

PHEROMONAL MEDIATION OF ALARM IN THE EASTERN
YELLOWJACKET (HYMENOPTERA: VESPIDAE)

P. J. LANDOLT¹, R. R. HEATH¹, H. C. REED², AND K. MANNING²

¹Insect Attractants, Behavior, and Basic Biology Research Laboratory
Agricultural Research Service, U.S.D.A.
P.O. Box 14565, Gainesville, FL 32604

²Department of Biology, Oral Roberts University,
7777 S. Lewis Ave., Tulsa, OK, USA, 74171.

ABSTRACT

Eastern yellowjacket, *Vespula maculifrons* (Buysson), workers exhibited alarm responses to a target sphere treated with an extract of conspecific workers or an extract of the wasp sting apparatus. Workers of *Vespula squamosa* also responded to extracts of the sting apparatus of *Vespula maculifrons*, indicating some cross reactivity of their alarm pheromones. N-3-methylbutylacetamide, a known alarm pheromone of the southern yellowjacket, *Vespula squamosa* (Drury), was found in the extract of the eastern yellowjacket venom sac and also elicited alarm in worker *V. maculifrons*, although only when presented at an unnaturally high dose.

Key Words: Insecta, social, communication, defense, behavior, wasp

RESUMEN

Las obreras de la avispa del este, *Vespula maculifrons* (Buysson), mostraron respuestas de alarma ante un blanco contituido por una esfera tratada con un extracto de obreras conspecificas o con un extracto de su aparato picador. Las obreras de *Vespula squamosa* (Drury) también respondieron a los extractos del aparato picador de *V. maculifrons*, indicando cierta reacción cruzada de sus feromonas de alarma. La N-3-metilbutilacetamida, una conocida feromona de alarma de la avispa del sur, *V. squamosa*, fue encontrada en el extracto del saco del veneno de la avispa del este y además provocó alarma en las obreras de *V. maculifrons*, aunque solamente cuando se presentó a una dosis más alta que la natural.

Several species of social wasps (Vespidae) in different genera are known to use pheromones to communicate alarm at the nest site evoking recruitment, attraction, and/or attack. Alarm responses to venom or extracts of venom have been demonstrated for the yellowjackets *Vespula vulgaris* (L.), *Vespula germanica* (Fab.) (Maschwitz 1964), *Vespula squamosa* (Drury) (Landolt & Heath, 1987) and *Dolichovespula saxonica* (Fab.) (Maschwitz 1984), the hornet *Vespa crabro* L. (Veith et al. 1984), the paper wasps *Polistes canadensis* (L.) (Jeanne, 1982), *Polistes exclamans* Viereck and *Polistes fuscatus* (F.) (Post et al 1984), and the polybiine *Polybia occidentalis* (Olivier) (Jeanne, 1981). Despite these reports, it is not known if pheromonal communication of alarm is widespread within the Vespidae, or if most social wasps rely primarily on substrate vibration to communicate alarm (Akre & MacDonald 1986).

Alarm pheromones have been identified from only two species of Vespidae; both isolated from venom. Veith et al. (1984) isolated and identified methyl-3-butene-2-ol

from venom of *Vespa crabro* and demonstrated that it elicits wing buzzing, defense flights, and departure from the nest in worker *V. crabro*. Heath & Landolt (1988) identified N-3-methylbutylacetamide as an alarm pheromone of the southern yellowjacket, *V. squamosa*, and demonstrated attraction and attack responses in worker *V. squamosa*. This compound was found by Aldiss (1983) in extracts of venom sacs of *V. vulgaris*, but its function in that species has not been experimentally tested.

We report here pheromonal mediation of alarm and attack by workers of another species of yellowjacket, *Vespula maculifrons* (Buysson), as a response to a solvent extract of conspecific worker wasps and an extract of the worker sting apparatus, including the venom sac. We also demonstrated alarm and attack by *V. squamosa* workers in response to extract of *V. maculifrons* sting apparatus, indicating some cross reactivity and overlap in the chemical makeup of alarm pheromones of the two species. Subsequently, we confirmed the presence of N-3-methylbutylacetamide, an alarm pheromone of *V. squamosa* (Landolt & Heath 1987) and venom component of *V. vulgaris* (Aldiss 1983), in the venom sac of *V. maculifrons* workers.

MATERIALS AND METHODS

Colonies of *V. maculifrons* and *V. squamosa* used in this study were located in Tulsa, Oklahoma and Gainesville and Sarasota, Florida. All were terrestrial, or underground, nests. Voucher specimens have been retained in the collection of the first author (P.JL).

Wasps used for extracts were either collected by placing a glass jar over the nest entrance, or were vacuumed from colony entrances, using a device similar to that of Akre et al. (1973). The jar or collector trap with wasps was then placed in an insulated chest with dry ice. Wasps were stored at -60°C until extraction (1-30 d). Whole wasp extracts were obtained by grinding groups of 50 yellowjackets in 10 ml methylene chloride, using a mortar and pestle. Extract was then pipetted into a glass vial for storage in a freezer at -10°C. Sting apparatuses for extracts were removed from wasp abdomens by pulling the sting out with forceps and disconnecting the rectum from the sting chamber. This included the sting, sting chamber, venom and Dufour's glands and the venom sac. Groups of 100 were placed in one ml of methylene chloride for one to 2 h. Solvent extracts were then pipetted into clean glass vials and were stored at -10°C until used in bioassays. Sting apparatus extract aliquots (measured in wasp-equivalents) were applied as 200 µl amounts on 5.5 cm diam filter papers in bioassays.

Chemical Analysis

Extracts of the venom sac were prepared as described above, using six groups of 5 venom sacs placed in vials containing 200 µl solvent. Gas chromatographic analysis was conducted using a Hewlett Packard 5890 GC with a flame ionization detector, a splitless capillary injector, and a 50 m (0.25 mm i.d.; 0.25 µm film of BP-1) apolar fused silica capillary column (Supelco Corp., Bellefonte, PA). Helium was used as the carrier gas at a linear flow rate of 18 cm per sec. The structure of the principal compound evident in the chromatograms (Fig. 1) was confirmed with methane chemical ionization mass spectra (CI-MS), using a Nermag Model R1010 mass spectrometer.

Alarm Bioassays

Four experiments were conducted to test for alarm and attack by worker yellowjackets in response to extracts or to candidate alarm pheromone. Two experiments us-

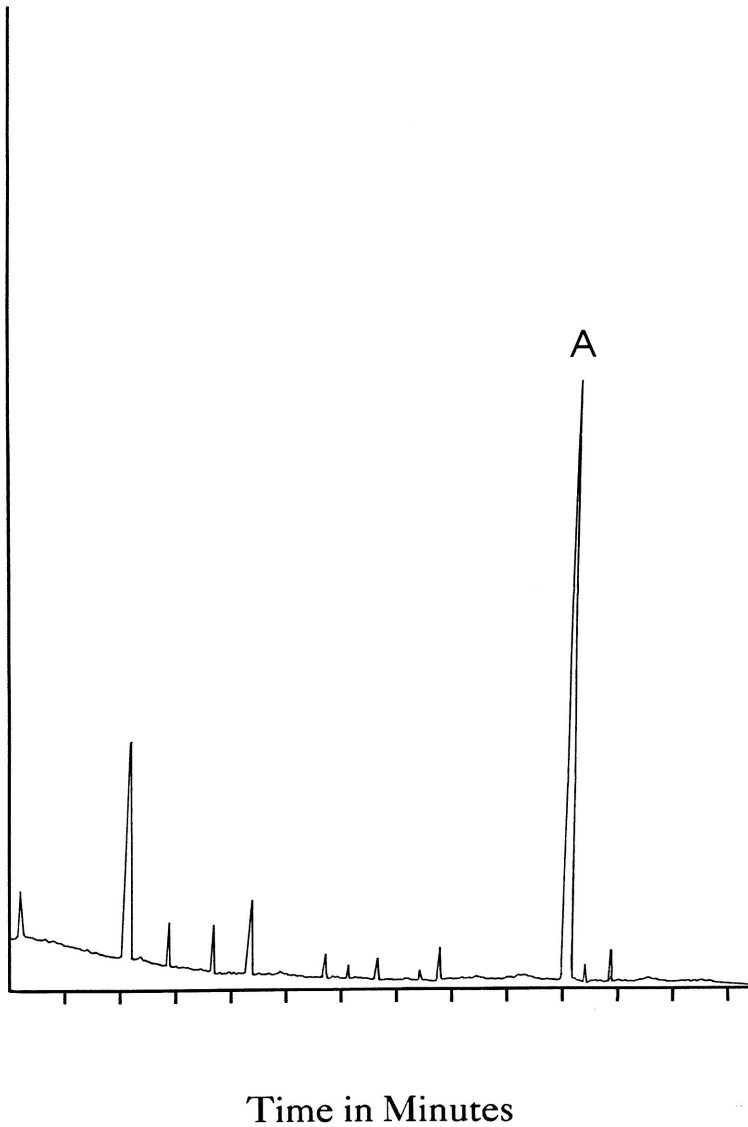


Fig. 1. Chromatogram of a methylene chloride extract of the sting apparatus of worker *Vesputula maculifrons*. Peak A is N-3-methylbutylacetamide, confirmed by GC-MS.

ing different assay designs were conducted initially to assess *V. maculifrons* alarm responses to an extract of conspecific workers and to an extract of conspecific worker sting apparatuses. The third experiment tested for heterospecific alarm responses of *V. squamosa* workers to the sting apparatus extract of *V. maculifrons*. Lastly, an experiment was conducted to determine if *V. maculifrons* workers respond to N-3-meth-

ylbutylacetamide, which was found in venom sac extracts of both species. For most colonies one-minute counts were made of numbers of wasps entering and exiting the colony as a relative assessment of colony size and activity (Malhalm et al. 1991). Colony responses to disturbances and to alarm pheromone are likely to be dependent in part on colony size.

In the first experiment, a whole *V. maculifrons* wasp extract was tested. Forty-three wasp-equivalents of extract (8.6 ml methylene chloride) were applied to a 5.5 cm diam filter paper in a glass petri plate 6-8 cm upwind of the colony entrance (as per Maschwitz 1964) and seven wasp-equivalents of the whole wasp extract (1.4 ml) were placed on a 5.5 cm diam filter paper on a 14 cm diam target sphere 0.5 cm upwind of the entrance and 0.5 m above ground on a wooden stake. Counts were then made of numbers of wasps hitting the target for a 5 min period following application of the extract. Similar applications of methylene chloride were conducted first as a control. This experiment was conducted 6 times, using 2 colonies of eastern yellowjackets, during October and November 1991 in Tulsa, Oklahoma. Bioassays were conducted between 1100 and 1600 hrs, with full sun and temperatures of 24-25°C.

In the second experiment, *V. maculifrons* sting apparatus extract was applied to 5.5 cm diam filter papers placed directly on the top of the target only, as described by Landolt & Heath (1987). The target (14 cm diam black sphere) was coated with an adhesive (Tangle Trap, The Tanglefoot Company, Grand Rapids, Michigan, USA) to capture wasps contacting the target. The target sphere was placed on a wooden dowel 0.2 m above ground and was positioned one m upwind of the entrance of a *V. maculifrons* colony. Treatments consisted of a solvent control and extract dosages of 1, 5, and 25 wasp equivalents in 200 µl methylene chloride. Wasps captured on the target adhesive were counted 2 min after application of the extract sample to the filter paper. Each sphere was removed from the test area at 2 min to end the test. A test series (consisting of the 4 treatments in increasing dosage order) was conducted three times on 2 different colonies in Gainesville, Florida. Spheres were not reused following an assay.

In the third experiment, dosages of *V. maculifrons* sting apparatus extract were tested for responses by worker *V. squamosa*, using the same bioassay procedure as in the second experiment. Treatments consisted of a solvent control and extract dosages of 1, 5, and 25 wasp equivalents. The target sphere was positioned one m upwind of the entrance of a *V. squamosa* colony entrance and numbers of wasps captured on the target adhesive were counted 2 min after application of the extract. This experiment was conducted seven times in November and December 1992, using 3 colonies of *V. squamosa* in Gainesville and Sarasota, Florida.

The fourth experiment was a test for stimulation of alarm in *V. maculifrons* workers by synthetic N-3-methylbutylacetamide. Synthesis and purification procedures are reported in Heath & Landolt (1988). Dosages were applied in 200 µl aliquots of hexane to 5.5 cm diam filter papers placed on the top of 14 cm diam black spheres coated with Tangle Trap. Spheres were positioned one m upwind of a *V. maculifrons* colony entrance. For each replicate, a series of treatments were tested, with increasing dosages of N-3-methylbutylacetamide (0, 2, 10, and 50 µg, or 0, 23, 120, and 600 wasp equivalents). Numbers of wasps contacting the sphere and captured in the adhesive were counted 2 min after each treatment. Spheres that captured wasps were replaced between treatment dosages tested. This experiment was conducted 3 times on different days during November 1992, using one colony of *V. maculifrons*.

Statistical analyses of the responses of wasps to bioassay treatments were determined for all 4 experiments using the Mann-Whitney U test as described in Spiegel (1991).

RESULTS

Workers from colonies of eastern yellowjackets contacted a target sphere when methylene chloride extracts of conspecific workers were placed both near the colony entrance and on the target sphere, and did not hit the sphere when the solvent control was applied (significantly different at $p \leq 0.01$, $z = 2.9$) (Table 1). Similar results were obtained when sting apparatus extracts were placed only on a filter paper on the target sphere, and not near the entrance (Table 2). Again, wasps hit the sphere and were captured in the adhesive in response to the extract (at 5 wasp equivalents) and not in response to the solvent control (significantly different at $p \leq 0.05$, $z = 1.96$).

Workers of *V. squamosa* also responded to 25 wasp equivalents of sting apparatus extract of *V. maculifrons* workers with circling flights and direct hits on the treated target sphere (significantly greater than the control at $p \leq 0.05$, $z = 2.05$) (Table 2).

One principal peak was evident in chromatograms of *V. maculifrons* venom sac extract, with a Kovats Index (Kovats, 1965) of 1102 (Fig. 1). This peak co-eluted with that of synthetic N-3-methylbutylacetamide, an alarm pheromone of the southern yellowjacket (Heath & Landolt, 1988). The structure of the compound in *V. maculifrons* sting apparatus extract was confirmed to be N-3-methylbutylacetamide with chemical ionization mass spectra. Quantitative gas chromatographic analysis of sting apparatus extract of *V. maculifrons* workers from 6 batches of 5 wasps showed an average (\pm SEM) of 84.2 ± 17.7 ng N-3-methylbutylacetamide extracted per wasp.

Eastern yellowjackets responded to synthetic N-3-methylbutylacetamide in the bioassay employed with direct hits on the target sphere and did not respond to the solvent controls. Numbers captured in response to the 50 μ g dosage were significantly greater than those responding to the control ($p \leq 0.05$, $z = 1.96$) (Table 3).

TABLE 1. ALARM RESPONSE (HITS ON TARGET) OF EASTERN YELLOWJACKETS TO DOSAGES OF CONSPECIFIC WHOLE WASP EXTRACT PLACED SIMULTANEOUSLY NEAR THE NEST ENTRANCE (43 WASP EQUIVALENTS) AND ON A TARGET SPHERE 0.5 M FROM THE NEST ENTRANCE (7 WASP EQUIVALENTS), AND TO SOLVENT CONTROLS. DURATION OF ASSAY WAS 5 MIN. AI (ACTIVITY INDEX) IS WASPS ENTERING AND EXITING THE COLONY PER MINUTE.

Trial	Date	AI	Treatment	No. Hits
1	29 Oct 91	42	Control	0
			Extract	12
2	2 Nov 91	11	Control	0
			Extract	36
3	2 Nov 91	11	Control	0
			Extract	109
4	3 Nov 91	53	Control	0
			Extract	8
5	4 Nov 91	32	Control	0
			Extract	82
6	4 Nov 91	32	Control	0
			Extract	3

TABLE 2. ALARM RESPONSE (HITS ON SPHERE) OF *V. MACULIFRONS* OR *V. SQUAMOSA* WORKERS TO DOSAGES OF *V. MACULIFRONS* VENOM SAC EXTRACT APPLIED TO A TARGET SPHERE ONE M FROM A COLONY ENTRANCE. DURATION OF ASSAY WAS 2 MIN. AI (ACTIVITY INDEX) IS THE NUMBER OF WASPS ENTERING AND EXITING THE COLONY PER MINUTE.

Trial	Date	AI	Dosage in Wasp Equivalents			
			0	1	5	25
<i>V. maculifrons</i>						
1	26 Dec 87	-	0	0	4	168
2	18 Nov 92	182	0	0	8	0
3	19 Nov 92	148	0	0	1	11
<i>V. squamosa</i>						
1	18 Nov 92	40	0	7	1	0
2	19 Nov 92	44	0	0	1	7
3	23 Nov 92	98	0	0	1	1
4	14 Dec 92	240	0	-	-	0
5	14 Dec 92	240	0	-	-	2
6	16 Dec 92	300	1	-	-	7
7	21 Dec 92	240	1	-	-	27

DISCUSSION

The results of these experiments indicate that *V. maculifrons* workers possess an alarm pheromone associated with the sting apparatus that elicits attraction and attack. The behavior of eastern yellowjackets that we observed in response to wasp and sting apparatus extracts was qualitatively similar to that reported for the southern yellowjacket (Landolt & Heath 1987). These included circling flights (either around the nest entrance or the target spheres), casting and zigzagging upwind flight patterns towards the target, and direct flights to contact the target. Most responding wasps came from the nest entrance and foragers returning to the nest did not appear to respond to the extracts. In these assays, only the numbers of wasps trapped in the target adhesive were quantified. However, in all cases, this appeared to be the end result of attraction and attack; typical of alarm responses in other social wasps.

Numbers of wasps hitting the target sphere in response to a 5 wasp-equivalent dosage of extract in these studies were variable. This may have been due in part to differences in colony size, as indicated by the range of activity indices, as well as general activity levels at the nest entrance. We also experienced problems with changing wind direction in some experiments with a target placed one m from the nest entrance. An attempt was always made to position the sphere upwind of the entrance so that treatment odors would be carried to the nest entrance. Wind direction shifted during the course of some assays, however.

Observed alarm responses of *V. squamosa* workers to *V. maculifrons* extract suggest overlap in pheromone chemistry between the two species. The chemical analyses of *V. maculifrons* venom sac extract revealed the presence of N-3-methylbutylacetamide, a known alarm pheromone of *V. squamosa* (Heath & Landolt 1988). This compound was the principal volatile found in those extracts of *V. maculifrons*. Subsequent

TABLE 3. ALARM RESPONSE (HITS ON SPHERE) OF *V. MACULIFRONS* WORKERS TO DOSAGES OF N-3-METHYLBUTYLACETAMIDE APPLIED TO A TARGET SPHERE ONE M FROM A COLONY ENTRANCE. DURATION OF ASSAY WAS 2 MIN. AI (ACTIVITY INDEX) IS THE NUMBER OF WASPS ENTERING AND EXITING THE COLONY PER MINUTE.

Trial	Date	AI	Dosage in Micrograms			
			0	2	10	50
1	22 Oct 92	60	0	0	5	11
2	27 Oct 92	62	0	13	0	29
3	29 Nov 92	50	0	0	0	38

bioassay results demonstrated that this compound elicited attraction and attack in conspecific workers when applied to target spheres.

The doses of N-3-methylbutylacetamide needed to evoke eastern yellowjacket alarm responses in our bioassay were much higher than that found in wasp venom sacs, indicating that additional chemicals from the venom sac, or possibly the Dufour's gland, may be used by *V. maculifrons* to communicate alarm. In analyses of venom sac extracts, an average of 84.2 nanograms N-3-methylbutylacetamide was found per wasp (Fig. 1). However, while only about 420 ng of this compound should have been present in the 5 wasp equivalent samples of extract that elicited an alarm response (Table 2), much more of the synthetic N-3-methylbutylacetamide (2 to 50 ug) was required to stimulate alarm behavior (Table 3). Although no other alarm pheromone compounds have been isolated from yellowjackets, it seems likely that a multi-component alarm pheromone may exist in *V. maculifrons*.

N-3-methylbutylacetamide was also found by Aldiss (1983) in the venom of *V. vulgaris* and may be an alarm pheromone of this species also. The sharing or overlap of chemicals comprising alarm pheromones is not unusual, possibly due to the limited complexity of hydrocarbon compounds in the required range of volatility for alarm pheromones (Wilson & Bossert 1963) and the absence of a need for species or functional specificity (privacy referred to by Holldobler & Wilson 1990). *V. squamosa* is considered to be a member of a different species group or genus (Duncan 1939; Carpenter 1987) than *V. maculifrons*. However, since *V. squamosa* is a facultative social parasite of *V. maculifrons* (MacDonald & Matthews, 1975), it may be particularly adaptive for *V. squamosa* to share and respond to the same alarm pheromone as its host, since workers of both species may occupy the same nest. Additional work is needed to determine if N-3-methylbutylacetamide is present in the venom of other Vespidae and if, indeed, it functions as an alarm pheromone among other species of yellowjackets and social wasps.

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