DEPARTMENT FOR ENVIRONMENT, FOOD AND RURAL AFFAIRS

Research and Development

Annual/Interim Project Report - Financial Year

(Not to be used for LINK projects)

Section 1 : Project details

1. (a) DEFRA Project Code: **HH3114TTF**

(b) Project Title: SEMIOCHEMICALS IN MANAGEMENT OF APPLE LEAF MIDGE

(c) Project start date: 01/07/2003

(d) Project end date: 30/06/2005

(e) DEFRA Project Officer: Dr Richard Bradburne

(f) Name and address of contractor:

Prof David R HALL
Natural Resources Institute, University of Greenwich
Central Avenue
Chatham Maritime
Postcode ME4 4TB

(g) Contractor's Project Officer:

Section 2 : Scientific objectives

2. Please list the scientific objectives as set out in CSG 7 (ROAME B). If necessary these can be expressed in an abbreviated form. Indicate where amendments have been agreed with the DEFRA Project Officer, giving the date of amendment.

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.
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.
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.
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.
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 work will aim to provide improved control of apple leaf midge within the three years of the project.

Section 3 : Summary of progress

3. Please summarise, in layperson's terms, scientific progress since the last report/start of the project and how this relates to policy objectives set out in ROAME A. Please provide information on actual results where possible rather than merely a description of activities.

Although the project did not start until half-way through the 2003 season for apple leaf midge, it was possible to collect large numbers of midge larvae and rear them through to adults at EMR (previously HRI). Pheromone was collected by passing purified air over virgin female midges and trapping volatiles on a synthetic resin, Porapak Q. This method has been used to collect pheromone from other insects, but not previously from midges. Volatiles were collected from over 2000 females and a similar number of males for comparison. The collections were analysed at NRI by gas chromatography (GC) linked to electroantennographic (EAG) recording from the antenna of a male midge, a difficult procedure bearing in mind the small size of the insect. However, with previous experience on midges and other insects, it was possible to record an EAG response to a single component in the female volatile collections. No response was detected from the collections of male volatiles, confirming that the active compound was likely to be a component of the female sex pheromone. In spite of the fact that this component was estimated to be produced at only 1.5 picograms per hour by the female midge, it was detected by GC analysis linked to mass spectrometry (MS), and mass spectra were obtained in electron impact and chemical ionisation modes. The spectra did not correspond to those of pheromone components of other midge species or related compounds. However, careful interpretation of the spectra and comparison of the GC and MS data with those of a large library of compounds at NRI eventually made it possible to determine the basic structure of the active component. Three candidates were synthesised and the third had chromatographic, spectrometric and electrophysiological properties identical to those of the natural compound. This is a novel compound whose structure is rather different from those of other known midge pheromones, although there probably is a biogenetic relationship with these.

Trapping experiments were carried out with the synthetic compound in orchards at EMR, and it was shown to be highly attractive to male apple leaf midge. In fact, in our experience it is one of the most potent pheromones known: in the first trial, rubber septa impregnated with 100 μg of the pheromone and lreleasing it at less than 1 ng/hr caught an average of over 4,000 male midges over a period of 14 days. Doses as low as 1 μg are significantly attractive, and a septum loaded with 3 μg of pheromone has been adopted as standard for monitoring experiments as it is highly attractive but not so attractive as to saturate the traps. The traps were also shown to catch apple midges at 50 m distance from the nearest orchard.

Experiments are in progress to investigate other trap designs and to optimise the placement of the trap. As required in the next project milestone, experiments are in progress to determine the relationship between catches of male midges in pheromone traps with infestation of growing tips and egg laying by female midges. In view of the unexpectedly high attractive properties of the pheromone, the possibilities of using the pheromone for control of the pest are being reconsidered. A small trial of "lure-and-kill" has already been established using panels coated with microencapsulated lambdacyhalothrin provided by Agrisense Ltd.

In the laboratory, release rates of the pheromone from different dispensers are being measured to optimise lures for monitoring and for planned trials of control by mass trapping and/or mating disruption. The rubber septa dispensers used currently are predicted to remain attractive for over 10 years under field conditions! The pheromone has one chiral centre. The initial synthesis gave racemic material, and synthesis of the two enantiomers is under way. It is anticipated that only one of these will be attractive, such that the dosages and release rates for the racemic material mentioned above must all be halved to give effective amounts of active material.

As mentioned in the project document, given the success of the pheromone identification and synthesis, work is starting on identification of components of apple leaf volatiles which are attractive to female apple midges.

Section 4 : Amendments to project

4. Are the current scientific objectives appropriate for the remainder of this project? YES

If **NO**, explain the reasons for any changes giving the financial, staff and time implications.

Contractors cannot alter scientific objectives without the agreement of the DEFRA Project Officer

Section 5 : Progress in relation to targets

5. (a) List the primary milestones for the year/period under report as on CSG 7 (ROAME B).

It is the responsibility of the contractor to check fully that ALL primary milestones have been met and to provide a detailed explanation if this has not proved possible

Milestones		Target date	Milestones met?	
Number	Title		in full	on time
01/01	Components of the female sex pheromone of the apple leaf midge identified.	31/12/2003	YES	YES
01/02	Components of the female sex pheromone of the apple leaf midge synthesised.	31/03/2004	YES	YES
01/03	Synthetic pheromone shown to attract male apple leaf midge in field.	05/04/2004	YES	YES

Section 5 : Progress in relation to targets (continued)

5. (b) Do the remaining primary milestones look realistic? YES

(c) If you have answered **NO** at (a) or (b), please provide an explanation.

Section 6 : Project costs and staffing input

6. In this reporting period, what was:

(a) the approved expenditure?

(b) the actual expenditure?

(c) * the approved staff input?

(d) * the actual staff input?

*** staff years of direct science effort**

Section 7 : Publications and other outputs

7. (a) Please give details of any outputs, e.g. published papers/presentations during this reporting period.

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.

2. An article on the project appeared in Kent on Sunday, 9 November 2003.

3. A summary of the project has been submitted to the Worshipful Company of Fruiterers for a possible award as a contribution to organic fruit-growing in the UK.

4. An abstract has been submitted for a poster at the IOBC meeting on orchard pest control in Baselga di Pine, Italy, 26-29 September 2004.

Section 7 : Publications and other outputs (continued)

7. (b) Have oppportunities for exploiting Intellectual Property arising out of this work been identified ?
If you have answered **YES**, please give details.

YES

In view of the unexpected novelty of the chemical structure of the apple leaf midge pheromone and its very strong attractiveness, it was decided to make a patent application. Two patent applications on the type of compound and its use for monitoring and control of apple leaf midge were file on 25 June 2004.

(c) Has any other action been taken to initiate Technology Transfer?

YES

If you have answered **YES**, please give details.

Identification of the pheromone and possible commercial exploitation have been discussed with Agrisense Ltd., Pontypridd, UK. The company has provided insecticide-treated panels for a trial of lure-and-kill against apple leaf midge, free of charge.

Section 8 : Future work

8. Please comment briefly on any new scientific opportunities which may arise from the project.

As mentioned above, given the success of the pheromone identification work and the highly attractive nature of the pheromone, it is planned to include investigation of use of the pheromone in control of apple leaf midge, as well as for monitoring the pest, within the existing project. A small trial of "lure-and-kill" has already been started and possibilities for mass trapping and/or mating disruption will be considered.

Section 9 : Declaration

9. I declare that the information I have given is correct to the best of my knowledge and belief.
I understand that the information contained in this form may be held on a computer system.

Signature		Name	David R Hall
Date	09/07/2004	Position in Organistation	Professor of Chemical Ecology