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DEVELOPMENT OF LURES FOR DETECTION AND DELIMITATION OF INVASIVE *ANASTREPHA* FRUIT FLIES

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ABSTRACT: Development of female-biased synthetic attractants for fruit flies offers considerable opportunities for fruit fly management programs. Traps baited with a food-based synthetic attractant composed of ammonium acetate, putrescine and trimethylamine are being used to detect and delimit populations of the Mediterranean fruit fly, *Ceratitis capitata*. Current research is being conducted in 14 countries via an FAO/IAEA-sponsored Coordinated Research Project to determine the utility of this synthetic attractant for detection of other fruit flies, particularly those in the genus *Anastrepha*. These include tests of four species of concern to the Caribbean basin: the Mexican fruit fly, *A. ludens*; the West Indian fruit fly, *A. obliqua*; the guava fruit fly, *A. striata*; and the sapote fruit fly, *A. serpentina*. Results of tests conducted in Columbia, Costa Rica, Honduras, and Mexico found that the highest capture tended to be in traps baited with liquid protein (11 out of 15 tests); in the other four tests highest capture was in traps baited with ammonium acetate-based synthetic attractants. The role of ammonia release rate from preferred baits and the development of improved attractants for these species are discussed.

KEY WORDS: trapping, synthetic attractant, *Anastrepha ludens*, *Anastrepha obliqua*

INTRODUCTION

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) and *Anastrepha* spp. fruit flies are pests of major economic importance that threaten fruit and vegetable production and export. The medfly is considered a major economic pest worldwide because of its wide distribution and large host range, encompassing over 240 species of fruits and vegetables (Liquido et al., 1991). California, Texas, Arizona and Florida maintain traps for detection of Mediterranean, Mexican and other exotic fruit flies. *Anastrepha* species of particular importance to the Caribbean Basin include the Caribbean fruit fly (caribfly), *A. suspensa* (Loew); the West Indian fruit fly, *A. obliqua* (Marquart); the Mexican fruit fly (mexfly), *A. ludens* (Loew); the guava fruit fly, *A. striata* Schiner; and the sapote fruit fly, *A. serpentina* (Wiedemann). Geographic distributions and host plant lists have been published (e.g., Stone, 1942; Hernández-Ortiz and Aluja, 1993; Zucchi et al., 1996) and recent detection of pest fruit flies is documented in the United States Department of Agriculture, Animal Plant Health Inspection Service (USDA/APHIS), National Agricultural Pest Information System (NAPIS, http://www.ceris.purdue.edu/napis).

Specifically, the caribfly occurs in Puerto Rico and Florida. Its presence impacts guava production in Florida and questions of host status impact marketability of other tropical fruit crops (Simpson, 1993). The West Indian fruit fly occurs in Puerto Rico and Mexico, and is detected periodically in Texas and California. Establishment of this fly in the continental US would pose a serious threat to mango production as well as cause quarantine restrictions for affected States. This fly was first detected in Grenada, West Indies, in the spring 2002 and has

now become established in that country (Pest Management Unit, Ministry of Agriculture, St. George's, Grenada). Mexflies occur in Mexico and throughout Central America. Presence of breeding populations of mexfly in southern California in 2003 resulted in widespread quarantine and control activities to eradicate flies in areas currently infested as well as to prevent movement to other agricultural regions of California and the US. Mexfly larvae were intercepted in infested peppers in Florida in 2003 (Steck, 2003). This species poses a direct threat to citrus production and, as with presence of medfly, would cause widespread quarantine measures that greatly concern growers in potentially affected areas. The guava fruit fly and the sapote fruit fly occur in Mexico, Central, and South America and are occasionally detected in southern Texas.

Availability of highly effective traps for these and other exotic fruit flies is essential for suppression of fruit flies in areas in which they occur and for early detection in areas currently free of these pests. The earliest trapping systems for pest fruit flies relied on the use of baits made from proteins (needed by flies for reproductive maturation) and fermenting sugar (Gurney, 1925). Traps baited with these substances capture both females and males of a number of pest tephritid species, with the same as or greater numbers of females captured than males. These baits are usually deployed in McPhail traps (Newell, 1936), which are bell-shaped invaginated glass traps with a water reservoir, or other bucket-type traps (Cunningham, 1989a). Aqueous solutions of the corn hydrolysate Nulure and borax (Gilbert et al., 1984) and of torula yeast and borax (Lopez-D. and Becerril, 1967) are liquid protein baits used for medfly and *Anastrepha* detection. Several compounds were found that are potent lures for male medflies (Cunningham, 1989b). This finding culminated in the development of trimedlure (TML; Beroza et al., 1961). TML dispensers are typically mounted in Jackson traps (Harris et al., 1971), which are triangular cardboard traps that contain a sticky insert, or are attached to yellow panels that are coated with sticky material (Cunningham, 1989a).

In research of female-targeted trapping systems, the International Pheromone's McPhail trap (International Pheromone Systems, South Wirral, England) baited with liquid protein bait was found to be as effective as any of the McPhail-type traps tested (Katsoyannos, 1994). A food-based synthetic attractant that uses ammonium acetate (AA) and putrescine (Pu), a cylindrical closed-bottom plastic trap used with a toxicant panel (Heath et al., 1995; Epsky et al., 1995) and a cylindrical open-bottom plastic trap used with a sticky insert (Heath et al., 1996) were developed for pest fruit flies that are captured with liquid protein-baited traps. These female-targeted trapping systems were as effective as liquid protein baited traps for capture of medfly females. This synthetic lure also captures mexflies and caribflies (Thomas et al., 2001), although results for *Anastrepha* spp. tend to be more variable. Subsequent research found that trimethylamine (TMA) synergized capture of female medflies in traps baited with ammonium acetate and putrescine (Heath et al., 1997), and captured fewer non-target species than liquid protein baited traps (Katsoyannos et al., 1999). TML-baited traps have been used world wide for detecting and monitoring populations of male medflies; however their use for detection and delimitation trapping is no longer recommended because female-targeted trapping systems are more effective in detecting the presence of very low medfly populations (Papadopoulos et al., 2001).

Although the three-component synthetic food-based attractant (AA+Pu+TMA) is highly effective for capture of medflies (Epsky et al., 1999), studies are ongoing in several countries with endemic populations of *Anastrepha* and *Bactrocera* fruit flies under a Coordinated Research Project (CRP) funded by FAO/IAEA to optimize female-biased lures for these flies. Reported herein are results of initial field tests of several species of *Anastrepha* that were conducted as part of the CRP.

MATERIALS AND METHODS

Traps and Lures. Multilure McPhail traps (Better World, Miami, FL) were used in all studies. Liquid protein-baited traps had 300 ml of an aqueous solution of 9% Nulure (vol/vol; Miller Chemical & Fertilizer, Hanover, PA) + 3% borax (wt/vol; sodium tetraborate decahydrate) or torula yeast/borax (3 pellets in 300 ml water; ERA Intl., Baldwin, NY). Synthetic attractants included a solid formulation of ammonium bicarbonate (AB; Agrisense-BCS Ltd, UK) and individual membrane-based formulations of ammonium acetate, putrescine and trimethlyamine (BioLure, Suterra, LLC, Bend, OR). Traps baited with synthetic lures contained either 300 ml of water with 1-2 drops of Triton X-100 or 275 ml water with 25 ml polypropylene glycol (environmentally-safe car antifreeze). Comparisons were made among traps baited with Nulure/borax solution, torula yeast/borax solution, AA+Pu+TMA with water/triton, AA+Pu+TMA with water/polypropylene glycol, AA+Pu with water/triton, or AB+Pu with water/triton.

Protocol for Field Tests. Field tests were conducted in Columbia, Costa Rica, Honduras, and Mexico. The field plot design was a six treatment by six-trap Latin square in an area with a fairly uniform stand of host trees. No tests were conducted in areas where insecticide was being applied. There was at least 10-15 m between rows and 10-15 m between traps within a row. Tests were conducted for 8 wk, with fresh protein bait solutions made each week and the synthetic lures replaced after 4 wk. These tests were conducted in March-April in mango (Columbia and Costa Rica), in July-August in mango (Costa Rica), in April-June in mango and in August-September in grapefruit (Honduras), and in April-June in mango and in mamey (Mexico) in 2001. Traps were checked twice a week, and numbers of male and female flies recorded by species. Data for each species and each test were summarized separately by number of flies (males plus females) per trap per day, and the percentage of that total capture that was females was determined.

RESULTS AND DISCUSSION

The best lure for each species in each host/country tested is given in Table 1. Multiple lures are listed if two tied in number of flies captured, or if additional lures performed almost as well. Number of flies captured ranged from 0.3 to 19.7 per trap per day, and the percentage females ranged from 39.0 to 88.2% for the best of the lures in each test. Most of the captures were female-biased except for a male-bias in capture of *A. striata* in mango in Columbia, and little bias in capture of *A. serpentina* and *A. striata* in the spring tests in mango in Costa Rica, and *A. obliqua* in grapefruit in Honduras, and in mango in Columbia. The results from these tests showed considerable variation in lure effectiveness both within and among the different species. One of the liquid protein baits, however, was as good as, or better than, any of the synthetic lures in most of the studies. The ammonium bicarbonate plus putrescine lure was not the most effective in any test.

Among the many differences in volatile chemicals emitted from these baits, there are differences in release rates of ammonia. The Nulure/borax releases the highest amount of ammonia; the AA and AB lures intermediate amounts, and the torula yeast/borax releases the lowest amount (Heath et al., 1995; Heath unpublished). However, ammonia release rate alone does not explain the differences, and for several of the tests the highest capture was in both the Nulure/borax- and the TY/borax-baited traps, which represented the highest and lowest release of ammonia (Table 1). AA lures also release acetic acid, AB lures release carbon dioxide, and numerous other chemicals are released from the liquid protein baits. Therefore, differences in

capture among these lures may be due to attraction or repellency of any of these chemicals.

Field tests are ongoing by the CRP collaborators to further evaluate the role of ammonia release rate and synthetic formulation. In research being conducted at USDA, ARS, SHRS (Miami, FL), electroantennogram (EAG) techniques are being used to measure chemoreceptive response of antennae, using the caribfly. Antennal responses are a prerequisite for behavioral responses, making EAG a useful tool for screening potential attractants. We are quantifying antennal sensitivity to ammonia, the primary attractant released from liquid protein baits and commercial lures.

Since the protein baits have higher capture rates than ammonia-based lures, it seems likely that additional food-based attractants remain to be identified. For this identification, EAG coupled with gas chromatography (GC-EAG) will be conducted to screen volatile chemicals emitted from liquid protein baits. This method uses GC to separate complex mixtures of volatiles into component peaks, and then uses EAG to determine the physiologically active peaks. This strategy will facilitate isolation and identification of new attractants. The best candidates determined by GC-EAG will then be evaluated as attractants in flight tunnel bioassays (Heath et al., 1993) with caribflies and subsequently field tested in combination with other known synthetic attractants for effectiveness for the other *Anastrepha* pest species.

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Table 1. Lures used in McPhail traps that resulted in the highest captures of the fruit fly species in field tests conducted in several countries, the average number of flies (males plus females) per trap per day captured in that trap, the percentage of those flies captured that were female, and the relative release rate of ammonia from those baits.

Species	Country	Host	Fruit Fly Lure[a]	Flies/Trap/Day	Percent Females	Ammonia Rate
A. obliqua	Columbia	Mango	Nulure/borax	7.2	53.9	High
A. striata	Columbia	Mango	Nulure/borax, TY/borax	0.6, 0.5	39.0, 42.8	High, Low
A. ludens	México	Mango	Nulure, AA+Pu, TY/borax	0.3	83.3, 55.6, 88,2	High, Medium, Low
A. serpentina	México	Mango	Nulure, AA+Pu+TMA	1.0	73.7, 60.7	High, Medium
A. serpentina	México	Mamey	AA+Pu+TMA, TY/borax	5.5, 3.9	64.8, 69.5	Medium, Low
A. obliqua	México	Mamey	AA+Pu+TMA, AA+Pu	0.5, 0.4	65.5, 70.4	Medium
A. striata	Costa Rica	Mango, summer	AA+Pu+TMA	0.4	61.9	Medium
A. obliqua	México	Mango	AA+Pu, Nulure/borax	19.7, 15.2	63.8, 64.0	Medium, High
A. ludens	Honduras	Grapefruit	TY/borax	0.6	68.7	Low
A. ludens	Costa Rica	Mango, summer	TY/borax, Nulure	3.8, 3.5	63.8, 71.7	Low, High
A. obliqua	Honduras	Mango	TY/borax, AA+Pu	1.1, 0.9	61.2, 57.9	Low, Medium
A. obliqua	Honduras	Grapefruit	TY/borax	0.07	54.5	Low
A. obliqua	Costa Rica	Mango, spring	TY/borax	6.0	61.6	Low
A. serpentina	Costa Rica	Mango, spring	TY/borax	0.5	48.3	Low
A. striata	Costa Rica	Mango, spring	TY/borax	1.2	52.9	Low

[a]Lures include aqueous solutions of the liquid protein baits Nulure/borax and torula yeast (TY)/borax; and the food-based synthetic lure of ammonium acetate (AA), putrescine (Pu) and trimethylamine (TMA).