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Authors: Massa, Michelle J., Robacker, David C., and Patt, Joseph

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# IDENTIFICATION OF GRAPE JUICE AROMA VOLATILES AND ATTRACTIVENESS TO THE MEXICAN FRUIT FLY (DIPTERA: TEPHRITIDAE)

MICHELLE J. MASSA[1], DAVID C. ROBACKER[2] AND JOSEPH PATT[3]
[1]University of Texas-Pan American, Department of Biology, 1201 W. University Drive, Edinburg, TX 78539

[2]Crop Quality and Fruit Insects Research, USDA, Agricultural Research Service, Kika de la Garza Suptropical Agricultural Research Center, 2413 E. Highway 83, Building 200, Weslaco, TX 78596 USA

[3]Beneficial Insects Research, USDA, Agricultural Research Service, Kika de la Garza Suptropical Agricultural Research Center, 2413 E. Highway 83, Building 201, Weslaco, TX 78596 USA

ABSTRACT

Volatiles from a Concord grape juice produced in Mexico were identified, tested for attractiveness, and mixed into an attractive blend. Volatiles were sampled with solid phase microextraction (SPME). Chemicals were analyzed by gas chromatography and identified by mass spectrometry (GC-MS). Identified chemicals were ethanol, ethyl propionate, ethyl butyrate, ethyl 2-methylbutyrate, ethyl decanoate, ethyl dodecanoate, D-limonene, sorbic acid, benzoic acid, methyl anthranilate, and dimethyl anthranilate. Chemicals were tested at 2 concentrations, 1 µg and 100 ng, for attractiveness to Mexican fruit flies (*Anastrepha ludens*) in laboratory cage-top bioassays. All test chemicals except sorbic acid were attractive to either sugar-fed or sugar-starved flies over both concentrations tested. A nine-component synthetic grape essence mixture was developed that matched the headspace volatiles profile of the grape juice. Attractiveness of the mixture was equal to that of the grape juice in laboratory bioassays. The mixture was 70% as attractive as the juice in traps in field tests. Results demonstrate that most of the attractive principals of the juice were identified.

Key Words: *Anastrepha ludens*, attractants, lures, grape, juice

RESUMEN

Volatiles de jugo de uva Concord producido en México fueron identificados, evaluados como atrayentes y conjuntados en una mezcla. Los volatiles fueron obtenidos mediante una microextraccion de fase solida (SPME). Los químicos fueron analizados por cromatografía de gases e identificados por espectrometria de masa (GC-MS). Los químicos identificados fueron etanol, propionato de etilo, butirato de etilo, etil 2-metilbutirato, etil decanoato, etil dodecanoato, D-limoneno, acido sorbico, acido benzoico, metil antranilato, y dimetil antranilato. Los químicos fueron evaluados a dos concentraciones, 1 µg y 100 ng como atrayentes para la mosca mexicana de la fruta (*Anastrepha ludens)* en ensayos de laboratorio utilizando jaulas. Todos los químicos excepto acido sorbico ejercieron atracción de moscas alimentadas con azucar o no, en las dos concentraciones usadas. Se desarrollo una mezcla sintetica de esencia de uva formada por nueve químicos, semejante a los volatiles encontrados en el jugo de uva. La atracción ejercida por esta mezcla sintetica fue igual a la del jugo de uva en ensayos de laboratorio. La mezcla fue 70% atractiva en comparación con el jugo de uva en ensayos de campo. Los resultados demuestran que la mayoria de los principales atrayentes del jugo de uva fueron identificados.

Translation provided by the authors.

The Mexican fruit fly, *Anastrepha ludens* (Loew), is an economically damaging agricultural pest of citrus and mango in Mexico and Central America, where commercial mango and citrus production is infested seasonally with high economic losses occurring each year (Aluja 1994). In addition to the actual damage caused in Mexico and Central America, the fly also has the potential to invade subtropical citrus growing areas of the United States, including Texas, Florida, Arizona, and California (Citrograph 1989).

An important component in the management of insect pests, such as the Mexican fruit fly, is the development of lures to attract them to traps or poisoned baits. Studies have shown that some tephritids are attracted to mixtures of synthetic compounds based on the aroma emitted by their host fruit. Fein et al. (1982) developed a synthetic attractant for *Rhagoletis pomonella*, the apple maggot fly, based upon volatiles identified from Red Delicious and Red Astrachan apples. Robacker et al. (1990b) developed a synthetic attractant mixture

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for the Mexican fruit fly that was modeled upon volatiles identified from fermented fruit of a native host. Development of synthetic host odor and plant volatiles attractants of many of the tephritid agricultural pests remains a high priority.

A non-host fruit juice, grape juice, has been studied in field tests in Mexico for attractiveness to *A. ludens*. Loera-Gallardo et al. (2006), in presentation of research results at the annual meeting of the Rio Grande Valley Horticulture Society (Weslaco, TX), reported that grape juice (variety not specified) was 2 times more attractive than Biolures (Suterra LLC, Bend, OR) in field tests in Mexico. Also, preliminary research with Frutibases (Frutibases S.A. de C. V., Monterrey, Nuevo Leon, MX) grape juice concentrate suggested this juice was highly attractive to Mexican fruit flies in field tests conducted in Mexico. While these natural products are attractive, their usefulness is limited by fermentation that results in aroma changes and by buildup of debris and non-target insects. A synthetic mixture of chemicals derived from grape juice would provide a consistent level of attraction by emitting volatiles at a specific rate over a long period of time without the potential loss in attractiveness from fermentation. Also, buildup of debris and attraction of non-target insects would probably be reduced.

The objectives of this research were to identify the components of Frutibases grape juice volatiles and provide evidence that the identified chemicals are the attractive components of the aroma. The work was conducted in 4 phases: Identification of the chemicals in grape juice odor; testing of attractiveness of each chemical; preparation of a synthetic mixture of the chemicals that mimics the odor of grape juice by gas chromatographic comparisons; and evaluation of the synthetic mixture in laboratory and field experiments. Frutibases grape juice concentrate was chosen for study because of its known attractiveness to Mexican fruit flies in Mexico.

## Materials and Methods

### Chemistry Methods

*Volatiles Sampling and GC Analyses.* Volatiles in the headspace above Frutibases grape juice were analyzed by gas chromatography (GC). The analyses were carried out with a Shimadzu GC-17A (Shimadzu Scientific Instruments, Inc., Columbia, MD) that was equipped with flame ionization (FID) and flame thermionic (Model FTD-17) detectors. FID was used for quantification of chemicals in grape juice volatiles, comparisons of retention times with those of standards, and quantification of chemicals in a synthetic grape essence mixture (described below). FTD was used to determine if chemicals contained C-N bonds. Measurement of GC peak areas was accomplished with Empower 2 Chromatography Data software (Waters Corporation, Milford, MA). Sampling of volatiles was carried out by SPME with a fiber coated (100µm coating) with polydimethylsiloxane (PDMS) (Supelco, Inc., Bellefonte, PA). A 1-mL aliquot of 17% grape juice (1 part Frutibases concentrate + 5 parts water) was put into a 4-mL glass vial sealed with a plastic ring cap and septum and allowed to equilibrate for 1 h before sampling. 7teen percent juice was chosen because it was the concentration used successfully to trap Mexican fruit flies in preliminary tests in Mexico. The PDMS fiber was inserted into the headspace through a pin-sized hole in the top of the septum. The hole punctured in the septum was just slightly larger than the fiber sheath in order to reduce loss of volatiles during the sampling period of 1 h at ≈22°C. On-column injection of volatiles was performed by thermal desorption from the SPME fiber at 220°C in a 10-cm retention gap (0.53 mm ID deactivated fused-silica) attached to the GC column by a GlasSeal™ connector (Supelco). The analytical column was a DB-1 (60 m, 0.32 mm ID, 5 µm film) (Agilent Technologies, Inc., Santa Clara, CA). A three-step temperature program was used as follows: Initial oven temperature at 50°C held for 5 min, then 5°C/min until 200°C, held for 30 min. Carrier gas was helium at a linear velocity of 30 cm/sec.

*GC-MS of Chemicals in Frutibases Grape Juice*. Gas chromatography-mass spectrometry (GC-MS) data were obtained with a Hewlett Packard 6890 GC (Hewlett Packard Company, San Fernando, California) with a HP 5973 Network Mass Selective Detector (EI) (electron energy = 70eV) over a mass range of 20-550 amu. An HPMS Chemstation (Hewlett Packard) controlled the system.

Volatiles from grape juice were collected by SPME for 60 min and then injected onto a DB-1 column (60 m, 0.25 mm ID, 0.25 µm film) (Agilent Technologies). The sample was thermally desorbed from the SPME fiber for one minute in a split/splitless injector in the splitless mode at 250°C. Purging of the injector occurred after 1 min. The linear velocity of the helium carrier gas was 26 cm/sec. Oven temperature programming was the same as described above. Unknowns were identified by matching their mass spectra to those in the NIST 98 Library of Mass Spectra and Subsets (Hewlett-Packard).

*Test Chemicals*. Ten chemicals identified by GC-MS in volatiles of Frutibases grape juice concentrate were obtained from various sources: ethyl propionate (Sigma-Aldrich Chemical Co., Milwaukee, WI, 99% purity); ethyl butyrate (Sigma-Aldrich, 99%); ethyl 2-methylbutyrate (Sigma-Aldrich, 99%); ethyl decanoate (Sigma-Aldrich, 99%); ethyl dodecanoate (Pfaltz & Bauer, Inc., Waterbury, CT, 98%); D-limonene (Sigma-Aldrich, 97%); sorbic acid (Sigma-Aldrich, 99%); benzoic acid

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(Fisher Scientific, Fair Lawn, NJ, 99%); methyl anthranilate (ABCR GmbH & Co., Karlsruhe, Germany, 99%); and dimethyl anthranilate (ABCR GmbH & Co., 93%). By GC analysis, dimethyl anthranilate contained 7% methyl anthranilate.

Behavioral Assays

*Insects.* Both fertile (non-irradiated) and sterile (irradiated) *A. ludens* pupae were obtained from the USDA-APHIS Rearing Facility in Mission, Texas. Approximately 200 mixed-sex fertile or sterile pupae were maintained in 473-mL cardboard cartons with soft screen tops until used in laboratory tests or released in the orchard, respectively. Sucrose and water were the only nutrients provided to the adult flies. Conditions in the laboratory were consistent at 22 ± 2°C, 50 ± 20% relative humidity, and photophase from 0630 to 1930 h.

*Cage-Top Bioassay Procedure.* Cage-top bioassays were used to determine the level of attractiveness of individual chemicals, synthetic grape essence mixture, and diluted Frutibases grape concentrate by procedures modified from Robacker et al. (1990b). The basic test was to apply test samples and control solvents or solutions on filter papers on the tops of insect cages and to count the flies that visit the papers. Specifically, the protocol was to place a filter paper triangle (3 cm/side) in each corner on the top of an insect cage (30 cm/side, aluminum-screened, removable clear glass front). The 4 filter paper triangles were placed upon plastic rings that raised them 5 mm above the screened cage top. This ensured that flies could not touch the filter papers and that fly response to the test samples was based on olfaction and not contact chemoreception. Two paper triangles contained test samples and 2 contained controls. The 2 filter papers containing the test chemicals were positioned diagonally across from each other as were the control filter papers. The numbers of flies underneath each filter paper were counted and recorded at 1-min intervals for 10 min total time. One carton containing ≈200 flies was used for each bioassay. Flies tested were between the ages of 4-20-d-old with all tests being conducted between the hours of 1030 and 1830. Previous experiments with chemicals from host fruit indicated that Mexican fruit fly responses varied only slightly over these ages and during these hours of the day (Robacker et al. 1990a).

*Cage-Top Bioassays of Individual Grape Chemicals.* The purpose of these tests was to analyze the level of attractiveness of 10 chemicals identified from volatiles of Frutibases grape juice. An 11$^{th}$ chemical, ethanol, found in grape juice was not tested. Eight of the 10 chemicals were mixed in hexane. Two of the chemicals, benzoic acid and sorbic acid, were mixed in water. Two sets of bioassays were conducted for each chemical: 1 µg of a chemical (in 10 µL of solvent) per filter paper vs. solvent; and 100 ng (in 10 µL of solvent) per paper vs. solvent. Control papers each received 10 µL of appropriate solvent, either hexane or water depending on the solvent used for the test chemical.

Four experiments were conducted for each chemical: low concentration with sugar-fed flies; high concentration with sugar-fed flies; low concentration with sugar-starved flies; and high concentration with sugar-starved flies. For all sugar-starved bioassays, sugar was removed from flies 48 h prior to testing. Eight to 10 replications were completed for all chemicals at each concentration for both sugar-fed and sugar-starved flies.

*Preparation of Synthetic Grape Essence Mixture.* The preliminary synthetic grape essence mixture was prepared by adding 100 µL (100 mg for benzoic acid) aliquots of 9 of the 10 aforementioned chemicals into 100 mL of 10% Tween 85 (Sigma-Aldrich, Inc., St. Louis, MO) aqueous solution. Sorbic acid was not included. SPME-GC analysis was conducted on the preliminary mixture, and the chromatogram was compared with the chromatogram obtained from analysis of 17% grape juice. A trial and error approach was taken where the concentration of each chemical in the synthetic grape essence was adjusted until peak areas matched the peak areas from the grape juice. The final synthetic grape essence mixture contained 3.0 µL ethyl propionate, 2.0 µL ethyl butyrate, 0.3 µL ethyl 2-methylbutyrate, 0.1 µL D-limonene, 0.16 g benzoic acid, 7.0 µL methyl anthranilate, 0.3 µL ethyl decanoate, 7.0 µL dimethyl anthranilate, and 4.4 µL ethyl dodecanoate dissolved 100 mL of a 10% Tween 85 in RO water.

*Cage-Top Bioassays of Grape Essence Mixture.* The purpose of these experiments was to evaluate the attractiveness of the synthetic grape essence mixture relative to Frutibases grape juice. Due to low attractiveness of 17% grape juice in preliminary bioassays, 34% grape juice was used in these tests. Accordingly, concentrations of chemicals in the synthetic grape essence were also doubled for these tests. Grape essence and grape juice were tested against 10% Tween in separate bioassay cages. Test quantities were 10 µL for both essence and juice.

Because Tween was present in the grape essence but not in the grape juice, it was necessary to determine whether Tween affected attractiveness. Four experiments were conducted to assess effects of Tween and also fly-hunger on attractiveness of grape essence and grape juice: non-starved flies without 10% Tween 85 on the grape juice filter papers; non-starved flies with a 10 µL aliquot of 10% Tween 85 on the grape juice filter papers; starved flies without 10% Tween 85 on the grape juice filter papers; and starved flies with a 10 µL aliquot of 10% Tween 85 on the grape juice filter papers. All control filter papers contained 10% Tween.

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*Field Test of Grape Essence.* A randomized complete block design was used for field tests. Eighteen multilure traps (Better World Manufacturing, Riverside, CA) were hung in 2 rows of citrus with 3 sets of 3 traps per row; with a tree separating each set of traps. Each trap in a set contained 100 mL of one of the following treatment mixtures: 17% grape juice, synthetic grape essence (based on 17% juice), and the control. The control trap contained 10% Tween 85 solution. The traps were hung within the shady interior of trees, slightly northeast of the center of the trunk about midheight, at the same spot in each tree for all replications of all treatments. In order to compensate for any attractive or repellent effect of Tween 85 in the synthetic grape essence mixture, each trap containing Frutibases grape juice also contained two 20-mL vials each containing 1 mL of 100% Tween 85. The estimate of how much Tween 85 to use was derived by calculating that the surface area of the liquid in the grape essence trap was 20 times the surface area of 1 mL of liquid in a 20-mL vial, and hypothesizing that 2 vials each with 1 mL of 100% Tween 85 would emit about the same amount of volatiles as the 10% Tween 85 in the grape essence trap. GC analyses of volatiles from 10% Tween 85 showed only very small peaks (largest peak < 1% of the size of methyl anthranilate in the Frutibases 17% grape juice dilution) suggesting that achieving the exact emissions of Tween 85 from the 2 trap types was probably not important. Tween 85 was not added directly to the grape juice concentrate because in preliminary tests adding the Tween 85 changed emission rates of the volatiles.

For each replicate, the traps were set-up and left in the orchard for 48 h after which counts of trapped male and female flies were recorded. Following each replicate, traps were returned to the lab and washed. Treatments were alternated to different trees within sets for each replication of the experiment. Twelve replications were completed. For 10 of the 12 field tests, volatiles from 1 mL samples of the 2 trap baits (17% grape juice and grape essence) were analyzed by GC to verify similarity of the grape essence to grape juice. Sterile (irradiated) 7-14-day-old flies were released in 1 row of trees directly adjacent to each row containing traps on the day the traps were put into the orchard. Flies were released at the rate of one carton (200 flies, approximately 50:50 males:females) per 2 trees in the row. The purpose of releasing flies in this manner was to acquire an even dispersal of flies into the row containing traps.

### Statistics

Paired *t* tests (Sokal & Rohlf 1995) were used to compare the counts of flies at test chemical filter papers with the counts of flies at solvent-control filter papers for all individual chemicals. Paired *t* tests also were used to analyze results from cage-top bioassays comparing synthetic grape essence to the control and Frutibases grape juice to the control with and without Tween 85 added to the filter papers containing grape juice. Paired *t* tests were conducted with JMP:Analyze:Matched Pairs analysis (JMP 2002).

Analysis of variance was conducted to compare grape juice with grape essence in both laboratory and field experiments by SuperAnova (Abacus Concepts 1989). For laboratory bioassays, total fly counts at treatment papers were divided by total counts at control papers for each bioassay and the resulting ratios, transformed by square root to normalize variance, were used as data points in ANOVA. Ratios were used to show relative attractiveness of the treatments to the Tween controls. For field tests counts of flies in traps were transformed by square root to normalize variance, and used as data points in the ANOVAS. Effects of replication were partitioned out of the sum of squares for both laboratory and field tests. Means separations were conducted by Fisher's Protected LSD (Abacus Concepts 1989).

## RESULTS

### Chemistry

*Chemical Identifications.* All 11 chemicals identified by GC-MS have above 90% computer matches to mass spectra in the NIST 98 Library of Mass Spectra and Subsets except ethyl propionate which had a 64% match (Table 1). Identifications were confirmed by matching GC retention times of the unknowns in grape juice volatiles to those of standards. The presence of very large peaks by GC-FTD at retention times of methyl anthranilate and dimethyl anthranilate showed that these 2 compounds contain C-N bonds, substantiating their identifications as amines.

*Quantification by SPME-GC-FID.* The mean peak areas (mV/sec) determined from GC samplings of the grape juice used in field tests, and the percentage each volatile contributed to the total volatiles identified in the headspace of Frutibases grape juice, are shown in Table 1. Five chemicals, ethyl butyrate, benzoic acid, methyl anthranilate, dimethyl anthranilate, and ethyl dodecanoate contributed the highest percentages to the total volatiles. The volatiles contributing the lowest percentages to the total were ethyl 2-methylbutyrate, sorbic acid, D-limonene, and ethyl decanoate. These 11 chemicals constituted 98.7% of the total volatiles in the headspace.

### Attractiveness of Individual Chemicals: Sugar-Fed Flies

Results from bioassays testing the attractiveness of individual chemicals to sugar fed flies show that ethyl decanoate and ethyl dodecanoate

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TABLE 1. IDENTIFICATION AND QUANTITATION OF CHEMICALS FROM FRUTIBASES GRAPE JUICE VOLATILES BY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY[1].

| Chemical | GC-MS match(%)[2] | RT juice (min)[3] | RT standard (min)[3] | Mean peak area (mV)[4] | % of total volatiles[5] |
|---|---|---|---|---|---|
| Ethanol | 90 | 4.2 | 4.2 | 78.6 | 5.2 |
| Ethyl propionate | 64 | 14.9 | 14.9 | 81.8 | 5.4 |
| Ethyl butyrate | 97 | 19.0 | 19.0 | 223.5 | 14.8 |
| Ethyl 2-methylbutyrate | 96 | 21.4 | 21.4 | 41.2 | 2.7 |
| Sorbic acid | 96 | 28.9 | 29.0 | 39.0 | 2.6 |
| D-Limonene | 96 | 29.5 | 29.5 | 31.3 | 2.1 |
| Benzoic acid | 91 | 32.8 | 33.0 | 244.0 | 16.2 |
| Methyl anthranilate | 96 | 40.4 | 40.4 | 276.8 | 18.4 |
| Ethyl decanoate | 98 | 42.4 | 42.4 | 37.7 | 2.5 |
| Dimethyl anthranilate | 95 | 44.0 | 44.0 | 204.3 | 13.6 |
| Ethyl dodecanoate | 98 | 56.8 | 56.8 | 227.4 | 15.1 |

[1]Frutibases grape juice prepared as 17% dilution of concentrate.
[2]Matching mass spectra of unknowns in grape juice to the NIST 98 Library of Mass Spectra and Subsets (Hewlett-Packard).
[3]GC retention times.
[4]Mean peak areas of chemicals in grape juice used in field tests by GC-FID. $n$ = 10 samplings.
[5]Mean peak area for each chemical in grape juice divided by the sum of peak areas of all chemicals in the chromatogram, times 100.

were significantly more attractive than solvent controls ($P < 0.05$) at the lower concentration of 100 ng (Table 2). Fly responses to the other 8 chemicals were not significantly different from responses to solvent controls.

At the higher concentration of 1 µg, 4 chemicals were significantly more attractive than controls ($P < 0.05$) (Table 2). These were ethyl propionate, ethyl dodecanoate, benzoic acid, and sorbic acid. The other 6 chemicals were not significantly different from solvent controls in attractiveness.

Overall (high and low concentration combined), 7 chemicals were significantly more attractive than controls ($P < 0.05$) (Table 2). These were ethyl propionate, ethyl butyrate, ethyl decanoate, ethyl dodecanoate, D-limonene, benzoic acid, and dimethyl anthranilate. The other 3 chemicals were not significantly different from controls in attractiveness.

### Attractiveness of Individual Chemicals: Sugar-Starved Flies

Results from bioassays testing the attractiveness of individual chemicals to sugar-starved flies show 7 of the 10 chemicals were significantly more attractive than solvent controls ($P < 0.05$) at the lower concentration of 100 ng (Table 3). The 7 chemicals were ethyl propionate, ethyl butyrate, ethyl 2-methylbutyrate, D-limonene, benzoic acid, methyl anthranilate, and dimethyl anthranilate. Fly responses to the other 4 chemicals were not significantly different from solvent controls.

At the higher concentration of 1 µg, 7 chemicals were significantly more attractive than controls ($P < 0.05$) (Table 3). These were ethyl propionate, ethyl butyrate, ethyl 2-methylbutyrate, ethyl decanoate, D-limonene, benzoic acid, and methyl anthranilate. The other 3 chemicals were not significantly different from controls in attractiveness.

Overall (high and low concentrations combined), all of the chemicals except sorbic acid were significantly more attractive than controls ($P < 0.05$) (Table 3).

### Evaluation of Synthetic Grape Essence

*Composition*. Nine chemicals that proved attractive to either sugar-fed or sugar-starved flies over both test amounts (100 ng and 1 µg combined) were included in the synthetic grape essence mixture. Sorbic acid was not attractive to either hunger group of flies over both test concentrations so it was not included. The synthetic grape essence mixture contained amounts of the chemicals as previously described in the Methods section.

Similarity of grape essence volatiles to grape juice volatiles was determined by comparison of peak areas from GC analyses of the 1 mL samples taken from the field tests. A similarity index (%) for each chemical was calculated as the smaller mean GC-FID peak area (mV/sec) (either juice or essence mean) divided by the larger mean (juice or essence), and multiplying the resulting ratio by 100. A negative sign was assigned to the index if the grape essence mean was smaller than the grape juice mean. Similarities were calculated as: ethyl propionate, 42.0; ethyl butyrate, 60.7; ethyl 2-methyl butyrate, 54.2; D-limonene, 43.6; benzoic acid, -30.9; methyl anthranilate, -42.7; ethyl decanoate, 89.8; dimethyl anthranilate, 56.1; and ethyl dodecanoate, -36.6. The mean similarity index summed over the absolute values of the nine chemicals was 50.7%.

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TABLE 2. ATTRACTION OF SUGAR-FED MEXICAN FRUIT FLIES TO CHEMICALS IDENTIFIED FROM FRUTIBASES GRAPE JUICE VOLATILES IN CAGE-TOP BIOASSAYS.

| Chemical | Amount tested | $n$[1] | Mean counts at: T[2] | C[2] | T - C[3] | $t$[4] |
|---|---|---|---|---|---|---|
| Ethyl propionate | 100 ng | 10 | 21.2 | 15.7 | 5.5 ± 5.1 | 1.08 |
| | 1 µg | 10 | 29.3 | 14.8 | 14.5 ± 3.7 | 3.97** |
| | overall | 20 | 25.3 | 15.3 | 10.0 ± 3.2 | 3.10** |
| Ethyl butyrate | 100 ng | 10 | 31.5 | 23.5 | 8.0 ± 4.6 | 1.75 |
| | 1 µg | 10 | 23.5 | 17.9 | 5.6 ± 3.8 | 1.48 |
| | overall | 20 | 27.5 | 20.7 | 6.8 ± 2.9 | 2.34* |
| Ethyl 2-methylbutyrate | 100 ng | 10 | 24.1 | 23.3 | 0.8 ± 4.3 | 0.18 |
| | 1 µg | 8 | 23.4 | 19.9 | 3.5 ± 5.8 | 0.60 |
| | overall | 18 | 23.8 | 21.8 | 2.0 ± 3.4 | 0.59 |
| Ethyl decanoate | 100 ng | 10 | 27.4 | 19.7 | 7.7 ± 3.2 | 2.37* |
| | 1 µg | 9 | 30.2 | 21.2 | 9.0 ± 5.9 | 1.52 |
| | overall | 19 | 28.7 | 20.4 | 8.3 ± 3.2 | 2.60* |
| Ethyl dodecanoate | 100 ng | 10 | 33.4 | 15.7 | 17.7 ± 4.8 | 3.70** |
| | 1 µg | 9 | 22.9 | 15.4 | 7.4 ± 2.4 | 3.06* |
| | overall | 19 | 28.4 | 15.6 | 12.8 ± 3.0 | 4.34** |
| D-Limonene | 100 ng | 10 | 25.4 | 19.5 | 5.9 ± 3.3 | 1.81 |
| | 1 µg | 8 | 23.6 | 15.4 | 8.3 ± 3.9 | 2.12 |
| | overall | 18 | 24.6 | 17.7 | 6.9 ± 2.4 | 2.84* |
| Sorbic acid | 100 ng | 10 | 29.3 | 27.8 | 1.5 ± 3.9 | 0.39 |
| | 1 µg | 10 | 26.4 | 18.7 | 7.7 ± 2.6 | 2.94* |
| | overall | 20 | 27.9 | 23.3 | 4.6 ± 2.4 | 1.92 |
| Benzoic acid | 100 ng | 10 | 28.3 | 22.7 | 5.6 ± 3.2 | 1.76 |
| | 1 µg | 10 | 20.8 | 14.7 | 6.1 ± 2.3 | 2.65* |
| | overall | 20 | 24.6 | 18.8 | 5.9 ± 1.9 | 3.06** |
| Methyl anthranilate | 100 ng | 10 | 27.5 | 21.7 | 5.8 ± 5.0 | 1.15 |
| | 1 µg | 10 | 24.2 | 18.5 | 5.7 ± 3.2 | 1.77 |
| | overall | 20 | 25.9 | 20.1 | 5.8 ± 2.9 | 1.98 |
| Dimethyl anthranilate | 100 ng | 10 | 23.7 | 15.2 | 8.5 ± 4.0 | 2.10 |
| | 1 µg | 10 | 22.9 | 18.3 | 4.6 ± 2.5 | 1.90 |
| | overall | 20 | 23.3 | 16.8 | 6.6 ± 2.3 | 2.79* |

[1]$n$ = number of bioassay replications.
[2]T = treatment; C = solvent blank.
[3]Mean ± standard error.
[4]Paired $t$ test of T - C. *Indicates $P < 0.05$, ** indicates $P < 0.01$.

*Laboratory Evaluation of Essence and Juice vs. Tween 85.* Results from laboratory cage-top bioassays testing the attractiveness of synthetic grape essence and grape juice relative to Tween 85 controls are shown in Table 4. Both synthetic grape essence and grape juice were significantly more attractive than Tween 85 controls ($P < 0.05$) for both non-starved and starved flies with or without Tween 85 added to the grape juice filter paper.

*Laboratory Evaluation of Essence vs. Juice.* Results of the same cage-top bioassays discussed in the previous section were also analyzed to test the attractiveness of synthetic grape essence relative to the attractiveness of grape juice (Table 5). For non-starved flies, essence and juice did not differ in attractiveness ($P < 0.05$) whether or not Tween 85 was added to the filter papers containing grape juice. For sugar-starved flies, essence was significantly more attractive than juice when Tween 85 was not present on the filter papers containing juice. However, essence and juice were not significantly different when Tween 85 was added to the papers with grape juice. Results of the 4 experiments could not be compared with each other because time of year when experiments were con-

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TABLE 3. ATTRACTION OF SUGAR-STARVED MEXICAN FRUIT FLIES TO CHEMICALS IDENTIFIED FROM FRUTIBASES GRAPE JUICE VOLATILES IN CAGE-TOP BIOASSAYS.

| Chemical | Amount tested | $n$[1] | Mean counts at: T[2] | C[2] | T - C[3] | $t$[4] |
|---|---|---|---|---|---|---|
| Ethyl propionate | 100 ng | 9 | 40.0 | 24.8 | 15.2 ± 2.1 | 7.20** |
| | 1 µg | 9 | 43.3 | 27.1 | 16.2 ± 2.6 | 6.31** |
| | overall | 18 | 41.7 | 25.9 | 15.7 ± 1.6 | 9.71** |
| Ethyl butyrate | 100 ng | 9 | 44.6 | 33.7 | 10.9 ± 4.5 | 2.41* |
| | 1 µg | 10 | 38.5 | 22.2 | 16.3 ± 3.3 | 4.91** |
| | overall | 19 | 41.5 | 27.8 | 13.7 ± 2.8 | 5.24** |
| Ethyl 2-methylbutyrate | 100 ng | 9 | 39.1 | 22.8 | 16.3± 4.9 | 3.33** |
| | 1 µg | 10 | 28.5 | 21.5 | 7.0 ± 2.8 | 2.53* |
| | overall | 19 | 33.5 | 22.1 | 11.4 ± 2.9 | 3.97** |
| Ethyl decanoate | 100 ng | 9 | 35.2 | 26.7 | 8.6 ± 5.9 | 1.45 |
| | 1 µg | 8 | 43.1 | 27.9 | 15.3 ± 6.0 | 2.52* |
| | overall | 17 | 38.9 | 27.2 | 11.7 ± 4.2 | 2.80* |
| Ethyl dodecanoate | 100 ng | 9 | 47.7 | 36.4 | 11.2 ± 6.6 | 1.70 |
| | 1 µg | 8 | 34.0 | 26.1 | 7.9 ± 3.3 | 2.36 |
| | overall | 17 | 41.2 | 31.6 | 9.6 ± 3.8 | 2.57* |
| Sorbic acid | 100 ng | 9 | 33.4 | 32.1 | 1.3 ± 4.0 | 0.33 |
| | 1 µg | 10 | 33.3 | 24.4 | 8.9 ± 4.7 | 1.88 |
| | overall | 19 | 33.4 | 28.1 | 5.3 ± 3.2 | 1.68 |
| D-Limonene | 100 ng | 9 | 46.8 | 26.7 | 20.1 ± 4.4 | 4.6** |
| | 1 µg | 10 | 27.3 | 18.1 | 9.2 ± 1.8 | 5.06** |
| | overall | 19 | 36.5 | 22.2 | 14.4 ± 2.6 | 5.61** |
| Benzoic acid | 100 ng | 9 | 41.2 | 30.5 | 10.6 ± 2.8 | 3.83** |
| | 1 µg | 10 | 32.9 | 22.6 | 10.3 ± 3.2 | 3.17* |
| | overall | 19 | 36.8 | 26.4 | 10.5 ± 2.1 | 4.99** |
| Methyl anthranilate | 100 ng | 9 | 43.9 | 34.1 | 9.8 ± 3.4 | 2.81* |
| | 1 µg | 10 | 36.0 | 26.4 | 9.6 ± 3.3 | 2.87* |
| | overall | 19 | 39.7 | 30.1 | 9.7 ± 2.3 | 4.13** |
| Dimethyl anthranilate | 100 ng | 9 | 52.0 | 35.0 | 17.0 ± 4.8 | 3.57** |
| | 1 µg | 9 | 36.4 | 29.2 | 7.2 ± 5.7 | 1.28 |
| | overall | 18 | 44.2 | 32.1 | 12.1 ± 3.8 | 3.20** |

[1]$n$ = number of bioassay replications.
[2]T = treatment; C = solvent blank.
[3]Mean ± standard error.
[4]Paired $t$ test of T - C. *Indicates $P < 0.05$, ** indicates $P < 0.01$.

ducted was confounded with both feeding status and the use of Tween 85 with grape juice.

*Field Tests.* Results from field tests are shown in Figure 1. Both grape juice and synthetic grape essence captured significantly more male and female Mexican fruit flies than control traps containing 10% Tween 85 ($P < 0.05$), capturing at least 4 times more males and 8 times more females than the control traps. Grape juice was significantly more attractive than synthetic grape essence to both males and females. The juice captured about 40% more of each compared with grape essence.

## DISCUSSION

### Chemical Identification and Quantification

Identifications of the chemicals in the aroma of Frutibases grape juice were based upon several criteria. The primary method was by computer matching of mass spectra of the chemicals in the headspace with standard spectra in the 98 NIST Library of Mass Spectra and Subsets. The low match for ethyl propionate (64%)is not unusual in these types of analyses because interfering peaks in complex samples, such as grape juice, contrib-

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TABLE 4. ATTRACTIVENESS OF FRUTIBASES GRAPE JUICE AND SYNTHETIC GRAPE ESSENCE RELATIVE TO 10% TWEEN 85 CONTROLS IN CAGE-TOP BIOASSAYS[1].

| Experiment | Treatment | Mean counts per bioassay at: $n$[2] | T[3] | C[3] | T - C[4] | $t$[5] |
|---|---|---|---|---|---|---|
| Not starved | juice | 11 | 51.7 | 22.6 | 29.1 ± 4.1 | 7.0* |
| | essence | 11 | 45.0 | 15.4 | 29.6 ± 3.3 | 9.0* |
| Not starved, grape juice w/Tween | juice | 19 | 56.0 | 29.5 | 26.5 ± 3.2 | 8.2* |
| | essence | 19 | 51.1 | 27.6 | 23.5 ± 3.1 | 7.6* |
| Starved | juice | 17 | 90.5 | 43.7 | 46.8 ± 7.3 | 6.4* |
| | essence | 17 | 100.7 | 37.2 | 64.5 ± 3.7 | 17.6** |
| Starved, grape juice w/Tween | juice | 13 | 76.0 | 53.3 | 22.7 ± 4.0 | 5.7** |
| | essence | 13 | 85.1 | 55.2 | 29.8 ± 5.8 | 5.1** |

[1]Frutibases grape juice prepared as 34% dilution of concentrate (2 times concentration used in field tests); Grape essence at 2 times concentration used in field tests.
[2]$n$ = number of bioassay replications.
[3]T = treatment paper; C = 10% Tween 85 paper.
[4]Mean ± standard error.
[5]Paired $t$ test of T - C; * Indicates $P < 0.05$, ** indicates $P < 0.01$.

ute to the MS of the targeted peaks making matching difficult.

The matching of retention times of standard chemicals purchased and analyzed by GC-SPME to the retention times of the chemicals identified in Frutibases grape juice also helped confirm the chemical identifications. For sorbic acid and benzoic acid, asymmetrical shapes in which the leading edges of the peaks were tailed, and retention times that increased as their concentrations increased, were consistent with the identifications of these compounds as carboxylic acids.

Comparison with Published Data on Grape Juice Aroma

Numerous chemicals have been identified in previous research into the aroma of different varieties of grape. Methyl anthranilate, the chemical that gives concord grapes their characteristic aroma (Morris 1989) was first identified as a component of the aroma of *Vitis labrusca* grapes nearly a century ago (Power & Chesnut 1921). Holley et al. (1955) later identified methyl anthranilate from concord grape juice along with ethanol, methanol, ethyl acetate, methyl acetate,

TABLE 5. ATTRACTIVENESS OF SYNTHETIC GRAPE ESSENCE RELATIVE TO FRUTIBASES GRAPE JUICE IN CAGE-TOP BIOASSAYS[1]

| Experiment | Treatment | $n$[2] | Mean T/C per bioassay[3] | $F$[4] |
|---|---|---|---|---|
| Not starved | juice | 11 | 4.5 ± 1.9 | 0.01 |
| | essence | 11 | 4.0 ± 0.8 | |
| Not starved, grape juice w/Tween | juice | 19 | 2.1 ± 0.2 | 0.04 |
| | essence | 19 | 2.2 ± 0.2 | |
| Starved | juice | 17 | 2.2 ± 0.2 | 7.0* |
| | essence | 17 | 3.0 ± 0.2 | |
| Starved, grape juice w/Tween | juice | 13 | 1.5 ± 0.1 | 0.60 |
| | essence | 13 | 1.6 ± 0.1 | |

[1]Frutibases grape juice prepared as 34% dilution of concentrate (2 times concentration used in field tests); Grape essence at 2 times concentration used in field tests.
[2]$n$ = number of bioassay replications.
[3]For each bioassay, the ratio "counts at treatment papers/counts at control (10% Tween 85) papers" was calculated. "T/C" = the mean ± standard error.
[4]*Indicates $P < 0.05$.

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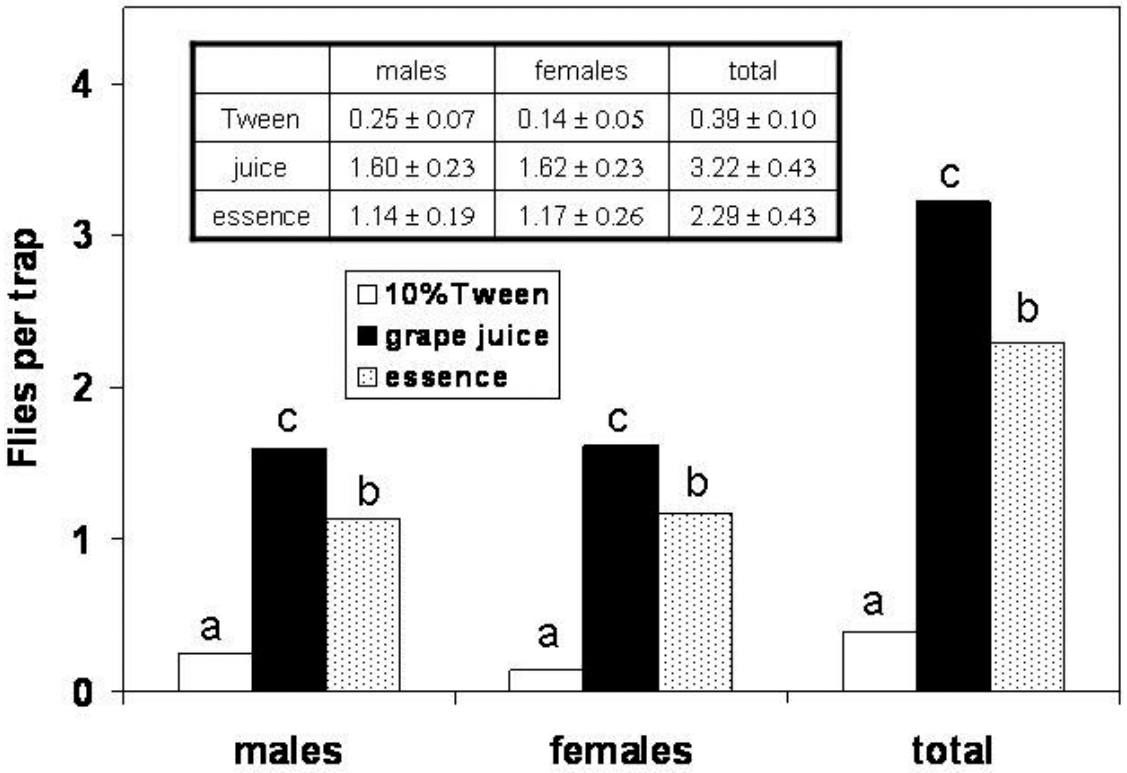



Fig. 1. Mean captures (_ standard error) of irradiated Mexican fruit flies in traps baited with Frutibases grape juice and synthetic grape essence in a citrus orchard. Bars in the same sex with different letters are significantly different by Fishers Protected Least Significant Difference Test ($P < 0.05$).

acetone, acetaldehyde and acetic acid. Stevens et al. (1965) found additional compounds including isopropyl acetate, isopropanol, ethyl propionate, propyl acetate, propanol, 2-methyl-3-buten-2-ol, ethyl butyrate, isobutanol, butanol, 2-methylbutanol, 3-methylbutanol, and ethyl hexanoate. Morris (1989) reported benzoic acid as a component of concord grape juice. Finally, Viñas et al. (1993) identified dimethyl anthranilate in addition to methyl anthranilate in the aroma and flavor of concord grapes. These previous reports indicate that concord grape juices contain many compounds that were not found in aroma of Frutibases grape juice or were present in trace amounts undetected by analyses used in this work. The most important similarities among the early reports and the results presented here are the presence of ethyl propionate, ethyl butyrate, methyl anthranilate, and dimethyl anthranilate.

Generally, the aromas of white and Muscat grapes differ markedly from those of concord grapes. In Muscat grape varieties, Ribereau-Gayon et al. (1975) identified D-limonene in the aroma of Alexandria grapes. Rocha et al. (2000) identified propionic acid, butyric acid, decanoic acid, and dodecanoic acid in aroma of two White grape varieties. Baek et al. (1997) identified ethyl butyrate and ethyl 2-methylbutyrate as predominant compounds in the aroma and juice of Muscadine grape juice. While these 2 chemicals were also found in the Frutibases grape juice, the absence of methyl anthranilate as a major component of these other grape varieties probably accounts for the noticeable difference in aroma.

### Similarity of Volatiles of Frutibases Grape Juice and Synthetic Grape Essence

The mean similarity index for volatiles from synthetic grape essence and grape juice was only 50.7%. Bartelt & Hossain (2006) identified attractive compounds from peach juice and constructed a synthetic peach essence as an attractant. Although the authors did not evaluate the similarity of chemical emissions, they did provide emissions data for the synthetic mix and the juice. Based upon the similarity index definition developed for grape in the present research, the mean similarity index for their synthetic mix was 76.2%. This is better than the mean index obtained in the current study. However, the mixture made by Bartelt & Hossain (2006) contained only

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alcohols, esters, and 1 aldehyde. It did not contain carboxylic acids and amines that interact in solution and greatly impact each other's emissions. Their mixture also did not contain a wide range of molecular weights nor an adjuvant, such as Tween 85. Thus it is not surprising that they were able to make mixtures that more closely matched their model juice solution.

### Attractiveness of Chemicals from Grape Aroma

All of the chemicals from the Frutibases volatiles that were tested were attractive in at least 1 of our experiments. Ethanol was not tested in this work because Baker et al. (1944) stated that ethanol was only "feebly" attractive to Mexican fruit flies. Also for this reason, a decision was made not to include ethanol in the synthetic grape essence. As reported in the Results, sorbic acid was not attractive to either hunger group of flies over both test concentrations, so it was not included in the synthetic grape essence. D-limonene was tested previously by Robacker et al. (1990b) for attractiveness to sugar-starved Mexican fruit flies but not to sugar-fed flies. D-limonene was attractive in those tests at a test amount of 0.4 µg. All of the other chemicals tested in this work had not been previously tested as attractants for the Mexican fruit fly.

### Attractiveness of Synthetic Grape Essence

Both the synthetic grape essence mixture and Frutibases grape juice were more attractive than Tween 85 in laboratory cage-top bioassays. Also, the T/C ratios for synthetic grape essence and Frutibases grape juice were similar in 3 of the 4 experiments testing their attractiveness under different conditions of fly hunger and presence or absence of Tween 85 on filter papers containing grape juice. The exception was that the synthetic grape essence was more attractive than grape juice to starved flies in the experiment in which there was no Tween 85 added to the grape juice filter papers. This suggests a weak increase in the attractiveness of the grape juice by addition of Tween 85. Any type of attractive response that may have been generated by the addition of Tween 85 could not be determined by statistical analysis, and therefore no conclusions could be drawn. Overall, there was no difference between the attractiveness of the synthetic grape essence and grape juice in the laboratory cage-top bioassays.

In the field, the Frutibases grape juice was more attractive than the synthetic grape essence, however, there were environmental variables such as temperature fluctuations that might have affected the emission rates of volatiles from the synthetic mixture and juice differently. For example, it was observed that on very cold days the synthetic mixture attracted very few flies relative to the juice. When the 3 replications during which daytime high temperatures were below 20°C were deleted from the data set, traps with juice captured only 12% more flies than those with the synthetic mixture and the difference was not statistically significant. Also, the buildup of dead moths and other insects in the juice traps over the 2-d period could have had an attractive effect due to volatiles released from decomposing insects.

Generally, synthetic grape essence was more attractive in laboratory bioassays but grape juice was more attractive in field tests. In addition to possible effects of environment and decomposing insects, differences between the laboratory and field results may be attributable to the different delivery methods used (filter paper vs. liquid reservoir in trap). Also, physiological state of field flies may have differed from that of lab flies causing different responses to grape juice compared with the grape essence mixture. This latter effect may have been enhanced by response to minor chemicals in grape juice that were not present in the grape essence mixture.

Based upon the laboratory bioassays and field tests, the most prudent conclusion is that most of the critical attractive principals of Frutibases grape juice were identified and incorporated into the synthetic grape essence mixture. Also, the concentrations used in the synthetic mixture apparently were adequate to elicit attractiveness similar to that of the grape juice. With this work, it should be possible to develop a grape-based lure for the Mexican fruit fly and perhaps other species of fruit flies.

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