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Fire Ant Alarm Pheromones

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The red imported fire ant, *Solenopsis invicta*, has several purported sources of alarm pheromones. Crushed heads from workers and worker Dufour's glands produce an alarm reaction; however, for this paper we will concentrate on the excitant pheromone produced by sexuals during mating flights.

Reproduction in the *S. invicta* begins with nuptial flights during which male and female alates (winged reproductives) leave their nests and mate several hundred meters in the air (Markin *et al.* 1971). Males take flight before females and form large aggregations into which the females fly (Markin *et al.* 1971). Mating flights may take place year round but occur primarily during the summer months, when suitable conditions most frequently arise.

The surface of a fire ant mound normally has no entrance or exit holes (Markin *et al.* 1975). However, just prior to a mating flight, workers create holes in the mound surface from which workers and winged alates emerge (Markin *et al.* 1971). At this time, workers swarm excitedly over the mound and exhibit many characteristics of what has been classified as alarm behavior, including frenzied running, rapid back and forth movement, and increased aggression (Markin *et al.* 1971, Obin and Vander Meer 1994). Workers often aggregate around alates as they climb up vegetation to take flight and sometimes attempt to pull the alates back down.

Obin and Vander Meer (1994) induced *S. invicta* flights in the laboratory and showed that chemical cues from both male and female alates but not from workers, attracted workers, induced alarm-recruitment behaviors in the workers, and promoted alate retrieval by workers. They proposed that volatile substances produced by the alates were responsible for eliciting the worker reactions. We report here the glandular source(s) of chemical substances responsible for inducing worker excitement in *S. invicta* workers prior to mating flights.

Methods and Materials

Source of Alates. Colonies were induced to fly in the laboratory by watering the soil of each colony one day prior to testing in order to simulate rainfall. On the day of each test we increased the temperature in the lab room to 30°C and augmented the available light with additional incandescent lamps (see Obin and Vander Meer 1994). Alates were collected and flown from colonies collected year round.

"Mating flight activated" (MFA) alates were obtained from these laboratory colonies after a flight was initiated. Alates were collected as they climbed up tongue depressors placed in the tubs, and were weighed and tested immediately.

Bioassay Procedure. Our bioassay consisted of a set of worker groups from mature *S. invicta* colonies maintained in the laboratory. Workers and test alates were always collected from different colonies. Approximately 100 workers and a small amount of brood from each colony were placed in small covered petri dishes with moist Castone® bottoms. Red cellophane placed over the petri dishes induced the workers to stay with their brood in the dishes. These sub-colonies were maintained in individual plastic trays that were coated with Fluon® on the sides to prevent escape. Ants were allowed to acclimate to the dishes for at least two hours before the lids and cellophane were removed.

The bioassay was conducted by an observer and assistant. The assistant prepared test samples for the observer so that the observer did not know the sample identity. Test samples consisted of 3 ml of air drawn by a syringe from a control or sample vial (samples are defined below). The assistant then assigned each sample arbitrarily to a worker group, such that all worker groups were tested with each of the test samples and controls. The observer positioned the syringe 1-2 cm directly above a part of the selected worker group, then slowly released 1 ml of air over the workers. Reactions were characterized by the observer either as no reaction, when workers did not change their behavior in any way or simply raised their heads and antennated the air, or as an excited reaction, in which at least one worker reacted with rapid movement. If workers moved rapidly toward the source of the airstream, the reaction was noted as possible attraction. A Y-tube bioassay was used to measure attraction (see Vander Meer et al. 1988). Only overwintering and summer female MFA alate poison sac extracts were evaluated for attraction.

Sample Preparation. Each sample to be tested in the bioassay was placed in a 7-ml glass scintillation vial and tightly capped. Tests with live alates consisted of five live alates placed in a vial and shaken immediately before each air sample was drawn. Vials were shaken to disturb the alates and induce them to release the excitant pheromone. Tests of alate body parts included one individual head, thorax, or gaster. Each body part was obtained from different alates to minimize cross contamination. Alates were chilled to 8°C before body parts were separated with micro dissecting scissors. Each body part was placed toward one end of a thin strip of filter paper (Whatman #1, qualitative, 3 cm x 0.5 cm), then the filter paper was folded over and the body part was crushed with a hammer. The filter paper with the crushed body part was immediately placed in a sample vial for bioassay.

Solutions of glandular products were made from mandibular glands, post-pharyngeal glands, and poison sacs excised in water under a binocular dissecting microscope from alates that had initiated pre-flight activity. Mineral oil was used as the solvent to slow the rate of release of the chemical compounds. For each test, five ul of solution (1.6 alate equivalents) was applied to a thin strip of filter paper (dimensions above) and placed in a sample vial.

Data Analysis. Data were analyzed using the McNemar test for significance of changes (Sokal and Rohlf 1981). This statistic is used for comparisons in which the same individuals are tested repeatedly and is appropriate for our analyses because the same worker groups were exposed to several test samples and a control in each bioassay. This statistic compares the number of worker groups that displayed an excited reaction to the test sample but not to the negative control to the number of groups that reacted to the negative control but not to the test sample.

Results and Discussion

Air from vials containing live mating flight activated (MFA) female alates elicited highly significant excited reactions in *S. invicta* worker groups in summer and winter tests. In addition, workers reacted with excitement to summer male alates, as well as to winter non-flying female and male alates. Live winter MFA female alates that were not shaken before testing also elicited significant reactions.

Crushed heads of all alate categories stimulated highly significant excitement in the workers. Results for crushed female thoraces were variable while crushed male thoraces elicited excitement at a lower level of significance than crushed heads ($p<0.05$ vs. <0.001, respectively). It is not clear yet whether the thorax results are due to contamination by substances released from the mandibular glands during separation of the body parts or if a glandular source from the thorax is responsible. Crushed gasters of all female alate categories but not male gasters produced some level of excitement in the workers, as did crushed poison gland solutions from winter MFA females. Overwintering female alates are abnormal in that they produce a queen pheromone in the poison gland, which is usually only produced and released by inseminated queens or dealated virgin females (Vander Meer *et al.* 1980). Our Y-tube bioassay results showed significant worker

attraction to overwintering but not summer female MFA poison sac extracts. Thus, our excitant bioassay results for this treatment category could be confounded by worker attraction.

Mineral oil extracts of mandibular glands from MFA female and male alates produced significant excitement in workers. Tests with winter MFA females were heterogeneous, with four highly significant replicates (80%, each with $p<0.005$) and one with an equal number of reactions to the test sample as to the control. Crushed heads without mandibular glands did not elicit excitement in workers ($p>0.05$) while heads with intact glands tested at the same time elicited excitement at a highly significant level ($p<0.001$). No excitement was elicited by post-pharyngeal gland solutions from any of the alate categories.

Our results show that the mandibular glands are a source of an excitant pheromone in both female and male *S. invicta* alates. In our bioassay, *S. invicta* workers consistently reacted with rapid movement and frantic running when exposed to live alates, crushed heads, and mandibular gland solutions. These results support Obin and Vander Meer's (1994) suggestion that the "alarm" and recruitment reactions exhibited by workers towards alate residues during mating flights were likely derived from the alate mandibular glands.

Although other crushed body parts from male and female alates elicited some excitement, only the head elicited a strong reaction from both sexes of alates. This is significant because within a fire ant population, colonies may produce only males, females, or both sexes. It is likely that the alates are responsible for initiating mating flight activity in response to environmental conditions, because the opening of the mound surface and swarming of workers are only associated with mating flights. It is also probable that the glandular source for this very specific activity is the same for both sexes. Thus, all evidence points to the mandibular glands as the source of mating flight excitant pheromones.

Studies of chemical communication associated with mating flights have mainly focused on sex attraction pheromones (e.g. Hölldobler 1971 and Hölldobler 1976). Pheromones involved in pre-flight activity have received little attention but most likely are as important to the mating process as are those responsible for mate attraction.

Mandibular glands have been identified as the source of alarm pheromones in many ant species, particularly in the subfamily Myrmicinae (see review by Hölldobler and Wilson 1990). Despite the small size of *S. invicta*'s mandibular glands, we have demonstrated that the chemical contents of alate mandibular glands elicit significant excitement in workers. We are currently working to identify the chemical compounds in the mandibular glands responsible for producing the excited reactions.

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