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Antennal Olfactory Responsiveness of the Texas Leaf Cutting Ant (Hymenoptera: Formicidae) to Trail Pheromone and its Two Alarm Substances[1]

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ABSTRACT Electroantennograms (EAGs) were recorded from major workers, queens, and males of the Texas leaf cutting, *Atta texana* (Buckley) (Hymenoptera: Formicidae) in response to serial dilutions of two alarm substances, 2-heptanone and 4-methyl-3-heptanone, and its trail pheromone, 4-methylpyrrole-2-carboxylate. The lower EAG threshold for major workers relative to queens and males for both alarm substances correlated well with previously reported behavioral bioassays which showed workers to be most responsive to these odorants. Although laboratory behavioral bioassays showed minor workers, queens, and males to have a similar behavioral threshold for the trail pheromone, minor workers were more responsive to higher concentrations of the trail pheromone. However, EAGs revealed queens significantly more sensitive and responsive to the trail pheromone than the other castes. These seemingly enigmatic results are discussed with regard to *A. texana* biology and receptor physiology.

KEY WORDS Leaf cutting ant, *Atta texana*, olfaction, trail pheromone, electroantennogram, EAG, trail pheromone, behavior.

The Texas leaf cutting ant or town ant, *Atta texana* (Buckley), is the northernmost species of the genus and the largest of the fungus growing ants (Weber 1972). These ants are found in Louisiana and Texas (Cameron and Riggs 1985).

Studies on the chemical communication systems of *A. texana* (Moser 1983), and other ants (Bradshaw and Howse 1984) have primarily concerned the identification of various pheromones, and determination of the behavioral effects of the pheromones in laboratory bioassays and field tests. In this regard, the trail following pheromone of *A. texana* was identified as 4-methylpyrrole-2-carboxylate (Tumlinson et al. 1971), and 2-heptanone and 4-methyl-3-heptanone were shown to function as alarm substances (Moser et al. 1968). Until the study reported here, no investigations have been conducted to determine the antennal olfactory response of *Atta texana* to its trail and alarm pheromones. In fact, in only a few instances have electrophysiological studies been conducted to determine receptor responses to pheromones in any ant species (Dumpert 1972; Masson and Friggi 1971, 1974; Payne et al. 1975; Glancey and Dickens 1987).

The purpose of the study presented here was to determine the antennal olfactory response sensitivity of castes of *A. texana* to the trail pheromone, 4-

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methylpyrrole-2-carboxylate, and the alarm pheromones, 2-heptanone, and 4-methyl-3-heptanone, in order to gain insight into detection of the pheromones and their potential roles in the behavior of the castes. In addition, bioassays of queens and males in response to the trail pheromone were conducted, since in previously reported behavior studies only workers were tested.

Materials and Methods

Winged queens, males, and major workers obtained from Brazos and Grimes counties, Texas, were sexed and kept in Petri dishes with moistened filter paper at 4° C until use.

4-Methylpyrrole-2-carboxylate obtained from Dr. P. E. Sonnet, USDA-ARS Animal Biomaterials Research Laboratory, Philadelphia, PA, and 2-heptanone and 4-methyl-3-heptanone obtained from Chemical Samples Co., Columbus, OH, were each > 98% chemcially pure as determined by gas chromatography. Serial dilutions of each odorant were prepared in hexane from 0.1 - 100 μg/μl.

Methodology for recording electroantennograms (EAGs) was derived from an earlier technique (Schneider 1957) and is described in detail elsewhere (Payne 1975; Dickens and Payne 1977). Ag-AgCl glass capillary electrodes filled with an insect Ringer solution were used. The recording electrode was inserted into the distal end of the antenna following puncture with a sharpened tungsten needle. The indifferent electrode was inserted into the head capsule.

Serial dilutions of each odorant were delivered as 10-μl aliquots placed on filter paper (20 mm × 7 mm) inserted into glass cartridges (5 mm I.D. × 75 mm length) oriented toward the preparation from a distance of 1 cm. A one 1/min flow of air which had been filtered through activated charcoal carried odor molecules over the preparation. Stimulus duration was 2 sec. At least 4 min were allowed between each stimulation, which was adequate for recovery of EAG activity.

Hexane (10 μl on filter paper) was used as a control. Responses to hexane by a given preparation were subtracted from subsequent responses to experimental stimuli. 2-Heptanone at 100 μg served as a standard to normalize responses from different preparations and to monitor the viability of individual preparations. Stimulation with the standard followed the control and every two test stimuli. Responses to test stimuli were represented as a percent of the mean of the two nearest (in time) responses to the standard which preceded and followed response to a given test stimulus.

The amplitude of the EAG depolarization was considered a measure of the size of the population of responding acceptors (Payne 1975; Dickens and Payne 1977). The threshold for a given odorant was defined as the dosage at which the standard error for that dosage was greater than zero. Responses of various castes were compared by a Mann-Whitney U-test (Ostle 1963).

Behavioral bioassays of the trail pheromone utilized the "minor worker technique" (Moser and Silverstein 1967). The technique is based on the fact that minor workers are not as excitable as major workers and were, therefore, much better for the bioassay used. Similar results may be obtained with major workers in an artificial nest, which was not available in this study. In brief, 10 ants at a time were placed in the center of a circular trail of pheromone applied to paper file dividers. After 10 min, the number of ants on the trail were counted, and the

results tabulated. Data collected for virgin queens and males are presented for the first time in the current study. Data for minor workers are taken from results of a previously published study (Robinson et al. 1974). Because major workers were used in the EAG studies, they may have been more ideal subjects for the trail bioassays than minor workers. However, the use of major workers for this purpose is only possible with a laboratory colony enclosed in a large container similar to that described by Sonnet and Moser (1973). Such a colony was not available when this study was done.

Results and Discussion

Mean EAGS to the standard 2-heptanone at 100 μg were 1.23 mV (S.E. = 0.03; n = 15) for males, 1.38 mV (S.E. = 0.03; n = 15) for queens, and 1.53 mV (S.E. = 0.05 mV; n = 15) for major workers.

Dosage-response curves constructed from EAGs of the three castes to the alarm substances, 2-heptanone and 4-methyl-3-heptanone, showed antennae of both major workers and males to be at least 10 X more sensitive than queens (Figures 1 and 2). After reaching threshold at 100 μg on filter paper, substantial EAGs were elicited from queens to both compounds, but they were not significantly different from responses of the other two castes. The source of the large variability around some of the means is uncertain. It may be due to the fact that the data were obtained from different preparations over a period of many months.

The low EAG threshold of the major workers for both alarm substances relative to the queens correlates well with results from previously reported behavioral bioassays (Moser et al. 1968). Those studies showed queens had a higher threshold than workers for detection of both odorants, and while queens did detect both odorants, alarm behavior was not elicited as in workers. Males did not respond overtly to either odorant in those bioassays.

Results of the trail following bioassays presented here for virgin queens and males, and those documented in earlier experiments with minor workers (Robinson et al. 1974) show that of the concentrations tested the behavioral threshold for all three castes was 4.0 pg (Table 1). Increasing amounts of 4-methylpyrrole-2-carboxylate led to increased numbers of ants which followed the trail, until a maximal response was reached for minor workers at 0.4 ng at which point minor workers were considerably more responsive than either virgin queens or males. Further increases in pheromone concentration beyond 0.4 ng led to a precipitous decrease in the response of minor workers, while the decrease in response of queens and males was not as pronounced.

Results of the EAG studies were in contrast with the behavioral bioassays. The electrophysiological studies revealed both queens and males not only to be more sensitive (i.e., lower thresholds and steeper response curve slopes), but also to have a larger population of acceptors responsive (i.e., higher response maxima) to the trail pheromone than the major workers (Figure 3).

Data from our behavioral studies correlate well with *A. texana* biology. Since workers lay down the trail pheromone and follow it in the field, one might expect them to be more responsive than the other two castes. Results of the electrophysiological studies are somewhat of an enigma since it might be expected that major workers have receptors more sensitive and responsive to the trail pheromone. However, the trail of *A. texana* is rather long-lived and trail pheromone, 4-

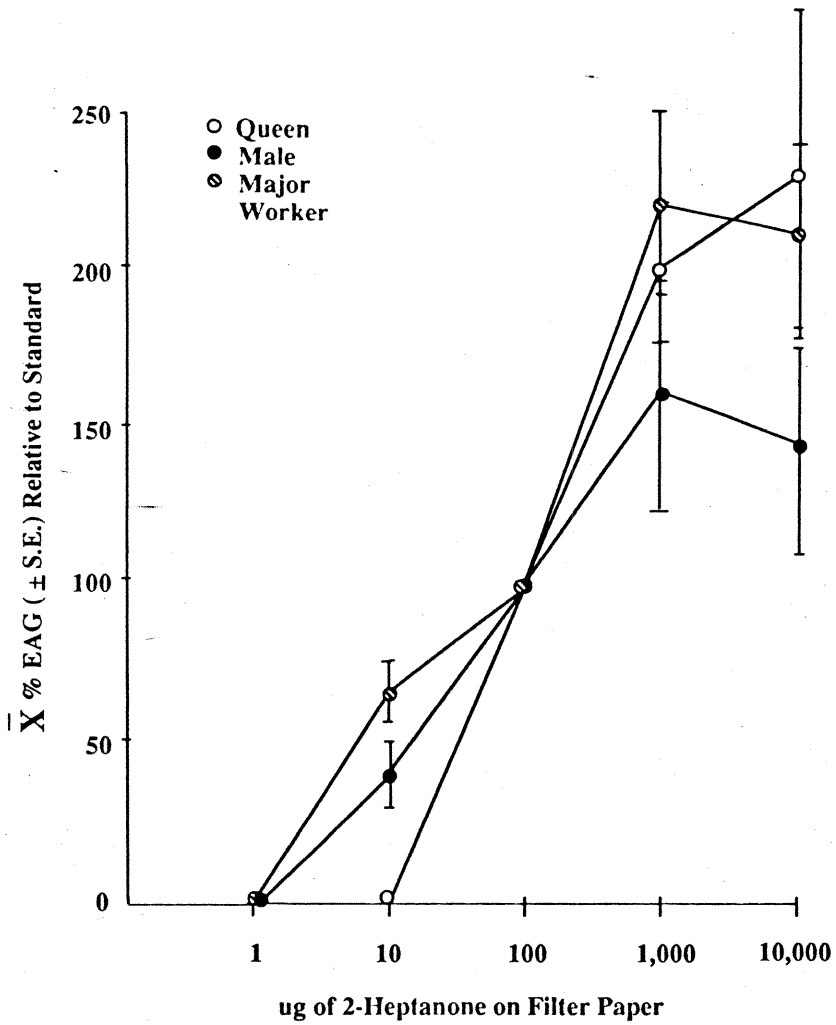

Fig. 1. Dosage-response curves constructed from EAGs($\bar{X} \pm$ S.E., n = 4) of *A. texana* queens, males, and major workers to serial dilutions of 2-heptanone. Vertical lines represent standard errors.

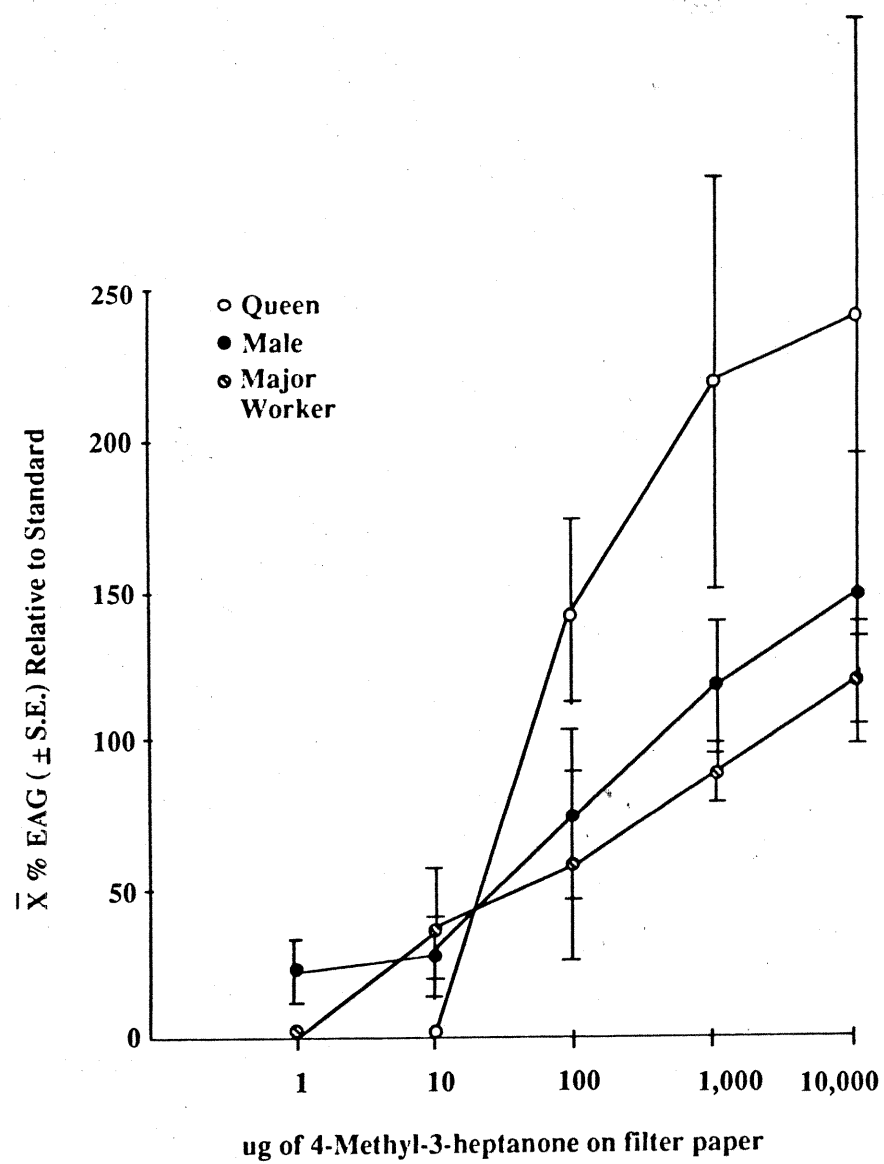

Fig. 2. Dosage-response curves constructed from EAGs($\bar{X} \pm$ S.E., n = 4) of *A. texana* queens, males, and major workers to serial dilutions of 4-methyl-3-heptanone. Vertical lines represent standard errors.

Table 1. Percent of *A. texana* per caste responding to trail pheromone at various concentrations. Data for minor workers taken from Robinson et al. (1974).

Caste	# Tested	Blank	Concentrations (4X $\log_{10}$ng)						
			-4	-3	-2	-1	0	1	10
Minor worker	20	0	0	65	85	95	65	15	0
Virgin queens	70	0	0	11	50	59	57	41	0
Males	60	0	0	3	15	30	35	28	0

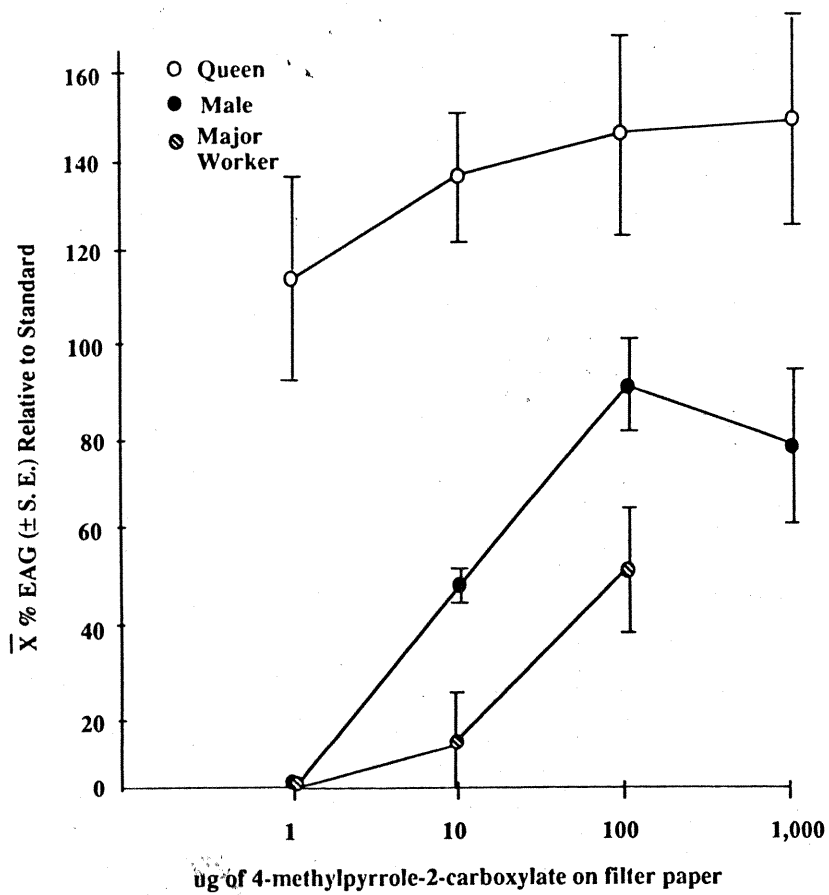

Fig. 3. Dosage-response curves constructed from EAGs($\overline{X} \pm$ S.E., n = 4) of *A. texana* queens, males, and major workers to serial dilutions of 4-methyl-3-heptanone. Vertical lines represent standard errors.

methylpyrrole-2-carboxylate, is not very volatile (Tumlinson et al 1971). It may be that the pheromone is detected mostly by olfaction by queens and males, while dectection of the trail pheromone by workers is primarily by contact chemoreceptors with some olfactory capabilities, as reported for the tobacco hornworm, *Manduca sexta* L. (Lepidoptera: Sphingidae) (Stäedler and Hanson 1975). The trail pheromone could serve a dual purpose: it might function as a trail pheromone for workers and a sexual attractant for reproductives. This explanation would take into account a previously published report (Echols 1966) that new colonies of *A. texana* are frequently found on the nest structure of existing colonies.

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