

Pheromone Production by Virgin Queens of *Solenopsis invicta* Buren^{1,2}

by
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ABSTRACT

Mechanically dealated virgin females of the red imported fire ant, *Solenopsis invicta* Buren, produce a pheromone that attracts workers in the same manner as a pheromone reported from mated queens. The virgin queens become attractive 9 to 12 days after dealation, by which time the wing muscles have been histolyzed and large numbers of eggs are developing in the ovaries. Since the pheromones that induce this behavior towards virgin and mated queens seem to be identical, virgin queens provide a readily available source of material for studies on isolation, identification, and synthesis.

INTRODUCTION

While observing the behavior of the red imported fire ant, *Solenopsis invicta* Buren, in the field, we have often noted dealated queens surrounded by an entourage of workers. We previously assumed that the dealate was a fertile queen, since the production of a pheromone attractant by *S. invicta* queens has been well documented (Jouvenaz et al., 1977; Glancey, 1978; Vander Meer et al., 1980). However, on many occasions we found no sperm in the female's spermatheca, which suggested to us that uniseminated females could also produce a pheromone attractant. We have found no reports in the literature of this for other ant species, although attraction of workers of other species to their mated ant queens is well documented (Wheeler, 1910; Stumper, 1956; Carr, 1962; Watkins and Cole, 1966; Brian, 1973). We report here results of recent research conducted to determine whether the attraction

¹Hymenoptera: Formicidae

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of workers to virgin dealated females is due to the same pheromone(s) produced by mated queens of the red imported fire ant.

MATERIALS AND METHODS

The female alate queens and workers used in these experiments were obtained from laboratory-reared colonies. These colonies were maintained at 25-27°C with irregular illumination. Newly mated queens were collected during a nuptial flight in Alachua County, Florida. Some were tested immediately, whereas others were held for 10 days to ensure dealation and wing muscle histolysis. Other mated queens were obtained from laboratory colonies, and extracts were obtained from field-collected mated queens. We prepared extracts by crushing queens in a tissue grinder with hexane as the solvent and made assays at concentrations of either 0.5 or 0.2 queen equivalents (QE's). The olfactometer used in our bioassays will be described in detail in a future report. Basically, it is an open arena (Fig. 1) into which air that has passed over a queen or queen extract is released. The system consists of an uncovered Wilson cell with all but 1 port sealed and the base covered with Castone® (plaster material for making dental impressions; Ranson and Randolph, P.O. Box 905, Toledo, Ohio 43691). A glass pipette that houses the queen or a small piece of filter paper, either treated or untreated with the queen extract, is inserted in the open port. A hollow, plastic-stemmed cotton swab (Johnson & Johnson, Skilman, N.J. 08558) is cut in half, and one half is placed over the tip of the pipette and positioned so that the swab rests on a glass slide placed on the floor of the Wilson cell. Compressed air is blown through the pipette and the swab at the rate of 0.5 l/min. Worker ants (20) are placed in the cell and attractiveness determined according to the number of ants within a 2 cm² area around the swab at 5 consecutive one-minute intervals. Untreated controls consisting of a pipette/swab without an extract, are conducted with each set of tests.

TESTS AND RESULTS

Test 1. In the first study, we compared the attraction of workers to queens prior to and at 1 to 3 weeks after dealation. The virgin queens were dealated by removal of the wings with small surgical scissors. Six to 8 replications of each alate condition were made.

The results of these tests are presented in Table 1. Live newly eclosed alate queens, live 3-week-old alate queens, live mechanically-dealated 1-week-old

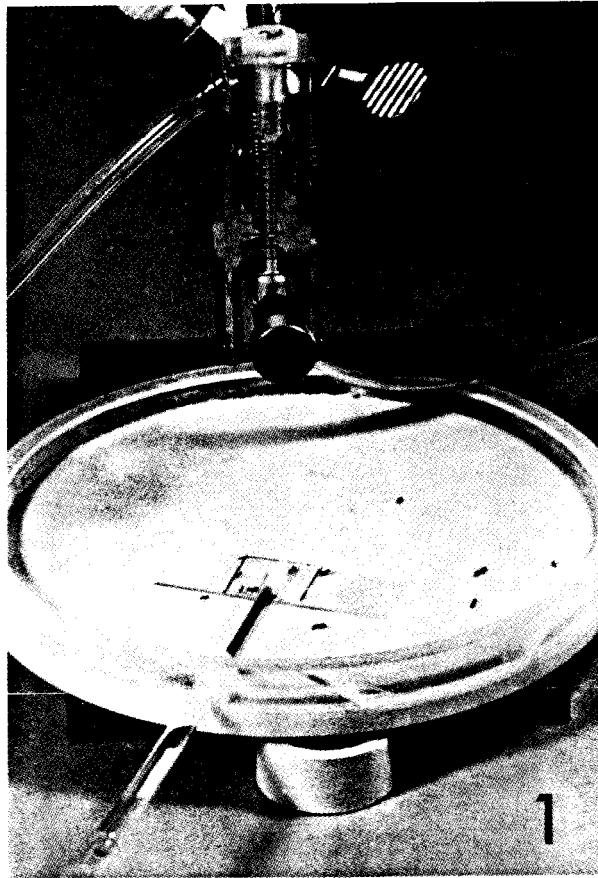


Fig. 1. Olfactometer used to determine attractiveness of virgin queens or extracts to workers of *S. invicta*. Compressed air is blown through the pipette containing the queen or piece of paper containing the extract. Ants respond by clustering around the cotton swab.

queens, or live newly mated queens captured and tested immediately after a nuptial flight exhibited little attraction. Likewise, extracts of these queens exhibited little attraction. However, extracts of unmated dealated queens that had completed wing muscle histolysis and had filled their thoracic crop were just as attractive as live aged (1 yr) mated colony queens and extracts of such queens. These extracts were just as attractive as extracts of newly mated queens that had undergone dealation and wing muscle histolysis (10 days).

Test 2. Since the data from Test 1 demonstrated that production of a pheromone by virgin and mated queens appeared dependent on wing muscle histolysis, our second test was designed to determine the age at which the queens begin to release pheromone and to correlate this with other physiological events. We caused virgin alate queens to dealate by placing them individually in small vials containing a moistened cotton swab (33 of 50 had dealated after 24 hrs). Then at intervals of 0, 3, 6, 9, and 12 days, groups of 6 females were individually assayed in the olfactometer for attractiveness, after which they were dissected to determine degree of wing muscle histolysis, ovariole development, and amount of fluid in the gastral and thoracic crops and the postpharyngeal gland. Past research has shown that newly mated queens, having undergone dealation and wing muscle his-

Table 1. Response of *Solenopsis invicta* workers to volatile emanations from live queens or from chemical extracts of queens.

Test insect or extract	Mean number of workers responding ^{a,b}	SD	Mean number of Workers responding to blank	SD
Live, mated colony queens, 1 yr old	25a	10.0	6	5.1
Extracts of live, mated colony queens, 1 yr old (0.2 Queen Equivalents/20 l)	24a	7.0	6	5.1
Extracts of newly-mated queens after wing muscle histolysis and thoracic crop formation (0.2 QE/20 l)	23a	6.1	3	3.3
Extracts of virgin, dealated queens after wing muscle histolysis and thoracic crop formation (0.2 QE/20 l)	22a	7.4	8	2.8
Live, newly-mated queens tested immediately after nuptial flight	11b	5.0	6	3.2
Live newly-eclosed alate queens	11b	7.5	11	5.9
Extract of newly-eclosed alate queens (0.5 QE/20 l)	7b	5.1	11	5.9

Table 1. (Continued)

Test insect or extract	Mean number of workers responding ^{a,b}	SD	Mean number of Workers responding to blank	SD
Live, 3-wk-old alate queens	4b	2.8	7	5.7
Extract of 3-wk-old alate queens (0.2 QE/20 l)	7b	3.4	8	2.8
Mechanically-dealated 1-wk-old queens	7b	1.8	7	5.7
Extract of mechanically dealated 1-wk-old queens (0.5 QE/20 l)	11b	3.6	11	5.9

^aAverage of 6 to 8 tests; means followed by the same letter are not significantly different from each other at the 0.05 level as determined by Duncan's multiple range test.

^bThe mean responses to the blank air streams ranged from 5 to 11.

tolysis, contain ovarioles with eggs at all stages of development and a yellow, oily fluid that fills the gastric crop, the thoracic crop, and the postpharyngeal gland.

The results of these tests appear in Table 2. It is apparent that virgin queens began to release the pheromone from 6 to 9 days after dealation, and by the twelfth day they attract workers as readily as fertile physogastric colony queens.

Table 2. Response of *S. invicta* workers to mated queens, unmated queens at the indicated number of days after dealations, and untreated controls.

Number of days after dealation of unmated queens	Response to unmated queens		Response to ^a aged (1 yr) mated queen	Response ^a to control
	x	SD		
0	5	4.0	32	3
3	4	6.1	22	3
6	8	4.6	22	7
9	16	13.0	22	5
12	25	9.9	26	11

^aA single aged queen (not always the same) and control were tested in each series of tests with unmated queens. Mean response to mated queen = 25; SD = 4.4. Mean response to control = 6; SD = 3.3.

Results of our dissections of the virgin queens showed that wing muscle histolysis and egg production were well under way after 6 days but were not complete until 9 to 12 days after dealation. All of the postpharyngeal glands were filled within 6 days. The first evidence of enlargement of the thoracic crops occurred after 6 days, but even after 12 days, not all of the thoracic crops were filled. Two of the alate queens dissected after 12 days lacked fluid in either the thoracic or gastric crops. Since the alate queens were taken at random from laboratory colonies, we had evidently selected some alates that had not filled their gastric crops. However, these queens were found to be as attractive to workers as queens with full crops.

DISCUSSION

Carr (1962) showed that virgin queens of the genus *Myrmica* were unattractive to workers whether they were alates or dealates. Passera (1969) found that virgin queens of *Plagiolepis pygmaea* were not attractive to workers but that they behaved differently from fertile queens, and that workers were

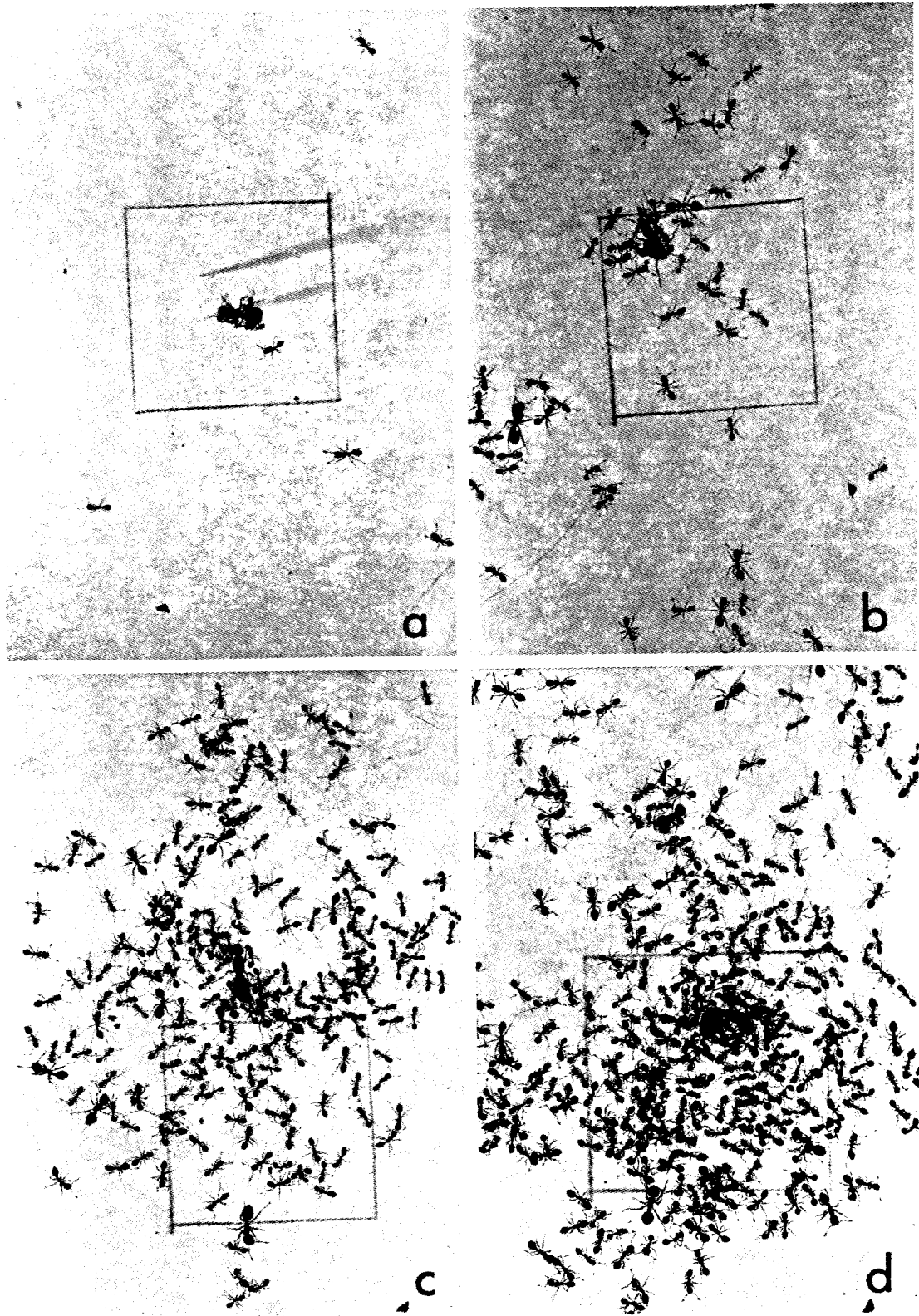


Fig. 2. Response of workers to virgin dealate which has undergone wing muscle histolysis. Series of photos taken at 10-min intervals.

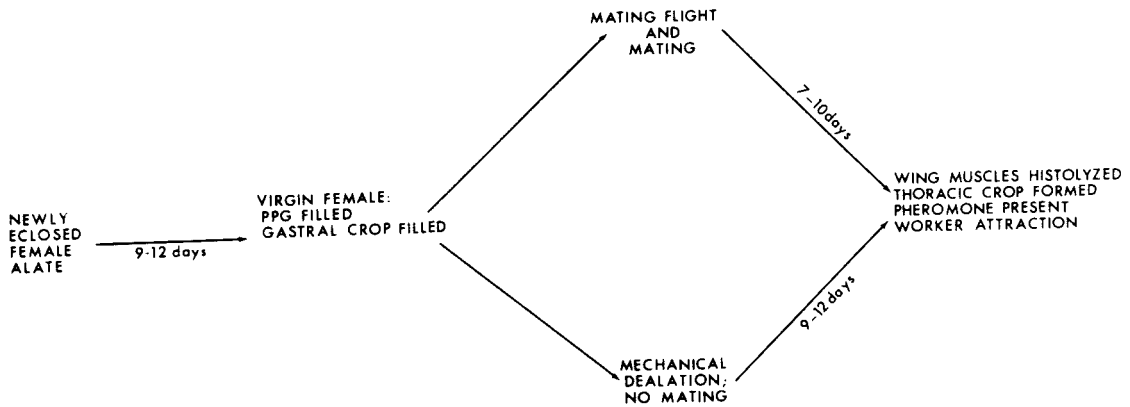


Fig. 3. Schematic diagram of events leading to the production of the recognition pheromone in mated and unmated females of *S. invicta*.

able to recognize a fertile queen by her behavior.

In our experiments, dealated virgin queens or their extracts were found to be as attractive to workers as mated queens. Development of attractiveness was correlated with histolysis of wing muscles and the presence of large numbers of eggs in the ovaries.

It is apparent that attraction results regardless of the method of dealation. If a virgin female that is dealated and forced to undergo the histolytic process is separated from the parent colony, the workers will, upon finding her, begin to groom her, establish a trail of workers to her, and guide or force her along the trail until she enters the nest. Figure 2 (A-C) shows workers being attracted to a virgin dealated queen. If she is newly dealated, the workers ignore her.

The production of the recognition pheromone and subsequent attraction must be linked to the biochemical, physiological, or hormonal changes associated with histolysis of the wing muscles and initiation of egg development. Normally, this histolysis does not begin until the virgin female has mated during a nuptial flight. Prerequisites for mating and flight include filling of the postpharyngeal gland and the gastral crop with a clear, oily material. This "filled" condition is found in females collected as they leave the mound for a nuptial flight and in newly mated females captured immediately after the nuptial flight. This sequence of events is depicted in Figure 3.

In summary, our research has shown that virgin females of *S. invicta* are capable of producing the queen pheromone that has been reported previously only for mated queens. The discovery of queen pheromone production in virgin queens will be of inestimable value in our efforts to isolate, identify and synthesize this pheromone.

REFERENCES

- Brian, M. V. 1973. Queen recognition by brood-rearing workers of the ant, *Myrmica rubra* L. Anim. Behav. 21, 691-698.
- Carr, C. A. H. 1962. Further studies on the influence of the queen ants of the genus *Myrmica*. Insectes Soc. 9, 197-211.
- Glancey, B. Michael. 1978. Biological studies on the queen pheromone of the red imported fire ant. Proc. of Tall Timbers Research Conf. 1978. (In Press)
- Jouvenaz, D. P., W. A. Banks, C. S. Lofgren. 1977. Fire ants: Attraction of workers to queen secretions. Ann. Entomol. Soc. Am. 67:442-444.
- Passera, L. 1969. Biologie de la reproduction chez *Plagiolepis pygmaea* Latr. et ses deux parasites sociaux *Plagiolepis grassei* Le Masne et Passera et *Plagiolepis xene* St. (Hym. Formicidae). Ann. Sci. Nat., Zool. Biol. Anim. 11, 327-482.
- Stumper, (R.) 1956. Etudes myrmecologiques. LXXVII. Les secretions attractives des reines de fourmis. Mitt. Schweiz. Entomol. Ges. 29, 373-380.
- Vander Meer, R. K., B. Michael Glancey, C. S. Lofgren, A. Glover, J. Tumlinson, and J. Rocca. 1980. The poison sac of red imported fire ant queens: source of a pheromone attractant. Ann. Entomol. Soc. Am. (In Press)
- Watkins, J. F., and T. W. Cole. 1966. The attraction of army ant workers to secretions of their queen. Tex. J. Sci. 18, 254-265.
- Wheeler, W. M. 1910. Ants: Their structure, development and behavior. Columbia University Press. New York. 663 pp.

