The alfalfa weevil, *Hypera postica* (Gyllenhal), and the clover head weevil, *H. meles* (Fab.), are important pests of alfalfa, *Medicago sativa* (L.), and clovers, *Trifolium* spp., respectively. In spite of their economic importance, pheromones have not been isolated or identified for either insect. Aggregative behavior has been documented for alfalfa weevils by Hamlin et al. [2] and Simpson and Welborn [3], and have been observed in clover head weevils (Ellsbury, unpublished). We report the identification by GLC-MS of a number of volatile constituents of these insects as part of a search for pheromones.

Methods and materials. Adult clover head weevils (1200 F_1 and 485 overwintered, mixed sexes), and 330 adult alfalfa weevils (mixed sexes) were covered with 500–1000 ml hexane, and stored at 4 °C until they were poured without concentration on to a 5 × 14 cm silicic acid column (Bio-Sil A, 200–400 mesh Bio-Rad Lab., Richmond, CA) that had been slurried in hexane. The column was sequentially eluted with 500 ml hexane, 500 ml methylene chloride/hexane: 1/9, 500 ml methylene chloride/hexane: 1/1, and 500 ml methylene chloride. The eluates were concentrated under reduced pressure to ca 20 ml, examined by TLC, and combined to give 3–5 composited fractions. Each was examined by GLC-MS, on a DB-1 fused silica column (15 m × 0.322 mm) that was interfaced to a Hewlett Packard 5985-B^R quadrupole mass spectrometer. An approximation of relative concentrations of components was obtained by comparing the MS data system total abundance count of the ion chromatogram with that of the appropriate standards available from our previous work [4, 5].

Results and discussion. Found in the clover head weevil (both F_1 and overwintered) were 11 oxygenated monoterpenes and 3 fatty acids. Most prominent were 3 of the 4 boll weevil, *Anthonomus grandis* (Boh.), pheromone components previously identified by us [4], (Z)-3,3-dimethylcyclohexane-$\Delta^{1,\beta}$-ethanol **9**, and (Z)-and (E)-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetaldehyde **10** and **12** (table, fig.). The other 8 oxygenated monoterpenes have recently been identified by us as boll weevil pheromone intermediates or by-products [5] (table, fig.).

Identified from the alfalfa weevil (F_1) were the 2 boll weevil pheromone aldehydes ((Z)- and (E)-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetaldehyde), **10** and **12**, six of the oxygenated monoterpenes found in the clover head weevil, isogeraniol, nepetalactone, and 3 fatty acids (table, fig.). The yield of volatiles from the alfalfa weevil was lower (about 20%) than from the clover head weevil (table).

The identities of the compounds were confirmed by comparison of the GLC retention volumes and mass spectral fragmentation patterns to those of the standards that had been synthesized in previous work [4, 5].

It would not be unexpected for the clover head and alfalfa weevils to biosynthesize components similar or identical to the sex pheromones of the boll weevil, since all three are members of the family Curculionidae. Several other curculionids have sex pheromone components in common with the boll weevil [6]. However, the identification of the complete pheromones of these two insects awaits additional studies and associated chemical work [7].

1 Mention of a trademark, proprietary product or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

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The contents of the pygidial gland of the primitive ant *Nothomyrmecia macrops* (Hymenoptera: Formicidae)

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Summary. The principal constituent of the pygidial gland of *Nothomyrmecia macrops* is 3,7-dimethyloct-6-en-2-one, a substance not previously identified in insects. Also identified were 2,6-dimethylhept-5-enal, 2-nonanone, indole, γ-dodecalactone, and the hydrocarbons pentadecane, heptadecane, heptadecene and heptadecadiene, all in low nanogram quantities.
Key words. Ant; pygidial gland; *Nothomyrmecia;* dimethyloctenone; γ-dodecalactone.

We have recently collected specimens of the extremely elusive and primitive ant *Nothomyrmecia macrops* Clark at Poochera, South Australia. This is considered the most primitive living ant, and because of some peculiar anatomical features, has been placed by itself in the subfamily Nothomyrmeciinae [1]. It has therefore been the subject of several recent studies of its anatomy and phylogenetic position [2], its behaviour [3], genetics [4], and sting morphology [5]. We have now undertaken a combined study of the ultrastructure [6] and chemical contents of its exocrine glands. We have already described the large number of substances identified in its Dufour gland [7].

We describe here the contents of the pygidial gland and our attempt to study the mandibular gland. The pygidial gland (fig. 1), is associated with the intersegmental membrane between the 6th and 7th abdominal tergites of ants. Large pygidial glands are found in most species of the subfamily Dolichoderinae (where they were erroneously called 'anal glands') and in a number of species scattered throughout other subfamilies [8]. Excepting the dolichoderines, they have

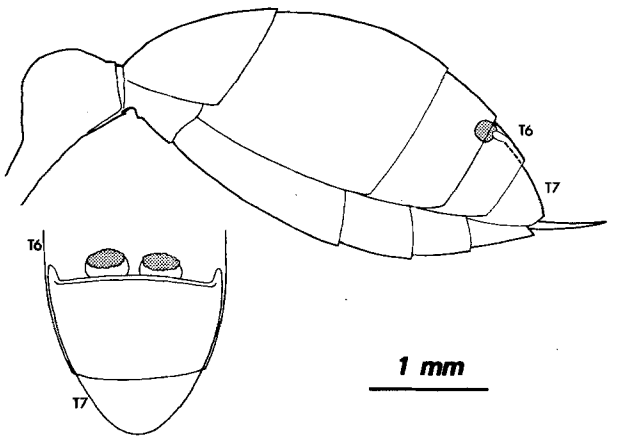

Figure 1. Longitudinal section of the abdomen and dorsal section of the abdominal tip of *N. macrops* showing the 6th and 7th tergites (T6, T7) and the location of the pygidial glands.

been little studied. The pygidial glands occur laterally in pairs and are relatively large in *N. macrops*. Pairs of glands were dissected out and analysed chemically and found to contain nanogram quantities of volatile substances.

Material and methods. Workers of *Nothomyrmecia macrops*, identified by Dr R. W. Taylor, were collected in South Australia, on 24 and 25 February 1987 and taken immediately to Canberra (CSIRO) and there anaesthetized and killed by placing for 3 min in a Biofreezer at − 50 °C. The ants were then dissected under water. Pygidial glands were removed with some cuticle and sealed immediately in soft-glass capillaries (2 mm × 2 cm) in pairs and one group of 4 pairs. Samples of cuticle from the 2nd abdominal tergite, pairs of mandibular glands, and whole heads were similarly sealed. The samples were analysed by GC-MS on a Hewlett Packard 5890 Gas Chromatograph and 5970B Mass Selective Detector with HP59970C ChemStation. A fused silica capillary column (12 m × 0.2 mm) coated with HP-1 (cross linked methyl silicone gum ≅ OV-1) of 0.33 µm film thickness was used. The carrier gas was helium at 10 psi column head pressure.

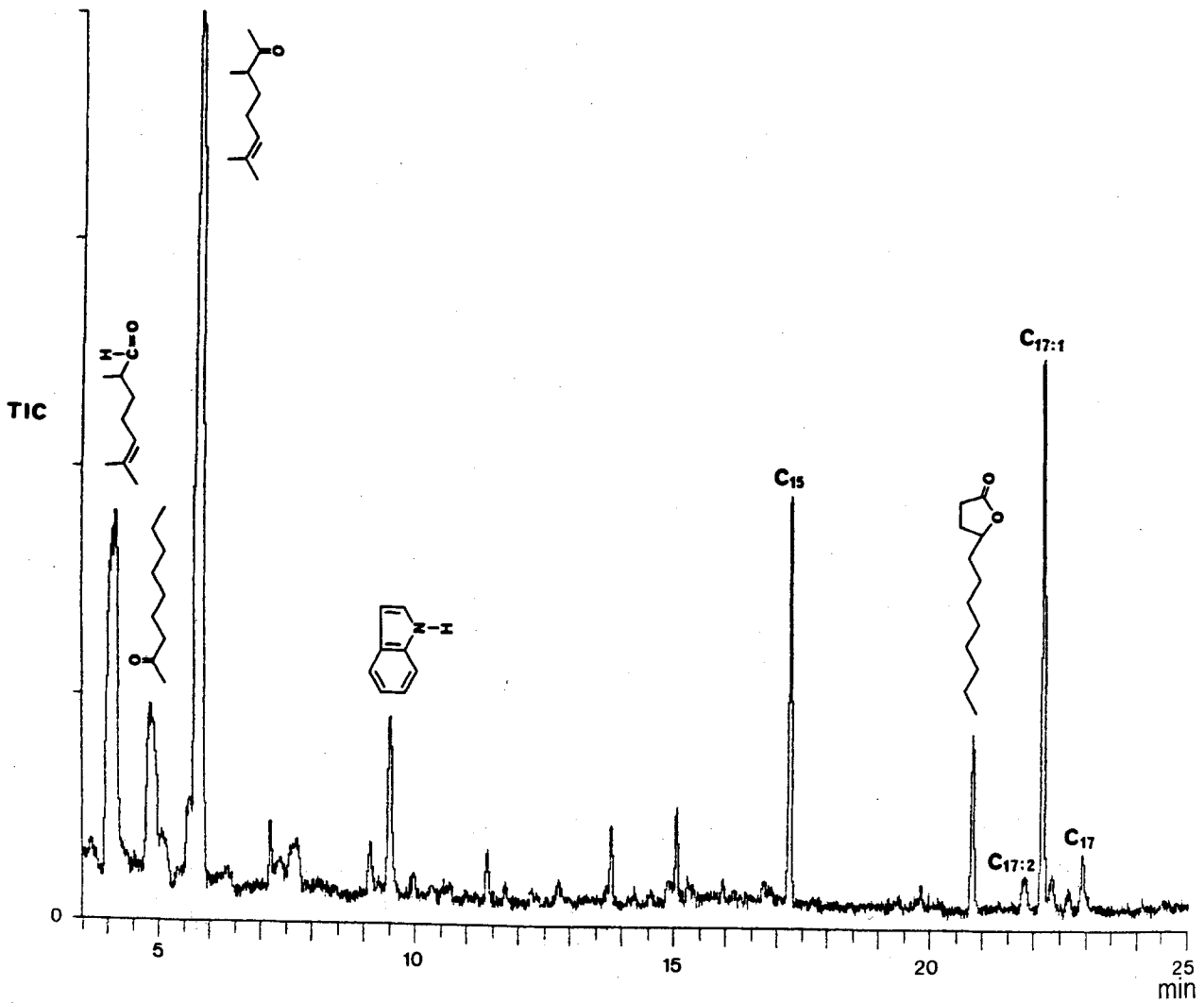

Figure 2. Typical total ion chromatogram of the contents of the pygidial glands of a single worker of *N. macrops*. The peak at 11.4 min is methyl decanoate. This, and other unlabelled peaks did not appear in other samples.

Mean amount of substances found in the pygidial glands of a single worker of *N. macrops*, together with the sample standard deviation (σ_{n-1}, n = 8)

Substance	Mean composition by weight ng/ant	SD	Mass spectral data m/z (%)
2,6-Dimethylhept-5-enal	7	17	140 (M^+, 2), 82 (73), 69 (30), 67 (63), 55 (31), 41 (100), 39 (35)
2-Nonanone	6	5	142 (M^+, 2), 71 (14), 59 (17), 58 (72), 57 (21), 43 (100), 41 (31)
3,7-Dimethyloct-6-en-2-one	33	29	154 (M^+, 5), 83 (27), 82 (61), 72 (37), 67 (57), 43 (100), 41 (73)
Indole	2	4	118 (8), 117 (M^+, 100), 116 (3), 90 (53), 89 (38), 63 (19), 62 (11), 58 (12), 39 (11)
Pentadecane	16	12	212 (M^+, 1), 113 (3), 99 (6), 85 (31), 71 (52), 57 (100), 43 (89)
γ-Dodecalactone	3	2	†128 (6), 114 (3), 85 (100), 69 (9), 55 (19), 43 (17), 41 (27)
Heptadecadiene	2	2	236 (M^+, 3), 109 (19), 95 (42), 81 (63), 67 (100), 55 (55), 41 (83)
Heptadecene	38	32	238 (M^+, 4), 111 (20), 97 (42), 83 (55), 69 (74), 55 (95), 43 (83), 41 (100)
Heptadecane	4	3	240 (M^+, 1), 113 (6), 99 (9), 85 (32), 71 (56), 57 (100), 43 (90)
Total	111	69	

† 198 (M^+, 0.03) synthetic compound only.

The capillary tubes containing the samples were kept in the solid injector[9] in the injection port at 200 °C for 2–3 min before crushing. A splitless injection was performed with the injection port purge turned on after 0.5 min. The oven temperature was initially 30 °C for 2 min then increased at a rate of 4 °C min^{-1} to 250 °C. The mass selective detector was set to monitor m/z 35–350 in the scan mode (≅ 1.5 scans s^{-1}) under "Autotune" conditions using 70 eV ionization. The quantity of each component was determined by external standards.

Results and discussion. Insects were transported live to Canberra, and there the pairs of glands from each of four workers were dissected and sealed in glass capillaries and brought to Keele. Glands from another four workers were sealed together in a single capillary. Whole worker heads and mandibular glands were prepared similarly.

These samples were analysed by combined gas chromatography-mass spectrometry using our solid sampling method[9]. Because a certain amount of cuticle has to be included with the dissected pygidial glands, comparable samples, free of glandular tissue were taken from the 2nd abdominal tergite to serve as controls.

Nine substances were recognized in the pygidial gland. A typical gas chromatogram of a single worker's gland is given in figure 2. The major substance was 3,7-dimethyloct-6-en-2-one, a compound which has not previously been identified in insects. Also present in smaller amounts were 2,6-dimethylhept-5-enal, 2-nonanone, indole and γ-dodecalactone. None of these substances has been identified before in pygidial glands of ants, though all have been found in other glands of ants or other insects[10]. 2,6-Dimethylhept-5-enal has been found in the mandibular glands of the ants *Acanthomyops claviger, Lasius alienus* and *L. carniolicus*[10]. 2-Nonanone has been identified in the pygidial ('anal') gland of *Azteca* spp. and in the mandibular glands of *Trigona* bees[10]. Indole is the major constituent of the paired intersegmental sternal glands of the trichopteran *Pycnopsyche scabripennis*[10] and γ-dodecalactone is the major substance of the pygidial glands of the staphylinid beetles *Bledius mandibularis* and *B. spectabilis*[10]. Also found were the four hydrocarbons pentadecene, heptadecane, heptadecene and heptadecadiene. These have already been encountered in *N. macrops* Dufour glands[7]. Indeed, they were present in roughly the same proportions as in the Dufour gland, though in less than 1% to 0.1% of the amount found there. The hydrocarbons, but not the other substances, were also found in the samples of tergal tissue. It is possible that they arise as contaminants during dissection or they are distributed over the cuticle by discharge of the Dufour gland. Still smaller amounts were found in the heads (see below). The mean quantities of all these substances found in the glands are given in the table.

The hydrocarbons are already familiar from many other species examined. For the other substances, synthetic specimens were obtained to confirm their identification from mass spectra and retention times. 2,6-Dimethylhept-5-enal, indole and 2-nonanone were available commercially. γ-Dodecalactone was prepared in high yield, accompanied by a little δ-lactone via a Knoevenagel condensation of decanal and malonic acid followed by acid-catalysed ring closure[11]. 3,7-Dimethyloct-6-en-2-one was prepared from 6-methylhept-5-en-2-one and ethyl 2-chloropropionate via a Darzens glycidic ester condensation followed by hydrolysis and decarboxylative rearrangement[12].

The large pygidial glands of dolichoderine ants, which contain monoterpene iridoids, have been extensively studied first by Pavan and co-workers and later by Cavill and co-workers (reviewed by Blum and Hermann[13] and ourselves[14]). The iridoids are used as defensive secretions. Apart from the work on dolichoderines, there is only one other chemical examination reported, that on the pygidial gland of the ponerine ant *Rhytidoponera metallica*[15], where 3-hydroxybenzaldehyde, isogeraniol, heptadecane and heptadecene were found.

Hölldobler and Taylor carried out simple tests on the behaviour of *N. macrops* workers towards their glandular secretions, and found the pygidial gland secretion caused some response, weaker than their reaction to their mandibular glands and Dufour gland, but the pygidial gland secretion was the only one that caused a positive repellant response in three species of *Camponotus*[3]. They concluded that the pygidial gland secretion has an alarm-defense function, perhaps in encounters with other ants. γ-Dodecalactone may contribute to this effect, since γ-decalactone, a component of the anal exudate of the thrips *Bagnalliella yuccae* (Thysanoptera), repels small predators such as ants (*Monomorium minimum, Iridomyrmex humilis*)[16].

Mandibular glands are probably present in all ants, and in many, but by no means all species, the glands contain volatile chemicals. The type of substance varies with the sub-family, but generally they have a defensive or pheromonal function. We have analysed individual heads, dissected glands, with and without mandibles attached, of workers of *N. macrops* and one sample consisting of six heads, and another of eight, and found no volatile material in any of them, other than still smaller traces of Dufour gland hydrocarbons. This was surprising since Hölldobler and Taylor found the greatest response of all the glandular secretions from the mandibular glands in simple behavioural tests on *N. macrops* workers. We must consider the possibility that the ants had discharged these glands in the disturbance of travelling.

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Determination of diel periodicity of sex pheromone release in three species of Lepidoptera by 'closed-loop-stripping' [1]

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Summary. By means of 'closed-loop-stripping' and subsequent GC analyses the diel periodicity of release of (*Z*)-11-hexadecenyl acetate, (*E*)-8-dodecenyl acetate, and (*Z*)-9-tetradecenyl acetate, the main constituents of the respective sex pheromone blends of *Mamestra brassicae, Cryptophlebia leucotreta* and *Spodoptera sunia* females, was determined.
Key words. Closed-loop-stripping; sex pheromone release; diel periodicity; *Mamestra brassicae; Cryptophlebia leucotreta; Spodoptera sunia.*

Production of sex pheromones by female moths and the subsequent release during 'calling' depends, among other factors such as age of the females, temperature, neural and hormonal conditions, mainly on the photoperiod experienced by the insects. The mating behavior, of which calling and pheromone release represents an essential part, usually occurs during a discrete period of the day/night cycle, and is often crepuscular or nocturnal. A knowledge of diel periodicity of pheromone release thus becomes an important factor to those interested in pheromone identification in order to maximize the yield of pheromone isolation.

We report here the results of laboratory experiments conducted to determine the effect of the photoperiod on sex attractant release of three different lepidopteran species, *Mamestra brassicae* L. (Noctuidae), *Cryptophlebia leucotreta* Meyr. (Tortricidae) and *Spodoptera sunia* Guenée (Noctuidae) by collecting the airborne volatiles from a 'closed-loop-stripping' [2] system.

Material and methods. M. brassicae females use (*Z*)-11-hexadecenyl acetate (Z-11-16:Ac) as the main component of their sex pheromone [3–7]. The sex attractant of *C. leucotreta* has been reported as a mixture composed mainly of (*Z*)- and (*E*)-8-dodecenyl acetates, depending on the geographical origin of the insects [8–11], and the strain investigated in our laboratory had (*E*)-8-dodecenyl acetate (E-8-12:Ac) as the major component [8]. In *S. sunia,* (*Z*)-9-tetradecenyl acetate (Z-9-14:Ac) represents the main constituent of the pheromone blend [12]. The quantification of the respective main components, Z-11-16:Ac, E-8-12:Ac and Z-9-14:Ac, of the collected volatiles, was performed by gas chromatography (GC).

Insects used in this study were provided by Hoechst AG (Frankfurt) and the Institut für Biologische Schädlingsbekämpfung (BBA Darmstadt). Pupae were sexed and kept at 22 °C in plastic containers lined with moistened filter paper. A reversed 14-h light:10-h dark cycle was maintained throughout the study. Adults were collected daily after eclosion and transferred to plastic boxes containing wicks with 5% sugar solution.

Closed-loop-stripping. The closed-loop-stripping system used for this study is a modification of that of Boland et al. [2]. Air was circulated (1.5 l/min) continuously through the closed system by a membrane pump (Antechnica, Karlsruhe, FRG). The air from the insect container was purged through a small charcoal filter (Brechbühler AG, Schlieren, Switzerland; 1.5 mg charcoal) which retained volatiles (fig. 1, A). The adsorbed volatiles were removed subsequently by solvent extraction. The filter was sealed inside a glass tube as shown in figure 1, C. Both sides of the glass tube ended in ball joints in order to obtain an airtight fit, and provide some flexibility to the system to withstand the vibrations of the pump.

The volume of the insect container was such that up to eight moths could move independently without interfering with one another. The air stream was not sweeping directly across the insects (fig. 1, B). The whole system was kept at an ambient temperature of 25–27 °C and was subjected to a 14:10-h light:dark cycle, which was identical to that maintained in the room where the insects emerged.

Two- to three-day-old female moths, 3–8 individuals per experiment, were placed in the insect container during the dark period and aerated for 10 h. After a 45-min adsorption period the pump was stopped and allowed to cool for 15 min, and the filter was removed for extraction. This procedure was repeated every hour.

Extraction of the filter. A 10-µl droplet CS_2 (CS_2 for IR-spectroscopy) was placed on top of the filter and moved up-and-down about 10 times through the charcoal zone by gentle cooling (ice bath) or heating (hand temperature) with a small Schlenckrohr or bulb attached to the filter tube (fig. 1, D). Afterwards, the droplet was sucked to one end of the coaxial inner tube (fig. 1, C) and taken up with a GC syringe. The