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Title: Response of Fire Ant Antenna to Odorant and Pheromone Stimulation

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Major Accomplishments:

Fire ant antenna anatomy:

1) In collaboration with Dr. S. B. Vinson, Texas A & M, we have found secretory glands in the 9th antennal segment of workers. Ducts from these glands pass through the cuticle and emerge as a ring of approximately 50 pores. Similar pores are seen in the 9th and 10th segments of queens. On queens, the 9th antennomer has 18+/-6 pores (N=7), whereas the 10th antennomer has 81+/-8 pores (N=7). The pores on the 9th antennomer are not evenly distributed, with more on the dorsal surface. The pores secrete a viscous material which may contain pheromones. It will be interesting to test this secretion as an attractant in future studies.

2) We have prepared three dimensional maps of sensory hairs (sensilla) on the antenna. Small worker's antennae (tenth segment less than 0.35 mm) appear to have different patterns of basiconic sensilla ("pegs") and coeloconic sensilla ("champagne corks") than large worker's antennae (tenth segment greater than 0.40 mm). This suggests the possibility of a hard-wired sensory component to behavioral diversity in workers. The male antenna has no basiconic sensilla and contains 3-4 times as many coeloconic sensilla as workers. Thus, if the function of the coeloconic sensilla could be determined, this might be used to disrupt mating.

3) Sensilla are distributed asymmetrically on the workers' antenna, with fewer basiconic sensilla on the surface that normally is oriented closest to the head. This suggests the basiconic sensilla are contact chemoreceptors. The right and left antennae have mirror image patterns. By contrast, the pattern on the male antenna appears to be symmetric.

4) The most abundant type of porous sensilla is a curved wall-pore sensilla. On the workers, these are most numerous on the club, but a few wall-pore sensilla are present on all segments. The males have wall-pore sensilla evenly distributed on all segments.

5) We have observed 3-4 circular plate or dome shaped depressions in the pedicel (antennomer 2) of females (workers, alates and queens).

Electrophysiology

1) Electroantennograms were measured across whole antennae from the tip of the club to the head. We found responses to vapor of a variety of substances including hexanol, octanol, acetophenone, citral, acetonitrile, triethylamine, and triolein. Dose-response curves were measured for these substances. In addition, electrical responses were detected from a change in relative humidity from 70% to 90% at constant temperature, and also from passing air over a

crushed worker on filter paper.

2) In order to localize the olfactory receptors, we compared electroantennograms across detached antennae and antenna fragments. The response to triethylamine appeared to be the same with intact antennae or with antennae lacking the club (segments 9 and 10). However, the response to hexanol was reduced by a factor of two when the club was removed.

Fluorescence microscopy

1) We developed a method for delivering fluorescent dyes into porous sensilla. Highly specific dye labeling of basiconic and wall-pore sensilla occurred after soaking a live ant in a mixture of dye and CHAPS detergent for a few minutes. The only other dye uptake observed with this procedure was in the glossa (tongue) and the distal tip of the tarsus (foot). After soaking, the ants are easily revived and display normal electroantennograms.

2) After longer soaking times, dye accumulated inside segment 10, indicating a transport mechanism clears substances taken up by contact chemoreceptors. This suggests limits on possible strategies for use of repellants that act through contact chemoreceptors. These repellants would have to be combined with transport inhibitors in order to have a long-lasting effect.

3) We labeled porous sensilla with the calcium-sensitive dye calcium green and observed changes in fluorescence intensity of individual sensilla in the presence of acetophenone vapor.

Bioassays

1) We developed a bioassay in order to test attractants and repellants in parallel with electroantennograms. Attraction to peanut oil was taken as a standard. Frozen pupae were ground to a powder and extracted with chloroform/methanol/water. Workers did not respond to the organic phase of this extract but they were attracted to the aqueous phase. Future experiments will examine HPLC fractions of the pupa extract in the bioassay.

2) An entire fire ant hive was extracted with chloroform/methanol/water. Workers were strongly attracted to the organic phase of this extract. Fractionation by gas chromatography revealed numerous high boiling components, which will be individually tested in bioassays and by electroantenograms.

3) Using the bioassay, we also tested several potential repellants or inhibitors, including 7,8-dihydroxy-6-methoxycoumarin, dinitrophenol and concanamycin A. The coumarin derivative had no effect. Dinitrophenol or concanamycin A were added to peanut oil, but they did not inhibit the ants' attraction to the oil.

4) Workers displayed attraction to individual antennae freshly removed from female alates.

Goals Achieved:

We have measured the electroantennogram response of fire ants to a variety of substances. We have developed a fluorescence microscopy assay for olfactory reception. We have identified extracts and secretions of fire ants that are likely to be sources of attractant pheromones.

Relevance to the Texas Imported Fire Ant Research and Management Plan:

Knowledge of the structure of the fire ant antenna and its electrical response to odors and pheromones