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SEMIOCHEMICALS RELEASED BY ELECTRICALLY STIMULATED RED IMPORTED FIRE ANTS, *Solenopsis invicta*

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Abstract—The red imported fire ant *Solenopsis invicta* Buren, has evolved sophisticated chemical communication systems that regulate the activities of the colony. Among these are recruitment pheromones that effectively attract and stimulate workers to follow a trail to food or alternative nesting sites. Alarm pheromones alert, activate, and attract workers to intruders or other disturbances. The attraction and accumulation of fire ant workers in electrical equipment may be explained by their release of pheromones that draw additional worker ants into the electrical contacts. We used chemical analysis and behavioral bioassays to investigate if semiochemicals were released by electrically shocked fire ants. Workers were subjected to a 120 V, alternating-current power source. In all cases, electrically stimulated workers released venom alkaloids as revealed by gas chromatography. We also demonstrated the release of alarm pheromones and recruitment pheromones that elicited attraction and orientation. Arrestant behavior was observed with the workers not electrically stimulated but near those that were, indicating release of unkown behavior-modifying substances from the electrically stimulated ants. It appears that fire ants respond to electrical stimulus by generally releasing exocrine gland products. The behaviors associated with these products support the hypothesis that the accumulation of fire ants in electrical equipment is the result of a foraging worker finding and closing electrical contacts, then releasing exocrine gland products that attract other workers to the site, who in turn are electrically stimulated.

Key Words—Semiochemicals, pheromones, alarm, attraction, electric field, fire ant, behavior, recruitment.

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INTRODUCTION

The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), occurs in densities at least five times greater in the southern and southeastern United States than in its native South American habitat (Porter and Savignano, 1990; Porter et al., 1992). This large biomass of aggressive fire ants dominates the ecological community (Porter and Savignano, 1990) and interferes with human activities (Lofgren, 1986). One unusual effect on human activities is fire ant damage to electrical equipment, in which large numbers of ants invade outdoor electrical apparatuses, create short-circuits, foul conductive materials, and jam internal mechanisms (Eagleson, 1940; Vinson and MacKay, 1990), all of which can lead to destruction of circuitry.

Alternating-current frequencies, heat, ozone, magnetic fields, and wire insulation have no effect on ants (MacKay et al., 1989). Fire ants were initially reported to be attracted to electrical fields along with several other ant species (MacKay et al., 1992a,b). However, Slowik et al. (1996) reported that fire ants are not attracted to electrical fields, but instead probably release chemicals that excite and attract other ants to the release site after ants contact bare, bridgeable conductive surfaces, such as exposed wires or contact points.

Electrical stimulation of worker ants causes incapacitation, death, and aggression towards sister workers (Slowik et al., 1996). In addition, both voluntary (not induced by electrical stimulation) and involuntary (displayed by electrically stimulated ants) "gaster-flagging" was observed (Obin and Vander Meer, 1985; Slowik et al., 1996). Gaster flagging signals the release of defensive venom alkaloids (Obin and Vander Meer, 1985). Therefore, the release of semiochemicals by electrically shocked worker ants in the vicinity of infested circuitry may cause destructive aggregations of fire ants in electrical equipment (Slowik et al., 1996).

Pheromonal chemical cues are the most important form of communication among ants (Vander Meer and Alonso, 1998), and several pheromone systems of *S. invicta* have been investigated. For example, an alarm/excitant pheromone is involved in mating flight activity (Obin and Vander Meer, 1994; Alonso and Vander Meer, 1997). A recruitment pheromone is produced by the Dufour's gland and is released via the sting apparatus (Wilson, 1959). This complex recruitment pheromone system has several concentration- and context-related functions. Pheromones attract workers (Vander Meer et al., 1988), modulate their general responsiveness [i.e., orientation induction (Vander Meer et al., 1990)], and orient workers along a pheromone trail to food (Vander Meer et al., 1981). Fire ant venom is primarily composed of piperidine alkaloids, readily analyzed and well characterized (MacConnell et al., 1971; Leclercq et al., 1994). Venom functions in defense against intruders, in procurement of prey, and as an antimicrobial agent to maintain hygienic conditions within a colony (Obin and Vander Meer, 1985).

Venoms of other social hymenopterans such as bees, wasps, and hornets can be experimentally collected by electrical stimulation (O'Connor et al., 1963). In the field, honeybees (*Apis mellifera* L.) housed near extra-high-voltage power lines are induced to sting and release venom when electrified by as little as 900 nA of current after alighting on wet hive entrances (Bindokas et al., 1988). Fire ants, like honeybees, may also release venom and perhaps other semiochemicals when electrically shocked. These released chemicals might contribute to the observation that large masses of fire ants are often found in electrical circuitry, causing the equipment to malfunction (Vinson and MacKay, 1990). We report here the results of experiments that investigate, through behavior-specific bioassays and chemical analyses, what pheromones or semiochemicals are released by electrically stimulated fire ants.

METHODS AND MATERIALS

Source of Monogyne Solenopsis invicta Colonies. Queenright monogyne *S. invicta* colonies were obtained by rearing colonies from newly mated queens or by excavation of field nests. Monogyne colonies from the field were characterized by low mound density, well-developed large nests, polymorphic characteristics of colony workers (Greenberg et al., 1985), no more than one queen found, and a high level of intraspecific aggression (Morel et al., 1990). Excavated field colonies were brought to the laboratory only if the colony queen was found, and then the colony workers and brood were isolated by a standard float-out procedure (Banks et al., 1981). All colonies isolated after excavation were placed in plastic rearing trays (52 × 39 × 7.5 cm). Inside walls of trays were painted with Fluon (Dupont, Wilmington, Delaware, USA) to keep ants from escaping. Each colony was provided with Petri dish nest cells (14 cm diam. × 2.5 cm deep) with a Castone substrate (Dentsply Co., York, Pennsylvania, USA) and cotton-stoppered water tubes (Obin, 1986). Colonies were reared on crickets and 10% sugar water absorbed in wads of tissue. Rearing temperatures were maintained between 21 and 28°C, and the light–dark cycle was variable.

Preparation of Electrical Wire. A standard receptacle plug was attached to one end of common electrical cord (ca. 2 m). At the other end of the cord, ca. 2 cm of insulation was removed. Separately, insulation was stripped from 18-gauge wire, except for 5 mm on one end. The 18-gauge wire was soldered to the available end of the common electrical wire. Heat-shrink Teflon tubing was placed over each of soldered connections and wrapped with electrical tape. A piece of heat-shrink Teflon tubing was also placed over the wire ends that still had a small amount of insulation remaining. This functioned to hold the wires ca. 1 mm apart. The bare wires (5 cm long) were bent appropriately to better contact the floor and side of the test vial (20-ml scintillation vials), taking care to keep them 1 mm apart and

not touching each other. Wires were energized by plugging the electrical cord plug into an adjustable power supply containing a 1-A fuse that was connected to a standard 120 V, alternating-current power source.

General Experimental Procedure. Test worker ants (ca. 50) were randomly selected from laboratory-maintained monogyne *S. invicta* colonies (see above) and placed into 20-ml scintillation vials that had their inside lip painted with Fluon to help keep the ants inside the vial. The vial was clamped to a ring stand for stability. The wire was plugged into the adjustable power supply and gently inserted into the vial of ants. Ants were allowed to settle down without electrical stimulation for at least 15 min. The power was turned on and adjusted to 110 V. Ants walking across both bare wires would complete the electrical circuit and become electrically stimulated. After 2 min, the power was turned off and the ants were removed from the wire and vial with feather forceps. The bent end of the wire was placed on the lip of a clean and labeled 20-ml scintillation vial. Hexane (Burdick and Jackson, Muskegon, Michigan, USA; GC^2 grade) was added to the top of the bare wire one drop at a time with a disposable Pasteur pipet and allowed to run down the length of the wire to the bend where it dropped into the vial. This was repeated several times to ensure that all potential compounds released by the ants were removed from the wire. The hexane wire rinse was transferred to an autosampler vial for analysis by GC or for bioassay. The vial in which the worker ants were electrically stimulated was also carefully rinsed with hexane and subjected to chemical analysis or bioassay. If headspace samples (air in the vial) were needed, the vial in which the ants were electrically stimulated was immediately capped after the ants were removed. The procedure for the alarm bioassay is described in detail below.

Analysis of Venom Alkaloids and Cuticular Hydrocarbons. Immediately after an electrical stimulation treatment or control was complete and the worker ants were removed, 10 μl of a 100 ng/μl hexane solution of *n*-tetracosane were added to the test vial as an internal standard. Similarly, prior to rinsing the treatment or control electrical wire with hexane, 10 μl of a 100 ng/μl hexane solution of *n*-tetracosane were added to the collection vial as an internal standard. After addition of the internal standard, the treatment or control samples were treated as described above. Prior to GC analysis, all samples were brought to a volume of 100 μl and transferred to 300-μl autosample inserts.

Gas chromatography (GC) was carried out with a Varian 3700 (Varian, Palo Alto, California, USA) gas chromatograph equipped with a flame ionization detector and a split/splitless injector operated splitless for 0.33 min after which the splitter was turned on (90:1 ratio). Injector and detector temperatures were 300°C. Samples were injected into the system using an autosampler (Leap Technologies, Chapel Hill, North Carolina, USA). Analyses were performed on a DB-1 fused silica column (0.32 mm ID $\times$ 30 m; 0.25-μm film thickness; J & W Scientific, Folsom, California, USA). The oven was programmed from 150 to 285°C at 10°C/min with a 2-min hold. Data were analyzed with Turbochrome Workstation

Version 6.1.0.1 (Perkin Elmer, Norwalk, Connecticut, USA). Peak assignments for the venom alkaloids and cuticular hydrocarbons were based on the GC analysis of authentic *S. invicta* alkaloids and hydrocarbons obtained by overnight soaking of *S. invicta* workers in hexane (see Vander Meer et al., 1985; Ross et al., 1987).

Preparation of Poison and Dufour's Gland Extracts. The poison gland and the Dufour's gland are attached to the sting apparatus. The poison gland produces the piperidine alkaloids that are used by workers in defense and prey procurement. The Dufour's gland is located at the base of the sting and its products exit to the outside via the sting. The Dufour's gland is the source of the fire ant recruitment pheromone. The worker sting apparatus was removed in water with the aid of a dissecting microscope (Leica MZ-8, Heerbrugg, Switzerland) by placing and holding the ant in the water ventral side up. With another pair of forceps, the sting apparatus was removed by grabbing the last segment of the abdomen and pulling it out along with the sting apparatus. Once removed, the fat bodies were taken away, exposing the two glands. At this point, either gland could be obtained by pinching it off where it attaches to the sting apparatus. Excess water was removed by touching the gland carefully to a piece of tissue. The gland was transferred to a probe, then into a vial containing hexane where it was macerated. The volume of hexane was adjusted to give the required concentration in terms of gland equivalents.

Alarm Bioassay. Worker groups were taken from each of 15 mature monogyne *S. invicta* colonies maintained in the laboratory. Approximately 100 workers and a small amount of brood from each colony were placed in small plastic tray ($7 \times 22 \times 5$ cm), whose inner sides were painted with Fluon to prevent escape. The bottom of one half of the tray was covered with a thin layer of moist Castone. Red cellophane was placed over the Castone end of the tray to induce the workers to settle down with their brood. After the ants settled down (at least 30 min), the red cellophane was carefully removed. Tests were carried out only on subcolonies that were calm.

The bioassay required an observer and an assistant. The assistant prepared test samples for the observer so that the observer did not know the sample identity. Test samples consisted of 3 ml of air or headspace (vapor above a sample contained in a vial) drawn into a syringe (5 ml, plastic, Henke-Sass Wolf Co., Tuttlingen, Germany). The assistant randomly assigned each sample to a worker group, so that all worker groups were tested with each of the test samples and controls. The observer positioned the syringe 1–2 cm directly above a selected quiescent worker group, then 1 ml of vapor was slowly released over the workers. Prior to the actual test, the observer determined the blank air release rate that did not initiate a reaction from the worker ants. Reactions were rated on a scale of 1–4 (1 = no reaction, 2 = no more than raised heads and/or antennation of the air, 3 = one worker reacted with rapid movement, and 4 = several workers exhibiting an excited reaction). All tests were conducted with a room temperature of 28°C and standard overhead fluorescent lighting.

Air from an empty vial was tested as a negative control, and 10–25 workers (shaken) were used as a positive control. Shaking disturbed the workers and induced them to release alarm pheromone. Only those series of tests in which appropriate reactions to the negative and positive controls were obtained were used for analysis. Each replicate from a treatment was assayed at least five times, as were the negative and positive controls. We found that greater consistency was obtained for the positive control if there was a vial of workers for each replicate. The following treatment samples were evaluated: Electrically stimulated ants (stimulation time 120 sec); electrically stimulated ants (20 sec); shaken ants (with vial lid off for 120 sec); shaken ants (vial lid off $<$10 sec); and poison sac contents (1 gland equivalent/10 μl hexane absorbed onto a filter paper strip, solvent allowed to evaporate and placed in the bottom of a bioassay vial, headspace taken immediately).

The McNemar test for significance of changes (Sokal and Rohlf, 1981) was used for analysis of the alarm reaction bioassay data. For this analysis, scores of 1–2 were considered no reaction, and 3–4 were counted as alarm. The number of worker groups that displayed an alarm reaction to the test sample but not to the negative control was compared to the number of worker groups that reacted to the negative control but not to the test sample.

Orientation Bioassay. The orientation bioassay presumably measures the ability of workers to detect and follow a chemical trail. The procedure was a modification of that described by Barlin et al. (1976) and Jouvnenaz et al. (1978). Queenright, monogyne colonies were used in the bioassay. The orientation bioassay apparatus consisted of a ramp leading to a test platform (7.5 $\times$ 10 cm), which was connected by a tongue depressor to a similar size platform on which were food items, e.g., crickets or a cotton ball saturated with honey–water (1:1). The legs of the two platforms were painted with Fluon, which forced the ants to move up the ramp and across the test platform to the food platform. A piece of plain paper (7.5 $\times$ 10 cm) was placed on the test platform. The above-described apparatus was set up on a colony tray floor, and worker ants were given time to develop a natural trail to the food platform. Test pieces of paper were prepared by lightly marking in pencil two arcs on opposite sides of the paper from the center of one end to the center of the other end. Test solutions (10 μl) were streaked evenly along one of the arcs, and the appropriate solvent control streaked along the other arc. The test paper was air-dried for about 20 sec, then the natural trail paper on the test platform was replaced with the test paper. If, within 3 min, one ant followed the treatment arc from start to finish, the test was considered positive. A negative test was characterized by confusion at the two trail ends. Samples evaluated were the wire rinse (A) and the vial rinse (B) from electrically stimulated workers in the vial, the wire rinse (C) and vial rinse (D) from nonelectrically stimulated workers in a vial, and (E) the hexane vial rinse of worker ants in a vial without the wire. There were five replicate samples for each of the treatments. All samples

from electrically stimulated ants were adjusted to 250 μl total volume of hexane prior to use. Samples that did not give a positive orientation bioassay were concentrated to 50 μl volume and retested in the bioassay. If the samples did not show activity at this concentration, they were assumed to not have measurable activity.

The Dufour's Gland Equivalents Released by Electrically Stimulated Ants. Samples that showed positive orientation bioassay activity were systematically diluted with hexane until the samples were inactive. Dufour's gland extracts were prepared at a starting concentration of 1 Dufour's gland equivalent (DE) per 10 μl. Serial (1:2) dilutions were made with hexane, and orientation bioassays conducted until the extract was inactive. This was repeated three times. The Dufour's gland equivalent deposited by electrically stimulated ants on the wire and in the vial was determined by comparing the last active concentration of Dufour's gland extracts with the last active dilution of the wire and vial samples from electrically stimulated ants. Knowing the starting volume and the dilution regime allowed the estimation of Dufour's gland equivalents in the wire and vial samples.

Olfactometer Bioassay. A Y-tube olfactometer was used to determine if attractant compounds were released by electrically stimulated worker ants. The bioassay apparatus was similar to that described by Vander Meer et al. (1988). The positive standard was 0.3 queen equivalents of dissected and extracted queen poison sacs (poison sacs from 10 polygyne queens in 300 μl of hexane), the source of the queen attractant pheromone. A null hypothesis of equal numbers of ants in each arm was tested with chi-square analysis. Samples collected from electrically stimulated worker ants were evaluated in the olfactometer bioassay only if a positive response was obtained in the orientation bioassay, since workers are 200–300 times more sensitive to recruitment pheromone in the orientation bioassay than in the olfactometer bioassay (Vander Meer, 1986).

RESULTS

The Ant Response. Worker ants calmly walked on the vial surfaces prior to energizing the bare copper wires that were in the test vials. On energizing the system, worker ants bridging the two wires responded by gaster flagging, curling their gaster toward the wire in a stinging movement, and rapid, random movement in the vial. Other workers that were not initially electrically stimulated, reacted with alarm behaviors, including gaster flagging, and moved excitedly toward the wires, where if they made contact with both wires, they too were induced to exhibit the defensive behaviors described above. Interestingly, before the end of the bioassay (2 min), worker ants not on the wire aggregated quietly away from the wire ends. This was in sharp contrast to their rapid movement at the beginning of the test. Approximately 15–25 workers were electrically stimulated during the 2-min course of the experiment (mean $\pm$ SE: 22.8 $\pm$ 1.26 workers, $N = 8$). Some

of these workers did not survive the treatment. The observed reaction of the ants to electrical stimulation suggested that we could expect to find venom alkaloids and evidence for the release of alarm pheromones.

Venom Alkaloids and Cuticular Hydrocarbons. Both classes of compound can be readily analyzed by GC in a single chromatogram (Ross et al., 1987). All expected piperidine alkaloid components (six) characteristic of *S. invicta* were found. The quantitative results for the venom alkaloids are shown in Figure 1. The total amount of venom alkaloid from the wire rinse of electrically stimulated ants was greater than the treatment vial rinse (mean ± SE: 3.33 ± 0.56 μg vs. 0.23 ± 0.04 μg; $N = 5$ and 4, respectively; unpaired t test, $df = 7$; $t = 4.905$, $P = 0.002$) and by inference any of the controls (see Figure 1). This is a direct reflection of the observed worker defensive behaviors induced by electrical stimulation. The wire was the main target of the ants, but gaster flagging and venom-throwing may have also distributed venom onto the inner walls of the vial. In addition, some worker ants not on the wire may have been stimulated to release venom into the vial. The amount of venom found in the treatment vials also was greater than any of the control samples (treatment vial rinse vs. control wire rinse: mean ± SE: 0.23 ± 0.04 μg vs. 0.06 ± 0.01 μg; unpaired t test, $df = 7$, $t = 5.939$, $P = 0.002$). The amount of alkaloid found on the control wire and in the control vial were not

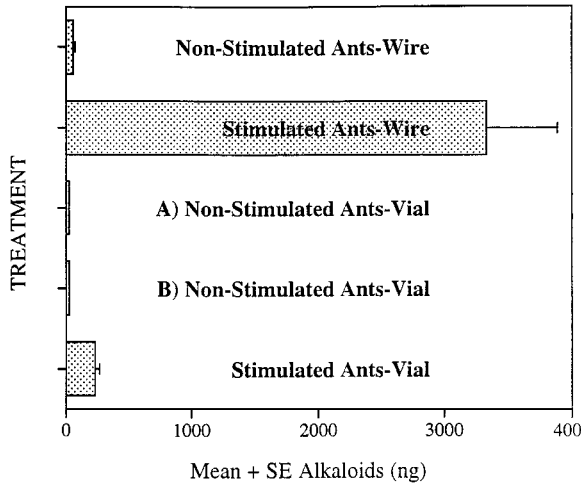

FIG. 1. Results are shown for the quantitative analysis of *S. invicta* venom alkaloids found on the wire or the vial for the control and treatment samples indicated. (A) "Non-stimulated ants-Vial" is the vial rinse that matches the "Non-Stimulated Ants-Wire" sample. This control had the wire inserted as in the electrically stimulated samples but the wire was not energized. (B) "Non-stimulated Ants-Vial" was a control that had worker ants, but did not have the wire inserted. $N = 5$.

different (unpaired t test, $df = 7$, $t = 2.127$, $P = 0.066$). Perhaps the disturbance of putting the wires into the vials with ants induced some of the workers to attack and release some venom on the wire. The mean amounts of venom in control vials were the same regardless of whether the wires were inserted or not (mean $\pm$ SE: 0.03 ± 0.01 μg vs. 0.03 ± 0.01 μg, respectively).

The principal source of cuticular hydrocarbons are from the cuticle of the insect; however, in most ants, including the fire ant, a cephalic lumen, called the postpharyngeal gland, is a copious source of the same hydrocarbon components that are found on the cuticle (Soroker et al., 1994). In our electrical stimulation experiments, the hydrocarbons could be passively transferred from worker cuticle to the wire and/or vial or they could be regurgitated from the postpharyngeal gland through the pharynx and mouth to the wire or vial substrate. Treatment and control vials did not differ in the total amount of cuticular hydrocarbons deposited in the vial during the 15-min acclimation time and the 2-min treatment time (mean $\pm$ SE: 77.0 ± 18.3 ng vs. 78.5 ± 10.4 ng; unpaired t test, $df = 7$, $t = -1.451$, $P = 0.944$).

Alarm Bioassay. Figure 2 shows the results of initial bioassays aimed at determining if electrically stimulated ants released alarm pheromone. Headspace

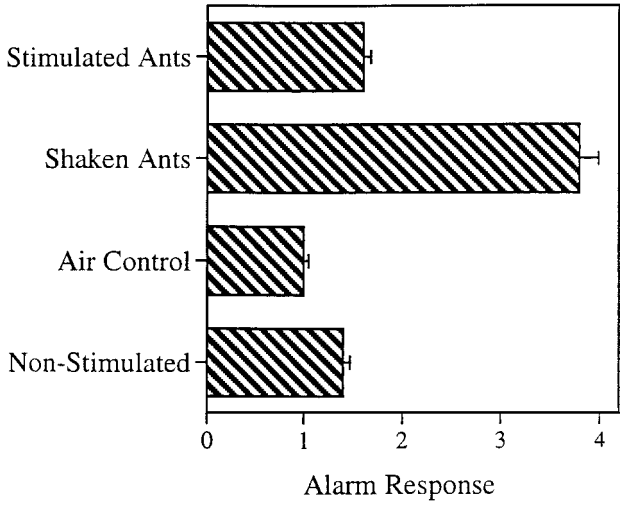

FIG. 2. Alarm bioassay results for headspace from vials in which worker fire ants were electrically stimulated and three controls. Shaken ants represents a positive control. Non-stimulated worker samples are a negative control, and laboratory air is another negative control. The results show that under standard experimental conditions the headspace above electrically stimulated ants does not elicit an alarm response in the alarm bioassay. Results are shown as the mean and SE of 10 replicates.

samples from electrically stimulated ants did not elicit a significantly different alarm reaction from workers than the nonelectrically stimulated controls (McNemar test: $G = 1.04$, $P > 0.05$; $N = 25$), but was different from the shaken ant positive control (McNemar test: $G = 18.5$, $P < 0.001$; $N = 25$). These results did not correspond to expectations based on behavioral observations.

Figure 3 shows the results of shaking worker ants in the vial (positive control) and then not sampling the opened vial for 2 min, the normal duration of the electrical stimulation. Allowing the volatiles generated from the shaken ants to dissipate for 2 min gave a result different from the positive control of shaken ants with the headspace sample taken immediately (McNemar test: $G = 13.86$, $P < 0.001$; $N = 10$). The results for shaken ant samples allowed to stand for 2 min was no different from the unshaken ant negative control (McNemar test: $G = 0$; $P > 0.05$; $N = 10$). Based on this result, we modified our electrical stimulation time to 20 sec, immediately after which the wire was removed and headspace samples were withdrawn for evaluation in the alarm bioassay. The results are shown in Figure 4. The electrically stimulated (20 sec) headspace samples elicited greater alarm response than the appropriate nonstimulated control (McNemar test: $G = 13.86$, $P < 0.001$; $N = 10$; mean $\pm$ SE: 3.9 ± 0.10 vs. 1.4 ± 0.16, respectively). However, there were no differences between the headspace samples of electrically

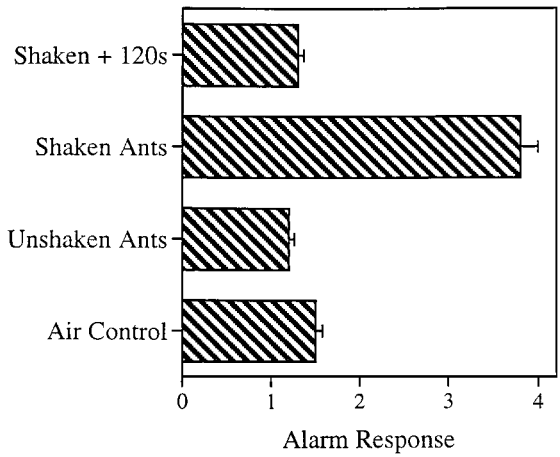

FIG. 3. Alarm bioassay results for headspace from vials in which worker fire ants were shaken to release alarm pheromone. For shaken ants (positive control) the headspace sample was taken immediately after shaking. Samples represented by shaken + 120 sec were shaken as above but the vial was left open for 120 sec prior to taking the headspace sample. Unshaken worker samples were a negative control as was laboratory air. The results show that while shaken ants release alarm pheromone, the pheromone components are highly volatile and evaporate by 120 sec after shaking. Results are shown as the mean and SE of 10 replicates.

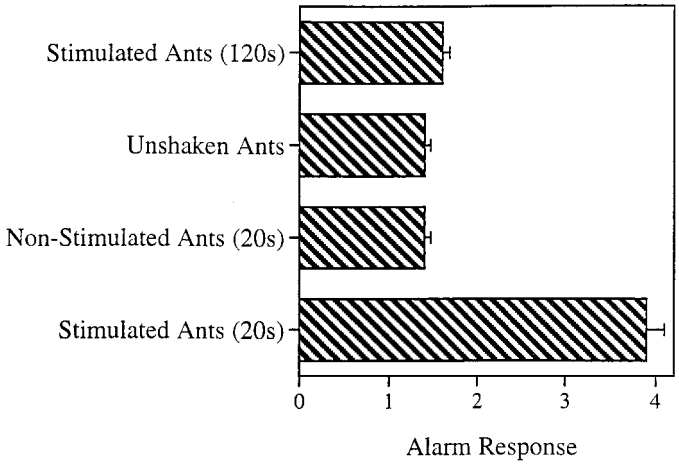

FIG. 4. Alarm bioassay results for headspace from vials in which worker fire ants were electrically stimulated for 120 sec and 20 sec. Unshaken ants was a negative control. Non-stimulated ants (20 sec) was another control where the nonenergized wire was inserted into the vial with worker ants and the headspace sample was taken after 20 sec. The results demonstrate that the alarm pheromone is released early during the electrical stimulation and evaporates out of the vial by the end of the standard 120-sec electrical stimulation time period. Results are shown as the mean and SE of 10 replicates.

stimulated workers and the headspace from the positive control, shaken workers (McNemar test: $G = 1.39$, $P > 0.05$; $N = 10$).

To test whether or not the venom alkaloids were responsible for the observed alarm activity, we applied one poison gland equivalent to a piece of filter paper, allowed the hexane to evaporate, and placed the paper into a typical bioassay vial (20 ml). The vial was capped and the headspace associated with that vial was tested for alarm activity. There was no difference between the air control and the headspace from above the poison sac samples (McNemar test: $G = 1.39$, $P > 0.05$; $N = 10$). Thus, we demonstrated that the contents of the poison sac (primarily venom alkaloids) were not responsible for the alarm response.

Orientation Bioassay. The orientation bioassay results are shown in Figure 5. All hexane rinse samples (wire and vial, $N = 5$) from electrically stimulated ants gave positive orientation bioassay results. The assay provides a "yes" or "no" answer to orientation activity. We calculated the DE by comparing electrically stimulated ant sample (wire and vial hexane rinses) bioassay results with results from Dufour's gland extracts at known volume and DEs. Both electrically stimulated ant samples and the Dufour's gland samples were systematically diluted until orientation activity was lost. The mean last positive bioassay for the Dufour's gland samples was 0.0015 DE (SE $= \pm 0.0005$). This corresponds to ca. 0.9 pg/cm

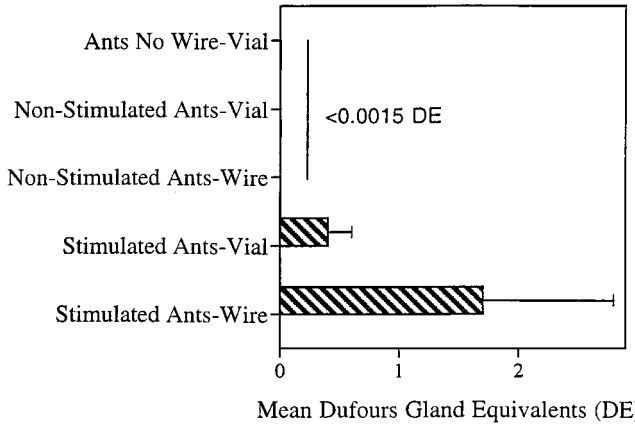

FIG. 5. Results from a recruitment orientation bioassay. Serial dilutions of treatment samples were carried out until the samples no longer gave positive results in the orientation bioassay. These results were compared with serial dilutions of Dufour's gland extracts (the source of the pheromone) containing known Dufour's gland equivalents (DE). The number of DE could then be calculated for each of the treatment samples. Only electrically stimulated workers released detectable quantities of the orientation pheromone. All other samples had to have <0.0015 DE—the lowest detectable amount. Results are shown as the mean and SE of 5 replicates.

of (*Z*,*E*)-α-farnesene, the recruitment orientation pheromone (Vander Meer et al., 1981). Knowing the starting volume of the electrically stimulated ant samples and monitoring their systematic dilution (or initial concentration) to the last positive orientation bioassay response, we related this to the results with the Dufour's gland extracts and calculated the DE of each sample (Figure 5). The initial bioassay of the controls (vial and wire rinses from samples that had ants, wire inserted, but not electrically stimulated) were negative even after fivefold concentration; therefore, they had to contain <0.0015 DE (Figure 5). Similar results were obtained with vial samples that had ants but no wire inserted (Figure 5). Like the venom alkaloids, more Dufour's gland products were deposited on the wire than on in the vial, although there was no significant difference between the two sets of samples (wire, 1.7 ± 1.1 DE vial, 0.4 ± 0.2 DE; $df = 8$, $t = 1.094$, $P = 0.359$; $N = 5$).

Olfactometer Bioassay. Each wire hexane rinse ($N = 5$) was tested in the olfactometer bioassay three times. Chi-squared analysis of the two-choice olfactometer showed that each of the wire hexane rinse replicates was attractive to worker fire ants (for each of the five replicates, $df = 1$, $\chi^2 > 3.85$, $P < 0.05$; null hypothesis of equal response to treatment and control). The mean percent of the ants responding to the treatment side of the olfactometer was 67.5 (SE = 1.5, $N = 5$). In contrast, there was not enough recruitment pheromone deposited by

electrically stimulated ants in the vial to release a response in the olfactometer bioassay ($df = 1$, $\chi^2 < 3.85$, $P > 0.05$). The mean percent of ants responding to the treatment side of the olfactometer was 56.7 (SE = 1.1, $N = 5$). The response of worker ants to the queen poison sac extract positive control was $75.5 \pm 2.6\%$ ($N = 5$, 0.33 queen equivalents/10μl hexane).

DISCUSSION

Fire ants use a wide array of semiochemicals for defense and intraspecific communication (Vander Meer and Alonso, 1998). Our results demonstrate that electrically shocked fire ants indiscriminately release exocrine gland products. Venom alkaloids from the poison sac and recruitment pheromone from the Dufour's gland were released through the sting apparatus. Alarm pheromone was released, presumably, from mandibular glands (Alonso and Vander Meer, 1997). Although not considered an exocrine gland, postpharyngeal gland contents can be regurgitated through the mouth during trophallaxis (Soroker et al., 1994). Hydrocarbons are the major class of compound found in the postpharyngeal gland, and these compounds are easily analyzed by GC (Lok et al., 1975; Vander Meer et al, 1985). Chemical analysis for hydrocarbons specific to *S. invicta* (Ross et al., 1987) from electrically stimulated fire ant workers failed to show release of postpharyngeal gland products. Release of exocrine gland products from other glandular sources, e.g., maxillary and labial glands, or the hindgut are probable, but not detectable due to our lack of knowledge regarding the behaviors and chemistry associated with these potential semiochemical sources. Interestingly, by the end of the electrical stimulation period, workers that had not been directly affected by the electrical current were inactively aggregated away from the electrified wire. This behavior may have been released by an unknown pheromone or was the result of exposure to high concentrations of known semiochemicals, e.g., venom alkaloids.

Our previous investigations into the behaviors associated with fire ant mating flights (Obin and Vander Meer, 1994; Alonso and Vander Meer, 1997) showed that the alate excitant pheromone used to initiate mating flights was produced by the mandibular glands. The products were further shown to be extremely short-lived and were released in small quantities and/or were of high volatility. Similarly, an alarm pheromone response was released from the crushed heads of workers (Wilson, 1962) and was reproducible in our laboratory (R.K.V.M., unpublished data). Our work here supports the ephemeral nature of the fire ant alarm pheromone, which, to be effective, must quickly dissipate when the threat is no longer detected (see alarm pheromone review in Vander Meer and Alonso, 1998).

Release of recruitment and alarm pheromones by an electrically stimulated worker has direct implications toward the accumulation of fire ants in electrical equipment. The estimated number of worker Dufour's gland equivalents released

by electrically stimulated fire ant workers was almost 1000 times the amount needed for significant worker attraction in the olfactometer (Vander Meer, 1986; Vander Meer et al., 1988) described here. In addition, one of the behaviors associated with alarm pheromones is attraction toward the worker releasing the alarm pheromone (Vander Meer and Alonso, 1998). Taken together, it is reasonable to assume that other fire ant workers in the vicinity of the semiochemical-releasing worker would be drawn toward the live contacts.

There are many species of ants that maintain large populations (see Hölldobler and Wilson, 1990), but none rival the high population densities of *S. invicta* in the United States. After its accidental importation into the United States in the 1930s (Lofgren, 1986), the population densities of the ant are about five times greater than found in their native South America (Porter et al., 1992), and in the United States can be as high as 100–120 mature monogyne colonies per hectare. Each colony maintains up to 250,000 workers or 30 million ants per hectare (3000 worker ants/m^2). Even if one assumes that only 5–10% of the workers are foraging, there are still 150–300 workers/m^2. Thus, it is likely that a foraging worker accidentally bridges live contacts in electrical equipment, releasing pheromones that attract nearby workers to the live contacts. These workers, in turn, release exocrine gland products, which increase pheromone concentration and the probability of attracting additional workers to the live contacts. We believe that the high population densities and the "weedy" nature (Tschinkel, 1987) of *S. invicta* is why this ant presents such a significant problem relative to electrical equipment.

Earlier work investigated the effect of electrical fields on *S. invicta* and several other ant genera (MacKay et al., 1992a,b). The authors concluded that workers from all species tested ($N = 10$) were attracted to electrical fields and that the number of ants accumulated at the electrical contacts increased with increasing voltage (directly related to electrical field strength). Our results for the fire ant present a pheromone-based alternative to their potential attraction to electric fields. All ant species are social and have evolved pheromone systems that help to maintain the health and social structure of the colony (see Vander Meer and Alonso, 1998; Hölldobler and Wilson, 1990). Therefore, there is a high probability that other ant species will also release exocrine gland products in response to electrical stimulation, which would then explain their apparent attraction to electrical fields (MacKay et al., 1992b). The differences in our conclusions may be based in semantics, since MacKay et al. (1992a) state in their discussion that while the ants accumulated at the electrically activated disks, "visual observations did not suggest the ants oriented to the powered disks." Orientation of the workers toward the powered disks would constitute "attraction," not simply that there were a number of ants at the disk after 10 min (see Vander Meer and Morel, 1988; Vander Meer and Alonso, 1998). Thus, our results support the report by Slowik et al. (1996) that electrically stimulated fire ants are not attracted to electrical fields, but may release pheromones that result in aggregations of ants at the site of electrical stimulation.

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