AN ALARM PHEROMONE FROM HEADS OF WORKER *VESPULA SQUAMOSA* (HYMENOPTERA: VESPIDAE).

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Several species of yellowjacket wasps (*Vespula* and *Dolichovespula* spp.) possess alarm pheromones associated with the venom that elicit recruitment, attraction, and stinging attacks if a suitable visual target is provided. Maschwitz (1964a & b) demonstrated alarm responses in workers of the common yellowjacket *Vespula vulgaris* L. and in the German wasp *Vespula germanica* (F.) in response to squashed venom sacs and solvent extracts of conspecific venom sacs, identifying the venom as the source of an alarm stimulus. His findings were confirmed by Aldiss (1983). Alarm responses to extracts of the venom sac and associated glands have also been observed for *Dolichovespula saxonica* (F.) (Maschwitz 1984), the southern yellowjacket *Vespula squamosa* (Drury) (Landolt and Heath 1987) and the eastern yellowjacket *Vespula maculifrons* (Buysson) (Landolt et al. 1995). The chemical N-3-methylbutylacetamide was identi-

fied as an alarm pheromone from the venom of the southern and eastern yellowjackets (Heath and Landolt 1988, Landolt et al. 1995).

There is some evidence that an additional alarm pheromone may be produced by yellowjackets, in glands associated with the head. Yellowjacket workers possess up to eight sets of exocrine glands in the head (Landolt and Akre 1979), but little is known of the functions or chemistry of their exocrine secretions (Downing 1991, Jeanne 1993). Aldiss (1983) demonstrated significant numbers of hits to a target by worker *V. vulgaris* in response to crushed heads of conspecific workers applied to cotton dental rolls. However, Maschwitz (1964b, 1984) did not observe an alarm response by workers of *V. vulgaris* or *D. saxonica* to crushed heads of conspecific workers, and Landolt and Heath (1987) found no response by southern yellowjackets to a methylene chloride extract of heads of conspecific workers. These disparities may be due to differences in experimental methods, including assay criteria and methods of preparing and presenting stimuli.

We hypothesize that an alarm pheromone from the head is used by the southern yellowjacket, in addition to the venom alarm pheromone N-3-methylbutylacetamide (Heath and Landolt 1988). In previous experiments with this species (Landolt and Heath 1987), a distinctive odor was evident from wasps vacuumed from nests and from objects and clothing that had been attacked by wasps. This odor is not similar to that of the alarm pheromone N-3-methylbutylacetamide and seems to be most potent in the head of the southern yellowjacket worker. Also, observations of southern yellowjackets attacking small corks presented near their nest entrances indicated that these wasps bite and chew in addition to stinging, when they attack. We report here alarm and attack responses from southern yellowjackets to extracts of conspecific heads, indicating a second alarm pheromone in this species originating from the head.

All studies were conducted in Alachua County and Sarasota County, Florida, with vigorous underground colonies of the southern yellowjacket. Workers were collected from active colonies using a shop vacuum with an attached trap. Wasps collected were stored for several weeks in a freezer at -60°C until extracted. Heads of 200 wasps were severed with a razor blade and placed in 4 ml of methanol in a pestle and were ground into a paste. The supernatant was pipetted into a 5 ml glass vial and was subsequently brought back up to a 4 ml volume with methanol. The treatment protocol was the application of 100 microliters of this solution (5 wasp equivalents) to a 5.5 cm diam filter paper attached to a 15 cm diam black sphere coated with tanglefoot and attached to a 0.5 m long wire. After the evaporation of the solvent (ca 30 sec.) from the filter paper, the sphere was moved to a one m distance from a wasp colony entrance, with the wire implanted into soil. Numbers of attacking wasps captured on a sphere were counted after a period of 2 min. As a control, 100 microliters of methanol was applied to a filter paper on an identical sphere presented in the same manner prior to each treatment replicate. This assay was conducted 10 times, using 4 different colonies of the southern yellowjacket. It was noted that the head extract used for this assay and the treated filter papers did possess the distinctive odor referred to above.

The pooled methanol extract of yellowjacket heads was analyzed by GC-MS for the presence of N-3-methylbutylacetamide using the methods of Heath and Landolt (1988). This analysis was conducted to determine if worker heads may have been contaminated with alarm pheromone from venom during wasp collection.

Significant numbers of southern yellowjackets were attracted and contacted the spheres in response to the head extract ($t = 2.35$, $p < 0.05$, $df = 9$) (Table 1). Numbers of wasps captured on the black 15 cm diam sphere coated with tanglefoot ranged from 0 to 224 when baited with a 5 wasp equivalent dose of methanol extract of conspecific heads, while 0 to 2 were captured on control spheres baited with methanol alone. N-3-methylbutylacetamide was not detected in the methanol extract of heads, indicating

that the alarm pheromone activity of those extracts was due to an additional pheromone. Estimated limit of detection was 0.2 ng per wasp equivalent of head extract.

Our results with the southern yellowjacket provide the second account (see Aldiss 1983) of alarm activity in a vespid originating from heads. Aldiss (1983) elicited alarm responses in the common yellowjacket with crushed conspecific heads applied to cotton dental wicks. Like the southern yellowjacket, the common yellowjacket also possesses an alarm pheromone in the venom. The failure of previous studies with the southern yellowjacket to obtain responses to extracts of heads (Landolt and Heath 1987) may have been due to the solvent used in the extractions. Previous studies were conducted using methylene chloride (Landolt and Heath 1987) whereas we used methanol in this study. Methanol was chosen after determining that the distinctive odor present in southern yellowjackets and evident following their attacks on corks, clothing, and assay spheres, was not readily extractable with hexane or methylene chloride, but was extracted with methanol. However, it is not known if this odor, similar to butterscotch to the human nose, is part of the alarm pheromone from the heads of southern yellowjackets.

Technical assistance was provided by K. M. Davis and B. Dueben. We thank S. L. Clement, J. Hansen, and R. Zack for suggestions to improve the manuscript.

SUMMARY

Workers of the southern yellowjacket, *Vespula squamos* (Drury) responded to methanolic extracts of conspecific worker heads with attraction and attack, evidenced by significant numbers captured on treated black spheres presented near nest entrances. This species appears to have alarm pheromones that originate both in the venom and in glands in the head. The venom pheromone N-3-methylbutylacetamide

TABLE 1. NUMBERS OF WORKER SOUTHERN YELLOWJACKETS ATTACKING A TREATED 15 CM DIAM BLACK SPHERE TREATED WITH METHANOL AS A CONTROL OR WITH A 5-WASP EQUIVALENT DOSE OF A METHANOL EXTRACT OF CONSPECIFIC WORKER HEADS (2 MIN ASSAY). ALACHUA AND SARASOTA COUNTIES, FLORIDA, 1992-1994.

		Numbers of "Hits"		
Rep.	Date	Control	Head Extract	Colony
1	14 Dec. 1992	0	24	A
2	15 Dec. 1992	0	108	A
3	21 Dec. 1992	0	0	B
4.	21 Dec. 1992	0	1	B
5	21 Dec. 1992	0	0	B
6	21 Dec. 1992	0	3	B
7.	21 Dec. 1992	1	66	B
8.	21 Dec. 1992	1	24	B
9.	4 Nov. 1993	0	224	C
10	17 Mar 1994	2	86	D
$\bar{x}$ ± SE		0.4 ± 0.2	53.6 ± 22.6	

was not detected in GC-MS analyses of *Vespula squamosa* head extracts. Behavior of attacking wasps suggests possible alarm pheromone application by the mandibles.

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