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# GRAPEFRUIT OIL ENHANCES ATTRACTION OF MEXICAN FRUIT FLIES TO A SYNTHETIC FOOD-ODOR LURE

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**Abstract**—We investigated the attractiveness of grapefruit oil to the Mexican fruit fly. Only high concentrations were attractive in laboratory wind-tunnel bioassays. Attraction of flies to grapefruit oil was not enhanced if they had previous experience with grapefruit. In citrus orchard experiments, undiluted grapefruit oil attracted Mexican fruit flies and enhanced attraction to traps baited with a synthetic food-odor lure emitting ammonia and other nitrogenous chemicals. This is the first demonstration of host fruit odor increasing attraction to another type of attractive blend in Mexican fruit fly. These results indicate differences in the way the flies respond to undiluted grapefruit oil compared with previously tested fruit odors.

**Key Words** V Diptera, Tephritidae, *Anastrepha ludens*, attractant, grapefruit oil.

## INTRODUCTION

It is well known that host plant odors are generally attractive to phytophagous insects. Further, host plant odors often enhance attraction to pheromone in Lepidoptera, Coleoptera, and Diptera (Landolt and Phillips, 1997). In Tephritidae, host fruit odors were commonly demonstrated to be attractive for oviposition, feeding, and mating (Fletcher and Prokopy, 1991; Landolt et al., 1992; Jang and Light, 1996). Additive or synergistic effects from combining host or plant odors with pheromones or bacteria odors were reported for several

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tephritids including Mediterranean fruit fly (*Ceratitis capitata*) (Dickens et al., 1990), papaya fruit fly (*Toxotrypana curvicauda*) (Landolt et al., 1992), and apple maggot (*Rhagoletis pomonella*) (MacCollom et al., 1994). Combinations of host or plant odors with other types of attractive blends sometimes were less attractive than expected in Tephritidae. For example, traps containing both host fruit and Nulure baits were less attractive than traps with only host fruit bait to protein-fed female Oriental fruit flies (*Bactrocera dorsalis*) (Cornelius et al., 2000).

Combinations of fruit odors with other types of attractants have always been less attractive than the more attractive of the two lure types in the Mexican fruit fly (*Anastrepha ludens* Loew) (Diptera: Tephritidae). Robacker and Garcia (1990) reported that fermented-fruit odor decreased attraction of sexually active females to pheromone, and that pheromone decreased attraction of hungry females to the fruit odor. Robacker (1991) showed that combinations of fermented-fruit chemicals with bacterial odor were less attractive to sugar-hungry flies than fruit chemicals alone. Finally, Robacker and Heath (1997) found that fermented-fruit chemicals decreased attraction to a mixture of ammonia, methylamine, and putrescine (AMPu) tested in a citrus orchard.

In this work, we tested grapefruit oil in laboratory wind-tunnel bioassays to determine the most attractive concentration and to investigate whether attraction of flies to the oil would increase after flies were exposed to grapefruit. Two experiments were conducted in citrus orchards to measure the attractiveness of grapefruit oil by itself and in combination with a synthetic food-odor lure containing ammonia, methylamine, and putrescine.

## METHODS AND MATERIALS

*Insects and Test Conditions.* Mexican fruit flies were obtained from a culture that originated from yellow chapote fruit (*Casimiroa greggii*), a native citrus host of the fly, collected in Nuevo Leon, Mexico, in 2000. Laboratory conditions for holding and testing flies were 22 ± 2°C, 50 ± 20% relative humidity, and photophase from 06:30 to 19:30 hr. Laboratory tests were conducted between 09:00 and 16:00 hr using 3- to 13-d-old flies.

*Experiments 1A and 1B: Wind-Tunnel Bioassay of Grapefruit Oil Dose*Y*Response Relationship.* The purpose of these experiments was to relate test quantity of grapefruit oil to attractiveness. Grapefruit oil (product code 7553, no lot numbers given by the manufacturer) was obtained from Now Foods (Bloomingdale, IL, USA). Grapefruit oil was tested undiluted, and as serial 1 to 10 dilutions down to 0.01% oil in hexane.

Bioassays were conducted in a Plexiglas wind tunnel with the dimensions of 0.3 × 0.3 × 1.2 m. Each end of the wind tunnel was screened to allow

airflow. The downwind end contained a baffle system to create a uniform airflow through the chamber. Air was pulled through the chamber at 0.4 m/sec by an exhaust fan connected to the downwind end. Air exiting the chamber was directed into an exhaust hose and removed to the outdoors. In addition to the direct exhaust from the wind tunnel, this room contained inlet and outlet vents to bring new air into the room from outdoors and remove air from the room to the outdoors. Air entered and exited the room at the rate of eight complete air replacements per hour. The top of the chamber had two circular openings (12.8 cm diam) with Plexiglas covers, located at each end of the chamber, to allow easy access to the chamber's interior. A 75-W "soft white^ light bulb (General Electric Co., Cleveland, OH, USA) in a reflecting lamp was positioned 17 cm above the downwind end of the chamber. The purpose of this light was to minimize random flying into the upwind end of the chamber by using the flies' positive phototactic reaction. Overhead lighting was provided by fluorescent "cool white^ lights (F40CW, General Electric).

Bioassays were conducted by using flies of two feeding regimes: (1) sugar-fed, protein-starved (from eclosion) (Experiment 1A) and (2) sugar-starved (for 2 d), protein-starved (Experiment 1B). Mixed-sex groups of 25Y40 flies (overall sex ratio was 1:1 but individual cartons varied) were kept in 473-ml cardboard cartons with screen tops until used in tests. To conduct a trial, 100 μl of sample (containing various amounts of grapefruit oil) were put onto a 3 × 3 cm piece of filter paper that was attached to the upwind side (not visible to approaching flies) of a yellow plastic card (10.5 × 15.5 cm) suspended in the upwind end of the wind tunnel. One carton of flies was placed under the downwind opening. Flies were given 5 min to leave the carton, fly or walk upwind, and contact the yellow card. Upwind movement was scored if flies passed a point two-thirds of the distance from the release carton to the card. Seven replications of each experiment were conducted.

*Experiment 2: Wind-Tunnel Bioassay of Effect of Experience with Grapefruit on Attraction to Grapefruit Oil.* The purpose of this experiment was to determine if attraction of flies to grapefruit oil would increase if the flies had previous experience with grapefruit. The grapefruit oil lure used in this experiment was a 4-ml glass vial containing 1 ml of undiluted grapefruit oil with a cotton wick. The lure was fastened to the upwind side of a yellow plastic card. A plastic card without a lure was used as control for testing both naïve and grapefruit-experienced flies. Bioassays were conducted as in Experiments 1A and 1B.

Test flies were from two groups: naïve flies and grapefruit-experienced flies. Both groups were held in Plexiglas cages (20.5 × 20.5 × 20.5 cm) (200 flies per cage) with screen tops containing a diet mixture of sugar and yeast hydrolysate (U. S. Biochemical, Cleveland, OH, USA) and water supplied separately. Half of the cages were provisioned with a grapefruit (*Citrus paradisi*,

variety Rio Red) from a local orchard beginning when flies eclosed. Grapefruits had a 2-cm-diam piece of peel removed to enhance the release of peel volatiles. All cages were kept in the same laboratory where bioassays were performed, but those with grapefruits were kept near an exhaust fan to vent grapefruit odor to outdoors. Test flies were older than 4 d to allow at least several days for flies to learn the odor of grapefruit. The four treatments (2 fly types × 2 lure treatments) were tested in random order in each replication. Thirteen replications of the experiment were conducted.

*Experiments 3A and 3B. Citrus Orchard Evaluation of Grapefruit Oil, Anastrepha Lure, and their Combination.* The purpose of these experiments was to determine the attractiveness of grapefruit oil and its combination with nitrogenous food odors in the field. The grapefruit oil lure used in these experiments was a 4-ml glass vial containing 1 ml of undiluted grapefruit oil with a cotton wick. *Anastrepha* fruit fly lures obtained from IPM Tech (Portland, OR, USA) were used as synthetic food odor lure. These lures contain ammonia, methylamine, and putrescine, and are based on an attractant for the Mexican fruit fly reported by Robacker and Warfield (1993).

The experiments were conducted in two citrus orchards located near the laboratory in Weslaco, TX, USA. The first orchard contained several varieties of oranges, lemons, and tangerines. One row of Valencia sweet oranges (*Citrus sinensis*) and one row of Dancy tangerines (*C. reticulata*) were used for tests. The other orchard contained only Rio Red grapefruit from which two rows were used for experiments. Within each row, four linear blocks of four trees each were chosen with one buffer tree between each block. Testing was done during autumn of 2003 in the first orchard and winterŸspring of 2004 in the second. All trees were laden with nearly ripe or ripe fruit initially, but fruits were stripped from the grapefruit orchard with three to four replications remaining during spring testing.

Four lure treatments were tested in each experiment: grapefruit oil, *Anastrepha* lure, their combination, and no lure. *Anastrepha* lures were used for the duration of the autumn replications, then replaced with new lures that were used for the duration of the winterŸspring replications. Grapefruit oil lures were replaced weekly. One each of the four treatments was tested in each block. Positions of treatments within each block were randomized for the first test of each series. A trial lasted 1 wk, after which flies were counted and traps were serviced as necessary. Positions of treatments in consecutive trials were not randomized but were moved sequentially within each block. Traps were hung one to a tree, north of center, at 1Ÿ2 m height. Each week, approximately 2000 flies were distributed uniformly onto rows of trees adjacent to the test rows. Mixed-sex groups of 180Ÿ200 flies were kept in 473-ml cardboard cartons under laboratory conditions (described above) until released in the test orchard. Flies were irradiated, due to quarantine laws, with 70Ÿ92 Gy (Cobalt 60) 1Ÿ2 d

before adult eclosion. Flies were fed sugar and water until they were released in test plots 3Y12 d after eclosion.

Experiment 3A was a test of the lures on Intercept AM traps (IPM Tech). Intercept traps are yellow cardboard rectangles (14 × 23 cm) coated with Stickem Special (Seabright Laboratories, Emeryville, CA, USA) on both sides. Lures were fastened onto trap hangers above traps such that the bottom of the lure contacted the top of the trap. Traps were replaced each week. Seventeen replications (weeks) were conducted; eight during autumn in the tangerines section of the first orchard, and nine during winterYspring in grapefruits of the second orchard.

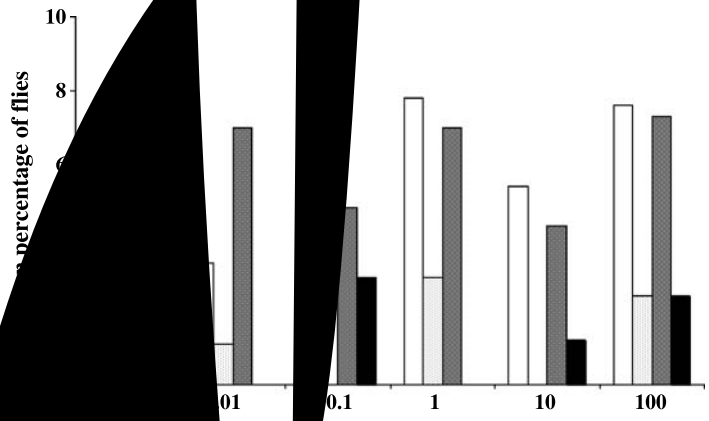
Experiment 3B was a test of the lures in yellow-bottom Multilure traps (Florence Agri Investment, Inc., Miami, FL, USA). Multilure traps are plastic McPhail-like traps with a clear, colorless top and an opaque, colored bottom that serves as a liquid reservoir for drowning captured flies. Grapefruit oil lures were taped inside the tops. *Anastrepha* lures were modified by removing the two plastic bags containing the attractants from the factory supplied mesh bag. The two plastic bags were inserted into the lure basket of the multilure traps. Traps contained water with 0.01% Triton®. (Rohm and Haas Co., Philadelphia, PA, USA) as a wetting agent. The no-lure trap contained only water and Triton. Water was replaced each week in all traps. Fourteen replications (weeks) were conducted; six during autumn in the oranges section of the first orchard, and eight during winterYspring in grapefruits of the second orchard.

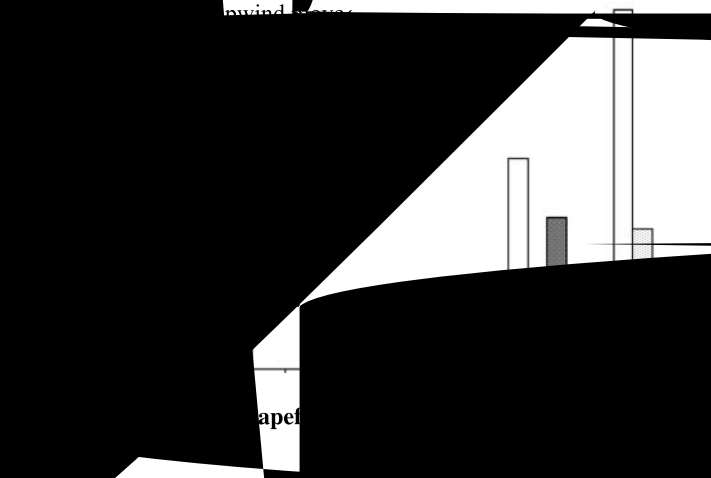
*Statistical Analyses.* All experiments were conducted as randomized complete blocks. For wind-tunnel data, the numbers of males and females that moved upwind or landed on the odor source were divided by the total numbers of males or females in the carton used in the trial. These proportions were transformed by arcsin of the square root for data analyses (Snedecor and Cochran, 1967). Proportions of 0 were replaced with $1/4N$ before transformation ($N$ is the number of males or females in the carton). For field tests, replications over time were treated like replications over space (blocks of trees) for the purpose of statistical analyses. Transformed percentages from the wind tunnel bioassays, and the numbers of flies captured on traps in field tests, were subjected to analysis of variance using SuperANOVA (Abacus Concepts, 1989). Means separations were done by Fisher's protected least significant difference method (LSD) (Snedecor and Cochran, 1967).

## RESULTS

*Experiments 1A and 1B: Wind-Tunnel Bioassay of Grapefruit Oil DoseYResponse Relationship.* Sugar-fed flies were not attracted to grapefruit

ny test quantity ( A). Sugar-starved males were attracted to the ghest test quantit apef t oil but not to any lower quantities d movement: $F =$ $P < 0.001$; landings: $F = 4.3$; $df = 5, 44$; .01) (Figure 1B). -star emales showed a similar trend but no dual test quantities re sign tly more attractive than blanks or other ities (Figure 1B). However, -*df* contrasts of the means for the two



highest test quantities vs. the blank and lower quantities were significant (upwind movement: $F = 5.6$; $df = 1, 44$; $P < 0.05$; landings: $F = 7.8$; $df = 1, 44$; $P < 0.01$).

*Experiment 2: Wind-Tunnel Bioassay of Effect of Experience with Grapefruit on Attraction to Grapefruit Oil.* Experience with grapefruit did not enhance attraction of either male or female flies to grapefruit oil. The treatment effect (for the four test sample × experience treatments) was significant only for upwind movements by males ($F = 2.9$; $df = 3, 36$; $P < 0.05$). However, in this instance, the percentage of naïve males to move upwind toward the blank yellow card (12.3%) was significantly greater than response to either of the grapefruit oil treatments (grapefruit oil/naïve males, 2.7%; grapefruit oil/experienced males, 4.5%) but not significantly greater than response by experienced males to the blank yellow card (7.1%).

*Experiment 3A. Citrus Orchard Evaluation of Grapefruit Oil, Anastrepha Lure, and their Combination on Interceptor Traps.* Results are shown in Table 1. Analysis of the complete dataset indicated that traps with grapefruit oil were not significantly more attractive than unbaited traps. However, when traps with *Anastrepha* lures and those with the combinations were not included, traps with grapefruit oil were significantly more attractive than unbaited traps to both males ($F = 9.5$; $df = 1, 114$; $P < 0.01$) and females ($F = 7.0$; $df = 1, 114$; $P < 0.01$). Traps with both lures were significantly more attractive than those with only *Anastrepha* lures to males but not to females (complete dataset). Traps with either *Anastrepha* lures or the lure combinations were more attractive than unbaited traps and traps with only grapefruit oil to both males and females. Results from the autumn replications did not differ from those from the winterYspring replications. Also, results from winterYspring replications in which trees had ripe fruit did not differ from those in which trees had no fruit.

*Experiment 3B. Citrus Orchard Evaluation of Grapefruit Oil, Anastrepha Lure, and their Combination in Multilure Traps.* Results are shown in Table 2. Traps with grapefruit oil were more attractive than unbaited traps to females.

TABLE 1. ATTRACTION OF MEXICAN FRUIT FLIES TO INTERCEPTOR AM TRAPS BAITED WITH GRAPEFRUIT OIL, IPM TECH *Anastrepha* FRUIT FLY LURES, OR THEIR COMBINATION IN A CITRUS ORCHARD

| | Males | Females | Total |
|---|---|---|---|
| Blank | 0.2 ± 0.05 a | 0.2 ± 0.05 a | 0.4 ± 0.08 a |
| Grapefruit oil | 0.5 ± 0.08 a | 0.6 ± 0.12 a | 1.1 ± 0.17 a |
| *Anastrepha* lure | 1.8 ± 0.22 b | 2.0 ± 0.30 b | 3.8 ± 0.47 b |
| Combination | 2.7 ± 0.41 c | 2.5 ± 0.33 b | 5.2 ± 0.65 c |

Values are mean flies captured per week (±SE). Means in the same column followed by different letters are significantly different at the 5% level by Fisher's protected LSD (males: $F = 29.4$; $df = 3, 24$; $P < 0.001$; females: $F = 29.3$; $df = 3, 34$; $P < 0.001$).

TABLE 2. ATTRACTION OF MEXICAN FRUIT FLIES TO MULTILURE TRAPS BAITED WITH GRAPEFRUIT OIL, IPM TECH *Anastrepha* FRUIT FLY LURES, OR THEIR COMBINATION IN A CITRUS ORCHARD

| | Males | Females | Total |
|---|---|---|---|
| Blank | 0.2 ± 0.08 a | 0.2 ± 0.08 a | 0.5 ± 0.14 a |
| Grapefruit oil | 0.9 ± 0.23 a | 1.2 ± 0.21 b | 2.1 ± 0.36 b |
| *Anastrepha* lure | 2.9 ± 0.38 b | 3.4 ± 0.40 c | 6.3 ± 0.70 c |
| Combination | 3.1 ± 0.41 b | 4.3 ± 0.47 d | 7.4 ± 0.79 c |

Values are mean flies captured per week (±SE). Means in the same column followed by different letters are significantly different at the 5% level by Fisher's protected LSD (males: $F = 29.3$; $df = 3, 21$; $P < 0.001$; females: $F = 42.6$; $df = 3, 21$; $P < 0.001$).

When traps with *Anastrepha* lures and the lure combinations were not included in the analysis, traps with grapefruit oil were also significantly more attractive than unbaited traps to males ($F = 9.4$; $df = 1, 97$; $P < 0.01$). Traps with both lures were significantly more attractive than those with only *Anastrepha* lures to females but not to males (complete dataset). Traps with either *Anastrepha* lures or the lure combinations were more attractive than unbaited traps and traps with only grapefruit oil to both males and females. As in Experiment 5, time of year and presence of fruit did not affect results.

## DISCUSSION

Grapefruit oil was only weakly attractive to male and female Mexican fruit flies in wind-tunnel bioassays and citrus orchard experiments. In contrast, fermented chapote fruit and low concentrations of chemicals from the fermented chapote fruit previously proved highly attractive to Mexican fruit flies in laboratory experiments (Robacker et al., 1990a,b; Robacker, 1991) and in one field test (Robacker and Heath, 1996), although chapote chemicals were not very attractive in another field test (Robacker and Heath, 1997). Considering similarities in experimental protocols in at least some of the work with chapote odor and grapefruit oil, the most prudent conclusion is that grapefruit oil is not very attractive to Mexican fruit flies.

Despite its weak attractiveness, grapefruit oil enhanced attraction of both males and females to *Anastrepha* lures in citrus orchard experiments. All previous experiments in which fruit odors were added to either bacterial odors or to a chemical blend similar to that emitted by the *Anastrepha* lures resulted in negative interactions of the odor types (see Introduction).

Grapefruit oil was not more attractive to grapefruit-experienced than to naïve flies. Previous experiments demonstrated that Mexican fruit flies caged

with peel-damaged grapefruits learned the odor of grapefruits and subsequently increased their attraction to low concentrations of grapefruit peel extracts (Robacker and Fraser, 2005). In the current work, the results suggest that flies that learned grapefruit odor did not recognize grapefruit oil as the same odor. The apparent lack of recognition could be due to quantitative differences (low vs. high concentrations) or qualitative differences. The grapefruit oil used in this work probably was qualitatively different from the odor of Rio Red grapefruit to which flies were exposed. The oil was purchased from Now Foods and most likely was not derived from Rio Red grapefruit. However, exposure to grapefruits increased attraction to oranges in a previous work (Robacker and Fraser, 2003) even though the odor of oranges is noticeably different from that of grapefruit.

The nature of the attraction response to grapefruit oil is not known at this time but it appears to be different from the nature of responses of Mexican fruit flies to fruit odors tested in earlier work. To summarize the evidence for this assertion: (1) attraction to grapefruit oil was weak compared with attraction to fermented chapote odor; (2) grapefruit oil enhanced attraction to a blend of attractive nitrogenous chemicals but a combination of chapote-odor chemicals inhibited attraction to the same blend of nitrogenous chemicals; and (3) attraction to grapefruit oil did not increase after exposure to grapefruit whereas attraction to a low concentration of grapefruit-peel extract did increase after exposure to grapefruit.

Although the nature of the attraction is not known, it is interesting that the origin of the attraction is a plant essential oil. The "parapheromone^ methyl eugenol, a powerful attractant to males of numerous *Bactrocera* species, was discovered as a minor constituent of citronella oil, another plant essential oil (Howlett, 1915). As recently as 15 years ago, parapheromones such as methyl eugenol were considered by some as "one of the great mysteries of tephritid biology^ (Cunningham, 1989). The physiological basis of the attraction to methyl eugenol was not unraveled until the last decade led in part by the efforts of Shelly, Tan, and Nishida (Shelly and Dewire, 1994; Tan and Nishida, 1996). What seemed mysterious attraction just 15 years ago now has been explained as attraction of males to a chemical that serves both as a precursor to a pheromone that attracts females and as an allomone that deters predation (Tan and Nishida, 1998). While the nature of the attraction of Mexican fruit flies to grapefruit oil is very different from the attraction of *Bactrocera* to parapheromones, the possibility exists that the ultimate source of the attractiveness may be a minor component of the oil that may be highly attractive at greater concentrations.

Also relevant to our work is the finding that female papaya fruit flies respond to papaya odor differently depending upon the odor concentration and their mating status (Landolt et al., 1992). Papaya odor, mixed with a constant amount

of pheromone, was most attractive to unmated females at a relatively low concentration but most attractive to mated females at the highest concentration tested. These results suggest the physiological basis of attraction of unmated and mated females to papaya may differ. A similar phenomenon could be occurring in Mexican fruit fly in that attraction to high concentrations of grapefruit odor may have a different physiological basis than attraction to low ones.

We propose three tentative explanations for the results reported here. First, attraction to undiluted grapefruit oil may be an aberrant behavior caused by overloading receptors that normally function to receive lower concentrations of odorants. Second, a minor component of grapefruit oil that has an unknown role in the ecology of Mexican fruit flies may be responsible for the unusual attraction properties. Finally, it is possible that an as yet undiscovered physiological/motivational state may exist in Mexican fruit fly (and other Tephritidae) that causes attraction to fruit odors at high concentrations.

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