Project Title	Chemical diversity of midge pheromones
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Project leader:	Prof. David Hall, Natural Resources Institute
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Location of project:	NRI, University of Greenwich at Medway, EMR, East Malling
Project coordinator:	Rupert Hargreaves
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The results and conclusions in this report are based on a series of experiments conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

I declare that this work was done under my supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

PROF DAVID R HALL
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Signature ..... Date .....

CP 38

THE CHEMICAL DIVERSITY OF MIDGE PHEROMONES: GROWERS' SUMMARY

Headline

A synthetic pheromone lure to attract pear leaf midge has been discovered.

Good progress has also been made on identification of pheromones for pear midge and blackcurrant leaf midge.

Background and Expected Deliverables

Many horticultural crops, especially perennial fruit crops, are subject to pest attack by various species of leaf-curling or other gall midges (Diptera: Cecidomyiidae). Some species are currently controlled with broad-spectrum organophosphate or pyrethroid insecticides, but use of these is undesirable because they may be harmful and toxic and will disrupt integrated pest management. These midges are thus likely to continue as significant pests and will affect the economic viability of their host crops. For other species, such as the apple and pear leaf midges, there are currently no effective control methods and they cause extensive damage, especially in nurseries and young orchards. Alternative, non-pesticidal control methods are required.

Plant-feeding midges are typically very short-lived as adults and highly specific for their host-crop. In several species there is evidence for production of highly potent sex pheromones by virgin female adults and strong attraction of mated females to volatiles from host plants. Identification of these attractants could provide means of manipulating pest behaviour at critical stages in their life cycle. However, identification of the attractants has proved extremely challenging due to the very small amounts of chemical involved and the difficulties of carrying out laboratory bioassays with the small and delicate insects.

The pheromones of midge species identified previously are molecules with a linear chain of an odd number of carbon atoms with one or two ester functionalities, neither at the ends of the carbon chain. In recent work at NRI and EMR the pheromones of the apple leaf midge, *Dasineura mali* and the raspberry cane midge, *Resseliella theobaldi*, were identified as members of a novel group of ketoesters.

The aim of this work is to explore further the chemical diversity of midge pheromones in the context of identifying the pheromones of midges of importance to the UK horticulture industry. These include the pear leaf midge, *D. pyri*, the pear midge, *Contarinia pyrivora*, and the blackcurrant leaf midge, *D. tetensi*.

Deliverables will be:

- synthetic pheromone lures for the above three species for use by growers in monitoring and control of these pests;
- further information on the chemical diversity and patterns in midge pheromones that may assist in identification of the chemical structures of pheromones of other midge species.

Summary of the Project and Main Conclusions

Pear leaf midge
Large numbers of pear leaf midge adults were reared from pupae and late larvae which had been collected from leaves of infested pear trees. Volatiles given off by virgin females and males were collected separately and analysed by gas chromatography (GC) coupled to electroanennographic (EAG) recording from the antenna of a male midge. Two compounds in volatiles from female midges were found to elicit an EAG response from the male midge. These were not present in collections from the male midges and were assumed to be components of the female sex pheromone. Only nanogram quantities of the compound causing the larger EAG response were available, but this was identified as (*Z*)-2,13-diacetoxy-8-heptadecene by comparison of its GC retention times and mass spectra with those of a wide range of synthetic standards available at NRI. The compound was synthesised and shown to be identical with the naturally-produced material and to elicit an EAG response from male pear leaf midges. The compound in volatiles from female midges causing the smaller EAG response was present at even smaller amounts and its chemical structure has not yet been determined.

The synthesised (*Z*)-2,13-diacetoxy-8-heptadecene was tested for attraction of male pear leaf midges in field trapping tests in growers' orchards towards the end of 2006 but no midges were attracted. The compound can exist as four stereoisomers and the synthesised compound is racemic, containing equal amounts of all four isomers. These were separated using a new technique of high performance liquid chromatography on a chiral column. When these were evaluated in field trapping tests, one isomer was highly attractive to male pear leaf midges and the other three showed no attractiveness. When the attractive isomer was mixed with each of the other three isomers, one of the latter was found to inhibit its attractiveness completely, explaining why the racemic mixture was unattractive. These results were confirmed in preliminary trials in the UK and in subsequent experiments carried out by collaborators at HortResearch in New Zealand.

Pear midge
The pear midge has only one generation per year and proved very difficult to collect and rear through to adulthood. Nevertheless, sufficient adults were reared to make possible collection of volatiles from virgin females and analysis of the collections by GC-EAG and GC-MS. Two components of the volatiles from females elicited EAG responses from male midges and these were identified and synthesised as 2,7-diacetoxyundecane and 7-acetoxy-2-undecanone. These are novel structures, the latter belonging to the new ketoester class of midge pheromone structures previously found only in the apple leaf midge and raspberry cane midge. The two components exist as four and two stereoisomers respectively. Separation is in progress for testing in growers' orchards during March-April 2008.

Black currant leaf midge
It was possible to collect large numbers of larvae and pupae of blackcurrant leaf midge from leaves of infested bushes. Collection and analysis of volatiles from adults showed two components in volatiles from female midges which caused an EAG response from the antennae of male midges. The more abundant of these was identified as (Z)-2,12-diacetoxy-8-heptadecene. The minor component is as yet unidentified, but may be another member of the novel pheromone class of ketoesters. Separation of the four stereoisomers of the major pheromone component by HPLC on a chiral column is in progress. It is planned to evaluate these in traps in growers' plantations during the whole 2008 growing season.

Conclusions and Future Plans

The major component of the female sex pheromone of the pear leaf midge has been identified and one stereoisomer has been shown to be highly attractive to male midges in field trapping tests. One of the other three stereoisomers inhibits this attractiveness while the other two isomers seem to be behaviourally inactive. Further work is required to establish the absolute configuration of the attractive isomer using resolution with enzymes and chemical synthesis. This will hopefully lead to development of a route to synthesise larger quantities of attractive material for use by growers. Further work is also required to identify the minor EAG-active component in volatiles from the female midges, although clearly the major component is highly attractive on its own.

Two components of the female sex pheromone of the pear midge have been identified and synthesised. Separation of the isomers of these is in progress to make possible field evaluation during March-April 2008. Initial results show both components are attractive to male pear midge. Further work will be required to find the optimum blend of components and then to determine absolute configurations of the active stereoisomers and to develop routes to synthesise these for use by growers.

The major component of the female sex pheromone of the black currant leaf midge has been identified and separation of the four stereoisomers of this compound is in progress to provide material for evaluation by growers during 2008. Further work is required to establish the absolute configuration of attractive isomers and to complete identification of the minor pheromone component.

Thus good progress has been made on identification of the female sex pheromones of three midge species of importance to UK horticulture. The components identified all have novel structures and at least one of these belongs to the new class of ketoester midge pheromone components. The field trapping tests carried out so far are showing extremely interesting relationships between stereochemistry and behavioural activity.

During the last year the student contributed a poster and paper to the IOBC Workshop on Integrated Soft Fruit Production at EMR, 24-27 September 2007, title “Identification of black currant leaf midge, *Dasineura tetensi* (Rübsaamen) female sex pheromone” by Lakmali Amarawardana, David Hall, Jerry Cross and Csaba Nagy (poster attached)

Financial Benefits

The three midges are pests of their respective host crops, the blackcurrant leaf midge being particularly commercially important. Growers will gain financial benefit from improved control and better targeted use of pesticides.

Action Points for Growers

- Experimental sex pheromone traps for blackcurrant leaf midge are to be evaluated by seven UK growers during 2008.
- Sex pheromone traps for pear leaf midge, pear midge and blackcurrant leaf midge will be available commercially for use by growers from 2009
- These pheromone traps can be used to determine the phenology and relative abundance of the different species in different crops, and will be useful for timing insecticide sprays for control.