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The Poison Sac of Red Imported Fire Ant Queens: Source of a Pheromone Attractant[1,2]

R. K. VANDER MEER, B. M. GLANCEY, C. S. LOFGREN, A. GLOVER, J. H. TUMLINSON[3]
AND J. ROCCA[3]

Insects Affecting Man and Animals Research Laboratory, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Gainesville, Florida 32604

ABSTRACT

The poison sac of queens of the imported fire ant, *Solenopsis invicta* Buren, has been identified as the novel storage site of a queen pheromone. The pheromone elicits orientation and attraction in workers and promotes the deposition of brood. Alkaloids normally associated with the venom of imported fire ants are not responsible for the behavior produced by the queen pheromone.

Ant queen pheromones have been reported in the literature since 1910, when Wheeler noted the soothing queen odor on *Formica consocians* Wheeler (=*F. difficilis* Emery). Subsequent investigators have shown that queens of *Lasius alienus* Foerster and *Pheidole pallidula* Nylander, and their extracts, are attractive to their respective workers (Stumper 1956). Queens of several species of army ants are also attractive to workers (Watkins and Cole 1966), a behavior that contributes to the cohesiveness of the colonies (Schneirla 1957). Queen recognition in *Myrmica rubra* L. is controlled by both nonvolatile chemical and topographical stimuli, the presence or absence of which has a profound influence on caste determination (Brian 1973). These studies have established the existence of ant queen pheromones, but they have not determined where the pheromones are synthesized, stored, or how they are distributed.

A queen pheromone that attracts and arrests worker ants has been reported for the imported fire ant, *Solenopsis invicta* Buren. The pheromone activity is transferrable to surfaces on which queens have been confined (Jouvenaz et al. 1974). Glancey (1980) further observed that air drawn over mated queens and pentane extracts of queens are highly attractive to workers, and when applied to inanimate objects the extracts induce attraction, clustering, and movement of the object into the nest; behavior patterns suggesting that the pheromone has potential for the development of species specific control methods. Here, we present evidence establishing the poison sac as the queen pheromone storage site for *Solenopsis invicta* and that the piperidine alkaloids associated with the queen venom sac contents are not responsible for the pheromone activity.

Materials and Methods

Queens used in this study were taken from laboratory-maintained multiple queen *S. invicta* colonies; such polygyny was first reported by Glancey et al. in 1973. In addition to being morphologically identical, the queens from single and multiple queen colonies are equally attractive to workers (Glancey, unpublished results).

Whole queens and extirpated parts were mechanically ground or broken up in hexane to give a concentration of 1 queen equivalent (QE) per 50 µl of solution. Live queens were taken directly from their colony prior to testing. All queen extracts were tested at 0.2 QE. Venom was obtained directly from individual queens by the capillary method of Brand et al. (1973).

The bioassay chamber[4] developed to detect and measure the response of worker ants to volatile attractants consisted of a lucite Wilson cell (20 cm diam) with a Castone bottom and one open port. The end of a 14.5 cm disposable Pasteur pipet was put into the hollow stem of a cotton swab that had been cut in half. (The swab was necessary to disperse the air stream.) Live queens were isolated in the pipet with small pieces of screening. The extracts and venom samples were applied to a piece of filter paper (0.3 × 3 cm) located inside the pipet. After the solvent evaporated, the pipet assembly was inserted into the open port of the Wilson cell so that the swab rested in the center of a premarked 2.5 cm square. The pipet was connected to a source of compressed air (flow rate 0.5 liter/min). Immediately thereafter, 20 worker ants were introduced into the bioassay chamber. The assay was scored by recording the number of ants in the marked square at 1-min intervals for 5 min. The total 5-minute response constituted one trial. Results are presented as the mean and standard deviation of six trials.

Another bioassay measured the reaction of worker ants to samples of extracts absorbed onto surrogate queens. The test arenas were portless Wilson cells (9 cm diam), with Castone bottoms and observation areas (2.5 × 2.5 cm) marked in their centers. Extracts were applied to surrogate queens prepared by quartering cylindrical rubber seal septa (Applied Science, Inc.). After drying for 10 min in a fume hood, a treated surrogate queen was placed in the center of the marked area. Twenty worker ants and 3 pieces of brood were introduced into the arena, and the number of workers in the square was recorded at 1-min intervals for 5 min. Results are recorded as the mean and standard deviation for six replicates. In addition, the total number of brood in the survey area at the end of the 5-min period for the six replicates is recorded, as well as the number of replicates in which brood was deposited beside the surrogate queen at any time during the test period.
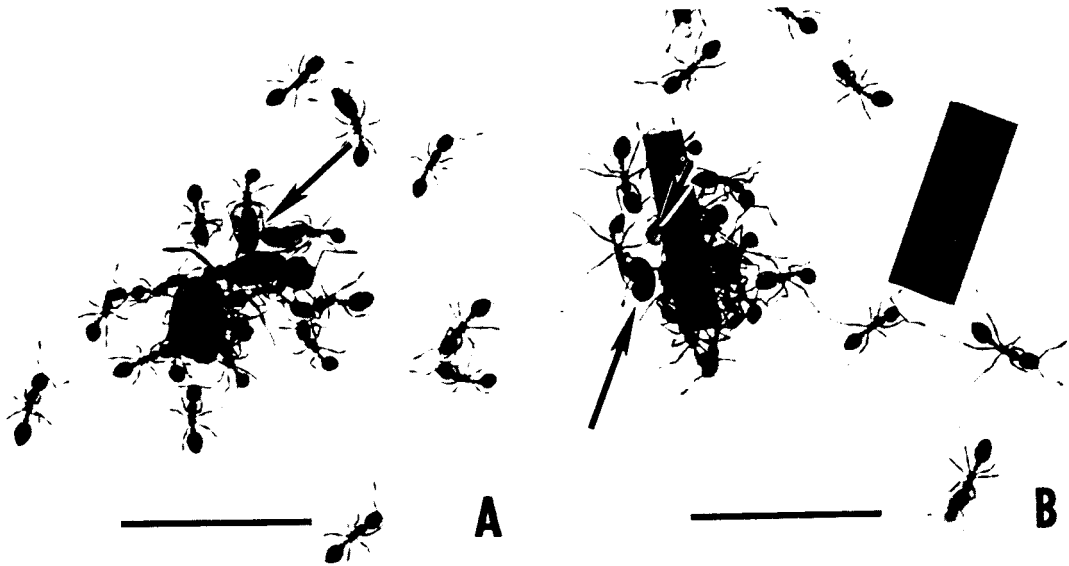

FIG. 1.—(A) Workers responding to their live queen. (B) Workers responding to surrogate queen blank (right) and a queen poison sac extract applied to a surrogate queen. Arrows indicate brood being deposited by workers near the queen and surrogate queen. Reference bars are 1 cm.

Results and Discussion

The results of both bioassays (Table 1) clearly show that the previously reported *S. invicta* queen pheromone that induces attraction of workers has its origin in the poison sac and that the mode of pheromone release is via the sting. None of the other queen parts showed activity significantly greater than the hexane blank. The deposition of brood that occurred near the surrogate queen in both whole queen and queen poison sac extracts implies that a queen-worker recognition factor is also present in the contents of the poison sac. If a live queen is substituted for a rubber septum in the surrogate queen bioassay, the workers will deposit brood near the queen as they did with the whole queen and queen poison sac extracts (Fig. 1). Therefore, we have established for the first time the site of storage of an ant queen pheromone and two of its behavioral responses.

The non-attractiveness of poison sac extracts of alate females and major workers (Table 1) agrees with observations in the laboratory that workers and female alates are not overtly attractive to workers. These results emphasize the importance of both the subtle and radical physiological changes that occur after the mating flight and wing muscle histolysis (Markin et al. 1972).

The major constituents of fire any venom have been characterized as 2-methyl-6-alkyl or alkenyl substituted piperidines. The venom of *S. invicta* workers consists of five alkaloids with the 6-substituents and the methyl group *trans* to each other (MacConnell et al. 1971), whereas female alates contain primarily one *cis*-alkaloid (Brand et al. 1973). Since the alkaloids are present in alate females, which are not attractive, it is unlikely that they are responsible for the queen pheromone activity. As confirmation of this, a sample of queen poison sacs was extracted with 0.5N H_2SO_4 to remove the basic alkaloids. Bioassays of the recovered alkaloids and the

non-alkaloid fraction showed a positive response only to the non-alkaloid fraction. Therefore the queen pheromone must consist of minor non-alkaloid components of the poison sac contents.

As the name implies, the primary function of the poison sac is to store venom for use in defense and for killing prey. However, in many ant species, worker poison glands produce a trail substance and/or an alarm pheromone as well (Gabba and Pavan 1970). The multiple role of the poison gland and reservoir is also evident in the imported fire ant, although at two different levels. The initial difference in role occurs at the caste level. So far no function other than defense and the securing of prey has been attributed to the poison gland of workers, which also have a well-developed Dufour's gland that produces the trail pheromone (Walsh et al. 1965). Queens have a degenerated Dufour's gland since they do not lay trails, but they do have a fully developed poison sac, comparable in size to a worker's. Queens are not aggressive and do not sting, which points to a different function for their poison sac contents. The evolutionary divergence of alkaloid composition in aggressive workers and placid queens also corresponds with functional differences, since venoms rich in *trans*-isomers have much greater necrotic activity (Brand et al. 1973).

We have determined two behavioral responses by workers to extracts of queens and queen poison sacs that can be interpreted functionally as queen recognition and worker recruitment. However, the pheromonal parsimony may be much more extensive than that indicated by our limited bioassays. Many hymenopteran pheromones are known to serve a multiple function depending on concentration and the physiology of the recipient (Blum 1970, Brough 1978). Therefore, at the caste level we have a difference in function for the same gland, and within the queen caste the poison gland produces pher-

Table 1.—Results of olfactometer and surrogate queen bioassays.**

| Sample* | Olfactometer bioassay | Surrogate queen bioassay | | |
	Number of workers responding mean (SE)†	Number of workers responding mean (SE)†	BS/18‡	BD/6§
1 Whole mated queen extract	27 (3.0)	32 (3.4)	12	6
2 Poison sacs	24 (2.5)	30 (3.7)	11	6
3 Ovarioles	8 (0.8)	3 (1.1)	0	1
4 Spermatheca	8 (1.4)	2 (0.5)	0	0
5 Digestive tract	10 (2.0)	3 (0.7)	0	0
6 Fat bodies	5 (1.5)	2 (0.5)	0	0
7 Dufour's gland	4 (1.4)	2 (0.8)	0	0
8 Abdominal exoskeleton	9 (1.0)	3 (0.6)	0	0
9 Thorax	12 (1.8)	4 (0.7)	0	0
10 Head	5 (1.1)	4 (1.1)	0	0
11 Post pharyngeal glands	4 (1.5)	4 (1.1)	0	0
12 Alate queen poison sacs	5 (1.4)	5 (1.5)	1	0
13 Major worker poison sacs	5 (1.6)	2 (0.7)	0	0
14 Hexane blank	7 (1.6)	4 (1.4)	0	0
15 Live queens	29 (3.56)			
16 Queen venom (direct collection)ψ	31 (3.3)			

* Samples were tested at 0.2 QE (10 μl of hexane solution) except major worker poison sac extracts (0.5 worker equivalents).
† Mean and standard errors of six replicates.
‡ Brood in survey area at the end of the 5 minute test period. Total for six replicates.
§ Number of replicates in which brood was deposited beside the surrogate queen at any time during the 5-minute test period.
ψ Three replicates concentration unknown.
** The results were analyzed statistically by an analysis of variance procedure followed by Duncan's multiple range test for variable results. Olfactometer bioassay: 95% confidence, DF = 75, treatments 1, 2 and 15 were significantly different from the others. Surrogate queen bioassay: 95% confidence, DF = 70, treatments 1 and 2 were significantly different from the others.

omones responsible for at least two and possibly more behavioral functions. Also of interest in this respect is the report by Hölldobbler (1971) that poison sacs from *Xenomyrmex floridanus* Emory alate females contain a sex pheromone.

The venom producing apparatus of the fire ant consists of a pair of free filaments that actively absorb biosynthetic precursors from the hemolymph. The absorbed materials are transported through the filaments to the convoluted poison gland, where venom and other compounds are synthesized. The products then flow into the venom reservoir where they are available for discharge through a duct to the sting (Blum and Hermann 1969). As expected from this scenario, bioassays of the free filaments (up to 1 QE) were negative, whereas bioassays of the poison sac and gland were positive. It is not possible to separate the poison gland from the surrounding poison sac. However, considering the negative bioassay results from other glands and queen parts, it is most probable that the poison gland is the point of pheromone biosynthesis, and the poison sac is the site of storage.

The queen poison gland and sac is a unique place to produce the queen pheromone. It allows the queen to dispense the pheromone, via the sting apparatus (Table 1), at whatever time and concentration a given situation dictates. Although two functions have been established for the material, other behavioral implications of this unusual pheromone dispensing system are under investigation, as well as the isolation and identification of the pheromone system and its potential as an aid in fire ant control.

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