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MALE-PRODUCED AGGREGATION PHEROMONE OF *Carpophilus mutilatus* (COLEOPTERA: NITIDULIDAE)

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Abstract—Males of *Carpophilus mutilatus* Erichson produce an aggregation pheromone to which both sexes respond. The pheromone includes two hydrocarbon components, (3*E*,5*E*,7*E*)-5-ethyl-7-methyl-3,5,7-undecatriene **(1)** and (3*E*,5*E*,7*E*)-6-ethyl-4-methyl-3,5,7-decatriene **(2).** These were emitted in a 10:1 ratio and in a total amount of ca. 5 ng per feeding male per day. All tested doses of **1** and **2,** from 0.03 to 30 ng, were more attractive than controls in wind-tunnel tests, but there was no evidence of synergism between these trienes. Dramatic synergism between the pheromone and a food-type coattractant occurred in the field, however. In a date garden in southern California, traps with a combination of synthetic **1** and fermenting whole-wheat bread dough attracted 22 times more beetles than dough by itself and 295 times more than **1** by itself. Volatile collections from males also contained three oxygenated compounds that were absent from females. One of these was tetradecanal (ca. .5 ng per male per day), but the structures of the other two are presently undetermined (0.8 and 1.1 ng per male per day). No function for these was demonstrated. One compound originating in the artificial diet, 2-phenylethanol, was particularly attractive in the wind-tunnel bioassay, as was the chromatographic solvent, methanol.

Key Words—*Carpophilus mutilatus*, sap beetle, Coleoptera, Nitidulidae, aggregation pheromone, hydrocarbon, triene, date, host volatiles.

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INTRODUCTION

The confused sap beetle, *Carpophilus mutilatus* Erichson (Coleoptera: Nitidulidae), is a small (<4 mm) brown sap beetle that occurs throughout the tropical, subtropical, and milder temperate regions of the world. In North America it ranges as far north as California (Tehama County) and Virginia (Connell, 1991). *C. mutilatus* belongs to a complex of five very similar species, along with *C. dimidiatus* (Say), *C. freemani* Dobson, *C. fumatus* Boheman, and *C. pilosellus* Motschulsky (Connell, 1975). Until *C. mutilatus* was redescribed by Dobson (1954), it was confused in the literature with *C. dimidiatus*.

C. mutilatus feeds primarily on fallen and decomposing fruits, but it will also infest fruit as it ripens on the plant, especially if it has been damaged. The species is a pest in crops such as dates (Lindgren and Vincent, 1953; Warner et al., 1990; Kehat et al., 1983); figs (Hall et al., 1978; Smilanick, 1979); peaches and plums (Gaven, 1964; Tate and Ogawa, 1975); and corn (Connell, 1975). Like other nitidulids, *C. mutilatus* can transmit fruit-degrading microorganisms, such as brown rot [*Monilinia fructicola* (Wint.) Honey] in stone fruits (Tate and Ogawa, 1975).

Male-produced aggregation pheromones have been identified in three other *Carpophilus* species: *C. hemipterus* (L.), *C. lugubris* Murray, and *C. freemani* Dobson. In each case, the pheromone is a blend of tetraene or tetraene plus triene hydrocarbons (Bartelt et al., 1990a,b, 1991, 1992b). The obvious abundance of *C. mutilatus* in a date garden in southern California (Bartelt et al., 1992a) prompted us to investigate its pheromone also. The resulting pheromone identification was guided by wind-tunnel bioassays and was later corroborated by a field test.

METHODS AND MATERIALS

Beetles. The *C. mutilatus* culture was started from insects captured in a date garden near Oasis, California. The beetles were reared on the diet reported by Dowd (1987), except that additional brewer's yeast replaced the pinto beans. Insects from the culture were used both for pheromone production and for wind-tunnel pheromone bioassays.

Pheromone Collection and Analysis. Beetles were separated by sex and placed with diet medium in volatile-collection flasks as described earlier for *C. hemipterus* (Bartelt et al., 1990a). Volatiles were adsorbed onto Tenax. Each flask contained ca. 70 beetles, but twice as many collectors were set up for males because all of the previously studied, related species had male-produced pheromones. Counts were kept so that amounts of volatiles could be expressed in "beetle-days" (the average amount of material collected per beetle per day).

The pooled collections over a three-week period amounted to 8800 beetle-days from males and 4400 from females.

Except for small bioassay aliquots, these collections were fractionated on open columns of silica gel (1 × 5 cm, column void volume = 3.8 ml). Ten-milliliter fractions were collected for each of the following elution solvents: hexane; 5%, 10%, and 50% ether in hexane (by volume); and 10% methanol in methylene chloride.

After these fractions were bioassayed, some were fractionated further by HPLC. For the hexane fractions (hydrocarbons), a $AgNO_3$-coated silica column was used (eluted with 10% toluene in hexane). For the active 5% and 50% ether–hexane fractions, a 50 Å size-exclusion column was used (eluted with hexane).

All fractions were analyzed by GC (15 m × 0.25 mm ID DB-1 capillary column with 1.0 μm film thickness). Kovats indices (KI), relative to *n*-alkanes, were determined for some GC peaks. The indices were calculated by linear interpolation for GC runs beginning at 100°C and temperature programming at 10°/min.

Positive ion, electron impact mass spectra were obtained for compounds of interest on a Hewlett-Packard 5970 MSD instrument, with sample introduction through a DB-1 capillary GC column. Proton NMR spectra were obtained at 300 MHz for synthetic compounds and for one beetle-derived hydrocarbon, which was isolated in sufficient quantity. The samples were dissolved in deuterobenzene. The chromatographic and spectroscopic instrumentation were as described previously (Bartelt et al., 1990a,b).

Wind-Tunnel Bioassay. The wind-tunnel methods were reported earlier (Bartelt et al., 1990a). Briefly, ca. 1000 beetles were placed into the wind tunnel without food. After 1–2 hr, they became active and began to fly. Bioassays were begun when ca. 100 beetles were in flight at any instant and were continued for as long as the flight activity persisted (typically 3–4 hr). For each bioassay test, two baits (pieces of filter paper treated with extracts, fractions, or solvents) were hung in the upwind end of the tunnel for 3 min, and the numbers of beetles flying to the baits and landing were recorded. The baits were then removed, and after a pause of 2–3 min, the next pair of test baits was put in. When chromatographic fractions were being screened against controls, an appropriate, active, parent material was employed every fourth or fifth test to confirm that the beetles remained responsive. Disposable rubber gloves were worn while handling test baits because the beetles usually responded to any filter papers (including controls) touched directly. Responses to some preparations such as the 50% ether–hexane fractions were inconsistent from day to day; addition of a drop of water to each filter paper (including controls) improved reproducibility without unduly increasing control responses.

Synthetic Hydrocarbons. Two synthetic hydrocarbons (Figure 1) were used

FIG. 1. Hydrocarbons used in this research and assigned structure numbers.

in this project: (3*E*,5*E*,7*E*)-5-ethyl-7-methyl-3,5,7-undecatriene **(1)** and (3*E*,5*E*,7*E*)-6-ethyl-4-methyl-3,5,7-decatriene **(2).** The synthesis of **1** involved seven steps. Butanal was the starting material. Wittig-Horner olefinations, first with triethyl 2-phosphonopropionate and second with triethyl 2-phosphonobutyrate, were the key reactions; these introduced both side chains and created the trisubstituted double bonds. In preparation for subsequent olefinations, the ester product of each Wittig-Horner reaction was reduced to the corresponding alcohol with $LiAlH_4$ and then converted to the aldehyde with MnO_2. A final Wittig reaction using propyl(triphenyl)phosphonium iodide formed the third double bond and completed the hydrocarbon.

The synthesis of **2** required only four steps because the aldehyde, (*E*)-2-methyl-2-pentenal, is commercially available as a starting material. The same final four steps used in the preparation of **1,** beginning with the olefination with triethyl 2-phosphonobutyrate, produced **2** from this starting material. Conditions for these reactions were as described previously for analogous hydrocarbons (Bartelt et al., 1990c). The mass and NMR spectra of these compounds were published earlier (Bartelt et al., 1990b).

The synthetic hydrocarbons were purified on silica gel and, for wind-tunnel tests, by $AgNO_3$ HPLC as well. Purity was 95% for both **1** and **2.** Impurities were primarily geometrical isomers. Aliquots were then diluted to between 0.015 and 15 ng/μl with hexane, the final concentration depending on the nature of the experiment. Only the silica gel purification was used in preparation for the field bioassay. Compound **1** was applied to rubber septa (200 μg/septum) with 300 μl of CH_2Cl_2.

Field Bioassay. Enough synthetic **1** was on hand late in 1990 so that an initial field test could be conducted. This was done in a date garden near Oasis, California. The location and methodology were as described previously for an experiment with *C. hemipterus* (Bartelt et al., 1992a). The wind-directed pipe traps (Dowd et al., 1992) were hung 1 m above the ground, and trap spacing was ca. 20 m. The traps were baited either with pheromone **(1)** only, fermenting whole-wheat dough only, a combination of pheromone plus dough, or were left unbaited as controls. There were two replications of each treatment in a completely randomized design. Beetles were collected from the traps weekly. The pheromone septa were replaced every two weeks, and the dough baits were replaced weekly. The test ran from August 31, 1990, until February 14, 1991. Whole-wheat bread dough is a commonly used nitidulid attractant, and its major volatile emissions have been characterized (Lin and Phelan, 1991).

RESULTS AND DISCUSSION

Chemical Analysis and Wind-Tunnel Bioassays. Volatile collections from both males and females of *C. mutilatus* were attractive in wind-tunnel tests, although the female-derived samples were less so (Table 1). The activity was distributed over several silica gel fractions (Table 1), suggesting the attractive compounds spanned a broad range of polarities. Further analysis was concentrated on the fractions eluted with hexane and with 5% and 50% ether in hexane. These fractions are discussed below in order of increasing polarity. Both 10% MeOH–CH_2Cl_2 fractions also attracted many beetles, but this activity was essentially due to the methanol in the solvent (note control, Table 1); methylene chloride was not attractive in the wind tunnel.

Hexane Fraction. The hydrocarbons from both sexes were further fractionated by $AgNO_3$ HPLC, and the bioassay results for these fractions are listed in Table 2. For both males and females, the eluant between 7 and 9 ml after injection contained essentially all of the activity. By GC, both the 7- to 8- and 8- to 9-ml fractions from males contained a compound (KI = 1393) that was not initially detected in the female-derived fractions (a total of 4.5 ng per beetle day). By GC retention, MS, and proton NMR spectrum (15-μg sample), the compound from the male beetles was identical with **1** (Figure 1). Compound **1** had been encountered previously as a minor volatile constituent from *C. freemani*, but it was behaviorally inactive in that species (Bartelt et al., 1990b).

Two additional hydrocarbons were present in the male-derived fractions

TABLE 1. WIND-TUNNEL ACTIVITY OF VOLATILE COLLECTIONS FROM FEEDING MALE AND FEMALE *C. mutilatus* AND SILICA GEL FRACTIONS DERIVED FROM THESE COLLECTIONS

Bioassay treatment[a]	Mean bioassay count[b]			N
	Male-derived	Female-derived	Control	
Whole collection	22.5 a	9.6 b	0.1 c	8
Silica gel fractions				
Hexane	25.0 a	8.9 b	2.1 c	24
5% Ether–hexane	23.6 a	4.3 b	2.1 c	8
10% Ether–hexane	2.2 a	0.9 a	1.6 a	8
50% Ether–hexane	30.3 a	15.1 b	1.5 c	16
10% MeOH–CH_2Cl_2	28.5 a	40.2 b	29.4 ab	12

[a] Treatments bioassayed at 7 beetle-days per test.
[b] Bioassay counts are the numbers of beetles flying upwind to the filter paper baits and alighting during the 3-min tests. In each row, means followed by the same letter are not significantly different [LSD, 0.05 level, balanced incomplete block analysis, log $(X + 1)$ scale].

TABLE 2. WIND-TUNNEL ACTIVITY OF $AgNO_3$ HPLC HYDROCARBON FRACTIONS DERIVED FROM MALE AND FEMALE *C. mutilatus*[a]

HPLC retention volume (ml)	Mean bioassay count[b]		Mean bioassay count	
	Male-derived fraction	Control	Female-derived fraction	Control
3–4	0.4	0.7	1.5	0.9
4–5	1.1*	0.2	1.8	0.7
5–6	0.6	0.1	1.3	0.9
6–7	0.7	0.3	1.1	0.4
7–8	17.2***	0.1	15.1***	0.4
8–9	17.2***	0.9	10.3***	0.4
9–10	1.2**	0.1	1.6*	0.3
10–11	0.5	0.2	0.7	0.5
11–12	0.6	0.2	0.6	0.4
12–13	0.6	0.0	2.8***	0.2
13–14	0.2	0.2	1.4	0.8
14–15	0.2	0.1	0.8	0.2

[a] Fractions tested at 7 beetle-days per trial; $N = 8$ for each fraction. The male-derived and female-derived fractions were tested on different days; thus the data cannot be compared quantitatively between sexes.

[b] Bioassay counts are the numbers of beetles flying upwind to the filter paper baits and alighting during the 3-min tests. Differences from the control at the 0.05, 0.01, and 0.001 levels denoted by *, **, and ***, respectively [t tests in log $(X + 1)$ scale, using pooled error for each sex].

that were not evident in the corresponding female fractions (KI = 1300 and KI = 1352). These were less abundant than **1** (0.4 and 0.3 ng per beetle-day, respectively). The former occurred along with **1** in the two most active HPLC fractions (Table 2). It was identical by GC retention and MS to **2** (Figure 1), another compound found previously in *C. freemani* (Bartelt et al., 1990b). The other minor hydrocarbon in *C. mutilatus* males (KI = 1352) was found only in an inactive HPLC fraction 6-7 ml after injection. The mass spectrum of this compound was indistinguishable from that of **1.** From previous experience (Bartelt et al., 1992b), this compound was believed to be a geometrical isomer of **1** with a *Z* configuration at one of the trisubstituted double bonds. Due to the bioassay inactivity, structure determination was not pursued further.

Compounds **1** and **2** fully accounted for the activity of the 7- to 9-ml $AgNO_3$ HPLC fractions. By GC, the 7- to 8-ml fraction contained 3.6 ng of **1** and 0.14 ng of **2** per beetle-day. This fraction (7 beetle-days per test), a solution of synthetic **1** and **2** in corresponding amounts, and controls were compared in the wind tunnel, and the resulting mean responses were 15.4, 15.7, and 1.2 beetles per test, respectively ($N = 12$). For the 8- to 9-ml fraction, having 0.94 ng of

1 and 0.26 ng of **2** per beetle-day, the wind-tunnel results for the fraction (7 beetle-days per test), the corresponding synthetic mixture, and controls were 16.2, 20.6, and 0.3, respectively ($N = 12$). For both experiments, the natural and synthetic mixtures did not differ from each other ($P > 0.4$), but all mixtures differed from the control ($P << 0.01$).

Both synthetic **1** and **2** were active by themselves in wind-tunnel tests (Table 3). The beetles responded to the lowest dose, 30 pg, of either **1** or **2** at levels significantly above controls, and the responses increased with higher doses for both compounds. At the lower doses (≤ 3 ng), **1** appeared somewhat more active than **2.** [In the table, the activity of each dose is expressed as a percentage of the standard blend (3 ng of **1** plus 0.3 ng of **2**), corrected for controls. Overall means for the standard and the control in this experiment were 35.7 and 1.1 landings per test]. There was no evidence for synergistic activity of **1** and **2.** Three treatments, in bold type in the table, had **1** and **2** in the natural ratio, ca. 10:1, but these were not superior to adjacent treatments with similar amounts of triene in other ratios. The main feature of the table is that at the higher doses, **1** and **2** and their blends appeared interchangeable. No treatments containing 3–30 ng of **1** and/or 30 ng of **2** were significantly different from the standard.

No female-specific hydrocarbons were detected, based upon careful GC analysis of the fractions. However, these analyses did reveal minute amounts

TABLE 3. RELATIVE ACTIVITY[a] OF DOSES AND COMBINATIONS OF **1** AND **2** IN WIND TUNNEL

	Dose of **1** (ng)				
Dose of **2** (ng)	0	0.03	0.3	3	30
0	0 c[b]	7 b	46 b	81 a	98 a
0.03	7 b	9 b	**39**[c] b	107 a	113 a
0.3	21 b	18 b	64 b	**100** a	91 a
3.0	58 b	69 a	69 b	88 a	**89** a
30	106 a	140 a	99 a	87 a	108 a

[a] Each dose treatment was compared with the control and the standard dose (3 ng **1** plus 0.3 ng **2**) in a balanced incomplete block experiment ($N = 8$, two baits per test). Numbers of beetles flying upwind and alighting on the filter paper baits during the test periods were recorded. Relative activity, R, of each treatment is expressed as a percentage of the standard dose, corrected for responses to controls: $R = [(\text{treatment} - \text{control})/(\text{standard} - \text{control})] \times 100$. By definition, R for the standard dose = 100, and R for the control = 0.

[b] Each incomplete block experiment was analyzed separately [log $(X + 1)$ scale]. Treatments followed by "a" were not significantly different from the standard. Treatments followed by "b" were different from both the standard and the control. All treatments were significantly more attractive than the control, marked "c" (t tests, 0.05 level).

[c] Responses in bold type represent the natural proportions of **1** and **2** (ca. 10:1).

(ca. 50 pg per beetle-day) of **1** in the two most active fractions (7–9 ml after injection). Compound **2** may have been present also, but it was below the limits of detection in the female-derived fractions. While it is possible that female beetles produce a pheromone, the presence of **1** in the fractions was more likely due to errors in sorting the beetles by sex under the microscope when the volatile collectors were set up. An error rate of about 1% could have accounted for the observed amount of male-specific compound in the female sample. Thus, there is presently no compelling evidence for a potent, female-produced, hydrocarbon attractant, despite the initial bioassay tests (Table 1). The minor activity in the 12- to 13-ml $AgNO_3$ fraction remains unexplained.

5% Ether–Hexane Fraction. By GC, the active, 5% ether–hexane fraction from males contained over 100 compounds. Three of these (KI = 1546, 1551, and 1598 in amounts of 1.1, 0.8, and 5.0 ng per beetle day, respectively) were absent from females. These compounds were relatively abundant, the KI = 1598 peak representing 12% of the material in the 5% ether–hexane fraction. On the size-exclusion HPLC column, all three compounds occurred in a fraction 11.2–12.4 ml after injection. The major compound (KI = 1598) was also detected in the next earlier fraction, 10.6–11.2 ml after injection.

Surprisingly, the HPLC fractions at 10.6–11.2 and 11.2–12.4 ml were essentially inactive in the wind tunnel (20 beetle-days per test). The mean bioassay counts were 0.8 and 1.5 for the fractions and 0.5 and 0.4 for the respective controls ($N = 4$, $P > 0.05$). The activity eluted entirely in the 8.4- to 9.5- and 9.5- to 10.2-ml fractions, which is where hydrocarbon **1** elutes. The mean bioassay counts were 29.2 and 19.7, and for the respective controls, 0.6 and 1.4 ($N = 4$, $P < 0.001$). After rechromatography of the active fractions on silica gel, the hexane fractions were very active in the wind tunnel at 20 beetle-days per test (means of 13.0 for both and 0.0 and 0.5 for their respective controls), but the subsequent, 5% ether–hexane fractions were not (no beetles alighted on either the fractions or the controls). Careful GC revealed a minute amount of **1** in the hexane fraction (ca. 60 pg per beetle-day). The original 5% ether–hexane silica gel fraction was probably active in the wind tunnel only because the hydrocarbon **1** tailed slightly in the silica gel chromatography.

The oxygenated compound at KI = 1598 was identified as tetradecanal by a GC and MS comparison to an authentic standard. The other two compounds remain unidentified. The mass spectrum of the compound at KI = 1546 was obtained [*m/z* (% of base peak): 206(M^+, 23%), 192(15%), 191(7%), 177(56%), 167(8%), 163(26%), 149(23%), 135(10%), 125(19%), 123(17%), 121(42%), 109(19%), 107(17%), 105(16%), 95(23%), 93(29%), 91(20%), 83(53%), 77(21%), 71(21%), 69(22%), 67(28%), 57(79%), 55(100%), 43(79%), 41(84%)]. The compound at KI = 1551 had an almost identical spectrum and is probably a geometrical isomer. The spectra suggest a molecular weight of 206, which probably corresponds to $C_{14}H_{22}O$. The similarity of these spectra

to that of **1** (Bartelt et al., 1990b) suggests these are oxygenated analogs of **1.** Several branched, 14-carbon, triene aldehydes prepared during the course of this project had similar, but not identical, mass spectra and GC retentions.

Although no pheromonal activity was demonstrated for the male-specific, oxygenated compounds, it is possible that a function would be revealed with a different type of bioassay or in combination with other chemicals. The existence of oxygenated, sex-specific compounds in *Carpophilus* beetles raises new questions to be investigated.

50% Ether–Hexane Fraction. The 50% ether–hexane fractions derived from males and females were both quite active in the wind tunnel (Table 1). After fractionation of the male-drived material by size exclusion HPLC, the activity was located primarily in an HPLC peak 30–35 ml after injection (73.6 beetles per test and 0.9 for controls, $N = 4$; no other fractions exceeded 3.7). For females, the peak eluted slightly earlier and was contained in two fractions, 20–30 and 30–35 ml after injection. Both of these fractions were active (21.1 and 31.7 beetles per test and 1.3 and 0.3 for respective controls, $N = 4$; no other fractions exceeded 3.1). By GC, all of these fractions shared one obvious, common peak (KI = 1095), and in the male-derived HPLC fraction, it represented 85% of the total material (ca. 40 ng per beetle-day). From the mass spectrum and a subsequent library search, the compound was determined to be 2-phenylethanol [*m/z* (% of base peak): 122(M^+, 24%), 104(3%), 103(4%), 92(60%), 91(100%), 78(5%), 77(6%), 65(24%), 63(7%), 51(11%), 50(6%)]. The sample matched an authentic standard in GC and MS properties. Subsequent volatile collection from a diet blank revealed that the compound originated in the artificial diet medium, and the compound is, therefore, not believed to be a pheromone component. In wind-tunnel comparisons such that weights of 2-phenylethanol were equal, the synthetic alcohol accounted for between 49% and 59% of the activity of the HPLC fractions. Thus, other, less abundant compounds in the HPLC fractions probably have activity as well. Food-related attractants were not pursued further here. No sex specific compounds were detected in these HPLC fractions.

Initial Field Study with 1. Synthetic **1** was very active in the test in the date garden, but as with the other *Carpophilus* species we have studied, the activity was obvious only when a food-type coattractant was present with the pheromone (Table 4). The pheromone alone attracted only 16 *C. mutilatus* during the study, but in combination with fermenting whole-wheat bread dough, it attracted 4722 *C. mutilatus*. The dough by itself attracted only 211 beetles. For all treatments, both sexes were captured, and the sex ratios favored females slightly. Trap catches were variable (see ranges in Table 4) primarily because of seasonal fluctuations in flight activity. Activity peaked in February, but moderate catches were also recorded during November and January (Figure 2).

Summary. C. mutilatus is like the three previously studied *Carpophilus*

TABLE 4. INITIAL FIELD TEST WITH SYNTHETIC PHEROMONE FOR *C. mutilatus* IN DATE GARDEN IN SOUTHERN CALIFORNIA, AUGUST 31, 1990 TO FEBRUARY 14, 1991[a]

Treatment	Overall total catch	Mean catch per week per trap[b]	Range in trap catch
1 plus dough	4722 (65% females)	45.8 a	0–668
Dough only	211 (53% females)	2.8 b	0–20
1 only	16 (69% females)	0.2 c	0–5
Control	0	0.0 c	0–0

[a] There were two traps per treatment; weekly trap catches were analyzed in log $(X + 1)$ scale. Factors in two-way ANOVA were treatment and week.

[b] Means followed by the same letter not significantly different (LSD, 0.05 level). Means calculated in log $(X + 1)$ scale and returned to numerical scale for presentation.

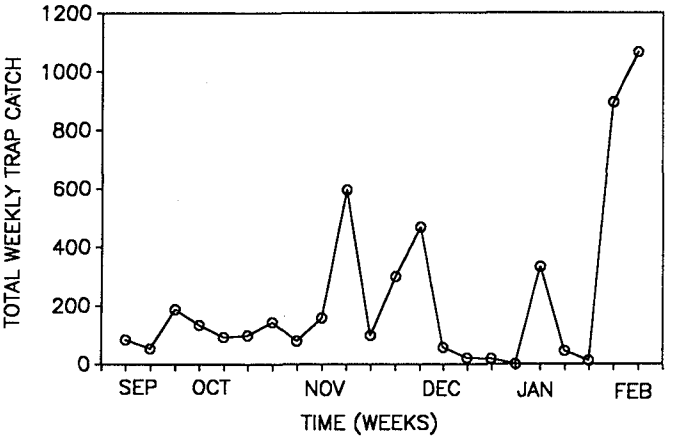

FIG. 2. Trap catch pattern over time for *C. mutilatus* in a date garden near Oasis, California, August 31, 1990, to February 14, 1991. Each point is a weekly catch, totaled over all traps and treatments.

species in having a male-produced, hydrocarbon pheromone to which both sexes respond. The pheromone includes two conjugated trienes (**1** and **2**), which were previously identified in the volatile emissions from *C. freemani*.

As with the other species, *C. mutilatus* responds best to a combination of food odors and pheromone. One especially attractive compound, 2-phenylethanol, was isolated from the artificial diet, and the chromatographic solvent, methanol, is also attractive. Additional work remains to be done on food-type attractants for this species. Male-specific, oxygenated compounds were also

isolated from *C. mutilatus* volatiles, but no pheromonal function has yet been demonstrated for these.

An initial field bioassay established that **1** is very active under natural conditions, as long as a food-type coattractant is also present. Further field studies involving pheromone dose, component blends of **1** and **2**, and trap height are under way and will be reported later.

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