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SID 5 Research Project Final Report

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Project identification

1. Defra Project code: HH3114TTF
2. Project title: SEMIOCHEMICALS IN MANAGEMENT OF APPLE LEAF MIDGE
3. Contractor organisation(s): Natural Resources Institute, University of Greenwich

   East Malling Research
4. Total Defra project costs (agreed fixed price): £ 104,493
5. Project: start date .... 01 July 2003

   end date .... 30 June 2006

6. It is Defra's intention to publish this form.
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Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

The apple leaf curling midge, *Dasineura mali* Kieffer, is a pest of apples in Europe, North America and New Zealand and in the UK it is widespread and abundant. It is particularly damaging in nurseries and newly-planted or re-grafted orchards. Natural enemies are very important in regulating leaf midge numbers, especially egg parasitoids. There is currently no satisfactory method for controlling the pest. All apple varieties are susceptible and it is unlikely that resistant varieties can be developed. This project aimed to identify the female sex pheromone and investigate its use for monitoring and control of the pest.

For identification of the female sex pheromone of apple leaf midge, volatiles were collected from over 2,000 virgin female midges which proved more effective than gland extraction methods used previously. Analysis of collections by gas chromatography (GC) coupled to electroantennographic (EAG) recording from the antenna of a male midge antenna showed a single active component which was not present in similar collections from virgin male midges and was assumed to be the sex pheromone. Although this was present at less than 20 pg per female, mass spectra were obtained and the compound was identified as (*Z*)-13-acetoxy-8-heptadecen-2-one by comparison of GC retention times and mass spectra with those of synthetic standards and microanalytical reactions. The synthetic compound had GC and MS data identical with those of the natural compound and elicited a strong EAG response from a male *D. mali* midge. A convenient route was developed for synthesis of the compound from giving 63% yield in eight steps. The two enantiomers of the compound were separated and isolated by high performance liquid chromatography on a chiral column. The first-eluting enantiomer was predicted to be the *S* enantiomer by nuclear magnetic resonance spectroscopy of the (*R*)-2-methoxy-2-trifluoromethyl-2-phenylacetyl esters.

The pheromone racemate was found to be highly attractive to male apple leaf midge in the field with rubber septa lures containing 1 μg being significantly attractive. Rubber septa dispensers were shown to give a relatively constant release over at least 374 days under laboratory conditions while sealed polyethylene vial dispensers showed a lag period before release began followed by relatively rapid release which then declined. Only one enantiomer of the pheromone, probably that with *S* configuration, was attractive, but the racemic mixture was equally attractive, the latter being much cheaper and easier to synthesise. A range of traps was evaluated and white sticky delta traps found to be most effective. Trap colour did not affect catches of midges in delta traps, but there were indications that red traps were less contaminated by non-target insects and this needs to be further investigated. The height of deployment of traps was shown to have a large effect on midge catches with highest catches in traps at ground level. Traps were shown to attract midges over a range of at least 50 m. White sticky delta traps baited with a rubber septum containing 3 μg of the racemic pheromone positioned at a height of 0.5 m above ground are recommended for monitoring of apple leaf midge by growers.

Pheromone trap catches can be used to monitor apple leaf midge populations in commercial crops. It was shown that apple leaf midge male flight has a strong diurnal periodicity. There were no catches in pheromone traps during the night. Catches started two hours after dawn, peaked at 08.00-09:00 hrs and then declined steadily through the day. Conversely, numbers of ovipositing females were very low at 09:00 hours but increased steadily, reaching a peak at 11:00 – 12:00 hrs and declining thereafter. These results imply that mating occurs in the earlier part of the morning and that oviposition occurs mainly in the middle of the day. Monitoring of populations in commercial crops in southern England showed three full generations of apple leaf midge per annum with probably an incomplete partial generation in the autumn. There were strong correlations between numbers of males caught in traps and the proportion of shoots with eggs and the numbers of galls that formed in shoots. Thus the pheromone traps can be used for crop damage prediction.

Two field experiments investigated whether use of the pheromone traps for more accurate timing of foliar sprays of synthetic pyrethroid insecticides deltamethrin or cypermethrin improved control of larval infestations of apple leaf midge in commercial orchards. Previous trials indicated that synthetic pyrethroids applied as foliar sprays were the only insecticides out of a wide range of materials tested with significant efficacy against the pest. Soil applications of chlorpyrifos were shown to be ineffective. The results showed that even when applied early during the midge emergence as indicated by the pheromone traps, treatment with six sprays at 3-4 day intervals was required to be highly effective, and fewer sprays at longer intervals gave only partial control. Further work is needed to validate these results and to investigate intermediate spray intervals between 3-4 days and 7 days. The results provide an effective treatment for apple leaf midge for nursery growers, and possibly in orchards where established trees have been re-grafted. Elimination of the first generation may keep midge levels low for the rest of the season, although continued monitoring of the sex pheromone trap would detect resurgences. Cypermethrin and deltamethrin are low cost insecticides of low toxicity to humans although they are highly disruptive to IPM. For this reason, use of multiple sprays in cropping orchards is undesirable.

In view of the high biological activity of the pheromone of apple leaf midge and poor results with insecticides, use of the pheromone for control of the pest was investigated using attract-and-kill (A&K) or mating disruption (MD) approaches. In a preliminary experiment during 2004 A&K devices were 20 x 20 cm squares of plastic laminated cardboard surface coated on both sides with a microencapsulated formulation of lambda cyhalothrin and baited with a rubber septum lure impregnated with 100 µg of apple leaf midge pheromone. Bioassays showed that midges that momentarily touched the cards were unable to make coordinated flights after 10 minutes and were dead within 3 hr. This activity was retained for at least 2 months in the field. The devices were deployed at a density of 100/ha in two heavily infested isolated orchards. Catches in pheromone traps in the treated plots were much lower than catches in untreated plots, but no substantive control was achieved. During 2005, a very large scale field trial was done on 30 plots of 1 ha in commercial apple orchards in Kent. Treatments were a comparison of MD and A&K, each at deployment densities of 500 or 2000 devices per ha, and an untreated control. MD devices were polyethylene caps each initially loaded with 500 µg of the apple leaf midge pheromone. The A&K target devices were 10 x 6.7 cm cards treated with lambda cyhalothrin baited with a polythene cap lure containng 100 µg of the pheromone. A fully randomised dispersed plot design with 6 replicates was used. Half the plots were newly planted orchards with low populations of the apple leaf midge, half were established orchards with high populations. The MD and A&K treatments gave high levels of suppression of pheromone trap catches from April to July but this broke down in August. There were no differences in infestations of apple leaf midge in the treated and untreated plots at any sampling occasion. The open polyethylene cap dispensers used in both MD and A&K treatments gave a constant release rate of pheromone over a period at least 270 days in a laboratory wind tunnel. However, at the end of the season no pheromone was found in the lures sampled from the field, and this was subsequently shown to be due to degradation of the pheromone when exposed to direct sunlight. This could explain the loss of effectiveness of the MD and A&K treatments during the season. A further experiment is in progress in 2006, using a dye to protect the pheromone. Trials of mass trapping for control of apple leaf midge were carried out by collaborators in New Zealand using pheromone supplied by NRI.

In order to promote transfer of these technologies to growers, an international patent application has been made on use of novel keto esters, including the apple leaf midge pheromone, in management of midge pests. Apple leaf midge pheromone traps and lures have been made available on a semi-commercial basis to UK growers for evaluation in the 2006 growing season. Depending on the results, it is anticipated that the pheromone trapping system will be made fully available to growers in 2007. The UK company Agrisense has been involved in provision of materials during this project and another company, International Pheromone Systems Ltd. Has expressed interest in marketing the pheromone. Collaborations have been established with research institutes in Italy, New Zealand and The Netherlands to test and develop the apple leaf midge pheromone for pest monitoring and control.

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:

- the scientific objectives as set out in the contract;
- the extent to which the objectives set out in the contract have been met;
- details of methods used and the results obtained, including statistical analysis (if appropriate);
- a discussion of the results and their reliability;
- the main implications of the findings;
- possible future work; and
- any action resulting from the research (e.g. IP, Knowledge Transfer).

OBJECTIVES AND EXTENT TO WHICH THEY HAVE BEEN MET

1. This project will aim to identify the chemical structures of the components of the sex pheromone produced by virgin females of the apple leaf midge. The components will be synthesised.

This objective has been met in full. The female sex pheromone of the apple leaf midge has been shown to consist of a single component, identified as one enantiomer of (Z)-13-acetoxy-heptadecen-2-one, most probably the S enantiomer. A practical route for synthesis of the racemic compound has been developed and this material is highly attractive to male midges in the field.

2. The blend composition, controlled release dispenser and trap design will be optimised for trapping of male apple leaf midges in orchards. If this work proceeds rapidly, similar work will be carried out to identify components of apple volatiles attractive to mated female apple leaf midge and to evaluate their use as attractants in traps alone or in combination with the synthetic pheromone.

This objective has been met in full. As a result of numerous comparative experiments, sticky delta traps baited with rubber septa loaded with 3 μg of the pheromone are recommended for monitoring apple leaf midge. They have been provided to growers in the UK for evaluation on a semi-commercial basis, as well as to collaborators in Italy, New Zealand and The Netherlands. In view of the success of the pheromone work, only preliminary studies were carried out on collection and analysis of apple volatiles using gas chromatography linked to electroantennographic recording from female midges and no active compounds were found.

3. The traps will be used as simple, highly specific tools to monitor emergence of apple leaf midge in orchards, and the results correlated with other methods of measuring adult populations and oviposition levels.

This objective has been met in full. Pheromone traps have been used to monitor diurnal behaviour and seasonal emergence patterns. Catches of male apple leaf midges in pheromone traps correlate well with oviposition by the females and infestation levels.

4. The traps will be used as monitoring devices to improve timing of the application of insecticides for killing adult apple leaf midge and deterring oviposition. Insecticides found to be effective previously will be evaluated as well as new, potentially more active products such as Trigard (cyronazine) which is currently registered in France against dipterous leafminers. Other factors may be investigated such as placement of the spray at the base of the trees to kill emerging apple leaf midge adults and minimise effect on egg parasites.

This objective has been met in full. Previous work established that foliar sprays of synthetic pyrethroid insecticides were the only effective approach and an effective regime has been established.

5. The project will liaise with manufacturers and growers to ensure that the pheromone traps are commercialised and made available to growers, along with protocols for their use in monitoring of apple leaf midge. Results of the insecticide trials will be demonstrated and made available to growers.

This objective has been met in full. A PCT application has been submitted on use of the apple midge pheromone and related compounds for management of midge pests. Pheromone traps are being evaluated by growers in the UK on a semi-commercial basis. Insecticide trials and those on control of apple leaf midge by attract-and-kill and mating disruption approaches have been carried out on commercial farms with participation by growers and manufacturers.

METHODS USED AND RESULTS OBTAINED

Objective 1. Identification and synthesis of female sex pheromone of apple leaf midge

Identification of pheromone

During July-August 2003, leaves with galls containing late larvae and pupae of apple leaf midge, *D. mali,* were collected from apple orchards and emerging larvae were reared individually to adult midges. Volatiles were collected from the adults by passing charcoal-purified air over groups of female or male midges separately and trapping volatiles on Porapak Q resin. In all volatiles were collected from 2191 females and 806 males. Trapped volatiles were eluted from the Porapak with dichloromethane and analysed by gas chromatography (GC) coupled to electroantennographic (EAG) recording from the antennae of male midges. A single component eliciting an EAG response was observed in collections from female midges. This was not present in collections from male midges and was assumed to be the female sex pheromone. Although at best less than 20 pg of this component was obtained from each female midge, mass spectra (MS) in electron impact and chemical ionisation modes were obtained. Comparison of MS data and GC retention times on polar and non-polar columns with those of standards from the library of compounds at NRI indicated the pheromone was a 17-carbon acetoxyketone with one double bond. After microhydrogenation to remove the double bond the positions of the acetoxy and keto functionalities were deduced from the MS and synthesis. The position of the double bond was worked out by interpretation of the original MS and the proposed compound, (*Z*)-13-acetoxy-8-heptadecen-2-one (Figure 1) was synthesised. The synthetic compound had GC and MS data identical with those of the natural compound and elicited a strong EAG response from a male *D. mali* midge.

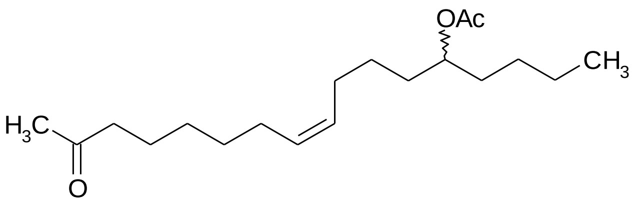

Fig. 1. Structure of (*Z*)-13-acetoxy-8-heptadecen-2-one, female sex pheromone of *D. mali*.

Synthesis of pheromone

The initial synthesis provided milligram quantities of material for field testing during 2004. Subsequently an improved route was developed which gave the racemic compound in 8 steps and 63% overall yield from readily available 6-bromohexanoic acid. This provided 20 gm quantities for field testing in both 2005 and 2006.

(*Z*)-13-acetoxy-8-heptadecen-2-one exists as two enantiomers. These could not be separated by GC on a chiral Cyclodextrin column at NRI. However, they were separated by colleagues at Tokyo University of Agriculture and Technology using chiral high performance liquid chromatography (HPLC), and the pure enantiomers made available for field testing. Examination of the ^{1}H nuclear magnetic resonance (NMR) spectra of the corresponding (*R*)-2-methoxy-2-trifluoromethyl-2-phenylacetyl esters indicated the first-eluting enantiomer has the *S* configuration, and this was subsequently shown to be the attractive enantiomer (below). Synthesis of the separate enantiomers to confirm this is in progress but has not been completed. Unless otherwise stated, the racemic mixture was used in all experiments described below.

Objective 2. Optimisation of pheromone dispenser and trap for apple leaf midge

Optimisation of pheromone dispenser and loading

In a first trapping experiment carried out in May 2004, sticky delta traps were baited with rubber septa or polyethylene vial dispensers containing 10 µg or 100 µg of synthetic (*Z*)-13-acetoxy-8-heptadecen-2-one. Results (Table 1) showed that the synthetic pheromone racemate dispensed from rubber septa was highly attractive to male *D. mali*, but not when dispensed from polyethylene vials during the course of this experiment.

TABLE 1. Catches of male *D. mali* in traps baited with racemic (*Z*)-13-acetoxy-8-heptadecen-2-one (6-20 May 2004; 5 replicates in randomised complete block design)

Treatment	Mean catch/trap/day[a]	
100 µg vial	0.7	c
10 µg vial	0.6	c
100 µg septum	288.3	a
10 µg septum	85.8	b
blank	0.2	c

[a] Means followed by same letter are not significantly different in LSD test (*P* = 0.05) after analysis of variance of log transformed data

In fact the high numbers of midges caught in traps baited with the septum containing 100 µg of pheromone meant that the traps were saturated with midges after a few days. In a second experiment, lower loadings of 1 µg and 3 µg were compared with the 10 µg loading. The results (Table 2) indicated that even traps baited with the 1 µg loading of the racemate caught significantly more than the unbaited traps, and there was a positive correlation between catch and pheromone loading.

TABLE 2. Catches of male *D. mali* in traps baited with racemic (*Z*)-13-acetoxy-8-heptadecen-2-one (20-27 May 2004; 5 replicates in randomised complete block design)

Treatment	Mean total/trap/day[a]	
100 µg vial	0.2	d
10 µg septum	55.9	a
3 µg septum	25.5	b
1 µg septum	3.05	c
blank	0.2	d

[a] Means followed by same letter are not significantly different in LSD test (P=0.05) in analysis of variance of log transformed data

Collaborators in New Zealand carried out similar experiments during October 2005 with a wider range of loadings of pheromone supplied by NRI in rubber septa, i.e. 3 µg, 10 µg, 30 µg, 100 µg, 300 µg, 1,000 µg, 3,000 µg, 10,000 µg and , 30,000 µg. There were three replicates at two sites using red delta traps. Cumulative catches were plotted against loading on log-log scales. Similar regular curvilinear dose responses were obtained in both experiments and catches were still increasing slightly at the highest loading tested. However, whereas increasing loading from 3 µg to 100 µg increased catch 100-fold, further increase of loading to 30,000 µg only increased catch by 10-fold.

In order to measure pheromone release rates, closed polyethylene vials, open polyethylene vials and rubber septa loaded with 100 µg were held in a laboratory wind tunnel at NRI at 27°C and 8 km/hr windspeed. Release rates were measured at intervals up to 574 days by aeration and trapping of volatiles on Porapak Q for 24 hr under the same conditions, followed by GC analysis against hexadecyl acetate as internal standard. Mean release rates for two replicates are shown in Figure 2. For the closed polyethylene vials the pheromone took several days to penetrate through the walls of the vial, which probably explained the low catches in the field tests. Release rates remained generally high at 6-7 ng/hr for about 100 days, and then steadily declined. The open vials did not show the initial lag but otherwise the release profile was similar. The rubber septa released the pheromone at a much lower but steadier rate of 0.5-1 ng/hr over the 574 day measurement period. This steady, low release rate makes rubber septa ideal dispensers for the pheromone for pest monitoring purposes. As release rates are proportional to loading, a lure loaded with 3 µg would be predicted to release pheromone at approximately 15-30 pg/hr and have a field life of >10 years.

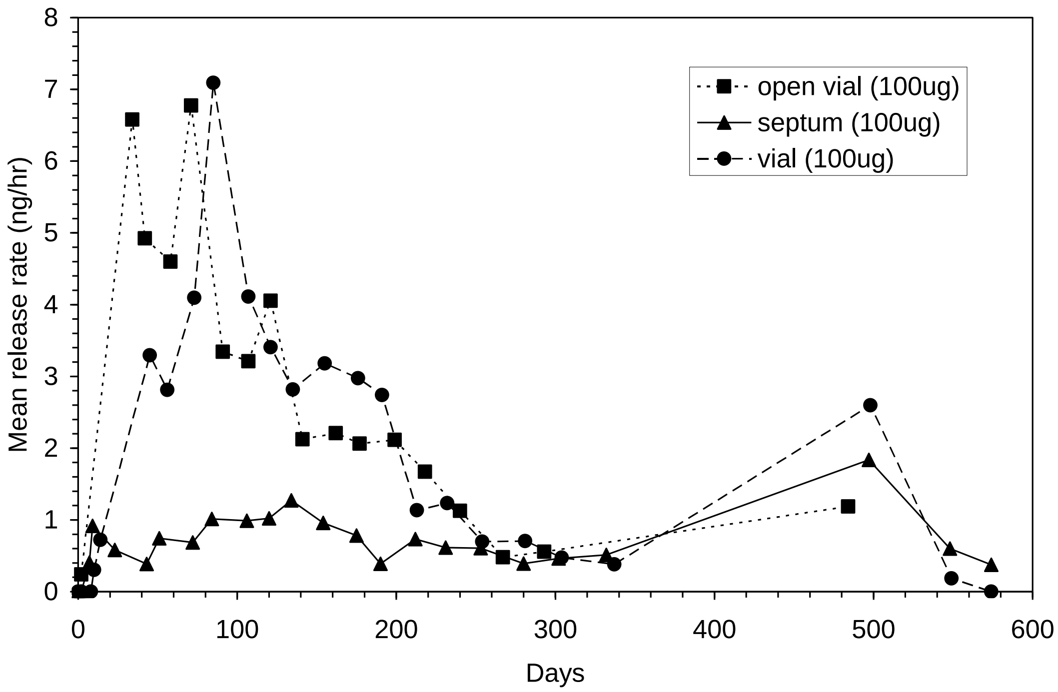

FIG. 2. Mean release rates of apple leaf midge pheromone in a laboratory wind tunnel at 27°C and 8 km/hr windspeed (n = 2).

Enantiomeric composition of pheromone

Two trapping experiments were carried out to compare attractiveness of the enantiomers of (*Z*)-13-acetoxy-8-heptadecen-2-one separated by HPLC (above) with two samples of the racemic compound. White sticky delta traps and rubber septum lures were used. Results in Table 3 show that the first eluting *S* enantiomer and the two racemic samples were highly attractive to male *D. mali*, and the second-eluting *R* was significantly less attractive. It is thus probable that the female midge produces the *S* enantiomer. From a practical point of view the most important result is that the racemic mixture is just as attractive as the single *S* enantiomer, the former being much easier and cheaper to produce.

TABLE 3. Catches of male *D. mali* in traps baited with enantiomers or racemic (*Z*)-13-acetoxy-8-heptadecen-2-one (10-24 July 2005; 5 replicates each experiment in Latin Square design).

	(*Z*)-13-Acetoxy-8-heptadecen-2-one in lure	Loading (µg)	Experiment 1 Gala orchard		Experiment 2 Bramley orchard	
			n	$\log_{10}(n+1)$	n	$\log_{10}(n+1)$
A	Enantiomer 1[a]	5	495	2.41	5528	3.728
B	Enantiomer 2[a]	5	5	0.69	867	2.309
C	Racemic (>95% pure)	10	998	3.01	6169	3.765
D	Racemic (>99% pure)	10	858	2.84	5699	3.734
E	No lure	0	5	0.57	10	1.005
	Fprob			<0.001		<0.001
	SED (12 df)			0.352		0.1200
	LSD (P = 0.05)			0.784		0.2641

[a] Fractions from HPLC separation of enantiomers

As a result of these experiments a rubber septum loaded with 3 µg of racemic pheromone was adopted as a standard lure for monitoring traps, providing a significant catch even at low populations and avoiding saturation of the traps at higher populations.

Optimisation of trap design

In July 2004, five different trap designs were compared for monitoring apple leaf midge. These were: a commercially-available dark green, funnel-type "Unitrap" (Agrisense); a bottle trap made from a two-litre clear plastic drinks bottle suspended vertically with four rectangular windows cut in the side and a layer of antifreeze solution in the bottom to retain insects; a transparent 10 cm Petri dish base held horizontally and containing antifreeze solution with a central pillar on which the lure was held approximately 2 cm above the surface of the antifreeze, as used for experiments with aphids; a standard 20 x 20 cm white delta trap containing a white sticky base (Agrisense); and a green, open Wota –T dish trap, 25 cm in diameter, 7 cm deep filled with antifreeze solution (Pest Control (India) Private Ltd). All traps were baited with a rubber septum containing 3 µg pheromone and five replicates were run in separate orchards at EMR and Broadwater farm.

Results in Table 4 show that the dish trap caught most midges although not significantly more than the delta trap. However, when catches in the different designs were ranked at each recording date, the overall rankings of these two traps were essentially the same. Furthermore if catches in the different designs were expressed as a percentage at each recording date, the mean percentage caught in the delta trap was clearly higher than in the dish trap. This was because when catches were low the delta trap always caught more male midges than the dish trap. Only when catches were high did the sticky base of the delta trap become saturated and more midges were caught in the dish trap. Other trap designs were less effective in catching midges.

TABLE 4. Catches of male *D. mali* in different designs of pheromone trap (9-30 July 2004; 5 replicates in randomised complete block design; catches recorded on 4 occasions; traps baited with rubber septum containing 3 µg pheromone)

Treatment	Mean total catch per trap	Mean √ total catch per trap	Mean % total catch per sampling date	Total of plot rankings
A. Funnel	193	25.4	15.3	72
B. Bottle	273	32.1	13.3	62
C. Petri	399	38.2	17.5	51
D. Delta	526	44.6	35.2	35
E. Dish	651	49.2	27.7	34
Fprob		0.003		
SED (13 df)		5.05		

In practical terms, the open Petri dish and Wota-T traps were not shielded from rain and were prone to overflowing. Also, it was difficult to clearly see the individual midges when submerged in liquid, a difficulty which also occurred with the funnel and bottle traps. It was thus concluded that for monitoring *D. mali* the standard delta trap is the best design for use by growers. The delta trap is robust and weatherproof; the sticky base is shielded from dust and rain and can be removed for accurate counting of trapped midges. Moreover the delta trap is readily available and widely used by growers for monitoring other insect pests. Monitoring of *D. mali* will be most critical at low populations where the problem of saturation of the sticky base does not occur. For mass trapping of midges where maximum catch and low maintenance are important, some form of bottle or dish trap with water as the catching agent may be more suitable.

It was thought that trap colour would be important for the day-flying *D. mali*. Delta traps were constructed from red, green, yellow, black and blue Correx sheet and compared with the standard white traps. The traps were suspended from a low branch in the tree at a height of approximately 0.5 m and spaced 15 m apart.

Results (Table 5) showed no significant difference in catches of midges in the different coloured traps, but numbers of non-target species were lower in the red, green and black traps compared with numbers in the yellow and white traps. The majority of these were small Diptera. Green traps are probably impractical to use in orchards because of difficulty in locating them. Consideration should be given to using red or black traps, but more work is needed to investigate the effects of trap colour on specific groups of non-target arthropods, especially bees and bumble bees.

TABLE 5. Catches of male *D. mali* in different coloured delta traps (3-11 July 2006; 3 replicates in randomised complete block design; traps baited with rubber septum containing 3 µg pheromone)

Trap colour	Mean no. midge/trap		Mean no. non-targets/trap		
	n	$\log_{10}(n+1)$	n	$\log_{10}(n+1)$[a]	
Red	203	2.266	4.0	0.661	a
Green	239	2.379	3.7	0.667	a
White	215	2.207	9.7	1.011	b
Yellow	223	2.337	8.7	0.982	b
Black	180	2.258	3.7	0.661	a
Blue	237	2.343	10.3	1.029	b
Fprob		0.932		0.002	
SED (10 df)		0.1853		0.0899	
LSD (P = 0.05)		0.4128		0.2002	

[a] Means followed by the same letter do dot differ significantly (P = 0.05) in a Duncan's Multiple Range Test

Optimisation of trap deployment

The effect of trap height on catches of male *D. mali* midges in pheromone traps was investigated by comparing catches in standard white delta traps positioned at 0 m, 0.5 m, 1 m, 1.5 m, 2 m and 2.5 m above ground level and baited with a rubber septum containing 3 µg pheromone.

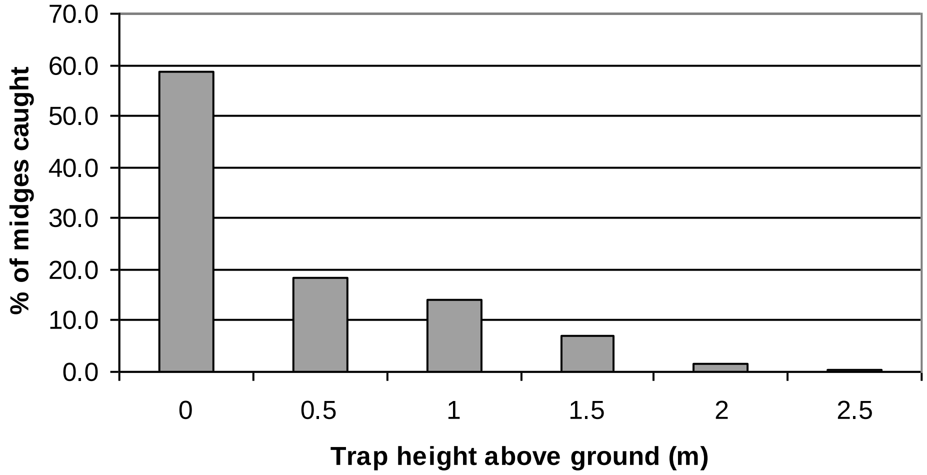

FIG. 3. Effect of trap height on catch of apple leaf midge (13-29 July 2004; 3 replicates).

Height of trap deployment had a strong effect on the number of midges caught (Figure 3) with nearly 60% of midges caught in the traps deployed at ground level, 20% caught at 0.5 m above ground, and progressively decreasing percentages at increasing height. Very few were caught at 2.5 m, the height of the canopy. In view of these results, it was decided that it was important to standardise the height of trap deployment for pest monitoring. A standard deployment height of 0.5 m was chosen, enabling traps to be hung from suitable low hanging branches. Deployment on the ground was considered to be impractical.

The range of attraction of the pheromone traps was investigated in a simple experiment with standard white delta traps baited with a 3 µg rubber septum lure deployed in a cereal field at EMR at distances of 10 m, 20 m, 30 m, 40 m and 50 m from an isolated apple orchard infested with apple leaf midge. The orchard chosen was a long rectangular one and the traps were positioned relative to the orchard and the prevailing light south westerly wind so that trap skipping was unlikely. The results clearly show that midges were caught at all distances up to 50 m from the edge of the apple orchard, with a clear trend to decreasing catch with distance (Table 6).

TABLE 6. Numbers of male *D. mali* caught in delta traps at varying distances from an isolated apple orchard (18-27 May 2004; single replicate, 4 sampling occasions).

Sampling	Distance of trap from edge of apple orchard				
date	10m	20m	30m	40m	50m
19 May	210	174	84	200	70
20 May	81	65	27	30	13
24 May	182	69	22	22	14
27 May	90	40	15	14	6
Mean/day[a]	91.6 a	67.4 b	30.4 de	60.0 cd	22.1 e

[a] Means followed by the same letter do not differ significantly (LSD test, $P = 0.05$)

Objective 3: Development of pheromone-baited traps for monitoring apple leaf midge

Diurnal correlation of trap catches of male D. mali and oviposition by females

An experiment was carried out in 2004 in which catches of male *D. mali* in standard white delta pheromone traps baited with a septum containing 3 µg of pheromone were recorded every hour as were numbers of eggs laid by female midges in nearby apple trees (five shoots on 80 trees). The experiment was done in two commercial Bramley apple orchards at Broadwater Farm. The results (Figure 4) showed that large numbers of apple leaf midge males were caught immediately at 09:00 hrs but that thereafter numbers declined steadily through the day. Conversely, numbers of ovipositing females were very low at 09:00 hours but increased steadily, reaching a peak at 11:00–12:00 hrs and declining thereafter.

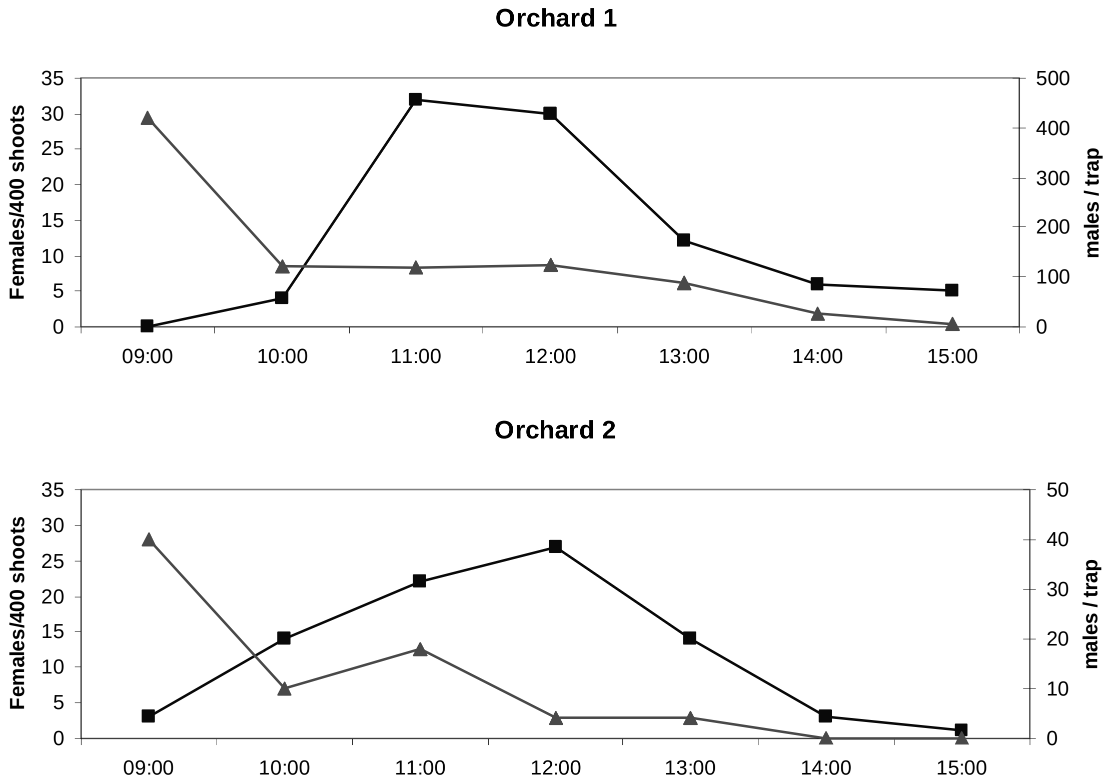

FIG. 4. Numbers of ovipositing apple leaf midge females (■) and males caught in pheromone traps (▲)in two Bramley orchards (22 July 2004; Broadwater farm, West Malling)

A second experiment was done in 2005 with three widely-spaced, standard white delta traps baited with a rubber septum containing 100 µg of pheromone. Catches were recorded at two-hourly intervals during a 24-hr period. Numbers were zero throughout the night from 22:00–07:00 hrs. They rose steeply in the morning starting at 07:00 hrs (2 hours after dawn) and rapidly reached a peak at 09:00 hrs. Numbers steadily declined throughout the day thereafter, confirming the previous year's results.

These results imply that mating occurs in the earlier part of the morning and that oviposition soon afterwards towards the middle of the day.

Population monitoring in orchards

In 2004, two standard white delta traps baited with standard 3 µg rubber septa lures were deployed in each of 6 commercial apple orchards at Broadwater Farm, West Malling, Kent and monitored at 3-4 day intervals from 28 May – 1 September. The orchards were of different varieties, rootstocks and ages and thus had widely varying populations of apple leaf midge: 1) a newly planted intensive M9 Braeburn orchard which had very low midge populations; 2) a 1 year old intensive M9 Braeburn orchard which had slightly higher populations of apple leaf midge; 3) established M26 Bramley orchard with moderate populations of leaf midge; 4) young established Gala/Meridian orchard with moderate populations of midge; 5) established Gala orchard with moderate midge populations; 6) a vigorous old established Bramley orchard with MM106 rootstocks and high populations of apple leaf midge. On each monitoring date, 100 shoots in the centre of each orchard (5 selected at random on each of 20 trees) were examined for the presence of ovipositing females and the numbers of leaves with apple leaf midge galls were counted. Then the distribution of galls in an additional 25 new shoots (5 from 5 trees) was scored.

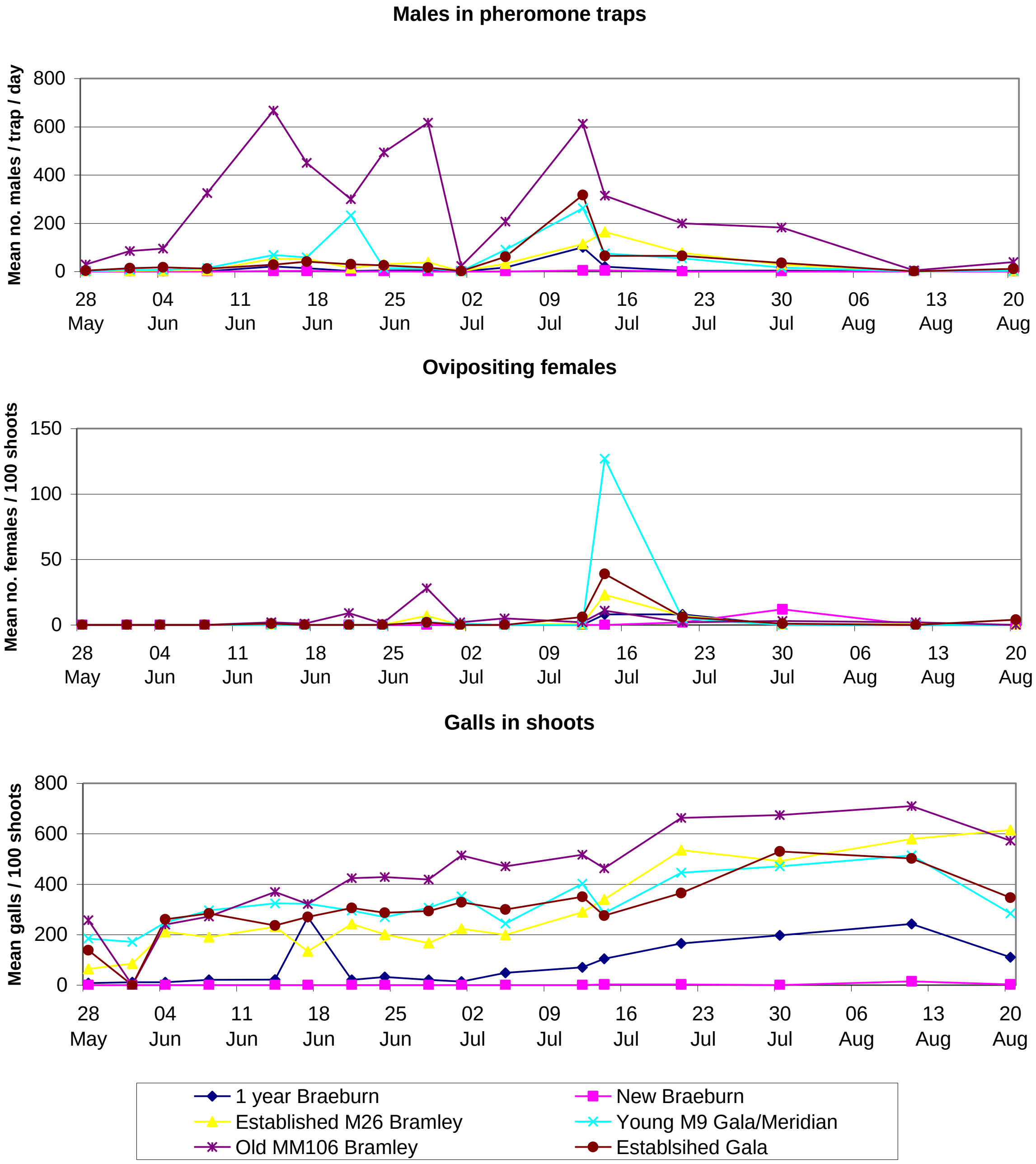

FIG. 5. Mean numbers of apple leaf midge males captured per day (top), numbers of ovipositing females per 100 shoots (middle) and numbers of galls per 100 shoots (bottom) in six orchards at Broadwater farm, West Malling in 2004.

The pheromone trap catches showed two clear generations of apple midge after 28 May 2004 (the first generation was missed because the synthetic pheromone was not available earlier) (Figure 5). The second generation occurred in June and the third in July and August. There were clear, large differences in total numbers of males caught, which reflected the population density in the particular orchard. A mean total of 67 males were captured in the newly planted Braeburn orchard, mostly in July and August. A mean total of 718 males were caught in the lightly infested 1 year old Braeburn orchard. Mean totals of 3090 – 3377 males were captured in the three moderately infested orchards and a mean total of 20,400 males was caught in the heavily infested old vigorous Bramley orchard. Numbers of ovipositing females in shoots followed similar trends, peak numbers broadly coinciding with the latter part of the peaks of the male flights and total numbers correlating with the total numbers of males. Total numbers of galls per 100 shoots showed an upward trend throughout the monitoring period in the moderately and heavily infested orchards. Numbers of galls broadly correlated with the numbers of midges caught. Only in the lightly infested 1 year old Braeburn orchard was a second generation peak in galling damage clearly apparent.

These results, taken together, were the first indication that the pheromone traps could be used for pest monitoring purposes and that that catches reflected the degree and timing of damage.

In 2005, single standard white delta traps baited with standard 3 µg rubber septa lures were deployed at 0.5 m above ground in the centre of each of 6 plots in the IPM trial in Wiseman orchard, EMR. Two of the six plots were completely untreated with insecticides, two received a standard conventional full insecticide and fungicide programme and two of the plots received the EMR zero residues IPM programme. In addition, double sided yellow sticky traps were deployed nearby at mid tree height to monitor populations of adults of the apple leaf midge egg parasitoid, *Platygaster demades.* Catches of male apple leaf midge and adult male and female *P. demades* were recorded weekly and the percentages of growing shoots damaged by apple leaf midge larvae were recorded once for each generation on 19 May, 4 July and 2 September 2005 respectively.

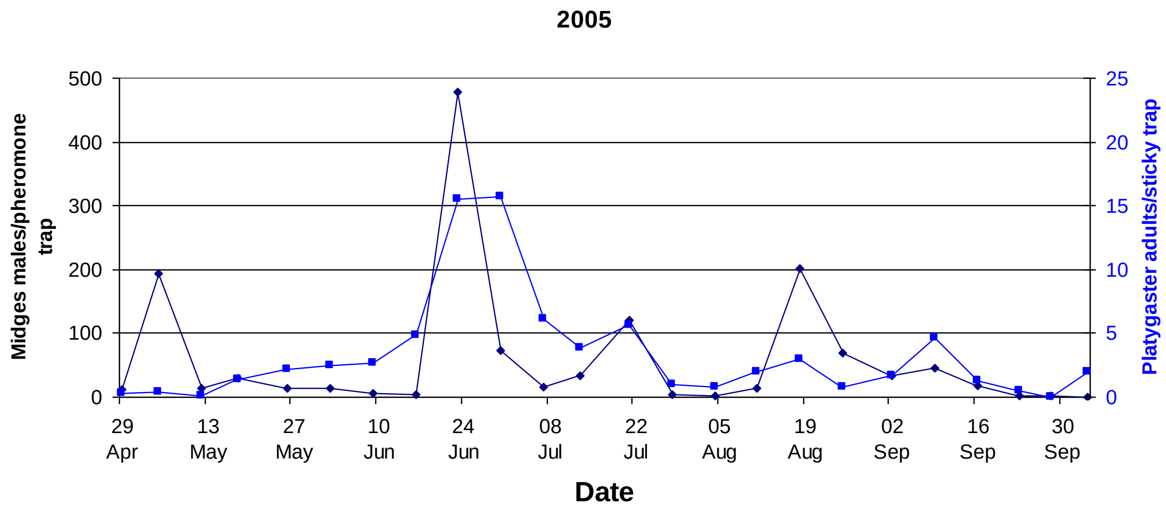

FIG. 6. Weekly grand mean numbers of apple leaf midge males (black ■) caught in standard white delta sex pheromone traps and *P. demades* adults (blue ♦) caught in yellow sticky traps in plots in the zero residues IPM experiment in Wiseman orchard, EMR, in 2005

Grand mean numbers of midges caught in the IPM plots (1019/trap) were somewhat lower than in the untreated (1512/trap) or conventionally sprayed (1672/trap), though these differences were small and the data was not subjected to statistical analysis (two replicates only). The flight of the male midges indicated three generations, the first in early May, the second in late June and early July and the third in late August and early September (Figure 6). The first generation attack led to an average of 7.8% of shoots being galled, the second generation to 27. 8% of shoots galled and the third generation to 78% of shoots being galled. However, at this latter generation, shoot growth had more or less terminated and numbers of growing shoots were very small.

Totals of 237 *Platygaster demades* males and 263 females were captured. There were no obvious differences in the timings of the fights of the two sexes. No *P. demades* adults were recorded at the peak of the first generation flight of apple leaf midge but subsequent peak numbers more or less coincided with those of apple leaf midge male catches (Figure 6).

Further investigations into the phenology of apple leaf midge in relation to its parasitoid and the relationship between pheromone trap catches and oviposition, were done in 2006. Single standard apple leaf midge delta traps and yellow sticky traps for *P. demades* adults were deployed in the centres of three apple plots at EMR on 18 April 2006 and were monitored at 3-4 day interval throughout the season. Two of the plots were untreated controls in the Wiseman zero residues IPM experiment (records taken on cv Gala), the third plot was a young, conventionally sprayed Bramley orchard. Thirty growing shoots were examined in the centre of each plot on each monitoring occasion for the presence of apple leaf midge eggs. Counts of the numbers of galls in the terminals were made on 9 June and 14-16 July and 100 mature larvae were sampled from each plot on 13 June and 16 July 2006 and dissected to determine whether or not they were parasitised by *P. demades*.

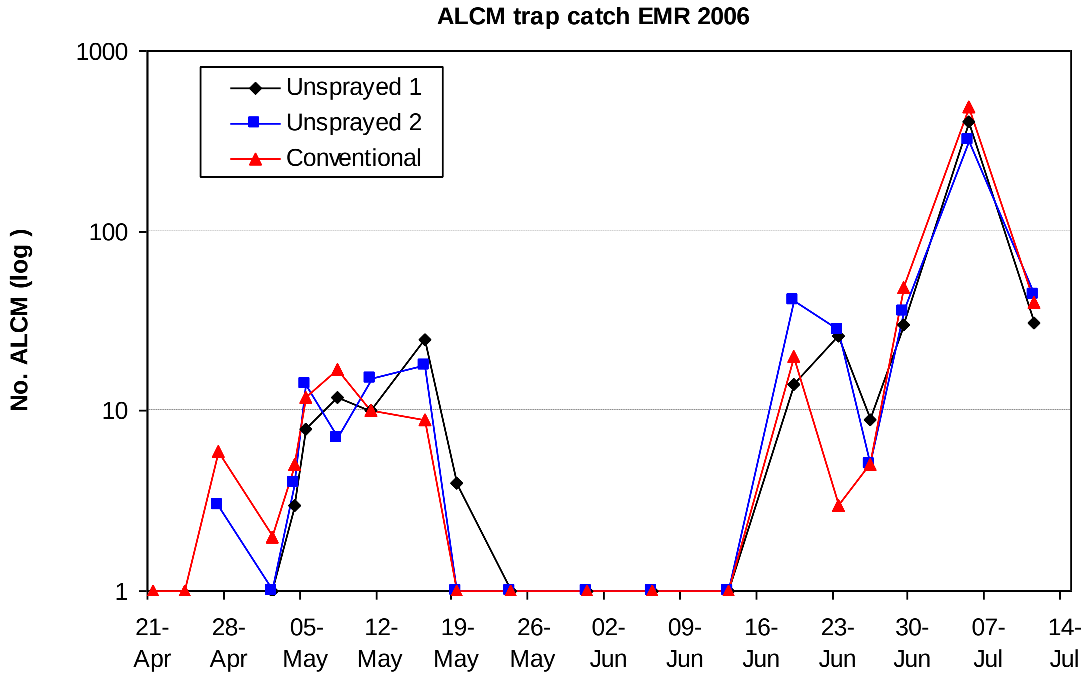

FIG. 7. Mean total numbers of apple leaf midge males captured in single pheromone traps in two unsprayed IPM plots in the Wiseman zero residues IPM experiment and in a conventional sprayed orchard at EMR in 2006.

The first generation flight of males started on 24 April ending on 19-26 May and the second generation flight started on 19 June 2006. The pattern and sizes of emergence was very similar in all 3 plots (Figure 7). The percentage shoots infested with eggs followed a remarkably similar pattern, demonstrating the close correlation between trap catches of males and oviposition by females (Figure 8). Peak catches of 11 males per day resulted in 6% of shoots infested with eggs in the first generation. Peak catches of 5 males per day on 23 June correlated with 19% of shoots infested with eggs on that day and peak catches of 67 males per day on 5 July correlated with 90% of shoots being infested with eggs on that day. Thus the ratio of daily catches of males to percentage shoots infested varied considerably. These results do however, indicate that a very low pheromone trap threshold (<< 5 males/trap/day) would be appropriate as an indicator of low levels of ovipostion (<10% shoots infested).

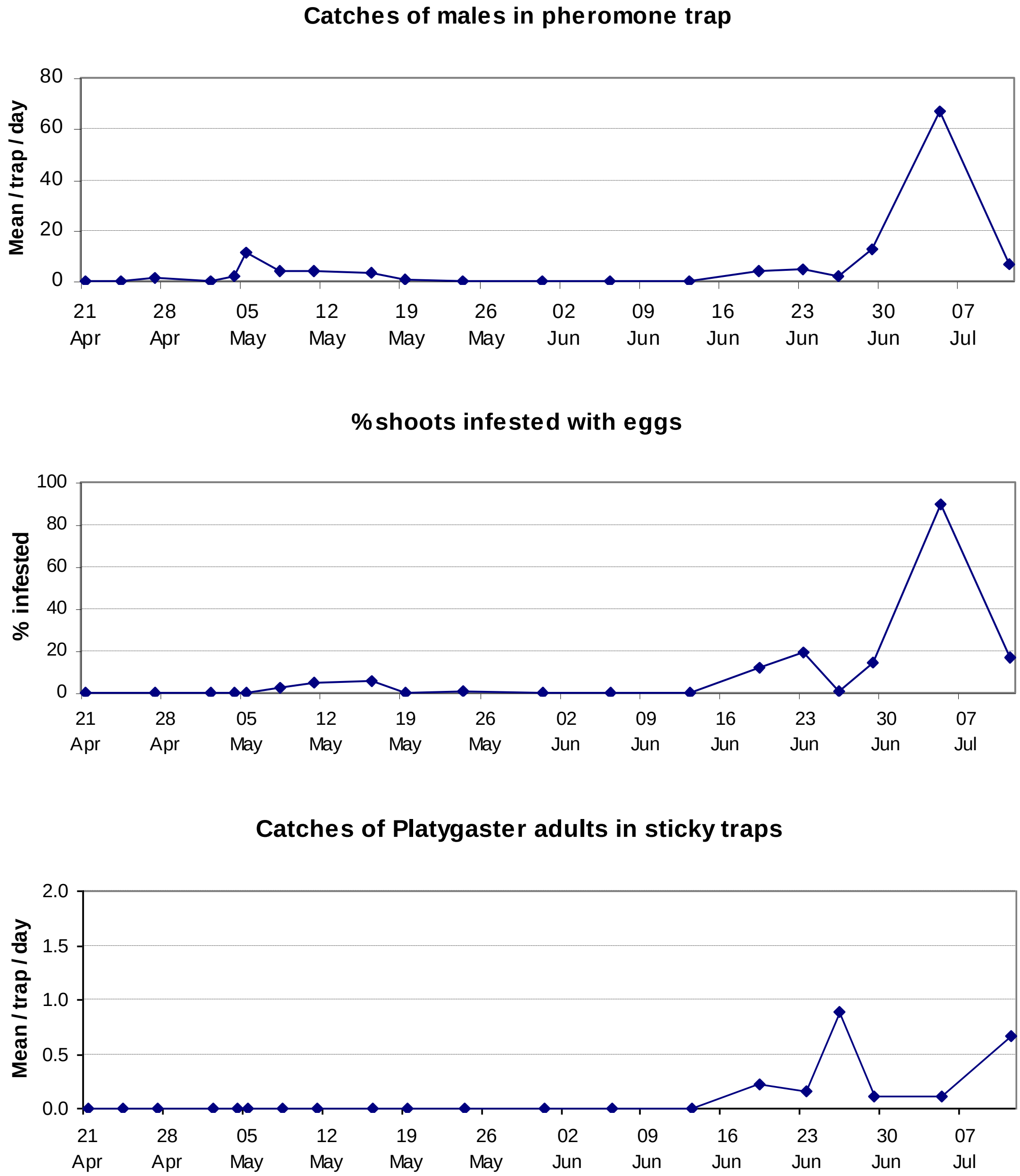

FIG. 8. Grand mean catches of apple leaf midge males in sex pheromone traps per day (upper), percentage shoots infested with apple leaf midge eggs (middle) and catches of *P. demades* adults in yellow traps (lower) in 3 plots (2 unsprayed in Wiseman zero residues experiment and one conventionally sprayed orchard) at EMR in 2006.

Objective 4: Development of control strategies for apple leaf midge

Use of pheromone traps to time insecticide sprays

Previous work at EMR showed that foliar sprays of synthetic pyrethroid (SP) insecticides were the only approach that provided any significant control of apple leaf midge. In 2005, a simple experiment was done to examine the use of apple leaf midge sex pheromone traps to time sprays of SP insecticides to control galling damage by apple leaf midge. Four orchards were selected in the Faversham area of Kent, ranging in size from 0.6 ha to 3.0 ha. Two of the orchards were newly planted and had low populations of apple leaf midge. Two of the orchards were established and had high populations. A single standard white delta trap with standard 3µg rubber septum lure was located in the centre of each orchard and the numbers of apple leaf midge captured were recorded at approximately weekly intervals through the season. Sprays of the SP insecticide deltamethrin (Decis) were applied on 4 May and 24 June 2005 in response to increases in pheromone trap catches in an attempt to control apple leaf midge. Galling damage was assessed on 23 May (first generation), 20 June and 6 July (second generation) and 31 August (third generation)

TABLE 7. Numbers of shoots assessed, numbers infested with apple leaf midge galls and total pheromone trap catches in the insecticide experiment (27 April-30 September 2005).

	A. Untreated low population		B. Untreated high population		C. Treated low population		D. Treated high population	
Date	Assess	Dam	Assess	Dam	Assess	Dam	Assess	Dam
23 May	120	0	120	14	120	1	120	28
20 June	302	2	200	4	477	4	259	2
6 July	263	59	200	117	235	16	327	210
31 August	262	245	235	235	234	214	168	160
Total catch	83		921		85		362	

The first generation flight of the midge was recorded on 3 May, the second generation on 12 July and the third generation on 10 August 2005. Catches reflected the population in the particular orchard with low catches in the newly planted orchards and high catches in the established orchards (Table 7). However, damage levels as measured by the percentage of shoots infested with *D. mali* showed no obvious differences between the different plots during the season, and there was essentially 100% infestation by the end of the season.

In 2006, a replicated field experiment was done to determine whether apple leaf midge sex pheromone trap catches can be used to time sprays of the SP insecticide cypermethrin to control the first generation of apple leaf midge and to examine the numbers and timings of sprays required to achieve good control. The experiment was done in a young apple plantation at EMR with alternating rows of Bramleys Seedling and Jonagold on the M9 rootstock. A standard white delta pheromone trap with 3 µg rubber septum lure was deployed in the centre of the plantation at a height of 0.5 m on 18 April and catches monitored every 3-4 days. Treatments were 1, 2, 3 or 6 applications of the synthetic pyrethroid insecticide cypermethrin (Toppel 10, United Phosphorous) applied at a rate of 350 ml product in 500 l water per ha per spray with a motorised air-assisted knapsack sprayer, and an untreated control. A 5 x 5 Latin square experimental design was used for the experiment. Each plot consisted of 4 adjacent Bramley trees which received the spray treatments, but only the central two trees were used for assessment. Plots were separated by 4 unsprayed guard trees and a guard row on each side on the variety Jonagold.

The first spray applications were made on 4 May, at the beginning of the flight of apple leaf midge males, as indicated by catches in the sex pheromone trap (Figure 9).

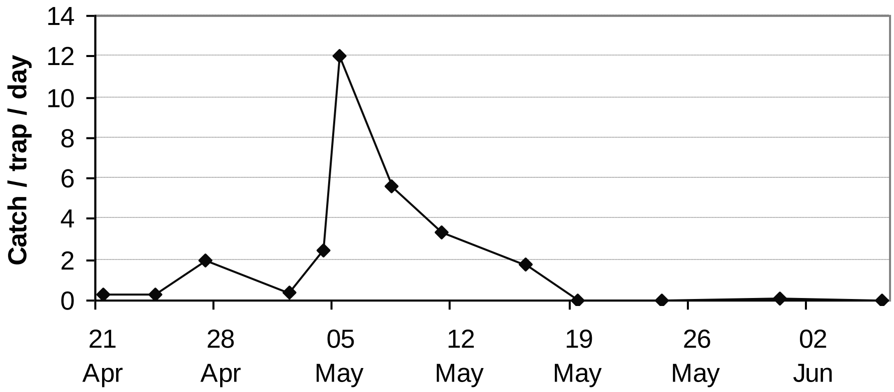

FIG. 9. Catches of first generation male apple leaf midge in the sex pheromone trap in the insecticide trial in 2006.

Counts of eggs and ovipositing females were made on 5 shoots on each of the two central trees in each plot on 8 May (50% bloom), 16 May (50% petal fall), 25 May (end of petal fall) and 7 June (early fruitlet). A full assessment of galling damage on 20 shoots per plot was done on 7 June 2006. Weather conditions were not favourable for midge development and the first generation flight of *D. mali* was small. On the untreated control, only 3.4 % of shoots were damaged by apple leaf midge galls on

7 June with a mean of 12.8 galls per tree (Table 8). The treatment with 6 sprays of cypermethrin virtually eliminated the gall infestation, but the treatments with 1, 2 or 3 sprays performed similarly, reducing gall numbers by 75% on average.

TABLE 8. Shoot and gall damage by apple leaf midge in insecticide trial (measured on 7 June 2006; means of 5 replicates)).

	No. shoots per tree			No. galls per tree	
No. sprays cypermethrin (dates 2006)	n	No. damaged	% damaged	n	$\log_{10}(n+1)$
0	54.2	5.0	3.40	12.8	1.059
1 (4 May)	47.1	1.9	4.45	5.1	0.629
2 (4,15 May)	49.2	0.9	2.32	1.9	0.308
3 (4,11,18 May)	46.4	1.6	3.72	3.5	0.403
6 (4,8,11,15,18,23 May)	48.6	0.1	0.14	0.1	0.030
Fprob					0.002
SED (12 df)					0.1882
LSD (P=0.05)					0.410

Use of pheromone for control of apple leaf midge by attract-and-kill and mating disruption

In July 2004, a preliminary field experiment was done at EMR to investigate use of the apple leaf midge sex pheromone for control of apple leaf midge using an attract-and-kill (A&K) strategy. A&K devices were 20 x 20 cm squares of plastic laminated cardboard surface coated on both sides with a microencapsulated formulation of the SP insecticide lambda cyhalothrin developed for control of olive fly (Agrisense). These were positioned 5 cm above ground level and baited with a rubber septum lure impregnated with 100 µg of apple leaf midge pheromone fixed centrally.

Four small heavily infested apple orchards at EMR were selected for the experiment: On 5 July, a single standard white delta trap baited with a standard 3 µg rubber septum lure was deployed in the centre of each plot. Catches of males were counted on six occasions between 7-27 July 2004. On 7 July, 28 and 12 A&K devices respectively were deployed in lattices in two of the plots to give a density of approximately 100/ha. On 27 July, 100 shoot terminals in the centre of each plot were examined for ovipositing females and presence of apple leaf midge galls.

TABLE 9. Pheromone trap catches of male *D. mali* and damage assessments in preliminary attract and kill experiment at EMR (pheromone trapping 7-27 July; damage assessments on 27 July 2004)

Plot	DM153	DM167	DM169	WM135
Variety	Bramley	Cox	Fiesta	Mixed dessert
Area	0.28 ha	0.08 ha	0.26 ha	0.09 ha
No. A&K devices	28	12	0	0
Trap catch/day	40.5	7.6	122.2	409.1
% shoots galled	100	84	100	100
No. females/100 shoots	3	0	2	5

The two orchards where the A&K treatment was deployed had considerably lower trap catches than the untreated plots, but the treatments failed to shut down catches completely (Table 9). The shoots assessment on 27 July revealed that 100% of shoots were galled on 3 of the plots with 84% galled on the other. Small numbers of ovipositing females were also recorded. The results showed the treatment was not able to adequately suppress mating and that a much higher dosage of pheromone would be required with many more devices per ha and/or a higher pheromone dose.

Bioassays of the effect of contact of apple leaf midge adult males with the lambda cyhalothrin target devices were conducted on 1 and 2 September 2004. A&K devices were observed in the field and at intervals 10 attracted male midges were pootered from the surface of the device or shortly after they had made contact, and held in tubes. Midges were pootered in a similar way from the surface of a similar device not treated with lambda cyhalothrin. After 1 hr, all the midges that had been exposed to the lambda cyhalothrin card, even for 5 min, were severely affected by the insecticide. They were unable to fly and lay trembling in the bottom of the tube. After 2 hr, all were trembling or moribund. After 3 hr, all were dead. Similar results were obtained with A&K devices that had previously been exposed for two months in the field.

A very large scale field trial was carried out in commercial apple orchards in Kent during 2005 to evaluate the use of the apple leaf midge sex pheromone for control of apple leaf midge by mating disruption (MD) or attract and kill (A&K) approaches. MD devices were polythene caps each initially loaded with 500 µg of the apple leaf midge sex pheromone. These caps each released the pheromone at approximately 10 ng/hr at 27 ºC in the laboratory. The attract and kill target devices were 10 cm x 6.7 cm oblongs of the microencapsulated lambda cyhalothrin surface treated cardboard with a polythene cap lure containing 100 µg of the apple leaf midge sex pheromone fixed to the centre with a drawing pin. These caps each released the pheromone at approximately 2 ng/hr at 27 ºC in the laboratory. Both MD and A&K devices were deployed at 500 devices/ha or 2000 devices/ha, fixed to tree stakes so that the lure was at a height of approximately 15 cm above the ground in a regularly spaced lattice. Thus for the MD treatments, pheromone application rates were 0.25 g/ha or 1 g/ha respectively and release rates were approximately 5 µg/ha/hr and 20 µg/ha/hr. For the A&K treatments, pheromone application rates were 0.05 g/ha or 0.2 g/ha respectively and release rates were approximately 1 µg/ha/hr and 4 µg/ha/hr.

A fully randomised experimental design was used with six replicate 1 ha plots of each treatment, requiring 30 plots of 1 ha in 11 orchards on six different fruit farms in Kent. Three of the plots for each treatment were in newly planted orchards where leaf midge populations were low and three in established orchards where leaf midge populations were high. Untreated plots were well separated from those which had MD or A&K treatments which themselves were adjacent. Experimental Approvals allowing this work to proceed without crop destruction were applied for and granted by PSD.

The effectiveness of the treatments was assessed by weekly monitoring of catches of adult male midges in a delta trap baited with a 3 µg rubber septum lure in the centre of each plot and by counting the number of galls present in 200 shoots in the centre of each plot for each of the three main generations, at the peak of damage expression on 17-23 May, 20-25 June, 4-6 July and 30-31 August 2005.

TABLE 10. Pheromone trap catches of male *D. mali* and damage assessments in mating disruption (MD) and attract-and-kill (A&K) trials (April-September 2005; 1 ha plots, 3 replicates per treatment)

	Mean pheromone trap catch				Mean % shoot damage			
Treatment	Apr-May	Jun-July	Aug-Sept	Total	17-23 May	20-25 Jun	4-6 Jul	30-31 Aug
Heavily-infested orchards								
MD 500/ha	3	5	43	52	50	1	73	99
MD 2000/ha	6	1	30	36	28	2	62	99
A&K 500/ha	7	29	73	109	43	37	58	95
A&K 2000/ha	7	1	9	16	21	13	83	93
Untreated	429	4094	817	5340	29	5	29	94
Newly-planted orchards								
MD 500 /ha	0	0	3	3	0	0	3	87
MD 2000/ha	0	1	2	2	0	0	3	87
A&K 500/ha	0	0	4	4	0	0	5	88
A&K 2000/ha	0	0	0	0	1	0	4	85
Untreated	0	11	7	18	0	0	14	90

In the analyses of variance of the log transformed total counts, the only differences of significance were whether or not any 'treatment' had been applied, with no differences between type and number of lures. All the MD and A&K treatments suppressed the catches of males in the traps in the centres of the plots compared to the untreated control (Table 10). In the established orchards with higher populations, catches were decreased by > 98% in April-May, by > 99% in June-July, but by only >91% in August-September (Table 10). In the newly planted orchards with very low populations of the midge, trap catches were zero in April-May in all plots, were very low but suppressed by 90% by the MD and A&K treatments in June-July, but rose somewhat in August September being suppressed by about 80% in the treated plots (Table 10).

Regrettably, there was no evidence that either the MD or A&K treatments were suppressing numbers of galls in shoots in the established orchards (Table 10). In the newly planted orchards, it appeared that the MD and A&K treatments were failing in July and certainly by August, no suppression of galling damage was evident.

On 9 September 2005, bioassays of the effectiveness of target devices which had been deployed in the field since the start of the A&K trials were conducted in a similar way to that described above for the preliminary experiment. The results were less clear cut than in the previous bioassays with substantial mortality in the untreated controls. However, the experiments did show that the devices maintained their activity.

Measurement of release of pheromone from the open polyethylene caps used in these trials for both MD and A&K treatments showed relatively uniform release for at least 270 days under laboratory conditions. However, lures recovered from the field at the end of the above experiments were found to contain no detectable pheromone. This has subsequently been shown to be due to degradation of the pheromone, the lures being unprotected from direct sunlight in both MD and A&K treatments.

Use of pheromone for control of apple leaf midge by mass trapping
Control of apple leaf midge by mass trapping is under investigation in New Zealand using pheromone supplied by NRI. In a trial carried out during 2005, water traps baited with rubber septa containing 3 mg of pheromone were used at 500 traps/ha. Numbers of midges caught in treated areas were reduced by 96% relative to those caught in untreated areas. However, shoot infestations were uniformly very low that season, probably due to the dry summer, and it was not possible to determine any effect of the treatments. This work is to be repeated during 2006 if pheromone is available.

Objective 5. Transfer of technologies

- An international patent application has been made on the new group of midge pheromone, the keto esters, including the apple leaf midge pheromone and that of the raspberry cane midge, *Resseliella theobaldi*, subsequently discovered by the NRI/EMR team (PCT/GB2005/002504).

- The apple leaf midge sex pheromone trap and lure was made commercially available to UK growers in the 2006 growing season who have registered to take part in a Beta test of the trapping system. A detailed instruction sheet was provided with the system describing setting up and maintenance of the traps as well as interpretation of the results. Depending on the results, it is anticipated that the pheromone trapping system will be made fully available to growers in 2007.

- The UK company Agrisense provided the insecticide-treated cards for the A&K trials, and the polyethylene caps used in both MD and A&K trials were purchased from them. Another UK company, International Pheromone Systems Ltd., has also expressed interest in commercialising the pheromone.

- Collaborations have been set up to test and develop the apple leaf midge pheromone for pest monitoring and control with research institutes in Italy (Istituto Agrario di S. Michele all'Adige, IASMA), New Zealand (HortResearch) and The Netherlands (Applied Plant Research, Lisse).

MAIN IMPLICATIONS OF THE FINDINGS

Objective 1. Identification and synthesis of female sex pheromone of apple leaf midge

The female sex pheromone of the apple leaf midge, *D. mali*, has been identified as the novel compound, (*Z*)-13-acetoxy-8-heptadecene-2-one. Because of its importance, internationally-renowned groups in New Zealand, Canada and the USA have previously tried to identify this pheromone but failed. The NRI/EMR team was successful by bringing together their experience in both the chemical and entomological aspects of the work. Techniques such as collection of pheromone by trapping of volatiles rather than gland extraction and use of highly-sensitive ion trap mass spectrometry were particularly important.

The pheromone is chiral and only one of the two enantiomers is attractive to male midges, probably the *S* enantiomer. However, the racemic mixture of both enantiomers is just as attractive. If the pure *S* enantiomer was required for attraction, use of this in control of *D. mali* by mass trapping, lure-and-kill or mating disruption approaches could probably not be considered for economic reasons. However, a convenient synthetic route to the racemic compound has been developed and 20 gm quantities synthesised at NRI for field trials of mating disruption and lure-and-kill in 2005 and 2006.

Objective 2. Optimisation of pheromone dispenser and trap for apple leaf midge

The pheromone of *D. mali* is incredibly active – the most active compound encountered by the author of this report in over 30 years of work on semiochemicals. Rubber septa dispensers impregnated with only 1 µg of the racemic pheromone (0.5 µg of the active enantiomer) are significantly attractive to male midges in the field, and this is even more remarkable because the actual release rate of active material is less than 100 pg/day.

Rubber septa dispensers impregnated with only 3 µg of racemic pheromone and white, sticky delta traps are recommended for use in monitoring *D. mali*. Lures and traps will last at least a season if the sticky bases are replaced regularly. This monitoring package is being tested during 2006 by volunteer growers who have purchased the system from NRI/EMR.

Traps should be placed as near the ground as possible: 0.5 m above ground is recommended. The traps attract male midges from at least 50 m.

The delta traps can become saturated if midge populations are high or traps not emptied for long periods. For catching large numbers of midges, as in control by mass trapping, traps using water as the trapping medium may be more suitable. Dish and bottle traps were evaluated during this project and the latter have been used for mass trapping in New Zealand.

Objective 3: Development of pheromone-baited traps for monitoring apple leaf midge

Pheromone-baited traps provide a highly sensitive means for detection of adult male apple leaf midge, and catch of the male midges is closely correlated with subsequent oviposition by the females. Furthermore, the results provided the first demonstration that the pheromone traps could be used for pest monitoring purposes and that that catches reflected the degree and timing of damage. The results indicate that a very low pheromone trap threshold (<< 5 males/trap/day) would be appropriate as an indicator of low levels of ovipostion (<10% shoots infested).

The traps showed that there typically at least three generations of apple leaf midge per year in southern England. Adults of the apple leaf midge egg parasitoid, *P. demades,* could not be detected during the first generation of apple leaf midge, suggesting that other interventions are required to control this generation.

Objective 4: Development of control strategies for apple leaf midge

The pheromone-baited traps can be used to time interventions against the first generation of apple leaf midge. Previous trials indicated that synthetic pyrethroids (SP), applied as foliar sprays, were the only insecticides out of a wide range of materials tested with significant efficacy against the pest. They appear to act by killing adults and deterring oviposition. Soil applications of chlorpyrifos were shown to be ineffective. The aim of the experiments reported here was to investigate whether more accurate timing of foliar sprays of synthetic pyrethroids improved control. However, results indicate that even when spraying is initiated at the first appearance of apple leaf midge, up to six sprays of cypermethrin at 3-4 day intervals are required for control, and a seven day interval is too great. Although SP insecticides have good persistence, midges attack the new growth in the growing shoot which needs continually protecting with deposits of insecticide.

The results do point to an effective treatment for apple leaf midge for nursery growers, and possibly in orchards where established trees have been re-grafted. Elimination of the first generation may keep midge levels low for the rest of the season, although continued monitoring of the sex pheromone trap would indicate resurgences. Further work is needed to validate these results and to investigate intermediate spray intervals between 3-4 days and 7 days. Cypermethrin is a low-cost insecticide of low toxicity to humans but it is highly disruptive to IPM. For this reason, use of multiple sprays in orchards is undesirable.

Control of apple leaf midge with insecticides will always be difficult and disruptive to IPM programmes. In view of the high biological activity of the pheromone, experiments have been carried out on 1 ha plots to investigate use of the pheromone for control of apple leaf midge by attract-and-kill or mating disruption. High levels of trap catch suppression were achieved, at least initially. However, this suppression was not complete and no reduction in galling was observed in treated plots relative to those in untreated plots in these trials.

Pheromone application rates used in these trials were low compared with those typically used against other insects, and, because of the low release rate of the pheromone the actual atmospheric concentrations would have been very low. In the attract-and-kill experiments pheromone was applied at 0.05 g/ha and 0.2 g/ha, and 0.25 g/ha and 1 g/ha in the mating disruption, each with 500 and 2000 devices/ha respectively. Furthermore, it was found that no pheromone remained in the dispensers by the end of the trials due to degradation by direct sunlight on the unprotected dispensers. This explains the reduced trap catch suppression in the later stages of the trials compared with the very high levels at the beginning.

Control of apple leaf midge by mass trapping is under investigation in New Zealand using pheromone supplied by NRI. In a trial carried out during 2005, water traps were used at 500 traps/ha. Numbers of midges caught in treated areas were 96% lower than those caught in untreated areas, but shoot infestations were uniformly very low and it was not possible to see any effect of the treatments. This work is to be repeated during 2006 if pheromone is available.

Objective 5. Transfer of technologies

A patent application has been made to cover use of the apple leaf midge pheromone and related structures in management of midge pests. It is anticipated that this protection will aid commercial development of the pheromone.

The pheromone traps and lures have been made available to growers on a semi-commercial basis for evaluation during 2006. It is hoped to make them commercially available for 2007. At least two UK companies have expressed interest in commercialising the pheromone.

Collaborations have been set up to test and develop the apple leaf midge pheromone for pest monitoring and control with research institutes in Italy, New Zealand and The Netherlands.

FURTHER WORK REQUIRED

- Further work is required to confirm the configuration of the attractive enantiomer pheromone (in progress).
- Further work is required to secure commercial production of the pheromone.

- Although the trap and lure are considered to be optimised for apple leaf midge, there is scope for further investigation of the effect of colour on reducing catches of non-target insects.
- Further work is required to calibrate the pheromone traps for timing of specific interventions against apple leaf midge, and develop their use by growers. The latter is in progress with the traps and lures being evaluated by growers during 2006 and expected to be fully available in 2007.
- Further work is needed to validate the results using multiple sprays of SP insecticides against the first generation of apple leaf midge and to investigate intermediate spray intervals between 3-4 days and 7 days.
- Although multiple sprays of SP insecticides applied as indicated by monitoring with pheromone traps can provide effective control of the first generation of apple leaf midge, this is highly disruptive for IPM programmes in orchards. As shown by this work, the apple leaf midge parasitoid, *P. demades*, only really appears in significant numbers during the second generation of the midge and SP insecticides applied during the first generation must severely reduce this build-up of populations. Thus alternative approaches not dependent upon SP insecticides are still required for control of apple leaf midge. In view of the high biological activity of the pheromone, use of it in control should be investigated further. Following on from the work described here, further work is required to develop formulations that protect the pheromone and to test these, probably at higher application/release rates than used previously.

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

1. Following the Defra press release at the start of this project, interviews were given to BBC South East television, Radio 4 Today programme, BBC Farming Today and BBC Radio Suffolk by David Hall and/or Jerry Cross.

2. An article on the project appeared in Kent on Sunday, 9 November 2003 following an interview with Jerry Cross.

3. Item with video footage on BBC South East Today, 9 September 2004. http://www.gre.ac.uk/~hd18/chemecol/bbc0909.mpg

4. "The Sex Pheromone of the Apple Leaf Midge" by J.V. Cross, D..R. Hall and P.J. Innocenzi, poster at the IOBC meeting on orchard pest control in Baselga di Pine, Italy, 26-29 September 2004.

5. "Attracting the midges". *Plant It*, Issue 6, October 2004, pp 2-3. http://defrafarmingandfoodscience.csl.gov.uk/unit/downloadpagedoc.cfm?id=179

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11. PCT/GB2005/002504 Application: Pheromone by J V Cross and D R Hall (25 June 2005)

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16. Sex pheromones of gall midges of UK fruit crops. Jerry Cross & David Hall. Presentation at Joint Meeting of Royal Entomological Society and British Plant Gall Society, London 5 April 2006.

17. Mass Trapping Apple Leaf Curling Midge (Diptera: Cecidomyiidae). D.M. Suckling, J.T.S. Walker, P.W. Shaw, L.A. Manning, P.Lo, R. Wallis, V. Bell, W.R.M. Sandanayaka, D. R. Hall, J.V. Cross & A.M. El-Sayed. Abstract for presentation at 22nd Annual Meeting of International Society of Chemical Ecology, Barcelona, Spain, 15-19 July 2006.

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