FIRE ANT, *SOLENOPSIS INVICTA*, WORKER ALARM PHEROMONES ATTRACT *PSEUDACTEON* PHORID FLIES

Robert K. Vander Meer and Sanford D. Porter

USDA- ARS, Center for Medical, Agricultural, & Veterinary Entomology, 1600 SW 23rd Drive, Gainesville, FL 32608

Abstract. The parasitic phorid fly, *Pseudacteon tricuspis* was found to be attracted to the volatile compounds released by shaken fire ant workers. Among the compounds released were alarm pheromones, venom alkaloids, and recruitment pheromones. In subsequent bioassays we eliminated poison sac contents and recruitment pheromones as possible attractants. We have demonstrated the involvement of alarm pheromones in the attraction of this phorid fly to their host fire ant.

Introduction. The imported fire ants, *Solenopsis invicta* and *S. richteri*, currently inhabit over 125 million hectares in Puerto Rico and twelve southern states from Texas to Virginia Lofgren 1988). *Solenopsis invicta* has also become established in limited areas in California, Arizona, and New Mexico. Annual damage, treatment, and medical costs are reported to exceed a billion dollars. Fire ant populations in the U.S. are 5-7 times higher than in their native range of Brazil and Argentina, likely the result of escape from natural enemies left behind in South America (Porter et al. 1992).

At least 18 species of *Pseudacteon* flies have been found attacking fire ants in South America (Porter and Pesquero 2001). The flies are highly specific in their host preferences and they have been shown to stop fire ant foraging and may shift the local competitive balance to other ant species (Porter et al. 1995).

Maggots of these miniature flies develop in the heads of fire ant workers, decapitating their host upon pupation. The maggot then pushes the mouthparts aside and pupates within the empty head capsule using it as a pupal case. The worker ants respond to this "dead ant" by taking it out of the colony onto a refuse pile. Here the adult fly ecloses and starts the cycle all over again (Porter 1998).

The flies are able to locate their fire ant hosts. Fire ant pheromones (kairomones in the context of parasite host finding) are probably involved in phorid fly flight activation, attraction, and initiation of attack. Elucidation of the fly/host semiochemical interactions could lead to better utilization of this suite of fire ant parasites.

Previous investigations demonstrated that electrically stimulated fire ant workers release all detectable semiochemicals from their exocrine glands, including recruitment pheromone, alarm pheromone, and defensive chemicals from the poison gland (Vander Meer et al. In Press). We also determined that *P. tricuspis* phorid flies were attracted to the semiochemicals released by electrically stimulated fire ants. The objective of this work was to determine the source of the fire ant semiochemicals that attract phorid fly parasites.

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Materials and Methods. We previously developed an alarm bioassay to investigate mating flight excitants (Alonso and Vander Meer 1996). In these bioassays worker ants and brood were placed in a small "Fluoned" tray where they quickly settled into a quiet clump. The air (headspace) above control and treatment samples was drawn (3 ml) into a syringe, and then 1ml of the headspace sample was carefully expressed over the quiet worker ants. Their reaction was scored on a scale of 1-4, with 1 & 2 measures of antennation (non-alarm) and 3 & 4 measures of rapid worker movement (alarm).

Automated phorid fly rearing chambers contained two rows of seven fire ant colony trays, each with workers and brood. Centrally located trays were selected as Treatments (2) and Controls (2). Prior to the experiment, pre-counts were made of resting phorid flies and those attacking in the treatment and control trays. Two, five, and 10 minutes after introduction of the Treatment and Control, the flies at rest and those attacking were again counted. Only 5 min attacking fly results are presented.

The quantity of venom alkaloids released by shaken and unshaken ants in the bioassay vial was determined by standard gas chromatographic techniques using an appropriate internal standard. Poison sacs were extirpated and crushed in hexane. The extract was diluted with hexane to allow easy application of two poison sac equivalents. This is over 100 times the amount of venom deposited by shaken ants in the alarm bioassay. The sample was applied to a small petrie dish and the solvent evaporated. Simultaneously, a control dish was treated with hexane and evaporated. The control and treatment dishes were immediately placed in the rearing boxes to determine their affect on the number of attacking phorid flies.

The quantity of recruitment pheromone released by shaken and unshaken ants in the bioassay vial was determined in terms of Dufour's gland equivalents (DE) by an orientation bioassay. Dufour's glands were extirpated and extracted in hexane to provide doses of 12.5 DE. This solution was used in the phorid fly attack boxes to evaluate their ability to attract phorid flies (see attack box description above).

Results and Discussion. Behavioral observations of the ants and flies suggested that the fire ant alarm pheromone may be involved in the attraction of the fly parasites to their host. Previous studies demonstrated that shaken ants release alarm pheromones that can be measured in an alarm bioassay. Shaking ants in a vial and immediately placing the vial in phorid fly-rearing boxes resulted in an increased number of attacking flies (Figure 1). While at first this appears to support the involvement of an alarm pheromone, it is possible that the shaken ants are in fact releasing additional compounds.

We analyzed vial solvent rinses for venom alkaloids and found that shaken fire ant workers release on average over 200ng of total alkaloids, which is a small amount compared to what is in a poison sac (ca. 10μg). The alkaloids represent the amount of poison sac contents that were released by the ants. Non-alkaloid compounds are in the poison sac and may elicit a behavioral response from the phorid flies. This was tested in phorid fly rearing boxes where poison sac extracts (two worker equivalents, 20 ug) were presented in a manner similar to the shaken ants.

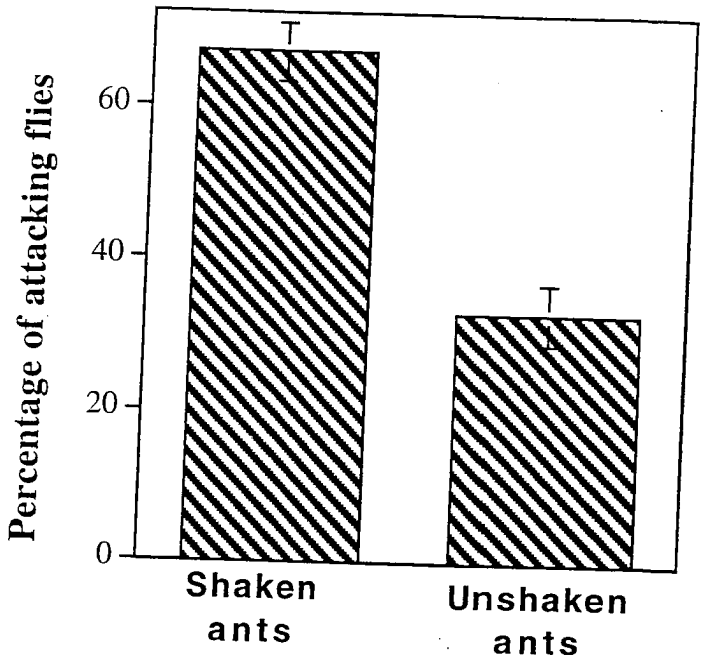

Figure 1. The effect of the volatiles from shaken ants on the phorid fly rate of attack. Many flies are resting while others are attacking. The effect of the worker volatiles was to increase the percentage attacking flies and therefore decrease the percentage of resting flies.

The results demonstrated that even at this high concentration the poison sac contents did not elicit phorid fly attraction.

Recruitment orientation bioassays demonstrated that in one of three replicates extracts of vials, in which ants had been shaken, gave a positive bioassay result. The concentration was at the lowest detectable concentration and activity was lost with the next 1:2 dilution. Phorid fly attraction bioassays with much higher concentrations of Dufour's gland extracts (12.5 DE) demonstrated that the phorid flies were not attracted to the very small amounts of Dufour's gland products released by shaken ants (Vander Meer 1986).

All of the above indicate that of the compounds released by shaken ants, it is the alarm pheromone that is responsible for phorid fly attraction. The function of alarm pheromones dictate that they be highly volatile. Further evidence for their involvement in phorid fly attraction comes from the fact that if the shaken ant vial is left open for four minutes all activity is lost. Future research will focus on the isolation and identification of the compounds responsible for phorid fly attraction to their fire ant host.

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