Behavior of Disrupted Colonies of *Solenopsis invicta* Towards Queens and Pheromone-Treated Surrogate Queens Placed Outside the Nest

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ABSTRACT

Disruption of a fire ant colony and placement of the colony queen a short distance from the nest elicits a series of behavioral responses by the workers as follows: workers respond to the displaced queen by showing an intense attraction, clustering, touching of the queen with brood or depositing brood next to her, formation of a trail from the nest to the queen, and guidance of the queen along the trail back to the nest. Surrogate queens (pieces of rubber septa) impregnated with extracts of queens containing the queen recognition pheromone elicited the same behavioral patterns, whether tested in the field or in the laboratory.

INTRODUCTION

According to Wilson (1971), a social insect society can function only if the workers are able to distinguish the different castes and life stages of nestmates and act accordingly. Of prime importance is recognition of the nest queen. She is treated very differently from other female sexuals and in large colonies is attended by large numbers of workers who constantly feed and groom her. All this attention infers the existence of a queen recognition pheromone, and indeed, such pheromones have been reported for a number of ant species (Stumper, 1956; Watkins and Cole, 1966; Brian, 1973; Glancey, 1980).

Observation of the red imported fire ant, (RIFA) *Solenopsis invicta* Buren, (Glancy, 1980) showed that worker ants from colonies responded to their queen when she was removed from the nest by clustering about her and forming a trail over which she was guided back to the nest. The fact that this behavior was pheromonally-controlled was demonstrated in the laboratory by Vander Meer et al. (1980) and Lofgren et al. (1982). The former author

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reported on a queen recognition pheromone attractant from the poison sac of the queen while both sets of authors demonstrated that isolated workers of *S. invicta* responded to the pheromone in olfactometer bioassays and to surrogate queens treated with purified queen extracts or poison sac extracts.

Since most of the tests in the previous studies were conducted under laboratory conditions with cohorts of adult workers separated from their colony, we extended the research by studying the response of worker ants of disrupted field and laboratory colonies to their queen or pheromone-treated surrogate queens when placed outside the nest.

METHODS AND MATERIALS

Our initial field observations of RIFA worker responses to their queen were made during the winter and spring on cold (4 to 15 C) mornings when the queen is usually located close to the upper surface of the tumulus of the mound. A shovelful of soil was quickly removed from the sunny side of the nest and scattered thinly over the surface of a hardtop road or sidewalk. We were able to remove the queen by this technique more than 50% of the time. Observations were made then on the behavior of the workers towards their queen.

This "toss and look" method had rather simplistic advantages, but we found that the coloration of the queen and workers often blended with the road surface making location of the queen very difficult. Resolution of the problem came with the development of a portable wooden observation tray. The tray was constructed with a floor of plywood (81.3 X 121.9 cm) and 3 sides of Formica® (7.6 cm high). The Formica sides were coated with Fluon® to prevent the ants from climbing on them and the floor was painted light green to improve ant visibility. During a test, the open end of the tray was placed against a mound and a shoveful of the tumulus and ants scattered on the tray. The colony queen was usually quite visible against the light background and the responses of the workers easily observed. This method worked very effectively in the winter and spring, but not in the summer because high temperatures and/or dry soil caused the queen to move more deeply into the nest. Nevertheless, we were able to utilize the technique in hot weather for bioassays with pheromone-treated surrogate queens. On bright sunny days we found it necessary to use a beach umbrella to shade the ants from the sun's rays. Without this shade the worker ants sought immediate shelter under clods of dirt rather than returning to the nest or responding to the queen extracts.

Also, we found that the technique could be used in the laboratory by closing the open end of the tray and placing a laboratory nest cell with brood, workers and queen in one end of the tray. Then the queen or surrogate

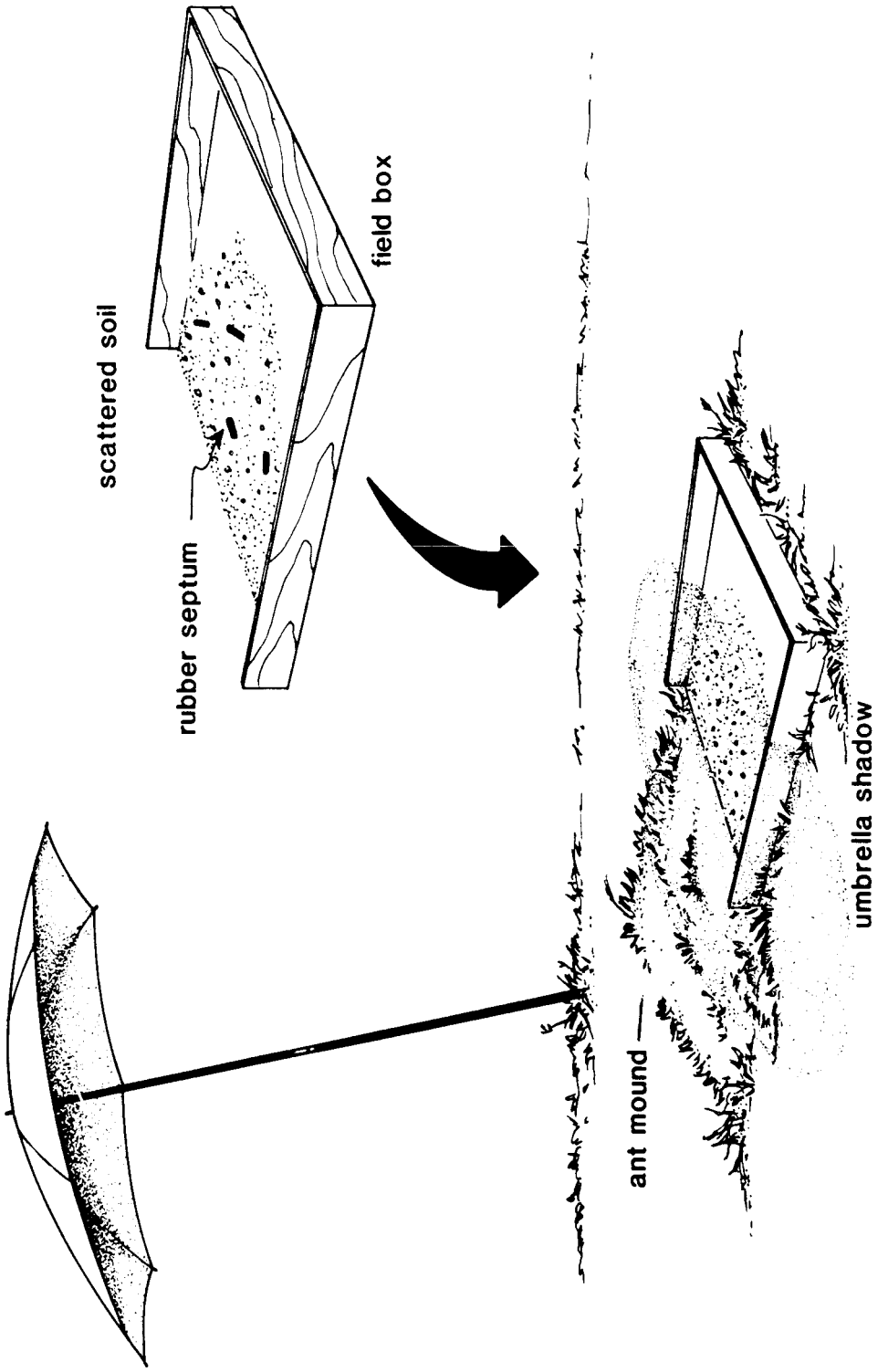

Fig. 1. Drawing of the field setup used to evaluate surrogate queen septa.

queens were placed outside the cell, several hundred workers were scattered over the tray, and the response of the colony was observed.

Crude queen extracts were evaluated by impregnating pieces of rubber septa and placing these on the soil or tray about 18 inches from the nest or mound. The extracts were obtained by crushing queens vigorously under solvent, filtering to remove insoluble material, and drying the resultant solution over anhydrous sodium sulfate. Concentrations were adjusted to 100 mg body weight (ca. 10 queen equivalents (QE) per ml). Pieces of rubber septa (8 mm) were quartered lengthwise, extracted with methanol, methylene chloride and hexane and then dried at ca. 80°C for several hours. A given amount of the extract solution (20-30 μl) was placed onto each "surrogate queen" in an ampule and the ampule sealed until testing. At the time of the test the septa were removed and allowed to dry for 15 to 30 min. Assays were run with up to 8 septa in any given trial. Control septa were treated with the solvent used to apply the extract (benzene or hexane). Figure 1 shows the experimental apparatus and design used in the field studies.

RESULTS

The ants' response to the disruption of their colony was immediate. In the field all the brood and injured workers were quickly collected and hidden beneath clods of dirt while in the laboratory they were carried directly to the nest or stacked in piles from which they later were returned to the nest. At the same time, if a queen was present, the workers immediately began to cluster about her and place brood near or next to her. Within 10 min., the ants usually had established trails back to the nest and were collecting and moving the brood along these trails.

The full sequence of behaviors toward the queen included the following:

(1) Intense initial attaction;

(2) Formation of a dense cluster about the queen;

(3) Transport of brood to the queens and touching of the queen with the brood and/or depositing the brood next to her;

(4) Formation of trails to the nest; often several of the trails coalesced into an unusually wide, heavy trail ending at the queen, just as if it were a "royal carpet" leading the way for the queen back to the mound; and

(5) Guidance of the queen along one of the trails into the nest; if she did not readily follow the trail workers dragged her along the trail, back to the nest.

These five behaviors occurred consistently in all of our tests with live queens, and thus, were used as criteria to judge whether or not purified chemicals obtained from the queen extracts elicited "queen-like" responses. A total of 17 trials was made with field colonies and 35 with laboratory colonies. The workers in the field tests responded to the pheromone-treated

surrogate queens by attraction and clustering (100% of the time), touching brood to the septum (100%), forming trails (95%) and returning the septum to the nest (95%). In the laboratory tests the responses were: attraction and clustering (100%), touching brood (91%), forming trails (94%) and returning the surrogate to the nest (89%). Extracts of major workers, male alates, and sexual pupae and controls (hexane, benzene and soybean oil) failed to induce any responses (4 to 24 trials); however, in the field tests some of the septa treated with extracts of female alates and sexual larvae were carried back to the nest by a single worker ant, but not along an established trail (7 of 12 and 2 of 4 replicates, respectively). The lack of response of the workers to septa treated with soybean oil indicates the workers were not responding to the surrogate queens as food.

DISCUSSION

When a colony of RIFA is disturbed and the queen or a surrogate queen placed outside the nest, a series of observable behavioral responses are elicited. These responses include immediate attraction and clustering, brood touching or brood deposition, trail formation and the return of the queen or carrying of the surrogate queen to the nest. Attraction and clustering are considered to be the most important of these behavioral responses since they indicate queen recognition which then put into motion the other responses. The brood touching or deposition behavior is probably related to the clustering behavior and represents a means of protecting the immatures by placing them near the central element of the colony. However, not all brood is brought to the queen. Much of it is returned directly to the nest or placed in caches under lumps of soil or at various open sites prior to returning it to the nest. Interestingly, the ants seem to move the surrogate queen or their own queen to the nest only after most of the brood has been collected.

Trail laying and recruitment are given secondary status as queen recognition behaviors, but they are necessary steps in the safe return of the queen to the nest. Rapidity of recruitment and trail formation varies considerably from colony to colony, but eventually a large well-defined trail leading to her is formed and at some point in time, she starts to return to the nest. In some tests the workers physically pulled her back to the nest. When a surrogate queen septum is contacted by the workers and a cluster begins to form, a single worker may occasionally pick up the surrogate queen and drag it immediately back to the nest, thus, precluding the formation of a trail. Normally, however, a trail is formed to the surrogate queen which is of the same type and intensity as trails laid to living queens.

The final act in the behavioral sequence is the entry of the queen or surrogate queen into the nest. As one would expect, a living queen is always

returned to the nest, but in a few tests the ants moved the surrogate towards the nest, only to abandon it or leave it at the edge of the board. In some cases, one might suspect that the pheromone had evaporated, and thus, the ants were no longer stimulated to tend the surrogate. It is also possible that the ants moving the surrogate queen recognize their colony queen's sphere of influence at the boundary of the mound, and thus, lose interest in the "new" queen.

In summary, we have observed five basic behaviors which are characteristic of the workers' response to the queen when placed outside the nest. The same hierarchy of responses are exhibited when they encounter a surrogate queen treated with the queen "recognition pheromone". This fact enables us to utilize them as a bioassay for the isolation and identification of the chemical components of the pheromone.

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