loew). *A. suspensa* is a quarantine pest of citrus in Florida, but preferred hosts include guava, *Psidium guajava* L., loquat, *Eriobotrya japonica* (Thunb.) Lindl. and Surinam cherry, *Eugenia uniflora* L. Populations are generally low in citrus but high numbers can be found in preferred hosts. McPhail traps baited with the liquid protein bait torula yeast/borax (TYB) were traditionally used to detect and monitor *A. suspensa* as well as other *Anastrepha* spp. (Heath et al. 1993a). Food-based synthetic attractants comprised of ammonium acetate (AA) and putrescine (Pt) (Heath et al. 1995, Epsky et al. 1995, Thomas et al. 2001, Hall et al. 2005, Holler et al. 2006) or ammonium bicarbonate (AB), Pt and methylamine (Robacker and Warfield 1993) have been developed based on volatile chemicals released from liquid protein baits. Response of *Anastrepha* spp. to traps baited with these synthetic lures versus liquid protein bait tends to be variable and host/population level may be factors in this variation (Epsky et al. 2004, Thomas et al. 2008). Identification of additional attractant chemicals from protein baits that could be added to food-based synthetic lures may provide more effective trapping systems for *A. suspensa* as well as other pest Tephritidae that are captured in higher numbers in traps baited with liquid protein solutions.

A multidisciplinary approach was used to investigate response of *A. suspensa* to synthetic lures as well as to several liquid protein baits. Field tests were conducted to evaluate effects of ammonium dosage and formulation on *A. suspensa* response in field tests in preferred hosts. Laboratory studies were conducted to evaluate response of *A. suspensa* to volatiles from other liquid protein baits in comparison with response to TYB, and to test the effect of age of bait solution on this response. In addition, electroantennogram (EAG) analysis was used to measure antennal sensitivity to volatile chemicals emitted from liquid protein baits to further understand the electrophysiology that underlies behavioral response. This information will be used in chemical analysis for identification of new attractants from preferred bait formulations.

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MATERIALS AND METHODS

Traps and Lures. Multilure traps (Better World Manufacturing Inc., Fresno, CA, USA) were used in field studies and in tests conducted in the large flight tunnel. Liquid protein baits included aqueous solution of TYB (ERA Intl., Baldwin, NY, USA), Nulure (Miller Chemical & Fertilizer, Hanover, PA, USA) or CPH protein bait (Süsbin, Mendoza, Argentina). Traps baited with Nulure or CPH had 3% borax (wt/vol; sodium tetraborate decahydrate) added to the aqueous solution. Liquid protein baits in the small and large flight tunnel tests were added to 100 ml water. Synthetic attractants included a solid tablet formulation of ammonium bicarbonate (AB) (Agrisense-BCS Ltd, UK) and membrane-based lure formulations of AA and Pt (BioLure, Suterra LLC, Bend, OR). Release rate of ammonia from the AB tablets is ~50% of the AA lures (Heath et al. 2006), therefore traps baited with 2 AB tablets release approximately the same amount of ammonia as the traps baited with AA . To reduce the release rate from the AA lures, metallic tape (United Tape Co., Cummings, GA, USA) was used to cover 50 or 75% of the membrane surface. This decreased the release of ammonia to ~50 or 25 %, respectively, of the uncovered AA lures (Heath et al. 2007), and a 50% AA lure released approximately the same amount of ammonia as an AB tablet. Unbaited traps and traps baited with synthetic lures contained 200 ml 10% polypropylene glycol (LowTox, Prestone, Danbury, CT, USA) aqueous solution to retain captured flies.

Field Tests. Field tests were conducted from March 18 - June 3, 2004 at USDA/ARS, SHRS in Miami, FL (experiment 1) and June 17 - July 15, 2005 at Univ. FL, TREC in Homestead, FL (experiment 2). Experiment 1 examined the effect of ammonium acetate release rate on trap capture and had 4 treatments: 1) unbaited, 2) baited with 25% AA + Pt, 3) baited with 50% AA + Pt, and 4) baited with AA + Pt. Traps baited with all four treatments were placed in individual *A. suspensa* host trees including grapefruit, *Citrus paradisi* Macfad., black sapote, *Diospyros digyna* Jacquin, starfruit, *Averrhoa carambola* L., loquat, and Surinam cherry. Only one tree per host species was available, and the trees with traps were widely separated. The experiment was conducted for 8 wk, although some trees had host fruit present for only 6 wk of the test. Experiment 2 was conducted in a grove of common guava. The field plot design was a randomized complete block with five treatments placed in five rows (blocks) of trees. There were at least 10 m between rows and 10 m between traps within a row. The treatments were traps baited with 1) 2 TYB pellets in 200 ml water; 2) 50% AA + Pt, 3) AA + Pt, 4) AB tablet + Pt, and 5) 2 AB tablets + Pt. Tests were conducted for 4 wk, with fresh protein bait solutions made each week. Synthetic lures were replaced after 4 wk. For both experiments, traps were sampled every 7 d, and numbers of male and female flies were recorded. Traps were rotated sequentially to the next position within a host tree or block at time of sampling. Host trees (experiment 1) and blocks (experiment 2) were used as replicates, and there were five replicates for each experiment.

Flight Tunnel Tests. Insects used in flight tunnel tests were obtained from a laboratory colony maintained at SHRS, Miami, FL. Sexually mature 8 - 10 d old females were obtained from mixed sex cages and used for all tests. Females were protein-starved for 24 h prior to the start of a test. All flight tunnel tests were conducted as choice tests, with females given the choice of two test substrates. Tests in experiment 3 were conducted in small flight tunnels (30.2 by 30.2 by 122 cm) with volatile chemicals from the test substrates piped into the tunnels from chambers placed outside of the tunnels (Heath et al. 1993b). Tests in experiment 4 were conducted in a large flight tunnel (61 by 61 by 180 cm). Multilure traps baited with test substrates were hung 9 cm apart side by side in the upwind end of a large flight tunnel, with the trap openings 17 cm apart. Experiment 3 compared response to volatile chemicals from 100 ml aqueous solutions of either 5% or 10% solutions of CPH with 3% borax with response to standard TYB (1 pellet in 100 ml water). To determine if choice between the two test substrates was affected by age of the bait solution, baits were tested 0, 2, 4 and 6 d after preparation, and there were 6 replicate tests for each bait solution age. Experiment 4 compared response to Multilure traps baited with 100 ml of aqueous solutions of either standard TYB (1 pellet) or 9% Nulure with 3% borax. Effect of age of bait solution from 0 to 8 d after preparation was determined, and there were 3 replicates for each time period. Twenty females were released per tunnel, and number of flies per choice was recorded after ~20 h. Flies responding to volatile chemicals from substrates placed outside of the tunnels were captured in insect isolation traps within the small flight tunnels and did not contact the test solutions. Flies were captured in the test substrates in the Multilure traps used in the large flight tunnels. Percentage of flies captured was determined by dividing the number captured per treatment by the total number of flies captured by either treatment. Bait solution pH was measured at the start of each trial in the large flight tunnels using a pHep 4 pH meter (Northern Brewer, St. Paul, MN).

Electrophysiological Response. The effect of age of bait solution on antennal response to TYB and Nulure was examined by electroantennogram methods. Electroantennogram signals were recorded with a Syntech EAG system (Hilversum, Netherlands) from sexually mature, laboratory-reared females, using methods described previously (Kendra et al. 2005a, 2005b). The test substrates consisted of 300 ml aqueous solutions of TYB (3 pellets) and 9% Nulure with 3% borax. Solutions were prepared at room temperature (~24°C) in 1000 ml flasks, which were capped loosely to permit venting of gases. Three hours prior to sampling, flasks were plugged with foam stoppers. Using gas-tight syringes, headspace samples were withdrawn from the plugged flasks and presented to the fly antennae. EAG responses were measured initially in millivolts (peak height of depolarization), and then normalized with a standard reference sample (2-butanone) by the Syntech EAG 2000 software. Normalization corrected for time-dependent variability in antennal performance, and test responses were expressed as a percentage of the standard response. The normalized values were used to make comparisons between different test substrates and different doses of each substrate. Four volumetric doses of headspace were evaluated: 0.5, 1.0, 2.0, and 4.0 ml. The 2 ml dose was then used to determine the effect of age of bait solution (0-8 d after preparation) on EAG response. For each test substrate, EAG responses were measured from 3-5 insects, and response from each insect was the average of three measurements.

Statistical Analysis. Numbers of female and male flies per trap per day (ftd) per treatment in field tests were summed per host/block over all sample dates, and were either used in analysis directly or were converted to relative trapping efficiencies (rte) per host/block (Epsky et al. 1999). Data (rte for experiment 1 and ftd for experiment 2) were analyzed by one-way analysis of variance (ANOVA) using PROC GLM (SAS Institute 1985). The Box-Cox procedure, which is a power transformation that regresses log-transformed standard deviations (y + 1) against log-transformed means (x + 1), was used to determine the type of transformation necessary to stabilize the variance before analysis (Box et al. 1978). Separate analyses were conducted for females and males for each experiment. Significant ANOVAs were followed by least significant difference test (LSD, $P = 0.05$) for mean separation. Two-sample t-tests using PROC TTEST were used for all two-at-a-time laboratory comparisons over the whole experiment and/or with separate comparisons made for each age of bait solution test. Response variables for *t*-tests were either percentage of flies trapped per treatment (experiments 3 and 4) or percentage of standard antennal response (EAG recordings).

RESULTS AND DISCUSSION

Field Tests. In experiment 1, numbers of flies trapped in tests conducted in starfruit, black sapote and grapefruit were very low, with only 19, 20 and 28 flies captured, respectively. Numbers were higher in tests conducted in Surinam cherry and loquat, with 1004 and 1170 flies captured, respectively. Data were converted rte to allow comparisons among the range of number of flies captured in the different hosts (Epsky et al. 1999). There was no effect of AA dosage on percentage of females captured (Fig. 1), although all baited traps captured more flies than unbaited traps ($F = 22.73$; df = 3, 16; $P < 0.0001$). There was no effect of AA dosage on percentage of males captured (Fig. 1), but only the two higher dosages of AA captured a higher percentage of males than unbaited traps ($F = 3.75$; df = 3, 16; $P = 0.0326$). There were higher percentages of females captured in traps with 50% AA + Pt versus AA + Pt, but further reducing ammonia release to 25% resulted in decreased capture.

Numbers of flies captured in guava in experiment 2 were also very low, with only 41 flies captured, so data used for analyses were flies per trap per day. Treatment significantly affected capture of females ($F = 3.68$; df = 4, 20; $P = 0.0211$) but not males ($F = 1.93$; df = 4, 20; $P = 0.1211$). More females were captured in traps baited with either dosage of AA + Pt than with either dosage of AB + Pt (Fig. 2). Capture in TYB-baited traps was intermediate to both AAPt- or ABPt-baited traps.

Flight Tunnel Tests of TYB and CPH/borax Solutions. There was no difference in response of females to volatiles from aqueous solutions of 5% CPH/borax and TYB (27.8 ± 3.03% and 29.5 ± 3.22%, respectively; $t = 0.36$, df = 44; $P = 3.23$), but fewer females were captured in response to volatiles from aqueous solutions of 10% CPH/borax than in TYB (20.0 ± 2.48% and 32.0 ± 2.86%, respectively; $t = 3.18$, df = 46, $P = 0.0027$). A comparison of response by age of bait solution (Fig. 3) shows that age had no effect in the 5% CPH/borax test, but the difference in capture of TYB versus 10% CPH/borax was observed mainly in the first 4 d since bait

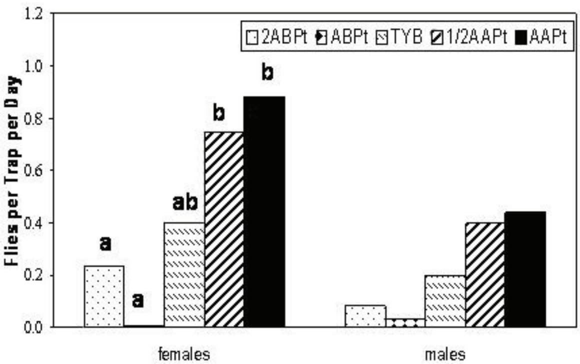

Fig. 1. Average relative trap efficiency (%) for female and male *A. suspensa* in Multilure traps that were unbaited (open bars), or baited with a putrescine lure and either an ammonium acetate lure with release reduced to 25% (dotted bars), with release reduced to 50% (diagonal lined bars), or with release not reduced (solid bars). Field tests were conducted in *A. suspensa* host trees in Miami, FL in spring 2004. Bars headed by the same letter within a sex are not significantly different (LSD test, $P = 0.05$)

preparation. Similar results were observed in field tests of TYB versus several formulations of mazoferm/borax (Corn Products, Argo, IL, USA) (Epsky et al. 1994).

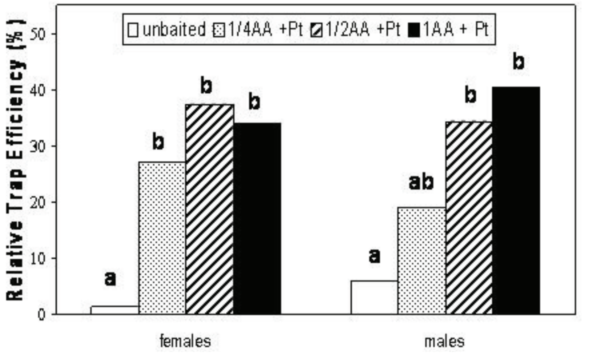

Fig. 2. Average number of female and male *A. suspensa* per trap per day in Multilure traps that were baited with two ammonium bicarbonate tablets and a putrescine lure (dotted bars), one ammonium bicarbonate tablet and a putrescine lure(diamond hatch bars), standard torula yeast/borax aqueous solution (horizontal lined bars), an ammonium acetate lure with release reduced by 50% and a putrescine lure(diagonal lined bars) or an ammonium acetate lure and a putrescine lure (solid bars). Field tests were conducted in *A. suspensa* host trees in Homestead, FL in summer 2005. Bars headed by the same letter within a sex are not significantly different (LSD test, $P = 0.05$)

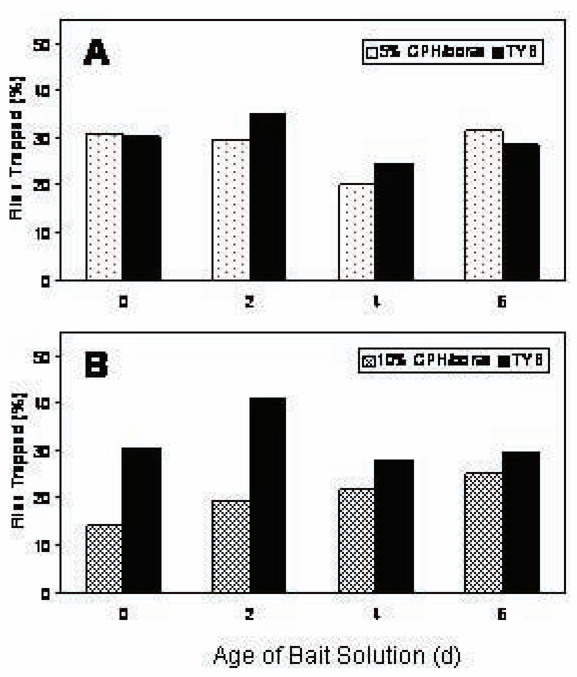

Fig. 3. Average percentage of female *A. suspensa* captured in flight tunnel choice tests in response to volatile chemicals from A) aqueous solutions of 5% CPH protein bait with 3% borax (light dotted bars) or standard torula yeast/borax aqueous solution (solid bars), or B) aqueous solutions of 10% CPH protein bait with 3% borax (hatched bars) or standard torula yeast/borax aqueous solution (solid bars). There were 20 females per test and 6 replicate tests for each age of bait solution.

Flight Tunnel Tests and EAG Measurements with TYB and Nulure/borax Solutions. An effect of age of bait solution was also observed in the comparison between aqueous solutions of TYB and Nulure/borax (Fig. 4). Although TYB tended to capture more females over all bait ages, the differences were greatest in the first few days after bait preparations. Similar results were observed in field tests of TYB versus several formulations of Nulure/borax (Epsky et al. 1993). In those studies, TYB solutions captured more flies the first 3 - 4 d than 4 - 7 d after deployment, while bait age had no effect on capture with Nulure/borax formulations. The pH (mean ± std. dev.) of the TYB was higher than the pH of the Nulure/borax (8.22 ± 0.856 versus 7.36 ± 0.805), but there was no change in pH over the time period of the test. In parallel EAG tests (Fig.5), higher EAG responses were obtained from Nulure/borax than TYB for the first 5 d, but there was no difference over the last 3 d. Antennal response to TYB increased while response to Nulure decreased as the bait solutions aged. This trend in EAG response was consistent over the range of doses evaluated.

Previous studies have found a positive correlation between choice among food-based attractants for laboratory-reared flies in flight tunnel bioassays and wild flies in field tests (Epsky et al. 1993), and for sterile flies and wild flies in field tests (Robacker and Thomas 2007). Laboratory-reared flies may be less discriminating than wild flies, and may respond to tests substrates in laboratory bioassays that wild flies do not respond to in the field, however we have not observed response in the field that was not also observed in the laboratory (N.D.E. and R.R.H., unpublished data). Thus, laboratory bioassays provide a valuable tool for screening potential attractants. Perhaps more interesting is the negative correlation between EAG response and choice between fresh TYB and fresh Nulure/borax solutions. Kendra et al. (2005b) observed that sexually immature females had higher antennal sensitivity to ammonia but lower response to high ammonia dosages in flight tunnel bioassays than sexually mature females, indicating that the high levels of ammonia were possibly repellant to the immature flies. Robacker and Czokajlo (2005) found that optimal ammonia dosage in food-based lures was affected by use in open traps, such as sticky traps, versus closed traps, such as Multilure traps. Thus showing that high dosage of an attractant may be repellant in closed traps. Volatile attractant chemicals from Nulure/borax solution may be released at levels too high for effective fly capture when freshly prepared but have dropped to non-repellent levels by 4-5 d after preparation. This information will used to direct further studies into additional attractant chemicals released from these protein baits.

Summary. Traps baited with AAPt captured more *A. suspensa* than traps baited with ABPt even when the ammonia release rates were similar. Reducing dosage of ammonia by 50% of the commercially available AA lure slightly increased female capture, but reducing dosage to 25% tended to decrease female capture. The 5% CPH/3% borax bait captured the same number of flies as TYB, and was more effective than 10% CPH/3% borax. Further decreasing the amount of borax added to CPH may improve its effectiveness. As has been observed in field tests, fresh TYB captures more *A. suspensa* than fresh Nulure/borax but this difference decreases as the bait solutions age. EAG analysis indicates that volatiles from fresh Nulure/borax elicit a higher antennal response than TYB, but this difference decreases as the TYB solution ages. Chemical analysis will be needed to determine the nature of reduced capture by fresh Nulure/borax and to identify additional attractive chemicals emitted by these protein baits.

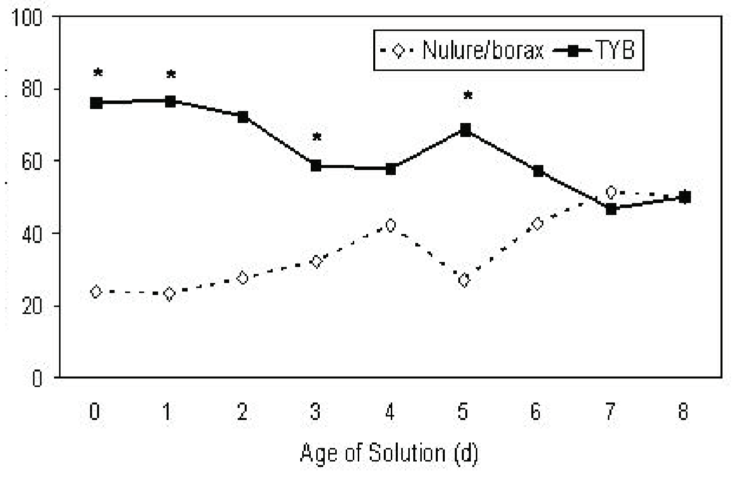

Fig. 4. Average percentage of female *A. suspensa* captured in flight tunnel choice tests in Multilure traps baited with aqueous solutions of 9% Nulure with 3% borax (dotted line) or standard torula yeast/borax aqueous solution (solid line). There were 20 females per test, 3 replicates for each age of bait solution, and an asterisk indicates significant difference in response (*t*-test, $P < 0.05$) within age of bait solution treatment.

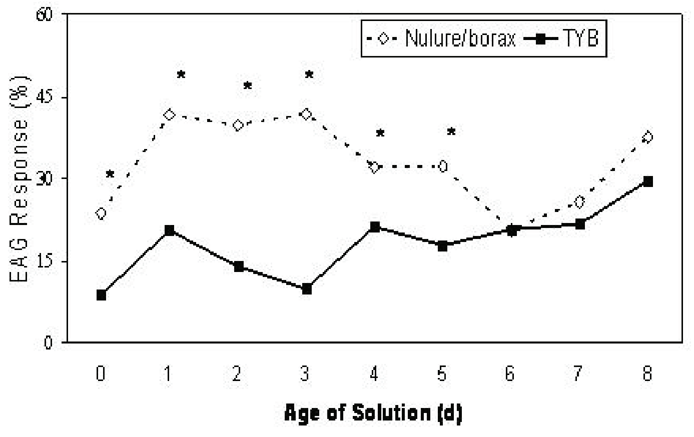

Fig. 5. Average percentage EAG response of female *A. suspensa* to 2 ml headspace samples of 9% Nulure with 3% borax (dotted line) or standard torula yeast/borax aqueous solution (solid line). Measurements were recorded from 3-5 females for each age of bait solution and an asterisk indicates significant difference in EAG response (*t*-test, $P < 0.05$) within age of bait solution treatment.

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