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IDENTIFICATION OF ODORS FROM OVERRIPE MANGO THAT ATTRACT VINEGAR FLIES, *Drosophila melanogaster*

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Abstract—Bioassays with a variety of overripe fruits, including mango, plum, pear, and grape, and their extracts showed that odors from overripe mango were most attractive to adult vinegar flies, *Drosophila melanogaster*. Combined gas chromatography–electroantennographic detection (GC-EAD) analyses of solid-phase microextraction (SPME) and Tenax extracts of overripe mango odors showed that several volatile compounds, including ethanol, acetic acid, amyl acetate, 2-phenylethanol, and phenylethyl acetate elicited significant EAG responses from antennae of female flies. Most of the volatile compounds in the extracts were identified by mass spectral and retention index comparisons with synthetic standards. In cage bioassays, lures with a blend of ethanol, acetic acid, and 2-phenylethanol in a ratio of 1:22:5 attracted six times more flies than any single EAG-active compound. This blend also attracted four times more flies than traps baited with overripe mango or unripe mango. However, in field trials, the blend was not as attractive as suggested by the laboratory bioassay.

Key Words—Overripe fruit, mango odors, SPME, GC-EAD, behavioral response, vinegar fruit fly, *Drosophila melanogaster*.

INTRODUCTION

Although the vinegar fly, *Drosophila melanogaster*, has never been considered a serious pest, it is a nuisance in households and in commercial food marketing and handling areas. Volatile chemicals associated with fermentation, such as ethanol, acetic acid, ethyl acetate, and acetaldehyde, either as single components or in mixtures, previously have been reported to attract several *Drosophila* species (Barrows, 1907; Hunter et al., 1937; West 1961). However, there have been no

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recent studies using contemporary analytical chemistry techniques to analyze fruit sources for further attractants for *D. melanogaster*.

In the present study, we report on the attraction of *D. melanogaster* to various overripe fruit sources in cage bioassays; the identification of volatiles from overripe mango that elicited electroantennographic responses from antennae of *D. melanogaster*; and the results of bioassays testing the attractiveness of a formulated blend containing those active components to *D. melanogaster*.

METHODS AND MATERIALS

Insects. Vinegar fruit flies, *Drosophila melanogaster* (wild type, Oregon), were obtained from a laboratory colony maintained at the Fruit Fly Genetics Laboratory, Iowa State University, Ames, Iowa. Adult flies were provided with a standard corn meal–agar diet containing hydrolyzed protein (ISU-Lephardt) and water until they were used in experiments.

Fruit Volatiles Collection. Six different types of overripe fruit, including mango, strawberry, banana, grape, pear, and plum, were collected from fruit sorting areas of three local grocery stores. Approximately 250 g of each overripe fruit was placed in glass jars (1 liter) for odor analysis. For fruit volatile collection, activated-charcoal-purified air was blown at 200 ml/min through a glass jar containing one type of overripe fruit, and the odors collected on a Tenax trap connected to the outlet. The trap consisted of a Pasteur pipet (5 cm long × 0.5 cm diam.) packed with 300 mg of precleaned Tenax (20–35 mesh, Alltech, Deerfield, Illinois, USA) held in place by glass wool plugs. Volatiles were continuously collected for 1–2 days, eluted with 2 ml of HPLC-grade hexane (Burdick & Jackson High Purity), and concentrated to 200 μl under a gentle nitrogen stream. Extracts (2 μl) were injected for analysis with either coupled gas chromatography–electroantennographic detection (GC-EAD) or gas chromatography-mass spectrometry (GC-MS).

For solid-phase microextraction (SPME), a SPME fiber (100 μm polydimethylsiloxane, Supelco, Bellefonte, Pennsylvania, USA) was preconditioned for 1 hr at 250°C. During collections, the fiber was exposed approx. 2–3 cm above the overripe fruit in the glass jar for ca. 12 hr at room temperature, which gave sufficient time for equilibration of all volatiles. The loaded SPME fiber was then desorbed in the injection port of either a GC-EAD or a GC-MS system.

Chemical Analyses. For GC-EAD analysis, Hewlett-Packard (HP) 5890 Series II gas chromatograph equipped with a DB-5 column (30 m × 0.25 mm ID, J & W Scientific, Folsom, California, USA), and a 50:50 effluent split allowed simultaneous flame ionization (FID) and EAD of the separated fruit odors. Helium was used as the carrier gas with a flow rate of approximately 30 ml/min for both FID and EAD. Extracts were injected in splitless mode, injector temperature 250°C, and split valve delay 1 min. The temperature program was 5°C/min, then 15°C/min to 250°C. The outlet for the EAD was continuously supplied with a

purified, moisturized airstream flowing over the antennal preparation at 0.5 m/sec. A fruit fly was restrained with thin copper wires (32 gauge) for EAD recordings. An Ag–AgCl glass capillary recording electrode filled with saline (0.1 M KCl) was placed in contact with the distal segment of the antenna. The ground electrode, filled with the same solution, was placed in the eye. The EAD amplifier (a high-impedance DC amplifier with automatic baseline drift compensation) was used (Syntech, Hilversum, The Netherlands). A GC-EAD program (Syntech version 2.3) was used to record and analyze the amplified EAD and FID signals on a PC computer.

GC-MS analyses of the fruit volatiles were performed with an H-P 5890 Series II GC interfaced to a HP 5972 Mass Selective Detector (MSD). The GC-MS was equipped with either a DB-5 column (identical to the one used in the GC-EAD system described above) or a Carbowax column (30 m × 0.25 mm ID, J & W Scientific). The temperature program was the same as that described for the GC-EAD analyses, except the maximum temperature for analyses using the Carbowax column was set at 230°C. Mass spectra were recorded from 30 to 550 amu with electronic impact ionization at 70 eV. Identifications of fruit volatiles were confirmed by comparison of retention indices and mass spectra with those of authentic standards.

Chemicals. Most synthetic standards were purchased from Sigma/Aldrich (St. Louis, Missouri, USA). Purities ranged from 98% to 99.5%. α-Copaene was purchased from Fluka with 95% chemical purity.

Cage Bioassays. Trapping tests were conducted in screen cages (90 × 48 × 36 cm) either in the greenhouse or in the laboratory under conditions of room temperature (23°C ± 3°C) and daylight. About 150 mixed-sex, mixed-age flies (age differences not more than 2 days) were released into a cage containing randomly placed traps with different treatments. Traps were constructed from 18 ml clear plastic cups (Fill-Rite Inc., Newark, New Jersey, USA) covered by a white paper lid with a hole (2.5 mm diam.) drilled at the center. For experiments involving extracts or synthetic compounds, a medical cotton wick (2 cm long) was used as a dispenser. Our standard blend was comprised of 100 mg of ethanol, acetic acid, and 2-phenylethanol (in a ratio of 1:22:5) in 1 ml of water (water was critically important for vinegar fly attraction; no flies were attracted to traps loaded with chemicals alone) and was placed on the bottom of the cup. Compounds that were not water soluble were loaded onto a cotton wick and placed in a treatment cup with a second wick dosed with 1 ml of water. Controls contained only water. For the trapping tests involving a variety of overripe fruits, approx. 5 g of each fruit was used for each test. The ratio of the naturally emitted blend from mango was determined by SPME analyses of the attractive traps containing mango ($N = 3$). For testing volatiles collected on Tenax, we used approx. 50 μl of extract (out of a total volume of 200 μl) on the wick in each trap, with hexane (50 μl) for a control. Wicks were allowed to dry for 30 min before being used. For the experiment testing

the attractiveness of the synthetic blend versus overripe and unripe mango, traps were baited with 100 mg of the synthetic blend (in 1 ml water) or 5 g of chopped overripe and unripe mango. The control used only water. In the dose–response test, serial dilutions of the synthetic blend were made in water at doses ranging from 0.1 to 100 mg (total amount of three components). Treatments were tested in a randomized complete-block design. The numbers of flies caught in traps were counted 8 hr after release for each experiment. Experiments were usually conducted from 9:00 AM to 5:00 PM daily. Trap positions were rerandomized every 2–3 hr to minimize positional effects. Each experiment was replicated 3–5 times.

RESULTS AND DISCUSSION

Attraction of D. melanogaster to Overripe Fruits. Traps baited with overripe fruits were attractive to *D. melanogaster* in cage bioassays conducted in a greenhouse. Traps baited with mango caught significantly more flies than other treatments. No flies were caught in the control traps (Figure 1). A second bioassay using Tenax extracts of overripe fruits showed similar results, with traps baited with mango extracts catching significantly more flies than those baited with strawberry, plum, and grape extracts, all of which were not significantly different than the control (Figure 2).

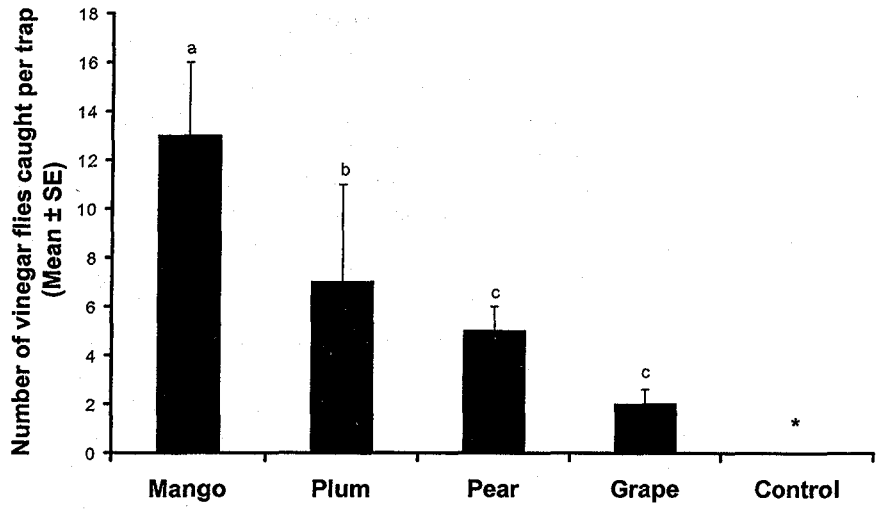

FIG. 1. Mean catches of *Drosophila melanogaster* in traps baited with a variety of overripe fruits. Different letters on top of bars indicate significant differences ($N = 3$, ANOVA followed by Fisher test, $P < 0.05$). *Control traps caught no flies and were not included in the ANOVA.

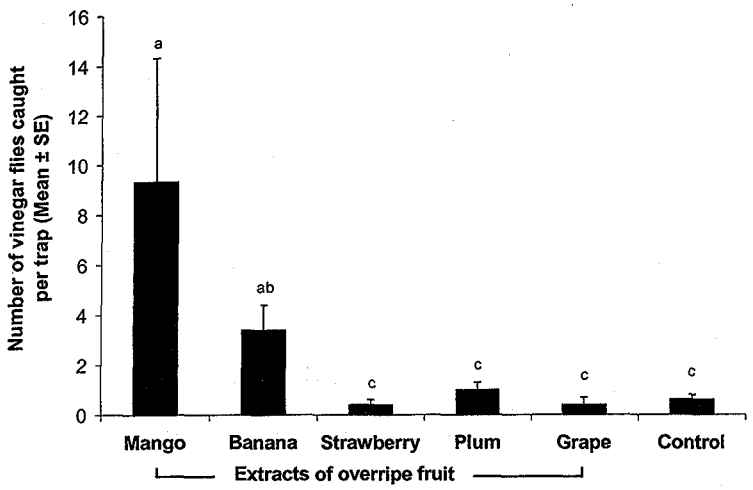

FIG. 2. Mean catches of *Drosophila melanogaster* in traps baited with solvent extracts of different overripe fruits. Different letters on top of bars indicate significant differences ($N = 5$, ANOVA followed by Fisher test, $P < 0.05$).

Analysis and Bioassay of Volatiles from Overripe Mango. GC traces of SPME or Tenax extracts of overripe mango showed more than 20 compounds in extracts, 13 of which were identified (Figures 3 and 4). These consisted primarily of monoterpene and sesquiterpene hydrocarbons (Table 1) that are considered important volatile compounds contributing to mango flavor (MacLeod and Snyder, 1985; Sakho et al., 1985; Bartley and Schwede, 1987; Winterhalt, 1991). Among them, 3-carene was consistently the most abundant volatile present, accounting for more than 75% of total terpene hydrocarbons. Another typical mangolike odor, α-copaene, found in trace amounts, has also been recorded as a minor component from a number of fruits, including citrus, guava, litchi, and peach (MacLeod and de Troconis, 1982; MacLeod et al., 1988; McInnis and Warthen, 1988).

Among the other aliphatic and aromatic hydrocarbons present in overripe mango odor, 2-phenylethanol is a common floral volatile of most roses, and various tropical fruits including mango (Wong and Siew, 1994; Ollé et al., 1998; Boulanger and Crouzet, 2001). This aromatic alcohol may be a product of fermentation of overripe fruits, because it has been identified from various fermentation products such as wine and cider (Fischer et al., 2000; Picinelli et al., 2000). Phenylethyl acetate has not been reported previously from mango.

Characterization of fruit aroma can be problematic because certain fruit odors are highly volatile and may be lost during extraction or can co-elute with the solvent used for extraction during GC analysis. Solid-phase microextraction (SPME) (Zhang and Pawliszyn, 1993) to some extent circumvents these problems and can

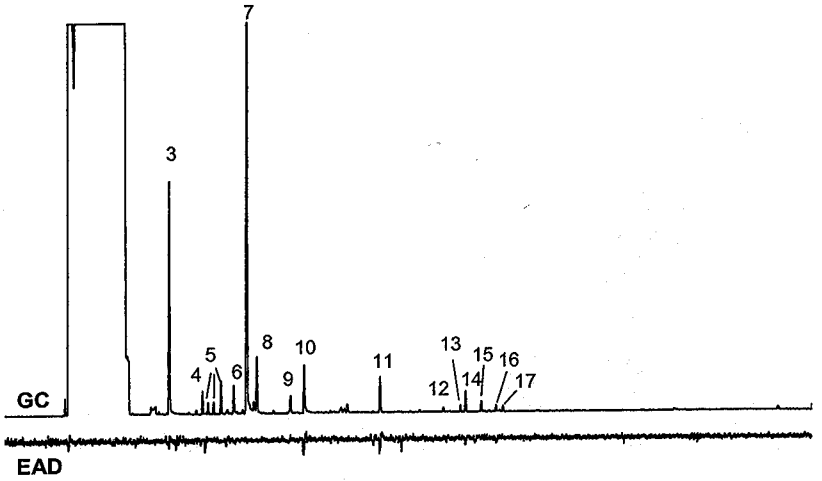

FIG. 3. GC-EAD analysis of overripe mango extract using an antenna from a female *Drosophila melanogaster*. Thirteen compounds were identified after GC-MS analysis. Number on each peak refers to compounds listed in Table 1.

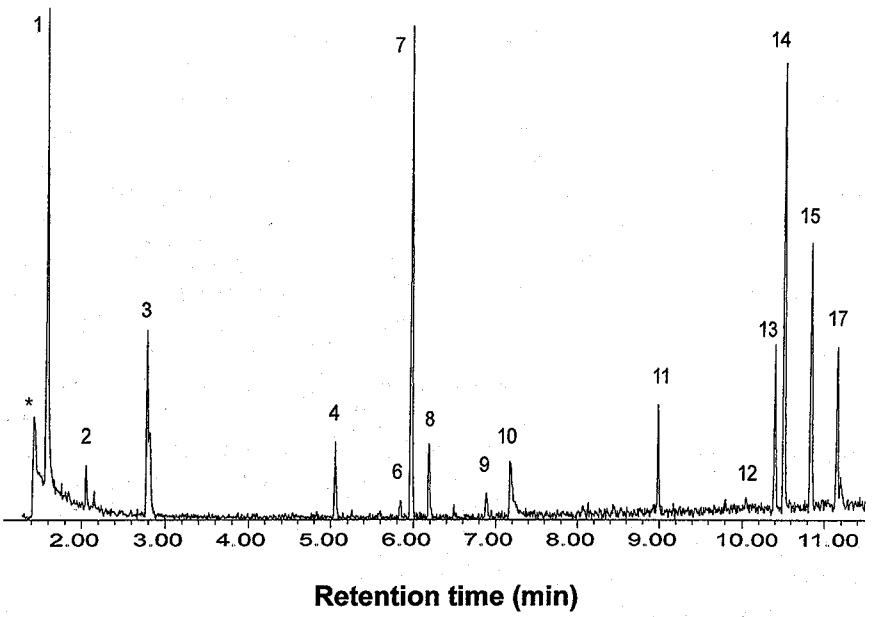

FIG. 4. Chromatogram of SPME headspace collection of overripe mango. Numbered peaks refer compounds listed in Table 1. *The compound was tentatively identified as acetaldehyde.

TABLE 1. VOLATILES IDENTIFIED FROM OVERRIPE MANGO WITH TENAX AND SPME COLLECTION AND THEIR EAG ACTIVITY

Peak	Volatile	Tenax extract	SPME collection	EAG
1	Ethanol	[a]	+	+++
2	Acetic acid	[a]	+	++
3	Amyl acetate	+	+	++
4	α-Pinene	−	+	−
5	unknowns	+	−	−
6	β-Myrcene	+	+	−
7	3-Carene	+	+	−
8	dl-Limonene	+	−	−
9	Terpinolene	+	+	−
10	2-Phenylethanol	+	+	+++
11	Phenylethyl acetate	+	+	++
12	α-Copaene	+	−	−
13	Sesquiterpene	−	+	−
14	*E*-Caryophyllene	+	+	+
15	Sesquiterpene	+	+	−
16	Sesquiterpene	+	+	−
17	Butylated hydroxytoluene (contaminant)	+	−	−

[a] Coelutes with the solvent peak.

be used to check for additional volatiles not collected by Tenax. SPME analysis of overripe mango revealed three fermentation volatiles, acetaldehyde, ethanol, and acetic acid that may have been hidden by the solvent peak of the Tenax extract in GC analyses or which were not efficiently trapped by the Tenax. Acetaldehyde, another volatile commonly associated with fermentation, also was tentatively detected from overripe mango at a low level (Figure 4).

In GC-EAD analyses, the two strongest EAD responses were elicited by 2-phenylethanol and phenylethyl acetate. In separate EAG trials with synthetic standards, ethanol and acetic acid also elicited strong EAG responses from antennae of *D. melanogaster* (Table 1).

In bioassays using synthetic standards, all compounds were tested as aqueous solutions, or with a source of water in the treatment cup, because responses were inhibited in the absence of water. Adult *D. melanogaster* were maximally attracted to an aqueous solution of three EAD active compounds, ethanol, acetic acid, and 2-phenylethanol, at their naturally emitted ratio of 1:22:5 (Figure 5). Aqueous solutions of ethanol and acetic acid were more attractive than the other single components, all of which were significantly more attractive than the controls. The attraction of *D. melanogaster* to ethanol was reported previously by Reed (1938) and West (1961), at dosages similar to those tested in the present study. Barrows (1907) showed that another *Drosophila* species, *D. ampelophila* was attracted to

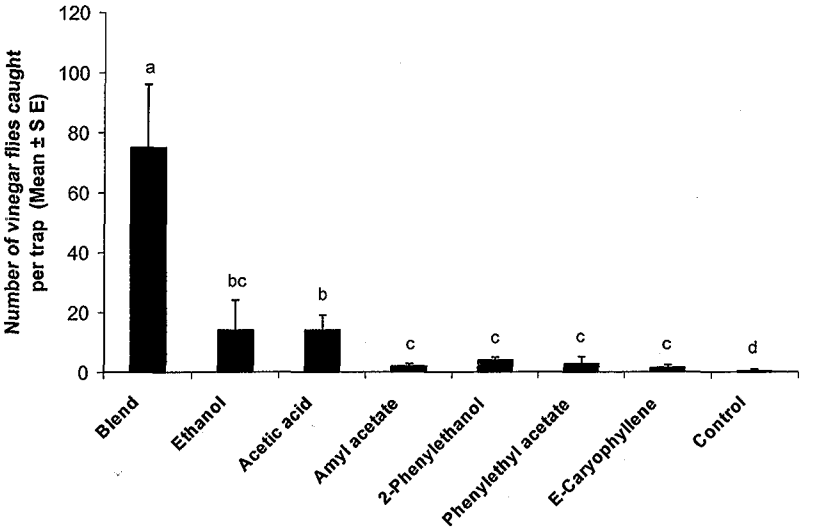

FIG. 5. Mean catches of *Drosophila melanogaster* in traps baited with standards of EAG-active compounds and the reconstructed blend identified from overripe mango. Different letters on top of bars indicate significant differences ($N = 5$, ANOVA followed by Fisher test, $P < 0.05$).

a mixture of ethanol and acetic acid. In Barrows' study, only 8% of released flies were caught, whereas our aqueous synthetic blend attracted over 70% of the flies released inside the cage, while also competing with the other treatments. The three compounds are generally considered to be fermentation-related products, although ethanol has been reported from fresh mango (TNO, 1976). 2-Phenylethanol has been reported to be attractive to several other insect species, such as cabbage looper moths (Haynes et al., 1991), pineapple beetles (Zilkowski et al., 1999), lady beetles (Nout and Bartelt, 1998), and green lacewings (Zhu et al., 1999).

The efficacy of the synthetic blend as an attractant was compared to volatiles from chopped overripe and unripe mango in a cage bioassay. Although more flies were caught in traps baited with aqueous formulations of the synthetic blend (Figure 6) than in mango-baited traps, these results must be treated with caution because the comparative release rates of volatiles from the mango baits and the synthetic lure were not determined. A subsequent dose–response test with the synthetic blend demonstrated that the highest dose tested (100 mg) was most attractive to *D. melanogaster* (Figure 7).

Although we have demonstrated the efficacy of this blend in capturing *D. melanogaster* in cage bioassays, its attraction was not as great as expected when tested under more natural conditions. Traps set on fruit shelves at a grocery store caught only 30% of the available flies, based on a 5-day observation period. Further

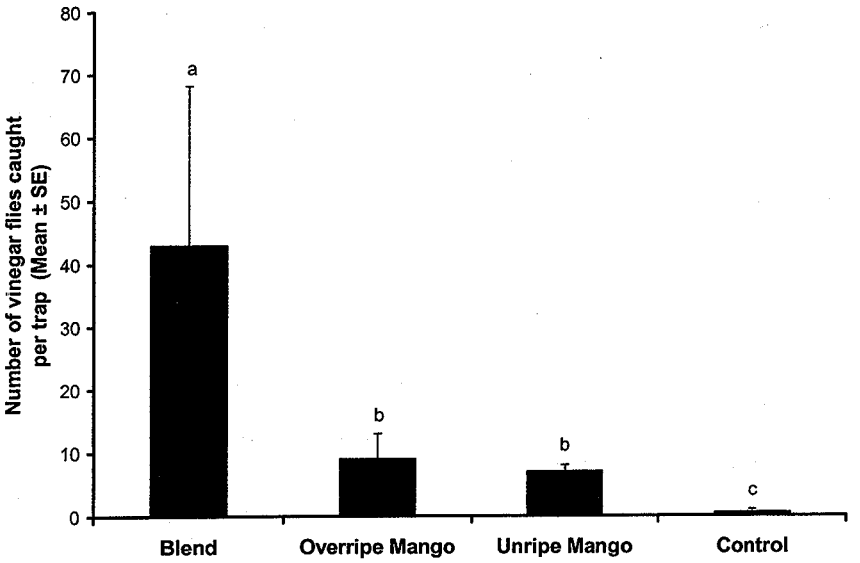

FIG. 6. Mean catches of *Drosophila melanogaster* in traps baited with synthetic mango blend, and chopped overripe or unripe mango. Different letters on top of bars indicate significant differences ($N = 3$, ANOVA followed by Fisher test, $P < 0.05$).

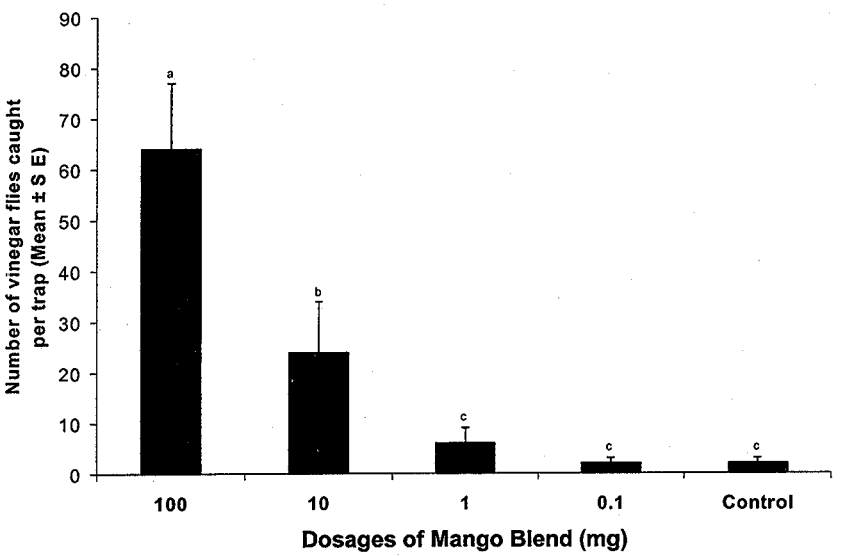

FIG. 7. Mean catches of *Drosophila melanogaster* in traps baited with different doses of the synthetic blend of overripe mango volatiles. Different letters on top of bars indicate significant differences ($N = 5$, ANOVA followed by Fisher test, $P < 0.05$).

development of this blend by the addition of synergistic compounds from other fruit sources is in progress.

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