Project title:	Blueberry gall midge: sex pheromone monitoring and control with insecticides
Project number:	SF126
Project leader:	Prof. Jerry V Cross, EMR
Report:	Final Report, March 2014
Previous report:	Annual Reports 2012, 2013
Key staff:	Dr Michelle Fountain, EMR Adrian Harris, EMR Dr Philip Brain, EMR Prof. David Hall, NRI Paul Douglas, NRI Dudley Farman, NRI
Location of project:	EMR, East Malling NRI, Chatham Maritime
Industry Representative:	Laurie Adams
Date project commenced:	1 April 2011
Date project completed (or expected completion date):	31 March 2014

The results and conclusions in this report are based on an investigation conducted over a three-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.

Adrian Harris

Entomologist

East Malling Research

Signature ..... Date ...7 April 2014

Prof. David Hall

Chemical Ecology Group

Natural Resources Institute

Signature ..... Date .....

Report authorised by:

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Leader of the Pest and pathogen Ecology for Sustainable Crop Management science programme

East Malling Research

Signature .....**J V Cross**..... Date ...7 April 2014

CONTENTS

GROWER SUMMARY

Headline

A sex pheromone trap has been developed for monitoring populations of blueberry gall midge in the UK.

Background and expected deliverables

The blueberry gall midge (*Dasineura oxycoccana* (Johnson 1899), syn *Dasineura vaccinii* (Smith, 1890)) is a damaging invasive pest of highbush blueberry (*Vaccinium corymbosum*) in the UK. It is also a serious pest of blueberry in the USA and Canada where it originated and where it is known as the cranberry tipworm or blueberry gall midge. Recent work in Canada has suggested that populations of this species on cranberry and on blueberry are different and may represent different, cryptic 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 2-3 years of establishment.

The midge lays its eggs in the tender growing points of shoots and the larvae live in leaf galls in the shoot tip, causing leaf distortion and blackening of buds which are killed by the attack. The growth habit of the blueberry occurs in flushes which end with the death of the terminal meristem and the next growth flush starts from the next bud or buds below. Midge attack 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 gall 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 was 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 sex pheromone of the blueberry gall midge found in the UK has been shown to correspond to that of the form infesting blueberry in Canada, which was identified by Canadaian workers. Traps baited with this pheromone attracted large numbers of male midges in UK growers' plantations. The pheromone identified for the form infesting cranberry in Canada did not attract any midges in blueberry, cranberry or wild bilberry in the UK.

The pheromone has been synthesised and traps and lures will be commercially available in the UK during 2014. Traps should be deployed before any signs of damage are present as populations of the midge can build rapidly. Traps should be placed as near to the ground as possible for maximum catches. A trap threshold of 10 male midges per trap per week is advised as a suitable level at which to initiate control with plant protection products.

Two trials were conducted to investigate the efficacy of a range of insecticides for control of blueberry leaf midge. Thiacloprid (Calypso) and pyrethrins (Spruzit) are the only products currently approved for use against gall midge on blueberry. Of these only thiacloprid proved effective but accurate timing of application is essential. Chlorpyrifos and cypermethrin reduced numbers of larvae and resulting leaf galls. Lambda cyhalothrin also reduced some larvae and galling, but results were not consistent between spray dates and varieties.

The coded product UKA285a gave similar control to chlorpyrifos. Being systemic, this gave the greatest degree of control of both damage and larvae. It also offered the largest window of application.

Financial benefits

No detailed financial information on the cost to growers of the blueberry gall midge has been made in the UK. In Latvia, the midge has been shown to reduce growth and yields of large fruited cranberry by 60% (Apenite, 2010). In the USA, the blueberry gall midge causes losses in excess of $20 m per annum to rabbiteye blueberries (*Vaccinium ashei*) where the pest feeds in the flowers leading to premature floral bud abscission, or aesthetically compromised fruit when mature (Dernisky et al., 2005).

Action points for growers

- Pheromone traps for monitoring blueberry midge will be commercially available from early 2014.
- Crops should be monitored from early in the season before the pest is active.
- A trap catch of 10 male midges per trap is recommended as the threshold for application of approved products.
- Thiacloprid is currently approved for use on blueberry and is effective.

SCIENCE SECTION

Introduction and Summary of Work during Years 1 and 2

General 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 (Collins *et al*., 2010; Collins and Eyre, 2010). It is also a serious pest of blueberry in the USA and Canada where it originated and where it is known as the cranberry tipworm. It is abundant and widely distributed in UK blueberry crops having spread from nurseries on planting material and is most important in newly planted crops and during the first 2-3 years of establishment.

The gall midge, *Jaapiella vacciniorum* (Kieffer, 1913) occurs in the wild on bilberry (*Vaccinium myrtillus*) and is a native to Britain. It is not clear whether this is the same species as *D. oxycoccana* or a distinct species. There is no type material for comparison in the British Museum. In Italy, Grassi and Forno (2004) have identified *J. vacciniorum* as a pest of highbush blueberries, but it is probable that this is *D. oxycoccana.*

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

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).

Currently, UK growers attempt to control the midge by applying a spray of thiacloprid (Calypso) when galling damage is first seen in spring. Commercial experience also

indicates that a weekly programme of sprays of pyrethrum prevents midge attack. However, on other crops including blackcurrant, blackberry, apple and pear, thiacloprid (Calypso) has been shown to be at best only partially effective for leaf midge control, and it is likely this is the case with the blueberry midge. Thus effective methods for monitoring the pest and controlling it with insecticides are needed.

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

Other work by EMR has shown that an EC formulation of spirotetramat is very effective for control of the leaf midge pests and it is likely to be effective against blueberry gall midge. The OD formulation of spirotetramat, Movento, has recently been approved for use on vegetable crops in the UK, and the approval of the SC formulation on apple is pending. Other workers have investigated *Bacillus thuringiensis* Berliner subsp. *israelensis* (Bti), chlorantraniliprole, flubendiamide, metaflumizone and spirotetramat and diazinon but found that none of the alternative insecticides provided consistent significant control on par with diazinon.

EMR have demonstrated that 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 was 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.

Work during Year 1

Pheromone Identification

Samples of pheromone were collected from over 1,000 virgin male and female blueberry gall midge by aeration and by trapping of volatiles on a solid adsorbent and by solid-phase microextraction (SPME). These were analysed by gas chromatography coupled to mass spectrometry (GC-MS) and by GC linked to electroantennographic recording (GC-EAG) from the antenna of a male midge.

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

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

Insecticide Trials

Trials were carried out to evaluate the efficacy of Calypso, chlorpyrifos, Hallmark, Toppel 10, pyrethrum and UKA385a for control of first and second generation blueberry midge. Because most of the active ingredients were not registered for use on blueberry, a nursery plantation was chosen so that fruit destruction was not required. Treatments were two sprays of the products tested applied at an interval of 14 days (not exceeding the maximum number of applications permissible) at their full recommended rate (Table 2).

The effects of the treatments were assessed 14 days after the 1st application, immediately prior to the second application (15 September 2011), and the second assessment was conducted 13 days later (28 September 2011). At the first assessment 50 shoots per plot were assessed for presence or absence of blueberry midge damage. Those damaged were collected and brought back to the laboratory so that the numbers of larvae per gall could be assessed. At the second assessment every damaged shoot per plot was recorded.

At the first assessment, ANOVA of the untransformed data showed significant reductions in the percentage of infested shoots in all treatments when compared to the double replicated untreated control (P = 0.012). However after angular transformation of the data, the mean for Calypso was not significantly less than the control, though all the other treatments gave significant reductions. No larvae were present in the galls at this stage.

At the second assessment there were no significant reductions in the percentage of infested shoots when compared to the double replicated untreated control (P = 0.069) and the analysis was not improved by angular transformation (P = 0.128).

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

However, most of the insecticides tested have broad-spectrum activity and are likely to be very harmful to the midge's natural enemies and anthocorid predatory bugs, as well as to the natural enemies of other blueberry pests. UKA385a is selective insecticide which work on other midge pests has shown to control larvae inside the galls and which is less likely to have harmful effects, especially persistent ones, on natural enemies. The efficacy of control of larvae by all treatments is unknown as no larvae could be found at either assessment date.

Modification of Objectives

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 Prof Gries kindly provided lures of the two sub-species for evaluation in the UK. In light of these developments, it was agreed with the Project Industry Representative that objectives of the project required some 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 our cranberry species if a suitable plantation can be found, and for our native blueberry midge*, Jaapiella vacciniorum*, if a suitable wild bilberry site can be found.
- 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 G 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.

Work during Year 2

Pheromone Identification

During the second year of the project, lures provided by Prof Gries for the cranberry and blueberry versions of *D. oxycoccana* were extracted, purified and analysed and a route to synthesise the pheromone was developed

Two field tests were carried out on growers' farms. Experiment 1 aimed to test the blueberry gall midge and cranberry gall midge lures provided by Prof Gries for attractiveness to their target species and to the UK native bilberry gall midge *Dasineura*

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

Experiment 2 was to determine the optimum height of the pheromone trap. The experiment was carried out in commercial blueberry Hall Hunter Farms, Tuesley Farm, Milford. Traps were red delta traps at 0.5 m, 1 m or 2 m above ground level.

From analyses of the lures it was worked out that the pheromones of the blueberry and cranberry versions of *D. oxycoccana* identified by Prof Gries were (*R,R*)-2,14-diacetoxyheptadecane and (8*Z*,2*S*,14*S*)-2,14-diacetoxy-8-heptadecene respectively. This was subsequently reported in Fitzpatrick et al. (2013).

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. However, an occasional EAG response that had been observed in GC-EAG analyses of pheromone collections was consistent with that of the compound from the blueberry midge lures.

Synthesis of (*R,R*)-2,14-diacetoxyheptadecane was carried out at NRI (Figure 1)

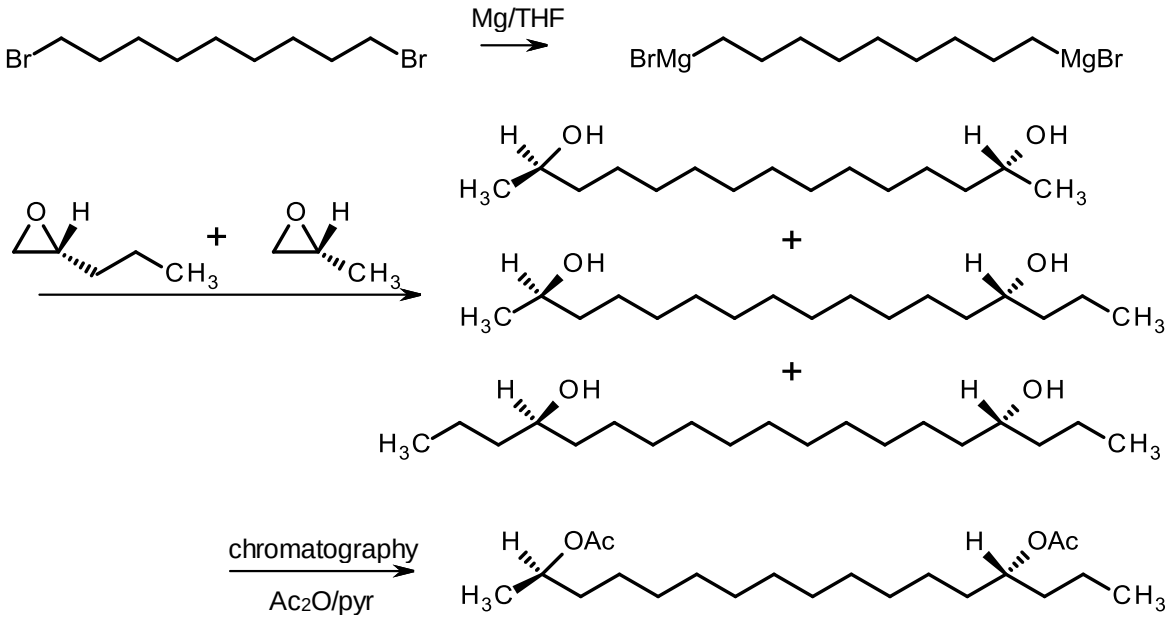


Figure 1. Synthesis of (*R,R*)-2,14-diacetoxyheptadecane

In trapping Experiment 1, there were significantly more blueberry midge males ($P < 0.05$; Figure 2) 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. 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.

In Experiment 2, there were significantly more midges captured closer to the ground in the crop with more than 3 times the number at 0.5 m compared to 1.0 m and very few midges at 2 m (Table 1).

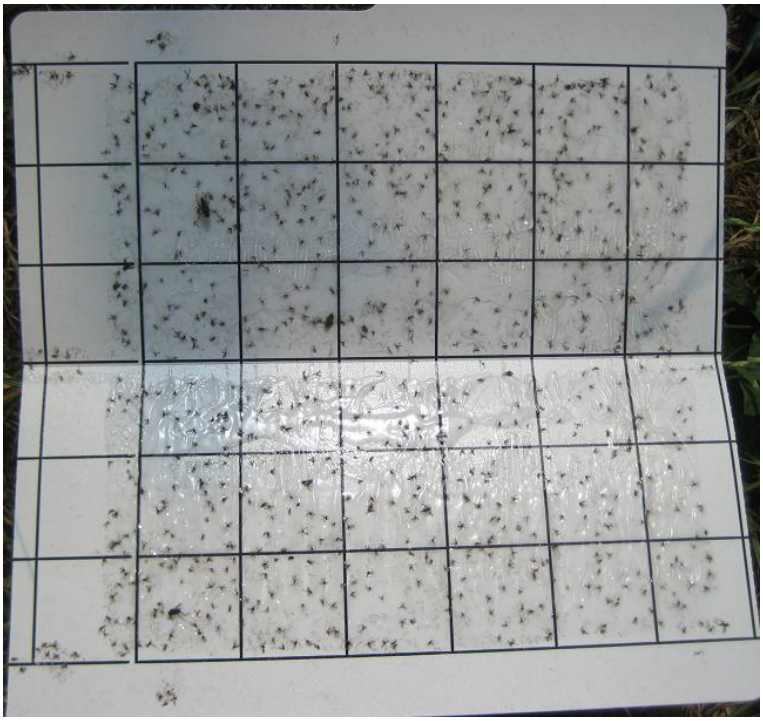


Figure 2. Sticky insert from trap baited with blueberry midge pheromone at Tuesley Farm after one week

Table 1. 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
F pr		<.001
s.e.d.		0.2237
l.s.d.		0.5290

Insecticide Trial

Traps were deployed in a commercial protected crop in Essex in mid-summer 2012 with the intention of carrying out 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 it was decided not to do the trial in the absence of the pest.

Materials and Methods

Optimisation of Pheromone Loading in Lure

Sites and Experimental Design

Two highly infested blueberry plantations with a history of the pest were selected. One plantation was open and one under polythene protection. The plantations used were approximately 1 ha each with rows 200 m long. Two rows in each location were used for the deployment of the traps and the rows used were separated by at least 20 m to prevent any interaction between the pheromone plumes between traps. A randomised complete block design with 4 replicates of 5 treatments was used.

Traps and Lures

Plots comprised a single red delta trap, deployed on or close to the ground. Lures were rubber septa impregnated with the pheromone, (*R,R*)-2,14-diacetoxyheptadecane, at NRI.

For Experiment 1 lures were loaded with 1 µg, 10 µg, 100 µg or 1,000 µg and an untreated control. For Experiment 2 lures were loaded with 1,000 µg, 3,000 µg or 10,000 µg and an untreated control.

Meteorological records

Two lascar USB-502 data loggers were placed in a Stevenson screen and deployed in the centre of each trial area to record hourly temperature and humidity data throughout the duration of the trial (Appendix 1. and 2).

Assessments

Counts of numbers of midges caught in each trap were made. Populations of the midge were so high that an initial assessment could be made 1 hour after initial deployment. Subsequent readings were taken every 7 days when the sticky base in each trap was replaced with a fresh one.

Assessment of the traps for the efficacy and timing of plant protection products to control blueberry gall midge

Experimental design and layout

Four of the active ingredients used in this trial were not registered for use on blueberry (Table 2). In a normal grower trial this would mean crop destruction and subsequent loss of income. To avoid this an experimental plantation was set up at East Malling Research in a twin span polytunnel on 24 April 2013 (Fig. 3) and an experimental approval was acquired for all non-approved products.

Figure 3. Layout of blueberry plantation at East Malling Research

For Experiment 1 a randomised block experiment with 6 replicates of 7 treatments was used with three replicates of the variety Spartan and three of the variety Ozark Blue. Each plot consisted of 12 plants arranged in two rows of six. A gap of 0.5 m was left between plots to reduce spray drift.

For Experiment 2 a randomised block experiment with 4 replicates of 7 treatments was used. The plots were of a split plot design with each plot containing 14 plants, 7 of each variety, Spartan and Ozark Blue. Each plant was 1 m from each of the treatment blocks from the previous experiment. This prevented any effects of pesticide carry-over from the previous experiment. A gap of 0.5 m was left between plots to allow for spray drift.

Treatments

Treatments were two sprays of the test products, for Experiment 1 applied at an interval of 14 days and Experiment 2 at 7 day intervals (not exceeding the maximum number of applications permissible) at their full recommended rate (Table 2).

Table 2. Products and their rates of application

Trt	Product	Active substance and formulation	Dose rate /ha	Conc.	Approved on blueberry
1	Equity	chlorpyrifos 480 g/l EC	1.5 l	1.5 ml/l	no
2	Hallmark	lambda cyhalothrin 100 g/l CS	100 ml	0.1 ml/l	no
3	Pyrethrum 5 EC	pyrethrum 50 g/l EC	1.1 l	1.1 ml/l	yes
4	Permasect C	cypermethrin 100 g/l EC	350 ml	0.35 ml/l	no
5	UKA385a	novel 100 SC	750 ml	0.75 ml/l	no
6	Calypso	thiacloprid 480 g/l SC	250 ml	0.25 ml/l	SOLA 0335/06
7	Untreated				

Treatment Application

Treatments were applied at a volume rate of 1000 l/ha using a knapsack sprayer with a hand lance (not air-assisted). This minimised inter-plot contamination by spray drift. The accuracy of application of each treatment was calculated based on the volume of spray left after each application and the calculated target volume.

For Experiment 1 applications were generally within 17% of the target (Table 3), although some larger deviation occurred (two applications were within 22% of target).

For Experiment 2 applications were generally within 17% of required (Table 4).

Table 3: Accuracy of spray application estimated from the amount of sprayate remaining in the spray tank after spray application for Experiment 1

Spray round and date		Treatment No:	Accuracy (%)
1.	13 May	1	88%
		2	93%
		3	83%
		4	83%
		5	78%
		6	93%
2.	25 May	1	93%
		2	78%
		3	93%
		4	83%
		5	88%
		6	83%

Table 4. Accuracy of spray application estimated from the amount of sprayate remaining in the spray tank after spray application for Experiment 2

Spray round and date		Treatment No:	Accuracy (%)
1.	9 July	1	83%
		2	83%
		3	86%
		4	93%
		5	94%
		6	98%
2.	16 July	1	83%
		2	83%
		3	89%
		4	95%
		5	93%
		6	89%

Assessments

At each assessment 25 shoots per plot were collected and brought back to the laboratory. They were assessed for the number of leaves per shoot damaged by blueberry gall midge. Those damaged were dissected so that the numbers of larvae per gall could be counted.

The effects of the treatments in Experiment 1 were assessed 12 days after the 1st application, immediately prior to the second application (25 May 2013). The second assessment was conducted 12 days later (06 June 2013).

For Experiment 2 the assessment dates were at 7 day intervals .The 1st assessment was, immediately prior to the second application (16 July 2013), and the second assessment was conducted 7 days later (23 July 2013) a final assessment was conducted after a further 7 days (30 July 2013).

Meteorological records

Dry and wet bulb temperature, wind speed and direction were recorded before and after each spray occasion (Table 5). RH% was estimated from the dry and wet bulb temperature readings. In addition 2 lascar USB-502 loggers were deployed inside a Stevenson's screen to take hourly temperature and humidity readings inside the polytunnel (Appendix 3).

Table 5. Weather conditions at the time of spray application **(**N/A = Not applicable)

Expt No	Date	Time	Air temperature			Wind speed (Kph)	direction
			°C dry	°C wet	% rh		
1	13 May	09:38	17	10.5	47	0	N/A
1	25 May	10:37	11	10	89	0	N/A
2	09 July	07:27	15	13.5	80%	0	N/A
2	16 July	08:30	22	17	61%	0	N/A

Phytotoxicity

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

Quality Assurance

East Malling Research is an officially recognised efficacy testing organisation (Certificate no. 0321). The work was done according to GEP quality standards and according to East Malling Quality Assurance (EMQA) procedures and requirements (Experiment no. GEP13/017).

Results

Optimisation of Pheromone Loading in Lure

Statistical analysis

Analysis was conducted to show that it was justified to link the two experiments. Initially the data was square root transformed and analysed by analysis of variance (ANOVA), followed by nonlinear regression analysis of dose-response to summarise the counts into suitable summary statistics. These were then analysed by ANOVA (Appendix 3.)

Initial Analysis

The initial ANOVA showed that there were significant interactions between dose, date and if the traps were in protected or unprotected crops ($P < 0.001$)

Nonlinear regression

A dose-response analysis was used to summarise the counts into suitable summary statistics. The dose response curve has the following equation:-

$$Response = A + C\frac{\left(\frac{Dose}{\exp(logIC50)}\right)^{B}}{\left(1+\left(\frac{Dose}{\exp(logIC50)}\right)^{B}\right)}$$

where:

A is the zero-dose response (so the undosed response)

C is the maximum change from the zero-dose response caused by an infinite dose

logIC50 is ln(Dose) that gives 50% of the maximum change from baseline (i.e. C/2)

B is the "slope" of the dose response curve

As the same Blocks were used for the two experiments there is a linkage between them, so it is possible to use both the data from both Experiments pooled in each Date x Block x Protection combination. The model used to fit the relationship for a given combination would then assume that for a given Date x Protection x Block combination B and logIC50 would be the same for both experiments, but that A and C could be different. In practice it was not possible to fit separate B's for the different Date x Protection x Block combinations, so a common B was used for all fits.

To check that using this linkage in modelling was justified the most general model was fitted with common B's, but with separate IC50's for every Date x Protection x Block x Experiment combination, and compared with the model fitted above. There was no evidence that the more complex model fitted significantly better than the previous model, so the linkage approach is reasonable.

There was evidence that the IC50's were different between the different Date x Protection x Block combinations, so a common IC50 was not fitted. All models were fitted using a weighted regression with weights being 1/(Count + 0.375). This was used as the variance of Count is likely to be proportional to mean(Count).

Once the summary statistics had been obtained then they were analysed using an appropriate ANOVA and results reported.

Analysis of the regression model parameters using ANOVA

Although there is evidence from the regression analysis that the IC50's differ from the ANOVA there is no significant difference between them. Therefore the regression lines for all the experiments, sites and dates were not significantly different from each other.

Analysis of the maximum change from the baseline (C) using log-transformed data showed that the 3 way interaction “Experiment-Protected-Date” was significant: (*P* = 0.012) this means that the midge population within the two fields was different at each date of assessment and for the two experiments (Figure 4).

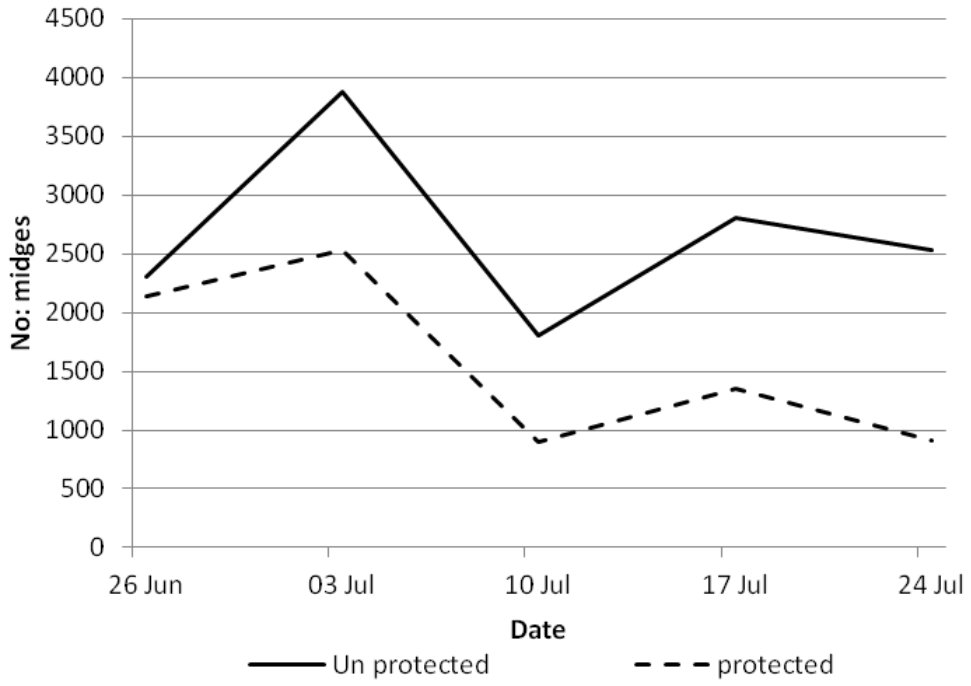


Figure 4. Mean background midge population as recorded in a 1000 µg lure for the duration of the experiment

Experiment 1

Significant catches of male blueberry midge were obtained within 1 h of deployment of the traps and after 7 d the sticky bases of the traps were becoming saturated at the higher doses (Figure 5). For both assessments, catches with the 1 µg lure were not significantly different from those in unbaited traps. At the higher doses catches increased significantly with increase in loading of pheromone in the septum (Figure 5).

Experiment 2

In Experiment 2, further increasing the loading of pheromone above 1,000 µg did not give a significant increase (or decrease) in catch (Figure 6).

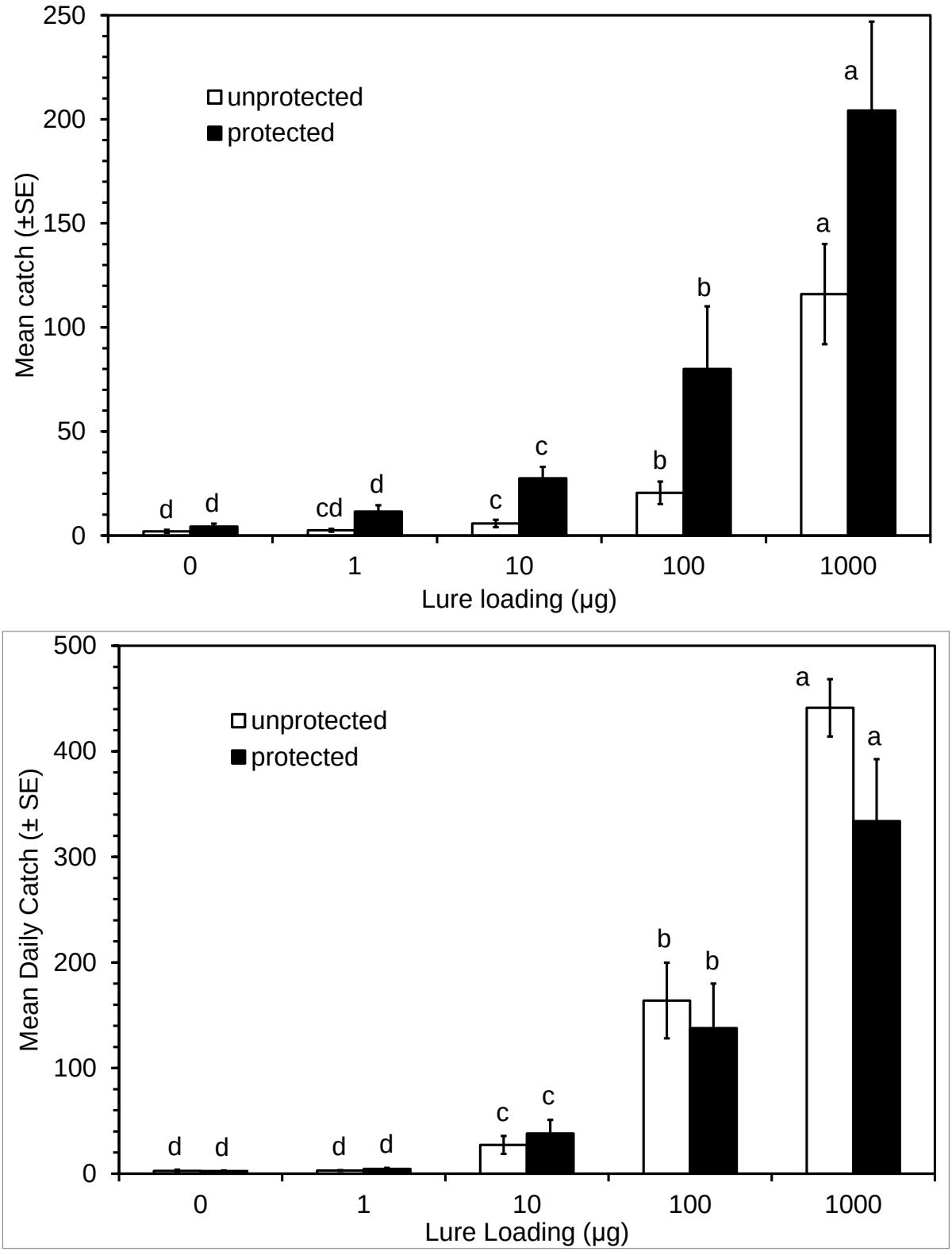


Figure 5: Mean catches of male blueberry midge in traps baited with different loadings of heromone in protected and unprotected crops in Experiment 1 (upper after 1 h, lower after 14 d 19 June – 3 July 2013; *N* = 4; means with different letters in each assessment are significantly different at *P* < 0.05 after analysis of variance on data transformed to log(x+1))

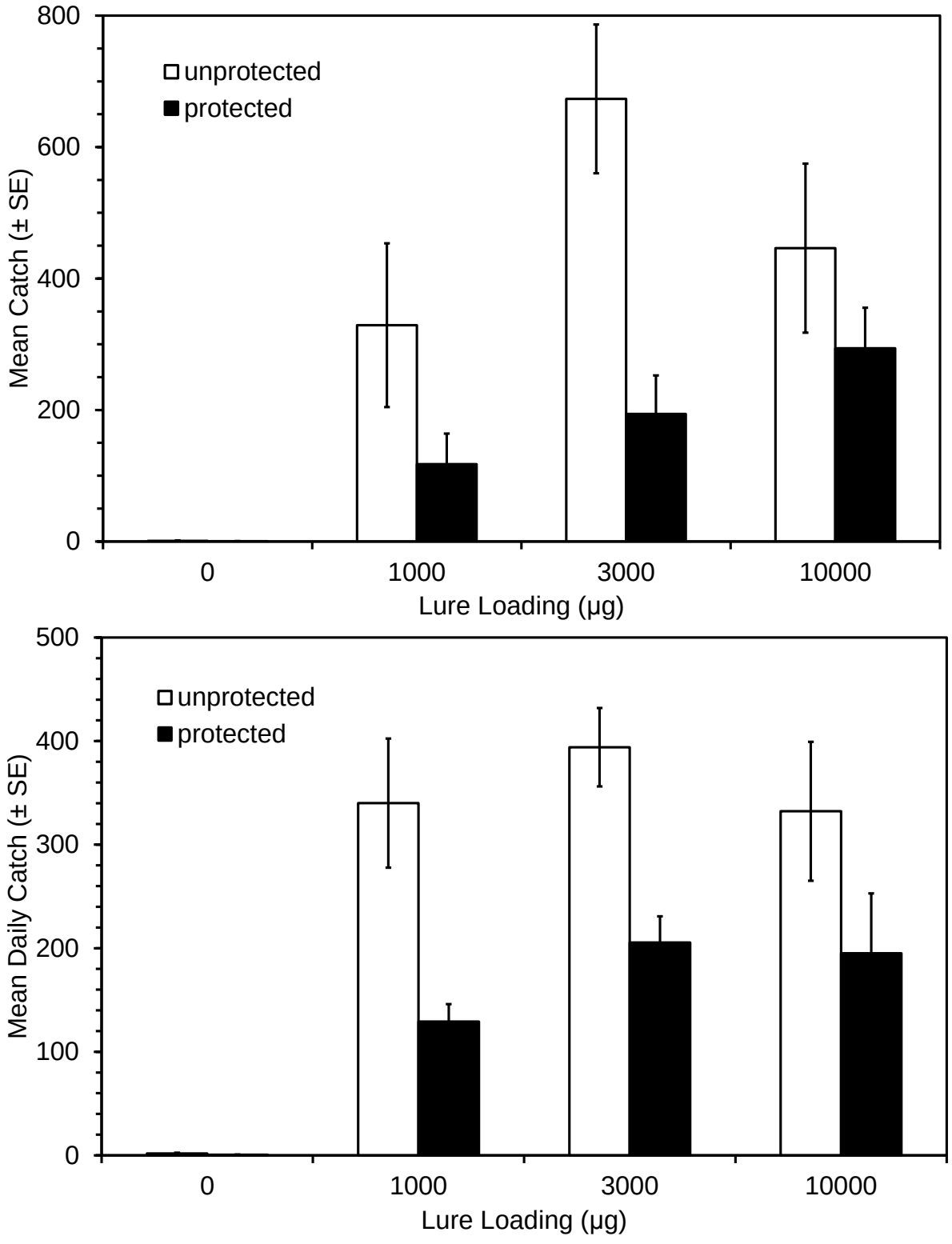


Figure 6: Mean catches of male blueberry midge in traps baited with different loadings of pheromone in protected and unprotected crops in Experiment 2 (upper after 1 h, lower after 21 d 3-24 July 2013; *N* = 4; catches in baited traps significantly different from catches in unbaited *(P* < 0.05), but no significant difference in catches in baited traps after analysis of variance on data transformed to log(x+1))

Combined data

When all the weekly assessments were combined and averaged the protected and unprotected sites followed the same pattern, with the pheromone trap catching increasingly more midges up to 1000 µg, then levelling off and dropping slightly at 10,000 µg (Fig. 7).

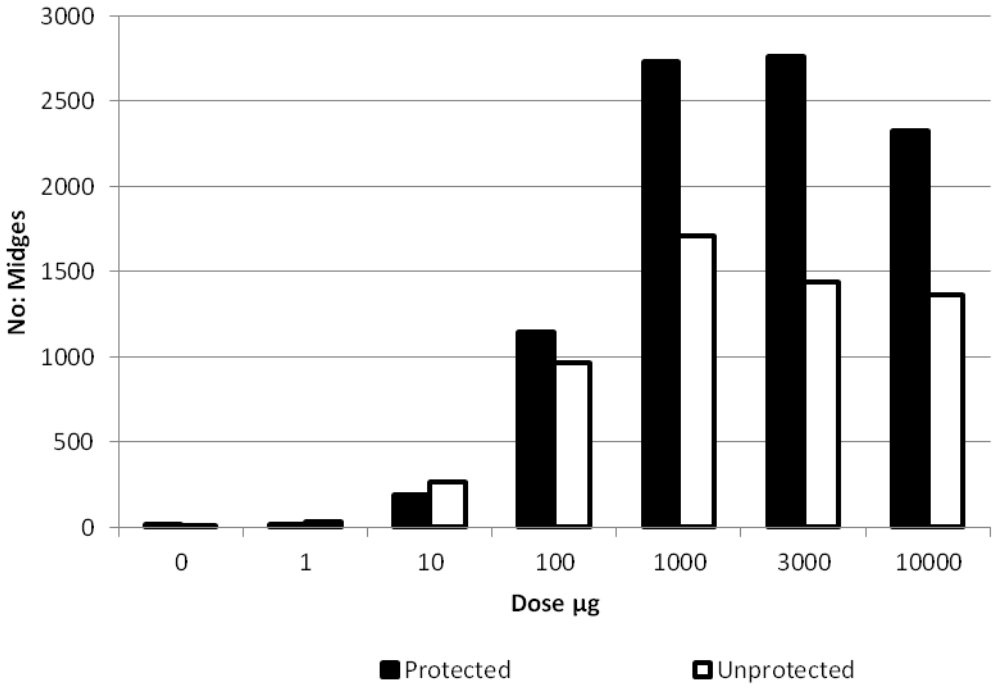


Figure 7: Mean weekly catch after for each lure dose for both experiments in the protected and unprotected sites.

Assessment of the traps for the efficacy and timing of plant protection products to control blueberry gall midge

Phytotoxicity

No signs of phytotoxicity were observed at any of the assessment dates or treatment dates for either of the two experiments.

Midge populations

A single monitoring trap was deployed in the centre of the experimental plot in an untreated control plot. The midge population from the date of trap deployment was higher than the nominated threshold of 10 male midges per trap per week (Figure 8).

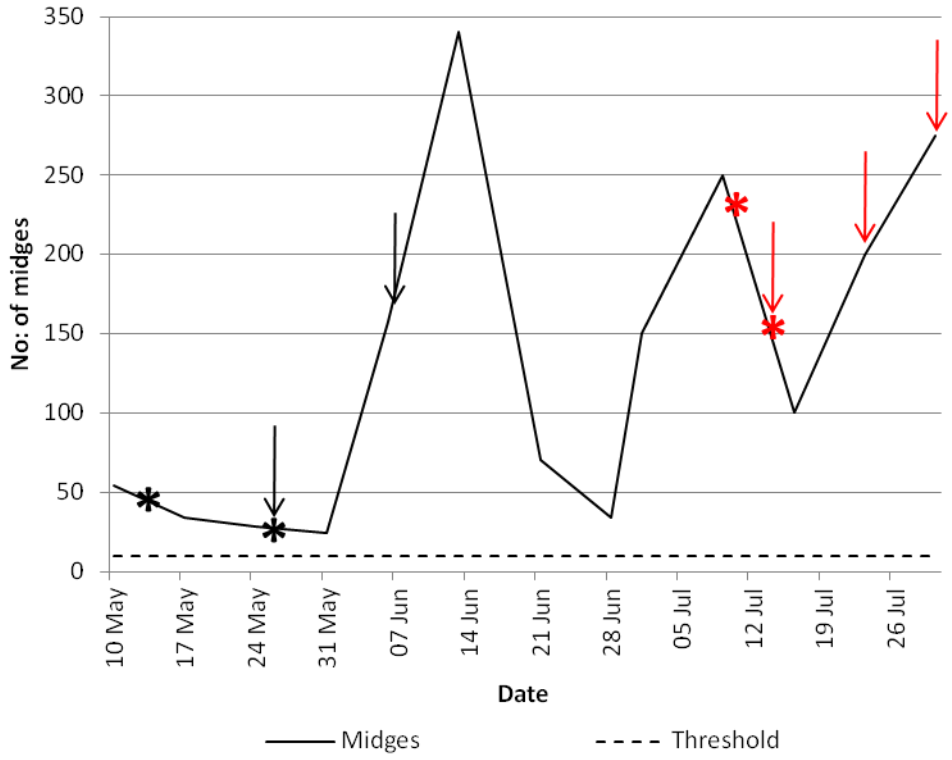


Figure 8. Midge populations and spray application and assessment dates for the duration of the two insecticide experiments. * indicate spray applications and arrows indicate assessments (black = experiment 1 and red = experiment 2

Statistical analysis

The data was expressed as numbers of leaves damaged, and numbers of larvae present. Because this was count data, it required square root transformation prior to undergoing statistical analysis by ANOVA.

Experiment 1

Preliminary analysis showed significantly different numbers of larvae between the two varieties at each analysis (*P*=0.008). Because of this the data from the two varieties was analysed independently.

First assessment. On 25 May there were no significant reductions in the number of damaged leaves when compared to the replicated untreated control (Table 6). Several of the treatments did, however, significantly reduce the levels of larvae within the damaged shoot tips, i.e. chlorpyrifos, thiacloprid and UKA385a for the variety Spartan. For the Ozark Blue the same three treatments reduced the numbers of larvae but so did lambda cyhalothrin and cypermethrin.

Table 6: Mean and square root transformed numbers of leaves galled by blueberry gall midge and the number of larvae within those galls on 25 May, two weeks after the first spray application (* = significantly different from untreated control)

Treatment	Spartan				Ozark Blue			
	Galls		Larvae		Galls		Larvae	
	mean	√mean	mean	√mean	mean	√mean	mean	√mean
Chlorpyrifos	11.3	3.28	4.67	*1.72	8.7	2.88	2.67	*1.22
λ-cyhalothrin	29.0	4.99	30.00	5.35	3.3	1.49	2.00	*0.82
Pyrethrum	16.7	4.06	15.00	3.83	10.0	3.04	9.67	3.05
Cypermethrin	20.3	4.24	18.67	4.19	13.3	3.34	3.00	*1.62
UKA385a	15.0	3.05	4.00	*1.63	11.0	1.91	1.33	*0.67
Thiacloprid	11.0	2.61	3.33	*1.46	25.3	4.64	6.33	*1.75
Untreated	29.0	5.08	24.33	4.43	26.0	5.02	11.33	3.24
Fprob		0.104		0.003		0.104		0.003
SED (52 df)		0.720		0.514		0.720		0.514
LSD (P=0.05)		1.487		1.061		1.487		1.061

Second assessment. The numbers of leaves damaged by blueberry gall midge on 06 June were not significantly different from the replicated untreated control (Table 7). For the Variety Spartan the UKA385a did reduce the amount of larvae as did all of the treatments applied to the Ozark Blue.

Table 7: Mean and square root transformed numbers of leaves galled by blueberry gall midge and the number of larvae within those leaves on 06 June 2013, two weeks after the 2nd application spray application (* = significantly different from untreated control)

Treatment	Spartan				Ozark Blue			
	Galls		Larvae		Galls		Larvae	
	mean	√mean	mean	√mean	mean	√mean	mean	√mean
Chlorpyrifos	10.3	3.16	0.33	0.33	11.7	2.28	0.00	0.00*
λ-cyhalothrin	29.3	5.32	1.67	1.24	19.7	4.36	0.00	0.00*
Pyrethrum	32.0	5.45	6.00	2.00	24.7	4.49	0.67	0.47*
Cypermethrin	24.3	4.83	2.00	0.82	6.0	1.95	0.00	0.00*
UKA385a	9.7	2.98	0.00	0.00*	17.0	3.90	0.00	0.00*
Thiacloprid	17.7	4.07	1.33	0.67	7.3	2.67	0.67	0.67*
Untreated	30.7	5.39	3.67	1.39	22.3	4.15	7.00	1.82
Fprob		0.104		0.003		0.104		0.003
SED (52 df)		0.720		0.514		0.720		0.514
LSD (P= 0.05)		1.487		1.061		1.487		1.061

Combined analysis. There were no significant date, date x variety, and date x variety x treatment interactions for damage (*P*= 0.652, *P*=0.581 and *P*=0.383) or larvae (*P*=0.112, *P*= 0.624 and *P*=0.579) and because of this the data for variety and date could be combined to create a more robust analysis (Table 8). There were no significant differences for the extent of observed damage. However, after square root transformation of the numbers of larvae observed, analysis showed significant levels of control by three of the applied treatments; chlorpyrifos, thiacloprid and the coded product UKA385a (Fig. 8).

Table 8: Mean (and square root transformed) numbers of leaves galled by blueberry gall midge and the number of larvae within those leaves combined by date and variety (* = significantly different from untreated control)

Treatment	Damaged		Larvae	
	data	√ data	data	√ data
Chlorpyrifos	10.5	2.90	1.92	*0.82
λ-cyhalothrin	20.3	4.04	8.42	1.85
Pyrethrum	20.8	4.26	7.83	2.34
Cypermethrin	16.0	3.59	5.92	1.66
UKA385a	13.2	2.96	1.33	*0.57
Thiacloprid	15.3	3.50	2.92	*1.14
Untreated	27.0	4.91	11.58	2.72
Fprob		0.104		0.003
SED (42 df)		0.382		0.603
LSD (P = 0.05)		0.783		1.511

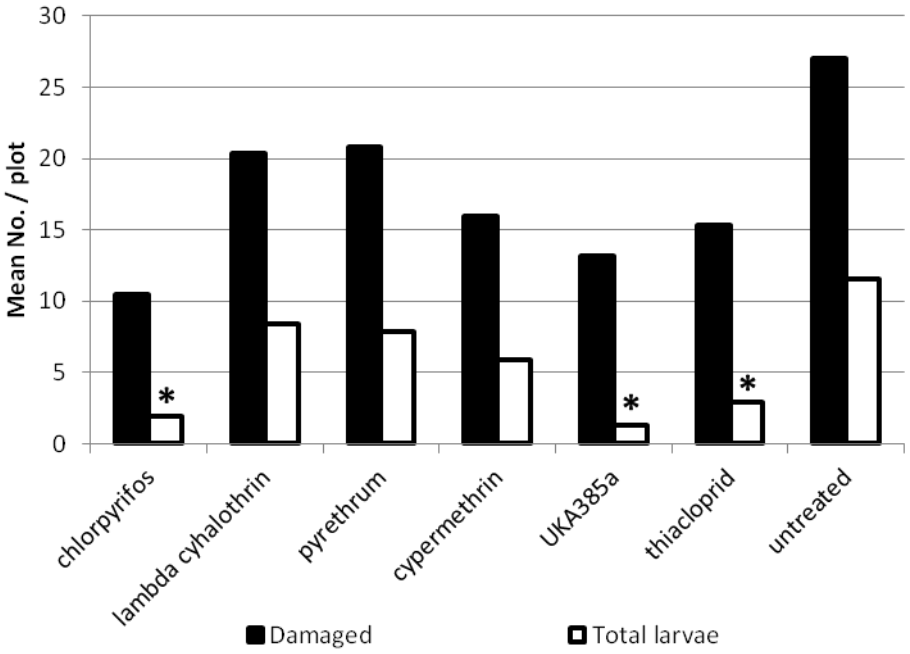


Figure 8: Mean number of larvea with galled damage and mean numbers of larvae within those leaves averaged for date and variety. * = significantly different from the untreated control.

Experiment 2

First assessment. For the variety Spartan the ANOVA of the square root transformation data showed significant reductions in the number of damaged leaves for 3 treatments when compared to the untreated control (Table 9), these reductions in damage did not correspond to a reduction in larval numbers. Only UKA385a significantly reduced these. The results for Ozark Blue were very different to those for Spartan with none of the treatments having a significant effect on larval numbers and only UKA385a reducing damage.

Table 9: Mean (and Square route transformed) numbers of leaves galled by blueberry gall midge and the number of larvae which develop within those galled leaves on 16 July, seven days after the first spray application, * = significantly different from untreated control.

Treatment	Spartan				Ozark Blue			
	Damaged		larvae		Damaged		larvae	
	Data	√ data	data	√ data	data	√ data	data	√ data
Chlorpyrifos	14.00	*3.34	88.5	9.29	8.25	2.76	141.0	11.44
λ-cyhalothrin	36.75	5.92	114.7	10.66	7.25	2.28	104.8	10.07
Pyrethrum	41.50	6.08	142.7	11.90	13.5	3.56	118.0	10.78
Cypermethrin	45.00	6.32	128.2	11.14	12.75	3.47	128.2	11.17
UKA385a	7.00	*2.45	61.2	*7.72	3.00	*1.62	125.5	10.92
Thiacloprid	26.00	*4.04	139.0	11.63	10.25	2.72	104.5	10.10
Untreated	37.50	5.76	131.7	11.19	11.75	3.37	151.0	12.22
Fprob		0.003		0.003		0.003		0.003
SED (101.75 df)		0.705		1.397		0.705		1.397
LSD (P=0.05)		1.399		2.776		1.399		2.776

Second assessment. The ANOVA of the square route transformed data showed significant reductions for both chlorpyrifos and the coded product reducing levels of damage to leaves for Spartan (Table 10). Four treatments significantly reduced the numbers of larvae in the damaged shoots, chlorpyrifos, lambda cyhalothrin, cypermethrin and the coded product. These same four treatments also reduced the levels of damage to the Ozark Blue and three of them also reduced the numbers of larvae. However cypermethrin had no significant effect on larvae in this variety.

Table 10: Mean and square root transformed) numbers of leaves galled by blueberry gall midge and the number of larvae which develop within those galled leaves on 23 July 2013, two weeks after the 1st application spray application (* = significantly different from untreated control).

Treatment	Spartan				Ozark Blue			
	Damaged		larvae		Damaged		larvae	
	data	√ data	data	√ data	data	√ data	data	√ data
Chlorpyrifos	1.5	*0.87	87.5	*8.48	4.00	*1.39	89.0	*9.24
λ-cyhalothrin	8.75	2.64	75.2	*8.53	5.25	*2.22	81.0	*8.94
Pyrethrum	4.75	1.60	198.8	12.09	12.75	3.29	161.0	12.62
Cypermethrin	7.75	2.60	92.2	*9.30	5.5	*2.02	95.0	9.73
UKA385a	1.25	*0.93	28.5	*4.46	1.00	*0.68	48.8	*6.73
Thiacloprid	8.00	2.00	136.5	11.45	7.75	2.64	111.2	10.43
Untreated	6.00	2.41	165.3	12.26	11.50	4.03	156.5	12.22
Fprob		0.003		0.003		0.003		0.003
SED (52 df)		0.705		1.397		0.705		1.397
LSD (P=0.05)		1.399		2.776		1.399		2.776

Third assessment. Only the coded product UKA385a was still having any significant effect reducing both the numbers of larvae and their resultant damage in both varieties (Table 11).

Table 11: Mean (and Square route transformed) numbers of leaves galled by blueberry gall midge and the number of larvae which develop within those galled leaves on 30 July 2013, two weeks after the 2nd application spray application (* = significantly different from untreated control).

Treatment	Spartan				Ozark Blue			
	Damaged		larvae		Damaged		larvae	
	data	√ data	data	√ data	data	√ data	data	√ data
Chlorpyrifos	8.25	2.84	98.2	9.84	3.5	1.80	50.0	6.78
λ-cyhalothrin	9.00	2.57	60.0	7.29	5.25	2.15	36.7	5.79
Pyrethrum	10.00	3.10	102.2	9.93	10.00	3.11	59.0	7.63
Cypermethrin	8.75	2.37	82.2	8.80	6.00	1.91	47.8	6.64
UKA385a	0.25	*0.25	19.3	*3.32	0.50	*0.50	16.8	*3.44
Thiacloprid	10.00	2.57	80.2	8.74	9.00	2.72	49.0	6.06
Untreated	11.75	3.39	75.2	8.43	11.50	3.19	36.7	7.85
Fprob		0.003		0.003		0.003		0.003
SED (52 df)		0.705		1.397		0.705		1.397
LSD (P=0.05)		1.399		2.776		1.399		2.776

Combined analysis. There were no significant date, date x variety and no date x variety x treatment interactions for damage (P= 0.652 and P=0.581) and larvae (P= 0.378 and P=0.254). Then the data for variety and date could be combined to create a more robust analysis (Table 12).after square root transformation there were significant differences for the amounts of observed damage; chlorpyrifos, lambda cyhalothrin, thiacloprid and the coded product significantly reducing levels of damage. For the larvae only 2 treatments had a significant effect on observed levels of larvae; lambda cyhalothrin, and the coded product (Fig. 9).

Table 18: Mean and square root transformed numbers of leaves galled by blueberry gall midge and the number of larvae which develop within those galled leaves for both date and variety combined. (* = significantly different from untreated control).

Treatment	Damaged		larvae	
	data	√ data	data	√ data
Chlorpyrifos	6.6	*2.16	92.4	9.18
λ-cyhalothrin	12.0	*2.96	78.7	*8.55
Pyrethrum	15.4	3.46	130.3	10.82
Cypermethrin	14.3	3.12	95.6	9.46
UKA385a	2.2	*1.07	50.0	*6.10
Thiacloprid	11.8	*2.78	103.4	9.73
Untreated	16.2	3.69	124.0	10.69
Fprob		<0.001		<0.001
SED (18 df)		0.316		0.830
LSD (P = 0.05)		0.663		1.687

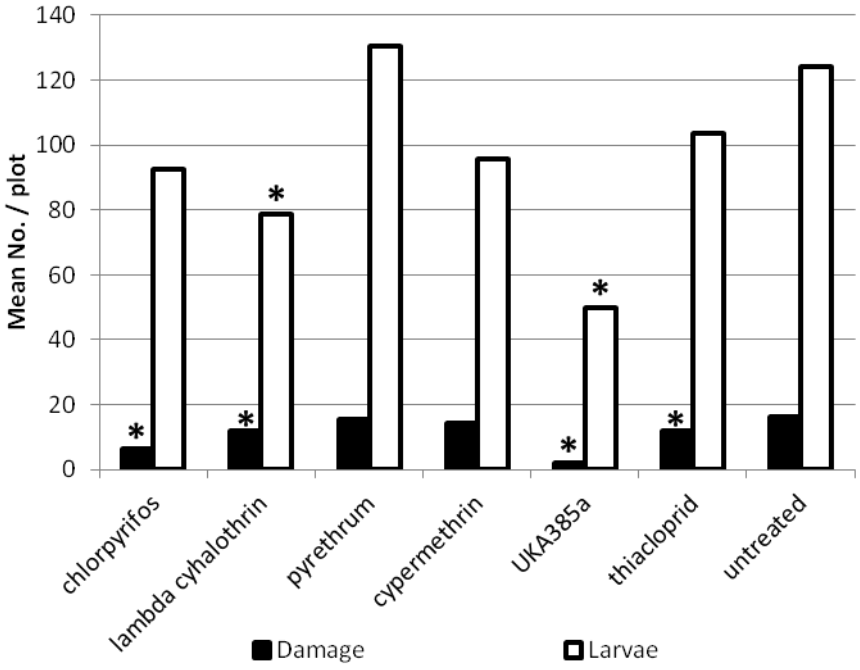


Figure 9: Mean number of leaves with galled damage and total numbers of larvae within those leaves averaged for date and variety (* denotes significantly different from the untreated control)

Discussion

Optimisation of Pheromone Loading in Lure

Trapping experiments carried out during the third year of this project confirmed that the synthetic pheromone is highly attractive to male blueberry midge in the UK. Even lures containing only 10 µg of pheromone attracted high numbers of midges. Increasing the loading of pheromone in the lure increased the numbers caught up to 1,000 µg. Further increases in loading did not significantly increase catches, or decrease them.

Significant numbers of midges were caught within 1 h of deploying the traps, and saturation of the sticky bases of the traps was probably occurring within the 7 d intervals between assessments at the higher loadings. In view of these factors, lures loaded with 100 µg are

recommended for monitoring this pest. Traps and lures for blueberry midge will be available from Agralan for the 2014 season.

For other midge species in the UK, a trap threshold of 10 midges per trap per week to trigger a spray application has been suggested. This threshold should be effective for blueberry midge. However, it has been noted that midge populations can be very high and can increase rapidly. Because of this it is imperative that traps are deployed early, before any signs of leaf galling are observed. From this trial it is also apparent that populations can differ significantly between protected and unprotected crops and that separate monitoring traps should be deployed in each situation.

Assessment of the traps for the efficacy and timing of plant protection products to control blueberry gall midge

Experiment 1

Pyrethrum and thiacloprid (Calypso) are the only insecticides currently approved for use on blueberry, and only thiacloprid gave control of blueberry gall midge larvae after 7 days. After 14 days pyrethrum was giving control of larvae but only on the Ozark Blue. Thiacloprid gave consistent control of blueberry gall midge larvae over time and between varieties, indicating that pyrethrum has a much smaller window of opportunity when application will have the desired effect.

Chlorpyrifos, cypermethrin, lambda cyhalothrin and the coded product also gave significant reduction in the numbers of larvae but this was not consistent between varieties, indicating that the midge populations were developing at different rates on the 2 varieties.

As the larvae develop the shoot tip becomes more distorted twisting around the larvae and protecting them and only products with a level of systemic ability have any chance of affecting the larvae once this damage has developed. The developmental rate of the damaging population was faster than expected probably due to the elevated temperature within the polytunnel, meaning that the two week interval between application and assessment was too large.

The efficacy of control of larvae by all treatments except pyrethrum at the first assessment indicates that all the treatments have the potential to control blueberry gall midge, however

the issue was clouded by the low number of replicates of each variety (three per variety) and the high variability within the data. By the second assessments midge populations were decreasing so only UKA385a showed any significant effect at controlling midge larvae on the variety Spartan. On the Ozark Blue all of the treatments applied (except pyrethrum) had significantly reduced the larval population of the midge.

Combining the data of the 2 assessments and the 2 varieties did increase the robustness of the data and showed that, chlorpyrifos, cypermethrin thiacloprid and the coded product significantly reduced the numbers of larvae present, but that they did little to reduce the amount of galling. The amount of galling is due to the larvae feeding and prevention of this damage is mainly due to correct timing of the application.

Experiment 2

Thiacloprid was still the only approved product that gave significant control of blueberry gall midge larvae and only at the first assessment and only on the variety Spartan. This may be because the midges develop at different rates on the two varieties.

Chlorpyrifos, lambda cyhalothrin, thiacloprid and the coded product significantly reduced the levels of damage. However, this was highly variable across date and variety with only the coded product consistently reducing levels of damage across both date and variety.

Numbers of larvae proved difficult to control with only the coded product having a rapid effect on their numbers. By the date of the second assessment (23 July) chlorpyrifos, lambda cyhalothrin and cypermethrin were also having significant effects on the number of larvae. However cypermethrin was inconsistent between the two varieties.

By the third assessment only the coded product was still showing levels of control against both larvae and the damage they cause.

Pooling of the data increased its robustness and showed that lambda cyhalothrin and the coded product both significantly reduce numbers of larvae and levels of damage, while chlorpyrifos and calypso reduced only the levels of damage.

General discussion

The differences in results between experiment 1 and 2 indicates that there is another level of complexity here that these trial did not address. The most likely explanation is the timing of the insecticide application relative to the life cycle stage of the midge. The significant differences between the effects of treatment on the midge populations for the two varieties indicate that the midges were at different developmental stages on the two varieties. This suggests that different blueberry varieties should be monitored independently in order to time spray applications effectively.

Monitoring the midge population was useful in that it gave positive proof that midges were present in the blueberry plot. However the fact that from the moment the blueberry plants were potted up there was a large midge population that was higher than the threshold, meant that timing the applications of insecticides to get best control was difficult. The majority of the products tested were targeted against the vulnerable life stages; the motile adults, exposed eggs and freshly emerged larvae. Once feeding has commenced and the leaves have galled around the larvae these products lose their effectiveness. Chlorpyrifos has a measure of vapour activity which explains is continued effectiveness against the larvae even inside the galls.

The coded product was a selective systemic insecticide which is effective against other midge pests and controls larvae inside the galls. This product is likely to be compatible with IPM programmes as it only affects insects that feed directly upon the plant. Priority should be given to its development for control of blueberry gall midge and possibly other fruit pests. The parent company of the product will not be undertaking relevant crop specific studies on bees and therefore they request, on the grounds of responsible stewardship, that applications are timed post flowering in the absence of such information.

A further experiment to investigate the ideal timing of applications in relation to the midge population needs to be conducted. Ideally with only a single variety or with two varieties well separated (separate polytunnels) to prevent midge populations interacting between the varieties.

Acknowledgements

We are grateful to Tim Sobie. Andre Miranda We would also like to thank Bethan Shaw, Claudia Carvalho, Zsuzsanna Hajdú, Matteo Maltese, Esther Sala of EMR, who assisted with the spraying, sampling and gall counts.

Conclusions

Overall conclusions from the project are as follows.

- In the UK, female blueberry midges, *Dasineura oxycoccana*, produce (*R,R*)-2,14-diateoxyheptadecane as pheromone and this compound is highly attractive to the males. This compound was identified by Canadian workers as the pheromone of the cryptic species of *D. oxycoccana* found on blueberries.
- The pheromone identified by the Canadian workers for the cryptic species found on cranberry did not attract any male midges in the UK when deployed in blueberry, cranberry or wild bilberry.
- Red sticky delta traps baited with rubber septa proved effective for monitoring blueberry midge. A loading of 100 µg is recommended to give significant catches but avoid saturation of the sticky base.
- Traps should be placed as near the ground as possible for maximum catch
- For monitoring of midge populations, traps should be deployed well before the first flight. A weekly threshold of 10 midges per trap per week is suggested for initiation of control measures.
- Traps and lures will be available from Agralan for the 2014 season.
- Thiacloprid is the most effective product that is approved for the control of blueberry gall midge. Chlorpyrifos, thiacloprid, and lambda cyhalothrin controlled larvae and damage but with inconsistencies between varieties and experiment.
- UKA385a gave consistent control of damage and larvae of blueberry gall midge on both blueberry varieties used in this trial and should be investigated further.

References

Apenite, I. (2010). Biology, ecology and economical significance of the cranberry tipworm, *Dasineura vaccinii* (Smith) in Latvia. Doctoral Thesis, Latvia University of Agriculture, 46 pp.

Collins D.W. and Eyre, D. (2010). Blueberry gall midge *Dasineura oxycoccana.* FERA plant pest factsheet. 3pp

Collins, D.W., Harris, K.M., and Glover, R.H. (2010). *Dasineura oxycoccana* (Johnson, 1899) (Diptera, Cecidomyiidae), a pest of cultivated blueberries, new to Britain. Dipterists Digest 17: 53-59.

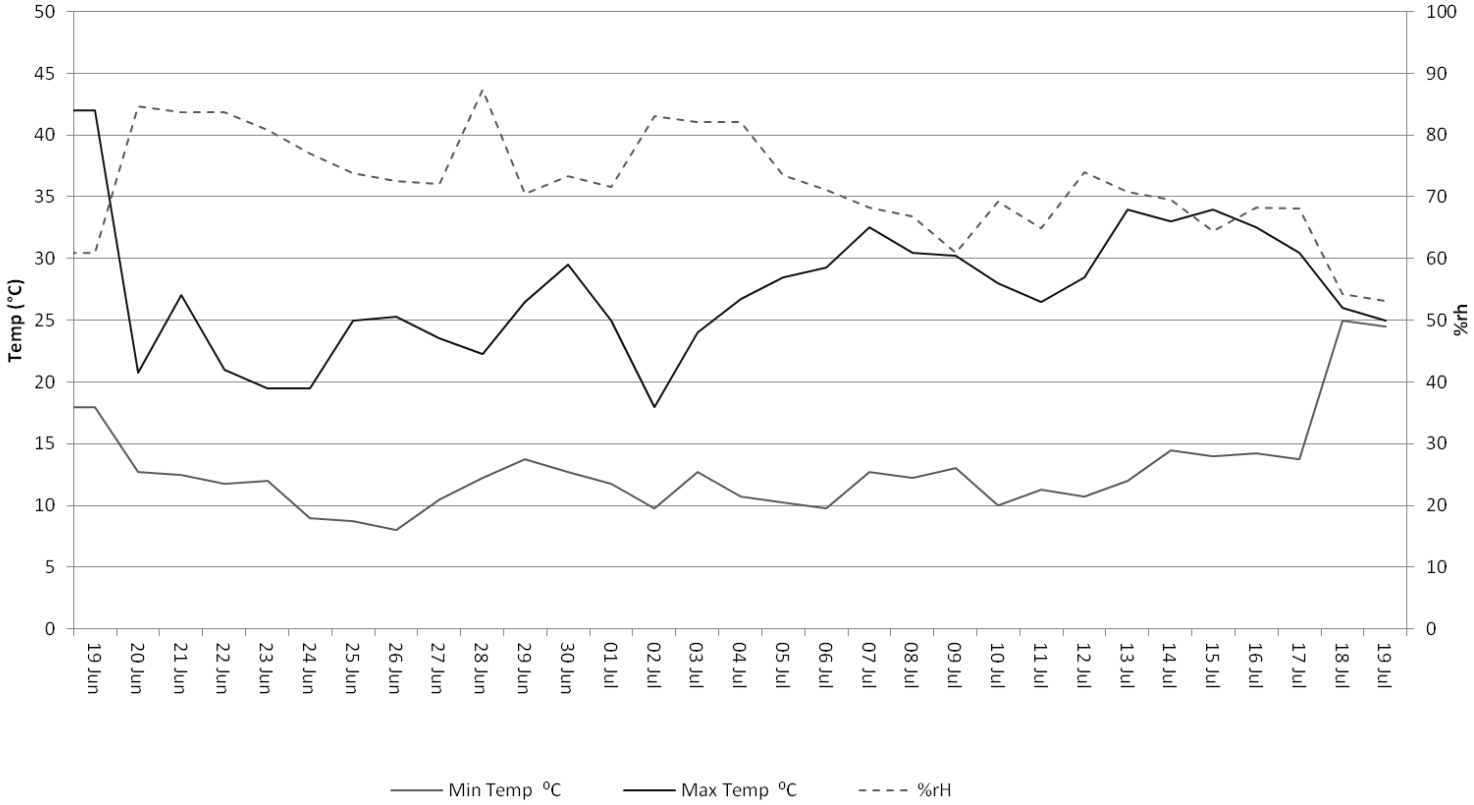
Dernisky A.K, Evans R.C, Liburd O.E, and MacKenzie K. (2005). Characterization of early floral damage by cranberry tipworm (*Dasineura oxycoccana* Johnson) as a precursor to reduced fruit set in rabbiteye blueberry (*Vaccinium ashei* Reade). International Journal of Pest Management 51(2), 143-148.

Fitzpatrick, S.M, Gries R, Khaskin G, Peach D.A.H, Iwanski J and Gries G. (2013). Populations of the gall midge *Dasineura oxycoccana* on cranberry and blueberry produce and respond to different sex pheromones. Journal of Chemical Ecology 39:37-49.

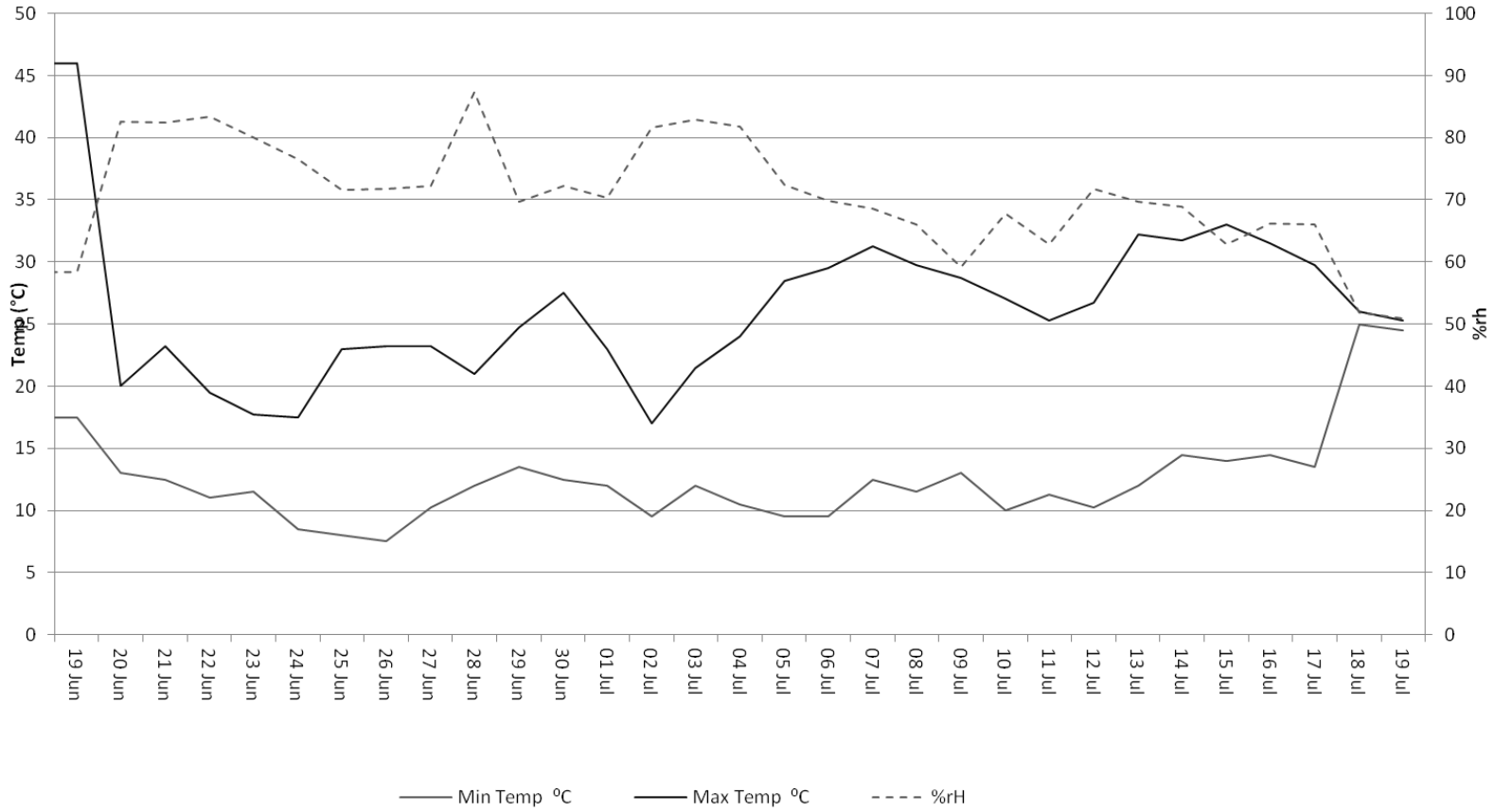
Grassi, A. and Forno, F. (2004). *Jaapiella vacciniorum* Kieffer (Diptera: Cecidomyiidae); a new pest of highbush blueberry (*Vaccinium corymbosum*) in Trentino, Italy. IOBC Bulletin 27(4), 167-172.

Appendices

Appendix 1. Weather data for protected blueberry dose response trial



Appendix 2. Weather data for unprotected blueberry dose response trial



Appendix 3. Weather data for twin span polytunnel at East Malling Research