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DISCLAIMER

AUTHENTICATION

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

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GROWER SUMMARY

Headline

This project has identified and synthesised the major components of the female sex pheromones of the pear leaf midge, the pear midge and the blackcurrant leaf midge, with lures now being made available to growers for pest monitoring.

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 therefore 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 are highly specific to 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 a 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 these 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 further explore the chemical diversity of midge pheromones and identify the midge pheromones of importance to the UK horticulture industry. These include the pear leaf midge, *D. pyri*, the pear midge, *Contarinia pyrivora*, the blackcurrant leaf midge, *D. tetensi*, and the blackberry midge, *D. plicatrix*.

The project will deliver:

- synthetic pheromone lures for the above species for use by growers in monitoring of these pests;
- potential for control of these pests using the pheromones for mass trapping, mating disruption or lure-and-kill approaches;
- 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

Summary

The project has investigated the female sex pheromones of four species of midge which are important pests of horticultural crops in the UK. These are the pear leaf midge, *Dasineura pyri*, the pear midge, *Contarinia pyrivora,* the blackcurrant leaf midge, *D. tetensii*, and the blackberry midge, *D. plicatrix*. For the first three of these, components of the female sex pheromones have been identified and synthesised, and lures containing only a few micrograms of the synthetic pheromone have been shown to be highly attractive to conspecific male midges in field trapping experiments. Significant progress has been made on identification of two components of the female sex pheromone of the blackberry midge, but this is not yet complete.

New techniques for pheromone research

In achieving these results, the project has made significant advances in improving several of the techniques used in isolation and identification of pheromones, particularly those of midges.

- ***Collection of insects*** - Previously midge pupae have been isolated by time-consuming sieving of soil. In this project, large numbers of insects were collected relatively easily as late larvae in galls, and reared to adults in individual tubes to ensure they were unmated – e.g. 20,000 pear leaf midge larvae were collected.

- ***Collection of pheromone*** - Other workers have collected pheromones by dissecting out the pheromone glands and extracting them with solvent. This is difficult and time-consuming and introduces impurities which interfere with the analysis. In this project, pheromones were collected by trapping the volatiles emitted by the insects on a solid adsorbent. Large numbers of insects can easily be processed and the collections are cleaner and can be concentrated without interfering with the analysis. This is particularly important with midge pheromones that are generally produced in very small quantities, typically less than 10^{-9} gm per insect.

- ***Electroantennography (EAG)*** - In this project, pheromone components were detected by electrophysiological recording of the responses from an insect antenna when exposed to the pheromone collections. This is obviously a challenge with such small, delicate insects. Various approaches are described in the literature, and a convenient, reproducible technique was developed during this work which is applicable to a wide range of midges and similar small insects.
- ***Separation of stereoisomers*** -The pheromone components identified in this project consist of two or four stereoisomers, and it is very time consuming to synthesise all the individual stereoisomers in pure state for field testing. In this project, high performance liquid chromatography (HPLC) with a chiral column was developed as a very convenient method for separating and isolating the individual isomers from the racemic mixture of all the isomers.

Progress on pheromone identification

Pear leaf midge, D. pyri

Over 20,000 larvae were collected in the UK and New Zealand, although only about 30% survived to adulthood. Volatiles were collected separately from virgin male and female insects. Two potential pheromone components were detected in analyses of volatiles from female midges by gas chromatography (GC) coupled to EAG recording from a male midge. The major component was identified as (*Z*)-2,13-diacetoxy-8-heptadecene by interpretation of the mass spectrum and comparison of GC retention times with those of a large library of standards. The minor component was present in very small quantities and could not be identified.

The major component was synthesised at NRI in 10 steps as the racemic mixture of all four stereoisomers, but this did not attract any male pear leaf midge when tested as a lure in traps in pear orchards. The four stereoisomers were separated by HPLC as above and the one eluting first, proved to be highly attractive to male pear leaf midge in field tests. The other three isomers were not attractive, but when the second-eluting isomer was added to the first it completely inhibited the attractiveness of the latter. Field tests were carried out in both UK and New Zealand pear orchards with similar results.

It was possible to synthesise the stereoisomers of the analogue of the pheromone with no double bond and comparison of these with the attractive isomer of the pheromone to determine the absolute configuration showed that this was (2*R*,13*R*,8*Z*)-2,13-diacetoxy-8-heptadecene. The inhibitory isomer was (2*R*,13*S*,8*Z*)-2,13-diacetoxy-8-heptadecene.

Pear midge, C. pyrivora

This was a very difficult insect to collect and rear, having only one generation per year. However, sufficient adults were obtained to make collections of pheromone from virgin females, and analysis of these by GC-EAG indicated the presence of two active components that stimulated receptors on the antennae of males. The most abundant of these was identified as 2,7-diacetoxyundecane and the relatively minor component as 7-acetoxy-2-undecanone. The compounds were synthesised at NRI in three and five steps respectively and separated into four and two stereoisomers respectively by HPLC.

Two main field trapping tests were carried out during 2008. In both of these, the first eluting isomer of the major component caught male pear midge. However, in the first test the fourth isomer was also attractive whereas in the second test this was unattractive and the third-eluting isomer was attractive. It was subsequently found that another species was also caught in the traps. It was not then possible to go back to examine all the catches, but this probably explains the anomalous results. A further experiment in 2009 confirmed that the first and third eluting isomer are attractive. The racemic mixture of all four of the isomers of the major component was not attractive, and this is probably due to the presence of the second-eluting isomer. The minor pheromone component was also attractive, although less so than the major component. In this case one of the two stereoisomers and the racemic mixture of both were equally attractive. The other isomer was unattractive.

Blackcurrant leaf midge, D. tetensii

Approximately 12,600 larvae were collected and 36% survived to adulthood. Volatiles were collected from virgin females and analysed by GC-EAG. Two active components were detected and the most abundant of these was identified as (*Z*)-2,12-diacetoxy-8-heptadecene which differs from the major component of the pheromone of the pear leaf midge only in the position of the second acetate group which is shifted by one carbon atom. Potential structures for the minor pheromone component have been proposed but not confirmed.

The major pheromone component was synthesised at NRI in 10 steps and separated into its four stereoisomers by HPLC. For this species, the third-eluting stereoisomer was highly attractive to males. The other isomers were not attractive themselves and only had a weakly inhibitory effect when mixed with the attractive isomer. Thus the racemic mixture of all four stereoisomers is significantly attractive to male midges and can be used for monitoring this pest.

Blackberry midge, D. plicatrix

Approximately 10,000 larvae were collected from farms in UK and The Netherlands and reared to adulthood. GC-EAG analyses of collection of pheromone from females indicated the presence of two pheromone components. Identification of these is not yet complete but preliminary work has demonstrated they are both 15-carbon compounds with an acetate group in the 2-position. The major component probably has two double bonds and the minor component one, but the positions and configurations of these have not been established.

Pheromone structures

All the pheromone components are novel structures, but they conform to patterns emerging as more midge pheromones are identified. They all have an odd number of carbon atoms, although the components of the pheromone of the blackberry midge are the first to be identified with 15 carbon atoms. All have an oxygenated functionality in the 2-position. Some have another oxygenated functionality, as in the di-acetates and ketoacetates, and some have one or two double bonds, but the positions and configurations of these are much more variable. Interestingly, one other example of the new ketoacetate structure was discovered in this work – the minor pheromone component of the pear midge.

Further work

- For pear leaf midge, small numbers of lures can be made available to growers for monitoring using HPLC to separate the stereoisomers. Now we know the absolute configurations of the isomers, development of a route for synthesising the attractive stereoisomer uncontaminated by the inhibitory isomer is required..
- Similarly for pear midge, small numbers of lures containing the attractive isomer of the major pheromone component only, can be made available. Further work is required to establish the absolute configuration of this compound, but synthesis should be relatively straightforward for this pheromone.

- Lures containing the attractive stereoisomer of the pheromone of the blackcurrant leaf midge have been deployed by 8 growers during 2009 to monitor the pest. Further work is required to determine the absolute configuration of the attractive isomer and develop a synthetic route to the attractive isomer.
- Further work is required to complete identification of the two components of the pheromone of the blackberry midge.
- For all these species, further work is required to calibrate trap catches against populations of the midge and the damage it causes. As indicated, this work is in progress with growers for the blackcurrant midge.

Financial benefits

The four midges are pests of their respective host crops, the blackcurrant leaf midge being particularly commercially important. As lures continue to be made commercially available to growers, they will gain financial benefit from improved monitoring and better targeted use of pesticides, leading to improved control.

Action points for growers

- Subject to at least some of the further work above being completed, it is hoped to make the sex pheromone traps available commercially to growers.
- 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.