Project number:	SF012 (GSK217)
Title:	Developing a sex pheromone lure and trap for blackcurrant leaf midge 2008
Project leader:	Jerry Cross East Malling Research
Final report:	*Contract report for GlaxoSmithKline and the Agriculture & Horticulture Development Board* Annual report, 2008
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Location of project:	Stonebridge & Wellbrook

DISCLAIMER

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.

Signature .... Date ....

Report authorised by:

Signature .... Date ....

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GROWER SUMMARY

Headline

- Good progress is being made in developing a sex pheromone lure and trap for blackcurrant leaf midge control in commercial blackcurrant plantations.

Background and expected deliverables

The chemical structure of the blackcurrant leaf midge pheromone, a mono-unsaturated 17 carbon diacetate, has been determined by EMR and NRI in previous work. The compound has 4 stereoisomers but until now, it was not known which one of the four is attractive or whether the other stereoisomers have inhibitory activity. The pheromone racemate (a mix of the 4 stereoisomers) had also been synthesised and the 4 individual stereoisomers separated by High Pressure Liquid Chromatography (HPLC).

The project work expected to demonstrate which stereoisomer is attractive and which are inhibitory, to identify a suitable dispenser, optimise pheromone blend and release rate, develop a practical effective trap and demonstrate its use for monitoring the pest in commercial plantations.

Summary of the project and main conclusions

- The third stereoisomer eluting from the HPLC column ‘C’ was shown to be the natural attractive stereoisomer and the other 3 isomers were shown to be unattractive.

- The first and fourth eluting stereoisomers A and D are partially inhibitory of the natural attractive stereoisomer C, whilst the second eluting, B, was not.

- The racemate is attractive but significantly less so than the single natural stereoisomer. It would be possible to use the racemate in monitoring lures, but sensitivity would be low.

- Rubber septa lures loaded with 1 µg of the racemate showed maximum attractiveness, higher dose lures being progressively less attractive and those with > 30 µg of the racemate being virtually unattractive.

- Lures loaded with the natural attractive stereoisomer C were much more attractive, though the attractiveness of high lure loadings (> 10 µg C/lure) was not investigated. A lure loading of 10 µg C is proposed as a standard for sex pheromone monitoring traps, though further work is needed to confirm that this gives adequate sensitivity without leading to excessive catches and trap saturation.

- Pheromone traps deployed at a height of 3 cm above the ground caught the greatest numbers of midges with strongly (exponentially) decreasing catches at greater heights. Traps at 30 cm caught < 30% of the numbers of midges compared to those at 3 cm and traps at 1 m height caught very few midges. A height of trap deployment of 3 cm is recommended for maximum sensitivity.

- No significant effects of trap or sticky base colour on leaf midge catches in sex pheromone traps were found. Catches of non-target insects were affected by trap colour, black traps having the smallest catches, yellow ones the greatest.

- Sex pheromone traps deployed in 7 different commercial blackcurrant plantations in England and Scotland showed >10 fold variation in catches. The date of first catches in spring also varied between the week of 21 April and the week of 26 May. Sprays of chlorpyrifos and/or thiacloprid (Calypso) applied at most sites in April to May failed to prevent continuing catches of midges in the sex pheromone traps or the occurrence of a high percentage shoot damage at some sites.

- Overall, the results indicate that blackcurrant leaf midge sex pheromone traps will prove useful for monitoring the timing and numbers of midges attacking different blackcurrant plantations and could lead to an improvement in the control of midge if they are used to time sprays.

- A practical, free-standing 20 x 20 cm base red delta trap with a fine mesh grid at each entrance to prevent non-target contamination is proposed for future development, as a standard blackcurrant leaf midge sex pheromone monitoring trap.

Work proposed in 2009

Although the experiments carried out during 2008 show that stereoisomer C of the blackcurrant midge pheromone is the attractive isomer, the absolute configuration of this is unknown. Work is ongoing at NRI to determine the absolute configuration of stereoisomer C as part of Ms Lakmali Amarawardana's PhD studies. Once this is known, it will be necessary to devise a synthetic route to this stereoisomer as separation of commercial quantities of this isomer by HPLC would be impractical. It is hoped that an application for a new 3 year PhD studentship recently made by EMR and NRI will be supported for continued work on the sex pheromones of blackcurrant leaf midge as well as blackberry midge and blueberry leaf midge. However, such a studentship would not effectively start until 2010.

The following work could be considered for 2009:

- Field testing the proposed new blackcurrant leaf midge delta trap with vs. without grids at entrances.

- Examining the relationship between trap catches and numbers of galls formed in different commercial crops to establish a trap threshold.

- Conduct a spray trial to examine how sex pheromone traps can be used for timing of sprays of insecticides that are known to be effective against blackcurrant leaf midge (e.g. chlorpyrifos and a pyrethroid).

- Conduct a spray trial to examine the efficacy of novel insecticides for control of blackcurrant leaf midge, with sprays timed according to sex pheromone trap catches.

- Establish the relationship between lure load and male midge attraction for stereoisomer C.

- Prepare mating disruption and 'attract and kill' formulations of the racemate and stereoisomer C for field evaluation for control of the midge in 2010.

Financial benefits

Once the sex pheromone trap has been refined and trap thresholds developed, growers will improve their monitoring of the pest in blackcurrants, helping them to improve the timing of spray application, potentially reducing the number of insecticide applications required to gain control.

Action points for growers

- At present, there are no direct action points emanating from this work, but growers should follow the results of further work with an expectation of acquiring pheromone traps on a commercial basis.

SCIENCE SECTION

General introduction

The chemical structure of the blackcurrant leaf midge pheromone, a mono-unsaturated 17 carbon diacetate, was determined by us in previous work (see 2007 report). The compound has 4 stereoisomers but before this work was done it was not known which of these is attractive or whether the opposite isomers have an inhibitory activity. A substantive quantity of the pheromone racemate (a mix of the 4 stereoisomers) had been synthesised and the 4 individual stereoisomers separated by High Pressure Liquid Chromatography. A possible minor component of the pheromone, the corresponding acetoxy ketone, had also been identified and synthesised. Work was needed to demonstrate male midge attraction, identify a suitable dispenser, optimise the pheromone blend and release rate, develop and calibrate a practical effective trap for monitoring the pest by UK blackcurrant growers and timing of sprays.

The overall aim of the proposed work was to develop an effective sex pheromone lure and trap for practical monitoring of blackcurrant leaf midge populations. The contracted work was divided into 4 objectives:

1. Determination of the best attractive blend
2. Determination of the effects of release rate and a standard lure loading for pest monitoring
3. Determine effects of height of deployment
4. Determine seasonal flight pattern in commercial plantation

In addition to the contracted work, additional work was done to determine

5. Effects of trap colour
6. Improve trap design

Pheromone synthesis and preparation of stereoisomers

The racemic minor pheromone component was synthesised from 6-bromohexanoic acid in eight steps and this was converted to the major component in two further steps. The four stereoisomers of the major component were separated by high performance liquid chromatography using a Chiralpak AD-H column (150 mm x 4.6 mm i.d.; Daicel Chemical Industries Ltd.) with a PU-2080 *plus* pump (Jasco) and a manual injector with a loop capacity of 20 µl. Elution of compounds was monitored with a UV-2075 *plus* detector (Jasco) at 200 nm and the data was captured and processed with EZChrom Elite software. Baseline separation of three peaks was achieved by isocratic elution with 0.4% of propan-2-ol/hexane and flow rate of 0.6 ml/min. Two (A & B) of the four stereoisomers were eluted together and the resolution of above were carried out with 0.15% isopropane-2-ol in hexane in 0.3 ml/min.

A volume of 10 µl of a racemic mixture (1 mg/ml in hexane) was separated at a time, giving approximately 2 µg of each isomer. The isomers were collected, by hand, separately into sample vials and each isomer quantified by gas chromatography.

Objective 1. Determination of the best attractive blend

Tasks 1.1 and 1.2. Demonstration of attractiveness and identification of the active stereoisomers and identification of any inhibitory stereoisomers

Introduction

The blackcurrant leaf midge sex pheromone has four stereo isomers, only one of which is likely to be the natural attractive one. The objective of this experiment was to determine whether or not the racemate (an equal mixture of all 4 stereo isomers) is attractive, which of the 4 individual stereoisomers is attractive and which if any are inhibitory. Note that the best outcome would have been that the racemate is attractive and that the minor component is unnecessary as this is the simplest and least costly in terms of chemical synthesis. However, in some midges (e.g. the pear leaf midge) the racemate is not attractive because the activity of the attractive (natural) stereoisomer is inhibited by one of the other isomers. Results from the grower monitoring sites indicated that the third stereo isomer eluting from the HPLC column ‘C’ was the attractive isomer so further treatments investigated whether the racemate was attractive and whether any of the other 3 isomers inhibited the C isomer.

Methods and materials

A replicated experiment was done in two midge infested commercial blackcurrant plantations from 12 May – 9 June 2008 to test the attractiveness to blackcurrant leaf midge males of lures containing different stereoisomer blends. Treatments comprised the 4 individual stereo isomers, the racemate, the 3 two way blends including stereoisomer C and an untreated (no lure) control (Table 1.1). The commercial plantations were a Ben Alder blackcurrant plantation at Stonebridge, Horsmonden (Location NGR TQ 719 399) (kind permission of Tom Maynard) and a Ben Gairn blackcurrant plantation at Wellbrook Farm, Faversham (Location NGR TR 028 593) (kind permission of Steven Holmes). Treatments were applied as rubber septa lures in white delta traps with sticky bases. The traps were set at a height of 3 cm above the ground and spaced >20 m apart.

Table 1.1. Treatments in blends experiment

Treatment name	**Description**	**Amount (µg / lure)**
A	first HPLC eluting stereoisomer	5
B	second HPLC eluting stereoisomer	5
C	third HPLC eluting stereoisomer	5
D	fourth HPLC eluting stereoisomer	5
R	Racemate	20
AC	1 + 3 above	5 + 5
BC	2 + 3 above	5 + 5
DC	3 + 4 above	5 + 5
Blank	No lure	0

Randomised complete block designs with 3 replicates were used at each of two sites. Plots comprised 1 standard white delta trap hung with its base at approximately 3 cm above the ground on canes adjacent to the bush rows so that machinery access in the alleys was not restricted. Each block comprised of 9 traps arranged in one row of blackcurrant bushes. At Stonebridge, the traps were spaced 23 m apart in the row and at 8 row intervals (= 24 m). At Wellbrook, the traps were spaced 20 m apart in the row and at 7 row intervals (= 21 m). The number of blackcurrant leaf midge males (and females, if present) in each trap were counted on 15 May, 26 May, 2 June and 9 June 2008. Analysis of variance was done on the total counts per trap after square root transformation to stabilise variances. Means were compared using simple least significant difference (LSD) tests (at p=0.05, p=0.01, p=0.001).

Results

The results clearly showed that the third stereoisomer eluting from the HPLC column ‘C’ was the natural attractive one and the other 3 stereoisomers were unattractive (Table 1.2, Figure 1.1). The racemate was also attractive, but significantly less so than C. In the two way blends with C, both AC and DC caught significantly smaller numbers of midges (59% and 32% less) than C alone, though BC did not differ significantly from C alone. Further statistical analysis indicated that A had strong inhibitory activity on the natural C stereoisomer (p<0.001). There was also an indication that D was inhibitory (p=0.067), but that B was not (p=0.451). Both inhibitory stereoisomers occur in the racemate and the accumulated effect of this is that the racemate captured significantly less than AC or DC.

Table 1.2. Mean total numbers of male blackcurrant leaf midges captured per trap at each site in the blends experiment, overall mean and the mean square root transformed numbers per trap subjected to analysis of variance

Treatment	Total catch per trap (n)			Mean(√n)	
	Stonebridge	Wellbrook	Mean		
A	4.7	10.0	7.3	2.65	
B	2.7	4.3	3.5	1.68	
C	217.0	189.0	203.0	13.84	***
D	7.0	2.7	4.8	1.95	
R	33.3	37.7	35.5	5.83	**
AC	78.3	88.0	83.2	8.93	***
BC	187.3	278.0	232.7	14.85	***
DC	193.3	83.3	138.3	11.42	***
Blank	8.7	3.7	6.2	2.38	
Fprob				<0.001	
SED(40 df)				1.180	
LSD (p=0.05)				2.385	

* significantly greater than control p≤0.05
** significantly greater than the control p≤0.01
** significantly greater than the control p≤0.001

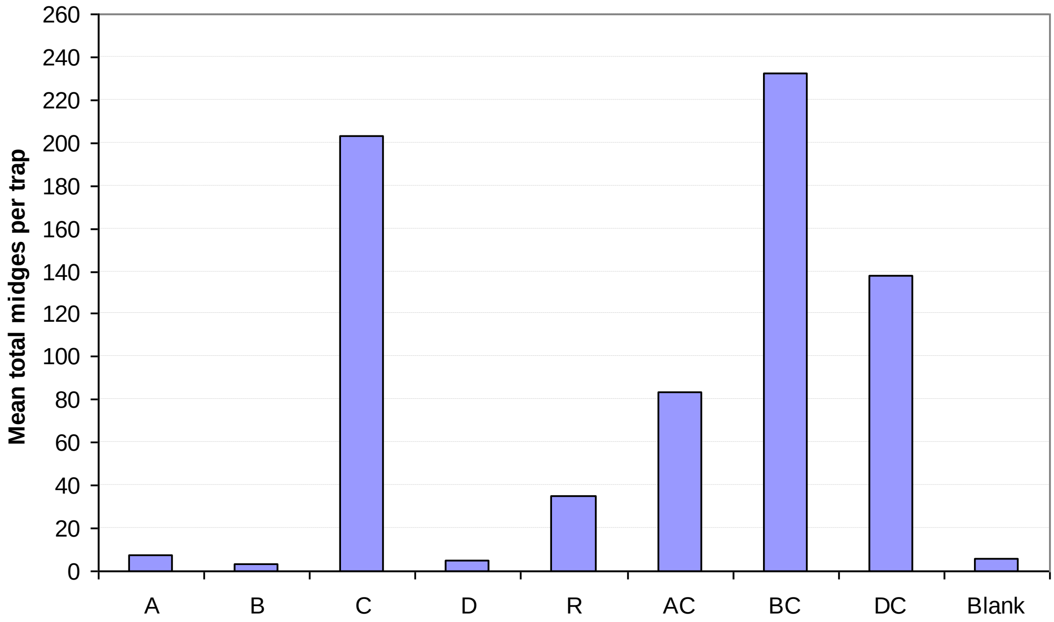


Figure 1.1. Mean total numbers of male blackcurrant leaf midges captured per trap in the blends experiment. [*, **, *** significantly greater than control (blank) at p≤0.05, p≤0.01, p≤0.001 respectively]

Conclusions

- The third stereoisomer eluting from the HPLC column, C, is the natural attractive stereoisomer and the other 3 isomers are unattractive
- The first and fourth eluting stereoisomers A and probably D are partially inhibitory of the natural attractive stereoisomer C, the second eluting, B, was not. The inhibitory activity of A was much stronger than that of D
- The racemate is attractive but significantly less so than the single natural stereoisomer. It would be possible to use the racemate in monitoring lures, but sensitivity would be reduced

Task 1.3. Determining the benefits of addition of the minor component

Once the best major component blend has been determined in tasks 1.1 and 1.2, a further experiment will be conducted to test whether or not addition of the minor component is beneficial. There are two stereoisomers of the minor component and addition and each of these and the racemate will be evaluated at two ratios.

Objective 2. Determination of the effects of release rate and a standard lure loading for pest monitoring

Task 2. Determining effects of release rate

Introduction

The rate of release of pheromone from rubber septa dispensers commonly used for midges is proportional to the amount of pheromone in the lure. The effect of the rate of release of pheromone on attraction varies greatly between different midge species. In apple leaf midge, increasing the release rate increases male attraction and no maximum has yet been found. In raspberry cane midge, there is a sharp rise in attraction with increasing release rate, but a maximum is reached (at the rather low loading of 100 µg/lure) beyond which the number of males captured greatly decreases. Determining this relationship for the blackcurrant leaf midge is of fundamental importance. Note that standard white 20 x 20 cm delta traps have proved effective for apple leaf midge and raspberry cane midge and are likely to be suitable for blackcurrant leaf midge.

In the first blends experiment reported above under objective 1, the blackcurrant leaf midge sex pheromone racemate was shown to be attractive, though significantly less so than stereoisomer ‘C’. The objective of this study was to determine the effect of release rate of the racemate on attractiveness.

Methods and materials

Experiment A: Effects of racemate over wide range of lure loadings

A replicated experiment was done in the same two midge infested commercial blackcurrant plantations from 9 June – 7 July 2008 to test the effect of release rate of the racemate, as determined by lure loading, on attractiveness to blackcurrant leaf midge males. Treatments comprised the 8 serial lure loadings, the racemate and a zero loading control (Table 2.1). The commercial plantations were the same as used in the blends experiment (objective 1); Ben Alder blackcurrant plantation at Stonebridge, Horsmonden (Location NGR TQ 719 399) (kind permission of Tom Maynard) and a Ben Gairn blackcurrant plantation at Wellbrook Farm, Faversham (Location NGR TR 028 593) (kind permission of Steven Holmes). Treatments were applied as rubber septa lures in white delta traps with sticky bases. The traps were set at a height of 3 cm above the ground and spaced >20 m apart.

Table 2.1. Treatments in experiment A

Treatment name	Initial lure loading (µg/rubber septum)	Estimated release rate of racemate (pg/h)†
R0	0	
R1	1	0.4
R3	3	1.2
R10	10	4
R30	30	12
R100	100	40
R300	300	120
R1000	1000	400
R3000	3000	1200

† Based on preliminary measurements of ~10 ng/day released from 1 mg racemate loaded rubber septa in a laboratory wind tunnel at 27˚C and 8 km/h windspeed

Randomised complete block designs with 3 replicates were used at each site. Plots comprised 1 standard white delta trap hung with its base at approximately 3 cm above the ground on canes adjacent to the bush rows so that machinery access in the alleys was not restricted. Each block comprised 9 traps arranged in one row of blackcurrant bushes. At Stonebridge, the traps were spaced 23 m apart in the row and at 8 row intervals (= 24 m). At Wellbrook, the traps were spaced 20 m apart in the row and at 7 row intervals (= 21 m). The number of blackcurrant leaf midge males (and females, if present) in each trap were counted on 16, 23, 30 June and 7 July 2008. Analysis of variance was done on the total counts per trap after $\log_{10}(n+1)$ transformation to stabilise variances. Means were compared using a simple least significant difference (LSD) test (p=0.05).

Experiment B: Comparison of low release rates of racemate versus attractive stereoisomer C

In the first blend experiment the blackcurrant leaf midge sex pheromone racemate was shown to be attractive, although significantly less so than stereoisomer ‘C’. In experiment A, the lowest release rate of the racemate (initial dose = 1 µg/lure) was shown to be most attractive. The objective of this study was to determine the relative attractiveness of four very low release rates of the racemate in comparison with equivalent rates of the natural attractive stereoisomer C (Table 2.2.).

Table 2.2. Treatments in experiment B

Treatment name	**Lure**		**Estimated release rate of C stereoisomer (pg/h)**
	Component	**Initial loading (µg/rubber septum)**	
0	-	-	-
R0.01	Racemate	0.01	0.001
R0.1	Racemate	0.1	0.01
R1	Racemate	1	0.1
R10	Racemate	10	1
C0.0025	C stereoisomer	0.0025	0.001
C0.025	C stereoisomer	0.025	0.01
C0.25	C stereoisomer	0.25	0.1
C2.5	C stereoisomer	2.5	1

† Based on preliminary measurements of ~10 ng/day released from 1 mg racemate loaded rubber septa in a laboratory wind tunnel at 27 ˚C and 8 km/h windspeed

Results

In experiment A, the greatest attraction occurred at the lowest dose (lure dose 1 µg/lure) with progressively lower attraction at increasing doses to R30 ((lure dose 30 µg/lure) Table 2.2, Figure 2.1). Greater lure loads (i.e. 100, 300, 1000 or 3000 µg/lure) did not catch more midges than the untreated control.

In experiment B, all the lures loaded with the racemate (0.01, 0.1, 1, 10 µg/lure) caught significantly more midges than the control (0 loading). There was a progressive increase in attraction with increasing load but the rate of increase reduced to near zero between the highest loadings. i.e. the R10 lure only caught fractionally more midges than the R1 lure. This results ties in with that from experiment A where the greatest attraction with the racemate occurred with a lure loading of 1 µg, but where the 10 µg racemate lure caught significantly less midges. In experiment B, the lowest two C component lure doses (0.0025 and 0.025 µg/lure) did not catch significantly more than the untreated control lure, there was a strongly increasing catch with increasing dose. The lure loadings were chosen such that the same amount of the natural stereoisomer C was present in the lures loaded with C and the lures loaded with 4 times the amount of racemate. At the lowest two doses, R0.01 and R 0.1 caught significantly more midges that C0.0025 and C0.025. Catches at R1 and the C0.25 lures did not differ significantly, but at the highest dose the C2.5 caught significantly greater numbers of male midges (3.5 times as many) than the R10 lure.

Further work is needed to explore the attractiveness of higher loadings of C.

Table 2.3. Mean total numbers of male blackcurrant leaf midges captured per trap at each site, overall mean and the mean square root transformed numbers per trap in experiment A

Treatment	Total catch per trap (n)			Mean(√n)
	Stonebridge	Wellbrook	Mean	
R0	59	110	85	1.881
R1	785	398	592	2.720 ***
R3	464	222	343	2.497 ***
R10	424	208	316	2.452 ***
R30	258	182	220	2.335 ***
R100	130	100	115	2.036
R300	103	57	80	1.887
R1000	72	43	57	1.741
R3000	65	45	55	1.728
Fprob				<0.001
SED(40 df)				0.0888
LSD (p=0.05)				0.1795

*** significantly greater than unbaited control R0 p≤0.001

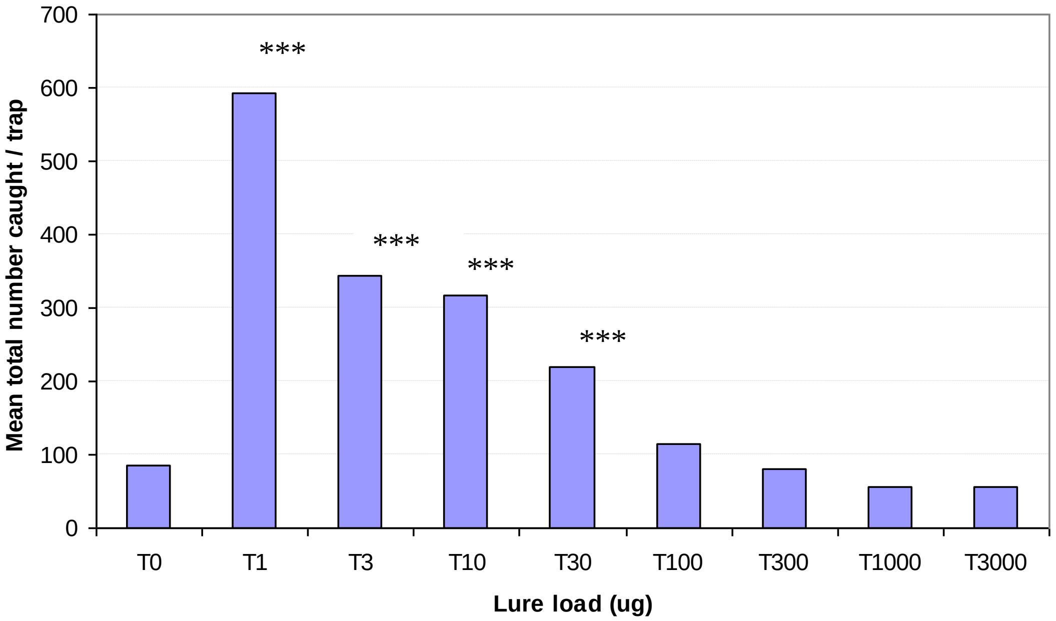


Figure 2.1. Mean total numbers of male blackcurrant leaf midges captured per trap in experiment A [*** significantly greater than unbaited control R0 p≤0.001].

Table 2.4. Mean total numbers (n) of male blackcurrant leaf midges captured per trap and mean $log_{10}(n+1)$ transformed numbers per trap in experiment B

Treatment	n	Mean(log10(n+1))
0	3	0.460
R0.01	15	1.165 **
R0.1	49	1.667 ***
R1	80	1.892 ***
R10	85	1.906 ***
C0.0025	4	0.667
C0.025	7	0.831
C0.25	129	2.069 ***
C2.5	298	2.449 ***
Fprob		<0.001
SED(16 df)		0.1867
LSD (p=0.05)		0.3959

** significantly greater than control p=0.01

*** significantly greater than control p=0.001

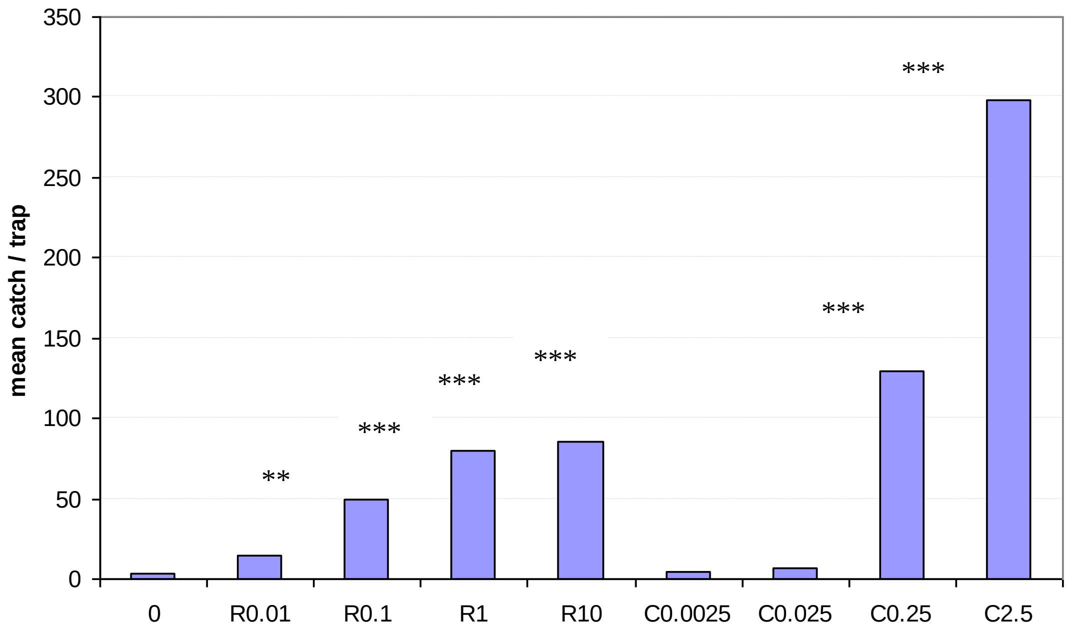


Figure 2.1. Mean total numbers of male blackcurrant leaf midges captured per trap in experiment B

[**, *** significantly greater than control '0' p=0.01, p=0.001 respectively]

Conclusions

In the first experiment, the greatest attraction of the racemic pheromone to male midges occurred at the lowest load (1 µg/lure) with progressively lower attraction at increasing doses (up to 30 g/lure). Greater lure loads (i.e. 100, 300, 1000 or 3000 µg/lure) did not catch more midges than the untreated control.

In the second experiment, there was a progressive increase in attraction with increasing racemate load but the rate of increase reduced to near zero between the highest loadings. i.e. the 10 µg racemate lure only caught fractionally more midges than the 1 µg lure. The 0.0025 and 0.25 µg C/lure loadings did not catch significantly more than the untreated control but there was a strongly increasing catch with increasing dose at the highest loadings. At the lowest two doses, the racemate 0.01 and 0.1 µg/lure caught significantly more midges than the 0.0025 and 0.025 µg. At the highest dose the 2.5 µg C lure caught 3.5 times as many midges as the 10 µg racemate lure.

Objective 3. Determine effects of height of deployment

Task 3. Determining the effects of height of deployment

Introduction

In apple leaf midge and raspberry cane midge, height of trap deployment has a profound effect on attraction to midges, with traps on the ground catching 4 times as many midges as those at 0.5 m and 8 times as many as at 1 m height. Determining the effect of height of deployment is of fundamental importance. The objective aims to test the effect of the height of deployment of blackcurrant leaf midge sex pheromone traps on the numbers of midges caught.

Methods and materials

A replicated experiment was done in the same midge infested commercial Ben Alder blackcurrant plantation at Stonebridge, Horsmonden, (Location NGR TQ 719 399) from 12 May – 9 June 2008 to test the effect of height of trap deployment on attractiveness of rubber septa lures loaded with 5 µg of the natural attractive stereoisomer C to blackcurrant leaf midge males. Treatments comprised the 4 heights of deployment on a half $\log_{10}$ scale (Table 3.1). Standard white delta traps with 20 x 20 cm sticky bases were used. The traps were spaced were 20 m apart.

Table 3.1. Treatments. Lure are rubber septa containing 5 µg of stereoisomer C (third eluting from HPLC)

Treatment No	Treatment Name	Height of trap base above ground (cm)
1	H3	3
2	H10	10
3	H30	30
4		100

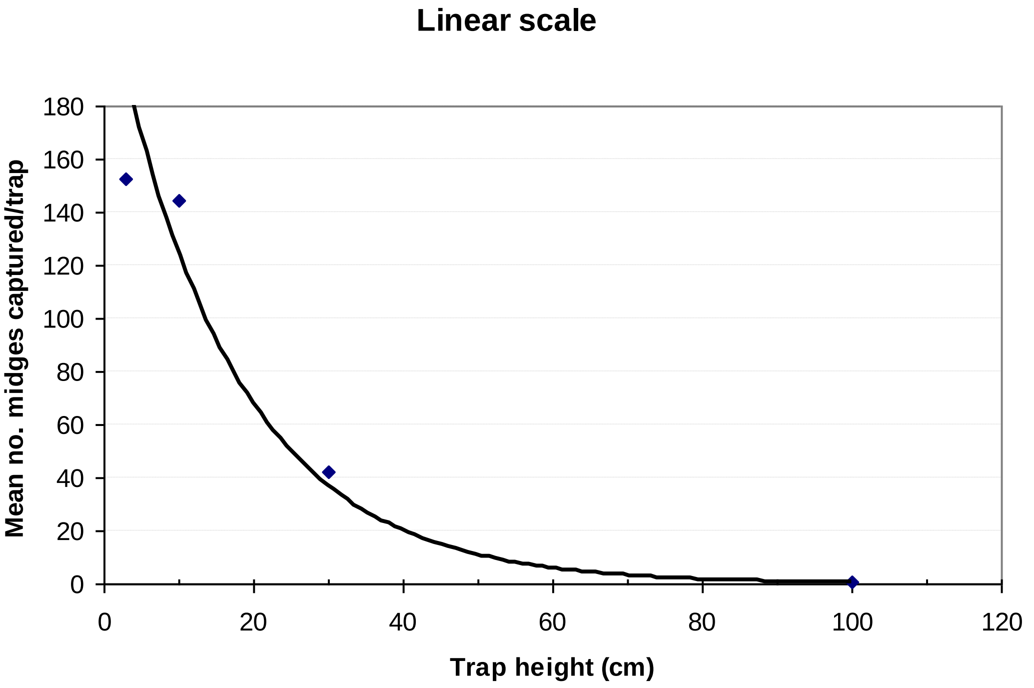
A randomised complete block experiment design with 4 replicates was used. Plots comprised one delta trap suspended on canes at the appropriate height for the treatment. The number of blackcurrant leaf midge males (and females if any present) in each trap was counted on 15 and 26 May, 2 and 9 June 2008 when the experiment was terminated as sufficient numbers had been captured for statistical analysis to determine the effects of the treatments.

Results

Greatest numbers of midges were caught in the traps deployed at 3 cm height with progressively decreasing numbers at greater height (Table 3.2, Figure 3.1). Plotting the catches against deployment height on a log scale showed that catches decreased exponentially with height. Though traps at 10 cm caught only slightly fewer midges than those at 3 cm, traps at 30 cm caught < 30% of the numbers of midges as those at 3 cm and traps at 1 m caught very few midges.

Table 3.2. Mean numbers of midges captured at the different heights.

Height	19 May	26 May	2 June	9 June	Average
3	22.5	84.3	161.8	341.5	152.5
10	8	70.5	97.3	401.8	144.4
30	0.25	15.8	32.5	121.3	42.4
100	0	0	0.3	1.8	0.5



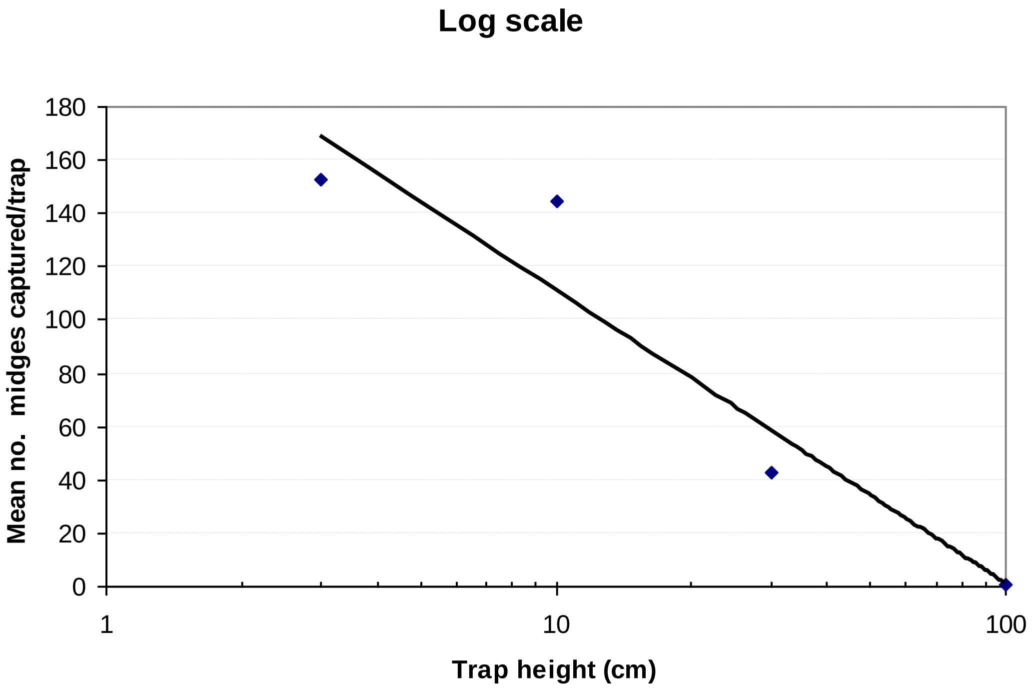


Figure 3.1. Mean catches of midges per week at different heights of trap deployment plotted on a linear scale (upper chart) and a log scale (lower chart)

Objective 4. Seasonal flight pattern in commercial plantations

Task 4. Grower monitoring

Introduction

The aim of the work done for objective 4 was to demonstrate the usefulness of the sex pheromone trap for monitoring and quantifying blackcurrant leaf midge populations in commercial blackcurrant plantations. The flight of leaf midge males was monitored in 7 commercial blackcurrant plantations in the UK, one on each of 7 farms, through the 2008 season. The data generated provided information on the timing of occurrence and relative abundance of the midge in different plantations. Such information is likely to be valuable as an aid to timing of sprays.

Methods and materials

Blackcurrant leaf midge sex pheromone traps were deployed in each of 7 commercial blackcurrant plantations throughout England and Scotland (Table 4.1) and were monitored weekly through the 2008 season. At the outset of this study, it was not known which of the 4 stereoisomers of the blackcurrant leaf midge sex pheromone is the natural one, nor whether the racemate (equal mixture of all 4) is attractive. For this reason, five pheromone traps were deployed in each plantation, one with each of the 4 stereoisomers as lures, one with the racemate. Once the best lure had been identified, records from the other traps were discontinued.

Initial lures and height of trap deployment

The 4 single stereoisomers were prepared by separation of the racemate on a chiral HPLC column at NRI. In the week of 14 April 2008, 5 standard white delta traps were deployed by the host grower at a spacing of approximately 10 m in the centre of the designated field (Table 4.1). They were placed adjacent to the bushes rather than amongst them so they could be easily accessed. The traps were suspended so that the base was 0.5 m above the ground from a pair of canes. Each trap was baited with one lure (A, B, C, D, R) as shown in Table 4.2. Lures were known to have a very long life and to easily last a whole season.

Numbers of blackcurrant leaf midge males were recorded weekly throughout the growing season in 2008. Sticky bases were refreshed each time, unless only a very small number of midges were present in which case they were scraped from the base and the base re-used for a second week. Crop growth stage was recorded and an estimate of % shoot terminals attacked by midge made weekly. The sticky bases had a 6 x 6 or a 7 x 6 grid of squares to aid counting. Sticky bases were sent to EMR to confirm identification where necessary.

Table 4.1. Sites and contact details of participating growers

Farm name & location	Contact person	Field name	Variety	Area (ha)	Age (years)
Mile Oak Farm	Ian Overy	’12 acre’	Tirran	4 ha	7
Wellbrook Fruit Farm	Steven Holmes	’36 acres’	Lomond	4 ha	7
Red House Farm	Andrew Youngman	‘The old orchard’	Hope	3.5 ha	8
Street Farm	Alastair Whyte	’17 acre meadow’	Avon	5.4 ha	?
Oxhouse Farm	Richard Bowen (manager)	‘Telegraph’ first block	Tirran	1.8 ha	4
Bradfields Farm	William Price	‘IPM Hope’	Hope	1.2 ha	10
Balmyle	Ian McDiarmid	‘Balmyle field’ Tirran 2002	Tirran	4 ha	5

Table 4.2. Lures (rubber septa dispensers)

Lure	Component	Amount (µg)
A	first eluting stereoisomer	4
B	second eluting stereoisomer	4
C	third eluting stereoisomer	4
D	fourth eluting stereoisomer	4
R	racemate	16

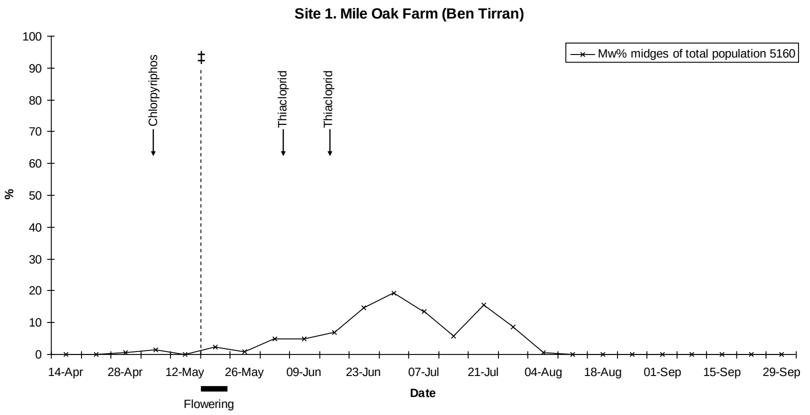
Changed lure and height of deployment

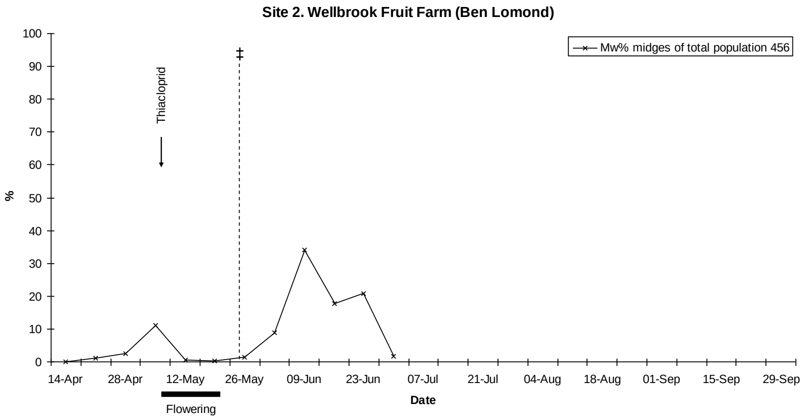
The results up to 16 May clearly showed that the C stereoisomer was the attractive one, but that only rather small numbers of midges were being caught and not sufficiently in advance of the appearance of galling. This was considered to be probably because the 4 µg C lure contained too little pheromone and the traps were set too high at 0.5 m above the ground. Therefore, from the week starting Monday 19 May all the traps except C were removed, and the lures in C were replaced with a new higher dose lure loaded with 10 µg of C. The height of the trap was lowered so its base was 3 cm above the ground. Monitoring the 10 µg trap was continued weekly for the rest of the season.

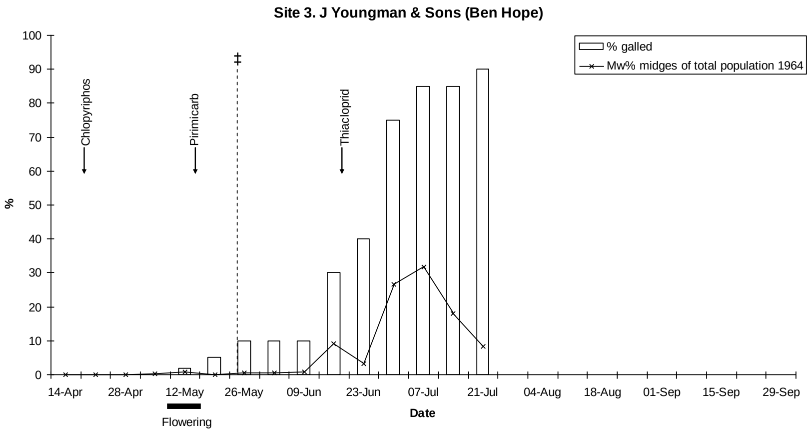
Results

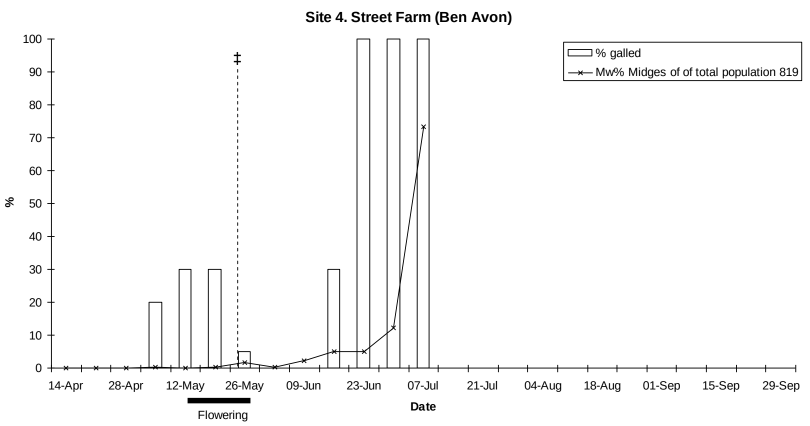
Total numbers of midges captured over the season varied between crops by >10 fold, the lowest numbers of midges (456) being captured at site 2 (Wellbrook) and the greatest numbers (5160) at site 1 (Mile Oak) (Figure 5.1). Note that recording was not continued throughout the season at several sites so the records are no strictly comparable. The date of first catches in spring also varied between the week of 21 April at site 2 (Wellbrook) and the week of 26 May at site 4 (Street Farm). However, the work reported above indicates that the initial height of deployment of 0.5 m was too high, greatly reducing sensitivity and it is probable that the initial flight was missed at several sites. At least two generations of midge flight can be discerned in general, but at sites where recording was continued (e.g. site 5, Oxhouse Farm), three generations seemed to occur. Records of shoot galling were not made at sites 1 or 2. However, at three of the other five sites (sites 3 - Youngman, 4 - Street Farm and 6 - Bradfields) the percentage shoots attacked reached or approached 100% and there was little evidence of effective control of the midge, despite applications of insecticides. The percentage shoots galled was lower (~30 and 10% respectively) at sites 5 (Oxhouse) and 7 (W P Bruce). Sprays of chlorpyrifos and/or thiacloprid (Calypso) applied at most sites in April to May failed to prevent continuing catches of midges in the sex pheromone traps or the occurrence of a high percentage shoot damage at some sites.

Overall, the results indicate that the traps will prove useful for monitoring the timing and numbers of midges attacking different blackcurrant plantations and could lead to an improvement in the control of midge if they are used to time sprays.


Site 1. Mile Oak Farm (Ben Tirran)
Mw% midges of total population 5160
Chlorpyriphos
‡
Thiacloprid
Thiacloprid
%
Date
Flowering
14-Apr 28-Apr 12-May 26-May 09-Jun 23-Jun 07-Jul 21-Jul 04-Aug 18-Aug 01-Sep 15-Sep 29-Sep


Site 2. Wellbrook Fruit Farm (Ben Lomond)
Mw% midges of total population 456
Thiacloprid
‡
%
Date
Flowering
14-Apr 28-Apr 12-May 26-May 09-Jun 23-Jun 07-Jul 21-Jul 04-Aug 18-Aug 01-Sep 15-Sep 29-Sep


Site 3. J Youngman & Sons (Ben Hope)
% galled
Mw% midges of total population 1964
Chlopyriphos
Pirimicarb
‡
Thiacloprid
%
Date
Flowering
14-Apr 28-Apr 12-May 26-May 09-Jun 23-Jun 07-Jul 21-Jul 04-Aug 18-Aug 01-Sep 15-Sep 29-Sep


Site 4. Street Farm (Ben Avon)
% galled
Mw% Midges of of total population 819
‡
%
Date
Flowering
14-Apr 28-Apr 12-May 26-May 09-Jun 23-Jun 07-Jul 21-Jul 04-Aug 18-Aug 01-Sep 15-Sep 29-Sep

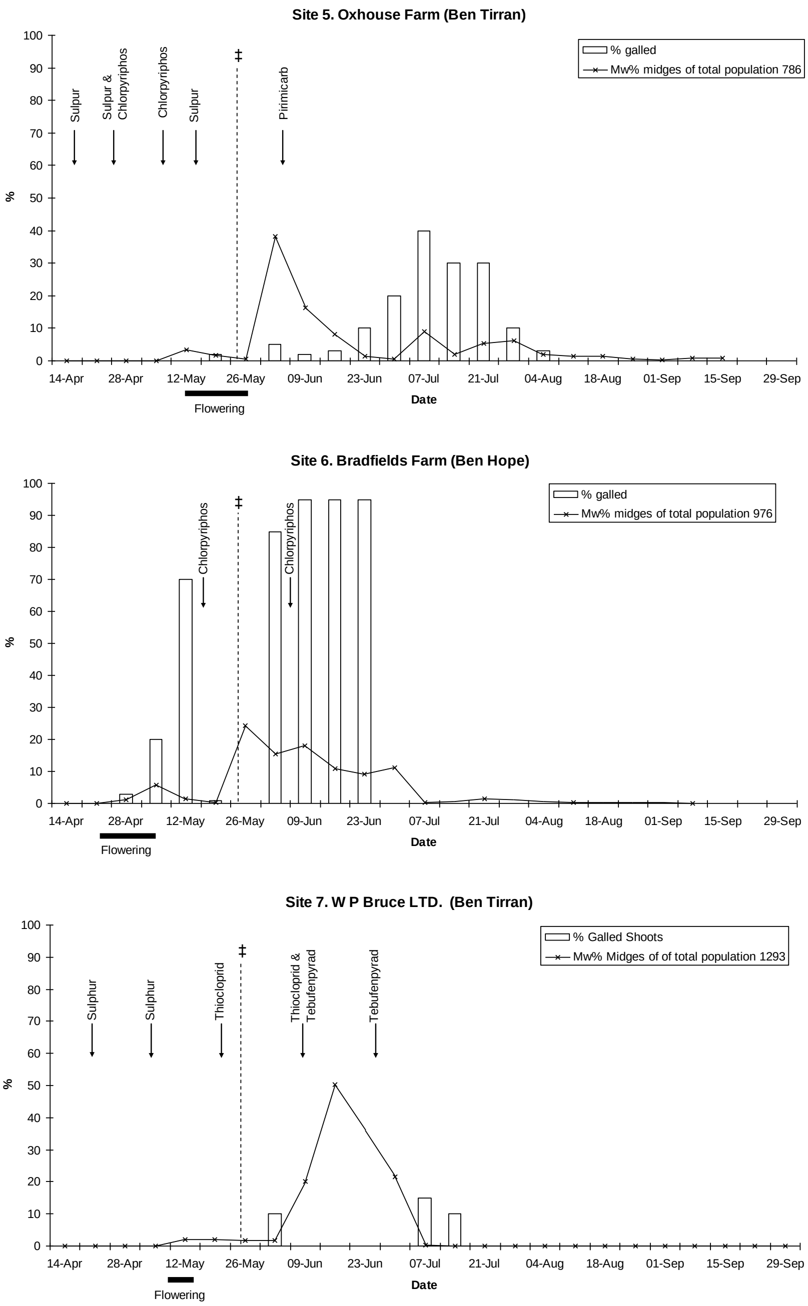


Figure 5.1. Graphs of Williams mean % catches (catch expressed as percentage of seasons total) of blackcurrant leaf midge adults in single sex pheromone traps baited with rubber septa lures loaded with the C stereoisomer in 7 commercial blackcurrant plantations in England and Scotland in 2008. Estimates of the percentage shoot terminals galled are showed as histograms. Note the changeover from the initial trap height of 0.5 m and loading of 4 µg to a height of 3 cm and 10 µg is indicated by a vertical dashed line in the third week of May.

Additional Objective 5. Effects of trap colour

Introduction

In the previous experiments, significant numbers of non-target insects were caught in the traps as well as blackcurrant midges and this required a trained entomologist to assess the catches with certainty. The objective of this experiment was to determine the effect of the colour of sex pheromone traps and their sticky inserts on blackcurrant leaf midge sex pheromone trap catches and of catches of non-target insects.

Methods and materials

A replicated experiment was done in two midge infested commercial blackcurrant plantations from 16 – 30 June 2008 to test how trap colour and the colour of the sticky base trap insert affected the attractiveness of traps baited with 100 µg of the sex pheromone racemate to blackcurrant leaf midge males. Treatments comprised 6 different colours of delta trap, with either white or matching coloured sticky bases. However, treatment 7 was a white delta trap with a blue sticky base, to avoid replicating the White_White treatment and to see whether sticky base colour has an influence on commonly used white delta traps (Table 5.1). The commercial plantations were Ben Alder blackcurrant plantation at Stonebridge, Horsmonden (Location NGR TQ 719 399) (kind permission of Tom Maynard) and a Ben Gairn blackcurrant plantation at Wellbrook Farm, Faversham (Location NGR TR 028 593) (kind permission of Steven Holmes). Each trap was baited with a rubber septum lure loaded with 100 µg of the sex pheromone racemate. The traps were set at a height of 3 cm above the ground and were spaced >20 m apart.

Table 5.1. Treatments

Treatment No	Treatment name	Trap colour	Insert colour
1	W_W	White	White
2	B_W	Blue	White
3	R_W	Red	White
4	G_W	Green	White
5	Y_W	Yellow	White
6	Blk_W	Black	White
7	W_B	White	Blue
8	B_B	Blue	Blue
9	R_R	Red	Red
10	G_G	Green	Green
11	Y_Y	Yellow	Yellow
12	Blk_Blk	Black	Black

A randomised complete block experimental design was used with 2 replicates at each of the two sites. Plots comprised 1 delta trap hung with the base at approximately 3 cm above the ground on canes adjacent to the bush rows so that machinery access in the alleys was not restricted. Each block comprised 12 traps arranged in one row of blackcurrant bushes. The traps were deployed on 16 June and counts of the blackcurrant leaf midges and other contaminating insects sorted into broad taxa were made on 23 and 30 June. Most contaminating insect taxa contained few individuals so the total number of non-target arthropods caught was calculated and subject to analysis of variance after $\log_{10}(n+1)$ transformation to stabilise variances. A factorial structure for (trap colour)*(matching vs. non-matching insert) was added to the analysis.

Results

Treatment effects in the analyses of variance of the $\log_{10}(n+1)$ transformed numbers of blackcurrant leaf midge males captured were not statistically significant on either sampling date (Table 5.2).

At the first assessment the numbers of non-target insects caught were similar to those of the blackcurrant midge, but at the second assessment the numbers of non-target insects caught were much greater. For non-target totals for the first assessment, none of the individual match vs. non-match comparisons was statistically significant. For non-target totals for the second assessment, there was an overall significant treatment effect (p=0.005). The factorial structure showed that the effect of trap colour was statistically significant (p=0.002) but not the match vs. non-matching insert. However there were no really clear-cut trap colour differences shown up overall. Yellow traps had the highest catch and black the smallest. Black also had the lowest catches for both types of insert, but the main difference for yellow was the higher catch with the white insert.

Table 5.2. Mean and mean log10(n+1) transformed numbers of blackcurrant leaf midge males captured in the different coloured traps

Trt	**23 June**		**30 June**	
	n	**$log_{10}(n+1)$**	**n**	**$log_{10}(n+1)$**
W_W	31.0	1.489	34.5	1.445
B_W	35.3	1.533	23.8	1.233
R_W	17.0	1.225	22.0	1.157
G_W	16.5	1.091	16.0	1.184
Y_W	28.0	1.419	64.5	1.700
Blk_W	31.8	1.435	17.5	1.236
W_B	11.3	1.083	28.8	1.183
B_B	15.8	0.981	17.3	0.999
R_R	37.3	1.353	24.0	1.343
G_G	14.0	0.981	27.0	1.435
Y_Y	12.8	1.009	41.0	1.440
Blk_Blk	16.5	1.159	24.5	1.207
Fprob		0.087		0.443
SED (33 df)		0.2157		0.2576
LSD (p=0.05)		0.4388		0.5242

Table 5.3. Mean numbers of non-target arthropods captured in the coloured traps on 23 June 2008

Trt	bumble bees	flies	thrips	Hymenoptera	beetles	aphids	lacewings	syrphids	Lepidoptera	spiders	earwig	Hemiptera	total	
													n	$\log_{10}$ (n+1)
W_W	0.0	32.8	0.0	0.3	1.3	0.8	0.0	0.3	0.0	0.0	0.0	0.3	35.5	1.824
B_W	0.0	32.8	0.0	0.5	0.5	0.0	0.0	0.3	0.0	0.5	0.0	0.3	34.8	1.831
R_W	0.0	15.8	0.0	1.3	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18.0	1.538
G_W	0.0	18.5	0.0	0.5	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	20.5	1.504
Y_W	0.0	42.5	0.0	1.0	0.8	1.5	0.0	0.0	0.0	0.8	0.3	0.5	47.3	1.880
Blk_W	0.0	25.0	0.3	0.3	0.5	0.0	0.0	0.0	0.0	0.0	0.3	0.0	26.3	1.741
W_B	0.3	26.8	0.0	2.5	0.8	0.0	0.0	0.3	0.0	0.0	0.3	0.0	30.8	1.626
B_B	0.5	30.8	0.0	0.8	0.5	0.3	0.0	0.8	0.0	0.3	0.0	0.3	34.0	1.682
R_R	0.0	20.0	0.0	1.8	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	22.0	1.716
G_G	0.0	27.0	0.0	2.5	0.3	2.0	0.0	0.0	0.0	0.0	0.0	0.5	32.3	1.664
Y_Y	1.0	35.8	0.3	3.8	1.3	1.8	0.0	0.0	0.0	0.0	0.0	0.5	44.3	1.730
Blk_Blk	0.0	14.8	0.0	0.3	0.0	0.0	0.0	0.0	0.3	0.3	0.0	0.3	15.8	1.494
													Fprob	0.076
													SED (33 df)	0.1311
													LSD (p=0.05)	0.2667

Table 5.4. Mean numbers of non-target arthropods captured in the coloured traps on 30 June

Trt	other midges	bees	large flies	small flies	Hymenoptera	beetles	syrphids	Lepidoptera	spiders	earwigs	Hemiptera	ants	harvestmen	Total	
														n	$log_{10}(n+1)$
W_W	86.3	0.0	23.8	78.3	0.5	1.0	2.0	0.0	0.0	0.0	0.0	0.0	0.3	192.0	2.180
B_W	78.8	0.8	10.0	25.8	0.5	0.8	0.3	0.0	0.0	0.0	0.0	0.0	0.0	116.8	2.009
R_W	74.3	0.0	7.5	26.8	0.5	3.5	1.3	0.5	0.5	0.0	0.0	0.0	0.5	115.3	2.059
G_W	52.5	0.0	2.8	8.8	0.0	3.0	1.3	0.3	1.0	0.0	0.0	0.0	0.0	69.5	1.804
Y_W	113.0	0.0	19.5	95.0	0.0	2.3	2.5	0.0	0.0	0.0	0.0	0.0	0.3	232.5	2.347
Blk_W	47.5	0.0	5.5	12.0	0.0	1.5	0.0	0.0	0.3	0.0	0.0	0.0	0.0	66.8	1.738
W_B	32.5	0.0	18.0	27.3	0.5	1.8	4.5	0.3	0.0	0.0	0.0	0.0	0.0	84.8	1.884
B_B	57.3	0.0	12.0	6.0	0.0	2.3	2.8	0.0	1.0	0.0	0.0	0.0	0.3	81.5	1.950
R_R	83.3	0.0	22.3	0.0	2.8	0.8	0.0	0.0	0.3	0.3	0.5	0.3	0.5	110.8	2.025
G_G	68.5	0.0	7.5	11.8	1.3	0.8	0.0	0.3	0.3	0.0	0.5	0.0	0.0	90.8	1.936
Y_Y	50.0	0.5	18.8	37.3	1.3	0.3	3.5	0.0	0.5	0.0	0.5	0.5	0.0	113.0	2.045
Blk_Blk	26.0	0.5	7.7	13.0	0.8	0.5	0.0	0.3	0.5	0.0	0.0	0.0	0.0	49.2	1.700
														Fprob SED (31 df) LSD (p=0.05)	0.005 0.1440 0.2937

Additional Objective 6. Improved trap design

Introduction

The objective of this work was to develop a design of blackcurrant leaf midge sex pheromone trap suitable for use by UK blackcurrant growers. The following features were desirable:

1. Standard 20 x 20 cm base delta trap format with easily changeable sticky base
2. Height of deployment 3 cm, free standing but held in position by the soil
3. Red colour to minimise contamination by non-target arthropods, but still readily visible to growers
4. Fine mesh grid at entrances to minimise contamination by non-target arthropods, debris and to prevent bird ingression
5. Re-useable for several seasons to minimise costs and waste

Methods and results

Various prototype designs were prepared in the laboratory at East Malling Research. The design shown in Figure 6.1 was found to be robust and effective. The materials used are red Correx, 2 x bulldog clips, 4 x groundsheet pegs (Homebase), 2 mm modelling mesh (Cross' Art Shop), binding spine, small self tapping screws and washers, Agrisense sticky base with cross grid, bent paper clip to suspend rubber septa lure.

A trap with grids at the entrance and one without were deployed in a plantation at East Malling Research for 2 weeks in early September. Six blackcurrant leaf midge were captured in the trap with the grid, 7 in the trap without. The trap without the grids was heavily contaminated with non-target arthropods and debris. The trap with the grids had no large contaminating arthropods or debris. However, both traps contained small numbers of non-target midges and other small insects.

Thus, a practical, free-standing 20 x 20 cm base red delta trap with a fine mesh grid at each entrance to prevent non-target contamination is proposed for future development as a standard blackcurrant leaf midge sex pheromone monitoring trap.

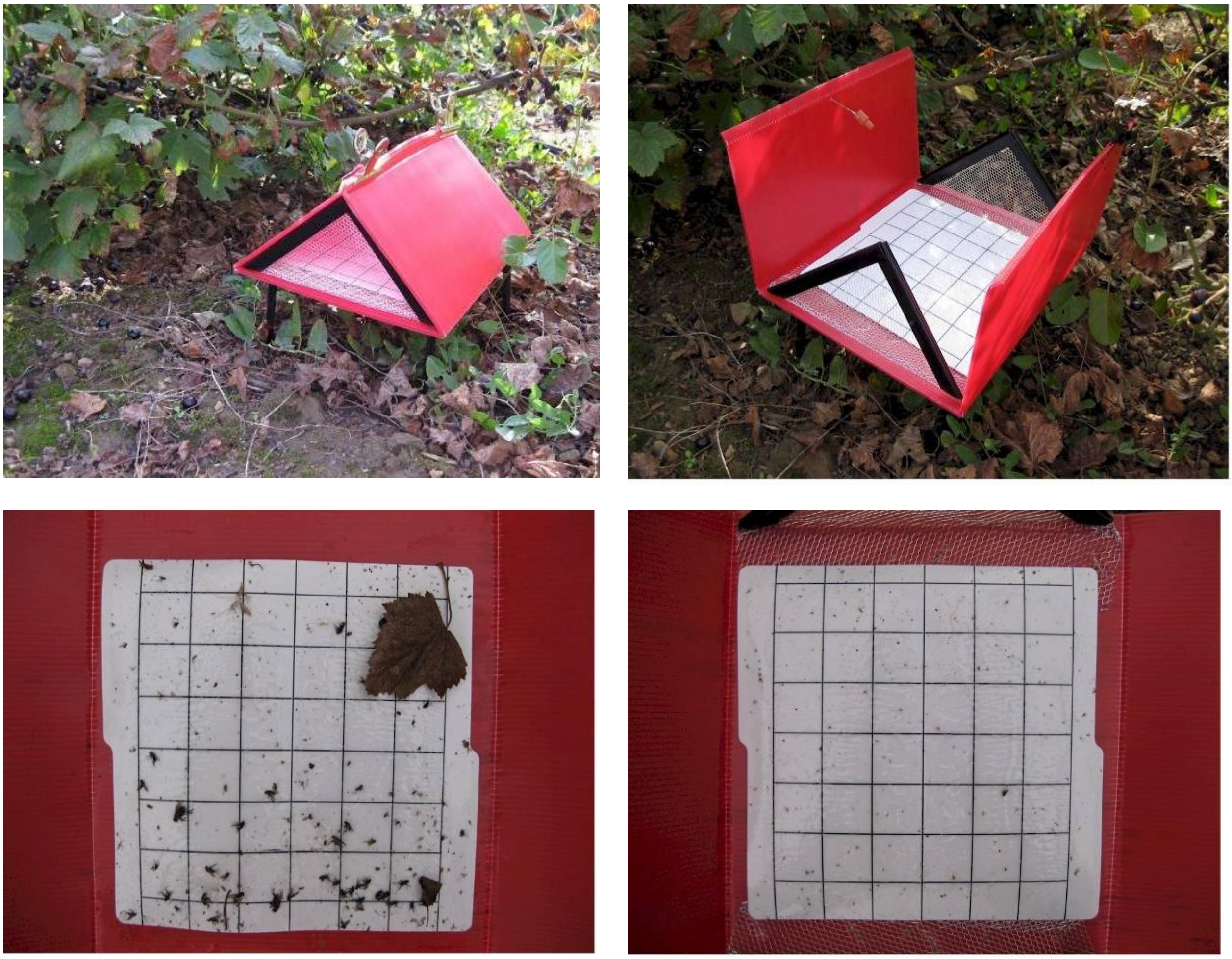


Figure 6.1. Prototype blackcurrant leaf midge sex pheromone monitoring traps in operation (top left), opened to change sticky base (top right). The sticky bases from traps deployed for 2 weeks in early September at East Malling Research without grids at entrances (bottom left) with grids at entrances (bottom right).

Work proposed in 2009

Although the experiments carried out during 2008 show that stereoisomer C of the blackcurrant midge pheromone is the attractive isomer, the absolute configuration of this is unknown. Work is ongoing at NRI to determine the absolute configuration of stereoisomer C as part of Ms Lakmali Amarawardana's PhD studies. Once this is known it will be necessary to devise a synthetic route to this stereoisomer as separation of commercial quantities of this isomer by HPLC would be impractical. It is hoped that an application for a new 3 year PhD studentship recently made by EMR and NRI will be supported for continued work on the sex pheromones of blackcurrant leaf midge as well as blackberry midge and blue berry leaf midge. However, such a studentship would not start effectively till 2010.

The following work could be considered for 2009:

- Field testing the proposed new blackcurrant leaf midge delta trap with versus without grids at entrances
- Examining the relationship between trap catches and numbers of galls formed in different commercial crops to establish a trap threshold
- Conduct a spray trial to examine how sex pheromone traps can be used for timing of sprays of insecticides that are known to be effective against blackcurrant leaf midge (e.g. chlorpyrifos and a pyrethroid)
- Conduct a spray trial to examine the efficacy of novel insecticides for control of blackcurrant leaf midge, with sprays timed according to sex pheromone trap catches
- Establish the relationship between lure load and attractiveness for stereoisomer C
- Prepare mating disruption and attract and kill formulations of the racemate and stereoisomer C for field evaluation for control of the midge in 2010

Acknowledgements

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