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0014-4754/89/050484-04$1.50 + 0.20/0

3-Ethyl-2,5-dimethylpyrazine, a component of the trail pheromone of the ant *Messor bouvieri*

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Received 8 November 1988; accepted 24 January 1989

Summary. 3-Ethyl-2,5-dimethylpyrazine, found in the poison gland of workers, induces trail following in the Mediterranean harvester ant *Messor bouvieri.* The poison gland contains on average 9 ng of this trail pheromone component. The alkaloids anabasine and anabaseine are also present in this gland, but induce no reaction in workers. Trail following is also induced by substances present in the Dufour gland, but the combined effect of both glands shows no synergism.
Key words. Messor bouvieri; Formicidae; 3-ethyl-2,5-dimethylpyrazine; trail pheromone; poison gland; anabasine; Dufour gland; *Tapinoma simrothi.*

The genus *Messor* of Old World harvester ants contains about 40 species[1], many of which are common in the south Mediterranean region. Workers collect large numbers of seeds, stores of up to 100 l being known[1]. Localized crop damage has been reported in Saudi Arabia[2] and significant economic damage to crops is found in North Africa[1].

Little is known of the foraging or recruitment mechanisms in this genus[3], although Bernard[1] noted the extensive use of chemical trails. Hahn and Maschwitz[3], in a study of foraging in *M. rufitarsus,* a species occupying an enclave north of the Alps in western Germany, found that chemical trails were used, the source of which was the Dufour gland. In contrast, Coll et al.[4] were unable to determine clearly the source of trail pheromones in *M. ebeninus* from Israel. Trail pheromones are used by many ant species for recruitment to food sources and new nest sites. However, the identity of the chemicals employed is known only for a limited number of species[5]. We report here the first identification of a trail pheromone component of a species of *Messor.*

M. bouvieri Bondroit is a common south European species found in the Balkans, Malta, the Balearic Islands[6] and North Africa. Workers were collected from Hammamet on the coast of Tunisia in August 1988. Field observations indicated that workers used an efficient chemical trail system for recruitment to food sources. The trail is laid by spotting substances from the tip of the gaster onto the ground surface.

In order to determine the source of these substances bioassays were performed, using the method of Pasteels and Verhaeghe[7], with hexane extracts of glands and other abdominal parts. The extracts were laid on a circular track of 5-cm radius and the behaviour of the workers observed (table).

The trail made from the hexane extract of one poison gland was followed by workers of *M. bouvieri.* The medi-

Response of *Messor bouvieri* workers to various extracts and to synthetic EDMP using the trail following test of Pasteels and Verhaeghe[7]. The number of 1-cm arcs followed by individual ants were counted. At least 20 ants were observed for each test and the median number of arcs followed was calculated.

Test extract/Compound	Median number of arcs run
Whole gaster	7
Poison gland	7
Dufour gland	6
Dufour gland (after 20 min in N_2)	8
Dufour gland (after 20 min in air)	1
EDMP	
300 ng/trail	1
100 ng/trail	4
30 ng/trail	4
10 ng/trail	3
1 ng/trail	0
0 ng/trail (control)	0

an distance followed on the artificial trail without deviation (7 cm) was not as great as has been found in some other ant species (e.g. *Tetramorium caespitum*[8], which followed hexane extract trails from its poison gland for a median of 33 cm), but was comparable with that of *Myrmica rubra*[9] and its poison gland (7 cm).

More interestingly, the *M. bouvieri* trail was followed by workers of *Myrmica rubra*. A similar trail made from the poison gland of a worker of *Myrmica rubra* was also followed by workers of *M. bouvieri*. Workers of *Myrmica rubra* are known to use 3-ethyl-2,5-dimethylpyrazine (EDMP) as their trail pheromone[9].

A trail laid with synthetic EDMP (prepared in this laboratory by a new method[10]) also induced trail-following in workers of *M. bouvieri*. Maximum activity in the bioassay was produced with 10 to 100 ng per trail, i.e. 0.3–3.0 ng cm^{-1} (table). This was slightly less, although not significantly so, than the activity induced by the extract of a single poison gland. The use of EDMP as a trail pheromone has also been reported in other myrmicine ants. It is known to be used by 13 species of *Myrmica*[9,11,12], *Manica rubida*[13], *Atta sexdens*[14,15], *Tetramorium caespitum*[8] and *Pheidole pallidula*[16]. It is apparent that the use of EDMP as a trail pheromone is of widespread occurrence in myrmicine ants.

The contents of the poison gland were analysed by gas chromatography-mass spectrometry (GC-MS). An excised gland was sealed in a glass capillary and injected without solvent, by the solid sampling method of Morgan and Wadhams[17]. The analysis was carried out using a Hewlett-Packard 5890 gas chromatograph linked to a Hewlett-Packard 5970B MSD, with a fused silica capillary column (15 m × 0.32 mm) coated with SE-54. The carrier gas was helium (1 ml min^{-1}) and the temperature programme was 30 °C for 2 min then increased at 6 °C min^{-1} to 250 °C.

Three volatile compounds were identified in the poison gland. The major component was anabasine (3-(piperidinyl)pyridine, or neonicotine) (3.0 µg per gland) accompanied by the related compound anabaseine (3,4,5,6-tetrahydro-2,3′-bipyridine) (40 ng per gland). This is the second report of anabasine in insects. It has recently been found as the major volatile compound in the poison gland of *Messor ebeninus*[4]. The finding of anabaseine earlier reported from *Aphaenogaster* species[18] may be of chemotaxonomic significance since the genus *Messor* was once considered a subgenus of *Aphaenogaster*[19]. The only other volatile compound detected was EDMP (mean value of 9 ng per gland), representing only 0.3% of the total.

Synthetic anabasine (racemic, purchased from Sigma) which also contained 2% anabaseine had no effect on the trail following of *M. bouvieri* when added to the synthetic EDMP in the same proportion as in the glandular contents. Also, a small piece of filter paper impregnated with the synthetic anabasine elicited no response. In contrast, when the same piece of paper was presented to workers of the ant *Tapinoma simrothi*, which were collected from the same area as the *M. bouvieri*, it elicited an extremely aggressive response. Pharmacologically, anabasine is a highly active compound and toxic to a wide range of animals, and indeed was formerly used as an insecticide.

Trails made from extracts of the hind gut and its contents and the terminal sternite did not produce any significant trail-following behaviour. However, the trail produced from the contents of the Dufour gland did elicit trail following of a degree similar to that of the poison gland extracts. The Dufour gland of *M. bouvieri* is relatively small, containing chiefly heptadecadiene with other linear alkanes, alkenes and alkadienes[20].

The alkenes of ant Dufour glands are, from experience, closely related to the common fatty acids of lipids. For example (Z)-8-heptadecene is a common alkene of Dufour glands, related by a supposed decarboxylation of oleic acid. This heptadecadiene would be expected to be (Z,Z)-6,9-heptadecadiene by relation to linoleic acid. Two attempts to find the position of the double bonds through reaction with dimethyl disulphide catalysed by iodine, followed by GC-MS were unsuccessful because of the very small size of the Dufour glands and the limited size of the colony at this time. Further work on the Dufour gland will have to await the collection of more material.

The heptadecadiene, if derived from linoleic acid, will contain a methylene group between two double bonds, an arrangement highly susceptible to atmospheric oxidation. In order to test the stability of the trail pheromone in the Dufour gland, two circular trails were made from the extract of a single Dufour gland. One of these trails was kept in an inert nitrogen atmosphere for 20 min while the other was exposed to the atmosphere. After 20 min the trail exposed to the atmosphere had lost almost all of its activity, but the trail kept under the atmosphere of nitrogen maintained its activity. This indicated that the components responsible for trail following are not very volatile, but degrade on exposure to oxygen.

Trails made from the poison gland and Dufour gland together, or from the whole gaster, showed the same level of trail following as the single glands. This is the first chemical investigation of an ant species that uses components of both its poison secretion and its Dufour secretion for trail following.

Acknowledgements. We thank C. A. Collingwood for identification of the ants and the SERC for a grant to EDM for the purchase of the GC-MS equipment and for a research studentship to BDJ.

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0014-4754/89/050487-03$1.50 + 0.20/0

Host tree unsuitability recognized by pine shoot beetles in flight

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Received 3 October 1988; accepted 20 January 1989

Summary. In spring, the landing rate of flying European pine shoot beetles, *Tomicus piniperda* L., on injured Scots pine diminishes as colonization continues. This is due to olfactory cues that indicate progressive host degradation. Verbenone was shown to play a role in the beetle's recognition of this unsuitability of a formerly suitable host, since the compound was increasingly released from colonized tree sections as they aged, but not from uninfested sections. Also, the release of verbenone at natural rates in the forest inhibited the attraction of beetles to host monoterpenes.
Key words. Semiochemical; verbenone; *Tomicus piniperda*; Scolytidae; bark beetle; *Pinus sylvestris*; host selection.

The question of how insects select their host plants is one of the important areas of investigation into insect herbivory [1]. A related topic which has been much less investigated is how insects may recognize the relative suitability of a food resource which has become increasingly unsuitable due to competition from established conspecifics, or to general microbial degradation leading to a decrease of food quality [2]. Several theories on how bark beetles terminate their aggregation concern olfactory mechanisms which are based on observations of a reduced response to attractants by a specific inhibitor associated with the beetle [3-5]. However, the rates of release are not known for either the natural inhibitor or for the synthetic inhibitor, or both [3-5]. Thus, the theories remain unproven. In this study, we attempt to quantify the relationship between the attractant and inhibitory semiochemicals in the natural system so that they can be tested experimentally at a natural release rate.

Pine shoot beetles aggregate in large numbers on fallen Scots pine within tens of minutes after the temperature rises above 12 °C on the first swarming day of spring [6,7]. The species is unusual compared to other aggregating bark beetles in that it does not appear to use a long-range pheromone but instead is strongly attracted to volatiles from host logs [6]. At least three monoterpenes are responsible for this attraction to the host, (+/−)-α-pinene, (+)-3-carene and terpinolene, which are released in relatively large quantities from wound oleoresin exudations [6]. This olfactory mechanism allows the beetle both to select the host tree from among non-hosts and to recognize an injured tree as being especially susceptible to attack colonization, due to a lower resinous exudation capacity as a result of storm damage [6].

In the earlier stages of aggregation when the densities of attack are lower, there are few adverse effects of competition [9,10], but as the density of colonization increases over time, the detrimental effects of competition become increasingly apparent in the reduced brood output per female [10,11]. This relationship has been found in several species [11] and is a logical expectation of ecological theory. What is poorly understood, however, is the qualitative and quantitative nature of the olfactory mechanism that plays a role in terminating the aggregation due to the individual's avoidance of fully colonized areas.

To determine whether *T. piniperda* beetles recognize when a host tree becomes less suitable as colonization progresses, we followed the relative attractiveness of colonized trees through time compared with traps releasing constant rates of monoterpenes (fig. 1). Twenty flight-barrier traps, consisting of a transparent plastic pane (17 × 35 cm high) with funnel and collection bottle, were placed next to six Scots pines, *Pinus sylvestris* (L.), which had fallen in winter storms and had subsequently been colonized by *T. piniperda*. The barrier traps were sampled daily (April 18–May 6, 1986) and the catches of beetles were compared to catches on four perforated cylinder