Fire Ants: Attraction of Workers to Queen Secretions[1,2]

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ABSTRACT

Workers of *Solenopsis invicta* Buren and *S. geminata* (F.) were attracted to areas (squares of grid) of blotter paper to which queens of their species had been confined. The attraction persisted for at least 72 h. Workers of *S. invicta* were more strongly attracted to squares previously occupied by the mother queen than to those previously occupied by foreign queens. Workers of *S. invicta* were strongly attracted to squares previously occupied by queens of *S. geminata*, but workers of *S. geminata* were poorly attracted to squares previously occupied by queens of *S. invicta*. The responsible secretion(s) were obtained in organic solution.

The attraction of workers of 6 species of army ants, *Nieramyrmex* and *Labidus* spp., to their queens was studied by Watkins and Cole (1966). They found that the workers were attracted to the area (square within a grid) of absorbent paper where the queen had been previously confined. Squares that had been occupied by the mother queen were more strongly attractive than squares occupied by conspecific queens (foreign queens of the same species). There was some degree of interspecific attraction, and the squares remained attractive for 72 h. Thus, queens of army ants seemed to secrete pheromones that were attractive to their workers.

We have maintained many colonies of *Solenopsis invicta* Buren in the laboratory for varying periods of time, and fewer colonies of the native fire ant, *S. geminata* (F.), and we have observed that fire ant queens are usually surrounded by a cluster of workers that presumably care for them. Therefore, we investigated the existence of attractive secretions in these 2 species of fire ants.

MATERIALS AND METHODS

The test procedure was a modification of that of Watkins and Cole (1966). The test chamber was a lidless plexiglass box (10.2×10.2×3.2 cm ID) whose inner walls were dusted with inert talc to prevent escape of the workers. A grid measuring 7.6×7.6 cm with 16 squares (1.9 cm^2) surrounded by a 1.2 cm border was drawn with pencil on white blotter paper and placed in the bottom of the chamber (Fig. 1). A queen (or a virgin alate female or 10 workers from the same colony) was confined in a bottomless aluminum cylinder on one of the 16 squares for 1 h. In tests in which 2 queens were placed in competition, the queens were confined separately on squares on opposite sides of the grid.

After removal of the queen(s) or other ants and the cylinder(s), 50 workers that had been confined in a 30-ml plastic medicine cup (inner walls were dusted with talc) were placed in the center of the grid. In tests in which *S. invicta* and *S. geminata* queens competed, the workers were from a different colony than the queen of their species. This was necessary because the mother queen proved more attractive to her offspring than conspecific queens. The reaction of the workers was determined after 30 s from photographs taken with a 35-mm camera mounted ca. 45 cm above the test chamber. In every test but one (duration of attraction), the worker ants were anesthetized with CO_2, removed from the test chamber, and replaced with 50 new workers after each photograph was taken. Twenty exposures (Kodak® Tri-X Pan film, ASA 400) were made with normal room light for each test; thus, a total of 1000 workers was used in each test (50 workers/photograph × 20 photographs). After prints were made from the film, the total number of ants on each square in the 20 photographs was counted. Control tests were conducted in the same manner by using grids that had not been exposed to queens or other ants.

Two tests, each using a different queen and her workers, were made to determine the persistence of the attraction. After the initial determination of attractiveness of a grid, the ants were removed and the grid was allowed to remain in the test chamber. The grid was aged and retested after 24, 48, and 72 h. A new group of worker ants (from the same colony as the queen originally confined on the grid) was used at each time interval. However, the ants were not removed after each photograph but were used throughout the particular test being photographed sequentially 20 times at 30-s intervals.

To determine whether the attractive secretion of the queens is soluble in organic solvents, 200 queens were collected after a mating flight, soaked in purified hexane for 1 h and washed twice. Then all the hexane was pooled and concentrated under a stream of N_2 to 1.0 ml. This preparation was tested by treating squares on opposite sides of the grid with 0.02 ml of the queen secretion solution and with 0.02 ml of a 10% (v/v) solution of once-refined soybean oil in hexane, a highly attractive food for workers (Lofgren et al. 1964).

The data obtained in tests other than persistence of attraction were analyzed with the Normal Distribution (Z) Statistic.

RESULTS

Squares occupied for 1 h by queens of *S. invicta* were attractive to their workers and remained so for at least 24 h, though attractiveness declined with time (Table 1). Also, squares treated with the hexane solution of the queen secretion were highly at-

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[2] Mention of a proprietary product in this paper does not constitute an endorsement of this product by the USDA.

FIG. 1.—*S. invicta* workers on grid in plexiglass test chamber. The mother queen had been confined for 1 h on the square on which the workers are clustered.

tractive (P=0.0005), while squares treated with soybean oil were not. In one test, a total of 145 workers was attracted to the square treated with the queen secretion solution, while only 14 workers were attracted to the square treated with soybean oil solution. In 2 other tests, these numbers were 303 vs. 20 and 125 vs. 11, respectively.

Workers of *S. invicta* were attracted about twice as strongly to squares previously occupied by their mother queen than they were to squares previously occupied by conspecific queens. In one test, 134 workers were attracted to the mother queen square and 62 workers were attracted to the conspecific queen square. In the 2 other replicates these numbers were 229 vs. 112 and 99 vs. 41, respectively. Thus, in all 3 replicates, the numbers of workers attracted to the mother queen square were significantly higher (P=0.0005) than the numbers attracted to the conspecific queen squares. Also, in all 3 tests, the numbers found on the conspecific queen square were significantly higher (P=0.005) than the mean numbers found on the control squares.

Confinement of a virgin alate female to a square resulted in attraction of workers from the same colony in only one of 3 tests. In this instance the test square attracted 54 ants as contrasted with an average of 12.7±4.6 ants for the other squares (P= 0.0005). The number of ants on the test square in the other 2 tests was not significantly higher than the other squares (23 vs. 17.1±6.7 and 20 vs. 19.7± 4.8, respectively).

Confinement of 10 workers to a square resulted in attraction (P=0.005) of workers from the same colony in only one of 3 tests. In this instance the test square attracted 34 ants as contrasted with an average of 14.6±2.4 ants for the other squares. The number of ants on the test square in the other 2 tests was not significantly higher than the other squares (38 vs. 27.9±12.2 and 25 vs. 20.5±6.4, respectively).

Table 1.—Attraction of *S. invicta* workers to squares previously occupied by mother queens.

Hours queen removed	Total numbers of workers on squares[a]	
	Queen	Controls[b]
	Test No. 1	
0	101	8.7±3.8
24	46	7.5±2.8
48	51	7.8±1.6
72	27	9.4±3.0
	Test No. 2	
0	64	7.9±4.6
24	52	8.2±3.3
48	39	8.5±3.5
72	33	10.9±3.0

[a] Total in 20 consecutive photographs.
[b] Mean and standard deviation.

Workers of *S. invicta* were attracted to squares that had been occupied by queens of *S. invicta* or *S. geminata*, and, in 3 of 5 tests, were significantly more attracted to squares previously occupied by a queen of *S. geminata*. In one test, *S. invicta* workers were more strongly attracted to the square previously occupied by a queen of *S. invicta*, and, in the remaining test, the difference in attraction was not significant. Workers of *S. geminata* were attracted to squares previously occupied by queens of *S. geminata* in all 5 tests and were attracted to squares previously occupied by queens of *S. invicta* in only 2 tests, but much less strongly (Table 2). Ants placed on control grids were distributed randomly (χ^2 test).

DISCUSSION

The secretions of fire ant queens are probably important to them in eliciting care and protection from their workers. Accordingly, we refer to the secretions as "queen-tending pheromones," though they may prove to be merely attractants, and secondary

Table 2.—Attraction of workers of *S. invicta* or *S. geminata* to squares previously occupied by queens of either species.

No. of workers on square occupied by queen of		
S. invicta	*S. geminata*	Probability
	S. invicta workers	
148	83	0.0005
80	103	NS
79	136	.0005
39	55	.05
57	83	.05
	S. geminata workers	
19	48	.0005
24[a]	124	.0005
12[a]	72	.0005
56	181	.0005
9[a]	27	.005

[a] Numbers of workers on test squares was not significantly higher than mean numbers on control squares.

stimuli may elicit the appropriate caretaking behavior. Since queens were more attractive to their own offspring than they were to workers from another colony, we think there may be a basic pheromone for each species upon which a specific colony odor is superimposed. The limited attraction of workers to a square previously occupied by 10 workers from the same colony (one test of 3) indicates that ants can detect chemicals from other workers, but attraction is very weak. The attraction of workers to one square, but not to 2 others that had been occupied by virgin alate females, may reflect age differences between the females. The attractive female may have been approaching readiness for a mating flight.

The distribution of the worker ants on the grid at the time of photographing could, theoretically, bias their distribution in a subsequent photograph; therefore, after each photograph in a test series the workers were replaced with new ants in all but one test. In the test for persistency of attraction, we got inconsistent results on successive days if the ants were replaced after each photograph. Thus, the same group of ants was used for a series of 20 photographs (30-sec intervals) and then were removed. A new group of ants was used for the evaluation on the following day. Because of this procedure, no statistical conclusions are drawn; however, the persistence of attractancy of the square is evident from the data.

The strong attraction of *S. invicta* workers to squares previously occupied by queens of *S. geminata* was most surprising and may represent an aggression response. Small colonies of *S. geminata* that have been deprived of a queen for 3 wk will often accept or tolerate a queen of *S. invicta;* however, *S. invicta* colonies cannot be induced to tolerate a queen of *S. geminata*. Small colonies of either species deprived of a queen for this period readily accept a conspecific queen (our unpublished data). Thus workers of either species are able to sense secretions of queens of the other species, even though the secretions may not be strongly active as queen-tending pheromones. *S. invicta* workers may respond much more aggressively to sensing the foreign queen than *S. geminata* workers.

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REFERENCES CITED

Lofgren, C. S., F. J. Bartlett, and C. E. Stringer. 1964. The acceptability of some fats and oils as food to imported fire ants. J. Econ. Entomol. 57: 601–2.

Watkins, J. F., II, and T. W. Cole. 1966. The attraction of army ant workers to secretions of their queens. Texas J. Sci. 18: 254–65.