Developing a sex pheromone lure and trap for blackcurrant leaf midge 2007

Customer: GlaxoSmithKline Blackcurrant Growers Research Fund
Contractors: East Malling Research (EMR) and Natural Resources Institute (NRI)

SUMMARY

- The chemical structure of the blackcurrant leaf midge pheromone, a mono-unsaturated 17 carbon diacetate, has been determined.
- The compound has 4 stereoisomers (enantiomers) but it is not yet known which of these is attractive or whether the opposite isomers have inhibitory activity.
- A substantive quantity of the pheromone racemate (a mix of the 4 stereoisomers) has been synthesised and the 4 individual stereo isomers are currently being separated by High Pressure Liquid Chromatography.
- A possible minor component of the pheromone, the corresponding acetoxy ketone, has also been identified and synthesised.
- Work is needed in 2008 to demonstrate attractancy, identify a suitable dispenser, optimise pheromone blend and release rate, develop a practical effective trap and calibrate it for use for monitoring the pest by UK blackcurrant growers and timing of sprays.
- In the longer term, the possibility of exploiting the pheromone for control of the midge by mating disruption, mass trapping or attract and kill approaches could be explored in a substantive, longer term project.

INTRODUCTION

The blackcurrant leaf midge, *Dasineura tetensi* (Diptera: Cecidomyiidae), is a pest of blackcurrant whose importance in the UK is increasing with the decline and withdrawal from use of the synthetic pyrethroid (SP) insecticide fenpropathrin (Meothrin) against blackcurrant gall mite. Although alternative SPs are available for control of the midge on blackcurrant, they are broad-spectrum insecticides that are harmful to the natural enemies of several important blackcurrant pests. They are highly disruptive to IPM and growers would like to avoid using them. The severity and timing of midge attacks are difficult to monitor or predict. The aim of this work was to identify the sex pheromone of blackcurrant leaf midge, to synthesise it and demonstrate its activity in the field. Sex pheromone traps could then be developed to provide a monitoring method for the midge which is likely to be very valuable for determining the need for and timing sprays. Furthermore, it might prove possible to exploit the pheromone for control by mating disruption, mass trapping or attract and kill methods.

Production of a sex pheromone by female *D. tetensi* that attracts males was demonstrated over 20 years ago by scientists at Rothamsted. In previous work by EMR and NRI pheromone was collected from virgin female midges and analysed by gas chromatography (GC) coupled to electroantennographic (EAG) recording from a male midge antenna. A single EAG response was observed and the GC retention times and mass spectrum indicated

that this was due to a 17-carbon diacetate with one double bond. While this work was in progress, the female sex pheromone of the apple leaf midge, *D. mali*, was identified by EMR and NRI as (*Z*)-13-acetoxy-8-heptadecen-2-one, and it was thought the pheromone of *D. tetensi* might be the corresponding diacetate, i.e. (*Z*)-2,13-diacetoxy-8-heptadecene (Figure 1). The latter compound was synthesised and was found to have slightly different GC retention times and mass spectrum to that of the *D. tetensi* pheromone, although we subsequently showed the above compound to be the female sex pheromone structure of the pear leaf midge, *D. pyri*.

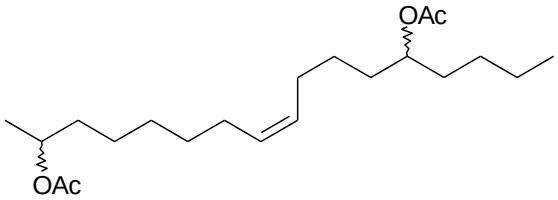

FIG. 1. Structure of (*Z*)-2,13-diacetoxy-8-heptadecene, major component of the female sex pheromone of the pear leaf midge, *D. pyri*.

Unfortunately the mass spectra of these unsaturated diacetates are not particularly diagnostic in terms of determining the positions of the acetoxy substituents and the position and configuration (*Z* or *E*) of the double bond, and there are 1,800 possible isomers of this apparently simple structure. In addition, the diacetates have two asymmetric centres and so even having fixed the positions of the acetoxy groups and the position and configuration double bond, there are still four possible stereoisomers to distinguish.

The pheromone is produced in minute quantities by the female midges – less than 10^{-10} g per female - and the aim of this phase of the work was to collect larger amounts of pheromone to make it possible to carry out various microanalytical reactions which would make possible a more accurate prediction of the structure of the pheromone which would then be synthesised.

RESULTS

Collection and analysis of pheromone

Blackcurrant shoots infested with *D. tetensi* were collected from a field in Horsmonden, UK, in May 2007 and approximately 12,300 larvae were collected and reared in individual tubes.

Only 36% of the larvae developed through to adults, but 14 collections of pheromone from batches of up to 688 virgin female midges were made. Five collections were made from similar batches of males.

In analysis of the collections from female midges by GC-EAG the presence of a major EAG-active component was confirmed and in some analyses a second response was observed (Figure 2).

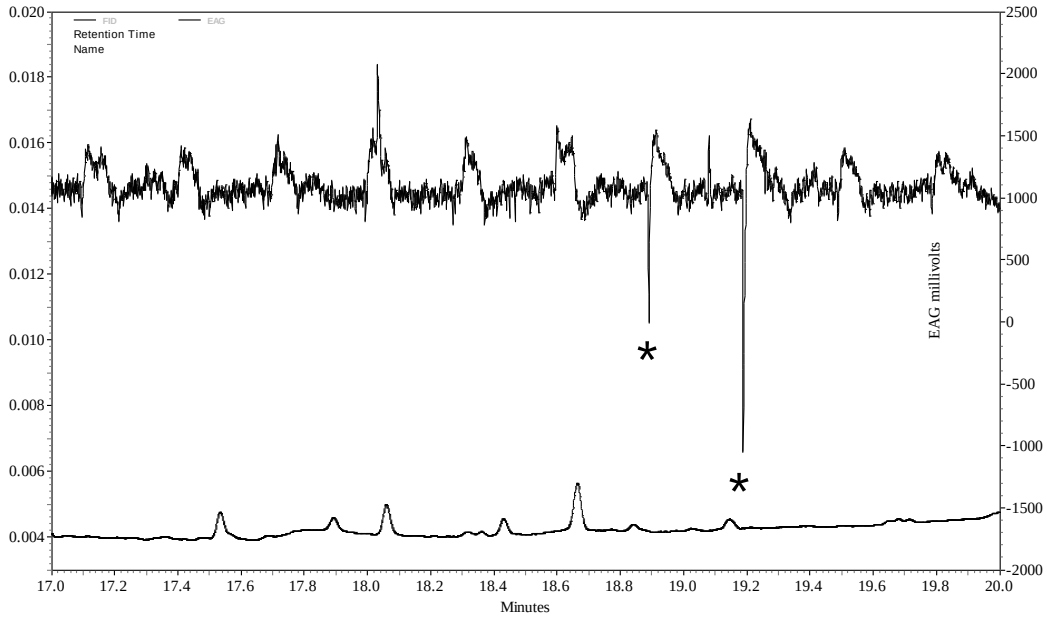

FIG. 2. GC-EAG analysis of volatiles from female *D. tetensi* on a polar GC column (upper trace is EAG, lower is GC; * marks EAG responses)

Major component

The mass spectrum of the major EAG-active component was confirmed to be consistent with that of a 17-carbon diacetate with one double bond. It was possible to hydrogenate the compound to remove the double bond, and the mass spectrum of the product indicated this was a 17-carbon diacetate with acetoxy groups in known fixed positions.

This compound was synthesised in ten steps and found to have identical mass spectrum and GC retention times on polar and non-polar GC columns to those of the major EAG-active compound from female *D. tetensi*.

The four stereoisomers could be separated on a chiral high performance liquid chromatography (HPLC) column, a new technique for midge pheromones developed at NRI.

Minor component

The GC retention times of the minor EAG-active component observed in analyses of volatiles from female *D. tetensi* were consistent with those of a 17-carbon acetoxyketone with one double bond, although different from those of the pheromone of *D. mali*, (*Z*)-13-acetoxy-8-heptadecen-2-one. Insufficient material was present to obtain a mass spectrum of this component, even using chemical ionisation which is highly-sensitive for these compounds. However, synthesis of the corresponding acetoxy ketone of the pheromone was an intermediate in the synthesis of the diacetate and had similar GC retention times to the minor EAG-active component.

CONCLUSIONS

This project made possible collection of large numbers of blackcurrant leaf midge, *D. tetensi*, which allowed determination of the structure of the major component of the female sex

pheromone. The compound has been synthesised and the four stereoisomers are being separated by chiral HPLC for evaluation in the field by during 2008. Experience with other midge pheromones of similar structure suggests that only one stereoisomer will be attractive to male midges and that one or more of the others will inhibit attraction. It should then be possible to devise a synthetic route to minimise the content of inhibitory isomer(s) and produce an attractive lure without resort to HPLC.

The work also indicated the presence of a potential minor pheromone component. This is probably the corresponding acetoxy ketone of the major component which has also been synthesised and will be evaluated in the field during 2008. Experience with other midge pheromones indicates this may not be necessary for attraction.

WORK NEEDED IN 2008 (and beyond)

Replicated field tests are needed as soon as possible to optimise the pheromone blend and release rate. The following tests of attractancy are needed:

- The pheromone racemate and each of the 4 enantiomers to determine which is attractive
- The 2 way blends of the attractive enantiomer with the other 3 enantiomers to determine which are inhibitory
- The benefits of addition of the minor component
- The effects of lure load (release rate) over a very wide range of factors of 10, i.e. from 0.01 μg/lure to 10 mg/lure
- Suitable dispensers and trap designs (rubber septa with standard delta traps have proved successful for other midges)

Once a suitable standard trap has been developed, further work is needed to

- Test the effects of height of deployment
- Evaluate the range of attraction
- Monitor populations in commercial plantations through the season to determine the seasonal flight pattern of the midge and the range in populations that occur in commercial plantations.
- Determine the relationships between the numbers of midges caught and the numbers of galls that develop to calibrate the traps.
- Examine the use of the trap for timing of sprays.

The possibility of exploiting the pheromone for control of the midge by mating disruption, mass trapping or attract and kill approaches could be explored in a substantive, longer term project.