and rubbed them with their antennae. When the colony was disturbed by sharp tapping that caused alarm reaction, the worker ants picked up the treated grits and ran about with them in the same manner as they normally do with brood. After 2-3 hours the grits were usually removed from the nest to the refuse pile.

(II) Tubercles and surrounding cuticle

The grits were placed in the arena near the entrance to the nest and after approximately 30 seconds, the ants foraging within 3.5cm became alerted Their rate of locomotion increased, they started making short fast runs with frequent changes of direction with mandibles usually held open and considerable antennal movement. The ants within 2cm were attracted directly towards the grits. Initially, as with the head extract of the ants, the ants were repelled from touching the grits, but after 5-10 minutes they began investigating them with their antennae. The ants then (in 40% of cases) picked up the grits and carried them into the nest. These grits were usually carried to the refuse pile after 1-2 hours. The grits not carried into the nest with the brood were usually investigated and tended by the ants for up to an hour. Some of these grits were then carried to the refuse pile.

C. Negative controls

Grits soaked in pure dichloromethane were ignored by the ants. If placed directly into the nest they were removed to the refuse pile after being discovered. Grits which were not treated in any way were completely ignored by the ants. If placed directly into the nest they were removed to the refuse pile after being discovered.

4.2.1.4 Gas chromatography of the volatile secretions of *A. capensis* and *A. dentatis*

The peaks on the chromatograms obtained from the two *A. dentatis* extracts were numbered and then compared using relative retention times with those of *A. capensis* (Table 6). The relative amounts of each chemical were also compared by taking the areas under the peaks and calculating the percentage of each (Table 7). For the abdominal extract of *A. capensis* (fig. 23), because of the large number of peaks, only those corresponding to the ones found on the *A. dentatis* chromatograms

Table 6. Gas chromatographic peaks with relative retention times for the extracts of *Acantholepis capensis* and *Aloeides dentatis* run on a Carbowax 20M column.

Peaks	Extracts				
	A. capensis			*A. dentatis*	
	Head	Abdomen	Brood	Thoracic area	Tubercles and surrounding area
1			0.386	0.381	
2			0.560	0.555	
3	0.668	0.660	0.670	0.665	0.670
4	0.707		0.710	0.705	0.709
5	0.748	0.748	0.750	0.743	0.750
6			0.856		0.859
7	0.892	0.887	0.893	0.888	0.895
8					0.933
9	0.960	0.955	0.958		0.962
10	1.000	1.000	1.000	1.000	1.000
11		1.022	1.025		1.025
12	1.060	1.052	1.060	1.058	1.062
13	1.113	1.105	1.108	1.100	1.113
14	1.170	1.168	1.175	1.168	1.174
15	1.203		1.210	1.200	1.200
16	1.241	1.243	1.248	1.243	1.243
17		1.268		1.268	
18	1.315	1.310			1.312

were numbered.

The retention times and the areas of the peaks as given by the Autolab Minigrator and the calculations for the tables can be found in Appendix II.

Table 7. The area of each peak on the chromatograms of *Acantholepis capensis* and *Alpeides dentatis* expressed as a percentage.

Peaks	Extracts			
	A. capensis		*A. dentatis*	
	Head	Brood	Thoracic area	Tubercles & surrounding area
1		7.4	10.1	+
2		7.5	17.0	+
3	4.8	1.3	1.4	0.9
4	2.4	1.2	4.5	1.0
5	4.9	7.0	3.9	2.0
6		5.2		4.0
7	20.2	7.0	4.9	3.9
8				2.8
9	4.5	3.3		3.4
10	2.2	7.8	5.0	3.8
11		6.7		7.4
12	17.2	7.8	6.2	14.4
13	10.2	7.8	12.0	8.7
14	12.4	12.1	10.0	9.7
15	6.3	4.4	12.2	12.1
16	3.2	13.5	5.8	14.1
17			7.0	
18	11.7			11.8
Total	100.0	100.0	100.0	100.0

A comparison of the chromatograms showed that a number of the peaks were common to all the extracts (Table 6), these are 5, 7, 10, 12, 13, 14, and 16. The abdominal extract (fig. 23) had relatively few peaks in common with the *A. dentatis* extracts. All the peaks on the head

(fig. 24) and brood (fig. 25) extract chromatograms has an equivalent in *A. dentatis*.

A. dentatis thoracic area chromatogram (fig. 26)

The peaks on this chromatogram correspond most closely to the peaks obtained on the brood extract chromatogram of *A. capensis* (Table 6). Peaks 1 and 3 were only found on the thoracic area and brood extract chromatograms and not at all in the others. Peaks 3, 4 and 15 were also common to the head extract chromatogram. Peaks 6, 9 and 11 were found in the brood extract but not in the thoracic area extract.

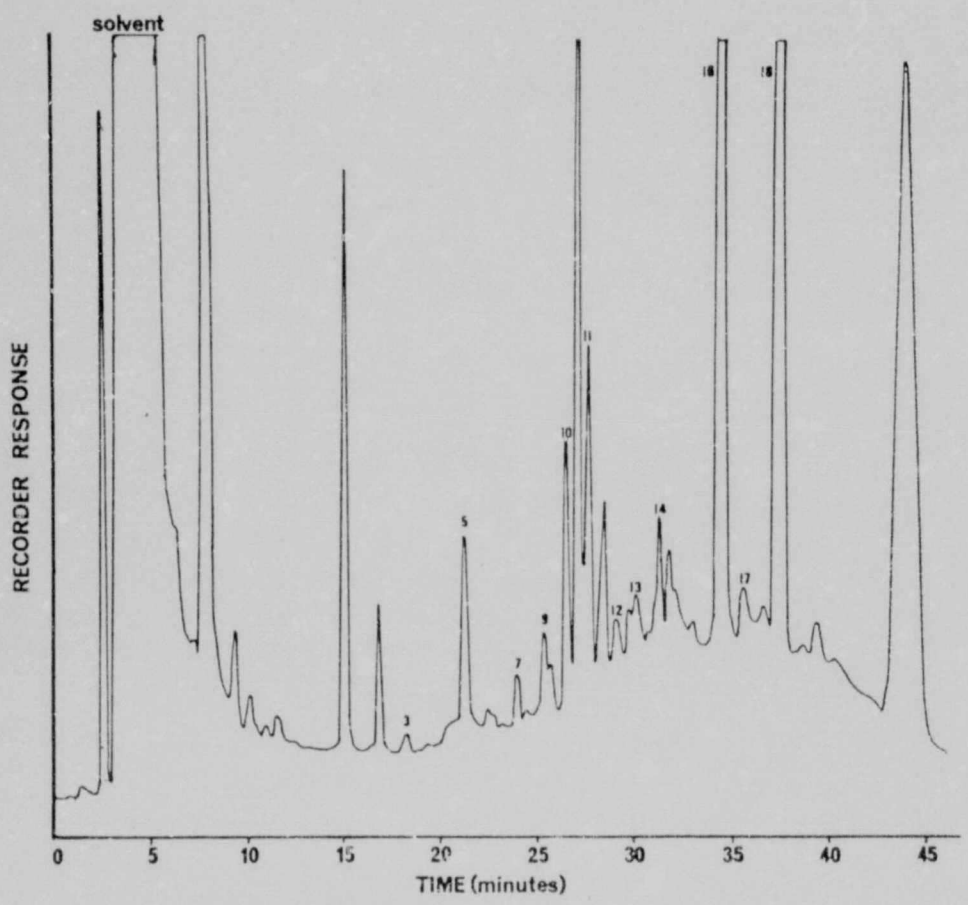


Fig. 23. Gas chromatogram of the volatile material from the abdomen of *A. capensis*.

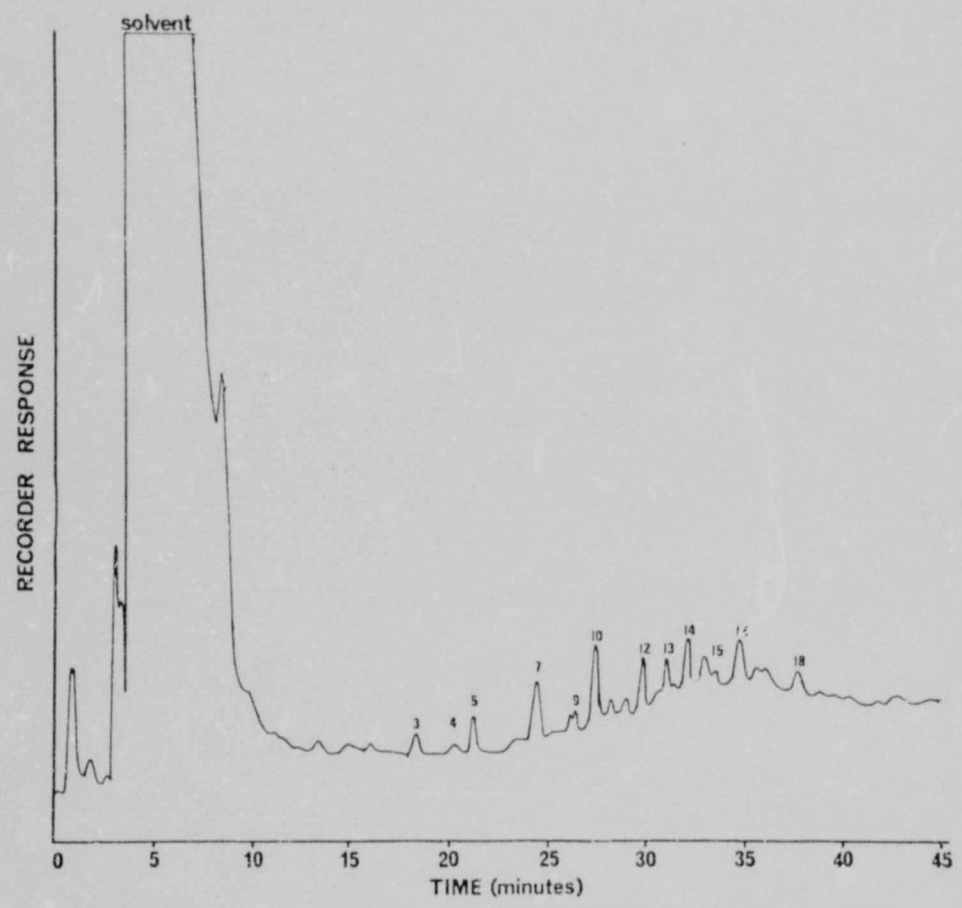


Fig. 24. Gas chromatogram of the volatile material extracted from the head of *A. capensis*.

The relative amounts of each chemical producing the peaks on the thoracic area and brood extract chromatograms can be seen on Table 7. Considering the crudity of the extracts and the fact that all the peaks are not common to both the relative amounts of each chemical in the brood and thoracic area extracts are remarkably similar. Therefore from these results it appears that the crude extracts of the volatile secretions from the thoracic area of *Aloeides dentatis* appear to mimic the crude extracts of the brood pheromone of *A. capensis*.

A. dentatis tubercles and surrounding cuticle (fig. 27)

The peaks obtained on this chromatogram appear to be made up of a mixture of those from the head and brood extract chromatograms. All the peaks on the head extract chromatogram are to be found on the tubercle area chromatogram. The remaining peaks on the latter are to be found on the brood extract chromatogram (Table 6). Peaks 1 and 2 found in the brood extract chromatogram are usually at too low a concentration to give a response in the tubercle area extract, although they do appear in two of the ten chromatograms run.

As this chromatogram appears to mimic both the alarm and brood

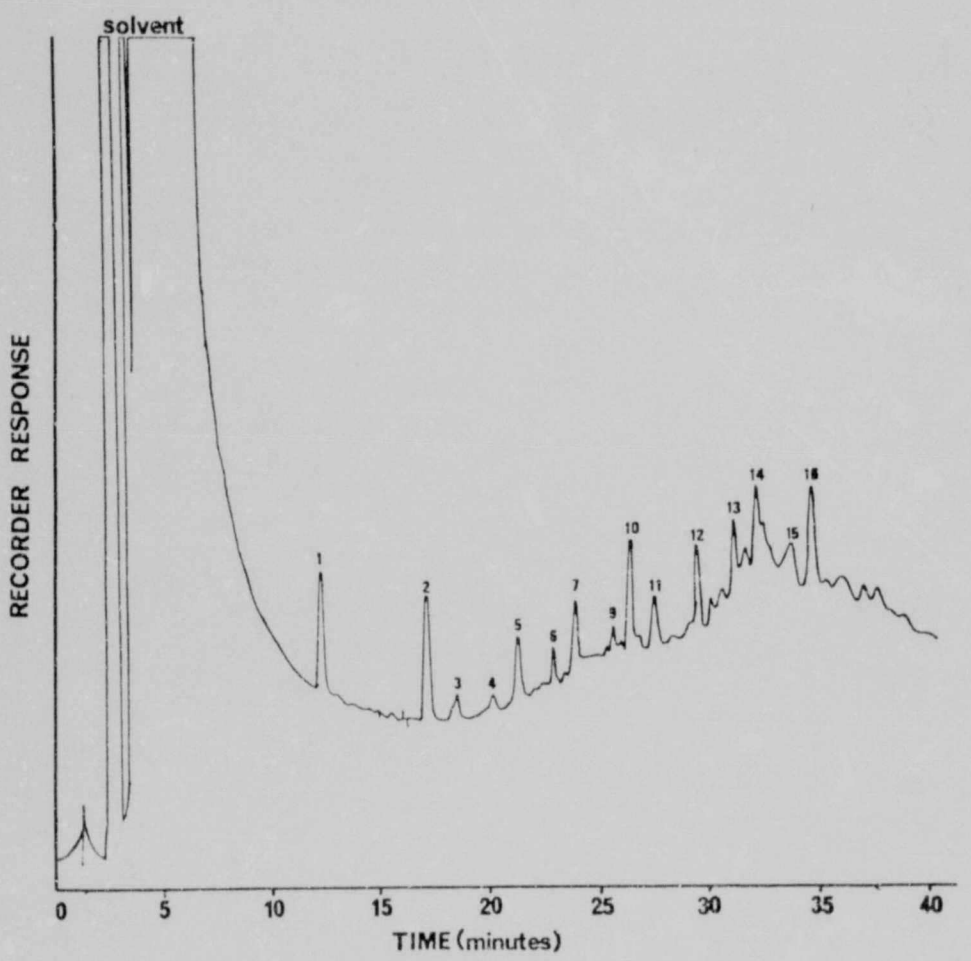


Fig. 25. Gas chromatogram of the volatile material extracted from the brood of *A. capensis*.

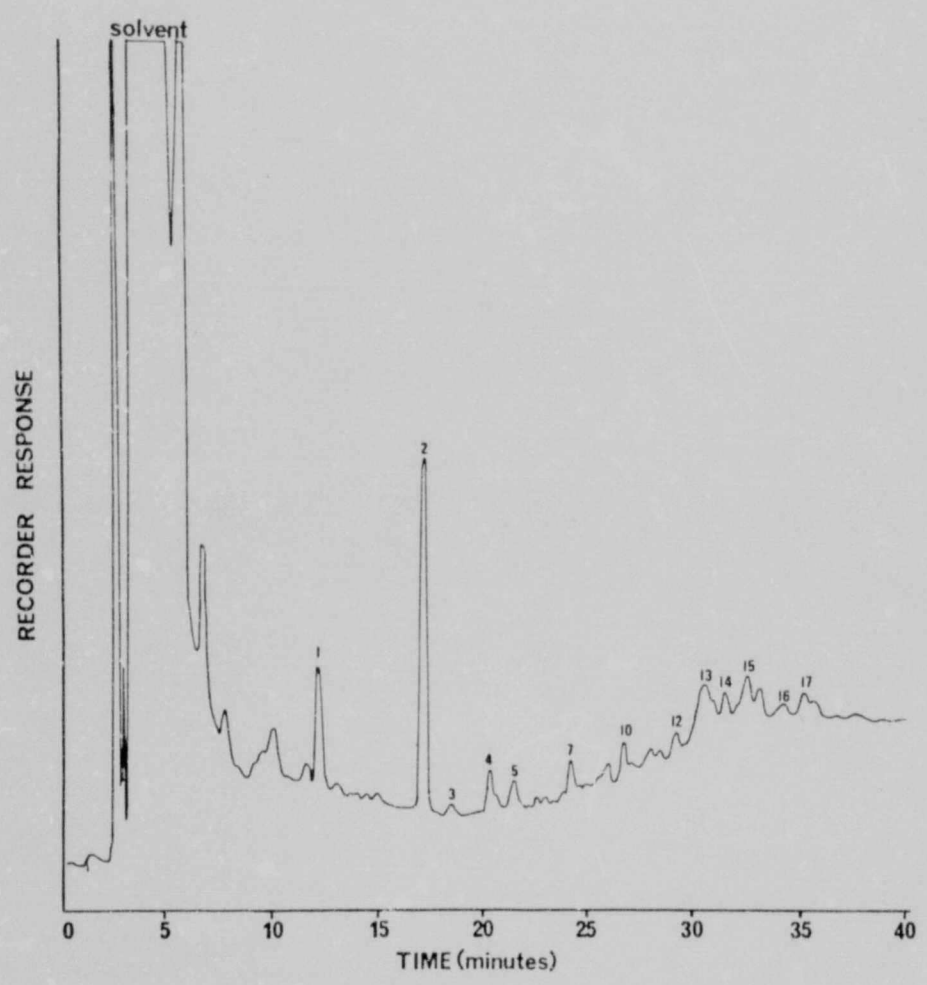


Fig. 26. Gas chromatogram of the volatile material from the thoracic area of *A. dentatis*.

extracts of *A. capensis* , the relative amounts of each chemical were difficult to compare, although the results are not too inconsistent from what would be expected from a mixture of these extracts (Table 7). One must also remember that the behavioural response may be released by only one or two of the chemicals indicated on the chromatograms

From these results then it appears that the crude extract of the tubercle area of *A. dentatis* produces a volatile secretion which appears to be a mixture of the alarm and brood pheromones of *A. capensis*. The

alarm component eminating from the tubercles themselves, the brood component from the perforated cupolas surrounding the tubercles.

4.2.1.5 Conclusion

From the above study it appears as if the association between *A. dentatis* and *A. capensis* is mediated by mean_ of chemical signals. From the experiment with the corn cob grits it was demonstrated that *A. dentatis* produces a volatile secretion which releases a behavioural response in the ants similar to the one shown by them to their own brood. This volatile chemical compound of *A. dentatis* also gave a chromatographic

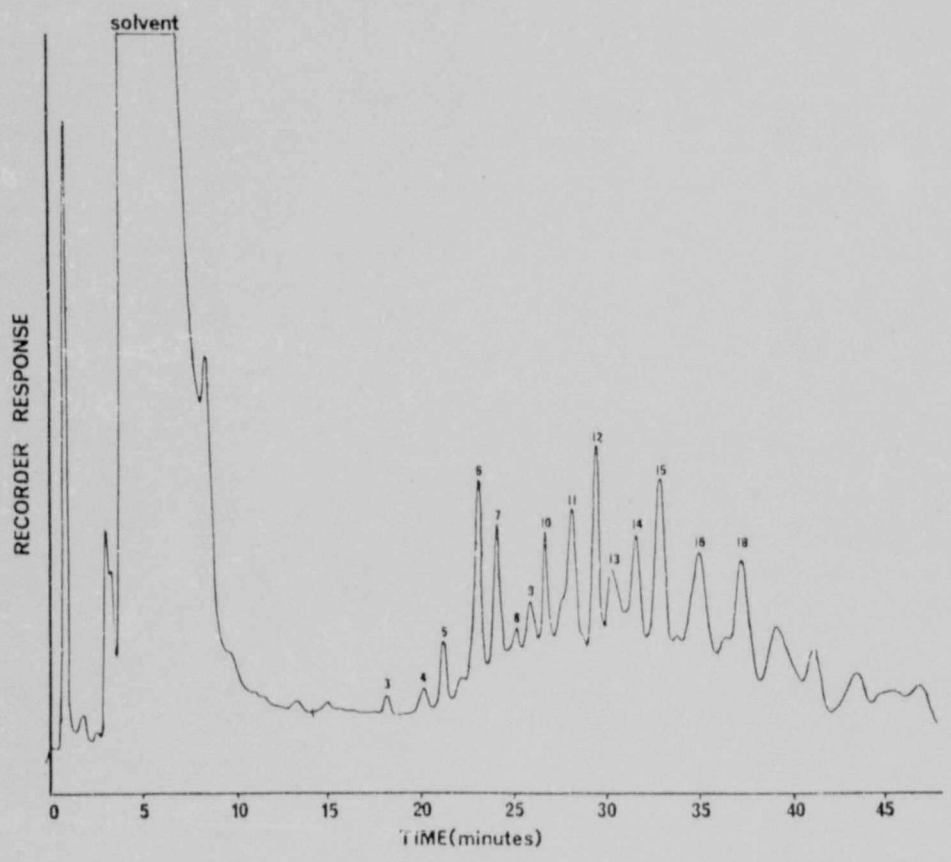


Fig. 27. Gas chromatogram of the volatile material extracted from the tubercles and surrounding cuticle of *A. dentatis*.

fingerprint pattern rather similar to that of the brood extract chromatogram of *A. capensis*. It was also found that a volatile chemical compound produced by the tubercles of *A. dentatis* elicited an alarm reaction in the ants and had a gas chromatographic picture rather similar to that of the head extract chromatogram of *A. capensis*.

The 'mimic' of the brood pheromone is most likely produced by the perforated cupolas which were the only glands common to both areas of cuticle under study. The gland that produced the alarm pheromone was not located (see p. 34), but there are probably pyriform secreting cells at the base of the setae of the tubercle of the type described by Ehrhardt (1914). Ehrhardt and other authors suggested that the pyriform cells secreted an odiferous substance, but were never able to prove it (see p. 34).

4.2.2 *Lepidochrysops ignota* (Trimen) and *Camponotus niveosetosus* Mayr.

4.2.2.1 Field observations

A. *Lepidochrysops ignota* (Trimen)

(I) Eggs and phytophagous larval instars

In the study area the foodplant is *Becium obovatum* (Benth) N.E.Br. (Labiatae) which flowers from September to November. The eggs are usually laid singly on the flowers, the seeds of which the young larvae are later to feed. The eggs take about six days to hatch. The first and second instars feed on the seeds (achenes) enclosed within the dry persistent calyx of the plant. The only indication that there is a larva within the calyx is the hatched egg on the outside. After the moult to the third instar, the larvae disappear from the *B. obovatum* inflorescences and become associated for the remainder of their annual cycle (some 10-11 months) with the host ants.

(II) Myrmecophilous larval instars

Unfortunately no specimens were found in the field. Unlike the *Camponotus maculatus* Fabr. in the area, the host ant *C. niveosetosus*

did not appear to bring their brood to the surface beneath stones, but kept them some distance below the soil surface, which made investigation of their nests rather difficult. Attempts were made to dig up nests but without success.

(III) Pupae

No pupae were found in the field for the above reasons.

(IV) Egg laying behaviour of female

In the study area the distribution of *L. ignota* is rather limited, the species being confined to one short rocky ridge and adjacent grassland. The host ants *C. niveosetosus* were also only found along this ridge although the host plant *B. obovatum* is common throughout the whole area. The flowers of *B. obovatum* growing along the ridge were being continually visited by *C. niveosetosus* workers. It was mainly on these flowers that *L. ignota* females were seen to oviposit and where the majority of eggs were found.

A *L. ignota* female would flutter around the flowers. She would alight briefly and pass her antennae over the buds and flowers and if conditions were satisfactory, would curve her abdomen around and lay a single egg. To establish whether it was the presence of the ants that induced the laying a similar experiment to the one carried out on *A. dentatis* was tried.

Twenty female *L. ignota* were captured. Ten were placed in a container with the foodplant alone and ten with foodplant plus host ants. The results were not as clear cut as in the case of *A. dentatis*, because *L. ignota* proved to be rather fragile. *A. dentatis* lived as long as three weeks in captivity, while not a single *L. ignota* female lived longer than five days. With *A. dentatis* some females took as long as a week to settle down before starting to lay.

Of the ten *L. ignota* females housed with foodplant alone not a single egg was laid. Out of the ten females housed with the foodplant and ants three of them laid eggs. It appeared that the presence of the ants plus foodplant may be necessary to induce the *L.ignota* female to lay.

B. *Camponotus niveosetosus* Mayr

This is a dull black ant with characteristic coarse, bristly white pilosity present mainly on the abdomen. The major workers are 8mm long and the minor workers are 6mm. The queen is similar in colour and pilosity to the workers and is about 11mm long. The pupae are enclosed in thin creamy-white cocoons.

It nests in the soil and the colonies are never very large, usually consisting of some hundred individuals. In the study area *C. niveosetosus* was not observed to bring the brood up under stones after rain.

This is a diurnal species and the workers can be observed visiting flowers in the vicinity of their nests. The workers appear to find the flowers of *Becium obovatum* particularly attractive.

C. Mode of transport of third instar *L. ignota* larvae to the nest of *C. niveosetosus*

Camponotus niveosetosus is diurnal and, in the field, third instar *L. ignota* larvae are encountered during the day by foraging workers. During the course of the study five third instar *L. ignota* larvae were placed on *Becium obovatum* inflorescences seen to be frequented by *C. niveosetosus* workers. It was observed that a foraging worker upon encountering one of the larvae on the inflorescences would touch and stroke it with its antennae usually in the vicinity of the honey-gland but also in the thoracic region. The ant would then proceed to make drumming movements (palpation) with its antennae in the vicinity of the honey-gland. It was not observed whether the larvae responded to this treatment by exuding liguid form the honey-gland as Claassens (1976) found with *L. trimeni* and *L. methymna*.

The third instar larvae used in these observations had already stopped feeding and had wandered off their foodplants. All five of these larvae were picked up and carried away by the ants. The larvae rolled themselves up when the ants attempted to pick them up by closing their mandibles between the last thoracic and first abdominal segments. This allowed the ants to carry the larvae with ease (fig. 28). One worker carried a larva from the flower-head to its nest some 3 metres away. The other larvae were carried to nests within a 2 metre radius of the flowers.

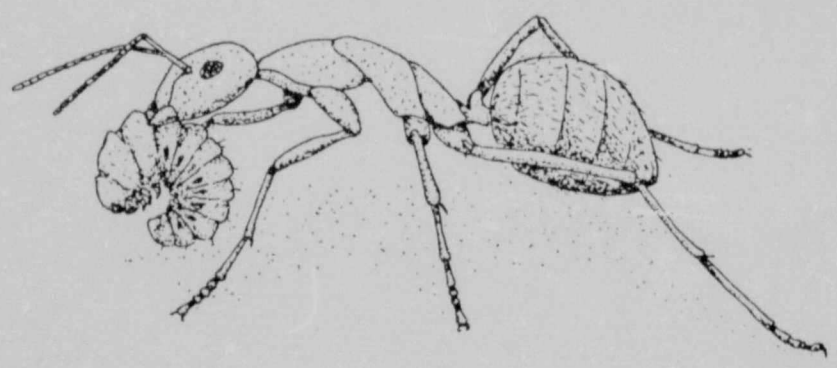


Fig. 28. *Camponotus niveosetosus* Mayr worker carrying a third instar *Lepidochrysops ignota* (Trimen) larva.

4.2.2.2 Formicarium observations

A. *Lepidochrysops ignota* (Trimen)

(I) Description of early stages

For a full description of the egg, larval and pupal stages see Appendix III.

(II) Interaction between *L. ignota* larvae and *C. niveosetosus*

The third instar *L. ignota* larvae that had stopped feeding and had started wandering around were either placed directly on the floor of the arena, or the inflorescence they were on was placed in the arena. The larvae responded to handling by rolling themselves into tiny spheres as they do when being picked up by the ants.

The third instar larvae were treated in a similar manner by the ants as were those in the field observations. Upon encountering an *L. ignota* in the arena an ant would touch and stroke it with its antennae usually in the vicinity of the honey-gland, but also in the thoracic region. The ant would then proceed to make drumming movements (palpation) with its antennae in the vicinity of the honey-gland. One of the larvae under observation was seen to respond by exuding a liquid from the honey-

gland. The ant would then grasp the larva with its jaws between the last thoracic and first abdominal segments while the larva rolled itself up and was carried into the nest.

Larvae placed directly into the nest were usually picked up immediately and carried to the brood without preliminary trok This is probably in part due to the fact that the ants wer. -i at the opening of the nest and were running about carrying brood.

Having been deposited amongst the ant brood the larvae remain in the 'rolled up' position for some time. Later they crawl around between the brood of the ants. The larvae are at first investigated by some ants, but thereafter no more attention is paid to them than to the rest of the brood. While investigating the lycaenid larvae with their antennae the ants appear to concentrate on the thoracic and honey-gland areas. It has been suggested in the past that the ants pay more attention to the guests than to their own brood, but I was unable to confirm this from my own observations. The *L. ignota* larvae feed on ant larvae and pupae.

One example of what appeared to be trophallaxis was observed between an *L. ignota* larva and a worker ant. The larva raised its head so that its mouth-parts became visible. An ant was observed putting its mandibles on the mouth-parts for approximately thirty seconds, but whether there was actual exchange of liquid could not be determined. Claassens (1976) observed similar behaviour in *Lepidochrysops methymna* when brood was scarce in the nest. The larvae of several non-South African species of lycaenid have been observed to be fed by ants, including *Maculinea alcon* (Elfferich, 1963; Malicky, 1969).

4.2.2.3 Reaction of the ants to the glandular extracts absorbed on to corn cob grits

The following behavioural responses were obtained from worker ants of *C. niveosetosus* which discovered the grits.

A. *C. niveosetosus* extracts

(I) Head extract (Table 8)

The grits were placed in the ar ıa and after some 30-45 seconds the

worker ants within 3-6cm were alerted. Their rate of locomotion increased, the mandibles were opened and there was considerable antennal movement. The ants within 3cm were also alerted, but they were attracted towards the grits. Upon reaching the grits the ants explored the air around them, stretching forward and palpating with their antennae, but not actually touching the grits. Occasionally they would run off a short distance, but they soon returned to the grits palpating in the air with their antennae the whole time. This behaviour lasted approximately 15 minutes until most of the material on the grits had dispersed. After this time the ants approached closer and passed their antennae over the surface of the grits. In several instances the grits were picked up and carried to the brood in the nest, but usually they were just left after a brief investigation. The grits carried into the nest were usually removed to the refuse pile after about an hour.

(II) Abdominal extract (Table 8)

The grits were placed in the arena and after some 30-60 seconds the workers within approximately 2cm raised their antennae and swept them through the air and then ran in the general direction of the grits. They would approach the grits and investigate them with their antennae. A single worker ant would often spend as long as five minutes passing its antennae over the grits. After this time the ant would appear to lose interest and wander off, occasionally returning to investigate the grits once more. The grits were generally given a cursory examination by the worker ants for about 25-30 seconds. After about an hour the grits were being ignored or had been removed to the refuse pile.

(III) Brood extract

The grits were placed in the arena near the entrance of the nest. After 30-60 seconds the worker ants within a radius of some 2cm had made their way towards the grits palpating the whole time in the air with their antennae. The ants briefly examined the grits with their antennae and then picked them up and carried them into the nest as if they were brood. The grits were deposited amongst the brood in the nest. The worker ants appeared to care for the treated grits as they did their brood. They groomed the grits and rubbed them with their antennae and

Table 8. The reactions of *Camponotus niveosetosus* workers to various glandular extracts absorbed on to corn cob grits.

Glandular extracts on grits	n	Reactions of *C. niveosetosus* workers			
		no reaction	Alarm Behaviour	Investigation	Carrying & brood tending behaviour
C. niveosetosus					
Mandibular gland	30		30		8'
Abdominal extract	30	2		28	
Brood extract	30			1	29
L. ignota					
Thoracic area	30	1		1	28
Honey-gland & surrounding area	30	1		2	27
Dichloromethane	30	30			
Untreated	30	30			

n = number of individual grits

' = After alarm behaviour

palpi. After about six hours the ants started removing the grits from the nest to the refuse pile.

When the colony was disturbed by making a sharp tapping that caused an alarm reaction, the worker ants picked up the treated grits and ran about with them in the same manner as they normally do with brood.

B. *L. ignota* extracts

(I) Thoracic area extract (Table 8)

The grits were placed in the arena near the entrance to the nest and after some 30-50 seconds the worker ants within 2cm raised and extended their antennae and swept the air with them. They then approached the grits and investigated them by passing their antennae over the surface. The ants then picked up the grits and carried them to the brood in the nest. In the nest the grits were treated as if they were ant brood. The ants groomed the treated grits, rubbed them with their antennae and palpi.

When the colony was disturbed by sharp tapping that caused alarm reaction the worker ants picked up the treated grits and ran about with them in the same manner as they normally do with brood. After about six hours the grits were removed from the nest to the refuse pile.

(II) Honey-gland and surrounding cuticle (Table 8)

The response obtained with the grits treated with this extract was essentially identical to the response obtained with the thoracic area extract. The treated grits were picked up and carried into the nest by the worker ants and deposited amongst the brood. The worker ants treated the grits as they did their own brood. The grits were removed to the refuse pile after about six hours.

C. Negative controls (Table 8)

Grits soaked in pure dichloromethane were ignored by the ants. If placed directly in the nest they were removed to the refuse pile after being discovered. Grits which were not treated in any way were completely ignored by the ants. If placed directly into the nest they were removed to the refuse pile after being discovered.

4.2.2.4 Gas chromatoraphy of the volatile secretions of *Camponotus niveosetosus* and *Lepidochrysops ignota*

The peaks on the chromatograms from the two *L. ignota* extracts were numbered and compared using relative retention times with those of *C. niveosetosus* (Table 9). The relative amounts of each chemical were

Table 9. Gas chromatographic peaks with relative retention times for the extracts of *Camponotus niveosetosus* and *Lepidochrysops ignota* run on a Carbowax 20M column.

Peaks	Extracts				
	C. niveosetosus			*L. ignota*	
	Head	Abdomen	Brood	Thoracic area	Honey-gland & surrounding area
1	0.416				
2	0.496			0.494	0.492
3	0.550				
4	0.592				
5	0.670	0.675		0.672	0.673
6	0.712		0.706	0.714	0.713
7	0.875	0.880		0.882	0.881
8	0.895		0.891		
9	0.908	0.913	0.908	0.914	0.913
10	1.000	1.000	1.000	1.000	1.000
11		1.074	1.069	1.075	1.072
12			1.090	1.098	

also compared by taking the areas under the peaks and calculating the percentage of each (Table 10).

The retention times and the areas of the peaks as given by the Autolab Minigrator and the calculations for the tables can be found in

Appendix III.

Comparison of the chromatograms showed that only two peaks were common tc all the extracts (Table 9), these are peaks 9 and 10.

L. ignota thoracic area chromatogram (fig. 32)

The thoracic area chromatogram has all but one of the peaks found in the brood extract chromatogram of *C. niveosetosus*. The peaks common to the brood extract chromatograms are 6, 9, 10, 11, 12 and the one

Table 10. The area of each peak on the chromatograms of *Camponotus niveosetosus* and *Lepidochrysops ignota* expressed as a percentage.

Peaks	Extracts			
	C. niveosetosus		*L. ignota*	
	Head	Brood	Thoracic area	Honey-gland & surrounding cuticle
1	2.8			
2	5.6		1.5	1.4
3	4.8			
4	4.3			
5	8.6		1.7	1.6
6	4.7	1.6	1.2	1.4
7	2.6		4.1	5.3
8	4.1	7.5		
9	23.9	6.7	6.2	8.9
10	38.6	10.8	22.3	17.2
11		47.8	46.7	64.2
12		19.6	16.3	
Total	100.0	100.0	100.0	100.0

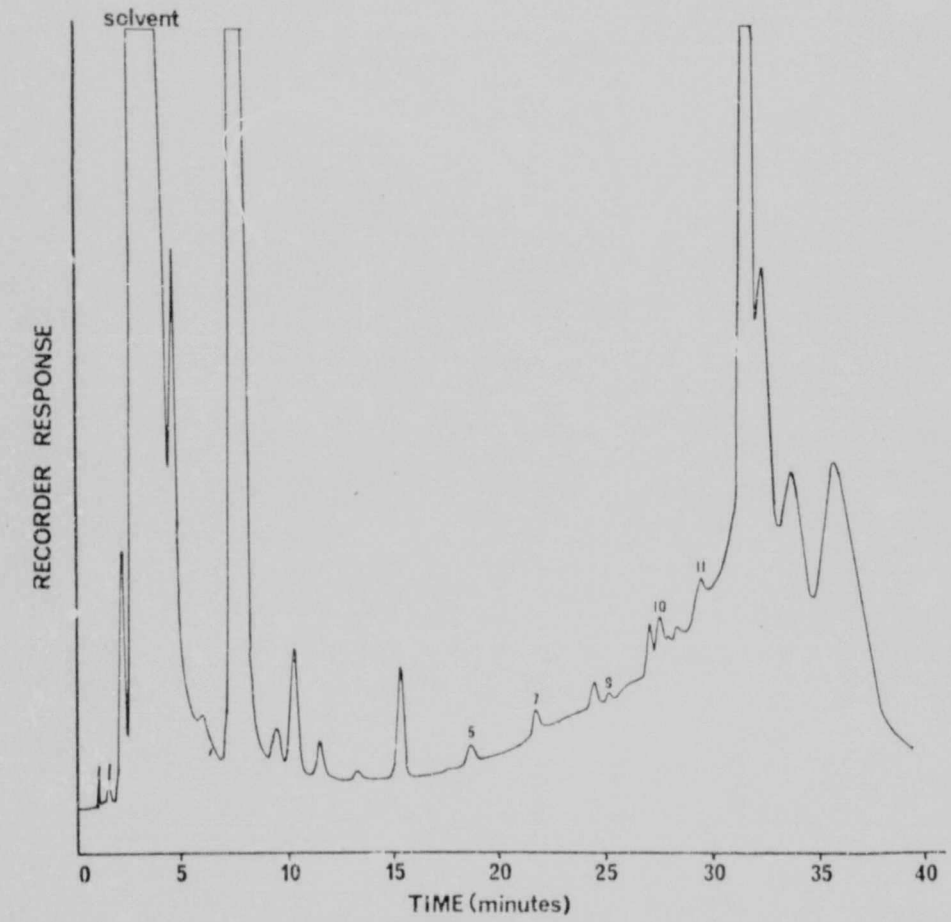


Fig. 29. Gas chromatogram of the volatile material from the abdomen of *C. niveosetosus*.

missing is 8 (Table 9). The other peaks on this chromatogram are common to that of the head extract (fig. 30).

The relative amounts of the chemicals involved in this chromatogram are remarkable similar to that of the brood extract chromatogram of *C. niveosetosus* (Table 10). The major peak on both chromatograms is peak 11 and it makes up nearly fifty percent of the extract. Two other peaks 10 and 12 make up the majority of the rest of the extract, both being about 20 %.

From t[illegible]esults one can say that the crude extract of the

volatile secretions from the thoracic area of *Lepidochrysops ignota* gives a similar "fingerprint pattern" to the crude extract of the brood of *Camponotus niveosetosus* (fig. 31) and thus appears to mimic it.

L. ignota honey-gland and surrounding cuticle (fig. 33)

The peaks obtained in this chromatogram are identical to those on the thoracic area one, except for peak 12 which is missing. The peaks common to the brood extract chromatogram (fig. 31) are 6, 9, 10, 11 and those missing are 8 and 12. Peaks 2, 5 and 7 are again common to the head extract (Table 9).

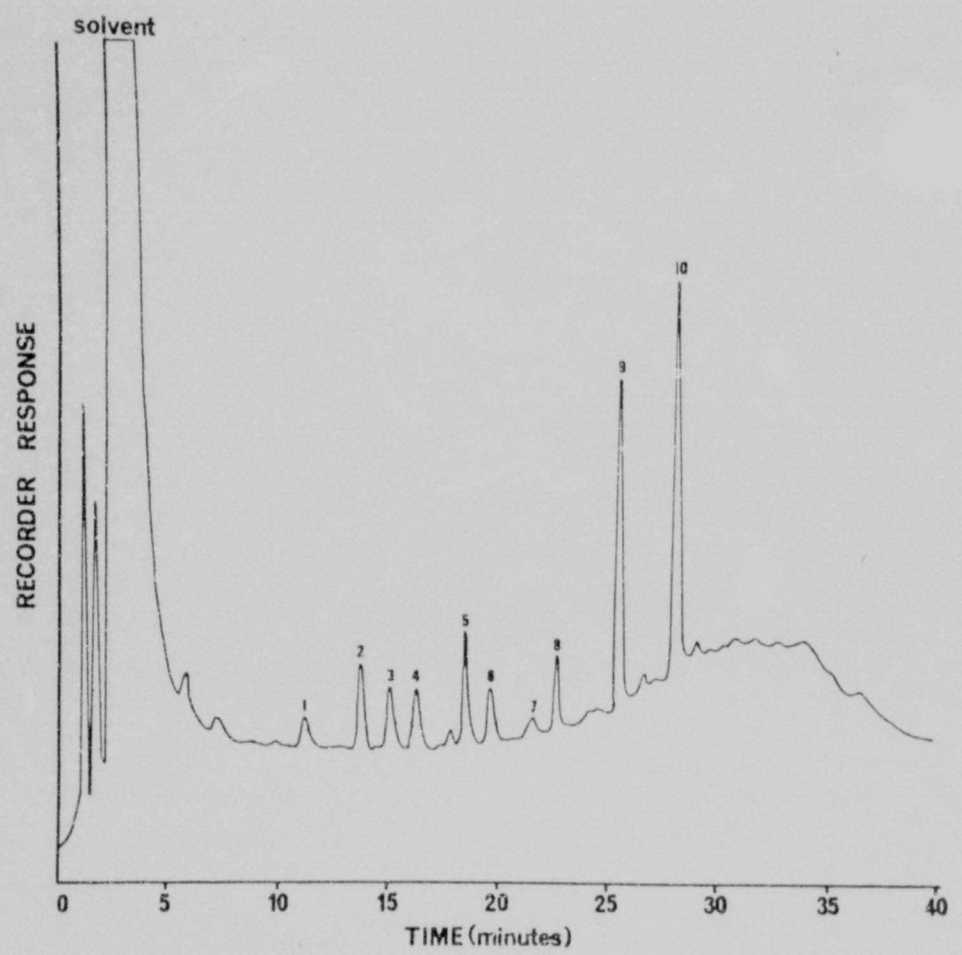


Fig. 30. Gas chromatogram of the volatile material extracted from the head of *C. niveosetosus*.

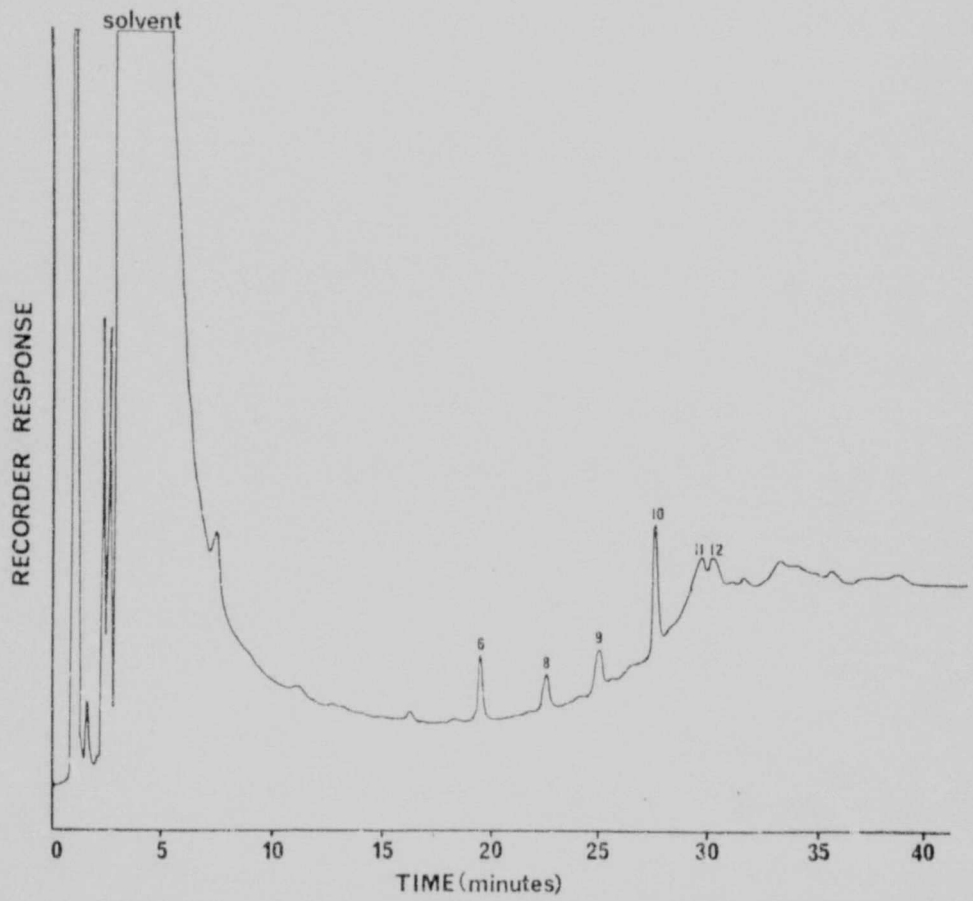
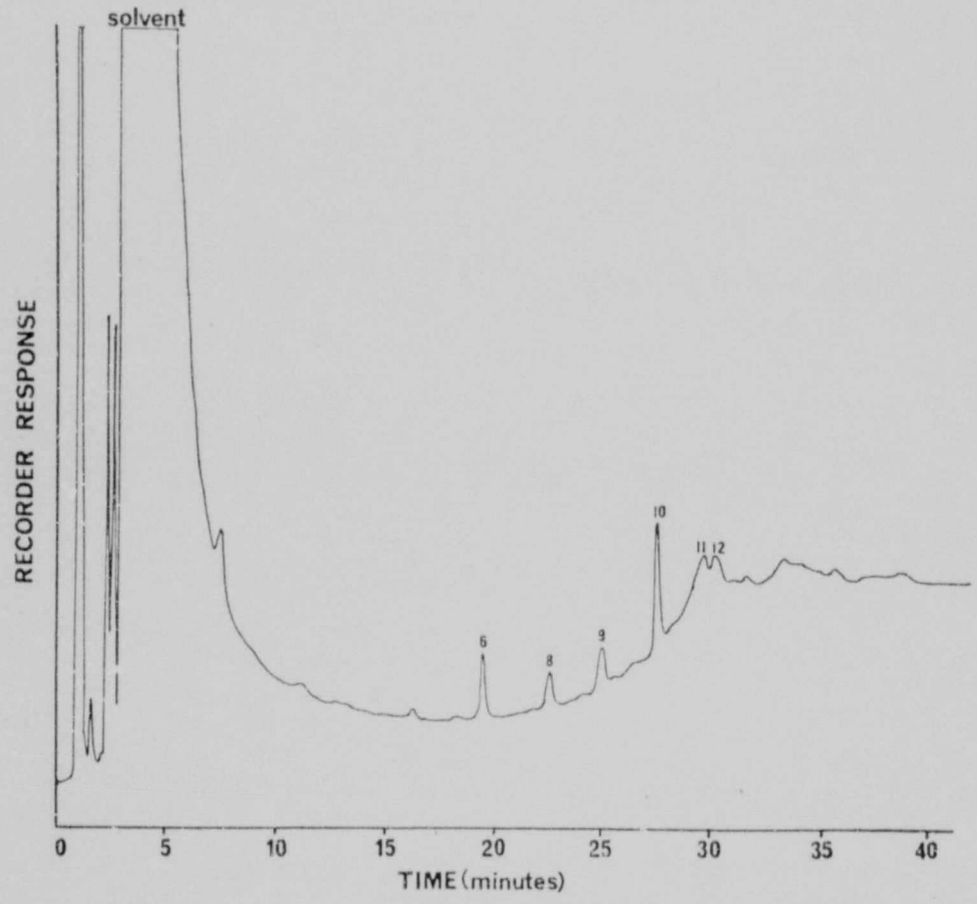


Fig. 31. Gas chromatogram of the volatile material extracted from the brood of *C. niveosetosus*.

The percentage areas of the peaks involved are again remarkably similar to that of the brood extract (Table 10). Peak 11 provides 64.2% of the volatile chemical produced which is a far higher proportion than in the brood and thoracic area extracts. The missing peak 12 lies very close to peak 11 therefore these two chemical substances may well have passed through the coloumn together giving this high result, because if you combine the areas of peaks 11 and 12 in the brood and thoracic extracts they are 67.4% and 63% respectively (Table 10).



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From these results it appears that the crude extract of the honey-gland and surrounding cuticle gives a similar picture to that of the crude extract of the brood of *C. niveosetosus* indicating that the larva may produce a volatile chemical that mimics the brood pheromone of its host ant.

4.2.2.5 Conclusion

From the above study it appears as if the association between *L. ignota* and *C. niveosetosus* is mediated by chemical signals. The

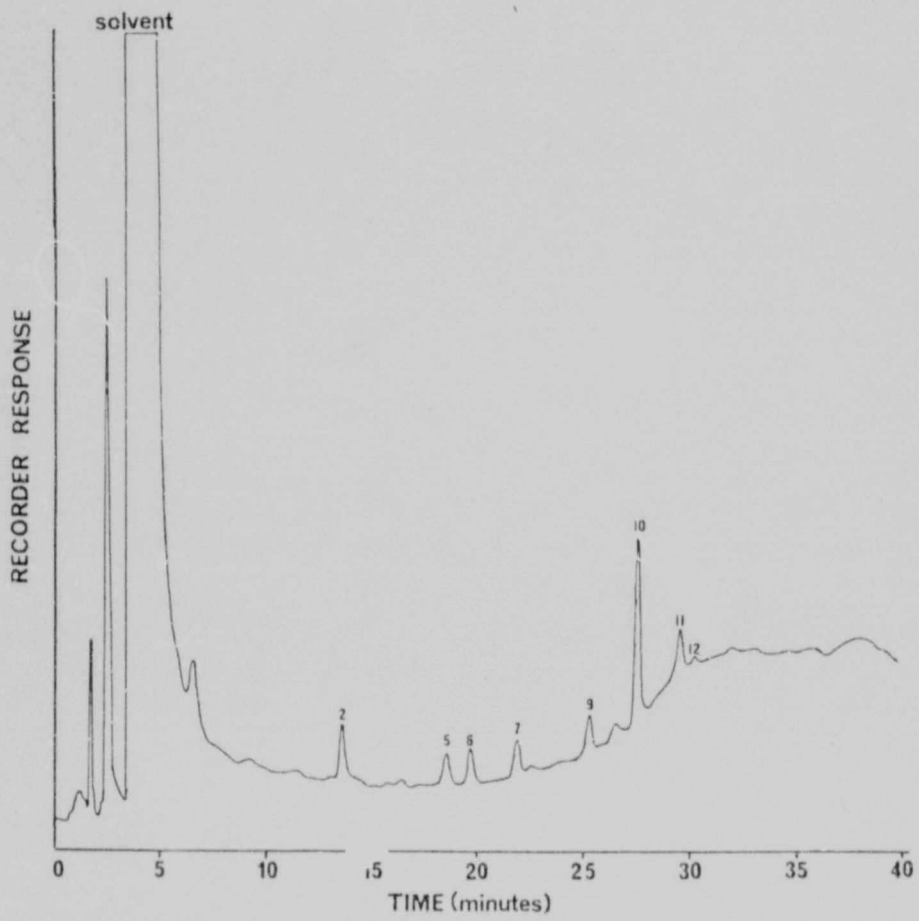

Fig. 32. Gas chromatogram of the volatile material extracted from the thoracic area of *L. ignota*.

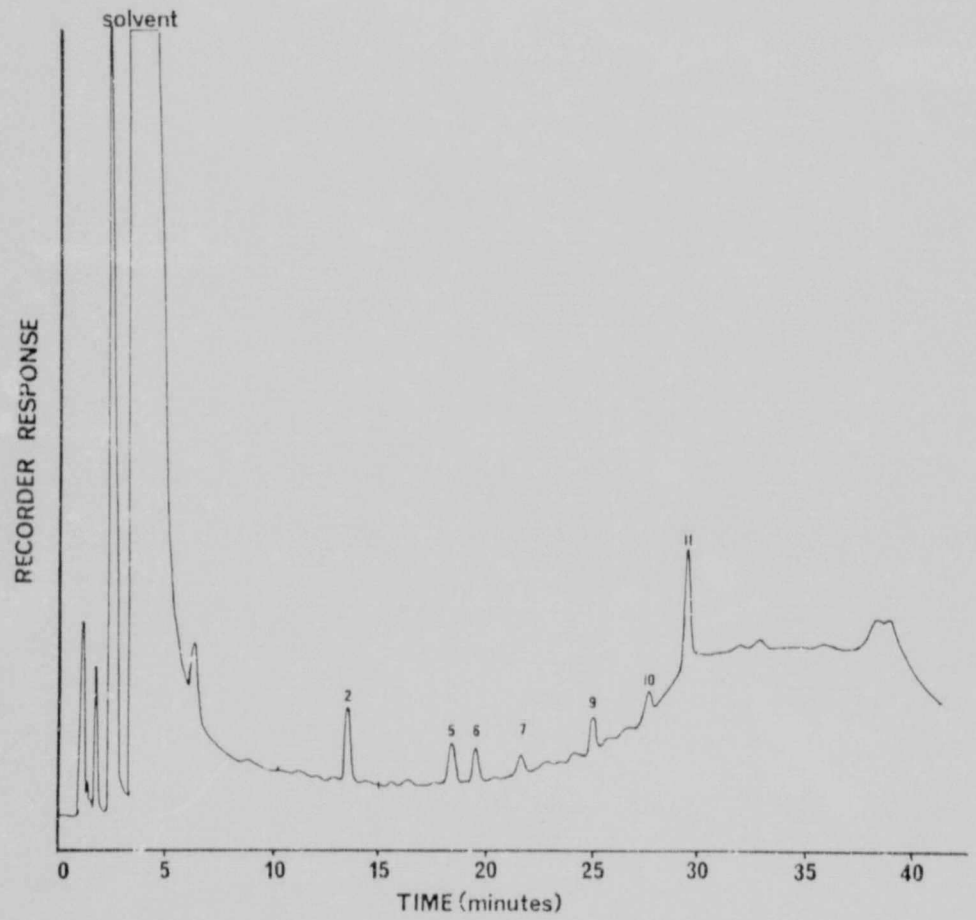

Fig. 33. Gas chromatogram of the volatile material extracted from the honey-gland and surrounding cuticle of *L. ignota*.

volatile chemical secretion produced by the *L. ignota* larvae gives a gas chromatographic fingerprint pattern rather similar to that of the brood extract chromatogram of *C. niveosetosus*. There are also several peaks in common with the head extract chromatogram which suggests that it may also mimic certain components of their hosts alarm pheromones, i.e. ones that are involved in attraction (see p. 108).

The behavioural experiments showed that this volatile secretion attracted the ants and induced them to carry the larva as it would its