Fire Ants and Leaf-Cutting Ants

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The Queen Recognition Pheromone of *Solenopsis invicta*

B. M. Glancey

Wheeler (1910) was the first to suggest that ant queens elicited unique behavior from their workers. Later, other researchers demonstrated the existence of queen pheromones in particular ant species. For example, Stumper (1956) reported that Lasius alienus and Pheidole pallidula queens produced attractant pheromones that originated from glands in their thorax or abdomen. The pheromones induced specific licking or grooming behaviors. Solvent extracts of these queens elicited queen-like responses from workers when placed on "surrogate queens" made of paper, sponge, wood, or even bodies of dead worker ants. Schneirla (1957, 1971) reported that army ant queens produced worker attractants that were important in maintaining colony cohesiveness. Watkins and Cole (1966), working with six species of Neivamyrmex, found that workers were attracted to portions of filter paper on which their queen had been confined. Brian (1973) described tests in which he found evidence for what he called a "queen recognition pheromone" in Myrmica rubra. The compound was non-volatile and was probably produced in the thorax or abdomen.

The first observations of a worker attractant produced by queens of Solenopsis invicta were made in 1971, when co-workers and I noticed that an open jar containing field queens was highly attractive to fire ant workers (Glancey 1980). Subsequent experiments showed that: (1) workers responded to air drawn over a mated queen confined in a syringe; (2) pentane extracts of mated queens applied to filter paper were attractive to workers; (3) virgin alates were not attractive; and (4) the queen abdomen was more attractive than the thorax or head. In other tests, S. invicta queen extracts painted on worker ants of S. richteri, S. geminata, Camponotus caryae discolor, and C. pennsylvanicus offered temporary protection from attack when placed in S. invicta colonies (Glancey 1980).

Jouvenaz et al. (1974) found that S. invicta and S. geminata workers aggregated within squares on absorbent paper in which their

queens had been confined. S. invicta workers were more attracted to squares previously occupied by their mother queen than to areas occupied by a conspecific queen. In addition, one-way species specificity was noted, since S. invicta workers were attracted to areas previously occupied by their own or S. geminata queens, but S. geminata workers were attracted only to areas occupied by queens of their own species.

Over the past several years, we have concentrated on isolating and identifying the chemicals in S. invicta queens that mediate the behavioral responses of workers towards their queen. The following is a review of the status of this research.

LABORATORY TESTS

Over a period of several years, laboratory bioassays for the S. invicta "queen pheromone" evolved into those described briefly below and in detail by Lofgren et al. (1983). They referred to the pheromone as the "queen recognition pheromone" even though this terminology was not completely descriptive of all the associated behaviors. The same terminology will be used in this paper.

Olfactometer Bioassay

The olfactometer consisted of a Wilson cell (9 cm diam) with three of the four entrance/exit ports closed and the sides dusted with talc to prevent escape of the ants. Live queens or queen extracts were tested by placing them in a Pasteur pipet (extracts were absorbed onto a piece of filter paper). The narrow end of the pipet was inserted into a hollow plastic tube (ca 2 mm ID; 8 cm in length). The other end was rolled in cotton to form a tight swab. The swab end was inserted into the open port of the olfactometer and the space around the swab filled with clay. The volatiles were conveyed through the pipet and swab into the test arena with an air stream (0.5 L/min). The efficacy of the test materials was measured by introducing either a single worker or groups of 20 workers into the olfactometer. In the case of one worker, the total time that it spent at the swab over a 10-min period was determined. With 20 workers, we counted the number of ants attracted to the swab at 1-min intervals for 5 minutes. The sample results were compared to solvent controls in each set of experiments.

Surrogate Queen Bioassay

Surrogate queens were made of rubber septa cut to the size of a physogastric queen (8 by 3 mm; ca 20 mg); quartered lengthwise; extracted with methanol, methylene chloride, and hexane; and oven-dried at ca 80°C. Pretreatment of the septa was necessary because

it increased its absorptive capacity and changed, for the better, its pheromone release properties.

The test arena was a Wilson cell with all the exit/entrance ports closed. The treated septum was placed in a 2-cm observation square in the center of the cell. Twenty worker ants with a small amount of brood were then placed in the cell. The data were quantified by counting the number of ants clustered in the square at 1-min intervals for 5 minutes. Responses to a control were determined for each series of tests.

Results

Our results demonstrated conclusively that S. invicta queens produce a worker attractant. Release of the attractant ceased when the queen was separated from her colony for more than 30 min; however, activity returned immediately after she again came in contact with her workers. Extracts of live queens were attractive in the olfactometer and gave a queen-like response when applied in the surrogate queen bioassay. The maximum response occurred at concentrations of 0.5 to 5.0 queen equivalents. The responses of workers to extracts of sexual immatures and adults were significantly less than to queen extracts (Lofgren et al. 1983).

WHOLE COLONY AND FIELD BIOASSAYS

Evaluation of test materials against queenright laboratory colonies and field colonies required a different protocol than that used in bioassays with groups of isolated workers. A three-sided tray (81 x 122 x 7.6 cm) with Fluon®-painted sides was used as a field observation arena (Glancey et al. 1983). In the summer season, it was necessary to shade the box to prevent heat kill of the ants. In each test, a shovelful of nest tumulus was scattered on the floor of the box. Treated septa were then placed on the soil and observations made of the responses of the ants. Assays were run with up to 8 septa at one time.

Workers from a colony disrupted as described above immediately collected and hid their brood beneath pieces of soil. If the queen was thrown out with the soil, the workers would immediately cluster about her and place brood near her. In about 10 min, the workers established several trails along which they carried the brood back to the nest and, if the queen was present, they also guided her back to the nest along one of the trails.

Similar trays were also used in laboratory assays after closing their open ends. A laboratory nest cell with brood, workers, and queen was placed at one end of the tray, and a live caged queen or surrogate queen was placed at the opposite end. Workers from the nest cell were then scattered over the tray floor and their behavior

towards the queen or test samples noted. Our observations of the responses of ants in both laboratory and field tests were categorized into five behaviors. These were used to evaluate the effectiveness of the natural queen extracts and synthetic compounds. These behaviors were as follows:

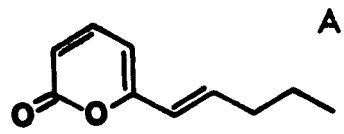
1. Intense initial attraction toward the queen;
2. Formation of a dense cluster of workers about the queen;
3. Transport of brood to the queen and touching of the queen with the brood and/or depositing the brood next to her;
4. Formation of trails to the nest, often with several of the trails coalescing into a very wide trail terminating at the nest;
5. Guidance of the queen along one of the trails (if the queen did not follow the trail by herself, the workers dragged her into the nest).

In 17 field trials, workers responded to the pheromone-treated surrogate queens by attraction and clustering in 100% of the trials, touching brood to the septum 100%, forming trails 95%, and returning the septa to the nest in 95% of the trials. The ants ignored septa that were treated with extracts of ant larvae or pupae or soybean oil. In a few cases the workers did pick up and carry septa treated with extracts of female alates and sexual brood. However, they did not carry them along an established trail (Glancey et al. 1983).

In the laboratory, the responses of attraction and clustering occurred in 100% of the trials, touching brood 91%, forming trails 94%, and returning the surrogate to the nest in 89% of the trials. Septa treated with extracts of major workers, male alates, sexual pupae, and solvent controls failed to produce any responses.

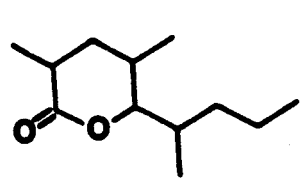
TESTING OF SYNTHETIC COMPONENTS OF THE PHEROMONE

Chemical fractionation of extracts of S. invicta queens coupled with the bioassays described above led to the isolation of three compounds (Fig. 1) that were identified through spectral data and tests of synthesized materials (Rocca et al. 1983a, b). Blends of compounds A + B and A + B + C were as active as extracts of live queens in eliciting responses from workers at four of five geographical locations (Florida, Georgia, Mississippi). At one site in Georgia, the two blends were equal in the response they elicited but were statistically less effective than the queen extract. At the Mississippi site, a combination of component C with A was also equal in attraction to the queen extract. None of the components were active by themselves. The role of component C is not clear at this time. The synthetic pheromone blends of A + B and A + B + C were most active between 5 and 10 ng per surrogate queen. In field bioassays, S. geminata, S. xyloni, and S. richteri did not respond to either the extracts of live queens or to the synthetic compounds (Glancey et al. 1984).



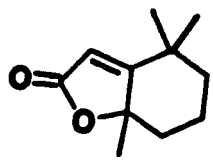
A

(E)-6-(1-pentenyl)-2H-pyran-2-one



B

tetrahydro-3,5-dimethyl-6-(1-methylbutyl)2H-pyran-2-one



C

dihydroactinidiolide

FIGURE 1. Chemical structure of three of the components of the red imported fire ant queen recognition pheromone as determined by Rocca et al. 1983a, b.

DISCUSSION

The queen recognition pheromone of S. invicta is the first ant pheromone system of its kind to be described. The pheromone was reported by Vander Meer et al. (1980) to originate in the poison sac. However, the three compounds isolated were obtained from whole body extracts, thus we cannot at this time affirm that all three originated in the poison sac or that other components might not be discovered. Jones and Fales (1983) reported the isolation of several compounds from the mandibular gland of the carpenter ant, C. pennsylvanicus, one of which was identical to compound A from S. invicta queens. They do not report any behavioral responses controlled by this chemical.

Glancey et al. (1981) found that virgin dealated queens also produce the pheromone as part of the biochemical and physiological changes that occur after dealation. Maximum queen pheromone

production was determined by bioassays to occur 9 to 12 days after dealation.

The pheromone stored in the poison sac of the queen appears to remain active after her death. Glancey (unpublished results) observed a dead queen's abdomen that was maintained and tended for up to 8 months. Williams et al. (1981) reported that the gasters of queens killed with Amdro® were retained by surviving workers for up to 9 weeks. The head and thorax were never kept but discarded onto the refuse pile. Recently Glancey (unpublished results) has shown that once a colony has adopted a mated or a dealated virgin queen for its colony queen, the workers will tend the gaster of that queen after death if the poison sac remains inside the gaster. Removal of the poison sac results in rejection of the gaster. Also, in seven trials, colonies that were tending a gaster that contained a poison sac were offered a live mated physogastric queen. Only two of the colonies accepted the live queen.

Fletcher and co-workers (see Chapter 15) discovered a primer pheromone produced by the queen that prevents female alates from competing for resources with the queen. This primer pheromone inhibits dealation and oviposition of virgin queens and appears to be produced in the queen's abdomen. It is not known if the pheromone is the same as the recognition pheromone. In any event, the grooming and licking induced by the recognition pheromone dictates that it may be involved in the distribution of the primer pheromone (Vander Meer 1983).

Fowler and Roberts (1982) concluded that queens of C. pennsylvanicus release an "entourage" pheromone. They based their conclusion on worker attraction to marked squares on a paper on which a queen had been confined. This technique is similar to that used by Jouvenaz et al. (1973) with S. invicta. These investigators referred to chemicals released on the paper as "queen tending pheromones." They noted, however, that the worker response might be simply attraction rather than tending.

Another subject that needs investigation is the relationship between the queen's age and the quality and quantity of pheromones she produces. Brian (1980) found that the influence of M. rubra queens over their workers varied with queen age and season of the year. The queens communicated their presence to the workers by both chemical and topographical stimuli. The abdomen was postulated as the most likely source of the chemical stimulus.

Passera (1980) found that queens of Plagiolepis pygmaea produce an epicuticular pheromone that inhibits egg-laying by workers. He found that the pheromone could be removed by dipping the queen in acetone daily for 15 seconds.

A different case of inhibition exists in the weaver ants, Oecophylla longinoda and O. smaragdina (Holldobler and Wilson 1983). The queens apparently produce a material in a cephalic

organ(s) which induces her attendant workers, at frequent intervals, to regurgitate ingested food and produce trophic eggs for her consumption. The workers are prevented from laying viable eggs, evidently as a result of pheromones produced by the queen. These pheromones persist in the corpses of dead queens for as long as 4 months. The origin of the queen pheromones may be the cephalic organs acting in conjunction with abdominal intersegmental glands.

In conclusion, it is obvious that the repertoire of pheromones produced by ant queens, and S. invicta queens in particular, has barely been scratched. The queen recognition pheromone and the inhibitory primer pheromone (Fletcher, Chapter 15) are the only queen pheromones described thus far for S. invicta. However, integration and regulation of the complex social behavior of the colony dictates the presence of other queen-produced pheromones. Perhaps the key to effective safe control techniques awaits the elucidation of one or more of these pheromones. It is to this end that part of our current research program involves the isolation and identification of other queen pheromones that control specific behaviors or physiological processes.

REFERENCES CITED

Brian, M. V. 1973. Queen recognition by brood-rearing workers of the ant, Myrmica rubra L. Anim. Behav. 21: 691-698.

Brian, M. V. 1980. Social control over sex and caste in bees, wasps and ants. Biol. Rev. 55: 379-415.

Fowler, H. G., and R. B. Roberts. 1982. Entourage pheromones in carpenter ants (Camponotus pennsylvanicus) (Hymenoptera: Formicidae) queens. J. Kans. Entomol. Soc. 55: 568-570.

Glancey, B. M. 1980. Biological studies of the queen pheromone of the red imported fire ant. Proc. Tall Timbers Conf. Ecol. Anim. Control Habitat Manage. 7: 149-154.

Glancey, B. M., A. Glover, and C. S. Lofgren. 1981. Pheromone production by virgin queens of Solenopsis invicta Buren. Sociobiology. 6: 119-127.

Glancey, B. M., C. S. Lofgren, J. R. Rocca, and J. H. Tumlinson. 1983. Behavior of disrupted colonies of Solenopsis invicta towards queens and pheromone-treated surrogate queens placed outside the nest. Sociobiology. 7: 283-288.

Glancey, B. M., J. Rocca, C. S. Lofgren, and J. H. Tumlinson. 1984. Field tests with synthetic components of the queen recognition pheromone of the red imported fire ant, Solenopsis invicta Buren. Sociobiology. 9: 19-30.

Holldobler, B., and E. O. Wilson. 1983. Queen control in colonies of weaver ants (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 76: 235-238.

Jones, T. H., and H. M. Fales. 1983. E-6-(1-pentenyl)-2H-pyran-2-one from carpenter ants (Camponotus spp.). Tetrahedron Lett. 24: 5439-5440.

Jouvenaz, D. P., W. A. Banks, and C. S. Lofgren. 1974. Fire ants: Attraction of workers to queen secretions. Ann. Entomol. Soc. Am. 67: 442-444.

Lofgren, C. S., B. M. Glancey, A. Glover, J. Rocca, and J. Tumlinson. 1983. Behavior of workers of Solenopsis invicta (Hymenoptera: Formicidae) to the queen recognition pheromone: Laboratory studies with an olfactometer and surrogate queens. Ann. Entomol. Soc. Am. 76: 44-50.

Passera, L. 1980. La fonction inhibitrice des reines de la fourmi Plagiolepis pygmaea Latr.: Role des pheromones. Insect. Soc. 27: 212-225.

Rocca, J. R., J. H. Tumlinson, B. M. Glancey, and C. S. Lofgren. 1983a. The queen recognition pheromone of Solenopsis invicta, preparation of (E)-6-(1-pentenyl)-2H-pyran-2-one. Tetrahedron Lett. 24: 1889-1892.

Rocca, J. R., J. H. Tumlinson, B. M. Glancey, and C. S. Lofgren. 1983b. Synthesis and stereochemistry of tetrahydro-3,5-dimethyl-6-(1-methylbutyl)2H-pyran-2-one, a component of the queen recognition pheromone of Solenopsis invicta. Tetrahedron Lett. 24: 1893-1896.

Schneirla, T. C. 1957. Theoretical consideration of cyclic processes in doryline ants. Proc. Am. Philos. Soc. 101: 106-133.

Schneirla, T. C. 1971. Army ants. W. N. Freeman and Co. San Francisco, CA. 349 pp.

Stumper, R. 1956. Etudes myrmecologiques. LXXVII. Les secretions attractives des reines de fourmis. Mitt. Schweiz. Entomol. Ges. 29: 373-380.

Vander Meer, R. K. 1983. Semiochemicals and the red imported fire ant Solenopsis invicta Buren (Hymenoptera: Formicidae). Fla. Entomol. 66: 139-161.

Vander Meer, R. K., B. M. 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. 73: 609-612.

Watkins, J. F. II, and T. W. Cole. 1966. The attraction of army ant workers to secretions of their queen. Texas Sci. 18: 254-265.

Wheeler, W. M. 1910. Ants: Their structure, development and behavior. Columbia Univ. Press, N.Y., NY. 663 pp.

Williams, D. F., C. S. Lofgren, and R. K. Vander Meer. 1981. Tending of dead queens by workers of Solenopsis invicta Buren (Hymenoptera: Formicidae). Fla. Entomol. 64: 545-547.