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FOOD-BASED LURE PERFORMANCE IN THREE LOCATIONS IN PUERTO RICO: ATTRACTIVENESS TO *ANASTREPHA SUSPENS*A AND *A. OBLIQUA* (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Lures based on odors released by hydrolyzed protein were assessed for their attractiveness to *Anastrepha obliqua* and *A. suspensa* at 3 locations in Puerto Rico in Aug through Oct 2009. Lures compared included ammonium acetate combined with putrescine, hydrolyzed corn protein (Nulure) with borax, freeze-dried Nulure, freeze-dried Nulure in combination with ammonium acetate, freeze-dried Nulure in combination with ammonium acetate and putrescine, and the Unipak lure, a single lure containing ammonium acetate and putrescine. Where the distribution of trapped flies departed significantly from what would be expected given an equal attraction of the baits, Nulure and freeze-dried Nulure always attracted fewer flies than the other baits tested, regardless of species, sex, or location. Although all of the baits or bait combinations containing ammonium acetate attracted more flies than the Nulure or freeze-dried Nulure baits, there was a distinct trend of ammonium acetate and putrescine and the Unipak lures to attract more flies after the 4th week of the study and for the freeze-dried Nulure with ammonium acetate or in combination with ammonium acetate and putrescine to attract more flies in the 1st 4 weeks of the study. This trial is unique in that it was conducted in orchards of carambola, *Averrhoa carambola* (Oxalidaceae), a poor host for both fly species. Our results are compared with other studies on lures of *A. obliqua* and *A. suspensa* and the implications for monitoring/detecting pest Tephritidae are discussed.

Key Words: ammonium acetate, putrescine, Nulure, McPhail trap

RESUMEN

Trampas que trabajan a base de olores liberados por proteína hidrolizada se evaluaron como atrayentes de las moscas *Anastrepha obliqua* y *A. suspensa* en tres localidades en Puerto Rico durante agosto a octubre de 2009. Las trampas utilizadas en el estudio incluyeron acetato de amonio en combinación con putrescina, proteína hidrolizada de maíz (NuLure) con bórax, NuLure liofilizado en combinación con acetato de amonio y putrescina, y la trampa Unipak la cual contiene acetato de amonio y putrescina en una sola mezcla. Las trampas NuLure y NuLure liofilizada atrajeron menos moscas que el resto de las trampas irrespectivamente de la especie, sexo, o localidad. Aunque todas las trampas o combinaciones de estas con acetato de amonio atrajeron más moscas que las trampas NuLure o NuLure liofilizada, hubo una clara tendencia de las trampa de acetato de amonio y putrescina y la trampa Unipak a atraer más moscas después de la cuarta semana a partir de comenzado el estudio y de las trampas NuLure liofilizadas con acetato de amonio o en combinación con acetato de amonio y putrescina a atraer más moscas en las primeras cuatro semanas del estudio. Este estudio es único en que se llevó a cabo en huertos de carambola *Averrhoa carambola* (Oxalidácea), un cultivo que es un pobre hospedero de ambas especies de moscas. Nuestros resultados se comparan con otros estudios con trampas de *A. obliqua* y *A. suspensa* y las implicaciones para el monitoreo y detección de plagas Tephritidae son discutidos.

Translation provided by the authors.

Although less than 10% of the 199 described species of *Anastrepha* are considered economically important (White & Elson-Harris 1992; Aluja 1994; Norrbom 2004), the occurrence of any of these economically important species in a region has a negative impact on growers. Growers may be restricted from exporting their produce to certain markets, or may have to subject their fruit to expensive post-harvest sterilization measures (Simpson 1993).

The island of Puerto Rico contains populations of 2 economically important species; the Caribbean fruit fly, *A. suspensa* (Loew) and the West Indian fruit fly, *A. obliqua* (Macquart) (Jenkins & Goenaga 2008). Although there are populations of *A. suspensa* in Florida, there are no populations of *A. obliqua* there, making it risky to transport some Puerto Rican produce to Florida. Establishment of *A. obliqua* in Florida could jeopardize mango and other subtropical fruit crops.

Regulatory agencies spend considerable effort and expense monitoring large areas for these and other potentially invasive *Anastrepha* spp. (Anonymous 2010). The need for effective monitoring/detection devices has resulted in a long history of studies on attractants for *Anastrepha* spp. (Heath et al. 1993). Females of all frugivorous species of Tephritidae that have been studied, including *Anastrepha* spp., are anautogenous, i.e., they need to consume protein as adults for ovary development (Drew & Yuval 2000). Exploiting this need for protein, a variety of potential lures based on odors released from hydrolyzed proteins have shown some degree of attractiveness, including ammonia (released from ammonium acetate, ammonium bicarbonate and urine) (Bateman & Morton 1981; Burditt et al. 1983), and hydrolyzed torula yeast (Lopez et al. 1971; Burditt 1982). For many years hydrolyzed torula yeast in a liquid suspension, along with borax to reduce cadaver decay, was used in 1 piece glass McPhail traps to monitor and detect populations of *Anastrepha* spp. (Anonymous 1989; Heath et al. 1993). A series of modifications to the trap and the lures have improved the utility and effectiveness of the trap (Epsky et al. 1993; Heath et al. 1995; Thomas et al. 2001). Heath et al. (1995) identified some common volatiles from baits and decomposing fruit that were attractive to *A. suspensa*. These included ammonia, acetic acid (both released from ammonium acetate) and putrescine. Although not attractive when deployed alone (Heath et al. 2004), putrescine has been shown to be a potent synergist to ammonium acetate for capture of both *A. ludens* and *A. suspensa* (Kendra et al. 2008). Thomas et al. (2001) pointed out that the design of the 1-piece glass McPhail trap was difficult to service, especially with the new lures, and prone to damage. A 2-piece plastic version of the McPhail trap has since been widely adopted by regulatory agencies. However, despite many studies, no single bait has been identified to satisfy the needs of regulatory agencies. Ideally, a bait would be easy to apply, long-lasting, attractive to target species (often multiple target species; regulatory agencies in Florida are currently monitoring for *A. obliqua* and the Mediterranean fruit fly, *Ceratitis capitata* Wied., among many others) combined with low non-target attractiveness.

APHIS-PPQ in Puerto Rico currently deploys a battery of traps and lures to detect and monitor pest Tephritidae; trimethylamine and ammonium acetate plus putrescine are used in Multilure traps (2-piece plastic McPhail traps) to detect *C. capitata*; methyl eugenol is used in Jackson traps (tent-shaped sticky cards) to detect Oriental fruit flies, *Bactrocera dorsalis* (Hendel) and carambola fruit flies, *B. carambolae* Drew & Hancock, and Cuelure is used in Jackson traps to detect melon fruit fly, *B. cucurbitae* (Coquillett), and Queen-

sland fruit flies, *B. tryoni* (Froggatt) (Anonymous 2010). In addition, torula yeast is still used at some trap sites (Saez, personal communication).

Current lures for detecting/monitoring pest *Anastrepha* spp. include Nulure (Miller Chemical & Fertilizer, Hanover, PA.), a hydrolyzed corn protein lure (Gilbert et al. 1984), a freeze-dried preparation of Nulure (Heath et al. unpublished), ammonium acetate combined with putrescine (Biolure, Suterra LLC, Bend, Oregon), and the Unipak (Suterra LLC), a single bait dispenser containing ammonium acetate and putrescine (Holler et al. 2009). Our objective in this study was to compare these lures, as well as certain combinations (freeze-dried Nulure combined with ammonium acetate, or combined with both ammonium acetate and putrescine) for relative attractiveness to populations of *A. suspensa* and *A. obliqua* in Puerto Rico. We chose to conduct these trials in carambola, *Averrhoa carambola* (Oxalidaceae), because orchards of this fruit were available to the researchers in 3 different regions of Puerto Rico. Additionally, this is a poor host of both *A. obliqua* and *A. suspensa*; collections of thousands of carambola fruit yielded no pupae of *A. suspensa* and relatively few pupae of *A. obliqua*, principally when preferred hosts, such as mango, were not available (Jenkins & Goenaga 2008). Most lure trials are conducted in orchards of preferred hosts; by conducting these trials in a poor host environment we evaluated efficacy of lures for detection of pest *Anastrepha* at low population levels.

MATERIALS AND METHODS

Study Sites

Field trials were conducted in Sep and Oct of 2009, a time we had determined to be peak season for both fly species (Jenkins, unpublished). All trap blocks were set in experimental orchards of carambola located at the USDA-ARS Tropical Agriculture Research Experimental Station in Isabela, PR, and at the University of Puerto Rico Agricultural Experiment Stations in Corozal and Juana Diaz, PR. All orchards were planted in 1999 and were composed of 10 rows, each row containing 22 trees. Trees were planted in a quincunx system 3.7 m apart with 5.5 m between rows. All of the 6 internal rows had 9 varieties of tree planted randomly throughout the row. The varieties, grafted onto Goldenstar rootstock, were Arkin, B-10, B-16, B-17, Kajang, Kari, Lara, Sri-Kembangan, and Thai Knight. The 2 rows of trees on either side of these 6 internal rows were composed entirely of Arkin grafted onto Goldenstar rootstock. The first 2 trees and the last 2 trees of each row were also Arkin grafted onto Goldenstar rootstock. We have never recovered *A. suspensa* from thousands of carambola fruit and relatively low numbers of *A. obliqua* have been recovered

from carambola fruit (Jenkins & Goenaga 2008); nonetheless past experience has demonstrated that both species can be trapped in relative abundance from orchards of this fruit with no demonstrable effects of fruit variety on trap catch (Jenkins, unpublished).

Traps and Lures

All baits were tested using plastic 2-piece Multilure™ traps (Better World Manufacturing, Inc., Fresno, CA). Commercial lures (Suterra, LLC, Bend, OR) consisted of ammonium acetate and putrescine (Biolure MFF), and the newly formulated Unipak.

A total of 6 lures or lure combinations were tested in each block as follows:

1. Ammonium acetate + putrescine (=AAPt)
2. Nulure with borax
3. Freeze-dried Nulure 7 (=FDN7)
4. Freeze-dried Nulure 7 + ammonium acetate (=FDN7 + AA)
5. Freeze-dried Nulure 7 + ammonium acetate + putrescine (=FDN7 + AAPt)
6. Unipak

For all treatments except the 9% Nulure with borax, the trap fluid consisted of 200 mL of a 10% solution of propylene glycol (Qualichem Technologies, GA) and water. The trap fluid for the 9% Nulure lure (18 mL) consisted of 3% Borax (6 g) mixed with 182 mL of water. The Nulure and freeze-dried Nulure (9 g) were dissolved in the respective trap fluid. Nulure and freeze-dried Nulure baits were changed every 2 weeks (3 times during the study). The ammonium acetate and putrescine lures were changed every 4 weeks (once during the study).

Trap Block Design

Traps were deployed in 3 rows of each orchard. Rows with traps were at least 2 rows from the orchard border and separated by at least 1 trap-less row from another row with traps. All 6 treatments were represented in each of the 3 rows. Trees with traps were separated from other trees with traps by at least 1 tree. Traps were rotated to subsequent positions within rows each time they were checked. Traps at all sites were checked for fruit flies on Monday and Friday of each week between 24 Aug 2009 and 16 Oct 2009. All fruit flies were returned to the laboratory for identification and stored in 95% EtOH.

Statistical Analyses

The large number of independent variables we were comparing combined with the low number of

replicates we were forced to use made the use of an ANOVA unsuitable for our purposes. Chi-square analyses were used to compare the observed number of flies of each species and sex trapped in each treatment to the expected number of flies under the assumption that flies would be equally distributed among the treatments if there was no difference in attraction. These analyses were conducted for each sex of each species for each week of the study (a total of 8 weeks) and for the total number of flies trapped throughout the study for each site. Chi-square probabilities exceeding 0.05 were labeled as insignificant. When the total number of flies trapped during a given week or at a given site was less than 30, i.e., an expected distribution among the treatments would be less than 5 (30 flies divided by 6 treatments = 5), the result was regarded as too weak to make inferences, even if the analyses indicated significant departure from the null hypothesis (= there was no difference in the distribution of flies among the treatments).

RESULTS

Too few *A. suspensa* were caught in traps at the Isabela site (9 females and 1 male, total) to merit analysis. A total of 294 *A. obliqua* flies were caught in Isabela, 167 of which were females (58.0%) and 127 of which were males (42.0%). Throughout the 8 weeks of the trial, the percentage of females trapped averaged $57.9\% \pm 4.7$ (SEM). Of the 294 *A. obliqua* trapped at the Isabela site during the experiment, 23% were in traps baited with freeze-dried Nulure and ammonium acetate and putrescine, 19% were in traps baited with freeze-dried Nulure and ammonium acetate, 19% in traps baited with UniPak lures, 17% were in traps baited with ammonium acetate plus putrescine, 14% were in traps baited with freeze-dried Nulure, and 7% in traps baited with Nulure. Chi-square analyses indicated that the distribution of *A. obliqua* (combined sexes) among the treatments departed significantly from the null hypothesis, although this was not true for every week of the study (Table 1).

A total of 231 *Anastrepha* spp. individuals were trapped at the Corozal site. One hundred and fifty nine (69%) of these were identified as *A. obliqua*, of which 109 (69%) were females and 50 (31%) were males. Of the 72 *A. suspensa* identified from traps at Corozal, 54 (75%) were female and 18 (25%) were male. Throughout the 8 weeks of the trial, the percentage of female *A. obliqua* trapped averaged $70.2\% \pm 3.0$ (SEM) and the percentage of female *A. suspensa* trapped averaged $75.3\% \pm 3.8$ (SEM).

Of the 159 *A. obliqua* trapped at the Corozal site during the experiment, 30% were in traps baited with ammonium acetate and putrescine, 28% were in traps baited with freeze-dried Nu-

TABLE 1. NUMBER OF FLIES CAPTURED BY WEEK AND BY BAIT AT THE ISABELA SITE. CHI-SQUARE ANALYSES WERE PERFORMED ON COMBINED SEXES WITHIN A SPECIES.

Week	Sex	Number of flies												χ^2 value	χ^2 prob					
		AAPt		Nulure		FDN7		FDN7 + AA		FDN7 + AAt		UmiPak				Total flies				
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female			Male	Female	Male	Female	
1	<i>A. obliqua</i>	0	1	3	5	1	3	7	3	2	2	0	2	0	0	2	13	16	12.6	0.0280
	<i>A. suspensa</i>	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	NA	NA
2	<i>A. obliqua</i>	3	3	3	1	3	8	4	14	11	16	4	3	28	45	3	28	45	31.8	<0.0001
	<i>A. suspensa</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	NA
3	<i>A. obliqua</i>	4	1	2	0	4	1	3	3	9	8	6	3	28	16	3	28	16	18.7	0.0020
	<i>A. suspensa</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	NA
4	<i>A. obliqua</i>	2	5	2	2	4	3	6	1	2	0	3	4	19	15	4	19	15	4.1	0.5320
	<i>A. suspensa</i>	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	NA	NA
5	<i>A. obliqua</i>	0	1	0	0	0	1	1	3	2	1	0	0	3	6	0	3	6	9.0	0.1090
	<i>A. suspensa</i>	0	1	0	0	0	0	0	1	0	2	0	0	0	0	0	0	4	NA	NA
6	<i>A. obliqua</i>	3	1	1	0	2	3	3	1	2	6	5	10	16	21	10	16	21	19.3	0.0020
	<i>A. suspensa</i>	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	NA	NA
7	<i>A. obliqua</i>	1	4	2	0	0	1	2	4	1	3	0	9	6	21	9	6	21	9.2	0.1000
	<i>A. suspensa</i>	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	2	NA	NA
8	<i>A. obliqua</i>	9	13	0	2	2	4	0	1	2	2	1	5	14	27	1	14	27	28.3	<0.0001
	<i>A. suspensa</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	NA
Total	<i>A. obliqua</i>	22	29	13	10	16	24	26	30	31	38	19	36	127	167	25.4	127	167	25.4	<0.0001
	<i>A. suspensa</i>	0	1	0	1	0	0	1	4	0	3	0	0	1	9	0	1	9	NA	NA

lure and ammonium acetate and putrescine, 13% were in traps baited with UniPak lures, 12% were in traps baited with freeze-dried Nulure and ammonium acetate, 11% were in traps baited with freeze-dried Nulure, and 6% were in traps baited with Nulure.

Of the 72 *A. suspensa* trapped at the Corozal site during the experiment, 33% were in traps baited with ammonium acetate and putrescine, 26% were in traps baited with UniPak lures, 25% were in traps baited with freeze-dried Nulure and ammonium acetate and putrescine, 8% were in traps baited with freeze-dried Nulure and ammonium acetate, 6% were in traps baited with freeze-dried Nulure, and 1% were in traps baited with Nulure. As at the Isabela site, ammonium acetate and putrescine, freeze-dried Nulure combined with ammonium acetate or combined with ammonium acetate and putrescine, and the Uni-pak trapped more flies than the Nulure or the freeze-dried Nulure (Table 2). This was true for both *A. obliqua* and *A. suspensa*.

A total of 157 *Anastrepha* spp. individuals were trapped at the Juana Diaz site. Ninety four (59.9%) of these were identified as *A. suspensa*, of which 78 (83.0%) were female and 16 (17%) were male. A total of 63 (40.1%) *A. obliqua* were trapped at the Juana Diaz site, of which 49 (77.8%) were female and 14 (22.2%) were male. Throughout the 8 weeks of the trial, the percentage of female *A. obliqua* trapped averaged $80.0\% \pm 4.2$ (SEM) and the percentage of female *A. suspensa* trapped averaged $75.3\% \pm 7.3$ (SEM).

Of the 63 *A. obliqua* trapped at the Juana Diaz site during the experiment, 24% were in traps baited with freeze-dried Nulure combined with ammonium acetate and putrescine, 21% were in traps baited with freeze-dried Nulure, 17% were in traps baited with UniPak lures, 16% were in traps baited with freeze-dried Nulure and ammonium acetate and putrescine, 13% were in traps baited with ammonium acetate and putrescine, and 10% were in traps baited with Nulure.

Of the 94 *A. suspensa* trapped at the Juana Diaz site during the experiment, 27% were in traps baited with freeze-dried Nulure and ammonium acetate, 26% were in traps baited with ammonium acetate and putrescine, 21% were in traps baited with freeze-dried Nulure and ammonium acetate and putrescine, 16% were in traps baited with UniPak lures, 6% were in traps baited with Nulure, and 4% were in traps baited with freeze-dried Nulure. Generally, too few flies were captured of either species to make a confident analysis except when captures were summed for the duration of the experiment (Table 3). No significant departures from the null hypothesis were detected in the distribution of *A. obliqua* flies among the different baits.

At all 3 sites there was a consistent temporal pattern in capture; freeze-dried Nulure combined

with ammonium acetate or combined with ammonium acetate and putrescine caught more flies in the first 4 weeks, whereas ammonium acetate plus putrescine or the Uni-pak lures caught more flies after the fourth week (Tables 1-3). The only exception occurred at the Juana Diaz site when the ammonium acetate and putrescine combination caught more *A. suspensa* than expected in the first week (Table 3).

DISCUSSION

For all locations, species and sexes, where a Chi-square analysis detected significant departure from equal distribution among the different baits (and at least 30 flies were captured) Nulure or freeze-dried Nulure consistently attracted the fewest flies. This would suggest that the higher attractiveness of the Uni-pak, ammonium acetate and putrescine lures and the freeze-dried Nulure in combination with either ammonium acetate or ammonium acetate and putrescine is attributable to the common factor of these lures, namely, the presence of ammonium acetate in all of these lures. However, bait attractiveness was not constant over time, with a general trend of freeze-dried Nulure in combination with ammonium acetate or in combination with ammonium acetate and putrescine attracting more flies in the first 4 weeks of the study and ammonium acetate and putrescine or Uni-pak lures attracting more flies in the fourth week and later. This appears to be consistent with the anecdotal reporting that freshly opened ammonium acetate lures are less attractive than those that have been out a week or more (Thomas et al. 2008). This is potentially due to the dosage of ammonia released; Thomas et al. (2008) demonstrated that higher doses of the ammonia significantly reduced capture of *A. suspensa* and *A. ludens* compared to lower doses. Also, Kendra et al. (2005) demonstrated that increased doses of ammonia decreased the capture of female *A. suspensa* with undeveloped ovaries. However, fresh ammonium acetate and putrescine lures were placed in the field on the 5th week of this study, approximately when they began to catch more flies. Also, the freeze-dried Nulure combined with ammonium acetate or ammonium acetate and putrescine caught more flies early in the study, suggesting that the freshly opened ammonium acetate packages are not too strong, or that combined with the freeze-dried Nulure, the ammonium acetate packages are attractive at higher doses.

Many studies have been conducted on the attractiveness of certain baits, but comparing these studies in a meaningful manner is difficult and subject to speculation. This is because these studies are often conducted in different regions, trap different species of flies, different strains of flies (wild versus lab-reared), test different combina-

TABLE 2. NUMBER OF FLIES CAPTURED BY WEEK AND BY BAIT AT THE COROZAL SITE. CHI-SQUARE ANALYSES WERE PERFORMED ON COMBINED SEXES WITHIN A SPECIES.

Week	Sex	Number of flies														χ^2 value	χ^2 prob	
		AAPt		Nulure		FDN7		FDN7 + AA		FDN7 + AAt		UniPak		Total flies				
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female			Male
1	<i>A. obliqua</i>	0	1	0	1	2	0	1	6	0	6	0	0	0	3	14	15.1	0.0100
	<i>A. suspensa</i>	0	0	0	0	0	0	0	0	1	1	0	2	1	3	3	8.0	0.1560
2	<i>A. obliqua</i>	0	1	2	0	0	0	3	3	0	4	1	0	3	8	5.9	0.3150	
	<i>A. suspensa</i>	0	0	0	0	0	0	2	0	0	0	0	0	1	2	15.0	0.0100	
3	<i>A. obliqua</i>	3	4	2	1	0	6	3	5	7	16	0	0	15	32	51.5	<0.0001	
	<i>A. suspensa</i>	0	1	0	0	1	0	1	1	0	5	0	1	1	8	10.3	0.0660	
4	<i>A. obliqua</i>	6	9	0	2	0	0	0	0	3	3	0	3	9	17	37.2	<.0001	
	<i>A. suspensa</i>	1	4	0	0	0	1	0	0	2	2	1	2	4	9	27.3	<0.0001	
5	<i>A. obliqua</i>	0	3	0	0	1	2	0	1	3	1	0	2	4	9	5.0	0.4160	
	<i>A. suspensa</i>	0	4	0	0	0	1	0	1	1	1	0	3	1	10	5.9	0.3150	
6	<i>A. obliqua</i>	2	1	0	0	0	2	0	0	0	0	0	0	2	7	17.0	0.0050	
	<i>A. suspensa</i>	2	2	0	0	0	1	0	0	0	2	0	0	2	5	11.0	0.0510	
7	<i>A. obliqua</i>	3	3	0	1	0	0	0	0	0	0	0	1	4	9	42.7	<.0001	
	<i>A. suspensa</i>	3	3	1	0	0	0	0	0	0	2	2	5	6	10	17.8	0.0030	
8	<i>A. obliqua</i>	4	8	0	0	2	3	0	0	1	0	3	2	10	13	27.9	<.0001	
	<i>A. suspensa</i>	1	3	0	0	0	0	0	1	0	1	1	2	2	7	9.0	0.1090	
Total	<i>A. obliqua</i>	18	30	4	5	5	13	4	15	14	30	5	16	50	109	48.3	<0.0001	
	<i>A. suspensa</i>	7	17	1	0	1	3	1	5	4	14	4	15	18	54	37.5	<0.0001	

TABLE 3. NUMBER OF FLIES CAPTURED BY WEEK AND BY BAIT AT THE JUANA DIAZ SITE. CHI-SQUARE ANALYSES WERE PERFORMED ON COMBINED SEXES WITHIN A SPECIES.

Week	Sex	Number of flies														χ^2 value	χ^2 prob	
		AApt		Nulure		FDN7		FDN7 + AA		FDN7 + AAt		UniPak		Total flies				
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female			Male
1	<i>A. obliqua</i>	0	3	0	1	0	3	1	2	1	3	0	0	0	2	12	4.9	0.4332
	<i>A. suspensa</i>	1	9	2	3	0	0	0	4	1	6	1	1	1	5	23	13.6	0.0190
2	<i>A. obliqua</i>	0	0	0	0	1	0	0	0	0	2	0	0	0	1	2	7.0	0.2206
	<i>A. suspensa</i>	1	3	0	0	0	0	1	7	0	7	0	2	2	2	19	17.0	0.0050
3	<i>A. obliqua</i>	0	1	0	1	2	2	1	3	1	2	1	2	1	5	11	3.5	0.6234
	<i>A. suspensa</i>	0	4	0	1	0	2	0	8	0	2	2	1	2	18	9.4	0.0940	
4	<i>A. obliqua</i>	0	1	1	0	0	0	1	1	0	5	1	0	3	7	9.2	0.1013	
	<i>A. suspensa</i>	0	0	0	0	1	1	1	3	0	1	0	3	2	8	8.0	0.1560	
5	<i>A. obliqua</i>	0	0	0	0	0	1	1	0	0	0	0	0	5	1	6	16.4	0.0057
	<i>A. suspensa</i>	1	1	0	0	0	0	0	0	0	0	1	2	2	3	10.6	0.0600	
6	<i>A. obliqua</i>	0	1	0	0	0	0	0	0	0	1	0	0	0	2	4.0	0.5494	
	<i>A. suspensa</i>	0	1	0	0	0	0	0	0	0	2	0	1	0	4	5.0	0.4160	
7	<i>A. obliqua</i>	0	0	1	2	0	0	0	0	0	0	0	2	1	4	10.6	0.0599	
	<i>A. suspensa</i>	0	1	0	0	0	0	0	0	0	0	1	0	1	1	4.0	0.5490	
8	<i>A. obliqua</i>	0	2	0	0	1	3	0	0	0	0	0	0	1	5	14.0	0.0156	
	<i>A. suspensa</i>	1	1	0	0	0	0	1	0	1	1	0	0	2	2	5.0	0.4160	
Total	<i>A. obliqua</i>	0	8	2	4	4	9	4	6	2	13	2	9	14	49	5.1	0.4038	
	<i>A. suspensa</i>	4	20	2	4	1	3	3	22	1	19	5	10	16	78	25.9	<0.0001	

tions of lures, are conducted in orchards of different crop species, and at different times of the year, all of which can impact the outcome. Nonetheless, it is useful to summarize these studies because baits will be used in a variety of conditions/locations/seasons to monitor/detect a variety of pest Tephritidae.

Regionally, the experiments most similar to the present study are those of Pingel et al. (2006) comparing the attractiveness of ammonium acetate plus putrescine with torula yeast plus borax in commercial orchards of 3 crop species in southern Puerto Rico, coinciding climactically and geographically with our Juana Diaz site. Conducted in Apr to May of 2002, their study found that the torula yeast outperformed the ammonium acetate and putrescine combination in orchards of mamey sapote and sapodilla, but the ammonium acetate and putrescine lure attracted more flies than the torula yeast in carambola orchards. The difference between the effectiveness of the 2 lures in the different orchards is striking and they point out the preponderance of *A. obliqua* in the carambola orchard (94% trapped flies in the carambola orchard were *A. obliqua*) whereas the other orchards had higher relative populations of *A. suspensa*. *Anastrepha suspensa* was more abundant in the carambola orchard at the Juana Diaz site during our study.

In a Colombian mango orchard *A. obliqua* was caught in traps baited with Nulure and borax more frequently than in traps baited with ammonium acetate with putrescine, torula yeast, or ammonium bicarbonate with putrescine (Epsky et al. 2003). However, in another study in a Mexican *Pouteria sapota* (Sapotaceae) orchard, traps baited with ammonium acetate with putrescine caught more *A. obliqua* than traps with the other baits, and in a Mexican mango orchard Nulure and ammonium acetate with putrescine both caught more *A. obliqua* than traps baited with other lures (Epsky et al. 2003). Furthermore, traps baited with torula yeast in Costa Rica and Honduras caught more *A. obliqua* than traps baited with the other lures. *Anastrepha suspensa* does not occur in any of the locations of the cited trial and so no comparison can be made with the *A. suspensa* results of our study. A similar study indicated that ammonium acetate was the best lure for detection of *A. suspensa* in Florida but that Nulure or torula yeast were the best lures for *A. obliqua* in the Dominican Republic, based on traps in mango orchards (Thomas et al. 2008).

In a study conducted in cages in Mexico, Diaz-Fleischer et al. (2009) found ammonium acetate and putrescine were more attractive to *A. obliqua* than Nulure, but the ammonium acetate and putrescine was not as attractive to *A. ludens*. *Anastrepha ludens* is an economic pest which regulatory agencies in the United States would like to be able to detect. They also found that attractive-

ness varied according to whether the flies tested were wild or reared in the laboratory for several generations.

There is strong evidence that the attractiveness of a particular lure to a given fly is based on that fly's physiological state, usually the stage of ovary development and a possible explanation for our results may be that populations varied physiologically over the duration of the experiment. Electroantennagram studies on *A. suspensa* indicated that immature females (females with little ovary development) were more responsive to ammonia and to ammonium bicarbonate lures, while females with mature ovaries were more responsive to putrescine and to carbon dioxide (Kendra et al. 2005; Kendra et al. 2009). Diaz-Fleischer et al. (2009) found that diet of the target fly did influence subsequent capture in traps, with more protein-starved individuals being captured by protein baits.

Nulure, followed by freeze-dried Nulure, consistently attracted the fewest flies in our study, regardless of species, sex, or location. This contrasts with the results obtained by Thomas et al. (2008) in mango orchards in Dominican Republic, where *A. obliqua* was most attracted to Nulure and torula yeast baits. It is conceivable that Nulure would attract more flies in different seasons. Liquid lures, including Nulure and torula yeast, have been shown to be more attractive in the dry season than in the wet season (Heath et al. 1997) and the Thomas et al. study was conducted at the beginning of the wet season.

Diaz-Fleischer et al. (2009) recently concluded that "there is no magic fruit fly trap," based on the complex interactions of fly species, physiological state and bait "preference," aggravated by low trap efficiency. One particular short-coming was the number of flies that entered a trap and successfully escaped from it. It is certainly true that these interactions are complex and that no single bait or trap will suffice for all target species in all regions. It has long been known that different tephritid species respond differently to hydrolyzed protein from different sources; *A. ludens* was more attracted to hydrolyzed cottonseed oil than to hydrolyzed corn protein (Lopez and Becerril 1967). *Anastrepha striata*, *A. serpentina*, *A. obliqua* and *A. balloui* preferred baits of hydrolyzed soy protein to torula yeast hydrolysate (Jiron and Soto-Manitiu 1989). Despite all of the improvements to the traps themselves and the lures, estimates of percent capture have not changed over more than 20 years; Calkins et al. (1984) and Diaz-Fleischer et al. (2009) came up with estimates of about 10% of the available population. Kendra et al. (2010) were able to recapture up to 35% of released *A. suspensa*, though. The results of this study confirm what has been suspected; that trap baits will have to be tailored based on regional and seasonal use.

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