PROCESSORS & GROWERS RESEARCH ORGANISATION

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Project Leader:	Dr. A. J. Biddle
Project Collaborator:	Professor J. Lofqvist (Lund)
Location of Project:	Processors and Growers Research Organisation Great North Road Thornhaugh Peterborough
and	Swedish University of Agricultural Sciences Lünd Sweden
Project Coordinator:	P. Shepherd
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CONTENTS

RELEVANCE TO GROWERS

The objective of the project was to develop a pheromone based monitoring system for the control of pea midge in vining peas. Because timing of application of insecticides is critical for effective control of the adults before eggs are laid, a simple to use monitoring system would allow the susceptible crops to be identified and to determine the correct spray timing.

Identification of the full pheromone compound was incomplete although some preliminary field trials with a trapping system were carried out.

Work on the biology of the midge showed the times of peak activity thereby identifying the time of day at which midge migrate into the pea crop.

Trials with a pyrethroid insecticide showed its effectiveness in control of pea midge, when applied just before peak midge infestation.

Further work is required before a pheromone-based monitoring system for pea midge is available.

SUMMARY

Studies on the morphology of the antennae of the pea midge (*Contarinia pisi*) showed differences in the structure of male and female sensory organs. This work formed the basis of techniques used for electroantennograph studies with pheromone compounds.

Females of the pea midge were shown to emit a sex pheromone that attracted males. The daily activity of males and females was studied using freshly emerged insects. Techniques were developed to isolate and confirm the biological activity of the pheromone by gas chromatography and an electrophysiological method; the coupled GC-EAD. Methods were also developed to show behavioural responses in a wind-tunnel.

Preliminary identification of a sex pheromone was made, but field tests with sticky delta traps containing lures dosed with the compound were inconclusive. Further work using the techniques developed in the project is necessary to identify all the active components in the pheromone.

The pyrethroid insecticide, lambda cyhalothrin, was shown in field trials to give effective control of pea midge in vining peas when applied just prior to peak midge infestation.

ACTION POINTS FOR GROWERS

* In areas where pea midge is known to have been a problem in the past, crops should be examined as they reach the susceptible stage.

* Peas at the enclosed bud stage are susceptible to damage.

* Crops should be examined for the presence of midge **from mid to late afternoon** after pinching together the leaves surrounding the developing flower buds.

* Spray an insecticide as soon as possible after finding midge adults in the crop.

* A second spray applied after 7 days is essential for maximum control.

INTRODUCTION

The pea midge (*Contarinia pisi*), is a serious pest of peas in the UK especially in Yorkshire, Humberside, Lincolnshire, parts of Cambridgeshire and East Anglia and throughout much of northern Europe. Its local, sporadic and sudden attacks make control by insecticide sprays before they oviposit inside the flower buds and must, therefore, be timed accurately, (Biddle, 1992).

The present system of warning of attack issued regionally by PGRO is based on time-consuming soil sampling carried out during the early summer. The stage of development of overwintering midge cocoons is monitored up to the time of pupation. However, such a system cannot predict the time of emergence, nor can it take into account local variations in both the incidence and timing of attacks. This results in inaccurate and unnecessary insecticidal spraying of peas. It has been shown previously, that the female midge attracts the male by means of a sex-pheromone (Wall *et al*, 1985) and preliminary work using captive females in a water trap showed the potential of such a pheromone trap as a means of detecting newly emerged adult males in an overwintering site (Wall *et al*, 1994).

Work began in 1990 to develop a monitoring system based on the employment of pheromone traps to determine the presence of midge and the time of adult emergence. The project had three main objectives which were, to isolate the pheromone sex-attractant from the pea midge, to identify the active components of the compound and to utilize a synthesized compound in the field.

The work involved the cooperation of the Pheromone Research Group of the Agricultural University of Sweden, based at Lund and field work based in the UK and carried out by PGRO.

METHODS

1. Supply of insects

a) Overwintering cocoons. (PGRO)

During the four years of the project, a supply of overwintering cocoons was maintained in a cold store at 4°C. The cocoons were collected by soil sampling of fields known to have been infested with pea midge the previous season. Surveys were carried out in fields in Norfolk, Lincolnshire and North Humberside to establish sites with high populations of overwintering cocoons.

Soil cores were taken with 10 x 20 cm corer in a 'w' pattern across each field. Soil was then washed through a series of sieves which retained the fine organic matter. After immersion in a saturated solution of magnesium sulphate, the midge cocoons were floated off and removed to a damp chamber held at 4°C.

Larger quantities of soil from fields which contained the highest population of midge were also collected and deposited in a cold store at 4°C for future supplies of cocoons.

The soil was held for a minimum period of 3 months after which time batches of up to 2000 freshly extracted cocoons were sent on a weekly basis throughout the year by air-post to Lund, for rearing to adults.

b) Collection of summer larvae. (PGRO)

In 1990, midge pupation was monitored in fields in Norfolk, which were infested the previous year, by periodic examination of freshly extracted cocoons. When the start of pupation was observed, water traps were placed at soil level and at crop height in the emergence sites. Traps were examined daily until the first adults had been caught. In adjacent pea fields, larvae from infested shoots were observed and a method of collection of these larvae was devised. Shoots from infested plants were collected in polythene bags and transported, in cooled insulated containers, to the laboratory. Shoots were then laid on the surface of seed trays containing a peat and sand compost, covered with clear plastic hoods to maintain humidity and held in a growth room at 23°C with an 18h light cycle. After 3 days the compost containing the cocoons was sent to Sweden for insect rearing.

2. Insect rearing. (Lund)

Midge cocoons sent by PGRO to Lund were placed individually in glass tubes containing moist sand and peat. Tubes were maintained in a growth room at 25°C, 75% RH with a 18h light cycle. Individual adult midge were removed from the tubes, and the sexes determined. Males were stored in tubes at 18°C at 70% RH in a light cycle of 12h and at 12°C at 80% RH during the dark phase. Females were selected for either behavioural studies or for pheromone extraction.

3. Behavioural studies (Lund)

a) Emergence

In order to determine the emergence pattern and the sex ratio, six batches of midges were studied in 1991. The numbers emerging per day of each sex was noted. The median emergence days for males and females respectively, were calculated by setting the day of the first midge which emerged, as Day 1.

b) Female calling frequency

Cocoons were placed into glass tubes and emerged females were observed every 15 minutes for 6h and 45 minutes, starting 1h after the beginning of the photo phase. The observations were made under a microscope to avoid disturbance of the midge. Female calling activity was studied when they were newly emerged, 1 day and 2 days old.

4. Morphology of the antennae (Lund)

Antennae from both sexes of newly emerged midge were dipped for a few seconds in 70% ethanol to reduce surface tension and then transferred to 3% glutaraldehehyde in 0.15 M cacodylate buffer. After dehydration in ethanol, the preparations were brought to Epon via propyleneoxide. Polymerisation was carried out at 60°C for 48h. The blocks were trimmed and the ultra-thin sections on the grids were stained in uranyl acetate and lead citrate in a LKB ultra stainer. The sections were examined in a JEOL 1200 EX transmission electron microscope operated at 60 kV. Preparations fixed and dehydrated as above, were critical point dried, mounted on holders and spatter-coated with gold/palladium 40:60. The preparations were studied in a JEOL T330 scanning electron microscope.

5. Wind tunnel observations (Lund)

A plexiglass wind-tunnel, 45x15 cm was constructed. One end was filled with a pressure equaliser coal filter and fan. The laminar flow varied between 0.15 - 0.25 m/s and the temperature ranged between 18.5 and 22°C. A constant 2500 lux light was provided by a UV-lamp. Flight activity experiments were conducted between 9.00 and 12.20h.

Three live females, 1 day old, were used in each experiment. They were kept in an open glass tube, 5 x 2 cm, with both ends covered with a fine-meshed net. Females were placed in the tubes 30 minutes before each experiment and the tubes were introduced into the wind-tunnel to allow 10 minutes conditioning.

One day old males were released into the wind-tunnel after a 10 minute conditioning time. The males were used once for each experiment.

After each experiment the wind-tunnel was cleaned with 70% ethanol and the glass tubes were put in an oven at 400°C for 12h.

Preliminary tests were made with ovipositor extract. The extract was placed on a piece of hexane-washed filter paper and the solvent was allowed to evaporate before being placed in the wind-tunnel.

6. Pheromone identification (Lund)

a) Extracts

Ovipositor extracts were made in the morning, before 13.00. Extracts were made from 1-3 day old midges.

Calling females were caught with forceps and the ovipositors dissected and dropped into a glass dissection vial kept in liquid nitrogen. When the desired number of ovipositors had been collected in the vial, it was removed from the liquid nitrogen. While the ovipositors were thawing, redistilled hexane or heptane, was added. They were allowed to extract for one minute after thawing. The solvent was then removed and put into a micropipette, sealed in a flame, and stored at -18°C.

A second method was developed. Five virgin females (0-4 hours old) or five males of the same age, were placed in burned pipettes for 24h. The tip of each pipette was sealed with distilled water to maintain high humidity. The other end was closed with parafilm. Pipettes were stored in the growth chamber for 24h, before the insects were removed.

b) Gas chromatograpy and mass spectometry

Analyses of gland extracts were performed on a Hewlett Packard 5890A gas chromatograph with an SE 54 column (25 m, 0,25 mm i. d., Kupper & Co., Bonaduz, Switzerland) and a DB WAX column (30 m, 0,32 mm i. d., J & W Scientific, Folsom, CA, USA). The GC peak areas were calculated by a Hewlett Packard 3396A integrator.

The hydrogen flow on the SE 54 column was 2,6 ml/min, the injector temperature was 200°C, and the split valve was opened 60 s after injection. The column temperature was maintained at 60°C for 2 min following injection, then raised to 100°C using a program of 10°C/min and then raised to 230°C using a program of 5°C/min.

The hydrogen flow on the DB WAX column was 2.7 ml/min, the injector temperature was 200°C for 2 min following injection and then raised to 230°C using a program of 10°C/min.

Mass spectra were run on a Jeol SX 102 mass spectrometer.

c) Electrophysiology, GC-EAD

Insect antennae were prepared for GC-EAD recordings by cutting off the abdomen and placing the rest of the body in the indifferent electrode with the head and the antennae protruding. The head was fixed with water-soluble correction fluid. After removing the distal segments, the tip of the antenna was placed in the recording electrode. The electrodes were connected to a high impedance amplifier. Purified, humidified air was blown over the antenna at a flow of 0,5 m/s, the outlet 1 cm from the antenna.

As the male antennae were not considered to live for more than 15 min, and in order to cover the area where two preliminary recordings had indicated biological activity, the male antennal recordings were started 5 min after the extract was injected on the gas chromatograph. Four recordings were made using four different males. The extracts tested contained 170, 179, 179 and 250 ovipositors respectively.

The gas chromatograph used was a Hewlett Packard 5830A with a DB WAX column (30 m, 0,32 mm i.d., J & W Scientific, Folsom, CA, USA). The GC peak areas were calculated by a Hewlett Packard 18850 a GC terminal. The hydrogen flow of the column was 4 ml/min, the injector temperature was 250°, and the split valve was opened 60 s after injection. The column temperature was maintained at 80°C for 2 min after injection and then raised to 230°C following a programme of 10°C/min.

7. Pheromone studies in the field (PGRO)

A compound found to be active in the laboratory tests was applied to two lure types, cotton wadding and rubber. Three dose rates were used on each lure type, 0.1, 1.0 and 10.0 mg. The lures were placed in 'Delta' traps with a removable sticky base. Each trap was positioned 10 cm above the soil surface

and 10 m apart. Each dose and lure type was replicated twice together with undosed control traps. A water trap comprising a grey plastic seed tray 170 x 250 x 50mm containing water and a drop of non-ionic detergent was also placed in each field at the same height. The fields chosen were those with a known high overwintered population of cocoons and each field was currently cropped with winter wheat. The development of the midge cocoons was monitored at three day intervals from soil cores taken at each site and traps were deployed as soon as pupation had commenced. One site in Norfolk and two sites in North Humberside were monitored in 1993. Traps were examined daily and the number and sex of the pea midge determined.

8. Insecticide timing in the pea crop (PGRO)

In conjunction with Unilever Research, replicated small plot trials were carried out in 1994 at sites in Suffolk, Cambridgeshire and North Humberside. The core treatment was lambda cyhalothrin plus pirimicarb at a rate of 150 ml/ha + 280 g/ha, with pirimicarb as a control treatment. Sites chosen were as close as possible to overwintering sites where soil sampling to monitor pupation was carried out and water traps sited at the first stage of pupation.

The insecticide timings were applied to peas at around the green bud stage (201) and were as follows:-

Treatment	Timing
1. pirimicarb control	T_1 = at midge emergence in overwintered site
2. lambda cyhalothrin + pirimicarb	T_1 = midge emergence in overwintered site
3. pirimicarb control	T_2 = when midge first found in peas and T_3 = 7 days after T_2
4. lambda cyhalothrin + pirimicarb	T_2 & T_3
5. pirimicarb control	T_3
6. lambda cyhalothrin + pirimicarb	T_3

Each treatment was replicated 4 times. Plot size was 5 m x 2 m. Daily trap catches were recorded and crops were examined each day by sampling shoots from 50 randomly selected plants around the area of the trial.

Sprays were applied with Azo plot sprayers using 200-250 l water per ha.

Site details were as follows:-

Site 1. Metfield, Suffolk
cv. Waverex spray trial conducted by Unilever Research

Site 2. Thorney, Cambridgeshire
cv. Sancho spray trial conducted by PGRO

Site 3. Walkington, N. Humberside
cv. Darfon spray trial conducted by Unilever Research

Site 4. Lions Den, N. Humberside
cv. Waverex spray trial conducted by Unilever Research

Site 5. High Hunsley, N. Humberside
cv. Waverex spray trial conducted by Unilever Research

At the freezing stage of maturity, all plots were harvested and vined and the yield of the shelled peas recorded.

RESULTS

1. Emergence of laboratory reared insects

From the six batches of soil containing overwintered cocoons, the median emergence day for females was 9.3 and for males 9.2. There was no statistically significant difference between the sexes (Table 1). The emergence pattern of the two sexes was similar and the total insects emerged from each batch over a 30 day emergence period, is shown in Table 1.

Table 1. Female and male emergence days in 6 soil batches

Batch	median emergence day		daily emergence total		sex ratio
	females	males	females	males	
1	10	7	67	54	1:0.8
2	7	10	121	83	1:0.7
3	9	11	342	215	1:0.6
4	11	10	228	183	1:0.8
5	9	8	256	223	1:0.9
6	10	9	113	83	1:0.7
	9.3	9.2	1127	841	1:0.7
sig = p = 0.001		n.s.d.		sig different	

More females than males emerged in all batches. The difference was statistically significant. The ratio of females:males was 1:0.7

2. Female calling frequency

The percentage of females that were calling for each hour after a) newly emerged, b) one day old and c) two days old is shown in Figure 1.

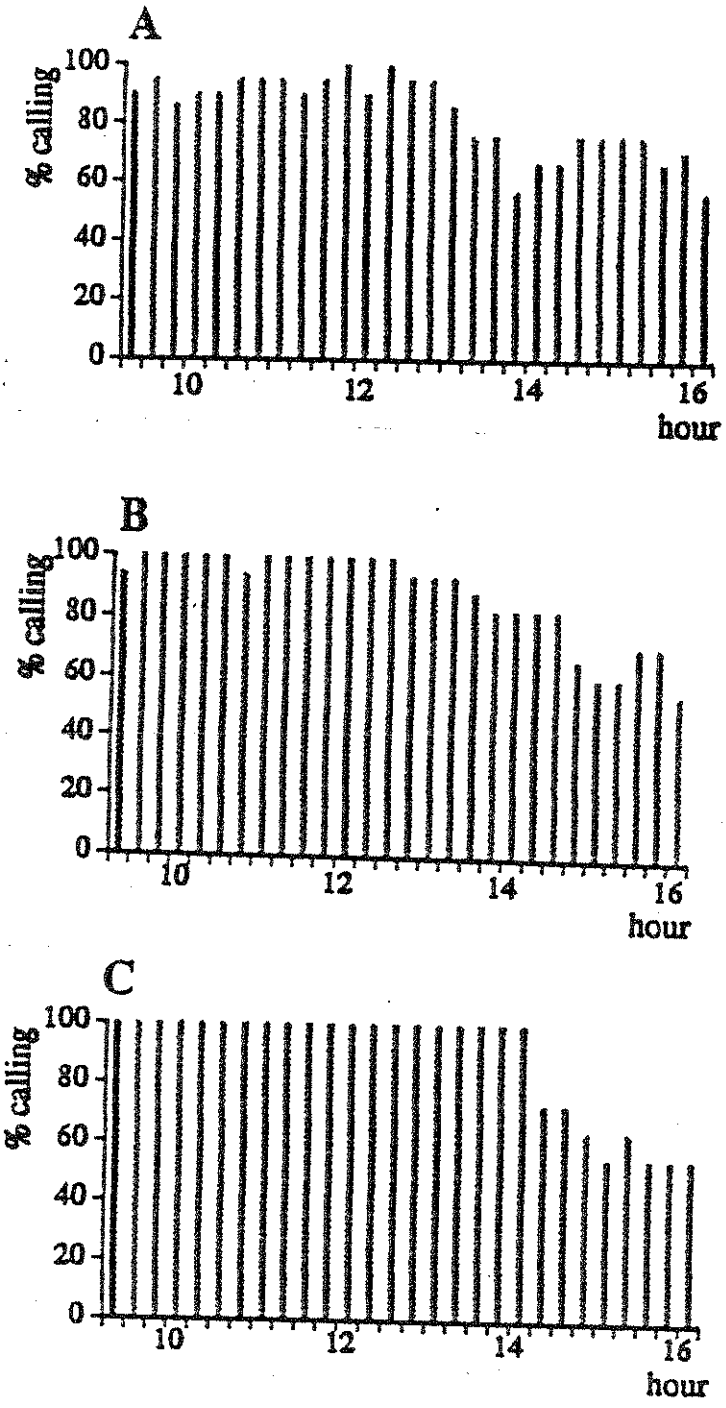
About 80% of newly emerged females were calling between 9.00 and 13.00h but the number increased by the time that the females were 1 day old. 100% of females were calling at 2 days old but they were more active; either flying or walking on the emergence day, than by the time that they were 2 days old.

In all three days, the mean percentage calling behaviour was higher between 9.15 and 13.00h (Table 2).

Table 2. The mean percentage of newly emerged (n.e.), 1 day old and 2 day old females in the intervals 9.15 - 13.00 and 13.15 - 16.00h

Interval	n.e.	1 day	2 day
09.15 - 13.00	93	99	100
13.15 - 16.00	70	75	61

Figure 1. Percentage females calling: A. newly emerged, B. 1 day old and C. 2 days old



3. Morphology of the antennae

The antennae have 12 flagellomeres. In the females, these are cylindrical, whereas in males they are dumb-bell shaped. There are four sensillar types on the antennae. These are, fused wall-pore sensilla (*sensilla cirumfila*), single wall-pore sensilla, double-walled sensilla and poreless sensilla. In the females, the *s. circumfila* of a flagellomere consists of two transverse rings connected with two long-longitudinal extensions. The total number of sensilla that forms a single circumfilum in the females is 25-30. In the males, the circumfila form wreaths, one on each node of the flagellomeres. There are about 10 individual sensilla in each. In the males, the circumfila branch basally and the tips of adjoining sensilla are fused apically. The other types conform to corresponding types found in other insects (Figures 2, 3 and 4).

4. Wind-tunnel

Observations on the orientation behaviour of males towards female pheromones were made during the experiments in the Wind-tunnel. Two types of male behaviour were distinguished:-

a) full response, with the males reaching the lure and wing-fanning or, wing-fanning with attempts to copulate with the lure.

b) no response, the males did not reach the lure and spent most of the time on the walls of the Wind-tunnel.

Fifty seven males were tested with 58% responding and 42% not responding to ovipositor extract on filter paper.

Ninety percent of males responded to caged females.

5. Pheromone identification

a) GC and GC-MS

The gas chromatograms of different ovipositor extracts showed a consistent pattern with constant relative retention times and about the same relative proportions of the different substances (Figure 5). This pattern was also seen when parts of the GC-EAD extracts were analysed on the same column (Figure 6). Moreover, these chromatograms were in accordance with those from the GC-EAD recordings and thus the substance that elicited a male antennal response could be traced in all the ovipositor extracts. An identification was made by mass spectrometry, from an extract containing 25 ovipositors. However, as the total amount of the compound in the extract was estimated to less than 1 ng, the identification was preliminary.

b) GC-EAD

In the GC-EAD recordings, the antennae responded to a compound eluating after 11-12 minutes. The chromatogram and the electroantennogram from that recording are shown in Figure 7.

c) Male antennal responses

Whole newly emerged insects were placed on a piece of adhesive tape. The tip of the antenna was cut and the recording electrode placed over the antenna. The reference electrode was either placed around one of the cut legs or in the abdomen. The flow of the main air-stream in the EAG was set to 0.5 ml/s. The stimulating pulse added to the main air-stream had a flow rate of 5.0 ml/s and a duration of 0.5 seconds.

Figure 2. Flagellomere from male consisting of two nodes showing sensilla circumfila and companion hairs

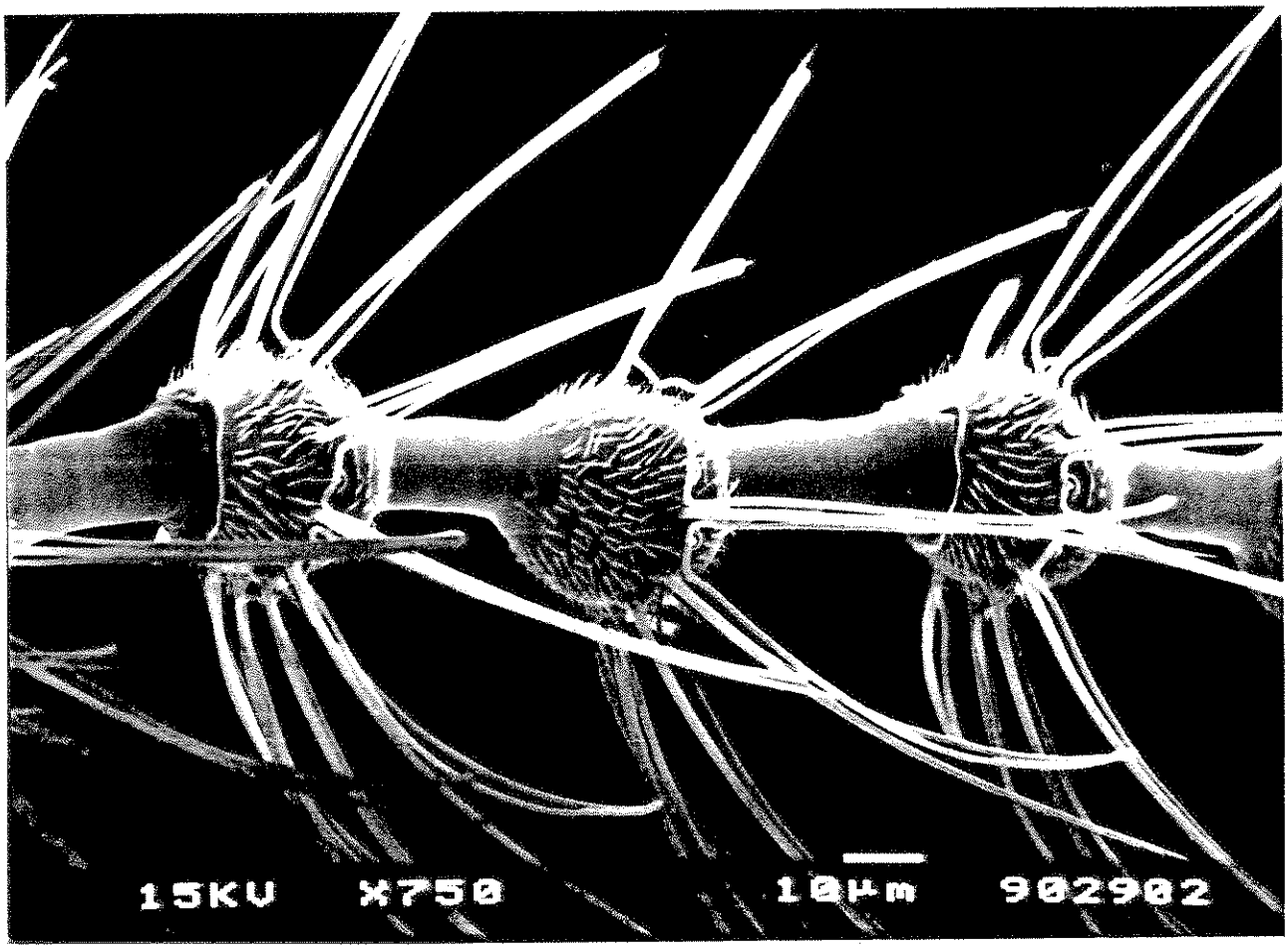


Figure 3. Male antenna: showing forked sensilla circumfila

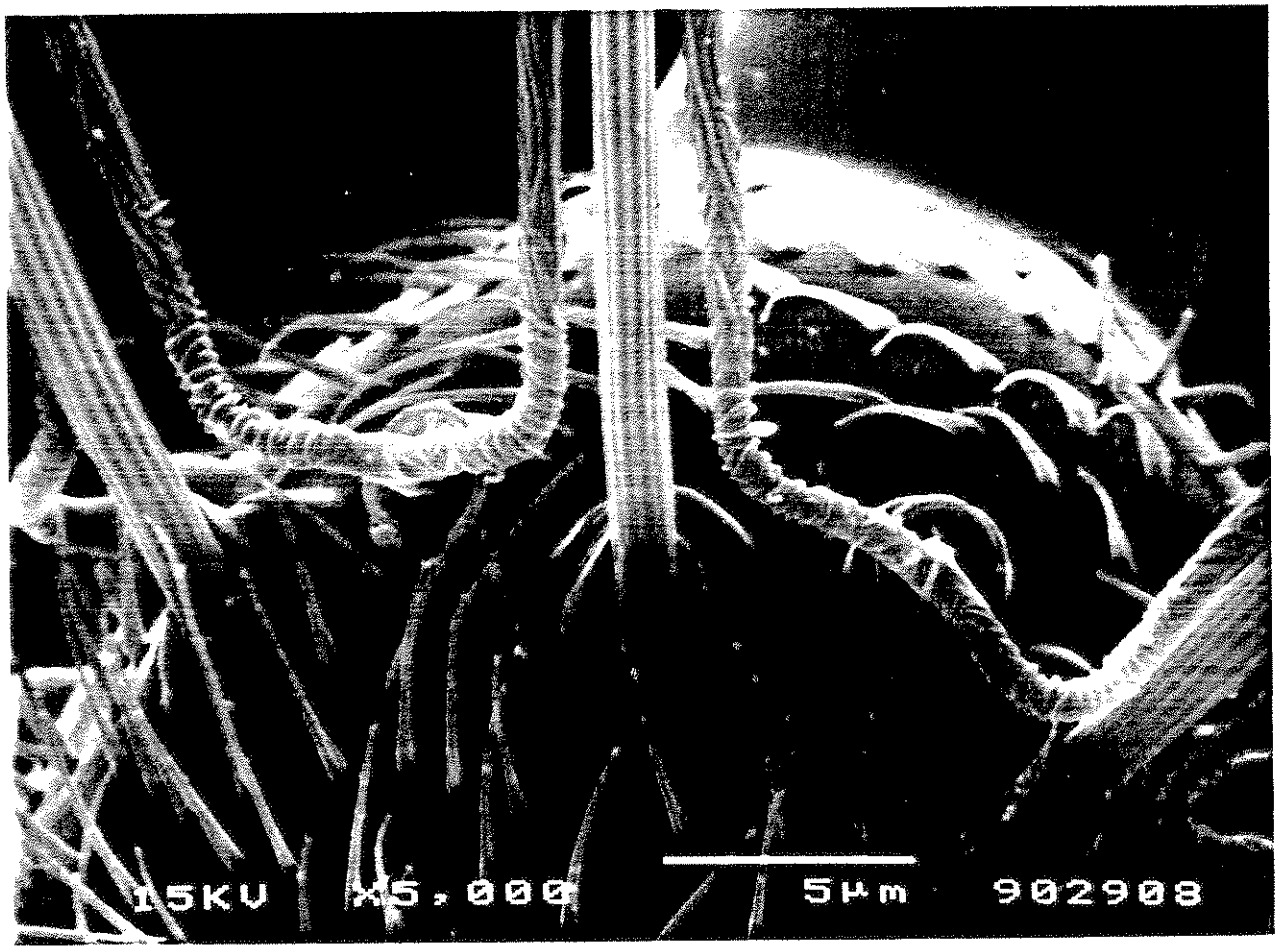


Figure 4. Flagellomere from female showing sensilla circumfila and companion hairs

Figure 5. Gas chromatogram of an extract containing 14 ovipositors analysed on a DB wax column

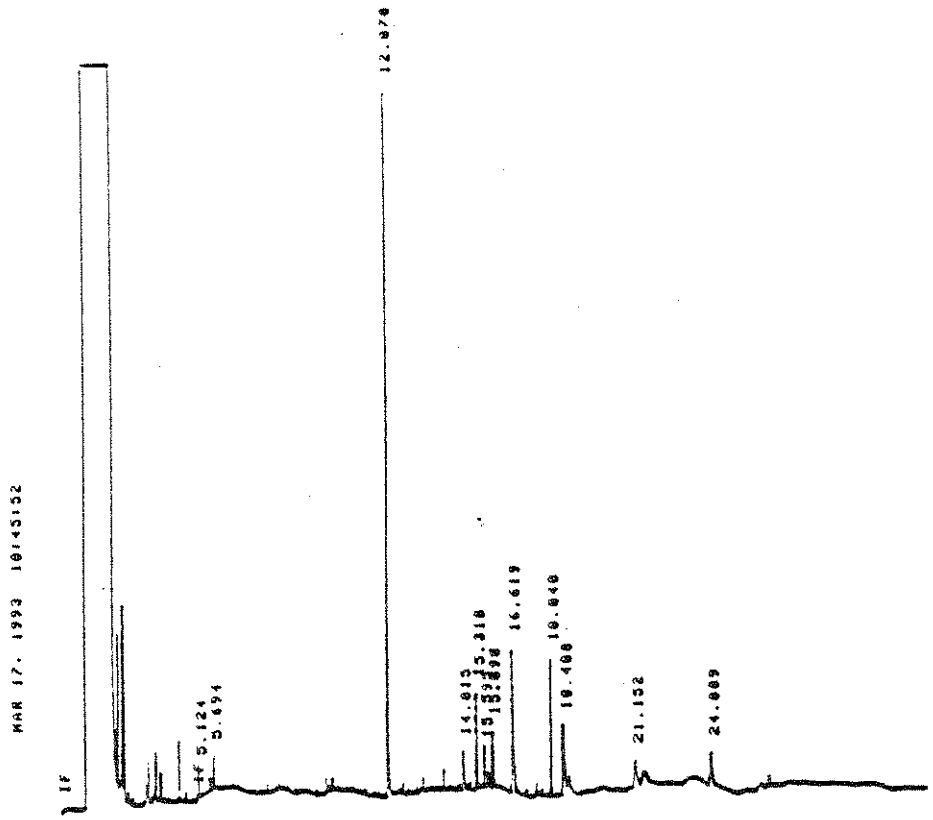


Figure 6. Gas chromatogram of a small part of the extract used in the GC-EAD recording

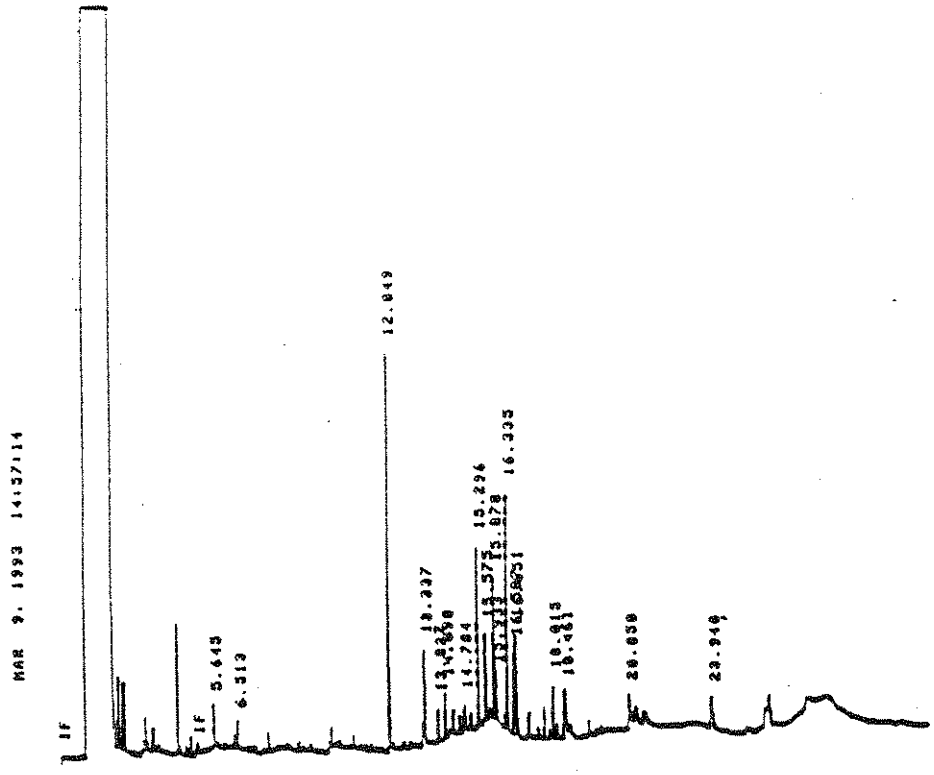
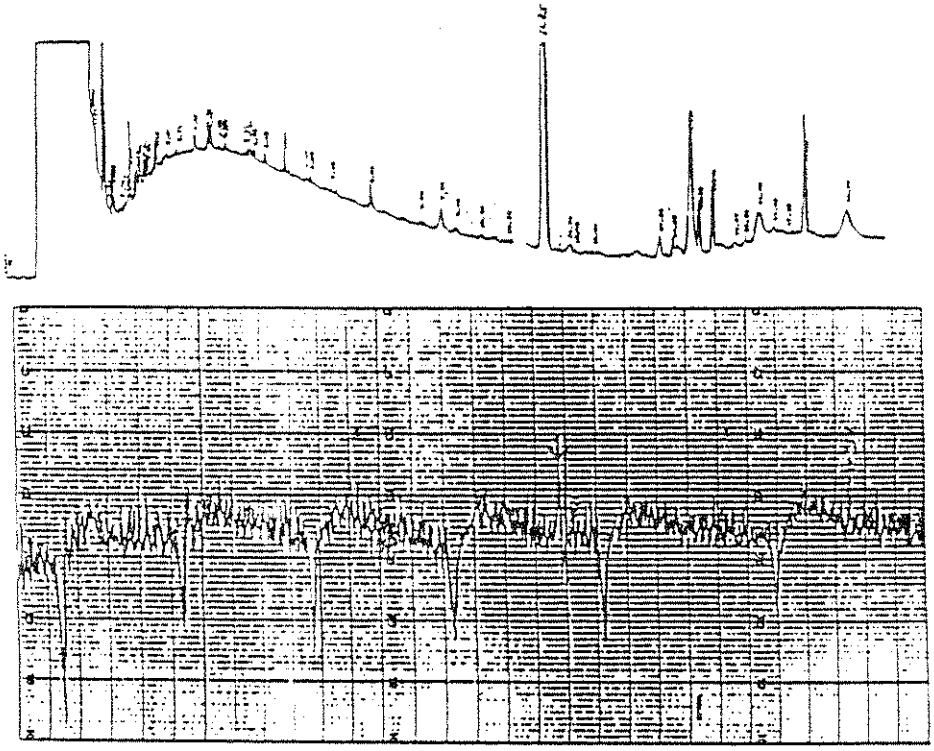


Figure 7. Gas chromatogram (above) and electroantennogram (below). The arrow in the EAG indicates the antennal response to a peak eluating after 11.75 minutes



The responsiveness of the males to olfactory stimulation was tested using a pipette containing a filter paper dosed with 10μl heptanol for calibration.

Pipettes which had contained females elicited an antennal response. Those of males, gave no response.

6. Pheromone studies in the field

Overall, the number of female midge caught was very low and was not considered further. The numbers of male midge caught in each trap were compared with those caught in the non-attractive water traps and a comparison was made between the dispenser types. The mean male midge caught per day at the three sites are shown in Table 3.

Table 3. Mean male midge caught per day 1993.

Trap type	Site 1. Norfolk (2 days)	2. Thwing (5 days)	3. Walkington (6 days)	Mean of sites 2 and 3
Water	1.5	32.6	5.5	19.1
Control delta trap	2.0	38.6	33.3	36.0
0.1 wadding	0.5	12.8	34.5	23.7
0.1 rubber	1.0	11.4	44.6	28.0
1.0 wadding	0.5	9.4	21.1	15.3
1.0 rubber	0	24.2	17.5	20.9
10.0 wadding	1.0	19.4	16.3	17.9
10.0 rubber	1.5	14.2	19.0	16.6

Catches at the Norfolk site were very low and trapping ceased after two days.

There appeared to be no differences in attractiveness between the rubber and wadding dispensers. However, there were more adult males caught in the control delta trap than those with the pheromone compound.

The daily trap catches at the Thwing and Walkington sites are shown in Tables 4 and 5. Because of the apparent lack of difference between the dispenser types, the results show the number of males caught per dose rate of pheromone compound.

Table 4. Total daily trap catches of male midge at Thwing

Trap:	water	control delta	0.1	1.0	10.0 dose
June 24	10.0	4.0	4.0	8.0	2.0
25	11.0	8.0	8.0	7.0	6.5
26	36.0	38.0	7.0	38.5	26.0
27	67.0	109.0	21.0	25.5	37.0
28	39.0	34.0	12.5	5.0	12.5
mean:	32.6	38.6	10.5	16.8	16.8

Table 5. Total daily trap catches of male midge at Walkington

	Trap: water	control delta	0.1	1.0	10.0 dose
June 24	0	10.0	8.0	7.5	4.0
25	3.0	6.0	13.0	5.5	4.5
26	15.0	54.0	46.0	22.0	23.0
27	7.0	24.0	46.0	17.5	5.0
28	8.0	13.0	22.5	9.5	17.0
July 1	0	93.0	102.0	57.5	52.5
mean:	5.5	33.3	39.6	18.8	17.7

Catches of midge on the delta traps were almost all males and very few non-target species of insects were trapped, however, midge numbers were generally low. Because of the small amount of compound available, the trap experiments could only be run with two replicates of each dose and lure type. The spacing, therefore, may not have been sufficient to compound the effects of any local aggregation of over-wintering cocoons.

Trap catches were very variable, both between the different doses and from day to day. Generally there were no differences in trap catches between the doses and the undosed control.

7. Insecticide timing in the pea crop

A summary of all four sites where midge was present is shown in Figure 8. The results of the insecticide trials carried out in North Humberside, where midge was highest are summarised in Figure 9.

Figure 8. Yield response to insecticide application - all sites 1994

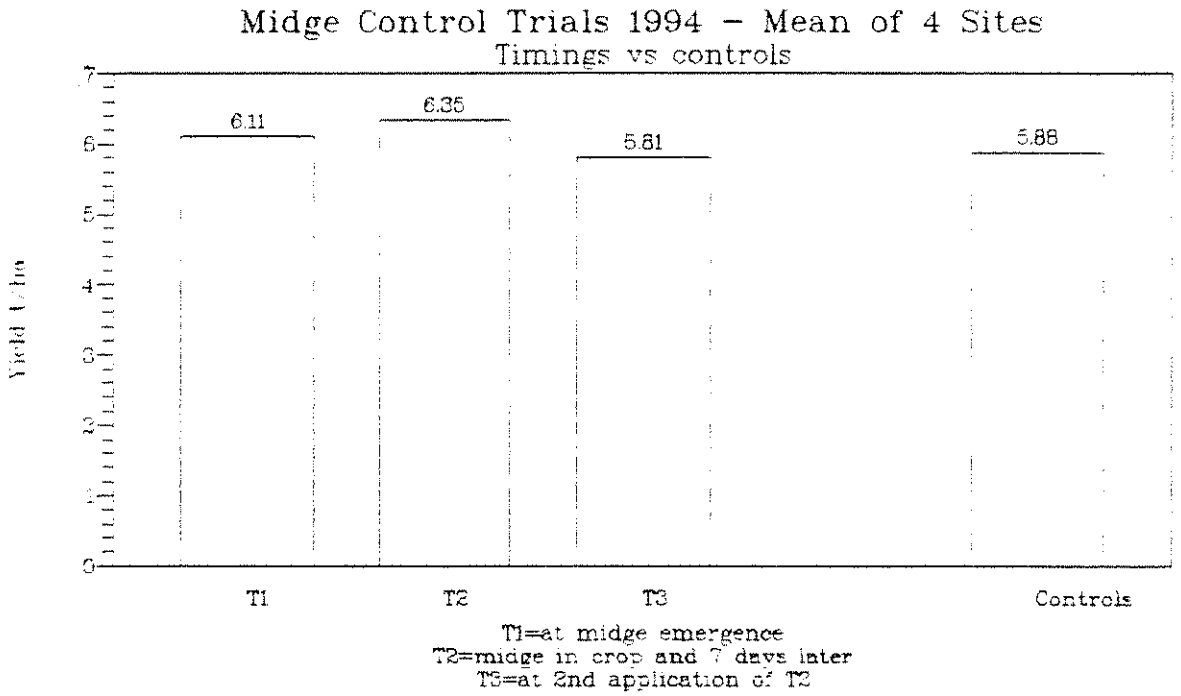
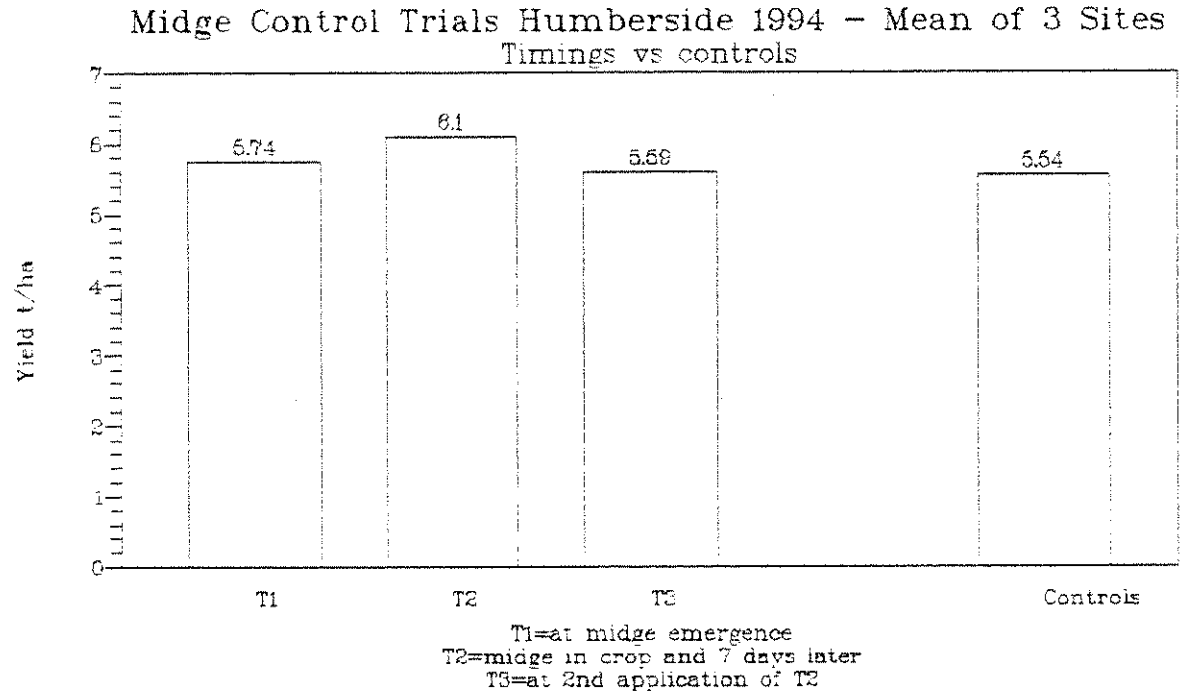


Figure 8. Yield response to insecticide application - N. Humberside - 3 sites 1994



Full details of the individual site results are shown in the Appendix. No midge were present in the crop at Thorney and the results are not presented.

At the North Humberside sites, there was a considerable time difference between the midge emergence in the overwintering site and their arrival in the pea crop. Table 6 shows the midge counts in the pea crops over the spray period.

Table 6. Midge catches in pea crops

	midge/50 plants			
Site:	1	3	4	5
date: 13.6	2	-	-	-
14.6	3	-	-	-
15.6	0	0	0	0
16.6	0	0	0	0
17.6	0	0	0	0
18.6	0	0	0	0
19.6	0	0	0	0
20.6	0	0	0	0
21.6	1	0	0	0
22.6	1	0	0	0
23.6	0	1	1	0
24.6	1	1	1	0
25.6	1	10	3	1
26.6	0	0	1	0
27.6	1	28	18	0
28.6	0	7	5	0
29.6	0	2	2	0
30.6	1	19	11	30
1.7	-	20	2	2
2.7	-	10	0	0
3.7	-	42	36	40

At all sites in North Humberside, the T_2 spray occurred just before peak invasion of midge. Control of midge was reflected by the yield increases compared with the pirimicarb control treatment. At two sites, the two spray programme produced significantly higher yields than the control indicating very good control of pea midge. At site 5, peak midge infestation occurred 4 days after the T_2 spray timing and yield increases were statistically significantly different to the control. There was therefore an indication that lambda cyhalothrin had some persistent activity in the control of pea midge.

CONCLUSIONS

1. Emergence and sex ratio

Emergence patterns under controlled conditions showed that slightly more females than males emerged, with peak emergence of both sexes coinciding on a daily basis. However, the study did not record any daily fluctuation in the sex ration of males to females.

2. Female sex pheromone

The female calling frequency established in the laboratory is in accordance with the field trapping results obtained by Wall *et al.* (1991). In the laboratory almost all of the females were calling when the observations started, about 1h into the photophase, and the calling intensity was continuously high for about 5h, when it started to decrease. This is reflected in the midge behaviour in the field. In the morning males, searching for calling females, were caught low down in the crop at the emergence sites. Whereas in the afternoon the then mated females were caught at a higher level in the crop and also at the edges of the emergence field, presumable migrating to the peas.

To verify the substance that elicited a male response in the GC-EAD recordings, is a component of the sex-pheromone, additional mass spectrometry analyses of ovipositor extracts have to be made. Furthermore, the biological activity of the synthetic substance has to be confirmed in Wind-tunnel experiments and by the use of electrophysiological methods.

3. Male pheromone response

The results of the Wind-tunnel tests, with 58% of the males responding to calling females, show that the Wind-tunnel, with some modifications, is suitable for further tests with extracts and, in the future, with synthetic attractants.

As the fragility of the midges makes them very sensitive to environmental conditions, the modifications of the Wind-tunnel mainly consider physical parameters. Sharma and Vidyasagar (1991) showed that both female calling and male activity in *C. sorghicola* decreased with an increase in temperature and a decrease in relative humidity. Compared to natural conditions, the temperature in the *C. pisi* Wind-tunnel tests, 18.5-22°C, was probably below any critical value. The relative humidity though, was not measured, but as there was no humidification of the air in the tunnel the RH-value was possibly low compared to the values one could expect in the field.

Apart from temperature and humidity, light intensity might be another factor affecting both female calling and male response *C. pisi* is day-active and full daylight by far exceeds the 2500 lux in which all the Wind-tunnel tests were conducted. For comparison, a light intensity of 80 000 - 100 000 lux was measured out-doors in the morning on a clear day at the end of May.

Female calling behaviour in the Wind-tunnel cage can also be affected by the number of females in the cage. If it is too crowded they can disturb each other and their normal calling activity can be disrupted.

The results of the GC-EAD recordings demonstrated the biological activity of the ovipositor extract. In addition, retention time of one active substance is known. However, because the antennae are so fragile and might die shortly after the injection is made; the method is time-consuming and unreliable for comparison of the activity of different extracts. Thus a new technique to prepare the antennae that has been successfully used on biting midges was tried. This involves using the whole body instead of just the head and the thorax in the preparation. This increases the time that the midge and the antennae can stay alive. Additional humidification, such as wet cotton wool underneath the midge, is also essential.

4. Field tests with the pheromone compound

The limited amount of material enabled only field observations to be made, which could not be statistically analysed. However, the compound did not appear to be active under field conditions, and there was an indication that, at high doses, the compound was repellent. However, midges were caught by the delta traps and were easily counted. The trap design and height positioning appeared to be satisfactory.

Further work is necessary to identify the full pheromone blend with the intention that it should be available for field testing in the future.

5. Insecticide trials

The correct timing of insecticide application is essential to achieve maximum control of pea midge in the crop. There was a delay of several days between first emergence of midge in the overwintering site and the arrival of female midge in the pea crop. This allowed a reasonable amount of time to carry out the spray treatments.

Lambda cyhalothrin was shown to be an effective insecticide for the control of midge and there was an indication that the persistency of the material allowed some additional flexibility in the timing of the application.

Further work in progress to obtain a full label recommendation for this product for pea midge control in the UK.

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APPENDIX 1

Site 1: Metfield, Suffolk

Treatment	Application timing*	yield t/ha	yield as % of control
1. control (pirimcarb)	T_1, T_2, T_3	6.91	100
2. lambda cyhalothrin + pirimicarb	T_1	7.21	104
3. "	T_2 & T_3	7.13	103
4. "	T_3	6.49	94
significance at p = 0.05			n.s.d.

* T_1 = 23.6.94
T_2 = 24.6.94
T_3 = 28.6.94

Site 3: Walkington, North Humberside

Treatment	Application timing*	yield t/ha	yield as % of control
1. control (pirimcarb)	T_1, T_2 & T_3	4.34	100
2. lambda cyhalothrin + pirimicarb	T_1	4.44	104
3. "	T_2 & T_3	4.52	103
4. "	T_3	4.04	94
	significance at p = 0.05		n.s.d.

* T_1 = 15.6.94
T_2 = 26.6.94
T_3 = 3.7.94

Site 4: Lions Den, North Humberside

Treatment	Application timing*	yield t/ha	yield as % of control
1. control (pirimcarb)	T_1, T_2 & T_3	3.67	100
2. lambda cyhalothrin + pirimicarb	T_1	3.83	104
3. "	T_2 & T_3	4.36	109
4. "	T_3	3.82	104

Treatment 2 was significantly different from the control @ p = 0.01
cv% 9.31

* T_1 = 15.6.94
T_2 = 26.6.94
T_3 = 3.7.94

Site 5: High Hunsley, North Humberside

Treatment	Application timing*	yield t/ha	yield as % of control
1. control (pirimcarb)	T_1, T_2 & T_3	8.61	100
2. lambda cyhalothrin + pirimicarb	T_1	8.94	104
3. "	T_2 & T_3	9.40	109
4. "	T_3	8.90	103

Treatment 2 was significantly different from the control @ p = 0.05

* T_1 = 15.6.94
T_2 = 26.6.94
T_3 = 3.7.94

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