Project title: Developing biocontrol methods and their integration in sustainable pest and disease management in blackcurrant production

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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

COMMERCIAL – IN CONFIDENCE

The following are members of the Consortium for LINK project HL 01105:

GlaxoSmithKline blackcurrant growers research fund

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East Malling Trust for Horticultural Research

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Bayer Crop Science Ltd

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AUTHENTICATION

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

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

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CONTENTS

GROWER SUMMARY

Headline

The first year of research has made good progress on the use of alternative, biological methods for the control of pests and diseases in blackcurrant

Background and expected deliverables

The overall aim of the proposed project is to develop new management methods for key pests and diseases of blackcurrants, giving priority to alternative, biological methods, and then integrate them into an Integrated Pest and Disease Management (IPDM) programme which will be evaluated and refined in large scale field experiments in the final two years of the project. Work will target Botrytis, the most important disease of blackcurrants which causes significant losses in fruit quality, and two important pest problems, blackcurrant leaf midge and sawfly which are currently controlled by routine insecticide applications. The aim will be to develop appropriate improved management methods for each target to improve control whilst reducing dependence on and unnecessary use of pesticides.

Summary of the project and main conclusions

Progress on each objective of the project is summarised below

Objective 1: *Botrytis cinerea*

18 genotypes have been identified, including both advanced breeding lines and a few commercial cultivars, which vary in their susceptibility to Botrytis. Propagated material from SCRI will be sent to EMR within the next few weeks. Controlled inoculation experiments will be conducted soon to assess whether susceptibility to botrytis is correlated with other morphological characters. Furthermore, latent botrytis has been assessed on nearly all cultivars/breeding lines at two cultivar trial sites. Other morphological characters recorded on these genotypes were also delivered to EMR. These data will be analysed to detect any association between susceptibility to botrytis and morphological characters. Historical samples will be analysed for the incidence of botrytis rot and to assess whether the incidence is

associated with filter blocking.

Preliminary investigations at SCRI have identified physical differences in epidermal strength and structure across a range of blackcurrant germplasm; these measurements will be correlated with evaluations of Botrytis infection over the next 2 years.

Field trials have shown that supplementing blackcurrant plantations with *Bombus terrestris dalmatinus* nest boxes at the point of flower opening increases yield and fruit size of blackcurrant (Ben Hope and Ben Gairn) and this is particularly important in periods of poor weather when naturally occurring pollinating insects are less active. Pathology results were unclear and more replication is needed to ascertain whether *Bombus* pollinated crops are less susceptible to Botrytis infection.

Objective 2: Blackcurrant leaf midge

Crop damage assessment in fruiting plantations

A 3 year, replicated large plot experiment was started in April 2010 in 7 commercial blackcurrant plantations in England, to investigate the effects of blackcurrant leaf midge attacks on crop growth and yield. The plantations included establishing versus fully established crops of Ben Alder, Ben Hope and Ben Tirran. Galling damage, yields and shoot growth were recorded in replicate plots treated with synthetic pyrethroid insecticides (bifenthrin and/or lambda cyhalothrin) where blackcurrant leaf midge attacks were low, versus untreated plots where populations were high.

Pheromone trap catches showed that the 7 different commercial plantations had widely varying levels of leaf midge at the outset. The first generation midge flight started in early April and reached a peak in late April to early May. A mean peak number of 25 midges per trap were captured for the first generation in the untreated plots. The second generation midge flight started in the last week of May to early June. Peak numbers captured averaged 81 per trap.

The insecticide treatments applied to the treated plots reduced numbers of galls per shoot by

67% and 79% for the first and second generations respectively. Catches of midges in the sex pheromone monitoring traps were also reduced by 38% and 80% for the two generations, respectively. Regressions between the numbers of galls recorded per shoot and the mean and peak numbers of midges caught in the sex pheromone traps in the untreated were not significant. More data is required over several seasons.

The gall midge attacks did not affect yield in the first year. The grand mean yield for the treated plots (7527 kg/ha) was very similar to the grand mean yield for the untreated plots (7583 kg/ha). Dormant season shoot growth measurements have yet to be made. The experiment will be continued for 2 further years to determine long term effects of blackcurrant leaf midge attacks on growth and yield.

Crop damage assessment in cut down bushes

The first of a series of 3 field experiments was done in leaf midge infested plantations at Adamston Farms Ltd, Scotland, to examine the effects of midge attack on extension growth in cut down bushes. The aim was to evaluate the effects on galling damage and extension growth, of control of both the first and second generation (1st+2nd gen treatment) of blackcurrant leaf midge, versus control of the second generation only (2nd gen treatment). These would be compared to an untreated control treatment. Sprays of bifenthrin were applied on 15 and 24 May for the first generation, and on 23 June for the second generation.

The 1st+2nd treatment reduced the numbers of galls by 84% and 93% at the two assessments, respectively. Photographs taken of representative plots on 10 and 24 June showed that the gall midge was stunting shoot growth, but dormant season shoot growth measurements to determine the full effects of the treatments have yet to be made.

Timing and efficacy of insecticides

A small plot replicated field experiment was done in 2010 to evaluate the efficacy of foliar sprays (500 l/ha) of UKA385a, Hallmark, Brigade or Calypso for control of first generation blackcurrant leaf curling midge. Treatments were a factorial comparison of the 4 products (UKA385a, Calypso, Hallmark, Brigade) at 3 timing combinations (1 spray at 5 days, 1 spray at 15 days, 2 sprays, one at 5 and one at 15 days, after a threshold catch of > 10 blackcurrant

leaf midge males had been captured per trap in the two sex pheromone monitoring traps deployed in the plantation) versus an untreated control (double replicated). A summary of the findings of the experiment is as follows:

Spray timing was found to be important for effective control of blackcurrant leaf midge with the insecticides tested. Insecticides worked best when they were applied at the 5 days after threshold timing i.e. during the early part of the midge attack. At this time, females were laying eggs in the shoots tips and the eggs were developing and hatching. Application was before or at the early stages of gall formation. Insecticide application 15 days after the pheromone trap threshold was largely ineffective. The numbers of galls already present were not reduced, and only at best partial reductions in the numbers of semi-mature and mature larvae present in the galls were achieved. Two sprays were, thus, not significantly better than one as the second spray was largely ineffective. Monitoring with sex pheromone traps was thus important to getting good results. The results suggest that pheromone traps need to be deployed well before the start of the midge flight, probably by early March at the latest, and it would be best if they were monitored more frequently, probably twice weekly until the threshold is reached.

Hallmark, Brigade and UKA385a all gave effective control of blackcurrant leaf midge when applied 5 days after pheromone trap threshold. Hallmark and Brigade performed rather similarly. They both reduced the numbers of galled leaves by > 80% and gave a very high degree (>98%) of control of larvae in galls. Hallmark may be an effective alternative to Brigade and other bifenthrin products when approval for bifenthrin is lost in spring 2011. However, these synthetic pyrethoids have very broad-spectrum activity and they are likely to be very harmful to the midge's natural enemies including the parasitoid *Platygaster demades* and anthocorid predatory bugs, as well as to the natural enemies of other blackcurrant pests. Ideally, use of these products should be avoided so that blackcurrant IPM programmes which exploit natural enemies can be developed.

A single spray of UKA385a gave virtually 100 % control of larvae in galls. UKA385a is selective insecticide which is less likely to have harmful effects, especially persistent ones, on natural enemies. It is likely to be compatible with IPM programmes and priority should be given to its development for control of blackcurrant leaf midge and possibly other pests in UK blackcurrant IPM. These results indicate that UK385a is slower acting and because of this it does not prevent leaf galling occurring. However, it did give a high degree of efficacy of control

of larvae. Bayer CropScience, the parent company of UKA385a, 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.

The Calypso treatment did reduce larval numbers significantly, but only by 47% and 66% at the two assessment dates, respectively - a notably lower degree of efficacy than the other treatments. The results suggest that Calypso has at best only very limited effectiveness for control of blackcurrant leaf midge.

The findings of this study are preliminary. Further work is needed to validate them and especially to explore timing of application of UKA385a and Hallmark in relation to pheromone trap catches more closely. For experiments in 2011, it is suggested that the pheromone traps are monitored twice weekly during the critical period and timings of application of UKA385a and Hallmark 1, 3, 7 and 10 days after threshold are evaluated.

Can unmated females reproduce?

It was demonstrated that unmated female midges were unable to produce viable offspring and this increases the potential of the pheromone as a mating disruption system to control populations in blackcurrant crops.

Objective 3: Blackcurrant sawfly

A method for culturing blackcurrant sawfly (*N. olfaciens*) has been tested and proven to be effective. Analyses of both hexane washes and collections of volatiles from male and female *N. olfaciens* showed a series of saturated and unsaturated, long-chain *n*-alkanes, as has been found in several other sawfly species. The major component was identified as (*Z*)-9-tricosene in both males and females. However, there were no obvious differences in the profiles of compounds from males and females, either qualitatively or quantitatively in that amounts of material found in hexane washes were not significantly different and amounts produced in collections of volatiles were not obviously different. In other sawfly species it has been reported that the female sex pheromone is actually the product of oxidative breakdown of one of the hydrocarbons. Oxidative cleavage of (Z)-9-tricosene would give nonanal and

tetradecanal, but significant amounts of these were not detected consistently in either hexane washes or collections of volatiles. Future work should focus on analysis of hexane washes and collections of volatiles by GC linked to electroantennographic (EAG) recording from the male sawflies to detect candidate pheromone components. If some of the minor poly-unsaturated hydrocarbons are being oxidised then amounts of the corresponding aldehydes could be very small and the structures of these would be more difficult to predict.

Financial benefits

New knowledge obtained in this project will enable growers to manage the important pests and diseases on blackcurrant more effectively with less reliance on pesticides. In particular:

1. Accurate predictions of *B. cinerea* infection risk may enable growers to time sprays and hence to increase spray efficacy.

2. Integration of biocontrol agents with fungicides may reduce Botrytis development without increasing fungicide use.

3. Potential correlation of physiological characters with Botrytis development may accelerate breeding of less susceptible cultivars.

4. Understanding fungi responsible for filter blockage may enable appropriate control measures to be developed and implemented.

5. Crop damage assessment of blackcurrant leaf midge would allow growers to focus control measures where they are needed and avoid spraying in plantations where damage is cosmetic.

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7. Establishment of thresholds for the newly developed leaf midge sex pheromone trap will enable sprays to be scheduled and timed to improve control and reduce insecticide use.

8. A pheromone based control method for leaf midge would allow growers to control the midge without use of insecticides.

9. A monitoring trap for blackcurrant sawfly and attendant treatment thresholds would allow growers to focus control measures where they are needed and avoid spraying in plantations unnecessarily close to harvest.

10. An improved Integrated Pest and Disease Management programme combining the above components would allow a substantive reduction in pesticide use, reduced incidence of residues and improved sustainability.

Action points for growers

- The trials are at a preliminary stage, but growers should consider supplementing pollination in blackcurrant plantations with commercially available boxes of the native bumblebee *Bombus terrestris audex* in the future.

- Growers interested in using the newly designed blackcurrant leaf midge trap to time spray applications should contact Jerry Cross (jerry.cross@emr.ac.uk).

SCIENCE SECTION

Objective 1: *Botrytis cinerea*

Task 1.1 To characterise variation in varietal susceptibility and to determine whether such varietal differences in susceptibility are correlated with physiological characters

This objective is to determine whether cultivar differences in susceptibility to B. cinerea (mainly in terms of fruit infection) are related to morphological or physiological characters.

Task 1.1.1 Cultivar trialling (year 1-3) (EMR, GSK)

Methods

We have assessed incidence of latent Botrytis in fruit from samples collected in the two variety trial sites in East Anglia and the other in the West Midlands. Fruit were sampled in June. At least 50 (usually 100) fruit were randomly sampled from each line at each site. All the sampled fruit per breeding line were then surface sterilised in 5% Domestos for 15 minutes and rinsed several times. Fruit were then placed well apart onto paraquat amended agar for incubation under ambient conditions. *Botrytis* development was assessed one to two weeks later. In addition, morphological and physical characters were assessed at the two sites for each line by ADAS.

Results

Table 1.1.1 shows the percentage of latent botrytis infection in blackcurrant fruit. Percentage of infection varied greatly, ranging from no infection to 93%. There is no apparent relationship between the incidence of infection and fruit maturity. Statistical analysis of the data will be conducted once we have another year data to determine (1) whether the differences in latent infection are consistent over two years and (2) whether the differences are related to morphological and physical characters.

Table 1.1.1 Incidence of latent botrytis fruit rot on fruit sampled from two variety trial sites (Norfolk and West Midlands). Fruit were collected on 22 June and 28 June for the West Midlands and Norfolk sites, respectively

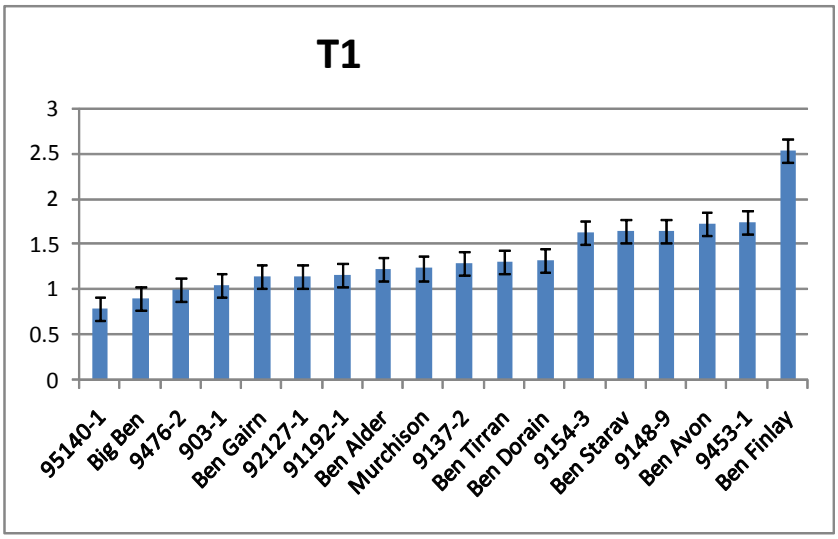
Cultivars / lines	Site	Row No.	Maturity (green to red)	Total No. fruit	No. infected fruit	Incidence
Ben Alder	Norfolk	1	1	101	66	65.35
Baldwin	Norfolk	2	3	97	65	67.01
2008-5	Norfolk	3	3	104	43	41.35
Ben Hope*	Norfolk	4	2	103	47	45.63
9253-1	Norfolk	5	2	96	26	27.08
2008-1	Norfolk	6	5	100	16	16.00
2008-4	Norfolk	7	3	100	55	55.00
2008-7*	Norfolk	9	1	100	22	22.00
2008-2	Norfolk	10	4	100	44	44.00
2008-6*	Norfolk	11	1	100	17	17.00
8962-1	Norfolk	12	4	100	36	36.00
92127-1	Norfolk	13	3	100	0	0.00
95141-3*	Norfolk	16	3	100	24	24.00
2008-3	Norfolk	17	4	100	49	49.00
9154-4*	Norfolk	19	3	86	14	16.28
9265-6	Norfolk	20	2	100	70	70.00
92105-13	Norfolk	21	3	100	27	27.00
9260-20	Norfolk	22	4	100	38	38.00
Various	Norfolk	23	2	100	35	35.00
9559-6*	Norfolk	24	3	100	67	67.00
9521-2*	Norfolk	27	3	100	24	24.00
91153-1	Norfolk	28	1	100	18	18.00
9453-1*	Norfolk	29	4	100	43	43.00
9443-3	Norfolk	30	2	100	41	41.00
Baldwin	West Midlands	41	1	102	90	88.24
Ben Gairn	West Midlands	42	7	100	53	53.00
Ben Hope*	West Midlands	43	1	100	38	38.00

9154-4*	West Midlands	44	2	98	49	50.00
9253-1	West Midlands	45	2	100	36	36.00
8962-1	West Midlands	46	3	103	50	48.54
92105-13	West Midlands	47	5	121	65	53.72
9260-20	West Midlands	48	6	100	13	13.00
9265-6	West Midlands	49	1	98	92	93.88
92127-1	West Midlands	50	2	100	37	37.00
Ben Tirran	West Midlands	51	1	100	31	31.00
Ben Starav(M)	West Midlands	52	5	95	56	58.95
Vane(M)	West Midlands	54	3	100	22	22.00
Vane(M)	West Midlands	55	3	89	8	8.99
Ben Starav(M)	West Midlands	56	6	103	14	13.59
9559-6*	West Midlands	57	4	128	53	41.41
9521-2*	West Midlands	58	7	100	3	3.00
9453-1*	West Midlands	59	4	98	29	29.59
95141-3*	West Midlands	60	6	100	28	28.00
91153-1	West Midlands	61	1	100	35	35.00
9443-3	West Midlands	62	2	100	23	23.00
2008-1	West Midlands	63	7	100	57	57.00
2008-2	West Midlands	64	5	50	13	26.00
2008-3	West Midlands	65	4	51	21	41.18
2008-4	West Midlands	66	3	61	12	19.67
2008-5	West Midlands	67	6	73	24	32.88
2008-6*	West Midlands	68	2	66	3	4.55
2008-7*	West Midlands	69	1	51	7	13.73

Task 1.1.2 Advanced breeding lines (year 1-3) (SCRI)

Initial studies were made to investigate genotypic variation in blackcurrant skin strength and thickness, with following work planned to relate any differences to blackcurrant losses through *Botrytis* infection. The study has developed methods for obtaining reliable assessments of skin strength. Additionally, the method for measuring skin thickness by cryosectioning frozen fruit, mounting the sections, imaging and calibrating for cell measurements, has been developed.

Blackcurrant varieties were harvested on two dates at ripe and overripe stages, and measurements of skin strength were taken using both penetration and compression protocols. Significant variation between cultivars was observed in skin strength, as measured by force required to pierce the berries (Fig. 1.1.2.1) or compress by 30% (Fig. 1.1.2.2). For the penetration study, clear and significant differences were found between the two harvest dates, with reduced force required to penetrate the fruit at the later date, while compression showed less harvest date effect. Some cultivars, e.g. Big Ben and Ben Alder, showed the easiest compression leading to the bursting of all berries tested (Fig. 1.1.2.3), whilst others e.g. Ben Avon and Ben Starav proved more resilient.



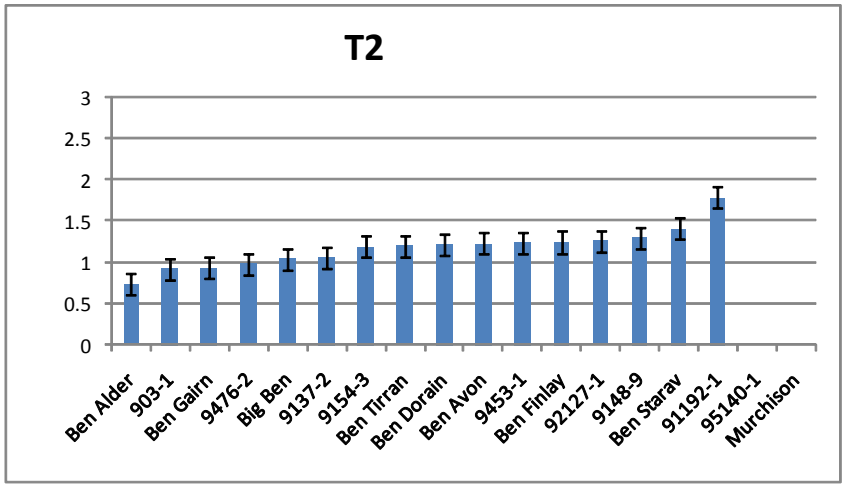


Fig. 1.1.2.1 Force (N) required to pierce berries of blackcurrant cultivars at two different harvest dates (T1 = ripe, T2 = ripe/overripe)

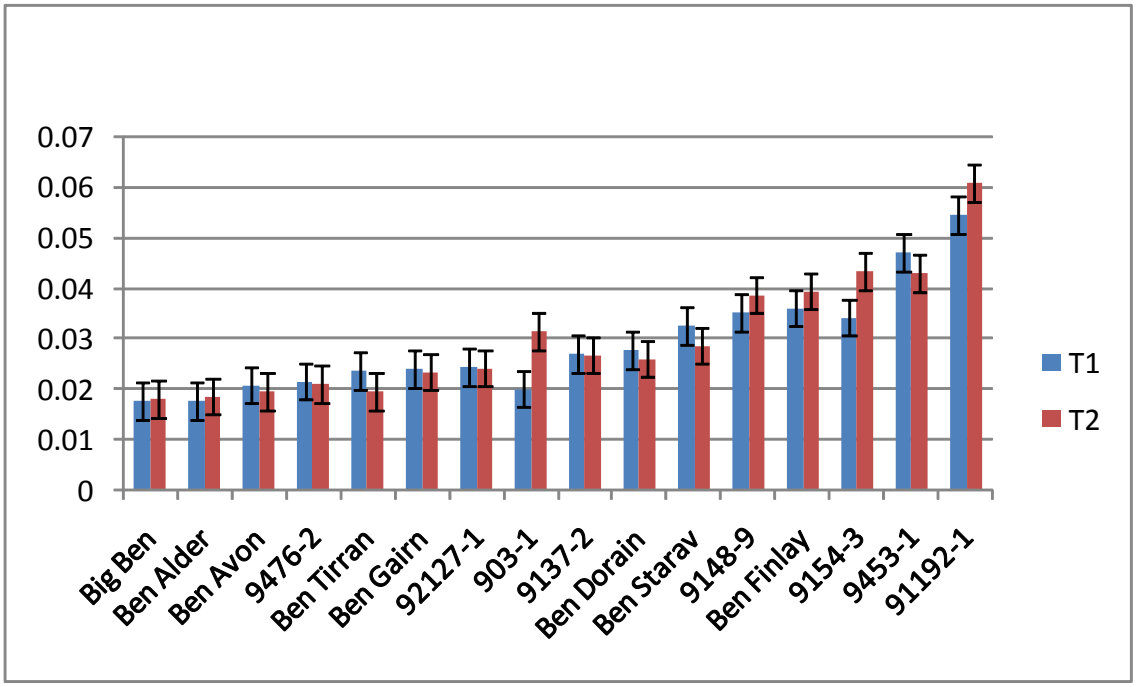


Fig. 1.1.2.2 Stress (Force (N) x πr^2) required to compress blackcurrant berries by 30% at two different harvest dates

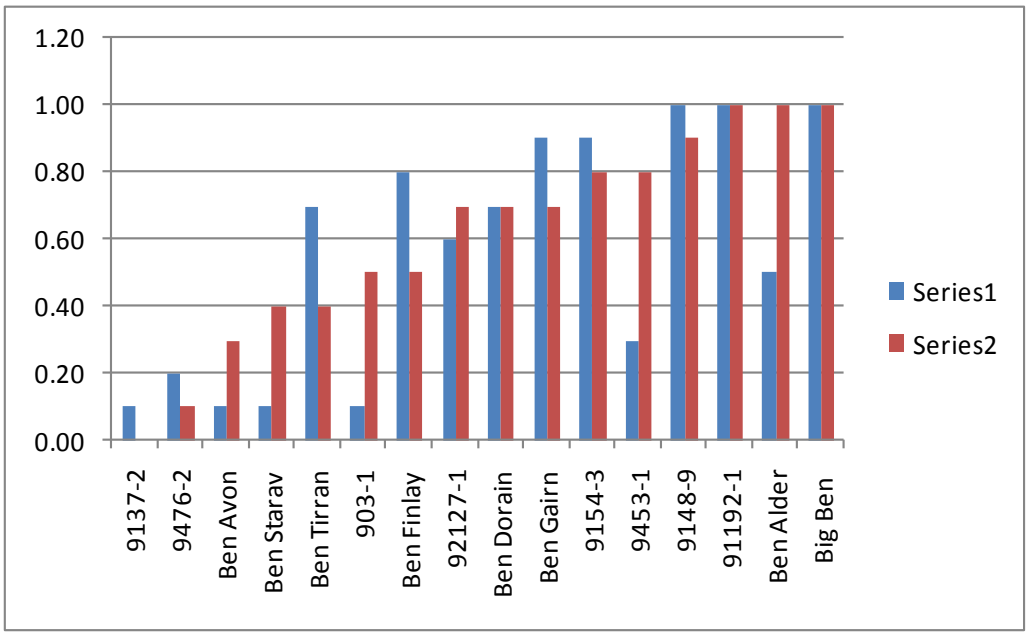


Fig. 1.1.2.3 Mean bursting of berries after 30% compression at the two different harvest dates. (0 = unburst, 1 = burst)

Using cryosectioning of blackcurrant berries, subsequent microscopic analysis revealed significant variation in skin thickness, both of the cuticle alone but also of the cuticle plus the epidermal layers beneath. Cultivars that are known to suffer from skin strength failure post-harvest, e.g. Ben Alder, were found to have the thinnest skin, whilst cultivars with good skin integrity after harvest, e.g. Ben Avon, were also identified (Fig. 1.1.2.5). There was no clear relation between berry size and skin strength.

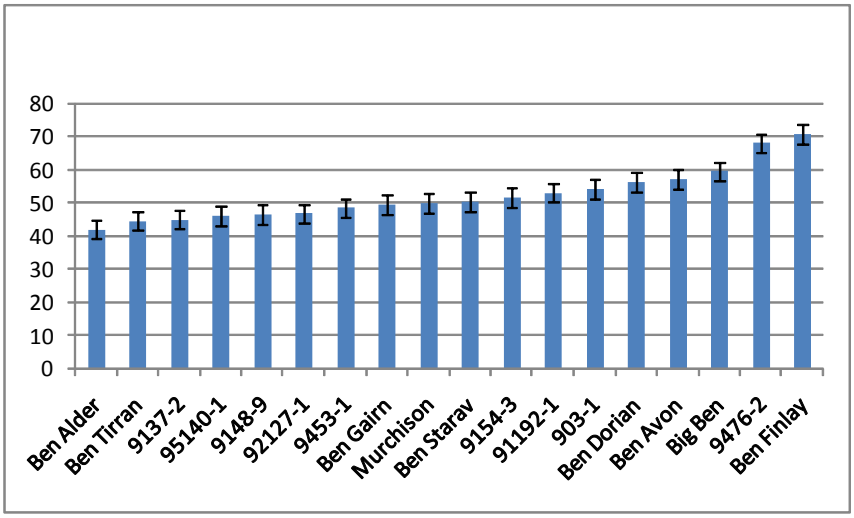


Fig. 1.1.2.4 Thickness (µm) of cuticle plus two epidermal cells of blackcurrant genotypes

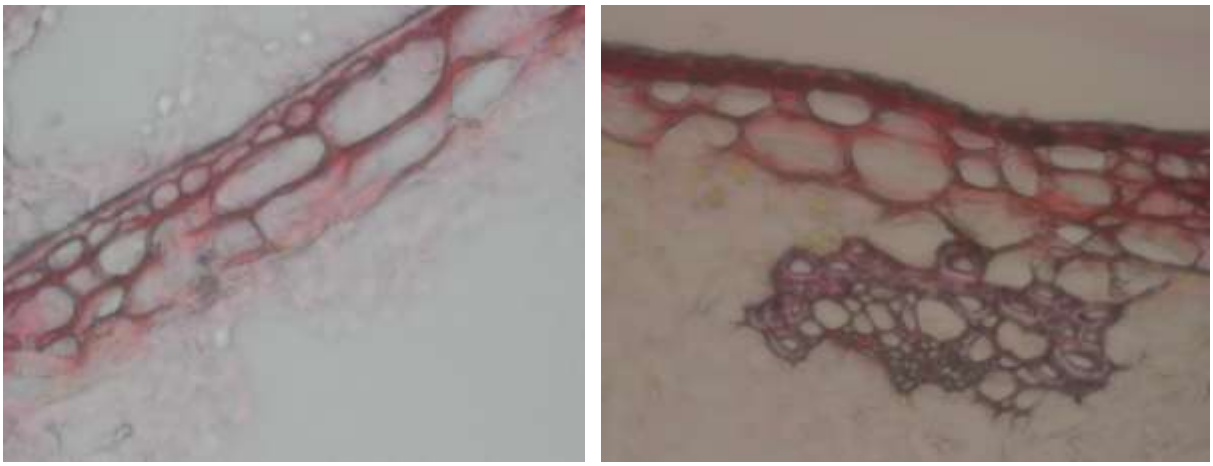


Fig. 1.1.2.5 Cryosections of blackcurrant berries from cvs. Ben Alder (left) and Ben Avon (right), showing differences in cuticle and epidermal layers

Task 1.1.3 Inoculation studies of selected lines (year 1-3) (EMR, SCRI)

SCRI, together industrial partners, has identified 15 cultivars (including advanced breeding lines) for inoculation studies in controlled conditions in 2011. Plants of these selected lines are being raised for study in 2011 at East Malling Research.

Task 1.2 Time fungicide application and supplementary sprays of BCAs during flowering to improve control

This objective is to determine whether current control of flower infection based on fungicides can be improved with additional use of biocontrol agents. Unlike *B. cinerea* on strawberry and raspberry, control of botrytis flower infection is necessary in order to prevent potential flower abscission. However, due to the nature of flowering, it is not possible to protect all flowers from fungal infection even with a 7-day spray interval. Nevertheless, an additional round of a biocontrol agent between two fungicide applications may significantly improve disease control. Experiments will begin next spring and currently we are finalising treatment details and locating trial sites.

Task 1.3 To enhance pollination by provision of pollinating insects in order to increase crop yield/uniformity and reduce infection by *B. cinerea*

Blackcurrant is susceptible to poor pollination because it is an early-flowering crop and therefore often subject to lower temperatures when pollinating insects are less active. It is possible that enhancing the provision of pollinating insects will increase crop yield and quality and reduce infection by *B. cinerea*, particularly in seasons when blackcurrant flowering occurs over an extended period. Premature fruit drop occurs more frequently in self-pollinated compared to honeybee-pollinated blackcurrant and this runoff is often attributed to botrytis infection. Research in New Zealand (report SFF06/007) has demonstrated that fruit set is doubled if crops are provisioned with bumblebees instead of honeybees. Ensuring that flowers are pollinated and fruit is set before the fungal infection can take hold may improve the resistance to fruit drop and the prevalence of latent infection later on. Honeybees are in decline across Europe and are less effective at pollinating early season crops as the colonies are not up to full size and the temperatures are generally too low for foraging. Alternative, more effective pollinating bee species (e.g. *Osmia*) are practically available, but have not been fully tested in UK blackcurrant plantations.

Methods

The field trial plot was two cultivars of blackcurrant that were expected to flower at approximately the same time, but have two different susceptibilities to *B. cinerea* (Ben Gairn botrytis tolerant and Ben Hope botrytis susceptible). The row spacing of the plantation at EMR (CE179) was 3 m with plants 0.5 m apart (20 plants/plot). The plantation consisted of cvs. Baldwin, Ben Gairn, Ben Hope, Ben Lomond and Ben Tirran as 10 4x4 Latin squares (two of each variety). The spacing between plots was 1.5 m (6667 bushes/ha). The plot was planted in March 2002.

There were four treatments:

1. Control (caged blackcurrant bushes excluding pollinating insects, four double sided yellow sticky traps and four single sided white sticky traps suspended from the U bolts in each tunnel to trap pollinating insects emerging within the nets)
2. Caged bumble bee (*Bombus terrestris dalmatinus*) mini colony (~50 worker bees) (sourced from Agralan, commonly used to supplement pollination in glasshouse vegetable crops)
3. Caged red mason bees (*Osmia rufa*) (nest of 25 male and 25 female bees)
4. Open pollination (no cage and open to natural pollinating insects)

Cages were constructed over 12 m length rows of blackcurrant before flowering (10-23 Mar 2010) and each treatment had four replicates (Fig. 1.3.1). Netted cages contained the bees and prevented them from escaping. Care was given to ensure that the net used did not impair botrytis conidia dispersal and was directly comparable to the netted (no insects) and un-netted treatments. The cages were removed at fruit-set. On 30 Mar 2010, four double sided yellow sticky traps and four single sided white sticky traps were hung from the top bar in the four ‘no insect’ tunnels to catch pollinating insects emerging within the nets. On 9 April the bumble bee nest boxes were installed on the floor of the tunnels facing west. Robin Dean installed the red mason bee nest boxes by anchoring with Gaffa tape to the top bar of the tunnel – all nest boxes faced east. Mason bees were seen to be mating on release into the tunnels. The mason bees were provisioned Ambrosia sugar syrup and the food chambers were opened to the bumblebees within the nest boxes.

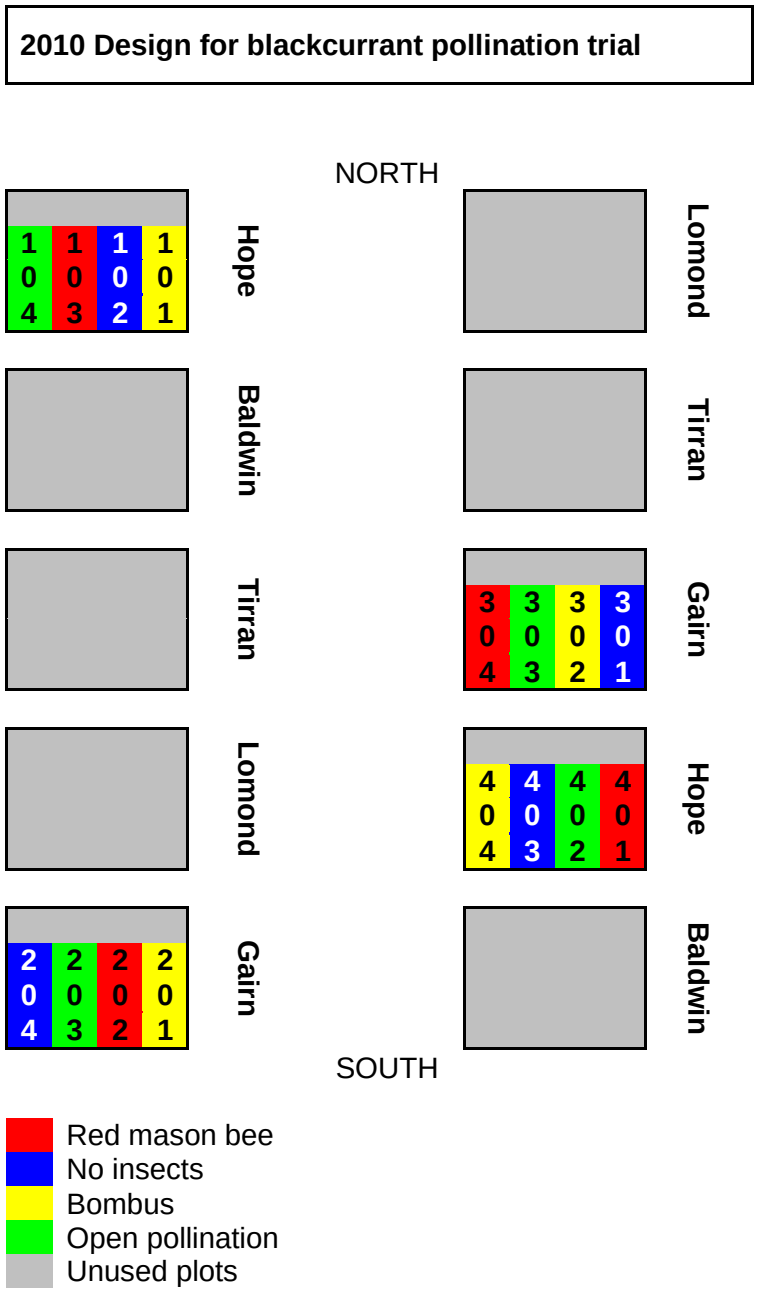


Figure 1.3.1 Plan of the EMR CE179 plot and location of the treatments (tunnels) within plantation

The site was visited on 20, 23 and 27 April to assess the behaviour and diversity of bees visiting the blackcurrant flowers. A note was made of any flowering plants on the ground inside

the tunnels and of any potential bumblebee nests in the tunnel. On 25 Jul 2010 pinned samples of bees taken from the plots were sent to Stuart Roberts at the University of Reading for identification.

Fruit-set was assessed just after pollination (Gairn 11 May, Hope 17 May) and fruit quality (BRIX), yield, fruit size and distribution of berries on bushes at harvest were assessed. Harvest yields were calculated by hand picking and weighing 6 m of the bushes in each plot (Gairn 5 July, Hope 14 July). Fifty fruits per plot were weighed and the diameter measured. The fallen fruit on the ground under each bush was counted. In addition, winter assessments were done to look at extension growth and new green growth in early summer in each plot (number of new shoots 11 June).

Five strigs of green and ripe bush fruit from the centre of each plot were selected for determining the incidence of latent fruit infection by *B. cinerea* (see Task 1.4.1). Samples were cultured and assessed at EMR using the paraquat agar method (fruit plated out; Gairn 12 May, Hope 17 May).

No fungicide sprays were applied to the plots but the plants were inspected regularly after flowering to determine if insecticide sprays against pests needed to be applied. On 11 May 2010 the whole plantation received sprays of Serenade (*Bacillus subtilis*)for mildew, Calypso (thiacloprid) for aphid and Challenge (glufosinate ammonium) for weeds.

A data logger was placed inside the tunnel and another outside to monitor for differences in and out of the tunnel. The East Malling Research meteorological station data was used for the analysis of pollination prediction.

Results

Ben Gairn was ahead of Ben Hope in development and hence flower opening (Table 1.3.1). Once the flowers in the bumblebee treatments had been exposed to bees for more than two days at 100% open flower the nest boxes were removed to prevent the large numbers of worker bees damaging flowers by over foraging.

Table 1.3.1 Timetable of blackcurrant development and removal bee nest boxes

Date	Hope	Gairn	
22-Mar	1st leaf – C1	1st leaf – C1	
30-Mar	2nd leaf – C2	2nd leaf – C2	
9-Apr	Grape just visible - D	Grape just visible - D	Bombus and Osmia nest boxes installed
15-Apr	All grapes emerged - E2	First flower - F1	
20-Apr	2 flowers open - F1	>50% flower open - F2	
23-Apr	>25% flower open	All flowers open - F3	
26-Apr	50% flower open - F2	All flowers open - F3	Bombus removed from Gairn
27-Apr	70% flower open	All flowers open - F3	
30-Apr	90% flower open	All flowers open - F3	
6-May	All flowers open - F3	first fruit setting - 11	
10-May	first fruit setting - 11	50% fruit set - 12	Bombus removed from Hope
11-May	50% fruit set - 12	All fruits set - 13 - green	
17-May	All fruits set - 13 - green	Fruits green	
11-Jun	Fruits green	Fruits beginning to ripen	
5-Jul		BRIX >15 harvest	
14-Jul	BRIX >15 harvest		

The weather at the time of 100% open flower was dry and warm in Ben Gairn compared to Ben Hope where there was a period of rainfall, low solar radiation and temperatures (Fig. 1.3.2).

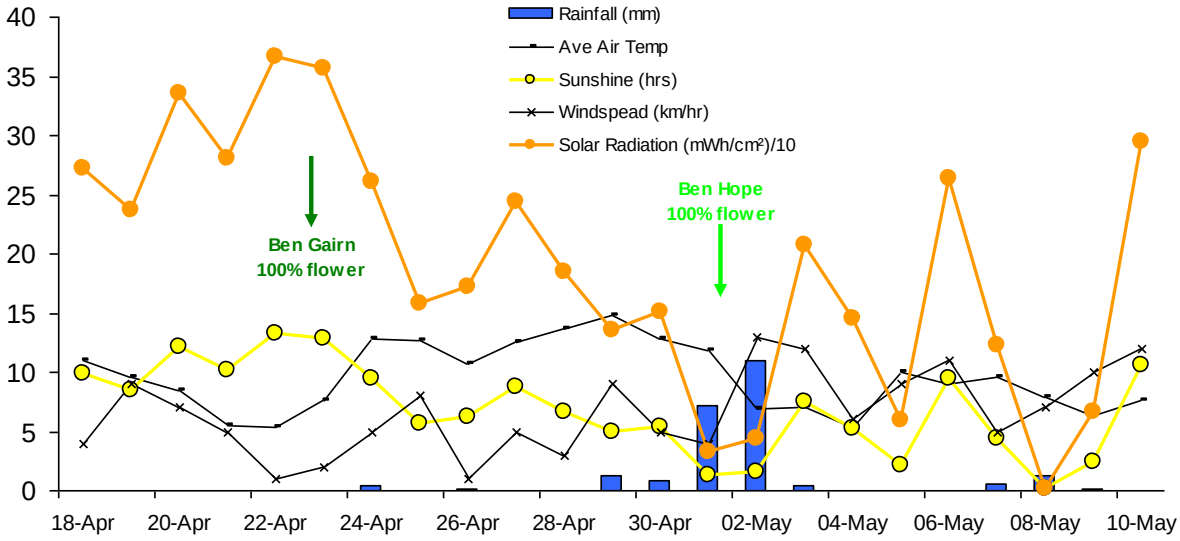


Figure 1.3.2 Weather data for the period of flowering of Ben Gairn and Ben Hope

The temperature and humidity inside the tunnels varied little compared to outside the tunnels (15 Apr – 11 May; inside tunnel 9.8 ^{o}C, 71.1 %RH, outside tunnel 9.2 ^{o}C, 71.7 %RH) (Fig. 3.1.3).

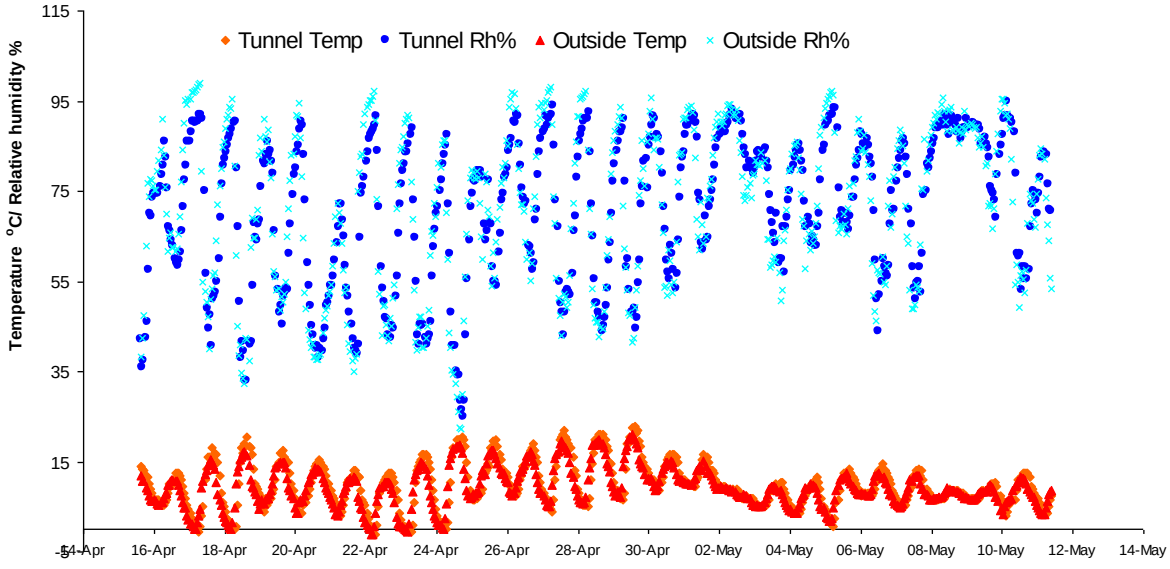


Figure 3.1.3 Temperature and humidity data inside and outside the insect netted tunnels

Bumblebees and solitary bees were sometimes found inside the ‘no bees’ tunnels. These were removed whenever possible. Solitary bees emerged from the bare soil underneath the blackcurrant bushes. Yellow sticky traps caught small numbers of solitary bees. Honeybees were only seen around the plants once the main flowering period was over – despite there being over 20 honeybee hives on site.

Small numbers of red mason bees were seen flying in the tunnels in good weather and were videoed entering and leaving the nest tubes with a peak of activity in the early afternoon. Many of the red mason bees were found dead at the south end of the tunnels either on the floor or attached to the mesh of the net with their jaws (roosting). In total 18 and 27 larvae survived to pupate in the Gairn and Hope tunnels, respectively.

Bumblebees were seen actively foraging on blackcurrant flowers on all of the dates observations were made and were often observed at the top of the north end of the tunnels on really hot days – trying to disperse.

Ten species of Apidae (*Bombus terrestris, B. vestalis, B. lapidarius, Andrena carantonica, A. nigroaenea, A. haemorrhoa, Halictus tumulorum, Lasioglossum morio, L. calceatum, Osmia rufa*) and four species of Syrphidae (*Epistrophe eligans, Eupeodes luniger, Platycheirus peltatus, P. albimanus)* were sampled from the blackcurrant plantation on only three sampling dates (Table 1.3.2.). The likelihood of the diversity of potential pollinating insects being higher is very probable.

Table 1.3.2 Potential blackcurrant pollinating insects found around the blackcurrant bushes on three sampling dates in April. Insects identified by Stuart Roberts, University of Reading

No.	Date	Treatment	Blackcurrant cultivar	Family	Genus	Species	Sex
3	20 Apr	Bombus	Ben Hope	Apidae	*Bombus*	*terrestris*	w
6	20 Apr	Bombus	Ben Hope	Apidae	*Andrena*	*carantonica*	f
1	20 Apr	Bombus	Ben Hope	Apidae	*Andrena*	*carantonica*	m
1	20 Apr	Bombus	Ben Hope	Syrphidae	*Epistrophe*	*eligans*	m
1	20 Apr	Bombus	Ben Gairn	Apidae	*Andrena*	*haemorrhoa*	f
1	20 Apr	Open	Ben Gairn	Syrphidae	*Eupeodes*	*luniger*	m
1	20 Apr	Open	Ben Gairn	Apidae	*Bombus*	*terrestris*	w
1	20 Apr	No bees	Ben Gairn	Apidae	*Bombus*	*terrestris*	w
1	20 Apr	Bombus	Ben Gairn	Apidae	*Andrena*	*haemorrhoa*	m
1	20 Apr	Open plots		Apidae	*Bombus*	*lapidarius*	f
3	27 Apr	No bees	Ben Hope	Apidae	*Bombus*	*terrestris*	w
3	27 Apr	Osmia	Ben Hope	Apidae	*Bombus*	*terrestris*	w
1	27 Apr	Osmia	Ben Hope	Apidae	*Bombus*	*terrestris*	f
1	27 Apr	Osmia	Ben Hope	Apidae	*Halictus*	*tumulorum*	f
1	27 Apr	Open	Ben Hope	Apidae	*Lasioglossum*	*morio*	f
1	27 Apr	Osmia	Ben Gairn	Apidae	*Andrena*	*haemorrhoa*	f
1	27 Apr	Osmia	Ben Gairn	Apidae	*Andrena*	*nigroaenea*	m
1	27 Apr	Open	Ben Gairn	Apidae	*Andrena*	*haemorrhoa*	f
1	27 Apr	No bees	Ben Gairn	Apidae	*Bombus*	*terrestris*	f
1	27 Apr	No bees	Ben Gairn	Apidae	*Andrena*	*haemorrhoa*	f
1	27 Apr	No bees	Ben Gairn	Apidae	*Bombus*	*vestalis*	f
1	27 Apr	Osmia	Ben Hope	Apidae	*Bombus*	*terrestris*	f
1	27 Apr	Osmia	Ben Gairn	Apidae	*Bombus*	*terrestris*	w
1	27 Apr	Osmia	Ben Gairn	Apidae	*Osmia*	*rufa*	f
1	27 Apr	Osmia	Ben Hope	Apidae	*Bombus*	*vestalis*	f
1	27 Apr	Osmia	Ben Hope	Apidae	*Andrena*	*haemorrhoa*	f
1	27 Apr	No bees	Ben Hope	Apidae	*Bombus*	*vestalis*	f
1	29 Apr	Open plots		Apidae	*Andrena*	*nigroaenea*	f
1	29 Apr	Open plots		Apidae	*Andrena*	*carantonica*	m
1	29 Apr	Open plots		Apidae	*Andrena*	*UNIDENTIFIED*	m
1	29 Apr	Open plots		Apidae	*Lasioglossum*	*calceatum*	f
1	29 Apr	Open plots		Syrphidae	*Epistrophe*	*eligans*	f
1	29 Apr	Open plots		Syrphidae	*Platycheirus*	*peltatus*	m
1	29 Apr	Open plots		Syrphidae	*Platycheirus*	*albimanus*	m
1	29 Apr	Open plots		Syrphidae	*Platycheirus*	*albimanus*	f

Very few flowering weeds were found in the tunnels, only single speciemens of *Veronica perscia*, *Stellaria media* or *Senecio vulgaris*.

Fruit set (at green fruit) was higher in Bombus and open pollinated plots in Ben Gairn, and Bombus pollinated plots in Ben Hope (ANOVA on angular transformed data; P=0.001, Fig.1.3.4). The low fruit set in Ben Hope was believed to be due to inclement weather at the peak of flowering for this variety (Fig. 1.3.2).

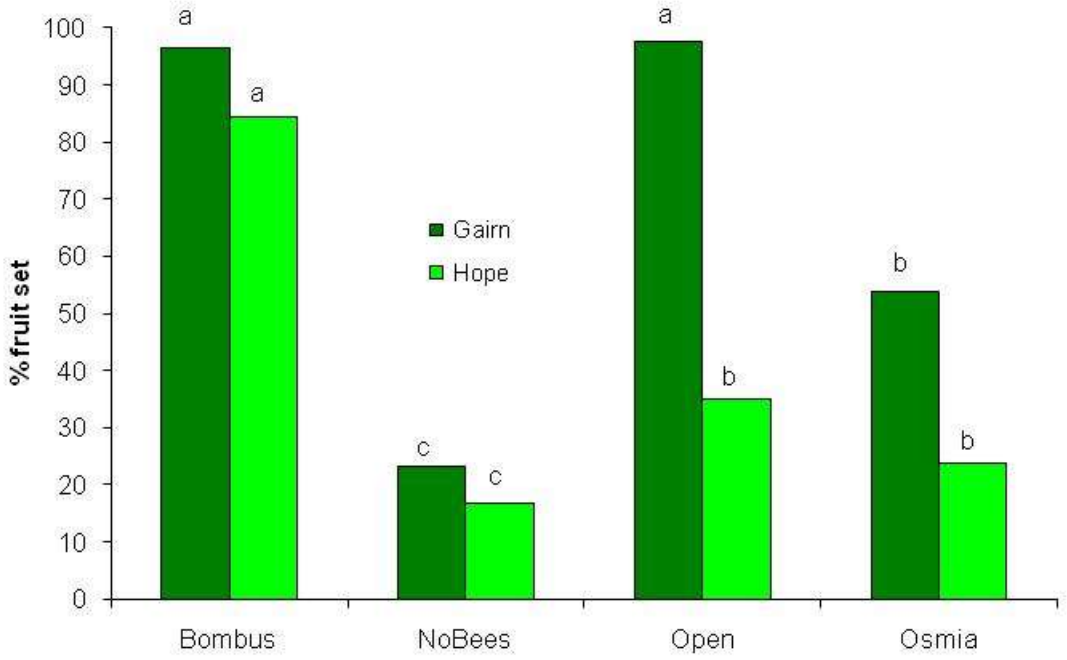


Figure 1.3.4 Fruit set of Ben Gairn and Ben Hope at the green fruit stage. Gairn was assessed on 11 May and Hope on 17 May

Fruit size was also effected by the treatments. Berries in the Ben Gairn and Ben Hope were larger in the Bombus, and the Bombus and open treated plots, respectively (Fig. 1.3.5; ANOVA, angular transformed data; Gairn P=0.022, Hope P=0.047).

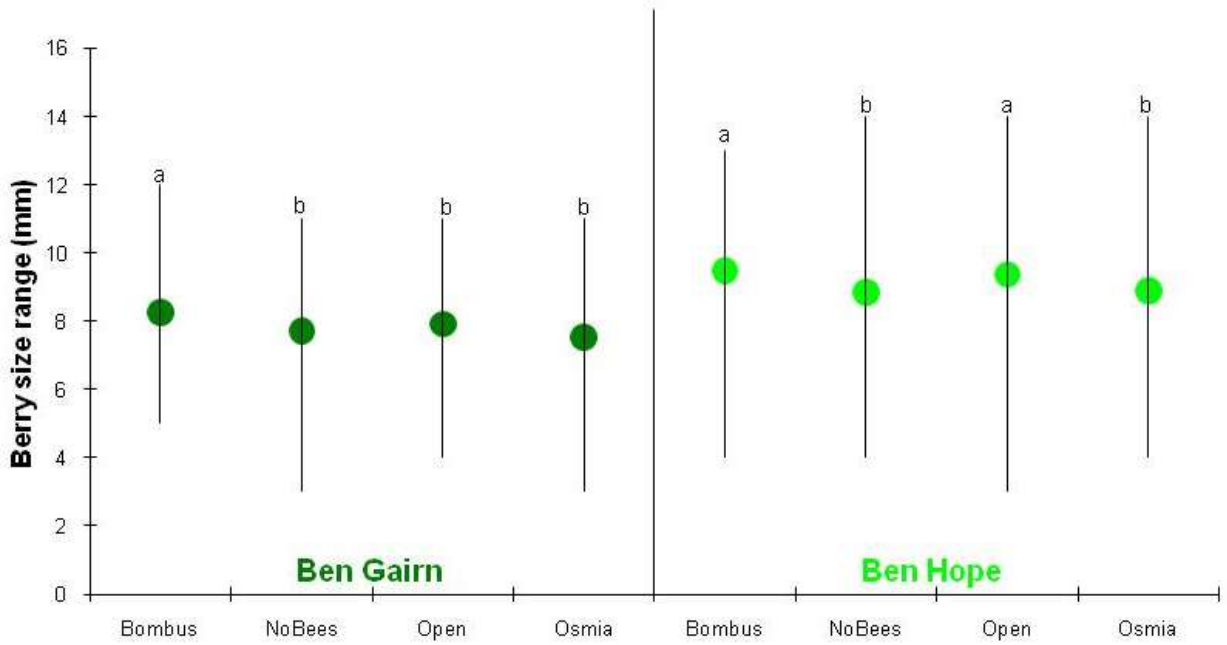


Figure 1.3.5 Mean diameter and range of size of berries at harvest from the treated plots

Ben Hope yielded significantly more fruit than Ben Gairn (ANOVA on $\log_{10}$ transformed data (Fig. 1.3.6)). The yield from the Bombus treated plots was significantly higher than that of all other treatments (Fig. 1.3.6) including the Open plots.

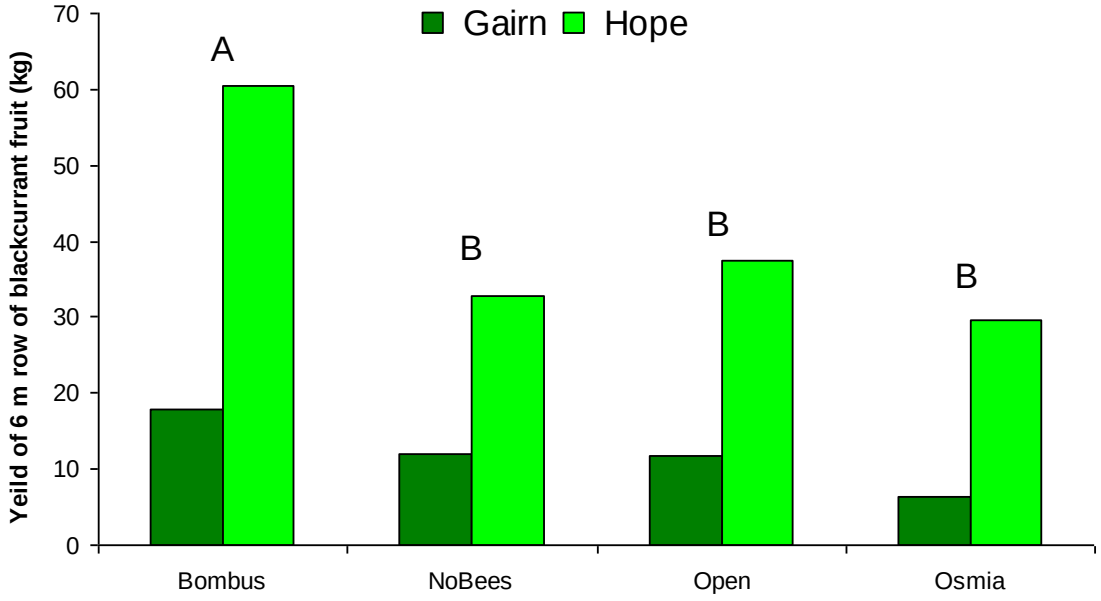


Figure 1.3.6 The yeild of 6 m of hand harvested blackcurrant from the 16 plots. Different letters denote significant differences in yield between the treatments

At harvest there was more dropped fruit in the Bombus and open plots in both blackcurrant varieties (Figs. 1.3.7. 1.3.8). This probably correlates to the yield and observed earlier ripening of these berries (statistics yet to be done)

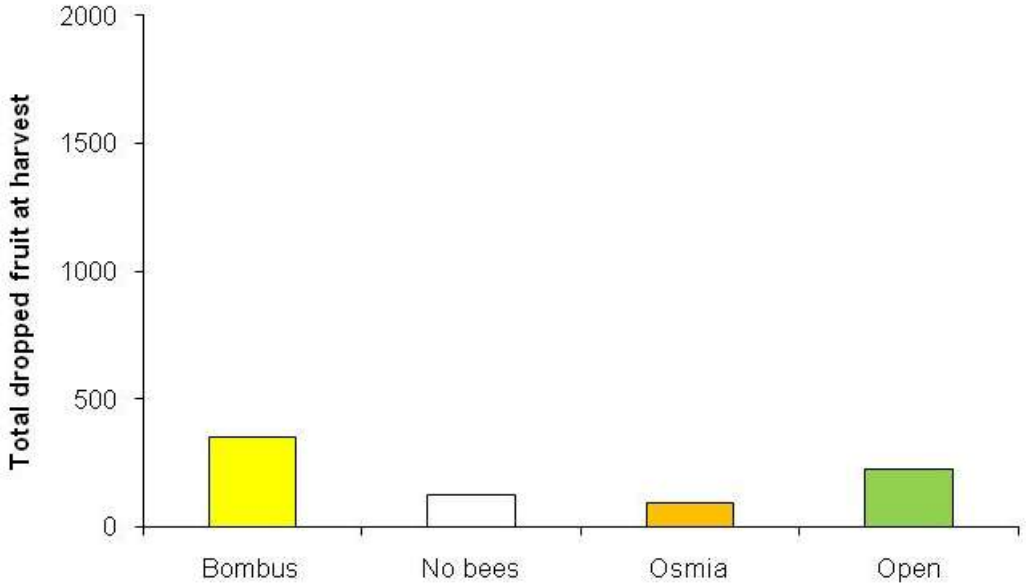


Figure 1.3.7 The number of dropped fruit at harvest in Ben Gairn

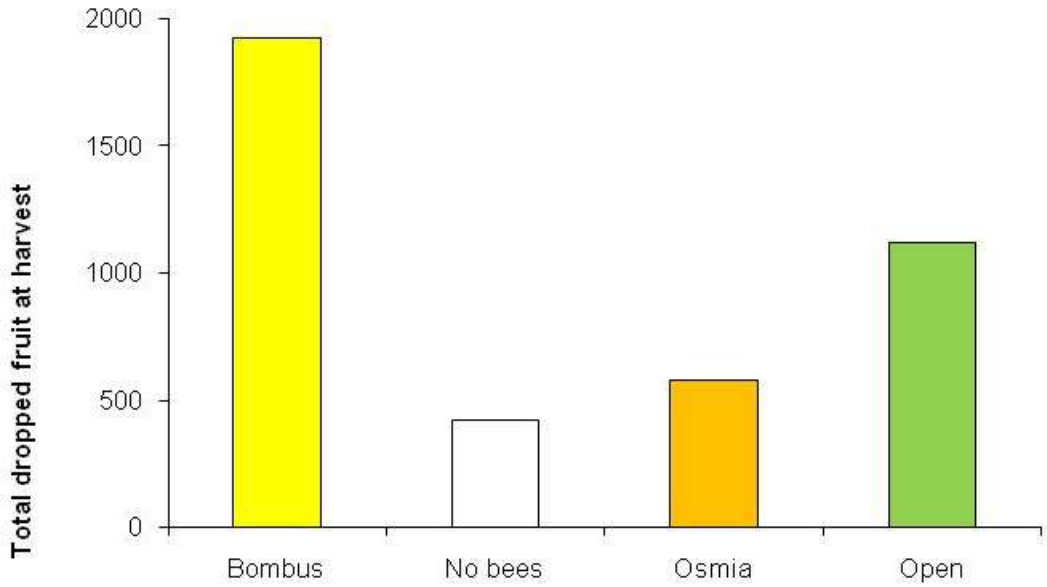


Figure 1.3.8 The number of dropped fruit at harvest in Ben Hope

Ben Gairn had higher numbers of berries per strig in the Bombus pollinated plots and Ben Hope in the Bombus and open plots (Figs. 1.3.9, 1.3.10). (ANOVA $\log_{10}$ data, P=0.005 Gairn

and P<0.001 Hope). Ben Hope also had significantly more berries per branch in the Bombus plots (ANOVA angular transformed data P=0.018).

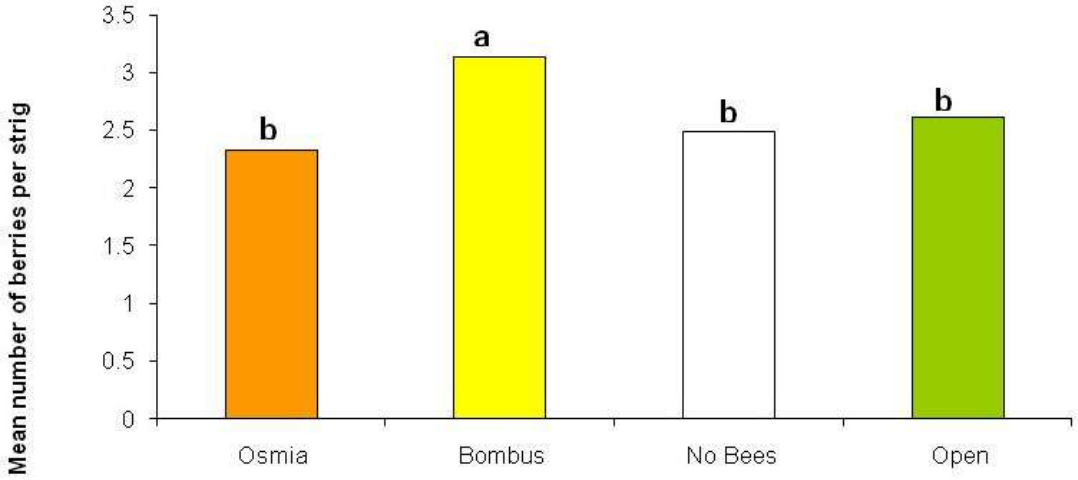


Figure 1.3.9 The number of berries per strig in Ben Gairn

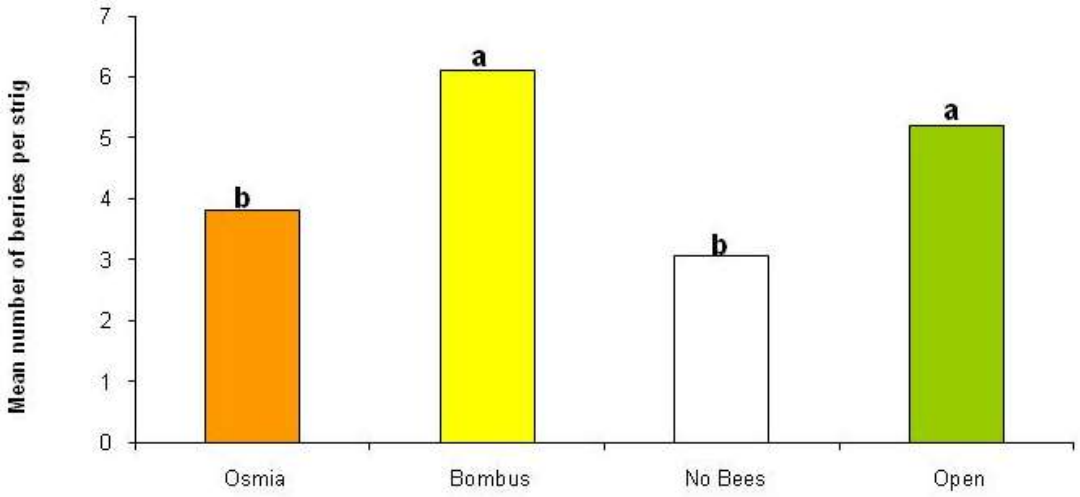


Figure 1.3.10 The number of berries per strig in Ben Hope

Neither BRIX measurements, the number of bunches of berries per branch nor the number of new shoots were significantly different between treatments. Measurements of extension growth are still to be done, but it is predicted that the no bees and Osmia treatments will have a higher extension growth measurement.

Disease assessments were variable and not replicated enough to draw firm conclusions. The only difference that was present was between the two varieties with Ben Hope expressing less latent botrytis infection than Ben Gairn (ANOVA, angular transformed data, P= 0.017, Fig.

1.3.11, 1.3.12). Other diseases found growing on the surface sterilised fruits, particularily at harvest included trichoderma, fusarium, penicillium and aspergillus (Fig. 1.3.13).

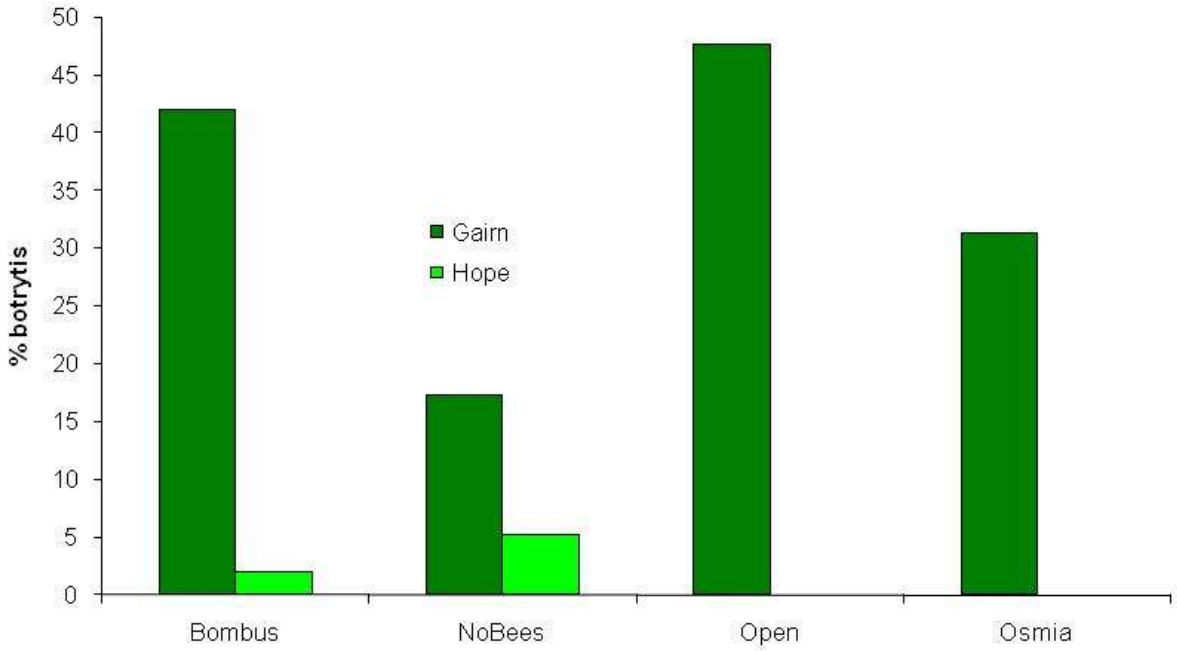


Figure 1.3.11 The percentage infection of blackcurrant berries by latent botrytis at the green fruit stage

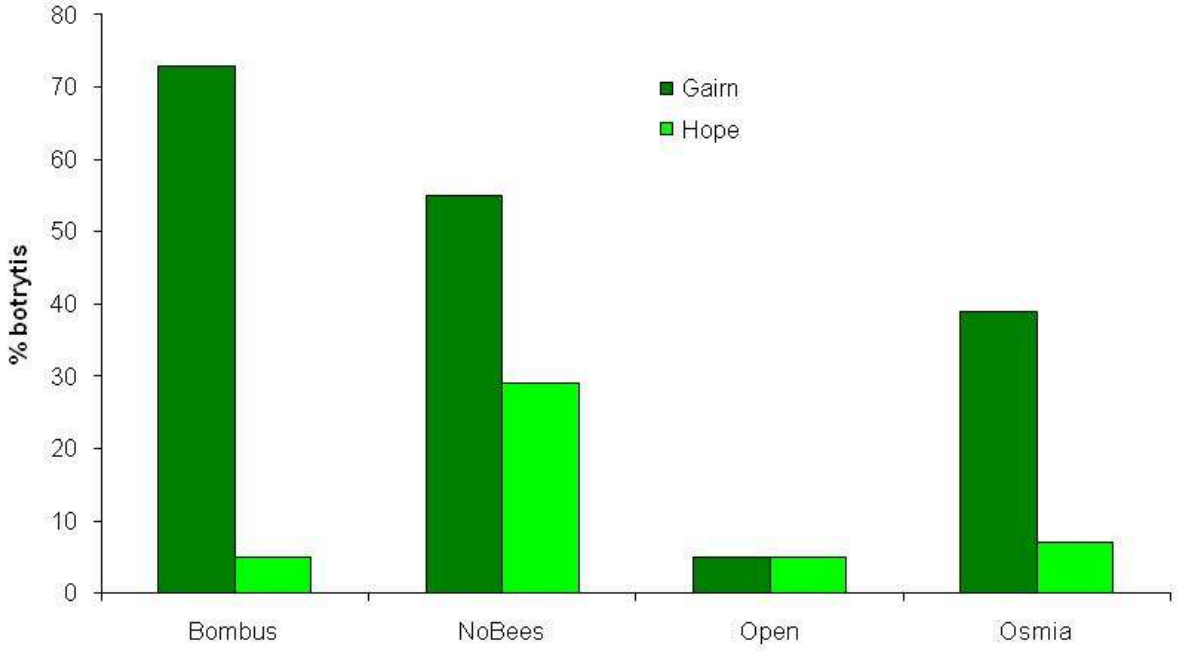


Figure 1.3.12 The percentage infection of blackcurrant berries by latent botrytis at harvest

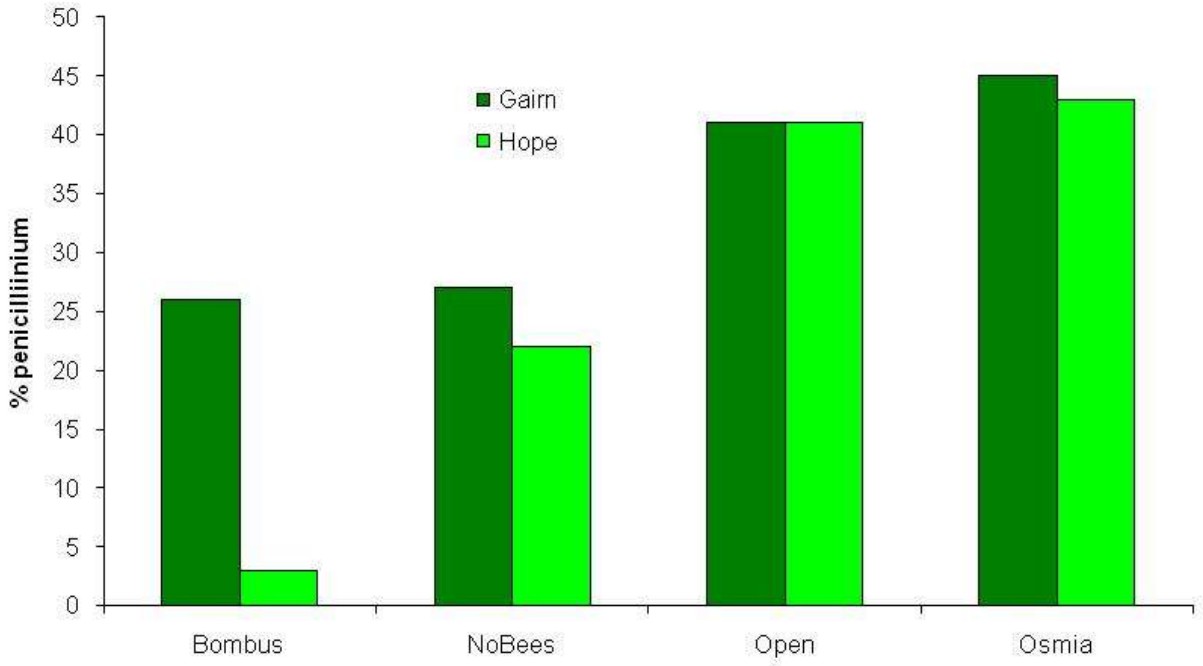


Figure 1.3.13 The percentage infection of blackcurrant berries by penicillium at harvest

Conclusions

- This study highlights the vulnerability of blackcurrant to poor pollination if the weather is not suitable for adequate insect activity
- Supplementing natural populations of pollinating insects with bumblebees may ensure a high yield
- The red mason bee performed less well than anticipated in all of the parameters measured and was no more effective than the 'no bees' treated plots
- More data with higher replication of each treatment is needed to quantify the effects of pollination on latent botrytis infection
- The next field trials should concentrate on large scale use of bumblebees for pollination and the quantification of the numbers needed for improved yield
- The role of bees in vectoring botrytis is unclear and needs further investigation

Task 1.4 To determine the role of *Botrytis cinerea* and/or other fungi in blocking filters and to evaluate the benefit of fungicide and/or BCA sprays applied at different times in reducing such filter blockage

In some years, filters may be blocked during juicing processes and it is suspected that fungal mycelia may be partially responsible for such blockage. It is often assumed that *Botrytis cinerea* is the causal agent, although to date there is no unequivocal evidence for this. The project aims to:

(i) identify whether fungi are responsible for blocking filters;
(ii) (ii) which species are involved;
(iii) (iii) if it is *B. cinerea*, how internal colonisation can be restricted.

Initially, we planned to estimate botrytis levels in frozen fruit samples kept by GSK and relate them to the extent of filter blocking of the batches of fruit. Having discussed with GSK, we felt this approach might not be appropriate because:
(1) frozen fruit sample is a very small bulk sample and hence is not likely to be true representation of field disease level;
(2) botrytis may have infected these samples post-harvest prior to freezing and thus may overestimate the level of infection.

Thus, we have decided to conduct artificial inoculation studies to study the possibility of fungal accumulation within latently infected fruit. This will be done next year since we do not have potted plants in time this year. Currently, we are establishing protocols for quantifying botrytis in latently-infected fruit.

Objective 2: Blackcurrant leaf midge

Task 2.1 To determine the relationships between the severity of galling damage caused by blackcurrant leaf midge and loss in growth and yield at different stages of crop growth and in different cultivars

and

Task 2.2 To determine the relationships between sex pheromone trap catches and numbers of galls that develop subsequently

A Blackcurrant leaf midge crop damage assessment and sex pheromone monitoring trap calibration in fruiting crops 2010-13

Preliminary report

(The first year's work is not yet complete as dormant season shoot growth measurements have not yet been done)

This work addressed sub-objectives 2.1 and 2.2 of Objective 2 of this HortLINK project. Each objective has one main task. The objectives and tasks as specified in the protocol for the project are as follows:

Objective 2.1 To determine the relationships between the severities of galling damage caused by blackcurrant leaf midge and loss in growth and yield (EMR)

Task 2.1.1 Crop damage assessment experiments in growing crops (EMR, Bradenham Hall Farms, Bradfield Farm Ltd, Corbett Farms Ltd, Ian Overy Farms, R Boucher & Son, J Youngman & Sons, Wellbrook Farms, GSK) (Yrs 1-3)

Replicated large plot experiments in plantations of six blackcurrant growers will investigate the effects of leaf midge attacks on growth and yield in establishing (1-2 year old) versus fully established commercial plantations of two different cultivars, Ben Alder and Ben Hope. These two cultivars have been chosen because the former is particularly susceptible to leaf midge attack, the latter because it has a weaker growth habit and does not readily throw new growing shoots and so is potentially seriously affected by leaf midge attacks.

Each experiment will be conducted for a period of three years to allow possible affects of leaf midge attack to accumulate. In total, at least six experiments will be conducted, each in a different blackcurrant plantation on the six different farms participating. Comparisons will be between insecticide treated plots with very low populations of the midge and untreated plots where the midge attack is likely to be high. Randomised block experimental designs with two

replicates will be used. Plots will each be at least 10 rows wide and are likely to comprise the whole length of the plantation. Sprays to control leaf midge will be omitted on the untreated (high midge) plots whereas the treated plots will receive an insecticide spray programme to control midge through the season. Adult midge populations will be monitored through the season in each plot by the use of a standard sex pheromone trap (5µg C isomer sex pheromone rubber septa lures in red delta traps with excluder grids deployed at 3cm above the ground). A record will be taken of the degree of galling damage (at the time of peak damage for that generation) for each generation on each plot. Yields will be taken from the central two rows in each plot separately by machine harvesting by the host grower. In the dormant period following treatment, bush size and growth will be measured for each plot by EMR staff. Data will be collated and subject to statistical analysis to determine the relationships between growth reductions, yield loss and the severity of midge galling damage to establish economic damage threshold for the two cultivars and ages of plantation.

Objective 2.2 To determine the relationships between sex pheromone trap catches and numbers of galls that develop subsequently

Task 2.2.1 Calibrate leaf midge sex pheromone traps (EMR, NRI, Bradenham Hall Farms, Bradfield Farm Ltd, Corbett Farms Ltd, Ian Overy Farms, J Youngman & Sons, R Boucher & Son, Wellbrook Farms, GSK) (Yrs 1-3)

The data collected in Task 2.1.1 will be collated and subject to regression analysis to determine the relationships between peak catches and the gall density results for that generation. If a significant relationship is found, it will be used to establish a preliminary trap threshold.

Methods

Seven commercial blackcurrant plantations of THREE varieties, Ben Alder, Ben Hope and Ben Tirran, were selected for the work (Table 2.1.1).

Table 2.1.1 The SEVEN commercial blackcurrant plantations selected for the work

Farm name & address	Field name and location	Variety	Area (ha)	Age (years)
Ian Overy Farms, Mile Oak Farm, Paddock Wood, Tonbridge, Kent TN12 6NG	House Meadow, Burrs Hill NGR TQ 689 404	Alder	1.26 spray ha	7-8
Wellbrook Fruit Farm, Boughton, Faversham, Kent ME13 9NA	Railway Gate NGR TR 042 585	Alder	2.4	7-8
J Youngman & Sons, Red House, Chasfield, Woodbridge, Suffolk IP13 7QE	Old Orchard NGR TM 240 566	Hope	4	~9
Robert Boucher & Son, Newlands Farm, Teynham, Sittingbourne, Kent ME9 9JQ	Provender Blackcurrants NGR TQ 971 611	Hope	4	7
Oxhouse Farm, Shobdon, Leominister, Hereford HR6 9LT	Telegraph Block 1	Tirran	1.7	4
Bradfields Farm, Bradford-on-Tone, Taunton, Somerset TA4 1HR	9 Acres	Tirran	3.2	Planted 1993, 3rd crop after flailing
Bradenham Hall Fruit Farm, West Bradenham, Thetford, Norfolk IP25 7QR	Rabbit Burrows NGR TF 933 110	Tirran	4	Planted winter 07/08. Cut down winter 08/09.

There were two treatments:

1) Sprayed with bifenthrin or lambda cyhalothrin to give good control of leaf midge (red plots);

2) Not sprayed for leaf midge (green plots). Insecticides applied to the blackcurrant leaf midge treated (Red) (Treatment 1) plots are given in Table 2.1.2.

Note that at Wellbrook Farm, Aphox (pirimicarb) and Calypso (thiacloprid) were applied to the Treated plots for aphid control (Table 2.1.3) and that chlorpyrifos was applied to the untreated plots at Bradenham at the same time bifenthrin (various products) was applied to the treated plots.

Table 2.1.2 Insecticides applied for blackcurrant leaf midge insecticide control to the treated (Red) plots in 2010

Site	Date	Gen	Product	Active substance and formulation	Dose (/ha)
Burrs Hill	20 Apr	1	Starion Flo	80 g/l bifenthrin SC	0.5 l
	22 Apr	1	Brigade	80 g/l bifenthrin SC	0.5 l
	03 Jun	2	Starion Flo	80 g/l bifenthrin SC	0.5 l
	15 Jun	2	Talstar	80 g/l bifenthrin SC	0.5 l
Wellbrook	29 Apr	1	Starion Flo	80 g/l bifenthrin SC	0.2 l
	09 Jun	2	Starion Flo	80 g/l bifenthrin SC	0.2 l
Chasfield	26 Apr	1	Brigade	80 g/l bifenthrin SC	0.5 l
	31 May	1	Brigade	80 g/l bifenthrin SC	0.5 l
	28 Jul	2	Hallmark	100 g/l lambda cyhalothrin CS	0.1 l
Provender	-	-	-	-	-
Shobdon	28 May	1	Starion Flo	80 g/l bifenthrin SC	0.5 l
	16 Jun	2	Hallmark	100 g/l lambda cyhalothrin CS	0.1 l
Bradfields	24 Apr	1	Alpha Chlorpyrifos	chlorpyrifos 480 g/l EC	1.0 l
	03 Jun	2	Starion Flo	80 g/l bifenthrin SC	0.5 l
Bradenham	22 Apr	1	Starion Flo	80 g/l bifenthrin SC	0.5 l
	15 Jun	2	Starion Flo	80 g/l bifenthrin SC	0.5 l

Table 2.1.3 Other insecticides applied to the insecticide treated (Red) plots in 2010 but targeted against other pests

Site	Date	Gen	Product	Active substance and formulation	Dose (/ha)
Wellbrook	21 May	1	Aphox	50% w/w pirimicarb WG	0.28 kg
	08 Jun	2	Calypso	480 g/l thiacloprid SC	0.26 l
Bradenham	27 Apr	1	Chlorpyrifos	chlorpyrifos 480 g/l EC	1.0 l
	4 Jun	2	Chlorpyrifos	chlorpyrifos 480 g/l EC	1.0 l
	20 Jun	2	Masai	20%ww tebufenpyrad	0.5 kg

Table 2.1.4 Insecticides applied to the insecticide treated (Green) plots in 2010 targeted against other pests

Site	Date	Gen	Product	Active substance and formulation	Dose (/ha)
Bradenham	27 Apr	1	Chlorpyrifos	chlorpyrifos 480 g/l EC	1.0 l
	4 Jun	2	Chlorpyrifos	chlorpyrifos 480 g/l EC	1.0 l
	20 Jun	2	Masai	20%ww tebufenpyrad	0.5 kg

In each field (experiment), there were four plots, two treated for leaf midge, two not treated for leaf midge, in a randomised block design. The plots were six to ten rows wide and ran the entire length of the plantation.

Monitoring leaf midge with pheromone traps

In early April, one red blackcurrant leaf midge sex pheromone trap was deployed at a height of ~3 cm above the soil in each plot (Figure 2.1.1).

Figure 2.1.1 Red delta sex pheromone monitoring traps deployed in each plot

Each trap was baited with one rubber septum lure (loaded with 5µg of blackcurrant leaf midge pheromone enantiomer C). The number of blackcurrant leaf midges in each trap was recorded weekly by the host grower. The crop growth stage and an estimate of % of shoot terminals attacked by midge were also recorded weekly.

The experimental plots at each site received overall the growers normal spray programmes of

sulphur for gall mite, fungicides, herbicides and nutrients.

Yields at harvest

The weight of blackcurrants harvested from one or two central two rows in each plot was recorded by the host grower. The area harvested (row length x spacing) was also calculated and the yield of currants per ha for each plot estimated.

Leaf midge galling

Counts were made *in situ* by EMR staff of the numbers of galls in a sample of ~100 shoots in the centre of the central row in each plot, except at Bradfields, at the peak of galling damage for the first and second generation

Extension shoot growth

In winter 2010/11, the lengths of the 2010 seasons extension growth were measured on 100 shoots in the centre of each plot. At the time of writing, these measurements have yet to be processed and analysed.

Statistical analysis

Analysis of variance (ANOVA) was done on the data, after $\log_{10}(n+1)$ transformation to stabilize variances. The block factor was site/block/plot as comparisons of sites was not considered interesting/worthwhile and so that maximum discrimination could be focused on the midge treated versus untreated factor. Linear regression analyses were done between the numbers of galls per shoot and the total midges per trap per day and the peak midges per trap for the first and second generation.

Results

Seasonal dynamics of catches of males in pheromone traps (Figure 2.1.2)

There were large differences in the numbers of midges captured at the different sites. Greatest numbers were caught in the untreated plots at Chasfield and Shobdon. Smallest numbers were caught at Provender followed by Bradenham (Figure 2.1.2, Table 2.1.5). The first generation flight started in early April and reached a peak in late April to early May. A mean peak number of 25 midges per trap were captured for the first generation in the untreated plots.

The second generation midge flight started in the last week of May at Burrs Hill, Shobdon and Bradfields and a week or so later at Wellbrook and Chasfield. The second generation flight could not be clearly distinguished at Provender or Bradenham. Peak numbers captured averaged 81 per trap.

Trap recording was stopped as harvest approached in late July - August. There was an upsurge in catches in mid-July at Chasfield and some evidence of what may have been a third generation flight at Bradfield and Bradenham. However, at this time shoot growth had stopped at most sites and it is suspected that this generation was unsuccessful due to a lack of oviposition sites.

Trap catches and galling damage in the insecticide treated and untreated plots (Table 2.1.5)
The insecticide treatments applied to the treated plots reduced, but did not eliminate, galling damage in the treated versus the untreated plots. For the first generation, the numbers of galls per shoot was reduced by 67% (from 1.18 to 0.39 galls per shoot) by insecticide treatment on average ($P = 0.001$). For the second generation, the numbers of galls per shoot was reduced by 79% (from 2.72 to 0.57 galls per shoot) by insecticide treatment on average ($P = 0.004$). Catches of midges in the sex pheromone monitoring traps were also reduced: by only 38% for the first generation ($P = 0.027$) but by 80% for the second generation ($P < 0.001$)

Relationships between pheromone trap catches and numbers of galls per shoot (Figure 3)
The linear regressions (constrained through the origin) of the numbers of galls recorded per shoot and the mean and peak numbers of midges caught in the sex pheromone traps in the untreated plots all showed positive slopes but no fits ($R^2 < 0$) (Figure 2.1.3). However, it is not surprising that the fits were poor considering that the data was taken from plantations of different ages and varieties.

Effects of pesticide treatment on yield (Table 2.1.5)
The grand mean yield for the treated plots (7527 kg/ha) was very similar to the grand mean yield for the untreated plots (7583 kg/ha) (Table 3) and the yields did not differ significantly ($P = 0.847$). Thus, insecticide treatment for blackcurrant leaf midge provided no yield benefit. Highest yields were recoded in the mature established plantations at Burrs Hill and Provender. Lowest yields were recorded at Bradfields and in the second year plantation at Bradenham.

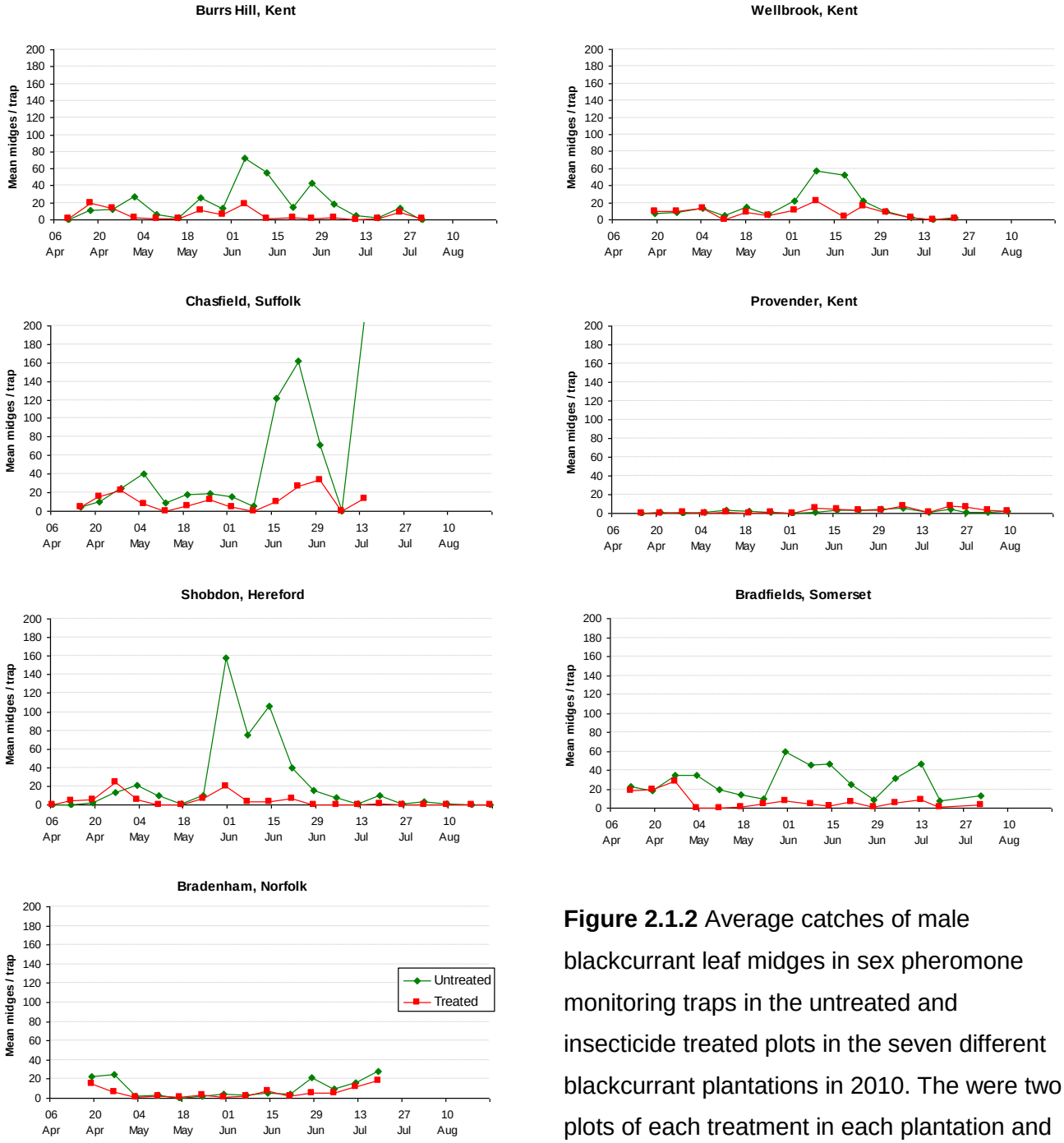


Figure 2.1.2 Average catches of male blackcurrant leaf midges in sex pheromone monitoring traps in the untreated and insecticide treated plots in the seven different blackcurrant plantations in 2010. The were two plots of each treatment in each plantation and one standard red 20 x 20 cm base delta trap with excluder grids and with a 5 µg enantiomer C rubber septa lure. The traps were deployed at a height of 3 cm in the centre of each plot

Table 2.1.5 Mean numbers of midges captured per day, mean peak numbers of midges per trap and numbers of galls per shoot for the 1st and 2nd generation of the blackcurrant leaf midge and the mean plot yields (kg/ha) on the insecticide treated and untreated plots. The overall mean values for each variate have been calculated and the $\log_{10}(n+1)$ transformed data subjected to ANOVA

Farm	Total midges/trap/day				Peak midges/trap				No. galls / shoot				Yield (kg/ha)	
	1st gen		2nd gen		1st gen		2nd gen		1st gen		2nd gen			
	Trt'd	Untrt'd	Trt'd	Untrt'd	Trt'd	Untrt'd	Trt'd	Untrt'd	Trt'd	Untrt'd	Trt'd	Untrt'd	Trt'd	Untrt'd
Burrs Hill	0.95	1.39	0.70	3.98	24.5	27.5	18.5	73.0	0.88	2.00	0.07	4.76	13199	12697
Wellbrook	1.16	1.17	1.16	2.80	14.0	15.0	22.0	58.0	0.78	1.40	1.14	3.13	9466	9274
Chasfield	1.47	2.57	1.67	9.91	22.0	40.0	34.5	161.5	0.03	0.71	0.89	4.68	8809	10682
Provender	0.01	0.00	0.43	0.34	1.5	4.0	9.0	5.5	0.00	0.00	0.07	0.11	11554	11375
Shobdon	0.99	1.15	0.66	6.59	24.5	21.0	20.0	178.5	0.56	2.38	0.09	2.52	5258	4277
Bradfields	1.93	3.70	0.65	4.48	35.0	40.0	8.5	67.5	0.15	0.02	0.94	0.05	2367	2564
Bradenham	0.89	1.89	0.63	1.07	15.5	27.5	7.0	22.0	0.35	1.78	0.80	3.77	2034	2212
Mean	1.06	1.70	0.84	4.17	19.6	25.0	17.1	80.9	0.39	1.18	0.57	2.72	7527	7583
Mean $\log_{10}(n+1)$	0.288	0.383	0.254	0.613	1.204	1.301	1.188	1.685	0.129	0.295	0.177	0.488	3.786	3.790
Fprob (P)	0.027		<0.001		0.199		<0.001		0.001		0.004		0.847	
SED(13 df)	0.0379		0.0760		0.0716		0.1170		0.0396		0.0873		0.0162	
LSD (P = 0.05)	0.0818		0.1642		0.1547		0.2528		0.0855		0.1887		0.0350	

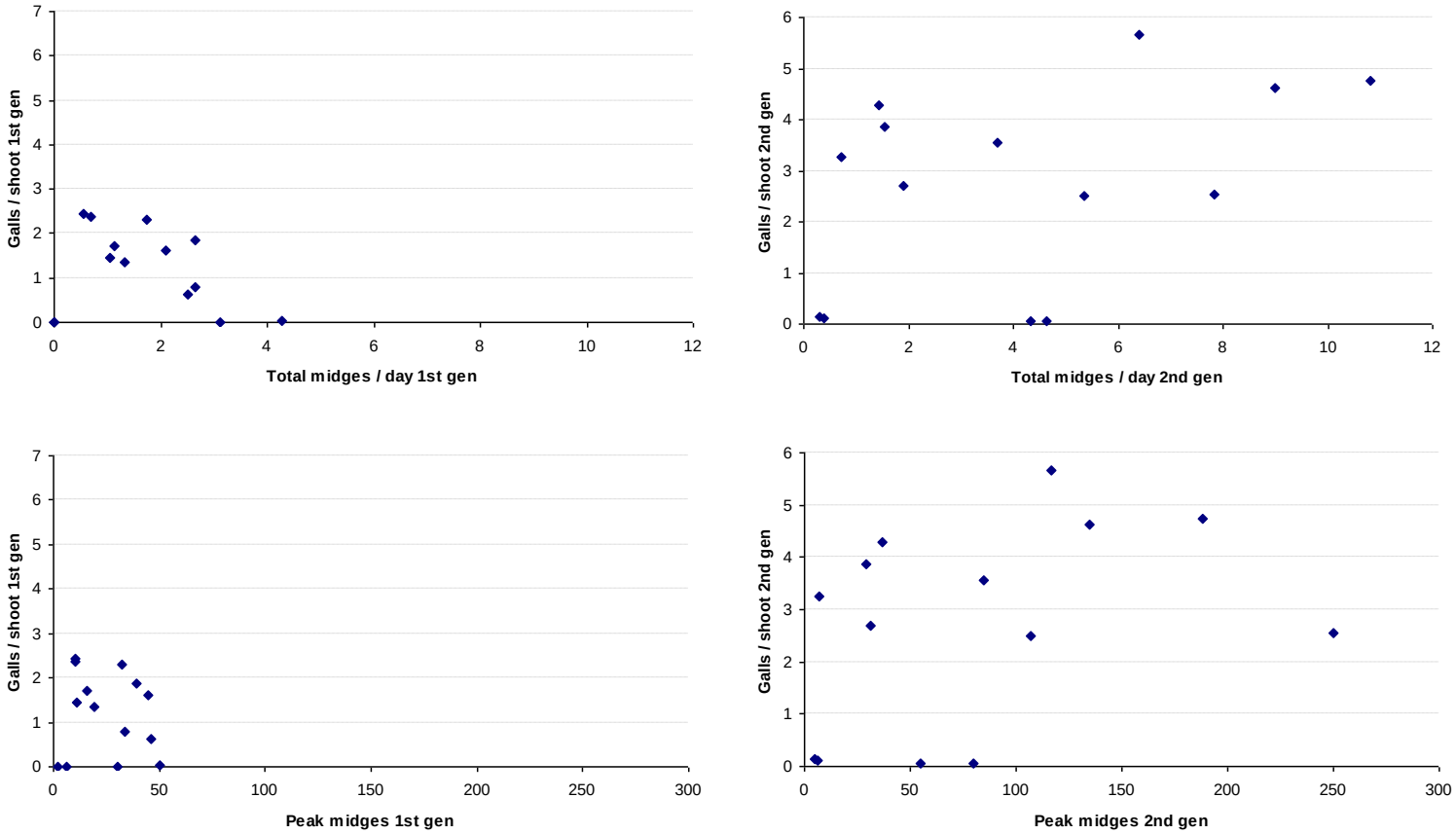


Figure 2.1.3 Regressions between peak and total numbers of midges in the sex pheromone traps and the mean numbers of galls per shoot for the untreated plots

Discussion

Overall this season's work was successful though the dormant season shoot growth measurements have yet to be processed. One limitation of the work was that only one plantation (Bradenham) was young in the establishment phase, the other six plantations being well established and in full crop. It proved difficult to find suitable young plantations of sufficient size.

The insecticide treatments with bifenthrin or lambda cyhalothrin greatly reduced, but did not totally eliminate, galling damage. It is suspected that four such pyrethroid applications, two against each generation, would be required to give a very high standard of control. Control was more successful for the second generation than the first. The insecticide treatments also reduced the numbers of midges caught in the pheromone traps. The fact that midge catches were reduced for the first generation suggests that the insecticide sprays were directly killing adults. As bifenthrin is no longer available, it will be necessary to use lambda-cyhalothrin for the remaining years of the experiment

It is disappointing, though not surprising, that the regressions between the numbers of galls in shoots and the numbers of midges in pheromone traps for each generation were poor - considering that the data was taken from plantations of different ages and varieties. More data is needed over several seasons. It is hoped that it will be possible to establish a firmer trap catch threshold than the nominal one of > 10 midges per traps used currently.

No effects of the leaf midge attacks on yield were apparent in the first year. Shoot growth measurement data when it is processed will indicate whether the midge attacks were affecting growth and continuing the experiments over three seasons should reveal whether leaf midge attacks have a longer term affect on yield.

Conclusions

- Pheromone trap catches showed that the seven different commercial plantations had widely varying levels of leaf midge at the outset. Chasfield and Shobdon

were the most heavily infested, Provender followed by Bradenham were the least heavily infested.

- The first generation midge flight started in early April and reached a peak in late April to early May. A mean peak number of 25 midges per trap were captured for the first generation in the untreated plots.
- The second generation midge flight started in the last week of May to early June. Peak numbers captured averaged 81 per trap.
- The insecticide treatments applied to the treated plots reduced but did not eliminate galling damage in the treated versus the untreated plots. Numbers of galls per shoot were reduced by 67% and 79% for the first and second generations respectively. Catches of midges in the sex pheromone monitoring traps were also reduced by 38% and 80% for the two generations, respectively.
- Regressions between the numbers of galls recorded per shoot and the mean and peak numbers of midges caught in the sex pheromone traps in the untreated were not significant. More data is required over several seasons
- The gall midge attacks did not affect yield in the first year. The grand mean yield for the treated plots (7527 kg/ha) was very similar to the grand mean yield for the untreated plots (7583 kg/ha)
- Dormant season shoot growth measurements have yet to be processed
- The experiment will be continued for two further years to determine any long term effects of blackcurrant leaf midge attacks on growth and yield

Acknowledgements

We are most grateful to Ian and Nick Overy, Steven Holmes, Hugh Boucher, Andrew Youngman, Chris Alhusen, Mike Thurley, William Price, Richard Corbett and Richard Bowen for hosting this experiment on their farms, for applying the insecticide treatments and taking the pheromone trap and yield records. We are also most grateful to Rob Saunders, Tom Maynard and the GSK Blackcurrant Growers' Association committee for their encouragement and support for this work.

B Blackcurrant leaf midge crop damage assessment on cut down, re-growing blackcurrant bushes 2010-13

This work addresses sub-objective 2.1, Task 2.1.2 of this HortLINK project as follows:

Sub-objective 2.1 To determine the relationships between the severities of galling damage caused by blackcurrant leaf midge and loss in growth and yield (EMR)

Task 2.1.2 Crop damage assessment experiments in cut down, re-growing crops (EMR, GSK, Adamston Farms Ltd) (Yrs 1-3)

A series of three field experiments, one per year for three years each on a different cultivar, will be done in leaf midge infested plantations at Adamston Farms Ltd, Scotland, to examine the effects of midge attack on extension growth in cut down bushes. For each experiment, a ~200m long length of row will be cut to the ground (by maceration) in the dormant period prior to the commencement of the experiment. At least ten 20m plots will be marked, end to end in the row. Standard pheromone traps will be deployed to monitor leaf midge attack through the season. Using a randomised block design, one third of these plots will receive a full spray programme of insecticides, timed according to pheromone traps catches, to maintain leaf midge attack at a very low level, one third of the plots will receive sprays against one generation of the midge only. The remaining plots will receive no insecticide sprays for leaf midge and so should be heavily attacked by leaf midge. A count of the numbers of galls in a sample of at least 25 shoots per plot will be made by the host grower at the peak of galling damage of the first and the second generation. In the dormant period following treatment, the length of 25 shoots will be measured in each plot by GSK. The data will be subject to statistical analysis to determine the effect of the midge attack on the length of extension growth on each cultivar.

Methods

The trial was done in the three most eastern rows of Field 24 Ben Hope blackcurrant plantation at Adamston Farms, near Dundee (Table 2.1.6, Figure 2.1.4).

Table 2.1.6 Site location and details

Farm name & address	Field name and location	Variety	Area (ha)	Age (years)	Total no. rows
Adamston Farms Ltd East Adamston, Muirhead Dundee, DD2 5QX Scotland	Field 24 NGR NO 328 353	Ben Hope	2 ha	6-7 years. Cut down winter 2009/10	Most eastern 3 rows, next to stone wall used

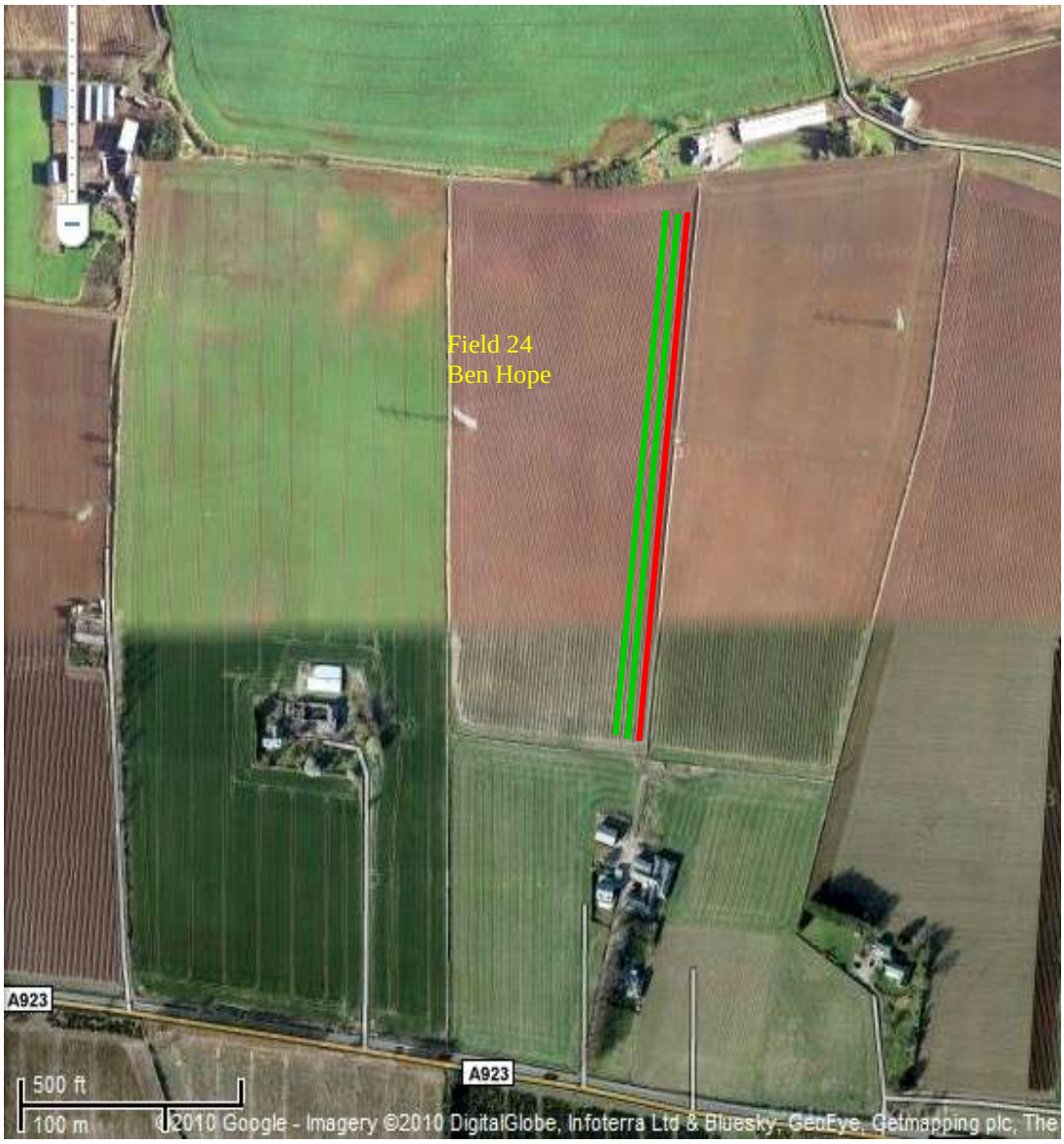


Figure 2.1.4 Field 24 (Ben Hope cut down winter 2009/10) showing the three most eastern rows of the plantation where the trial was done. The plots are tin the most eastern row (marked in red). The two adjacent rows (marked in green) are guard rows.

There were three treatments as shown in Table 2.1.7. The aim was to evaluate the effects on galling damage and extension growth of control of both the first and second generation of blackcurrant leaf midge and control of the second generation only, in comparison with an untreated control treatment.

Table 2.1.7 Treatments bifenthrin (Starion Flo)

Trt. No. (Colour code)	Trt. descriptor	Sprays against 1st gen		Sprays against 2nd gen	
		No. sprays	Application dates	No. sprays	Application dates
1. Red	1st+2nd	2	15 May, 24 May	1	23 June
2. Blue	2nd	0	-	1	23 June
3. Green	None	0	-	0	-

The sprays of bifenthrin 80 g/l SC (Starion Flo) were applied at a dose of 120 ml product in 300 l water per ha. The first spray for each generation was applied a few days after a cumulative threshold catch of > 10 midges per trap was captured for that generation, following the treatment regime set out in Table 2. A second spray 11 days later was applied to ensure a high standard of control. Note that the maximum approved number of sprays of bifenthrin per year on blackcurrant was two at the full dose. However, the experimental plot was non-fruiting and so this restriction did not apply.

The bifenthrin sprays for leaf midge were applied by the grower with his SFM sprayer at a volume rate of 300 l /ha. The plots and the two guard rows were not sprayed with other insecticides that could have affected leaf midge such as chlorpyrifos, thiacloprid (Calypso) or synthetic pyrethroids.

The whole experimental plot was treated overall with the normal spray programme of fungicides, herbicides and nutrients.

The most eastern row of the plantation (marked in red in Figure 2.1.4), which is approximately 380 m long, was divided into 15 plots, numbered 1-15 starting at the northern end. The 15 plots took the entire row and were each 25 m long. The

treatments were ascribed to plots in a randomised block design as shown in Table 2.1.8.

Table 2.1.8 Randomisation of treatments to plots

Plot No.	Block	Treatment	Colour code
1	1	1	red
2	1	3	green
3	1	2	blue
4	2	2	blue
5	2	3	green
6	2	1	red
7	3	3	green
8	3	1	red
9	3	2	blue
10	4	2	blue
11	4	3	green
12	4	1	red
13	5	1	red
14	5	3	green
15	5	2	blue

Blackcurrant leaf midge sex pheromone traps

At the end of March, two red blackcurrant leaf midge sex pheromone monitoring traps were deployed at a height of ~3 cm above the soil in between the two guard rows adjacent to plots 3 and 13. The numbers of midges in the traps was recorded on 11 May and 22 June.

Leaf midge galling

Counts were made of the total number of blackcurrant leaf midge galls in 50 shoots, 10 randomly selected from each of five bushes in the centre of each plot, at the peak of galling damage for second generation on 10 and 24 June 2010

Extension shoot growth

In dormant period after the trial (winter 2010/11), the lengths of the 2010 season's extension growth was measured to the nearest cm on 25-50 randomly selected shoots in the centre of each plot. The sample size was adjusted to give stable data and was the same on all plots.

Results

Catches in sex pheromone traps

Few midges were captured in the sex pheromone traps (Table 2.1.9.), but the threshold of > 10 per trap was just reached for each generation. The traps were recorded insufficiently frequently for proper resolution of the generations.

Table 2.1.9 Numbers of blackcurrant leaf midges captured in the sex pheromone traps

Date	Trap 1 (Plot 3)	Trap 2 (Plot 13)	Mean
11 May	14	8	11
22 June	10	10	10

Galling damage

The ANOVAs of the square root transformed counts of galls per 50 shoots on the 10 and 24 June both revealed highly significant (P < 0.001) treatment effects (Table 2.1.10). The '1st+2nd 'treatment (Treatment 1, red) reduced the numbers of galls by 84% and 93% at the two assessments, respectively. The '2nd' treatment (Treatment 2, blue) did not reduce the numbers of galls significantly at these times. This is not surprising because at the first assessment on 10 June, the sprays for that treatment had not been applied, and at the second assessment on the 24 June the sprays had only been applied one day previously.

Table 2.1.10 Mean and mean square root transformed numbers of galls per 50 shoots on 10 and 24 June 2010 (2nd generation attack)

Trt. no. (colour code)	Trt descriptor	1st assessment 10 June		2nd assessment 24 June	
		n	√n	n	√n
1. Red	1st+2nd	29.8	4.57	16.2	2.75
2. Blue	2nd	[192.8]	[13.88]	{262}	{16.17}
3. Green	None	189.2	13.62	250	15.79
Fprob (P)			<0.001		<0.001
SED (8 df)			1.720		1.389
LSD (P = 0.05)			3.967		3.202

[ ] values for treatment where sprays had not been applied
{ } values for treatment where sprays had only been applied one day previously

Shoot growth

Photographs taken of representative plots on 10 and 24 June showed that the gall midge was stunting shoot growth (Figure 2.1.5). The dormant season shoot growth measurements have yet to be taken but it is expected they will show dramatic differences, the leaf midge attack having caused severe stunting of the shoot growth.

Figure 2.1.5 Photographs of representative bushes in the treated and untreated plots on 10 and 24 June

Task 2.3 To identify new selective insecticides for control of leaf midge and to optimise timing of application in relations to sex pheromone traps catches

Timing of insecticide application for the control of blackcurrant leaf midge 2010

The objective of this study was to evaluate the efficacy of timings of application of UKA385a, Hallmark, Brigade and Calypso in relation to pheromone trap catches for control of first generation blackcurrant leaf curling midge.

Methods

Against the first generation of blackcurrant leaf midge in late April-May 2010

The experiment was done in Stonebridge blackcurrant plantation near Goudhurst, Kent by kind agreement of Tom Maynard, Windmill Hill, Ticehurst, E Sussex TN5 7HQ (Figure 2.3.1). The plantation was located at NGR TQ 718 398. It consisted of 23 rows of Ben Alder. The row spacing was 3 m and bushes were spaced 44 cm apart in the rows. Rows 7-9 of the plantation (counting from the eastern edge) were used for the trial. These rows were 232m long.

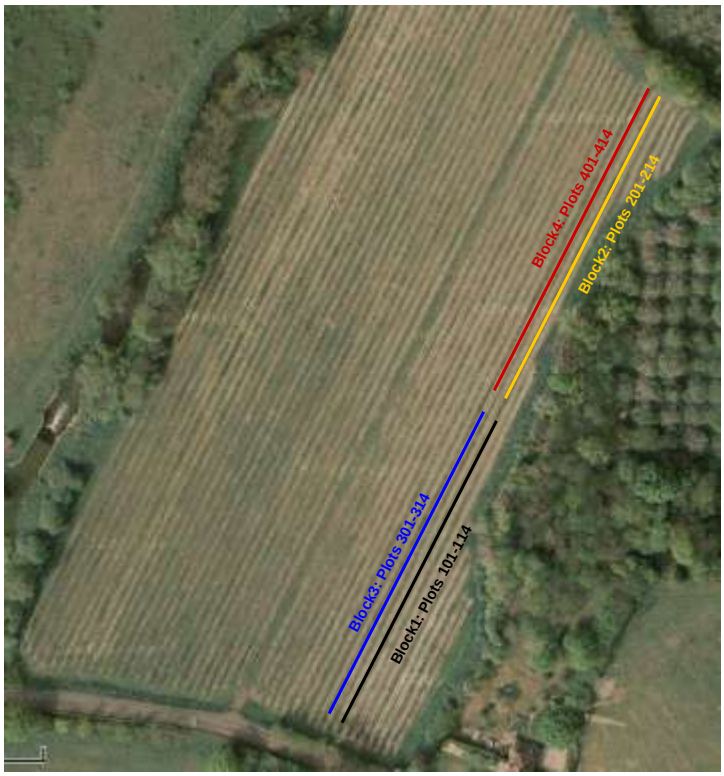


Figure 2.3.1 Location of the experiment in Stonebridge blackcurrant plantation

Treatments were a factorial comparison of 4 products (UKA385a, Calypso, Hallmark, Brigade) at three timing combinations (one spray at five days, one spray at 15 days, two sprays, one at five and one at 15 days, after a threshold catch of > 10 blackcurrant leaf midge males had been captured per trap in the two sex pheromone monitoring traps deployed in the plantation) versus a untreated control (double replicated) (Table 2.3.1).

Table 2.3.1 Treatments

Trt no.	Active substance and formulation	Product	Product dose rate /ha	No. sprays	Timing (Days after threshold catch)†
1	Novel 100 SC formulation†	UKA385a	750 ml	1	5
2	"	"	"	1	15
3	"	"	"	2	5, 15
4	Thiacloprid 480 g/l SC	Calypso	125 ml	1	5
5	"	"	"	1	15
6	"	"	"	2	5, 15
7	Lambda cyhalothrin 100 g/l CS	Hallmark	100 ml	1	5
8				1	15
9	"	"	"	2	5, 15
10	Bifenthrin 80 g/l SC	Brigade	500 ml	1	5
11	"	"	"	1	15
12				2	5, 15
13, 14	Untreated				-

† On 27 April and 7 May, respectively

Two sex pheromone traps (red Delta deployed at 3 cm above ground level) were deployed in the plantation on 15 April 2010 and the threshold catch of > 10 midges was exceeded when the traps were examined on 22 April 2010 (Figure 2.3.2).It was assumed that catches before this date would have been zero or very low due to cold weather conditions.

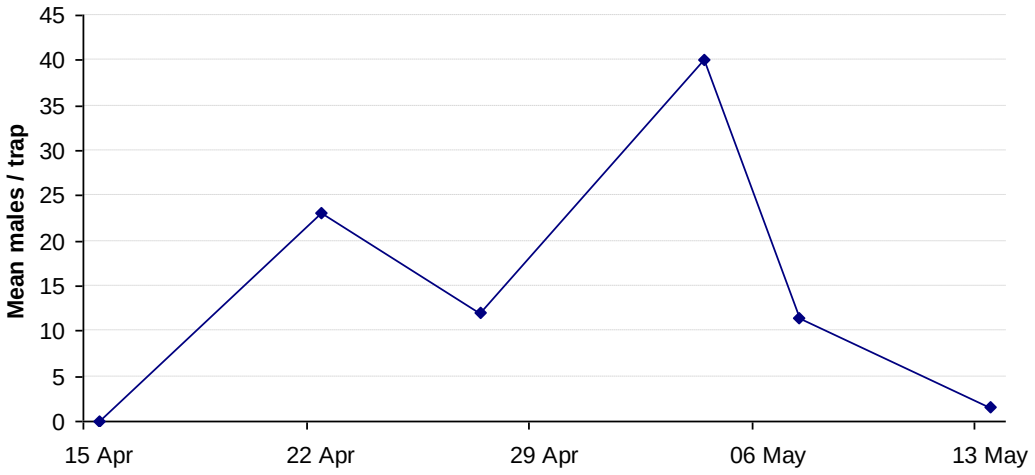


Figure 2.3.2 Mean numbers of blackcurrant leaf midge captured per trap in two sex pheromone traps

Sprays were applied by EMR staff with a motorised, air-assisted knapsack sprayer at 500 l/ha (a volume rate that is realistic for commercial application by growers), ensuring uniform coverage of foliage. The accuracy of application of each treatment was estimated by measurement of the amount of spray that had actually been applied (calculated from the initial minus the final volume of sprayate left in the tank, minus the amount that should have been left had the spray been applied at exactly the correct volume rate). Applications were generally within 5% of required (Table 2.3.2).

Table 2.3.2 Accuracy of spray application estimated from the amount of sprayate remaining in the spray tank after spray application

Spray round number and date	Treatment numbers	Accuracy of application (%)
1. 27 April 2010	1	99
	2	99
	4	100
	5	100
	7	96
	8	96
	10	102
	11	102
2. 7 May 10	2	96
	3	96
	5	100
	6	100
	8	97
	9	97
	11	99
	12	99

Wet and dry bulb temperature, wind speed and direction were recorded before and after each spray occasion (Table 2.3.3). In addition, USB-502 loggers were used to take hourly temperature and humidity readings (Appendix 2.3.1).

Table 2.3.3 Weather conditions at the time of spray application

Date	Time	°C dry	°C wet	%RH	Kph	DIR
27 Apr	12:30	20	14	52.5	0	N/A
27 Apr	14:30	20	16	67.5	0	N/A
07 May	10:15	9	7	85	0	N/A
07 May	12:30	13	9	57.5	2	N

A randomised block design with four replicates of 13 treatments including an untreated control (double replicated) was used (Table 2.3.4). Plots were 8 m lengths of row arranged end to end in a block with adjacent blocks separated by an unsprayed guard row to minimise inter-plot contamination by spray drift. Blocks 1 and 2 were in row 7 and blocks 3 and 4 were in row 9 of the plantation (counting from the eastern end).

Table 2.3.4 Randomisation of treatments to plots

Block 1		Block 2		Block 3		Block 4	
Plot	Trt	Plot	Trt	Plot	Trt	Plot	Trt
101	4	201	11	301	8	401	13
102	2	202	2	302	12	402	8
103	11	203	6	303	3	403	6
104	10	204	13	304	9	404	5
105	9	205	3	305	6	405	4
106	13	206	14	306	11	406	2
107	6	207	4	307	13	407	7
108	5	208	1	308	10	408	9
109	1	209	9	309	14	409	14
110	3	210	7	310	4	410	12
111	8	211	12	311	2	411	10
112	7	212	10	312	1	412	11
113	14	213	5	313	7	413	3
114	12	214	8	314	5	414	1

Counts were made of the numbers of galls that developed and the numbers of larvae they contained in a sample of 25 shoots per plot on 5 and 13 May 2010, eight and six days after the first and second spray treatments, respectively. The shoots were sampled into polythene bags in the field and the counts of larvae made in the lab at EMR.

UKA385a was not approved for use on blackcurrant though the other products were. An automatic experimental permit to use was obtained from PSD by EMR. The crop from the whole trial area was not harvested. The bushes were cut to the ground by the host grower after the trial.

A maintenance programme of fungicides was applied as for the rest of the plantation.

Table 2.3.5 Spray programme for insecticide sprays

Date	Brand name	Dose	Rate
18 Apr	Sulphur 80	8 l/ha	400 l/ha
26 Apr	Scala	2 l/ha	300 l/ha
06 May	Signum	1.1 l/ha	300 l/ha

Detailed weather data was available from the East Malling weather station (see Figure 2.3.3).

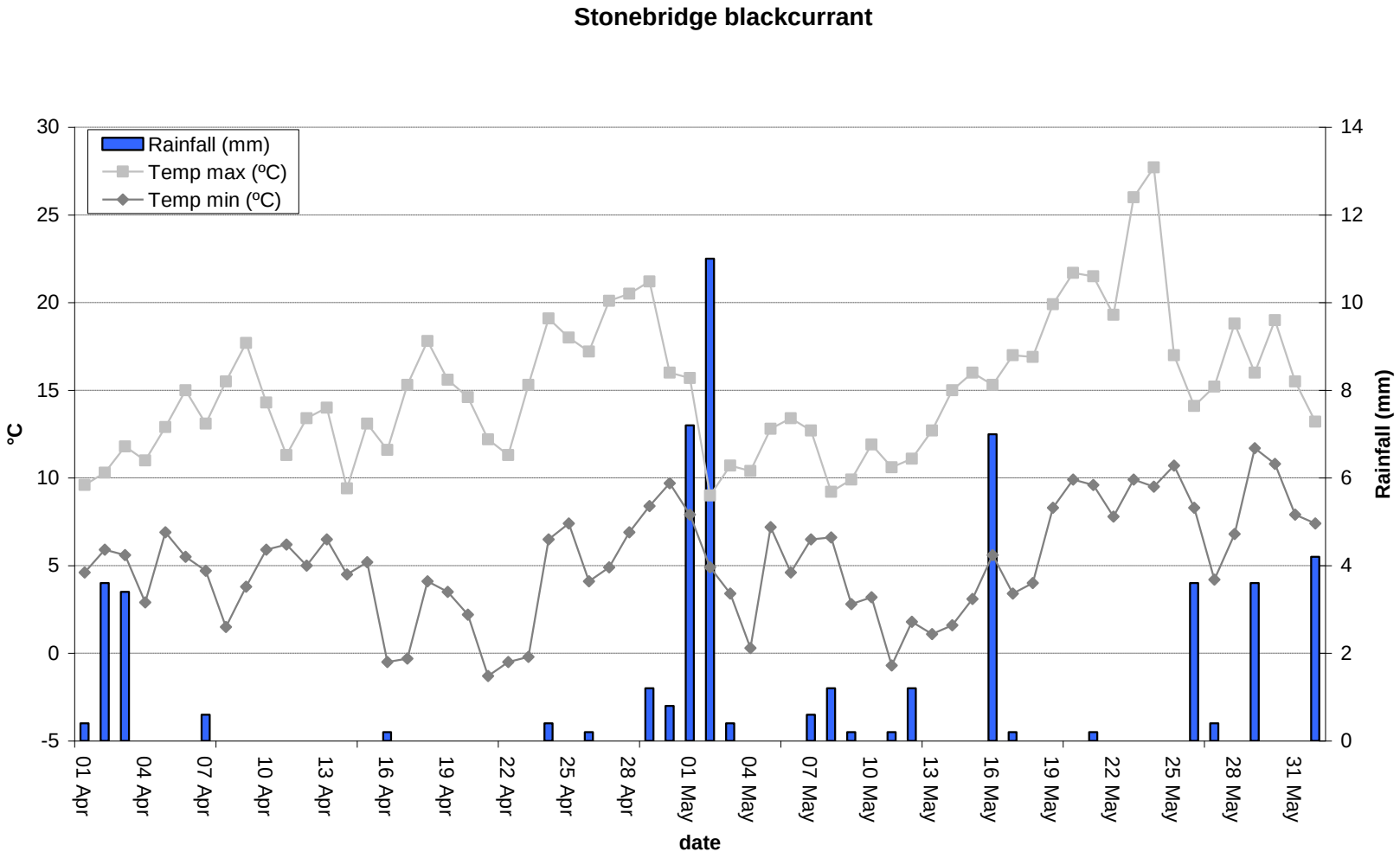


Figure 2.3.3 Meteorological records from EMR weather station for the duration of the trial at Stonebridge

Firstly, one way analysis of variance (ANOVA) with no factorial treatment structure was done on the counts after $\log_{10}(n+1)$ transformation to stabilise variances. LSD (P = 0.05) testing was done to determine which individual treatments had lower values than the untreated control. Factorial ANOVA of counts was then done after $\log_{10}(n+1)$ transformation to stabilise variances. Untreated control versus treatment (Contvstrt), Product (UKA385, Calypso, Hallmark, Brigade) and Timing (5 d, 15d, 5 + 15 d) were used as factors the treatment model being Contvstrt/(Product*Timing)

Results

Numbers of galled leaves in 25 shoots (Figure 2.3.4, Tables 2.3.6 and 2.3.7)

The one way ANOVAs of the $\log_{10}(n+1)$ transformed counts of infested leaves both showed highly significant (P < 0.001) treatment effects (Figure 2.3.4). The factorial ANOVAs of the $\log_{10}(n+1)$ transformed gall count data showed highly statistically significant effects for Contvstrt, Contvstrt.Product, Contrvstrt.Timing though the Contvstrt.Product.Timing three way interactions were not statistically significant (Tables 2.3.6 and 2.3.7).

On average, the insecticide treatments averaged across all timings reduced the numbers of leaves galled by 47% and 36% at the assessments on 5 and 13 May, respectively. Note, however, the overall grand means are averaged across timings which were ineffective and products which were less effective and thus are of little value in practical terms.

The 5 day spray timing and the 5 + 15 day timing performed similarly, reducing the numbers of galled leaves in 25 shoots by 65% and 58% at the 5 and 13 May assessments, respectively. The 15 day timing was ineffective and none of the products applied at this time reduced numbers of galled leaves per 25 shoots significantly compared to the control.

Brigade and Hallmark performed similarly, the treatments applied at the 5 day timing reducing numbers of galled leaves per 25 shoots by 85% and 80% on average on 5 and 13 May, respectively. Calypso was the least effective product. The treatment with Calypso at 5 days did not reduce the numbers of galled leaves significantly at either assessment. UKA385 gave mixed results. On 5 May, the 5 days treatment with UKA385a reduced the numbers of galled leaves per 25 shoots by 74% compared with the untreated control but at the 13 May assessment the reduction was only 30% and it was not statistically significant.

Numbers of larvae per 25 shoots (Figure 2.3.5, Tables 2.3.8 and 2.3.9)

The one way ANOVAs of the $\log_{10}(n+1)$ transformed larval count both showed highly significant ($P < 0.001$) treatments effects. The ANOVAs of the $\log_{10}(n+1)$ transformed larval count data showed highly statistically significant effects for Contvstrt, Contvstrt.Product, Contrvstrt.Timing and Contvstrt.Product.Timing (Tables 2.3.8 and 2.3.9).

On average, the insecticide treatments averaged across all timings reduced the numbers of larvae by 37% at the 5 May assessment but by 82% at the 13 May assessment. Note, however, the overall grand means are averaged across timings which were ineffective and products of mixed effectiveness and thus are of little value in practical terms.

The 5 day spray timing and the 5 + 15 day timing performed similarly, reducing the numbers of larvae per 25 shoots by 73% and 92% at the 5 and 13 May assessments, respectively. The 15 day timing was ineffective at the 5 May assessment, except for the UKA385a which reduced numbers of galls by 30%. At this assessment, none of the other products applied at the 15 day timing reduced numbers of galls in shoots significantly compared to the control. But at the assessment on 13 May, of the treatments applied at the 15 day timing, only the Hallmark and Brigade reduced numbers of larvae significantly, by 76% compared to the untreated control.

Brigade, Hallmark and UKA385a performed similarly, all greatly reducing the numbers of larvae per 25 shoots. On average they reduced numbers of larvae by 98% and 100% at the 5 and 13 May assessments, respectively. The Calypso treatment did reduce larval numbers significantly, but only by 47% and 66% at the two assessment dates, respectively - a notably lower degree of efficacy than the other treatments.

Table 2.3.6 Mean (n) and mean $log_{10}(n+1)$ numbers of leaves per 25 shoots galled by blackcurrant leaf midge on 5 May 2010. Note Fprob (P), DF, SED and LSD values are from ANOVA of $log_{10}(n+1)$ data

Timing	Insecticide treatment					Untreated
Product	UKA385a	Calypso	Hallmark	Brigade	**Mean**	control
			n			
5 d	5.8	16.0	1.5	5.0	**7.1**	
15 d	22.8	22.0	17.3	17.8	**19.9**	
5 + 15 d	11.3	11.8	1.3	9.5	**8.4**	
Mean	**13.3**	**16.6**	**6.7**	**10.8**	**11.8**	**22.1**
			$Log_{10}(n + 1)$			
5 d	0.663	1.141	0.345	0.470	**0.655**	
15 d	1.290	1.307	1.178	1.198	**1.243**	
5 + 15 d	0.765	1.034	0.195	0.717	**0.678**	
Mean	**0.906**	**1.161**	**0.573**	**0.795**	**0.859**	**1.286**

		LSD (P = 0.05)		
	Fprob	Min rep	Min - Max	Max rep
Contvstrt	0.002	-	0.2634	-
Contvstrt.Product	0.002	0.3449X	0.3149	0.2816
Contvstrt.Timing	<0.001	0.3449X	0.2987	0.2439
Contvstrt.Product.Timing	0.325	0.4878	0.4224	0.3449X

Note: No comparisons is categories where LSD marked with X

Table 2.3.7 Mean (n) and mean $log_{10}(n+1)$ numbers of leaves per 25 shoots galled by blackcurrant leaf midge on 13 May 2010. Note Fprob (P), DF, SED and LSD values are from ANOVA of $log_{10}(n+1)$ data

Timing	Insecticide treatment					Untreated
Product	UKA385a	Calypso	Hallmark	Brigade	**Mean**	control
			n			
5 d	16.8	15.5	6.3	3.5	**10.5**	
15 d	34.3	21.5	25.3	23.8	**26.2**	
5 + 15 d	12.5	15.8	8.0	4.0	**10.1**	
Mean	**21.2**	**17.6**	**13.2**	**10.4**	**15.6**	**24.3**
			$Log_{10}(n + 1)$			
5 d	1.043	1.132	0.626	0.450	**0.813**	
15 d	1.412	1.288	1.412	1.328	**1.360**	
5 + 15 d	1.082	1.212	0.569	0.537	**0.850**	
Mean	**1.179**	**1.211**	**0.869**	**0.772**	**1.008**	**1.351**

		LSD (P = 0.05)		
	Fprob	Min rep	Min - Max	Max rep
Contvstrt	0.026	-	0.3003	-
Contvstrt.Product	0.016	0.3932X	0.3589	0.3210
Contvstrt.Timing	<0.001	0.3932X	0.3405	0.2780
Contvstrt.Product.Timing	0.359	0.5560	0.4815	0.3932X

Note: No comparisons is categories where LSD marked with X

Table 2.3.8 Mean (n) and mean $log_{10}(n+1)$ numbers of blackcurrant leaf midge larvae per 25 shoots on 5 May 2010. Note Fprob (P), DF, SED and LSD values are from ANOVA of $log_{10}(n+1)$ data

Timing	Insecticide treatment					Untreated
Product	UKA385a	Calypso	Hallmark	Brigade	**Mean**	control
			n			
5 d	1.0	30.8	2.5	0.3	**8.6**	
15 d	41.0	65.5	118.5	101.3	**81.6**	
5 + 15 d	0.8	45.5	2.3	8.0	**14.1**	
Mean	**14.3**	**47.3**	**41.1**	**36.5**	**34.8**	**58.3**
			$Log_{10}(n + 1)$			
5 d	0.270	1.336	0.445	0.075	**0.532**	
15 d	1.258	1.693	1.973	1.872	**1.699**	
5 + 15 d	0.151	1.559	0.250	0.380	**0.585**	
Mean	**0.560**	**1.529**	**0.889**	**0.776**	**0.939**	**1.568**

		LSD (P = 0.05)		
	Fprob	Min rep	Min - Max	Max rep
Contvstrt	<0.001	-	0.3390	-
Contvstrt.Product	<0.001	0.4438X	0.4051	0.3624
Contvstrt.Timing	<0.001	0.4438X	0.3843	0.3138
Contvstrt.Product.Timing	0.010	0.6276	0.5435	0.4438

Note: No comparisons is categories where LSD marked with X

Table 2.3.9 Mean (n) and mean $log_{10}(n+1)$ numbers of blackcurrant leaf midge larvae per 25 shoots on 13 May 2010. Note Fprob (P), DF, SED and LSD values are from ANOVA of $log_{10}(n+1)$ data

Timing	Insecticide treatment					Untreated
Product	UKA385a	Calypso	Hallmark	Brigade	**Mean**	control
			n			
5 d	0.0	15.8	0.0	0.0	**3.9**	
15 d	24.8	19.8	11.8	10.0	**16.6**	
5 + 15 d	0.5	15.3	0.0	0.0	**3.9**	
Mean	**8.4**	**16.9**	**3.9**	**3.3**	**8.1**	**46.0**
			$Log_{10}(n + 1)$			
5 d	0.000	0.637	0.000	0.000	**0.159**	
15 d	1.274	1.258	1.076	0.960	**1.142**	
5 + 15 d	0.119	1.134	0.000	0.000	**0.313**	
Mean	**0.464**	**1.010**	**0.359**	**0.320**	**0.538**	**1.574**

		LSD (P = 0.05)		
	Fprob	Min rep	Min - Max	Max rep
Contvstrt	<0.001	-	0.2083	-
Contvstrt.Product	<0.001	0.2727X	0.2490	0.2227
Contvstrt.Timing	<0.001	0.2727X	0.2362	0.1929
Contvstrt.Product.Timing	0.010	0.3857	0.3340	0.2727X

Note: No comparisons is categories where LSD marked with X

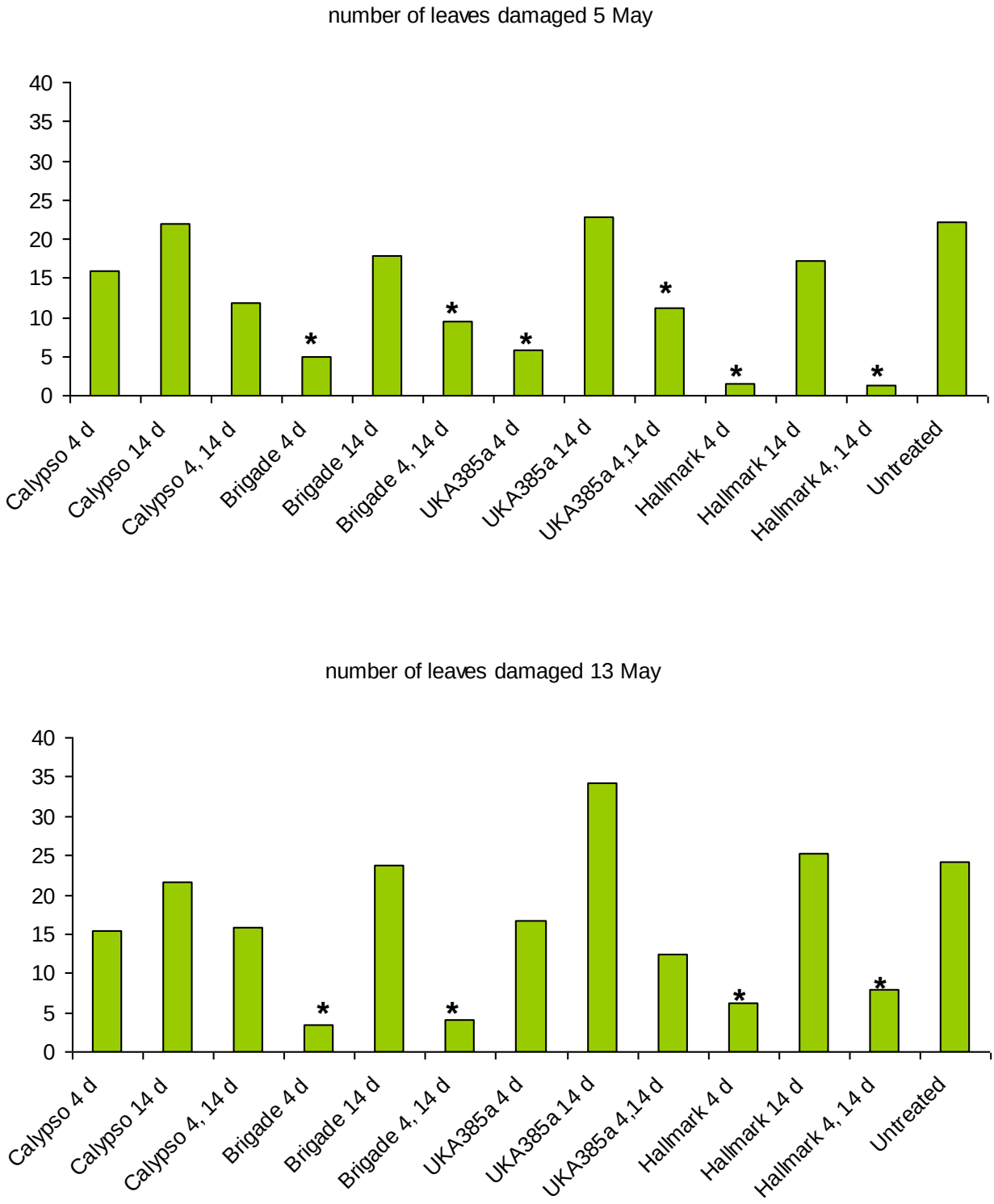


Figure 2.3.4 Mean numbers of leaves damaged per 25 shoots on 5 and 13 May, eight and six days after the first and second spray treatments, respectively. Bars that are significantly ($P \leq 0.05$) less than the untreated control in a simple one way ANOVA are marked with an asterisk

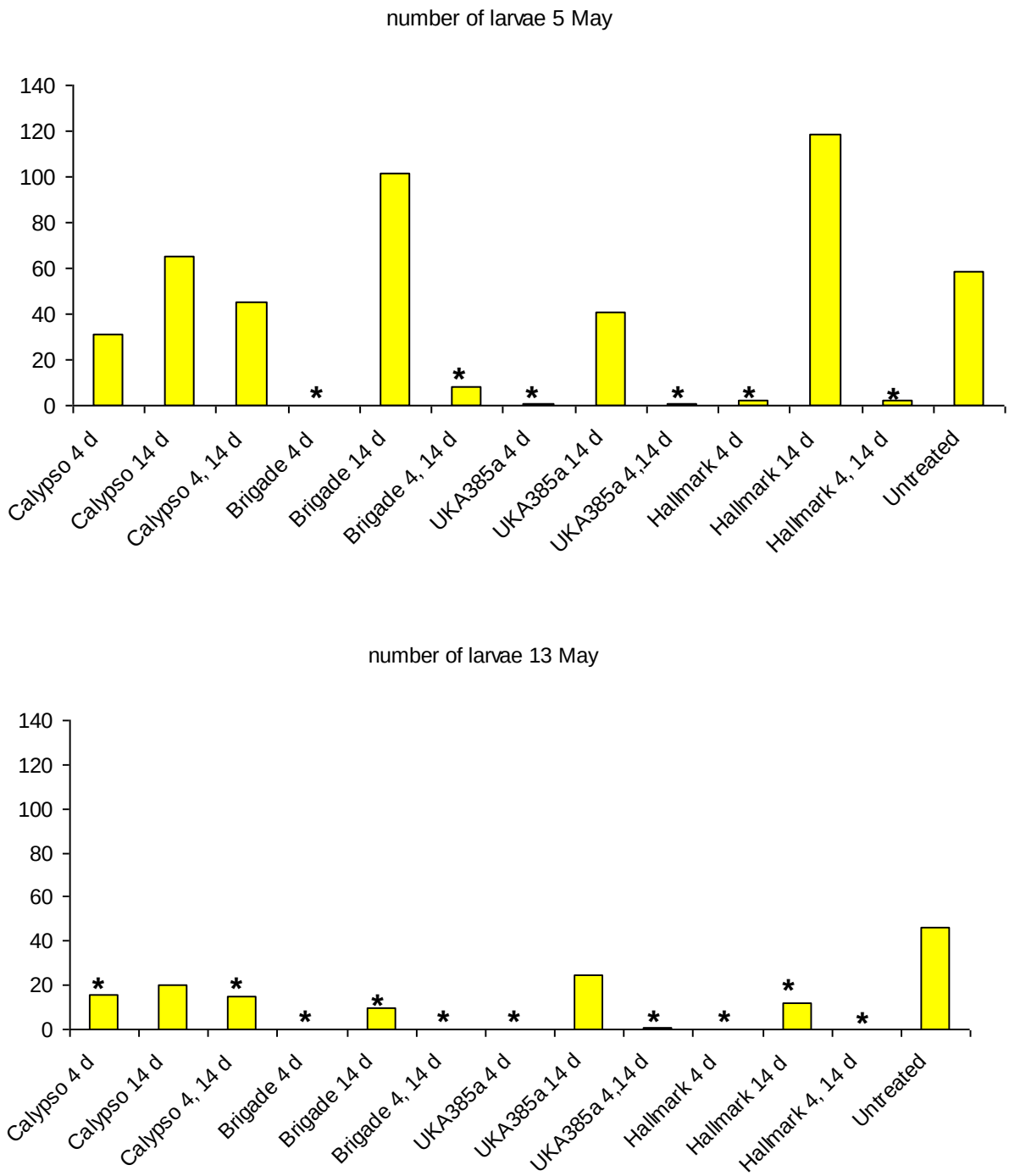


Figure 2.3.5 Mean numbers of larvae per 25 shoots on 5 and 13 May, eight and six days after the first and second spray treatments, respectively. Bars that are significantly ($P \leq 0.05$) less than the untreated control in a simple one way ANOVA are marked with an asterisk

Discussion

Spray timing

The pheromone trap catch threshold of >10 midges per trap is nominal and was chosen arbitrarily to indicate the start of the first generation flight period of the midges in spring. As the traps were only read approximately weekly, the 5 day and 15 day timings are somewhat arbitrary. However, the results do clearly show that the early '5 day' timing was much more effective than the later '15 day' timing. This suggests that the insecticides work best when they are applied during the early part of the midge attack, when females are laying eggs in the shoots tips and when eggs are developing and hatching before or at the early stages of gall formation. At the 15 day assessment date, the pheromone trap catches indicate that the midge flight and egg laying had mostly come to an end and most of the population was mature or semi-mature larvae in leaf galls. At this stage, the insecticide treatments clearly have little prospect of reducing the numbers of galls already present, though the results clearly indicate that the UKA385a, Brigade and Hallmark treatments were able to kill a high proportion of these larvae in galls.

These results show that spray timing is very important for effective control of blackcurrant leaf midge with insecticides. Early treatment at the start of the attack is vital to getting good results and preventing galling. The pheromone traps are thus vitally important for getting good results. Further work is necessary to explore the effects of spray timing, but the results suggest that pheromone traps need to be deployed well before the start of the midge flight, probably by early March at the latest, and it would be best if they were monitored more frequently, probably twice weekly until the threshold is reached.

Products

Hallmark and Brigade, both synthetic pyrethroids, performed rather similarly in this trial suggesting that Hallmark may be an effective alternative to Brigade and other bifenthrin products when approval for bifenthrin is lost in spring 2011. However, these synthetic pyrethoids are very broad-spectrum insecticides and they are certainly very harmful to the midge's natural enemies, including the parasitoid *Platygaster demades* and anthocorid predatory bugs, as well as to the natural enemies of other blackcurrant pests. Ideally, use of these products should be avoided so that blackcurrant IPM programmes which exploit natural enemies can be developed.

UKA385a, in contrast, is a much more selective insecticide which is less likely to have harmful effects, especially persistent ones, to natural enemies. It is likely to be much more

compatible with IPM programmes. These results indicate that it may be rather slow acting and because of this it does not prevent leaf galling occurring. However, it did give a high degree of efficacy of control of larvae.

The results suggest that Calypso has only very limited effectiveness for control of blackcurrant leaf midge.

Future work

The findings of this study can only be regarded as preliminary. Further work is needed to validate them and especially to explore timing of application of UKA385a and Hallmark in relation to pheromone trap catches more closely. In the experiments in 2011, it is suggested that the pheromone traps are monitored twice weekly during the critical period and that the timing of application of UKA385a and Hallmark at 1, 3, 7 and 10 days after threshold is evaluated.

Conclusions

- Spray timing was found to be important for effective control of blackcurrant leaf midge with the insecticides tested. Insecticides worked best when they were applied at the 5 days after threshold timing i.e. during the early part of the midge attack. At this time, females were laying eggs in the shoots tips and the eggs were developing and hatching. Application was before or at the early stages of gall formation
- Insecticide application 15 days after the pheromone trap threshold was largely ineffective. The numbers of galls already present were not reduced, and only at best partial reductions in the numbers of semi-mature and mature larvae present in the galls were achieved
- Two sprays were, thus, not significantly better than one as the second spray was largely ineffective
- Monitoring with sex pheromone traps was thus important to getting good results. The results suggest that pheromone traps need to be deployed well before the start of the midge flight, probably by early March at the latest, and it would be best if they were monitored more frequently, probably twice weekly until the threshold is reached
- Hallmark, Brigade and UKA385a all gave effective control of blackcurrant leaf midge when applied 5 days after the pheromone trap threshold
- Hallmark and Brigade performed rather similarly. They both reduced the numbers of galled leaves by > 80% and gave a very high degree (>98%) of control of larvae in galls

- Hallmark may be an effective alternative to Brigade and other bifenthrin products when approval for bifenthrin is lost in spring 2011. However, these synthetic pyrethoids have very broad-spectrum activity and they are likely to be very harmful to the midge's natural enemies, including the parasitoid *Platygaster demades* and anthocorid predatory bugs, as well as to the natural enemies of other blackcurrant pests. Ideally, use of these products should be avoided so that blackcurrant IPM programmes which exploit natural enemies can be developed
- A single spray of UKA385a gave virtually 100 % control of larvae in galls. UKA385a is a selective insecticide which is less likely to have harmful effects, especially persistent ones, on natural enemies. It is likely to be compatible with IPM programmes and priority should be given to its development for control of blackcurrant leaf midge and possibly other pests in UK blackcurrant IPM
- These results indicate that UK385a is slower acting and because of this it does not prevent leaf galling occurring. However, it did give a high degree of efficacy of control of larvae
- Bayer CropScience, the company that owns UKA385a, 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.
- The results suggest that Calypso has at best only very limited effectiveness for control of blackcurrant leaf midge
- The findings of this study are preliminary. Further work is needed to validate them and especially to explore timing of application of UKA385a and Hallmark in relation to pheromone trap catches more closely. For experiments in 2011, it is suggested that the pheromone traps are monitored twice weekly during the critical period and the timing of application of UKA385a and Hallmark at 1, 3, 7 and 10 days after threshold is evaluated

Acknowledgements

We are most grateful to Rob Saunders, Tom Maynard and Richard Meredith for their support for this work.

Task 2.4 To investigate the feasibility of exploiting the leaf midge sex pheromone for control by mating disruption (MD), attract and kill (A&K) or mass trapping (MT)

The overall aim of the experiment was to determine whether the female of the blackcurrant leaf midge can still give rise to viable offspring if she is unmated.

Methods

Midges were reared in the entomology controlled temperature rooms at EMR. Plants were kept in a glasshouse at the research centre. 40 potted plants were obtained from a grower in Hereford. The plants were root washed and re-potted into peat in the first experiment. However, the plants or glasshouse were still found to be contaminated with egg laying blackcurrant midge. For the second experiment (30 June) stems were stripped of leaves and fingers run over the stems to remove/kill any eggs remaining (Fig. 2.4.1). A perforated polythene bag was then tied close to the base with a twist tie.

Figure 2.4.1 Treatments of plants prior to introducing the adult midges

Larvae collected from infested leaves in the field were reared individually in tubes (6 July). See EMR SOP L812. The largest larvae were collected from the leaves with a moist paintbrush and transferred individually to a clean AA cup containing a small piece of moist filter paper (distilled water used to reduce microbial growth). The size and moisture of the paper are extremely important. Too dry and the midge larvae or pupae will dry out, too moist and the insect will drown in the condensation accumulated on the side of the tube. A piece of filter paper at least 1 x 1.5 cm was used, and after wetting it was folded in half and squeezed

to get rid of excess water. The tubes were sealed with a lid and placed on their side on a white plastic tray. The colour contrast between the tray and the midge makes viewing the midges much easier. The larvae/pupae were incubated at 20 ^{o}C, 16:8 hr L:D. The incubator lights were set to come on at 7 am. Freshly emerged midges (#2-6) were placed into the perforated bags by cutting a corner at the top, tapping midges out of the tubes and then resealing with a twist tie (9-22 July). The bag was labelled. Three treatments were compared:

- Virgin females / shoot (nine replicates)
- Virgin females + five males / shoot (eight replicates)
- No midges / shoot (control) (nine replicates)

10 days later the damaged leaves were collected and the number of larvae and eggs counted (20 July – 2 August). The leaves were placed in a Perspex box with moist tissue paper on the bottom.

Results

Only shoots that had male + female blackcurrant midges added had larvae on them 10 days later. The bagged shoots that had either no midges or females only did not have midge eggs or larvae (checked 6 August) (Table 2.4.1). Hence, reproduction in blackcurrant midges seems to require sexual reproduction.

Table 2.4.1 Numbers of midges added to bag sealed shoots and resulting offspring

	Added to bags		**Resulting larvae**			
	No. females	**No. males**	**No. Larvae**	**Offspring/ female**	**No. male midges from larvae**	**No. female midges from larvae**
Female + male	25	25	194	62.5	16	23
Control	0	0	0	0	0	0
Females only	33	0	0	0	0	0

Objective 3: Blackcurrant sawfly

Task 3.1 To demonstrate sex pheromone attraction and investigate mating behaviour

Between 16 July and 12 August 2010 traps were placed in a moderately infested blackcurrant plantation (Horsmonsden, Kent) by kind permission of Tom Maynard. The field trials, using 29 individually caged females and two males as bait in white delta traps with sticky bases, found no attraction of sawfly into the traps. Sticky stake traps set up in the same field for catching another insect did trap the occasional blackcurrant sawfly.

Task 3.2 To identify the sawfly sex pheromone

A method of culturing blackcurrant sawfly was devised. Larvae on blackcurrant shoots were posted to EMR from growers around the UK. Mature larvae (vivid green in colour) were placed into *Drosophila* culture tubes with 2 cm of compost and a blackcurrant leaf. The exuvia of the insect could be clearly seen in the tube once the larvae had entered the soil to pupate. 2 ml of water was added to each tube to provide moisture. Cultures were maintained at 20 ^{o}C on a light: dark programme of 16:8 hours. Some of the specimens that emerged were sent to Guy Knight at the National Museums, Liverpool to confirm the identification. Complete records were kept of the life history of the emerging larvae (Table 3.1.1).

Table 3.1.1 Details of the life history of blackcurrant sawfly in culture

	No. pupated	**No. Emerged**	**% emerged**	**Total individuals replicates)**	**Time to emerge range)**	**Average lifespan – adult (days)**
Male	288	141	48.96	10	16.1 (9.5-22.5)	4.9 (2.5-8.0)
Female				129	19.2 (12.0-46.0)	7 (1.5-15)

Chemical Studies

Methods

Pheromone Collection

Hexane washes were obtained by placing a single insect (1-2 d old) in hexane (pesticide residue grade; 1 ml) in a glass sample vial. After five minutes the hexane was carefully

transferred to another vial with a Pasteur pipette. Washes were made from 22 virgin females and two virgin males.

Volatiles were collected from individual insects (1-2 days old) held in a glass vessel (15 cm x 4 cm diameter) by drawing charcoal-filtered air (0.4 l/min) over them and trapping the volatiles on Porapak (200 mg) held between glass wool plugs in a Pasteur pipette (4 mm i.d.). Trapped volatiles were removed by eluting with dichloromethane (Pesticide grade; 3 x 0.5 ml). Collections were made for 1-3 days from 29 virgin females and four virgin males.

Analyses

Collections were analysed by gas chromatography (GC) with flame ionisation detection (FID) using polar or non-polar fused silica capillary columns. The former was coated with DBWax (Supelco; 30 m x 0.32 mm i.d. film thickness 0.25 µ) temperature programmed from 50ºC for two minutes then at 10ºC/min to 250ºC with injector temperature 220ºC, detector 250ºC and helium carrier gas (2.4 ml/min). The latter was coated with DB5 (Supelco; 30 m x 0.32 mm i.d. film thickness 0.25 µ) temperature programmed from 60ºC for two minutes then at 10ºC/min to 300ºC with injector temperature 250ºC, detector 300ºC and helium carrier gas (2.4 ml/min).

Analyses by GC coupled to mass spectrometry (MS) were carried out on a Varian 3500 GC coupled to a Saturn 2200 MS (Agilent) operated in electron impact mode. Polar and non-polar GC columns were used for the analyses except that the column diameter was 0.25 mm and in both cases the oven temperature was programmed from 50ºC for two minutes then at 6ºC/min to 250ºC with helium carrier gas (1 ml/min).

Compounds were identified by their mass spectra, their GC retention indices relative to the retention times of *n*-alkanes and comparison with synthetic standards.

Microanalytical reaction

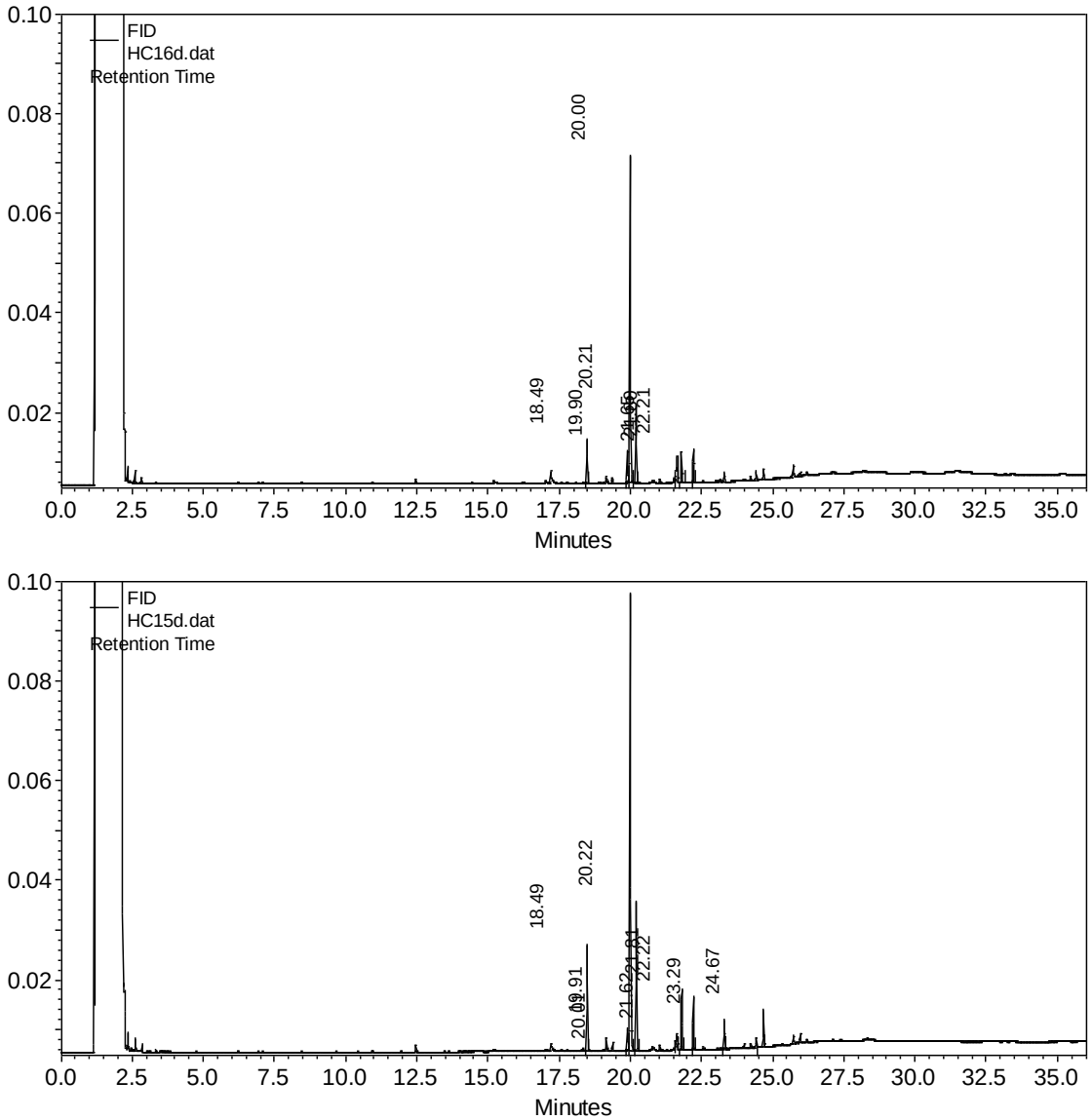
To prepare dimethyldisulphide derivatives, an aliquot of the natural or synthetic material in hexane (100 µl) was taken in a conical sample vial, the solvent removed under a gentle stream of nitrogen and then mixed with dimethyldisulphide (20 µl) and iodine (10 µl of 5% solution in ether). The vial was sealed and heated at 80ºC overnight. The mixture was dissolved in hexane (100 µl) and extracted with an aqueous solution of sodium thiosulphate (5%, three drops) to remove iodine and dried over magnesium sulphate. The product was analysed by GC-MS using a HP6890N GC and HP5973 MS (Agilent) fitted with a column

coated with non-polar DB5, oven temperature programmed from 60ºC for two minutes then at 10ºC/min to 300ºC with injector temperature 270ºC and helium carrier gas (1 ml/min).

Release rate study

(Z)-9-Tricosene (50 µg) was impregnated onto a cotton dental roll and exposed in a windtunnel at 27°C and 8 km/h windspeed. At intervals the dental roll was extracted in hexane (5 ml) containing eicosane (5 µg) as internal standard. The resulting solution was analysed by GC-FID on the polar GC column as above. Two replicates were analysed for each exposure time.

Results



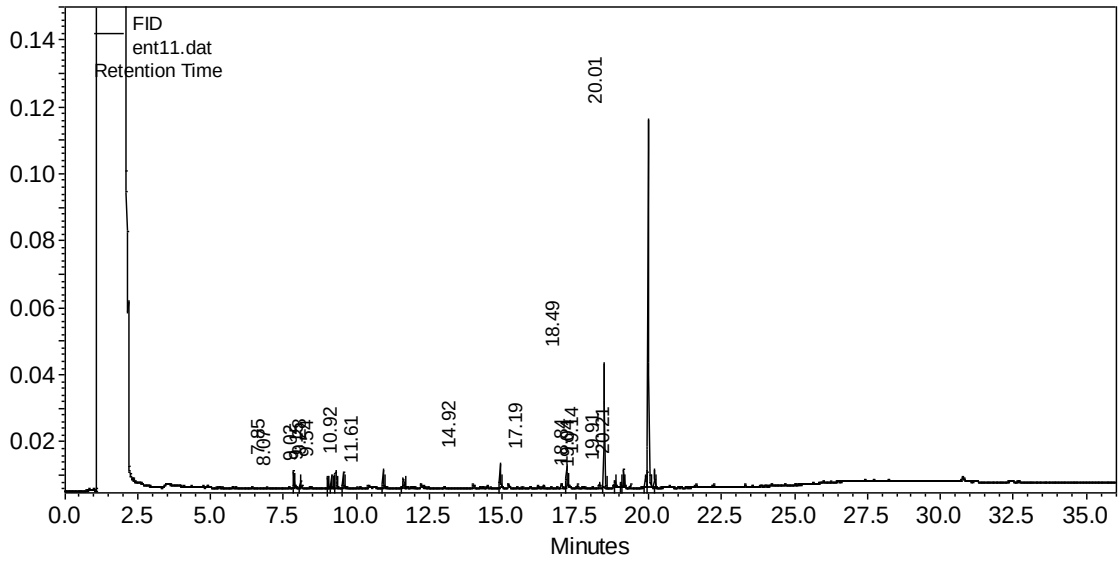
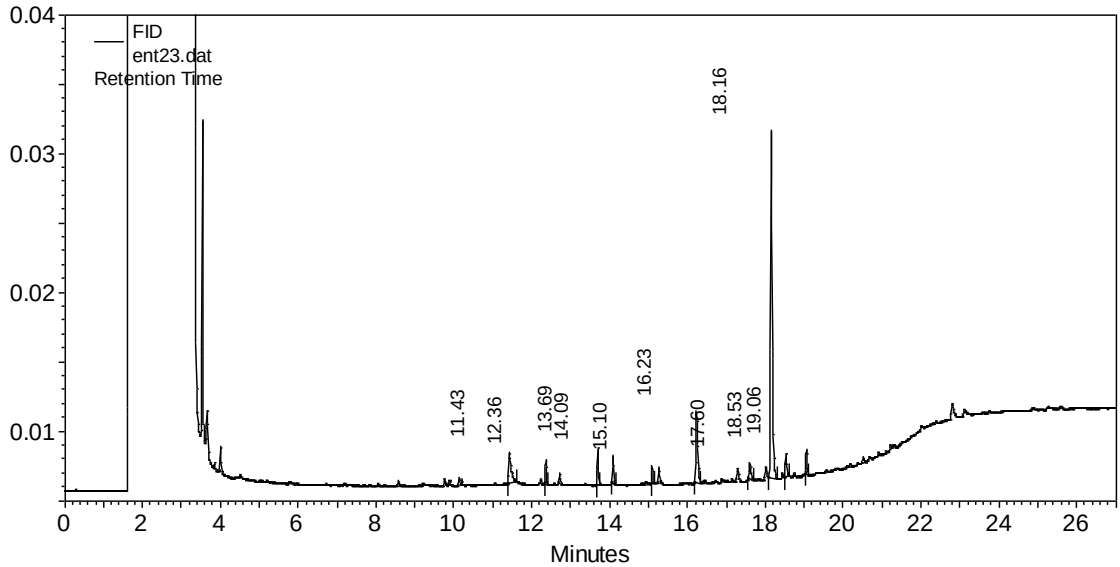


Figure 3.2.1 Analyses by GC-FID on non-polar column of hexane washes (upper male, middle female) and volatiles collected from female *N. olfaciens* (lower)

Analyses of hexane washes made from individual *N. olfaciens* were carried out by GC-FID with a non-polar column under conditions designed to analyse long-chain hydrocarbons up to 36-carbons. Washes from females and males were very similar with a single main peak (Fig. 3.2.1).

Analyses of volatile collections from *N. olfaciens* showed the same main peak and at least one of the minor components (Fig. 3.2.1 on non-polar GC column and Fig. 3.2.2 on polar column; Table 3.2.2).



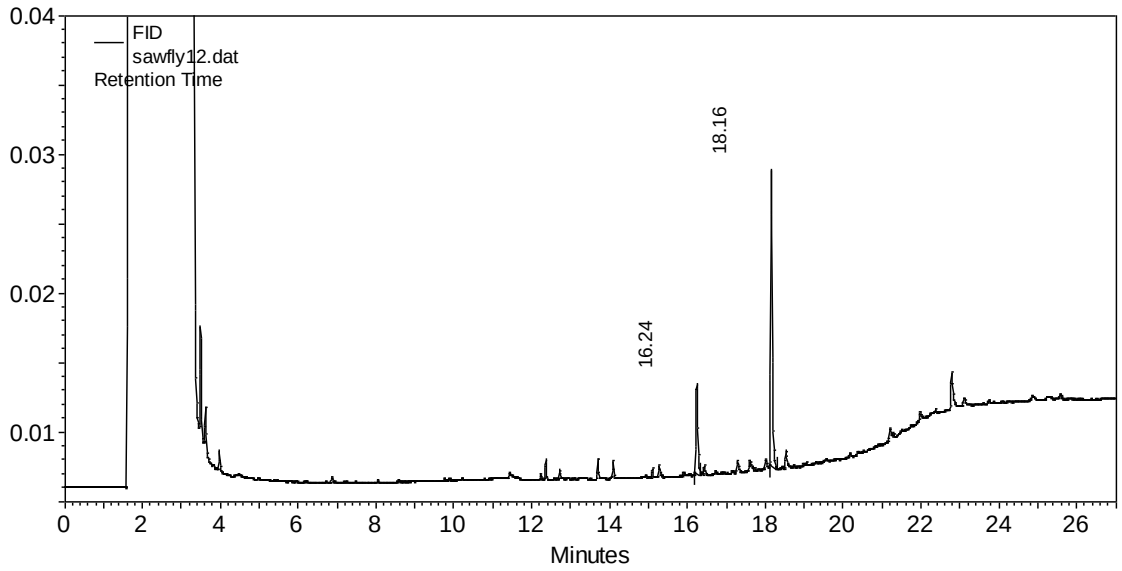
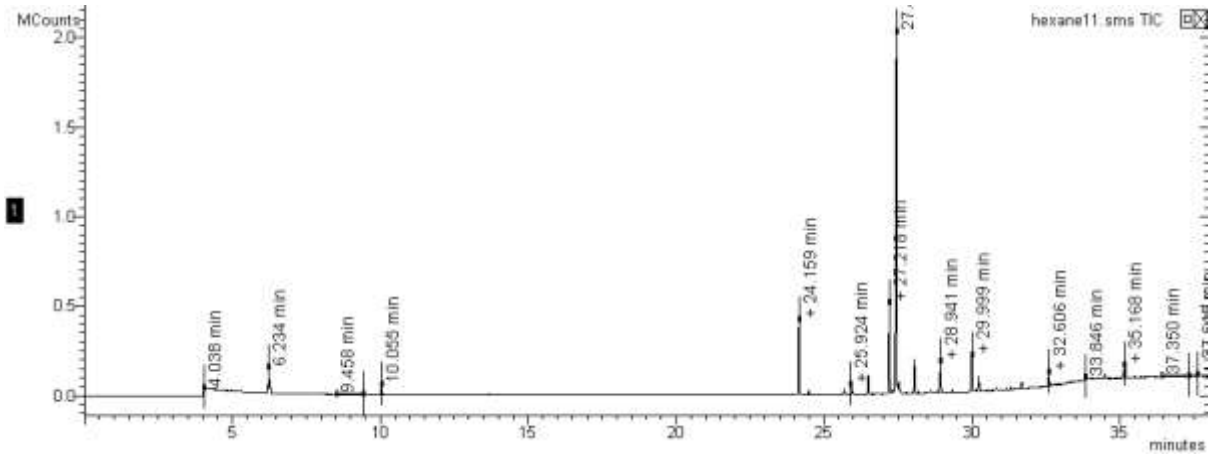


Figure 3.2.2 Analyses by GC-FID on polar column of volatiles collected from individual *N. olfaciens* (upper male, lower female; nonanal 8.55 min, tetradecanal 14.57 min)

Analyses of both washes and collections of volatiles by GC-MS (Fig. 3.2.3) suggested that the major component was a monounsaturated, 23-carbon hydrocarbon, and its retention time on both polar and non-polar columns fitted that of (*Z*)-9-tricosene.The next two most abundant compounds in the washes were identified as the saturated hydrocarbons heneicosane (21-carbons) and tricosane (23-carbons). The other minor compounds were identified as analogues with two, three and four double bonds. One of these had identical retention times to those of (Z,Z,Z)-3,6,9-tricosene (Table 3.2.2).



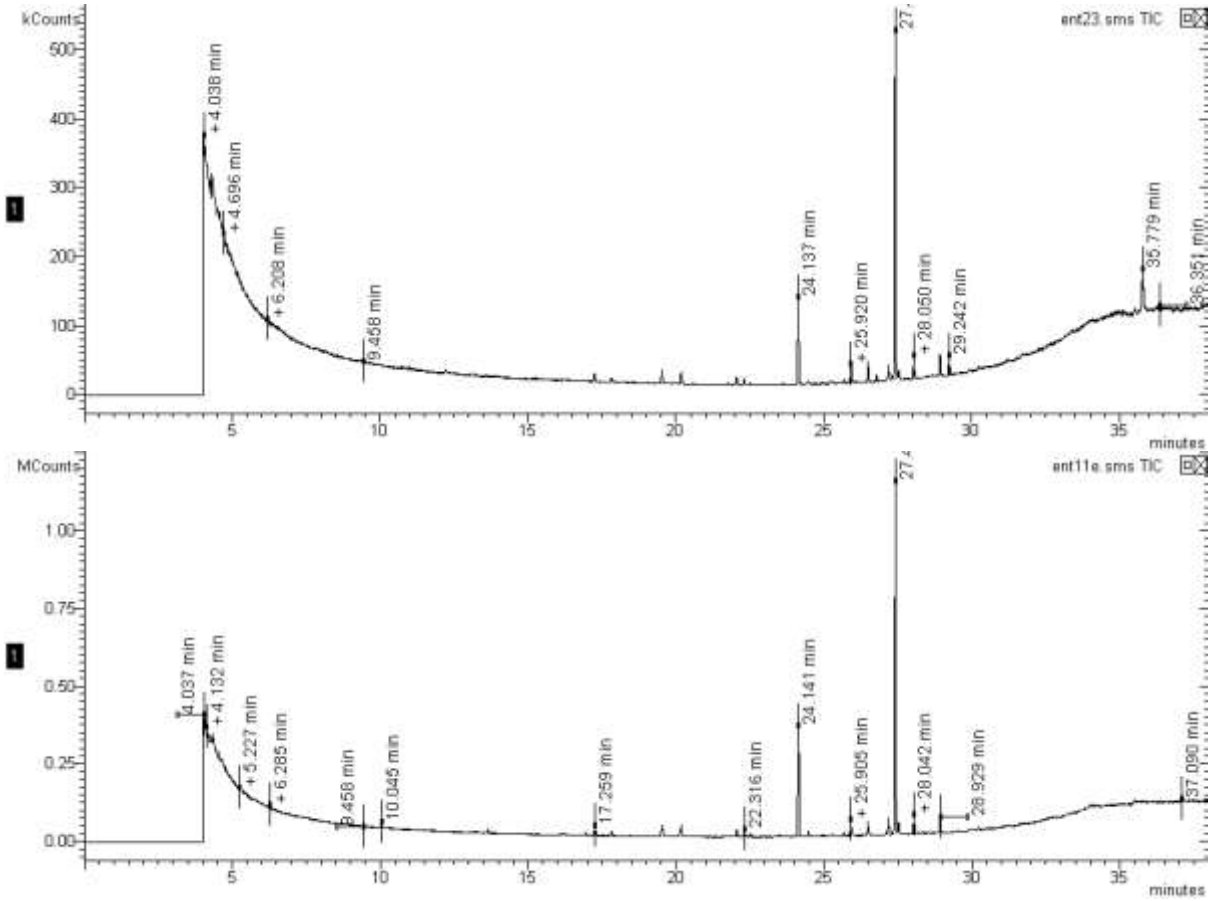


Fig. 3 Analysis by GC-MS on polar column of hexane wash from female *N. olfaciens* (upper) and volatiles from male (middle) and female (lower) *N. olfaciens*

In further work the position of the double bond in the major component was confirmed to be in the 9-position by conversion to its dimethyldisulphide derivative (RI 2930 on non-polar column) which has a characteristic mass spectrum diagnostic of the position of the double bond (Fig. 3.2.4). The same results were obtained with hexane washes from male and female *N. olfaciens*.

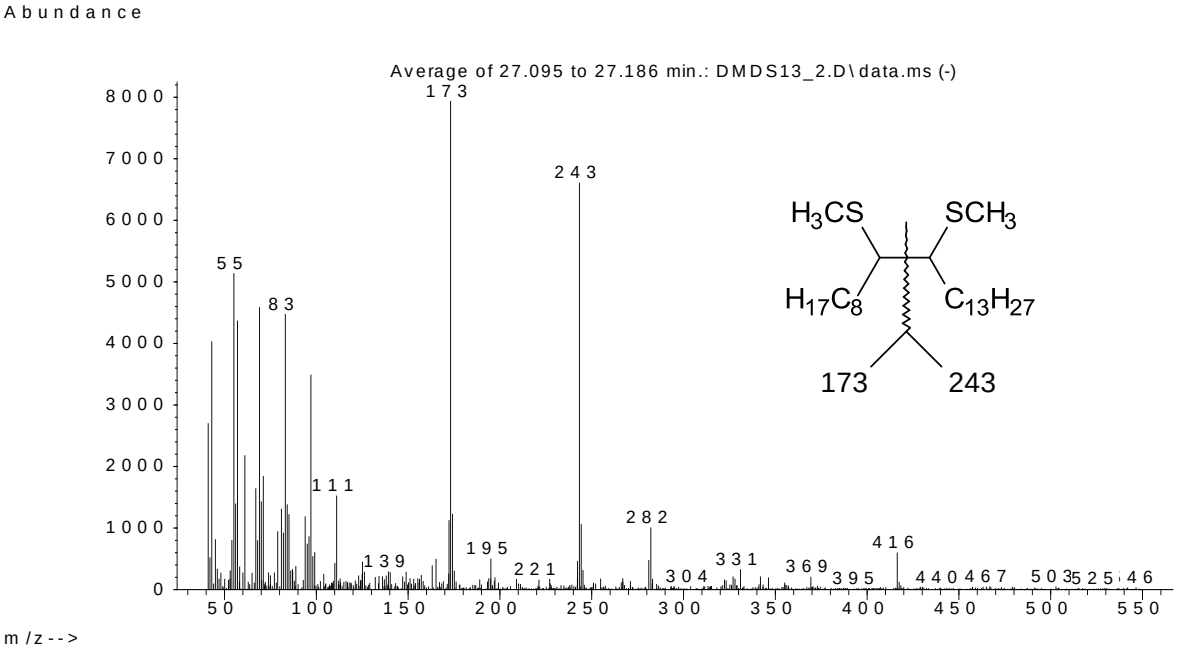


Figure 3.2.4 Mass spectrum of dimethyldisulphide derivative of major component in hexane wash from female *N. olfacien*.

It was also shown that the amounts of material obtained in hexane washes from females and males were not significantly different (female mean 21.4 µg SE 3.0 µg, *n*=8; male mean 24.2 µg SE 8.2 µg, *n*=2).

Potential products of oxidation of (*Z*)-9-tricosene would be the two aldehydes nonanal and tetradecenal (Fig. 3.2.5). GC-MS analyses of both collections of volatiles and hexane washes were examined for the presence of these compounds (Table 1). Tiny traces were found in some analyses of volatiles, particularly the nonanal.

Fig. 5. Oxidative breakdown of (Z)-9-tricosene to nonanal and tetradecanal

Release rate studies showed that for a dental roll impregnated with (Z)-9-tricosene and exposed in a windtunnel at 27°C and 8 km/h windspeed, 67% remained after 53 h and 6% after 124 h.

Table 3.2.2 GC Retention data for compounds and relative amounts in hexane washes and collections of volatiles

No.	Retention Time (min)			Retention Index[3]		Identification	Relative Amount	
	GC-MS[1]	GC-FID[1]	GC-FID[2]	Polar	Non-polar		Hexane Wash	Volatiles
1	24.16	14.80	18.54	2100	2100	heneicosane	+++	+++
2	24.49	15.00	18.36	2121	2079	(unsat 21H)	+	+
3	25.75	15.74	19.40	2200	2200	docosane	+	+
4	25.95	15.84	19.19	2214	2176	(unsat 22H)	+	++
5	26.50	16.17		2251		(sat hydrocarbon)	++	++
6	27.22	16.59	20.26	2300	2300	tricosane	+++	+
7	**27.45**	**16.72**	**20.06**	**2317**	**2277**	**(*Z*)-9-tricosene**	**++++++++++**	**++++++++++**
8	28.06	17.09		2360		di-unsat 23H	++	++
9	28.95		20.09	2424	2280	(*Z,Z,Z*)-3,6,9-tricosatriene (?)	++	++
10	30.00		21.83	2500	2500	pentacosane	++	
11	30.20		21.64	2515	2476	unsat 25H	+	
12	31.75			2453			+	
13	32.61		23.31	2700	2700	heptacosane	++	
14	35.17		24.70	2900	2900	nonacosane	++	
	10.98	8.55	6.91	1390	1107	nonanal		
	21.17	14.57	13.60	1923	1614	tetradecanal		

[1] Polar GC column
[2] Non-polar GC column
[3] Relative to retention times of *n*-alkanes

Task 3.3 To demonstrate pheromone activity, develop a lure and trapping system, and calibrate in the field

A small scale field trial at the end of the growing season (30 Aug - 6 Sep 2010) was set up to test the efficacy of the compounds found in Task 3.2. Dental rolls were placed onto white sticky bases which were suspended vertically from the blackcurrant bushes. A yellow sticky base was used as a control. Compounds tested included female (H13, H14, H15) and male extracts (H16, H19) each 1 insect equivalent, approx 30 µg or the 50 µg synthetic Z9-23:H compounds (Syn1, Syn2, Syn3 = (Z)-9-tricosene (23 carbons)). The hydrocarbon in the insect breaks down by oxidation to smaller, more volatile aldehydes which are the attractive chemicals. Three replicates of each treatment were tested. Only one female blackcurrant sawfly was trapped on a yellow sticky base.

Conclusions

- A method for culturing sawfly has been tested and proven to be effective.
- Analyses of both hexane washes and collections of volatiles from male and female *N. olfaciens* showed a series of saturated and unsaturated, long-chain *n*-alkanes, as has been found in several other sawfly species. The major component was identified as (*Z*)-9-tricosene in both males and females.
- However, there were no obvious differences in the profiles of compounds from males and females, either qualitatively or quantitatively in that amounts of material found in hexane washes were not significantly different and amounts produced in collections of volatiles were not obviously different.
- In other sawfly species it has been reported that the female sex pheromone is actually the product of oxidative breakdown of one of the hydrocarbons. Oxidative cleavage of (Z)-9-tricosene would give nonanal and tetradecanal, but significant amounts of these were not detected consistently in either hexane washes or collections of volatiles.
- Future work should focus on analysis of hexane washes and collection of volatiles by GC linked to electroantennographic (EAG) recording from the male sawflies to detect candidate pheromone components. If some of the minor poly-unsaturated hydrocarbons are being oxidised then amounts of the corresponding aldehydes could be very small and the structures of these would be more difficult to predict.

Objective 4: Integrated programme

To develop Integrated Pest and Disease Management strategies for blackcurrants combining the methods developed in objectives 1-3 with best crop husbandry practices and evaluate them including their economic and environmental impact

SIX MONTHLY REPORT TO HORTICULTURE LINK PROGRAMME MANAGEMENT COMMITTEE

Project Number:	HL01105
Project Title:	Developing biocontrol methods and their integration in sustainable pest and disease management in blackcurrant production
Project Partners:	*SCIENCE BASED PARTNERS* Scottish Crops Research Institute, Natural Resources Institute, East Malling Research *INDUSTRY PARTNERS* GlaxoSmithKline blackcurrant growers research fund, East Malling Trust for Horticultural Research, Bayer Crop Science Ltd, Fargro Ltd, Red Beehive Co Ltd, Ian Overy Farms, Wellbrook Farms, Robert Boucher and Son, Maynards, Bradenham Hall Farms, Bradfield Farm Ltd, Corbett Farms Ltd J Youngman & Sons, Adamston Farms Ltd East Malling Ltd
Report Written by:	Michelle Fountain & Xiangming Xu
Project Start/Completion Dates:	April 2010 – April 2015
Reporting Period:	12 month report (year 1)
Number of Months Since Commencement:	12 months
Date of Last Management Meeting:	13 December 2010
Date of next management meeting:	20 June 2011 & 14 December 2011

1. Project objectives: (from project proposal, or other more recently approved planning document)

Objective 1: *Botrytis cinerea*

1.1 To characterise variation in varietal susceptibility and to determine whether such varietal differences in susceptibility are correlated with physiological characters

1.2 To time fungicide application and supplementary sprays of BCAs during flowering to improve control

1.3 To enhance pollination by provision of pollinating insects in order to increase crop yield/uniformity and reduce infection by *B. cinerea*

1.4 To determine the role of *B. cinerea* and/or other fungi in blocking filters and to evaluate the benefit of fungicide and/or BCA sprays applied at different times in reducing such filter blockage

Objective 2: Blackcurrant leaf midge

2.1. To determine the relationships between the severity of galling damage caused by blackcurrant leaf midge and loss in growth and yield at different stages of crop growth and in different cultivars

2.2. To determine the relationships between sex pheromone trap catches and numbers of galls that develop subsequently

2.3. To identify new selective insecticides for control of leaf midge and to optimise timing of application in relations to sex pheromone traps catches

2.4. To investigate the feasibility of exploiting the leaf midge sex pheromone for control by mating disruption (MD), attract and kill (A&K) or mass trapping (MT)

Objective 3: Blackcurrant sawfly

3.1 To demonstrate sex pheromone attraction and investigate mating behaviour

3.2 To identify sawfly sex pheromone

3.3 To demonstrate pheromone activity, develop a lure and trapping system, and calibrate in the field

Objective 4: Integrated programme

To develop Integrated Pest and Disease Management strategies for blackcurrants combining

the methods developed in objectives 1-3 with best crop husbandry practices and evaluate them including their economic and environmental impact

2. Table showing overview of progress against milestones for project as a whole (from project proposal, or other more recently approved planning document)

Primary Milestones

Milestone	Target month	Title	
P1.1.1	30/09/2010	Advanced breeding lines identified and sent to EMR for investigation	Y
P1.1.2	30/09/2011	Historical data on cultivar trials analysed	
P1.1.3	31/03/2013	Relationships among physiological/morphological characters and susceptibility to *B. cinerea* established	
P1.2	31/03/2014	Efficacy of BCAs applied with fungicides during flowering against *B. cinerea* established	
P1.3	31/03/2015	Effects of additional pollinations on yield and quality established	
P1.4.1	31/03/2014	Key fungi responsible for blocking filters identified	
P1.4.2	31/03/2012	Incidence of important fungi in historical samples established	
P1.4.3	31/03/2015	Methods for reducing internal fungal colonisation evaluated	
P2.1.1	31/03/2013	Leaf midge crop damage on different cultivars and ages of plantation determined	
P2.1.2	31/03/2013	Leaf midge crop damage in cut down, re-growing crops determined	
P2.2	31/03/2013	Relationships between leaf midge sex pheromone trap catches and numbers of galls that develop subsequently determined	
P2.3	31/03/2013	Selective insecticides for leaf midge and optimised spray timing in relation to sex pheromone traps catches determined	
P2.4.1	31/03/2011	Necessity of mating in leaf midge determined	Y
P2.4.2	31/03/2012	Effects of pheromone on the mating behaviour of midges determined	
P2.4.3	31/03/2014	Formulation for pheromone control of leaf midge developed, if appropriate	
P2.4.4	31/03/2015	Field evaluation of pheromone control completed	
P3.2	31/03/2012	Sawfly sex pheromone identified	
P3.3	31/03/2015	Optimised pheromone trap for blackcurrant sawfly developed and protocol for use in monitoring produced	
P4.1.1	31/03/2013	IPDM programme for evaluation in years 4 and 5 devised	
P4.1.2	31/03/2015	IPDM programme in large scale grower trials evaluated in large scale commercial trials and refined	

P4.1.3	31/03/2015	Blackcurrant IPDM best practice guidelines prepared

Secondary Milestones

Milestone	Target month	Title	
S1.1.1	30/06/2010	Historical data on cultivar trials delivered to EMR	Y
S1.1.2	31/05/2011	CE experiments on cultivar comparison started	
S1.2.1	30/09/2010	Experimental products obtained for spray trial	Y
S1.3.1	31/03/2011	Year 1 Caged pollinating insect trials complete	Y
S1.3.1	31/03/2012	Year 2 Caged pollinating insect trials complete	
S1.3.1	31/03/2013	Year 3 Caged pollinating insect trials complete	
S1.4.1	30/09/2010	Historical samples delivered to EMR for analysis	Not available
S1.4.2	31/03/2012	Molecular methods for quantifying latent botrytis in fruit validated	
S1.4.3	31/03/2013	Experiments on restricting botrytis internal colonisation started	
S2.1.1	31/03/2011	1st year leaf midge crop damage assessment experiments completed	Y
S2.1.2	31/03/2012	2nd year leaf midge crop damage assessment experiments completed	
S3.1	31/03/2011	Sawfly sex pheromone attraction and mating behaviour investigated	N
S3.2	31/03/2012	Blackcurrant sawfly pheromone identified and synthesised	
S4.1.2	31/03/2014	1st year evaluation of IPDM programme in large scale grower trials complete	

3. Milestones for the six month period: (from project proposal, or other more recently approved planning document)

Primary Milestones

Milestone	Target month	Title	
P2.4.1	31/03/2011	Necessity of mating in leaf midge determined	Y

Secondary Milestones

Milestone	Target month	Title	
S1.3.1	31/03/2011	Year 1 Caged pollinating insect trials complete	Y
S2.1.1	31/03/2011	1st year leaf midge crop damage assessment experiments completed	Y
S3.1	31/03/2011	Sawfly sex pheromone attraction and mating behaviour investigated	N

4. Research report: (concise account including comments on whether targets are being met)

Objective 1: *Botrytis cinerea*

18 genotypes have been identified, including both advanced breeding lines and a few commercial cultivars, which vary in their susceptibility to botrytis. Propagated material from SCRI will be sent to EMR within the next few weeks. Controlled inoculation experiments will be conducted soon to assess whether susceptibility to botrytis is correlated with other morphological characters. Furthermore, we have assessed latent botrytis on nearly all cultivars/breeding lines at two cultivar trial sites. Other morphological characters recorded on these genotypes were also delivered to EMR. These data will be analysed to detect any association between susceptibility to botrytis and morphological characters. Historical samples will be analysed for the incidence of botrytis rot and to assess whether the incidence is associated with filter blocking.

Preliminary investigations at SCRI have identified physical differences in epidermal strength and structure across a range of blackcurrant germplasm; these measurements will be correlated with evaluations of Botrytis infection over the next 2 years.

Field trials have shown that supplementing blackcurrant plantations with *Bombus terrestris dalmatinus* nest boxes at the point of flower opening increases yield and fruit size of blackcurrant (Ben Hope and Ben Gairn) and this is particularly important in periods of poor weather when naturally occurring pollinating insects are less active. Pathology results were unclear and more replication is needed to ascertain whether *Bombus* pollinated crops are less susceptible to botrytis infection.

Objective 2: Blackcurrant leaf midge

Crop damage assessment in fruiting plantations

A 3 year, replicated large plot experiment was started in April 2010 in 7 commercial blackcurrant plantations in England to investigate the effects of blackcurrant leaf midge attacks on crop growth and yield. The plantations included establishing versus fully established crops of the cultivars Ben Alder, Ben Hope and Ben Tirran. Galling damage, yields and shoot growth were recorded in replicate plots treated with synthetic pyrethroid insecticides (bifenthrin and/or lambda cyhalothrin) where blackcurrant leaf midge attacks were low, versus untreated plots where populations were high.

Pheromone trap catches showed that the 7 different commercial plantations had widely varying levels of leaf midge at the outset. The first generation midge flight started in early April and reached a peak in late April to early May. A mean peak number of 25 midges per trap were captured for the first generation in the untreated plots. The second generation midge flight started in the last week of May to early June. Peak numbers captured averaged 81 per trap.

The insecticide treatments applied to the treated plots reduced numbers of galls per shoot by 67% and 79% for the first and second generations respectively. Catches of midges in the sex pheromone monitoring traps were also reduced by 38% and 80% for the two generations, respectively. Regressions between the numbers of galls recorded per shoot and the mean and peak numbers of midges caught in the sex pheromone traps in the untreated were not significant. More data is required over several seasons.

The gall midge attacks did not affect yield in the first year. The grand mean yield for the treated plots (7527 kg/ha) was very similar to the grand mean yield for the untreated plots (7583 kg/ha). Dormant season shoot growth measurements have yet to be made. The experiment will be continued for 2 further years to determine long term affects of blackcurrant leaf midge attacks on growth and yield.

Crop damage assessment in cut down bushes

The first of a series of 3 field experiments was done in leaf midge infested plantations at Adamston Farms Ltd, Scotland, to examine the effects of midge attack on extension growth in cut down bushes. The aim was to evaluate the effects on galling damage and extension growth of control of both the first and second generation (1st+2nd gen treatment) of blackcurrant leaf midge versus control of the second generation only (2nd gen treatment), in comparison with an untreated control treatment. Sprays of bifenthrin were applied on 15 and 24 May for the first generation, and on the 23 June for the second generation.

The ‘1st+2nd ‘treatment reduced the numbers of galls by 84% and 93% at the two assessments, respectively. Photographs taken of representative plots on 10 and 24 June

showed that the gall midge was stunting shoot growth, but dormant season shoot growth measurements to determine the full effects of the treatments have yet to be made.

Timing and efficacy of insecticides
A small plot replicated field experiment was done in 2010 to evaluate the efficacy of foliar sprays (500 l/ha) of UKA385a, Hallmark, Brigade or Calypso for control of first generation blackcurrant leaf curling midge. Treatments were a factorial comparison of the 4 products (UKA385a, Calypso, Hallmark, Brigade) at 3 timing combinations (1 spray at 5 days, 1 spray at 15 days, 2 sprays, one at 5 and one at 15 days, after a threshold catch of > 10 blackcurrant leaf midge males had been captured per trap in the two sex pheromone monitoring traps deployed in the plantation) versus a untreated control (double replicated). A summary of the findings of the experiment is as follows:

Spray timing was found to be important for effective control of blackcurrant leaf midge with the insecticides tested. Insecticides worked best when they were applied at the 5 days after threshold timing i.e. during the early part of the midge attack. At this time, females were laying eggs in the shoots tips and the eggs were developing and hatching. Application was before or at the early stages of gall formation. Insecticide application 15 days after the pheromone trap threshold was largely ineffective. The numbers of galls already present were not reduced, and only at best partial reductions in the numbers of semi-mature and mature larvae present in the galls were achieved. Two sprays were, thus, not significantly better than one as the second spray was largely ineffective. Monitoring with sex pheromone traps was thus important to getting good results. The results suggest that pheromone traps need to be deployed well before the start of the midge flight, probably by early March at the latest, and it would be best if they were monitored more frequently, probably twice weekly until the threshold is reached.

Hallmark, Brigade and UKA385a all gave effective control of blackcurrant leaf midge when applied 5 days after pheromone trap threshold. Hallmark and Brigade performed rather similarly. They both reduced the numbers of galled leaves by > 80% and gave a very high degree (>98%) of control of larvae in galls. Hallmark may be an effective alternative to Brigade and other bifenthrin products when approval for bifenthrin is lost in spring 2011. However, these synthetic pyrethoids have very broad-spectrum activity and they are likely to be very harmful to the midge's natural enemies including the parasitoid *Platygaster demades* and anthocorid predatory bugs, as well as to the natural enemies of other blackcurrant pests. Ideally, use of these products should be avoided so that blackcurrant IPM programmes which exploit natural enemies can be developed.

A single spray of UKA385a gave virtually 100 % control of larvae in galls. UKA385a is selective insecticide which is less likely to have harmful effects, especially persistent ones, on natural enemies. It is likely to be compatible with IPM programmes and priority should be given to its development for control of blackcurrant leaf midge and possibly other pests in UK blackcurrant IPM. These results indicate that UK385a is slower acting and because of this it does not prevent leaf galling occurring. However, it did give a high degree of efficacy of control of larvae. Bayer CropScience, the parent company of UKA385a, 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. The results suggest that Calypso has at best only very limited effectiveness for control of blackcurrant leaf midge.

The findings of this study are preliminary. Further work is needed to validate them and especially to explore timing of application of UKA385a and Hallmark in relation to pheromone trap catches more closely. For experiments in 2011, it is suggested that the pheromone traps are monitored more twice weekly during the critical period and timings of application of UKA385a and Hallmark 1, 3, 7 and 10 days after threshold are evaluated

Can unmated females reproduce?
It was demonstrated that unmated female midges were unable to produce viable offspring and this increases the potential of the pheromone as a mating disruption system to control populations in blackcurrant crops.

Objective 3: Blackcurrant sawfly

A method for culturing blackcurrant sawfly (*N. olfaciens*) has been tested and proven to be effective. Analyses of both hexane washes and collections of volatiles from male and female *N. olfaciens* showed a series of saturated and unsaturated, long-chain *n*-alkanes, as has been found in several other sawfly species. The major component was identified as (*Z*)-9-tricosene in both males and females. However, there were no obvious differences in the profiles of compounds from males and females, either qualitatively or quantitatively in that amounts of material found in hexane washes were not significantly different and amounts produced in collections of volatiles were not obviously different. In other sawfly species it has been reported that the female sex pheromone is actually the product of oxidative breakdown of one of the hydrocarbons. Oxidative cleavage of (Z)-9-tricosene would give nonanal and tetradecanal, but significant amounts of these were not detected consistently in either hexane washes or collections of volatiles. Future work should focus on analysis of hexane washes and collections of volatiles by GC linked to electroantennographic (EAG) recording from the male sawflies to detect candidate pheromone components. If some of the minor poly-unsaturated hydrocarbons are being oxidised then amounts of the corresponding aldehydes could be very small and the structures of these would be more difficult to predict.

5. Project changes: (proposed or agreed with the LINK programme, and including any changes to expected profile of grant claims)

None to date

6. Publications and technology transfer outputs: (including public presentations/talks given. Indicate additions since last report by use of bold type)

Technology transfer activities

M Fountain; Field talk to the Institute of Physics on the pollination part of the project (29 April 2010).

M Fountain; Talk to the blackcurrant growers in New Zealand (organised by Food and Plant Research) on the effectiveness of pollination by bumblebees for blackcurrant (28 October 2010).

M Fountain; Invited to give a talk to the Institute of Physics, 76 Portland Place, W1B 1NT on 13 Jan 2011 on bees, including the results pollination part of this project.

Publications

Poster: Michelle T Fountain, Robin Dean and Jerry V Cross. *The effect of pollinating insects on blackcurrant fruitset, yield and quality.*IOBC Working Group "Integrated Plant Protection in Fruit Crops"*,* Subgroup "Soft Fruits"*,* "Workshop on Integrated Soft Fruit Production"*,* 7th Meeting in Budapest, Hungary*.*

7. Exploitation plans: (give an update on perceived exploitation opportunities and future plans.)

The project is at a very early stage. Any exploitation plans will be discussed at the next winter meeting by the consortium.

