The *Solenopsis invicta* Alarm Pheromone

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As with most social insects, the red imported fire ant, *Solenopsis invicta*, utilizes a complex milieu of chemical signals to regulate the activities of the colony. Several of these pheromones, including the trail pheromone and queen recognition pheromones, have been identified. However, the identification of the alarm pheromone has proven to be more elusive. Generally, alarm pheromones are associated with the mandibular gland, but they have also been identified in the Dufour's and anal glands. Behavioral studies point to the mandibular gland as the source *S. invicta's* alarm pheromone. Several functions of the alarm pheromone have been suggested for *S. invicta*, from signaling the presence of a threat, to inducing worker activity during mating flights, as well as attracting eavesdropping parasitoids. Wilson demonstrated that frenzied behavior was elicited by exposing *S. invicta* workers to volatiles released from crushed heads of conspecifics. Similarly, workers responded with frantic, excited movements when exposed to live alates, crushed heads, and mandibular gland solutions. The morphology of the gland and the ephemeral nature of the components have complicated the identification of this pheromone. In *S. invicta*, the mandibular gland consists of only a few cells, making it difficult to successfully isolate without the loss of materials. The chemistry of ant alarm pheromones is incredibly diverse with identified alarm

pheromones spanning many structural families, including terpenoids, alcohols, aldehydes, ketones, esters, and nitrogen heterocycles. The compounds are highly volatile, a necessity of quick but transient information transfer. We employed two analytical techniques, solid phase microextraction (SPME) and purge and trap, to collect and analyze headspace contents about workers exhibiting alarmed behavior. These techniques also eliminate the need for solvent, which complicates analysis of highly volatile compounds using the gas chromatograph-mass spectrometer (GC-MS). Using these techniques, we identified a substituted pyrazine as a component of the fire ant alarm pheromone. We tested this chemical in a behavioral bioassay with *S. invicta* workers and we found no significant difference between the alarm response to the headspace volatiles collected above shaken ants and the pyrazine at a concentration of 100 ng/μL, supplying a headspace concentration of 0.50 pg/μL. Additionally, compared to the response to headspace volatiles collected above unshaken ants, we found a significant alarm response to concentrations as low as 1 ng/μL, with a corresponding headspace concentration below detection limits (less than 0.03 pg/μL). We are continuing to define the alarm pheromone source and chemistry.