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Short Communication

Potential of attractive toxic sugar baits for controlling *Musca domestica* L., *Drosophila melanogaster* Meigen, and *Megaselia scalaris* Loew adult flies

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ABSTRACT

Musca domestica, *Drosophila melanogaster* and *Megaselia scalaris* (Diptera: Muscidae, Drosophilidae, and Phoridae, respectively) are common urban pest flies. Potential control of these adult fly species using an attractive toxic sugar bait (ATSB) system was evaluated in the laboratory. ATSB, consisting of a combination of mango fruit syrup (as bait) and acetamiprid (as toxic agent), was evaluated and compared with a commercially available fly bait (EndZone™ Insecticide sticker, FMC Corp., USA) containing acetamiprid. Mango syrup without toxicant (ASB) served as the negative control. The ASB + acetamiprid bait was the most effective control mixture based on initial knockdown within 10 min for house flies and 200 min for phorids. The combination produced a higher percentage of mortality in house flies and phorids, respectively, than in fruit flies; however, there was no significant difference between the bait formulations as indicated by the mean mortality of house flies and phorids. Significant differences in *Drosophila* mean mortality were demonstrated between the ATSB combinations. These findings suggested a fruit-based ASB + acetamiprid system could be used as a cost-effective, alternative for adult fly control.

Introduction

Musca domestica (L.) (house fly), *Drosophila melanogaster* Meigen (fruit fly), and *Megaselia scalaris* Loew (phorid fly) (Diptera: Muscidae, Drosophilidae, and Phoridae, respectively) are common pest insects globally (Campobasso et al., 2004; Khan et al., 2012; Zhu et al., 2003). *Musca domestica* has been implicated as potential mechanical vectors of pathogens (viruses, bacteria), which may have detrimental health and economic impacts (Greenberg et al., 1970). Male and female flies feed on nectar and organic matter, so they are commonly attracted to waste receptacles and other forms of organic matter (Iqbal et al., 2014). *Drosophila melanogaster* are commonly associated with ripe and spoiled fruits and vegetables, and can have a devastating impact on food production (Markow and O'Grady, 2005). *Megaselia scalaris* is also known as the scuttle fly and feeds on various damp, decaying organic material, but also functions as a facultative predator (Disney, 2008). Phorid flies are potential mechanical vectors of pathogens and may be responsible for sporadic occurrences of facultative myiasis in humans (Carpenter and Chastain, 1992). In larger numbers, phorids can become serious nuisance pests by infesting various structures, breeding in moist food debris and in drains in food producing or food handling facilities (Disney, 2008).

Many fly species require a sugar source for sustenance and reproduction (Muller et al., 2010). This need for sugar is useful in the deployment of attractive toxic sugar bait (ATSB) systems as a means to control adult flies (Diclaro et al., 2012; Gahan et al., 1954; Hogsette et al., 2002; Yee, 2011) Insecticide resistance in houseflies has been documented with many active ingredients in common chemical classes used for control, including: organochlorines, organophosphates, carbamates and pyrethroids (Khan et al., 2013). Recently, there has been an increased emphasis on the development of new pesticide chemistries or re-purposing older active ingredients with novel applications as a means of combating or

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mitigating the development of insecticide resistance and providing alternatives in insect control programs (Hardy, 2014). Aside from an expanding list of various insect growth regulators, one of the most significant recent developments for fly control has been the use of neonicotinoid compounds (Palumbo et al., 2001). Acetamiprid, used in the previous study, is an alternative for fly control because significant resistant to imidacloprid has been observed in house flies (Kaufman et al., 2006). Acetamiprid is one of several neonicotinoids that have been introduced for insect vector and pest control; however, Kavi et al. (2014) reported high levels of resistance in house flies to imidacloprid and levels of low cross-resistance to acetamiprid. The combination of bait, sugar and chemicals has been used to control several pests. One example is the combination of sugar and acetamiprid on a plastic sheet that is applied as a sticker to windows (Beitzel et al., 2014).

In the current study, one objective was to compare the effectiveness of two alternative bait systems combined with acetamiprid compared to a commercial product. The other objective was to compare response (knockdown and mortality) of the ATSB systems against three different species of flies. Bait-coated plastic sheets containing a fruit-based bait combined with acetamiprid were tested. Ripe mango was selected as the preferred bait as it is commonly available in many countries and could serve as an easily sourced, inexpensive bait for devising local ATSB systems to control susceptible insect pests.

Materials and methods

Insects and rearing procedures

Three fly species were used in this study. All flies were laboratory-reared using standard procedures under environmentally controlled conditions (26 °C, 55% relative humidity) and an artificial 12:12 light:darkness photoperiod, at the Department of Entomology and Nematology, University of Florida, Gainesville, FL. USA. Adult *M. domestica* were collected in 2004 from the Horse Teaching Unit in Gainesville, FL, USA. The larvae were fed on nutritive medium made by combining 3 L of dry wheat bran, 1.5 L of water and 250 mL of dairy calf feed pellets (Calf Manna, Manna Pro. Corp., St. Louis, MO, USA) according to Chaskopoulou et al. (2009). House flies were reared by placing eggs in a basin containing freshly mixed larval media. Developing larvae took approximately 1 wk to pupate. Pupae were collected and placed in a screened cage (30 cm × 62.54 cm × 30 cm) together with granulated sugar, powdered milk and water mixture provided *ad libitum*. A long-established laboratory strain of *Drosophila melanogaster* was reared in 25 mL × 200 mL glass test tubes (Pyrex Labware; Corning NY, USA) and fed a mixture of potato, yeast and water. *Megaselia scalaris*, another long-established laboratory strain, was similarly reared in 25 mL × 200 mL glass test tubes and fed on preserved, dried insects such as crickets (Robinson, 1971) and cockroaches (Disney, 2008; Miller, 1978).

Fly bait preparation

Attractive sugar bait (ASB) concentrate was prepared using 150 mL of peeled, macerated ripe mango pulp (El Sembrador™; L and J General International; Miami, FL, USA), 150 g granulated sugar, and 0.9 mL 0.06% lime juice (ReaLime 100% Lime Juice; Mott's LLP; New York, NY USA). This mixture was heated to 100 °C until all ingredients had fully dissolved and the bait reached a paste-like consistency and was then allowed to cool at room temperature. Before use in experiments, the ASB concentrate was diluted with three parts distilled water (225 mL) for each part (75 mL) of ASB concentrate. A second ‘No-sugar’ ASB concentrate was produced using mango pulp only without the addition of granulated sugar or lime juice.

Acetamiprid ((E)-*N*-[(6-chloro-3-pyridyl) methyl]-*N*′-cyano-*N*-methylacetamidine) (BASF), an odorless neonicotinoid provided as a 10% water dispersible powder, was combined with two different natural fruit-based baits. For the Treatment 1 preparation, 12.5 mg of acetamiprid was mixed into 0.48 mL of the diluted ASB resulting in a 0.1052% weight per volume (w/v) concentration of active ingredient in the final ATSB. For the No-sugar treatment, 12.5 mg of acetamiprid and 0.55 mL of No-sugar ASB concentrate were combined. The negative control was ASB solution (Treatment 1 preparation) without acetamiprid. The positive control was a commercially available fly control product marketed as EndZone™ (FMC Corp.; Philadelphia PA, USA), an insecticide-treated plastic sticker containing a sugar-based attractant and 4.4% acetamiprid (approximately 11.9 mg active ingredient per unit).

In order to present the bait to the flies in a manner similar to the EndZone product instructions, transparent plastic sheets (3M™ Dual-Purpose Transparency Film, CG5000), each cut to 9 cm × 10.1 cm in size, were lightly abraded on the surface of one side using P600-grade sandpaper to allow for better adhesion of the ATSB to the plastic surface. For the ASB treatment and negative control, 0.3156 g of appropriate formulated baits were used to coat individual plastic sheets, which were then placed under a fume hood to allow the formulation to air-dry for approximately 12 h. Each treated plastic sheet was cut into five 8.55 cm^2 disks resulting in each disk having approximately 0.094 mL ATSB containing 2.46 mg of acetamiprid.

Bioassay

For each test, 20 *M. domestica* (between 3 and 5 day-old adults), and 10 *D. melanogaster* and *M. scalaris* (between 1 and 3 day-old adults) each were used in separate assays. Each assay was tested in five separate replications. All flies were randomly selected, regardless of sex or mating status. All experiments were performed under the same controlled laboratory conditions during daylight hours, using artificial illumination, with a mean room temperature of 25 ± 5 °C and relative humidity of 65 ± 10%.

The bait or control disks were placed (one per container) in the middle of a clean, 480 mL^3 plastic container. The container cover was fitted with plastic mesh netting with a 2 cm diameter opening in the center of the cover that allowed flies to be placed inside or removed from the container as needed (Fig. 1). A cotton wool plug was placed in the opening to prevent escape.

For handling of adult flies, a desired sample of each species was collected by aspiration using a modified hand vacuum device and then immobilized in a freezer compartment at 4 °C for 3–4 min, removed and counted on a cold plate. Individual flies were transferred into a glass tube and allowed the opportunity to recover for 2 h at 26 °C. Flies were held at room temperature in the laboratory under high moisture conditions (wet paper towel inside the holding tube) for another 6–8 h to ensure full recovery and a healthy state. Flies were then transferred to the test container using an aspirator and allowed free direct exposure to the treated or control disks.

Knockdown was defined as flies observed as moribund (incapacitated but presumed alive) and incapable of normal, sustained flight or ambulation. Initial knockdown response was recorded at intervals of 0.5 min, 2 min, 4 min, 6 min, 8 min and 10 min during the exposure period. Observations continued beyond 10 min until all flies were knocked down. The test period was concluded after all test flies were judged knocked down (thus, no longer actively feeding) in the treatment chambers and likewise in the paired negative controls with un-treated disks (whether flies were active

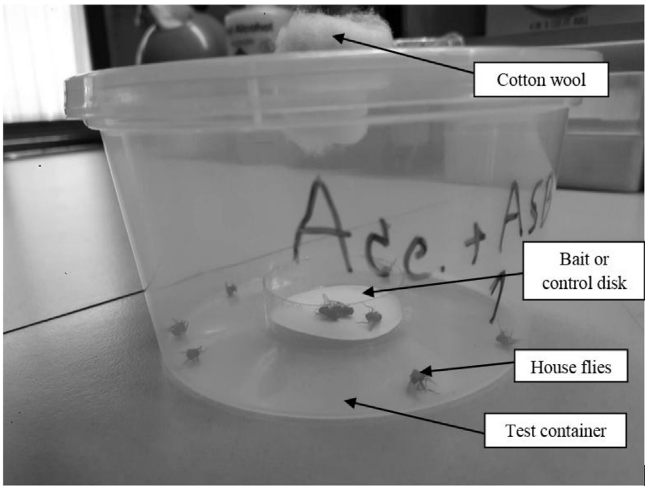

Fig. 1. Example experimental set-up used in attractive toxic sugar bait trials.

or not). Following the tests, flies from each treatment or control replicate were removed and placed in a clean 480 mL^3 plastic holding container under the laboratory conditions described above. Flies were held separately in the same type of clean containers topped with cotton pads soaked in a 10% sugar solution during the holding period. Containers were held for 24 h before recording final mortality.

Data analysis

All data were analyzed using one-way analysis of variance and Duncan's mean difference statistic with the statistical package SPSS (version 17.0; SPSS Inc.; Chicago, IL, USA). Mean mortalities were calculated for each treatment with significance set at 0.05% ($p < 0.05$). If applicable, Abbott's formula (Abbott, 1925) was used to adjust the final mortality if the number of dead flies in the paired negative control exceeded 5%.

Results and discussion

In all tests, mortality in the untreated controls (ASB alone) was negligible (between 0.6 and 1.2%), thus no adjustment of final mortality was needed (Table 1). All house flies were knocked down by all treatments and positive control within 10 min of exposure, whereas fruit flies and phorids required an exposure of 200 min to reach near complete knockdown. In all cases, the final mortality level seen in the negative controls was significantly different ($p < 0.05$) from the three baits containing acetamiprid. Within 4 min, more than 60% of *M. domestica* flies were knocked down after contact with the acetamiprid + ASB combination (Fig. 2) with complete knock down occurring within 10 min; however, final mortality after 24 h was 87% (Table 1), indicating recuperation in some of the treated flies. Differences in mean mortality of house flies were not statistically significant between the three toxic baits.

More than 50% *D. melanogaster* flies were knocked down at 30 min with the Acetamiprid + ASB combination and EndZone (Fig. 3). Knockdown gradually increased to reach 100% after 120 min exposure for both formulations. Final mortalities were significantly different ($p < 0.05$) between the bait systems with the highest mean mortality (39.6%) observed with Acetamiprid + ASB compared to the other two toxic combinations (Table 1). Percentage of knockdown for *M. scalaris* was similar for all treatments until reaching 100% after 200 min exposure (Fig. 4). There was no significant difference ($p > 0.05$) between bait formulations with active ingredient for *M. domestica* and *M. scalaris*.

Two combinations of natural sugar bait combined with acetamiprid, a nicotinoid class insecticidal compound, were tested for toxic response with three different fly species. Nicotinoids typically function as systemic poisons that act on the central nervous system causing irreversible blockage of postsynaptic nicotinergic acetylcholine receptors by mimicking (rather than inhibiting or binding) acetylcholine (Ware and Whitacre, 2004). This mode of action differs from many of the most common classes of chemicals used in pest control, most notably pyrethroids, and has pushed nicotinoid agents as one of the preferred alternatives for use against insects that have developed resistance to other insecticides (Furlan and Kreutzweiser, 2015). All three species tested showed various degrees of post-exposure recovery following initial knockdown.

One limitation of using the EndZone sticker and bait formulation has been the control of smaller-sized flies. Although the different treatments were effective in eventually achieving complete knockdown, the response was significantly delayed with phorids and fruit flies compared to house flies, as was the final mortality. All flies were found susceptible to the knock down effects of acetamiprid but the variations in response outcome may be related to the differing feeding behaviors or physiology (smaller volume intake, possibly shorter, less frequent feeding intervals) of the two smaller species compared to the larger bodied house flies. However, varying susceptibility patterns (innate or otherwise) between the insect species used in this study could not be ruled out. Susceptibility assays to acetamiprid to determine lethal concentration values (at either the 50% or 99% mortality concentrations) were not conducted before or after the study.

Previous study has evaluated ATSB systems for *M. domestica* control (Gahan et al., 1954) evaluating the effectiveness dry sugar baits containing malathion and diazinon, which killed 99% of house flies resistant to dichlorodiphenyltrichloroethane (DDT) and other chlorinated hydrocarbon insecticides within 16 h of ingestion. The current study showed that acetamiprid used at low concentrations in combination with sugar baits provided fast knock down action

Table 1
Mean mortality of *Musca domestica*, *Drosophila melanogaster* and *Megaselia scalaris*, after 24 h exposure to attractive toxic sugar bait and controls.

Treatment	Mean mortality ± SE		
	M. domestica	*D. melanogaster*	*M. scalaris*
Control	0.6 ± 0.34^b	1.2 ± 0.58^c	0.7 ± 0.25^b
FMC EndZone	71.1 ± 16.83^a	32.4 ± 2.87^b	58.1 ± 12.11^a
Acetamiprid + Mango	61.0 ± 11.89^a	30.8 ± 1.31^b	50.4 ± 8.82^a
Acetamiprid + ASB	87.2 ± 16.28^a	39.6 ± 1.83^a	69.1 ± 12.67^a
Duncan's test	$F = 8.29$ (df = 3)	$F = 84.08$ (df = 3)	$F = 9.52$ (df = 3)
	$p > 0.05$	$p < 0.05$	$p > 0.05$

ASB = mango syrup without toxicant.
[a,b,c] = different lowercase superscript letters in a column denote significant ($p < 0.05$) differences between comparisons.

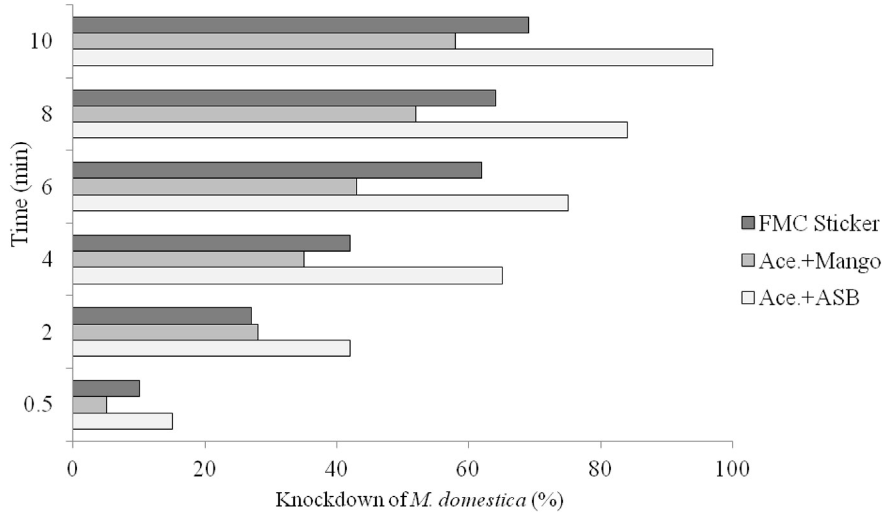

Fig. 2. Mean percentage knockdown of *Musca domestica* with 10 min exposure to different bait combinations versus contact time, where Ace. = acetamiprid, ASB = mango syrup without toxicant.

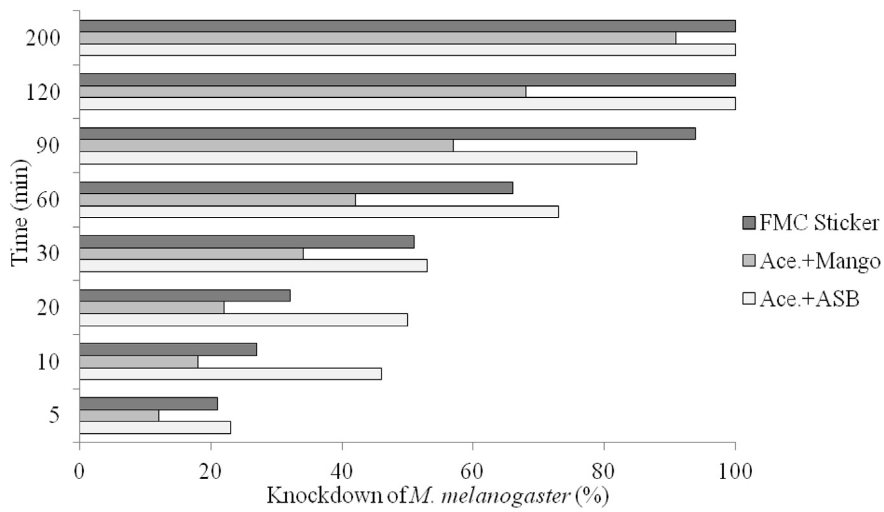

Fig. 3. Mean percent knockdown of *Drosophila melanogaster* with 200 min exposure to different bait combinations versus contact time, where Ace. = acetamiprid, ASB = mango syrup without toxicant.

against the three fly species tested. Compared to other active ingredients, acetamiprid would presumably be effective against various other fly species as well (Hogsette et al., 2002; Müller and Schlein, 2008; Xue et al., 2008, 2011; Yee, 2011).

The current results showed that acetamiprid was effective in killing only a proportion of each fly species, presumably because knockdown occurred before some individuals could ingest a sufficient amount of insecticide to be lethal. This apparent sub-lethal response might be overcome by either increasing the concentration of acetamiprid in the bait or by using alternative compounds that might offer advantages like slower knockdown to allow flies to feed for a longer period to produce a higher percentage kill. Other baits and active ingredients combinations are available, such as a spinosad bait system (a mix of a fermentation metabolite of the actinomycete, *Saccharopolyspora spinosa*, sugar, and other ingredients) combined with acetamiprid, or other nicotinoids, such as thiamethoxam or imidacloprid (Yee, 2011).

Nicotinoids provide advantages over more commonly used insecticides as one means to overcome or mitigate the development of resistance in insect pest populations. For example, a strain of house flies resistant to fipronil (a phenylpyrazole-class compound) showed very low probability of cross-resistance to acetamiprid with a heritability value of only 0.008, compared to other insecticides (Abbas et al., 2014). Despite some concerns, ATSB applications in the indoor urban environment would appear to have mostly minor impact on non-target arthropods. Understandably, when ASB (without a toxic element) is applied to flowering vegetation, non-target insects will be attracted to the baited plants. However, one study demonstrated that honeybees treated orally with sub-lethal doses of 0.1 and 0.5 μg acetamiprid showed no changed behavior (El Hassani et al., 2008). The low non-target impact and increased sustainability of the ATSB method evaluated in this report further supports this technique as a strategy that can be incorporated more widely into vector and pest fly control programs.

The current experiments were conducted exclusively in a laboratory and without the presence of other potential competing food sources or natural forms of sugar. The current study supports

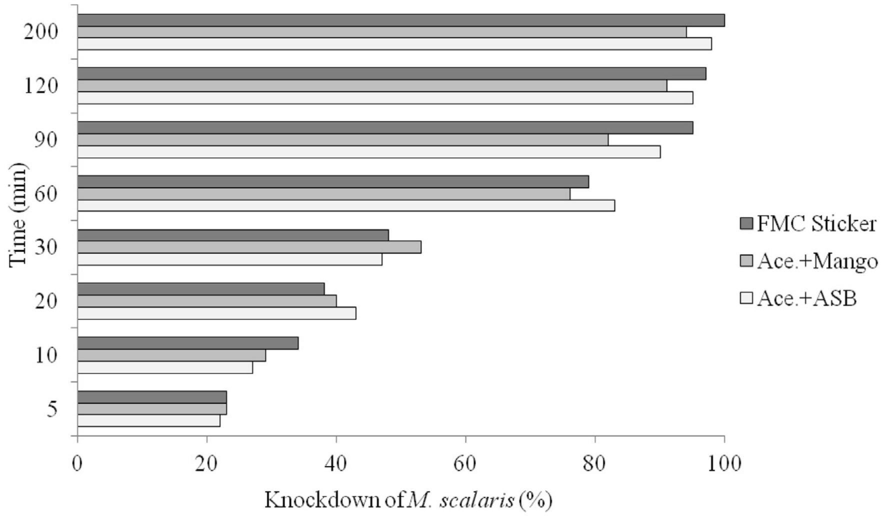

Fig. 4. Mean percent knockdown of *Megaselia scalaris* with 200 min exposure to different bait combinations versus contact time, where Ace. = acetamiprid, ASB = mango syrup without toxicant.

the design of an artificial, specially formulated sugar bait to be as or more attractive to adult flies than the majority of other natural food sources. However, this would have to be tested under different conditions and in open settings. The authors believe the ATSB system can be competitive in an open environment and further enhanced with additional improvements to boost attractiveness (such as visual and olfactory cues).

The ATSB approach has broader operational implications for a wide variety of dipteran species attracted to various forms of sugar. The ATSB system used in this study is especially promising because the bait combination has very low mammalian toxicity (Yamada et al., 1999) and would be economically feasible to use in both urban and rural settings. However, the impact of ATSB availability for non-target insects, such as beneficial hymenopteran species, must be carefully investigated. A better understanding of specific fly behaviors, such as requirements and preferences for a carbohydrate energy source, can provide strategic advantages for target-specific control measures. Thus, the importance placed on a better understanding of fly ecology and behavior for the development and implementation of more efficient control methods should not be underestimated. The expanded use of novel chemistries, delivery systems and pesticide alternatives for rotation to help mitigate development of resistance is essential for successful and sustained fly control. The various successes of the ATSB method provides evidence that flies are recurrently feeding on sugar throughout their adult life, which enables ATSB systems to be efficient and cost-effective control tools.

Conflict of interest

The authors declare no conflicts of interest.

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