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RESPONSES FROM SENSILLA ON ANTENNAE OF MALE *Heliothis zea* TO ITS MAJOR PHEROMONE COMPONENT AND TWO ANALOGS[1]

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Abstract—For some species, chemical analogs have been identified that can substitute for the major pheromone component and reduce pheromone-trap captures in atmospheric permeation experiments. The ability to substitute these analogs for the major pheromone component in field tests raises the question: Do the same set of olfactory receptor neurons on the insects' antennae respond to both the major component and the effective structural analogs? To investigate this question, extracellular responses were recorded from single sensilla on the antennae of male *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae) to stimulation with increasing doses of (Z)-11-hexadecenal, the major pheromone component, (Z)-9-tetradecen-1-ol formate, and (Z)-1,12-heptadecadiene. Both (Z)-9-tetradecen-1-ol formate and (Z)-1,12-heptadecadiene can substitute for (Z)-11-hexadecenal in trap disruption experiments and affect the sexual behavior of males, although neither has been identified in the volatiles released by calling females. All three compounds elicited responses from the same class of pheromone-sensitive receptor neurons over a range of stimulus concentrations. At equivalent stimulus concentrations, however, (Z)-11-hexadecenal elicited about a 10-fold greater response than (Z)-1,12-heptadecadiene. (Z)-9-Tetradecen-1-ol formate elicited a response slightly higher than, but not significantly different from, the response to (Z)-1,12-heptadecadiene.

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INTRODUCTION

Recently, much effort has been devoted to investigating the potential use of pheromones and other semiochemicals in the control of insect pest species (Mitchell, 1981). One control strategy involves the disruption of normal chemical communication between males and females by releasing large quantities of the major pheromone component into the atmosphere surrounding an agricultural crop or commodity (Shorey et al., 1974; Mitchell, 1975; Sanders, 1981). Ideally, this atmospheric permeation with pheromone prevents many males from being able to locate females, thereby drastically reducing insect mating and subsequent egg production. The efficiency of this approach is severely comprised in those species whose major pheromone component is unstable in storage or in the field. This has prompted evaluation of closely related analogs of major pheromone components with the view toward finding more stable mimics (Beevor and Campion, 1979; Carlson and McLaughlin, 1982a,b; Silk et al., 1985; Silk and Kuenen, 1986; Curtis et al., 1987).

Heliothis zea (Boddie) (Lepidoptera: Noctuidae), the corn earworm moth, is one insect where communication disruption using both pheromone components and analogs has been demonstrated (Mitchell, 1975; Mitchell et al., 1976; McLaughlin et al., 1981; Tingle and Mitchell, 1982; Mitchell and McLaughlin, 1982). Female *H. zea* produce a complex blend of pheromones that attract conspecific males for mating. The major component of their blend, (Z)-11-hexadecenal (AL), constitutes approximately 92% of the volatiles released by calling females (Klun et al., 1979). Mitchell et al. (1975) have demonstrated that in addition to disruption using AL, a structural analog, (Z)-9-tetradecen-1-ol formate (FO), can also disrupt this insect's communication. Carlson and McLaughlin (1982a,b) have shown a similar disruption ability with *H. zea* using another structural analog, (Z)-1,12-heptadecadiene (OL). Neither FO nor OL have been identified in gland extracts or volatiles released from this species.

The apparent ability of these analogs to substitute for the major pheromone component in disruption experiments raises an interesting question concerning the detection of these semiochemicals by the insect's peripheral sensory system. In most lepidopteran systems studied to date, receptor neurons sensitive to pheromone components show a high degree of selectivity for their target compounds. Consequently, one might question whether the effective mimics would stimulate the same or a different population of olfactory receptor neurons. Several researchers (Mitchell et al., 1975; Priesner, 1979) have suggested that such chemical mimics compete for activation sites on the same set of olfactory receptor neurons, which are responsive to the major component of the pheromone. To investigate the specificity of pheromone-sensitive neurons, we recorded responses using standard extracellular recording techniques from olfactory receptor neurons contained within sensilla on the antenna of male *H. zea* in response to increasing doses of Al, FO, and OL. We investigated those sexually dimorphic sensilla that are oriented in rows along the proximal 40 flagellar subsegments on the male antenna.

METHODS AND MATERIALS

Insects and Chemicals. Male *H. zea* were taken as pupae from colonies maintained at this laboratory. Insects were held at 75–85% relative humidity and 24–26°C under a 14 : 10 hr light–dark regimen. Neurophysiological recordings were conducted 24–72 hr following adult emergence. Chemical samples used in this study were provided by Dr. J. H. Tumlinson (AL and FO) and Dr. D. A. Carlson (OL). (*Z*)-11-Hexadecenal was analyzed by GLC on a OV-1, 36-m capillary column and found to be 99.3% pure. (*Z*)-1,12-Heptadecadiene was analyzed on a DB-1, 15-m capillary column, and no impurities, such as AL, were detected. (*Z*)-9-Tetradecen-1-ol formate was analyzed on 3% OV-1 on 100–120 mesh Gas Chrom-Q (1.8 m × 2 mm ID glass column) and found to >99% pure.

Recordings. Insects were secured for single sensillum recordings with low-melting-point wax and their antennae positioned to allow access with microelectrodes. The microelectrodes, electrolytically sharpened tungsten wire (tip diameters approx. 1 μm), were held and positioned under a compound microscope (E. Leitz, Inc., Rockleigh, New Jersey; 600×) with micromanipulators (E. Leitz). The indifferent electrode was inserted into the lumen of a flagellar subsegment on the distal one third of the antenna, and the recording electrode was positioned at the base of an individual sensillum trichodeum on a more proximal subsegment. Penetration of the sensillum cuticle with the recording electrode was accomplished by gently tapping the manipulator along the long axis of the electrode. The signals from the microelectrodes were amplified by a Grass P-18 preamplifier and sent concurrently to a Tektronix 5113 storage oscilloscope for observation and to a Digital PDP-11/23 minicomputer for data acquisition and analysis (Mankin et al., 1987).

Following penetration of the sensillum by the recording electrode, the preparation was allowed to stabilize for 10–15 min prior to the first stimulus presentation. During this period and throughout the recording session, purified carrier air (1000 ml/min) passed over the preparation to isolate the sensillum from potentially contaminated room air. The spontaneous activity of each neuron in the sensillum was measured before stimulation and was monitored at intervals during the recording session to assess the stability of the preparation.

Stimulation. The methods of dispenser preparation and stimulus delivery are described elsewhere (Mayer, 1973; Grant et al., 1989). Briefly, desired quantities of the chemical component, diluted in 0.5 ml of hexane, were distributed over the inner surfaces of the cylindrical glass dispensers. Following the evaporation of the hexane solvent, the ends of the assembly were attached by ground-glass joints to the stimulus delivery system. Control dispensers contained only 0.5 ml of hexane solvent. Delivery of the stimulus was accomplished by combining a stimulus airstream (200 ml/min), which passed through the pheromone-dosed dispenser, with a purified carrier airstream (1000 ml/min). The mixture of these two streams subsequently passed over the preparation. Initiation and termination of the stimulus period was accomplished by computer-controlled activation and deactivation of a solenoid valve attached to the stimulus airstream (Mankin et al., 1987; Grant et al., 1989). In all cases, 10 sec of neural activity were recorded. This 10 sec was composed of three periods: prestimulus (0–3.0 sec), stimulus (3.0–6.0 sec), and poststimulus (6.0–10.0 s). Responses are expressed as the mean frequency of impulses generated during the stimulus period minus the mean frequency of impulses generated during the prestimulus period.

Dose–Response Relationships. To establish mean dose–response relationships, increasing doses of AL were presented to at least 21 preparations. To establish the mean dose response relationships for FO and OL, each of 10 preparations was stimulated a single time at each dose. Each preparation was exposed to graded doses of only one stimulus compound. In all cases, the lowest dose stimulus was presented first, followed by stimuli of increasing dosages. Interstimulus intervals ranged from 4 to 10 min depending on the magnitude of the previous response. Control stimuli were presented approximately every five stimulations. The actual concentrations that stimulated the antenna were estimated from emission rate calibrations (Mayer et al., 1987).

Scanning Electron Microscopy. To determine the distribution of the sexually dimorphic sensilla on the male antenna, three antennae were examined using scanning electron microscopy. Micrographs were taken of every tenth subsegment beginning at the tenth flagellar subsegment from the pedicel. Prior to sputter coating with 60:40 gold–palladium, portions of the antenna were washed in spectrophotometric grade hexane, allowed to air dry, and grounded to the grid supports with silver paint. Coated specimens were immediately examined with a Hitachi 500 STEM microscope at 30 kV accelerating voltage and magnifications to 25,000×. Micrographs were recorded with Polaroid Type 55 P/N film.

RESULTS

Distribution of Sensilla. In agreement with a previous description of the antennae morphology of *H. zea* (Callahan, 1969), we found sexually dimorphic sensilla trichodea oriented in rows along the lateral surfaces of the proximal 40–50 flagellar subsegment (Figure IA,B). These sensilla were absent from the distal 20–30 subsegments (Figure IC) and appeared to be typical lepidopterous sensilla trichodea (e.g. Zacharuk, 1986), with their surfaces annulated and sparsely pitted by pores (Figure 1D). From our micrographs, we estimate that there are approximately 2200 of this type of sensillum on each male antenna.

Response Characteristics. Extracellular recordings from *H. zea* indicate that each sensilla trichodeum is innervated by at least two spontaneously active receptor neurons. The action potentials range in amplitude from 50 to 400 μV and have typical durations of approximately 2.5 msec. These impulses can be reliably discriminated from each other by their amplitudes and waveforms. The receptor neuron producing the larger impulse is designated as A and the neuron producing the smaller as B. In several of the preparations, very small impulses (< 30 μV) were observed that could not be reliably discriminated from the noise.

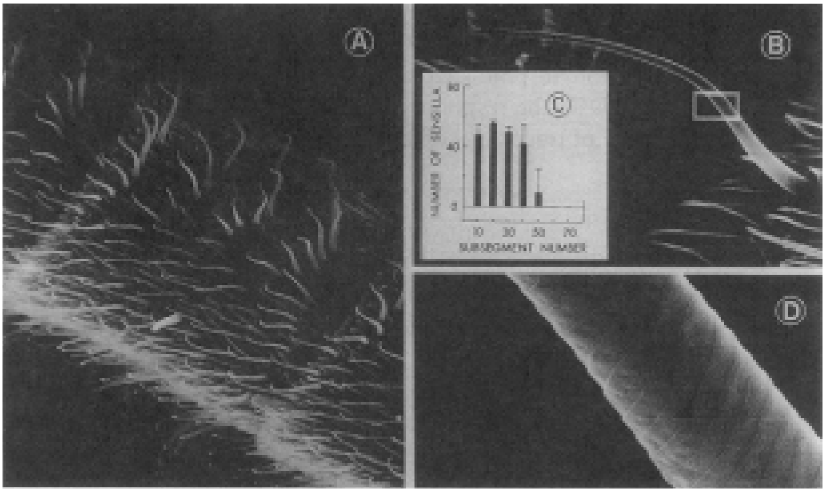

FIG. 1. Scanning electron micrographs of the antenna of a male *H. zea.* (A) A flagellar subsegment showing the rows of long, sexually dimorphic sensilla trichodea. (B) A single s. trichodeurn from the distal row located on one of the proximal subsegments. (C) A histogram of the mean (± SD) number of sensilla in rows on every tenth subsegment (*N* = 3) from the antennal base. (D) A higher magnification of the area enclosed by the square in Figure 1B illustrates the sensillar surface and pores.

For this reason, their responses were excluded from further analysis in this report.

Dose–Response Relationships. Responses from the A neuron were consistently observed to stimulation with each of the three compounds tested; AL, FO, and OL. No responses were observed from the B neuron to stimulation by any of these compounds at any of the doses tested. The observation that one class of receptor neuron responds to each of these three stimuli does not preclude the possible existence of other classes of receptor neurons in other morphological types of sensilla that may well have more pronounced differences in response selectivity with respect to these three compounds.

In all cases, responses from the A neuron were such that an increase in stimulus dosages led to an increase in the frequency of impulse production during the stimulus period. Mean responses of the A neurons to stimulations with different doses of AL, FO, and OL are shown in Figure 2A. In addition to similarities in the mean discharge frequencies, similarities also were observed in the averaged temporal discharge pattern in response to stimulation with equal doses of each compound (Figure 2B). With all these compounds, the initial part of the response was enhanced as stimulus intensity was increased. Since the OL was synthesized via a Wittig reaction with AL as the starting material (Carlson and McLaughlin, 1982b), the possibility exists that trace amounts of AL could be in the sample, yet go undetected by conventional GC analysis. However, since samples of the olefin produced via the synthetic route described above were shown to be >99%, the responses seen in Figure 2A cannot be attributed solely to the presence of trace AL contaminants in the OL sample.

DISCUSSION

The neurophysiological data presented here indicate that there is a class of receptor neurons on the antenna of male *H. zea* that responds to stimulation with the major pheromone component and to stimulation with two other analogs. The mean dose–response curves for the three compounds are quite similar (Figure 2A). However, the similarity in dose–response curves may be misleading, since these chemicals differ by over half an order of magnitude in vapor pressure (Carlson and McLaughlin, 1982b) and consequently may differ in the rate at which they are released from the surface of the dispenser. Therefore, comparisons among the responses from equivalently dosed stimuli should be evaluated in terms of the concentration differences among the compounds and not solely on the amount of material loaded into a stimulus cartridge.

To make comparisons on the basis of concentration, emission rates were calibrated from the stimulus dispensers for a series of long-chain hydrocarbons (Mayer et al., 1987), including the three compounds evaluated in this study.

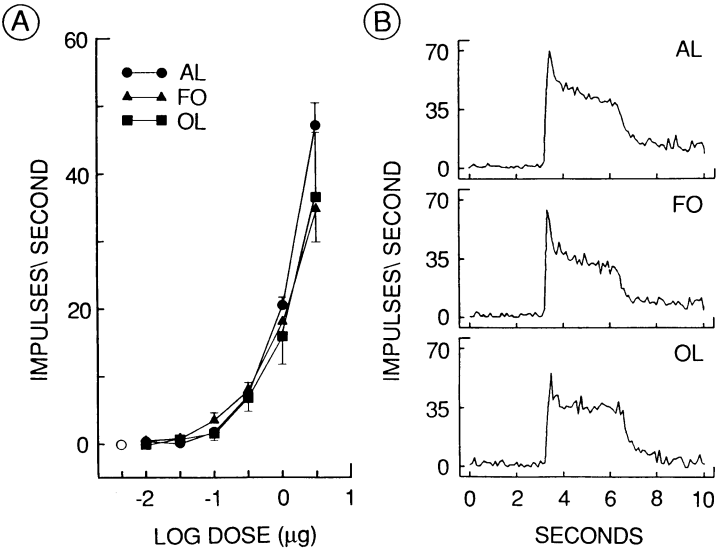

FIG. 2. (A) Mean (±SEM) dose-response relationships from the A neuron of s. trichodea on the antenna of male *H. zea* to stimulation with increasing doses of (Z)-11-hexadecenal (closed circles), (Z)-9-tetradecen-1-ol formate (closed triangles), and (Z)-1,12-heptadecadiene (closed squares). Standard error bars have been omitted from some mean values to enhance clarity of the figure. Doses on the x axis equal the amount of material loaded into the dispensers and not the amount reaching the preparation. The mean response to the hexane control stimulation is indicated by the open circle. (B) Averaged event-time histograms illustrating responses from the A neuron to stimulation with equal doses (3.16 μg) of (Z)-11-hexadecenal (N = 29); (Z)-9-tetradecen-1-ol formate (N = 11); and (Z)-1,12-heptadecadiene (N = 10). Responses are averaged in 100-msec intervals. The stimulus period began at 3.0 sec and ended at 6.0 sec.

From the measured concentration emitted from the dispenser at known doses, we calculated regression equations for each material, relating the amount of material loaded into the dispenser (dose in micrograms) to the amount of pheromone emitted from the dispenser outlet. Concentration in micromoles/per cubic centimeter can be calculated from emission rate, given the total rate of flow of stimulus-laden air over the antenna (concentration = emission rate/1200 cm^3/min). Due to the extreme sensitivity of olfactory receptor neurons to stimulations with semiochemicals, the lowest stimulus dose for which we could reliably quantify the emission rate was equal to the highest dose tested in these electrophysiological studies. It was necessary to estimate concentrations for lower doses (those effective in the electrophysiological studies) by extrapolating from the dose emission functions in Mayer et al. (1987). The resulting concentration estimates were then used to replot (Figure 3) the data shown in Figure 2. When replotted, there is a larger quantitative difference among the mean stimulus–response curves elicited by the three compounds. (Z)-11-Hexadecenal is a more effective stimulant than either (Z)-9-tetradecen-1-ol formate or (Z)-1,12-heptadecadiene. It should be noted, however, that these differences in sensitivity are relatively small when compared to the very large differences expected when pheromone-sensitive receptor neurons are stimulated with other nonpheromone compounds.

These neurophysiological responses are difficult to interpret unambiguously because the processes involved in disruption of insect chemical communication by atmospheric permeation with the major pheromone component are not well understood. For example, modification of the insect's sensory system by either adaptation of the peripheral receptor neurons or habituation of the central nervous system has been proposed to explain elements of the disruption process (Bartell, 1982). Alternatively, persistent activity in these pheromone-

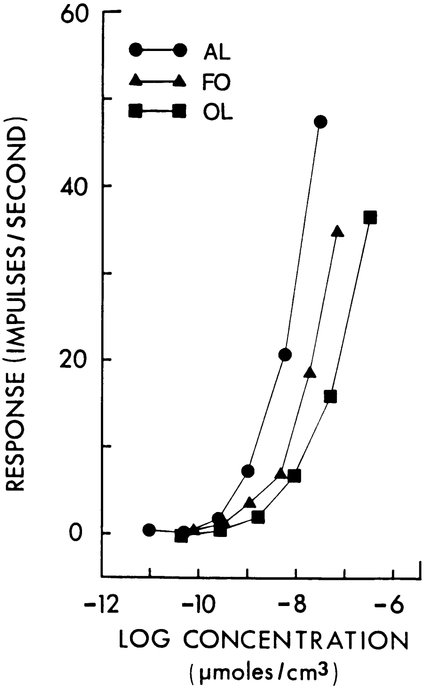

FIG. 3. Mean concentration vs. response relationships from the A neuron to stimulation with increasing concentrations of (Z)-11-hexadecenal (closed circles); (Z)-9-tetradecen-1-ol formate (closed triangles), and (Z)-1,12-heptadecadiene (closed squares).

sensitive receptor neurons may act to confuse or modify the normal behavioral response of the male and render him unable to orient to a calling female. A widely accepted theory proposes that individual trails of pheromone emanating from calling females are camouflaged by the large synthetic release of pheromone in disruption experiments (Cardé, 1981). Whatever the mechanism, we assume that in order for disruption to occur, the major pheromone component must interact in some fashion with the highly sensitive pheromone receptor neurons in the trichoid sensilla. Additionally, the present data suggest that the olefin and the formate analogs may achieve their disruptive effects via interaction with the same population of olfactory receptor neurons that are sensitive to the major pheromone component.

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