Project title:	Developing biocontrol methods and their integration in sustainable pest and disease management in blackcurrant production
Project number:	HL01105
Project co-ordinator:	Tom Maynard
Project administrator:	Prof. Jerry Cross (EMR)
Researchers:	Prof. Jerry Cross (EMR), Prof. David Hall (NRI), Dr Angela Berrie (EMR), Prof. Xiangming Xu (EMR), Dr Rex Brennan (James Hutton Institute), Dr Michelle Fountain (EMR), Dr Phil Brain (EMR), Adrian Harris (EMR)
Collaborators:	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:	Final report, 2015
Date commenced:	1 April 2010
Expected completion date:	31 March 2015
Key words:	Blackcurrant, *Botrytis cinerea*, pollination, processing filters, honeybees, bumblebees, *Bombus terrestris audax,* Blackcurrant leaf midge, *Dasineura tetensi*, Blackcurrant sawfly, *Nematus olfaciens*

DISCLAIMER

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

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.

[Name]

[Position]

[Organisation]

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

[Name]

[Position]

[Organisation]

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

Report authorised by:

[Name]

[Position]

[Organisation]

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

[Name]

[Position]

[Organisation]

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

TABLE OF CONTENTS

GROWER SUMMARY

Headline

Key components of an Integrated Pest and Disease Management programme for blackcurrant have been developed

Background and expected deliverables

The overall aim of the proposed project was 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 was to be evaluated and refined in large scale field experiments in the final two years of the project. Work targeted *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 was to develop appropriate improved management methods for each target to improve control whilst reducing dependence on and unnecessary use of pesticides.

Summary of project and main conclusions

Progress on each objective of the project is summarised below

Objective 1: *Botrytis cinerea*

Further morphological examination of the epidermal characteristics of diverse germplasm was made, focusing on the floral calyx area of the berry, but as previously no correlations were found with the rate or levels of *Botrytis* infection. As a result, the overall conclusion from the series of anatomical investigations and examination of detailed sections taken across a diverse genetic base is that differences in *Botrytis* susceptibility between the genotypes are unlikely to be due to anatomical or morphological differences in berry structure.

In the trial in 2013 the natural product Cropbiolife, based on flavonoids gave comparable control of *Botrytis* rot to the standard fungicide programme on cv. Ben Tirran. The incidence of *Botrytis* on Ben Hope was negligible which it was suggested could have been due to the amount of nitrogen fertilizer applied. In 2014 various programmes of Cropbiolife were compared with a standard fungicide programme on plots which received a high and low nitrogen fertilizer regime on Ben Hope and Ben Tirran for control of *Botrytis* fruit rot. Winter 2013/2014 was relatively mild which resulted in insufficient chilling for blackcurrants especially cv. Ben Tirran. Consequently bush development was very variable and yield also poor. For Ben Hope the standard fungicide programme resulted in significantly less *Botrytis* fruit rot in post-harvest tests compared to the untreated control (9% *Botrytis*). None of the other treatments were effective. The incidence of *Botrytis* in post-harvest tests was higher in Ben Tirran (20% in untreated plots). There were no significant effects of treatments on *Botrytis* fruit rot. There was no effect of nitrogen on fruit rot in either cultivar. The variable bush development resulting from insufficient winter chilling will have had an effect on the

performance of the various treatments. Further trials are needed to properly assess the effect of CropBiolife on fruit rots.

An experiment was conducted to investigate the effects of high nitrogen on fruit susceptibility to *B. cinerea*. A range of genotypes (commercial cultivars and advanced lines) received a standard N or high N (additional top dressing during the flowering period) treatment, and were inoculated with *B. cinerea* about 2-3 weeks after full bloom. Results provided some evidence to suggest that high N may lead to a high incidence of *B. cinerea*; however the incidence of total rotting did not differ much between the nitrogen treatments.

Tests to determine if insect pollination improved the nutritional status of fruit were done. Dry matter in blackcurrant fruit was improved by insect visits; this may be attributable to more effective pollen transfer than wind alone. The analysis of the nutrient levels in pollinated fruits were somewhat difficult to interpret. Fruits not pollinated by insects or by hand tended to be more acidic.

Objective 2: Blackcurrant leaf midge

No work was planned for 2014. It was found in previous year that there was no significant effect of the midge on established crops. This result is being used commercially. The blackcurrant midge pheromone is probably not viable to use as a mating disruption control strategy commercially.

Objective 3: Blackcurrant sawfly

In field trials we demonstrated that it was not necessary to have a window in the delta traps as previously thought. The two-component blend Z7-14iPr+Z7-16iPr was attractive to male blackcurrant sawfly and more attractive than Z7-14iPr alone or the three-component blend of Z7-14iPr + Z5-14iPr + Z7-16iPr tested previously. Addition of Z9-23H to the two-component blend increased the catches of male sawfly further and the blend was more effective if released from a polythene vial compared to a rubber septum.

Commercial 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:

Botrytis

- Identify differences in susceptibility between varieties but not the physical or biochemical basis
- The first three fungicide sprays applied from first flower are the most important treatments for *Botrytis* control
- If effective fungicide products are used there is no benefit from additional sprays near harvest

- BCAs can reduce *Botrytis* fruit rot significantly but efficacy is inconsistent from year to year
- Plant strengtheners / elicitors (e.g. Cropbiolife) can reduce *Botrytis* fruit rot and offer an alternative to BCAs but more work is needed to establish rates and timing for the best results
- Improved selection for *Botrytis* infection in commercial trials for potential new blackcurrant varieties
- The laboratory screening of surface-sterilised fruit for *Botrytis* infection is now part of the selection process within the breeding programme for blackcurrant funded by LR Suntory (and previously by GSK)

Pollination

- Supplement crops at flowering with bumblebees in poor weather conditions or if there has been a history of poor fruit set
- Wild bees were shown to contribute up to third of fruit set (7.5 tonne/ha, £650/tonne) = contribute up to 2.5 tonnes = £1625 /ha.
- Solitary bees were the main wild insect visitors to blackcurrant so numbers should be sustained and increased when plantations are not in flower, by providing alternative food sources
- Many were nesting in bare earth in the plantations so numbers could be increased by providing more nesting sites; undisturbed, south-facing areas of sparsely vegetated ground
- Bumblebees were also important flower visitors and areas to over winter and nest should be encourages (rotting wood, woodland, tussock grasses)

Midge

- Blackcurrant leaf midge should be controlled in newly planted, establishing or cut down blackcurrant bushes
- Control in established crops is less important and hence sprays targeted against the pest are unnecessary
- Lambda cyhalothrin (Hallmark) should be applied within a few days of threshold catches (>10 midges/trap) for the first and second generation where control is required
- UKA385a is promising new selective insecticide controls larvae in galls

Sawfly

- A new highly effective pheromone trap for sawfly will be made available commercially after calibration in 2015

Action points for growers

Botrytis

- Clear differences in susceptibility between varieties but physical or biochemical basis remains unclear
- The first three fungicide sprays applied from first flower are the most important treatments for *Botrytis* control
- If effective there is no benefit from additional sprays near harvest
- BCAs tested gave inconsistent control of *Botrytis* fruit rot and at present it is difficult to justify their use in blackcurrants for *Botrytis* control
- Plant strengtheners / elicitors gave promising results for rot control in 2013 but more work is needed to understand their possible use

Pollination

- Supplement crops at flowering with bumblebees in poor weather conditions or if there has been a history of poor fruit set
- Sustain and increase wild bee numbers when plantations are not in flower, by providing alternative food sources http://bumblebeeconservation.org/about-bees/habitats/
- Mow alleyways before spraying insecticides
- Provide undisturbed, south-facing areas of sparsely vegetated ground for solitary bees to nest in
- Leave untidy areas of rotting wood, preferably areas of woodland, and tussocky grasses for bumblebees to overwinter and nest

Midge

- Control blackcurrant leaf midge in newly planted, establishing or cut down blackcurrant bushes
- Control in established crops is less important
- Lambda cyhalothrin (Hallmark) should be applied within a few days of threshold catches (>10 midges/trap) for the first and second generation.
- UKA385a is a promising new selective insecticide which controls larvae in galls

Sawfly

- A new highly effective pheromone trap for sawfly will be made available commercially after calibration in 2015

SCIENCE SECTION

Introduction

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

N.B.: Key components of a biologically based integrated pest management (IPM) programme were identified (see report for objective 4 above) but biological methods (BCAs) for *Botrytis* were insufficient in the initial years and an alternative control needed to be identified. In addition, by year 3 the sawfly pheromone had not been confirmed and there was no selective insecticide for midge control available for testing. Some methods were found to be uneconomical for a processing crop, e.g. leaf midge mating disruptions using sew pheromones and *Botrytis* control using BCAs.

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

Examination of the floral calyx as a potential point of *Botrytis* entry into developing fruit was continued, with six berries of six diverse cultivars examined using protocols developed in Year 4. The berries were frozen and embedded in wax, from which 10 µm sections were cut using a Leica RA2265 microtome. The cultivars were chosen to represent the range of variation in *Botrytis* infection found in the storage tests and variations in skin thickness found in previous investigations, as follows:

Ben Alder	Thin skin but resistant to splitting, moderate shelf-life
Ben Starav	Resistant to fruit splitting, good shelf-life
Ben Dorain	Minimal fruit splitting, moderate to good shelf-life
Big Ben	Very large fruit, with high susceptibility to *Botrytis* infection, poor shelf-life
McWhite	New Zealand cultivar, very susceptible to *Botrytis* and fruit splitting, very small fruit
JHI 9154-3	Resistant to fruit splitting, moderate shelf life, small berries

Comparisons of the sections through the floral calyx remains on the berries showed considerable variability in skin thickness in all genotypes, but there were no consistent differences between genotypes, and no features or characteristics that could be associated with higher susceptibility to *Botrytis* infection or reduced shelf-life. Sections of two *Botrytis*-susceptible genotypes and two less-susceptible genotypes are presented in Fig. 1.1.1.

The conclusion from the series of anatomical investigations and examination of detailed sections taken across the available and diverse genetic base is therefore that differences in *Botrytis* susceptibility between the genotypes are unlikely to be due to anatomical or

morphological differences in berry structure.

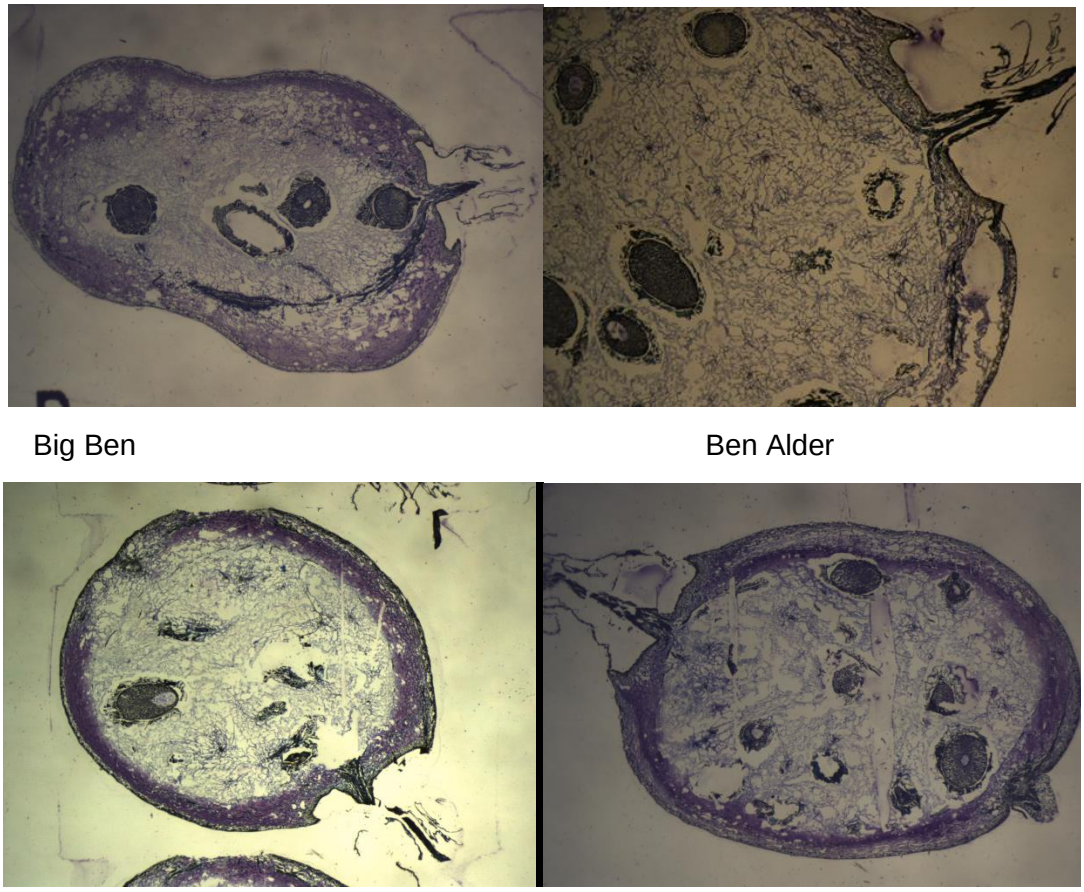


Figure 1.1.1. Sections through ripe fruit of four genotypes with differing susceptibilities to *Botrytis*

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

Task 1.2.1. The effect of nitrogen fertilizer on *Botrytis* fruit rot

Objectives

- To evaluate the effect of nitrogen fertilizer on *Botrytis* fruit rot
-

Materials and methods

An experiment was conducted to investigate the effects of high nitrogen on fruit susceptibility to *B. cinerea*. A range of genotypes (commercial cultivars and advanced lines) received a standard N or high N (additional top dressing during the flowering period) treatment, and inoculated with *B. cinerea* about 2-3 weeks after full bloom. Inoculated genotypes included:

Big Ben, Ben Hope, Ben Gairn, 9145-3, 9148-9, 9198-1, Murchinson, 91192, Ben Dorain, 9199-4, 92127-1, 9476-2, Ben Alder, Ben Avon, Ben McWhite, 903-1, Ben Tirran, and Ben Starov. Most genotypes had four plants, two of which randomly assigned to either the standard or the high nitrogen treatment. For the high nitrogen treatment, 35 g ammoniacal nitrogen was added to the top surface of the compost on 16 April 2014, around the earliest flowering time.

Plants were inoculated ca 2 weeks after full bloom. Flowering time was recorded for individual plants on two occasions; based on the recorded flowering time, plants were divided into two batches for inoculation on 30th April and 8th May 2014. *Botrytis* spore suspension was made, adjusted to the concentration of 10^6 conidia, and sprayed onto fruit clusters. Inoculated plants were misted for 24 h in a glasshouse compartment. All fruit were harvested and incubated at room temperature to assess fruit rot (if possible the cause of each rot was also recorded).

A generalised linear mixed model (GLMM) was applied to the data where the incidence of fruit rot is assumed to follow a binomial distribution. In addition, the cultivar factor was considered to be a random effect factor and nitrogen treatment to be a fixed effect factor.

Results

The overall predicted incidence of *B. cinerea* from the GLMM analysis was 36.5% and 27.3% for the high and standard nitrogen treatments, respectively. This difference was close to the statistical significance (P = 0.09). On the other hand, the incidence of total fruit rot did not differ significantly between the two nitrogen treatments (P = 0.28): 84.1% and 78.8% for the high and standard nitrogen treatment, respectively.

Task 1.2.2 Field evaluation of natural plant elicitors with or without fungicides or biocontrol agents for control of *Botrytis* in blackcurrants 2013

Background

Three experiments were conducted between 2011 and 2013. In 2011 various BCAs were compared for control of *Botrytis* to a conventional fungicide programme and a programme based on fungicide use during flowering and BCAs during fruit development. The results showed that all BCAs tested significantly reduced *Botrytis* fruit rot in ripe fruit but were not as effective as the conventional fungicide programme. However, there was some benefit in *Botrytis* control where the final fungicide was replaced by two pre-harvest sprays of the BCA Serenade ASO (*Bacillus subtilis*). Experiments reported in 2012 found no effect of BCAs on *Botrytis* control. In 2013 it was decided to evaluate alternative chemicals and elicitors for effects on *Botrytis* fruit rot.

Materials and methods

The blackcurrant plantation was located at East Malling Research and was planted in 2004 and consisted of separate blocks of the cvs. Ben Hope, an early flowering cultivar, and Ben Tirran, a late flowering cultivar. The trial was conducted on both cultivars. The plots contained six bushes spaced 0.5 m in the row and 1.5 m between rows. Treatments were replicated six times (two replicates of three blocks) in a randomised block design. Treatments were applied using a motorised air-assisted knapsack sprayer at 500 L/ha. Weather data was collected from a weather station adjacent to the plantation. The efficacy of various elicitors was compared to a standard fungicide programme, an experimental fungicide and a BCA (Table 1.2.2.1). Fungicides and BCAs were applied at 7-10 day intervals from first flower (BBCH 60). Elicitors were applied either at 2 week or 4 week intervals (Table 1.2.2.2).

Plots were assessed at harvest for visible *Botrytis* on fruit by counting all the berries on at least 5 branches per plot and recording the number with *Botrytis*. Three hundred berry fruit samples were collected from each plot weighed and then incubated post-harvest in high humidity in polythene-covered plastic trays at ambient temperature (approximately 20^{o}C) to encourage development of *Botrytis* rot if present. Numbers of *Botrytis*-rotted fruit were recorded after 7 days. Other fungal rots were also recorded but not reported here.

Table 1.2.2.1. Fungicides, BCAs and elicitors applied to blackcurrants cvs Ben Hope and Ben Tirran in 2013

Product	Active ingredient	Product rate per ha or per litre
Untreated control	-	-
Signum	boscalid + pyraclostrobin	1.5 kg
Switch	cyprodonil + fludioxonil	1.0 kg
Teldor	fenhexamid	1.5 kg
UKA386a	experimental	0.8 L
Serenade ASO	*Bacillus subtilis*	10 L
Pretect	Harpin protein	2 kg
Elicitor A	experimental	350 ml
Farmfos	potassium phosphite	10 L

Table 1.2.2.2. Treatment programmes applied to blackcurrant cvs Ben Hope and Ben Tirran in 2013

Treatment	First flower (BBCH 60)	7days after 1st spray	14 days after 1st spray	21 days after 1st spray	28 days after 1st spray	42 days after 1st spray	56 days after 1st spray (BBCH 79)
1	-	-	-	-	-	-	-
2	Signum	Switch		Teldor			
3	UKA386a	UKA386a		UKA386a			
4	Serenade	Serenade		Serenade	Serenade		
5	Pretect				Pretect		Pretect
6	Cropbiolife				Cropbiolife		Cropbiolife
7	Farmfos				Farmfos		Farmfos
8	Pretect		Pretect		Pretect	Pretect	Pretect
9	Cropbiolife		Cropbiolife		Cropbiolife	Cropbiolife	Cropbiolife
10	Farmfos		Farmfos		Farmfos	Farmfos	Farmfos

Results

2013 was characterised by relatively cool temperatures during spring with Ben Hope flowering approximately 2 weeks earlier than Ben Tirran. However, actual weather conditions, especially rainfall were similar during flowering for both cultivars (approximately 111 mm of rain) but conditions were wetter pre-harvest for Ben Tirran with 55 mm of rain in the week before harvest (compared to 6.4 mm for Ben Hope). *Botrytis* was visibly sporulating on mummified fruit from the previous year in the plantation on both Ben Hope and Ben Tirran before flowering in May. No *Botrytis* was observed during flowering or on fruit on the bushes before harvest.

The incidence of *Botrytis* in post-harvest tests on Ben Hope was negligible and sporadic with only 1.5 % rot recorded on fruit from untreated plots (Table 1.2.2.3). None the treatments had any significant effect on *Botrytis* incidence. By contrast the incidence of *Botrytis* rot in Ben Tirran was relatively high with over 36 % rot recorded in fruit from untreated plots. The most effective treatment was UKA386a. CropBioLife, applied as a three or five spray programme, was as effective in reducing *Botrytis* as the standard fungicide programme. None of the other

elicitors applied were effective in reducing *Botrytis* fruit rot. There were no significant effects of treatments on weight of 300 fruit.

Table 1.2.2.3 Incidence *of B. cinerea* (angular transformed) on harvested blackcurrant fruit incubated for 7 days at ambient temperature cvs Ben Hope and Ben Tirran following various spray programmes of fungicides, BCAs or elicitors in 2013. Figures in brackets are back transformed data

Treatment	Spray programme	% *Botrytis* fruit rot	
		Ben Hope	Ben Tirran
1	Untreated	5.3 (0.9)	36.9 (36.1)
2	Signum, Switch, Teldor	3.4 (0.4)	27.8 (21.8)
3	UKA386a x 3	4.4 (0.6)	18.6 (10.2)
4	Serenade x 4	7.0 (1.5)	34.9 (32.7)
5	Pretect x 3	6.4 (1.2)	33.7 (30.7)
6	Elicitor A x 3	5.8 (1.0)	30.1 (25.1)
7	Farmfos x 3	4.2 (0.5)	36.3 (35.1)
8	Pretect x 5	4.4 (0.6)	35.3 (33.5)
9	Elicitor A x 5	4.4 (0.6)	28.7 (23.0)
10	Farmfos x 5	5.4 (0.9)	33.7 (30.7)
	F Prob	0.984	<0.001
	SED (45 df)	3.11	2.93
	LSD (p=0.05)	6.26	5.90

Elicitors were evaluated as an alternative approach to BCAs. The results showed that Cropbiolife was as effective in reducing *Botrytis* fruit rot as the standard fungicide programme. This product was used at a low rate and at a reduced cost compared to the fungicide programme. The use of elicitors may therefore offer a viable alternative to fungicides but further work is needed to demonstrate that such effects on disease control are consistent from season to season. Trials in 2014 will evaluate further the efficacy of elicitors in control of *Botrytis* in blackcurrants and the effect of nitrogen fertiliser on *Botrytis* susceptibility.

Task 1.2.2. Field evaluation of natural plant elicitors and nitrogen effect on *Botrytis* fruit rot 2014

Background

In the trial in 2013 the natural product Cropbiolife, based on flavenoids gave comparable control of *Botrytis* rot to the standard fungicide programme on cv. Ben Tirran. The incidence of *Botrytis* on Ben Hope was negligible which it was suggested could have been due to the amount of nitrogen fertilizer applied. The aim of the trial in 2014 was to further explore the use of Cropbiolife and to look at the effect of nitrogen fertilizer on *Botrytis* rot in blackcurrants.

Experiment Title: Evaluation of natural plant elicitors with or without fungicides for control of *Botrytis* in blackcurrants 2014

Objectives

- To evaluate programmes of CropbioLife in comparison with fungicides for control of *Botrytis* fruit rot and other rots
- To evaluate the effect of nitrogen fertilizer on *Botrytis* fruit rot

Materials and methods

Site and experimental design: Blackcurrant plantation located at East Malling Research. The plantation (CE186) was planted in 2004 and consisted of six separate blocks, three each of either cvs. Ben Hope or Ben Tirran. The trial was conducted on both cultivars. Each plot contained 6 bushes spaced 0.5 m in row and 1.5 m between rows. Each treatment was replicated six times in a randomised block design with two replicates in each cultivar block.

Treatments: Two fertiliser treatments were applied – a standard rate of fertilizer of 80 kg/ha N applied to all plots. Half of the plots also received an additional amount of fertilizer applied by hand of 50 kg/ha N. Five spray treatments were then applied to these (Table 1.2.2.4) giving 10 treatments in total. The spray treatments consisted of a standard fungicide programme applied at 7-10 day intervals from first flower, three CropbioLife programmes and an untreated control.

Treatment application: Treatments were applied using a Stihl motorised air-assisted knapsack sprayer at 500 L/ha.

Assessments

Weather*:* Weather data was collected from a weather station adjacent to the plantation.

Disease: The incidence of *Botrytis* on flowers was assessed soon after flowering on 5 branches per plot. Plots were assessed at harvest for visible *Botrytis* on fruit by counting all the berries on at least 5 branches per plot and recording the number with *Botrytis*. At harvest 300 fruit samples were collected from each plot, weighed to give an indication of fruit size, and incubated post-harvest in high humidity to encourage development of *Botrytis* rot, if present. Numbers of *Botrytis*-rotted fruit and other rots were recorded.

Yield: Yield was assessed by comparing plots visually and scoring compared to the untreated control.

Results and discussion

Dates treatments were applied are shown in Tables 1.2.2.5 and 1.2.2.6. Weather during winter 2013/2014 was generally mild resulting in insufficient chilling for blackcurrants especially cv. Ben Tirran which has a high chilling requirement. Consequently, flowering and bush development was erratic especially in Ben Tirran making it difficult to time sprays for *Botrytis* control. Strig length and yield was also considerably reduced. The incidence of visible *Botrytis* at flowering and pre-harvest was very low for both cultivars and not assessed. Yield was very variable between bushes with no obvious differences in treatments. There was no significant effect of treatment on weight of 300 fruit (Tables 1.2.2.7 and 1.2.2.8). In post-harvest tests *Botrytis* and *Penicillium* rot were the main rots recorded. Mucor rot was also recorded on Ben Tirran.

Table 1.2.2.4. Spray treatments applied to blackcurrants in 2014

Treatment	Product	Active ingredient	Product rate per ha	Timing
1 Low N	Untreated control	-	-	
2 Low N	Signum Switch Teldor	pyraclostrobin + boscalid cyprodinil + fludioxonil fenhexamid	1.5 kg 1.0 kg 1.5 kg	3 sprays at 7-10 day intervals from 1st open flower
3 Low N	CropBioLife	flavonoids	300-500 ml	5 sprays from pre-flowering starting at 300 ml and increasing to 500 ml
4 Low N	CropBioLife	flavonoids	350 ml	3 sprays from pre-flowering
5 Low N	CropBioLife	flavonoids	350 ml	5 sprays from pre-flowering
6 High N	Untreated control	-	-	
7 High N	Signum Switch Teldor	pyraclostrobin + boscalid cyprodinil + fludioxonil fenhexamid	1.5 kg 1.0 kg 1.5 kg	3 sprays at 7-10 day intervals from 1st open flower
8 High N	CropBioLife	flavonoids	300-500 ml	5 sprays from pre-flowering starting at 300 ml and increasing to 500 ml
9 High N	CropBioLife	flavonoids	350 ml	3 sprays from pre-flowering
10 High N	CropBioLife	flavonoids	350 ml	5 sprays from pre-flowering

Table 1.2.2.5. Spray dates, growth stages and treatments applied to Ben Hope

Treatment	23 April First flower BBCH 60	30 April	6 May	13 May	22 May	10 June	26 June	Harvest
1	Untreated	-	-	-	-	-	-	1 August
2	Signum	Switch	-	Teldor	-	-	-	
3	CropBioLife 300 ml	-	CropBioLife 300 ml	-	CropBioLife 400 ml	CropBioLife 400 ml	CropBioLife 500 ml	
4	CropBioLife	-		-	CropBioLife		CropBioLife	
5	CropBioLife	-	CropBioLife	-	CropBioLife	CropBioLife	CropBioLife	
6	-	-	-	-	-	-	-	
7	Signum	-	-	-	-	-	-	
8	CropBioLife 300 ml	-	CropBioLife 300 ml	-	CropBioLife 400 ml	CropBioLife 400 ml	CropBioLife 500 ml	
9	CropBioLife	-		-	CropBioLife	-	CropBioLife	
10	CropBioLife	-	CropBioLife	-	CropBioLife	CropBioLife	CropBioLife	

Table 1.2.2.6 Spray dates, growth stages and treatments applied to Ben Tirran

Treatment	12 May First flower BBCH 60	22 May Full flower BBCH 65	27 May	5 June	10 June	26 June	9 July	Harvest
1	Untreated	-	-	-	-	-	-	20 August
2	Signum	Switch		Teldor	-	-	-	
3	CropBioLife 300 ml	-	CropBioLife 300 ml	-	CropBioLife 300 ml	CropBioLife 400 ml	CropBioLife 500 ml	
4	CropBioLife	-	-	-	CropBioLife		CropBioLife	
5	CropBioLife	-	CropBioLife	-	CropBioLife	CropBioLife	CropBioLife	
6	Untreated	-	-	-	-	-	-	
7	Signum	Switch	-	Teldor	-	-	-	
8	CropBioLife 300 ml	-	CropBioLife 300 ml	-	CropBioLife 300 ml	CropBioLife 400 ml	CropBioLife 500 ml	
9	CropBioLife	-	-	-	CropBioLife	-	CropBioLife	
10	CropBioLife	-	CropBioLife	-	CropBioLife	CropBioLife	CropBioLife	

The incidence of *Botrytis* rot and total rots in post-harvest tests was significantly reduced by the fungicide programme in cv. Ben Hope (Table 1.2.2.7). None of the other treatments had any significant effect. For Ben Tirran (Table 1.2.2.8) which was most affected by the lack of winter chill none of the treatments had any significant effect on fungal rots. This was most likely due to the erratic bush development which made it difficult to consistently time treatments to flowering time. There was no effect of nitrogen on *Botrytis* rot incidence for either cultivar.

The results for CropBioLife are rather disappointing compared to 2013. However the erratic development of the bushes and the poor yields was probably a major contributory factor. There is need to repeat the trial before any firm conclusions can be drawn on CropBioLife.

Table 1.2.2.7 Weight of 300 fruit and incidence *of B. cinerea, Penicillium and total rots* (angular transformed) on harvested blackcurrant fruit incubated for 7 days at ambient temperature cvs Ben Hope following various spray programmes of fungicides or elicitors in 2014. Figures in brackets are back transformed data

Treatment	Nitrogen applied	Products	Weight 300 fruit g	% *Botrytis* rot	% Penicillium rot	% Total rots
1	Low N	Untreated	315.8	17.5 (9.2)	14.7 (6.4)	23.7 (16.2)
2		Signum Switch Teldor	302.3	**10.2 (3.2)**	8.2 (2.0)	**13.5 (5.4)**
3		CropBioLife	294.9	16.8 (8.4)	13.5 (5.5)	22.4 (14.6)
4		CropBioLife	313.5	17.0 (8.5)	13.6 (5.6)	22.4 (14.5)
5		CropBioLife	326.4	19.9 (11.6)	15.0 (6.7)	25.5 (18.5)
6	High N	Untreated control	305.6	17.4 (8.9)	16.8 (8.4)	25.0 (17.9)
7		Signum	323.3	14.6 (6.4)	13.7 (5.6)	20.9 (12.7)
8		CropBioLife	307.7	14.4 (6.2)	13.9 (5.8)	20.5 (12.3)
9		CropBioLife	317.0	18.8 (10.4)	15.6 (7.2)	26.1 (19.4)
10		CropBioLife	315.5	19.4 (11.1)	14.2 (6.0)	24.8 (17.6)
		F Prob	NS	0.004	NS	0.004
		SED (45 df)	18.8	2.27	2.95	2.91
		LSD (p=0.05)	37.8	4.58	5.95	5.86

Table 1.2.2.8 Weight of 300 fruit and incidence of B. cinerea, Penicillium and total rots (angular transformed) on harvested blackcurrant fruit incubated for 7 days at ambient temperature cvs Ben Tirran following various spray programmes of fungicides or elicitors in 2014. Figures in brackets are back transformed data

Treat-ment	Nitrogen applied	Products	Weight 300 fruit g	% *Botrytis* rot	% Penicillium rot	% Total rots
1	Low N	Untreated	299.3	27.0 (20.6)	17.3 (8.8)	36.3 (35.0)
2		Signum Switch Teldor	293.5	24.0 (16.5)	19.3 (10.9)	34.3 (31.7)
3		CropBioLife	279.5	21.1 (12.9)	18.0 (9.5)	32.6 (29.0)
4		CropBioLife	303.1	23.9 (16.5)	18.8 (10.4)	35.7 (34.1)
5		CropBioLife	308.5	27.6 (21.5)	16.8 (8.4)	36.4 (35.2)
6	High N	Untreated control	300.5	26.2 (19.5)	17.8 (9.3)	38.2 (38.3)
7		Signum Switch Teldor	339.3	20.9 (12.7)	17.9 (9.5)	31.2 (26.8)
8		CropBioLife	299.7	22.7 (14.9)	18.4 (10.0)	34.1 (31.5)
9		CropBioLife	308.4	25.1 (18.0)	19.9 (11.6)	37.1 (36.4)
10		CropBioLife	285	23.2 (15.5)	18.6 (10.2)	34.6 (32.2)
		F Prob	NS	NS	NS	NS
		SED (45 df)	18.4	2.72	3.35	2.92
		LSD (p=0.05)	37.1	5.47	6.75	5.88

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

Task 1.3.2. Effect of pollinating insects on blackcurrant yield and quality (Years 1-3)

Objective for 2014

To determine if blackcurrant flowers pollinated by insects produce a higher quality (nutritional) fruit than self-pollinated flowers or optimally (hand) pollinated flowers.

Materials and methods

Site and site manager: Ian & Nick Overy, Burrs Hill, Brenchley, Ian Overy Farms. Contact Address: Mile Oak Farm Mile Oak Road Paddock Wood Tonbridge Kent TN12 6NG Plantation 'Matfield Big Bit', cv. Ben Hope, spacing 30 cm.

Treatments: Three blackcurrant flower treatments (Fig. 1.3.1).

1. Insect excluded – branches with mesh bags on
2. Open pollinated – left open to insect pollination
3. Optimum pollinated – hand pollinated with paint brush

Figure 1.3.1. Treatments applied to crop and marked with coloured tape and berries ripening in crop

Experimental design and statistical analyses: On each bush there were three treatments with 20 replicates; each treatment was one branch. This allowed for differences between bushes. There were an additional 10 replicates for dry weight analyses (Fig. 1.3.2)). The experiment was set up in three rows of the crop 10 m in from the edge. Plots were 10 m apart. Data was collated and statistically analysed by ANOVA as appropriate comparing treatments.

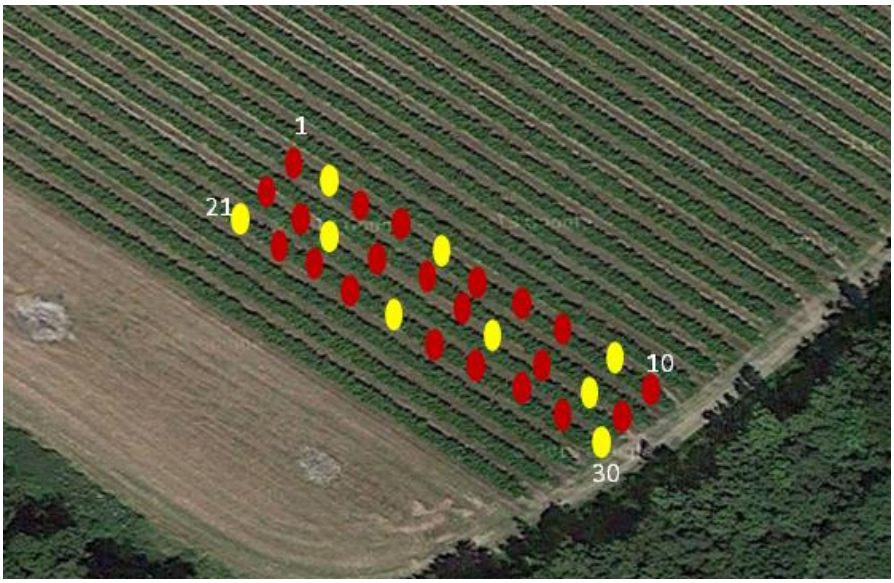


Figure 1.3.2. Approximate location of samples in crop. Red = fruit nutritional analyses samples, yellow = fruit dry wt samples.

Treatment application: Mesh bags which allowed pollen in by wind but excluded insects were applied to one treatment (7 Apr). The optimum pollinated treatment was visited on 5 occasions (14, 17, 23, 28 Apr and 6 May). On the first date, 10% of flowers were open; on 28 Apr 99% flowers were open and on 6 May there was approximately 40% fruit set. Flowers were pollinated by transferring pollen from flower to flower with an artist's paintbrush. Exclusion bags were removed on 12 May. No manipulation was done on the open pollinated branches.

Assessments: Plants were monitored weekly after fruit set and harvested once ripened (7 Jul). The fruit from the top 6 strigs was harvested and then assessed for fruit set, weight and ripeness. In addition a record was taken of dry weight of berries from plots 2, 5, 9, 12, 14, 18, 21, 25 and 30. The ripe fruit was frozen at -80^{o}C and then analysed at EMR laboratories for fruit quality (biochemical).

Sample preparation: Blackcurrant samples previously stored at -80^{o} C were dropped into liquid nitrogen and then ground to a fine power with a commercial waring blender. Sub-samples of 5 g were used for sugar, acid, antioxidant and anthocyanin analysis.

Sugars: Samples were homogenised in 25ml of ultra-pure water, shaken for 30 min at 4^{o}C on an orbital shaker, and then centrifuged at 5040*g* for 35 min. A five hundred micro-litre aliquot of sample was pipetted into a Thomson 0.45µm PTFE filter vial. A ten micro-litre injection of sample was made into a Waters Alliance 2690 HPLC. Sugars were separated on a Pinnacle II amino column 250 x 4.6 mm 5µm (Thames restek UK Ltd) and detected with a Waters 410 differential refractometer (RID). The mobile phase was [80:20] [acetonitrile: ultra-pure water] with a flow rate of 2.5 ml min^{-1}, column and RID temperature was 40^{o} C. Standards of known amounts of fructose, glucose and sucrose were injected into the HPLC, Millinium^{32} software was used to produce linear calibration curves in the range of 5 to 50µg with r^2 0.999. These calibration curves were used to determine the amount of fructose, glucose and sucrose in the samples.

Acids: Samples were homogenised in 25ml of 20mM potassium phosphate containing 5mM Tris (2-carboxyethyl) phosphine hydrochloride at pH 2.9, shaken for 30 min at 4^{o}C on an orbital shaker then centrifuged at 5040g for 35 min. A five hundred micro-litre aliquot of sample was pipetted into a Thomson 0.45µm PTFE filter vial. A five micro-litre injection of sample was made into a Waters Alliance 2690 HPLC. Acids were separated on an Allure organic acid column 5 µm 300 x 4.6 mm (Thames Restek UK Ltd) and detected with a Waters 996 photodiode array detector. Malic and Citric acid were detected at 220 nm, ascorbic acid was detected at 243 nm. The mobile phase was 20 mM tri-potassium

phosphate pH 2.9 with a flow rate of 1 ml min^{-1} with column temperature at 20°C. Standards of known amounts of malic, citric and ascorbic were injected into the HPLC, Millinium[32] software was used to produce linear calibration curves in the range of 2 to 10 µg for malic acid, 5 to 40 µg for citric acid and 1 to 5 µg for ascorbic acid all with r^2 0.999. These calibration curves were used to determine the amount of malic, ascorbic and citric in the samples.

Antioxidant capacity: The antioxidant capacity was measured, in triplicate, using the Trolox equivalent anti-oxidant capacity (TEAC) assay. Anti-oxidants were extracted by homogenising the sample in 25 ml of [80:20] [methanol: water] shaken for 30 min at 4°C on an orbital shaker then centrifuged at 5040*g* for 30 min. The solvent was decanted and an aliquot was diluted [1:8] with extraction solvent prior to measurement. A 7mM solution of 2, 2' –Azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) in ultra-pure water was converted to its mono-cationic form ($ABTS^+$) by the addition of 2.45 mM (final concentration) potassium persulphate ($K_2S_2O_8$). The $ABTS^+$ solution was left in the dark at room temperature for 24 h, it was diluted [1:100] [$ABTS^+$: ethanol] to give an absorbance in the range of 0.850 - 0.975, the solution was kept in the dark and maintained at a temperature of 30°C.

A 30µl aliquot of diluted extract was transferred into a 3 ml cuvette containing the $ABTS^+$ solution. The cuvettes were placed in a water bath at 30°C for 20 min. Measurements of absorbance were made with a Jenway 6705 uv/vis spectrophotometer at a wavelength of 734 nm. The absorbance of standards of 6-Hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) in a concentration range of 0 to 2 mM was measured to determine the % inhibition of $ABTS^+$. A standard curve was plotted (r^2 0.999) and used to determine the anti-oxidant capacity of the samples relative to the reactivity of Trolox.

Total anthocyanin: The total anthocyanin content was measured, in triplicate, by the pH differential method. Samples were homogenised with 25 ml methanol containing 0.1% hydrochloric acid. The samples were placed in a water bath at 35°C for 35 min, shaken on an orbital shaker for 10 min then centrifuged at 5040*g* for 35 min. Thirty microliters of sample was pipetted into cuvettes containing either 3 ml of 0.025M potassium chloride pH 1.0 and 0.4M sodium acetate pH 4.5. Absorbance was measured in a Jenway 6705 uv/vis spectrophotometer at 520 nm and 700 nm. Absorbance readings were converted to total milligrams of cyaniding 3-glucoside per g fresh weight of blackcurrant using the molar extinction coefficient of 26 900 and absorbance of $A = [(A_{520} - A_{700})_{pH\ 1.0} - (A_{520} - A_{700})_{pH\ 4.5}]$.

Results

On 23 Apr when the blackcurrants were visited for the 3rd hand pollination approximately 90% of the flowers were open. It was also noted that the majority of insect visitors on this date were honey bees (*Apis mellifera*). There were also some bumblebee workers. On 28 April most flowers were open and honeybees were absent but some bumblebees were foraging.

The flowers that were excluded from hand (optimum) or insect pollination resulted in at least 28% fewer fruits being set. This is comparable with earlier studies showing up to a 35% fruit-set deficit where insects are excluded from blackcurrant flowers at blossom. The number of ripe berries at harvest was significantly higher if the flowers had been insect- or hand-pollinated (Table 1.3.1). The percentage dry weight in the excluded treatment berries was lower than if hand- or insect-pollinated. This could be attributable to pollinated flowers having a better seed set which would increase cell replication, development and fruit growth (see also sugars in Table 1.3.2).

In general, acids in the insect excluded fruits were higher than in hand- or insect-pollinated fruits (wet weight). However, vitamin C (ascorbic acid) was higher in hand pollinated fruits compared to wind only pollinated, with insect pollinated between. Sugars (wet weight analysed) were, on the whole, higher in insect pollinated fruits than insect excluded fruits with the hand pollinated fruits somewhere between. There were no differences in the amounts of anthocyanins in the different treatments (Table 1.3.2).

Table 1.3.1. Analyses of fruit set and weights (top six strigs) of the three treatments. Capital letters denote significant differences

	Fruit set (%)	No. of ripe fruit	No. of unripe fruit	Fresh wt. (g)	Dry wt. (g)	% dry wt. (g)
excluded	36 A	21 A	1.8	19 A	3.4 A	21.11 A
optimum	64 B	37 B	3.5	31 B	8.6 B	22.85 B
open	66 B	38 B	3	35 B	7.6 B	22.88 B
F pr.	< 0.001	< 0.001	NSD	< 0.001	0.007	0.032
sed	4.795	3.631		3.911	1.492	0.6769
lsd	9.605	7.273		7.835	3.164	1.443

Adjustments for dry weight of fruits were calculated using the formulae;

% dry weight (Table 1.3.1) = mean of destructively sampled 10 replicate bushes (berries not used for nutrient analysis).

$$\text{Dry wt. of sample} = \frac{\text{Fresh weight of sample (g)}}{100} \times \text{\% Dry wt.}$$

$$\text{Dry wt. nutrient value} = \frac{\text{Fresh wt. of sample (g)}}{\text{Dry wt. sample}} \times \text{Dry wt. nutrient value.}$$

The effects on vitamin C and sugars was not apparent when fruits were analysed by dry weight, but the acids (citric and malic) in insect excluded fruit was still significantly higher than in insect or hand pollinated fruits (Table 1.3.2).

Discussion/conclusions

- Honey bees visited Ben Hope in large numbers on one of the dates. Ben Hope flowers later than Ben Gairn and so may have been more attractive to honeybees at this time.
- Bumblebees were seen as one of the main visitors and play an important role in blackcurrant pollination along with solitary bees identified in previous years of this study. The neighbouring woodland could have been good habitat for bumblebees.
- There did not seem to be a pollination deficit for blackcurrant in this small trial. In general the hand pollinated and the insect pollinated measures were the same. Whether this is the same for all blackcurrant crops and varieties is not known.
- We confirmed that insects contribute around a third of the fruit set of blackcurrant.
- Dry matter in blackcurrant fruit is also improved by insect visits; this may be attributable to more effective pollen transfer than wind alone. Effective pollen deposition onto the stigma of the flower can improve seed set in fruit and hence hormone release which contribute to cell replication and development.
- Fruits not pollinated by insects or by hand tended to be more acidic.
- Red Mason Bees placed in a crop were not found to be foraging blackcurrant pollen.

Table 1.3.2. Nutritional analyses of berries which were insect excluded, insect pollinated or hand pollinated at the time of flowering. Capital letters denote significant differences

Fresh wt.	mg/g fresh weight								
Treatment	**Anthocyanin**	**Ascorbic acid**	**Citric acid**	**Fructose**	**Glucose**	**Malic acid**	**Sucrose**	**Total acid**	**Total sugar**
excluded	1509	1.562 A	31.48 B	30.7 A	18.8	4.229	6.33 A	37.3 B	56 A
hand	1642	1.903 B	29.52 A	33.7 AB	19.8	3.963	7.08 A	35.4 A	61 AB
open	1542	1.703 AB	30.01 A	36.0 B	21.7	3.803	7.84 AB	35.5 A	66 B
F pr.	NSD	0.018	0.020	0.031	0.054	NSD	0.027	0.045	0.027
sed		0.114	0.697	1.965	1.195		0.544	0.819	3.508
lsd		0.232	1.414	3.985	2.424		1.102	1.661	7.115

Dry wt.	mg/g adjusted for dry weight								
Treatment	**Anthocyanin**	**Ascorbic acid**	**Citric acid**	**Fructose**	**Glucose**	**Malic acid**	**Sucrose**	**Total acid**	**Total sugar**
excluded	7143	7.4	149 B	145.7	89.06	20.0 B	29.98	176 B	264.7
hand	7186	8.3	129 A	147.6	86.74	17.4 A	31.00	155 A	265.3
open	6739	7.4	131 A	157.5	94.87	16.6 A	34.24	155 A	286.7
F pr.	NSD	NSD	< 0.001	NSD	NSD	0.008	NSD	< 0.001	NSD
sed			3.124			1.091		3.674	
lsd			6.336			2.213		7.452	

Objective 2: Blackcurrant leaf midge

No work was planned for 2014. It was found in previous year that there was no significant effect of the midge on established crops. This result is being used commercially.

The blackcurrant midge pheromone is probably not viable to use as a mating disruption control strategy commercially.

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

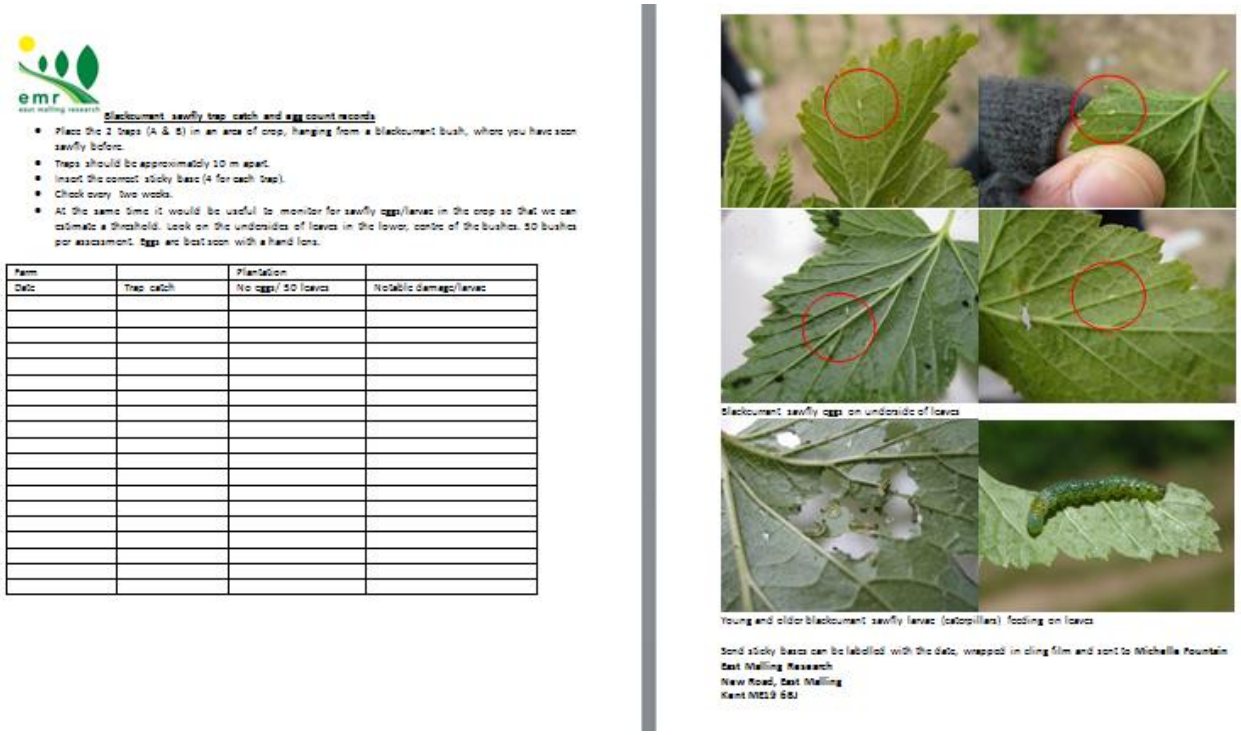
2014 Objectives

1. To test the blackcurrant sawfly trap in growers holdings
2. To optimise the lure and trap

Chemicals and Lures: Chemicals were synthesised at NRI and were > 98% pure. Dispensers were polyethylene vials (20 mm x 8 mm x 1.5 mm thick; Just Plastics) or white rubber septa (20 mm x 10 mm; International Pheromone Systems Ltd.)

Grower trials: Red delta traps with or without a clear plastic window in the top (Fig. 3.1.1) were baited with isopropyl (Z)-7-tetradecenoate only or isopropyl (Z)-7-tetradecenoate isopropyl + (Z)-5-tetradecenoate + isopropyl (*Z*)-7-hexadecenoate in 1:0.1:0.5 ratio. Two traps and lures were sent to growers on 26 March, with a record sheet (Fig. 3.1.2). Growers monitored the traps weekly and sent records to EMR when requested (Table 3.1.1). To test whether a window was needed in the trap half the growers received red delta traps with windows and half without windows. A second set of traps and lures were sent to growers on 3 June (Codes 2014-068, A-E). Traps were hung from a branch of the blackcurrant bushes in a place where the entrance of the traps is not obscured and the spray machinery would not damage the trap. Traps were at least 10 m apart along a row of bushes. Traps were provided to the growers with lures in place, but the grower needed to insert the white sticky bases provided.

Figure 3.1.1. Delta trap with window trialled with synthetic blackcurrant sawfly sex pheromone



emr

Blackcurrant sawfly trap catch and egg count records

- Place the 2 traps (A & B) in an area of crop, hanging from a blackcurrant bush, where you have seen sawfly before.
- Traps should be approximately 10 m apart.
- Insert the correct sticky base (4 for each trap).
- Check every two weeks.
- At the same time it would be useful to monitor for sawfly eggs/larvae in the crop so that we can estimate a threshold. Look on the undersides of leaves in the lower, centre of the bushes. 50 bushes per assessment. Eggs are best seen with a hand lens.

Farm		Plantation	
Date	Trap catch	No eggs/ 50 leaves	Notable damage/larvae

Blackcurrant sawfly eggs on undersides of leaves

Young and older blackcurrant sawfly larvae (caterpillars) feeding on leaves

Send sticky bases can be labelled with the date, wrapped in cling film and sent to Michelle Fountain
East Malling Research
New Road, East Malling
Kent ME19 6BJ

Figure 3.1.2. Record sheet and identifiaction guide sent to growers

Table 3.1.1. List of growers demonstrating use of monitoring traps with or without windows

Growers participating	Trap type
Tom Maynard, Windmill Hill, Ticehurst, East Sussex, TN5 7HQ	Window
Richard Corbett, Oxhouse Farm, Shobdon, Leominster, Hereford, HR6 9LT	Window
Andrew Husband, Adamston Farms Ltd. East Adamston Farm, Muirhead, Dundee, Angus, DD2 5QX	Window
Sandra Gordon, Carolyn Mitchell, The James Hutton Institute, Invergowrie, Dundee, DD2 5DA	Window
Andrew Youngman, J Youngman & Sons, Red House, Chasfield, Woodbridge, Suffolk, IP13 7QE	No window
Hugh Boucher, Robert Boucher & Son, Newlands farm, Teynham, Sittingbourne, Kent, ME9 9JQ	No window
Ian Ovary, Mile Oak Farm, Paddock Wood, Tonbridge, Kent, TN12 6NG	No window
Stephen Holmes, Wellbrook Fruit Wellbrook Fruit Farm, Boughton, Faversham, Kent, ME13 9NA	No window

In addition, traps and baits were also sent to D.C Williamson, Barn Farm, Wix Road, Bradfield, Manningtree, Essex, CO11 2UX (kind permission of Gail Marshall) and placed in a gooseberry crop and other traps were placed in Wiseman apple orchard at East Malling Research to examine for attraction to apple sawfly.

Lure and Trap optimisation: Two trials were carried out to test blends of the potential pheromone components for attracting male sawfly using randomised replicated block trials on growers holdings where there was known to be sawfly infestations in the past (Table 3.1.2). There were 4 replicates of each treatment in Scotland, 6 replicates in Norfolk and 5 replicates in E Sussex. Catches were counted and discarded weekly. Data was analysed using ANOVA with data transformations as appropriate and differences between means were tested for significance by a Least Significant Difference (LSD) test. Treatments with zero catches were omitted from the analyses.

Table 3.1.2. Farms used for testing pheromone blend and trap combinations

Traps deployed	Test	Contact
28 Mar – 12 May	1	Geoff Bruce, Bruce Farms, Balmyle, Meigle, Perthshire PH12 8QU
28 Mar – 29 Jul	1	Chris Allhewson, Bradenham Hall Fruit Farm, West Bradenham, Thetford, Norfolk IP25 7QR
3 Jun – 24 Jul 3 Jul – 18 Aug	1 2	Tom Maynard Windmill Hill, Ticehurst, East Sussex TN5 7HQ. Plantation at Horsmonden

In Test 1, catches of male sawfly with *iso*propyl (Z)-7-tetradecenoate (Z7-14iPr) alone were compared with those with the three-component blend of Z7-14iPr with *iso*propyl (*Z*)-5-tetradecenoate (Z5-14iPr) and *iso*propyl (*Z*)-7-hexadecenoate (Z7-16:iPr) in 1:0.1:0.5 ratio and with the three possible 2-component blends. Blends were dispensed from polyethylene vials as previously, and were tested in standard red delta traps and red delta traps with a transparent window in the roof (Table 3.1.3).

Table 3.1.3 Test 1 treatments: blends of (Z)-7-tetradecenoate (Z7-14iPr) with *iso*propyl (*Z*)-5 tetradecenoate (Z5-14iPr) and *iso*propyl (*Z*)-7-hexadecenoate (Z7-16:iPr) dispensed from polyethylene vials

Treatment	Loading (µg)			Trap type
	Z7-14iPr	Z5-14:iPr	Z7-16iPr	
A (none)	1000			Red delta
B (BLK)	1000	100	500	Red delta
C (RED)	1000		500	Red delta
D (BLUE)		100	500	Red delta
E (GRN)	1000	100		Red delta
F (YELL)	unbaited			Red delta
G (none)	1000			Window red delta
H (BLK)	1000	100	500	Window red delta
I (RED)	1000		500	Window red delta
J (BLUE)		100	500	Window red delta
K (GRN)	1000	100		Window red delta
L (YELL)	unbaited			Window red delta

In Test 2, the most attractive blend from Test 1 was compared with and without the addition of (Z)-9-tricosene (Z9-23H), the compound identified originally in both volatiles and hexane washes. These were tested dispensed from polyethylene vials and white rubber septa (Table 3.1.4) in standard red delta traps.

Table 3.1.4. Test 2 treatments: blends of (Z)-7-tetradecenoate (Z7-14iPr), isopropyl (Z)-7-hexadecenoate (Z7-16:iPr) and (Z)-9-tricosene dispensed from polyethylene vials or white rubber septa

Treatment	Loading (µg)			Dispenser
	Z7-14iPr	Z7-16iPr	Z9-23H	
A	1000	500		Vial
B	1000	500		Septum
C	1000	500	5000	Vial
D	1000	500	5000	Septum
E				unbaited

Results

Grower trials: Trap catch results were received from Sandra Gordon (JHI), Tom Maynard (Four Wents), Andrew Youngman (Suffolk) and Ian Ovary (Mile Oak Farm) by the end of July. No sawfly were captured in the Z7-14iPr only baited traps and no sawfly were trapped at Youngman's or Ian Ovary's farms. In total, three male and three female blackcurrant sawfly were captured in traps containing a lure with the three-way combination (Z7-14iPr + Z5-14iPr + Z7-16iPr) at JHI and Four Wents.

Interestingly, a small tortrix moth was attracted to Z7-14iPr on one occasion (around 4 April) at Four Wents. Identification was confirmed as *Cydia ulicetana*) as small moth associated with heathland (http://ukmoths.org.uk/show.php?id=2202).

No sawfly were captured in traps placed in the gooseberry plantation in Essex.

Lure and Trap optimisation: Test 1

In the apple orchard at East Malling Research 3 male apple sawfly were captured in the Z7-14iPr only baited traps and 2 males in the 3 way combination traps (Z7-14iPr+Z5-14iPr+Z7-16iPr) by 29 April.

There were no catches of sawfly at the Scotland site in 2014.

At the Norfolk site no male sawfly were captured in the unbaited traps. One male was captured in each of Z5-14iPr+Z7-16iPr, Z7-14iPr+Z5-14iPr, Z7-14iPr+Z5-14iPr+Z7-16iPr and 3 males in Z7-14iPr only baited traps. The majority were captured in the 2 way blend of Z7-14iPr+Z7-16iPr (total 6 males).

Trap catches at the Kent site were more encouraging. The two-component blend of Z7-14iPr+Z7-16iPr captured more male blackcurrant sawfly than any of the other tested baits and the untreated control. In the standard red delta traps, catches with this blend were much higher than those with the other treatments in all five weeks of the trial (Fig. 3.1.3) and this difference was significant in the overall catches (Fig. 3.1.4; F 15.98; df 5,20; $P < 0.001$). The three-component blend caught significantly more than the major component alone, Z7-14iPr. In the red delta traps with window, catches were highest with the two-component blend of Z7-14iPr+Z7-16iPr in four out of five weeks (Fig. 3.1.3). The high variability among replicates meant that overall catches with this blend were not significantly higher than catches with the three-component blend and actually not significantly higher than catches in unbaited traps (Fig. 3.1.4; F 2.69; df 5,20; P 0.05).

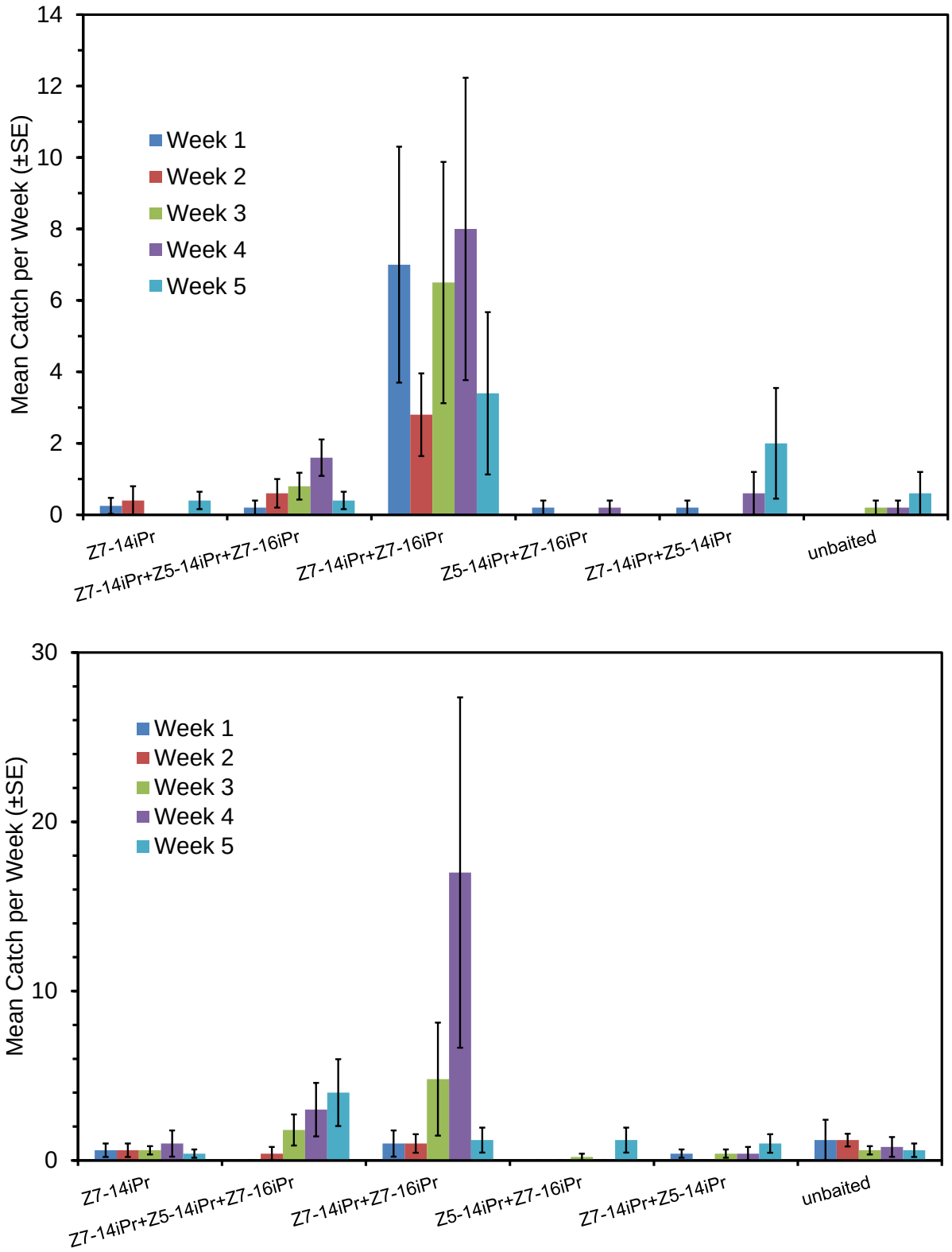


Figure 3.1.3. Mean weekly catches of male blackcurrant sawfly at the Kent site with blends of Z7-14iPr, Z5-14iPr and Z7-16iPr dispensed from polyethylene vials in standard red delta traps (upper) or red delta traps with window (lower) (3 June – 24 July 2014; *N* = 5)

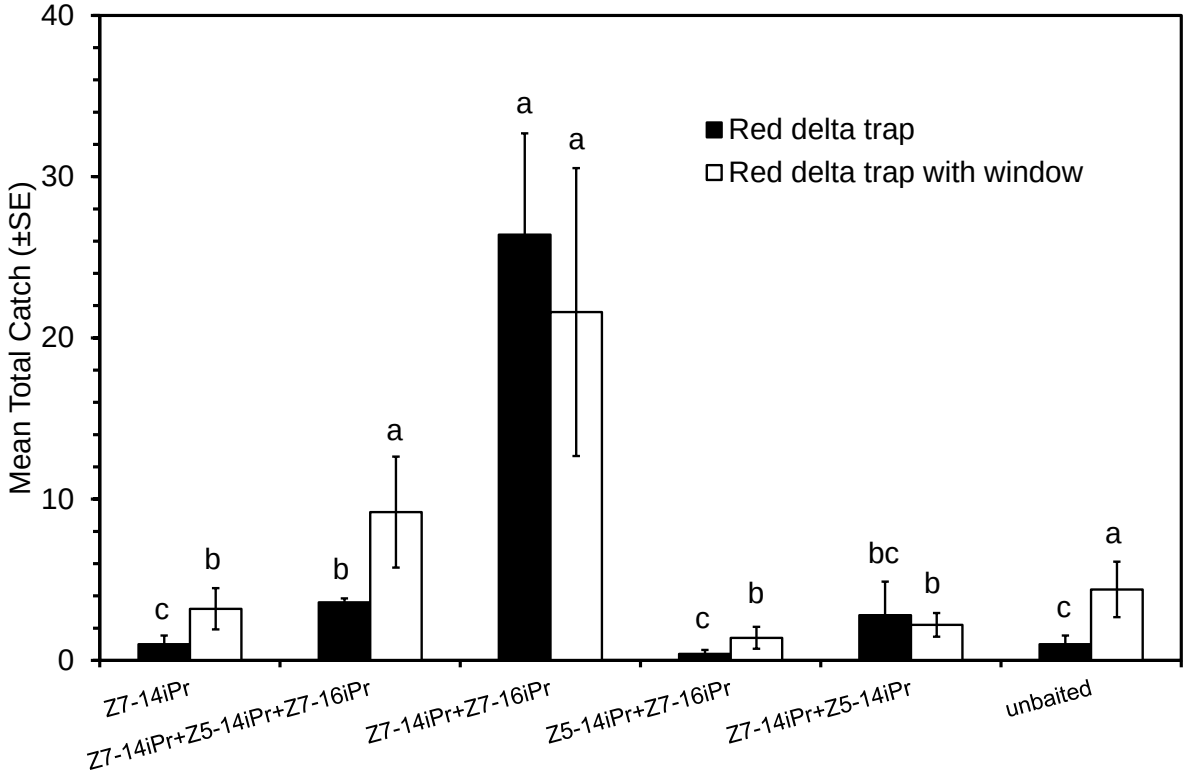


Figure 3.1.4. Total catches of male blackcurrant sawfly at Kent site with blends of Z7-14iPr, Z5-14iPr and Z7-16iPr dispensed from polyethylene vials in standard red delta traps or red delta traps with window (3 June – 24 July 2014; *N* = 5; for each trap type means with different letters are significantly different *P* < 0.05 after ANOVA on data transformed to log(x+1) and LSD test)

Lure and Trap optimisation: Test 2

Numbers in the field had begun to decline at the time that this test had begun. Addition of Z9-23H to the two component blend of Z7-14iPr + Z7-16iPr increased catches of male blackcurrant sawfly in both vials and septa, although only the former increase was statistically significant (Fig. 3.1.5; *F* 12.05; *df* 2,8; *P* < 0.004). This experiment ran from 3-24 July 2014. No males were captured in the first week, 17 in the second and virtually all (96) in the third week. Catches with the Z7-14iPr + Z7-16iPr + Z9-23H were highest in each replicate, but the results were distorted by two very high catches of 49 and 44 in two of the replicates during the last week. There was no significant difference between numbers of females captured in the traps with a total of 9 over the test period.

A significant number of grass moths (family Crambidae) were found in traps baited with Z7-14iPr+Z7-16iPr in a vial (Fig. 3.1.5; *F* 12.21; *df* 4,16; *P* < 0.001) but not with the same blend dispensed from a septum.

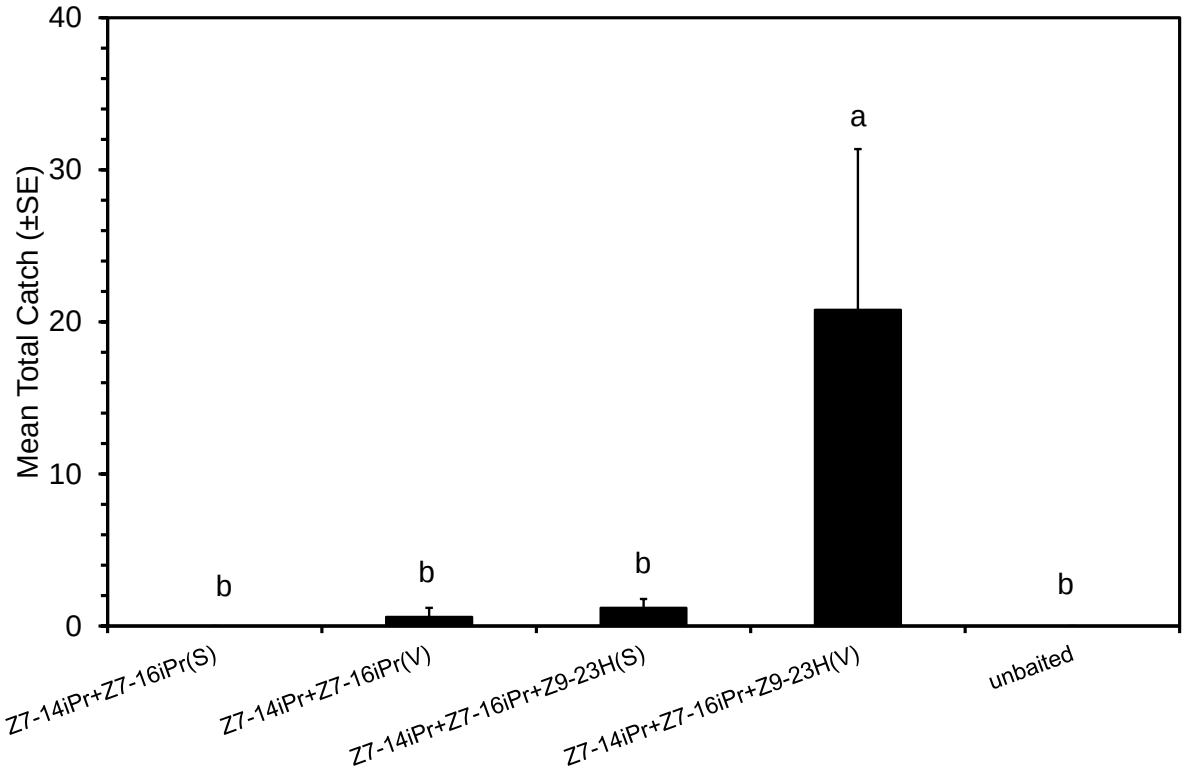


Figure 3.1.5. Total catches of male blackcurrant sawfly in Trial 2 at Kent with blends of Z7-14iPr, Z7-16iPr and Z9-23H dispensed from rubber septa (S) or polyethylene vials (V) in red delta traps (10-24 July 2014; *N* = 5 ; means with different letters are significantly different *P* < 0.05 after ANOVA on data transformed to log(x+1) and LSD test)

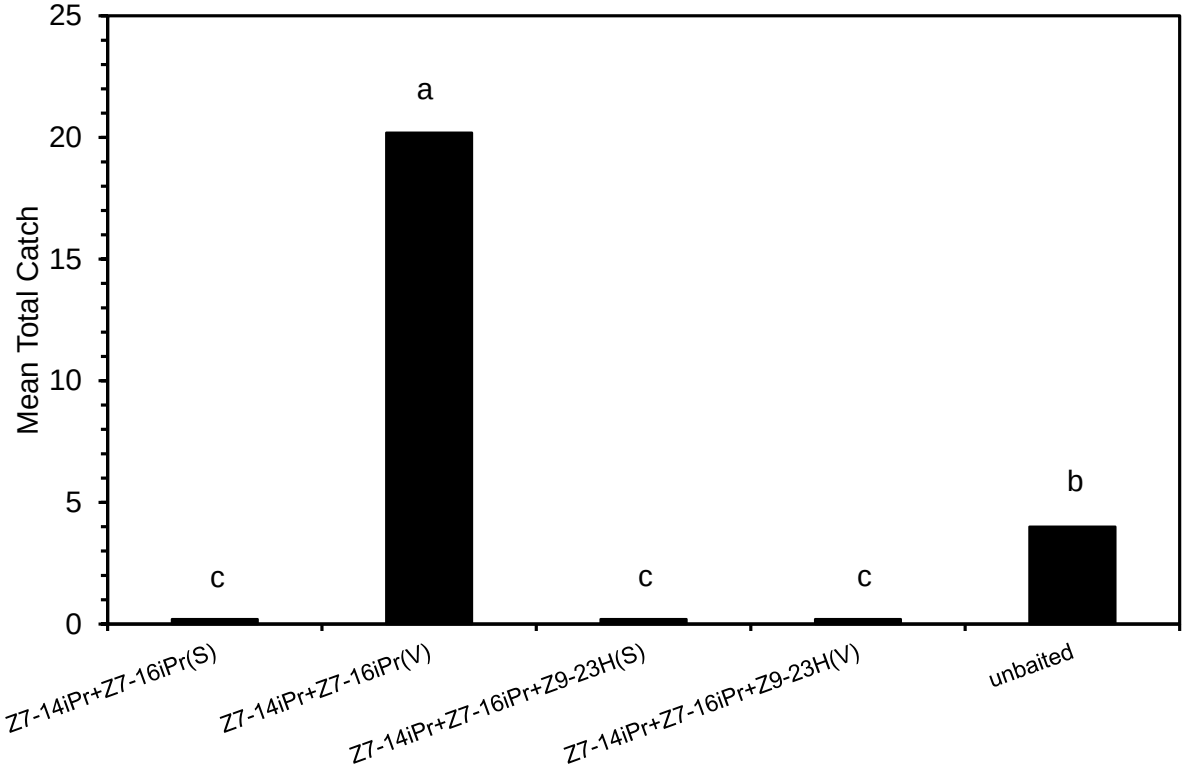


Figure 3.1.6. Total catches of grass moths in Trial 2 at Kent with blends of Z7-14iPr, Z7-16iPr and Z9-23H dispensed from rubber septa (S) or polyethylene vials (V) in red delta traps (3-24 July 2014; *N* = 5; means with different letters are significantly different *P* < 0.05 after ANOVA on data transformed to log(x+1) and LSD test)

Conclusions

- It is not necessary to have a window in the delta traps as previously thought.
- The two-component blend Z7-14iPr+Z7-16iPr is attractive to male blackcurrant sawfly and more attractive than Z7-14iPr alone or the three-component blend of Z7-14iPr + Z5-14iPr + Z7-16iPr tested previously.
- Addition of Z9-23H to the two-component blend increases catches further.
- The blend is more effective if released from a polythene vial compared to a rubber septum.

References

Bartelt, R.J., Jones, R.L., Kulman, H.M. 1982a. Evidence for a multi-component sex pheromone in yellow headed spruce sawfly *Pikonema alaskensis*. *Journal of Chemical Ecology* 8:83-94.

Bartelt, R.J., Jones, R.L., and Kulman, H.M. 1982b. Hydrocarbon components of the yellowheaded spruce sawfly sex pheromone: a series of (Z,Z)-9,19-dienes. *Journal of Chemical Ecology* 8:95-114.

Bartelt, R.J. and Jones, R.L. 1983a. (Z)-10-Nonadecenal: a pheromonally active air oxidation product of (Z,Z)-9,19-dienes in the yellow-headed spruce sawfly. *Journal of Chemical Ecology* 9:1333-1342.

Bartelt, R.J., Jones, R.L., and Krick, T.P. 1983b. (Z)-5-tetradecen-1-ol: a secondary pheromone of the yellowheaded spruce sawfly, and its relationship to (Z)-10-nonadecenal. *Journal of Chemical Ecology* 9:1343-1352.

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

Key components of a biologically based integrated pest management (IPM) programme were identified (see report for objective 4 above) but biological methods (BCAs) for *Botrytis* were insufficient in the initial years and an alternative control needed to be identified. In addition, by year 3 the sawfly pheromone had not been confirmed and there was no selective insecticide for midge control available for testing. Some methods were found to be uneconomical for a processing crop, e.g. leaf midge mating disruptions using sew pheromones and *Botrytis* control using BCAs.

SIX MONTHLY REPORT TO HORTICULTURE LINK PROGRAMME MANAGEMENT COMMITTEE (Due 31 March 2015)

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* James Hutton 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:		Jerry Cross, Rex Brennan, Michelle Fountain, Angela Berrie, David Hall & Xiangming Xu
Project Start/Completion Dates:		1 April 2010 – 31 March 2015
Reporting Period:		1 April 2014- 30 September 2014
Number of Months Since Commencement:		60 month report
Date of Last Management Meeting:		10 December 2014
Date of next management meeting:		17 March 2015 – talk practice 19 March 2015 - consortium
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		**Title**	
Milestone	**Target month**		
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 *Research work has been revised and hence this primary milestone is changed as well [see full description below]*	Y
P1.1.3	31/03/2013	Relationships among physiological/morphological characters and susceptibility to *B. cinerea* established *No correlations*	Y
P1.2	31/03/2014	Efficacy of BCAs applied with fungicides during flowering against *B. cinerea* established	Y
P1.3	31/03/2015	Effects of additional pollinations on yield and quality established	Y
P1.4.1	31/03/2014	Key fungi responsible for blocking filters identified *Fruits were not available so experiments done to look at Botrytis accumulation*	Y
P1.4.2	31/03/2012	Incidence of important fungi in historical samples established *Research work was revised and hence this primary milestone is changed [see full description below]*	Y
P1.4.3	31/03/2015	Methods for reducing internal fungal colonisation evaluated	Y
P2.1.1	31/03/2013	Leaf midge crop damage on different cultivars and ages of plantation determined	Y
P2.1.2	31/03/2013	Leaf midge crop damage in cut down, re-growing crops determined	Y
P2.2	31/03/2013	Relationships between leaf midge sex pheromone trap catches and numbers of galls that develop subsequently determined	Y
P2.3	31/03/2013	Selective insecticides for leaf midge and optimised spray timing in relation to sex pheromone traps catches determined	Y
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 *Cost was prohibitive for control strategies. Data being generated for other midge species.*	Y

P2.4.3	31/03/2014	Formulation for pheromone control of leaf midge developed, if appropriate	Y
P2.4.4	31/03/2015	Field evaluation of pheromone control completed	Y
P3.2	31/03/2012	Sawfly sex pheromone identified	Y
P3.3	31/03/2015	Optimised pheromone trap for blackcurrant sawfly developed and protocol for use in monitoring produced	Y
P4.1.1	31/03/2013	IPDM programme for evaluation in years 4 and 5 devised *Key components of a biologically based integrated pest management (IPM) programme were identified (see report for objective 4 above) but biological methods (BCAs) for Botrytis were insufficient in the initial years and an alternative control needed to be identified. In addition, by year 3 the sawfly pheromone had not been confirmed and there was no selective insecticide for midge control available for testing. Some methods were found to be uneconomical for a processing crop, e.g. leaf midge mating disruptions using sew pheromones and Botrytis control using Serenade.*	N
P4.1.2	31/03/2015	IPDM programme in large scale grower trials evaluated in large scale commercial trials and refined *SeeP4.1.1*	N
P4.1.3	31/03/2015	Blackcurrant IPDM best practice guidelines prepared *Publicity from year to year has been good and techniques have gone straight out to industry as the project has progressed. Some of the findings are already being practiced e.g. not spraying for leaf midge in established crops.*	N
5.	**Project changes:**	(proposed or agreed with the LINK programme, and including any changes to expected profile of grant claims)	
		See note in P4.1.1 above	
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: oral presentation, 20-23 September 2010. IOBC Working Group "Integrated Plant Protection in Fruit Crops", 7th Meeting in Budapest, Hungary. Poster: The effect of pollinating insects on blackcurrant fruit set, yield and quality

M Fountain: oral presentation, 4 March 2011. “To bee or not to bee”, Ashford Beekeepers

M Fountain: oral presentation, 13 Jan-2011, Institute of Physics “To bee or not to bee - Issues facing pollinating insects; the work of EMR on blackcurrant pollination; a year of bee keeping – a beginner’s point of view”

J Cross: oral presentation, overview of progress in the project at the International Blackcurrant Conference in Dundee,16 May 2012

The project consortium met for a site visit to view and discuss ongoing work on pollination on 17 April at a commercial farm in Sussex.

R Saville: “Blackcurrants: Control of *Botrytis* cinerea using biocontrol and fungicides”. Presentation to AAB

A Berrie: oral presentation: Control of *B cinerea* on blackcurrants using biocontrol and fungicides. Presentation to IOBC Biocontrol meeting, Reims, France, 24-27 June 2012

A Berrie: oral presentation: Integrated control of *B cinerea* on blackcurrants. Presentation to IOBC Soft Fruit Meeting, Vigalzano, Italy, 26-28 May 2014

M Fountain, S Roberts: Poster presentation to Kent University academic visitors. Bees and fruit pollination

M Fountain: International Blackcurrant Conference in Dundee on 16 May 2012. Led discussion session on – “Do blackcurrants need bees?”

J Cross gave a 40 minute presentation to the UK blackcurrant growers conference at Thatchers, Somerset on 13 March 2013

M Fountain: oral presentation at Royal Entomological Society conference (EMR) ‘Pollinating insects in UK perennial crops’ 13 November 2013

M Fountain: poster presentation (September 2013) EMR open day ‘Who is pollinating fruit crops?’

M Fountain: oral presentation, AAB Belgium 01 April 2014 Pollinating insects in UK perennial crops

M Fountain: oral presentation, 24 April 2014, HDC Tree Fruit day Pollinating insects in UK perennial crops

M Fountain: oral presentation, 8 May 2014, Pests, Predators and Pollinators, Warwick Crop Centre

M Fountain: oral presentation, Worshipful Company of Fruiterers, 04 June 2014 “Pollinating insects in UK perennial crops”

M Fountain: oral presentation, 25 September 2014, “Pests, Predators and Pollinators”, Ornamental Nursery Group, EMR

M Fountain: oral presentation, 5 February 2015, Northern Ireland Apple Growers Association – Pollination, Pest Control and Blastobasis in Orchards

M Fountain: oral presentation, 11 February 2015, Cider Growers Association – Pollination and Pest Control in Orchards

Publications

Berrie AM., Lower K & Passey T (2013). Control of *Botrytis cinerea* in blackcurrants using biocontrol as part of an integrated programme with conventional fungicides. IOBC-WPRS Bulletin Vol. 86, 9-14

Berrie AM, Lower K, Passey T & Saville R (2013). Integrated control of *Botrytis cinerea* in blackcurrants IOBC-WPRS Bulletin Vol. 109, 9-15

Hall DR, Amarawardana L, Hilbur Y, Boddum T & Cross, JV (2012). The chemical ecology of plant-feeding midges. *Journal of Chemical Ecology,* 38:2-22.

S Raffle article in HDC Soft Fruit magazine p. 21

M Fountain EMR Newsletter ‘Pollinating insects in UK perennial crops’ December 2013

Article in *HDC News* Annual Soft Fruit - Biocontrol methods in blackcurrants. HL01105 137 – Developing biocontrol methods and their integration in sustainable pest and disease management in blackcurrant production. October 2013

7.	**Exploitation plans:**	(give an update on perceived exploitation opportunities and future plans.)
These are documented in the final LINK completion form		