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VOLATILE SECRETIONS OF THE GREEN TREE ANT *OECOPHYLLA SMARAGDINA* (HYMENOPTERA: FORMICIDAE)

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Abstract—1. A comparison of the volatile exocrine secretions of *Oecophylla smaragdina* with published results on *O. longinoda* shows the two species are very similar.

2. The contents of Dufour glands of both major and minor workers are closely similar, with undecane the major substance in both castes of both species.

3. The mandibular glands were distinctly different, both between major and minor workers of *O. smaragdina* and between major workers of the two species. Major workers' mandibular glands contain chiefly hexyl hexanoate, 3-decanone and octyl hexanoate while minor workers contain nerol and 1-nonanol.

4. Males' mandibular glands contain chiefly 3-dodecanone, 3-decanone and 3-decanol, which is very different from the mixture of carboxylic acids in the heads of males of *O. longinoda*.

INTRODUCTION

Weaver ants represent one of the most spectacular examples of nest building known among insects. The arboreal ants use their larvae like miniature sewing machines, moving them back and forth between leaf margins to sew the leaves together with the silky secretion of their labial glands (Hölldobler and Wilson, 1983). This behaviour is found in species of the formicine genera *Camponotus*, *Dendromyrmex* and *Polyrhachis*. The specialized weavers *par excellence*, however, are *Oecophylla*, a genus comprising only two extant species; *O. longinoda* (Latreille) occurs in tropical forests of Africa, the closely related *O. smaragdina* (Fabr.), commonly known as the green tree ant, is found from India through Southeast Asia to Queensland in Australia. Both species are exceptionally abundant, aggressive and territorial, and are important in tropical forest ecology and agriculture (Leston, 1970).

Oecophylla longinoda has been the subject of extensive research dealing with the weaving behaviour (Hölldobler and Wilson, 1978, 1983), its glandular system and the corresponding behavioural aspects (Hölldobler and Wilson, 1978), and the glandular chemistry including reports on its alarm-communication system (Bradshaw *et al.*, 1979a–c). Hölldobler and Wilson (1978) believe that communication in this species was the most elaborate yet to be recognized in ants. They demonstrated the location of the source of the trail pheromone was the rectal gland, and that workers recruited nestmates by an attractant–arrestant pheromone from the sternal gland (Hölldobler and Wilson, 1978).

The sister species *Oecophylla smaragdina* has received less attention—no examination of its volatile exocrine secretions has yet been made. We therefore describe here the compositions of the Dufour glands and mandibular glands of major and minor workers and of the mandibular glands of males for comparison with those of *O. longinoda*.

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MATERIALS AND METHODS

Collection

All individuals for this study were collected from their tree nest in Colombo, Sri Lanka, in April 1990, and immediately transported to Leuven and maintained alive until dissection.

Dissection

Ants were killed by momentary immersion in liquid nitrogen. Dufour glands were obtained by gently pulling off the 7th abdominal segment, from which tissue was carefully removed until a cleanly dissected gland remained.

For mandibular glands, the exoskeleton of the cephalic capsule was carefully removed, starting from the posterior side. In this way, the mandible with attached gland remained. For two samples, only the cleanly dissected mandibular glands (without the sclerotized mandible) were prepared; other samples included the mandibles and surrounding tissues.

Mass spectrometry

The glands were analysed by gas chromatography–mass spectrometry using the solid sampling method of Morgan and Wadhams (1972) (see also Morgan, 1990) on a Hewlett-Packard 5890 gas chromatograph and 5970B Mass selective Detector with HP59970C Chemstation software. All samples were analysed on a polydimethylsiloxane (immobilized) capillary column (12 m × 0.2 mm i.d. × 0.3 μm) coated with OV-1. The injection port temperature was 140°C, the oven temperature was 30°C for 2 min and then increased 8°C/min to 260°C. The mass spectrometer was set to monitor m/z 35–350.

Quantification

Quantification was carried out by the use of 3-undecanone and nerol as external standards for the mandibular glands and heptadecane for the Dufour glands.

Identification

Commercial geraniol and nerol were used to confirm the identification of substances tentatively identified from their mass spectra.

The series of esters thought to be present within the major workers' mandibular glands was synthesized in the laboratory. A mixture of hexanoic and octanoic acids and nonanol, hexanol and octanol was heated at 120°C for 12 hr with a small drop of concentrated sulphuric acid, following the method of Attygalle *et al.* (1987), to give the corresponding mixture of esters, to confirm the retention times and mass spectra of the esters found in the mandibular glands.

Selective ion monitoring

Selective ion monitoring (SIM) was carried out on Dufour gland samples for ions of m/z 43, 55, 83, 224 and 41, 55, 236 which are the respective characteristic ions of a hexadecyl ester and heptadecadiene, main compounds in the Dufour glands of *O. longinoda*.

RESULTS

Dufour glands

The Dufour glands of major workers contain a mixture of hydrocarbons, dominated by undecane, heneicosane and pentadecane (Table 1). Only two minor workers were analysed, but both of these showed undecane also as the major substance (Table 1), but otherwise the mixture of hydrocarbons was rather different as illustrated by the gas chromatograms in Fig. 1. It is noteworthy that the average amount of secretion was considerably greater in the minor workers in spite of their smaller body size.

Table 1. Percentage composition of the volatile secretion of the Dufour gland of major and minor workers of *O. smaragdina*. Results for major workers are the means (with sample standard deviations) of the determinations of 10 individuals; only two minor workers were examined

Compound	Major workers %	Major workers SD	Minor workers 1	Minor workers 2
Octanal			t	t
Limonene			0.5	0.2
Nonane	2.3	0.7		
Decane	0.6	0.4	t	t
Nonanal			t	t
Decanol			t	t
Undecene	t		0.3	0.3
Undecane	41.4	5.4	36.8	38.8
Decanal	t		0.4	0.2
Dodecene			t	t
Dodecane	2.0	0.6	0.5	0.3
Tridecene	0.6	0.2	18.4	24.0
Tridecane	9.8	2.0	23.3	21.7
Tetradecane			0.2	0.2
Pentadecene			0.2	0.1
Pentadecane	11.1	1.7	5.7	0.2
Hexadecane			t	t
Dodecyl acetate	1.9	0.3		
Heptadecene	0.3	0.2	0.4	0.3
Heptadecane	0.7	0.5	2.9	2.0
Octadecene	1.7	0.7		
Octadecane	0.2	0.1		
Tetradecanol	t			
Nonadecene	0.4	0.3		
Nonadecane			1.6	1.1
7-Methylnonadecane	3.1	1.8		
Eicosane	0.9	0.9	0.3	0.3
7-Methyleicosane	1.2	0.3		
Heneicosene	0.6	0.3		
Heneicosane	13.8	2.9	3.7	3.9
Docosane	1.5	0.7	0.5	0.4
Tricosene	0.8	0.6		
Tricosane	6.2	2.6	1.6	2.7
Total amount (μg)	0.132		2.9	2.7

t = trace.

Table 2. The percentages of the compounds found in the mandibular glands of major workers, minor workers and males of *O. smaragdina*. Results are the means (with standard deviations) of 10 individual major workers, and one minor worker, and two males

Compound	Major workers %	Major workers SD	Minor workers	Males 1	Males 2
1-Hexanol			12.7		
Dimethyl trisulphide			t		
1-Heptanol			1.6		
3-Octanone				0.3	0.1
3-Octanol				t	
1-Octanol			0.3		
Limonene			1.3	0.4	0.2
1-Nonanol	4.0	1.9	29.2		
Nonanal	0.6	0.5	0.6		
3-Nonanone				0.1	
2-Nonanone				1.7	
Undecane				0.2	0.1
4-Methyl-3-heptanone				0.2	
1-Decanol	0.6	0.5			
Decanal			0.4		
3-Decanone	20.0	11.8		45.5	12.8
3-Decanol	8.4	4.7		11.9	15.2
3-Undecanone				t	t
3-Undecanone	0.7	0.6		7.5	4.4
3-Undecanol				2.1	1.1
Isogeraniol			1.9		
Nerol	3.4	1.9	46.2		
Geraniol	7.2	5.0	5.8		
Tridecane				0.2	
2-Dodecenol				0.4	0.5
3-Dodecanone				24.3	57.7
3-Dodecanol				0.6	7.7
Hexyl hexanoate	26.6	9.6			
Heptyl hexanoate	4.6	2.0			
Octyl hexanoate	14.4	8.4			
Nonyl hexanoate	1.9	1.6			
(Z,E)-α-Farnesol	1.1	1.3			
Geranyl butanoate	2.4	2.1			
Neryl butanoate	1.6	2.1			
Octyl octanoate	2.0	3.7			
Total amount (μg)	0.11		2.1	3.9	0.15

t = trace.

Mandibular glands

The mandibular glands of major workers contained chiefly the esters hexyl, heptyl, octyl and nonyl hexanoates (Table 2). The minor workers were quite different where it seems that the monoterpene nerol and 1-nonanol are the major substances, but only one sample has been given in Table 2, because there was a possibility of contamination in the second sample. The males contained 3-decanone and 3-dodecanone chiefly, together with their corresponding alcohols. Some samples of cleanly dissected mandibular glands of major workers were examined but most of the samples had other tissues attached. Both kinds of samples gave very similar gas chromatograms (Fig. 2).

DISCUSSION

The Dufour glands of major workers of *O. smaragdina* contain chiefly linear, saturated alkanes with

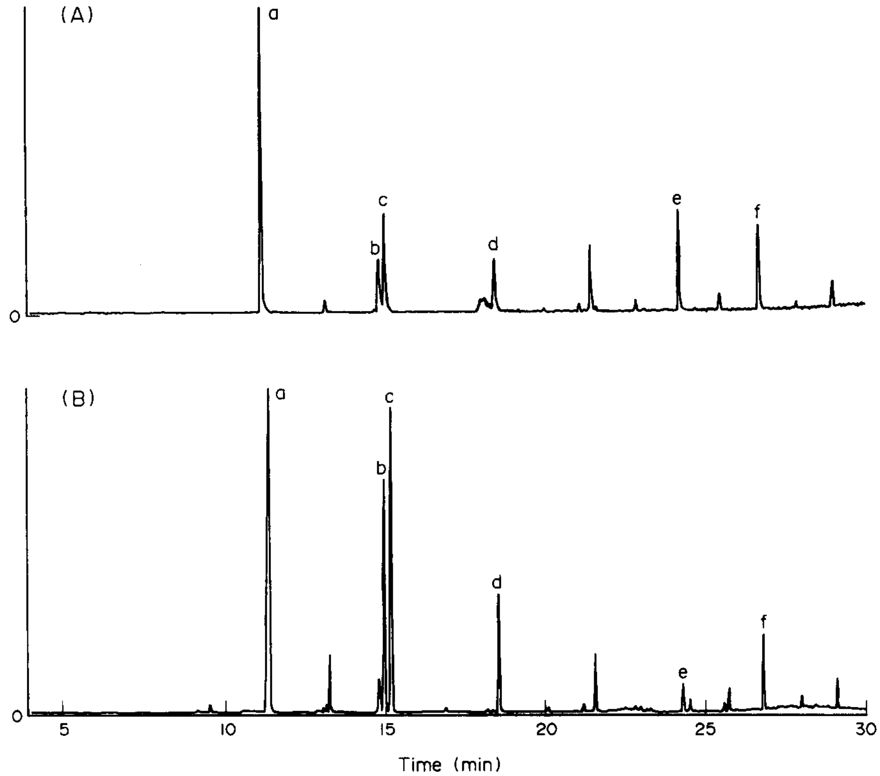

Fig. 1. Gas chromatograms of single Dufour glands of (A) a major worker of *O. smaragdina*, and (B) a minor worker. Labelled peaks are (a) undecane, (b) tridecene, (c) tridecane, (d) pentadecane, (e) nonadecane and (f) heneicosane.

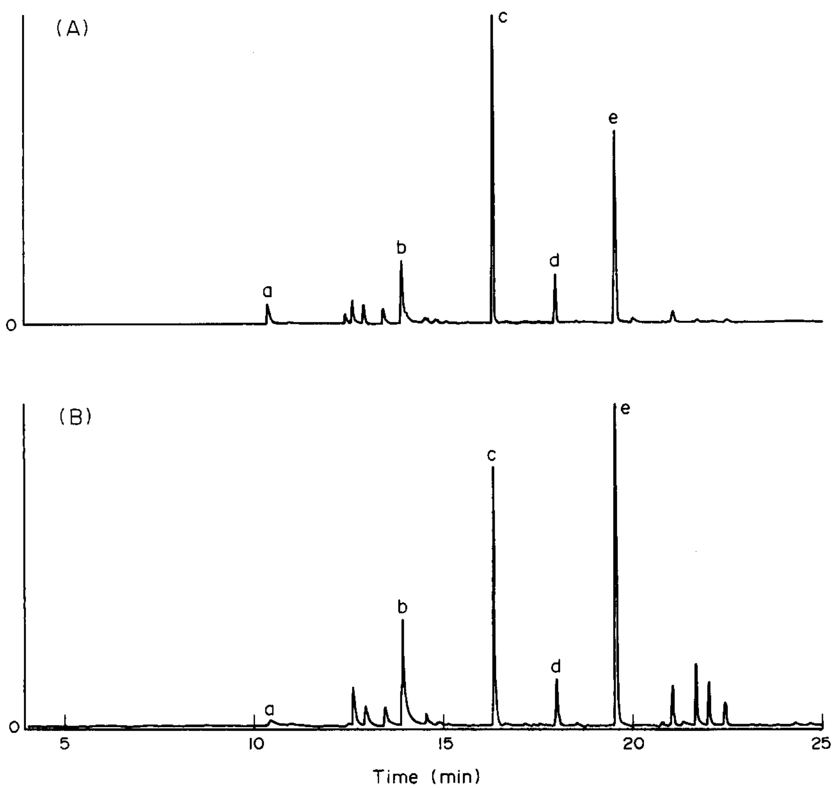

Fig. 2. Gas chromatograms of (A) a cleanly dissected pair of mandibular glands of a major worker of *O. smaragdina*, and (B) a similar pair of mandibular glands with other tissues attached. Labelled peaks are (a) 1-nonanol, (b) geraniol, (c) hexyl hexanoate, (d) heptyl hexanoate and (e) octyl hexanoate.

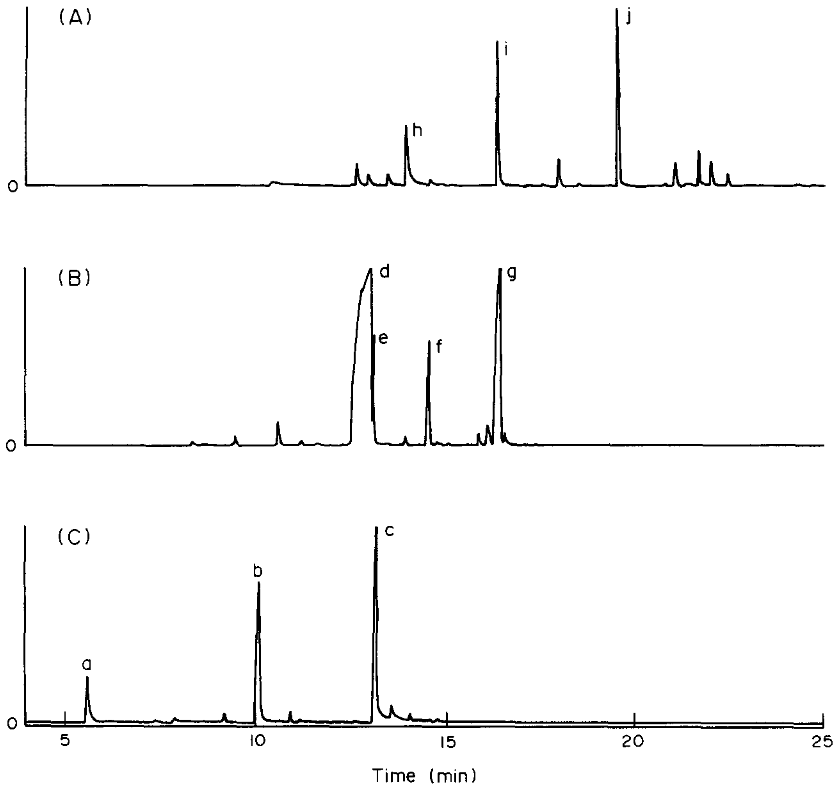

Fig. 3. Gas chromatograms showing the difference in composition of the mandibular glands of (A) a major worker, (B) a male, and (C) a minor worker, of *O. smaragdina* chromatographed under the same conditions. Labelled peaks are (a) 1-hexanol, (b) 1-nonanol, (c) nerol, (d) 3-decanone, (e) 3-decanol, (f) 3-undecanol, (g) 3-dodecanone, (h) geraniol, (i) hexyl hexanoate and (j) octyl hexanoate.

undecane (characteristic of formicine species) being the most important one. It is noteworthy that there are appreciable quantities (about 14%) of the relatively involatile heneicosane. The presence of higher mass, low volatile hydrocarbons is typical of species from tropical regions. There is a small but significant amount of the ester dodecyl acetate, again typical of a formicine species. Superficially the minor workers contained the same hydrocarbons, but there was less heneicosane and some tridecane and tridecene. There was no dodecyl acetate detectable in minor workers. This difference between major and minor workers has some resemblance to what we have reported in another formicine species *Camponotus aegyptiacus* (Ali *et al.*, 1988) where the major workers contain large amounts of esters, chiefly dodecyl acetate, while the minor workers contain none.

Bradshaw *et al.* (1979c) found undecane was the major substance in the Dufour glands of *O. longinoda* with heneicosane an important component. The differences were that they found a hexadecyl ester and large amounts of pentadecane and tricosane. With respect to major workers' Dufour glands, the two species can be said to be very similar. The minor workers of *O. longinoda* were very similar in composition to those of major workers of the same species but 10 times smaller and with 20 times less hexadecyl ester (Bradshaw *et al.*, 1979c). The contents of minor workers' Dufour glands can thus be said to be quite similar in the two species. Surprisingly, in spite of their smaller size, the minor workers of *O. smaragdina* contained rather large (μg) amounts of secretion in their Dufour glands (Table 1).

The mandibular glands of *O. smaragdina* and *O. longinoda* major workers contained some compounds in common (e.g. 3-undecanone) but there were also distinct differences, hexanol and hexanal of *O. longinoda* were not found in our work, nor was 2-butyl-2-octenal whereas Bradshaw *et al.* (1979a) did not find the esters which we found to be the major substances in *O. smaragdina*. Hexyl hexanoate has not previously been found in ants, but occurs in the metasternal scent of several species of Hemiptera (Aldrich and Yonke, 1975). It is interesting that hexyl hexanoate ($C_{12}H_{24}O_2$) the major substance of *O. smaragdina* has a similar volatility to the 2-butyl-2-octenol ($C_{12}H_{22}O$) of *O. longinoda* which Bradshaw *et al.* (1977, 1979a) showed was one of the major behavioural chemicals of the mandibular glands. Unfortunately we were unable to maintain our colony long enough to carry out behaviour tests on these substances.

The one good sample of a minor worker's mandibular glands of *O. smaragdina* with 1-nonanol and nerol as the principal components showed some correspondence to one sample of *O. longinoda* which had 1-octanol and nerol as principal components, but Bradshaw *et al.* (1979b) found considerable variations in the composition for different colonies. We must avoid concluding too much from our limited results on minor workers, for fear that *O. smaragdina* shows similar variability. The variation in amount of secretion in the mandibular glands is not unusual and similar to what we have found in some other species.

The mandibular glands of male *O. smaragdina* were very different from those of *O. longinoda*. The former contains 3-decanone, 3-dodecanone and smaller amounts of the corresponding alcohols, no acids were detected. The latter contained essentially 2,4-dimethylhexanoic acid and related saturated and unsaturated acids (Bradshaw *et al.*, 1979b). Evidently, it is in the male mandibular glands that the greatest difference between the exocrine secretions of these two species lies.

The distinctly different duties of major and minor workers of *O. smaragdina* is reflected in the very different volatile chemicals produced by the two castes and suggests very different roles for these chemicals in communication, and particularly for the substances of the mandibular glands.

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