OLFACTORY PERCEPTION OF THE SEX ATTRACTANT PHEROMONE OF THE GREATER WAX MOTH, *GALLERIA MELLONELLA* L. (LEPIDOPTERA: PYRALIDAE), BY THE HONEY BEE, *APIS MELLIFERA* L. (HYMENOPTERA: APIDAE)[1]

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ABSTRACT

Electrophysiological responses of worker honey bees were recorded to a series of six to eleven carbon saturated aldehydes (100 μg dosage) including nonanal and undecanal, components of the sex attractant pheromone of the greater wax moth. Peak responsiveness occurred to heptanal, octanal, and nonanal. Dosage response curves to serial dilutions of nonanal and undecanal revealed both greater sensitivity and a larger population of acceptors responsive to nonanal. The results are discussed with regard to honey bee/greater wax moth ecology.

Key Words: Honey bee, *Apis mellifera*, greater wax moth, *Galleria mellonella*, olfaction, coevolution, ectosymbiont, scavenger, sex attractant, pheromone.

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INTRODUCTION

The relationship of the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), to the honey bee is interesting not only because of the moth's scavenger (ectosymbiotic; Wilson 1971) relation to the bee, but also because of the role reversal in sex pheromone production where the male moth produces an attractant for the female (Barth 1937). This sex attractant pheromone, produced by the wing gland of the male, consists of two components, nonanal and undecanal (Röller et al. 1968; Leyer and Monroe 1973).

Male moths generally "call" females in the vicinity of the honey bee colony where mating sometimes occurs (Eischen, unpublished). After mating, females return to the hive to lay eggs. These females approach the hive, remain motionless, then enter the hive by running past the guard bees at the entrance (Nielsen and Brister 1977). Moths can be detected by the bees at very close range or by contact with the antennae or tongue (Eischen et al., 1986). If detected, the moth is attacked. Female moths are attacked in preference to male moths. Since the male produces the sex attractant pheromone, and calls in the vicinity of the hive, we thought this could be a factor in its camouflage from the honey bee.

The experiments presented here were conducted to determine whether or not the honey bee could perceive the sex attractant of the greater wax moth and to elucidate the sensitivity of the bee to the pheromone components and related compounds.

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METHODS AND MATERIALS

Insects

Worker honey bees used in this study were the Italian race, *Apis mellifera ligustica* (Spin). Bees of known age were obtained by placing sealed brood in an incubator at 35° ± 1°C. Newly emerged bees were marked with a small spot of quick drying paint on the mesothoracic notum and returned to their colony. Bees used were 21 days old which is the age at which workers assume guard duty at the entrance of the colony (Lindauer 1952).

Odorous Stimuli

Compounds used in this study are listed in Table 1.

Table 1. Source, purity, and presence of chemicals in volatile emission of honey or beeswax and/or component of *G. mellonella* sex attractant.

Compound*	Chemical purity (%)	Presence in honey or beeswax†	Component of *G. mellonella* sex attractant‡
Hexanal	99+	+	
Heptanal	95		
Octanal	99	+	
Nonanal	98	+	+
Decanal	98	+	
Undecanal	97		+

* All chemicals were obtained from Aldrich Chemical Company, Milwaukee, Wisconsin.

† Ferber and Nursten 1977; Maga 1983.

‡ Röller et al. 1968; Leyer and Monroe 1973.

Experimental Methodology

Techniques used for recording electroantennograms (EAGs) were modifications of earlier techniques (Schneider 1957) and are described in detail elsewhere (Dickens 1979, 1984). In brief, Ag-AgCl capillary electrodes were filled with physiological saline (Pantin 1948; Oakley and Schafer 1978). The recording electrode was inserted into the terminal antennal segment following prepuncture with a sharpened tungsten needle; the indifferent electrode was inserted into the scape. Signals were viewed on a Tektronix 5223 digitizing oscilloscope after conditioning and amplification by a Grass P-16 differential microelectrode preamplifier. For subsequent analyses and storage, EAGs were recorded using an X-Y plotter.

In order to elucidate the selectivity and sensitivity of the antennal receptors of the worker honey bee for the sex pheromone of the greater wax moth and related hive components, two experiments were performed. In the first experiment, the responsiveness of the antennal receptors to a saturated series of five to eleven carbon aldehydes was evaluated by recording EAGs to a 100 μg dosage of each. In the second experiment, the sensitivity of the antennal receptors to components of the sex attractant of the greater wax moth was investigated by recording EAGs to serial dilutions of each.

In all experiments, stimulus compounds diluted in nanograde pentane were delivered as 10 μl samples placed on filter paper (8 × 19 mm) inserted into glass cartridges (80 mm *l.* × 5 mm ID) and oriented toward the preparation from ca. 1 cm. Odor molecules evaporating from the filter paper were carried over the preparation by hydrocarbon free air (filtered and dried). Comparisons made between various stimuli are relative since the compounds tested do not have the same volatility.

Stimulus duration was 1 sec with an airflow of 1 m/sec as measured by a thermistor. EAGs from 3 worker bees were recorded for each stimulus. An interstimulus interval of at least 4 min allowed for complete recovery of the EAG. The atmosphere around the preparation was continuously exhausted.

A stimulus of 1-hexanol (100 μg) was used as a standard to allow for comparisons between different preparations (Dickens 1984). Stimulation with the standard either preceded or followed each stimulus by 4 min. Responses to test stimuli were represented as a percent of the mean of the 2 nearest responses to the standard (Dickens 1978, 1981). The magnitude of the EAG depolarization was considered to be a measure of the relative number of responding acceptors (Payne 1975; Dickens and Payne 1977). The threshold was taken as the dosage at which the standard error of the response did not overlap with the standard error for response to the pentane control.

RESULTS AND DISCUSSION

The mean response of *A. mellifera* workers to the 1-hexanol standard was -1.25 mV (S.E. = ± 0.14 mV).

Selectivity

EAGs of *A. mellifera* workers to a series of aldehydes of increasing carbon chain length (6 - 11 carbons) revealed a larger number of acceptors responsive to aldehydes of seven to nine carbons (Fig. 1). Response to the series decreased sharply with the ten and eleven carbon aldehydes.

Hexanal, octanal, nonanal and decanal have each been reported from extracts of honey and/or beeswax (Cremer and Riedmann 1965; Ferber and Nursten 1977). Hexanal is also a component of the green leaf volatile complex (Visser et al. 1979). The responsiveness of the worker bee to each of these odorants would facilitate orientation to the hive as well as to potential food sources in the environment. Furthermore, nonanal is also a component of the sex attractant pheromone of the greater wax moth (Leyer and Monroe 1973). Thus the heightened responsiveness to this odorant could aid in the detection and orientation to these scavengers.

Sensitivity

Dosage response curves constructed from EAGs of worker bees to the two components of the greater wax moth sex attractant pheromone revealed antennal receptors to be at least 10 × more sensitive to nonanal than to undecanal (Fig. 2). The threshold for nonanal was 1.0 μg and responses increased with increasing dosage through 1000 μg at a level 160% of the standard. Undecanal had a threshold of 10 μg with saturation occurring at 100 μg at a level only 70% of the standard. Thus not only are worker bees more sensitive to nonanal than undecanal, but they also possess a significantly larger population of acceptors for nonanal. Furthermore, responses to nonanal increase over a much larger dosage range than for undecanal (nonanal, 3 log steps; undecanal, 1 log step).

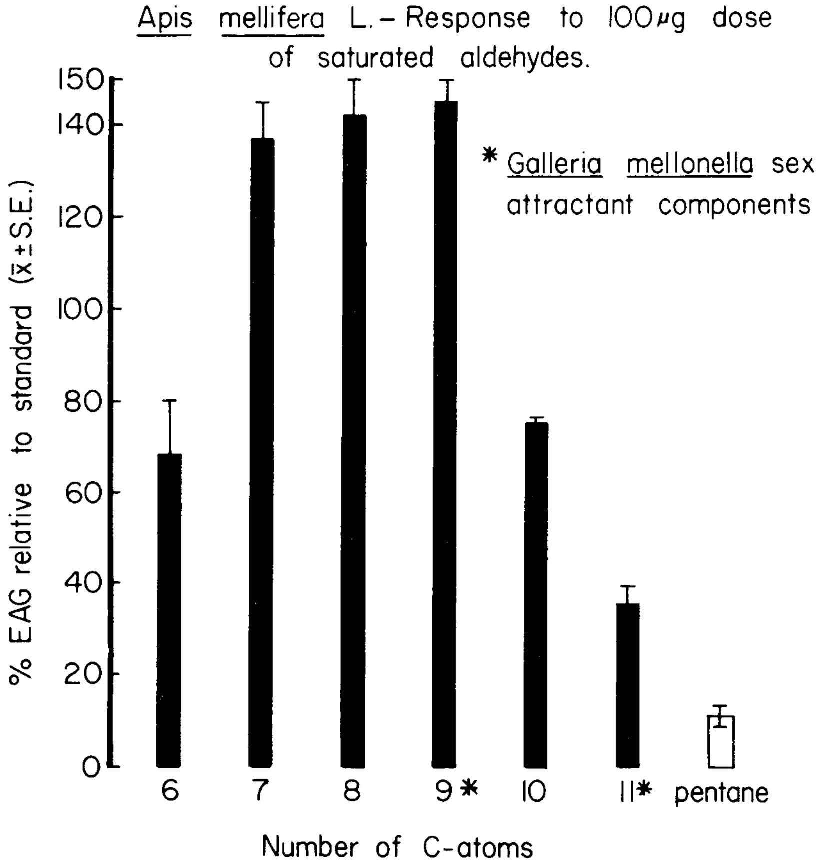

Fig. 1. Mean EAGs of worker bees, *A. mellifera,* to 100 µg dosage of saturated aldehydes of various carbon chain lengths. Horizontal bars represent standard errors.

Although the receptor population is obviously more sensitive and responsive to the nine carbon aldehyde, is this necessarily a response to the "pheromone"? Nonanal is a component of beeswax (Ferber and Nursten 1977). Thus nonanal could serve to facilitate orientation of worker bees to the hive or to communicate information to the workers on the condition of the hive. Since the bees have likely been associated with the hive considerably longer than with the moth, this latter explanation for the high sensitivity to nonanal seems appropriate. On the other hand, undecanal has not been identified from either honey or beeswax (see Maga 1983) and worker bees are relatively insensitive and have few acceptors responsive to this pheromone component. Thus, the utilization by the wax moth of a hive component, nonanal, and a compound with low sensitivity to the honey bee, undecanal, could serve to chemically camouflage calling male moths. Support for this communicative strategy may be found in preliminary behavioral bioassays in

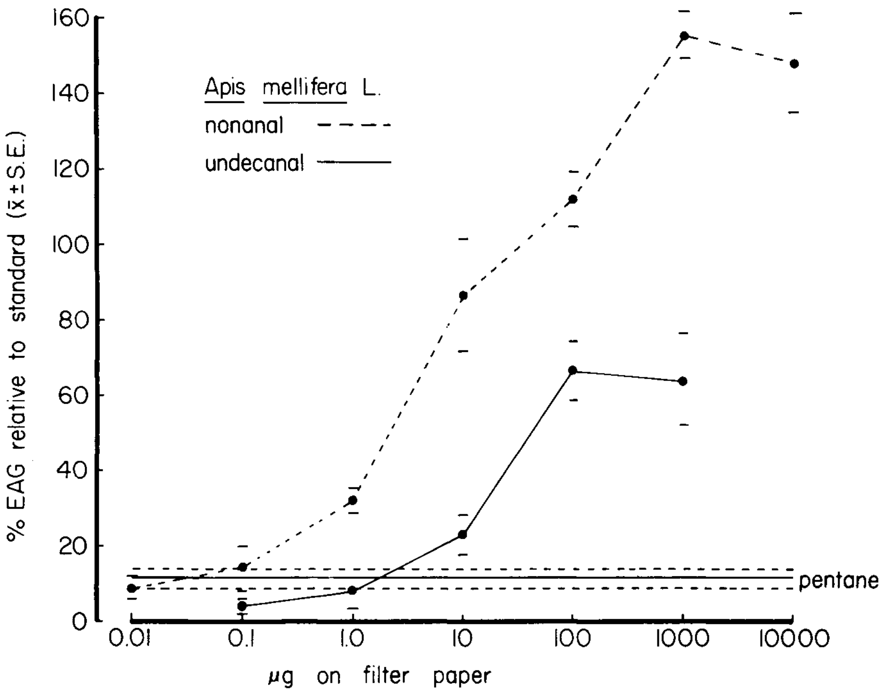

Fig. 2. Dosage response curves of worker bees, *A. mellifera,* to serial dilutions of greater wax moth, *G. mellonella,* sex pheromone components. Horizontal bars represent standard errors.

which worker bees exhibit only mild "interest" in volatiles emanating from wax moths (Eischen et al., unpublished). However, proof of this theory awaits more definitive behavioral experiments with individual pheromone components.

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