

Project title: Blueberry gall midge: sex pheromone monitoring and control with insecticides

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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.

AUTHENTICATION

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

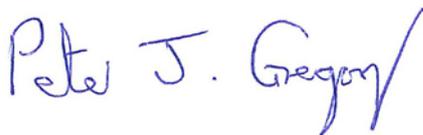
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Signature Date 28 March 2013

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Report authorised by:

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Signature Date 28 March 2013

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GROWER SUMMARY

Headline

The blueberry midge pheromone has been synthesised and is available for evaluation in the UK.

Background

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, though recent work in Canada has shown that *D. oxycoccana* on cranberry and blueberry produce and respond to different sex pheromones, and are therefore different species. 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-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 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 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. 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

The female sex pheromones of the two sub-species of *Dasineura oxycoccana* found on blueberry and cranberry have been identified and synthesised by Canadian researchers. Lures containing the two pheromones were provided for testing in the UK and the blueberry midge pheromone was shown to be highly attractive to male blueberry midges. No midges were attracted to the cranberry midge lure in the UK.

Analysis of the compounds in the lures suggested these were saturated and mono-unsaturated 2,14-diacetoxyheptadecanes respectively, and this was confirmed in a subsequent publication by the Canadian group. The pheromone of the blueberry midge has now been synthesised at NRI as both the racemic form and *R,R* stereoisomer and these are available for field testing.

A field trapping test showed that significantly more blueberry midges were caught in traps 0.5 m above ground level than in traps at 1.0 m, and few midges were caught in traps at 2.0 m. Further experiments are required to optimise the loading of pheromone in the lure.

It was not possible to carry out a trial of insecticides against the blueberry midge because of the absence of midges in the proposed trial plot, in spite of the fact that the field was heavily infested during 2011. These trials will be carried out during 2013 in a new dedicated experimental plantation at East Malling Research.

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

- No action points have been identified for growers 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-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 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 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, was approved for use on vegetable crops in the UK in 2010 and an Extension of Approval for Minor Use (EAMU) has been granted for Movento (number 1401/12) on blackcurrant, whitecurrant, gooseberry and blueberry. The

approval of the SC formulation on apple is pending. Other workers have investigated *Bacillus thuringiensis* Berliner subsp. *israelensis* (Bti), chlorantraniliprole, flubendiamide, metaflumizone, spirotetramat and diazinon but found that none of the alternative insecticides provided consistent significant control on a par with diazinon.

EMR has 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.

In Canada, Sheila Fitzpatrick (Entomologist, Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Agassiz, BC) and Gerhard Gries (Simon Fraser University, Vancouver, BC) reported that populations of *D. oxycoccana* in cranberry and highbush blueberry might be two cryptic species. Midges that develop on cranberry do not mate with those from highbush blueberry (Cook *et al.*, 2011), and there is divergence in the mitochondrial cytochrome oxidase I gene sequence between cranberry and blueberry populations (Mathur *et al.*, 2012), even though opportunity exists for gene flow between the populations (Cook *et al.*, 2012).

During a visit to Canada in the summer of 2012, Jerry Cross learnt that this group had identified the pheromones of the cranberry and blueberry versions of *D. oxycoccana*, and Professor Gries kindly provided lures of the two sub-species for evaluation in the UK. In light of these developments, it was agreed with the HDC Research Manager and the Project Industry Representative that the objectives of the project required a little modification and would be as follows:

- Test the lures provided from Canada for their attractiveness to blueberry gall midge, *D. oxycoccana*, which is considered to be an alien invasive species in the UK. The lures will also be tested against UK cranberry gall midge species if a suitable plantation can be found, and for our native bilberry gall midge *Dasineura vaccinatorum*, if a suitable wild bilberry site can be found. Note it is possible that this is the same species as the blueberry gall midge, *D. oxycoccana*, there being much taxonomic confusion in the Cecidomyiidae, even at generic level

- Investigate the collections of volatiles from females and males made as part of the HDC-funded project in 2011 for any traces of the compounds identified in the Canadian species. It may be possible to detect trace levels when we know exactly what to look for and to confirm which, if either, of the Canadian pherotypes we have in the UK
- In agreement with Gerhard Gries, to synthesise the appropriate pheromone so an adequate supply of material is available for use in the UK
- Carry out field trials to examine the effects of pheromone dispenser, lure loading, trap design and trap height on trap efficacy for the UK species
- Conduct an efficacy trial of candidate insecticide products using the lures to time sprays of insecticides for control of the blueberry midge

Materials and methods

Analysis of lures

Lures were supplied by Gerhard Gries, Simon Fraser University, Canada, as lures for blueberry midge and for cranberry midge. Two lures of each were extracted together in petroleum spirit (bp 40-60°C; 5 ml) at room temperature overnight. Each extract was then treated identically. The extract was pipetted off and the lures washed with more petroleum spirit (2 ml). The solvent was removed from the combined extracts on a rotary evaporator and the residue was chromatographed on silica gel (250 mg) packed in hexane in a Pasteur pipette (4 mm i.d.). The residue was applied to the column in a total of 1 ml hexane and then eluted with 1 ml aliquots of hexane and 25, 5%, 10%, 20%, 50% and 100% diethyl ether in hexane. Fractions of 1 ml were collected and analysed by gas chromatography linked to mass spectrometry (GC-MS).

Gas chromatography linked to mass spectrometry (GC-MS)

GC-MS Analyses were carried out with a 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). The carrier gas was helium (1 ml/min), injection splitless (220°C) and oven temperature programmed from 40°C for 2 min, then at 10°C/min to 250°C.

Synthesis

2,14-Diacetoxyheptadecane was synthesized as both the racemic mixture and *R,R* stereoisomer by reaction of the di-Grignard reagent from 1,9-dibromononane with a mixture of 1,2-epoxypropane and 1,2-epoxypentane. The required 2,14-dihydroxyheptadecane was isolated from the mixture of diols by chromatography on silica gel and acetylated with acetic anhydride in pyridine (Figure 1).

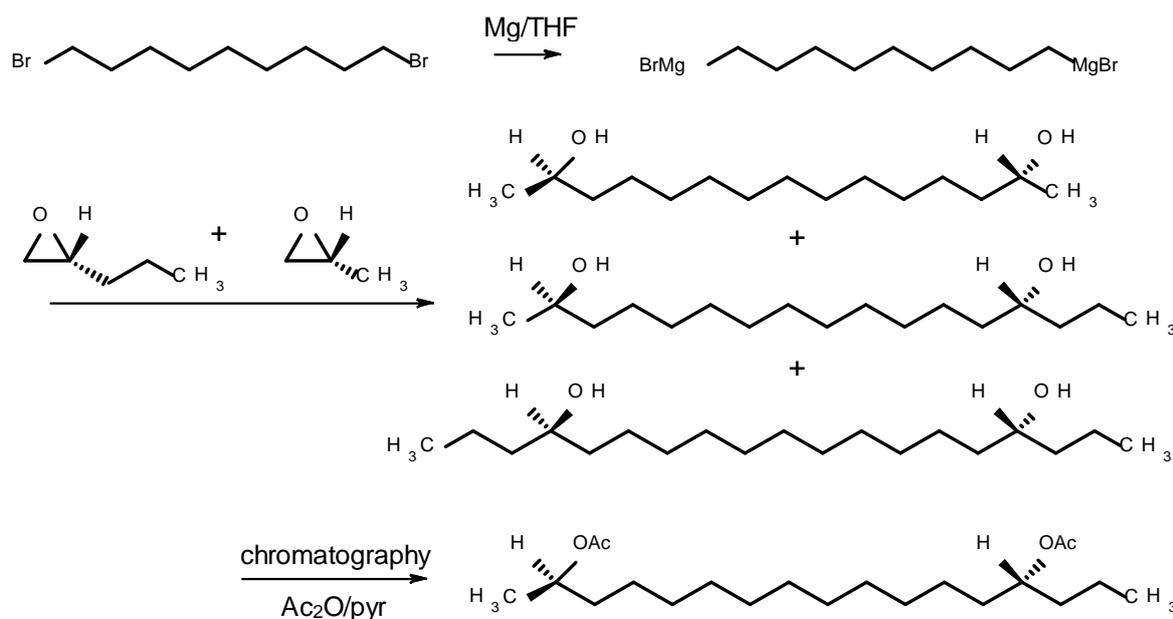


Figure 1. Synthesis of (*R,R*)-2,14-diacetoxyheptadecane

Field trapping tests

Experiment 1 aimed to test the blueberry gall midge and cranberry gall midge lures provided by Gerhard Gries, Simon Fraser University, Vancouver BC, Canada for attractiveness to their target species and to the UK native bilberry gall midge *Dasineura vacciniorum*.

The three sites were in protected blueberry tunnels at Hall Hunter Farms, Tuesley Farm, Milford, in wild bilberry at Witley National Trust reserve, near Guildford, Surrey, and in a confidential outdoor commercial cranberry plantation in Kent, organised by Lindrea Latham, Total Berry.

Traps were red delta traps with white inserts positioned 0.5 m above ground level spaced at least 12 m apart (Figure 2). The three treatments were a rubber septa lure for each species

and an unbaited control. The experimental design was a randomized complete block with five replicates at Tuesley and three at Witley.

Assessments were counts of numbers of male midges in traps and data was transformed to $\log_{10}(x+1)$ to normalize variances and subjected to analysis of variance.



Figure 2. Pheromone traps at Tuesley Farm

Experiment 2 was to determine the optimum height of the pheromone trap. The experiment was carried out in commercial blueberry tunnels at Hall Hunter Farms, Tuesley Farm, Milford. Traps were red delta traps with white inserts attached to every second post (12 m) at 0.5 m, 1 m or 2 m above ground level. Row spacing was 2.5 m with plants in pots 1.0 m apart. Lures were rubber septa containing the blueberry midge pheromone and there were five replicates.

Assessments were counts of numbers of male midges in traps and data was transformed to $\log_{10}(x+1)$ to normalize variances and subjected to analysis of variance.

Insecticide trial

Traps were deployed in a commercial protected crop in Essex in mid-summer 2012 where we intended to do an insecticide efficacy trial. The crop was heavily infested with the midge in the previous year. Regrettably, no midges were caught in the trap and there was no attack in the crop. It seems that the midge did not have a late summer generation in 2012 and we decided not to do the trial in the absence of the pest as it would have been a waste of resources.

Results

Analysis of lures

Lures for the cranberry and blueberry “sub-species” of *D. oxycoccana* supplied by Gerhard Gries were extracted, the extracts were fractionated on silica gel chromatography and the fractions analysed by GC-MS. For both types of lures, the fraction eluted with 20% diethyl ether in hexane contained a major peak with slightly different retention times for the two types of lure (Figure 3).

From previous experience, this fraction contains diacetates. The mass spectrum of the peak in the fraction from the cranberry midge lure was consistent with that of a 17-carbon diacetate with one acetate in the 2-position and a single double bond, by comparison with the mass spectra of (*Z*)-2,13-diacetoxy-8-heptadecene, the pheromone of the pear leaf midge, *D. pyri*, and (*Z*)-2,12-diacetoxy-8-heptadecene, pheromone of the blackcurrant leaf midge, *D. tetensi*, synthesised at NRI (Figure 4).

The mass spectrum of the peak in the fraction from the blueberry midge lure was consistent with that of a 17-carbon diacetate with one acetate in the 2-position by comparison with those of 2,13-diacetoxyheptadecane and 2,16-diacetoxyheptadecane, synthesised at NRI (Figure 5).

The GC retention data for the two compounds from the lures were also consistent with them being 17-carbon diacetates (Table 1).

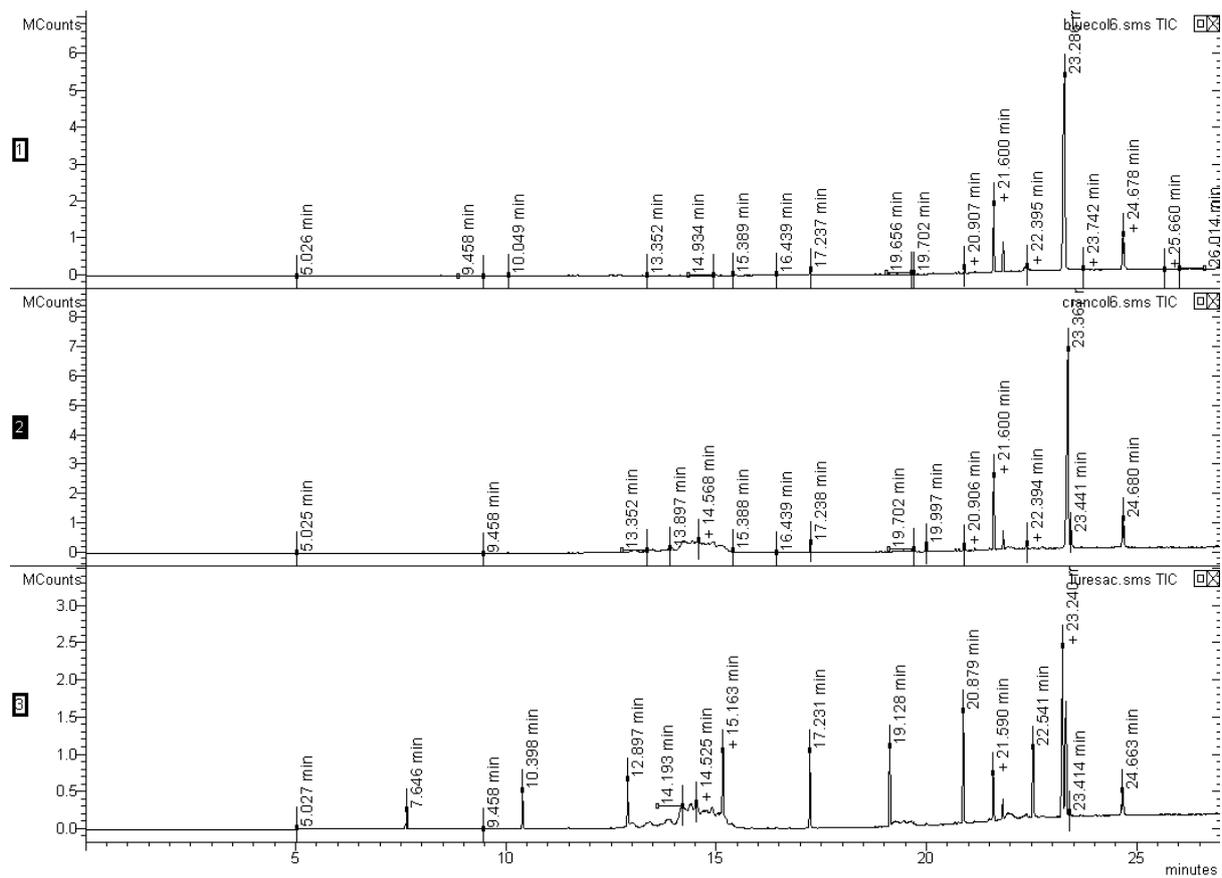


Figure 3. GC-MS traces (polar GC column) of fractions eluted with 20% ether in hexane from blueberry midge lure (upper), cranberry midge (middle) and both together (lower), showing a major component at 23.24 min and 23.32 min respectively

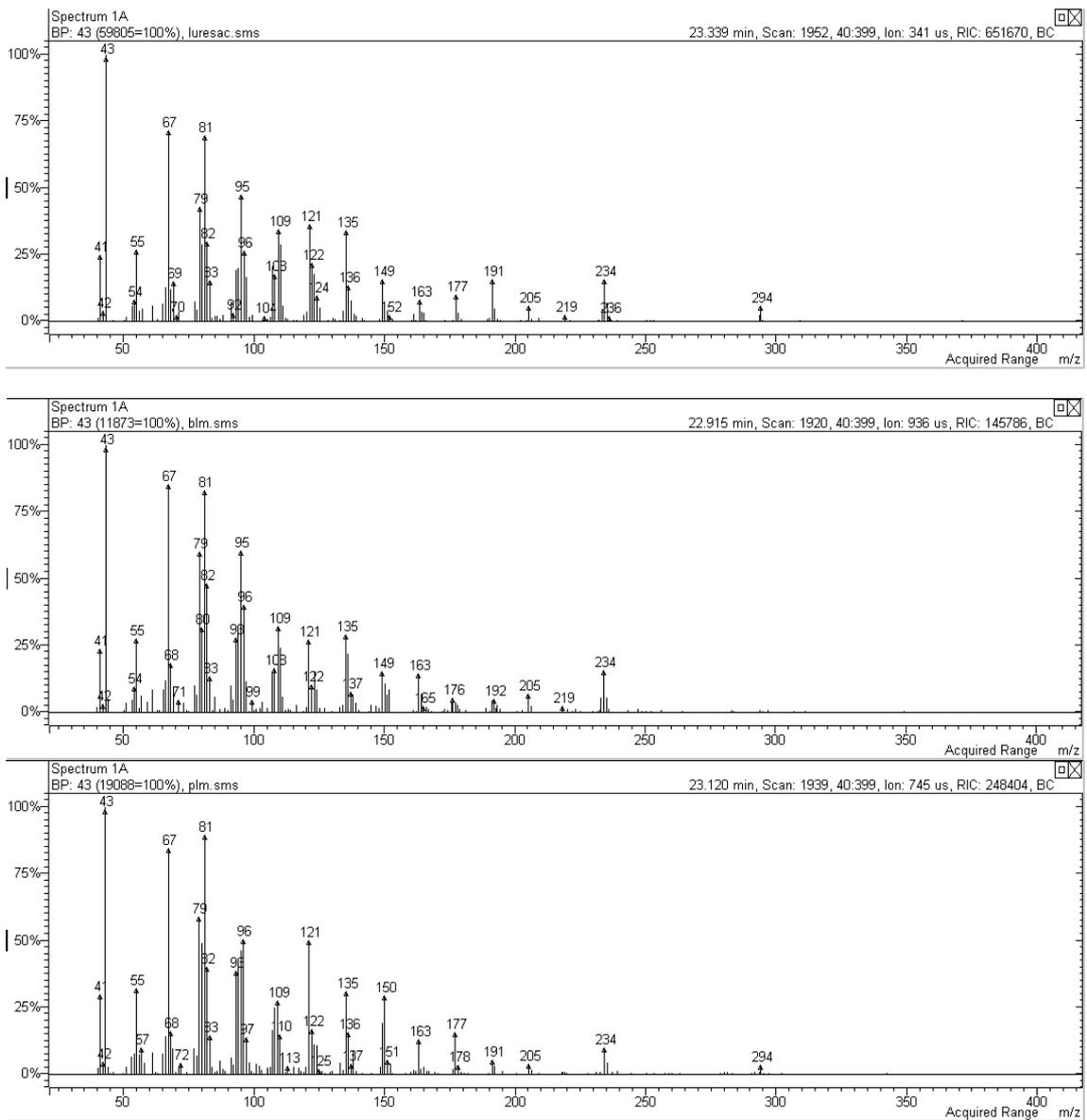


Figure 4. Mass spectra of compound from cranberry midge lure (upper), (*Z*)-2,13-diacetoxy-8-heptadecene, the pheromone of the pear leaf midge, *D. pyri* (middle) and (*Z*)-2,12-diacetoxy-8-heptadecene, pheromone of the blackcurrant leaf midge, *D. tetensi* (lower)

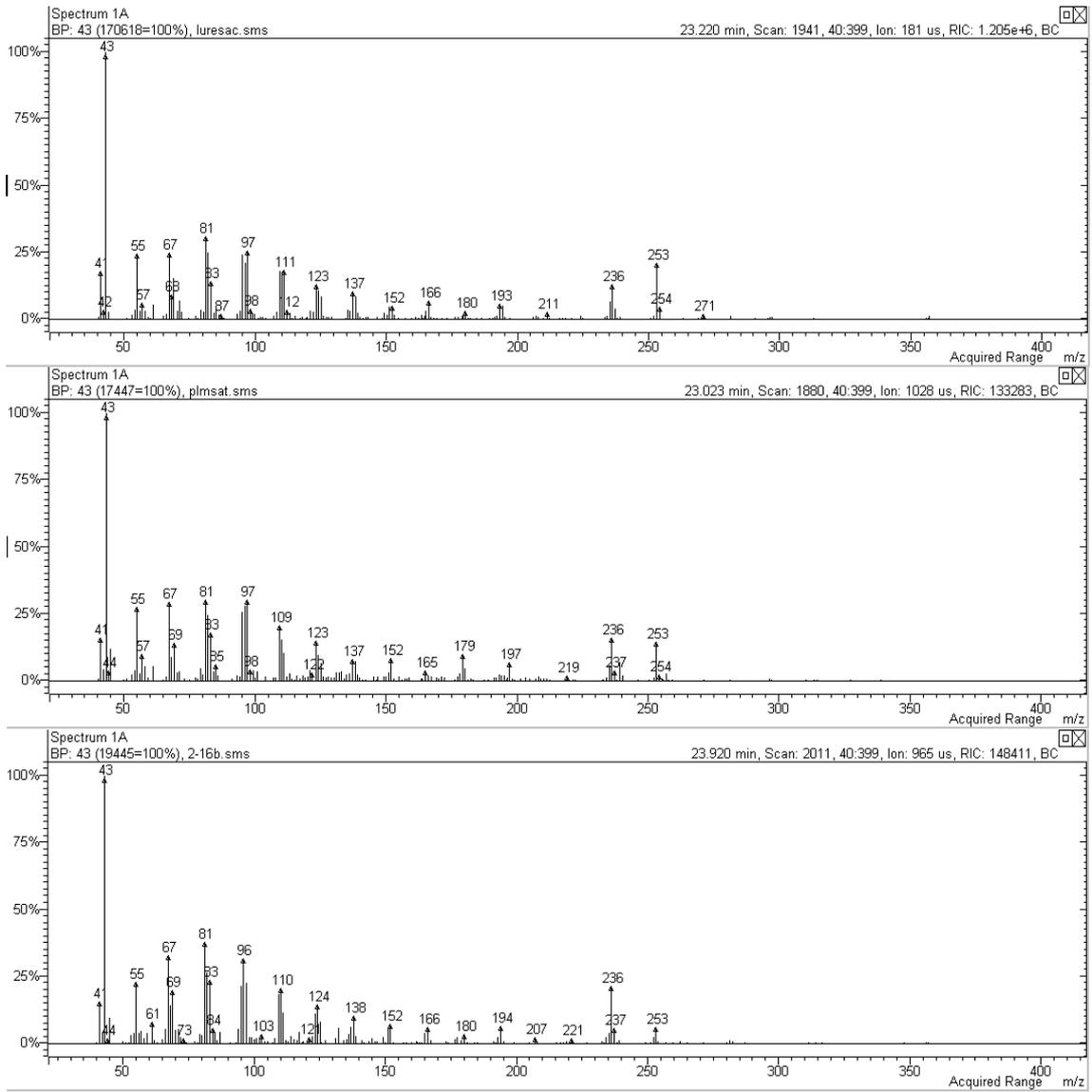


Figure 5. Mass spectra of compound from blueberry midge lure (upper), 2,13-diacetoxyheptadecane, (middle) and 2,16-diacetoxyheptadecane (lower)

Table 1. Retention data (RI relative to retention times of alkyl acetates) for compounds from cranberry and blueberry midge lures and synthetic standards

Compound	RI
Blueberry midge	2084
Cranberry midge	2094
Blackcurrant midge (Z8-2,12-17:diAc)	2047
Pear leaf midge (Z8-2,13-17:diAc)	2069
E8-2,13-17:diAc	2082
2,13-17:diAc	2061
2,16-17:diAc	2169

Further examination of the mass spectrum of the compound from the blueberry midge lures (Figure 4) showed enhancement of the ions at m/z 193/194, corresponding to enhancements of ions at m/z 179 in the spectrum of 2,13-diacetoxyheptadecane and m/z 221 in that of 2,16-diacetoxyheptadecane. This suggested that the compound in the blueberry midge lures was 2,14-diacetoxyheptadecane.

Similarly, the mass spectrum of the compound from the cranberry midge lures showed enhancement of the ion at m/z 191 compared with ions at m/z 177 and m/z 163 in the spectra of 2,13- and 2,12-diacetoxy-8-heptadecene respectively. This suggested that the compound was a 2,14-diacetoxyheptadecene with a single double bond. Previous work at NRI indicated that the most likely position of the double bond was the 8-position (Hall *et al.*, 2012), as suggested by the similarity of the mass spectra of the three compounds in Figure 3.

Subsequently Fitzpatrick *et al.* (2013) reported that the pheromones of the blueberry and cranberry versions of *D. oxycoccana* were indeed (*R,R*)-2,14-diacetoxyheptadecane and (8*Z*,2*S*,14*S*)-2,14-diacetoxy-8-heptadecene respectively.

GC-MS analyses of collections of pheromone from UK midges made during 2011 were re-examined, but no trace of either compound could be detected.

As reported previously (HDC project SF 126 Annual Report, 2011), an occasional EAG response was observed in GC-EAG analyses of pheromone collections (e.g. Figure 6) and the retention time of this was RI \leq 2090, consistent with that of the compound from the blueberry midge lures (Table 1).

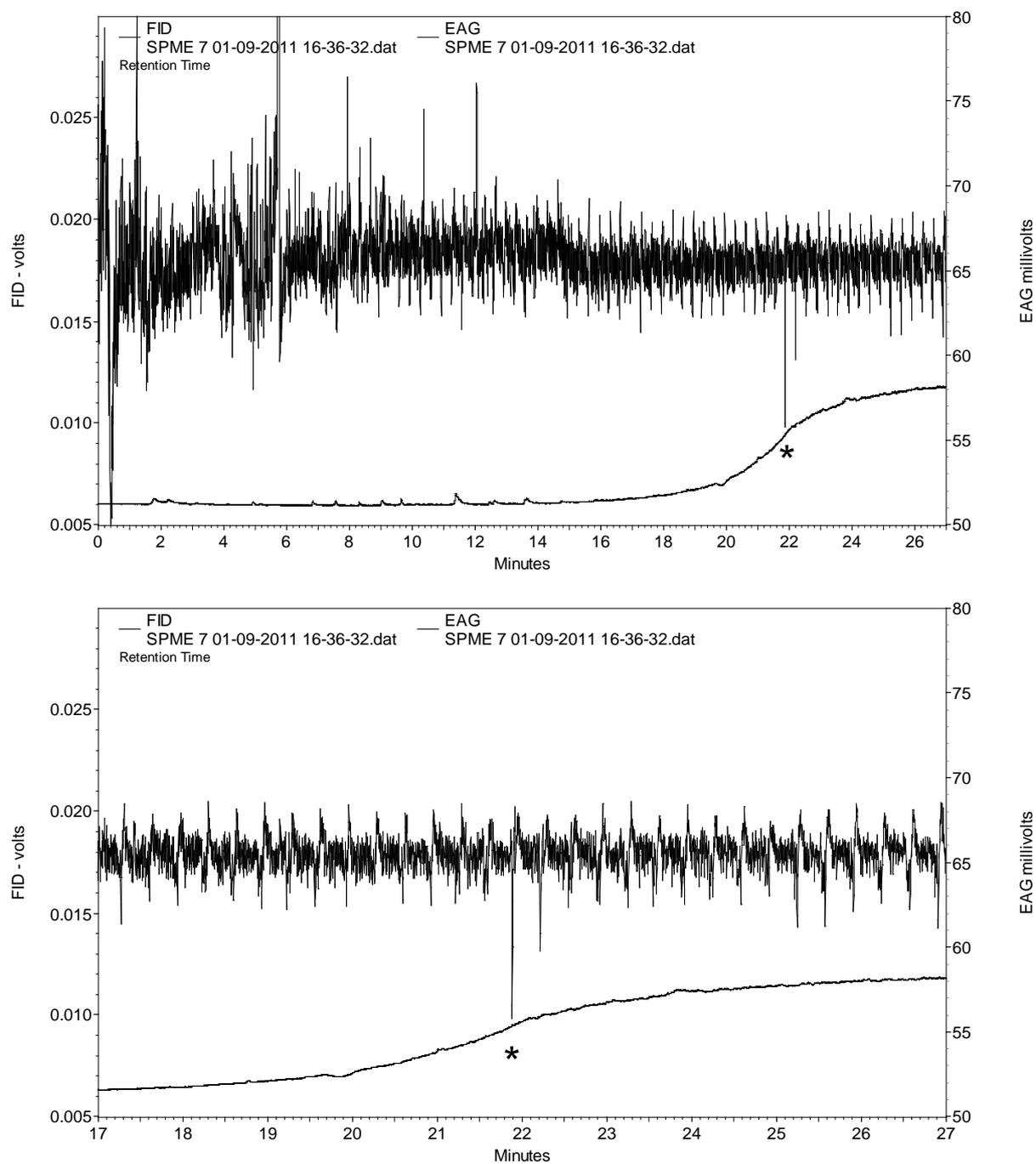


Figure 6. GC-EAG Analysis of SPME collection of volatiles from female *D. oxycoccana* with male EAG preparation on polar GC column made during 2011 (* indicates possible EAG response)

Field trapping tests

In Experiment 1 there were significantly more blueberry midge males ($P < 0.05$; Figure 7) in traps baited with blueberry midge pheromone than the unbaited or the cranberry midge lure at the blueberry plantation at Tuesley Farm. The traps baited with the cranberry midge lure did not catch significantly more than the unbaited traps (Table 2). Midges were attracted to the traps within two minutes of deployment.

No midges were captured at the wild bilberry or cranberry sites with either lure (Figure 8).

Table 2. Mean numbers of male blueberry midge found on sticky traps in the commercial blueberry plantation at Tuesley (7-14 July 2012; 5 reps).

Pheromone lure	Mean catch	Log₁₀(mean catch+1)
Blueberry	941	2.962
Cranberry	24	1.209
Untreated	16	1.190
<i>F pr</i>		<.001
s.e.d.		0.2166
l.s.d. ($P < 0.05$)		0.4995

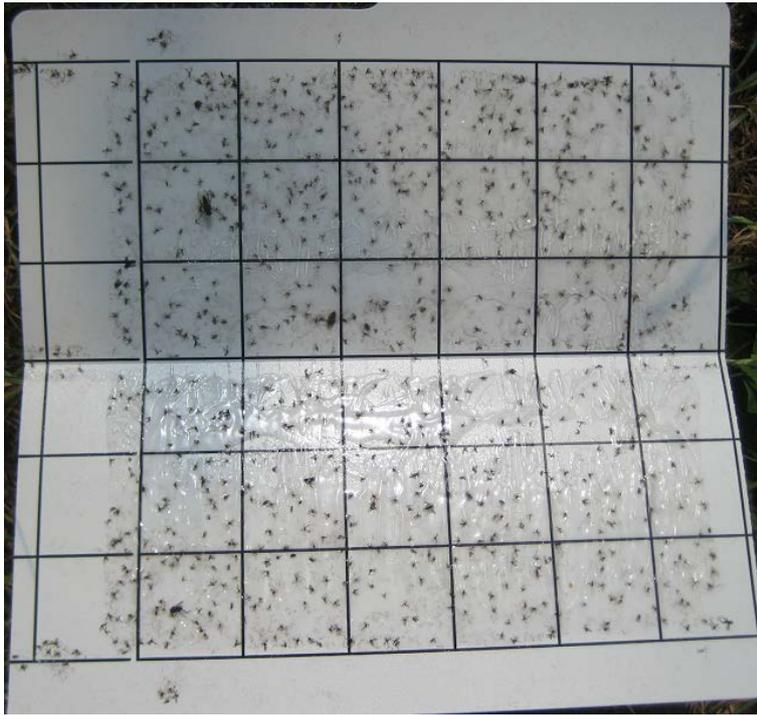


Figure 7. Sticky insert from trap baited with blueberry midge pheromone at Tuesley Farm after one week



Figure 8. Pheromone trap deployed in wild bilberry

In Experiment 2, there were significantly more midges captured closer to the ground in the crop with more than three times the number at 0.5 m compared to 1.0 m and very few midges at 2 m (Table 3).

Table 3. Mean numbers of male blueberry midge caught in sticky traps at different heights above ground at Tuesley Farm (13-19 July 2012; 5 replicates)

Height of trap (m)	Mean catch	Log ₁₀ (mean catch+1)
0.5	1047	3.003
1.0	344	2.314
2.0	7	1.133
<i>F</i> pr		<.001
s.e.d.		0.2237
l.s.d.		0.5290

Discussion

Pheromone identification

During 2011, pheromone was collected from virgin female and male blueberry midges by air entrainment and trapping on Porapak resin and by solid-phase microextraction (SPME). Analysis of these collections by GC linked to MS failed to show any consistent differences between the collections from males and females. Analysis of the collections from females by GC linked to EAG recording from the antennae of male midges showed an occasional EAG response late in the analysis, thought to be consistent with a 17-carbon diacetate, as in the pheromones of the pear leaf midge and blackcurrant leaf midge. However, the responses were only observed on a few occasions and no compounds could be detected by GC-MS at the corresponding retention times.

During 2012 it was learnt that the pheromones of “cryptic” species of *D. oxycoccana* found on blueberry and cranberry respectively had been identified by the Fitzpatrick/Gries group in Canada. Lures were provided for testing, but the structures were not revealed.

Isolation and analysis of the pheromones from the blueberry and cranberry midge lures at NRI indicated that these were the saturated 2,14-diacetoxyheptadecane and a mono-unsaturated 2,14-diacetoxyheptadecene respectively. Subsequently Fitzpatrick *et al.* (2013) reported that the pheromones of the blueberry and cranberry versions of *D. oxycoccana* were indeed (*R,R*)-2,14-diacetoxyheptadecane and (8*Z*,2*S*,14*S*)-2,14-diacetoxy-8-heptadecene respectively.

The pheromone of the blueberry midge was synthesised at NRI as both the racemic and *R,R* stereoisomer and these are now available for field evaluation during 2013.

Re-examination of the data from 2011 showed that the EAG response from the antennae of male blueberry midge observed occasionally in GC-EAG analyses of collections of pheromone from females was at retention times consistent with that of the blueberry midge rather than that of the cranberry midge. However, even knowing the structure of the pheromone, this could not be detected by GC-MS in our pheromone collections. These collections were all made by collection of volatiles, and it would seem that this is not a good approach for the very involatile 17-carbon diacetates. In fact collection of volatiles from the Gries lures under similar conditions during 2012 also failed to show any detectable amounts of pheromone. Pheromone collections were made during 2011 by whole-body extraction, but large amounts of impurities obscured any small amounts of the pheromone present.

Field trapping

Trapping tests in blueberry, cranberry and bilberry fields in the UK showed the blueberry midge lures were highly attractive to male blueberry midges.

No midges were caught in cranberry and bilberry fields, indicating either that no midges were present or that neither lures are attractive to the native cranberry species or our native blueberry midge, *Jaapiella vacciniorum*. Further evaluations will be carried out during 2013.

Pheromone traps at 0.5 m from the ground caught significantly more male blueberry midges than those at 1.0 m, and few midges were caught in traps at 2.0 m. Similar results have been found with other species of midge in horticultural crops (Hall *et al.*, 2012). Further experiments are required to optimise the loading of pheromone in the lure. Those from Gerhard Gries used so far contained 100 µg.

Insecticide trial

Traps were deployed in a commercial protected crop in Essex in mid-summer 2012 where we intended to do an insecticide efficacy trial. The crop was heavily infested with the midge in the previous year. Regrettably, no midges were caught in the trap and there was no attack in the crop. It seems that the midge did not have a late summer generation in 2012 and we decided not to do the trial in the absence of the pest as it would have been a waste of resources. Trials will be carried out in 2013.

Conclusions

The female sex pheromones of the two sub-species of *Dasineura oxycoccana* found on blueberry and cranberry have been identified and synthesised by Canadian researchers. Lures containing the two pheromones were provided for testing in the UK and the blueberry midge pheromone was shown to be highly attractive to male blueberry midges. No midges were attracted to the cranberry midge lure in the UK.

Analysis of the compounds in the lures suggested these were saturated and mono-unsaturated 2,14-diacetoxyheptadecanes respectively, and this was confirmed in a subsequent publication by the Canadian group. The pheromone of the blueberry midge has now been synthesised at NRI as both the racemic form and *R,R* stereoisomer and these are available for field testing.

A field trapping test showed that significantly more blueberry midges were caught in traps 0.5 m above ground level than in traps at 1.0 m, and few midges were caught in traps at 2.0 m. Further experiments are required to optimise the loading of pheromone in the lure.

It was not possible to carry out a trial of insecticides against the blueberry midge because of the absence of midges in the proposed trial plot, in spite of the fact that the field was heavily infested during 2011. These trials will be carried out during 2013.

Knowledge and Technology Transfer

None have taken place so far

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