

Alate Semiochemicals Release Worker Behavior During Fire Ant Nuptial Flights¹

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ABSTRACT Bioassays conducted during induced laboratory mating flights of *Solenopsis invicta* Buren investigated whether alate semiochemicals stimulate formation of the worker retinue that accompany alates during the preflight stage. In paired tests comparing worker response to alates vs response to other workers, workers preferentially entered, searched and recruited nestmates to vials that contained either an alate corpse or alate residue. Olfactory cues of both males and gynes (but not workers) attracted workers, induced alarm - recruitment, and promoted alate retrieval. Workers responding to these cues in our bioassays exhibited recruitment behavior previously unreported for fire ants, i.e., "back - and - forth jerking" and "group recruitment." We propose that the observed worker behavior is a response to alate mandibular gland - derived alarm pheromone modulated by less volatile caste recognition cues associated with the alate cuticle.

KEY WORDS Imported fire ant, *Solenopsis invicta*, nuptial flight, alates, semiochemicals, behavior, caste recognition.

Infestations of imported fire ants (*Solenopsis invicta* Buren, *Solenopsis richteri* Forel) (Formicidae: Myrmicinae) and their hybrid (Vander Meer et al. 1985) have increased in the southern United States and Puerto Rico during the last four decades (Lofgren 1986). These population expansions are due in part to the dissemination of hundreds of queens during fire ant nuptial flights (Markin et al. 1971). Fire ants exhibit the male aggregation syndrome (Hölldobler and Bartz 1985), a pattern of ant nuptial flight distinguished by the formation of male swarms into which females fly for insemination and from which they disperse to establish new colonies. A salient feature of the male aggregation syndrome is synchrony, i.e., flights occur at predictable times of day and within a particular range of conditions for any species (Kannowski 1959, 1963, Weber 1972, Boomsma and Leusink 1981). In addition, the behavior of colony members during the initiation of mating flights is itself stereotyped and temporally coordinated (Hölldobler and Wilson 1990).

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In *S. invicta*, the presence of unmated winged reproductives (alates), surface temperatures between 24 and 32° C, prior rainfall, and minimal surface winds are nuptial flight prerequisites (Rhoades and Davis 1967, Markin et al. 1971, Morrill 1974, Milio et al. 1988). During preflight activity, workers open the nest tunnels to the outside, and alates aggregate at these openings. At some as yet undetermined signal, alates exit the nest and begin to move about on the mound and surrounding vegetation, with frequent contact observed between alates and workers (Morrill 1974). Field and laboratory observations of this alate-worker contact (Obin and Vander Meer, unpublished) indicate that workers form retinues around individual alates and exhibit stereotyped behavior. Workers can be observed grabbing alates by the mandibles and neck and either leading or accompanying them around the nest surface and vegetation. As reproductives attempt to climb and take flight from vegetation, workers may pull at them, rendering flight impossible. In some instances, workers actually guide alates up vegetation before alates take flight. Workers exhibit elements of alarm - recruitment behavior that include orientation to alates, frenzied running, gaster flagging (Obin and Vander Meer 1985), rapid back-and-forth jerking, and heightened pugnacity. After a period that varies from several minutes to over one hour, this behavior subsides and alates fly from the nest. Temporal coordination is observed again, with the majority of males leaving before gynes (see also Markin et al. 1971, Milio et al. 1988). Similar patterns of tending by workers and males flying first have been noted for other species, including *Pogonomyrmex* harvester ants (Hölldobler 1976) and *Camponotus herculeanus* (Hölldobler and Maschwitz 1965).

This study addresses possible semiochemical control of preflight activity, with emphasis on chemical signals from alates that modulate worker behavior. We demonstrate that chemical cues from males and gynes attract workers, stimulate alarm-recruitment behavior, and induce workers to retrieve alate corpses. Our data suggest that attraction and alarm-recruitment are responses to volatile compounds, and that these volatiles can recruit workers in contexts other than nuptial flights.

Materials and Methods

Ant Collection and Rearing. Seven months prior to tests, large monogyne *S. invicta* colonies containing brood were collected in Alachua Co., Florida. The colony queen, a small portion of brood, and several thousand attendant workers were housed immediately in a Castone-lined Petri dish cell (14.0-cm diam) that was positioned on the floor of a large, plastic tray (11 × 60 × 75 cm). Approximately 0.1 m³ of original nest soil and the remaining workers and brood were then placed in the tray. Workers constructed a new nest over and around the queen cell within 24 h and moved the remaining brood inside. Colonies were maintained at 22° C on an *ad libitum* diet of honey-water, fly pupae, and roaches placed in ceramic foraging trays connected to the nest mound by tubing. Cotton-stoppered water tubes were placed around the mound and replenished when necessary. In addition, mounds were watered weekly with a watering can to further promote nest humidity as well as the growth of vegetation introduced with the nest soil. Photoperiod (fluorescent lighting) was variable

(10-14:14-10 h L:D). Eight colonies were selected for testing. A thin layer of white sand was applied to these mounds to provide an enhanced background for observing ant behavior.

Mating Flight Induction. Mounds were watered the day preceding the artificial induction of mating flights. At midmorning of the day of induction, the laboratory temperature was raised to 30° C. Unless otherwise specified, this temperature was maintained throughout the ensuing nuptial flight. Mating flights were initiated in only one colony each day, and at least four days separated nuptial flights in colonies tested more than once.

Bioassays. Experiment 1. Worker Response to Freeze-Killed Alates. Mating flights were induced and permitted to progress up to the point at which gynes began to leave the nest. Alates and major workers were collected with feather forceps from the nest surface and placed in individual 5-ml glass vials that had been left overnight on mounds to allow colony members to mark them with colony-specific territorial odors (Jaffe and Puche 1984). Collected ants were freeze-killed (-9° C), and the room was immediately cooled to 22° C. Alates and workers quickly returned into the nest, yielding a mound essentially devoid of ants, but a colony primed for nuptial flight interactions among members. After 30 min, vials were opened, thawed at room temperature (22° C) for 3 min, and placed 15-20 cm from and facing the nest entrance. Worker behavior was observed for 10 min. One vial was tested at a time, with a 15 min interval between tests. The order in which vials containing either an alate or a worker were presented to colonies was alternated among colonies. Eight assays testing gyne vs worker vials and four assays testing male vs worker vials were conducted.

Experiment 2. Alate and Worker Residues. Winged reproductives and workers were collected from mounds during laboratory mating flights and placed in vials. Vials had been previously marked by workers which contained either two gynes, two male alates, or five major workers (a control for ant mass). Vials were emptied by tapping after 15 min, and pairs of alate and worker vials were placed 10 cm apart (> 0.3 m between pairs) on the sides of the nest mound. Colony behavior directed at the vials was observed, and the number of workers entering each vial (including those exiting and reentering) was recorded for 2 min, beginning with the right-hand vial of each pair. The contents of the right-hand vial (alates or workers) was alternated for each assay. Twenty gyne/worker vial pairs were tested among four colonies, and eight male/worker vial pairs were tested among four additional colonies (Table 1). All tests in any one colony were conducted on the same day.

Experiment 3. Gyne and Worker Volatiles. Gynes and workers were collected as in Experiment 2 but were individually housed in tubular, wire-mesh cages (2.2 cm long, 0.75 cm diam) that were sealed at each end with glass wool and placed inside test vials. These cages prevented ants from contacting the glass sides of the vial. Vials were uncapped after 1 h, and following removal of cages and enclosed ants, tested as in Experiment 2. Twelve gyne/worker vial pairs were assayed among three colonies (Table 2).

Experiment 4. Alate Residues / No Mating Flight. Individual alate gynes and major workers were collected the day prior to assays and maintained in separate vials on moist filter paper. One hour prior to tests, ants were transferred to new, previously colony-marked vials and maintained at 30° C in another

room. The colony rearing room remained at 22° C. After 1 h, alate and worker vials were emptied and placed in pairs, 15-20 cm from a nest entrance on the side of the mound. Worker behavior was observed, and the number entering each vial was counted for 2 min. Nine gyne/worker vial pairs were assayed among three colonies (Table 3).

Results

Laboratory mating flights began within 90 min following establishment of 30° C temperature. As in nuptial flights observed in the field (Morrill 1974), nests were opened at several places near the apex of the mound. Alates clustered and milled about these openings, although in some instances males began to wander on the nest surface accompanied by small worker retinues. Within 60 min, the mound was swarming with alates of both sexes and frenzied retinues of workers. Bioassays were conducted during this period unless otherwise indicated. Males generally attempted flight first, followed by females within 15 min. Only a small proportion of alates actually became airborne, flying toward the fluorescent ceiling lights. The vast majority of reproductives returned to the nest when laboratory temperature was reset to 22° C at the conclusion of bioassays.

Experiment 1. Alate Corpses. Corpses of alates but not workers elicited typical worker behavior that included attraction, alarm-recruitment, and retrieval into the nest. Three of 8 male corpses (37.5%) and 8 of 20 gyne corpses (40.0%), but none of 20 worker corpses were retrieved. In two tests an alate corpse attracted workers from within the nest who ran *en masse* from the entrance and began a frenzied, sinusoidal search. Both corpses were carried back into the nest upon discovery. In tests of seven alates (two male, five gyne), individual workers on the mound rushed to the vial, antennated the corpse and ran back into the nest. Within seconds, as many as 11 workers streamed from the nest entrance in single file, ran directly to the vial and retrieved the corpse back into the nest. Other behavior observed on or near alate corpses included gaster flagging, 'bursts' of running with the tip of the abdomen on the substrate, and back-and-forth jerking. In the latter behavior, a worker approached and jerked her body rapidly back and forth several times directly in front of a nestmate for 1 or 2 s. She then turned and moved toward or into the test vial. Workers to whom these displays were directed began to move more quickly, and those that were not already searching the nest surface, began to do so. In contrast, worker corpses did not attract or excite workers, eliciting only prolonged antennation when discovered.

Experiment 2. Alate vs Worker Residues. Workers placed in vials attempted to escape, biting at the inside of the cap and moving rapidly back and forth from one end of the vial to the other. Gynes and males did not bite at the vial cap and were less frantic overall, with several becoming motionless within 15 min.

Significantly more workers entered vials that had contained alates (Table 1). Worker behavior in and around alate vials was distinguished by 'bursts' of running, rapid antennation of the vial interior, and repeated dragging of the tip of the abdomen over the vial opening. The proportion of workers entering alate or worker vials was independent of the order in which paired vials were counted

Table 1. Summary of Experiment 2, conducted after the initiation of mating flights, indicating the mean number (\pm SEM) of workers that entered vials formerly containing gynes, males or major workers.

colony	# pairs tested	gyne vial*	male vial**	worker vial
1	6	17.2 \pm 3.4	—	6.5 \pm 1.2
2	5	12.7 \pm 2.5	—	4.7 \pm 2.2
3	4	22.8 \pm 9.4	—	3.3 \pm 1.0
4	3	21.7 \pm 6.4	—	8.3 \pm 3.5
5	2	—	11.5 \pm 2.2	1.5 \pm 0.3
6	2	—	12.0 \pm 0.6	6.0 \pm 1.3
7	2	—	29.0 \pm 13.9	4.0 \pm 2.5
8	2	—	17.5 \pm 0.9	6.5 \pm 1.6

* Significantly different from "worker vial" by paired *t*-test ($t = 5.80$; $df = 17$; $P < 0.001$).

** Significantly different from "worker vial" by paired *t*-test ($t = 3.24$, $df = 7$; $P = 0.015$).

($X^2 = 0.91$; $df = 1$; $P = 0.37$). The mean number (\pm SEM) of workers entering 'gyne vials' (18.0 \pm 2.8) was not significantly different from the mean number of workers that entered 'male vials' (17.5 \pm 5.0) ($t = 0.09$; $df = 24$; $P = 0.96$).

Experiment 3. Gyne vs Worker Volatiles. Behavior of caged workers and gynes was similar to that observed in Experiment 2, i.e., workers attempted to escape, whereas gynes did not. As in Experiment 2, more workers entered gyne vials than worker vials (Table 2). Moreover, workers in and near gyne vials were visibly excited, as evidenced by bursts of running, prolonged and rapid antennation of the vial interior and marking of the vial opening with the tip of the gaster.

Experiment 4. Mating Flight Not Initiated. Because these assays were not conducted during mating flights, relatively few workers were observed on the nest surface of some colonies. However, vials containing gyne residues were attractive to these workers and were contacted significantly more often than vials containing worker residues (Table 3). In addition, workers approaching the mouth of gyne vials exhibited running 'bursts' and searched the inside of the vial. Some returned to the nest entrance. However, workers did not subsequently rush from nests as in Experiment 1.

Discussion

Studies of pheromonal regulation of ant nuptial flights have focused on interactions between reproductives. It has been demonstrated for example, that *C. herculeanus* males release mandibular gland contents that synchronize and trigger the mass flight of gynes (Hölldobler and Maschwitz 1965), and that alates of many species release pheromones to attract potential mates

Table 2. Summary of Experiment 3, conducted after the initiation of mating flights, indicating the mean number (\pm SEM) of workers that entered vials formerly containing caged gynes or workers.

colony	# pairs tested	gyne vial*	worker vial
1	3	28.7 \pm 11.0	7.0 \pm 1.5
2	4	54.3 \pm 13.0	7.5 \pm 2.1
3	5	79.2 \pm 19.0	38.0 \pm 7.6

* Significantly different from "worker vial" by paired *t*-test ($t = 4.87$; $df = 11$; $P < 0.001$).

Table 3. Summary of Experiment 4, conducted without the induction of mating flights, indicating the mean number (\pm SEM) of workers that entered vials formerly containing gynes or workers.

colony	# pairs tested	gyne vial*	worker vial
1	3	50.3 \pm 15.0	9.7 \pm 1.2
2	3	9.0 \pm 3.5	3.7 \pm 1.3
3	3	13.0 \pm 2.0	1.0 \pm 0.6

* Significantly different from "worker vial" by paired *t*-test ($t = 3.04$; $df = 8$; $P = 0.02$).

(Hölldobler 1971, 1976, Hölldobler and Wust 1973, Hölldobler and Haskins 1977). In contrast, the present study addressed interactions between workers and reproductives, in particular, the tending of alates that characterizes worker preflight activity in imported fire ants (and other species exhibiting the male aggregation syndrome). Our results demonstrate that chemical cues from both males and gynes can release components of worker tending behavior. These chemical cues are distinct from the compounds comprising the fire ant queen recognition pheromone (Jouvenaz et al. 1974, Glancey et al. 1983), as alates do not release these compounds (Vander Meer et al. 1980, Lofgren et al. 1983).

In Experiment 1 workers rushed from nest entrances in direct response to vials containing fresh alate corpses. Although we did not control for the potential effects of visual cues, the sinusoidal orientation exhibited by workers argues strongly that workers were responding to an odor plume emanating from the vial rather than to visual cues provided by alate corpses. This conclusion is supported by the results of Experiments 2 and 3 in which potentially confounding visual cues were excluded and in which workers preferentially entered, searched, and recruited other workers to vials that had contained alates. In addition, results of Experiments 1 and 3 (in which wire cages prevented test ants from touching the sides of vials) suggest that alate-derived olfactory cue(s) eliciting worker attraction and alarm-recruitment are volatile.

The paired mandibular glands are a likely source of these volatiles, because (1) both males and gynes possess mandibular glands, whereas only gynes possess a Dufour's gland and venom sac, (2) alates of other species release mandibular gland contents during nuptial flights (Hölldobler and Maschwitz 1965, Hölldobler 1976, see above), and (3) worker mandibular glands in numerous ant species (Bradshaw and House 1984) including *S. invicta* (Obin and Vander Meer, unpublished data) contain volatile attractants that release alarm behavior.

Indeed, worker preflight behavior might best be thought of as alate-induced alarm behavior (attraction, worker frenzy, and recruitment) modulated by caste recognition cues that, in the context of mating flights, promote worker protection of alates. Vulnerable to predation on the surface, alates recruit pugnacious worker retainues, perhaps while waiting for appropriate launch conditions (e.g., low wind). Upon contacting and 'recognizing' males and gynes as colony reproductives, workers attempt to pull them back to safety (i.e., retrieval). Results obtained in Experiment 4 suggest that workers may be programmed to protect and retrieve colony reproductives in contexts other than mating flight initiation (e.g., colony emigration, cave-ins). Although caste recognition cues may be contained in the volatile attractant released by alates, a 'cloud' of such cues on the nest surface would be inefficient as well as vulnerable to penetration by myrmecophiles. It is more likely that less volatile compounds associated with the alate cuticle provide the necessary caste - recognition information. However, we caution that visual and tactile caste recognition cues cannot be ruled out at this time.

Back - and - forth jerking (Experiment 1) has not been reported previously for fire ants, although similar motor displays in the contexts of alarm - recruitment and colony emigration have been documented in the Formicinae (Hölldobler et al. 1974, Hölldobler and Wilson 1978). Similarly, lines of recruited workers observed in Experiment 1 are to our knowledge the first evidence of "group recruitment" (Hölldobler 1974) reported for fire ants. The context(s) in which jerking and group recruitment are observed under field conditions and the role of the fire ant trail pheromone (Vander Meer 1986) in group recruitment are enticing subjects for comparative studies of signal ritualization and evolution.

In conclusion, we have demonstrated that alate - derived chemical cues stimulate the formation of worker retainues around winged reproductives during laboratory mating flights of *S. invicta*. Both males and gynes appear to release a substance(s) that attracts workers, facilitates caste recognition and induces alarm - recruitment, grabbing and retrieving of alates. The simplicity and replicability of our laboratory assay system should promote the isolation and identification of compounds involved in this response and in other aspects of fire ant nuptial flight biology. The potential rewards of identifying this compound(s) to the fields of insect behavioral ecology, evolutionary biology, and integrated pest management may be significant.

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