Project title:	Blueberry gall midge: sex pheromone monitoring and control with insecticides
Project number:	SF126
Project leader:	Professor Jerry V Cross, EMR
Report:	Annual Report, March 2012 (issued 3 April 12)
Previous report:	None
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Location of project:	EMR, East Malling NRI, Chatham Maritime
Industry Representative:	Laurie Adams
Date project commenced:	1 April 2011
Date project completed (or expected completion date):	31 March 2014

East Malling Research is an officially recognised efficacy testing station and the insecticide efficacy trial reported as part of this work is registered as study number ORETO 2011/004

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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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GROWER SUMMARY

Headline

- Work has begun to find an effective insecticide treatment for the blueberry gall midge, *Dasineura oxycoccana,* and to develop pheromone traps for more effective timing of control measures.

Background and expected deliverables

The blueberry gall midge (*Dasineura oxycoccana* -Johnson 1899, syn *Dasineura vaccinii* Smith, 1890) is a damaging invasive pest of highbush blueberry (*Vaccinium corymbosum*) in the UK. It is also a serious pest of blueberry in the USA and Canada where it originated and where it is known as the cranberry tipworm. It is abundant and widely distributed in UK blueberry crops, having spread from nurseries on planting material and is most important in newly planted crops and during the first 2-3 years of establishment.

The midge lays its eggs in the tender growing points of shoots and the larvae live in leaf galls in the shoot tip causing leaf distortion and blackening of buds which are killed by the attack. The growth habit of the blueberry occurs in flushes which end with the death of the terminal meristem and the next growth flush starts from the next bud or buds below. Midge attack caused termination more rapidly than it would otherwise occur. Serious attacks can affect the next season's crops because infested bushes develop few bud-bearing shoots. The pest is particularly troublesome on crops grown under protection.

Currently, UK growers attempt to control the midge by applying a spray of thiacloprid (Calypso) when galling damage is first seen in spring. Commercial experience also indicates that a weekly programme of sprays of pyrethrum prevents midge attack. However, on other crops including blackcurrant, blackberry, apple and pear, thiacloprid (Calypso) has been shown to be at best only partially effective for leaf midge control, and it is likely this is the case with the blueberry midge. Thus effective methods for monitoring the pest and controlling it with insecticides are needed.

EMR and NRI have successfully identified the female sex pheromones of other economically significant midge pests of UK fruit crops including apple leaf midge, pear leaf midge, pear midge, raspberry cane midge, blackcurrant leaf midge and blackberry leaf midge. Monitoring traps for several of these are currently in commercial use.

Other work by EMR has shown that an EC formulation of spirotetramat (Movento) is very effective for control of leaf midge pests and it is likely to be effective against blueberry gall midge, although not currently approved for use in blueberry. Best control of leaf gall midges on other crops is achieved with a spray of insecticide timed to coincide with the onset of the midge's first flight in spring, as indicated by catches in sex pheromone traps. The traps are highly sensitive and give good quality information and an early warning of the magnitude and timing of attacks. The aim of this project is to identify the female sex pheromone of the blueberry gall midge and establish an effective insecticide to provide the basis for development of a similar strategy against this pest.

Summary of the project and main conclusions

Samples of pheromone were collected from over 1,000 virgin female blueberry gall midge. Analyses by gas chromatography (GC) coupled to electroantennogram (EAG) recording from the antenna of a male midge gave strong indications that collections contained very small amounts of a compound related to the pheromone components of other *Dasineura* species. However, this was not detectable by GC coupled to mass spectrometry (MS) and could not be identified further. Future work will focus on increasing the amount collected.

Pyrethrins (Pyrethrum), lambda-cyhalothrin (Hallmark), cypermethrin (Toppel) and chlorpyrifos products all gave partial control of shoot galling by blueberry gall midge when applied in late August. The limited efficacy of these insecticides was probably due to the fact that they could not be properly timed in relation to the gall midge attacks, which occur more or less continuously as a result of overlapping generations later in the season. Much better control might be expected if insecticide applications, timed by use of a sex pheromone trap, were applied against the first or second generations in spring, which are likely to be more synchronised.

Thiacloprid (Calypso) did not reduce galling significantly, but a coded experimental product HDCI 034, a translaminar, selective insecticide which is known to control the larvae of other gall midge pests inside galls, shows promise.

Further trials will be carried out in 2012.

Financial benefits

No detailed financial information on the cost to growers of the blueberry midge has been made in the UK. In Latvia, the midge has been shown to reduce growth and yields of large fruited cranberry by 60% (Apenite, 2010). In the USA, the blueberry gall midge causes

losses in excess of $20 m per annum to rabbiteye blueberries (*Vaccinium ashei*) where the pest feeds in the flowers leading to premature floral bud abscission, or aesthetically compromised fruit when mature (Dernisky et al., 2005).

Action points for growers

- No action points have arisen from this work so far.

SCIENCE SECTION

Introduction

The blueberry gall midge (*Dasineura oxycoccana* (Johnson 1899), syn. *Dasineura vaccinii* (Smith, 1890)) is a damaging invasive pest of highbush blueberry (*Vaccinium corymbosum*) in the UK. It is also a serious pest of blueberry in the USA and Canada where it originated and where it is known as the cranberry tipworm. It is abundant and widely distributed in UK blueberry crops having spread from nurseries on planting material and is most important in newly planted crops and during the first two to three years of establishment.

The midge lays its eggs in the tender growing points of shoots and the larvae live in leaf galls in the shoot tip, causing leaf distortion and blackening of buds which are killed by the attack. The growth habit of the blueberry occurs in flushes which end with the death of the terminal meristem and the next growth flush starts from the next bud or buds below. Midge attack causes shoot termination more rapidly than it would otherwise occur. Serious attacks can affect the next season's crop because infested bushes develop few bud-bearing shoots. The pest is particularly troublesome on crops grown under protection.

Currently UK growers attempt to control the midge by applying a spray of thiacloprid (Calypso) when galling damage is first seen in spring. Commercial experience also indicates that a weekly programme of sprays of pyrethrum prevents midge attack. However, on other crops, including blackcurrant, blackberry, apple and pear, thiacloprid (Calypso) has been shown to be at best only partially effective for leaf midge control, and it is likely this is the case with the blueberry midge. Thus effective methods for monitoring the pest and controlling it with insecticides are needed.

EMR and NRI have successfully identified the female sex pheromones of other economically significant midge pests of UK fruit crops, including apple leaf midge, pear leaf midge, pear midge, raspberry cane midge, blackcurrant leaf midge and blackberry leaf midge. Monitoring traps for several of these are in use commercially.

Other work by EMR has shown that an EC formulation of spirotetramat is very effective for control of the leaf midge pests and it is likely to be effective against blueberry gall midge. The OD formulation of spirotetramat, Movento, has recently been approved for use on vegetable crops in the UK, and the approval of the SC formulation on apple is pending. Other workers have investigated *Bacillus thuringiensis* Berliner subsp. *israelensis* (*Bti*),

chlorantraniliprole, flubendiamide, metaflumizone and spirotetramat and diazinon but found that none of the alternative insecticides provided consistent significant control on par with diazinon.

EMR have demonstrated that the best control of leaf gall midges on other crops is achieved with a spray of insecticide timed to coincide with the onset of the midge's first flight in spring, as indicated by catches in sex pheromone traps. The traps are highly sensitive and give good quality information and an early warning of the magnitude and timing of attacks. The aim of this project is to identify the female sex pheromone of the blueberry gall midge and establish an effective insecticide to provide the basis for development of a similar strategy against this pest.

Materials and methods

Pheromone identification

Insect material

Late larvae and pupae of *D. oxycoccana* were collected at Driscolls' Farm, Tonbridge, Kent, on 6 May 2011. A further batch was obtained later from Peake Fruit, Ardleigh Colchester.

These were put individually into small plastic pots and maintained at ambient temperature and humidity until emergence. Adults were sexed on the basis of the longer antennae in the males.

Pheromone isolation

Pheromone was isolated by collection of volatiles, by making whole body washes in hexane at EMR (Table 1) and by solid-phase microextraction (SPME). Males and females were treated separately.

Table 1. Samples of blueberry midge pheromone collected 2011

NRI Ref No.	Dates		Number		Flow Rate (ml/min) or hexane (ml)
	Start	End	Male	Female	
2010-056-01	23/5/11	03/06/11	35		200 ml/min
2010-056-02	23/5/11	03/06/11		36	200 ml/min
2010-056-03	15/7/11	22/07/11	250		200 ml/min
2010-056-04	15/7/11	22/07/11		307	200 ml/min
2010-056-05[a]	15/7/11	22/07/11		307	200 ml/min
2010-056-06	22/7/11	29/07/11	131		200 ml/min
2010-056-07	22/7/11	29/07/11		221	200 ml/min
2010-056-08	29/7/11	02/08/11	28		200 ml/min
2010-056-09	29/7/11	02/08/11		130	200 ml/min
2010-056-10	05/08/11		1		HBB4 1ml
2010-056-11	05/08/11			15	HBB3 1ml
2010-056-12	06/08/11			45	HBB5 1ml
2010-056-13	09/08/11	15/08/11		81	HBB6-10 5ml
2010-056-14	04/08/11			32	HBB1 1ml
2010-056-15	04/08/11		4		HBB2 1ml
2010-056-16[b]	24/08/11	14/09/11	127		300 ml/min
2010-056-17[b]	24/08/11	14/09/11		137	300 ml/min
2010-056-18	18/09/11	29/09/11	61		300 ml/min
2010-056-19	18/09/11	29/09/11		231	/min

[a] Filter used for 2010-056-04 re-extracted
[b] Blueberry shoot added to entrainment vessel

For collection of volatiles, insects were maintained in a silanised glass vessel (12 cm x 4 cm diameter) and charcoal-filtered air was drawn in (200-300 ml/min) over the insects and out through a collection filter made from a Pasteur pipette (4 mm i.d.) containing Porapak Q (50/80 mesh; 200 mg) held between plugs of silanised glass wool. The Porapak was purified by Soxhlet extraction with chloroform (8 hr) and washing well with dichloromethane (Pesticide Grade) before use. Collections were made for periods of approximately one week, adding fresh midges as they emerged. Dead bodies were not removed until the end.

At NRI, trapped volatiles were removed with dichloromethane (3 x 0.5 ml). Samples were analysed unconcentrated and then after concentration approximately 10-fold under a gentle stream of purified nitrogen.

Hexane body washes were prepared by immersing up to 45 live individuals in hexane (Pesticide Grade; 1 ml) for at least 10 min and then removing the hexane with a syringe.

Samples collected are listed in Table 1. Volatile collections 01, 06 and 08 from males were subsequently combined, as were 02, 04, 05, 07 and 09 from females and hexane washes 11, 12, 13 and 14 from females.

For SPME collections, virgin female *D. oxycoccana* (35) were placed in a clean glass sample vial (5 ml) covered with aluminium foil. Collections were made with a fibre coated with PDMS/DVB (65 µm; Supelco) for 15 min.

Analysis by Gas Chromatography linked to Electroantennography (GC-EAG)

EAG recordings were made with a portable device consisting of micromanipulators, electrode holders and amplifier (INR-02; Syntech, The Netherlands) connected to the GC (HP6890, Agilent) as a second detector. Electrodes were fine glass capillaries filled with saline (0.1M KCl with 1% polyvinylpyrrolidine) and placed over silver wire electrodes.

The insect was anaesthetised with carbon dioxide or by cooling on ice and wings and legs were removed with a scalpel. The body was inserted into the base electrode and the end of one or two antenna inserted into the recording electrode.

The GC (HP 6890) was fitted with fused silica capillary columns (30 mm x 0.32 mm i.d. x 0.25 µm film thickness) coated with polar (DBWax, Supelco;) and non-polar (SPB1, Supelco) phases. The oven temperature held at 50°C for 2 min then programmed at 10°C/min to 240°C. The column effluent was split (1:1) with equal lengths of deactivated fused silica tubing leading to the flame ionisation detector and to a glass T-piece in the column oven. The contents of the T-piece were pulsed (3 sec) at intervals (17 sec) over the EAG preparation with humidified air (300 ml/min).

Data from both EAG and GC were collected and process with EZChrom Elite software.

Analysis by Gas Chromatography linked to Mass Spectrometry (GC-MS)

GC-MS Analyses were carried out with Varian 3800 GC coupled directly to a Varian Saturn 2200 instrument with fused silica capillary columns (30 m x 0.25 mm i.d. x 0.25 µm film thickness) coated with polar DBWax (Supelco) or non-polar VF5 (Varian). Carrier gas was helium (1 ml/min), injection splitless (220°C) and oven temperature programmed from 40°C for 2 min, then at 6°C/min to 250°C.

Insecticide trials

The aim of this trial was to evaluate the efficacy of Calypso, chlorpyrifos, Hallmark, Toppel 10, pyrethrum and a coded experimental product HDCI 034 for control of first and second generation blueberry midge.

Sites

Because most of the active ingredients are not registered for use on blueberry, a nursery plantation was chosen so that fruit destruction was not required. The trial was initially set up at Redbank Farm, Little Marcle, Ledbury, Hereford on blueberry nursery stock. The initial application was applied on 11 May, however no midge population developed. The site was monitored weekly but no midge infestation was found.

As an alternative a small plot replicated experiment comparing foliar sprays of the insecticidal products was carried out on blueberry gall midge infested nursery stock plants at Peake Fruit, Home Farm Lane, Ardleigh Colchester (National Grid Reference NGR TM 064 299; Landranger Sheet 168 – Colchester, Halstead and Maldon). Pot grown nursery stock blueberry cv. Duke in a polytunnel in Field 4 were used.

Experimental design and layout

The experimental blueberry plantation consisted of a poly tunnel 64 m long. A randomised block experiment with four replicates of eight treatments was used. Each plot consisted of a single bay of the tunnel (2 m), each bay contained 16 potted blueberry bushes in a double row. The entire plot was sprayed and central 14 plants were used for assessments.

Treatments

Treatments were two sprays of the products tested applied at an interval of 14 days (not exceeding the maximum number of applications permissible) at their full recommended rate (Table 2).

Table 2. Insecticide products and their rates of application

Product		Active substance and formulation	Dose rate/ha	Conc. (ml/l)	Approved on blueberry
1	Equity	chlorpyrifos 480 g/l EC	1.5 l	1.5 ml/l	no
2	Hallmark	lambda cyhalothrin 100 g/l CS	100 ml	0.1 ml/l	no
3	Pyrethrum 5 EC	pyrethrum 50 g/l EC	1.1 l	1.1 ml/l	yes
4	Toppel 100 EC	cypermethrin 100 g/l EC	350 ml	0.35 ml/l	no
5	HCDI 034	novel 100 SC	750 ml	0.75 ml/l	no
6	Calypso	thiacloprid 480 g/l SC	250 ml	0.25 ml/l	SOLA 0335/06
7, 8	Untreated				

Treatment application

Treatments were applied at a volume rate of 1000 l/ha using a knapsack sprayer with a hand lance (not air-assisted). This minimised inter-plot contamination by spray drift. The accuracy of application of each treatment was estimated by measurement of the amount of spray that had actually been applied (calculated from the initial minus the final volume of sprayate left in the tank, minus the amount that should have been left had the spray been applied at exactly the correct volume rate). Applications were generally within 10% of required (Table 3). Though some larger deviation occurred, 5 applications were within 23% of target.

Table 3. Accuracy of spray application estimated from the amount of sprayate remaining in the spray tank after spray application

Spray round and date		Treatment No:	Accuracy (%)
1.	30 Aug	1	103
		2	104
		3	91
		4	116
		5	113
		6	120
2.	15 Sep	1	123
		2	116
		3	101
		4	91
		5	101
		6	110

Assessments

The effects of the treatments were assessed 14 days after the first application, immediately prior to the second application (15 September 2011), and the second assessment was conducted 13 days later (28 September 2011). At the first assessment 50 shoots per plot were assessed for presence or absence of blueberry midge damage, those damaged were collected and brought back to the laboratory so that the numbers of larvae per gall could be assessed. At the second assessment every damaged shoot per plot was recorded.

Plot maintenance

All plants were trickle irrigated for the duration of the trial.

Meteorological records

Dry and wet bulb temperature, wind speed and direction were recorded before and after each spray occasion (Table 4). RH% was estimated from the dry and wet bulb temperature readings. In addition two lascar USB-502 loggers were deployed inside a Stevenson's screen to take hourly temperature and humidity readings inside the polytunnel (Appendix 1).

Table 4. Weather conditions at the time of spray application

Date	Time	Air temperature		% rh	Wind	
		°C dry	°C wet		speed (Km/h)	direction
30 Aug	08:30	14	13	90	0	N/A
15 Sep	10:00	17	14	70	0	N/A

N/A = Not applicable

Statistical analysis

The data was expressed as a percentage of shoots damaged, because this is a proportion, the data required angular transformation prior to undergoing statistical analysis by ANOVA.

Experimental approval and crop destruction

An experimental approval was acquired for all non-approved products by EMR. Nursery plantations were used so that no fruit was harvested and the experimental plants were destroyed at the end of the experiment.

Phytotoxicity

Determination of any phytotoxic effects of the treatments was not a central aim of this work. However, plots were inspected for any visual signs of phytotoxicity from the treatments on each sampling occasion.

Quality assurance

East Malling Research is an officially recognised efficacy testing organisation (Certificate no. 0232). The work was done according to GEP quality standards and according to East Malling Quality Assurance (EMQA) procedures and requirements (experiment no. GEP11/012).

Results

Pheromone identification

Sample collection

Volatile collections were made from totals of 1,062 virgin female and 632 male *D. oxycoccana*. During most collections females were observed with their ovipositor extended, typical of when midges are emitting pheromone. Hexane body washes were made from 173 female and five male midges.

GC-EAG analyses

The following GC-EAG analyses were carried out during 2011

- 37 analyses of volatile collections from female *D. oxycoccana* using a male EAG preparation and polar GC column;
- Nine analyses of volatile collections from female *D. oxycoccana* using a male EAG preparation and non-polar GC column;
- 12 analyses of hexane body washes from female *D. oxycoccana* using a male EAG preparation and polar GC column;
- 20 analyses of volatile collections from male or female *D. oxycoccana* using a female EAG preparation and polar GC column;
- Three analyses of a blend of synthetic pheromone components from other midge species using a male EAG preparation and polar GC column.

No consistent EAG responses from male midges were observed in these analyses.

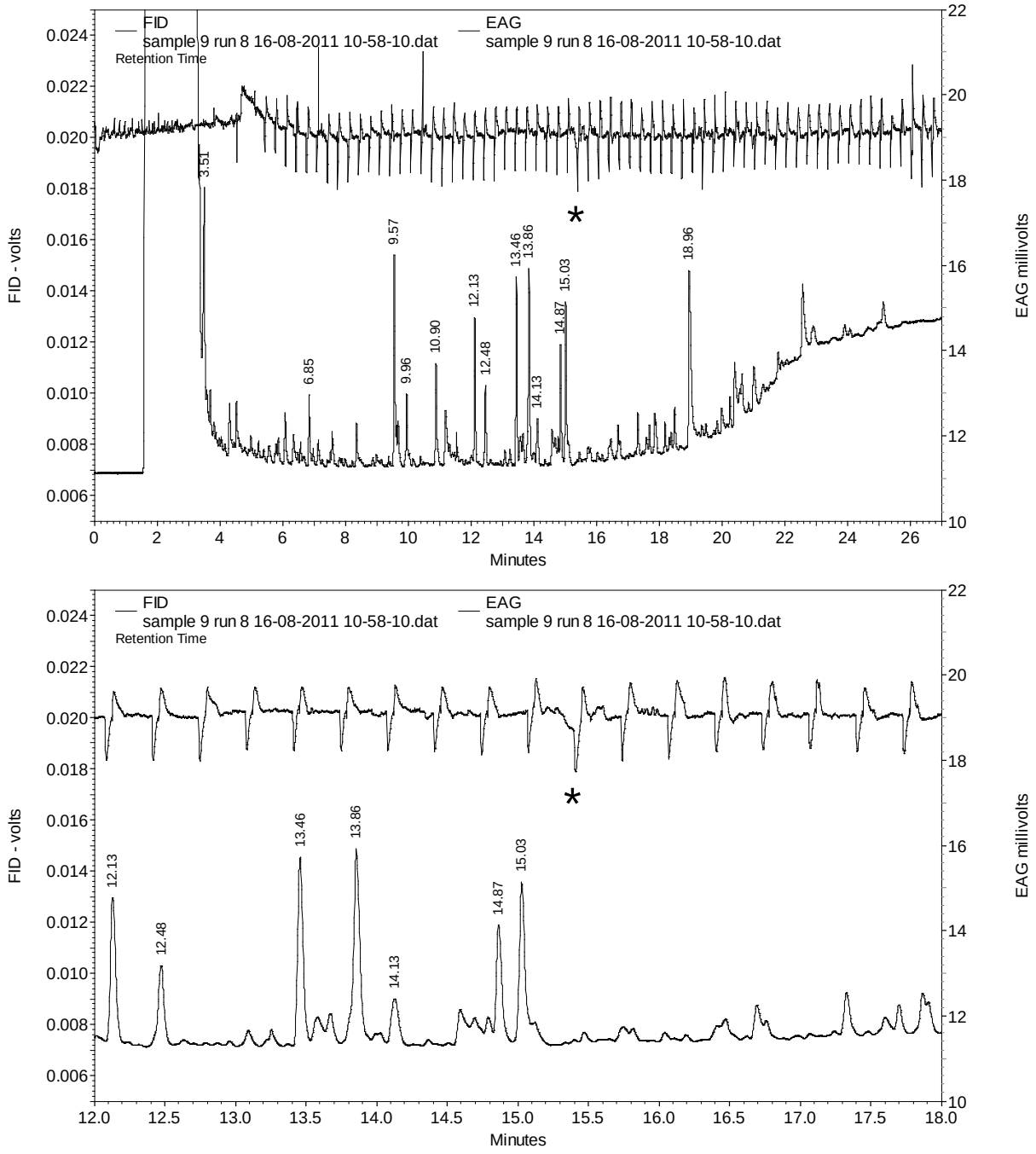

Figure. 1. GC-EAG analysis of volatiles from female *D. oxycoccana* (2010-056-09) with male EAG preparation on polar GC column (* possible EAG response).

In three analyses, there appeared to some activity round 15 min on the polar GC column, equivalent to retention indices (RI) of approximately 2000 relative to hydrocarbons and 1320 relative to acetates (e.g. Figure 1).

Similarly in three other runs there were more convincing responses at approx. 21.8 min on the polar GC column, i.e. RI 2090 relative to acetates (Figures 2 and 3).

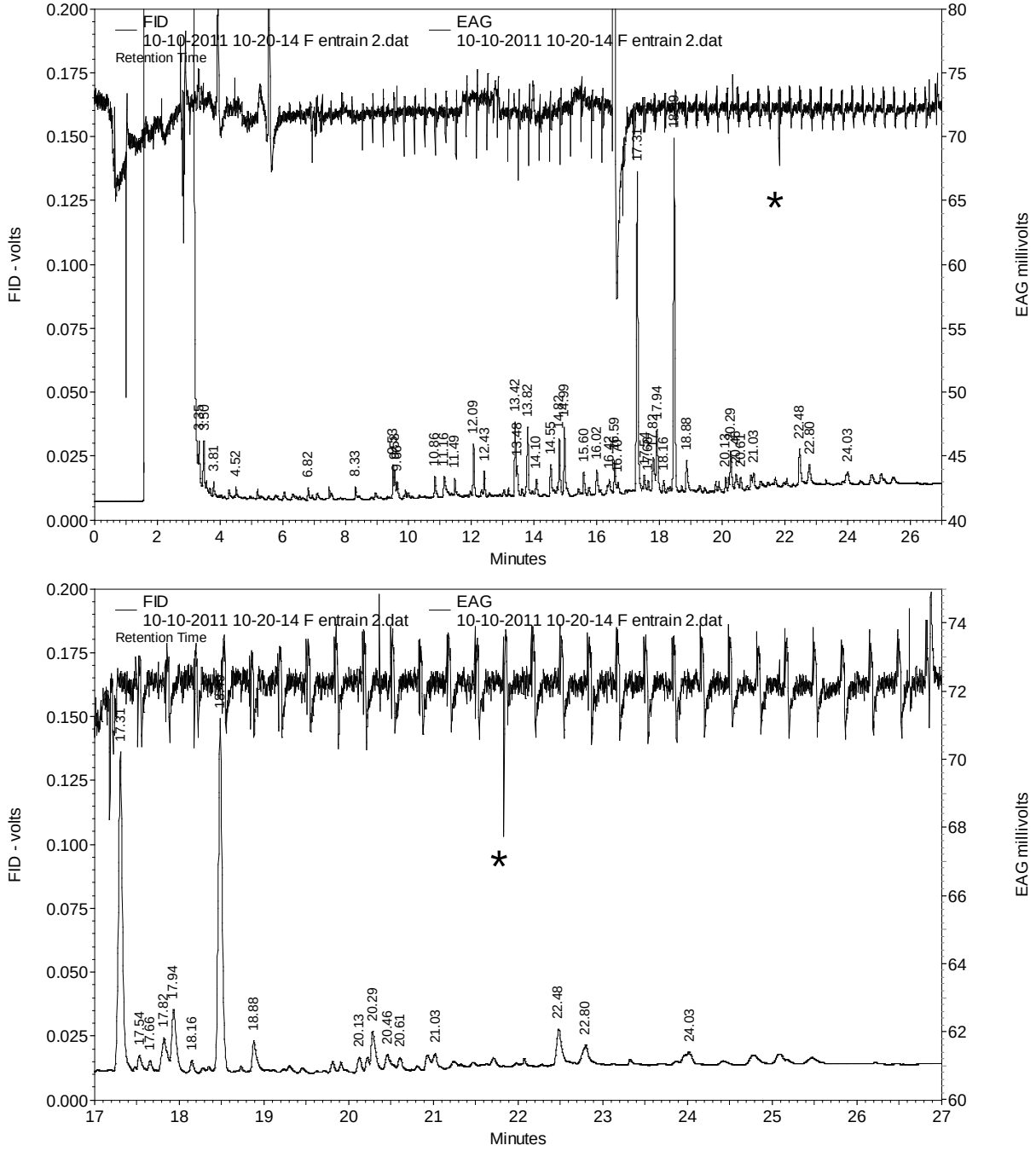

Figure. 2. GC-EAG analysis of volatiles from female *D. oxycoccana* (combined female collections) with male EAG preparation on polar GC column (* possible EAG response).

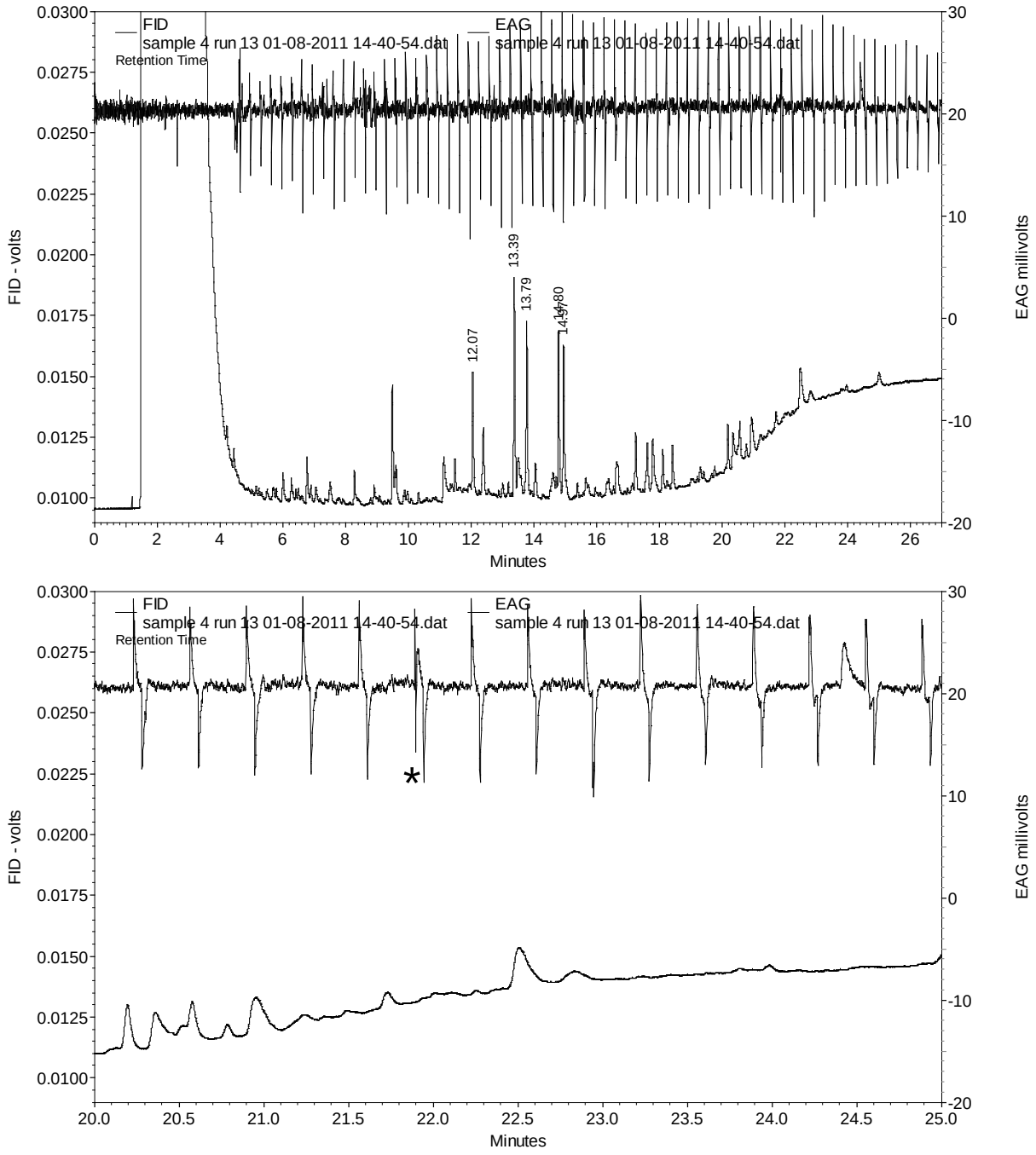

Figure. 3. GC-EAG analysis of volatiles from female *D. oxycoccana* (2010-056-04) with male EAG preparation on polar GC column (* possible EAG response)

However, no significant and consistent responses were ever observed in GC-EAG analyses on the non-polar GC column (e.g. Figure 4).

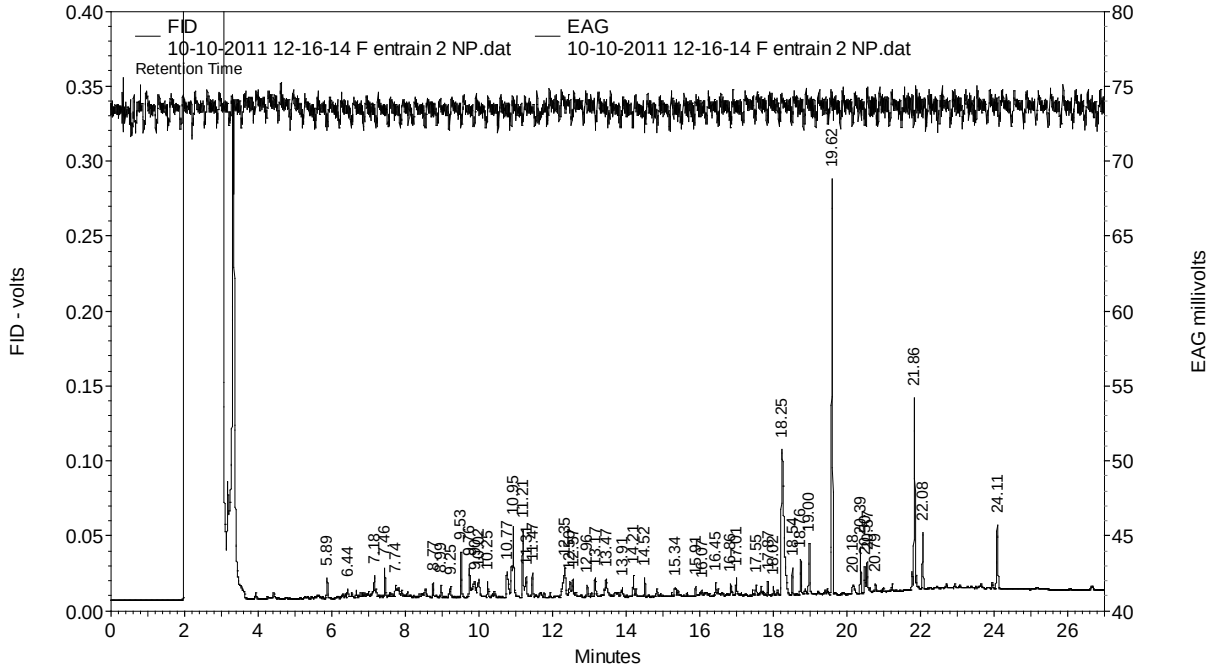

Figure 4. GC-EAG analysis of volatiles from female *D. oxycoccana* (combined female collections) with male EAG preparation on non-polar GC column

Nor were any responses observed in GC-EAG analyses of hexane body washes (e.g. Figures 5, 6).

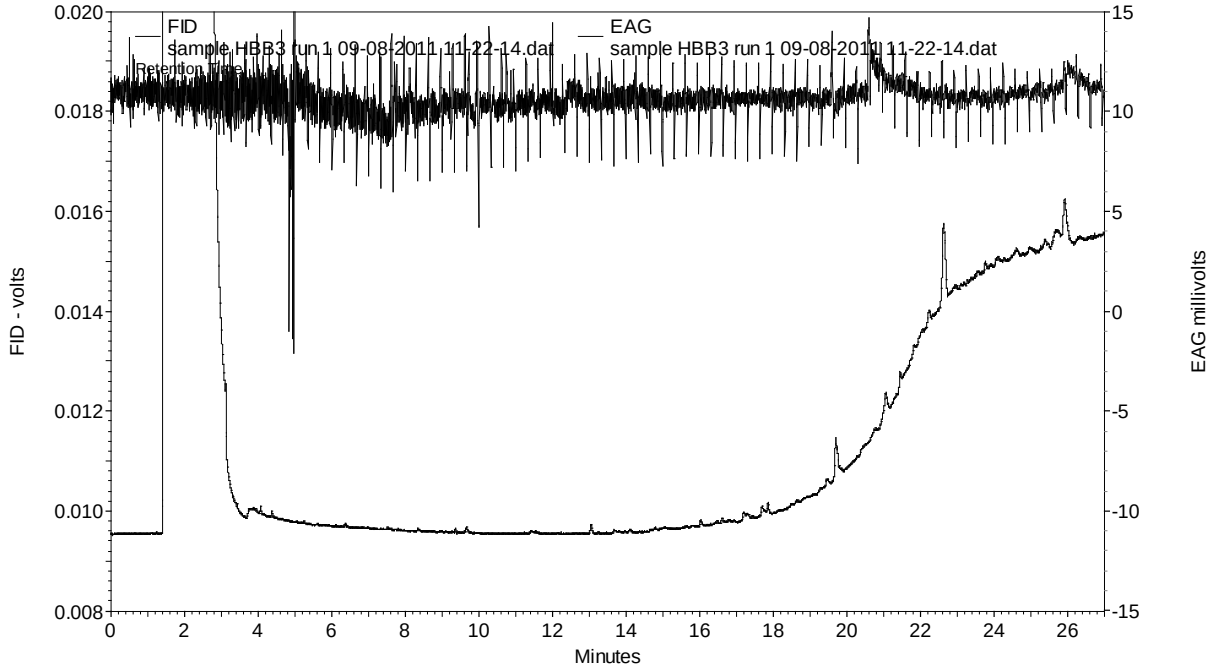

Figure 5. GC-EAG analysis of hexane body wash from female *D. oxycoccana* (2011-056-11) with male EAG preparation on polar GC column

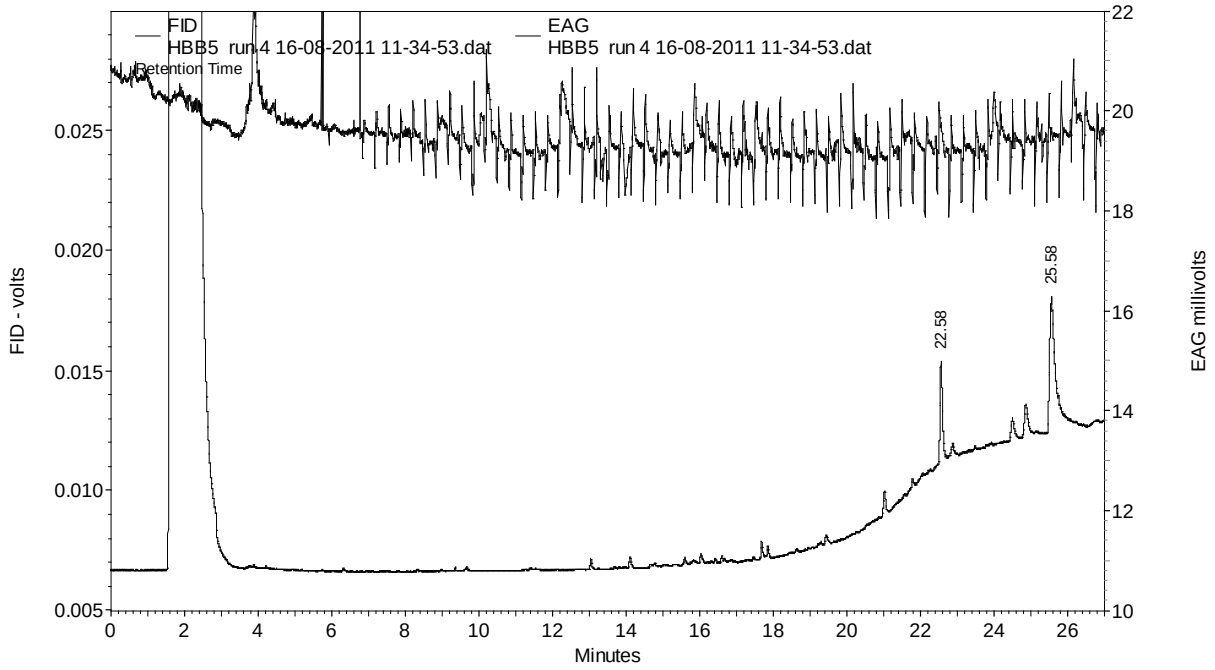

Figure 6. GC-EAG analysis of hexane body wash from female *D. oxycoccana* (2011-056-12) with male EAG preparation on polar GC column

In GC-EAG analyses of SPME collections from female *D. oxycoccana*, most runs showed no response (Figure 7) but in one run a convincing response was observed around 21.8 min on the polar GC column (cf. above) (Figure 8).

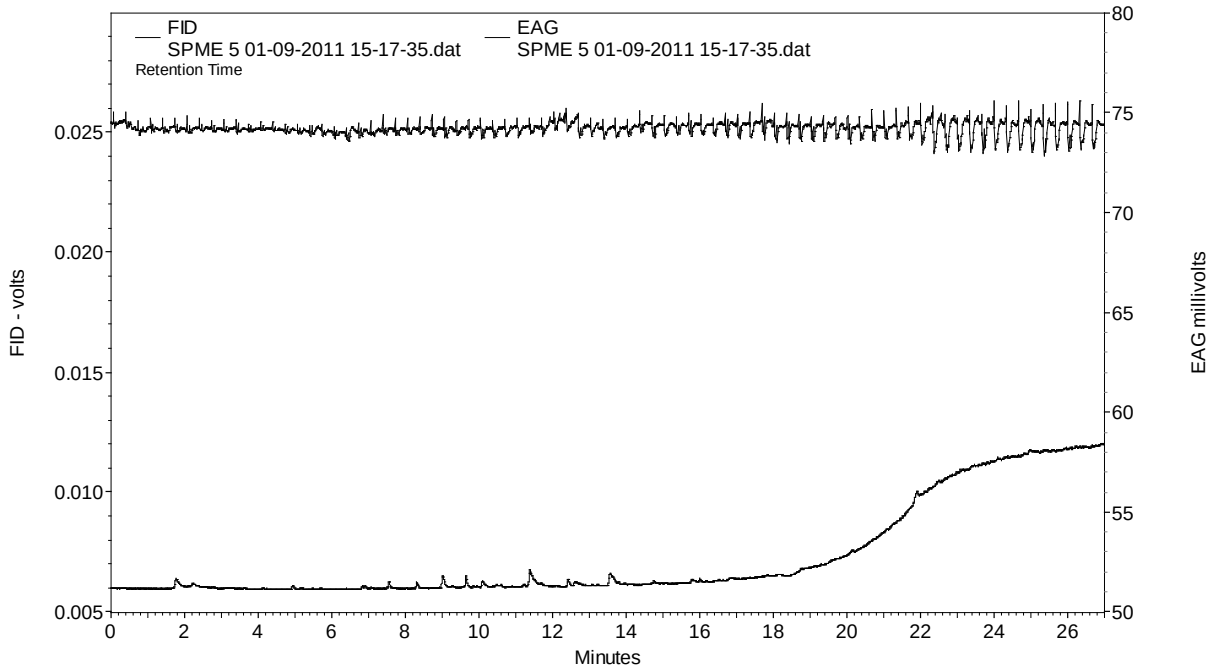

Figure 7. GC-EAG analysis of SPME collection of volatiles from female *D. oxycoccana* with male EAG preparation on polar GC column

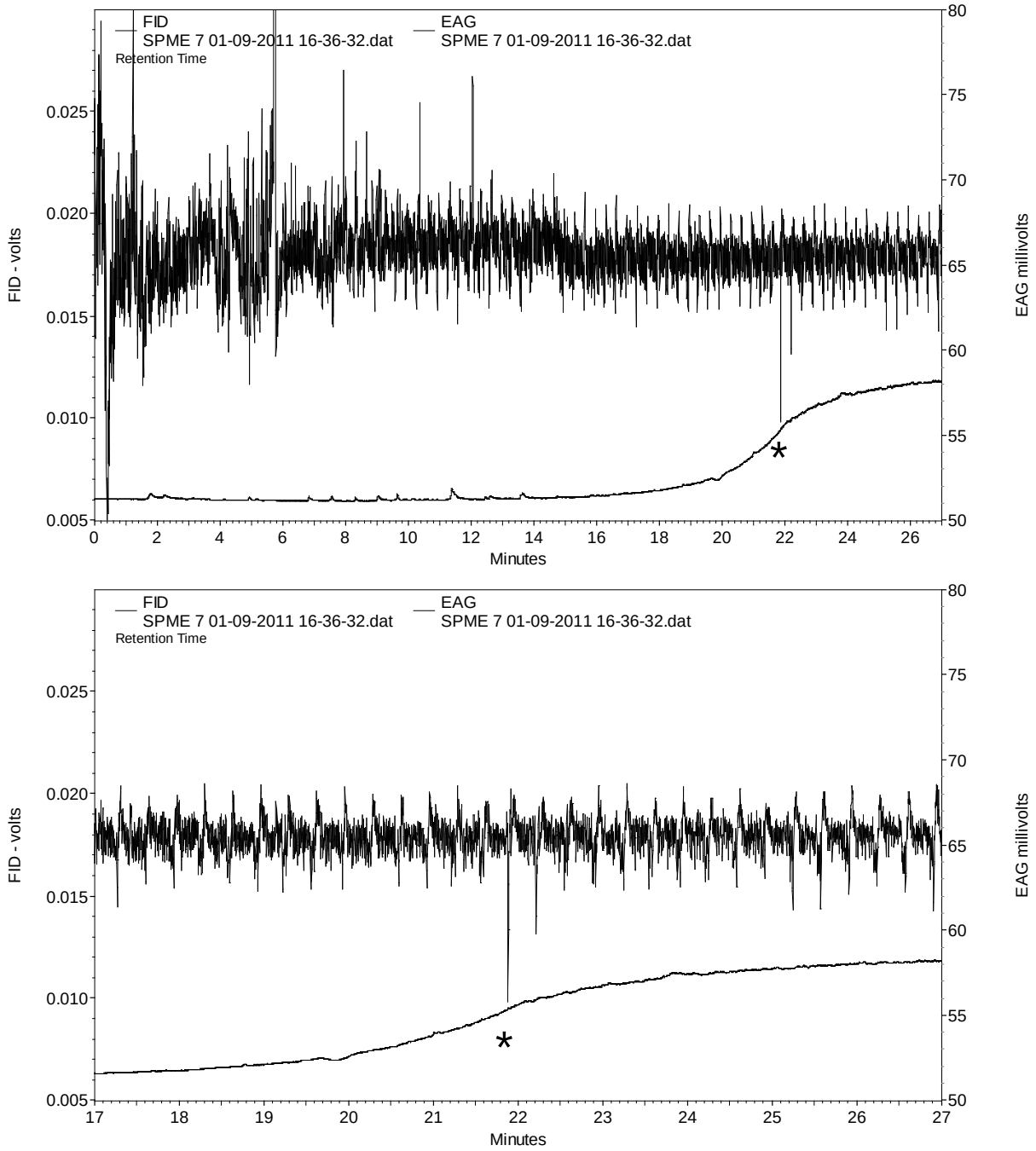

Figure 8. GC-EAG analysis of SPME collection of volatiles from female *D. oxycoccana* with male EAG preparation on polar GC column (* indicates possible EAG response)

A blend of pheromone components from other midge species was made up including (in order of elution on polar GC column):

- 2,6-diacetoxyheptane, pheromone of rice gall midge, *Orseolia oryzae;*
- (BHT – antioxidant);

- 2-acetoxy-5-undecanone, pheromone of raspberry cane midge, *Reseliella theobaldii*;
- (*Z*)-2-acetoxy-8-heptadecene, pheromone of honey locust midge, *Dasineura gleditchiae*;
- (Z)-2-butyroxy-8-heptadecene, pheromone of chrysanthemum midge, *Rhopalomyia longicauda;*
- (Z)-13-acetoxy-8-heptadecen-2-one, pheromone of apple leaf midge, *D. mali*.

No EAG responses to any of these compounds were observed in GC-EAG analyses with a male *D. oxycoccana* preparation (e.g. Figure 9).

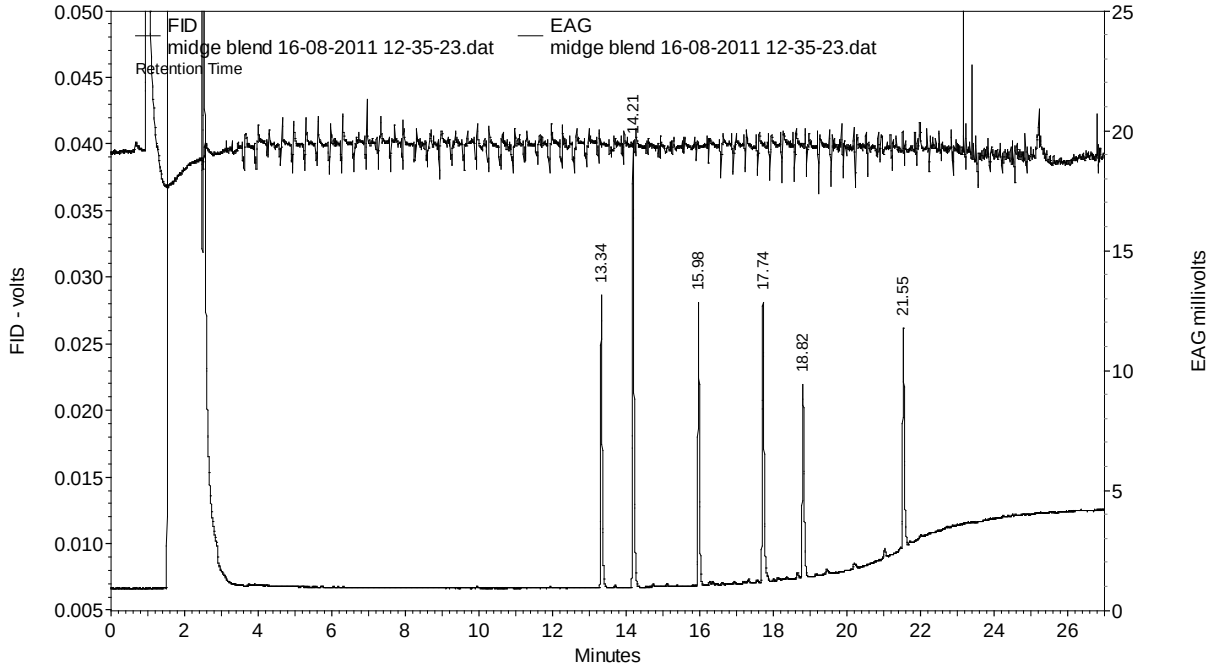

Figure 9. GC-EAG analysis of synthetic standards with male *D. oxycoccana* EAG preparation on polar GC column

In some collections of volatiles from *D. oxycoccana,* a blueberry shoot was added in case host-plant material was necessary for pheromone production (Table 1). In analyses of these by GC-EAG with a male EAG preparation, no EAG responses were observed. However, with a female EAG preparation responses to several of the plant volatiles were observed, particularly to (*Z*)-3-hexenyl acetate (7.31 min), linalool (10.22 min), caryophyllene (10.86 min) and α-terpineol (12.09 min) (Figure 10). This was interesting in itself as female midges are probably attracted to host plants for oviposition. It also confirmed that the GC-EAG equipment was working effectively.

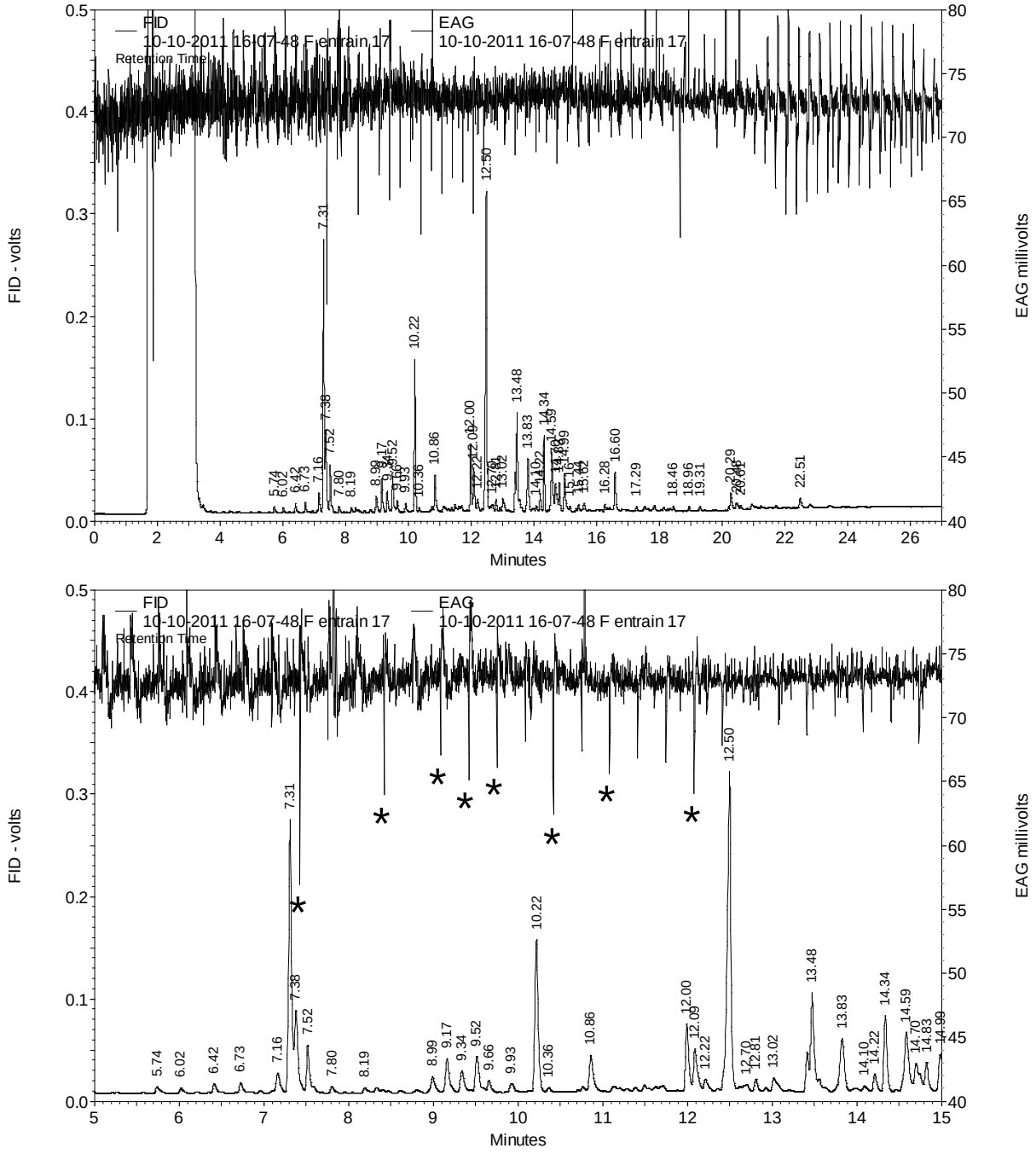

Figure 10. GC-EAG analysis of collection of volatiles from female *D. oxycoccana* with a blueberry shoot using female EAG preparation on polar GC column (* EAG responses)

GC-MS analyses

Detailed comparisons of GC-MS analyses of all the samples on both polar and non-polar GC columns showed no obvious and consistent differences between those from females and those from males that might be attributed to the presence of a female-specific component (e.g. GC-MS analyses of combined collections from female and male *D. oxycoccana* on polar GC column, Figure 11).

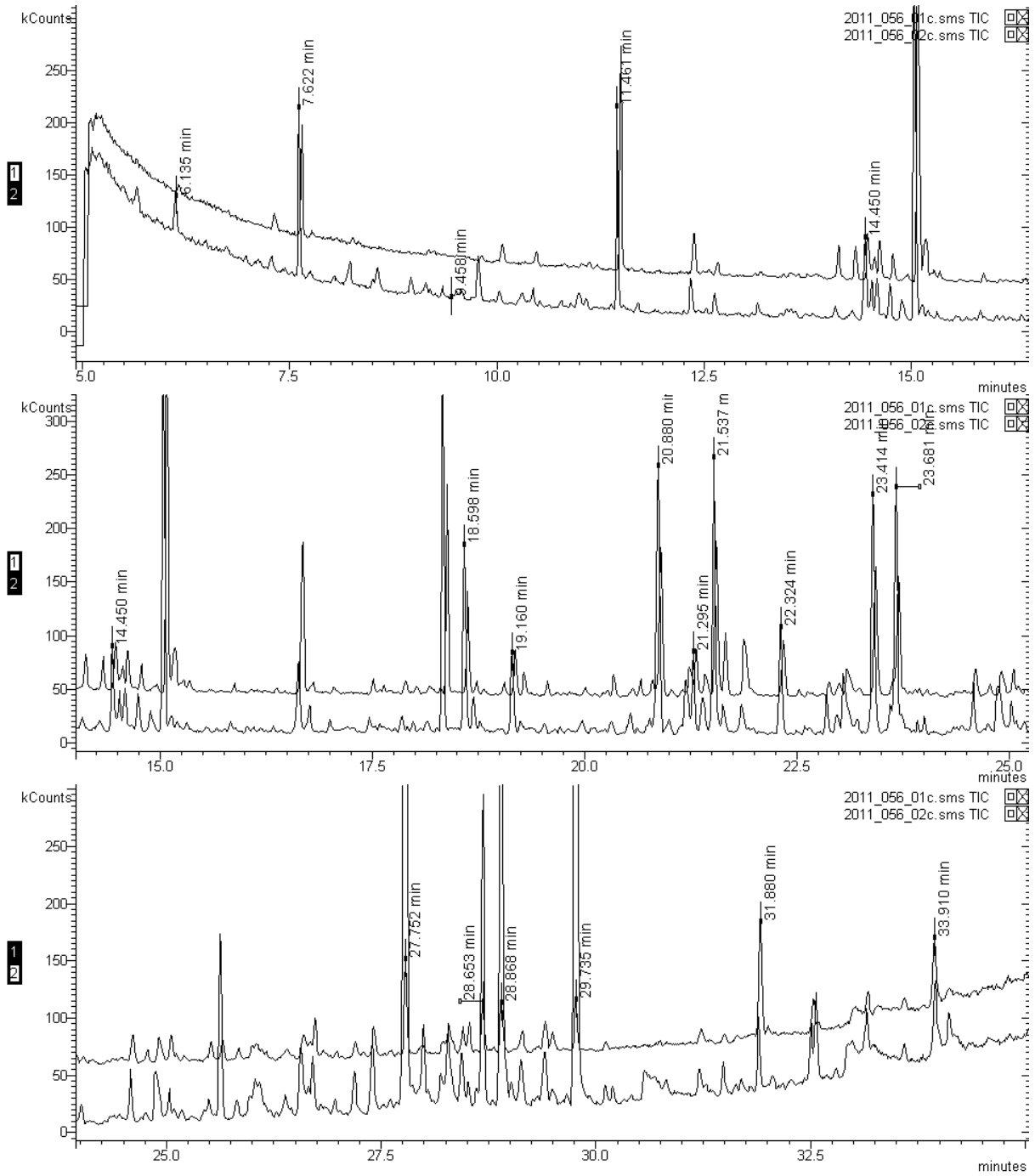

Figure 11. Comparison of GC-MS analyses of collection of volatiles from male (upper) and female (lower) *D. oxycoccana* on polar GC column

Comparisons in the region corresponding to the possible EAG responses observed above at RI 2090 (35.5 min) showed two peaks in this region, but both appeared to be largely simple hydrocarbons from their mass spectra (Figures 12, 13).

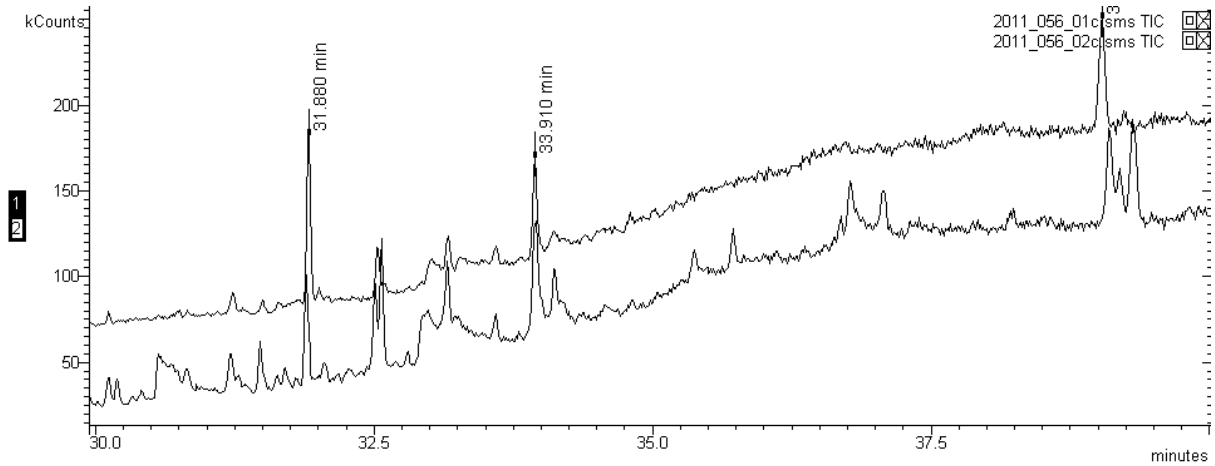

Figure 12. Comparison of GC-MS analyses of collection of volatiles from male (upper) and female (lower) *D. oxycoccana* on polar GC column (RI 2090 at 35.5 min)

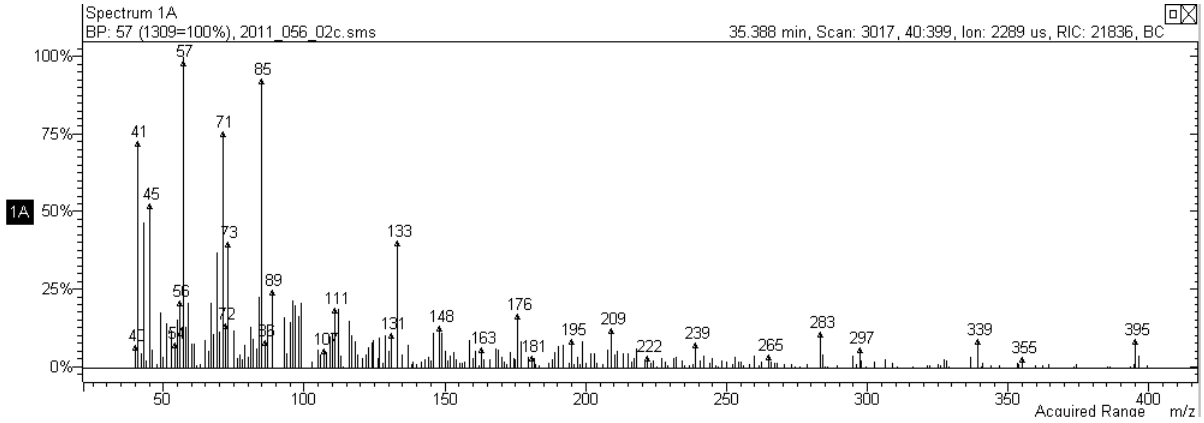

Figure 13. Mass spectrum of peak at 35.39 min in Figure 12.

Insecticide Trial

First assessment

The percentage of 50 shoots infested by blueberry gall midge on 15 September 2011 was analysed (Table 5). The ANOVA of the untransformed data showed significant reductions in the percentage of infested shoots when compared to the double replicated untreated control (P = 0.012). However after angular transformation of the data, the mean for Calypso was not significantly less than the control, though all the other treatments gave significant reductions. No larvae were present in the galls at this stage.

Table 5. Mean (and angular transformed) percentages of shoot terminals galled by blueberry gall midge at first assessment on 15 September 2012, two weeks after the first spray application

Treatment	% infested shoots	
	Untransformed	Angular transformed
Chlorpyrifos	11.0*	19.3*
Hallmark	8.8*	16.4*
Pyrethrum	13.6*	19.7*
Toppel 10	8.4*	15.4*
HDCI 034	11.5*	19.4*
Calypso	14.3*	21.7
Untreated	28.4	29.5
Fprob	0.012	0.02
SED (22 df)	5.31	4.60
LSD (P = 0.05)	11.02	9.54

Second assessment

The percentage of all of the shoots infested by blueberry gall midge on the 28 of September 2011 was analysed (Table 6). The ANOVA of the untransformed data showed no significant reductions in the percentage of infested shoots when compared to the double replicated untreated control (P = 0.069) and the analysis was not improved by angular transformation (P = 0.128).

Table 6. Mean (and angular transformed) percentages of shoot terminals galled by blueberry gall midge at second assessment on 28 September 2012, two weeks after the second spray application

Treatment	% Infested shoots	
	Untransformed	Angular transformed
Chlorpyrifos	3.72	10.92
Hallmark	2.95	9.52
Pyrethrum	5.04	12.84
Toppel 10	3.46	9.21
HDCI 034	2.90	9.28
Calypso	6.52	14.71
Untreated	4.57	12.07
Fprob	0.069	0.128
SED (22 df)	1.222	2.180
LSD (P = 0.05)	2.535	4.522

Discussion

Pheromone identification

Samples of pheromone were collected from over 1,000 virgin female blueberry gall midge. In detailed comparisons of GC-MS analyses of collections from female and male midges no consistent, female-specific component could be detected. Similarly, in GC-EAG analyses of collections from females using a male antenna for the EAG preparation, no consistent responses were observed. However, in three analyses of volatile collections and one of an SPME collection, possible EAG responses were detected around RI 2090 (relative to acetates) on the polar GC column. Unfortunately no such responses were observed in analyses on the non-polar column, which might have given valuable information on the type of structure responsible.

Nevertheless, this RI is in the region observed for the pheromones of other *Dasineura* species such as the apple leaf midge, *D. mali,* (RI 2070), the pear leaf midge, *D. pyri,* (RI 2087) and the blackcurrant leaf midge, *D. tetensi*, (2072). These are relatively involatile compounds. These data would all fit with the conclusion that the pheromone component(s) of the blueberry gall midge are di-functional, 17-carbon compounds such that only extremely small amounts were trapped in volatile collections which could not be detected in GC-MS analyses and were only occasionally detected in GC-EAG analyses.

Future work will build on these findings and endeavour to obtain larger amounts of pheromone for analysis.

Insecticide trials

Pyrethrum and Calypso are the only insecticides currently approved for use on blueberry, and, of these, only pyrethrum showed any sign that it gives useful control of blueberry gall midge at the first assessment. Hallmark, Toppel 10 and chlorpyrifos also reduced galling. The limited efficacy of the insecticides tested was probably due to the fact they could not be properly timed in relation to the gall midges attacks, which occur more or less continuously as a result of overlapping generations later in the season. By the second assessment populations of midge appeared to have crashed, or entered winter diapause. Much better control might be expected if insecticide applications, timed by use of a sex pheromone trap, were applied against the first or second generations, which are likely to be more synchronised.

However, all the above insecticides have broad-spectrum activity and are likely to be very harmful to the midge's natural enemies and anthocorid predatory bugs, as well as to the natural enemies of other blueberry pests. The coded experimental product HDCI 034 is a selective insecticide which works on other midge pests, will control larvae inside the galls and which is less likely to have harmful effects, especially persistent ones, on natural enemies. The efficacy of control of larvae by all treatments is unknown as no larvae could be found at either assessment date. HDCI 034 is likely to be compatible with IPM programmes and priority should be given to its development for control of blueberry gall midge and possibly other pests in UK. The parent company of HDCI 034, will not be undertaking relevant crop specific studies on bees and therefore they request, on the grounds of responsible stewardship, that applications are timed post flowering in the absence of such information.

A further trial to explore timing of application of HDCI 034, pyrethrum and the other insecticides tested in 2011 for control of blueberry gall midge is planned for 2012. Locating a site with early high level midge populations is essential for this. As a sex pheromone trap for this pest has not yet been developed, spray timing will have to be done on the first appearance of galls

Conclusions

- Pheromone samples were collected from over 1,000 virgin female blueberry gall midge
- There were strong indications that collections contained very small amounts of a compound related to the pheromone components of other *Dasineura* species, and future work will focus on increasing the amount collected
- Pyrethrum, Hallmark, Toppel 10 and chlorpyrifos all gave partial control of shoot galling by blueberry gall midge when applied in late August
- The limited efficacy of these insecticides was probably because sprays could not be properly timed in relation to the gall midges attacks, which occur more or less continuously as a result of overlapping generations later in the season. Much better control might be expected if insecticide applications, timed by use of a sex pheromone trap, were applied against the first or second generations in spring which are likely to be more synchronised
- Calypso did not reduce galling significantly
- The coded experimental product HDCI 034, a translaminar, selective insecticide which is known to control the larvae of other gall midge pests inside galls, shows promise

- A second insecticide screening trial will be carried out in 2012

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Knowledge and Technology Transfer

No technology transfer activities have yet taken place for this project

Glossary

None

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APPENDIX