BEHAVIORAL AND ELECTROPHYSIOLOGICAL ACTIVITY OF (Z,E)-7,9,11-DODECATRIENYL FORMATE, A MIMIC OF THE MAJOR SEX PHEROMONE COMPONENT OF CAROB MOTH, Ectomyelois ceratoniae

J.L. TODD*, J.G. MILLAR, R.S. VETTER, and T.C. BAKER

Department of Entomology
University of California
Riverside, California 92521

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Abstract—The behavioral and electrophysiological activity of a mimic [(Z,E)-7,9,11-dodecatrieny1 formate] of the major sex pheromone component [(Z,E)-9,11,13-tetradecatrienal] of carob moth was assessed. Wind-tunnel bioassays demonstrated that the formate was as effective as natural gland extracts, and significantly more effective than the trienal alone or than the trienal blended with two minor pheromone components, in evoking source contact. Dispensers containing the formate were as effective as trienal-containing blend lures in attracting males when placed at the same dosage in traps in date gardens. Single-cell recordings showed that at least two olfactory neurons, differentiated by spike amplitude, are located in the long trichoid hairs on male carob moth antennae. Dose-response relationships indicated that puffs from cartridges loaded with at least 0.1 µg of the formate or of the trienal were necessary to elicit spiking by either the small- or the large-spiking cell within a sensillum. Cross-adaptation studies demonstrated that both compounds stimulated the same large-spiking cell. The frequencies of spikes evoked from the large cell when stimulated by emissions from 0.1-µg, 1-µg, or 10-µg cartridges of either the formate or the trienal were not significantly different, suggesting that the formate is an effective mimic of the trienal at the antennal receptor cell level.

Key Words—Lepidoptera, Pyralidae, sex pheromone, (Z,E)-9,11,13-tetradecatrienal, mimic, (Z,E)-7,9,11-dodecatrieny1 formate, wind tunnel, attractant, electrophysiology, receptor cell.

*To whom correspondence should be addressed.
In the desert valleys of southern California, the carob moth, *Ectomyelois ceratoniae* (Zeller), is a serious pest of dates (Warner, 1988). If its range in this state expands northward towards the Central valley, economically important nut crops such as almonds and pistachios may be threatened, as the carob moth is a pest of these crops in other parts of the world (Dhouibi, 1982; Gothilf, 1984). In an effort to detect and monitor carob moth populations in southern California date gardens, synthetic lures placed in traps have been used with some success (Baker et al., 1991). The carob moth sex pheromone consists of a blend of three unsaturated aldehydes isolated and identified from female gland extracts (Baker et al., 1989b, 1991): (Z,E)-9,11,13-tetradecatrienal (trienal), (Z,E)-9,11-tetradecadienal (dienal), and (Z)-9-tetradecenal (monoenal). All three compounds evoked EAG activity from male antennae, and the major trienal component elicited upwind flight by males in the wind tunnel, with either minor component improving flight response when blended with the trienal (Baker et al., 1991). An 8:1:1 (trienal–dienal–monoenal) synthetic blend, approximating the ratio found in female gland extracts, was as effective as the natural gland extract in eliciting upwind flight and source contact (Baker et al., 1991).

In the field, Baker et al. (1991) demonstrated that the 8:1:1 blend loaded into a polyethylene Beem capsule, and placed unsealed in a Pherocon 1C trap captured significant numbers of carob moth males, although the numbers were low when compared to those trapped by live females. They suggested that this discrepancy might be due to decomposition of the trienal by oxidation and/or photodegradation under field conditions; without the trienal, attraction to the synthetic lure was negligible. Similar degradation problems have been reported for two other recently identified conjugated triene pheromones, 10E,12E,14Z-hexadecatrienal, a pheromone component of *Manduca sexta* (L.) (Doolittle et al., 1990), and 10E,12E,14Z-hexadecatrienyl acetate, a pheromone component of the mulberry pyralid, *Glyphodes pyloalis* Walker (Honda et al., 1990).

The likelihood of decomposition of the carob moth trienal in the field over periods of days or weeks prompted us to examine a potentially more stable mimic of this component that would successfully attract males when placed in traps. In the present study, we report the results of wind-tunnel bioassays and field-trapping studies that compare the attractiveness of (Z,E)-7,9,11-dodecatrienyl formate (formate) with that of the trienal. A formate compound was chosen as the trienal mimic because Mitchell et al. (1975) suggested that formates may be more stable under field conditions than unsaturated aldehyde pheromones, and because formates have been shown to disrupt mating communication in some moth species whose pheromones consist of blends of aldehydes (Mitchell et al., 1975, 1976). We also describe the single-cell responses of male carob moth olfactory neurons to the three sex pheromone components,
and to the formate, in order to determine if the formate is an effective mimic of the trienal at the antennal receptor cell level.

METHODS AND MATERIALS

Chemical Synthesis. The monoenal and dienal were prepared from the corresponding alcohols (available from Aldrich Chemical Co.) by oxidation with pyridinium dichromate molecular sieve (Herscovici et al., 1982) and by Swern oxidation (Mancuso et al., 1978). The crude products were purified by HPLC (RP-18; low-pressure gradient; methanol-water) or by flash chromatography on silica gel (5% ether in hexane) followed by Kugelrohr distillation (0.1 mm Hg, oven temperature 100°C). Syntheses of the trienal have been published elsewhere (Baker et al., 1989b, Millar, 1990). The triene formate was synthesized as described below (Figure 1).

Proton NMR spectra (CDCl₃) were recorded on a GE-300 NMR spectrometer at 300 MHz. Infrared spectra were recorded from films of neat compounds on NaCl plates with a Perkin-Elmer 727 spectrometer. Unit resolution mass spectra (electron impact at 70 eV) were recorded with a Hewlett-Packard (Avondale, Pennsylvania) 5970 mass selective detector interfaced to an H-P 5890 capillary GC. An HP-1 capillary column (20 m × 0.2 mm, Hewlett-Packard) was used. Routine GC runs were made on an H-P 5890 GC fitted with a DB-5 column (20 m × 0.32 mm, J & W Scientific, Folsom, California). Flash chromatography was run with 230–400 mesh silica gel (Aldrich Chemical Co.). Unless otherwise specified, reaction work-up solutions were dried over anhyd. Na₂SO₄ and concentrated under partial vacuum (ca. 100 mm Hg) on a rotary evaporator.

(1E)-1-Chloro-dec-1-en-3-yn-10-ol (3). 7-Octyn-1-ol (2) was prepared from 3-octyn-1-ol (1) in 84% yield by the modified acetylene zipper reaction (Abrams and Shaw, 1988). A dry 2-liter 3-neck flask was loaded with bis(triphenylphosphine)palladium(II) chloride and CuI (1 g each) and flushed

![Diagram](image)

**Fig. 1.** Synthetic route to (Z,E)-7,9,11-dodecatrienyl formate.
with Ar. Alkynol (2) (20 g, 159 mmol), freshly distilled trans-1,2-dichloro-ethylene (31 g, 356 mmol), and THF (200 ml) were added to the reaction flask via a 5-cm column of 3 Å activated molecular sieve. To the stirred mixture were added dropwise 40 ml of diisopropylamine (distilled from CaH₂), and the initially pale yellow solution rapidly turned brown, then black. The mixture was stirred overnight at room temperature, after which time the reaction was ca. 50% complete. A further 1 g each of CuI and bis(triphenylphosphine)-palladium(II) chloride was added, and stirring was continued until the reaction was complete (4 hr). Hexane (400 ml) was then added, and the mixture was stirred a further 10 min before filtering with suction. The filtrate was extracted with saturated aqueous NH₄Cl (3 × 100 ml), dried, and passed through a column of coarse silica gel (70–230 mesh), eluting with 40% EtOAc in hexane. The eluate was concentrated and distilled, bp 100°C (0.03 mm Hg), yielding 25.2 g (85%) of chloroalcohol (3). NMR δ: 6.43 (d, 1H, J = 13.7 Hz, H1), 5.92 (dt, 1H, J = 13.5, 2.2 Hz, H2), 3.64 (m, 2H, H10), 2.30 (td, 2H, J = 6.8, 2.2 Hz, H5), 1.5–1.65 (m, 4H, H6,9), 1.35–1.48 (m, 4H, H7,8), 1.22 (br. s, 1H, OH). IR cm⁻¹: 3360 (s, br.), 3100 (w), 3050 (w), 2965 (s), 2880 (s), 2250 (m), 1590 (m), 1240 (m), 1065 (m), 930 (s), 860 (m), MS m/z: 151 (2, M–Cl)⁺, 114 (72), 112 (52), 105 (45), 91 (84), 79 (100), 77 (57), 65 (55), 51 (37), 41 (99).

(9E)-Dodeca-9,11-dien-7-yn-1-ol (6). Chloroalcohol (3) was protected as the THP ether by treatment with dihydropyran (18 ml) and a few crystals of p-toluenesulfonic acid in ether overnight. The mixture was worked up by extraction with sat. aq. NaHCO₃ and brine, drying, concentration, and removal of solvent traces under vacuum. The protected alcohol (4) gave one spot on TLC (5% EtOAc in hexane) and was used without further purification.

Tetrakis(triphenylphosphine)palladium(0) and chloride (4) (36.92 g, 134 mmol) were added to 300 ml of degassed toluene under Ar. The mixture was cooled in an ice-bath while vinyl magnesium bromide (150 ml of a 1 M solution in THF) was added dropwise over 1 hr. The mixture was warmed to room temperature with stirring overnight. [Caution: the starting material and product were indistinguishable by TLC (5% EtOAc in hexane)]. The reaction mixture was poured into hexane (500 ml), extracted thoroughly with 2 M NH₄Cl and brine, dried, and concentrated. The residue was dissolved in MeOH (100 ml), 100 mg of p-toluenesulfonic acid was added, and the mixture was stirred at room temperature until transesterification was complete (36 hr). NaHCO₃ (2 g) was added, and the mixture was concentrated on a rotary evaporator. The residue was partitioned between water and hexane (200 ml each). The hexane layer was washed with brine, dried, and partially fractionated by passage through a 2.5-cm × 20-cm column of silica gel, eluting with 40% EtOAc in hexane, and cutting ca. 300-ml fractions. The fraction containing the purified product was concentrated and pumped under vacuum, giving dienynol (6) as a gold oil. NMR
(7Z,9E,11)-Dodecatrien-1-yl formate (8). Zinc granules (30 mesh, 100 g) were washed with 200 ml of 0.1 M HCl for 20 min under Ar. The acid was pipetted off, and the cleaned zinc was rinsed twice with distilled water. THF-water (80:20, 500 ml) was added, followed by a solution of dienynol (6) (13.35 g, 75 mmol) in 50 ml THF. The mixture was stirred vigorously under Ar for five days, then filtered, and the THF removed by rotary evaporation. The residue was extracted with hexane, the hexane layer backwashed with brine, dried, and concentrated, yielding 13.6 g of trienol (7) as a yellow oil. This oil was used without further purification.

Formic acetic anhydride was freshly prepared by addition of formic acid (20 ml of 96%) to 40 ml of acetic anhydride at 0°C. When the addition was complete, the mixture was slowly warmed to 50°C, held at this temperature for 20 min, then cooled to room temperature, and used immediately.

The mixed anhydride (30 ml) was added dropwise to a solution of trienol (7) (13.6 g, 75 mmol) and pyridine (30 ml) in ether (250 ml) at 0°C. The mixture was warmed to room temperature overnight, then cooled to 0°C, and 125 ml of sat. aq. NaHCO₃ were added cautiously (effervescence!) The layers were separated, the ether layer was extracted again with aq. NaHCO₃ and brine, then dried and concentrated, yielding 14.2 g of crude product (8). A portion (4 g) was purified by flash chromatography on silica gel (5 cm × 25 cm, 5% ether in hexane), yielding 2.47 g of purified product, which was contaminated with 4% of an unidentified isomer. The purified product showed signs of rapid degradation, with the formation of insoluble white deposits on the rims of the collection tubes. Consequently, the rest of the crude material was purified by rapid Kugelrohr distillation (oven temperature 110°C, 0.07 mm Hg). The distillate (8.79 g from 10.2 g of crude material) was immediately diluted to a concentration of 50 mg/ml with hexane, butylated hydroxytoluene (BHT) was added as an antioxidant (50 mg/g formate), and the solutions were flushed with Ar before storing in brown glass bottles at -20°C. Formate solutions prepared this way could be stored for several months with negligible deterioration.
Hz, H7), 5.22 (d, 1H, J = 16.6 Hz, H12-trans), 5.08 (d, 1H, J = 10.2 Hz, H12-cis), 4.17 (td, 2H, J = 6.6, 0.5 Hz, H1), 2.20 (m, 2H, H2), 1.67 (m, 2H, H6), 1.5-1.3 (m, 6H, H3-5). ^13C NMR δ: 25.68, 27.72, 28.44, 28.73, 29.43, 64.02, 117.00, 128.49, 128.54, 133.06, 133.15, 137.17, 161.18. IR cm⁻¹: 3110 (w), 3040 (m), 2965 (s), 2880 (s), 1725 (s), 1625 (m), 1585 (m), 1475 (m), 1190 (s), 1015 (s), 950 (m), 910 (m). MS m/z: 208 (21, M⁺), 133 (4), 119 (5), 105 (12), 93 (39), 91 (64), 80 (84), 79 (100), 77 (64), 65 (16), 41 (42).

Wind-Tunnel Bioassays. Moths were obtained from laboratory cultures started from individuals collected in 1985 from infested dates near Indio in the Coachella Valley of Riverside County, California. Larvae were reared on a honey and wheat-bran diet in 1-gallon jars (Warner, 1988). Pupae were segregated by sex, and adults emerged on a 14:10 hr light-dark photoperiod regime.

Bioassays were conducted in a 3.5 × 1.0 × 1.0-m wind tunnel described in detail in Kuenen and Baker (1982). Two- to four-day-old males were loaded singly into screen cages (4 cm long × 3 cm diameter) during the photophase and each cage was covered with a Petri dish lid. Cages with males were placed in an environmental chamber until being transferred to the wind tunnel 1 hr before bioassays in order to acclimate males to the wind-tunnel conditions (24–26°C, 0.3 lux, 30–70% relative humidity, 0.5 m/sec wind velocity). Bioassays were conducted 4–6 hr into the scotophase, the optimal sexual activity period for carob moth (Vetter, Tatevossian, and Baker, unpublished data). Two 8:1:1 blend ratios were used, representing 40 ng trienal, 5 ng dienal, and 5 ng monoenal (referred to as the trienal blend), or 40 ng formate, 5 ng dienal, and 5 ng monoenal, respectively, with the formate replacing the trienal (referred to as the formate blend). The trienal and the formate also were each tested alone at 40 ng. Solutions were checked on GC to confirm that the ratio and concentration of each treatment were correct. When freshly prepared, the trienal was >98% stereoisomerically pure by capillary GC (DB-5). However, the trienal was contaminated with 2–5% of a rearrangement product that coeluted with the trienal on silica gel flash chromatography or HPLC. The formate was >92% pure by capillary GC, and was contaminated with an uncharacterized impurity, also apparently due to rearrangement. A control consisted of 5 female equivalents (FE) obtained from excised pheromone glands according to methods described in Baker et al. (1991). For both the treatment and the control, 10 μl of a diluted solution prepared in hexane was delivered onto a 5-cm-diameter filter-paper disk (Whatman No. 1) affixed to a 6-cm-long metal clip that was stuck in a cork base. The cork wire holder for each disk was placed on a 15 × 15-cm sheet-metal platform 15 cm above the tunnel floor, and 30 cm from the tunnel’s upwind end. After completing bioassays, clips were cleaned with acetone and air-dried overnight.

Males were released from their cages 1 m from the odor source. A cage
containing a male was placed on the metal platform in the plume until the male took flight or until 1 min elapsed. If males did not fly, they were checked for flight ability by forcibly dislodging them from the cage. Forty flight-capable males were tested for each treatment and for the control. Males were scored for: (1) locking onto the plume and progressing upwind, (2) flying to within 50 cm of the source, and (3) contacting the source. Tests were conducted using a randomized, complete-block design so that all treatments were presented on a given day. The numbers of moths performing each behavior were compared by using a $\chi^2 2 \times 2$ test of independence with Yates' correction (Steel and Torrie, 1960). Statistical significance was determined at the 0.05 level.

**Field-Trapping Studies.** Two field studies were conducted in Deglet Noor date gardens located in the Coachella Valley in Indio or in Oasis, California, to determine the attractancy of the formate compared to that of the three-component aldehyde blend of carob moth. One study was conducted from late August through late September, 1990. Four different lures were placed in separate Pherocon 1C traps (Zoecon Corp., Palo Alto, California), and the traps were positioned in the date palm canopy, which was ca. 18 m above ground level, by using a rope and pulley system. Lures consisted of black, chopped, Celcon hollow fibers (1.5 cm × 0.02 cm ID) (Scentry Inc., Buckeye, Arizona) that were heat-sealed at one end and then vacuum-filled with either an 8:1:1 blend of the trienal–dienal–monoenal (three fibers), or with the formate (three and 10 fibers). Both the aldehyde blend and the formate were diluted by 90% with an inert diluent, methyl tridecanoate, before the fibers were filled. Fibers also were filled with neat formate (three fibers). Fibers were stuck into a 2-cm polyurethane foam cube and affixed to the bottom of a trap. Treatments were placed in a row in each of three replicates in a randomized, complete-block design. There were two trees separating treatments in each block and 10 trees between blocks. Traps were checked every three to four days, the males removed, and the traps re-randomized within blocks. Trap bottoms were replaced when they became too soiled or dusty. Trap counts were transformed using $(x + 0.5)^{1/2}$ before being subjected to analysis of variance (ANOVA). Treatment means were separated at the 0.05 level of significance using Tukey's HSD Studentized range test (Steel and Torrie, 1960).

Because this field study (as well as two others containing live females) seemed to indicate a significant interference with male capture even to females, and hence a general disruptive effect whenever a grid of more than a few traps was used, another field comparison was made between a single trap containing the synthetic lure (either trienal blend or formate), and a trap containing live females. This study was conducted in three Deglet Noor date gardens from mid-July through mid-August of 1990 and 1991. Both the aldehyde blend and the formate were diluted by 90% with methyl tridecanoate, and three fibers were filled as previously described and stuck into a 2-cm polyurethane foam cube.
that was affixed to the bottom of a Pherocon 1C trap. Five 1- to 3-day-old virgin female carob moths were placed in a screen cage in a separate Pherocon 1C trap as a control. A 2-dram glass vial containing a water-saturated dental wick was placed inside the screen cage to provide a water source for females. Traps were placed in the date palm canopy, which varied between 2 and 15 m above ground level, depending on the garden, by using a rope and pulley system. In 1990, a trienal blend-baited trap was placed in a date palm in each of the three date gardens, with a female-baited trap placed 5–10 trees away (ca. 30-m spread). In 1991, a formate-baited trap was placed in the same date palm in each garden as the previous year, with a female-baited trap also placed in the same tree as the trap from the previous year. The number of males caught in female-baited or in treatment-baited traps was recorded every three to four days. In both years, traps with females were rebaited twice a week, and fiber traps were rebaited once every two weeks. The trap catches of female-baited or of treatment-baited traps from each date garden were combined to obtain the mean (±SD) number of males captured per trap for each year. Trap catches from treatment-baited traps, either trienal blend-baited or formate-baited, and from female-baited traps were totaled within each garden, and the ratio between the number of males captured in a treatment-baited trap and in a female-baited trap was computed for each of the three date gardens. These six ratios were compared using ANOVA with the two chemical treatments blocked by date garden site. Statistical significance was determined at the 0.05 level.

Single-Cell Recordings and Stimulation. Recordings from olfactory antennal neurons of 1- to 3-day-old male carob moths were obtained by using a cut-sensillum technique (Kaissling, 1974; Van der Pers and Den Otter, 1978) that has been used on several other species of Lepidoptera (Baker et al., 1989a; Hansson et al., 1987, 1990; Löfstedt et al., 1990; Todd et al., 1992). An antenna was cut from the head, and its base placed in a micropipet (50 μl FISHERbrand disposable micropipets) that was filled with Beadle-Ephrussi Ringer solution (Ephrussi and Beadle, 1936), and grounded with an Ag–AgCl wire. Using a Leitz stereo microscope at 320× magnification, a single sensillum trichodeum was positioned over the blade of a vertically oriented, stationary glass knife by using a micromanipulator. The tip of the hair was then cut using a second glass knife whose edge was brought down over the vertical knife edge by means of a Leitz joy-stick type micromanipulator. The cut end of the hair was immediately contacted with a saline-filled Ag–AgCl pipet recording microelectrode. To prevent evaporation of the saline, a 10% aqueous solution of polyvinylpyrrolidone (Sigma Chemical Co., St. Louis, Missouri) was added to the saline (1:10 parts by volume), and the tip of the recording microelectrode was coated with a thin film of petroleum jelly. Recordings of both the AC and DC components of the signal were amplified on a Hansson model 103 amplifier, and stored on VCR tape using a Vetter model 420F four-channel FM recorder. Responses were...
The antenna was continuously bathed in a stream of purified, humidified air (10 ml/sec) that passed through a 17-cm-long glass tube (8 mm ID) whose outlet was positioned 2 cm from the antennal preparation. For each of the three sex pheromone components and the formate, 10 µl of a diluted solution prepared in hexane was pipetted onto a filter-paper strip held in a glass Pasteur pipet cartridge (15 cm long). Stimulus doses ranged from $10^{-3}$ to $10^1$ µg/cartridge in log_10 steps. Solutions were checked by GC to confirm that the amounts of respective compounds at a particular concentration were equal. Prior to exposure to any of the test compounds, the spontaneous activities of the receptor cell(s) within a sensillum were monitored for 60 sec. Cells were exposed to 30 msec puffs (Roelofs and Comeau, 1969) of each compound in random order, beginning with the $10^{-3}$-µg cartridges and working upward to the 10-µg cartridges, by manually injecting a 2-ml puff into the airstream through a hole in the glass tube 15 cm from the outlet. Interstimulus intervals ranged between 30 and 60 sec, or until the background firing approximated that observed prior to stimulus presentation. When action potentials (spikes) were elicited by a compound, their frequency was determined for 0.2 sec following the commencement of excitation, and peak-to-peak amplitude values (mV) of the bimodal spikes were obtained during this period. The spike frequencies of individual receptor cells that were excited by each dosage of the trienal and of the formate were compared using Student's $t$ test at the 0.05 significance level.

Cross-Adaptation Studies. Preliminary recordings indicated that the trienal and the formate consistently stimulated a large-spiking cell within each sensillum. Therefore, cross-adaptation studies were conducted to determine if the spiking activity could be attributed to the same cell or to different cells that produced similar-sized spikes (Kaissling et al., 1989). Recordings were made from the cells within 15 sensilla using four antennae of 1- to 3-day-old male carob moths as previously described. A 1-µg filter-paper-loaded cartridge of the trienal and of the formate provided the stimulus sources. To deliver a uniform stimulus with a well-defined onset and offset, a stimulus flow controller (model SFC-2, Syntech Inc., Hilversum, The Netherlands) that has been described in detail by Vickers and Baker (1992) was used. The stimulus valve was open for 0.5 sec, and two interstimulus intervals, i.e., the time between the end of the first stimulus and the beginning of the second stimulus, were used, either 0.1 sec or 1 sec. The stimulus regime consisted of the following four stimulus pairs presented at both interstimulus intervals: (1) trienal–trienal; (2) formate–formate; (3) trienal–formate; and (4) formate–trienal. Between 30 and 60 sec elapsed between exposure of the preparation to each stimulus pair.

Scanning Electron Micrographs. Antennae from newly emerged males were used for scanning electron micrographs. For fixation, antennae were placed in...
2% glutaraldehyde (in cacodylate buffer, pH 7.4). Postfixation involved placing the antennae in a 2% solution of osmium tetroxide for 24 hr, followed by dehydration to 100% EtOH. Glacial acetic acid was added to each dehydration step to maintain the natural size and shape of the antenna. Specimens were critical-point dried, mounted on stubs and coated under vacuum with 60:40 gold-palladium. Specimens were examined and micrographs prepared at 15 kV accelerating voltage, using 55 P/N film on a JEOL JSM-C35 SEM located in the Department of Nematology, University of California, Riverside.

RESULTS

Wind-Tunnel Bioassays. The 8:1:1 blend containing either the trienal or the formate, or either compound alone, was as effective as 5 FE of gland extract in eliciting upwind flight in male carob moths (Table 1). Similarly, all blends were equivalent to 5 FE of gland extract in causing males to fly to within 50 cm of the source ($P > 0.05, \chi^2 2 \times 2$ test of independence) (Table 1). The formate alone was significantly better than either the trienal alone or than the 8:1:1 trienal or formate blends in eliciting close approach to the source ($P < 0.05, \chi^2 2 \times 2$ test of independence) (Table 1). The formate alone also caused significantly more males to complete upwind flight and contact the source compared to either the trienal blend or to the trienal alone ($P < 0.05, \chi^2 2 \times 2$ test of independence). Both the formate alone and the formate-containing blend were as effective as the natural extract in evoking source contact (Table 1).

### Table 1. Percentages of Male Carob Moths Flying in Wind Tunnel in Response to 8:1:1 Blend of Trienal, Dienal, and Monoenal, or of Formate, Dienal, and Monoenal, or to Trienal or Formate Alone (8:0:0), Compared to 5 FE of Gland Extract

<table>
<thead>
<tr>
<th>Behavior</th>
<th>(%) Males responding to blend ratios</th>
<th>trienal 8:0:0</th>
<th>8:1:1</th>
<th>formate 8:0:0</th>
<th>8:1:1</th>
<th>Gland extract, 5 FE</th>
</tr>
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<tbody>
<tr>
<td>Upwind flight</td>
<td></td>
<td>50 a b</td>
<td>65 a</td>
<td>70 a</td>
<td>63 a</td>
<td>53 a</td>
</tr>
<tr>
<td>Flight to within 50 cm</td>
<td></td>
<td>38 b</td>
<td>38 b</td>
<td>65 a</td>
<td>38 b</td>
<td>43 ab</td>
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<td>of the source</td>
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<tr>
<td>Source contact</td>
<td></td>
<td>18 b</td>
<td>18 b</td>
<td>45 a</td>
<td>25 ab</td>
<td>40 a</td>
</tr>
</tbody>
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*Source loading on filter paper was 40 ng of trienal or formate in 10 μl of hexane. N = 40 flight-capable males.*

*Percentages in a row followed by the same letter are not significantly different at the 0.05 level according to a $\chi^2$ 2 × 2 test of independence with Yates' correction.*
**Field-Trapping Studies.** In the field study comparing the attractancy of the four synthetic lures to male carob moths, traps containing the three-fiber diluted formate lure captured as many males (2.6 ± 3.8, mean ± SD) per three- to four-day trapping period as those containing the three-fiber diluted trienal blend lure (0.7 ± 1.2, mean ± SD) (P > 0.05, Tukey’s test). Thus the formate was at least as active as the trienal blend in attracting males in the field. The 10-fiber diluted formate lure attracted significantly more males to traps (6.3 ± 6.8, mean ± SD) than the three-fiber neat formate lure (1.7 ± 2.2, mean ± SD), or the three-fiber diluted trienal blend lure (0.7 ± 1.2, mean ± SD) (P < 0.05, Tukey’s test), but a similar number of males as the three-fiber diluted formate lure (2.6 ± 3.8, mean ± SD). There were no statistically significant differences between the mean numbers of males caught per trap for all the other treatment comparisons (P > 0.05, Tukey’s test).

In the other field study, the female-baited traps caught significantly more males (42 ± 34.4, mean ± SD, N = 33) than the trienal blend-baited traps (3.7 ± 4.6, mean ± SD, N = 33) when placed in a date palm canopy (P < 0.001, Student’s t test). Similarly, female-baited traps captured significantly more males (67.2 ± 36.5, mean ± SD, N = 31) than the formate-baited traps (16 ± 10.9, mean ± SD, N = 30) (P < 0.01, Student’s t test). However, the formate was a significantly better lure relative to females than the trienal-containing blend, since the formate-to-female trap capture ratio (0.25 ± 0.07, mean ± SD) was significantly larger than the trienal-to-female trap capture ratio (0.07 ± 0.05, mean ± SD) (P < 0.01, ANOVA). Thus, the formate-baited traps caught 3–4 times as many male carob moths as the trienal-blend containing traps, relative to live-female traps.

**Single-Cell Recordings and Stimulation.** Single-cell recordings were obtained from 56 sensilla using 10 male antennae. The longer, curved trichoid hairs located on the ventral aspect of the antenna, and irregularly distributed over the surface of a subsegment, were easily accessible to cutting compared to the shorter, straighter hairs located closer to the dorsal scaled surface (Figure 2). Recordings from these longer trichoid sensilla showed that two spike sizes could be easily distinguished; there was a larger impulse of 13.6 ± 3.9 mV (mean ± SD), and a smaller impulse of 6.9 ± 0.8 mV (mean ± SD). The spike amplitude values obtained from cells responding to emissions from 10-μg cartridges of the dienal, trienal, and formate were not included in these mean values because the spike amplitudes of both cells were noticeably reduced by exposure to this dosage. Although transmission electron microscopy of male carob moth antennae has not yet been performed to confirm the number of receptor cells within the longer trichoid hairs, the two different spike amplitudes that we consistently observed indicate that at least two receptor cells are present, which we will refer to as the large and the small cell, respectively, even though at this point it would be possible for more cells of identical spike sizes to be present.
In all but two of the sensilla, background firing by one or both receptor cells was observed prior to stimulus presentation. In 34 of the sensilla, both cells fired spontaneously. The background activity was $0.3 \pm 0.1$ spikes/sec (mean $\pm$ SD) for the smaller-spiking cell, recorded from 43 sensilla, and $0.5 \pm 0.1$ spikes/sec (mean $\pm$ SD) for the larger-spiking cell, recorded from 45 sensilla.

Dose–response curves indicated that puffs from cartridges loaded with less than $0.1 \mu g$ of a pheromone component or of the formate did not elicit spiking activity from either the large cell (Figure 3A) or the small cell (Figure 3B). A few of the sensilla sampled contained a cell or cells that were excited by puffs from 0.1-$\mu g$ cartridges of the dienal, trienal, and formate, but not by the monoenal (Table 2). All three compounds stimulated the larger-spiking cell within a sensillum (Figure 4), although the smaller cell also was excitable in some sensilla (Table 2). There was no significant difference between the spike frequencies of the large-spiking cell when exposed to puffs from 0.1-$\mu g$ cartridges of the trienal or of the formate ($P > 0.05$, Student’s $t$ test) (Figure 3A). A statistical comparison between the number of spikes evoked by the small cell when exposed to emissions from 0.1-$\mu g$ cartridges of the trienal or of the formate could not be made because only one of the sensilla we sampled contained a small cell excited by the formate (Table 2).

The numbers of sensilla with receptor cells responding to puffs of the dienal, trienal, and formate increased most dramatically between the 0.1-$\mu g$ and 1-$\mu g$ dosages (Table 2). The first receptor cell responses to the monoenal also were recorded at the 1-$\mu g$ dosage (Figure 3A), although the number of sensilla that we sampled that contained such cells was low (Table 2). The monoenal
Fig. 3. Mean (± SE) dose–response relationship of the spiking activity of a large cell (A) and a small cell (B) in male carob moth antennal sensilla in response to puffs from cartridges loaded with graded amounts of one of the three sex pheromone components (monoenal, dienal, and trienal) or the trienal mimic (formate). Fifty-six sensilla were sampled. For the small-cell responses (B) at the 0.1-μg dosage, note the asterisk, which represents the spiking activity evoked by exposure to the dienal (65 spikes/sec, N = 1) and to the formate (65 spikes/sec, N = 1); no spiking activity was recorded from the small cell in the other 55 sensilla sampled.

typically stimulated the larger-spiking cell within a sensillum (Figure 4). The spike frequencies elicited by both the large and the small cell when exposed to puffs from 1-μg cartridges of the trienal were not significantly greater than those evoked by exposure to the formate (P > 0.05, Student’s t test) (Figure 3A and 3B).

Exposure to puffs from 10-μg cartridges of the trienal or of the formate elicited single-cell responses from nearly all the sensilla sampled (Table 2). The spike frequencies of the small and the large cells when exposed to puffs from 10-μg cartridges of the trienal were not significantly different from those evoked by exposure to the formate (Student’s t test, P > 0.05) (Figure 3A and 3B).
TABLE 2. PERCENTAGE OF SENSILLA ON MALE CAROB MOTH ANTENNAE WITH RECEPTOR CELL(S) RESPONDING TO EMISSIONS FROM CARTRIDGES LOADED WITH VARIOUS DOSAGES OF MONOENAL, DIENAL, AND TRIENAL (SEX PHEROMONE COMPONENTS), AND OF FORMATE (TRIENAL MIMIC)\(^a\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>0.001 (\mu g)</th>
<th>0.01 (\mu g)</th>
<th>0.1 (\mu g)</th>
<th>1 (\mu g)</th>
<th>10 (\mu g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>L</td>
<td>SM</td>
<td>L</td>
<td>SM</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
</tr>
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<td>0</td>
<td>1.8</td>
<td>3.6</td>
<td>0</td>
</tr>
<tr>
<td>Trienal</td>
<td>0</td>
<td>0</td>
<td>3.6</td>
<td>12.5</td>
<td>21.4</td>
</tr>
<tr>
<td>Formate</td>
<td>0</td>
<td>0</td>
<td>1.8</td>
<td>12.5</td>
<td>17.9</td>
</tr>
</tbody>
</table>

\(^a\)A total of 56 sensilla was sampled. SM = small cell, L = large cell.

![Graph showing single-cell responses](image)

FIG. 4. Typical single-cell responses of male carob moth antennal cells to emissions from 0.1-\(\mu g\), 1-\(\mu g\), and 10-\(\mu g\) cartridges of the monoenal, dienal, and trienal (sex pheromone components) and of the formate (trienal mimic). Arrows represent stimulus presentation. Note the spikes (2) produced by the smaller-spiking cell interspersed among the large spikes for 10 \(\mu g\) of monoenal.
Puffs from 10-μg cartridges of the dienal, trienal, and formate appear to overdrive the larger-spiking receptor cell, as suggested by the reduced interspike intervals and diminished spike amplitudes immediately following stimulus presentation (Figure 4). However, the spike amplitude of the large cell was not diminished by exposure to puffs from 10-μg cartridges of the monoenal (Figure 4). We also noted increased spiking activity from the small cell within a sensillum after puffs from 10-μg cartridges of each compound, compared to the lower dosages (Figure 3B).

**Cross-Adaptation Studies.** The single-cell responses of a large-spiking neuron located within the antennal sensilla of male carob moths showed that this neuron could be partially adapted by exposure to the trienal-trienal pair, with a 0.1-sec interstimulus interval (Figure 5A). A large-spiking cell was similarly partially adapted by exposure to the formate-formate pair at a 0.1-sec interstimulus interval (Figure 5E). For both the trienal-trienal (Figure 5C) and the formate-formate (Figure 5G) stimulus pairs, the large cell was capable of becoming markedly more disadapted after a 1-sec interstimulus interval because the second stimulus of each pair evoked action potential bursts that were nearly as high in frequency as those to the first stimulus of the pair. Exposing the neuron to the trienal-formate or to the formate-trienal pairs resulted in a similar pattern of responses as those evoked by the homogeneous pairs, whether it was for the 0.1-sec interstimulus interval (Figures 5B and 5F) or for the 1-sec interstimulus interval (Figures 5D and 5H). Initial exposure to the trienal seems to adapt the large cell more completely to the second puff in the stimulus pair, regardless of whether it is the formate or the trienal (Figure 5A-D), compared to when initial exposure is to the formate (Figure 5E-H).

**DISCUSSION**

If a mating disruptant is to be developed against the carob moth, it would be helpful to extend the field life of the blend of aldehydes identified by Baker et al. (1989b, 1991) or to develop a more stable mimic. The first step in developing a more stable mimic is ensuring that its ability to attract males is as good as the identified pheromone components. In this initial assessment of the activity of a potential trienal pheromone mimic, our results indicate that (Z,E)-7,9,11-dodecatrienyl formate is an effective replacement for (Z,E)-9,11,13-tetradecatrienal, the major sex pheromone component of the carob moth. We do not know why the formate seems to be as effective as, or more effective than, the blend of the trienal plus two other aldehydes in attracting carob moth males in the wind tunnel and in the field. However, the greater activity of a mimic compared to the natural pheromone is not unprecedented. Mimics have been found in other studies that are more active in attracting males than the natural...
FIG. 5. Histograms showing the spike frequency (mean ± SE) of the large-spiking neuron to four stimulus pairs (T = trienal, F = formate) during cross-adaptation studies. Each histogram shows a 1-sec prestimulus period, followed by a 3-sec period during which two 0.5-sec long stimulus puffs are directed over the sensillum. Horizontal lines above the bars indicate that the stimulus valve is open, and odor molecules are entering the airstream that is passing over the antennal preparation. Interstimulus intervals are 0.1 sec and 1 sec. Recordings are continued for at least 1 sec after the end of the second stimulus.
sex pheromone mimic

...
Trienial under the field conditions in the date gardens. In the field study comparing the attractancy of four synthetic lures to male carob moths, our frequent replacement of the lures every three to four days was designed to test the attractancy of fresh formate and trienial lures without the complication of potential long-term degradation. In the other field study, the fiber lures were replaced every two weeks, and the formate outperformed the trienial blend, relative to live females, in attracting males, thus suggesting that the formate may have a longer period of optimal attractivity than the aldehyde blend. The assessment of relative field longevity needs to be performed in future field studies in which traps have much longer replacement intervals.

The coupling between female-emitted sex pheromone components and the olfactory receptor cells located in male antennal sensilla is generally highly specific for moths (Baker, 1989), with one receptor type very selective for a particular component of the sex pheromone blend. Although the electrophysiological activity of a compound may be detrimentally affected by altering the double-bond position or geometry (Liljefors et al., 1985; Bengtsson et al., 1987), or by modifying functional groups (Liljefors et al., 1984), pheromone mimics can substitute for the natural pheromone component at both the neurophysiological and the behavioral levels (Löfstedt et al., 1990).

The electrophysiological activity of formates on the antennal receptors of several noctuid species was demonstrated by Priesner et al. (1975) when they showed that formate compounds could evoke moderate EAG responses when compared to natural sex pheromone components. Our single-cell recordings from male carob moth antennal neurons showed that the formate was as electrophysiologically active as the trienial in evoking action potentials. The effective concentrations of the two compounds reaching the sensilla during stimulation were likely to be very similar, based on the comparable vapor pressures of the trienial (206 MW) and the formate (208 MW). At all dosages tested, the spike frequencies elicited from receptor cells were similar for the trienial and for the formate, and cross-adaptation studies demonstrated that both compounds stimulated the same large-spiking cell. Grant et al. (1989) also found that the aldehyde major sex pheromone component of Heliothis zea (Boddie) and a formate mimicking this component stimulated the same large-spiking neuron, although the aldehyde elicited significantly higher spiking activity than the formate when tested at several dosages. Based on GC-single-cell analysis, Baker et al. (1991) initially concluded that the trienial was stimulating a smaller-spiking cell within a sensillum, whereas the monoenal and the dienal were stimulating the larger-spiking cell. However, the present, more detailed examination of the antennal receptor cell responses of male carob moths to various dosages of the trienial indicates this conclusion was probably in error. Excessive amounts of the trienial may have issued from the GC effluent in the GC-single-cell study by Baker et al. (1991), thus overdriving the larger-spiking cell, and causing a reduction in
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spike size to that more typical of the smaller-spiking cell (Figure 4). We now conclude that the major component and its mimic stimulate the larger-spiking cell within a male carob moth antennal sensillum, as is typical of all other moth species studied to date (Grant et al., 1989; Hansson et al., 1987, 1990; Löfstedt et al., 1990). Our results indicating that initial exposure to the trienal adapted the large cell more completely to the second puff in the stimulus pair, whether it was the trienal or the formate, may be explained by slight differences in the concentration emitted from the trienal pipets compared to the formate pipets, or perhaps to subtle differences in the time courses of adaptation–disadaptation in cells exposed to the trienal compared to the synthetic mimic. Nevertheless, our conclusion is that the two compounds stimulate the same receptor neuron based on the great similarities in adaptation of the large neuron.

Our data indicate that the behavioral and electrophysiological activity of the formate is equal, if not enhanced, compared to that of the trienal blend in attracting carob moth males. Although the formate has thus far outperformed the trienal blend under field conditions, more work needs to be done to improve the efficacy of the formate lure to make it competitive with females with regard to attractancy. We do not know why the ability of the formate to attract males in the wind tunnel is as good as the female extract, yet in the field it is only a fraction as good as live females.

In general, the high activity of synthetic blends in wind tunnels has been maintained in trapping tests in the field (Baker and Linn, 1984). However, full comparisons of the activity of synthetics against females in both the wind tunnel and the field are relatively rare, and so it is difficult to say how unusual such a decline in activity is, when the lures are taken into the field. It may be that the entire effect is due to degradation under field conditions, or perhaps some other inadequacy exists with respect to missing components that is not exposed in the relatively simple environment of the wind tunnel. In wind-tunnel bioassays with Manduca sexta (L.), Tumlinson et al. (1989) demonstrated that a synthetic blend of aldehydes evoked the same behavioral response level from males as gland rinses or calling females. One of the aldehydes necessary for completing the behavioral sequence, i.e., locating the source and attempting copulation, was (E,E,Z)-10,12,14-hexadecatrienal. Although a synthetic blend containing this trienal was not tested in the field, Tumlinson et al. (1989) suggested that aliphatic conjugated trienes are very labile and will decompose in nature when placed as components in lures or other pheromone dispensers.

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