Volatile Pheromonal Emissions from the Male Mediterranean Fruit Fly: Effects of Fly Age and Time of Day

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Pheromonal emissions from "calling" male Mediterranean fruit flies (*Ceratitis capitata* Wied.) were trapped in Tenax-packed traps, using 25-fly groups of laboratory-reared flies in a 5-L glass/Teflon/stainless steel chamber swept with purified air. Three different fly ages were used (5–6, 11–12, and 20–21 days old), and early-, mid-, and late-morning samples were collected from the first two age groups. Thirty-two components were identified; four had not been previously reported [propan-2-ol, hexanal, phenol, and (*Z,E*)-α-farnesene], and three others had been only partially identified in an earlier study [prop-2-yl (*E*)-3-octenoate, ethyl (*E*)-2-octenoate, and propyl (*E*)-3-octenoate]. Quantitatively, ethyl acetate, 1-pyrroline, ethyl (*E*)-3-octenoate, geranyl acetate, and (*E,E*)-α-farnesene were the most abundant emission components from 5–6- and 11–12-day-old flies. (*E*)-2-Hexenoic acid was also a major component but was not as readily quantified. Total emissions release appeared to peak in early morning with 5–6-day-old flies. With 11–12-day-old flies, the peak tended to move to mid- to late-morning, and limited semiquantitative data for 20–21-day-old flies suggest a late-morning maximum.

INTRODUCTION

The Mediterranean fruit fly, *Ceratitis capitata* Wied. (medfly), has the potential to cause major economic losses to agriculture in those portions of the United States where the climate approximates that of the Mediterranean region. This includes large portions of California, where favored hosts of the medfly such as citrus and stone fruit are grown extensively. Many other plant crops growing in such areas are also hosts of this tephritid, so the agricultural industry is very concerned about keeping the fly from becoming established. Economically, the threat posed by the medfly is twofold. In addition to the direct crop damage caused by fly larvae in fruit, secondary problems arise when numerous crops from infested regions are rejected by potential purchasers from fly-free parts of the country or world.

Since the medfly is presently established in Hawaii and in numerous other parts of the world, but not on the U.S. mainland, a major goal of state and federal agencies is to keep the fly from becoming established in the continental United States. If flies are found to have been introduced, major efforts are made to eradicate the introduced flies, to prevent them from becoming permanent residents on the mainland. The first line of defense in such efforts is detection, using arrays of traps placed around likely introduction points such as seaports and airports. The effectiveness of such arrays is directly related to the potency of attractants used in the individual traps. At present, the materials used are male lures such as trimedlure and ceralure. These are compounds identified empirically as attractants through screening tests and synthesis.

Several research groups have investigated the volatile pheromonal emissions released by the male medfly as a potential source for an effective virgin female attractant. No specific attractant for the female medfly is presently available, although such an attractant would be particularly useful for early detection of population outbreaks. If sufficiently potent, such an attractant might also find use in female annihilation programs and in mating disruption efforts. Féron (1959, 1962) described the "calling" behavior of the mature male medfly, which he associated with release of pheromonal volatiles attractive to the virgin female fly. Lhoste and Roche (1960) further describe this calling process and suggested that several abdominal glands present in the males were involved in production and release of the pheromone mixture. Jacobson et al. (1973) and Ohinata et al. (1977, 1979) reported the isolation and identification of methyl (*E*)-6-nonenoate, (*E*)-6-nonen-1-ol, and 15 carboxylic acids from cold-trapped male emissions. Laboratory and field bioassay results were ambiguous. Jacobson and Ohinata (1980) subsequently reported finding (−)-β-fenchol in male medfly emissions trapped on a porous polymer but could detect no attractiveness with the compound alone or in combination with previously identified components. Baker et al. (1985) identified an entirely different group of nine compounds in trapped male medfly emissions, including the somewhat uncommon 1-pyrroline. The three major components found by them were ethyl (*E*)-3-octenoate, geranyl acetate, and (*E,E*)-α-farnesene. In their laboratory bioassays of individual components, using an olfactometer and virgin female flies, only the 1-pyrroline was attractive, and it was judged highly attractive. A more extensive list of identified male emission components was published by some of the present authors (Jang et al., 1989). With the exception of (*E*)-2-hexenoic acid, all of the identifications made by Baker et al. (1985) were verified in this 1989 study. Baker et al. (1990) field-tested four compounds, linalool, geranyl acetate, and 2,3- and 2,5-dimethylpyrazines, which are reported by those authors to be male medfly emission components and to have biological activity in laboratory bioassays. The first two of these compounds are included among the pheromone components first reported by Baker et al. (1985). More recently, Heath et al. (1991) have monitored the release of three of the major

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Chart I. Blank Trappings

3:25 p.m. (afternoon before 5–6-day-old fly trapping sequence)	stainless steel trap 1	trapped 7 min at 100 cm^3/min	MS
3:34 p.m. (afternoon before 5–6-day-old fly trapping sequence)	glass trap 2	trapped 50 min at 100 cm^3/min	FID, MS
7:13 p.m. (evening before 20–21-day-old fly trapping sequence)	stainless steel trap 10	trapped 6 min at 100 cm^3/min	MS
7:28 p.m. (evening before 20–21-day-old fly trapping sequence)	glass trap 11	trapped 51 min at 100 cm^3/min	FID, MS

male medfly emission components [ethyl (*E*)-3-hexenoate, geranyl acetate, and (*E,E*)-α-farnesene] first reported by Baker et al. (1985), using Guatemalan medflies. They verified the identifications of these components and quantified their release rates as functions of time of day and fly origin (wild vs laboratory-reared). Formulations of the three components were prepared, and their field attractiveness to female medflies was demonstrated.

The present study was undertaken to provide better semiquantitative data on the release rates of male medfly emission components and to examine the effects of both male fly age and time of day on the emissions profile produced by calling males. Any efforts to develop a male emissions-based synthetic formulation for use as a virgin female attractant must necessarily taken into consideration both the overall emission rates from calling males and the relative amounts of individual components within the emission profile. Release rates of a number of the more volatile major components such as 1-pyrroline are of particular interest, in light of the statement by Baker et al. (1985) that 1-pyrroline is attractive to virgin female flies. By trapping from males of different ages, at several times during the morning, we can better understand how widely the two factors, overall emissions release rate and emissions profile makeup, might vary in a field situation.

EXPERIMENTAL PROCEDURES

Reference Compounds. Authentic samples of compounds tentatively identified by GC/MS examination of headspace-trapped emissions were obtained commercially, were isolated from known natural sources, or were synthesized by conventional methods. For example, 1-propyl and prop-2-yl (*E*)-3-octenoate were synthesized by acid-catalyzed esterification of (*E*)-3-octenoic acid, obtained via the synthetic method of Linstead et al. (1933). Ethyl (*E*)-2-octenoate was obtained from a commercial source (Aldrich-Bader Collection).

Insects. Mediterranean fruit fly pupae were obtained from the mass rearing colony at the USDA-ARS Tropical Fruit and Vegetable Research Laboratory in Honolulu, HI. They were sexed before adult emergence. After emergence, they were provided with water, sugar, and protein, and then 1–2 days before trapping, they were switched to sugar and water only. The flies were immobilized by slight chilling before being introduced into the sampling chamber and were allowed to equilibrate in the chamber for 30 min prior to trapping.

Trapping Chamber. The chamber used to contain the flies was a 5-L round-bottom flanged flask and head, essentially as described previously (Jang et al., 1989). Flies and emissions were exposed only to borosilicate glass, Teflon (both TFE and FEP), stainless steel, and Viton O-rings (minimal inlet and outlet port seals). The apparatus was completely disassembled before each trapping sequence. The parts were thoroughly washed, rinsed, oven-dried, and reassembled while hot. Breathing-quality air (cylinder) was passed through an activated charcoal filter before entry into the chamber. While cooling, the system was purged at ca. 1 L/min air flow. The system was further purged overnight at ca. 100 mL/min before each morning use. The flow rate during trapping sequences was 100 mL/min, measured at the outlet end of the system.

Trap Design. Two trap types were used, stainless steel and glass. The stainless steel (ss) traps [0.7 g of 35/60 mesh Tenax GC; 9.5 mm ($^3/_8$ in.) o.d.] have been described previously (Takeoka et al., 1988; Jang et al., 1989). The glass traps consisted of a 2.5 cm o.d. × 12 cm long borosilicate glass body, with an extra coarse 2 cm o.d. fritted glass disk sealed inside, near one end. A 6.3 mm ($^1/_4$ in.) o.d. tube was sealed on the inlet end (near the frit) and a 9.5 mm ($^3/_8$ in.) o.d. tube on the outlet end of the trap body. Each glass trap was filled with 6.5 g of 60/80 mesh Tenax GC, held in place by the glass frit and a loose plug of washed glass wool. Both trap designs were capped with stainless steel Swagelok caps, using ceramic-filled Teflon ferrules.

Trap Conditioning. Each trap was mounted vertically in a conditioning oven. Helium was passed through activated charcoal and oxygen scrubbing traps and then through the vertical trap from the inlet end (bottom) of the trap. The gas was then directed through an exit line to the outside of the oven for flow measurement. Each trap was conditioned at 225 °C for a minimum of several hours and then slowly cooled, with continued helium flow. While still warm, the trap was capped securely (exit end first).

Sampling Sequences. The sampling sequences used with each of three fly ages are shown as time lines in Figure 1. The trapping sequence with 20–21-day-old males was somewhat abbreviated, in comparison with those for the 5–6- and 11–12-day-old flies. This decision was made in part because the younger flies were of greater interest and also because insufficient numbers of ss traps were available to fully duplicate the other sequences. A number of blank trappings were also made, to check for background contaminants and workup artifacts. These blanks are listed in Chart I.

Trap Workup. *1. Stainless Steel Traps (Thermal Desorption).* Trapped volatiles were backflushed into a liquid nitrogen-cooled spiral stainless steel cryofocusing tube (1 mm i.d. × 15 cm long tube) using purified helium, while the ss trap was heated with an aluminum heating block (rt to 225 °C in 15–20 min). The spiral cryofocusing tube was switched to the head of the capillary column with the valving system, and then the LN trap was removed and the spiral tube heated to 225 °C in 15 s with a hot air gun, transferring the trapped volatiles to the head of the column.

2. Glass Traps (Solvent Desorption). Internal standard (ISTD) solution (10.1 mg of cyclodecanone in pentane) was placed on the inlet side of the trap bed. The trap was inverted, and the trapped volatiles and ISTD were eluted with two 25-mL portions of distilled ether/pentane (1:1 v/v). The eluant was concentrated by careful distillation to 0.1–0.2 g of solution.

Instrumentation. *1. Gas Chromatography.* A Hewlett-Packard 5830A GC fitted with a flame ionization detector (FID) and a laboratory-constructed valving system/cryofocusing trap was used for direct analysis of volatiles trapped in ss traps. A Hewlett-Packard 5890 Series II GC fitted with a flame ionization detector and a cool on-column injector was used for analysis of volatiles solvent-eluted from glass traps. A Hewlett-Packard 3365 Chemstation was used with the 5890 GC for data handling.

2. Gas Chromatograph/Mass Spectrometer/Data System. A FinniganMAT 4500 quadrupole mass spectrometer/SuperINCOS data system was fitted with a valving system/cryofocusing trap functionally identical to that on the HP 5830A GC. In addition, a cool on-column injector (Scientific Glass Equipment) was mounted on the Finnigan GC oven.

3. Capillary Columns. Identical 60 m × 0.32 mm i.d. (0.25-μm film thickness) bonded and cross-linked DB-1 fused silica columns (methylsilicone; J&W Scientific) were used in all instruments. All columns were fitted with ca. 1-m retention gaps. Gas chromatograph operating conditions were as follows: GC/FID headspace, −5 to 225 °C at 3 °C/min, 10 min isothermal; GC/FID on-column injection, 35–250 °C at 4 °C/min, 20 min isothermal; GC/MS headspace, −5 to 230 °C at 3 °C/min, no final period; GC/MS on-column injection, 35–235 °C at 4 °C/min, 20 min isothermal.

Internal Standard Calculations. Workup of glass trap contents included addition of an internal standard, so actual amounts of component trapped could be calculated directly from the initial GC/FID peak are values (ng/25 flies/50 min). Internal standard was not added to the stainless steel traps before thermal desorption, because of the practical problems of introducing small amounts of known compounds in a reproducible manner. Peak

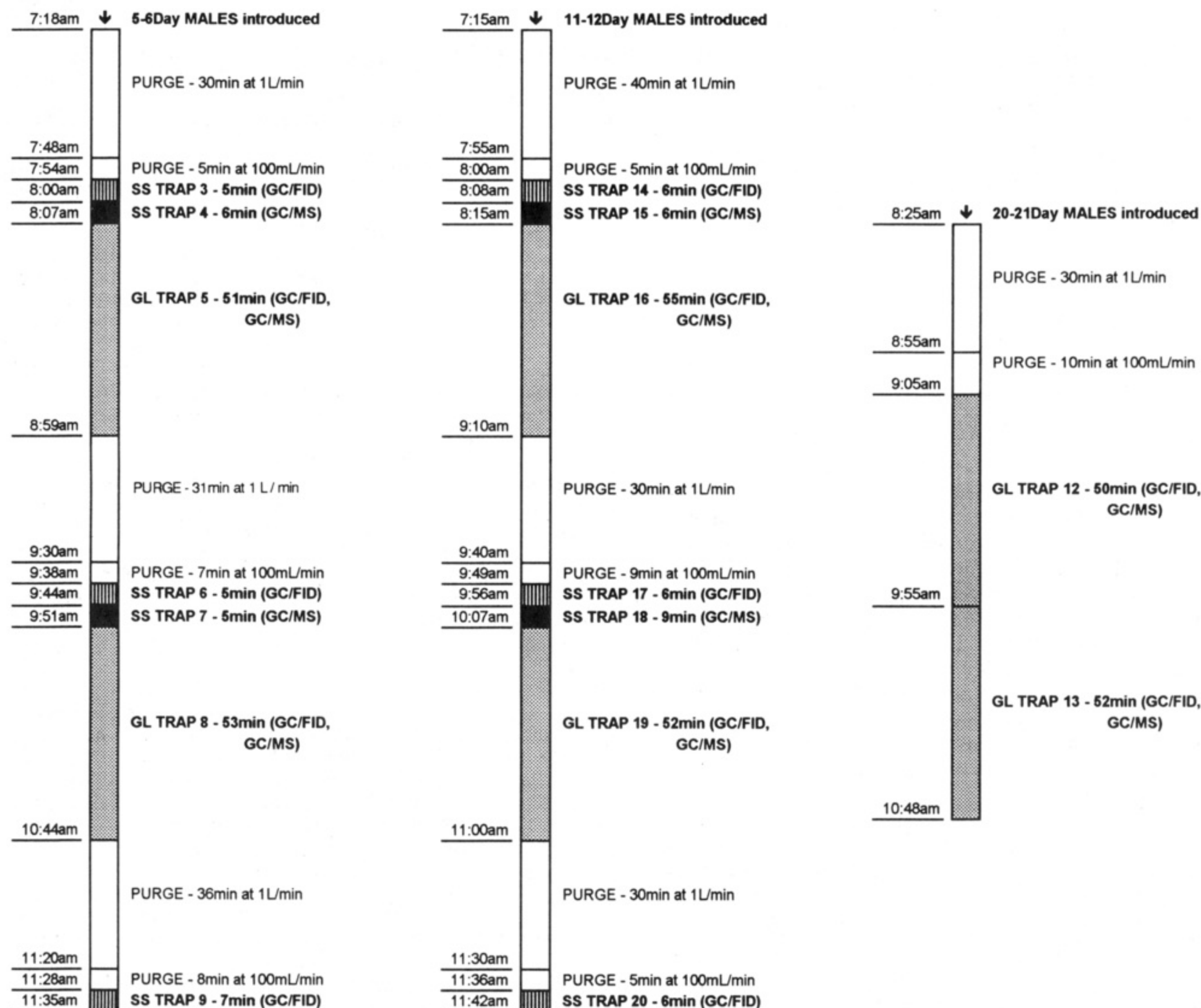

Figure 1. Time lines followed during male medfly emissions trapping. Blank trappings are not noted; see text (Sampling Sequences) for blanks information.

area counts from GC/FID runs of ss trap contents could not therefore be directly converted to similar component amount values (ng/25 flies/5 min). Ethyl (*E*)-3-octenoate, a major emission component trapped in both ss and glass traps, was therefore selected as a secondary standard, to permit conversion of the ss GC/FID peak area values into ng/25 flies/5 min units. Data from both 5–6- and 11–12-day-old fly trapping runs were used to calculate a conversion factor (ng/area count). Basically, ester area counts from two consecutive ss trap runs (e.g., SS3 and SS6) were averaged, and this average was divided into the ethyl (*E*)-3-octenoate nanogram value from the intervening glass trap run (GL5) (multiplied by 0.1 to adjust for a 5-min trapping interval). Repeating this process provided four intermediate factors (0.0053, 0.0050, 0.0081, and 0.0073 ng/area count) which, when averaged, gave an overall conversion factor of 0.0064 ng/area count. This was then applied to all ss trap area count values, yielding the ng/25 flies/5 min amounts listed in Table I.

RESULTS AND DISCUSSION

In any laboratory situation, attempts to reproduce a field environment require a number of compromises. The sampling chamber size and geometry in this study were chosen to provide sufficient room for some fly movement without making the chamber so large that it could not be purged between sampling intervals. Since lek formation, or gathering of males in a group, is typically involved in male calling behavior (Prokopy and Hendrichs, 1979; Arita and Kaneshiro, 1985), a multifly sample was selected. In a field lekking situation, the potential exists for modulation of emission production by individual calling males due to "feedback loops", as the male monitors the overall emissions level in its environment. Emission rates for an individual male would then be a function of the corresponding emission rates of other males in the lek. There is no unequivocal way to determine whether any such interaction occurred among the flies in the sampling chamber. Collection of volatiles from multifly subject groups does have the practical advantage of "averaging out" individual fly variations. McDonald (1987) reported that medfly males are stimulated to more frequent episodes of calling activity when they are able to detect the presence of other medfly males, but he attributed this interaction to visual and acoustic cues rather than to chemical communication.

Headspace trapping is the most appropriate sampling approach, since we were interested in the qualitative and quantitative aspects of the volatile emissions profile from calling males. Sampling times and sweep flows were selected to provide sufficient trapped material for examination, while avoiding component breakthrough—loss of volatiles from the trap exit end. During trapping, the trap exit flow was periodically checked by sniffing. While

Table I. Summary of SS and Glass Trap Results—Components Identified and Amounts Trapped (25 Flies, 5-min Trapping Interval, 100 mL/min Sweep)

	amounts trapped, ng											
	5–6 days old					11–12 days old					20–21 days old	
component[a]	SS3[b] 7:54–7:59 a.m.	GL5[c] 8:08–8:58 a.m. (prg)[d]	SS6 9:38–9:43 a.m.	GL8 9:15–10:44 a.m. (prg)	SS9 11:28–11:35 a.m.	SS14 8:00–8:06 a.m.	GL16 8:15–9:10 a.m. (prg)[c]	SS17 9:49–9:55 a.m.	GL19 9:05–0:55 a.m. (prg)[c]	SS20 11:36–11:42 a.m.	GL12 9:05–9:55 a.m.	GL13 9:56–10:48 a.m.
ethanol[f]	+[e]		+			+		+				
acetone[f]	+		+					+				
propan-2-ol			+			+		+				
ethyl acetate[f,g]	1430[n] (274)[p]		1011 (143)		234 (132)	673 (423)		887 (266)		426 (229)		
1-pyrroline[f,g]	294 (56)		276 (39)		88 (50)	75 (47)		351 (105)		246 (132)		
propyl acetate[f]	2 (<1)		2 (<1)		1 (1)	2 (1)		3 (1)		2 (1)		
pyrrole[f]	25 (5)		32 (5)		5 (3)	6 (4)		6 (2)		7 (4)		
hexanal	tr[q] (tr)		1 (<1)		1 (1)	1 (1)		13 (4)		4 (2)		
heptanal[f]	tr (tr)		1 (<1)		1 (1)	tr (tr)		1 (<1)		1 (1)		
unknown	2 (<1)		2 (<1)		1 (1)	1 (1)		2 (1)		2 (1)		
dihydro-3-methyl-2(3H)-furanone[f,g]	17 (3)	28 (5)	27 (5)	18 (5)	12 (7)	8 (5)	19 (6)	20 (6)	24 (8)	27 (15)	10 (29)	21 (17)
benzaldehyde[f]	6 (1)		8 (1)		5 (3)	5 (3)		8 (2)		3 (2)		
6-methylhept-5-en-2-one[f]	13 (2)	8 (<1)	12 (2)	3 (1)	2 (1)	5 (3)	tr (tr)	3 (1)	tr (tr)	4 (2)	tr (tr)	tr (tr)
phenol	2 (<1)		4 (1)		1 (1)			4 (1)		2 (1)		
hexanoic acid[h,j]												
myrcene[f]	51 (10)	1 (<1)	50 (7)	1 (<1)	11 (6)	10 (6)	1 (<1)	13 (4)	1 (<1)	12 (6)	2 (6)	1 (1)
(E)-2-hexenoic acid[g]		214 (42)		379 (95)			114 (37)		282 (95)		32 (91)	150 (121)
limonene[f]	10 (<1)		9 (1)		2 (1)	2 (1)		3 (1)		2 (1)		
(Z)-β-ocimene[f]	32 (6)	10 (2)	31 (4)	5 (1)	13 (7)	11 (7)	6 (2)	23 (7)	7 (2)	11 (6)	3 (9)	8 (7)
(E)-β-ocimene[f]	31 (6)	4 (1)	30 (4)	4 (1)	6 (3)	7 (4)	3 (1)	10 (3)	4 (1)	8 (4)	2 (6)	4 (3)
nonanal[f]	1 (<1)		1 (<1)		1 (1)	1 (1)		2 (1)		2 (1)		
linalool[f,g,k]	14 (3)	15 (3)	13 (2)	6 (2)	4 (2)	4 (3)	7 (2)	12 (4)	12 (4)	8 (4)	2 (6)	3 (1)
unknown	1 (<1)		1 (<1)		tr (tr)	tr (tr)		1 (<1)		tr (tr)		
ethyl (E)-3-octenoate[f,g,m]	522 (100)	511 (100)	708 (100)	397 (100)	177 (100)	159 (100)	310 (100)	334 (100)	296 (100)	186 (100)	35 (100)	123 (100)
prop-2-yl (E)-3-octenoate	1 (<1)		2 (<1)		1 (1)			tr (tr)		tr (tr)		
ethyl (E)-2-octenoate	1 (<1)		1 (<1)		tr (tr)			tr (tr)		tr (tr)		
linalyl acetate[f]	2 (<1)		3 (<1)		1 (1)			1 (<1)		1 (1)		
indole[f]	8 (2)	18 (4)	7 (1)	3 (1)	1 (1)		5 (2)	tr (tr)	7 (2)	4 (2)	tr (tr)	5 (4)
propyl (E)-3-octenoate	1 (<1)		2 (<1)		tr (tr)			1 (<1)		1 (1)		
methyl geranate[f]	3 (1)		3 (<1)		2 (1)	1 (1)		2 (1)		2 (1)		
neryl acetate[f]	2 (<1)		2 (<1)		tr (tr)	tr (tr)		tr (tr)		tr (tr)		
geranyl acetate[f,g,k,m]	223 (43)	269 (53)	462 (65)	208 (53)	115 (65)	56 (35)	236 (76)	153 (46)	360 (122)	221 (119)	23 (66)	79 (64)
unknown	tr (tr)		tr (tr)					1 (<1)		tr (tr)		
(Z,E)-α-farnesene	2 (<1)	2 (<1)	5 (5)	2 (<1)	1 (1)	1 (1)	4 (1)	1 (<1)	4 (1)	2 (1)		
(E,E)-α-farnesene[f,g,m]	143 (28)	51 (10)	241 (34)	15 (4)	45 (25)	47 (30)	203 (65)	66 (20)	269 (19)	149 (80)		30 (24)

[a] Mass spectra and retention indices match those of authentic samples. [b] Semiquantitative values from GC/FID headspace runs [ethyl (E)-3-octenoate = secondary standard]. [c] Semiquantitative values from glass traps (internal standard) were multiplied by 0.1 to obtain average 5-min values shown, for comparison with 5-min ss trap results. Lower-boiling components were lost during concentration of glass trap solvent extracts. [d] Half-hour purge interval (1 L/min) followed glass trap sample. [e] Detected by GC/MS but could not be quantified in the GC/FID runs; peaks overlapped at the FID chromatogram front. [f] Jang et al. (1989). [g] Baker et al. (1985). [h] Ohinata et al. (1977). [j] Appeared in GC/MS examinations of midmorning ss trap volatiles concentrates, but quantitative data could not be obtained from GC/FID runs (see text). [k] Baker et al. (1990). [m] Heath et al. (1991). [n] Nanograms of component/25 files/5 min at 100 mL/min sweep. [p] Normalized values; ethyl (E)-3-octenoate = 100. [q] tr = <0.6 ng.

small amounts of highly volatile materials such as acetone and ethanol might have broken through, there was no detection of 1-pyrroline breakthrough. Since the human nose can detect this compound at quite low levels, even if one is an anosmic (Amoore et al., 1975), this sniffing check is likely a valid one for breakthrough of one of the more volatile emission components.

The two different trap designs, and their application, tend to be complementary. The ss trap sequence provides maximum sensitivity for both GC/FID and GC/MS examination of trapped material, because the total trapped sample is used for one analysis. Sample manipulation is minimal, so the full range of components trapped can be monitored, and there is little opportunity for introduction of artifacts/contaminants. This approach is limited by three factors: there must be no trap breakthrough; the volatiles must be amenable to cryofocusing; and the components must be sufficiently stable to tolerate the elevated temperatures encountered during desorption and flash injection. The glass trap procedure, with solvent elution of trapped volatiles, minimizes the potential for thermal breakdown, facilitates direct introduction of internal standard, and provides enough concentrated solution for multiple runs, both FID and MS, with the same sample.

Qualitative Results. *Stainless Steel Traps (Thermal Desorption).* Component identifications from GC/MS analyses of ss trap samples are listed in Table I. They include all entries in the first column. For GC/MS identifications, two samples (SS4, SS7) were collected from 5–6-day-old males, two (SS15, SS17) were collected from 11–12-day-old males (none from 20–21-day-old males), and two (SS1, SS10) were system blanks. These were used for the component identifications listed. Identifications are based upon comparisons of both mass spectral data and GC retention indices (normal hydrocarbon series) with those of authentic reference compounds. Several components remain unidentified. Most of the unidentified run components were present at low concentrations, were found in only one or two of the four GC/MS runs, and were therefore thought to be artifacts or contaminants. Three unknowns were found in three or four of the four runs, however, and may be emission components. These are listed as "unknown" in the table. Because trapping times were short, at a moderate sweep rate, numerous minor components reported in Jang et al. (1989; 180 min at 150 mL/min) were not detected in headspace runs from these 5-min fly emission samples. However, two carboxylic acids, hexanoic and (*E*)-2-hexenoic, were detected in several of these 5-min samples by GC/MS. Hexanoic acid had been identified in male medfly emissions by Ohinata et al. (1977), and (*E*)-2-hexenoic acid had been reported by Baker et al. (1985), but neither was found during our previous study (Jang et al., 1989). In the current GC/MS examinations of 5–6- and 11–12-day-old fly emissions, no trace of either of the two acids could be found in the early-morning-trapped samples, although they appeared in relatively large amounts in both mid-morning emission samples. Their absence from the early-morning GC/MS ss trap samples may be due to two factors: adsorption of initially produced acid on the chamber walls and/or low initial acid production by the flies. In contrast with the GC/MS results, no peaks corresponding to the two acids were found in any of the GC/FID analyses. We were therefore unable to obtain any semiquantitative data for these two acids from any of the ss trap/thermal desorption runs for inclusion in Table I. Presumably the acids were adsorbed or destroyed in the transfer lines and/or valving of the GC/FID instrument but survived passage through the similarly constructed (but newer) GC/MS plumbing. A comparison of reconstructed chromatogram peak areas, based upon mass spectral responses, indicated that the hexanoic acid peak is roughly 10–15% as large as that of the unsaturated acid. Three additional headspace samples (SS3, SS6, SS9) were collected from 5–6-day-old males and three (SS14, SS17, SS20) from 11–12-day-old males for GC/FID examination (cf. Figure 1). These runs provided additional retention index information and also yielded peak area measurements (see Semiquantitative Results).

Glass Traps (Solvent Desorption). Corresponding identifications obtained via the glass trap/solvent desorption approach are also listed in Table I. Two samples (GL5, GL8) were collected from 5–6-day-old, two (GL16, GL19) from 11–12-day-old, and two (GL12, GL13) from 20–21-day-old males (each approximately 50 min at 100 cm^3/min). In addition, two blanks (GL2, GL11) were collected. With the 20–21-day-old flies, the second trapping interval directly followed the first. This differs from the sequence used with the two younger fly groups, where a high flow purge period intervened. Concentrate aliquots were used for parallel GC/MS and GC/FID runs. Again, identifications were based upon mass spectral data and GC retention behavior. Because of the eluant concentration step during workup, low-boiling components listed in Table I did not appear in the liquid injection gas chromatographic runs.

In the present study, (*E*)-2-hexenoic acid appeared at relatively high levels in the eluted volatiles solutions from glass traps. It was identified by GC/MS examination of the concentrated eluants and was quantified by GC/FID (see below). This is in marked contrast with the ss trap sample results, where the compound was detected in some of the GC/MS runs but not in any of the GC/FID results. The corresponding saturated hexanoic acid also appeared in the glass trap samples (GC/MS runs).

Most of the identified components listed in Table I were first found by Baker et al. (1985) or Jang et al. (1989). Three esters only partially characterized in the previous 1989 study have now been fully identified after comparison with authentic samples. These are prop-2-yl (*E*)-3-octenoate (DB-1 reference retention index = 1216), ethyl (*E*)-2-octenoate (=1223), and propyl (*E*)-3-octenoate (=1274). Propan-2-ol, hexanal, phenol, and (*Z,E*)-α-farnesene are new additions to the list of identified components. Two "intermediate" or "minor" components reported in Jang et al. (1989) were not cleanly identified in the present study. These are ethyl (*E*)-3-hexenoate and 3-methylbut-3-enyl acetate, respectively. There are indications that the two components are present at low concentrations (one or more major fragment ions at the correct retention times in several ss trap GC/MS runs), but extraneous fragment ions also appeared, so the identifications are tenuous at best and they are not included in the table.

Semiquantitative Results. Semiquantitative values are based upon flame ionization peak area integrations. An underlying assumption is that the FID response factors for individual compounds are all equal. This is not totally valid, especially with low molecular weight, high heteroatom content compounds, but is sufficiently correct for the purposes of this study.

Stainless Steel Traps. FID peak area counts were converted to ng/25 flies/5 min values by employing ethyl (*E*)-3-octenoate as a secondary standard (see Internal Standard Calculations under Experimental Procedures)

to provide the ss trap amounts listed in Table I. The three trappings from 5–6-day-old males and three from 11–12-day-old males were collected in early morning, mid-morning, and late morning (see Figure 1). Emissions from 20–21-day-old flies were not collected with ss traps, although this age group was included in the glass trap sequence (below). Five-minute ss trap sampling intervals were planned for this study, using 25 male flies. In some instances, the actual interval was slightly longer than 5 min, and 27 11–12-day-old flies were introduced into the sampling chamber, rather than 25. Correction factors were therefore applied to the initial peak area values, to adjust them to 5-min and 25-fly values.

Ethyl acetate was the most abundant emission volatile trapped from either 5–6- or 11–12-day-old flies with ss traps, and (*E,E*)-α-farnesene was consistently the fifth most abundant. The same three components—1-pyrroline, ethyl (*E*)-3-octenoate, and geranyl acetate—fell between ethyl acetate and farnesene in abundance. During the early-morning 5-min trapping, both 5–6- and 11–12-day-old flies produced ethyl (*E*)-3-octenoate > 1-pyrroline > geranyl acetate. By mid- and late-morning the 5–6-day-old flies were releasing more geranyl acetate than 1-pyrroline. Emissions from 11–12-day-old flies at mid- and late morning varied more in their relative composition; 1-pyrroline became the second most abundant emission component, surpassing both ethyl (*E*)-3-octenoate and geranyl acetate.

Considering the total amounts of volatiles trapped [other than (*E*)-2-hexenoic acid], as listed in Table I, the greatest total amount of material was trapped with the 5–6-day-old flies in early to mid-morning. With the 11–12-day-old flies, the maximum appeared to occur in mid- to late morning. Not all of the individual component concentrations followed this pattern, but the majority did.

Glass Traps. Aliquots of concentrated solutions obtained from the glass traps were submitted to GC/FID examination for component quantitation. These solutions contained known amounts of internal standard, so peak area values could be converted to amounts of component trapped. As was noted above under Internal Standard Calculations, the initially calculated values (ng/25 flies/50 min) were converted to average amounts (ng/25 flies/5 min) by multiplying each by 0.1. This permitted direct comparisons with the corresponding ss trap amounts. Although considerable care was taken to avoid contamination during workup, GC/FID examination of the concentrates showed evidence of more contaminants/artifacts than were seen in the corresponding ss trap/thermal desorption samples. However, these contaminants were also present in the blank run concentrates and so could be largely eliminated from consideration.

In contrast with the ss trap sampling sequences, which monitored emissions from 5–6- and 11–12-day-old flies only, all three fly ages were included in the glass trap trapping sequences, as were two blank samples. The identification and amounts (ng/25 flies/5 min) of each component found are listed in Table I.

(*E*)-2-Hexenoic acid appears to be one of the major volatile emissions from calling male medflies, on the basis of the glass trap results. Considering the typical chromatographic performance of free acids, the values listed are probably conservative. At all three fly ages, more of the acid was collected during the second sampling interval, starting at approximately 10:00 a.m. This parallels the results from the ss trapping sequences. Hexanoic acid was also detected in the glass trap samples but could not be quantified satisfactorily because the blanks contained significant (but variable) amounts of this acid (and other homologs).

In comparing the ss and glass trap semiquantitative data in adjacent columns of Table I (SS3–GL5; SS6–GL8; etc.), it is necessary to remember that the trappings were sequential; they were not run simultaneously. In addition, the glass trap amounts are 5-min averages derived from 50-min collections. With these points in mind, most values from the glass trap runs are in reasonable agreement with those from the ss runs. In addition, the component amounts measured in the glass trapped samples are largely consistent with emission maxima/fly age trends indicated by the ss trap data alone. Peak release of volatiles occurs earlier in the morning with 5–6-day-old flies than with 11–12-day-old flies. In the case of the 20–21-day-old flies, the abbreviated sampling sequence may confuse the situation a bit, but the shift appears to hold; higher production occurs still later in the morning.

The amounts listed in Table I are from 25 flies per 5-min period at a 100 cm^3/min sweep flow. These amounts can readily be converted to nanograms of compound trapped per fly per minute, but the resulting values might be misleading. The experimental design used in this study is not likely to provide a *maximum* release rate per isolated fly but rather a composite release rate when 25 male flies are exposed to one another in a somewhat constrained chamber, in an atmosphere containing volatile male emissions. During the course of the trapping sequences, several of the authors occasionally recorded the number of males who were visibly calling (anal ampulla everted and wings fanning). At no point did they observe more than 60% of the flies calling. More typically the percentage ranged between 30 and 60%.

Overall, the values (Table I) from a given stainless steel trap/thermal desorption and the following glass trap/solvent desorption (i.e., SS3–GL5; SS6–GL8; SS14–GL16; SS17–GL19) are in fair agreement, considering the potential for divergence. Some of the observed discrepancies, such as the absence of (*E*)-2-hexenoic acid from the ss trap results and the loss of highly volatile emission components during concentration of glass trap solvent washings, have been discussed above. Several additional differences are apparent in values for the monoterpene hydrocarbons and for (*E,E*)-α-farnesene. The monoterpenes myrcene, (*Z*)-β-ocimene, and (*E*)-β-ocimene were found at moderate levels in the stainless steel trap samples but appear at significantly lower levels in the glass trap washings. A solvent artifact, the monohydroperoxide of diethyl ether, was a major constituent of all of the concentrated trap washings, including those of blank traps. It was presumably formed during the slow distillation in air of the pentane/ether solvent mixture while concentrating solutions of the emission volatiles. Oxidation of the rather susceptible unsaturated monoterpenes by reaction with the monohydroperoxide may have reduced their concentrations to the levels observed. If true, this hypothesis should also hold for the easily oxidized farnesene. Data from the 5–6-day-old fly trappings are consistent with such an occurrence, but the 11–12-day-old fly results are just the reverse; more farnesene (5-min interval) was found in the glass trap concentrates than in the ss trap mixtures. Oxidation may still occur, but any resulting decreases in (*E,E*)-α-farnesene amounts appear to be more than offset by some other factors.

In addition to determining variations in amounts of a given component released per unit time, we also wished to look for any variations in the emissions profile (relative

amounts of individual components) from sample to sample. To better display such variations in total emissions profile among the trap samples, the amounts of individual components measured were normalized, setting ethyl (*E*)-3-octenoate at 100. This is preferable to simple calculation of percent composition for several reasons: First, the most abundant headspace component is ethyl acetate, but this component does not appear in the glass trap concentrates. Second, the percent concentration of ethyl acetate varies appreciably among the stainless steel trap results, distorting the percent values for other components. Third, since the (*E*)-3-octenoate ester was used as a secondary standard to convert area counts (stainless steel trap data) to nanograms of component, it seemed preferable to again use this compound as the common reference point for normalization. Normalized results are presented in parentheses immediately following the "amount" values (ng/25 flies/5 min) in Table I. In the total emissions samples from 11–12-day-old flies collected in glass traps, the concentration of α-farnesene increases, relative to ethyl (*E*)-3-octenoate. A similar increase is apparent in the ss trap samples, from mid to late morning. Normalized values for geranyl acetate show that this compound's presence in the trap samples follows a similar trend. These patterns are thought to be real, not artifacts of the experimental design, but in light of the variations in the α-farnesene values for 5–6-day-old flies, they must be considered with some caution.

Heath et al. (1991) have reported the mean amounts of pheromone components they collected from wild Guatemalan male medflies. Their efforts were limited to three of the major emission compounds—ethyl (*E*)-3-octenoate, geranyl acetate, and (*E*,*E*)-α-farnesene—and used an experimental approach similar in concept to that employed with glass traps in the present study, although it differed in specifics (8–12 vs 25 flies; 2-h vs 50-min trapping times; 0.9 vs 0.1 L/min sweep flows; 0.06 g of Porapak-Q vs 6.5 g of Tenax GC adsorbents). By summing the appropriate values (ng/25 flies/5 min) for these same three components from each column of Table I (for example, using the first column: 511 + 269 + 51 = 831 ng/25 flies/5 min) and then converting the individual sums to micrograms/male h [units used by Heath et al. (1991)], the following values are obtained: 5–6-day-old flies, 0.40 and 0.30; 11–12-day-old flies, 0.36 and 0.44; and 20–21-day-old flies, 0.03 and 0.11 μg/male h. In Heath et al. (1991; Figure 2 of the reference) a maximum yield of approximately 0.95 μg/male h from 5–10-day-old wild males is indicated, with other values ranging from approximately 0.07 to 0.50 μg/male h. Sweep rate differences (0.9 vs 0.1 L/min) were not factored into the values based upon our data, for it is not clear that there is a simple linear relationship between yield and sweep flow. The amount of released material available for trapping is ultimately determined by the flies, not directly by sweep flow (although production might indeed be increased somewhat, if emitted volatiles are continually removed from the fly locale). Quantitative totals for these three components are in fairly good agreement with those reported by Heath et al. (1991), especially considering the many opportunities for divergence (wild Guatemalan vs laboratory-reared Hawaiian; experimental design differences; etc.). The Guatemalan fly study did not consider other emission components reported by Baker et al. (1985) and Jang et al. (1989). We have found in the present study that several of these appear at levels which approach or exceed those of the two esters and sesquiterpene hydrocarbon. Ethyl acetate, 1-pyrroline, and (*E*)-2-hexenoic acid in particular are major emission components and should be considered as potential contributors to pheromonal activity. A recent publication by Baker et al. (1992) addresses the potential use of 1-pyrroline trimer as a long-term source of 1-pyrroline in evaluations of synthetic pheromone formulations, indicating a recognition by those authors that this emission component may also be a bioactive constituent.

The series of recent male medfly emission studies, beginning with that of Baker et al. (1985) and including Jang et al. (1989), Heath et al. (1991), and the present investigation, have fairly well identified the volatile organic compounds associated with male medfly calling activity. These findings cannot be reconciled with component identifications reported earlier by Jacobson et al. (1973) and Ohinata et al. (1977, 1979). The task of determining which components are necessary to trigger an attractive response in virgin female flies has been addressed to varying degrees in each study except the present one, which is primarily a qualitative and semiquantitative examination of the male emission complex. Ongoing laboratory evaluations of the major pheromone components identified indicate that several compounds contribute differentially but synergistically to the pheromone's attractiveness for virgin female medflies (Jang et al., unpublished results). Other intermediate- to low-concentration components may also be required to attain full parity with calling males. The potential exists for development of an effective and useful female attractant, especially if essential components and their optimum release rates can be pinpointed and reproduced.

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