

#34

ALARM PHEROMONES OF THE ANT *ATTA TEXANA*

J. C. MOSER, R. C. BROWNLEE, and R. SILVERSTEIN

Southern Forest Experiment Station, Forest Service, U.S. Department of Agriculture, Alexandria, Louisiana; and Stanford Research Institute, Menlo Park, California

(Received 13 October 1967)

Abstract—4-Methyl-3-heptanone (0.59 $\mu\text{g}/\text{head}$) and 2-heptanone (0.14 $\mu\text{g}/\text{head}$) are the main volatile components of the mandibular glands of major workers. In the laboratory, worker ants detected and were attracted by 4-methyl-3-heptanone at a concentration of $5.7 \times 10^{-13} \text{ g}/\text{cm}^3$ (2.7×10^7 molecules/ cm^3). They were alarmed by a concentration of $5.7 \times 10^{-12} \text{ g}/\text{cm}^3$ (2.7×10^8 molecules/ cm^3). 2-Heptanone is less effective by a factor of 1000. In the field, low concentrations attract and alarm; high concentrations repel and alarm.

INTRODUCTION

ACCORDING to BORGMEIER (1959), the fourteen species of *Atta* are so closely related that some can be readily identified only by male genitalia. Many species, however, can be identified in the field from the odour of a crushed head. All subspecies of *A. sexdens* L. smell strongly of citral. *A. laevigata* (Fr. Smith) and *A. robusta* Borg. have a similar but weaker odour, and *A. bisphaerica* (For.) smells of rancid coconut (GONÇALVES, 1945, and personal communication). The odour of *A. cephalotes* L. resembles stewed pomarac, a West Indies name of the fruit of *Eugenia malaccensis* (J. M. CHERRETT, personal communication). Crushed heads of *A. texana* (Buckley) workers as well as queens and males smell minty, and the same smell was detected from freshly opened fungus garden cavities in nests (MOSER, 1963).

BUTENANDT *et al.* (1959), identified citral (a mixture of neral and geranial) from the mandibular glands of *A. sexdens rubropilosa* Forel. MCGURK *et al.* (1966) reported 4-methyl-3-heptanone from mandibular glands of *Pogomyrmex barbatus* (F. Smith), *P. californicus* (Buckley), *P. desertorum* Wheeler, *P. occidentalis* (Cresson), and *P. rugosus* Emery. They found both 4-methyl-3-heptanone and 4-methyl-3-heptanol in *P. badius* (Latr.); single heads contained 5 nl of the ketone. 2-Heptanone is produced in mandibular glands of the honey bee (SHEARER and BOCH, 1965), as well as in the anal glands of two dolichoderine ants, *Iridomyrmex pruinosus* (Roger) (BLUM *et al.*, 1966) and *Conomyrma pyramica* (Roger) (BLUM and WARTER, 1966). Although 4-methyl-3-heptanone or 2-heptanone cause alarm in the latter ants, reports conflict on the effect of 2-heptanone on the honey bee (BUTLER, 1966).

We report the presence of 4-methyl-3-heptanone and 2-heptanone in the mandibular glands of *A. texana*, and the effects of these compounds and others on laboratory and field colonies.

ISOLATION, IDENTIFICATION AND QUANTITATIVE DETERMINATIONS

The heads from 500 major workers of *A. texana* were collected in July 1966 from the surface of a large nest in Grant Parish, Louisiana. Extraction of the heads (covered with benzene and stored for 3 months at -40°C) was carried out in a Waring Microblender (70 ml capacity) with three 50 ml amounts of pentane. After centrifugation, the supernatant solution was concentrated at reduced pressure to about 0.5 ml, and the residue was fractionated ($50\ \mu\text{l}/\text{injection}$) on an SE 30 gas chromatography column (5% on Chromosorb G 60/80 mesh, 5 ft \times $\frac{1}{8}$ in., 40 to 150°C at $2.5^{\circ}\text{C}/\text{min}$, $30\ \text{cm}^3\ \text{He}/\text{min}$, flame ionization detector, 1:10 stream splitter). The major components were collected as a single fraction (between 3 and 13 min), and this fraction was rechromatographed on an FFAP column (4% on Chromosorb G 60/80 mesh, 10 ft \times $\frac{1}{8}$ in., 60°C , $10\ \text{cm}^3\ \text{He}/\text{min}$, flame ionization detector, 1:10 stream splitter). Major fractions were isolated at 13.6 and 15.2 min.

The component that was eluted at 13.6 min was identified as 4-methyl-3-heptanone by congruence of its mass and i.r. spectra, and of its retention times on SE 30, FFAP, and Carbowax 20M with corresponding properties of an authentic sample. The compound that was eluted at 15.2 min was identified as 2-heptanone by congruence of its mass spectrum and retention times on the same three substrates with those properties of an authentic sample (Eastman purified by gas chromatography). 2-Heptanol, 4-methyl-3-heptanol, citronellal, geranial, and neral, some of which have been previously identified in Hymenoptera (WILSON, 1965), were not present.

Because losses were large in the isolation procedure described (presumably during distillation of large volumes of solvent), a small-scale extraction was carried out without further addition of solvent to that already present. The heads of 133 ants were ground with a glass mortar and pestle and short-path distilled at 100°C and $30\ \mu\ \text{Hg}$. The condensate was fractionated on an FFAP column (4% Chromosorb G 60/80 mesh, 10 ft \times $\frac{1}{8}$ in., initial temperature 40°C , programmed at $2.4^{\circ}\text{C}/\text{min}$, $20\ \text{cm}^3\ \text{He}/\text{min}$). Peak areas were compared with those calibrated for the appropriate reference compound. The peak areas of the three unidentified compounds were measured against 2-heptanone standards. The results were as follows.

Compound	Retention (min)	$\mu\text{g}/\text{head}$
4-Methyl-3-heptanone	11.7	0.59
2-Heptanone	12.5	0.14
Unidentified	17.1	0.15
Unidentified	19.2	0.10
Unidentified	27.1	0.057

THRESHOLD CONCENTRATIONS OF 4-METHYL-3-HEPTANONE AND RELATED COMPOUNDS

One cm^3 of headspace vapour was drawn at 25°C with a 10 ml glass syringe from a 250 ml flask that contained a saturated atmosphere of the vapour of a

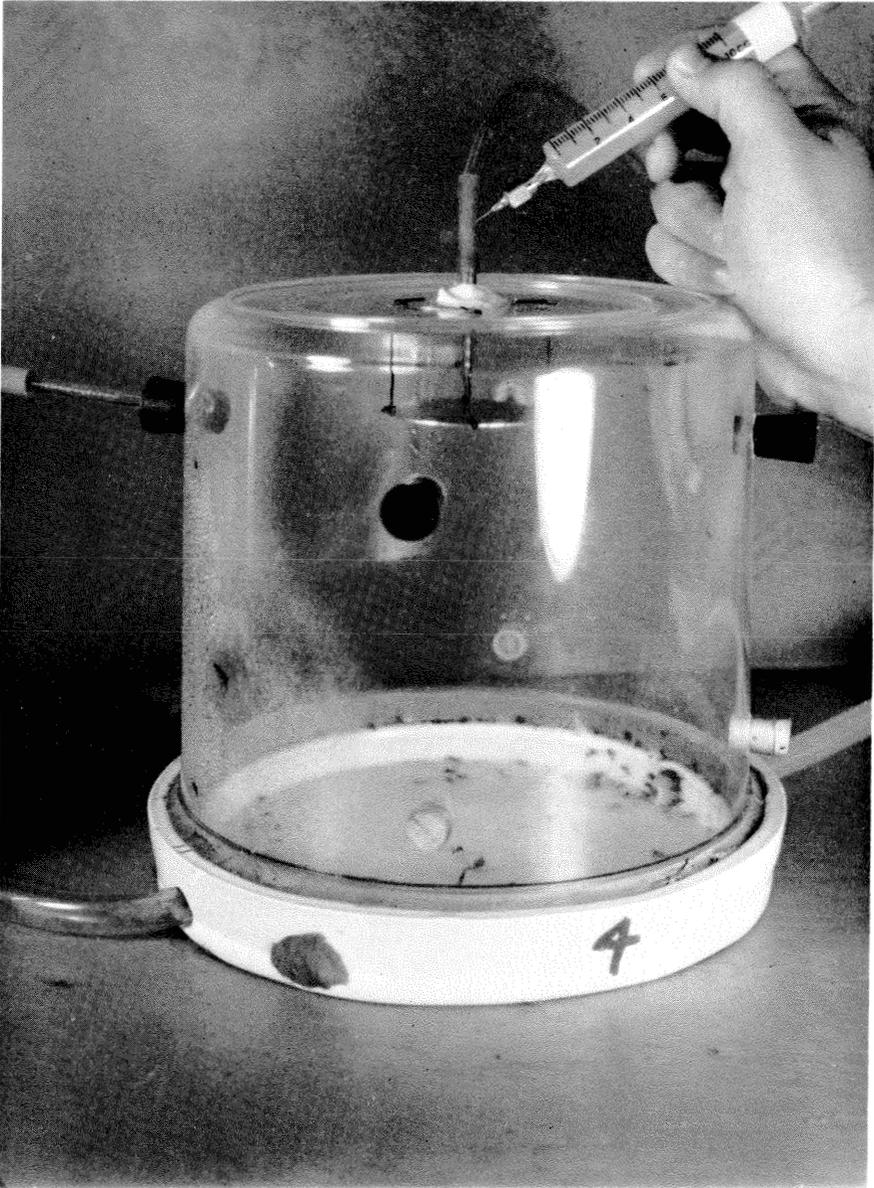


FIG. 1—Ketone vapours were injected into a tube through which a current of air was flowing into the laboratory canister. Tubes at bottom lead to the canisters containing the laboratory colony. Tube at upper left purges gases from the canister after each test.

compound or mixture of compounds in air. The vapour was then serially diluted with air in the syringe, and 1 ml of the diluted vapour was released into a 6 l. canister (Fig. 1), which was connected by plastic tubes to a large laboratory colony similar to that illustrated by MOSER (1962). The vapour was injected into a gum rubber tube just above the canister through which a current of air saturated with water vapour was passing at 25 cm³/min. The gas was further dispersed by an aluminium baffle suspended 3.5 cm below the canister top. The exhaust tube at the upper left in Fig. 1 was closed during bioassays, but air was permitted to escape through the nest tubes. Since ants in adjoining canisters never became excited, it was assumed that little if any of the ketones entered these tubes.

The temperature during laboratory tests was 25°C. As a control, 1 cm³ of pure air was injected into the canisters with a syringe before each bioassay. The ants never responded to these injections. After each test, the container was purged for 30 min with a water aspirator. Ants carrying leaves or detritus, or tending fungus gardens, were much less responsive to alarm substances than unoccupied workers. For this reason, only canisters without fungus gardens were used.

The gas took 45 to 60 sec after injection to reach the workers, who demonstrated three levels of response. At the lowest vapour concentration sensed by workers, they raised their heads and antennae (detection). At slightly higher concentrations of vapour, workers moved toward one side of the canister, and some climbed the walls to a height of 3 or more cm (probably attraction). We assumed that workers were attracted rather than repelled because of the experience of previous researchers. BOSSERT and WILSON (1963) showed that the initial behavioural response of *Pogonomyrmex badius* (Latr.) to low concentrations of 4-methyl-3-heptanone was attraction. BLUM and WARTER (1966) and BLUM *et al.* (1966) demonstrated a similar response of *Conomyrma pyramica* to 2-heptanone. Considerably increased concentrations (usually by a factor of ten) caused workers to run about at random with mandibles open, challenging other workers (alarm). When at least 50 per cent of the unoccupied workers responded in a particular way, the event was recorded.

Between 45 and 75 sec after being alarmed, workers closed their mandibles and slowed their activity. After about 3 min, activities returned to normal. Detection and attraction were caused by 4-methyl-3-heptanone at a syringe dilution of 1 : 1 million, and alarm at 1 : 100,000.* No behaviour was elicited at a 1 : 10 million dilution. Of a number of related carbonyl compounds tested (Table 1) none approached the effectiveness of 4-methyl-3-heptanone. Table 2 shows the effect of several other compounds.

* By gas-liquid chromatography 17 µg/cm³ of 4-methyl-3-heptanone were determined to be present in saturated air. This divided by the mol. wt. of 128 gives 1.328×10^{-7} moles/cm³. Multiplying $\times 6.022 \times 10^{23}$ gives 7.97×10^{16} molecules/cm³. For alarm, the syringe dilution is 1 : 100,000, and the total volume of colony space is 3×10^3 , assuming that the gas is diluted in at least 3 of the 6 l. Hence, 2.7×10^8 molecules/cm³ or 5.7×10^{-12} g/cm³ are required to trigger alarm. The former figure is termed *K* by BOSSERT and WILSON (1963), who theorized that *K* for *P. badius* would be 4.47×10^{13} molecules/cm³.

TABLE 1—RESPONSE TO CARBONYL COMPOUNDS

No. of carbon atoms and compound	Alarm level (cm ³ saturated air)
C ₃ 2-Propanone	10*
C ₅ 2-Pentanone	10
3-Pentanone	10
C ₆ <i>n</i> -Hexanal	†
2-Hexanone	10
3-Hexanone	1
2-Methyl-3-pentanone	1
4-Methyl-2-pentanone	10
C ₇ <i>n</i> -Heptanal	10*
2-Heptanone	10 ⁻²
3-Heptanone	10 ⁻²
4-Heptanone	10
4-Methyl-3-hexanone	10 ⁻²
C ₈ 2-Octanone	1
3-Octanone	10 ⁻²
4-Octanone	1
3,4-Dimethyl-2-hexanone	10
4-Ethyl-3-hexanone	1
2,2-Dimethyl-3-hexanone	10*
2,4-Dimethyl-3-hexanone	10
2,5-Dimethyl-3-hexanone	10*
4-Methyl-2-heptanone	1
5-Methyl-2-heptanone	10
6-Methyl-2-heptanone	10
2-Methyl-3-heptanone	10
4-Methyl-3-heptanone	10 ⁻⁵
5-Methyl-3-heptanone	10 ⁻²
6-Methyl-3-heptanone	1
3-Methyl-4-heptanone	10 ⁻¹
2-Methyl-4-heptanone	10
6-Methyl-5-hepten-2-one	10
C ₉ 2-Nonanone	10
3-Nonanone	10 ⁻¹

TABLE 1—cont.

No. of carbon atoms and compound	Alarm level (cm ³ saturated air)
C₉	
4-Nonanone	1
5-Nonanone	10
5-Ethyl-2-heptanone	10*
C₁₀	
3-Decanone	1

* Detection without alarm.

† Workers detected at 10⁻², but compound did not alarm at higher concentrations.

TABLE 2—RESPONSE TO MISCELLANEOUS COMPOUNDS

Compound	Alarm level (cm ³ saturated air)
4-Methyl-3-heptanol	1
2-Heptanol	1
Isoamyl acetate	10
Citral	10*

* Detection without alarm.

A 4 : 1 mixture of 4-methyl-3-heptanone and 2-heptanone released alarm at a dilution of 1 : 100,000. This might be expected since the same number of molecules of 4-methyl-3-heptanone are available to the ant in the gas of the mixture as in the gas of the pure compound alone. Obviously, 2-heptanone is not synergistic, but its function is not understood.

Crushed heads of males and winged queens smell similar to those of major workers, but the odour of a crushed queen head is more intense.

Single crushed heads of large workers and males dropped into the container alarmed workers about the same amount as did the 1 : 100,000 dilution of 4-methyl-3-heptanone. A queen head, however, elicited extreme alarm that lasted as long as 15 min. Crushed abdomens and thoraces of workers, males, and queens caused little or no alarm.

Thirty males were put in a canister similar to that in Fig. 1, but not connected to the colony. They did not respond to 10 cm³ injections of headspace vapour of 4-methyl-3-heptanone or 2-heptanone, or to crushed heads, thoraces, or abdomens of workers, females, or males.

Winged queens detected, but were not alarmed by 10 cm³ of 2-heptanone headspace vapour, 1 and 10 cm³ of 4-methyl-3-heptanone headspace vapour, and a crushed queen head. They did not respond to crushed heads, thoraces, or abdomens of workers and males, or to crushed thoraces and abdomens of queens.

Ten *Trachymyrmex septentrionalis* workers in the canister did not detect 10 cm³ of 4-methyl-3-heptanone or 2-heptanone headspace vapour.

FIELD TEST

Trails

To test the effects of chemicals in the field, pieces of filter paper 1 × 1 cm were saturated with 4-methyl-3-heptanone, 4-methyl-3-heptanol, 2-heptanol, a 4 : 1 mixture of 4-methyl-3-heptanone and 2-heptanone, and water, then pinned across trails. In addition, 0.125% 4-methyl-3-heptanone in water, 0.250% 2-heptanone in water, and citral were tested, along with heads, thoraces, and abdomens crushed on filter paper. The squares were placed linearly 1 cm apart on trails 6 cm wide so that ants could walk between them.

Squares saturated with the undiluted ketones or the 4 : 1 ketone mixture both alarmed and repelled laden and unladen workers at a distance of about 1 cm, creating traffic jams and often causing workers to drop their burdens. At trail temperatures near 20°C, detours were laid around the squares within 5 min. After about 12 min most of the ketone had evaporated, and 50 per cent of the workers again passed the squares. At trail temperatures near 30°C, no detours were made, presumably because the ketones evaporated before the detouring response occurred.

Aqueous solutions of ketones slightly repelled and alarmed workers but not enough to disrupt trail activities.

The alcohols were much less volatile than the ketones. They alarmed and repelled ants at 0.5 cm, blocked trails for at least 30 min, and caused detours.

Citral repelled workers at 1 cm, but caused no alarm. This liquid is relatively non-volatile, and permanent detours were formed around the barrier. GONÇALVES (1945) reports similar behaviour of *A. sexdens* in Rio de Janeiro, and CHERRETT (personal communication) of *A. cephalotes* in Trinidad.

Control and water-saturated squares were virtually ignored, but those with crushed heads, thoraces, and abdomens attracted large numbers of alarmed workers, who began cutting them. Within 3 min the crushed body part was grasped by a worker, carried at right angles to the trail, and dropped at a distance of at least 1 m. Workers carrying heads were often followed for the first 30 to 50 cm by a retinue of excited workers. When a crushed head was placed on a trail, it was quickly removed, but since some of the pheromone had undoubtedly leaked into the soil, the spot continued to repel and alarm workers for about 10 min.

Nest openings

Workers immediately rushed out in alarm when filter-paper flags (1 cm²) soaked with the ketones or alcohols were waved about 5 cm over nest openings.

Blowing air into a hole with the mouth produced similar results, but releasing grains of dirt into holes only elicited slight alarm.

Slight alarm was also produced by flags saturated with solutions of 0.125% 4-methyl-3-heptanone in water and 0.250% 2-heptanone in water, and by crushed heads and crushed abdomens. Flags alone or saturated with distilled water or citral produced no alarm. Flags saturated with the ketones and alcohols placed 25 cm upwind from holes triggered only slight alarm, and did not attract the ants.

Acknowledgements—Drs. E. J. EISENBRAUN and W. A. DREW, Oklahoma State University, furnished an authentic sample of 4-methyl-3-heptanone as well as twenty-two ketones, and provided a copy of their manuscript prior to publication. Dr. M. S. BLUM, Louisiana State University, furnished five ketones.

REFERENCES

- BLUM M. S. and WARTER S. L. (1966) Chemical releasers of social behavior—VII. The isolation of 2-heptanone from *Conomyrma pyramica* (Hymenoptera:Formicidae: Dolichoderinae) and its *modus operandi* as a releaser of alarm and digging behavior. *Ann. ent. Soc. Am.* **59**, 774–779.
- BLUM M. S., WARTER S. L., and TRAYNHAM J. G. (1966) Chemical releasers of social behavior—VI. The relation of structure to activity of ketones as releasers of alarm for *Iridomyrmex pruinosus* (Roger). *J. Insect Physiol.* **12**, 419–427.
- BORGMEIER T. (1959) Revision der Gattung *Atta* Fabricius (Hym., Formicidae). *Studia ent.* **2**, 321–390.
- BOSSERT W. H. and WILSON E. O. (1963) The analysis of olfactory communication among animals. *J. theoret. Biol.* **5**, 443–469.
- BUTENANDT A., LINZEN B., and LINDAUER M. (1959) Über einen Duftstoff aus der Mandibeldrüse der Blattschneiderameise *Atta sexdens rubropilosa* Forel. *Arch. Anat. micr. Morph. exp.* **48**, 13–20.
- BUTLER C. G. (1966) Mandibular gland pheromone of worker honeybees. *Nature, Lond.* **212**, 530.
- GONCALVES C. R. (1945) Saúvas do sul e centro do Brasil. *Bolm fitossanit.* **2**, 183–218.
- MCGURK D. J., FROST J., EISENBRAUN E. J., VICK K., DREW W. A., and YOUNG J. (1966) Volatile compounds in ants: identification of 4-methyl-3-heptanone from *Pogonomyrmex* ants. *J. Insect Physiol.* **12**, 1435–1441.
- MOSER J. C. (1962) Probing the secrets of the town ant. *Forests & People* **12**(4), 12–13, 40–41.
- MOSER J. C. (1963) Contents and structure of *Atta texana* nest in summer. *Ann. ent. Soc. Am.* **56**, 286–291.
- SHEARER D. and BOCH R. (1965) 2-Heptanone in the mandibular gland secretion of the honey bee. *Nature, Lond.* **206**, 530.
- WILSON E. O. (1965) Chemical communication in the social insects. *Science, N.Y.* **149**, 1064–1071.