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ANALYSIS, SYNTHESIS, FORMULATION, AND FIELD TESTING OF THREE MAJOR COMPONENTS OF MALE MEDITERRANEAN FRUIT FLY PHEROMONE[1]

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Abstract—Three major components, ethyl-(*E*)-3-octenoate, geranyl acetate, and (*E,E*)-α-farnesene, emitted as volatiles by laboratory-reared and wild male medflies were collected and analyzed qualitatively and quantitatively. Peak emission of these compounds occurred during the third to fifth hours of the photophase and differences were observed in the ratios of the three components emitted by male laboratory-reared and wild flies. These three compounds were synthesized, and a method was developed to formulate a synthetic blend that released the compounds in a ratio similar to that emitted by wild male medflies. Attractiveness of the blend to female medflies was demonstrated under field conditions by comparing trap catches. Black spherical traps, baited with the synthetic blend to release 1.6 male equivalents, caught significantly more females than blank traps and traps from which the blend released was 0.3, 3.2 or 6.4 male equivalents.

Key Words—Insecta, Diptera, Tephritidae, Mediterranean fruit fly, pheromone, attractant, *Ceratitis capitata*, ethyl-(*E*)-3-octenoate, geranyl acetate, (*E,E*)-α-farnesene.

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[1] This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or recommendation for its use by USDA.

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INTRODUCTION

The Mediterranean fruit fly, *Ceratitis capitata* (Wied.) (medfly), is a worldwide pest of many deciduous and tropical fruits. Although not established in the continental United States, small infestations in Florida and California have necessitated multimillion dollar eradication programs. It is estimated that in California an infestation of the medfly could result in a $206,000,000 loss in crops and would require a $156,000,000 control program, which would include the use of 1,818,000 kg of pesticide (Dowell and Wange, 1986). The most effective monitoring tool available for the medfly is trimedlure (Beroza et al., 1961), and it is routinely used in California, Texas, and Florida. Trimedlure predominantly attracts males, although a small percentage of females do respond to this chemical (Nakagawa et al., 1970). The lack of a useful attractant for female medflies prompted us to analyze the male-produced pheromone (Féron, 1962) and evaluate it as a lure for females.

Baker et al. (1985) reported the identification of nine compounds emitted by male medflies. However, they gave no behavioral data to substantiate pheromonal activity. Subsequently, Jang et al. (1989) determined electroantennogram responses of male and female medflies to those nine compounds and to an additional 56 compounds collected from air passed over male medflies. The latter were identified by gas chromatography–mass spectroscopy (GC-MS) by matching the spectra obtained with a mass spectral library. We report in this paper the qualitative and quantitative analyses of three principle components of the volatiles emitted by laboratory-reared and wild male medflies during the photophase. Additionally, we synthesized these compounds, developed a method to formulate each of the compounds individually so as to release these three compounds as a synthetic blend in a ratio similar to that emitted by wild male medflies, and investigated the attractiveness of the pheromone blend at four release rates by trapping wild female medflies in the field.

METHODS AND MATERIALS

Insects. Laboratory-reared *C. capitata* pupae were obtained from the USDA-APHIS mass-rearing facility located in Petapa, Guatemala. Sexually immature 2- to 3-day-old adults were sexed and maintained in 30 cm^3 screen cages containing a mixture of brown sugar and yeast hydrolysate. Flies were held indoors under natural light conditions and ambient temperature and humidity. Wild flies were obtained from infested coffee beans (*Coffee arabica*) collected near Antigua, Guatemala. Beans were held on a screen over soil until maggots left the fruit to pupate in the soil. Emerged flies were maintained in cardboard 1-liter cups with a cotton wick descending from the floor of the containers into a second, water-bearing, cup. When adults were 1–2 days old, they

were sexed and maintained as described above. The yeast hydrolysate food source was replaced with sugar water 24 hr prior to the collection of volatiles from the insects. Volatiles were collected on Porapak-Q (see later) from wild and laboratory-reared male medflies 5–10 days old in groups of 8–12 flies/chamber for 2-hr periods throughout the photophase. Similar collections of volatiles from female medflies were made.

Collection of Emitted Pheromone. The push–pull system used to collect volatiles emitted by the insects has been described previously (Landolt and Heath, 1987). Briefly, compressed air, purified by passing through two charcoal filters, entered two glass chambers 5 cm OD × 30 cm long, each containing 8–12 flies. A frit near the upwind end of the chamber produced a laminar airflow over the insects, and this flow was then pulled through a collector trap by vacuum. Each chamber contained three collector traps, any one of which could be selected for volatile collection without disturbing the flies. The airflow was 0.9 liters/min with a slight positive pressure (+0.2 cm H_2O) established in the insect holding chamber (incoming air to vacuum). Vent flow was monitored with a flowmeter from a vent in the chamber, and the vent flow was maintained at 2% of the airflow through the filter. Pressure drop in the insect chamber due to the collector trap was reduced to near zero by the use of vacuum downwind of the collector filter.

In preliminary experiments, volatiles emitted by male and female medflies were collected on traps using charcoal or Porapak-Q (Alltech Assoc., Deerfield, Illinois) as the adsorbent. Most collector traps initially were extracted in Guatemala as described below, and the extracts were sealed in glass ampoules and shipped to Gainesville, Florida. Some traps were sealed with Teflon tape and transported in glass vials to be extracted in Gainesville, Florida. Analyses of extracts obtained by both methods determined that the filters could be sealed with Teflon tape without loss of the collected volatiles. Based on results of this initial work, subsequent analyses were done on Porapak-Q collector traps that were sealed with Teflon tape and extracted after arrival in Gainesville, Florida.

Charcoal traps (2 mm × 4 mm ID) were prepared as described by Tumlinson et al. (1981). After collection of volatiles, each collector trap was extracted with three 20-μl portions of CH_2Cl_2 followed by three 20-μl extractions with hexane. A 200-ng internal standard of hexadecane was added, and each sample was concentrated to ca. 50 μl under argon for analysis as described below. Porapak-Q traps were prepared by packing ca. 60 mg of the adsorbent in 4-cm-long × 4.0-mm-ID glass tubes, resulting in a bed length of 15 mm. Silanized glass wool was used to contain the adsorbent. The Porapak-Q traps were cleaned by Soxhlet extraction with CH_2Cl_2 for 24 hr prior to use. Volatiles collected on the Porapak-Q traps were eluted with 500 μl of hexane–ether (90:10) and then 200 ng of hexadecane was added as internal standard for subsequent analyses. The average amount of each volatile component collected

per male medfly per hour, as determined by GC analyses (see later), was designated as one male-hour-equivalent (MHe).

Chemical Analyses. Gas chromatographic (GC) analyses were conducted using a Hewlett-Packard model 5890 gas chromatograph, equipped with splitless capillary and cool-on column injectors and flame ionization detectors. Helium was used as the carrier gas at a linear flow velocity of 18 cm/sec. Columns used in this investigation were: 50 m × 0.25 mm ID BP-1, 50 m × 0.25 mm ID Supelcowax-10, and 50 m × 0.25 mm ID CPS-1, a high-polarity cyano-silicone column. Each was operated at 60°C for 2 min, then temperature programmed at 30°C/min to 160°C. The chromatographic data were stored and analyzed in a Nelson 4000 data system.

The identities of the compounds were confirmed by GC-MS analyses using the BP-1 capillary column, operated as described above, coupled to a Nermag model R1010 mass spectrometer in the chemical ionization mode. The reagent gas in the mass spectrometer was either methane or isobutane. Spectra of the natural products were compared with those of candidate synthetic compounds. Additionally, 300-MHz proton magnetic resonance (PMR) spectra were obtained on both natural and synthetic compounds. Carbon-13 spectra (75.4 MHz) were obtained on all synthetic compounds.

Chemicals. Geranyl acetate was purchased from ICN Biomedicals Inc. (Plainview, New York). Prior to use, the material was purified by $AgNO_3$ high-performance liquid chromatography (HPLC) using previously described methods (Heath et al., 1977). Analysis by capillary GC on the columns described above indicated a purity of >99%.

The ester ethyl-(*E*)-3-octenoate was prepared from (*E*)-3-octenoic acid (ICN Biomedicals) by the following method. The acid (1.42 g, 10 mmol) was placed in a 50-ml flask together with 2.00 ml (30 mmol) of absolute ethanol, 0.120 g of *p*-toluenesulfonic acid, and 25 ml of benzene. This mixture was heated under reflux overnight, with the water produced collected in a Dean-Stark trap. When 10 mmol of water had been collected, the mixture was diluted with ether and washed (separating funnel) with water-saturated sodium bicarbonate and dried over anhydrous sodium sulfate. Removal of the drying agent and solvent left a yellow oil that was vacuum distilled, bp 80°C/8 mm Hg, to give a clear liquid (1.53 g, 90% yield). The ester was purified by $AgNO_3$ HPLC. Mass spectra and NMR spectra were consistent with the structure of ethyl-(*E*)-3-octenoate. Capillary GC analysis on columns described above indicated a purity of >98%.

(*E,E*)-α-Farnesene was prepared by the method described by Negishi and Matsushita (1984) with modification. The 1-buten-3-yne used in this procedure was either purchased from Wiley Organics (Columbus, Ohio) or prepared by the method of Brandsma (1971). The toluene solution of trimethylaluminum

used in this procedure was purchased from Aldrich Chemical Company (Milwaukee, Wisconsin). The geranyl chloride used in the preparation was prepared from geraniol (Aldrich Chemical Company) by the method of Calzada and Hooz (1974). Briefly, 1-buten-3-yne is carboaluminated with trimethylaluminum using a catalytic amount of dichlorobis-(N^5-cyclopentadienyl) zirconium. Without isolation, the resulting alkenylalane is coupled with geranyl chloride in the presence of a catalytic amount of tetrakis(triphenylphosphine) palladium. The ratio of 1-buten-3-yne to geranyl chloride should be at least 2 : 1. The use of a smaller ratio resulted in excessive amounts of geranyl chloride remaining after work-up. While it is possible to add the geranyl chloride as a solution in THF, it was more convenient to add the catalyst as a solid via a piece of Gooch tubing. It was necessary to chill the contents of the reaction flask in a dry ice–isopropanol bath during the trimethylaluminum addition as this step is exothermic. Because of difficulty in maintaining a temperature of 0°C with an ordinary ice bath during the hydrolysis with 3 N hydrochloric acid, the hydrolysis was begun with the flask contents at −25°C (CO_2/CCl_4). Due to the large amount of gas evolved during the hydrochloric acid addition, it was necessary to add the acid slowly while at the same time maintaining the low temperature.

The removal of unreacted geranyl chloride from the crude product was very important, since its presence during distillation causes extensive decomposition of the product. Geranyl chloride and catalyst residues were removed as follows: after concentration on a rotary evaporator at aspirator vacuum, the residue (about 125 ml for the scale described in the reference) was diluted 2 : 1 with hexane and allowed to stand in the refrigerator (6–7°C) overnight, which resulted in a yellow precipitate. This was removed by filtration and the filtrate passed first through a short (15–20 cm) silica-gel column (60–200 mesh, EM Science, New York, New York) to remove the catalyst residue, and then through a short (15–20 cm) basic alumina column (Woelm, 60–200 mesh, activity grade I) for removal of the unreacted geranyl chloride. The solvents then were removed and the residue vacuum distilled as a colorless or slightly yellow liquid (bp ≈60° at 0.05 mm). The yields were in the range of 60–70%, which is lower than that reported by Negishi and Matsushita (1984). It is suspected that the catalyst that we used may have deteriorated and thus resulted in reduced yields. The (*E,E*)-α-farnesene was found to be 96% geometrically pure with an overall purity of 86% based on analysis by capillary GC. This material was purified by $AgNO_3$ HPLC to provide a compound that was of >98% purity. Infrared and NMR spectra were in complete agreement with those reported by Negishi and Matsushita (1984). Infrared reported (Negishi and Matsushita, 1984) (neat) cm^{-1} 3080 (m), 2960(s), 2900(s), 1664(m), 1635(m), 1601(m), 981(m), 883(s); [^{1}H]NMR reported (Negishi and Matsushita, 1984) [$CDCl_3$, $(CH_3)_4Si$] δ = 1.59 (S, 3H), 1.63 (S, 3H), 1.66 (S, 3H), 1.74 (S, 3H), 2.03 (m, 4H), 2.82 (t, J =

6, 2H); [^{13}C]NMR [$CDCl_3$, $(CH_3)_4Si$] δ = 11.62, 16.07, 17.63, 25.69, 26.89, 27.35 , 39.88, 110.37, 122.36, 124.50, 131.10, 131.74, 133.79, 135.55, 141.69.

Formulations. In the first field test, all three synthetic components were formulated in glass capillaries of different inside diameters and with different lengths of the vapor–air column above the liquid in the capillary in a manner similar to that reported by Weatherston et al. (1984, 1985a,b). Capillaries (A.H. Thomas Company, Philadelphia, Pennsylvania #7707-B20 series) were sealed at the bottom and release rates were measured by the methods previously described using Porapak-Q as the adsorbent (Landolt and Heath, 1987). Regression analysis of the release rate versus the cross-sectional area of the capillary divided by vapor column height then was obtained. Using the resulting equation from this curve, we obtained the capillary inside diameter and vapor column height required for the desired release rate. Capillary formulations used in this study are reported in Table 1. Further validation of this method was obtained by the collection of volatiles emitted from capillaries formulated in the same manner as those loaded for field bioassays. Similarly, random samples of capillaries used in field bioassays were returned to the laboratory and release rates were measured and compared to the predicted release rate for the given exposure time. Release rates reported are based on measurement at an ambient room temperature of 22.5°C. No attempt was made to adjust for temperature effects on the release rate of the pheromone in the field.

(*E,E*)-α-Farnesene was formulated on rubber septa (Heath et al., 1986) for the second field test, while geranyl acetate and ethyl octenoate were formulated as described for the first field test. Septa were loaded with 10, 50, 100, 500, and 1000 μg of (*E,E*)-α-farnesene in 200 μl hexane and aired in the hood for two days. The load amount required for the desired release rate was determined from the linear equation obtained from release rate versus septum load.

Field Tests. Tests were conducted during March 1987 and March 1988. Traps used were 7.5-cm-diam. polypropylene hollow spheres painted black and coated with Stickum Special. Such a design was found to be very attractive visually to both sexes of medfly and was recommended by Nakagawa et al. (1978) for the testing of attractants. Pheromone dispensers were mounted on a 1-cm-long piece of 0.95-cm-ID Tygon tubing glued to the sphere ca. 2 cm down from the sphere top (trap hanger point). An "umbrella" also was mounted over pheromone dispensers on each trap to prevent rain from contaminating the capillary tubing. Each umbrella was a white plastic vial snap-cap (2 cm diam.) mounted above the lures with an insect pin stuck into the tubing. Traps were set up in north-to-south rows of coffee shrubs in a coffee farm near Antigua, Guatemala (Finca Portrero, at Ciudad Viejo). Dosages were initially randomized within each block and traps were rotated one position daily through the duration of the experiment. Each replicate block was placed in one row or hedge

TABLE 1. RELEASE RATE OF MEDFLY PHEROMONE FROM CAPILLARIES

	Capillary		Capillary formulations			
			Day 5 release rate		Day 30 release rate	
Release (MHe)	ID (mm)	Vapor height (mm)	Predicted[a] (ng/hr)	Measured laboratory[b] (ng/hr ± SD)	Predicted (ng/hr)	Measured field[c] (ng/hr ± SD)
	Ethyl octenoate					
0.3	0.59	20	198	167 ± 6	166	164 ± 8
1.6	0.84	9	881	862 ± 32	700	704 ± 12
3.2	1.19	9	1763	1627 ± 82	1290	1415 ± 120
6.4	1.68	9	3527	3360 ± 68	3280	
	Geranyl acetate					
0.3	1.19	20	68	76 ± 1	76	76 ± 1
1.6	1.68	8	339	351 ± 9	290	295 ± 16
3.2	1.68	4	677	700 ± 21	520	532 ± 23
6.4	2.49	4	1485	1500 ± 85	1380	
	(*E*,*E*)-α-Farnesene					
0.3	1.19	10	80	85 ± 13	78	95 ± 8
1.6	1.68	5	320	333 ± 34	310	410 ± 8
3.2[d]	1.68	5	640	642 ± 42	620	630 ± 68
6.4	3.49	6	1151	1130 ± 67	1090	

[a] Predicted based on regression analysis as follows: Ethyl octenoate release rate (ng/hr) = 14.3 × area of capillary ÷ length of vapor height (r^2 = 0.98, N = 10); geranyl acetate release rate (ng/hr) = 1.22 × area of capillary ÷ length of vapor height (r^2 = 0.97, N = 10); (*E*,*E*)-α-Farnesene release rate (ng/hr) = 0.72 × area of capillary ÷ length of vapor height (r^2 = 0.99, N = 10).

[b] Mean of three analyses.

[c] Mean of three analyses. Samples of 6.4 MHe used in field were lost.

[d] Release rate of 3.2 MHe was obtained by using two of the 1.6 MHe capillaries.

of coffee shrubs. Traps within blocks were 7.6 m apart. Blocks were roughly 8 m apart and traps were hung from coffee branches at heights of 1–1.5 m. Each day, flies were removed, sexed, and counted, and each days' catch was considered a replicate. In the first series of tests (1987), the attractiveness of the three major chemicals identified from wild medfly male volatiles was evaluated using a release rate equivalent to 0.3, 1.6, 3.2, and 6.4 MHe in the same ratio emitted by the wild male medflies. Spheres without chemicals were used as blanks. All chemicals were formulated in glass capillaries as described. Jackson traps (Harris et al., 1971) with a cotton wick containing 2 ml of standard trimedlure, used in USDA APHIS monitoring programs, were placed within test plots to provide an indication of fly populations. Traps and trimedlure were obtained from USDA, APHIS, PPQ, Guatemala City, Guatemala. Trimedlure baits are the

standard means of monitoring medfly populations. Three blocks of the six treatments were randomized daily for a six-day period. The second series of tests (1988) were set up in a similar manner with the following changes. The (*E,E*)-α-farnesene was dispensed from rubber septa, and no trimedlure-baited traps were placed in test plots. The duration of this test was eight days. Trap catch data were subjected to ANOVA and daily means separated using Duncan's (1955) multiple-range test.

RESULTS

Analysis of Volatile Constituents. Analysis of volatiles collected from 5- to 10-day-old virgin males and females indicated that three major compounds were present and were produced only by male medflies (Figure 1). These compounds were identified as geranyl acetate, ethyl-(*E*)-3-octenoate, and (*E,E*)-α-farnesene, based on retention times on the BP-1 and Supelcowax columns and the comparison of NMR and EI and CI mass spectra with synthetic compounds. These compounds accounted for 90% of total peak areas seen in the chromatograms and were previously reported by Baker et al. (1985) and Jang et al. (1989) to be present in the volatiles emitted by male medflies. Additionally, in

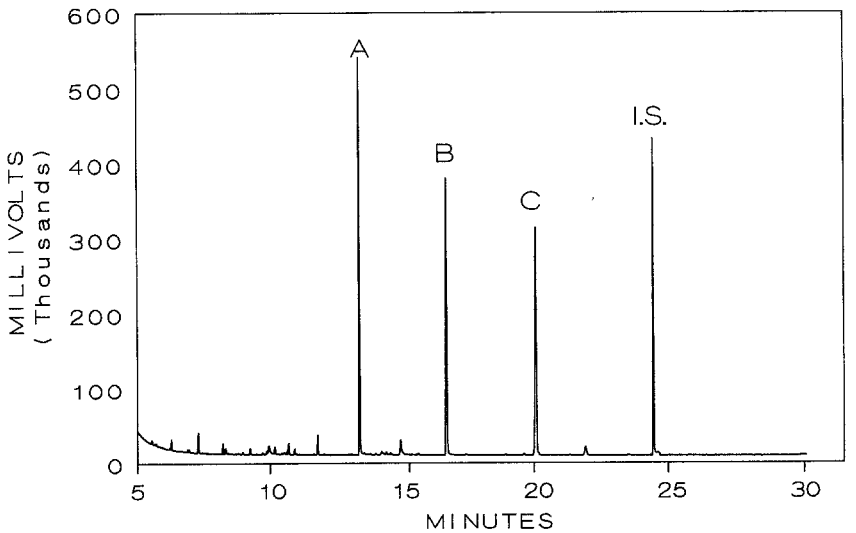

FIG. 1. Volatiles collected from eight wild male medflies during the third to fifth hour of the photophase, analyzed on a 50 m × 0.25 mm ID BP-1 (SGE) fused silica capillary GC column. Peak A = ethyl-(*E*)-3-octenoate, B = geranyl acetate, C = (*E,E*)-α-farnesene, and I.S. = internal standard, 8 μg of hexadecane.

the preliminary experiments, volatiles extracted from Porapak-Q traps immediately after collection from male medflies in Guatemala and sealed in ampoules and analyzed later in Gainesville were identical to those from collector traps sealed with Teflon tape, returned to Gainesville, and then extracted and analyzed. We confirmed that these compounds do not degrade when adsorbed on Porapak-Q for 20 days by analysis of synthetic material that was collected and extracted, and analyzed immediately compared with material that was collected, stored under ambient conditions in the laboratory for 20 days, and then extracted. It was noted during the preliminary experiment ($N = 10$) that less (E,E)-α-farnesene was recovered from volatiles collected on charcoal traps than from volatiles collected on Porapak-Q collector traps. This also was confirmed by the collection of volatilized synthetic compound, which indicated that a 40 $\pm$ 5.2% ($\bar{X} \pm$ SD) loss of (E,E)-α-farnesene occurred when charcoal was used in the collector trap compared with Porapak-Q ($N = 6$).

Periodicity of Pheromone Production. The results of the analyses of volatiles collected on Porapak-Q for 2-hr periods throughout the photophase from wild male medflies 5–10 days old is shown in Figure 2. Peak collection of volatiles occurred 3–5 hr into the photophase, with less material collected in the earlier and later 2-hr periods. No differences were observed in the patterns

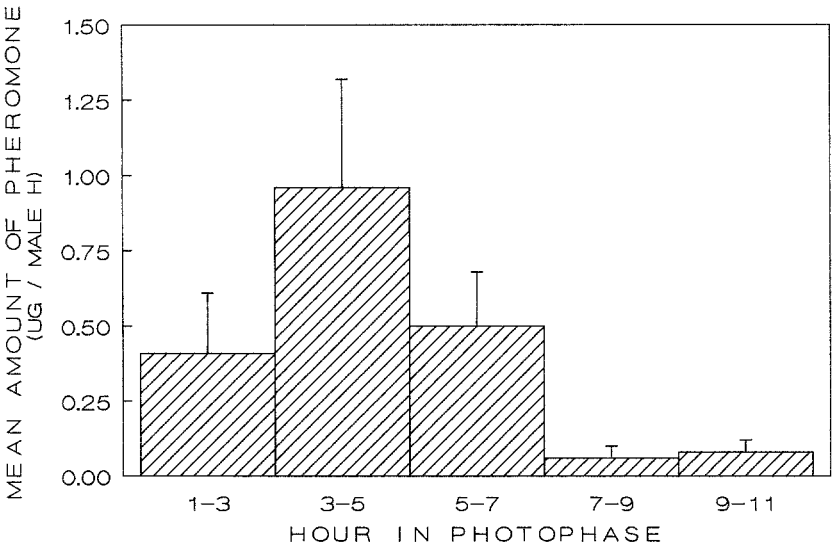

FIG. 2. Mean amount of 18 pheromone collections using Porapak-Q from wild male medflies 5–10 days old in groups of 8–12 flies/chamber for 2-hr periods throughout the photophase. Small bars indicate standard deviation.

of pheromone released by wild (N = 16) versus laboratory-reared (N = 11) flies.

Comparison of Volatiles. Significant differences in the ratios of ethyl-(E)-3-octenoate, geranyl acetate, and (E,E)-α-farnesene were found when the volatiles collected on Porapak-Q traps from wild medflies were compared with volatiles obtained from laboratory-reared flies (Figure 3) during the third to fifth hour of the photophase. Average percentages of ethyl-(E)-3-octenoate, geranyl acetate, and (E,E)-α-farnesene from wild males were 50.3 $\pm$ 5.6 SD, 24.7 $\pm$ 2.7 SD, and 25.0 $\pm$ 4.0 SD, respectively (N = 18), while average percentage releases from the laboratory-reared males were 29.6 $\pm$ 5.9 SD, 38.3 $\pm$ 2.7 SD, and 32.1 $\pm$ 3.8 SD, respectively (N = 10). The mean percent of ethyl-(E)-3-octenoate in wild fly volatiles was significantly greater than that in lab fly volatiles (t = 9.04, P = <0.00001), while the mean percent of geranyl acetate was significantly less in wild fly volatiles (t = 26.0, P < 0.001). The total amount of the three components released by the 5- to 10-day-old laboratory-reared flies during the 2-hr period of peak production was 360 $\pm$ 180 SD ng/male/hr (N = 6) while the 5- to 10-day-old wild flies released 960 $\pm$ 240 SD

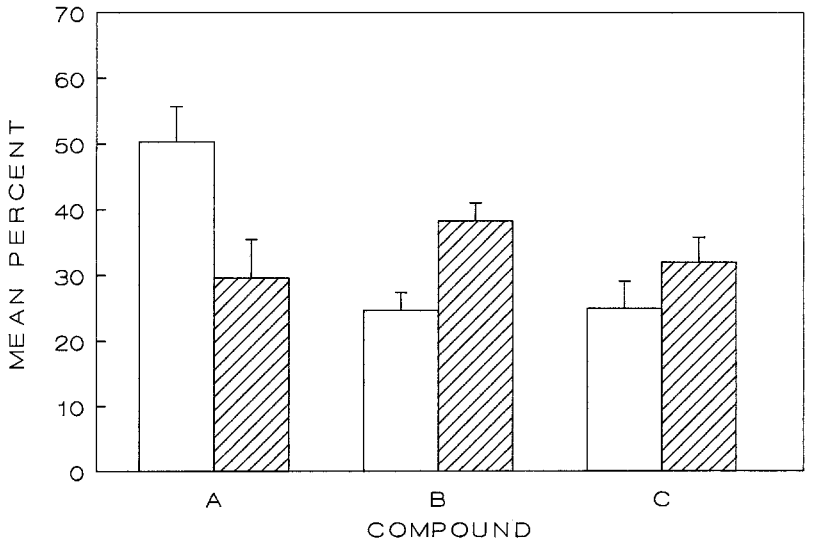

FIG. 3. Comparison of volatiles collected on Porapak-Q traps from wild medflies (N = 18) with volatiles obtained from laboratory reared flies (N = 10) during the 3- to 5-hr period of the photophase. Open bars indicate the percent of compound emitted by wild flies and slash bars the percent from laboratory reared flies. Percents are normalized to 100. Small bars indicate standard deviation. Letter designation as follows: A = ethyl-(E)-3-octenoate, B = geranyl acetate, and C = (E,E)-α-farnesene.

ng/male/hr ($N = 6$). The release rate used for subsequent formulation of synthetic material was based on the amount released by the wild flies (960 ng/hr).

Formulations. Regression analyses of release rate versus the area of the capillary–vapor column height was obtained for each of the three components used in the field bioassay. Results from the linear regression analyses are shown in Table 1. Capillaries then were formulated from these data to provide the desired release rate of each compound at approximate released percentages of 55.2 ± 0.6 SD, 23.9 ± 0.8 SD, and 21.0 ± 1.3 SD for ethyl-(*E*)-3-octenoate, geranyl acetate, and (*E,E*)-α-farnesene, respectively. Prior to use in the field, release rates and ratios of the formulations were determined in the laboratory by GC analysis of collected volatiles and compared to the desired predicted values (Table 1). Release rate and ratio data obtained from laboratory studies indicated that the longevity of the formulation was in excess of two weeks with less than a 5% decrease in emission rate of any of the three compounds investigated. After field use, capillaries were returned to the laboratory, and the change in vapor column height was measured. Additionally random samples of the capillaries used in the field were taken, and the release rates from these capillaries were measured and were found to be in close agreement with the predicted rate for the time they were used (Table 1). These measurements were in agreement with measurements obtained under laboratory conditions. It was noted that use of large (3.49 mm ID) capillaries for the formulation of (*E,E*)-α-farnesene resulted in what appeared to be movement of the compound towards the top of the capillary. Although the release rates from the capillaries were consistent with the predicted release rates, this observation prompted us to formulate (*E,E*)-α-farnesene in rubber septa for the second series of field tests. Based on regression analysis of release rate of (*E,E*)-α-farnesene versus increased load on rubber septa, a release rate approximately the same as that from the capillaries used the previous year was obtained. Release rates were measured in the laboratory from septa after two to nine days of exposure in the fume hood. The results of these analyses are shown in Table 2. Delays in shipment of the septa used in the field precluded the analysis of release rates from these septa.

Field Tests of Three Component Pheromone Blends. In the 1987 field tests, we compared a control unbaited sphere to spheres baited with the three component blend formulated to release at 0.3, 1.6, 3.2, and 6.4 MHe (Figure 4). A total of 259 female medflies were caught in this test. Females were trapped with all treatments and the control trap; however, the sphere baited with 1.6 MHe of pheromone was significantly more attractive. It should be noted in this test that traps with pheromone released at 0.3 and 3.2 MHe caught significantly fewer females than the spheres baited with pheromone that released 1.6 and 6.4 MHe. In field tests conducted during 1988, the same general trend in female trap captures on spheres loaded with different amounts of the three-component

TABLE 2. RELEASE RATE OF (*E*,*E*)-α-FARNESENE FROM RUBBER SEPTA

	Rubber septa formulations (*E*,*E*)-α-Farnesene			
			Measured[b] release rate (ng/hr ± SD)	
MHe	Desired release rate (ng/hr)	Septa[a] load (μg)	Day 2	Day 9
0.3	80	20	70 ± 7	65 ± 8
1.6	320	100	345 ± 40	326 ± 43
3.2	640	200	690 ± 50	652 ± 58
6.4	1151	400	1208 ± 72	1142 ± 67

[a]Septa load based on release rate (ng/hr) = 34.54 × load amount (μg), $r^2 = 0.991$ (septa were loaded with four increasing amounts of compound and release rates from each septum were determined three times).
[b]Mean of three analyses.

male pheromone was observed (Figure 4); traps baited with pheromone released at 1.6 and 6.4 MHe caught more females than the blank and the 0.3 and 3.2 MHe pheromone traps. In this test, a total of 368 females were trapped. Statistically, only the spheres baited with pheromone released at 1.6 MHe were significantly better than the other treatments. This may be due in part to the use of rubber septa formulation for (*E*,*E*)-α-farnesene in this test. Release rates from rubber septa are wind dependent, and this may account for the high variance in capture of flies in traps baited with pheromone as was found in the statistical analysis of data.

DISCUSSION

The first report of a putative medfly pheromone was published by Jacobson et al. (1973). Recent findings do not support their findings, and it is suggested that due to limited analytical methodology available at the time the report is incorrect. Baker et al. (1985) identified nine volatile compounds emitted and/or extracted from sexually mature male Mediterranean fruit flies. The identified components in addition to those reported here included 3,4-dihydro-2H-pyrrole, (*E*)-2-hexenoic acid, dihydro-3-methylfuran-2(3,H)-one, 2-ethyl-3,5-dimethylpyrazine, linalool, and ethyl acetate. It was claimed that the 3,4-dihydro-2H-pyrrole is active, but no data were reported to support pheromonal activity of this or other identified compounds. The amounts and ratios of the nine identified components released by male flies were not reported. Jang et al. (1989) detected 69 compounds using GC analysis of collections of headspace from sexually mature male laboratory reared medflies and identified 56 of these compounds.

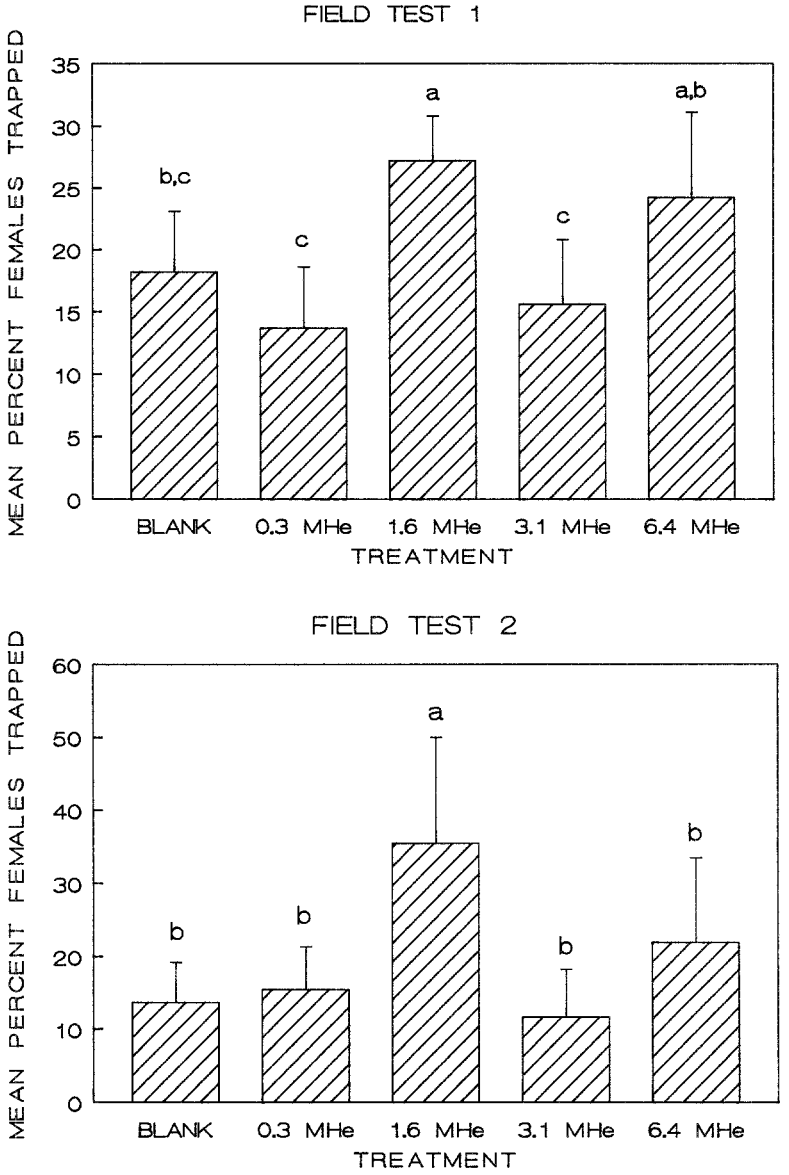

FIG. 4. Results of the field test 1 conducted in 1987 and field test 2 in 1988. Comparison of a blank sphere with spheres that had the three-component blend formulated to release pheromone in increased amounts. Release rate is in male hour equivalents (MHe) based on amount produced by wild male medflies. Bars with the same letter are not significantly different, Duncan's (1955) multiple range test ($P < 0.05$, $N = 6$ for test 1 and $N = 8$ for test 2).

Evaluation of six compounds [ethyl-(*E*)-3-octenoate, geranyl acetate, (*E,E*)-α-farnesene, 3,4-dihydro-2H-pyrrole, linalool, and ethyl acetate individually and as a blend] showed significant activity for all compounds and the blend compared to blanks. Baker et al. (1990), using sterile medflies, tested racemic linalool, 2,3-dimethylpyrazine, 2,5-dimethyl pyrazine, and geranyl acetate individually and in combination. Based on the results of female capture data using baited yellow delta traps and yellow squares, it was determined that the traps containing large amounts of the compound(s) singularly and in combination were more attractive to released female medflies than the control trap.

It is apparent from the studies reviewed that there are a considerable number of putative pheromonal compounds produced by male Mediterranean fruit flies. Our research effort was to provide some focus regarding the release, synthesis, formulation, and testing of three major compounds released by male medflies. A principal problem in interpreting the results of previous published studies on medfly pheromone is the lack of quantitative data and control over chemical release ratios and rates, both in analysis of male volatiles and in testing of compounds for pheromonal activity.

In this paper we demonstrated that ethyl-(*E*)-3-octenoate, geranyl acetate, and (*E,E*)-α-farnesene are three major compounds emitted by virgin male medflies and are released in a precise ratio within a population or group from one source, although this ratio differs significantly between laboratory-reared and wild flies. We have developed a formulation method that releases the three synthetic pheromone compounds in the same ratio released by wild males. Field tests demonstrated the attractiveness of the formulated blend to medfly females at 1.6 MHe but indicate a need for precise control of release rate. Thus we demonstrated that female medflies can be captured in the field with a three-component synthetic pheromone blend. In the first field evaluation of the pheromone, it was found that the black sphere baited with pheromone at a release equivalent to 1.6 MHe captured 25 times more female medflies than the trimedlure in the Jackson trap (11 females/pheromone trap/day versus 0.44 females/trimedlure trap/day). Conversely, the trimedlure-baited Jackson trap caught 14 times more male medflies than the pheromone-baited trap that released at 1.6 MHe (29 males/trimedlure trap/day versus 2.1 males/pheromone trap/day). Differences in lure type and trap design, however, preclude statistical comparison of this data.

The apparent bimodal response distribution observed on traps baited with pheromone at different release ratios is similar to that observed for the papaya fruit fly attraction to 2-methyl-6-vinylpyrazine (Landolt et al., 1988; Landolt and Heath, 1990). The apparent bimodal nature of the numbers of papaya fruit fly females captured at different pheromone release rates was likely due to different responses by mated versus virgin females to the pheromone (Landolt and Heath, 1990). Female medflies captured in these tests were not preserved and

dissected to determine their mating status. More field tests and behavioral studies are planned to investigate the nature of the dose–response relationship, the efficacy of the three-component pheromone system, and whether other compounds reported and yet to be identified as released by males will enhance attraction and capture of female medflies.

REFERENCES

BAKER, P.S., HOWSE, P.E., ONDARZA, R.N., and REYES, J. 1990. Field trials of synthetic sex pheromone components of the male Mediterranean fruit fly (Diptera: Trypetidae) in Mexico. *J. Econ. Entomol.* 6:2236–2245.

BAKER, R., HERBERT, R.H., and GRANT, G.G. 1985. Isolation and identification of the sex pheromone of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.). *J. Chem. Soc. Chem. Commun.* 1985:824–825.

BEROZA, M., GREEN, N., GERTLER, S.I., STEINER, L.F., and MIYASHITA, D.H. 1961. Insect attractants. New attractants for the Mediterranean fruit fly. *J. Agric. Food Chem.* 9:361–365.

BRANDSMA, L. 1971. Page 120, *in* L. Brandsma (ed.). Preparative Acetylenic Chemistry. Elsevier, New York.

CALZADA, I.G., and HOOZ, I. 1974. Geranyl chloride [(*E*)-1-chloro-3,7-dimethyl-2,6-octadiene]. *J. Org. Synth.* 54:63–67.

DOWELL, R.V., and WANGE, L.K., 1986. Process analysis and failure avoidance in fruit fly programs, pp. 43–65, *in* M. Mangel, J.R. Carey, and R.E. Plant (eds.). Pest Control. NATO ASI Series, Springer-Verlag, New York.

DUNCAN, D.B., 1955. Multiple range and multiple *F* tests. *Biometrics* 11:1–41.

FÉRON, M. 1962. L'instinct de reproduction chez la mouche Mediterranéene des fruits *Ceratitis capitata* Wied. (Diptera: Trypetidae). Comportement sexuel-comportement de ponte. *Rev. Pathol. Veg. Entomol. Agric. Fr.* 41:1–129.

HARRIS, E.J., NAKAGAWA, S., and URAGO, T. 1971. Sticky traps for detection and survey of three tephritids. *J. Econ. Entomol.* 64:62–65.

HEATH, R.R., TUMLINSON, J.H., DOOLITTLE, R.E., and DUNCAN, J.H. 1977. Analytical and preparative separation of geometrical isomers by high efficiency silver nitrate liquid chromatography. *J. Chromatogr. Sci.* 15:10–13.

HEATH, R.R., TEAL, P.E.A., TUMLINSON, J.H., and MENGELKOCH, L.J. 1986. Prediction of release ratios of multicomponent pheromones from rubber septa. *J. Chem. Ecol.* 12(12):2133–2143.

JACOBSON, J., OHINATA, K., CHAMBERS, D.L., JONES, W.A., and FUJIMOTO, M.S. 1973. Insect sex attractants. 13. Isolation, identification and synthesis of sex pheromones of the male Mediterranean fruit fly. *J. Med. Chem.* 16:248–251.

JANG, E.G., LIGHT, D.M., FLATH, R.A., NAGATA, J.T., and MON, T.R. 1989. Electroantennogram responses of the Mediterranean fruit fly, *Ceratitis capitata* to identified volatile constituents from calling males. *Entomol. Exp. Appl.* 50(1):7–19.

LANDOLT, P.J., and HEATH, R.R. 1987. Role of female-produced sex pheromone in behavioral reproductive isolation between *Trichoplusia ni* (Hübner) and *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae, Plusiinae). *J. Chem. Ecol.* 13:1005–1018.

LANDOLT, P.J., and HEATH, R.R. 1990. Effects of pheromone release rate and time of day on catches of male and female papaya fruit flies (Diptera: Tephritidae) on fruit-model traps baited with pheromone. *J. Econ. Entomol.* 83:2040–2043.

LANDOLT, P.J., HEATH, R.R., AGEE, H.R., TUMLINSON, J.H., and CALKINS, C.O. 1988. A sex

pheromone-based trapping system for the papaya fruit fly, *Toxotrypana curvicauda* Gerstaecker (Diptera: Tephritidae). *J. Econ. Entomol.* 81(4):1163–1169.

NAKAGAWA, S., FARIAS, G.J., and STEINER, L.F. 1970. Response of female Mediterranean fruit flies to male lures in the relative absence of males. *J. Econ. Entomol.* 63:227–229.

NAKAGAWA, S., PROKOPY, R.J., WONG, T.T., ZIEGLER, J.R., MITCHELL, S.M., URAGO, T., and HARRIS, E.J. 1978. Visual orientation of *Ceratitis capitata* flies to fruit models. *Entomol. Exp. Appl.* 24:193–198.

NEGISHI, E., and MATSUSHITA, H. 1984. Palladium-catalyzed synthesis of 1,4-dienes by allylation of alkenylalanes: α-Farnesene (1,2,6,10-dodecatetraene, 3,7,11-trimethyl). *Org. Synth.* 62:31–38.

TUMLINSON, J.H., TEAL, P.E.A., and HEATH, R.R. 1981. Chemical ethology: A holistic approach to the study of insect pheromones, pp. 19–31, *in* Proceedings 1st Japan/USA Symposium on IPM. Tsukuba, Japan, September 29–30.

WEATHERSTON, I., MILLER, D., and DOHSE, L. 1984. Release of volatile materials from glass capillaries—a reinvestigation, pp. 114–115, *in* Proceedings 11th International Symposium Controlled Release Bioactive Materials. Fort Lauderdale, Florida.

WEATHERSTON, I., MILLER, D., and DOHSE, L. 1985a. Capillaries as controlled release devices for insect pheromones and other volatile substances—a reevaluation: Part I. Kinetics and development of predictive model for glass capillaries. *J. Chem. Ecol.* 11:953–965.

WEATHERSTON, I., MILLER, D., and LAVOI-DORNIK, J. 1985b. Capillaries as controlled release devices for insect pheromones and other volatile substances—a reevaluation: Part II. Predicting release rates from Celcon and Teflon capillaries. *J. Chem. Ecol.* 11:967–968.