Project title:	Development of a Sex Pheromone Monitoring Trap for Gooseberry Sawfly
Project number:	SF 147
Project leader:	Dr Michelle Fountain, NIAB EMR, New Road, East Malling ME19 6BJ
Report:	Year 3 Final Report
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Key staff:	Madeleine Cannon (NIAB-EMR) Prof David Hall, Dudley Farman (NRI)
Location of project:	EMR, NRI
Industry Representative:	Richard Stanley
Date project commenced:	April 2014
Date project completed (or expected completion date):	March 2017

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.

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

Deputy Head of Pest and Pathogen Ecology

NIAB EMR, New Road, East Malling, Kent, ME19 6BJ

Signature ..... Date ..13 Apr 17.....

Prof David Hall

Research Leader in Semiochemical Ecology

Natural Resources Institute, University of Greenwich

Signature ..... Date .....13 Apr 17.....

Report authorised by:

Prof Jerry Cross

Science Group Leader

NIAB EMR, New Road, East Malling, Kent, ME19 6BJ

Signature ..... Date ....

CONTENTS

AUTHENTICATION **3**

CONTENTS **4**

GROWER SUMMARY **1**

Headline 1

Background and expected deliverables 1

Summary of the project and main conclusions 1

Financial benefits 2

Action points for growers 3

SCIENCE SECTION **4**

Introduction 4

Materials and Methods 5

Collection of sawfly 5

Culturing 6

Collection of volatiles 6

Analysis of collections of volatiles by coupled gas chromatography - electroantennography (GC-EAG) 7

Analysis of collections of volatiles by coupled gas chromatography – mass spectrometry (GC-MS) 8

Fractionation of collections of volatiles 8

Synthesis 8

Release Rate Study 8

Field testing 9

Results 9

Collection of sawfly 9

Collection of volatiles 9

Analysis of collections of volatiles by GC-EAG 9
Fractionation of collections of volatiles and analysis by GC-MS 16
Field tests.....24
Discussion 25
Conclusions 28
Knowledge and Technology Transfer 28
References 28

GROWER SUMMARY

Headline

- A potential component of the female sex pheromone of the gooseberry sawfly has been identified and synthesised and shown to be attractive to male gooseberry sawfly in gooseberry crops.

Background and expected deliverables

The common gooseberry sawfly, *Nematus ribesii*, is a sporadic, localised and unpredictable pest of gooseberry, as well as, red- and white-currants. If not detected and controlled in a timely manner, the feeding larvae are able to defoliate whole gooseberry bushes. The monitoring of the pest relies on the detection of the eggs on the underside of leaves in the centre of the bushes. Larval damage begins in the centre of the plants, low down, and radiates outwards to 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 aimed 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 has been reported by other scientists but never identified. In other work by NRI and NIAB 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 by growers and reared through to adults in the laboratory at NIAB EMR. 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, 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 the second year of the project, gooseberry sawfly larvae were again collected by growers and reared through to adults at NIAB EMR. Collections of volatiles were made from virgin females and analysed by gas chromatography coupled to mass spectrometry (GC-MS) at NRI. Similar results were obtained to those in the first year, and again there were indications of the presence of a mono-unsaturated, 16-carbon isopropyl ester. The collections were also analysed by gas chromatography coupled to electroantennographic recording (GC-EAG) from antennae of male gooseberry sawfly. Strong EAG responses were obtained to the isopropyl esters previously identified as components of the sex pheromone of female blackcurrant sawfly. However, in analyses of volatile collections from female gooseberry sawfly no EAG responses were observed at the retention times corresponding to these compounds. Instead a single EAG response was observed at a later retention time on the polar GC column. This seemed to be associated with one of the hydrocarbon components, ZZ6,9-23:H, but neither the synthetic compound nor the purified natural compound elicited an EAG response. Unfortunately, no EAG response was observed in GC-EAG analyses of volatile collections on a non-polar GC column.

In the third year collections of volatiles from virgin female gooseberry sawfly were analysed by GC-EAG on both polar and non-polar GC columns. The previous results were confirmed and extended to show that the EAG response was due to a very minor component co-chromatographing with the hydrocarbon on the polar GC column. On the basis of its mass spectrum, GC retention time and product after hydrogenation, this EAG-active compound was shown to be a 16-carbon isopropyl ester with three double bonds, and proposed to be isopropyl (*Z,Z,Z*)-7,10,13-hexadecatrienoate. This was synthesised and provided to growers for evaluation in traps. Although it was late in the season, significant numbers of male gooseberry sawfly were trapped, and, although further testing is required, it is proposed that this compound is the major, if not the only, component of the sex pheromone of female gooseberry sawfly.

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 spray applications. Most growers apply at least one prophylactic spray at the beginning of the season regardless. Targeting products better, usually 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 AHDB growing gooseberries in the UK. At least three growers supply to supermarkets and 11% of fruit is grown for processing. Around 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 was 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

- It is planned to produce a monitoring trap for gooseberry sawfly using a lure containing isopropyl (*Z,Z,Z*)-7,10,13-hexadecatrienoate. Growers should use this as soon as it becomes commercially available.
- Look or monitor for adults flying in April and May and target with approved products 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.
- Consult with your agronomist on the latest product approvals for gooseberry.

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 5-7 mm long with the females being larger (Figure 1). Female sawfly lay eggs on the underside of the leaves in rows (Figure 2) and the larval damage starts as small holes (also seen in Figure 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 at first, but soon spreads throughout the whole plant. Feeding attacks are often sporadic and unpredictable.

Figure 1. Adult common gooseberry sawfly female

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 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 NIAB 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. In the first two years of the project, communication was established with several growers who provided insect material and carried out testing. Virgin males and females were reared in the laboratory and collections of volatiles made at NIAB EMR. Analysis of these at NRI showed no obvious differences between collections from females and males that might be due to the presence of components of a female sex pheromone. Analysis of volatiles from female gooseberry sawfly by gas chromatography coupled to electroantennographic recording (GC-EAG) from antennae of male gooseberry sawfly showed a single strong response, but it was not possible to identify the structure of the compound responsible. The objectives for this third year of the project were to repeat and extend the GC-EAG analyses to detect the compound responsible for the response more conclusively and, if possible, to identify and synthesise it for field testing.

Materials and Methods

Collection of sawfly

A list of 25 gooseberry growers and gardeners were contacted (including the project industry representatives) on 13 April 2016. Contacts were asked to communicate with us as soon as they detected eggs, larvae or adult sawfly in their crop. Larvae were sent by post for culture to NIAB EMR.

Culturing

N. ribesii larvae were housed in ventilated, transparent push-fit insect rearing boxes (20 cm x 10 cm x 10 cm, Figure 3). The boxes contained 2 cm of standard compost and fresh gooseberry leaves to allow the larvae to continue feeding. Boxes were stored at 20°C on 16:8 h light: dark (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. A total of 33 females and 10 males emerged. In addition a high number (31) of hymenopteran (wasp) parasitoids emerged from culture.

Figure 3. Gooseberry sawfly larvae feeding and developing in laboratory culture.

Collection of volatiles

Entrainments of sawfly were done in room CT2 at NIAB EMR within the quarantine building at 20°C on 16:8 h L:D cycle. Insects were contained in silanised glass vessels (12 cm x 5 cm) and air was drawn in (1000 ml min^{-1}) 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 in diameter, Figure 4).

Before entrainment the apparatus was cleaned by passing a continuous air flow through for 24 h. Collections were made for varying lengths of time but ended at the death of an insect. Females were entrained separately in varying group sizes. The filters were connected and the pumps were switched on for 30 min 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 passing a continuous air flow through for 24 h. During May and June 2016 seven collections were made from groups of three to six females. Males were sent to NRI for EAG.

Figure 4. Sawfly females in collection chamber and equipment for collection of volatiles from gooseberry sawfly

Analysis of collections of volatiles by coupled gas chromatography - electroantennography (GC-EAG)

GC-EAG analyses were carried out with an HP 6890 instrument (Agilent) with two capillary GC columns (30 m × 0.32 mm in diameter with 0.25 µm film thickness) coated with polar (Wax10; Supelco) and non-polar (SPB1; Supelco) phases respectively. Carrier gas was helium (2.4 ml min^{-1}) and the oven temperature was programmed from 50°C for 2 min, then at 10 °C min^{-1} to 250°C. Injection was splitless (220°C) on the polar column and by a Programmed Temperature Vaporiser (PTV) on the non-polar column. The PTV temperature was held at 50°C for 0.2 min then programmed at 600°C min^{-1} to 220°C with the split opened after 1 min. The GC column effluent was split (1:1) with a zero dead volume connector and equal lengths of deactivated fused silica capillary column between the FID (250 C) and a heated transfer line (250°C; Syntech, Hilversum, The Netherlands). The outlet from the transfer line was delivered into a flow of air (500 ml/min) through a glass tube (4 mm i.d.) to within 3 mm of the EAG preparation.

For the EAG preparation, the sawfly was anaesthetised by refrigeration for 10 min before excising the head. The reference electrode (0.1 M potassium chloride with 1% polyvinylpyrrolidone) was inserted into the back of the head and attached to silver electrode held in micromanipulators on a portable EAG device (INR-02; Syntech, Hilversum, The Netherlands). The end of one antenna was inserted into the recording glass electrode. Both FID and EAG signals were collected and analyzed with EZChrom software (Elite v3.0; Agilent).

Analysis of collections of volatiles by coupled gas chromatography – mass spectrometry (GC-MS)

At NRI, adsorbed volatiles were extracted from Porapak collection filters with dichloromethane (Pesticide Residue Grade; 1 ml). Extracts were analysed un-concentrated and then concentrated by a factor of approximately ten times under a gentle stream of purified nitrogen.

Collections 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 in diameter 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^{-1} 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.

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

Fractionation of collections of volatiles

One of the collections 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 (4 mm in diameter) in a total of 0.5 ml hexane. The column was 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.

Synthesis

Iso-propyl esters of (*Z,Z,Z*)-9,12,15-octadecatrienoic acid (linolenic acid; SigmaAldrich, Gillingham, Dorset, UK) and (*Z,Z,Z*)-7,10,13-hexadecatrienoic acid (Laradan Fine Chemicals, Sweden) were prepared in high yield by reaction with iso-propanol in dichloromethane in the presence of N,N'-dicyclohexylcarbodiimide and a catalytic amount of 4-dimethylaminopyridine.

Release Rate Study

Lures containing isopropyl (Z,Z,Z)-7,10,13-hexadecatrienoate alone (A, 100 µg) or isopropyl (Z,Z,Z)-7,10,13-hexadecatrienoate + (Z)-9-tricosene (B, 100 + 500 µg), dispensed from low-density polyethylene vials (22 mm x 8 mm x 1 mm thick; Just Plastics, London, UK) were maintained in a laboratory wind tunnel at 27°C and 8 km/h windspeed and

collections of volatiles released were made in the same controlled-temperature room. Individual dispensers were held in a glass vessel (8 cm x 3 cm) and air drawn in at 2 l/min through a charcoal filter (20 cm x 2 cm; 10-18 mesh) and out through a collection filter (4 mm i.d.) containing Porapak Q (200 mg, 50-80 mesh) for 2-3 h. Volatiles were eluted with dichloromethane (Pesticide Residue Grade, 1ml). Tetradecyl acetate (14:Ac 2 µg) was added as an internal standard and the solutions were analyzed by GC-MS and GC-FID after concentration approximately ten-fold under a gentle stream of nitrogen. Amounts of pheromone components were quantified by comparison of peak areas with that of the internal standard. Because of the scarcity of material, only one vial of each treatment was measured.

Field testing

Following laboratory analyses at NRI three traps and lures were sent to six growers/gardeners known to have found common gooseberry sawfly in 2015. Traps were in place from August to October. Traps were red delta traps with a sticky glue card insert and colour coded so that treatments remained anonymous. Treatments were isopropyl (Z,Z,Z)-7,10,13-hexadecatrienoate (A, 100 µg), isopropyl (Z,Z,Z)-7,10,13-hexadecatrienoate + (Z)-9-tricosene (B, 100 + 500 µg), both dispensed from low-density polyethylene vials as above, and unbaited (C).

An instruction sheet, record sheet and freepost address was also provided. Traps were placed near to the edge of a plantation adjacent to woody hedgerow/woodland about 10 m apart. Growers were requested to take photographs or send in sticky inserts with sawfly for confirmation by EMR staff.

Results

Collection of sawfly

A total of 33 females and 10 males emerged. In addition 8 hymenopteran (wasp) parasitoids emerged.

Collection of volatiles

During May and June 2016 seven collections were made from groups of three to six females. Males were sent to NRI for EAG.

Analysis of collections of volatiles by GC-EAG

Collections of volatiles made in 2015 were analysed by GC-EAG with continuous delivery of the GC effluent over the EAG preparation rather than the intermittent delivery used

previously. Analyses on a polar GC column confirmed results obtained in 2015 with a single strong EAG response coinciding with a peak in the GC trace that had been identified as an unsaturated hydrocarbon, (*Z,Z*)-3,6-tricosadiene (ZZ6,9-23:H) (Figure 5). However, this year it was also possible to record a response in GC-EAG analyses on the non-polar GC column (Figure 6).

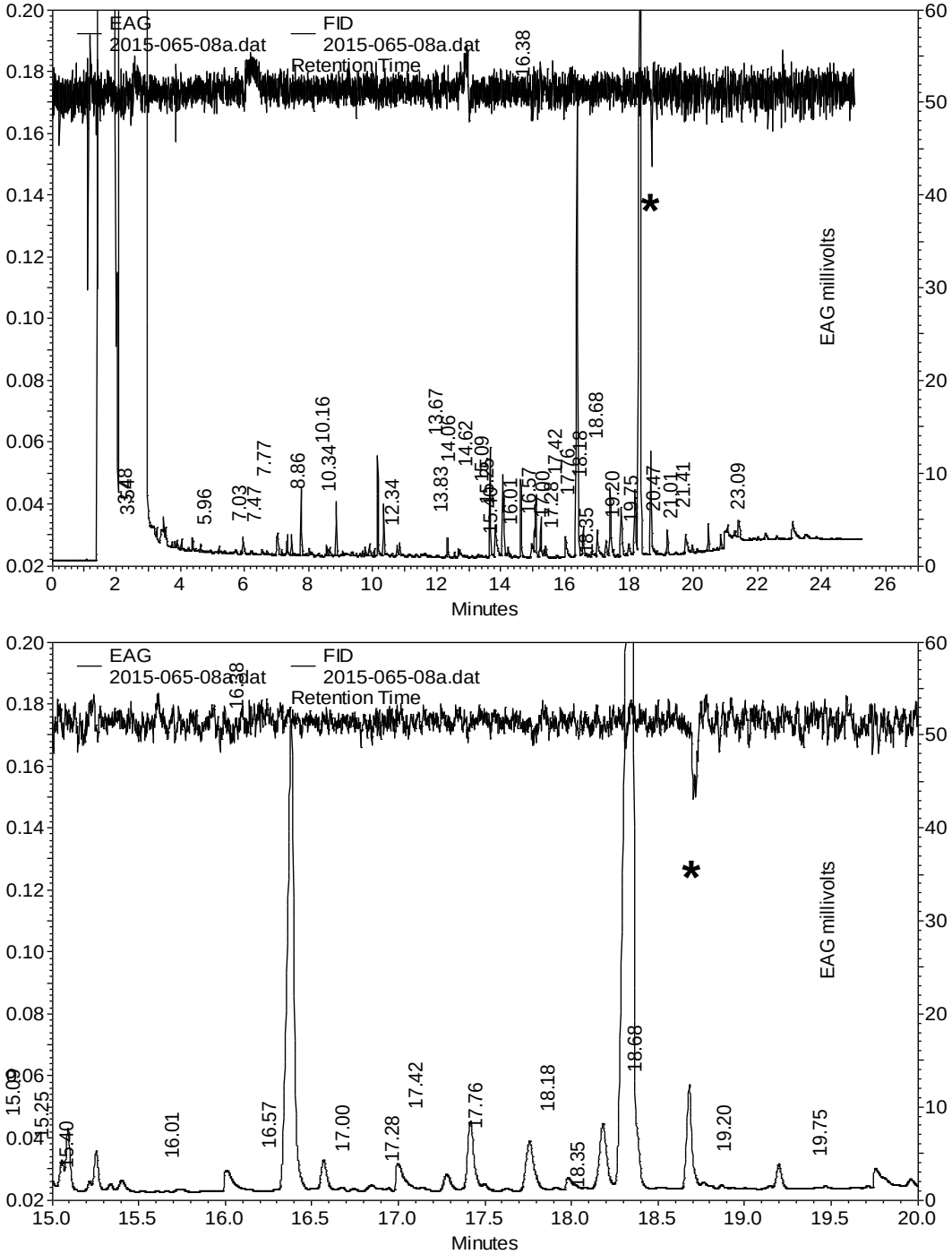

Figure. 5. GC-EAG analysis of volatiles from female gooseberry sawfly (2015/065/08) with male EAG on polar GC column. Lower panel is expansion of upper and in each lower trace is GC and upper is EAG. Response (*) and ZZ6,9-23:H at 18.68 min, RI 2363

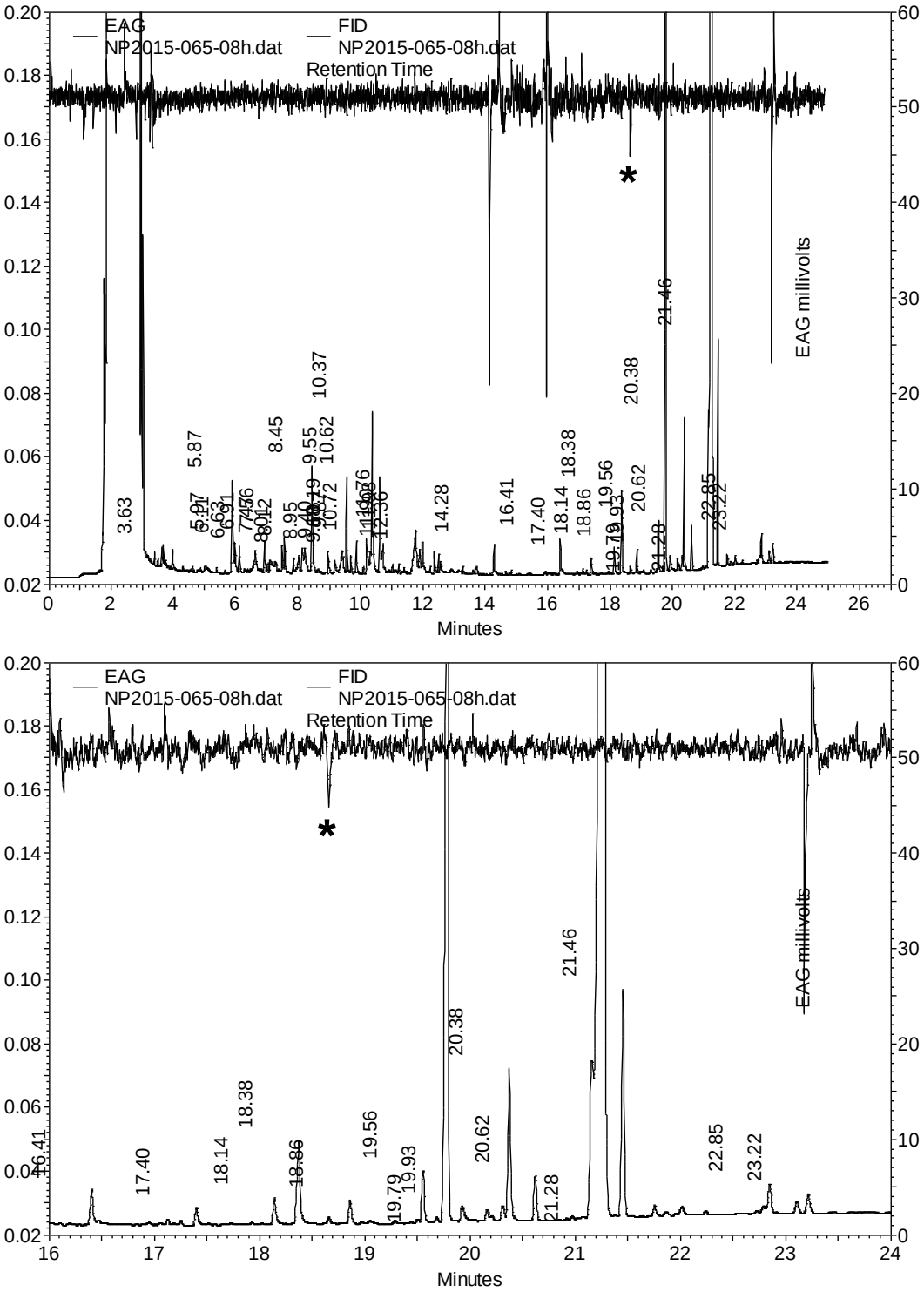

Figure 6. GC-EAG analysis volatiles from female gooseberry sawfly (2015/065/08) with male EAG on non-polar GC column. Lower panel is expansion of upper and in each lower trace is GC and upper is EAG. Response (*) at 18.66 min, RI 1978; ZZ6,9-23:H at 21.46 min

Using this new system of continuous delivery of the GC column effluent to the EAG preparation, it was also confirmed that the antennae of male gooseberry sawfly responded to components of the female sex pheromone of the blackcurrant sawfly (Figure 7)

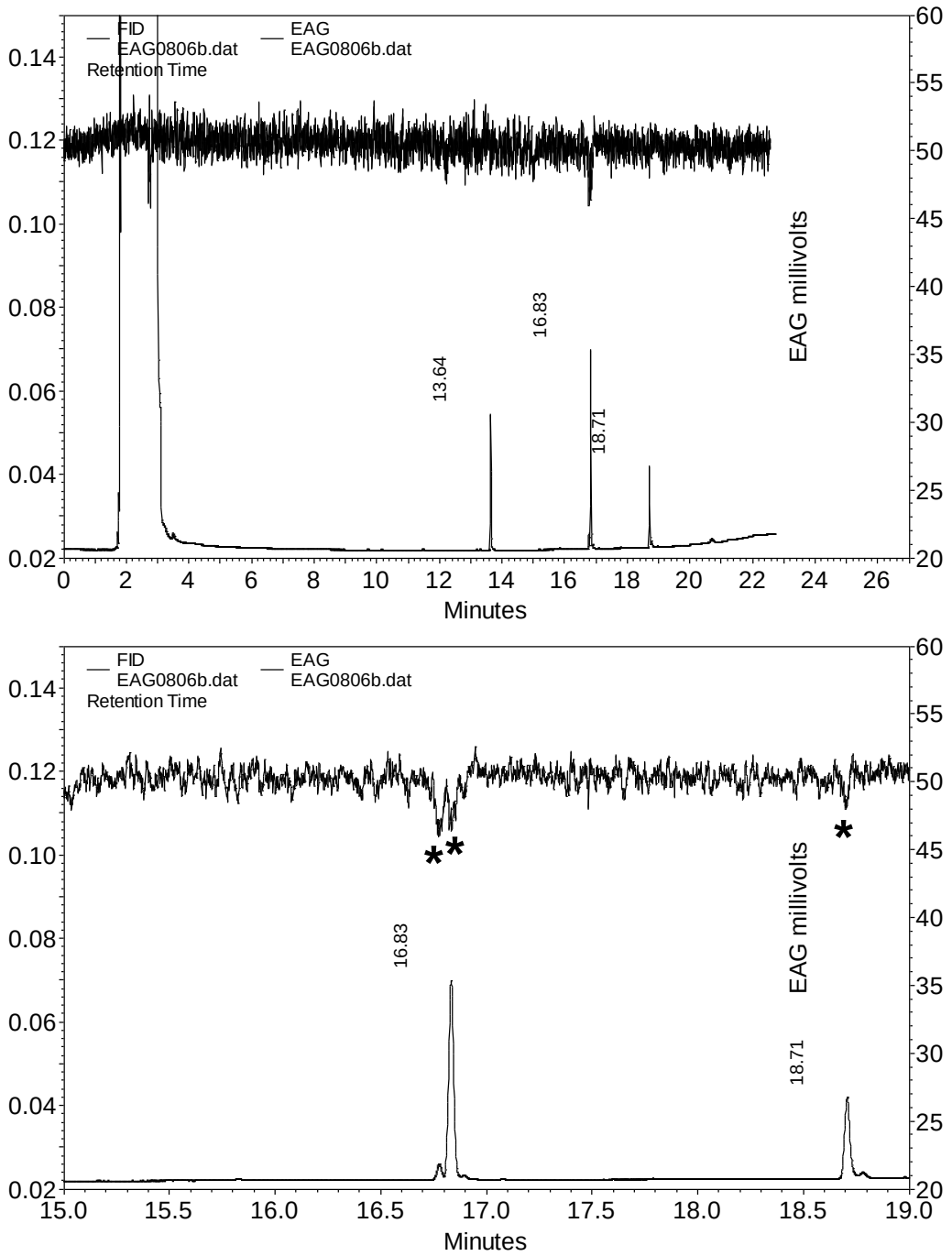

Figure 7. GC-EAG analysis components of sex pheromone of blackcurrant sawfly with male gooseberry sawfly EAG preparation on polar GC column. Lower panel is expansion of upper and in each lower trace is GC and upper is EAG. EAG responses (*) to Z5-14:iPr at 16.77 min, Z7-14:iPr at 16.83 min and to Z7-16:iPr at 18.71 min.

The EAG response in analyses on the non-polar column coincided with a very small peak at 18.66 min that was well separated from the peak due to ZZ6,9-23:H at 21.46 min (Fig. 6).

GC analyses of the seven remaining collections of volatiles from female gooseberry sawfly made during 2015 showed this peak to be present in three of them but essentially absent in the rest (Figure 8).

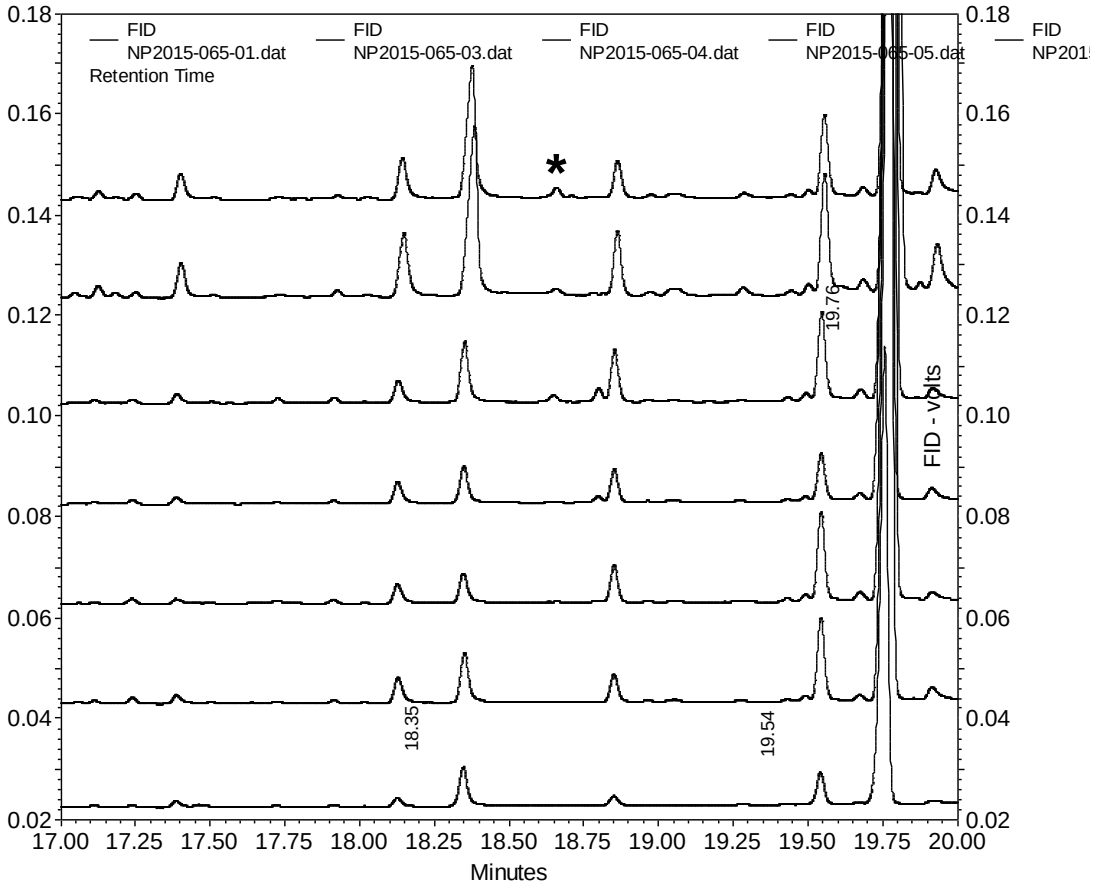

Figure 8. GC Analyses of collections of volatiles from female gooseberry sawfly made during 2015 on non-polar SPB1 column used for GC-EAG analyses (from bottom 2015-065-01, 03, 04, 05, 06, 07, 08; EAG-active peak *)

Similar screening of five collections made during 2016 showed significant amounts of this compound in two of them (Figure 9).

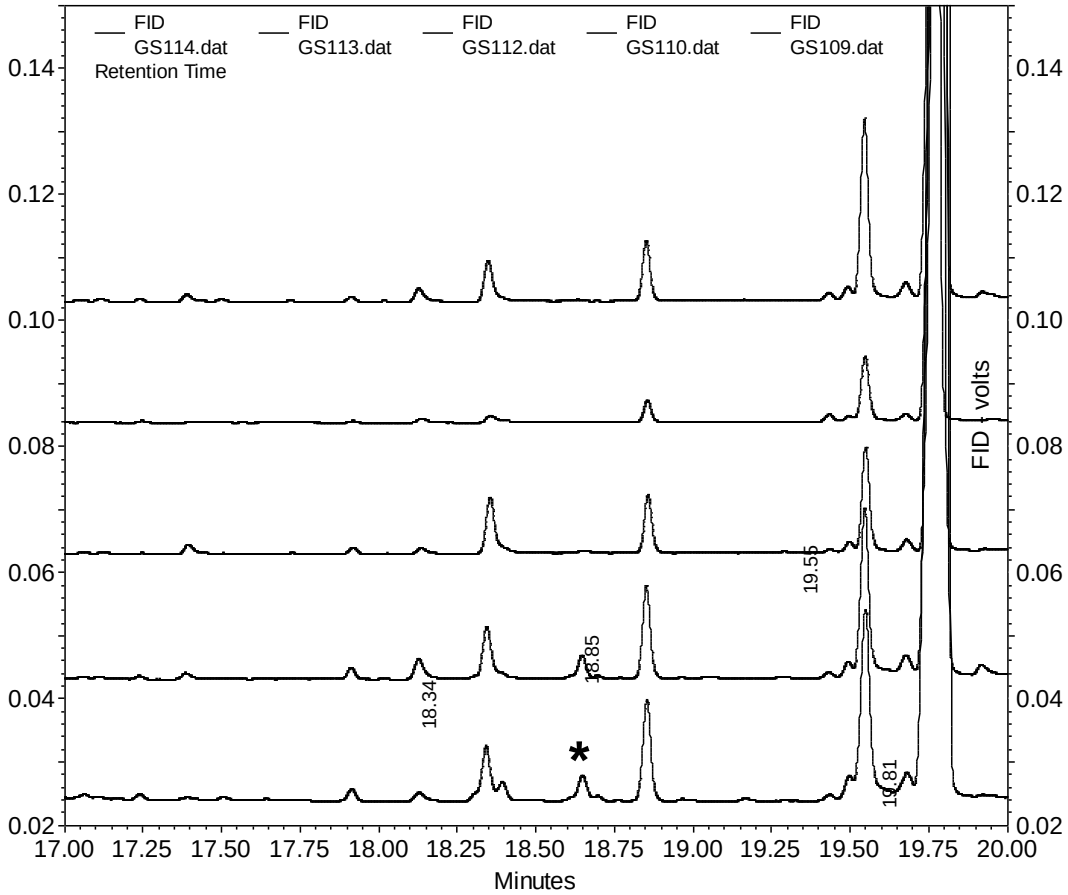

Figure 9. GC Analyses of collections of volatiles from female gooseberry sawfly made during 2016 on non-polar SPB1 column used for GC-EAG analyses (from top 2016-069-01, 02, 04, 05, 06; EAG-active peak *)

GC-EAG analysis of the collection that apparently had the most of this compound, 2016-069-06, confirmed that this peak did elicit an EAG response from the antenna of a male gooseberry sawfly (Figure 10).

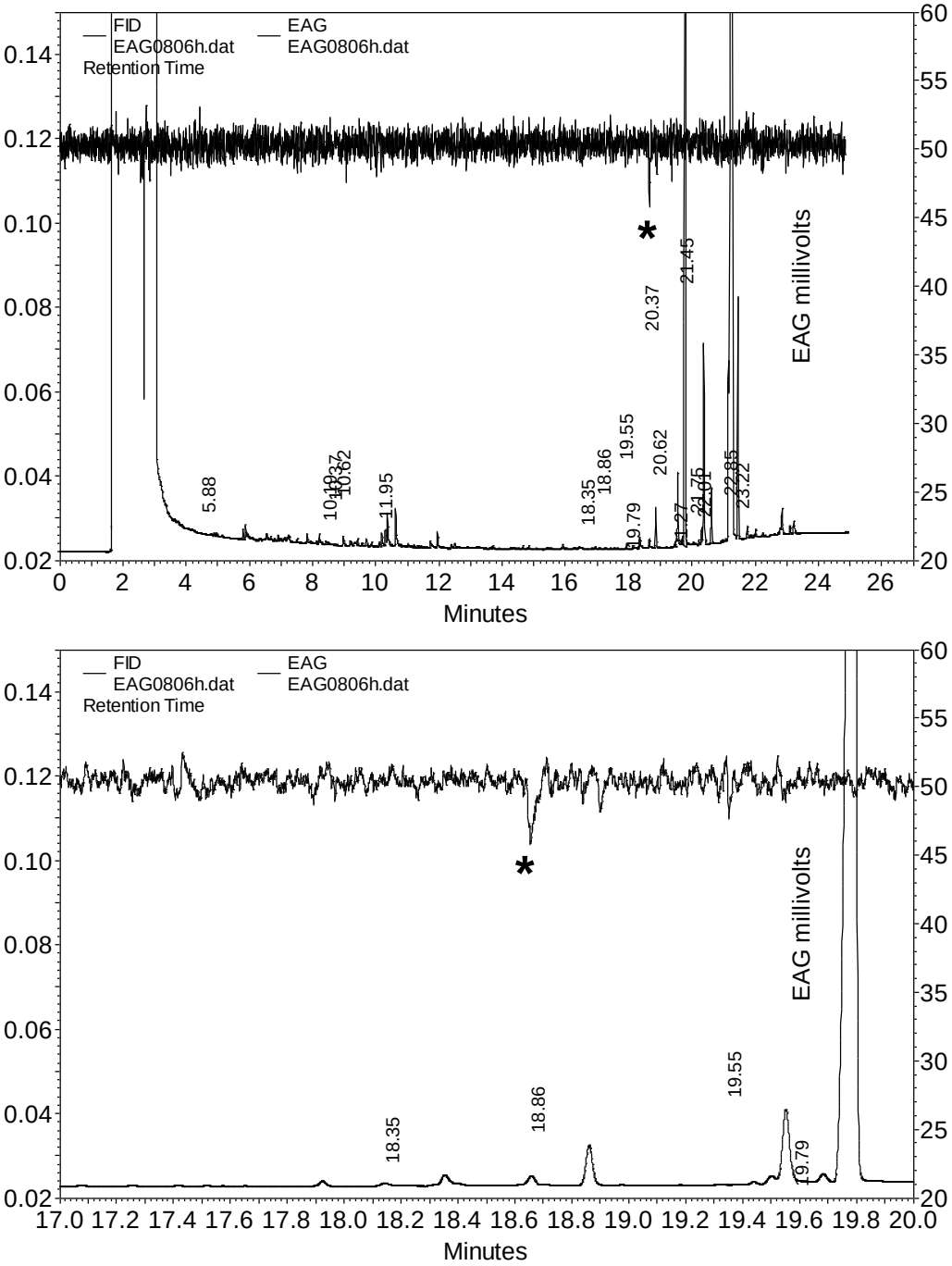

Figure 10. GC-EAG analysis of volatiles from female gooseberry sawfly (2016/069/06) with male EAG on non-polar GC column. Lower panel is expansion of upper and in each lower trace is GC and upper is EAG. Response (*) at 18.66 min, RI 1978; ZZ6,9-23:H at 21.45 min

Fractionation of collections of volatiles and analysis by GC-MS

When collections of volatiles from female gooseberry sawfly were analysed by GC-MS, on both the polar and non-polar GC columns the EAG-active peak was not clearly resolved from larger hydrocarbon peaks. The collection of volatiles containing most of the EAG-active compound, 2016-069-06, was fractionated on silica gel eluted with a gradient of diethyl ether in hexane, as used previously. The EAG active peak appeared in fraction 4, well separated from the hydrocarbons in fraction 1 and as expected for compounds such as esters (Figure 11).

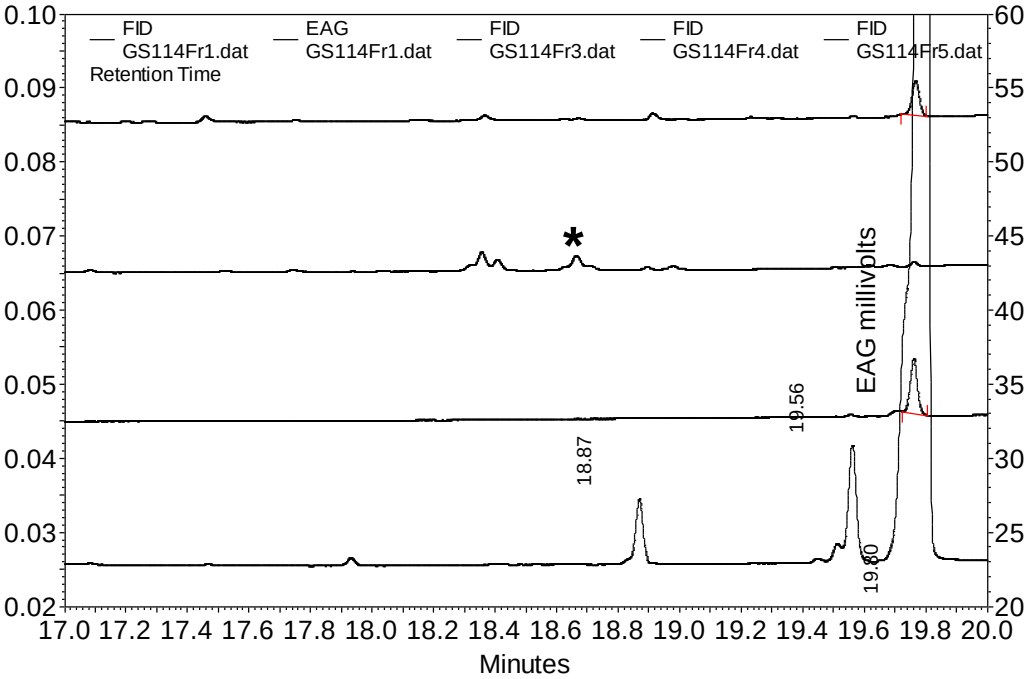

Figure 11. GC Analyses of fractions from liquid chromatographic separation of collection 2016-069-06 on non-polar SPB1 column. From bottom fractions 1, 3, 4 and 5; EAG-active peak at 18.66 min *)

GC-EAG analyses of this fraction 4 confirmed that the EAG-active compound was present and clearly separated from the hydrocarbon impurities on both non-polar (Figure 12) and polar (Figure 13) GC columns.

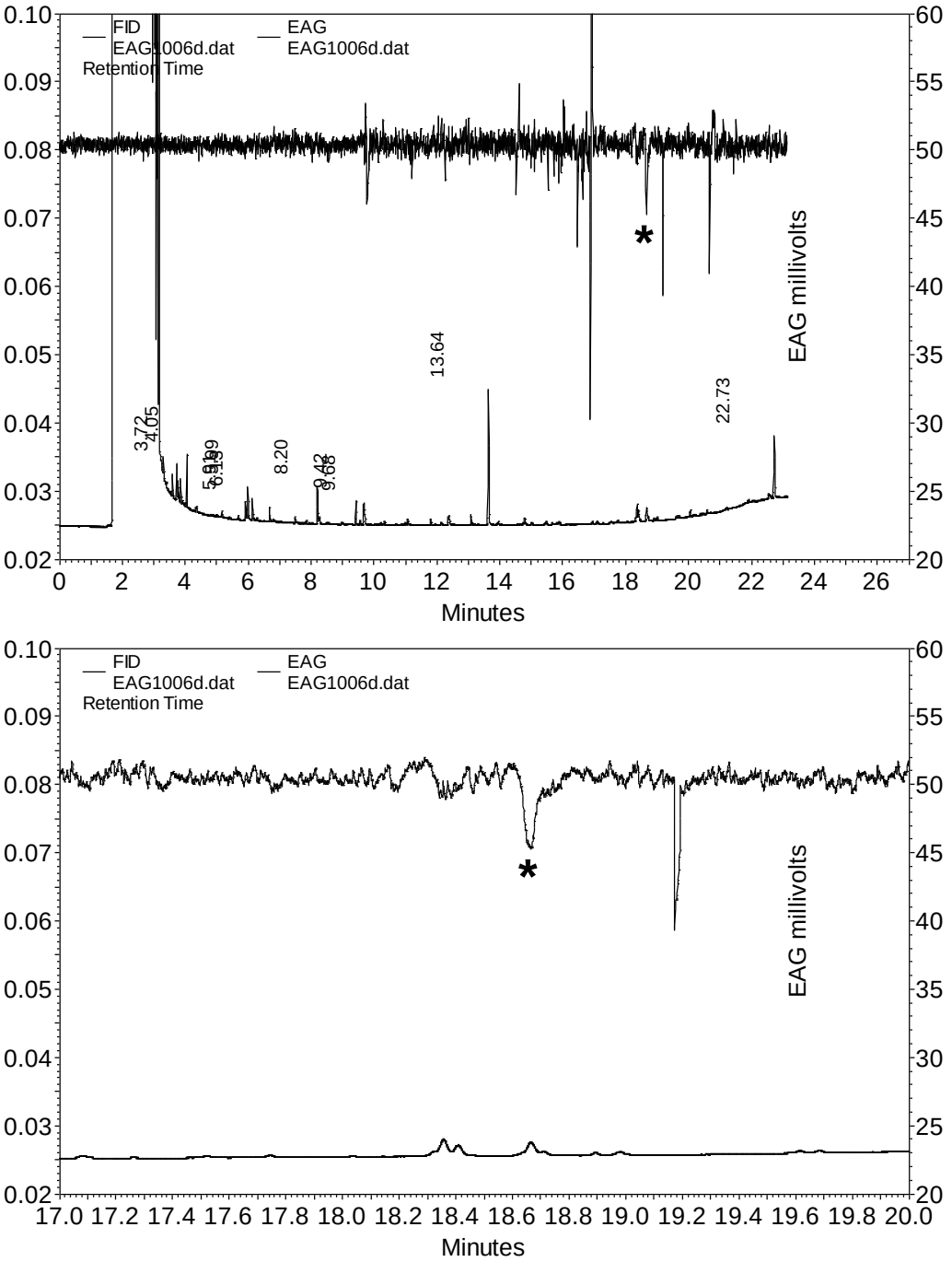

Figure 12. GC-EAG analysis of fraction 4 from liquid chromatography of volatiles from female gooseberry sawfly (2016/069/06) with male EAG on non-polar GC column. Lower panel is expansion of upper and in each lower trace is GC and upper is EAG. Response (*) at 18.66 min

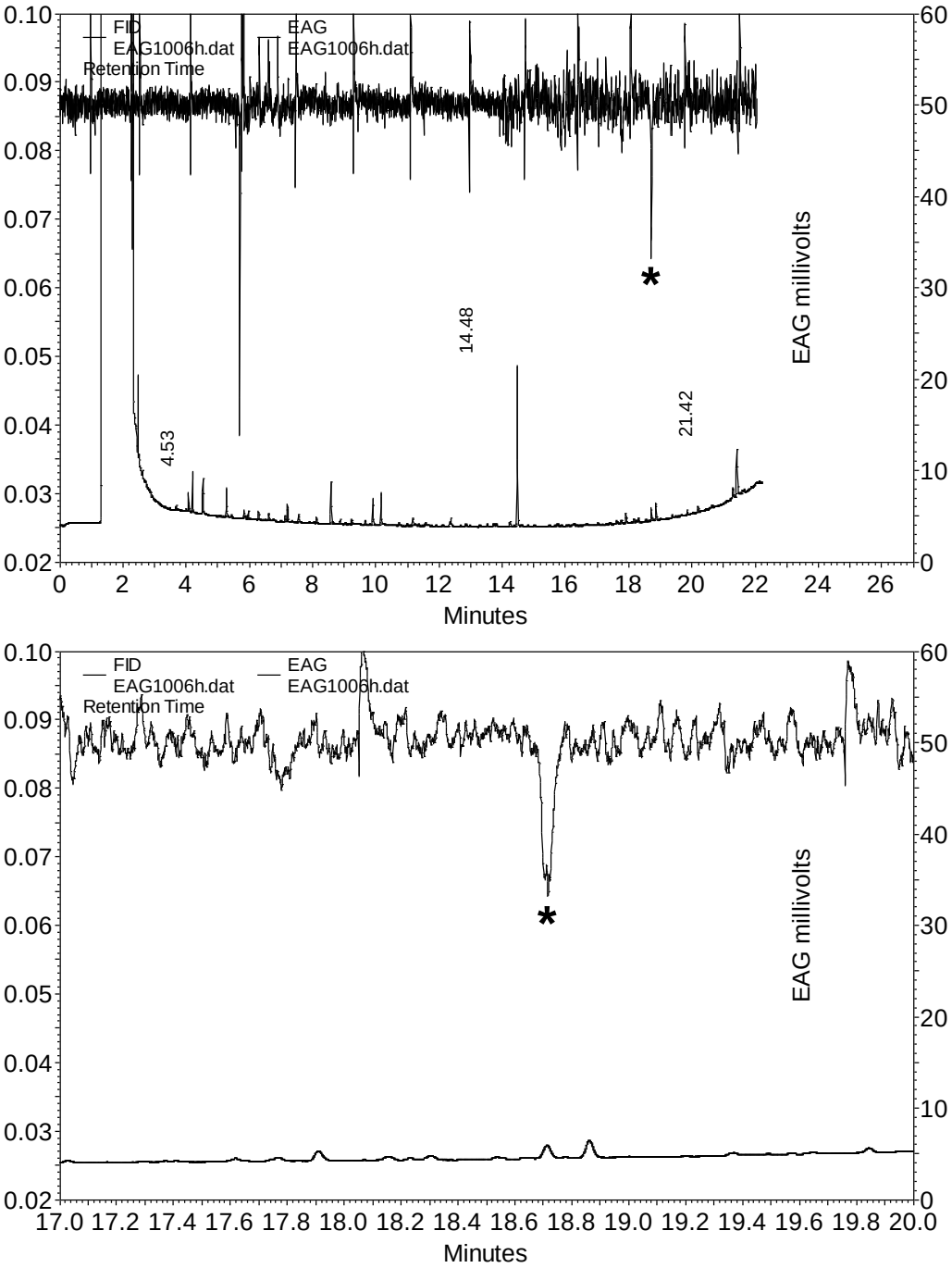

Figure 13. GC-EAG analysis of fraction 4 from liquid chromatography of volatiles from female gooseberry sawfly (2016/069/06) with male EAG on polar GC column. Lower panel is expansion of upper and in each lower trace is GC and upper is EAG. Response (*) at 18.72 min

The fraction 4 was analysed by GC-MS and the EAG-active compound located on both non-polar (Figure 14) and polar (Figure 15) GC columns.

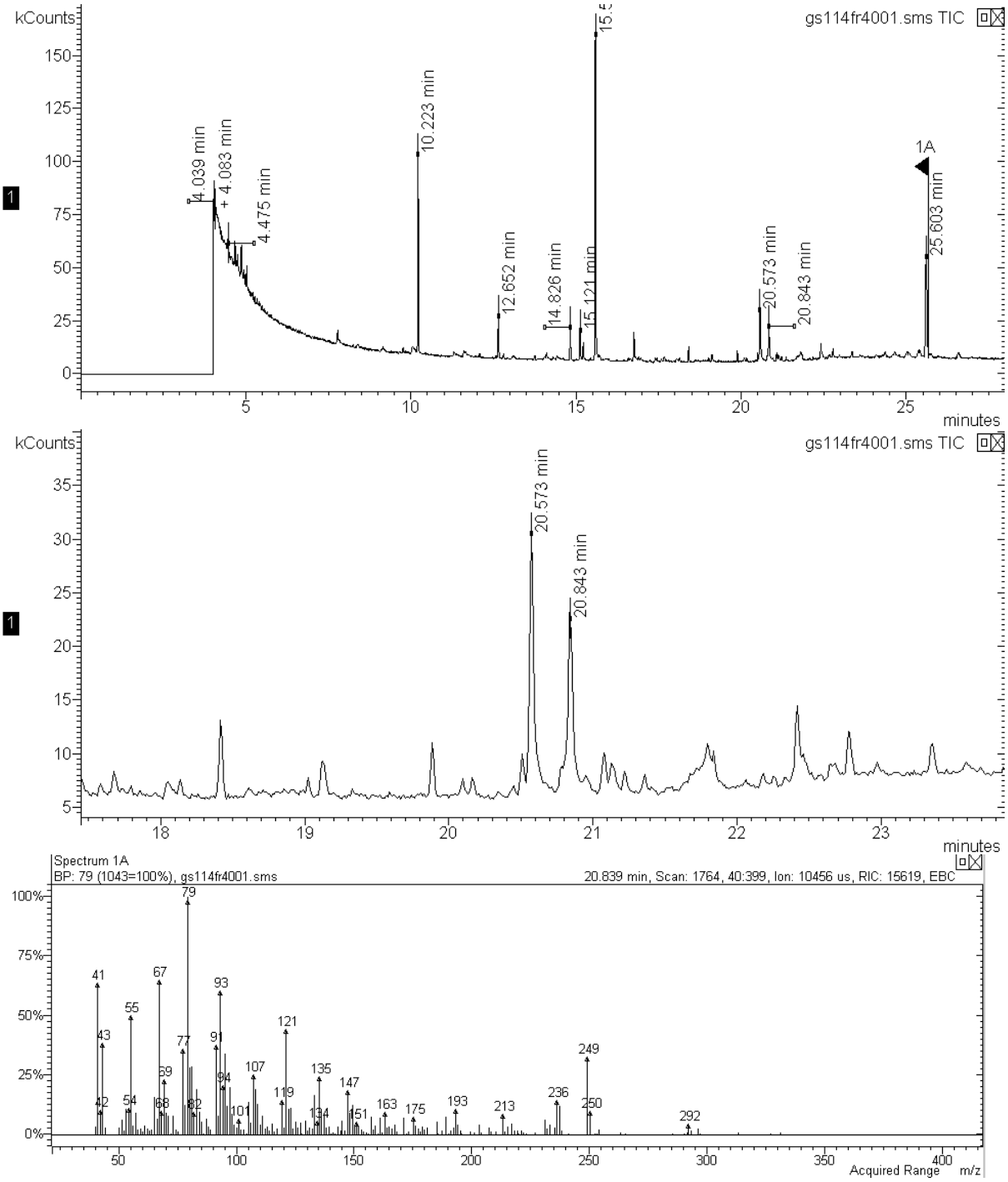

Figure 14. GC-MS analysis of fraction 4 from fractionation of collection of volatiles from female gooseberry sawfly on non-polar column showing (from top) GC-MS trace, expansion of region of trace, mass spectrum of peak at 20.84 min

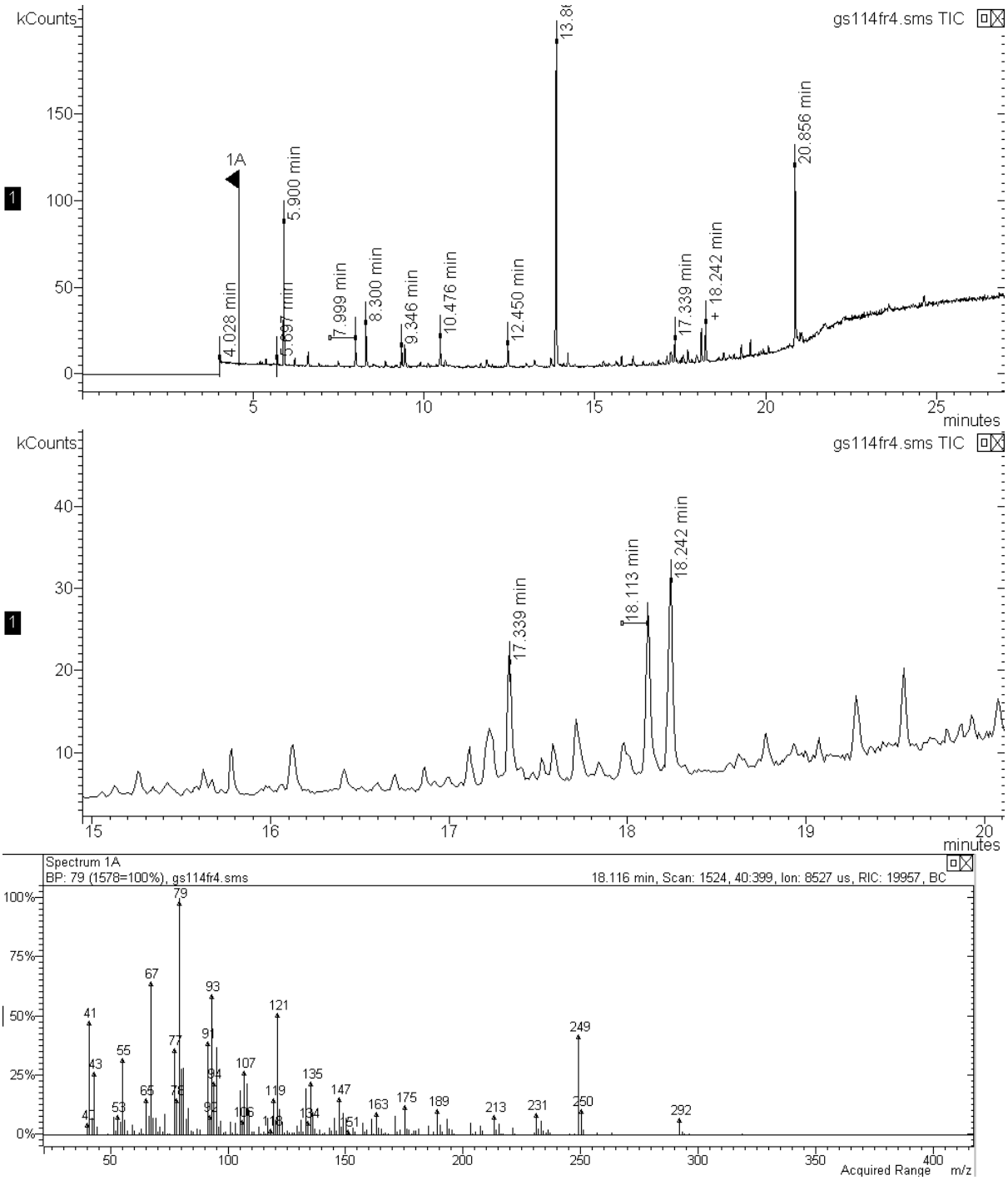

Figure 15. GC-MS analysis of fraction 4 from fractionation of collection of volatiles from female gooseberry sawfly on polar column showing (from top) GC-MS trace, expansion of region of trace, mass spectrum of peak at 18.11 min

The mass spectrum showed a molecular ion at *m/z* 292 and a fragmentation pattern consistent with that of a 16-carbon isopropyl ester with three double bonds. Catalytic hydrogenation of the fraction 4 from fractionation of volatiles from female gooseberry sawfly gave isopropyl hexadecanoate, further supporting this (Figure 16).

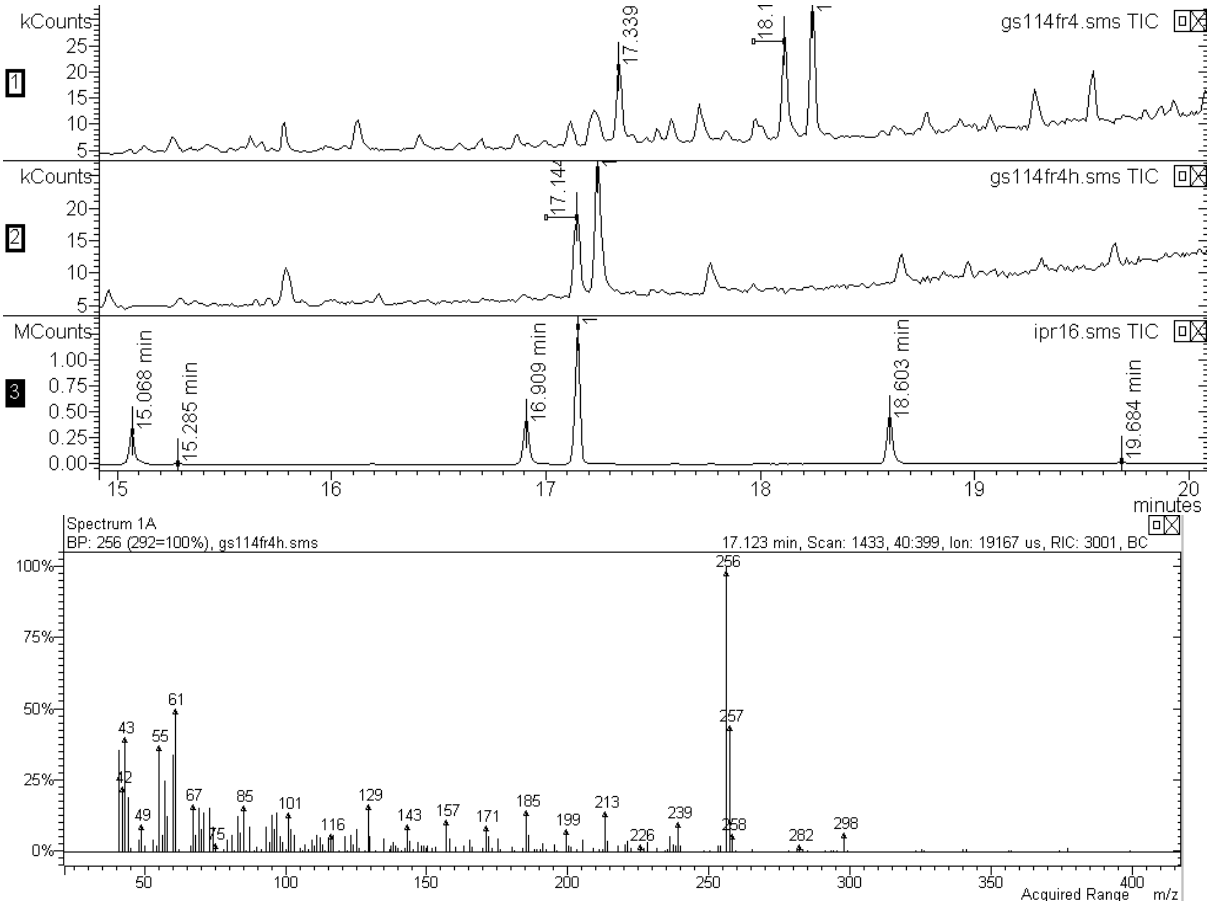

Figure 16. GC-MS analyses on polar GC column of (from top) fraction 4 from fractionation of volatiles from female gooseberry sawfly, fraction 4 after catalytic hydrogenation, isopropyl hexadecanoate and *n*-alkane standards, mass spectrum of isopropyl hexadecanoate (EAG-active compound at 18.11 min, isopropyl hexadecanoate at 17.14 min).

The base peak in the mass spectrum of the EAG-active compound at *m/z* 79 suggested that the three double bonds formed a skipped triene system at positions 3, 6 and 9 from the terminal end, which would be consistent with GC retention time data (Table 1). Thus the structure was proposed to be isopropyl (*Z,Z,Z*)-7,10,13-hexadecatrienoate (ZZZ-7,10,13-16:iPr; Figure 17). The corresponding acid is not readily available commercially and lengthy to synthesise, but the 18-carbon analogue, (Z,Z,Z)-9,12,15-octadecatrienoic acid, is readily available as linolenic acid. This was converted to the isopropyl ester (ZZZ9,12,15-18:iPr)

and had retention indices that further supported the proposed structure for the EAG-active compound (Table 1).

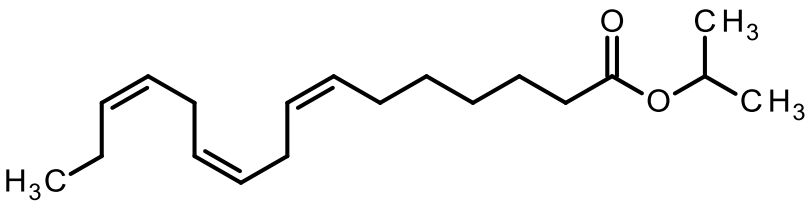

Figure 17. Structure of isopropyl (*Z,Z,Z*)-7,10,13-hexadecatrienoate (ZZZ-7,10,13-16:iPr)

Table 1. Retention indices (relative to retention times of *n*-alkanes) of compound in volatiles from female gooseberry sawfly eliciting EAG response from males, and synthetic compounds

	Retention Index	
Compound	Non-polar SPB1	Polar DBWax
EAG-active compound	1977	2369
Z7-14:iPr	1788	2061
Z7-16:iPr	1984	2271
16:iPr	2011	2241
ZZZ9,12,15-18:iPr	2178	2575
ZZZ7,10,13-16:iPr	1977	2369
ZZZ7,10,13-16:Et	1947	2386

Interestingly, the corresponding ethyl ester was detected in GC-MS analyses (20.57 min on non-polar column Figure 14; 18.42 min on polar column Figure 15) with mass spectrum in Figure 18.

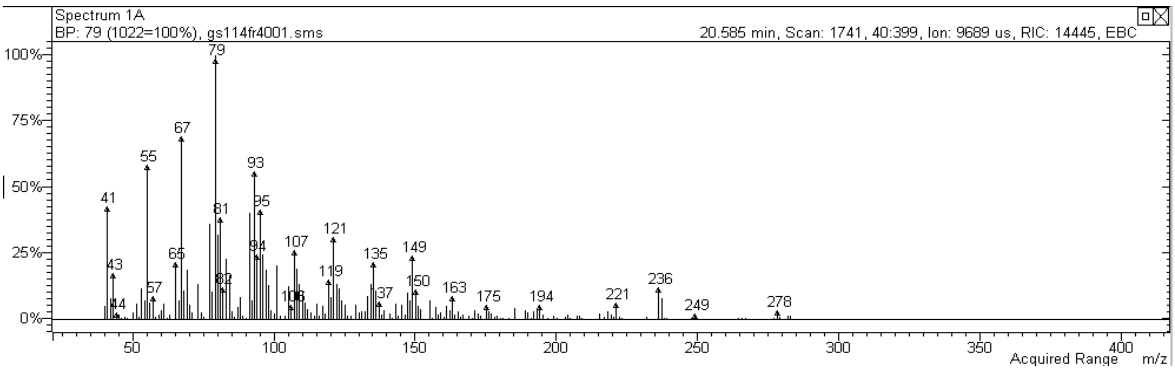

Figure 18. Mass spectrum of ethyl (*Z,Z,Z*)-7,10,13-hexadecatrienoate (20.57 min, Figure 14).

Isopropyl (*Z,Z,Z*)-7,10,13-hexadecatrienoate was synthesised from (*Z,Z,Z*)-7,10,13-hexadecatrienoic acid which was found to be available at high cost (200 euro for 5 mg), and shown to have identical retention times on non-polar and polar GC columns and mass spectrum to the EAG-active component (Table 1). Gooseberry sawfly were not available to confirm this compound elicited an EAG response, but as other unsaturated isopropyl esters elicited strong responses (Figure 7), it is highly likely that isopropyl (*Z,Z,Z*)-7,10,13-hexadecatrienoate does, and, in fact, would do so even if it was not exactly the correct structure.

Dispenser release rates

Lures for field testing were formulated in polyethylene vials as Blend A ZZZ7,10,13-16:iPr alone (100 µg) and Blend B ZZZ7,10,13-16:iPr with the unsaturated hydrocarbon (*Z*)-9-tricosene (100 µg + 500 µg). One lure of each blend was maintained in a wind tunnel at 27°C and 8 km/h windspeed and release rates were measured at intervals by collection of volatiles in the same controlled-temperature room. Results in Figure 18 show release rates increased as the compounds diffused through the polythylene wall and then declined, although significant amounts of both components were still being released after four months.

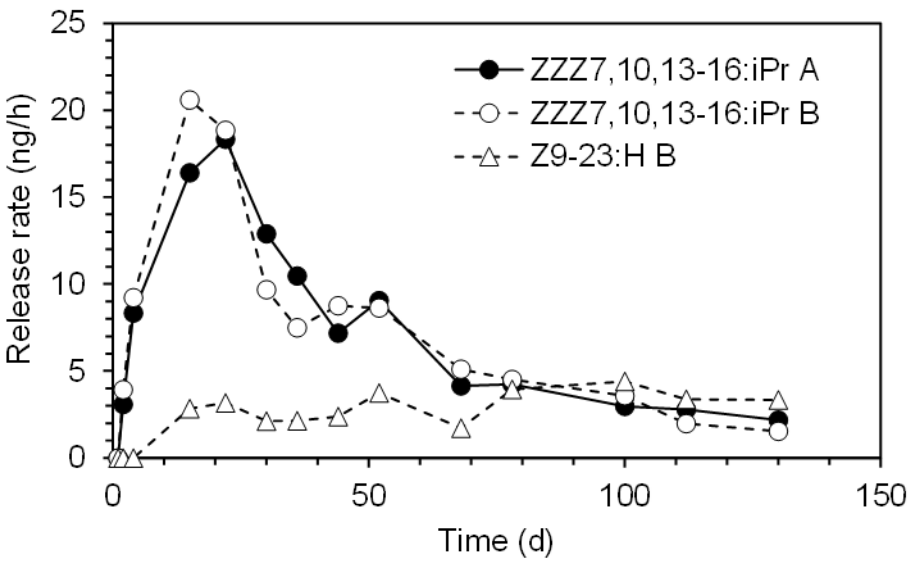

Figure 18. Release rates of ZZZ7,10,13-16:iPr from polyethylene vials alone (A; 100 µg) and in a blend (B) with the unsaturated hydrocarbon Z9-23:H B (100 µg + 500 µg). Vials maintained in a laboratory windtunnel at 27°C and 8 km/h windspeed and release rates measured at same temperature.

Field tests

Polyethylene vial lures containing the proposed pheromone component, with and without the major hydrocarbon found in volatile collections from both female and male gooseberry sawfly, (Z)-9-tricosene (Z9-23:H), were supplied to growers in August 2017. Even though this was late in the season, significant numbers of male gooseberry sawfly were trapped at some locations and high numbers at one site (Figure 19). The binary blend (B) seemed to be more attractive than the isopropyl ester alone (A) and total catches at four sites are shown in Figure 20.

Figure 19. Catches of male gooseberry sawfly at one site (treatment A ZZZ7,10,13-16:iPr (100 µg), B ZZZ7,10,13-16:iPr + Z9-23:H (100 µg + 500 µg), C unbaited)

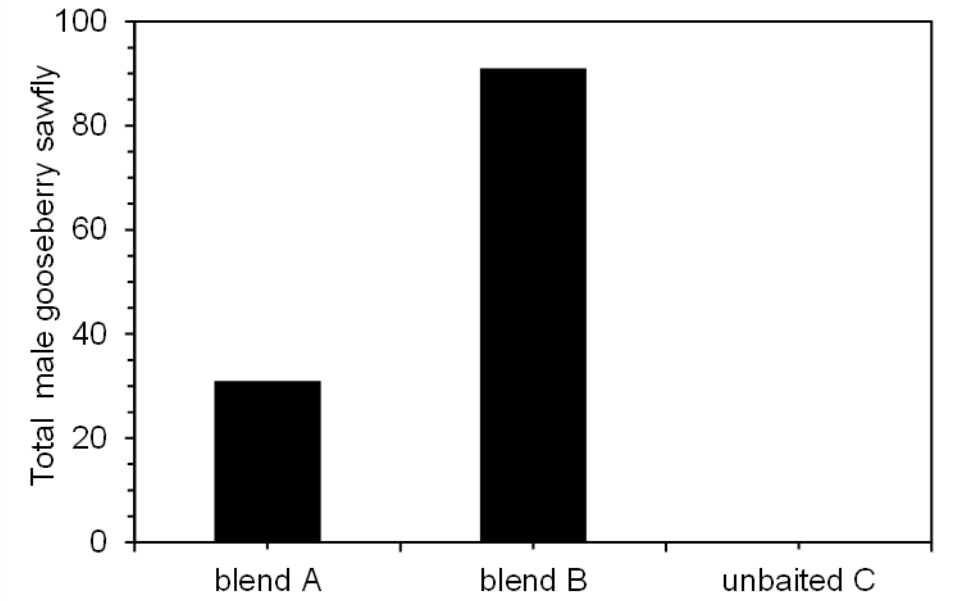

Figure 20. Total catches of male gooseberry sawfly at four sites (treatment A ZZZ7,10,13-16:iPr (100 µg), B ZZZ7,10,13-16:iPr + Z9-23:H (100 µg + 500 µg), C unbaited)

Discussion

Longhurst and 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, 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.

In the second year of the project these results were confirmed. Growers were requested to send in gooseberry sawfly larvae. These were successfully reared to adults in the laboratory at EMR and collections of volatiles made from virgin females. Analysis of these collections by GC-MS at NRI showed the same long-chain hydrocarbons as observed previously. Analysis with SIM of the collections failed to detect any isopropyl esters, at least in part because of the relatively large quantities of the hydrocarbons. Fractionation of a collection by liquid chromatography and analysis of the fractions by GC-MS with SIM indicated the possible presence of an unsaturated 16-carbon isopropyl ester as previously.

In the second year, sufficient insects were available to carry out analyses using GC-EAG. Male gooseberry sawfly showed strong EAG responses to the synthetic isopropyl esters previously identified by EMR and NRI as components of the female sex pheromone of blackcurrant sawfly. Analysis of collections of volatiles from female gooseberry sawfly on a polar GC column showed no responses at the retention time of 14-carbon and 16-carbon isopropyl esters but a consistent response at a longer retention time. This seemed to coincide with a hydrocarbon component identified as ZZ6,9-23:H. However, no EAG response was observed to the synthetic ZZ6,9-23:H, even in runs spiked with Z7-14iPr and Z7-16iPr which elicited EAG responses. Similarly, no EAG response was obtained to the ZZ6,9-23:H when the hydrocarbon fraction from volatile collections from female gooseberry sawfly was analysed.

In this third year of the project, the GC-EAG analyses were repeated and extended. A single EAG response was observed in analyses of volatiles from female gooseberry sawfly on both polar and non-polar columns, and, as suspected, it was shown that the compound responsible for the response was “hidden” by much larger amounts of hydrocarbon on the polar GC column. Analyses on both polar and non-polar GC columns and fractionation by liquid chromatography made it possible to isolate the EAG-active compound, and this was assumed to be the major component of the female sex pheromone. On the basis of its mass spectrum, GC retention times and product of catalytic hydrogenation, this was shown to be the isopropyl ester of a 16-carbon acid with three double bonds, and further consideration led to isopropyl (*Z,Z,Z*)-7,10,13-hexadecatrienoate being proposed as the structure. This was synthesised and shown to be identical analytically with the natural

EAG-active component in volatiles from female gooseberry sawfly. The compound was provided for testing in traps by growers and, although late in the season, significant numbers of male gooseberry sawfly were trapped. Combining the above compound with the main component of the cuticular hydrocarbons, (*Z*)-9-tricosene, seemed to give a more attractive blend than the isopropyl ester alone, although catches were very variable at the different sites and further, properly replicated, tests are required to confirm this.

Isopropyl (*Z,Z,Z*)-7,10,13-hexadecatrienoate is clearly chemically related to components of the sex pheromone of female blackcurrant sawfly identified previously by NRI and NIAB EMR as isopropyl esters of (*Z*)-7-tetradecenoic acid and (*Z*)-7-hexadecenoic acid, and indeed the latter two compounds also elicited strong EAG responses from gooseberry sawfly males. These are novel compounds not previously reported as components of sawfly pheromones, but this latest result provides added confidence the identifications are correct and might even call into question previous reports on other species. For instance, the only other female sex pheromone identified for a member of the Tenthredinae was that of the yellow-headed spruce sawfly, Pikonema alaskensis, which was proposed to consist of breakdown products of the cuticular hydrocarbons (Bartelt et al. 1982a,b; 1983a,b).

(*Z,Z,Z*)-7,10,13-Hexadecatrienoic acid is commercially available but at a prohibitive price. A lengthy synthetic route is available and it is hoped to develop this to produce larger amounts of the isopropyl ester for further field testing by growers to verify the current results and optimise the most attractive blend. If these tests are successful then Agralan have already expressed an interest in marketing the traps and lures to growers to help determine the need and timing of insecticide applications against gooseberry sawfly.

Conclusions

- The major, if not the only, component of the sex pheromone of gooseberry sawfly is proposed to be isopropyl (*Z,Z,Z*)-7,10,13-hexadecatrienoate.
- The synthetic compound attracted significant numbers of male gooseberry sawfly late in the 2016 season, and a blend of this compound with the major component of the cuticular hydrocarbons seemed to be more attractive than the isopropyl ester alone.
- Further work is required to develop a cost-effective route to synthesise be isopropyl (*Z,Z,Z*)-7,10,13-hexadecatrienoate and to optimise the blend, release rate and dispenser type for attractiveness to male gooseberry sawfly.
- Further work is also required to investigate how catches in the pheromone traps relate to subsequent populations of sawfly and damage caused in order to provide a robust monitoring system for growers.

Knowledge and Technology Transfer

February 2016 – David Hall - AHDB Soft Fruit Information Day, Winter Meeting, *Sex Pheromones of the Blackcurrant Sawfly,* Nematus olfaciens*, and the Gooseberry Sawfly,* N. ribesii *(Hymenoptera: Tenthredinae).*

16 Feb 2017 – Fountain - Soft Fruit Information Day, Winter Meeting, *Development of Sex Pheromone Monitoring Traps for Blackcurrant and Gooseberry Sawfly*.

References

Alford DV, 1984. *A colour atlas of fruit pests*. Wolfe Publishing Ltd., London, 105.

Bartelt, R. J., Jones, R. L. & Kulman, H. M. 1982a. Evidence for a multi-component sex pheromone in yellow headed spruce sawfly *Pikonema alaskensis*. J. Chem. Ecol. 8: 83-94.

Bartelt, R. J., Jones, R. L., & Kulman, H. M. 1982b. Hydrocarbon components of the yellowheaded spruce sawfly sex pheromone: a series of (*Z,Z*)-9,19-dienes. J. Chem. Ecol. 8: 95-114.

Bartelt, R. J. & Jones, R. L. 1983a. (*Z*)-10-Nonadecenal: a pheromonally active air oxidation product of (*Z,Z*)-9,19-dienes in the yellow-headed spruce sawfly. J. Chem. Ecol. 9: 1333-1342.

Bartelt, R. J., Jones, R. L. & Krick, T. P. 1983b. (*Z*)-5-tetradecen-1-ol: a secondary pheromone of the yellowheaded spruce sawfly, and its relationship to (*Z*)-10-nonadecenal. J. Chem. Ecol. 9: 1343-1352.

Longhurst, C. & Baker, R. (1980). Sex pheromones in the gooseberry sawfly, *Nematus ribesii*. Naturwissenschaften, 67: 146.

Mitchell, C., Brennan, R.M., Cross, J.V. & Johnson, S.N. (2011). Arthropod pests of currant and gooseberry crops in the UK: their biology, management and future prospects. *Agricultural and forest Entomology,* 13: 221-237

Raffle, S (2012). Gooseberries. A grower guide to production, variety choice and pest and disease problems. HDC Growers' Guide, 1-29.

