Project title:	**Developing biocontrol methods and their integration in sustainable pest and disease management in blackcurrant production**
Project number:	HL01105
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Report:	Annual report, year 4
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*

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 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 project and main conclusions

Progress on each objective of the project is summarised below

Objective 1: *Botrytis cinerea*

Effect of pollinating insects on blackcurrant yield and quality
Caged field trials showed that supplementing blackcurrant with *Bombus terrestris dalmatinus* nest boxes at the point of flower opening increased yield and fruit size of berries (Ben Hope and Ben Gairn). This was shown to be particularly important in a period of poor weather when the naturally occurring pollinating insects were less active. Bagging strigs of betties to exclude insects in open field conditions demonstrated that insect pollination contributes up to 35% fruit set of Ben Gairn.
Experiments indicated that bees may vector botrytis but that this was marginal and far outweighed by their contribution to pollination. Field trails with *B. t. audax* were inconclusive as the weather at flowering was good in 2001 and increased sampling of fruit set was needed. Wild bumblebees and solitary bees were important contributors to blackcurrant pollination; honeybees less so.
In 2012 at pre blackcurrant flowering no bees were seen visiting flowers. However, observations on flowering plants were not long enough to be conclusive. All bumblebees seen at this time were queens – mostly searching for nesting sites, although *B. lapidarius* was seem moving between blackcurrant bushes. Solitary bees were mostly seen basking on leaves or flying around bare earth – possibly males waiting for females to emerge. Bumblebee queens were particularly attracted to rough grass verges in headlands. Weather during the flowering period of Ben Garin in 2012 was poor with rainfall throughout. However, provisioning plantations with *Bombus terrestris audax* nest boxes did not improve the fruit in the blackcurrant plantations. High variability of crop management between farms and fields may have made it difficult to tease these effects apart. Bumble bees can forage for up to 2-3 km and so are not restricted to the plantations even though placed in the centres of the crops. However, bumblebees were observed foraging on blackcurrant flowers within a few minutes of opening the nest boxes. It is also likely that botrytis also affected fruit set as withered strigs of fruit were observed at the assessment time.
Although landscape scale surrounding land use varied between plantations provisions for nest sites could be manipulated within the plantation to bolster numbers of solitary and bumblebees (the main foragers of blackcurrant flowers). Many important blackcurrant forager bees were present before and after blackcurrant flowering within the vicinity of the crop. Hence provisioning with nest sites and diverse forage will help to foster better populations for pollination in future years. Most sites in this study were very forage poor, especially pre blackcurrant flowering, and some lacked numbers of bees and nest sites. *Osmia rufa* was a poor blackcurrant flower visitor and, therefore, not an important pollinator of blackcurrant.
In 2013 methods were developed that enabled us to test the nutritional quality of insect pollinated, hand pollinated (optimal pollination) and insect excluded fruits. Results were encouraging with but unclear. Experiments will be designed to further test these effects in 2014.

Objective 2: Blackcurrant leaf midge

Objective 3: Blackcurrant sawfly

A range of isomers of isopropyl tetradecenoate were synthesised and the isopropyl (Z)-7-tetradecenoate shown to have identical GC retention times and mass spectrum to the compound produced by female sawfly. The synthetic compound elicits a very strong EAG response from the males and is proposed to be the major component of the female sex pheromone. Similarly a range of isomers of the 16-carbon homologue has been synthesised and isopropyl (Z)-9-hexadecenoate shown to have identical GC retention times and EAG activity to one of the minor compounds produced by female sawflies. Lures containing these compounds were tested in the field in 2013 and were shown to attract large numbers of male blackcurrant sawfly. In 2014 we will optimise the pheromone blend and demonstrate the best trapping device for monitoring the pest.

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:

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

- Survey bee numbers in plantations and supplement with bumblebees where populations are low or where there has been a history of poor fruit set or fruit storage problems. This may also be necessary in poor weather conditions.
- If provisioning plantations with bumblebee nests, protect from badger attack.
- Sustain and increase bee numbers when plantations are not in flower, by providing alternative food sources.
    - Provide flowers with an open habit (e.g. Umbelliferae, Rosaceae, *Prunus*) to encourage a wider diversity of bees.
    - Blackthorn and Willow can be encouraged in hedgerows as an early source of forage.
    - Compositae/Asteraceae are foraged by solitary bees and can be encouraged in the alleyways. Alleyways can be most before spraying protective insecticides to discourage bees in the crop at that time.

    - Grow natural species, rather than plants that have been subject to horticultural breeding as nectar and pollen are more accessible in the former.
    - Make sure that you have a variety of plants that can provide season long flowers from mid March to late August.
    - Grow good sized patches of favoured plants as these are more attractive.
- Bees need to refuel after emerging from overwintering sites (February to March) and a good food supply (April to September) will help to ensure high populations into the following year.
- Provide undisturbed, south-facing areas of sparsely vegetated ground for solitary bees to nest in.
- Bare ground compacted by vehicles is also good, as long as these areas are in sunshine and not used too frequently.
- Avoid waterlogging soil, and loose, crumbly soil is best for bees to dig.
- Leave untidy areas of rotting wood, preferably areas of woodland, and tussocky grasses for bumblebees to overwinter and nest in (See http://bumblebeeconservation.org/about-bees/habitats/)

- Control of blackcurrant leaf midge in newly planted, establishing or cut down blackcurrant bushes is important, but control in established crops is less important providing there is adequate regenerative growth
- Lambda cyhalothrin (Hallmark) is the most effective currently available insecticide for blackcurrant midge. Sprays should be applied within a few days of threshold catches (>10 midges/trap) for the first and second generation.
- Growers interested in using the newly designed blackcurrant leaf midge trap to time spray applications should contact Jerry Cross (jerry.cross@emr.ac.uk).

- The first three fungicide sprays applied from first flower are the most important treatments for Botrytis control. If an effective fungicide programme is applied at this time then there is no benefit from additional sprays near harvest.

SCIENCE SECTION

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

1.1. To demonstrate sex pheromone attraction and investigate mating behaviour

1.2. To identify sawfly sex pheromone

1.3. 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 (IPDM) 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.

Objective 1: *Botrytis cinerea*

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

Task 1.1.2 JHI germplasm screening

Examination of the relative susceptibility to field infection by botrytis was extended for a further year due to the abnormally high incidence of the fungus in 2012. 20-berry samples were taken from the range of germplasm examined in previous years at full ripeness, and after surface sterilisation the samples were maintained in sterile Petri dishes at ambient temperature (20°C). The samples were examined for 21 days at 3-day intervals for signs of botrytis infection. Any berries showing symptoms were removed from the Petri dish.

Results show that the cultivars Ben Hope, Ben Klibreck, Ben Starav and the NZ cv. Murchison had the lowest levels of botrytis infection throughout the monitoring period, together with the breeding lines JHI 9148-9, JHI 9198-1 and JHI 91192-1. The highest levels of botrytis, and fastest development within the samples, were found in cvs. Ben Gairn, Ben Lomond, Ben Maia and the New Zealand cv. McWhite.

These results (Table 1.1.2) show broadly similar patterns to previous years, in that Ben Klibreck and Ben Hope have consistently been among the samples to show the lowest levels of botrytis, even after prolonged storage, and Ben Gairn has consistently been among the worst. However, there are some differences, e.g. the breeding lines 9198-1 and 9148-1, which were two of the worst samples in 2012.

Table 1.1.2a Botrytis incidence on 20-berry samples of existing cultivars

	3 days	6 days	9 days	12 days	15 days	18 days	21 days	Intact berries after 21 days
Ben Alder	1	10	15	18	18	18	18	2
Ben Avon	4	7	13	16	17	17	17	3
Baldwin	2	3	4	5	16	16	19	1
Ben Dorain	1	7	14	18	18	18	18	2
Ben Gairn	4	11	17	19	19	19	20	
Ben Hope	0	0	3	7	10	10	10	10
Ben Klibreck	1	1	3	3	3	3	3	17
McWhite	20	20	20	20	20	20	20	
Murchison	2	6	9	12	12	12	12	8
Ben Lomond	4	9	15	17	19	20	20	
Ben Starav	4	5	8	8	10	13	13	7
Ben Vane	9	12	18	19	19	19	19	1
Ben Maia	15	20	20	20	20	20	20	

Table 1.1.2b. Botrytis incidence on 20-berry samples of JHI breeding lines

	3 days	6 days	9 days	12 days	15 days	18 days	21 days	Intact berries after 21 days
903-1	2	5	11	20	20	20	20	
9137-2	6	17	20	20	20	20	20	
9148-9	3	5	8	8	10	13	14	6
9154-3	0	5	10	16	18	20	20	
9198-1	0	2	7	7	15	16	17	3
9199-4	2	7	12	20	20	20	20	
91192-1	0	0	0	0	13	15	18	2

There are clear and consistent differences in the development of botrytis between different genotypes. However, the correlation of these differences with traits concerning skin strength and thickness has already been shown in previous years to have proved impossible, although it is entirely possible that the skin traits may change in different environmental conditions. This is an area that should be considered for future evaluation, but at the present time we can state that the genetic differences in botrytis susceptibility are probably unrelated to skin thickness or durability.

Since the floral calyx remains may be a potential point of botrytis entry to developing fruit an examination of them was initiated. Six berries of each cultivar were frozen and embedded in wax, from which 10 µm sections were cut using a Leica RA2265 microtome. Work so far has aimed to develop suitable protocols, and sample sections are shown in Fig. 1. We are now continuing with a comparison of Ben Klibreck and Ben Gairn, to represent the range of variation in botrytis infection found in the storage tests.

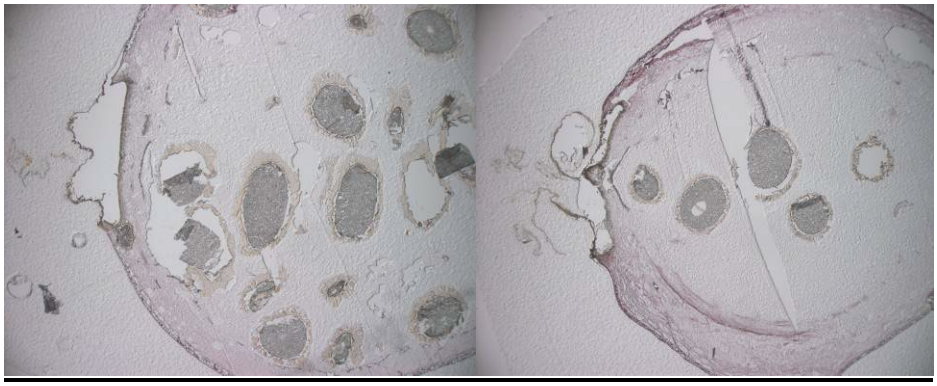

Fig. 1. Sections through ripe fruit of cv. Big Ben (left) and JHI 91192-1 (right)

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

Methods

We conducted the inoculation experiment again as in 2011 and 2012, using 22 cultivars (including advanced breeding lines) for inoculation studies in controlled conditions. Every plant was recorded for flower development. Plants were inoculated in two batches based on their flowering time: 21 and 24 May 2013. Plants of a single genotype were inoculated 4-5 weeks after mid-bloom.

Inoculation was conducted in a misting glasshouse compartment. A hand-held sprayer was used to deliver inoculum (1 x 10^5 conidia per ml) onto fruit clusters. Once inoculated, the plants were subjected to 24 h wet period, maintained by three misting nozzles in the compartment. Then, the plants were moved to the polytunnel. Ripe fruit were harvested, surface-sterilised, incubated for 1-2 weeks, and assessed for latent botrytis as described in Task 1.1.1 (Year 2 report).

Results

The percentage of fruit infection is presented in Figure 2. The level of botrytis infection ranged from 0 to 74%, higher than in 2012 but lower than in 2011. However, the correlation with the incidence in 2011 (< 0.01) and 2012 (-0.17) is very low and not statistically significant. This non-significant relationship can be clearly seen in Figure 1.

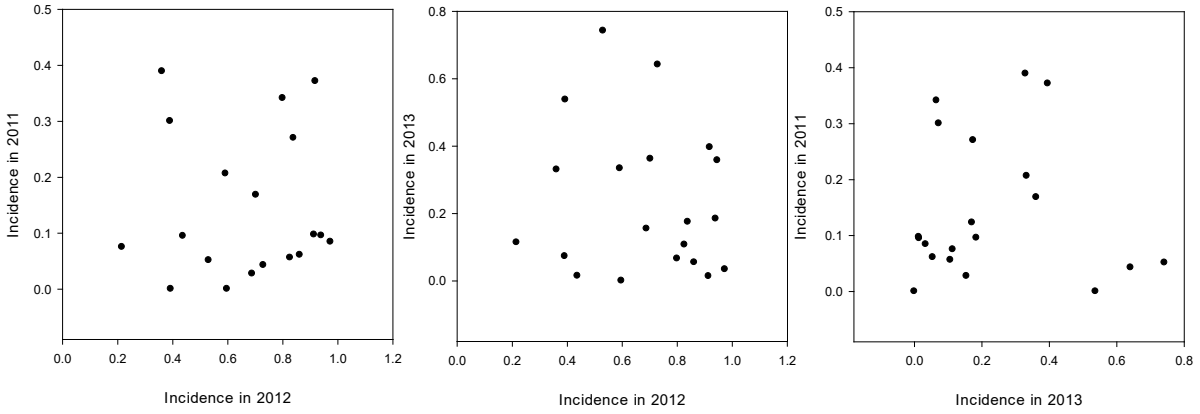

Figure 1.4. Incidence of latent botrytis on inoculated of fruit 4-5 weeks post-bloom on a number of selected genotypes

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)

Conclusions from 2010-13 studies

Caged field trials showed that supplementing blackcurrant with *Bombus terrestris dalmatinus* nest boxes at the point of flower opening increased yield and fruit size of berries (cvs. Ben Hope and Ben Gairn). This was shown to be particularly important in a period of poor weather when the naturally occurring pollinating insects were less active. Bagging strigs of berries to exclude insects in open field conditions demonstrated that insect pollination contributes up to 35% fruit set of Ben Gairn.

Experiments indicated that bees may vector botrytis but that this was marginal and far outweighed by their contribution to pollination. Field trials with *B. t. audax* were inconclusive. The weather at flowering was good and high variability of crop management between farms and fields may have made it difficult to tease out the effects of bees. Bumblebees can forage for up to 2-3 km and so are not restricted to the plantations, even though placed in the centres of the crops. Wild bumblebees and solitary bees were important contributors to blackcurrant pollination and honeybees less so.

At pre blackcurrant flowering no bees were seen visiting wild flowers, although observations on flowering plants were not long enough to be conclusive. All bumblebees seen at this time were queens – mostly searching for nesting sites, although *B. lapidarius* was seen moving between blackcurrant bushes. Solitary bees were mostly basking on leaves or flying around bare earth – possibly males waiting for females to emerge. Bumblebee queens were particularly attracted to rough grass verges in headlands.

Although the surrounding landscape varied between plantations provisions for nest sites could be manipulated within the plantation to bolster numbers of solitary and bumblebees. Many bees are present before and after blackcurrant flowering within the vicinity of the crop. Hence provisioning with nest sites and diverse forage will help to foster better bee populations for pollination in future years.

Objective

To determine if blackcurrant flowers pollinated by insects produce a higher nutritional quality fruit than self-pollinated flowers

Materials and Methods
Two experiments were done:

Hand pollinated
Potted (loam soil), drip irrigated, blackcurrant plants (cv. Ben Starev), courtesy of Harriet Roberts (ADAS) were caged in 0.5 x 0.5 x 1.0 m Bugdorm cages inside polytunnel 17 at EMR and either hand pollinated or left un-pollinated. (NB: initially an attempt was made to pollinate the plants using bumblebees but the bees did not forage inside the cages). Numbers of blackcurrant bushes in the cages differed, but the number of upright branches was consistent (n=7). Each plant was labelled with the treatment (hand /no bees), cage number and plant number (Fig. 1.3.1). By 15 April grapes were visible on the plants (crop stage E1). All hand pollinated plants were pollinated by transferring pollen with a small artists' paintbrush from flower to flower on 22, 25 and 29 April and 7 May. On 13 May, once fruits had set, the plants were moved outside and provided with drip irrigation.

Open pollinated
An additional experiment was set up to look at the effects of open vs. insect excluded fruit nutrient effects (Fig. 1.3.2). On 25 April a plot of cv. Ben Hope at EMR was used and branches labelled (red tape = bees, yellow tape = no bees); bushes were at stage D - grape just visible. Exclusion bags were placed on 20 replicates and there were 20 replicate 'open pollinated' branches. Exclusion bags were removed on 31 May.

Assessments
Once fruits were ripened estimates of fruit set per strig and other physiological and nutritional measurements were made (Table 1).

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, Millennium^{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^{o}C. Standards of known amounts of malic, citric and ascorbic acids were injected into the HPLC, Millennium^{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 $A = [(A_{52\,0} - A_{700})_{pH\ 1.0} - (A_{520} - A_{700})_{pH\ 4.5}]$.

Results

The results of the hand pollinated experiment give two extremes: unvisited was not visited by insects and the branches were not shaken by wind; the hand pollinated had pollen transferred directly from flower to flower. The field experiment bushes would have had wind pollinated flowers whether the bees were excluded or not. Studies in raspberry found that native bees in the crop were inadequate to increase fruit set and that the addition of bumblebees significantly increased crop yield (Lye GC, Jennings SN, Osborne JL, Goulson D. 2011 Impacts of the use of nonnative commercial bumble bees for pollinator supplementation in raspberry. J Econ Entomol. Feb;104(1):107-14).

In addition the fruit analyses were done by macerating the blackcurrants. In the processing of currants for juice the berries would be digested with pectin and then pressed and the skin and flesh of the fruit would be included in the end product, but not the seeds.

When analysed as nutrients per weight of fruit the values for many of the nutritional measurements was lower in the hand pollinated compared to the un-pollinated (not insect or wind pollinated) fruit (Table 1.3.1). This may have been because the size of the fruits in the latter were significantly smaller (Fig. 1.3.1) and hence contained less water thus concentrating the sugars and other nutritional values. No differences were seen in the open pollinated (insect visited) vs. insect excluded fruits in the field (Table 1.3.1).

When data was converted to nutrients per ha of crop there was significantly more nutritional value in hand pollinated fruit for all of the measurements (Table 1.3.2). For the open field pollinated fruits the anthocyanin and antioxidant levels were higher than if insects were not excluded from flowers (Table 1.3.2).

Figure 1.3.1. Cages in polytunnel for hand pollinated experiment and results of fruit set.

Figure 1.3.2. Insect exclusion bags on blackcurrant bushes.

Results

Table 1.3.1. Nutritional and fruit set analyses of blackcurrants either hand or open pollinated in two separate experiments. Nutritional values are given in mg/g except antioxidants mM/Kg

	Ben Starev					Ben Hope				
	Unvisited	Hand pollinated	P value	sed	lsd	Pollinator excluded	Open pollinated	F pr.	sed	lsd
Fructose	30.2	25.9	0.004	1.396	2.828	41.4	39.3	NSD		
Glucose	21.0	17.2	<.001	1.024	2.076	25.3	23.9	NSD		
Sucrose	3.8	3.6	NSD			13.6	13.1	NSD		
Total sugar	55.0	46.7	0.005	2.760	5.592	80.4	76.3	NSD		
Anthocyanin	350.0	284.0	0.023	28.100	57.000	2054.9	2205.2	NSD		
Antioxidant	59.7	58.5	NSD			42.8	44.9	NSD		
Malic	3.7	3.5	NSD			3.7	3.7	NSD		
Ascorbic	1.8	2.0	<.001	0.047	0.096	2.0	1.9	NSD		
Citric	24.5	23.6	0.011	0.359	0.727	27.7	30.0	NSD		
Total acid	30.0	29.1	0.040	0.432	0.875	33.3	35.6	NSD		
Mean Fruit set	48.7	72.9	<.001	4.745	9.614	46.8	55.2	NSD		
Green fruit No.	27.0	29.3	NSD			3.5	2.3	NSD		
Green fruit Wt. (g)	6.9	7.3	NSD			0.4	0.3	NSD		
Mid ripe fruit No.	17.0	48.5	<.001	5.453	11.050	2.0	3.1	NSD		
Mid ripe fruit Wt. (g)	5.4	21.4	<.001	3.307	6.701	24.0	26.3	NSD		
Ripe fruit No.	45.2	81.0	<.001	5.989	12.140	0.3	0.6	NSD		
Ripe fruit Wt. (g)	23.1	51.7	<.001	4.392	8.898	38.7	42.4	NSD		
Wt of single fruit (g)	0.5	0.6	<.001	0.037	0.075	0.6	0.6	NSD		

Table 1.3.2. Nutritional analyses of blackcurrants either hand or open pollinated in two separate experiments expressed at kg/ha. Values calculated from mean annual mean average for variety Ben Starev of 11 t/ha and Ben Hope of 6 t/ha.

	Ben Starev					Ben Hope				
	Unvisited (19 reps)	Hand Pollinated (20 res)	P value	sed	lsd	Pollinator Excluded (10 reps)	Open Pollinated (9 reps)	F pr.	sed	lsd
Fructose	184.6	285.3	<.001	11.610	23.520	231.1	235.8	NSD		
Glucose	128.6	189.5	<.001	8.611	17.450	141.0	143.7	NSD		
Sucrose	23.3	39.1	<.001	3.706	7.509	76.0	78.4	NSD		
Total sugar	336.4	513.9	<.001	22.810	46.230	448.2	457.9	NSD		
Anthocyanin	2.2	3.1	<.001	0.241	0.488	11.5	13.2	0.049	0.837	1.766
Antioxidant	365.4	643.0	<.001	26.430	53.560	238.6	269.3	0.016	11.460	24.180
Malic	22.6	38.9	<.001	0.977	1.979	20.5	22.0	NSD		
Ascorbic	11.2	22.4	<.001	0.383	0.776	11.1	11.6	NSD		
Citric	150.1	259.1	<.001	3.071	6.222	154.4	179.9	NSD		
Total acid	183.9	320.4	<.001	3.580	7.253	185.9	213.6	NSD		
sugar:acid ratio	1.8	1.6	0.038	0.106	0.215	2.5	2.2	NSD		

Task 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

Methods

As in 2012 and 2011, we have conducted artificial inoculation studies to study the possibility of fungal accumulation within latently infected fruit. Ben Hope plants were inoculated in a glasshouse compartment 4 weeks after mid blossom. Thereafter, at least 50 fruit were sampled weekly from these inoculated plants until harvest. In total, we have collated six samples. These samples are being stored in -80°C. Currently we are testing the real time PCR protocols to quantify fungal biomass. The protocols worked well when pure fungal culture was used. Now we are assessing the efficacy of several methods for extracting fungal DNA from blackcurrant fruit. Botrytis DNA in these latently infected fruit was quantified using the established the real-time quantitative PCR method.

Results

We have detected the presence of botrytis DNA in the sampled fruit which were inoculated 4 weeks after bloom. However, the amount of fungal DNA did not significantly increase over the sampling period, from inoculation to harvest, during which a total of six weekly samples was taken. Thus it is unlikely that botrytis mycelium could increase appreciably inside the latently infected fruit sufficient to block the filter. The inoculated samples of fruit in 2012 showed the similar results – the amount of fungal mycelium was very low. However, this low quantity of mycelia could be due to the difficulty in extracting DNA from fruit, a well-known problem.

Currently we are using 2013 samples to test different DNA extraction protocols (which were recently developed in 2013) to investigate whether these protocols will improve the extraction and subsequent quantification.

Objective 2: Blackcurrant leaf midge

Experimental protocol 2013

To investigate the feasibility of exploiting the blackcurrant leaf midge sex pheromone for control by mating disruption (MD), attract and kill (AK) or mass trapping (MT)

Methods

A large scale replicated field experiment was conducted at East Malling Research. Twelve cages (Image 2.1 a) were constructed, each 12 m x 1.5 m x 2 m (LxWxH) in size. These were separated by 24 m. Each cage contained 10 potted blackcurrants cv. Ben Dorain placed in a zig-zag linear formation. These were cut to 15 cm height and re-potted into compost on 29 May 2013 prior to introduction to encourage shooting. Plants were placed into the cages on 11 June 2013 and were watered by drip irrigation to each pot. No additional fertigation was applied. Dataloggers were placed inside a Delta trap in a central cage. External meteorological data was measured using the East Malling Research weather station for reference.

Image 2.1 a) Field cages and b) Sticky card with pheromone lure attached to the outer edge of a blackcurrant pot

The experiment was set up in randomised block design with three replicates of four treatments. Blocks were assigned from north to south of the plot. Treatments were:

1. Yellow sticky card (8 cm x 10 cm) + lure with 20 µg pheromone racemate
2. Yellow sticky card (8 cm x 10 cm) + lure with 5 µg pheromone enantiomer
3. Yellow sticky card (8 cm x 10 cm)
4. Untreated control

The double-sided yellow sticky cards (Image 2.1 b) were attached to the outer edge of each pot with the lure placed into a hole made by a single hole-punch in the centre top of the card. Treatments were deployed as soon as plants were introduced to the cages.

The number of curled shoots per plant was counted on 29 June and 9-11 July 2013. The numbers of leaf midges on the inside and outside of the sticky traps were counted for pot, in each cage for each generation of midge. This was done by removing each sticky card into a separate labelled plastic bag and identifying the midges under a binocular microscope in the laboratory. This was done on 2 and 16 July 2013. Data were analysed using ANOVA.

Results

There was no significant effect of either pheromone blends or yellow sticky traps on resultant damage by the blackcurrant leaf midge (Table 2.1). There were low midge numbers caught on the sticky traps (Table 2.2), although this may have been because traps coated with dry glue rather than wet glue had been used.

Table 2.1 The effect of pheromone blends and sticky traps on damage by blackcurrant leaf midge.

	29 June 2013	11 July 2013	11 July 2013
Treatment	**Mean curled leaves per plant**	**Mean curled leaves per plant**	**Mean no. shoots damaged per plant**
Sticky trap + racemate	2.0	6.2	3.7
Sticky trap + enantiomer	3.9	8.2	5.2
Sticky trap	3.5	6.8	4.0
Control	2.4	6.4	4.0

Table 2.2 Mean number of blackcurrant leaf midge caught on sticky traps per cage

Treatment	2 July 2013	16 July 2013
Sticky trap + racemate	4.6	0
Sticky trap + enantiomer	2.3	3
Sticky trap	2.3	2

This experiment should be repeated in 2014 with an improved sticky trap.

Objective 3: Blackcurrant sawfly

1.1. To demonstrate sex pheromone attraction and investigate mating behaviour
1.2. To identify sawfly sex pheromone
1.3. To demonstrate pheromone activity, develop a lure and trapping system, and calibrate in the field

Background
We aimed to develop a monitoring trap for blackcurrant sawfly based on the female sex pheromone, possibly coupled with a visual attractive component. In the first two years of the project we demonstrated, using virgin females as bait, that red delta traps with a top transparent window were effective for catching male sawfly.

In addition, volatiles and hexane body washes were collected from virgin female and male sawfly. In GC-EAG analyses of collections on polar and non-polar GC columns, at least two EAG responses from the antennae of male sawfly were recorded. No responses were recorded to volatiles collected from male sawflies. A small peak at the retention times of the strongest response in GC-MS analyses on both polar and non-polar columns had a mass spectrum indicating an *iso*-propyl tetradecenoate isomer. Synthetic *iso*-propyl (*Z*)-9-tetradecenoate elicited a small response from the antennae of male sawflies in GC-EAG analyses. Comparison of retention time shifts of tetradecenyl acetate isomers with double bonds in different positions indicated that iso-propyl (*Z*)-5-tetradecenoate was the most likely candidate for the candidate pheromone component. This would be a novel compound, although *iso*-propyl (*Z*)-9-tetradecenoate is a pheromone component of *Dermestes* spp. (Coleoptera: Dermestiidae).

During 2012 very clear EAG responses were obtained from the antennae of male sawfly to components of volatiles collected from female sawfly during GC-EAG analyses using continuous delivery of the GC effluent to the insect preparation. This resulted in more accurate determination of the retention times of the active components than was possible from analyses carried out during 2011 using intermittent "puffing" delivery of the GC effluent. Results obtained during 2012 were consistent with those obtained previously, although amounts of material in collections of volatiles were even lower than those obtained previously.

Analyses of collections by GC-MS confirmed the presence of isopropyl esters at the retention times of the EAG-active components. A range of isomers was synthesised and retention times of these compared with those of the EAG-active components on both polar and non-polar GC columns. Isopropyl (*Z*)-5- and (*Z*)-7-tetradecenoates and isopropyl (Z)-7-hexadecenoate had identical retention times to three of the active components on both columns and these compounds elicited very strong EAG responses from the male sawfly antenna.

Thus isopropyl (*Z*)-5- and (*Z*)-7-tetradecenoates and isopropyl (Z)-7-hexadecenoate were proposed to be three of the components of the female sex pheromone. The first EAG response observed in analyses on the polar GC column would correspond to the retention time of the saturated analogue, isopropyl tetradecanoate, and the synthetic compound was shown to elicit a strong EAG response in analyses on the polar column but not on the non-polar column. This difference may be because this compound is eluted first on the polar column but last on the non-polar. Analyses of volatiles from females on the non-polar column indicated the presence of a second isopropyl hexadecenoate, possibly the (Z)-5-isomer. Analyses on the polar GC column showed a response at the retention time expected for a isopropyl heptadecenoate ester, but this was not observed in analyses on the non-polar column.

Unfortunately, in field trials carried out during 2012 no male sawfly adults were attracted to traps baited with either virgin females or synthetic lures, even in a greenhouse when males were released. Lures contained isopropyl (Z)-7-tetradecenoate only or a blend of isopropyl (Z)-7-tetradecenoate + isopropyl (Z)-5-tetradecenoate + isopropyl (Z)-7-hexadecenoate in a ratio of 1:0.1:0.5.

These isopropyl esters are unusual pheromone components, although isopropyl (Z)-9-tetradecenoate is a component of the pheromones of dermestid beetles. Pheromone components identified previously in a Tenthredinidae sawfly, *Pikonema alaskensis*, (Bartelt et al., 1982a,b; 1983a,b) and other related sawflies have been saturated and unsaturated aldehydes formed by oxidative cleavage of the abundant cuticular hydrocarbons. Both volatile collections and body washes from the blackcurrant sawfly contain relatively large amounts of (*Z*)-9-tricosene, which could produce nonanal and tetradecanal by oxidative cleavage. However, nonanal and related aldehydes did not produce any EAG response from the male sawfly antenna under conditions when a strong response to isopropyl (*Z*)-7-tetradecenoate was observed. Interestingly, a highly synergistic minor pheromone component of *P. alaskensis* was subsequently identified as (*Z*)-5-tetradecenol which is much more closely related to the compounds described here.

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.

Materials and methods

Growers were contacted to take part in the use of prototype pheromone traps and lures (Table 3.1). Testing was done at seven farms in total. Most of the farms were in the West Midlands, Norfolk or Scotland as blackcurrant sawfly is not prevalent in Kent. At two farms sticky stakes (Fig. 3.4) were used to increase the trap catch, otherwise red windowed delta traps with sticky inserts were deployed (Fig. 3.1).

Figure 3.1. Delta trap with window trialled with synthetic sex pheromone

Table 3.1. Sites used for testing traps

Timing	Contact	Test	Consortium member
x	Tom Maynard, Windmill Hill, Ticehurst, East Sussex TN5 7HQ		Yes
March June	James at Richard Corbett Farms, Oxhouse Farm, Shobdon, Leominister, Hereford HR6 9LT 07974	1	Yes
March June	Geoff Bruce, Bruce Farms, Balmyle, Meigle, Perthshire PH12 8QU	1, 2	No
March	Edward Thompson, Pixley Berries, Pixley Court, Ledbury, HR8 2QA	1	No
x	Andrew Husband, Adamston Farms Ltd, East Adamston Farm, Muirhead, DD2 5QX Dundee		Yes
March June	James Wright, Whittern Farms Ltd / British Cassis, The Whittern, Lyonshall, Kington, Herefordshire HR5 3JA	1, 2	No
March June	Sandra Gordon, Carolyn Mitchell, The James Hutton Institute, Invergowrie, Dundee DD2 5DA Scotland UK	1, 2	Yes
June July	Chris Allhuson, Bradenham Hall Fruit Farm, West Bradenham, Thetford, Norfolk IP25 7QR	2, 3	Yes
June	Nigel Stangroom, Hamrow Fm, Whissonsett, Norfolk NR20 5SX	2	No

Test 1
This test aimed to compare catches in traps baited with the major candidate pheromone component, isopropyl (Z)-7-tetradecenoate (Z7-14:iPr), alone or a blend of the three candidates, isopropyl (Z)-7-tetradecenoate + isopropyl (Z)-5-tetradecenoate + isopropyl (Z)-7-hexadecenoate (Z7-14:iPr + Z5-14:iPr + Z7-16:iPr) dispensed from polyethylene vials. Ten replicates of red delta traps with a clear plastic window in the top, baited with the potential pheromone or no lure, were sent to growers and deployed in blackcurrant plantations. The traps were randomised and numbered so that treatments remained anonymous:

A. Z7-14:iPr (1 mg in polyethylene vial)
B. Z7-14:iPr + Z5-14:iPr + Z7-16:iPr (1 mg : 0.1 mg : 0.5 mg in polyethylene vial)
C. Un-baited

Traps were hung from a branch of the blackcurrant bushes in a place where the trap entrance was not obscured and the spray machinery would not damage the trap. Traps were >10 m apart along a row of bushes. Traps were provided to the growers with lures in place, but the grower inserted the white sticky bases. Traps were posted to growers on 25 March and were deployed by 20 April.

Test 2
This test was designed to compare catches in traps baited with the single component or 3-component blend as above dispensed from either polyethylene vials or rubber septa (10 replicates). Red delta traps (three farms) or sticky stake traps (Norfolk) were deployed with the following treatments. Traps were deployed on 17 June.

- 2013/064A vials (1 mg Z7-14:iPr; blue)
- 2013/064A septa (1 mg Z7-14:iPr; red)

- 2013/064B vials (1 mg : 0.1 mg : 0.5 mg Z7-14:iPr + Z5-14:iPr + Z7-16:iPr; yellow)
- 2013/064B septa (1 mg : 0.1 mg : 0.5 mg Z7-14:iPr + Z5-14:iPr + Z7-16:iPr; black)
- Un-baited (green)

Test 3
A third test aimed to compare catches in traps baited with the single major component, the 3-component blend as above and the three possible 2-component blends, all dispensed from polyethylene vials (10 replicates). Red window delta traps were deployed on 5 July.

- White vial (2013/098A; 1 mg Z7-14:iPr)
- Black vial (2013/098B; 1 mg Z7-14iPr + 0.1 mg Z5-14iPr + 0.5 mg Z7-16iPr)
- Red vial (2013/098C; 1 mg Z7-14iPr + 0.5 mg Z7-16iPr)
- Blue vial (2013/098D; 0.1 mg Z5-14iPr + 0.5 mg Z7-16iPr)
- Green vial (2013/098E; 1 mg Z7-14iPr + 0.1 mg Z5-14iPr)
- Green/Yellow stripes No lure

Assessments
Trap sticky bases or sticky stake traps were either collected by EMR staff or wrapped in cling film and posted to East Malling Research. Bases were examined for the presence of blackcurrant sawfly and examined microscopically to determine the sex of captured individuals.

Results

Test 1 (March to June)
Adult sawflies were only captured at one of the five farms involved in the first test. At this farm numbers of male blackcurrant sawfly were significantly higher in the traps baited with lure B (blend of isopropyl (Z)-7-tetradecenoate + isopropyl (Z)-5-tetradecenoate + isopropyl (Z)-7-hexadecenoate in 1:0.1:0.5 ratio), (Fig. 3.2, ANOVA, $\log_{10}(+1)$ transformed data, P<.001, sed 0.1626, lsd 0.3417).

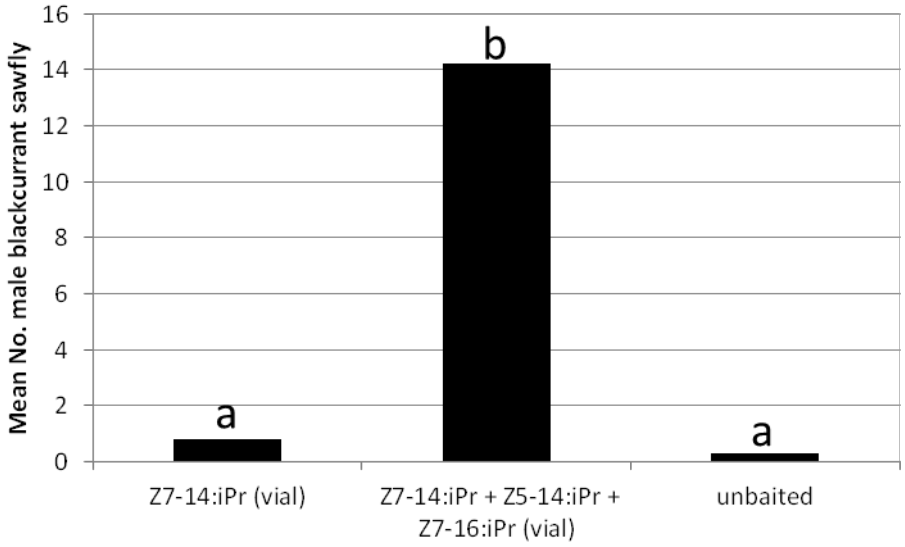

Figure 3.2. Mean numbers of blackcurrant sawfly males captured in Test 1.

Test 2 (June to July)
Numbers of male sawfly at one site were significantly higher in all of the treatments compared to the control, but 20-fold higher in treatment (Z)-7-tetradecenoate using a vial rather than rubber septa (Fig. 3.3, ANOVA, $\log_{10}(+1)$ transformed data, P<.001, sed 0.1982,

lsd 0.4011).

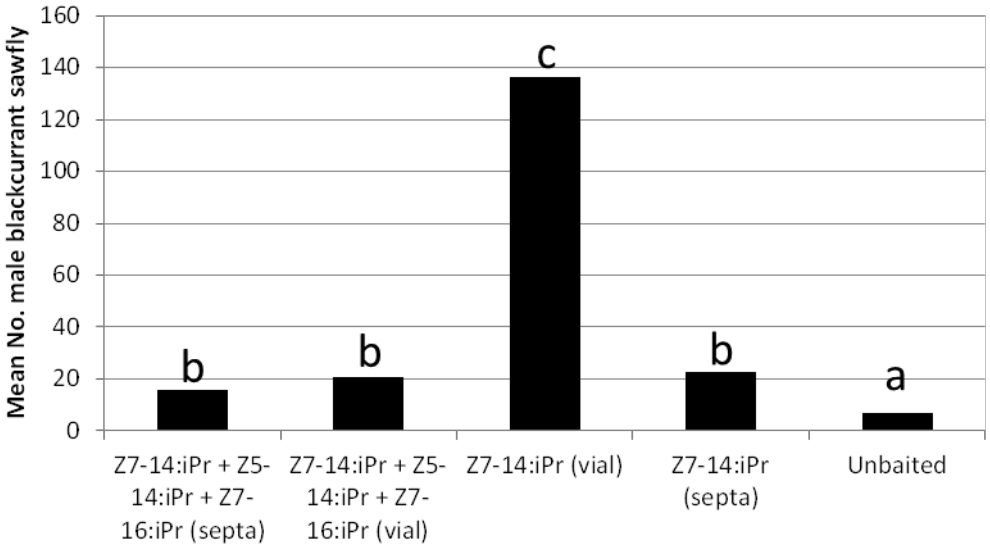

Figure 3.3. Mean numbers of blackcurrant sawfly males captured in Test 2

Figure 3.4. Rubber septa and polythene vial pheromone dispensers attached to sticky stake traps

A smaller trial using only the treatments Z7-14:iPr (vial), Z7-14:iPr + Z5-14:iPr + Z7-16:iPr (vial) and 'unbaited' was done using delta traps posted to 3 growers. Data was combined. There was no significant difference in the numbers of females found in the differently baited traps. Numbers of male sawfly were significantly higher in the Z7-14:iPr vials compared to the untreated control or three way mix vials (Fig. 3.5, 3.6, ANOVA, $\log_{10}(+1)$ transformed data, P=0.008, sed 0.2375, lsd 0.4770). In addition, a male noctuid moth species commonly known as Setaceous Hebrew Character (*Xestia c-nigrum*) was found in significantly higher numbers in the Z7-14:iPr + Z5-14:iPr + Z7-16:iPr (vial) baited traps at one site (Fig. 3.5, ANOVA, $\log_{10}(+1)$ transformed data, P0.045, sed 0.1184, lsd 0.2379).

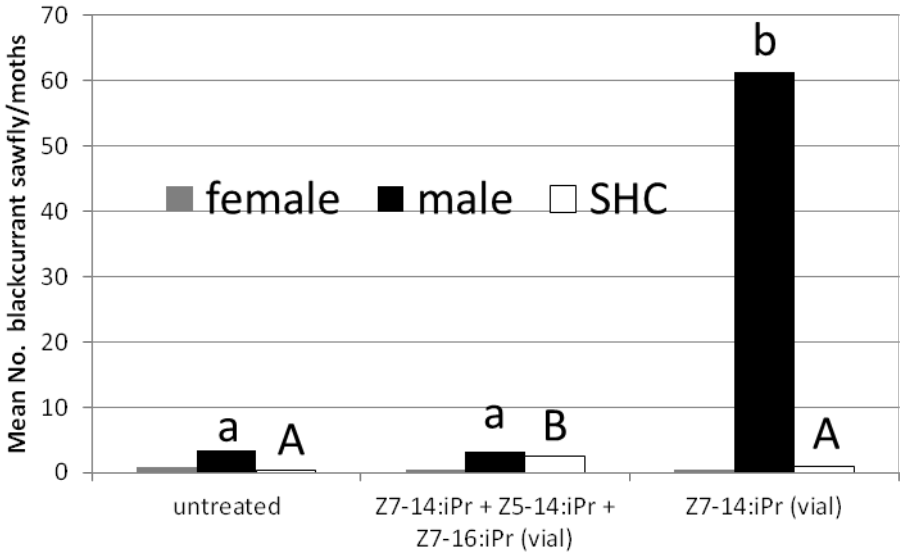

Figure 3.5. Mean numbers of blackcurrant sawfly and setaceous Hebrew character moth (SHC) captured in Test 2

Figure 3.6. Male blackcurrant sawfly on sticky base baited with Z7-14:iPr in a vial

The results from Test 2 indicated that the single component was attractive to male sawfly and the blend was not, whereas results from Test 1 indicated the opposite. The lures from Test 2 were checked by GC analysis of the remaining pheromone and found to be correctly labelled.

Attraction of *Xestia c-nigrum* was noted during tests in 2012. The pheromone of this moth is reported to be a 10:1 blend of (Z)-7-tetradecenyl acetate and (Z)-5-tetradecenyl acetate (Landolt et al., 2011; Steck et al., 1982). No traces of these acetates were found in the Z7-14:iPr and Z5-14:iPr used in these tests, but the latter isopropyl esters are clearly related to the acetate pheromone components.

Test 3 (July to August)
Traps were collected from the final assessment, but the grower had applied an insecticide so numbers of sawfly captured were too low to assess.

Landolt, P.J., Guédot, C., and Zack, R.S. 2011. Spotted cutworm, *Xestia c-nigrum* (L.) (Lepidoptera: Noctuidae) responses to sex pheromone and blacklight. *J. Appl. Entomol*. 135:593-600.

Steck, W., Underhill, E.W., Chisholm, M.D., and Bailey, B.K. 1982d. (Z)-5-tetradecenyl acetate, a trapping synergist for the major sex pheromone of the spotted cutworm, *Amathes c-nigrum*. Entomol. *Exp. Appl. 31*:328-330.

Objective 4: Integrated programme

To develop integrated pest and disease management (IPDM) 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

Due to the lack of economic efficacy of effective biologically based integrated disease management strategies for botrytis and lack of approval for UK385a for midge control, it was decided not to embark on this work but to focus on completing the work in other objectives. The key components of the pest management programme have been identified as follows:

- Gall mite: resistant cvs. and early season sulphur sprays;
- Leaf midge: no treatments in established crops;
- Aphids: autumn treatment with aphicide;
- Capsid bugs: autumn pheromone trap monitoring and treatment with insecticide where necessary)
- Botrytis: BCA treatments have been found to be insufficiently effective for Botrytis.

NB: Varietal considerations need to be made.

Appendix 1

SIX MONTHLY REPORT TO HORTICULTURE LINK PROGRAMME MANAGEMENT COMMITTEE (Due 31 March 2014)

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, Michelle Fountain, David Hall & Xiangming Xu
Project Start/Completion Dates:		1 April 2010 – 31 March 2015
Reporting Period:		1 April 2013- 30 September 2013
Number of Months Since Commencement:		36 month report
Date of Last Management Meeting:		12 Dec 2013 (consortium) Summer site visit: Tuesday 11 June 2013
Date of next management meeting:		Next meetings: 25 June 2014, 10 December 2014
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 *Research work has been revised and hence this primary milestone is changed as well [see full description below]*	N*
P1.1.3	31/03/2013	Relationships among physiological/morphological characters and susceptibility to *B. cinerea* established *The relationships have been investigated but have been found to be very variable. More work under controlled conditions is ongoing in 2014 and 2015*	N
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	
P1.4.1	31/03/2014	Key fungi responsible for blocking filters identified	ongoing
P1.4.2	31/03/2012	Incidence of important fungi in historical samples established *Research work has been revised and hence this primary milestone is changed as well [see full description below]*	N
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	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 *Work done but results were inconclusive and further work is ongoing in 2014 and 2015*	N

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	
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	
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 have been identified (see report for objective 4 above) but biological methods (BCAs) for Botrytis are insufficiently effective and alternative need to be identified. It was decided to start work to start evaluation of the IPM programme in autumn 2013. This milestone will therefore be delayed until 30/09/2013.*	N
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	Y
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 *The focus changed and whole field scale trials were done instead.*	Y
S1.3.1	31/03/2013	Year 3 Caged pollinating insect trials complete	Y
S1.4.1	30/09/2010	*Historical samples were not available so this work objective has been revised [see below]*	N
S1.4.2	31/03/2012	Molecular methods for quantifying latent botrytis in fruit validated	Y
S1.4.3	31/03/2013	Experiments on restricting botrytis internal colonisation started	Y
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	Y
S3.1	31/03/2011	Sawfly sex pheromone attraction and mating behaviour investigated	Y
S3.2	31/03/2012	Blackcurrant sawfly pheromone identified and synthesised	Y
S4.1.2	31/03/2014	1st year evaluation of IPDM programme in large scale grower trials complete *This milestone will be delayed until 31/03/2015, for the reasons explained above and below.*	

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

Primary Milestones			
P1.1.3	31/03/2013	Relationships among physiological/morphological characters and susceptibility to *B. cinerea* established *The relationships have been investigated but have been found to be very variable. More work under controlled conditions is ongoing in 2014 and 2015*	N
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
P4.1.1	31/03/2013	IPDM programme for evaluation in years 4 and 5 devised	N
Secondary Milestones			
S1.3.1	31/03/2013	Year 3 Caged pollinating insect trials complete	Y
S1.4.3	31/03/2013	Experiments on restricting botrytis internal colonisation started	Y

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

Objective 1: *Botrytis cinerea*

Determining whether varietal differences in susceptibility are correlated with physiological characters

Incidence of latent infection appeared to be increased with late development within a season, number of flowers per node, fruit losses on the floor, and decreasing fruit weight. However, it should be noted that the magnitude of correlation is generally very low. We have measured susceptibility of a number of selected advanced breeding lines for their susceptibility to botrytis and skin thickness. For both characters, there is considerable variability. Further data will be collected in 2012 to establish whether there is significant correlation between these two characters.

Characterising varietal variations in susceptibility – elite breeding lines

25 genetically diverse cultivars and elite breeding lines were selected for evaluation during the 2012 season for variations in natural susceptibility to Botrytis infection. Assessments of skin strength, using previously described methods, were aligned with the time taken for infection to occur in surface-sterilised berries, but no consistent correlations were found. Further comparisons of skin thickness will be made, together with an assessment of any year to year variations in susceptibility.

Results from controlled inoculation of 18-20 genotypes in 2011 and 2012 showed an insignificant correlation in the incidence of fruit infection between the two years. Thus, the response of cultivars to Botrytis infection is inconsistent between years, hence indicating the lack of intrinsic resistance.

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

A further replicated field experiment in 2012 examined the efficacy of programmes of pre-harvest sprays of the biocontrol agents and fungicides, alone or in combination, for control of Botrytis on fruit at harvest. The trial was done on Ben Hope and Ben Tirran. The weather conditions in 2012 were unusually wet and Botrytis was much worse on Ben Tirran, than Ben Hope, though results on the two varieties followed a similar pattern. Programmes of 3 or 4 sprays of a novel fungicide product gave by far the best control, performing significantly better than the programmes of similar numbers of sprays of the standard product (Signum). Programmes of 6 sprays of microbial BCA products gave a modest reduction in Botrytis but performed significantly less well than the fungicides. Additional sprays of BCA products on top of the fungicide programmes gave no discernible benefit.

Effect of pollinating insects on blackcurrant yield and quality

Caged field trials showed that supplementing blackcurrant with *Bombus terrestris dalmatinus* nest boxes at the point of flower opening increased yield and fruit size of berries (Ben Hope and Ben Gairn). This was shown to be particularly important in a period of poor weather when the naturally occurring pollinating insects

were less active. Bagging strigs of betties to exclude insects in open field conditions demonstrated that insect pollination contributes up to 35% fruit set of Ben Gairn.
Experiments indicated that bees may vector botrytis but that this was marginal and far outweighed by their contribution to pollination. Field trails with *B. t. audax* were inconclusive as the weather at flowering was good in 2001 and increased sampling of fruit set was needed. Wild bumblebees and solitary bees were important contributors to blackcurrant pollination; honeybees less so.
In 2012 at pre blackcurrant flowering no bees were seen visiting flowers. However, observations on flowering plants were not long enough to be conclusive. All bumblebees seen at this time were queens – mostly searching for nesting sites, although *B. lapidarius* was seem moving between blackcurrant bushes. Solitary bees were mostly seen basking on leaves or flying around bare earth – possibly males waiting for females to emerge. Bumblebee queens were particularly attracted to rough grass verges in headlands. Weather during the flowering period of Ben Garin in 2012 was poor with rainfall throughout. However, provisioning plantations with *Bombus terrestris audax* nest boxes did not improve the fruit in the blackcurrant plantations. High variability of crop management between farms and fields may have made it difficult to tease these effects apart. Bumble bees can forage for up to 2-3 km and so are not restricted to the plantations even though placed in the centres of the crops. However, bumblebees were observed foraging on blackcurrant flowers within a few minutes of opening the nest boxes. It is also likely that botrytis also affected fruit set as withered strigs of fruit were observed at the assessment time.
Although landscape scale surrounding land use varied between plantations provisions for nest sites could be manipulated within the plantation to bolster numbers of solitary and bumblebees (the main foragers of blackcurrant flowers). Many important blackcurrant forager bees were present before and after blackcurrant flowering within the vicinity of the crop. Hence provisioning with nest sites and diverse forage will help to foster better populations for pollination in future years. Most sites in this study were very forage poor, especially pre blackcurrant flowering, and some lacked numbers of bees and nest sites. *Osmia rufa* was a poor blackcurrant flower visitor and, therefore, not an important pollinator of blackcurrant.
In 2013 methods were developed that enabled us to test the nutritional quality of insect pollinated, hand pollinated (optimal pollination) and insect excluded fruits. Results were encouraging with but unclear. Experiments will be designed to further test these effects in 2014.

Filter blocking:
As in previous years, we have continued the study on investigating whether fungal botrytis increased substantially while it remains latent inside infected blackcurrant fruit. Fruit of cv. Ben Hope were inoculated 3-4 weeks after flowering. Thereafter, about 100 fruit were sampled every week for molecular quantification of fungal biomass. qPCR data revealed that fungal DNA did not appreciably increase over time from inoculation to harvest. Thus, mycelia inside the latently infected fruit by *B. cinerea* are unlikely to be responsible for blocking filters. This experiment was repeated in 2012 and samples of fruits are being quantified for *B. cinerea* DNA.

Objective 2: Blackcurrant leaf midge
Crop damage assessment in fruiting plantations and timing and efficacy of insecticides
Work on these topics was concluded in year 3. Two papers for publication in Crop Protection are currently being prepared.

Us of pheromone for control
A replicated cage (12 m x 1.5 m x 2 m LxWxH) trial in 2013 tested control of the midge on potted blackcurrant plants by deploying grids of rubber septa lures with 5 µg of the pheromone enantiomer vs 20 µg of the racemate on sticky cards, one card per plant. Untreated and sticky card only controls were included. Neither pheromone treatment gave good control of leaf midge galling.

Objective 3: Blackcurrant sawfly
A range of isomers of isopropyl tetradecenoate were synthesised and the isopropyl (Z)-7-tetradecenoate shown to have identical GC retention times and mass spectrum to the compound produced by female sawfly. The synthetic compound elicits a very strong EAG response from the males and is proposed to be the major component of the female sex pheromone. Similarly a range of isomers of the 16-carbon homologue has been synthesised and isopropyl (Z)-9-hexadecenoate shown to have identical GC retention times and EAG activity to one of the minor compounds produced by female sawflies. Lures containing these compounds were tested in the field in 2013 and were shown to attract large numbers of male blackcurrant sawfly. In 2014 we will optimise the pheromone blend and demonstrate the best trapping device for monitoring the pest.

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

		Due to the lack of progress in identifying effective biologically based integrated disease management strategies for Botrytis and lack of approval for UK386a for midge control, at the December 2013 consortium meeting it was decided to not to emabark on ork testing IPDM programmes. The key components of the pest management programme have been identified but BCA treatments have been found to be insufficiently effective for Botrytis and alternatives are being evaluated in 2014.
6.	**Publications and technology transfer outputs:**	(including public presentations/talks given. Indicate additions since last report by use of bold type)

Technology transfer activities

J Cross: gave a ½ hour talk presenting a comprehensive overview of progress in the project at the International Blackcurrant Conference in Dundee on 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: Control of *B cinerea* on blackcurrants using biocontrol and fungicides. Presentation to IOBC Biocontrol meeting, Reims, France

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

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

M Fountain: EMR Newsletter 'Pollinating insects in UK perennial crops' December 2013

M Fountain: Oral presentation at Royal Entomoligcal Society conference (EMR) 'Pollinating insects in UK perennial crops' 13 Nov 2013.

M Fountain: Poster presentation (September 2013) EMR open day 'Who is pollinating fruit crops?'

S Raffle: article in HDC Soft Fruit magazine p. 21

Publications

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

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

None at this stage of the project