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Project leader:	Dr Michelle Fountain East Malling Research
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Key staff:	Jamie Stozka, Bethan Shaw (EMR) Professor David Hall, Dudley Farman (NRI)
Location of project:	EMR, New Road, East Malling, Kent, ME19 6BJ NRI, University of Greenwich, Medway Campus, Chatham Maritime, Kent, ME4 4TB
Industry Representative:	Richard Stanley, Stanley & Pickford
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The results and conclusions in this report are based on an investigation 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.

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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.

Dr Michelle Fountain

Research Leader in Entomology

East Malling Research, New Road, East Malling, Kent, ME19 6BJ

Signature ..... Date .....

Professor David Hall

Research Leader in Semiochemical Ecology

Natural Resources Institute, University of Greenwich, Medway Campus, Chatham Maritime, Kent, ME4 4TB

Signature ..... Date .....

Report authorised by:

Professor Jerry Cross

Science Programme Leader

East Malling Research

Signature ..... Date .....

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GROWER SUMMARY

Headline

- A potential component of the female sex pheromone of the gooseberry sawfly has been identified and will be tested during 2015.

Background and expected deliverables

The common gooseberry sawfly, *Nematus ribesii*, is a sporadic, localised and unpredictable pest of gooseberry with lesser attacks on red and whitecurrant. The feeding larvae are able to defoliate whole gooseberry bushes if not detected in a timely manner. The monitoring of the pest relies on the detection of the eggs on the underside of leaves in the centre of the bushes. Damage begins in the centre of the crop low down and radiates outwards as the larvae devour the leafy areas of the bush. Crop scouting in plantations for eggs and larvae is not always the most reliable method as an adequate search is time-consuming. This project aims to identify the gooseberry sex pheromone which would lead to the development of a sex pheromone monitoring trap: a more sensitive and rapid monitoring method. The production of a sex pheromone attractive to males by virgin female gooseberry sawfly was reported by other workers but never identified. In other work by NRI and EMR, potential components of the female sex pheromone of the closely-related blackcurrant sawfly, *N. olfaciens,* have already been identified and synthesised.

Summary of the project and main conclusions

In the first year of the project (2014), larvae of common gooseberry sawfly, *N. ribesii*, were collected and reared through to adults in the laboratory. Volatiles were collected from unmated males and females and analysed by gas chromatography (GC) with flame ionisation detection (FID) or linked to a mass spectrometer (MS). Collections from both males and females contained large amounts of long-chain hydrocarbons, probably derived from the cuticle, but the pattern of these was identical in male and female gooseberry sawfly and very similar to those in collections from male and female blackcurrant sawfly. There were no obvious differences in the composition of volatiles from female or male gooseberry sawfly that might be attributable to potential components of a female sex pheromone. However, after fractionation of the collections to remove the hydrocarbons and analysis by GC-MS with selective ion monitoring to maximise sensitivity, traces of a mono-unsaturated,

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16-carbon isopropyl ester, similar to one of the compounds proposed to be a component of the pheromone of the blackcurrant sawfly, could be detected.

Future work will include further collections, analysis by GC with electroantennographic (EAG) detection, identification and synthesis and field testing in growers' fields to confirm this result.

Financial benefits

Gooseberry sawfly is a devastating pest of gooseberry which is difficult to predict and may lead to unnecessary plant protection products being applied for control. A trap and lure designed to accurately time and target sprays would decrease or even eliminate the use of unnecessary pesticide applications. Targeting products better, usually chlorpyrifos, thiacloprid, lambda-cyhalothrin or pyrethrins, would protect crops from defoliation and the related fruit losses.

'Invicta' and 'Careless' are the two principal varieties grown and it is estimated that there are at least 600 pick-your-own and farm shop growers (39% and 50% of fruit respectively), and 116 commercial growers registered with the HDC growing gooseberries in the UK. At least three growers supply to supermarkets and 11% of fruit is grown for processing. 238 hectares of gooseberries are grown in the United Kingdom. In 2013, the price for gooseberry was ~£3.79/kg, and approximately 4,000 kg of fruit are produced per ha making the UK gooseberry industry worth over £3.5 million revenue per annum (238 ha x 4000 kg x £3.79).

Action points for growers

Growers should:

- Look for adults flying in April and May and target with approved insecticides to prevent egg laying.
- Check for eggs on the underside of leaves in the centre of the bush.
- Check for larval damage low down in the centre of the bush.
- Contact michelle.fountain@emr.ac.uk if they would like to trial the test trap and lure.

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- Please send live gooseberry sawfly larvae, with leaves, in a crush proof box to Dr Michelle Fountain, East Malling Research, New Road, East Malling, Kent, ME19 6BJ.

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SCIENCE SECTION

Introduction

The common gooseberry sawfly, *Nematus ribesii*, is a sporadic, localised and unpredictable pest of gooseberry (Mitchell et al., 2011; Raffle, 2012). This species causes significant and devastating damage to gooseberry crops and also lesser attacks on red and white currant (Alford 1984). Adult gooseberry sawflies are between 5-7mm long with the females being larger (Fig. 1). Females lay eggs on the underside of the leaves in rows (Fig. 2) and the larval damage starts as small holes (also seen in Fig. 2). The larvae devour large sections of the leaf lamina, often leading to complete bush defoliation. Damage often occurs in the centre of the bush first, but soon disperses to feed on leaves throughout the whole plant. In addition, feeding attacks are often sporadic and unpredictable.

Figure 1. Adult common gooseberry sawfly female

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Figure 2. Gooseberry sawfly eggs along the leaf vein and larvae feeding on leaf lamina

Monitoring the pest relies on the detection of the eggs on the underside of leaves in the centre of the bushes and this egg laying may be aggregated within a plantation. Hence, crop scouting is not always reliable because doing an adequate search is time consuming. It is not uncommon for early infestations to be missed if the plantation is not well covered during an inspection.

The identification of the gooseberry sawfly sex pheromone and development of a pheromone-baited trap would allow growers to predict adult emergence in the crop accurately and time and target plant protection products better to control adults and larvae feeding on the foliage. Longhurst & Baker (1980) showed that male gooseberry sawfly adults were attracted to virgin females and to dichloromethane washes of virgin females, indicating the production of a sex pheromone by the females. In recent work at EMR and NRI, components of the female sex pheromone of the closely-related blackcurrant sawfly, *Nematus olfaciens,* have been identified, and it is likely that components of the pheromone of gooseberry sawfly will have related chemical structures.

The aim of this project was to confirm the production of a sex pheromone by female gooseberry sawfly, to identify and synthesise components of the pheromone and to evaluate these for attraction of males in the field.

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Materials and methods

Collection of sawfly

A list of gooseberry growers were contacted (including the project industry representatives) in February 2014. Growers were asked to contact EMR as soon as they detected eggs, larvae or adult sawfly in their crop. Larvae were sent by post for culture from the West Midlands (13 May and 6 June), Midlands (9 June) and from the South East (23 July).

Culturing

Sawfly larvae were housed in ventilated, transparent push-fit insect rearing boxes (20 cm x 10 cm x 10 cm). The boxes contained 2 cm of standard compost (EMR) and fresh gooseberry leaves to allow the larvae to continue feeding. Boxes were stored at 20°C on 16:8 hour L:D cycle. Larvae pupated in the soil and then boxes were checked daily for adult emergence. Males and females were separated into tubes and fed a drop of honey. Adult *P. pallipes* began emerging from seven days after pupation and *N. ribesii* from 12 days.

Collection of volatiles

Entrainments of sawfly were taken at 20°C on 16:8 hour (L:D) cycle. Insects were contained in silanised glass vessels (12 cm x 5 cm) and air was drawn in (1000 ml/min) through an activated charcoal filter (20 cm x 2 cm; 8-10 mesh) and out through a collection filter consisting of Porapak Q (200 mg; 50/80 mesh) held between glass wool plugs in a Pasteur pipette (4 mm i.d.) see Fig.3.

Before entrainment the apparatus was cleaned by passing a continuous air flow through for 24 hours. Collections were made for varying lengths of time but ended at the death of an insect. Males and females were entrained separately in varying group sizes. The filters were connected and the pumps were switched on for 30 minutes after placing the sawfly in the chamber to give the insects time to settle. This was to reduce the likelihood of collection of any potential alarm compounds. At the end of volatile collection, all chambers were wiped clean with 100% ethanol and a continuous air flow passed through for 24 hours. During August 2014, 14 collections were made from five-nine females, three from single males and three empty blanks.

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Figure 3. Equipment for collection of volatiles from gooseberry sawfly

Analysis of collections of volatiles

Adsorbed volatiles were extracted from Porapak collection filters with dichloromethane (Pesticide Residue Grade; 1 ml). Extracts were analysed un-concentrated and then concentrated approximately x 10 under a gentle stream of purified nitrogen.

Extracts were analysed by gas chromatography coupled to mass spectrometry (GC-MS) using a Varian 3500 GC coupled to a Saturn 2200 MS (Agilent) operated in electron impact mode. GC columns (30 m x 0.25 mm i.d. x 0.25 µ) coated with polar DBWax (Supelco) or non-polar VF5 (Varian) were used and the oven temperature was programmed from 40°C for 2 min then at 10°C/min to 250°C. Compounds were identified by their mass spectra, their GC retention indices relative to the retention times of *n*-alkanes and comparison with synthetic standards.

Extracts were also analysed by GC with flame ionisation detection (FID) using an Agilent 6850 GC fitted with a polar GC column as above.

Fractionation of collections of volatiles

The collection of volatiles in dichloromethane was evaporated almost to dryness under a gentle stream of nitrogen. Hexane (0.15 ml) was added and this was evaporated almost to dryness again. The residue was applied to the top of a silica gel column (230-400 mesh; 250 mg) held in a Pasteur pipette (i.d. 4mm) in a total of 0.5 ml hexane. The column was

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then eluted with 1 ml portions of hexane, 1%, 2%, 5%, 10%, 20% 50% diethyl ether in hexane and finally diethyl ether. Fractions of 1 ml were collected.

Results

Collections of volatiles from female or male gooseberry sawfly were analysed by GC-FID and GC-MS. Collections from both sexes were very similar and dominated by long-chain hydrocarbons (Z)-9-tricosene (Z9-23:H) and heneicosane (21:H) (Fig. 4).

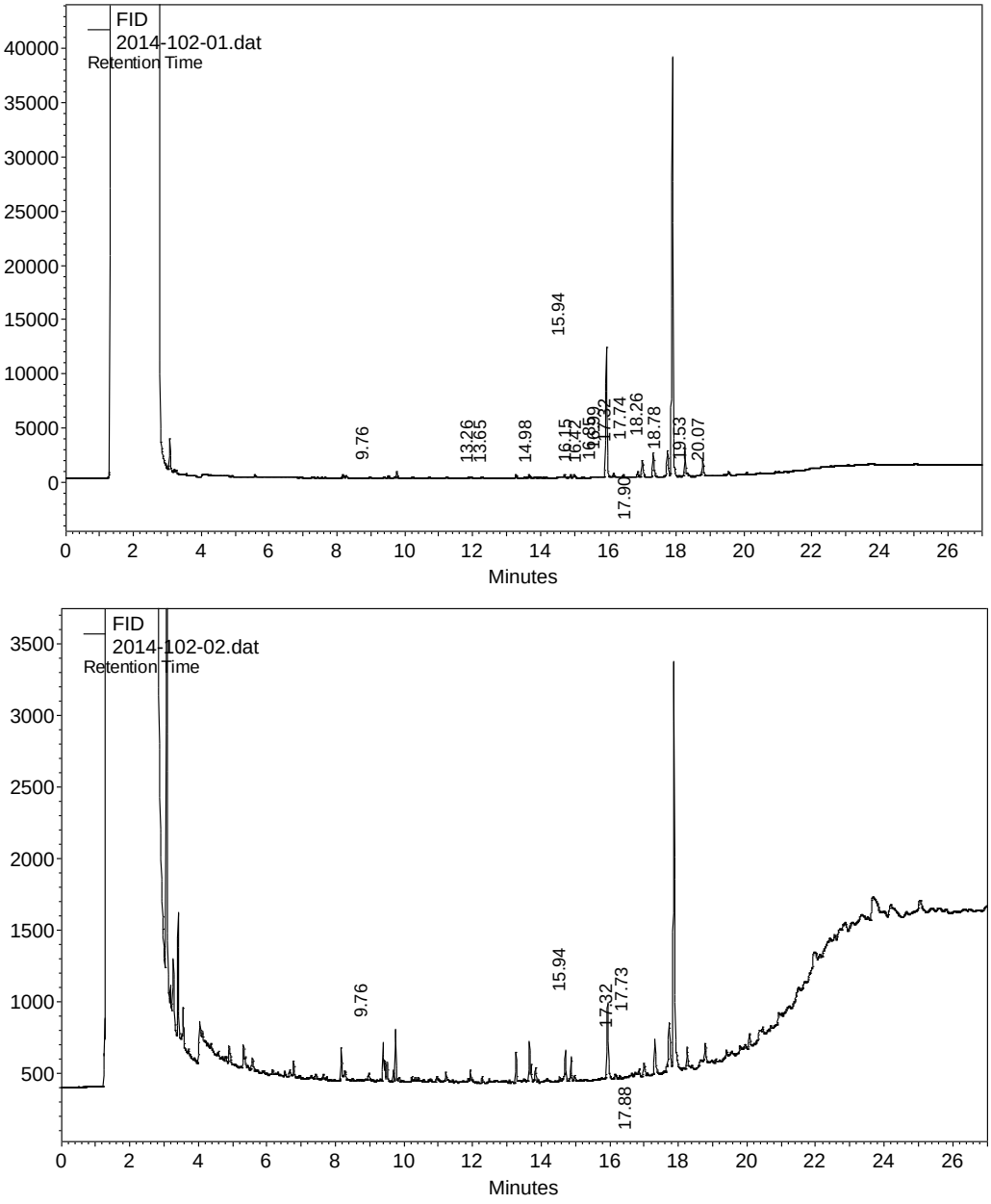

Figure 4. Analyses of volatiles collected from five female (upper) and one male (lower) gooseberry sawfly (GC-FID on polar column; Z9-23:H at 17.90 min; 21:H at 15.94 min)

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Detailed analysis of the traces showed no obvious differences between those from females and males that might be attributed to components of a female sex pheromone. They also confirmed that the pattern of long-chain hydrocarbons was essentially the same in both (Fig. 5). Furthermore this pattern was very similar to that in collections from male and female blackcurrant sawfly.

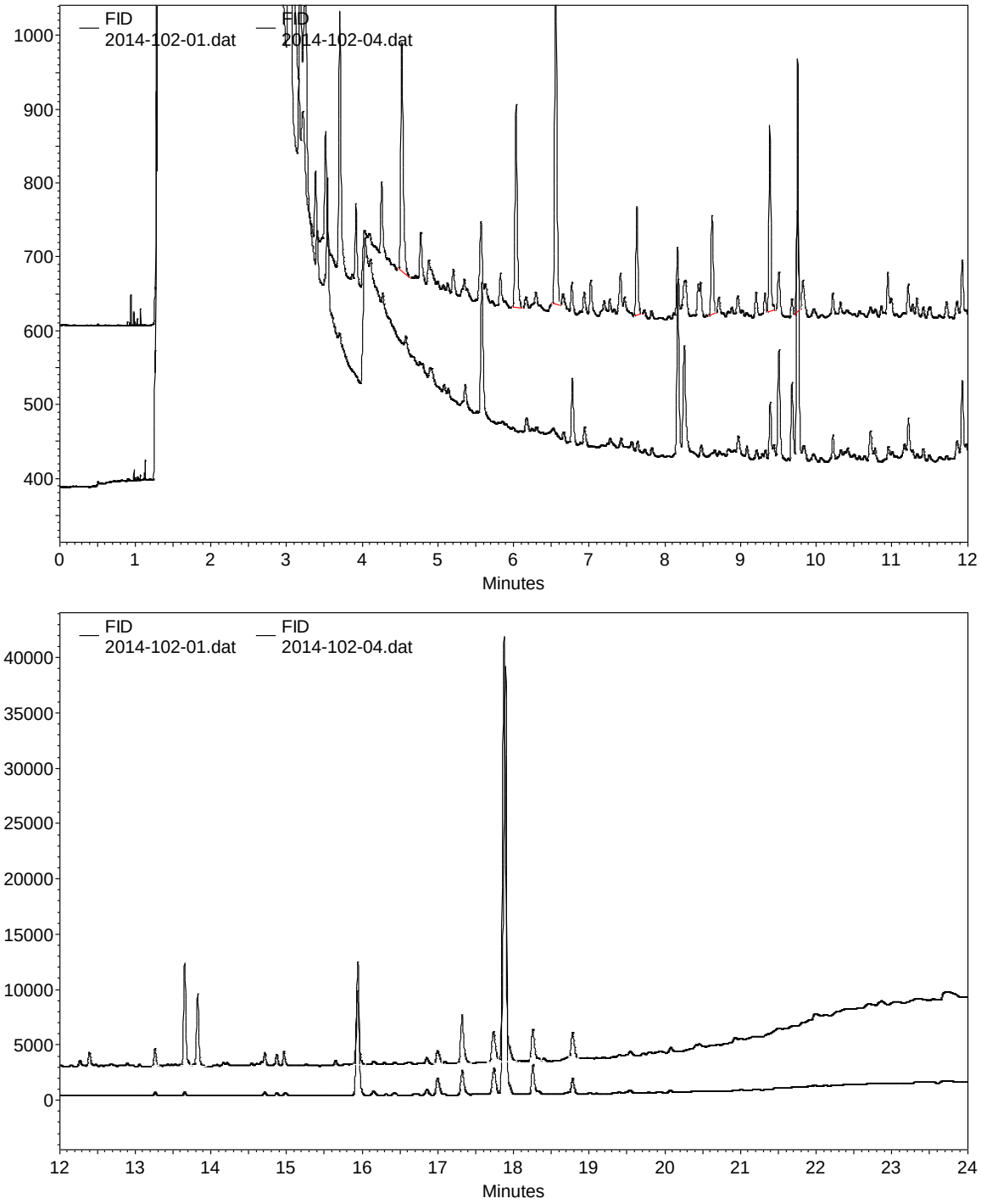

Figure 5. Comparison of GC-FID analyses of volatiles collected from female and male gooseberry sawfly (GC-FID on polar column; upper trace is male, lower female)

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GC-MS traces were examined for the presence of the compounds identified as potential pheromone components of the blackcurrant sawfly – the isopropyl esters of (Z)-5-tetradecenoic acid (Z5-14:iPr), (Z)-7-tetradecenoic acid (Z7-14:iPr) and (Z)-7-hexadecenoic acid (Z7-16:iPr). The mass spectra of the 14-carbon esters showed strong ions at *m/z* 209 and 227 and those of the 16-carbon ester at *m/z* 237 and 296 (Fig. 6).

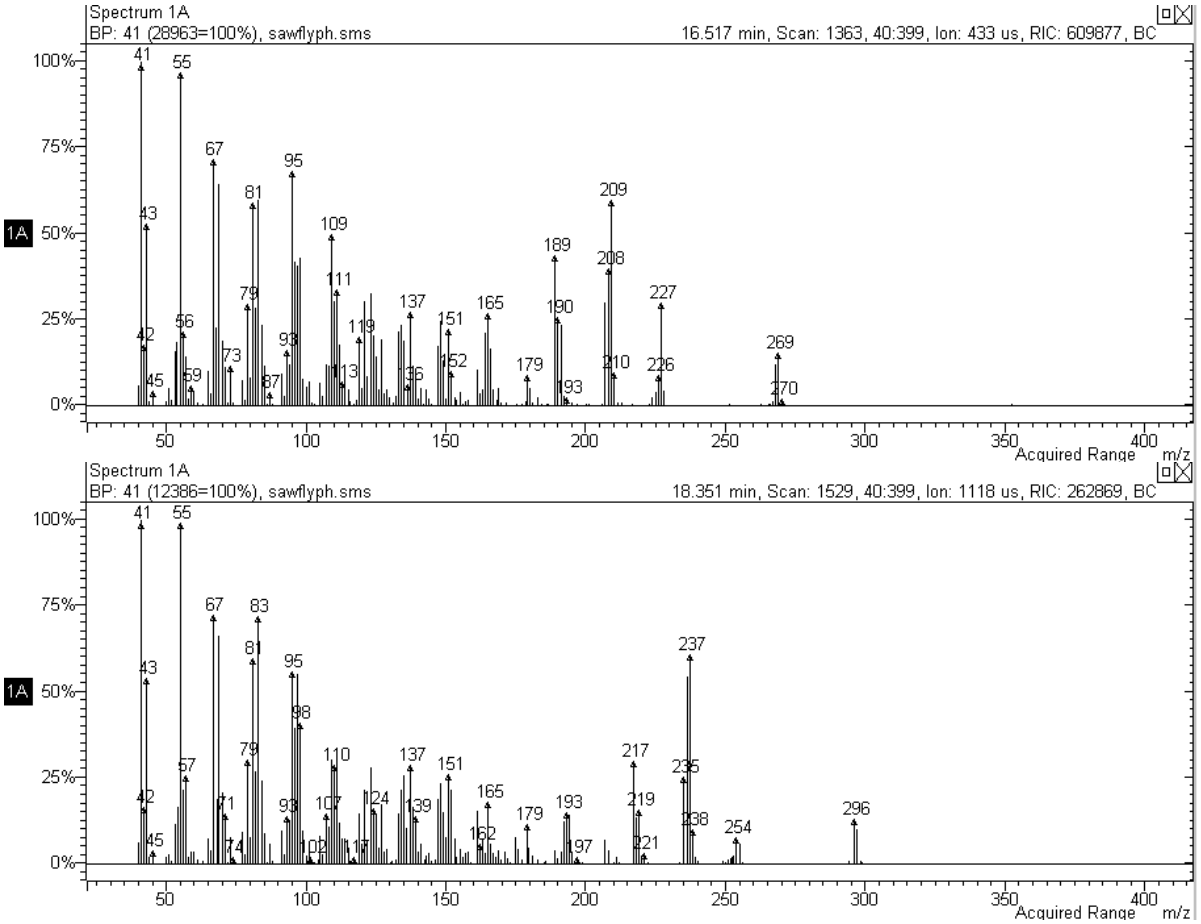

Figure 6. Mass spectra of Z7-14:iPr (upper) and Z7-16:iPr (lower)

Selective ion monitoring (SIM) for these ions showed no trace of the 14-carbon esters in collections either from males or females. There was a slight suggestion of the presence of a 16-carbon ester in collections from females, but this was obscured by one of the large hydrocarbon peaks on both polar and non-polar GC columns.

Some of the collections were then fractionated on silica gel columns which separated out the hydrocarbons (Fraction 1) from more polar compounds. Calibration with the synthetic isopropyl esters showed these were eluted mostly in Fraction 4 (5% ether; 90%) with a small amount in Fraction 3 (2% ether; 10%).

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Examination of the Fraction 4 from collections confirmed that the 14-carbon esters could not be detected. A trace of a mono-unsaturated, 16-carbon isopropyl ester could be detected in collections from female gooseberry sawfly (Fig. 7), but not in those from males (Fig. 8) or blank entrainments (Fig. 9).

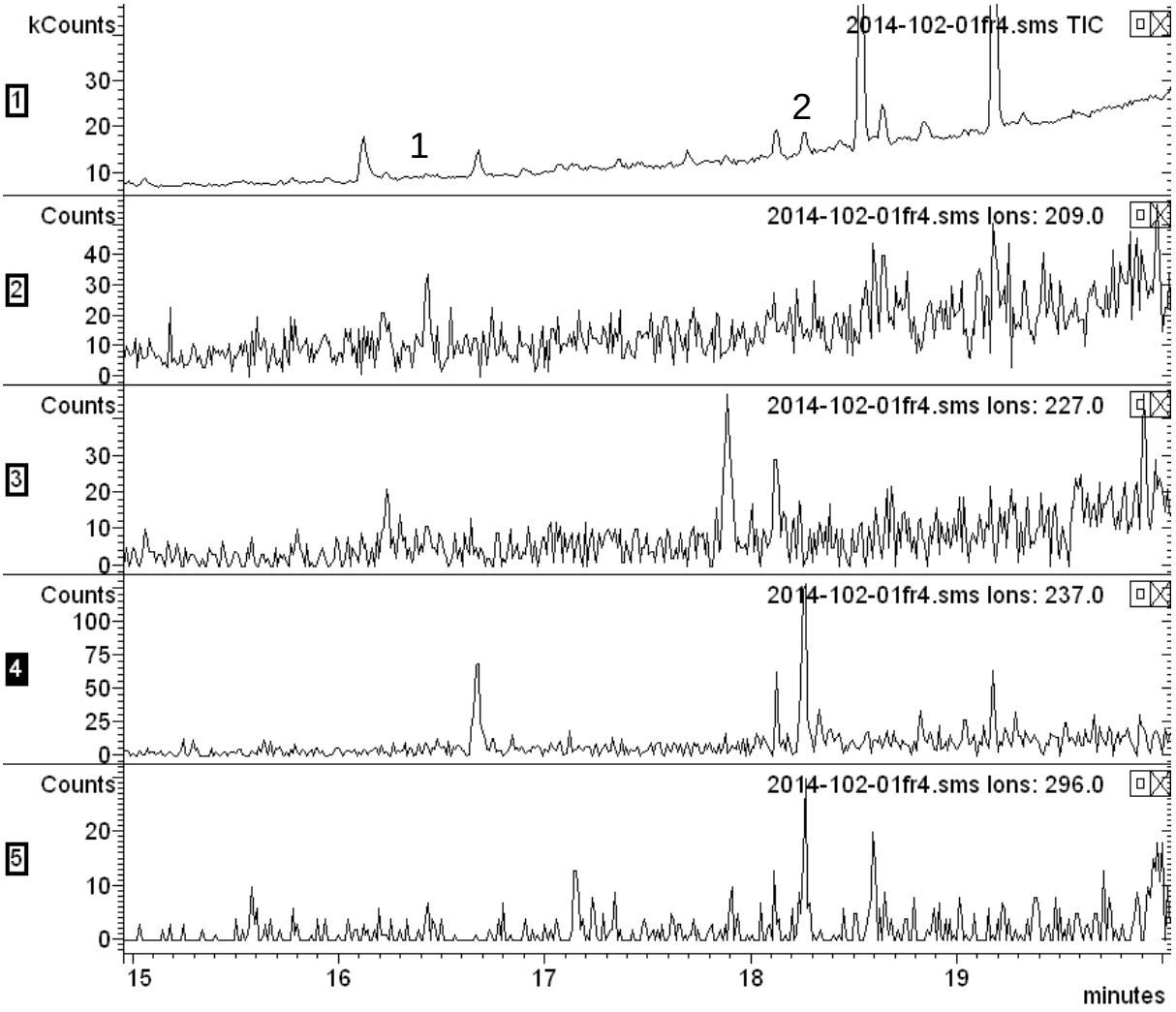

Figure 7. Segment of GC-MS analysis on polar GC column of volatile collection from five female gooseberry sawfly showing (from top) total ion current (TIC), selective ion monitoring (SIM) at *m/z* 209, 227 (characteristic of 14-carbon isopropyl esters), 237 and 296 (characteristic of 16-carbon isopropyl ester); Z7-14:iPr elutes at 16.40 min (1), Z7-16:iPr at 18.23 min (2)

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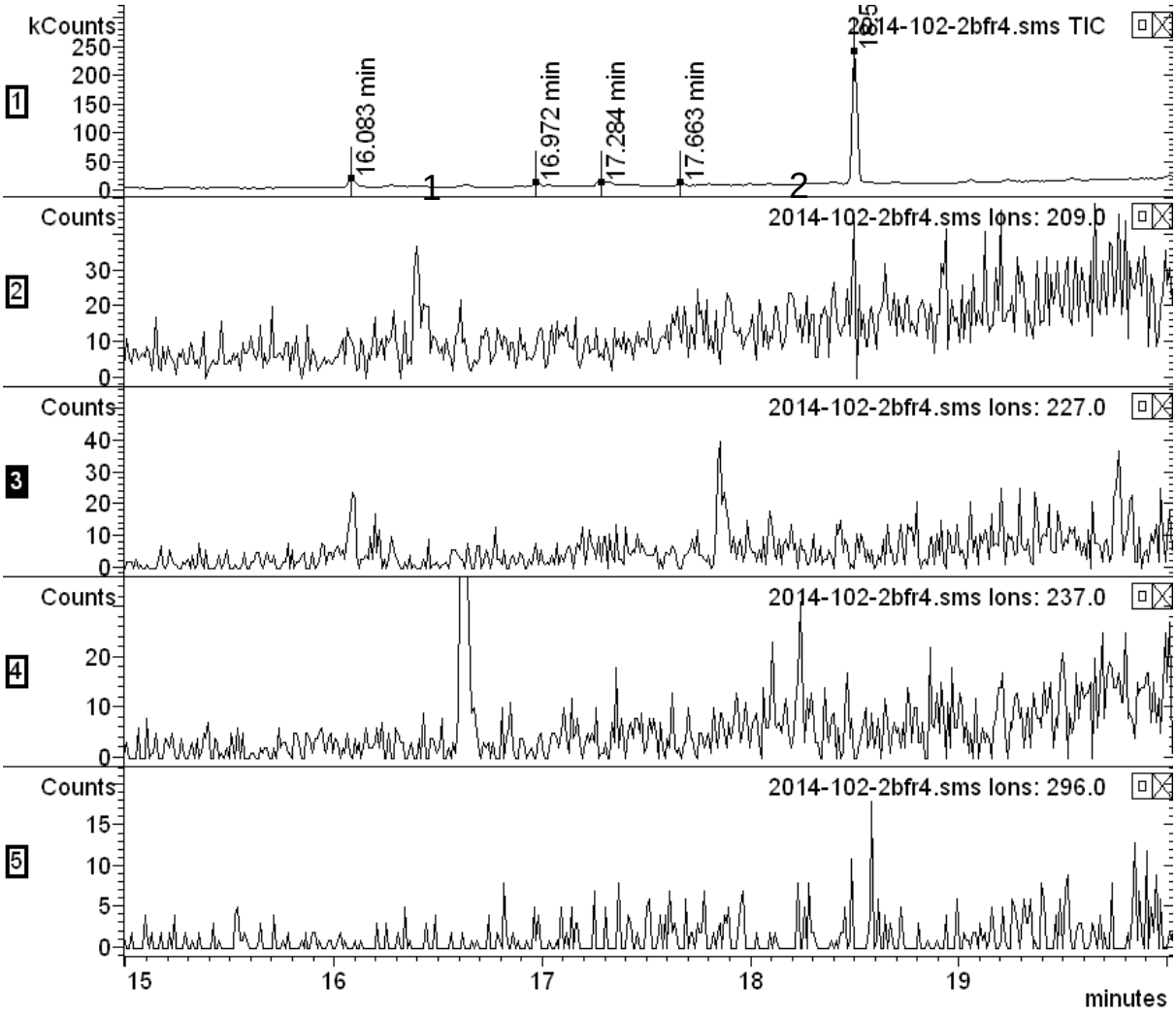

Figure 8. Segment of GC-MS analysis on polar GC column of volatile collection from one male gooseberry sawfly showing (from top) total ion current (TIC), selective ion monitoring (SIM) at *m/z* 209, 227 (characteristic of 14-carbon isopropyl esters), 237 and 296 (characteristic of 16-carbon isopropyl ester); Z7-14:iPr elutes at 16.40 min (1), Z7-16:iPr at 18.23 min (2)

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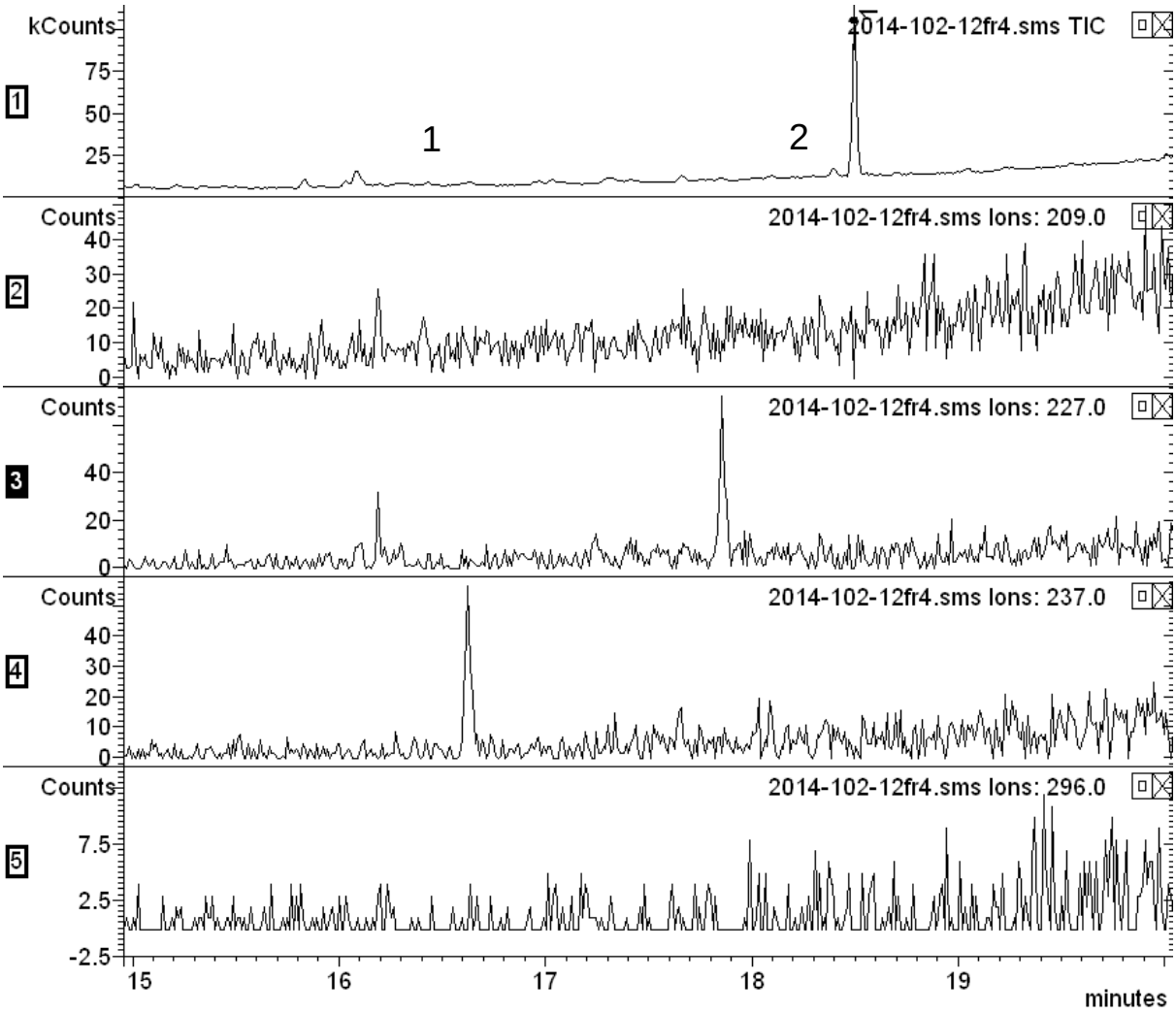

Figure 9. Segment of GC-MS analysis on polar GC column of volatile collection from blank chamber showing (from top) total ion current (TIC), selective ion monitoring (SIM) at *m/z* 209, 227 (characteristic of 14-carbon isopropyl esters), 237 and 296 (characteristic of 16-carbon isopropyl ester); Z7-14:iPr elutes at 16.40 min (1), Z7-16:iPr at 18.23 min (2)

Discussion

Longhurst & Baker (1980) showed that traps baited with a virgin female gooseberry sawfly attracted significant numbers of males. Traps baited with a dichloromethane wash of females applied to a rubber septum attracted far fewer males, but significantly more than unbaited traps.

In the first year of this project, larvae of the common gooseberry sawfly, *Nematus ribesii,* were collected and successfully reared through to adults in the laboratory. Volatiles were collected from virgin females or males and analysed by GC-FID and GC-MS. Collections from both males and females contained large amounts of long-chain hydrocarbons,

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probably derived from the cuticle, but the pattern of these was identical in male and female gooseberry sawfly. Interestingly, the patterns were very similar to those in collections from male and female blackcurrant sawfly with Z9-23:H and 21:H as major components and other saturated, monounsaturated and di-unsaturated long-chain hydrocarbons as minor components.

There were no obvious differences in the composition of volatiles from female or male gooseberry sawfly that might be attributable to potential components of a female sex pheromone. Examination for the presence of the compounds proposed to be components of the female sex pheromone of the blackcurrant sawfly was hampered by the large quantities of hydrocarbons which co-chromatographed on both non-polar and polar GC columns. However, an approach was developed to separate out the hydrocarbons by chromatography on a small silica gel column. The fraction that would contain the components of the blackcurrant sawfly pheromone could then be analysed by GC-MS with selective ion monitoring (SIM) to maximise sensitivity. No trace of the 14-carbon isopropyl esters could be found in any of the collections. However, traces of a mono-unsaturated, 16-carbon isopropyl ester, similar to one of the compounds proposed to be a component of the pheromone of the blackcurrant sawfly, could be detected in collections from female gooseberry sawfly, but not in those from males or blank collections.

Unfortunately, it was not possible to substantiate this result using analysis by GC coupled to EAG recording from the insect antenna because no insects were available after the analyses. In the second year of the project, GC-EAG analysis will be a priority in order to confirm the above result and to check for any other components detected by the antenna that would be potential components of the pheromone. The retention time of the compound detected above seems to be identical to that of the Z7-16:iPr produced by blackcurrant sawfly females. This isomeric composition needs to be confirmed, but it will also be possible to test this compound for attractiveness to male gooseberry sawfly in growers' fields.

Conclusions

- Gooseberry sawfly have been collected as larvae and successfully reared through to adults in the laboratory;
- Volatiles have been collected from male and female gooseberry sawfly and analysed by GC-FID and GC-MS;

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- Traces of one of the proposed pheromone components of the blackcurrant sawfly were detected in collections from female gooseberry sawfly and not in collections from males or blank collections;
- Further collections and analysis using GC-EAG are required to confirm this result, but it will be possible to test the proposed pheromone component for attractiveness to gooseberry sawfly males during 2015.

Knowledge and Technology Transfer

None to date.

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