Synergistic Trap Response of the False Stable Fly and Little House Fly (Diptera: Muscidae) to Acetic Acid and Ethanol, Two Principal Sugar Fermentation Volatiles

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ABSTRACT In an initial observation, large numbers of muscoid flies (Diptera) were captured as non-target insects in traps baited with solutions of acetic acid plus ethanol. In subsequent field experiments, numbers of false stable fly *Muscina stabulans* (Fallén) and little house fly *Fannia canicularis* (L.) trapped with the combination of acetic acid plus ethanol were significantly higher than those trapped with either chemical alone, or in unbaited traps. Flies were trapped with acetic acid and ethanol that had been formulated in the water of the drowning solution of the trap, or dispensed from polypropylene vials with holes in the vial lids for diffusion of evaporated chemical. Numbers of both species of fly captured were greater with acetic acid and ethanol in glass McPhail traps, compared to four other similar wet trap designs. This combination of chemicals may be useful as an inexpensive and not unpleasant lure for monitoring or removing these two pest fly species.

KEY WORDS Muscidae, fly, attractant, lure, trap, synergy

The dipteran family Muscidae includes species of flies that are a nuisance to people and livestock, and are vectors or carriers of pathogens of diseases, including dysentery, typhoid, and cholera (Hale et al 1960, Harwood and James 1979, Graczyk et al. 2001, Förster et al. 2007). Pestiferous muscid flies may occur in great numbers where suitable breeding sites are available, increasing their annoyance and the risk of disease transmission.

Baits and chemical attractants can be useful tools in the management of some fly species. Chemical attractants may be used as lures for trapping flies, or in formulations designed to kill attracted flies. Chemical attractants for flies in the family Muscidae include sex pheromones such as (Z)-9-tricosene produced by the female housefly (*Musca domestica* L.) (Carlson et al. 1971), volatiles from larval media such as pig manure (Cossé and Baker 1996), kairomones such as carbon dioxide from vertebrates that are attractive to biting muscid flies (Gerry and Mullens 2006) and 1-octen-3-ol (i.e., Vale and Hall 1985, Holloway and Phelps 1991), and adult fly feeding attractants (e.g., volatiles from materials high in sugar or protein, see Vanskaya 1942; Hwang et al. l978; Mulla et al. 1977, 1984; Qian et al. 2013). The efficacy and application of various lures and traps have been evaluated for the house fly by Burg and Axtell (1984), Beck and Turner (1985), and Lysyk and Axtell (1985). Bait formulations have been developed that are composed of an attractant, sugar, and a toxicant, for use in controlling house flies (Butler et al. 2007).

In a study of moth attraction to volatiles from fermented molasses solutions (Landolt 2000), numerous muscoid flies (Muscidae, Calliphoridae, Sarcophagidae, Anthomyiidae) were captured in traps baited with acetic acid and ethanol but were not reported. That study included a site near a commercial dairy where the false stable fly, *Muscina stabulans* (Fallén) and little house fly, *Fannia canicularis* (L.) were trapped in abundance. Both species are nuisance flies which breed in animal dung and can be a problem at and near animal farms such as poultry houses and dairies (Steve 1960). We took advantage of the local abundance of the false stable fly and little house fly to more thoroughly determine muscid attraction responses to acetic acid and ethanol. We evaluated the trap response of these flies to ethanol and acetic acid in the field and sought to determine if there is synergy between these two compounds. We then evaluated a method of dispensing these chemicals in traps, and compared several trap designs. Together, the results of these experiments provide strong evidence of synergistic fly responses to the two chemicals, and show methods for dispensing the chemicals as attractants for trapping the two species.

Materials and Methods

Trapping experiments were conducted in an apple orchard near a commercial dairy near Zillah, and on shrubbery near a commercial dairy near Moxee, both in Yakima County, Washington. Glass McPhail traps (Newell 1936) were used in all experiments except one test that was a comparison of trap designs. Traps were

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hung from apple tree branches at a height of 2 to 2.5 m, near the outside of the tree canopy (five experiments), or on shrubbery at a height of 1.5 to 2 m (one experiment). McPhail traps have an invagination in the bottom that permits insect entry from beneath and the invagination provides a reservoir in the trap bottom for holding liquid baits or drowning solutions designed to kill captured insects. A liquid detergent (Palmolive unscented concentrated dishwashing detergent, Colgate-Palmolive, New York) was used in water at 0.0125% by volume to enhance insect submersion and drowning. Two hundred ml of this drowning solution was placed within each trap. In apple orchards, traps within a treatment block were placed in a row of apple trees, with traps 20 m apart and treatment blocks in different tree rows 30 m apart. Traps placed in shrubbery were also 20 m apart and treatment blocks were placed on different lines of shrubs at least 30 m apart. For all experiments, males and females of *F. canicularis* and *M. stabulans* were counted. In addition, counts were made of all other Muscoidea, pooled together without sorting of species. We did not find other major pest fly species, such as *M. domestica* and *Stomoxys calcitrans* L., in these trapping tests.

Five trapping experiments evaluated *M. stabulans* and *F. canicularis* responses to acetic acid and ethanol. Four tests evaluated the two chemicals formulated in the trap drowning solution. These were tests of 1) the attractiveness of and synergy between acetic acid and ethanol; 2) varied amounts of ethanol in an ethanol and acetic acid aqueous solution, 3) varied amounts of acetic acid in an ethanol and acetic acid aqueous solution, and 4) five types of traps, all baited with an aqueous solution of acetic acid and ethanol. An additional test evaluated acetic acid and ethanol dispensed from two vials, with the release rates of the chemicals varied with the size of the hole in the vial lid. Within each experiment, the sex ratios of flies captured were similar among treatments and were combined for data analyses.

Glacial acetic acid (Baker Chemical, Phillipsburg, NJ) and 200 proof ethanol (Gold Shield Chemical Co., Hayward, CA) were used in all experiments.

Experiment 1. Synergy Between Acetic Acid and Ethanol. Traps were baited with either 1) 0.5% acetic acid in the trap drowning solution, 2) an ethanol dispenser mounted in the inside top of the trap, 3) both 0.5% acetic acid in the drowning solution and an ethanol dispenser in the trap, or 4) drowning solution alone. The ethanol dispenser was a 20-ml plastic canister containing 10 ml ethanol loaded on balls of cotton and with a 6.4-mm-diameter hole in the lid of the canister. A randomized complete block design was used with five blocks. Traps were maintained 6–15 May 1998. Flies were removed every 2–3 d, and traps were then moved one position within the block.

Experiment 2. Comparison of Concentrations of Ethanol in Combination with 0.5% Acetic Acid in Water. Ethanol was evaluated as a component of the drowning solution, at 0, 0.2, 1, 5, and 25% by volume. All traps also contained 0.5% acetic acid by volume in the drowning solution. A randomized complete block experimental design was used with four replications. Four blocks of this experiment were maintained 18–22 May, 25–29 May, 30 May to 1 June 1998, and five blocks were maintained from 10–12 July, and 14–16 July, 1999. This provided 22 replications of the experiment. However, data from two traps were lost due to broken traps, so trap catch data for those two blocks on those dates were not included in the analyses. Drowning solutions, including those with acetic acid and ethanol concentrations, were replaced weekly.

Experiment 3. Comparison of Concentrations of Acetic Acid With 5% Ethanol in Water. Concentrations of acetic acid relative to the concentration of ethanol were evaluated, with both chemicals placed in the drowning solution. All traps contained 5% ethanol in the drowning solution. The five concentrations of acetic acid added to 5% aqueous ethanol solution were 0, 0.03, 0.125, 0.5, and 2% by volume. A randomized complete block experimental design was used with four blocks. This experiment was maintained 8–14 July 1998. Traps were checked every 2–3 d, at which time captured flies were removed and traps were moved one position within the block.

Experiment 4. Comparison of Trap Designs. Five different trap designs were evaluated for capture of flies attracted to acetic acid and ethanol. Acetic acid and ethanol were placed in the drowning solution of the trap, at 2 and 5% respectively. The five traps were the glass McPhail trap, the Rescue Disposable Fly Trap (bag trap; Sterling International Inc., Veradale, WA), the Rescue Jacket Jar trap (Rescue jar trap; Sterling International), the Victor Magnet Fly Trap (Victor jar trap; Northern Sport Company, Elyria, OH), and the Trappit Dome Trap (dome trap; Agrisense BCS Ltd., Pontypridd, United Kingdom). A randomized complete block design was used with five replications. Traps were set up 19 July 1999 and were maintained for 5 d.

Experiment 5. Comparison of Hole Diameters of Both Acetic Acid and Ethanol Vials. Five release rates of acetic acid and ethanol were evaluated as fly attractants in McPhail traps. Acetic acid and ethanol were loaded into separate 15-ml polypropylene vials (Nalge Nunc International, Rochester, NY) that were mounted in the inside top of the traps. Three cotton balls were placed in the bottom of each vial and vials were then loaded with 10 ml of acetic acid or ethanol. Amounts of attractants released from vials were varied by the diameter of a hole drilled through the lid of each vial. Five such hole sizes were tested: 1) a 0.5-mm-diameter hole in both the acetic acid vial and the ethanol vial, 2) a 1.0-mm-diameter hole in each vial, 3) a 1.5-mm-diameter hole in each vial, 4) a 3.0-mm-diameter hole in each vial, or 5) a 6.4-mm-diameter hole in each vial. A randomized complete block design was used with five blocks. The experiment was conducted as five 24-h tests between 30 June and 9 July 1999, providing 25 replications.

Statistical Analyses. *M. stabulans*, *F. canicularis*, and other Muscoidea trap catches were analyzed using combined male and female counts. For all experiments comparing chemical amounts (effected by vial hole size or percent in the drowning solution), data were subjected to regression analysis, to determine if there was a significant positive relationship between trap catch and chemical amounts (either concentrations or relative release rates from dispensers). Fly catch data for other experiments were subjected to analysis of variance, with block as a random factor and treatment as a fixed factor using SAS Proc Mixed (SAS Institute 2009). Data were square root transformed to improve normality and homoscedasticity (Zar 1984). Treatment means were then compared using the Tukey–Kramer test ($P = 0.05$).

Results

Experiment 1. Synergy Between Acetic Acid and Ethanol. In this experiment, 98% of *M. stabulans* flies, 94% of *F. canicularis* flies, and 88% of other muscoid flies were captured in traps baited with the combination of acetic acid and ethanol (Table 1). The highest numbers of flies were captured in traps baited with the combination of acetic acid and ethanol (*M. stabulans*: $F_{3,12} = 123.39$, $P < 0.0001$; *F. canicularis*: $F_{3,12} = 98.88$, $P < 0.0001$; other muscoid flies: $F_{3,12} = 188.30$, $P < 0.0001$). Few *M. stabulans* were captured in traps baited with acetic acid alone or ethanol alone, and few *F. canicularis* and other muscoid flies were captured in traps baited with acetic acid. Totals of 1,324 male and 1,288 female *M. stabulans*, 801 male and 1,490 female *F. canicularis*, and 2,307 other Muscoidea were trapped in this experiment.

Experiment 2. Comparison of Concentrations of Ethanol With 0.5% Acetic Acid in Water. For *M. stabulans*, there was a significant regression of the natural log of numbers of flies captured with the natural log of percentages of ethanol tested ($r^2 = 0.96$, df = 4, $P = 0.0005$; Fig. 1a). For *F. canicularis* also, there was a significant regression of the natural log of numbers of flies captured with the natural log of percentages of ethanol tested ($r^2 = 0.96$, df = 4, $P = 0.0005$; Fig. 1a). For all other muscoid flies tallied, there was a significant regression of the natural log of numbers of flies captured with the natural log of percentages of ethanol tested ($r^2 = 0.95$, df = 4, $P = 0.0001$; Fig. 1a).

Totals of 687 male and 1,298 female *M. stabulans*, 1,604 male and 1,656 female *F. canicularis*, and 6486 other muscoid flies were captured in this test.

Experiment 3. Comparison of Concentrations of Acetic Acid With 5% Ethanol in Water. For *M. stabulans*, there was a significant regression of the natural log of numbers of flies captured with the natural log of percentages of acetic acid tested ($r^2 = 0.99$, df = 5, $P < 0.0000$; Fig. 1b). For *F. canicularis* also, there was a significant regression of the numbers of flies captured with the square root of percentages of acetic acid tested ($r^2 = 0.86$, df = 5, $P = 0.0017$; Fig. 1b). For all other muscoid flies tallied, there was a significant regression of the numbers of flies captured with the square root of percentages of acetic acid tested ($r^2 = 0.95$, df = 4, $P = 0.0001$; Fig. 1b).

Totals of 14 male and 54 female *M. stabulans*, 258 male and 955 female *F. canicularis*, and 2,829 other muscoid flies were captured in this experiment.

Experiment 4. Comparison of Trap Designs. Greatest numbers of *M. stabulans* were captured in glass McPhail traps, while numbers captured in dome traps were greater than in Victor jar trap, Rescue jar trap, and bag trap ($F_{4,16} = 22.14$, $P < 0.0001$; Table 2). For *F. canicularis*, greatest numbers were captured in glass McPhail traps and numbers captured in other trap designs were similar ($F_{4,16} = 40.80$, $P < 0.0001$). For all other Muscoidea combined, greatest numbers were captured in glass McPhail traps and numbers captured in dome traps were greater than numbers captured in Victor Jar, Rescue Jar, and bag traps ($F_{4,16} = 64.43$, $P < 0.0001$; Table 2). Totals of 39 male and 29 female *M. stabulans*, 80 male and 280 female *F. canicularis*, and 191 other muscoidea flies were captured.

Experiment 5. Comparison of Hole Diameters of Both Acetic Acid and Ethanol vials. When acetic acid and ethanol were formulated in separate polypropylene vials and chemical release rates were controlled by the size of holes in both vial lids, numbers of flies captured increased with hole diameter through the largest hole tested (6.4 mm; Fig. 2). There was a significant regression of the numbers of flies captured with the square root of the vial hole diameter for *M. stabulans* ($r^2 = 0.94$, df = 4, $P = 0.0012$), for *F. canicularis* ($r^2 = 0.99$, df = 4, $P < 0.0001$), and for all other muscoid flies tallied ($r^2 = 0.99$, df = 4, $P < 0.0001$; Fig. 2).

Totals of 151 male and 111 female *M stabulans*, 290 male and 1,025 *F. canicularis*, and 1,709 other muscoid flies were captured in this test.

Discussion

Results of these experiments show a strong synergy of acetic acid and ethanol in attracting muscoid flies, including two pestiferous species, into traps. For both *M. stabulans* and *F. canicularis,* numbers of flies captured in traps baited with acetic acid alone or ethanol

Table 1. Means (±SE) numbers of flies captured in glass McPhail traps baited with acetic acid, ethanol, and the combination of acetic acid and ethanol

Fly	Control	Acetic acid	Ethanol	Combination
M. stabulans	0.04 ± 0.04b	0.24 ± 0.10b	1.92 ± 0.62b	102.72 ± 21.78a
F. canicularis	0.0 ± 0.0b	0.08 ± 0.06b	6.24 ± 2.10b	91.64 ± 19.17a
Other Muscoidea	0.12 ± 0.12b	0.32 ± 0.15b	10.88 ± 1.92b	81.04 ± 11.63a

Means within a row followed by the same letter are not significantly different by the Tukey Kramer test at $P < 0.05$.

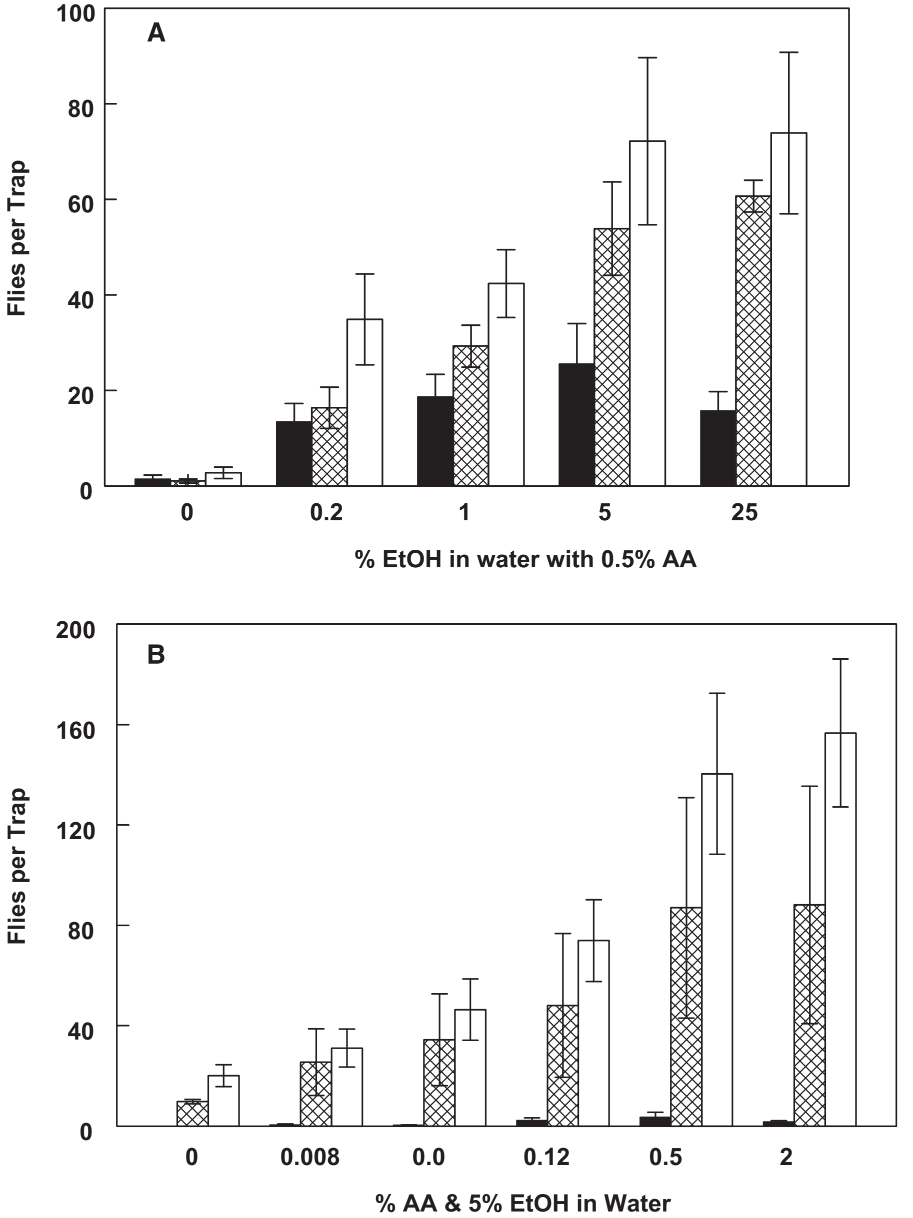

Fig. 1. Mean (± SE) numbers of *Muscina stabulans* (solid black bars), *Fannia canicularis* (cross-hatched bars), and other Muscoidea flies (open bars) captured in McPhail traps baited with: (a) 0.5% acetic acid and different concentrations of ethanol in the drowning solution and (b) 5% ethanol and different concentrations of acetic acid in the drowning solution.

Table 2. Mean (± SE) numbers of flies captured in different wet traps baited with 2% acetic acid and 5% ethanol in the drowning solutions of the traps

Trap type	*M. stabulans*	*F. canicularis*	Other muscoidea
McPhail	2.12 ± 0.59c	14.4 ± 1.83b	8.72 ± 1.11c
Trappit Dome	0.56 ± 0.14b	1.52 ± 0.33a	1.44 ± 0.34b
Victor Jar	0.08 ± 0.08a	1.08 ± 0.47a	0.56 ± 0.22ab
Sterling Jar	0.04 ± 0.04a	0.20 ± 0.10a	0.16 ± 0.09a
Sterling Bag	0.00 ± 0.00a	0.24 ± 0.09a	0.40 ± 0.15a

Means within a column followed by the same letter are not significantly different by Tukey Kramer test at $P < 0.05$.

alone were few in comparison to the numbers captured in traps baited with both compounds. The response pattern to acetic acid and ethanol was similar for other flies captured, with a strong response to the two compounds together and no response or a weak response to the compounds when presented separately. There are previous reports of muscid fly attraction to ethanol (Brown et al. 1961, Hwang et al. 1978, McIndoo 1933), but not to acetic acid. Hwang et al. (1978) showed attraction of both *M. stabulans* and *F. canicularis* to ethanol, which was isolated as an attractant from

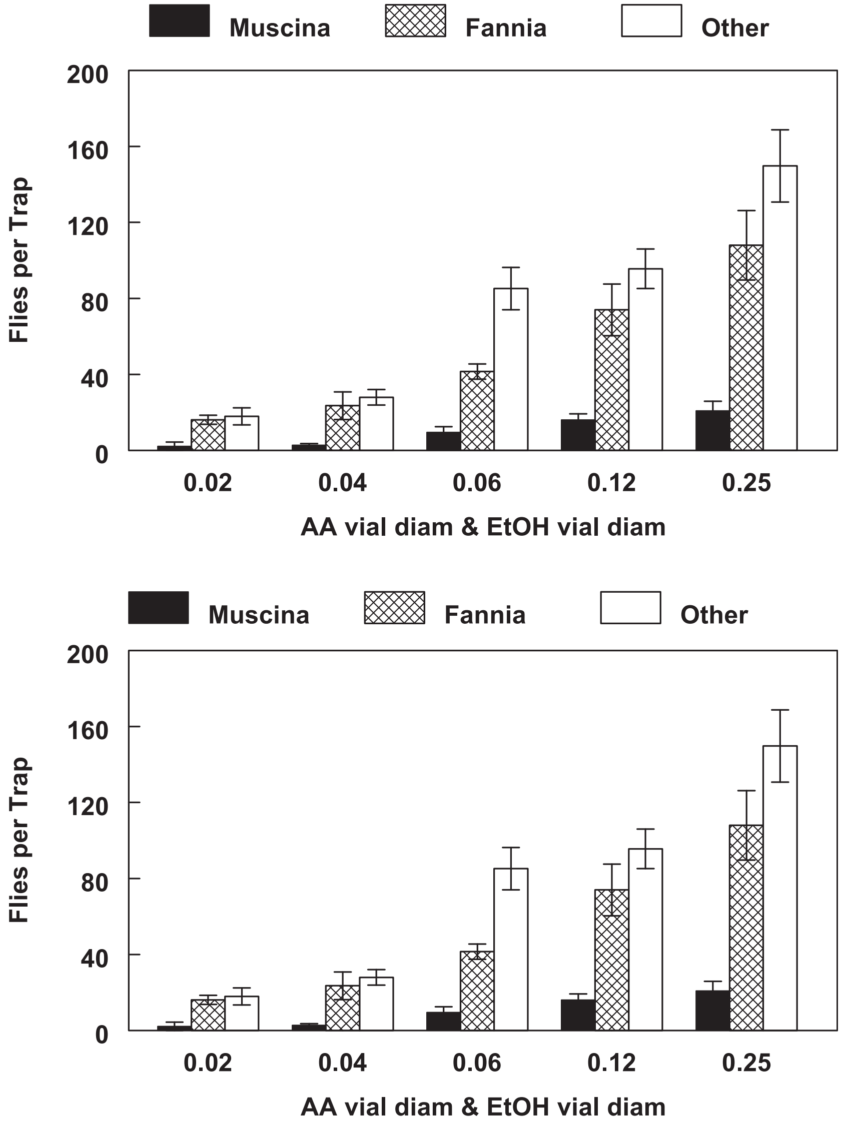

Fig. 2. Mean (± SE) numbers of *Muscina stabulans*, *Fannia canicularis*, and other Muscoidea flies captured in McPhail traps baited with acetic acid and ethanol in individual polypropylene vial dispensers with varying hole sizes.

fermented sucrose solutions. Our experimental results are consistent with the absence of reports of any response to acetic acid. Prior to this study, there were no reports of muscid attraction to the combination of ethanol with acetic acid. Landolt et al. (2012) showed synergistic attractiveness of ethanol and acetic acid to a non-muscoid fly, the spotted wing drosophila *Drosophila suzukii* Matsumura (Drosophilidae). These two compounds subsequently were the basis for a four-component lure for *D. suzukii* (Cha et al. 2014a) and a six-component lure for another drosophilid, the African fig fly *Zaprionus indianus* Gupta (Cha et al. 2014b).

We do not know why acetic acid and ethanol synergistically attract these flies, but suspect that the fly response may be food finding behavior when they seek sugar sources. Both acetic acid and ethanol are byproducts of microbial fermentation of sugars upon which flies probably feed. A lack of dietary sugar reduces the longevity of the house fly and biting stable fly and feeding on sugar greatly enhances house fly survival (Galun and Fraenkel 1957, Lysyk 1991, Müller et al. 2012), indicating the importance to flies of locating a suitable sugar source. Yet, sugar has a very low vapor pressure and is relatively odorless; thus flies are unlikely to be attracted to sugar. Other mechanisms must be involved in fly food-finding behavior to enable flies to locate and access sugar from a distance (Dethier 1955). While the work of Dethier (1955) indicated a pheromonal role in house fly recruitment to dry sugar, chemical cues originating from microbial colonization of sugar-rich sources in nature may also be involved in fly food-finding from a distance. Ethanol and acetic acid are abundant fungal and bacterial volatiles derived from sugar catabolism (Drysdale and Fleet 1988, Antonelli et al. 1999). We hypothesize that many muscoid flies, including some pestiferous species, may be attracted to acetic acid or ethanol as odor cues that indicate the presence of a sugar source.

It is also possible that muscid flies gain benefits from direct ingestion of microbes (Grübel et al 1997), as occurs for tephritid flies (Drew et al. 1983). Perhaps such microbes themselves are important for enhanced survival of these flies, either as food or as mutualists or symbionts in the midgut (Crotti et al. 2010). There are common and widespread yeasts that are symbionts of drosophilid flies and that affect fly physiology, behavior, and fitness Chandler et al. 2012). Similar relationships are also seen between widespread bacteria and tephritid fruit flies (Bateman 1972). Perhaps there are mutualistic or symbiotic relationships between microbes and muscid flies at work here.

Fermented sweet materials that are attractive to insects, including some man-made fermented food products, produce an abundance and diversity of volatile chemicals in addition to ethanol and acetic acid. For example, Utrio and Eriksson (1977) characterized the complex volatile chemistry of a fermented brew attractive to moths (Lepidoptera), El-Sayed et al. (2005) identified 40 chemicals from the headspace of four types of sweet baits for insects, and Carneiro and Nobrega (2003) identified numerous volatile chemicals from fermented sugar cane, among other studies. If indeed these flies are attracted to ethanol and acetic acid as a means to locate carbohydrate rich foods, then there may be additional volatile chemicals emitted by microbes on these materials that are attractive to the flies and co-attractive with acetic acid and ethanol. Such was found with the attraction of spotted wing drosophila attraction to wine and vinegar. The response of that fly was greatly increased with the microbial volatiles methionol and acetoin added to traps baited with acetic acid and ethanol (Cha et al. 2014a). Cha et al. 2015 showed a much stronger response of muscoid flies to a wine plus vinegar bait compared to the four-chemical attractant for *D. suzukii* that included acetic acid plus ethanol. However, that study did not identify pest species so we do not know how or if *M. stabulans* and *F. canicularis* responded in that test, and we do not know if the chemicals acetoin and methionol in that lure are attractive or repellent to these muscid flies. A more thorough assessment of additional microbial volatiles and fermented baits as attractants for pestiferous muscid flies might yield additional attractive compounds.

The attractiveness of a chemical mixture to insects might be influenced by the relative amounts or concentrations of the different chemical components (e.g., Cha et al. 2011). Since we did not know if the amounts of acetic acid and ethanol used in the first experiment were optimal, we attempted in experiments 2 and 3 to determine how the aqueous concentrations of acetic acid and ethanol impacted the numbers of flies captured in wet traps, with both chemicals incorporated into the trap drowning solutions. We expected that altered concentrations of the chemicals in the drowning solution would result in altered rates of evaporation and subsequently the concentrations of the compounds in air downwind of the trap. Reed (1938) found that *D. melanogaster* flies could be captured with a wide range of aqueous concentrations of either of these two chemicals, but that they became repellent with concentrations of ethanol above 25% and concentrations of acetic acid above 5%, with optimum concentrations of 5–20% ethanol and 0.1 to 5% acetic acid. For *F. canicularis* and for other muscoid flies, the numbers trapped increased over the range of concentrations tested; up to 25% ethanol and 2% acetic acid. For *M. stabulans*, the numbers trapped appeared to be highest at 5% ethanol and 2% acetic acid. We note that the rates of release of these compounds from aqueous solutions are not stable and should decrease with time.

In experiment 5, we sought to demonstrate a "dry" or nonaqueous method of dispensing acetic acid and ethanol, both to better control release rates of these chemicals for attracting flies and to permit additional research on evaluating dry trap or bait designs that lack the drowning solution. The dispenser system composed of polypropylene vials with holes in the lids has been demonstrated to be useful for a moth attractant composed of acetic acid with 3-methyl-l-butanol (Landolt and Alfaro 2001), and a yellowjacket wasp (*Vespula* spp.) attractant composed of acetic acid with isobutanol (Landolt et al. 2005). The captures of flies generally increased with vial hole size, but further increasing of the vial hole size would quickly deplete the chemicals in the vial. Additional studies will be required to determine if fly catch increases further with higher release rates of acetic acid and ethanol from larger vial holes. A larger reservoir for ethanol and acetic acid (a larger vial for example) would be required to support such high release rates for an extended period of time.

The comparison of trap designs indicated a very pronounced advantage to the use of the glass McPhail trap over other traps evaluated, including the dome trap which is similar in design. The glass McPhail trap has been used extensively to capture tephritid fruit flies that are attracted to various proteinaceous baits (Newell 1936). It is not known what aspect(s) of the McPhail trap is important to the capture of attracted flies. However, disadvantages of the glass McPhail trap are its limited availability, potential cost, and propensity to shatter when dropped or fallen.

Other pestiferous filth flies, such as *M. domestica* and *S. calcitrans*, were absent from this study site. Additional testing should be done at sites where other pest flies are present, in order to determine if they respond similarly to the combination of acetic acid and ethanol.

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