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GASTER FLAGGING BY FIRE ANTS (*Solenopsis* spp.): FUNCTIONAL SIGNIFICANCE OF VENOM DISPERSAL BEHAVIOR

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Abstract—Behavioral and chemical studies with laboratory colonies indicate that the imported fire ant *Solenopsis invicta* Buren (Myrmicinae) disperses venom through the air by raising and vibrating its gaster (i.e., "gaster flagging"). This mechanism of airborne venom dispersal is unreported for any ant species. Foraging workers utilize this air-dispersed venom (up to 500 ng) to repel heterospecifics encountered in the foraging arena, while brood tenders dispense smaller quantities (~1 ng) to the brood surface, presumably as an antibiotic. Brood tenders removed from the brood cell and tested in heteropspecific encounters in the foraging arena exhibited the complete repertoire of agonistic gaster flagging behavior. These observations suggest that airborne venom dispersal by workers is context specific rather than temporal caste specific and that workers can control the quantity of venom released.

Key Words—Ants, *Solenopsis invicta*, Hymenoptera, Formicidae, gaster flagging, alkaloids, defensive behavior, venom, antibiotic, caste.

INTRODUCTION

Functional parsimony is a recurring theme in the evolution of ant venoms, sting morphology, and related behaviors. Whereas social bees and wasps employ their stings almost exclusively for defense (but see Maschwitz, 1964; Post and Jeanne, 1983), ants use their stings for defense, prey capture, and pheromone dispersal (Wilson, 1971; Bradshaw and Howse, 1984). Here, we present a remarkable venom dispersal mechanism and behavioral correlates of venom alkaloid activity in the fire ant *Solenopsis invicta* Buren (Myrmicinae).

S. invicta is a South American ant that has attained introduced pest status in the southearstern United States (Lofgren et al., 1975). It is best known for its aggressive behavior and its painful sting. *S. invicta* venom is primarily (>95%) composed of piperidine alkaloids (2-alkyl or alkenyl 6-methyl piperidines). The in vitro insecticidal and antibiotic properties of the piperidine alkaloids have been established in laboratory assays (Blum et al., 1958; Jouvenaz et al., 1972). We have investigated the adaptive significance of these venom characteristics, particularly with respect to "gaster flagging" behavior (Adams and Traniello, 1981) in which workers raise and vibrate the gaster, frequently while extruding the sting. This behavior was noted during laboratory and field observations of *S. invicta* colonies by us and others (Bhatkar et al., 1972). We hypothesized that during gaster flagging, *S. invicta* workers dispersed venom through the air.

We chose to address two contexts during which gaster flagging is observed. *S. invicta* workers undergo age polyethism whereby young workers tend brood, while older workers do most of the foraging (Mirenda and Vinson, 1981; cf. Wilson, 1978). Gaster flagging was observed among workers tending brood as well as among foraging workers confronting heterospecifics in the foraging arena. It occurred to us that venom directed at heterospecifics encountered in the foraging arena could function as repellent, while venom directed to the brood or the surrounding brood chamber could reduce the likelihood of microbial infection.

In the following studies, we (1) more fully describe gaster flagging by *S. invicta*, (2) show that venom is dispersed through the air by gaster flagging during heterospecific confrontations, and (3) demonstrate that worker-derived venom is present on the surface of the brood.

METHODS AND MATERIALS

Analysis of Gaster Flagging Behavior. Ants tested were members of single queen colonies collected four to six months previously and maintained at the ARS, USDA Insects Affecting Man and Animals Research Laboratory, Gainesville, Florida. Colonies contained more than 10,000 individuals and included immatures at all developmental stages. Colonies were maintained in plastic Petri dish cells (diameter = 14.0 cm) with Castone® floors at 26–27°C on a diet of honey-water, fly pupae, and hard-boiled egg. These brood chambers were placed in plastic trays (52.0 × 39.0 × 7.5 cm) that served as foraging arenas (Banks et al., 1981). The sides of the tray were Fluon® coated.

The behavior of brood tenders in the brood chamber was observed in three different *S. invicta* colonies under an adjustable three diopter illuminated magnifier during a total of 4.3 h. Confrontation behavior was observed following introduction of individual *S. invicta* foragers or brood tenders into (1) the foraging arena of heterospecific (*S. geminata*) colonies (N = 11 trials) or (2) Petri

dish arenas (diameter = 5.4 cm) housing 8–12 *S. geminata* workers (N = 13 trials). Data were collected for 10 min post-introduction. Interspecific interactions in the Petri dish arenas were observed under a dissecting microscope fitted with an ocular micrometer. These interactions were also filmed with a Fujica ZC1000 Single-8 camera fitted with a Tamron Tele-Macro lens (1 : 1).

Airborne Venom Dispersal by Foragers. Two experiments were performed. In the first, encounters in Petri dish arenas were staged between groups of 8–12 *S. invicta* and *S. geminata* workers. When alarmed individuals began gaster flagging, a TLC plate impregnated with iodoplatinate reagent (Touchstone and Dobbins, 1978) was held 1–2 cm from the tip of the sting while the ant was prodded from the rear and side with forceps. (Iodoplatinate turns from red to either white or blue in the presence of alkaloids.) An impregnated plate was discarded whenever it was potentially contaminated due to contact with an ant, the arena surface, or forceps that had contacted either. The size and pattern of venom droplets on the TLC plate were analyzed under the dissecting microscope. Tests with alkaloid standards derived from extirpated *S. invicta* poison sacs indicated that as little as 5 ng/spot (minimum spot diameter = 0.02 mm) could be visualized by this method.

In the second experiment, lone *S. invicta* foragers were introduced into Petri dishes housing 10–12 *S. geminata* workers. During confrontations, the *S. invicta* intruder would invariably gaster flag at resident *S. geminata*. *S. geminata* workers making their initial approach to the introduced *S. invicta* worker were occasionally repelled without contacting the intruder's sting. Eight of these *S. geminata* were immediately collected and swirled in hexane (250 μl) for 30 sec. An internal standard (eicosane, Applied Science) was added and the sample analyzed for species-specific *S. invicta* piperidine alkaloids (Brand and Blum, 1973) by gas–liquid chromatography: DB-1 fused silica capillary column (J. and W. Scientific, Inc.), 15 m × 0.33 mm, splitless; Varian 3700, FID, temperature program = 150–200 at 2°C/min; DB-225 fused silica capillary column (J. and W. Scientific, Inc.), 30 m × 0.26 mm, splitless; Varian 3700, FID, 200°C isothermal.

Worker-Derived Venom on the Brood. A brood cell containing workers and brood was frozen and then the brood separated from brood tenders in an airstream (Stringer et al., 1972). The brood sample (N = 8000 immatures) was examined to ensure that workers were absent and then rinsed for 1 min in 2 : 1 chloroform–methanol (5 ml). This rinse was divided into two equal subsamples, each of which was reduced to 25 μl under a stream of nitrogen and then brought to 500 μl with hexane. Venom alkaloids were isolated and purified by (1) washing three times with 0.5 N sulfuric acid (200 μl) (2) separation of the aqueous phase followed by basification wth 1.5 N potassium hydroxide (250 μl), and (3) extraction of the alkaloids from the basified aqueous phase three times with hexane (200 μl). Internal standard was added to the hexane extract prior to GLC analysis (as above).

In order to assess potential contamination from endogenous larval and pupal alkaloids, we quantified the alkaloid contents of five extirpated pupal poison glands, and then determined the percent alkaloids extractable from whole workers by our rinse method. In addition, we quantified potential contamination from worker venom extruded in the airstream sorting device. We lined the apparatus used to separate brood from brood tenders with filter paper and conducted another brood/worker separation. The filter paper was then cut into strips, extracted with hexane, and vacuum-filtered. The filtrate was concentrated under nitrogen and analyzed by GLC for the presence of venom alkaloids.

RESULTS

Gaster Flagging Behavior. We recognized three distinct types of gaster flagging in *S. invicta*, hereafter referred to as the "headstand," "defensive flag," and "brood flag." The former two were exhibited only by workers during interspecific encounters.

Both brood tenders and foragers exhibited the headstand and/or defensive flag during the arena encounters. While performing the headstand (Figure 1), workers straighten the rear legs, elevate the petiole approximately 70–90° to the substrate, and extrude the sting. They then vibrate the gaster in the anteroposterior plane. A drop of venom up to 0.2 mm in diameter appears at the tip of the sting within several seconds. The "headstand" appears to be a nonoriented, general response to initial contact with a heterospecific or in response to grappling or other agonistic interaction between nestmate(s) and heterospecifics occurring within 2.0–3.0 cm. Individual *Solenopsis* of both species often gaster flagged in the headstand position near a grappling pair.

Defensive flagging is distinguished from the headstand by four criteria: (1) During defensive flagging, the gaster is clearly oriented toward specific intruders (Figure 2). (2) The gaster is elevated no more than 45°. (3) Although the sting is extruded, no distinct venom droplet is observed. (However, when defensive flagging follows or is interrupted by headstanding, venom may be present at the sting tip.) (4) Gaster vibration during defensive flagging is much more vigorous (i.e., the amplitude of gaster deflection is greater), and lateral as well as anteroposterior gaster deflection can be observed.

During our observations of brood chambers, we noted 161 incidents in which brood tenders raised and either vibrated or repeatedly shook their gasters while contacting or standing within 1.0 cm of brood. When they gaster flagged in the brood chamber, brood tenders elevated the gaster no more than 45°. However, the intensity of gaster vibration was highly variable. In nine instances, ants extruded their stings, but we observed no venom droplets.

Airborne Venom Dispersal by Foragers. Ants responded to prodding by either continuing to headstand or by flagging extruded venom at the TLC plate.

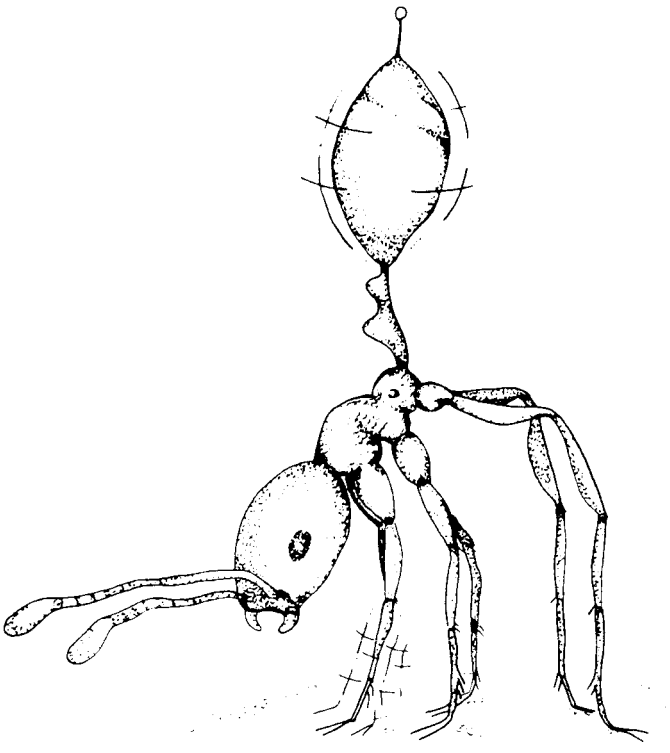

FIG. 1 The headstand or initial, nondirectional gaster flagging posture assumed during heterospecific encounters. Stereotyped elements include 90° gaster elevation, vertical gaster vibration, sting extrusion, venom droplet secretion, lowered head, open mandibles, and foretarsal tapping.

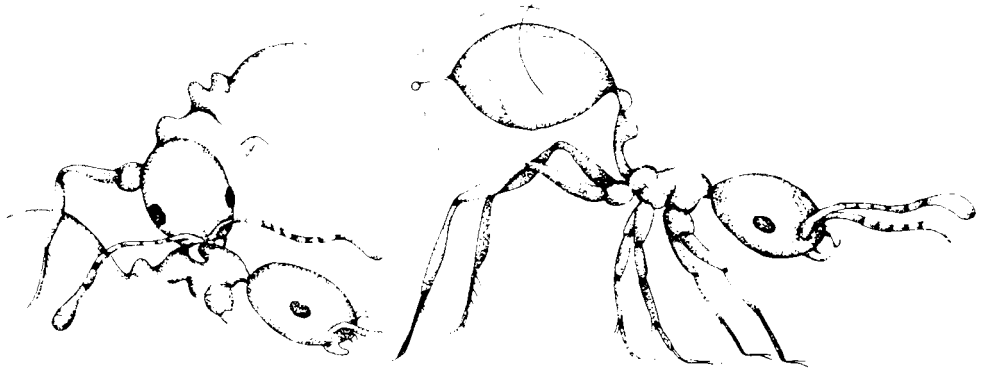

FIG. 2. The defensive flag, here directed at an immobilized heterospecific. This behavior is further distinguishable from the headstand in that the gaster is elevated much less than 90°, the venom droplet may or may not be present, and the gaster is vigorously shaken in both vertical and horizontal planes.

Plates at whch ants did not direct "defensive flagging" ($N = 6$) contained no visible evidence of venom alkaloids. Six of eight plates at which ants had directed defensive flagging did, however, contain venom alkaloids. Analysis of these plates revealed 4–11 venom droplets/mm^2 in the exposed areas. The droplets were assigned to two discrete size classes: (1) diameter = 0.08–0.13 mm, and (2) diameter = 0.02–0.03 mm. The larger droplets constituted from 9.9% (1 of 11) to 100% (4 of 4) of all venom droplets noted in a sampling area. No ordered pattern of droplets was apparent.

In our second experiment, *S. geminata* workers were occasionally repelled by gaster flagging *S. invicta* over a distance of up to 1.5 cm. Behaviors of repelled ants included rapid withdrawal, antenna dragging, and grooming. In addition, species-specific *S. invicta* venom alkaloids were recovered from the cuticle of *S. geminata* workers visibly repelled by gaster flagging (Figure 3A–C). Total venom present in the pooled sample of eight ants was between 3.5 and 4.0 μg.

Worker Derived Venom on the Brood. The two brood rinse subsamples yielded 1.13 ng alkaloids/immature and 0.92 ng alkaloids/immature (Figure 4). When *S. invicta* adults were subjected to the same rinse technique, they yielded less than 0.002% of the total available alkaloids from the surface and poison sacs. We assumed that all 4000 immatures per subsample contained the amount of alkaloids measured in the extirpated pupal posion sac (= 257 ng/pupa) and that our technique extracted 0.002% of these alkaloids (approximately 5 pg/immature). This figure represents 0.48% and 0.55% of the total alkaloid content in our two rinse samples. Venom alkaloids quantified in two samples of filter paper lining the ant sorting device were present at 13.0 and 16.3 pg/immature. Thus, between 1.25 and 1.48% of the venom in our brood rinses was potentially a result of contamination from worker venom extruded during the sorting process. Total venom on the brood surface excluding the maximum possible contamination from poison glands of workers and immatures was calculated to be 1.04 and 0.80 ng/immature, respectively.

DISCUSSION

It has been stated (Schmidt, 1978) that plasticity of venom composition and venom dispersal mechanisms in ants has been an important factor in ant domination of terrestrial habitats. Other species of myrmicine ants secrete venom droplet(s) to the sting tip. Volatile components of extruded venom are known to function in worker recruitment (i.e., "tandem calling") (Möglich et al., 1974; Möglich, 1979) and sexual calling (Buschinger, 1968, 1971). In addition, other species wipe venom droplets on antagonists as part of either raiding (Hölldobler, 1973; Blum et al., 1980) or interference competition strategies (Adams and

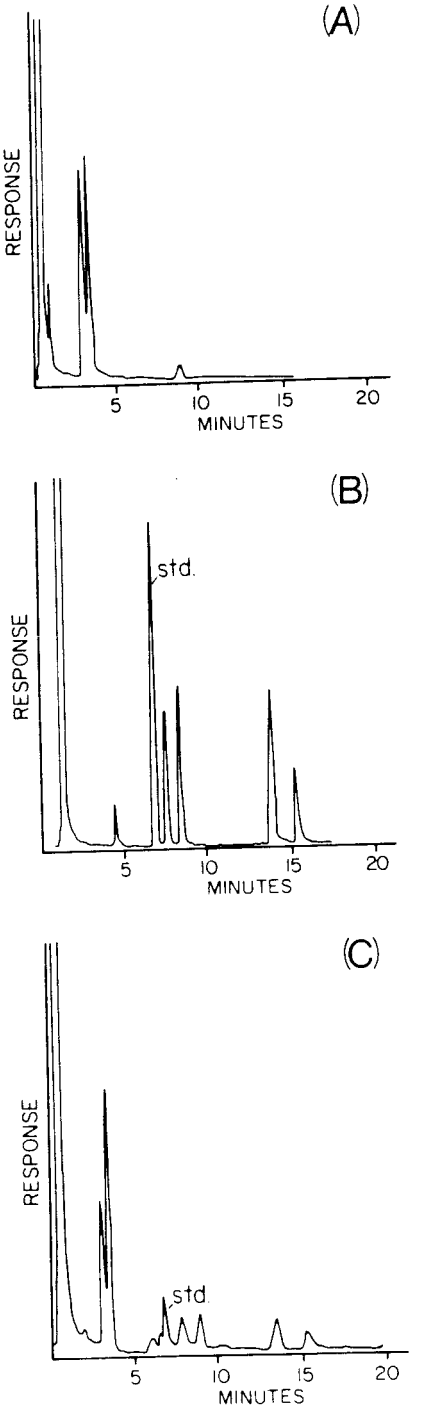

FIG. 3. Gas chromatogram demonstrating the presence of air-dispersed *S. invicta* venom alkaloids on *S. geminata* cuticle. (A) *S. geminata* cuticle rinse. (B) *S. invicta* venom alkaloids from extirpated worker poison sacs. (C) *S. geminata* cuticle rinse following arena encounter with gaster flagging *S. invicta*. Std = internal standard.

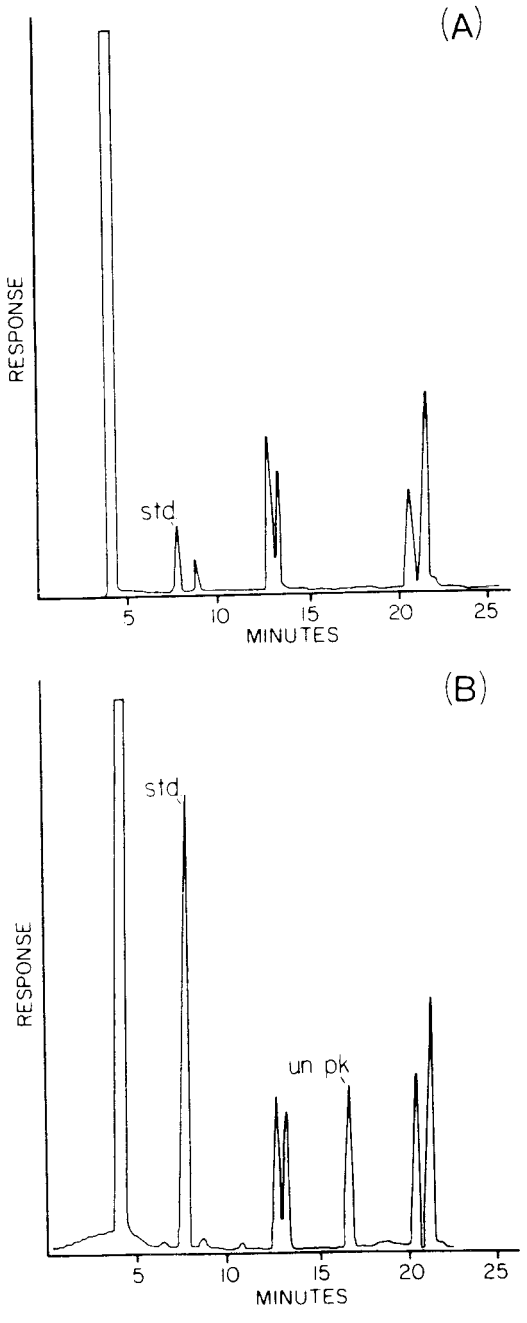

FIG. 4. Gas chromatogram demonstrating the presence of worker-derived venom alkaloids on the surface of *S. invicta* brood. (A) *S. invicta* venom alkaloids from extirpated worker poison sacs. (B) *S. invicta* brood rinse. Std = internal standard, un. pk. = unidentified peak.

Traniello, 1981; Hölldobler, 1982). Data presented here offer what is to our knowledge the first evidence of airborne dispersal of venom droplets by the sting apparatus of an ant, although we suggest that it may be a general phenomenon in this subfamily. However, a "spray sting type" of mechanism (Maschwitz and Kloft, 1971) has been previously observed in several species of vespid wasps (reviewed in Hermann and Blum, 1981).

The biomechanics of airborne venom dispersal by gaster flagging may have a precedent in man-made physical analogs. Rayleigh (1879) first proposed that directed streams of small droplets could be generated by a mechanically vibrated jet of liquid. More recently, Mason et al. (1963) produced controllable, reproducible streams of 30- to 500-μm droplets with a hypodermic needle (bore diameter = 100 μm) vibrated at resonant frequencies with an amplitude of several millimeters. We suggest that *S. invicta* (and congenerics) utilize a similar principle to produce a venom aerosol.

Venom dispersed by gaster-flagging *S. invicta* visibly repels heterospecifics and presumably functions as an antiseptic in the brood chamber. Our analysis of airborne venom dispersal by foraging workers suggests that during the "headstand" behavior workers pump large quantities of venom from the poison reservoir into the sting. Although not detected in this study, volatile components with potential pheromonal function may be released from the secreted droplet. Data indicate that as much as 500 ng of venom may be dispersed when directed at heterospecifics during defensive flagging. This figure represents 2.5% of the total alkaloid content of a typical *S. invicta* worker (Vander Meer, unpublished data).

In response to alarm, invasion, or other disturbance, brood tending *Solenopsis* workers typically pick up brood and flee the brood chamber. However, brood tenders removed from the brood chamber and introduced into arenas containing heterospecifics exhibited the full repertoire of confrontation gaster flagging behavior observed in foragers (i.e., the headstand and defensive flag). Although the product of artificial laboratory manipulation, this observation suggests that mechanisms of chemical defense in *Solenopsis* are not specific to a particular *S. invicta* temporal caste but, rather, are context specific.

With respect to venom on the brood surface, our data do not exclude the possibility that venom is transferred to the brood as a consequence of worker/brood contact (e.g., by grooming). However, if gaster flagging is the method of venom application, the small quantity of venom present ($\sim$1 ng, presumably evenly distributed on the brood surface) argues in favor of an aerosol with droplet diameters much less than 20 μm. Recall that our method of visualizing *Solenopsis* venom alkaloids permitted detection of no less than 5.0 ng venom/spot—a spot 20–30 μm in diameter. Based on physical models (Mason et al., 1963), it can be hypothesized that venom flow rate is the essential determinant of droplet diameter, although sting orifice diameter, vibrational frequency, and

amplitude may also play a role. Future efforts should include quantification and comparison of these features of gaster flagging mechanics. In this manner, a more complete understanding of how *S. invicta* workers regulate the amount of potentially lethal alkaloids that they apply to the brood surface may be achieved.

Selection pressures contributing to the evolution and maintenance of insecticidal repellents and antibiotic secretions in ants include (1) predation and/or competition due to other arthropods, especially ants and (2) conditions favoring bacterial and fungal growth. Although such pressures may vary in intensity, they should be ubiquitous among most soil-inhabiting ants species. Other myrmicine ants rely on repellents produced in the poison gland (Hermann and Blum, 1981), and antibacterial secretions derived from the metapleural gland (Maschwitz et al., 1970; Wilson 1971). Our data and those of Blum et al. (1958) indicate that the production and disperal of antibiotic exocrine materials in *Solenopsis* spp. is a function of the poison gland and sting apparatus. Furthermore, our laboratory (Alvarez, personal communication) has documented the absence of antibiotic activity in the metapleural gland of *S. invicta*. These findings argue in favor of an alternative role for the metapleural gland in fire ant ecology. Evidence in support of this hypothesis was recently presented by Jaffe and Puche (1984), who reported the production of colony-specific territorial pheromones in the metapleural glands of *S. geminata*. Antibiotic metapleural gland secretions of other Myrmicines tested by Maschwitz et al. (1970) did not function in either territoriality or nestmate recognition.

Lastly, we suggest that the internal sequestration and facultative dispersal of antimicrobial venom alkaloids by *S. invicta* may have implications for current theories of ant–plant interactions. Based on metapleural gland secretions of four geographically and ecologically different ant species (Schildknecht and Koob 1970, 1971), Beattie et al. (1984) suggested that antimicrobial secretions of ants interfere with pollen function (Iwanami and Iwadare 1978) and plant seed set. Since metapleural gland secretions are distributed over the entire surface of the ant body (Maschwitz et al. 1970), Beattie et al. (1984) hypothesized that antibiotic ant secretions have constrained the evolution of ant pollination. Quantification of venom alkaloids present on the body surface of *S. invicta* foragers in conjunction with knowledge of the effect of the venom alkaloids on pollen may provide one test of this hypothesis.

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REFERENCES

ADAMS, C.S., and TRANIELLO, J.F.A. 1981. Chemical interference competition by *Monomorium minimum* (Hymenoptera: Formicidae). *Oecologia* 51:265–270.

BANKS, W.A., LOFGREN, C.S., JOUVENAZ, D.P., STRINGER, C.E., BISHIP, P.M., WILLIAMS, D.F., WOJCIK, D.P., and GLANCEY, B.M. 1981. Techniques for collecting, rearing, and handling imported fire ants. U.S. Dep. Agric. Sci. Ed. Admin., AAT-S-21:1-9.

BEATTIE, A.J., TURNBULL, L., KNOX, R.B., and WILLIAMS, E.G. 1984. Ant inhibition of pollen function: A possible reason why ant pollination is rare. *Am. J. Bot.* 71:421-426.

BHATKAR, A., WHITCOMB, W.H., BUREN, W.F., CALLAHAN, P., and CARLYSLE, T. 1972. Confrontation behavior between *Lasius neoniger* (Hymenoptera: Formicidae) and the imported fire ant. *Environ. Entomol.* 1:275-279.

BLUM, M.S., JONES, T.H., HOLLDOBLER, B., FALES, H.M., and JAOUNI, T. 1980. Alkaloidal venom mace: offensive use by a thief ant. *Naturwissenschaften* 67:144-145.

BLUM, M.S., WALKER, J.R., CALLAHAN, P.S., and NOVAK, A.F. 1958. Chemical, insecticidal and antibiotic properties of fire ant venom. *Science* 128:306-307.

BRADSHAW, J.W.S., and HOWSE, P.E. 1984. Semiochemicals of ants, pp. 429-473, *in* W.J. Bell and R.T. Cardé (eds.). Chemical Ecology of Insects. Sinauer Assoc., Sunderland, MA.

BRAND, J.M., and BLUM, M.S. 1973. Biochemical evolution in fire ant venoms. *Insect Biochem.* 3:45-51.

BUSCHINGER, A. 1968. "Locksterzeln" begattungsbereiter ergatoider Weibchen von *Harpagoxenus sublaevis* NYL. (Hymenoptera, Formicidae). *Experientia* 24:297.

BUSCHINGER, A. 1971. "Locksterzeln" und Kopula der sozial parasitischen Ameise *Leptothorax* kutteri Buschinger (Hym., Form.). *Zool. Anz.* 186:242-248.

HERMANN, H.R., and BLUM, M.S. 1981. Defensive mechanisms in the social Hymensptera, pp. 77-197, *in* H.R. Hermann (eds.). *Social Insects, Vol II*. Academic Press, New York.

HOLLDOBLER, B. 1973. Chemische Strategie beim Nahrumgswerwerb der Diebameise (*Solenopsis fugax* Latr.) und der Pharaoameise (*Monomorium pharaonis* L.). *Oecologia* 11:371-380.

HOLLDOBLER, B. 1982. Interference strategy of *Iridomyrmex pruinosum* (Hymenoptera: Formicidae) during foraging. *Oecologia* 52:208-213.

IWANAMI, Y., and IWADARE, T. 1978. Inhibiting effects of myrmicacin on pollen growth and pollen tube mitosis. *Bot. Gaz.* 139:42-45.

JAFFE, K., and PUCHE, H. 1984. Colony-specific territorial marking with the metapleural gland secretion in the ant *Solenopsis geminata* (Fabr). *J. Insect Physiol.* 30:265-270.

JOUVENAZ, D.P., BLUM, M.S., and MACCONNELL, J.G. 1972. Antibacterial activity of venom alkaloids from the imported fire ant, *Solenopsis invicta* Buren. *Antimicrob. Agents Chemother.* 2:291-293.

LOFGREN, C.S. BANKS, W.A., and GLANCEY, B.M. 1975. Biology and control of imported fire ants. *Annu. Rev. Entomol.* 20:1-30.

MASCHWITZ, U. 1964. Gefahrenalarmstoffe und Gefahrenalarmierung bei Sozialen Hymenopteron. *Z. Vergl. Physiol.* 47:596-655.

MASCHWITZ, U., and KLOFT, W. 1971. Morphology and function of the venom apparatus of insects, bees, wasps, ants and caterpillars, pp. 31-60, *in* W. Bücherl and E.E. Buckley (eds.). Venomous Animals and Their Venoms. Academic Press, New York.

MASCHWITZ, U., and KLOFT, W. 1971. Morphology and function of the venom apparatus of insects, bees, wasps, ants and caterpillars, pp. 31-60, *in* W. Bücherl and E.E. Buckley (eds.). Venomous Animals and Their Venoms. Academic Press, New York.

MASCHWITZ, U., KOOB, K. and SCHILDKNECHT, H. 1970. Ein beitrag zur fumktion der metathoracaldrüse der ameisen. *J. Insect Physiol.* 16:387-404.

MASON, B.J., JAYARATRI, O.W., and WOODS, J.D. 1963. An improved vibrating capillary device for producing uniform water droplets of 15 to 500 μm radius. *J. Sci. Instrum.* 40:247-249.

MIRENDA, J.T., and VINSON, S.B. 1981. Division of labour and specification of castes in the red imported fire ant *Solenopsis invicta* Buren. *Anim. Behav.* 29:410-420.

MÖGLICH, M. 1979. Tandem calling pheromone in the genus *Leptothorax* (Hymenoptera: Formicidae): Behavioral analysis of specificity. *J. Chem. Ecol.* 5:35-52.