Project title:	Sex pheromone trap for monitoring blackberry leaf midge
Project number:	SF 117
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Report:	Final, March 2012
Previous report:	Annual, March 2011
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Location of project:	NRI/EMR
Industry Representative:	Tom Maynard
Date project commenced:	1 April 2010
Date project completed (or expected completion date):	31 March 2012

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AUTHENTICATION

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

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GROWER SUMMARY

Headline

The female sex pheromone of the blackberry leaf midge has been identified and synthesized. Traps baited with the synthetic pheromone are now commercially available from Agralan and should provide a new, specific method for growers to detect the presence of this pest in order to make timely application of control measures.

Background

Blackberry leaf midge (*Dasineura plicatrix*) has recently developed as a serious pest of blackberry and has now spread to raspberry in the UK and elsewhere in Europe. It attacks the primocane shoot tips, killing the terminals, stunting growth and causing branching. Growers consider that it significantly affects yield in both crops, although no crop damage assessment trials have yet been done. Growers currently have no method of predicting or monitoring attacks or timing sprays to control it. Timing of application is critical with midge pests as the larvae quickly become protected within the leaf rolls and it important not to disrupt natural biocontrol mechanisms.

The female midge produces a powerful sex pheromone attracting males. Identification of the pheromone would make it possible to develop sex pheromone traps for monitoring the pest and timing measures for its control, as has been done for the raspberry cane midge and blackcurrant leaf midge. The traps will be ideal for timing application of control programmes developed by ADAS in project SF102.

The female-produced sex pheromone of blackberry leaf midge was partially identified in an HDC-funded PhD project of Lakmali Amarawardana (CP38 'The Chemical Diversity of Midge Pheromones'). This project aims to complete identification of the chemical structures of the components of the pheromone, to synthesise them and develop effective lures and traps for use by growers. It is anticipated that the traps will prove invaluable in monitoring the pest to assess the need for control measures and to time the application of these more effectively.

Summary of the project and main conclusions

(2*R*,*Z*6,*Z*9)-2-Acetoxy-6,9-pentadecadiene was identified as the major component and (2*R*,*Z*6)-2-acetoxy-6-pentadecene as the minor component of the female sex pheromone of the blackberry leaf midge.

When the synthetic compounds were tested in the field the minor component alone was unattractive to male blackberry midges when tested as the racemic or the separate enantiomers at two different loadings. In contrast, the (*R*)-enantiomer of the major component was highly attractive. The (*S*)-enantiomer and racemic mixture were completely unattractive, indicating that the (*S*)-enantiomer actually inhibits the attractiveness of the (*R*)-enantiomer. Moreover, adding the minor component to the major at a 1:3 ratio significantly increased the attractiveness of the major component.

Further field trials in 2011 confirmed that blends of the two components from 1:1 to 10:1 were attractive to male *D. plicatrix*, and a 2:1 blend of (2*R*,6*Z*,9*Z*)-2-acetoxy-6,9-pentadecadiene and (2*R*,6*Z*)-2-acetoxy-6-pentadecene was adopted as standard. When different loadings of this blend were compared in rubber septa dispensers, loadings of 0.1 – 10 µg were more attractive then higher loadings. A loading of 10 µg was adopted as standard. The 2:1 blend at 10 µg loading of the major component was very significantly more attractive to male *D. plicatrix* than two virgin female midges. However, in this experiment the traps baited with female midges did not catch significantly more midges than unbaited traps suggesting that the females were not in good condition.

Pheromone traps baited with the synthetic lures were successfully used to monitor *D. plicatrix* in blackberry and raspberry fields in the UK and The Netherlands, although further work is required to correlate trap catches with actual adult numbers and subsequent infestations of larvae.

The traps and lures are now commercially available from Agralan.

Financial benefits

A leading grower estimates that attacks of blackberry midge can easily reduce yield of blackberry crops by 10% which would lead to losses of £3,000 per ha on a typical 15 t/ha blackberry crop. The pest is more serious on raspberry and can cause 60% loss in cane height on some modern primocane varieties. If the pest were not controlled and this occurred, 40% crop losses could be expected amounting to a loss of £12,000 per ha.

Action points for growers

Lures and traps for blackberry midge can be purchased from Agralan.

Their use to monitor populations and determine the need and timing for control measures against this pest should be evaluated.

SCIENCE SECTION

Introduction

Blackberry leaf midge (*Dasineura plicatrix*) has recently developed as a serious pest of blackberry and has now spread to raspberry in the UK and elsewhere in Europe. It attacks the shoot tips killing out the terminals, stunting growth and causing branching. Growers consider that it significantly affects yield in both crops, although no crop damage assessment trials have yet been done. Growers currently have no method of predicting or monitoring the severity of attacks or of timing application of control methods. Timing of application is critical with midge pests as the larvae quickly become protected within the leaf rolls and it important not to disrupt natural biocontrol mechanisms.

The female-produced sex pheromone of blackberry leaf midge was partially identified in the HDC-funded PhD project CP38 ‘The Chemical Diversity of Midge Pheromones’ which was recently completed Lakmali Amarawardana. The pheromone was shown to be a blend of mono-unsaturated and di-unsaturated 15-carbon mono-acetates with the acetate at C2. However, the exact positions and configurations of the double bonds in these compounds have not yet been determined.

This project aims to complete identification of the chemical structures of the components of the pheromone, to synthesise them and develop effective lures and traps for use by growers. It is anticipated that the traps will prove invaluable in monitoring the pest to assess the need for control measures and to time the application of these more effectively. Pheromone traps are now available for other bush fruit midges including the raspberry cane midge and blackcurrant leaf midge.

Materials and Methods

Pheromone Identification and Synthesis

Mono- and di-unsaturated 15-carbon acetates were synthesised as candidate pheromone components and their gas chromatographic (GC) retention indices and mass spectra (MS) were determined and compared with corresponding data on the naturally-produced pheromone components obtained in HDC Studentship CP38.

Mono-unsaturated acetates and (*Z,E*)-2-acetoxy-10,13-pentadecadiene were synthesised from the corresponding 14-carbon alcohols which are components of many Lepidopteran sex pheromones and were available in the NRI library of compounds (Figure 1). In some cases the (*E*)-isomers were obtained by isomerisation of the corresponding (*Z*)-isomers by heating at 120°C with a catalytic amount of thiophenol.

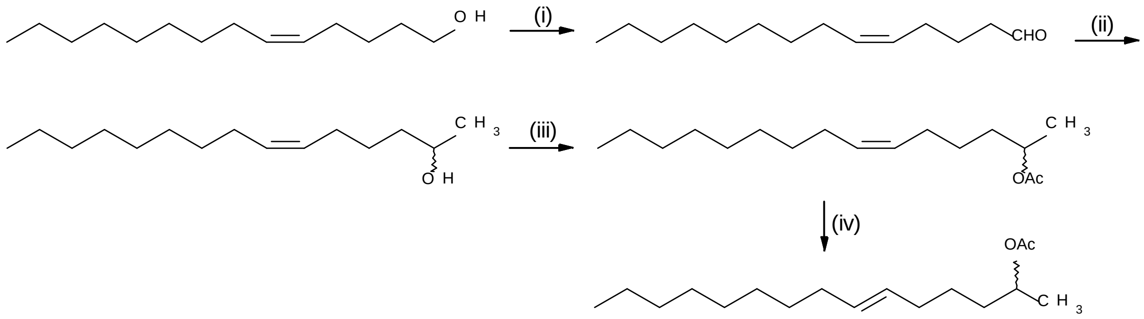


Fig. 1. Synthesis of (*Z*)- and (*E*)-2-acetoxy-6-pentadecene (Reagents (i) PCC/CH_2Cl_2; (ii) MeMgI/ether; (iii) Ac*2*O/pyridine; (iv) 1% thiophenol/120°C)

(*Z,Z*)-2-Acetoxy-6,9-pentadecadiene was synthesised in six steps from 2-octyn-1-ol and 5-hexyn-1-ol as shown in Figure 2. The (*Z,E*)-isomer was synthesised by a similar route replacing the 2-octyn-1-ol with (*E*)-2-octen-1-ol.

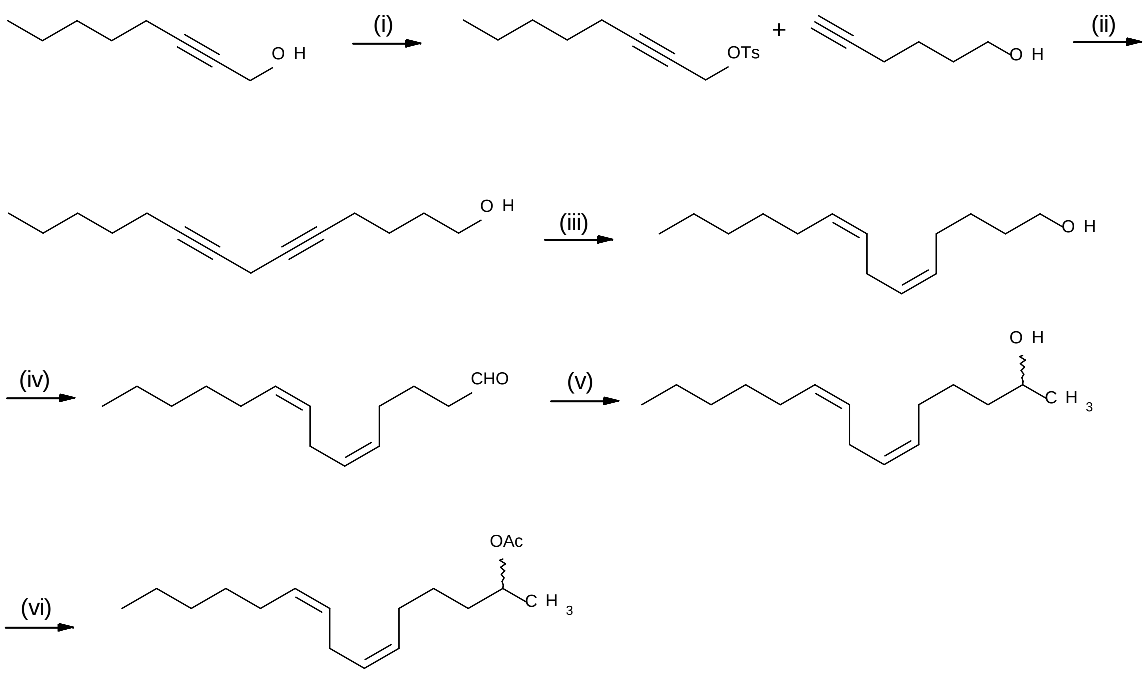


Fig. 2. Synthesis of (*Z,Z*)-2-acetoxy-6,9-pentadecadiene (Reagents (i) TsCl/pyridine; (ii) EtMgBr/THF/CuI; (iii) H_2/Lindlar catalyst or P2-nickel; (iv) PCC/CH_2Cl_2; (v) MeMgI/ether; (vi) Ac_2O/pyridine)

The above syntheses gave racemic products. The corresponding enantiomers were prepared in high enantiomeric purity (>99%) by acetylating the corresponding alcohol in the presence of lipase from Candida antarctica which gave the 2*R*-acetate and unreacted 2*S*-alcohol (Figure 3). These were separated by liquid chromatography and the alcohol acetylated with acetic anhydride and pyridine.

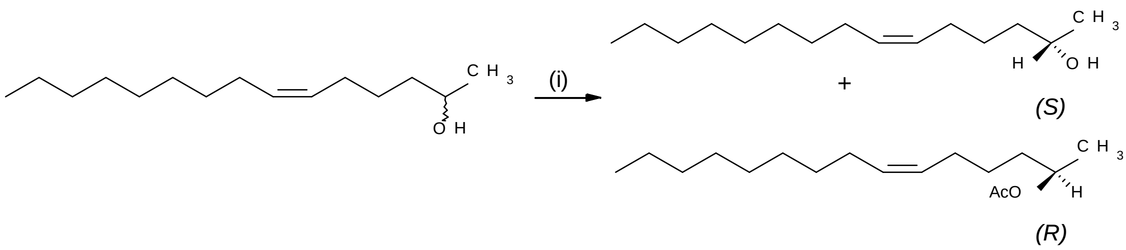


Fig. 3. Enzymatic resolution of enantiomers of (*Z*)-6-pentadecen-1-ol (Reagent (i) lipase from *Candida antarctica*/vinyl acetate/ether)

Pheromone Formulation

White rubber septa (International Pheromone Systems Ltd.) were impregnated with the minor pheromone component, (*Z*)-2-acetoxy-6-pentadecene (100 µg) and maintained in a laboratory wind-tunnel at 27°C and 8 km/hr wind-speed. At intervals volatiles were collected from duplicate septa onto Porapak resin and trapped volatiles quantified by GC analysis.

Field Tests

Field tests to optimise the pheromone blend were carried out during 2010 and 2011 in both blackberry and raspberry fields at Belks Farm and Salmons Farm, Kent. Traps were standard white sticky delta traps positioned 0.5 m above ground level and 25 m apart. Lures were white rubber septa impregnated with pheromone. Traps were set out in randomised complete block designs with up to ten replicates and catches were recorded and discarded weekly.

Total catches in each replicate were transformed to log(x+1), subjected to analysis of variance and differences between means were tested for significance with a Least Significant (LSD) Difference test ($P<0.05$).

Monitoring Trials

During 2011, traps and lures were provided to three growers in the UK and one in The Netherlands. UK growers were Paul Harrold of Sunclose Farm, near Cambridge and Tim Chambers of Langley, Maidstone. Unfortunately records from a third farm were lost as the person taking the records left the farm and did not notify us. In The Netherlands traps were maintained by Hermann Helsen of Applied Plant Research, Wageningen-UR at the BTG Fruit Farm, Zoelmond. Each grower was supplied with a standard red delta trap and also a red delta trap with excluder grids designed at EMR to minimize catch of other insects (Figure 4). Lures were contained the 2:1 blend of (2*R*,6*Z*,9*Z*)-2-Acetoxy-6,9-pentadecadiene and (2*R*,6*Z*)-2-acetoxy-6-pentadecene with 10 µg of the major component. Catches were recorded weekly.

Fig. 4. Red delta trap used in field trials: open (left) and with excluder grids (right)

Results

Pheromone Identification and Synthesis

In HDC Project CP38, analysis of volatiles collected from virgin female *D. plicatrix* by gas chromatography (GC) coupled to electroantennographic (EAG) recording from the antenna of a male midge showed two components eliciting an EAG response. One – the major component – was present in larger quantities than the other – the minor component – and these were assumed to be components of the female sex pheromone. Amounts of material obtained were small and the minor component could not always be detected.

The GC retention indices on polar and non-polar GC columns and the mass spectra indicated that the major component was a 15-carbon compound with an acetate function at C-2 and two non-conjugated double bonds. Data for the minor component suggested it was a similar compound with only one double bond. These proposals were further supported by showing that after catalytic hydrogenation of the collection of volatiles the two pheromone components had disappeared and 2-acetoxypentadecane was formed.

The mass spectra of the pheromone components gave no clear indication of the positions of the double bonds and the amounts present were too small for any further studies such as reaction with dimethyldisulphide, epoxidation or ozonolysis which can be used to determine the positions of double bonds.

Accordingly a number of candidate compounds were synthesised and their GC retention indices and mass spectra compared with those of the natural pheromone components.

For the minor component, mono-unsaturated 2-acetoxypentadecenes were conveniently synthesised from the corresponding tetradecen-1-ols many of which are components of Lepidopteran pheromones. Retention indices of these relative to the retention times of straight-chain acetates on both polar (DBWax) and non-polar (DB5) GC columns are shown in Table 1. The (*Z*6)-isomer had retention indices very similar to those of the minor pheromone component on both GC columns.

Table 1. Retention indices of synthesised mono-unsaturated 2-acetoxypentadecenes and the minor pheromone component relative to the retention times of straight-chain acetates on polar (DBWax) and non-polar (DB5) GC columns.

	Retention Index	
Double Bond	Polar	Non-Polar
Z5	1384	1387
E5	1396	1400
Z6	1392	1390
E6	1398	1397
Z7	1392	1390
E7	1383	1397
Z8	1400	1394
E8	1400	1400
Z9	1403	1398
E9	1402	1401
Z10	1411	1405
E10	1407	1405
Z11	1420	1410
E11	1408	1407
Z12	1417	1415
E12	1417	1410
Minor pheromone component	1391	1388

Comparison of the mass spectrum of (*Z*)-2-acetoxy-6-pentadecene with that of the minor pheromone component showed good agreement (Figure 5). In particular there was an ion at *m/z* 166 that was only present in the spectra of the 6-isomers and not in those of other positional isomers (e.g. Figure 6).

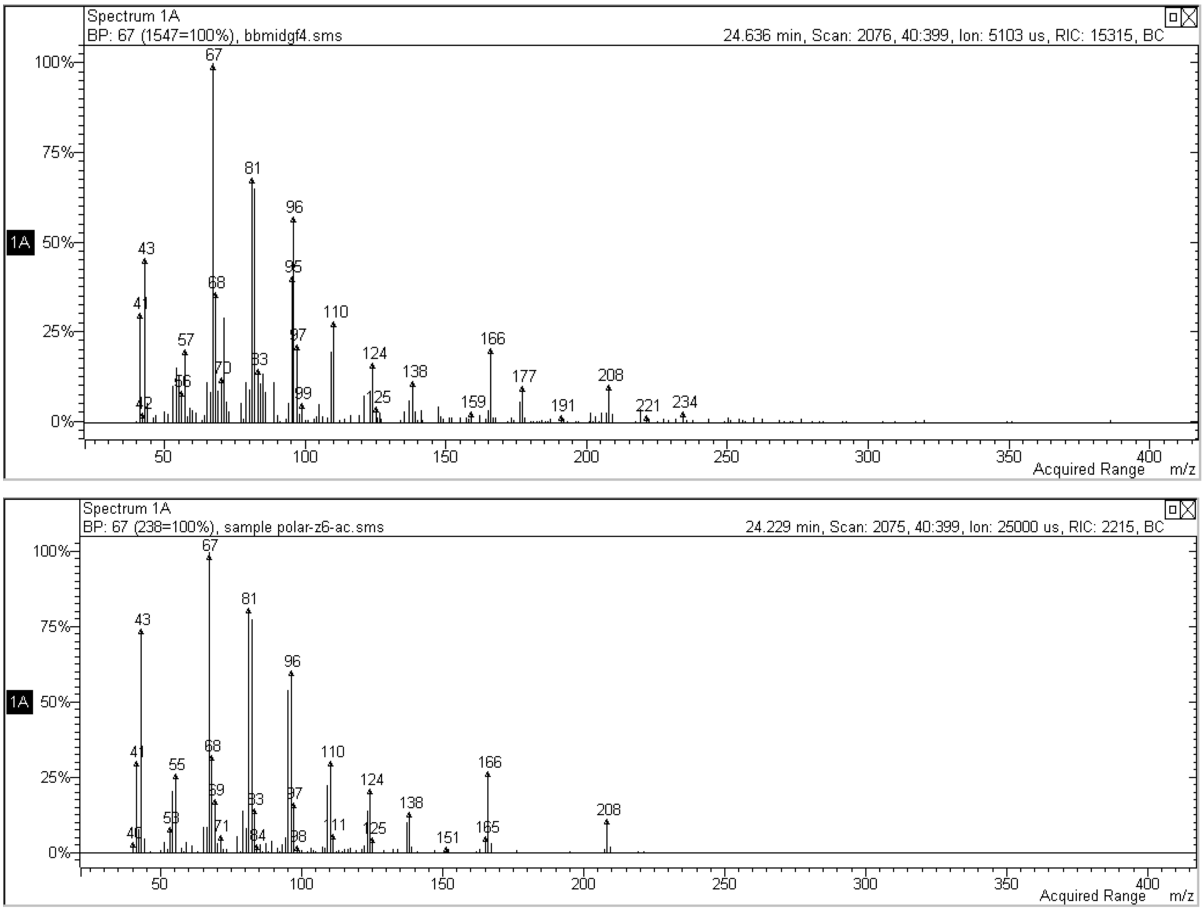


Fig. 5. Mass spectra of minor pheromone component (upper) and (*Z*)-2-acetoxy-6-pentadecene (lower).

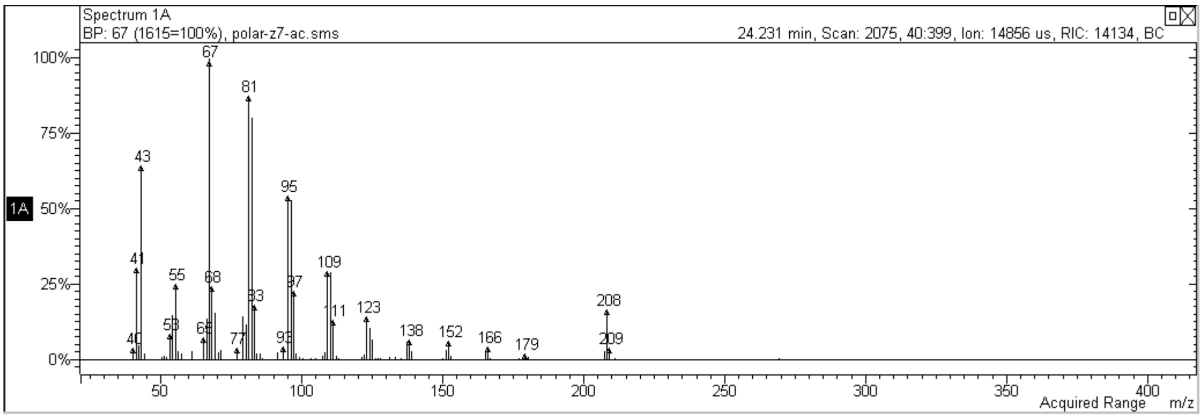


Fig. 6. Mass spectrum of (*Z*)-2-acetoxy-7-pentadecene.

Given the structure of the minor pheromone component, it was thought likely that one of the double bonds in the major component would also be in the *Z*6-position. Calculations based on the relative shifts in retention index for double bonds in the different positions suggested the 6,9-isomer as a candidate compound. The (*Z,Z*)-2-acetoxy-6,9-pentadecadiene was synthesised and shown to have identical retention indices and mass spectrum to those of the natural major pheromone component (Table 2 and Figure 7). The retention indices were different from those for the (*Z,E*)-isomer and also those of (*Z,E*)-2-acetoxy-10,13-pentadecadiene which were also synthesised (Table 2).

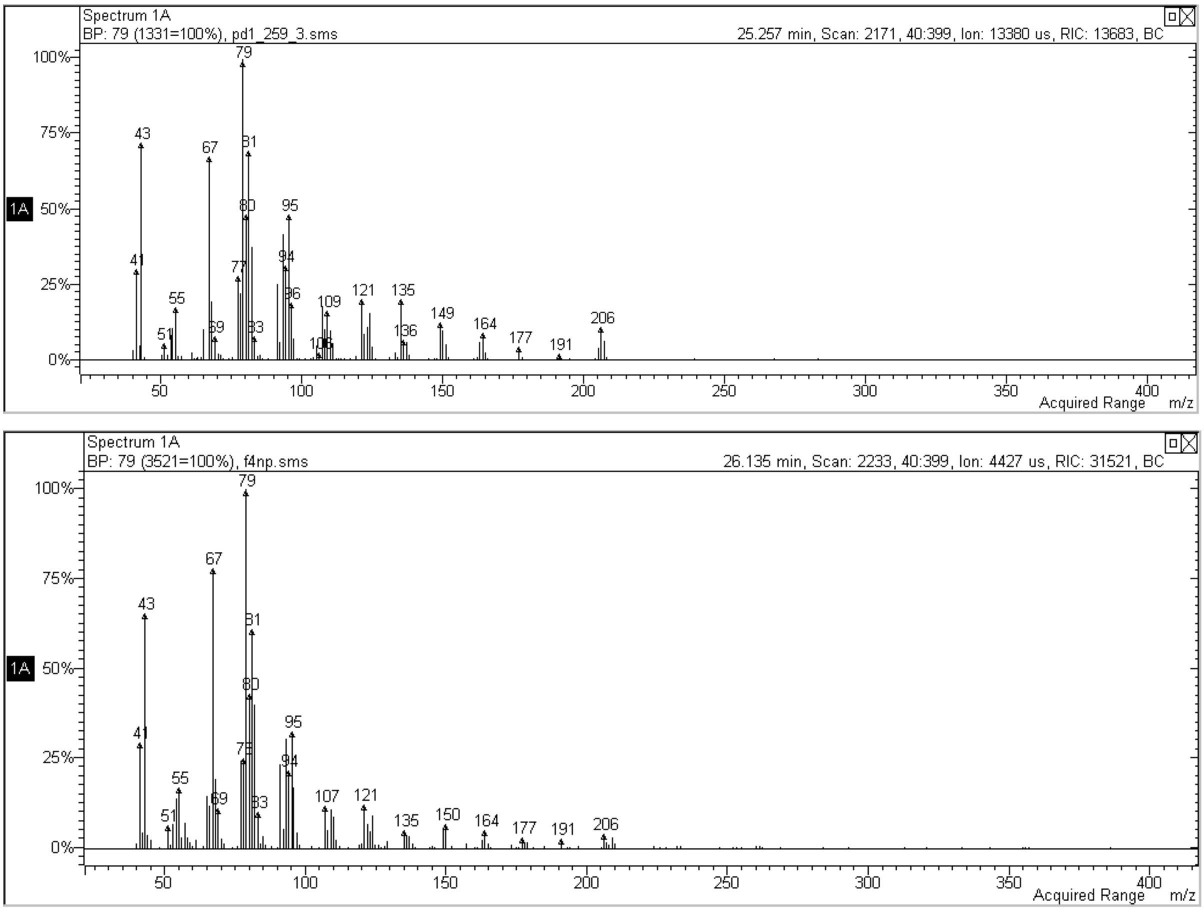
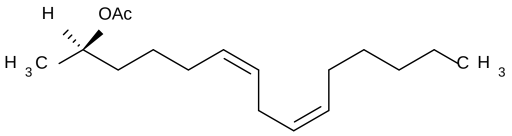


Fig. 7. Mass spectra of major pheromone component (upper) and (*Z,Z*)-2-acetoxy-6,9-pentadecadiene (lower).

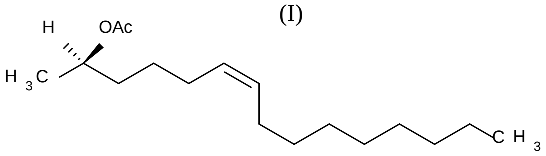
Table 2. Retention indices of synthesised di-unsaturated 2-acetoxypentadecadienes and the major pheromone component relative to the retention times of straight-chain acetates on polar (DBWax) and non-polar (DB5) GC columns.

	Retention Index	
Compound	Polar	Non-Polar
Z6,Z9-C15:2Ac	1435	1382
Unknown diene	1444	1393
Unknown diene	1452	1396
Z6,E9-C15:2Ac	1473	1379
Z10,E13-C15:2Ac	1435	1319
Major pheromone component	1434	1383

From these results, it was concluded that (*Z,Z*)-2-acetoxy-6,9-pentadecadiene and (*Z*)-2-acetoxy-6-pentadecene (Figure 8) were the most promising candidates for the major and minor pheromone components respectively. It was not possible to determine the chirality of the naturally-occurring pheromone components and the (*R*)- and (*S*)-enantiomers of both components were synthesised for field testing.



(I)



(II)

Fig. 8. Proposed structures for pheromone components (2*R*,6*Z*,9*Z*)-2-acetoxy-6,9-pentadecadiene (I) and (2*R*,6*Z*)-2-acetoxy-6-pentadecene (II)

Further evidence for the validity of these structures as pheromone components was obtained in 2011 when EAG responses were obtained from the antenna of a male *D. plicatrix* to both compounds in GC-EAG analyses (Figure 9).

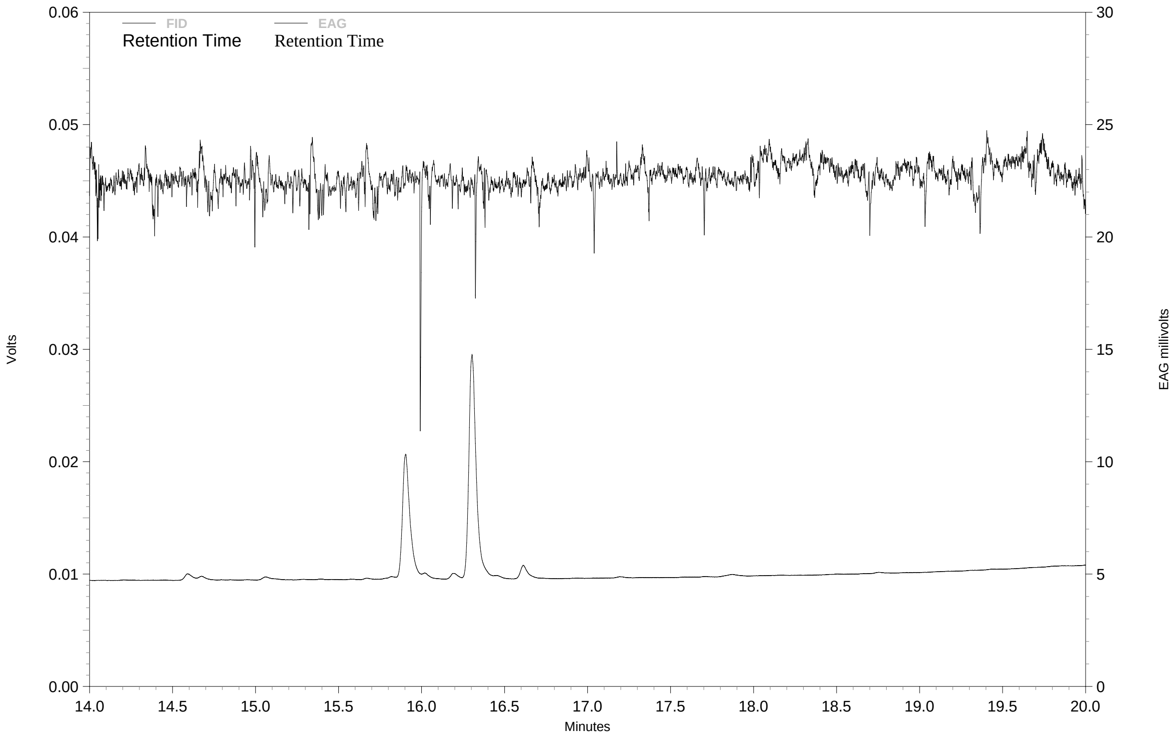


Fig. 9. GC-EAG analysis on polar GC column showing antennal responses (upper trace) to 1:2 mixture of synthetic (2*R*,6*Z*)-2-acetoxy-6-pentadecene (15.89 min) and (2*R*,6*Z*,9*Z*)-2-acetoxy-6,9-pentadecadiene (16.35 min) (lower trace).

Pheromone Formulation

Release of (*Z*)-2-acetoxy-6-pentadecene from rubber septa was initially rapid but then settled down to a relatively constant rate (Figure 10). Release continued for at least 51 days even at 27°C, so lures should last for at least two months in the field under normal UK conditions.

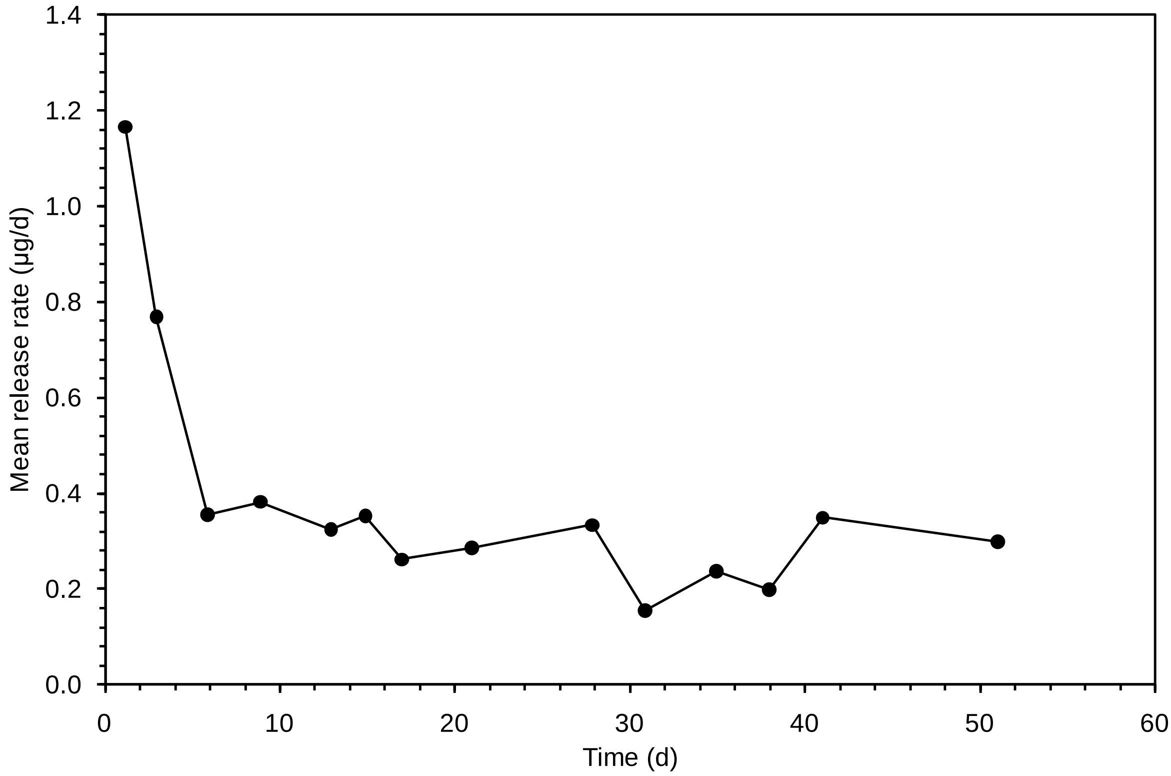


Fig. 10. Release rate of (*Z*)-2-acetoxy-6-pentadecene from rubber septa (initial loading 100 µg; 27°C, 8 km/h windspeed; mean of two replicates)

Field Tests 2010

In a first experiment, the minor pheromone component was tested alone as the racemic and the separate enantiomers at two different loadings. Only low numbers of blackberry midge were captured and there were no significant differences between catches with any of the treatments, including the unbaited control (Table 3).

Table 3. Mean total catches of blackberry midge, *D. plicatrix*, in traps baited with (*Z*)-2-acetoxy-6-pentadecene (10 replicates at Salmons, 10 replicates at Belks; 6 May – 8 June 2011)

Compound	Loading	Mean total catch/trap
racemic	10 µg	0.38
racemic	100 µg	0.75
2S	10 µg	0.65
2S	100 µg	0.55
2R	10 µg	0.63
2R	100 µg	0.35
control	unbaited	0.40

Although there was no “positive control” it was thought that blackberry midge was present and thus that the proposed minor pheromone component alone was unattractive, or the structure was wrong.

In a second experiment, the proposed major compound was tested at one loading as the racemic and separate enantiomers. Results from blackberry plots shown in Figure 11 showed that the (2*R*,6*Z*,9*Z*)-2-acetoxy-6,9-pentadecadiene was significantly attractive to male blackberry midge but the (*S*)-enantiomer and racemic material were not.

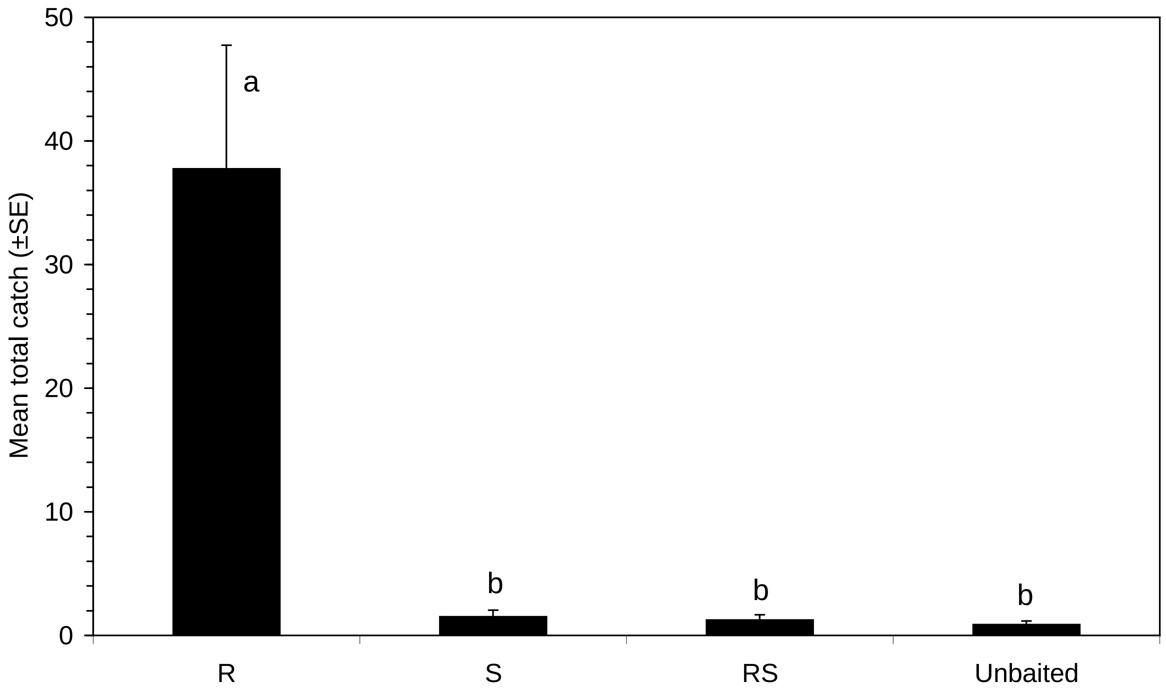


Fig. 11. Trapping of *D. plicatrix* with (*R*)- and (*S*)-enantiomers and racemic (6*Z*,9*Z*)-2-acetoxy-6,9-pentadecadiene (9 June – 19 August 2010; 4 replicates at Belks, 4 replicates at Salmons; bars with different letters are significantly different after ANOVA on data transformed to log(x+1) and LSD test, P<0.05)

A third field test was carried out to determine the effect of blending the two pheromone components together as their (2*R*)-enantiomers. A 3:1 blend of the major and minor components was used as this approximated to the ratio determined by analysis of volatiles from the female midge. Results in Figure 12 confirmed the previous results that the major component alone was significantly attractive to blackberry midge males but the minor component was not. Furthermore, a 3:1 blend of the major and minor components was significantly more attractive than the major component alone.

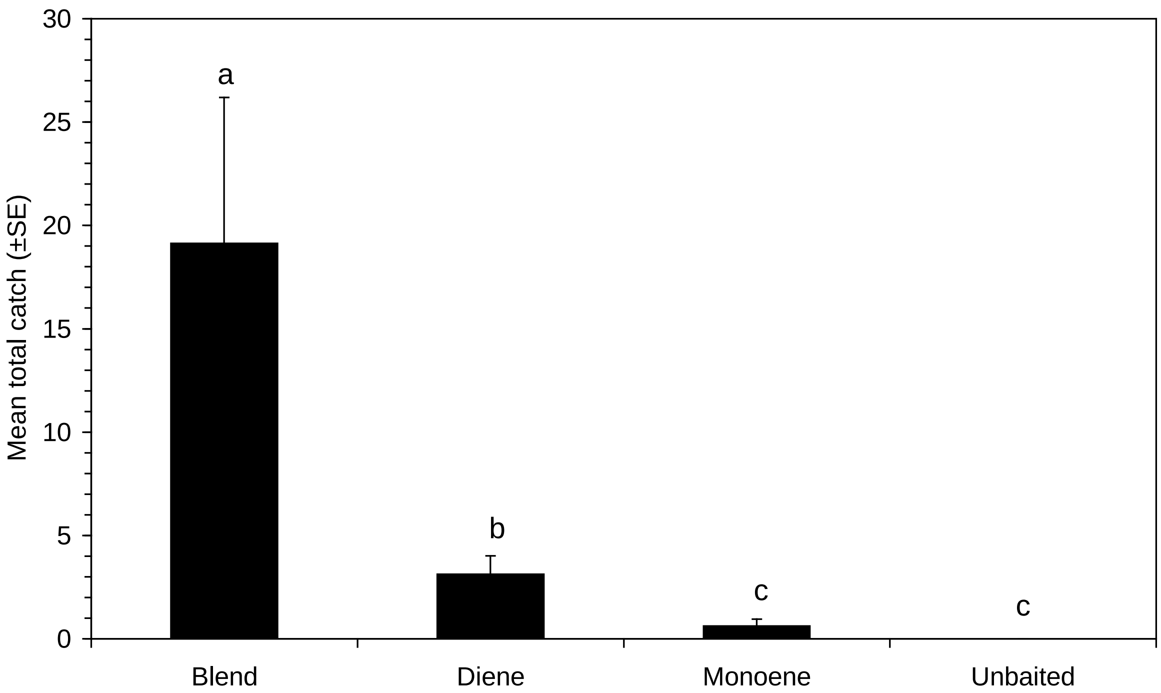


Fig. 12. Trapping of *D. plicatrix* with (2*R*,6*Z*,9*Z*)-2-acetoxy-6,9-pentadecadiene (diene), (2*R*,6Z)-2-acetoxy-6-pentadecene (monoene) and a 3:1 blend (19 August – 17 September 2010; 8 replicates; bars with different letters are significantly different after ANOVA on data transformed to log(x+1) and LSD test, $P<0.05$).

Field Tests 2011

Following on from the third experiment in 2010, a range of different blends of the two pheromone components was tested and blends between 1:1 and 10:1 were found to be equally effective (Figure 13). In this experiment the 2:1 blend of (2*R*,6Z,9*Z*)-2-acetoxy-6,9-pentadecadiene and (2*R*,6*Z*)-2-acetoxy-6-pentadecene caught most midges and this was adopted as the standard blend for further work.

In a second experiment, the 2:1 blend of (2*R*,6*Z*,9*Z*)-2-acetoxy-6,9-pentadecadiene and (2*R*,6*Z*)-2-acetoxy-6-pentadecene was tested at different loadings of the major component of 1-1000 µg. The results (Figure 14) showed that catches decreased as the loading was increased over this range.

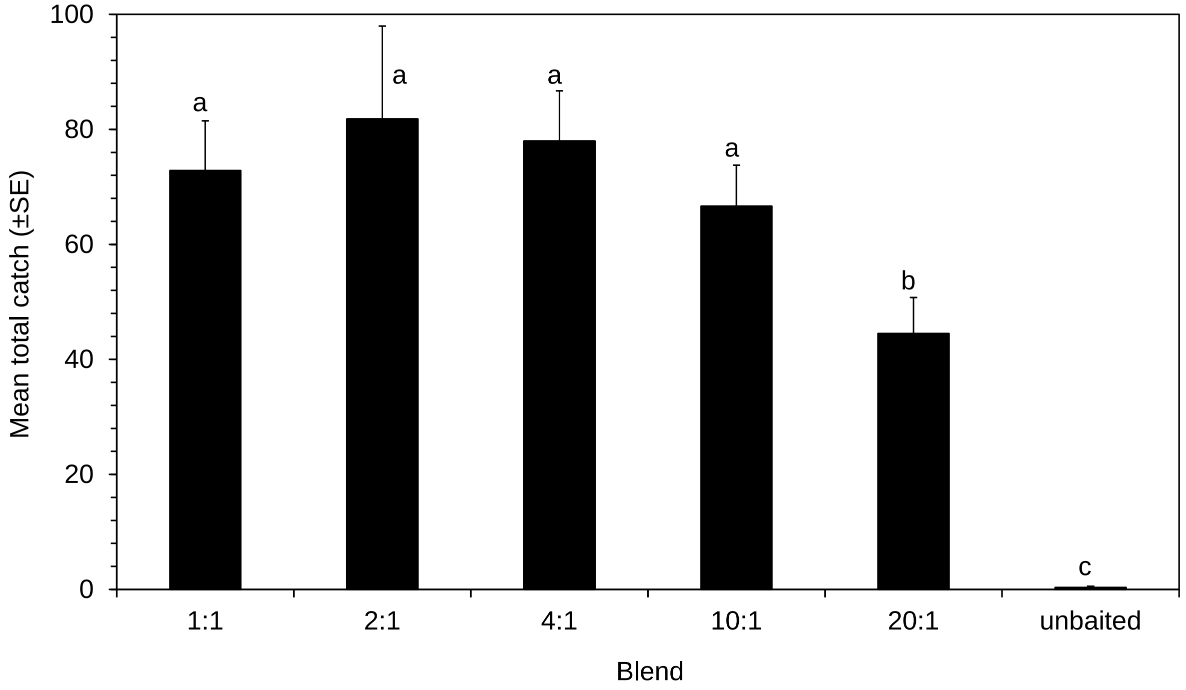


Fig. 13. Trapping of *D. plicatrix* with blends of (2*R*,6*Z*,9*Z*)-2-acetoxy-6,9-pentadecadiene and (2*R*,6*Z*)-2-acetoxy-6-pentadecene (12-26 April 2011; 6 replicates; bars with different letters are significantly different after ANOVA on data transformed to log(x+1) and LSD test, $P<0.05$)

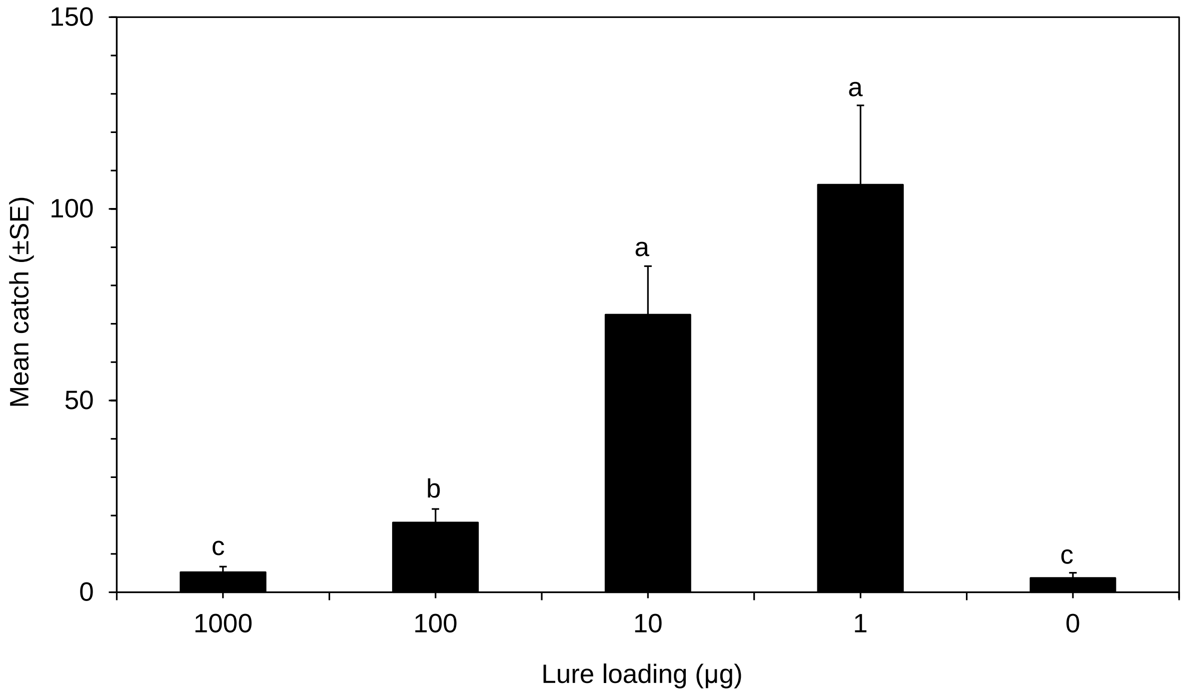


Fig. 14. Catches of *D. plicatrix* males with a 2:1 blend of (2*R*,6*Z*,9*Z*)-2-acetoxy-6,9-pentadecadiene and (2*R*,6*Z*)-2-acetoxy-6-pentadecene at different loadings relative to the major component (2-23 June 2011; 10 replicates; bars with different letters are significantly different P < 0.05 after ANOVA on data transformed to log (x+1) and LSD test)

The effect of pheromone loading was further examined in a third experiment. Results during the first two weeks (Figure 15) confirmed the previous results with highest catches obtained at loadings of 0.1 and 1 µg. However, in this experiment catches with the 0.3 µg loading seemed to be anomalously low and the lures were renewed. During the next week, traps baited with the 0.3 µg loading caught most male *D. plicatrix* (Figure 16), suggesting something was wrong with the lures used in the previous two weeks. There was no significant difference (*P* < 0.05) between the catches with loadings between 0.3 µg and 10 µg, and the latter loading was adopted as standard to ensure maximum longevity under field conditions.

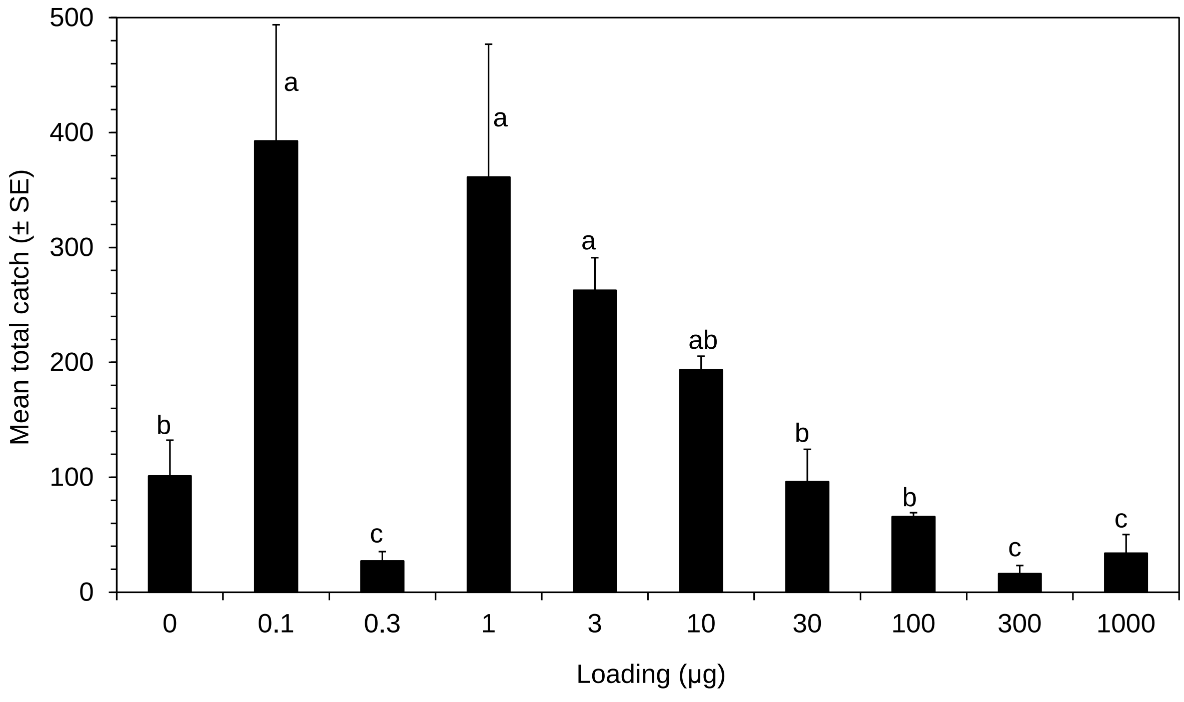


Fig. 15. Catches of *D. plicatrix* males with a 2:1 blend of (2*R*,6*Z*,9*Z*)-2-acetoxy-6,9-pentadecadiene and (2*R*,6*Z*)-2-acetoxy-6-pentadecene at different loadings relative to the major component (29 June – 13 July 2011; 4 replicates; bars with different letters are significantly different P < 0.05 after ANOVA on data transformed to log (x+1) and LSD test)

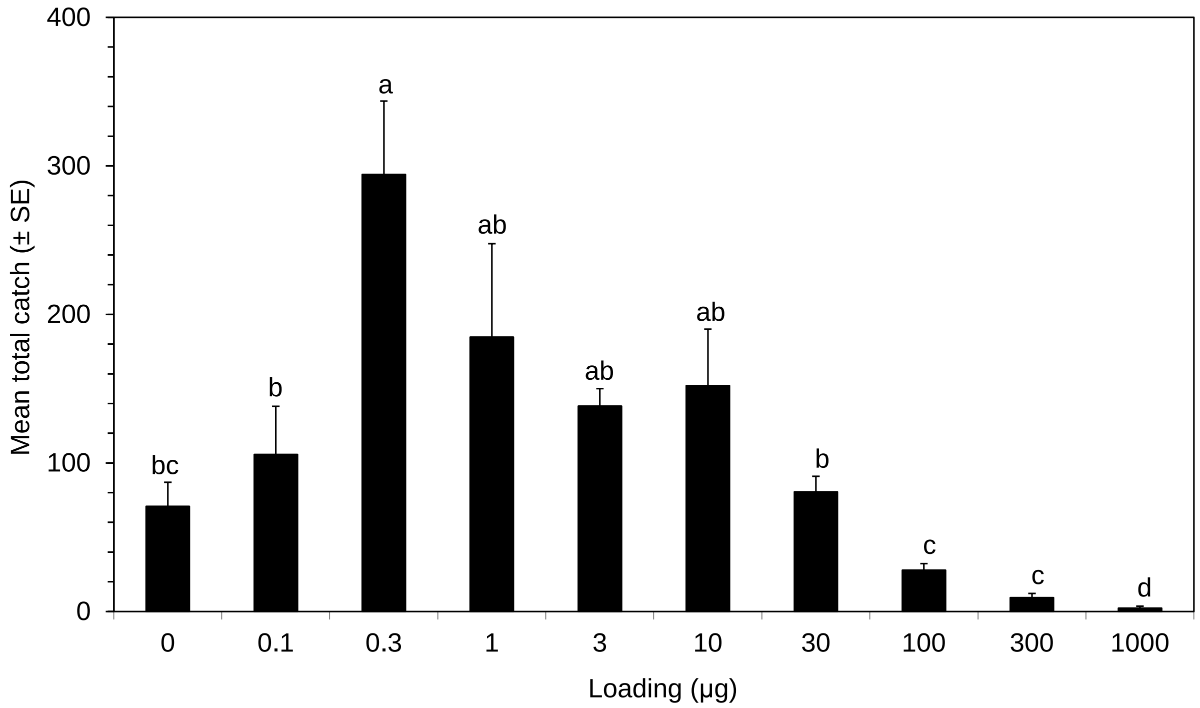


Fig. 16. Catches of *D. plicatrix* males with a 2:1 blend of (2*R*,6*Z*,9*Z*)-2-acetoxy-6,9-pentadecadiene and (2*R*,6*Z*)-2-acetoxy-6-pentadecene at different loadings relative to the major component (15-22 July 2011; four replicates; bars with different letters are significantly different P < 0.05 after ANOVA on data transformed to log (x+1) and LSD test)

The attractiveness of the optimised lure was compared with the attractiveness of two virgin female *D. plicatrix* midges. Ten replicates of each were deployed for one day and catches recorded. In the initial two trials only the virgin female and synthetic lure were compared and significantly more male *D. plicatrix* were caught with the synthetic lure than with the virgin females (Figure 17).

In the third trial an unbaited trap was included. Again significantly more male *D. plicatrix* were caught in traps baited with the synthetic lure than in those baited with virgin females (Figure 18). However, although more midges were caught in traps baited with females than in unbaited traps, the difference was not statistically significant ($P > 0.05$).

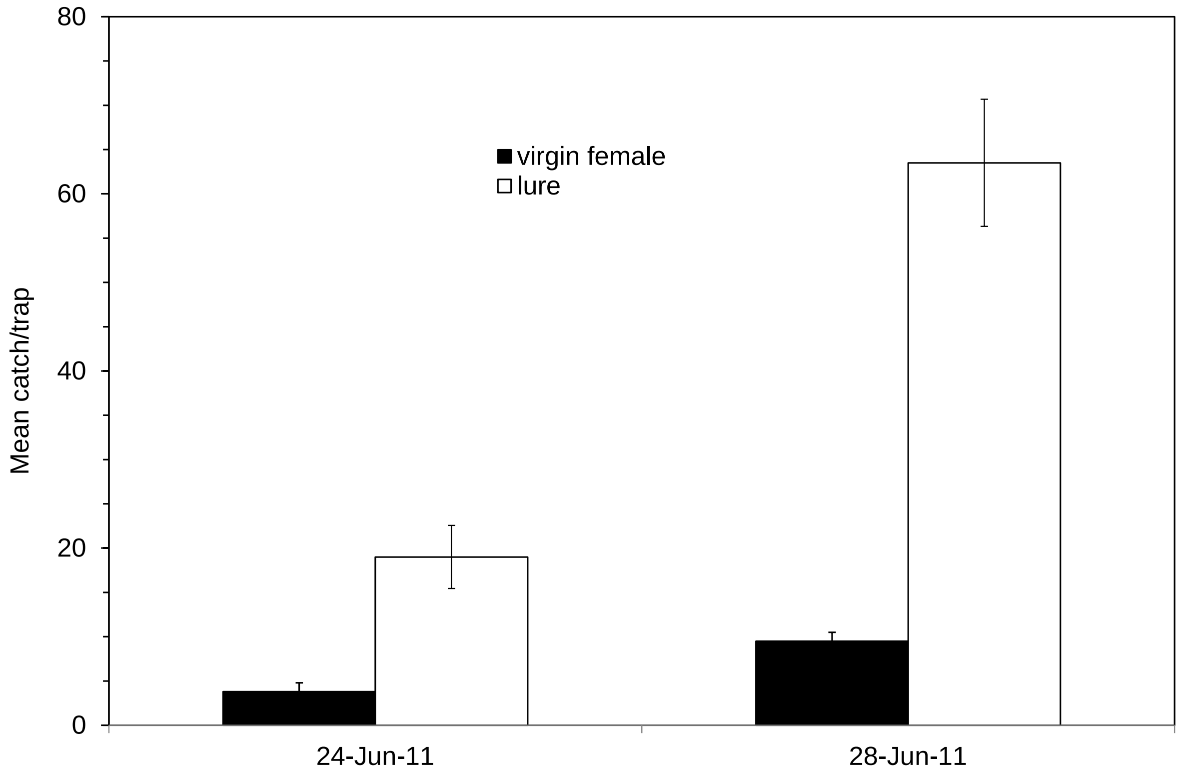


Fig. 17. Catches of male *D. plicatrix* in traps baited with two virgin females or a synthetic lure containing a 2:1 blend of (2*R*,6*Z*,9*Z*)-2-acetoxy-6,9-pentadecadiene and (2*R*,6*Z*)-2-acetoxy-6-pentadecene at 10 µg loading (10 replicates; bars with different letters are significantly different P < 0.05 after ANOVA on data transformed to log (x+1) and LSD test)

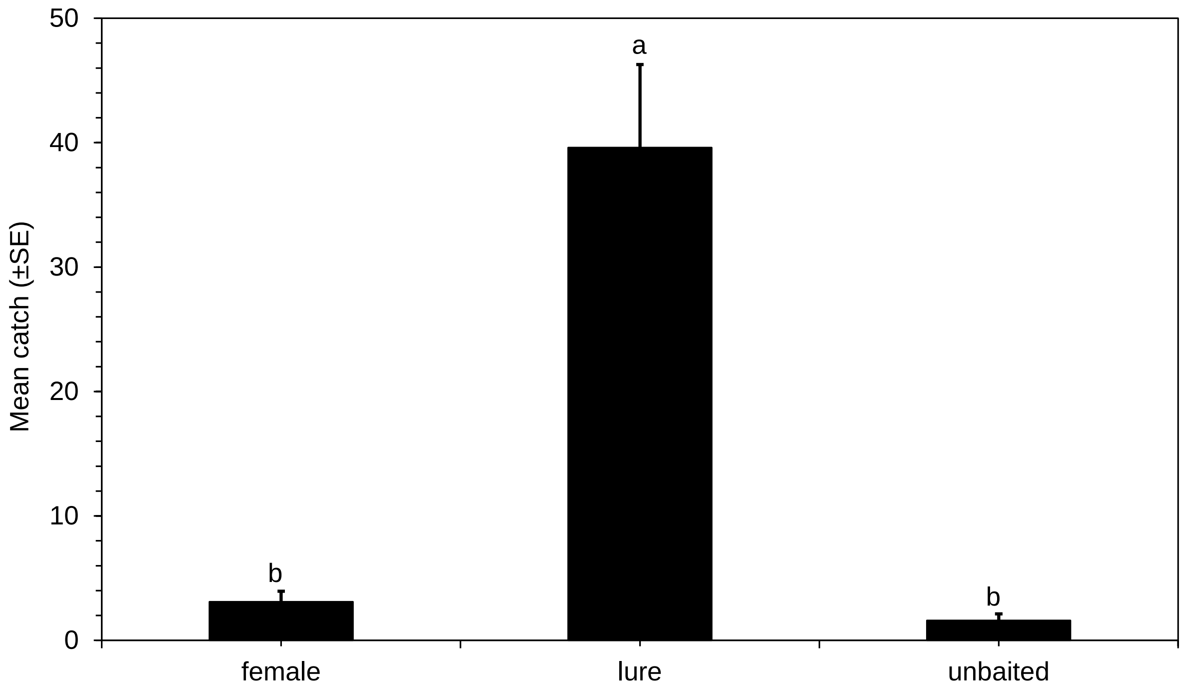


Fig. 18. Catches of male *D. plicatrix* in traps baited with two virgin females, a synthetic lure containing a 2:1 blend of (2*R*,6*Z*,9*Z*)-2-acetoxy-6,9-pentadecadiene and (2*R*,6*Z*)-2-acetoxy-6-pentadecene at 10 µg loading and an unbaited trap (10 replicates; bars with different letters are significantly different $P < 0.05$ after ANOVA on data transformed to log (x+1) and LSD test)

Monitoring Trials

In monitoring trials at Tim Chambers' farm at Langley, near Maidstone, males of *D. plicatrix* were caught in both raspberry (Figure 19) and blackberry fields (Figure 20). There were suggestions of two generations peaking at the end of April and end of June respectively, with numbers dropping off thereafter. In both trials numbers caught in the traps with excluder grids were much lower than those caught in open red delta traps. Lures were changed once on 4 June 2011. There were no obvious increases in catches at this time, suggesting no significant loss of attractiveness of the lures after two months in the field as the trials started on 1 April 2011.

At Sunclose Farm, Cambridge, male *D. plicatrix* were only caught after 30 June 2012 when the lures were renewed (Figure 21).

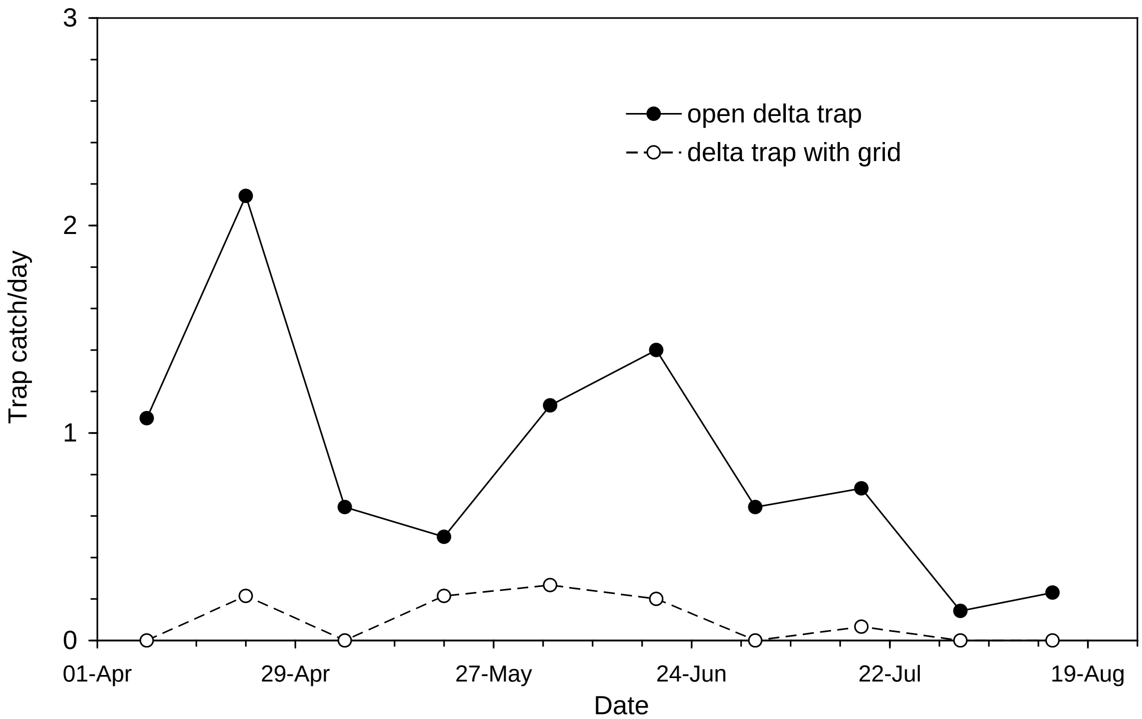


Fig. 19. Monitoring *D. plicatrix* male midges on raspberries (Tulameen) at Langley, near Maidstone, with pheromone traps (25 March - 14 August 2011).

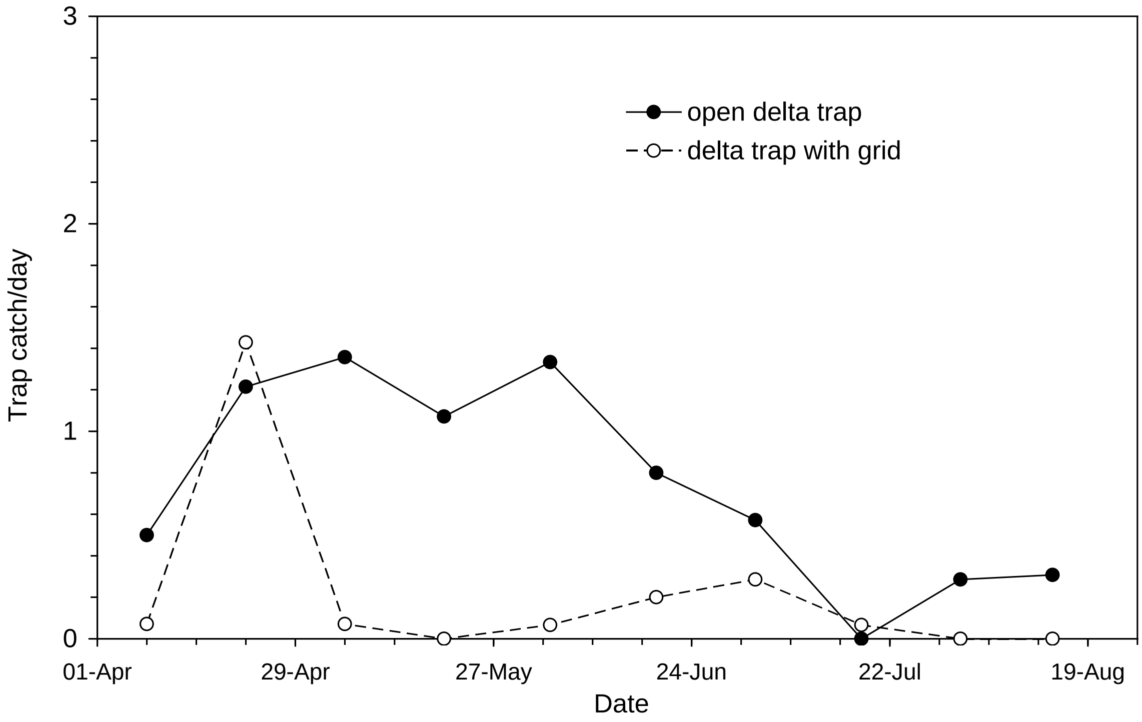


Fig. 20. Monitoring *D. plicatrix* male midges on blackberries (Loch Ness) at Langley, near Maidstone, with pheromone traps (25 March - 14 August 2011).

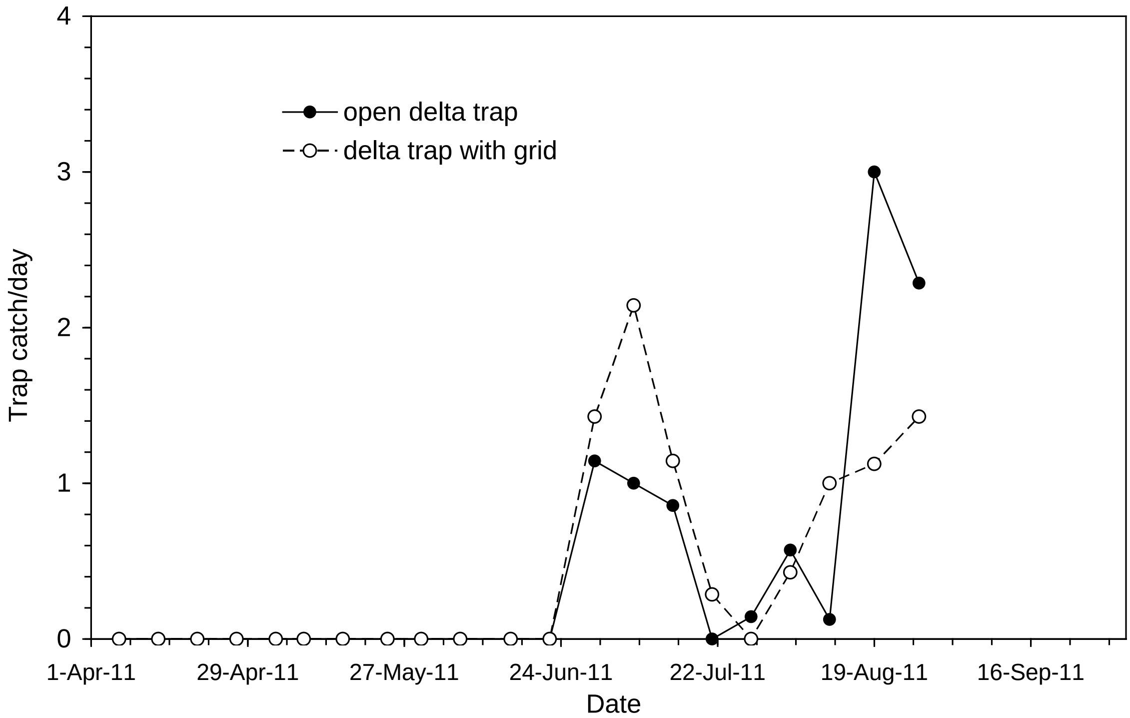


Fig. 21. Monitoring *D. plicatrix* male midges on blackberries at Sunclose Farm, Cambridge, with pheromone traps (6 April – 3 September 2011).

In The Netherlands, pheromone traps were deployed on blackberry grown under polytunnels. The results (Figure 22) suggested there were at least four generations occurring at monthly intervals. A spray of deltamethrin against blossom weevil on 30 May 2011 may have reduced catches at this time. As in the UK, catches in traps with excluder grids were much lower than those in open delta traps.

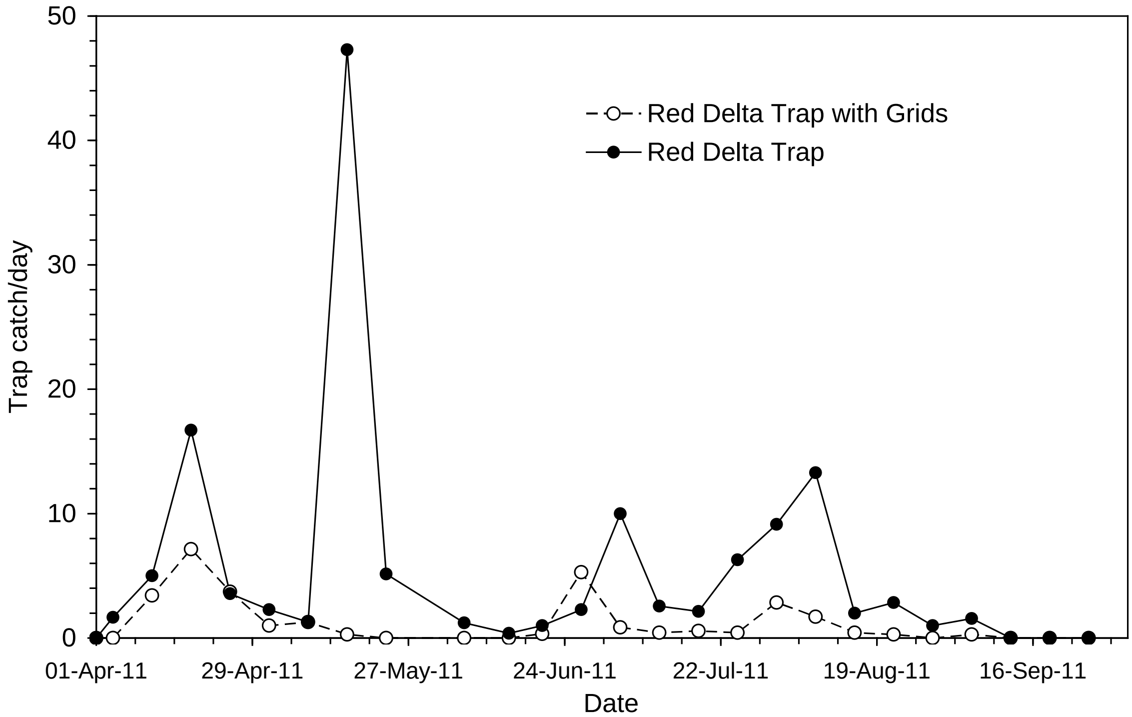


Fig. 22. Monitoring of male *D. plicatrix* in traps baited with pheromone traps at BTG Fruit Zoelmond, The Netherlands (1 April – 26 September 2011).

Discussion

Previous results indicated that the female-produced sex pheromone of the blackberry midge, *D. plicatrix*, consists of two components and that these were 15-carbon compounds with an acetate group at C-2 and two and one double bonds respectively. The current work provides good evidence that these are (2*R*,6*Z*,9*Z*)-2-acetoxy-6,9-pentadecadiene as the major component and (2*R*,6*Z*)-2-acetoxy-6-pentadecene as the minor. The GC retention indices of the synthetic compounds are very similar to those of the natural pheromone components on both polar and non-polar GC columns and their mass spectra match well. In particular, the mass spectrum of the minor pheromone component has a significant ion at *m/z* 166 which was only observed in 2-acetoxy-6-pentadecene isomers and not other positional isomers. Both compounds elicit strong EAG responses from the antenna of a male *D. plicatrix*.

When the synthetic compounds were tested in the field the minor component alone was unattractive to male blackberry midges when tested as the racemic or the separate enantiomers at two different loadings. In contrast, the (2*R*)-enantiomer of the major component was highly attractive. The (2*S*)-enantiomer and racemic mixture were completely unattractive, indicating that the (*S*)-enantiomer actually inhibits the attractiveness of the (*R*)-enantiomer. Moreover, adding the minor component to the major at a 1:3 ratio significantly increased the attractiveness of the major component. Only the (*R*)-enantiomer of the minor component was tested in the blend as it is assumed that the major and minor components have the same configuration.

Further field trials in 2011 confirmed that blends of the two components from 1:1 to 10:1 were attractive to male *D. plicatrix*, and a 2:1 blend of (2*R*,6*Z*,9*Z*)-2-acetoxy-6,9-pentadecadiene and (2*R*,6*Z*)-2-acetoxy-6-pentadecene was adopted as standard. When different loadings of this blend were compared in rubber septa dispensers, loadings of 0.1 – 10 µg were more attractive then higher loadings. A loading of 10 µg was adopted as standard. The 2:1 blend at 10 µg loading of the major component was very significantly more attractive to male *D. plicatrix* than two virgin female midges. However, in this experiment the traps baited with female midges did not catch significantly more midges than unbaited traps suggesting that the females were not in good condition.

Pheromone traps baited with the synthetic lures were successfully used to monitor *D. plicatrix* in blackberry and raspberry fields in the UK and The Netherlands, although further work is required to correlate trap catches with actual adult numbers and subsequent infestations of larvae.

The proposed structures for the pheromone components of *D. plicatrix* follow the pattern found in other midge species of having an unbranched chain of an odd number of carbon atoms with an oxygenated functionality at C-2. However, these are the only examples with 15 carbon atoms identified to date (Hall et al., 2012). The pattern of double bonds is similar to that in the homologous (2*S*,4*Z*,7*Z*)-2-acetoxy-4,7-tridecadiene, pheromone of the Douglas fir-cone gall midge, *Contarinia oregonensis* (Griess et al., 2002).

Conclusions

Components of the female sex pheromone of the blackberry midge, *D. plicatrix*, have been identified as (2*R*,6*Z*,9*Z*)-2-acetoxy-6,9-pentadecadiene and (2*R*,6*Z*)-2-acetoxy-6-pentadecene.

Rubber septa provide convenient dispensers for the pheromone components and should last for at least two months in the field.

The lures was optimised for attractiveness to male *D. plicatrix* as a 2:1 blend of the two pheromone components at 10 µg loading.

Traps baited with the synthetic lure were used to monitor male *D. plicatrix* in both the UK and The Netherlands during 2011. Further work is required to correlate trap catches with actual adult numbers and subsequent infestations of larvae.

Red delta traps with excluder grids caught lower numbers of blackberry leaf midges than red delta traps without grids. However, the use of excluder grids has the advantage of reducing the bicatch of non-target insects to near zero and reduces the risk of misidentification by growers. Traps with excluder grids are not available commercially currently, thought they are supplied to UK blackcurrant growers by East Malling Research.

Knowledge and Technology Transfer

An update on the work was provided for the Soft Fruit Agronomists' Handbook 2011.

Paul Douglas, David Hall, Lakmali Amaradana, Adrian Harris, Gloria Endredi, Jerry Cross and Tracie Evans (2011). Identification of the female sex pheromone of the blackberry leaf midge, *Dasineura plicatrix*. Poster presented at Annual Meeting of the Royal Entomological Society, University of Greenwich, Chatham Maritime, 7-9 September 2011.

Paul Douglas, David Hall, Lakmali Amaradana, Adrian Harris, Gloria Endredi, Jerry Cross and Tracie Evans (2011). Identification of the female sex pheromone of the blackberry leaf midge, *Dasineura plicatrix*. Presentation given at Fruit Focus, EMR, November 2011.

NRI will supply lures to Agralan for sale to growers as a package with an appropriate trap for season-long monitoring.

References

Gries, R., Khaskin, G., Gries, G., Bennett, R.G., King, S.G.G., Morewood, P., Slessor, K.N. and Morewood, W.D. (2002). (*Z,Z*)-4,7- tridecadien-(*S*)-2-yl acetate: sex pheromone of Douglas–fir cone gall midge, *Contarinia oregonensis*. *Journal of Chemical Ecology.* 28: 2283-2297.

Hall, D.R., Amarawardana, L., Hilbur, Y., Boddum T. and Cross, J.V. (2012). The chemical ecology of plant-feeding midges. *Journal of Chemical Ecology,* 38:2-22.