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Project co-ordinator:	Hugh Mowat (M&S)
Project administrator:	Jerry Cross (EMR)
Researchers:	Jerry Cross (EMR) Jean Fitzgerald (EMR) Angela Berrie (EMR) Xiangming Xu (EMR) Gillian Arnold (EMR) Tim O'Neill (ADAS) Janet Allen (ADAS) Erika Wedgwood (ADAS) Neil Boonham (CSL) Nicola Spence (CSL) David Hall (NRI)
Collaborators:	K G Growers Ltd (Richard Harnden) Berry World Ltd (Tim Newton) Summer Fruit Company (Rupert Carter) Mack Multiples Division (Tony Vallance) Marks & Spencer plc (Hugh Mowat) Sainsbury's plc (Theresa Huxley) International Pheromone Systems Ltd (David Hartley) Horticultural Development Company (Lindrea Latham) East Malling Trust (Jim Quinlan) East Malling Ltd (Ian Hardie) Jane & Paul Mansfield Soft Fruit Ltd (David Stanbury) Agralan Ltd (Mike Abel) Robert Boucher and Son (Hugh Boucher) Red Beehive Company Ltd (Robin Dean) Biological Crop Protection Ltd (Jennifer Lewis) Koppert UK Ltd (Willem Ravensburg)
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

GROWER SUMMARY

Headline

Some early progress has been made in developing novel, non-pesticide control methods for important pests and diseases of strawberry.

Background and expected deliverables

The overall aim of the project is to develop alternative, sustainable, non-pesticidal methods for managing powdery mildew, Botrytis, blackspot, capsids, aphids and blossom weevil on strawberry, so greatly reducing pesticide use and eliminating the occurrence of reportable pesticide residues on harvested fruit. The methods developed for the individual pests and diseases will be combined with existing non-chemical methods for other pests and diseases in an overall Integrated Pest and Disease Management (IPDM) system, and this will be tested and refined in commerical strawberry production over two seasons.

Summary of project and main conclusions

Progress on each objective of the project is summarised below

Strawberry powdery mildew

Inoculum in planting material

Experiments were conducted to investigate the extent of powdery mildew inoculum on initial planting materials (cold-stored runners and tray plants). Results so far suggest that the level of mildew in commercial planting materials is very low. The survival of mildew on runners in cold-store is currently being studied.

Alternative products

Preliminary results suggested that potassium bicarbonate and Serenade (a BCA, recently registered in the UK) can control mildew as effectively as Systhane when applied before inoculation. This will be reassessed in a field study to be conducted in 2009.

Powdery mildew forecasting

The prediction scheme developed by CSL and University of Hertfordshire has been developed as a computer programme. In 2009, this prediction scheme (and Botrytis model) will be evaluated on farms for its prediction accuracy and usefulness for disease management.

Botrytis

Latent infection in planting material

Botrytis cinerea was detected within the crown tissue of cold-stored runners of A+ grade cv. Elsanta received from five growers in May-June 2008. The incidence of infection determined by isolation onto agar ranged from 6% to 38% of plants. There was no obvious relationship between the incidence and quantity of *B. cinerea* in plants. *B. cinerea* was also detected in green petiole stubs in all samples, at levels ranging from 6–55% of plants. Further sampling will be done in 2009 to confirm these results.

Latent flower infection by Botrytis

Field sampling revealed a very low level of latent flower infection on the variety Elsanta but a very high level of infection on an everbearer variety. Further sampling in 2009 is necessary to confirm whether this is generally true or just a one-off event. These data will be used to validate a *Botrytis* prediction scheme developed at EMR based on open-field data.

Control of fruit infection using Binab (Trichoderma spp.)

Binab was applied once as a spray (Binab TF WP) and subsequently as a dry powder (Binab T-Vector), which is transmitted to flowers by honey bees and bumble bees. The efficacy was evaluated for control of flower infection, fruit infection and fruit rot in a replicated experiment at ADAS Boxworth. *Trichoderma* spp. were successfully transferred to flowers by the bees. The incidence of latent flower infection by *B. cinerea* was significantly reduced using the Binab products compared with untreated and fungicide-treated plants. However, Binab products did not significantly reduce either latent fruit infection or visible fruit Botrytis. The proportion of total fruit weight visibly affected by Botrytis rot was 4.0% in untreated plants and was not significantly reduced by any of the treatments, including use of Binab products (4.7%), a four-spray fungicide programme (2.9%) or a combined Binab/fungicide programme (4.8%) during flowering.

Black spot

Colletotrichum acutatum was isolated from the crowns of three of the five samples. Neither *C. acutatum* nor *Podosphaera aphanis* were detected in crown tissue by PCR tests for these fungi.

European tarnished plant bug

It was hoped to quantify the relative attraction of candidate herbaceous flowering plants and cover crops to the European tarnished plant bug (*Lygus rugulipennis*). *Chenopodium album* (fat hen) and *Matricaria recutita* (mayweed) were sown as they are known to attract *L. rugulipennis*; these plants then acted as a source in experiments to compare the attractiveness of other plant species to the pest. The chosen trap plants were *Artemisia vulgaris* (mugwort), *Matricaria recutita* (scented mayweed), *Medicago sativa* (lucerne- alfalfa), *Sinapsis arvensis* (charlock-mustard) and *Vicia sativa* (common vetch). All, except mugwort, germinated well. Samples of arthropods were collected from the trap plants and results showed that, overall, the highest numbers of *L. rugulipennis* nymphs were collected from mustard and mayweed. Lower numbers of *L. rugulipennis* adults were collected from lucerne than from the other three plant species.

A mark-recapture experiment was undertaken to quantify the attractiveness of the trap plants to *L. rugulipennis*. Source plants were sprayed with a protein marker and the trap plants sampled for *L. rugulipennis* adults 2 and 5 days after treatment. The ELISA analysis of these collected individuals is currently in progress.

In order to develop suitable lures for hexyl butyrate (a compound that will be tested as a repellent for *L. rugulipennis* in field experiments in 2009), measurement of release rates from different types of dispenser are in progress in a laboratory wind tunnel. Results obtained show that it will be possible to develop a vial dispenser that will continue to dispense hexyl butyrate for more than 77 days in the field.

Aphids

To evaluate the effectiveness of flowering plants to attract aphid predators and parasitoids, *Medicago sativa*, (lucerne), *Silene dioica* (red campion) *Galium verum* (lady's bedstraw), *Leucanthemum vulgare* (ox eye daisy), and a mixture of *Centaurea cyanus* (cornflower) + *Chrysanthemum segetum* (corn marigold) and *Anthemis arvensis* (corn chamomile) were sown

in a replicated block design around a commercial strawberry plantation. Unfortunately many of the plots became overgrown with thistles and it was only possible to sample the flower mix plots. Both predatory arthropods and the pest *L. rugulipennis* were found on these plots.

To evaluate the effectiveness of plant derived semiochemicals to attract aphid predators and parasitoids, laboratory experiments are assessing the effect of different release rates of four plant volatiles on *Orius laevigatus*, a predator that is often abundant in selectively sprayed plantations. One of the volatiles is methyl salicylate which has been shown to be attractive to beneficial species in other crops. This work is being done in olfactometry choice test experiments.

Suitable dispensers for methyl salicylate and one other attractive volatile are currently being developed. Initial work has focussed on determining release rates of methyl salicylate from two types of polyethylene sachet. Release of the compound was rapid; all the material (100 mg) was released within 10 days. Further work using polythene vials is in progress.

Strawberry blossom weevil super trap

Excellent progress has been made towards developing a highly attractive 'super' trap for strawberry blossom weevil that combines visual, host plant volatile and sex aggregation pheromone attractants.

Work in collaboration with Bioforsk, Norway has shown that the attractiveness of the strawberry blossom weevil aggregation pheromone can be increased by > 3 fold by synergising the pheromone with a volatile substance from strawberry flowers. Work in 2009 will test whether additional benefit can be gained from other common volatiles from strawberry foliage and flowers. Importantly, green funnel traps with white cross-vanes were shown to be more effective and practical than the sticky stake traps formerly used with the pheromone. Thus two important steps towards developing a 'super' trap were made in the first years work.

Financial benefits

Botrytis, mildew, black spot, aphids, blossom weevil and capsid bugs are very common problems wherever and however strawberries are grown in the UK. A very high percentage of strawberry plantations are infected by these pests and diseases. No quantitative data on losses

is available but conservatively assuming 10% of the crop is lost as a result of these infestations, this is equivalent to 5,074 tonnes of strawberries, worth £21 million.

To calculate the expected annual added value that might result from a successful project, it is assumed that it will lead to an average halving in losses in the current crop to 5%, i.e. an additional £10,623 million of UK sales. In addition, the improved consumer acceptability of UK strawberry growing compared to foreign competitors will reduce imports by 10%, yielding an additional £17 million of sales. It is possible that increased consumer confidence in strawberries will also grow the overall market marginally.

Action points for growers

There are no action points for growers at this early stage of the project.

SCIENCE SECTION

Objective 1. To develop an IPM system for powdery mildew through reducing initial inoculum levels in planting material, microbial biocontrol, use of natural products, and reducing plant susceptibility to disease through adjustment of N fertiliser application

Task 1.1 Detection and reduction of inocula in planting material (Y1-4)

We have conducted several experiments to investigate the extent and the importance of the infections in the production of planting material, and whether mildew can survive on runners in a cold store.

1.1.1 Cold stored runners

Methods

In order to examine whether there are viable mildew on cold stored runners that could act as initial inoculum after planting, we ordered two batches of Elsanta runners in April 2008, 80 from each of two producers. Then we potted them up and maintained each batch in separate controlled environment (CE) cabinets at 15°C. These plants were regularly examined for the presence of powdery mildew on young leaves.

Results

No single mildew lesion was observed on any of the 160 plants.

1.1.2 Module plants

Methods

We obtained two batches of module plants, 80 for Elsanta and 80 for Albion, to examine whether there was viable mildew in module plants that could act as initial inoculum after planting. Upon delivery, these plants were immediately placed into a CE cabinet at 15°C for one month for monitoring.

Results

No single mildew lesion was observed on any of the 160 plants.

1.1.3 Survival of mildew on runners in a cold store

Methods

We have obtained ~ 350 runners infected with powdery mildew; these runners were harvested from plants maintained in a glasshouse compartment at EMR. These runners were stored at -2°C on 20 October 2008. About 60 runners will be potted up in February, March and April 2009, and placed onto cabinets for frequent monitoring of mildew development.

Results

This study is still ongoing.

Task 1.2 Effect of nitrogen on the susceptibility to powdery mildew (Y3-4, EMR)

This task will start in Year 3.

Task 1.3. Determining the control efficacy of BCAs and alternative products

1.3.1 Alternative products

Methods

We have conducted one experiment in controlled environment conditions (15°C, and 75% rh) to determine the efficacy of several alternative products, as protectants, against powdery mildew. Five products were tested: Chitoclear, Enzicur, potassium bicarbonate, Serenade (a biocontrol agent), and Systhane. These products were applied with a hand-held sprayer to run-off 24 h before inoculation. In addition, there was an untreated control: inoculated but not sprayed with any product. There were two plants per treatment and number of mildews on each leaflet of at least two leaves per plant was counted 7-10 days after inoculation.

Results

Statistical analysis suggested that the overall differences in the number of mildew lesions among the six treatments were nearly statistically significant (P = 0.07). Of the five treatments, potassium bicarbonate, Serenade and Systhane resulted in fewer (P < 0.05) lesions than the control; there were no other significant differences among treatments (Fig. 1.3.1).

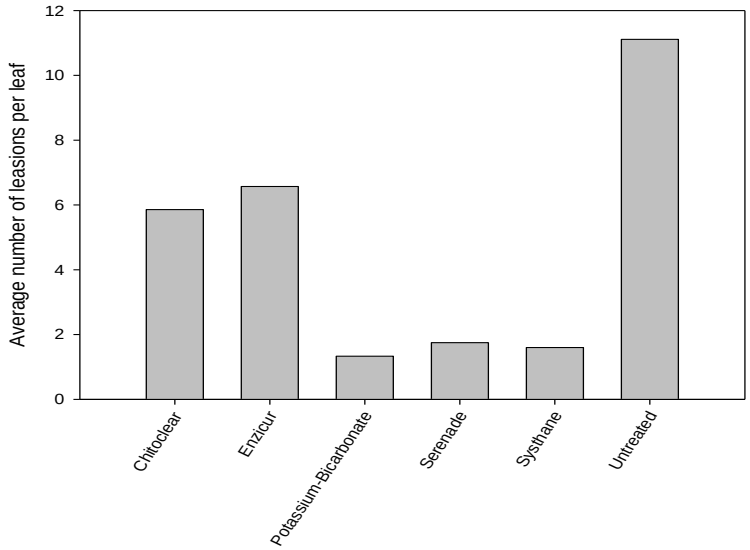

Figure 1.3.1 Average number of mildew lesions on leaves treated with alternative products 24 h before inoculation.

1.3.2 Survival of biocontrol agents

In a PSD-funded project, EMR is testing several BCA products against strawberry grey mould; one of the products tested is Serenade. The original aim of the PSD project was to investigate the efficacy of several commercial BCAs against botrytis on strawberry without considering the survival of BCAs on leaves. In view of this LINK project, we have modified experimental protocols for the PSD experiments such that we are also incorporating aspects of survival and spread of Serenade on strawberry plants under fluctuating conditions.

In the experiment, BCAs are applied 24 h before or after inoculation with *Botrytis*; disease was first assessed on one mature leaf. Then all plants are to be inoculated again; disease is to be assessed again on another old leaf (present in both inoculations as well as BCA application) and a young leaf (only present at the time of second inoculation). By comparing disease

incidences on the two old leaves and one young leaf on each plant, we may determine the likelihood of BCA survival and spread. This experiment is currently ongoing.

Task 1.4 Investigate the dynamics of pesticide dissipation under protection for improved determination of the persistence and the appropriate harvest interval (Y2-3, EMR)

This task will start in year 2.

Task 1.5 Evaluating a mildew prediction system (Yrs 2-4)

We have had a meeting with Dr Hall and Dr Dodgson to discuss their mildew prediction scheme. Based on this discussion, EMR has implemented this prediction scheme into a computer programme. This programme includes not only a prediction scheme from Drs Hall and Dodgson but also a new one developed on the basis of the EMR apple powdery mildew. This programme is able to read almost all formats of weather data files (as long as a text file).

We organised a workshop at EMR on 23 January to discuss how to evaluate and use both botrytis and mildew models. In addition, we have outlined the plan of model evaluation in 2009.

Objective 2. To develop an Integrated Crop Management (ICM) system for botrytis through reducing initial inoculum levels in planting material, accurate prediction of risk of flower infection, and the use of Biological Control Agents (BCAs) vectored by bees.

Task 2.1: Determine the occurrence of latent Botrytis cinerea, in commercial strawberry plants at planting

Introduction

Young strawberry plants raised in module trays at high density are sometimes affected by visible grey mould. Strawberry runners kept in cold-store until required for planting often have moribund petioles and leaf debris at planting that is susceptible to infection by *B. cinerea*. *B. cinerea* spore germination and mycelial growth potentially can occur on plants at the low temperatures found in cold-storage. In some other crop species, it has recently been found that some plants which appear healthy have symptomless (latent) infection by *B. cinerea*. The incidence of latent *B. cinerea* in strawberry planting material received by UK strawberry growers is unknown.

The objective of this work was to determine the occurrence of *B. cinerea* in strawberry runners as the first stage in seeking to reduce initial botrytis inoculum at planting. Ensuring that *B. cinerea*-free strawberry runners are planted would reduce fungicide requirements in the crop.

Methods

This work was conducted by ADAS in conjunction with CSL. Samples of 100 strawberry plants (12-14 mm crown diameter) were kept back from planting by growers after removal from cold-store and sent to ADAS. The plants originated from five different plant propagators. Sub-samples of 50 plants were taken for testing. Each plant was quartered through the crown, and eight pieces taken from internal tissue. Six sections were surface sterilised and placed on an agar plate, and the other two sections were put directly into a sample tube, together with two sections from each of another four crowns.

Where plants had green petioles from which the leaf blade had been trimmed prior to cold-storage, these stubs were sampled. The petiole ends were removed and surface sterilised before plating onto agar. The ends were not re-trimmed before plating, except to remove any rotted tissue to reduce isolation of secondary fungi.

Once plant sections had been taken, the remaining material was placed without surface sterilisation into a damp chamber. The composite parts of each plant (roots, crown and emerging shoots, mature green leaves and browning leaves) were laid out and assessed individually for fungal growth.

Tissue sections and damp chambers were examined for *B. cinerea*. The presence of *Colletotrichum* and other fungi was also recorded.

Molecular tests (TaqMan PCR) were carried out by CSL on the crown tissue only, to detect *B. cinerea*, *C. acutatum,* and *P. aphanis.* Tests were done on bulks of five plants.

DNA was extracted from each strawberry crown. Material was frozen in liquid nitrogen and ground to a fine powder followed by the addition of 10 volumes of CTAB homogenising buffer (20mM ethylenediaminetetraacetic acid, 100mM Tris-HCl pH 8.0, 1.4M NaCl, 2% hexadecyltrimethyl ammonium bromide, 1% Na_2SO_3, 2% polyvinylpolyrrolidone). After incubation at 65°C for 15 min, two chloroform/isoamyl alcohol (24:1) extractions were performed. Nucleic acid was precipitated from the aqueous layer by the addition of 0.5 volumes 5M NaCl and an equal volume of ice-cold isopropanol. After centrifugation the pellet was washed with 70% (v/v) ethanol, vacuum-dried and re-suspended in 50µl nuclease free water.

The primer and probe sequences used for the detection of *B. cinerea, C. acutatum, P. aphanis* in the Taqman assays, and the plant internal control, are shown in Table 2.1.2. A plant internal control assay was included with each test reaction to assess extraction efficiency. Results for *C. acutatum* and *P. aphanis* are purely presence or absence of the pathogen while results for *B. cinerea* are quantitative (mg botrytis DNA / g plant DNA).

Table 2.1.1: Sample details of strawberry planting material ex-cold storage sent by growers for disease testing; the five samples were from different propagators.

Sample	Variety	Location of grower	Date received	Date tested
BX08/47	Elsanta A+	Cambridgeshire	07/05/2008	08/05/2008
BX08/52	Elsanta A+	Staffordshire*	09/05/2008	12/05/2008
BX08/55	Elsanta A+	Staffordshire*	13/05/2008	16/05/2008
BX08/62	Elsanta A+	Nottinghamshire*	22/05/2008	23/05/2008
BX08/66	Elsanta A+	Scotland*	03/06/2008	03/06/2008

*Dutch propagator.

Table 2.1.2: Taqman primer and probe sequences used for the detection of *B. cinerea, C. acutatum*, *P. aphanis* and plant internal control by TaqMan PCR assay

Assay	Sequence (5'-3')	Reference
B. cinerea		Suarez *et al*., 2005
Bc3F	GCTGTAATTTCAATGTGCAGAATCCI	
Bc3R	GGAGCAACAATTAATCGCATTTC	
Bc3P (FAM labelled)	TCACCTTGCAATGAGTGG	
C. acutatum		Garrido *et al.*, 2008
F1	CGGAGGAAACCAAACTCTATTTACA	
R1	CCAGAACCAAGAGATCCGTTG	
PB (FAM labelled)	CGTCTCTTCTGAGTGGCACAAGCAAATAATTAAA	
P. aphanis		CSL assay
393F	GTTCGGCGTCCCCTAAACA	
473R	TCCCATCGCCACTCTGTCG	
429T (FAM labelled)	TGCTCTCCGCGTAGTCACGTATCTCG	
Plant ITS		Suarez *et al.*, 2005
ITSF	TMGGCTCWCGCATCGAT	
ITSR	ARACTCGATGGTTCRCGGGAI	
TSP (JOE labelled)	AAGAACGTAGCRAAATGCGATACTYGGTGTGAA	

Results and discussion

Botrytis

B. cinerea was detected in the crown tissue of all five samples, at varying levels, by both isolation onto agar (Table 2.1.3) and by a PCR test (Table 2.1.5). The planting material from growers originated from different propagators.

The five growers' samples differed significantly in the incidence of crowns infected by *B. cinerea* as determined by isolation, with one at a low incidence (6%), three at a moderate incidence (22-24%) and one with a relatively high incidence of infection (38%) (Table 2.1.3).

There was no statistically significant difference ($P > 0.05$) between the five samples in the mean quantity of *B. cinerea* DNA in strawberry crowns (both untransformed and log transformed data). The quantity of *B. cinerea* in bulked crown samples from five plants ranged from 0.002 to 0.100 mg/g, with one outlier value at 89.3 mg/g (Table 2.1.5).

Comparing the *B. cinerea* incidence and DNA quantity data for all five growers (10 sets of five plants per grower) (Table 2.1.7), the incidence of crowns with latent *B. cinerea* in each set determined by isolation plating did not obviously correlate with quantity of *B. cinerea* determined by PCR. The highest incidence from crown plating (38%) (Table 2.1.3) was in a sample which had been placed in cold storage when surplus at planting and was received for testing two months later, but the PCR test detected *B. cinerea* in only one of the 10 bulk samples. It is possible that there is localised distribution of the fungus within the crown (such as where petioles were attached) and that some sample sections did not include this mycelium. *Botrytis* mycelium may grow within the crowns of defrosted plants kept below 4°C and increase the detection rate by incubation (as might be the case for BX08/47), although conversely BX08/47 had fewest crown samples in which *B. cinerea* was detected by PCR (Table 2.1.5).

B. cinerea was also present in green petiole stubs which had been left after trimming at lifting. The number of plants with one or more petiole stubs present differed between samples. From 6% to 55% of petiole-sampled plants had botrytis in this tissue (Table 2.1.3). *B. cinerea* infection may have entered through wounds to petioles, but intact unwounded leaves were not tested. If infection is established during field growth, then conditions for disease development such as planting density may be important.

In damp chambers, the quantities of leaves and the stage of decay of old leaves differed between samples and so results were not strictly comparable. Crown infection may be under-represented as little internal material remained after sectioning for tissue samples. Incubated material was not surface sterilised and so after 10 to 20 days it was colonised by a number of secondary fungi such as *Fusarium* spp. Only sporulating botrytis was recorded as *B. cinerea* (Table 2.1.4) with other mycelium scored as either white or 'other' (results not shown). Old leaves produced the highest incidence of *B. cinerea.* The sample from extended cold storage (BX08/47) was one of the least infected by botrytis (internal or external growth). No botrytis developed in the damp chamber of BX08/66, and this sample also had a low incidence of latent botrytis in the plated crown and petiole tissue.

The number of propagation material sources sampled was relatively small, and material was only taken from A+ stock. This is the grade of planting material in the Plant Health Propagation Scheme most commonly used by growers, and plants are propagated at high density. A greater number of propagation sources, together with information on fungicide use during plant production and as a dip pre-storage, would be beneficial, but is outside the remit of this project.

Black spot

Colletotrichum was detected by isolation from tissue placed on agar in material from three growers (one UK, two Dutch sources), with a high incidence (56%) in crown tissue from sample BX08/62 when incubated for seven weeks (Table 2.1.8). This was probably *C. acutatum* from examination of the spores, but samples were not sent for PCR testing. Green petiole stubs were only found to be infected from sources with infected crowns.

There was no separate assessment for *Colletotrichum* sp. in the damp chambers. The orange spores of this fungus were only noted on two plants of BX08/66 when examined a month after the normal incubation period. This was a sample in which *Colletotrichum* sp. was not confirmed by isolation from crown or petiole tissue.

The PCR test for *C. acutatum* in internal crown tissue was negative for all samples.

Powdery mildew

No powdery mildew was seen in the plant samples received from growers, and the PCR test for *P. aphanis* in crown tissue was negative for all samples.

Table 2.1.3: Recovery of *B. cinerea* from within crown tissue and trimmed petiole stubs of 50 strawberry plants ex cold-store. The % infection of petiole stubs is shown as a proportion of the number of plants from which stubs were available.

Sample	Mean % plants (and 95 % confidence limits) with *B. cinerea* in:				Number of plants having petiole stubs
	Crown tissue		Petiole stubs		
BX08/47	38	(23.6 - 52.5)	6	(0.0 - 14.4)	47
BX08/52	22	(9.5 - 34.5)	20	(6.4 - 33.6)	40
BX08/55	22	(9.5 - 34.5)	55	(38.1 - 72.4)	38
BX08/62	24	(11.2 - 36.9)	23	(0.0 - 49.8)	13
BX08/66	6	(-1.6 - 13.6)	18	(5.5 - 30.1)	45

Table 2.1.4: Occurrence of *B. cinerea* on different tissues of strawberry plants after damp incubation

Sample	Mean number plants (of 50) with *B. cinerea* developing from:			
	Roots	Old leaves	Centre leaves	Crown core
BX08/47	1	6	1	0
BX08/52	0	12	0	0
BX08/55	5	27	2	4
BX08/62	8	41	1	2
BX08/66	0	0	0	0

Table 2.1.5: Detection of *B. cinerea* in internal crown tissue of strawberry plants by TaqMan PCR assay of 5-plant sample bulks and by individual isolation onto agar

Sample	Plant ID numbers	Mean quantity *B. cinerea* DNA (mg/g)	No. crowns (out of 5) with *B. cinerea* detected by isolation
BX08/47	1-5	0.000000	3
	6-10	0.001737	4
	11-15	0.000000	2
	16-20	0.000000	2
	21-25	0.000000	1
	26-30	0.000000	2
	31-35	0.000000	0
	36-40	0.000000	0
	41-45	0.000000	2
	46-50	0.000000	3
BX08/52	1-5	0.003922	0
	6-10	0.000000	0
	11-15	0.014712	1
	16-20	0.123981	1
	21-25	0.000000	1
	26-30	0.000000	0
	31-35	0.011291	2
	36-40	0.000000	2
	41-45	0.000000	1
	46-50	89.324615	3
BX08/55	1-5	0.000000	1
	6-10	0.000000	2
	11-15	0.022123	3
	16-20	0.000000	0
	21-25	0.004270	0
	26-30	0.000000	1
	31-35	0.000000	1
	36-40	0.002514	1

	41-45	0.002808	0
	46-50	0.005966	2
BX08/62	1-5	0.000000	2
	6-10	0.000000	0
	11-15	0.000000	1
	16-20	0.003181	0
	21-25	0.086863	1
	26-30	0.002778	1
	31-35	0.019953	2
	36-40	0.002168	2
	41-45	0.000343	0
	46-50	0.014162	3
BX08/66	1-5	0.001795	0
	6-10	0.000000	0
	11-15	0.005282	0
	16-20	0.000000	0
	21-25	0.000000	2
	26-30	0.000000	1
	31-35	0.000000	0
	36-40	0.000000	0
	41-45	0.000000	0
	46-50	0.000000	0

Table 2.1.6: Correspondence of occurrence of *B. cinerea* in crown tissue of strawberry plants as detected by a TaqMan PCR assay and isolation from the same 5-plant sample bulks plated onto agar.

Quantity ranges of *B. cinerea* in crown (mg/g plant DNA)	No. sample bulks per DNA range	Number of crowns (out of 5) with *B. cinerea* isolated, tabulated against the DNA quantity in tissue from the same crowns					
		0	1	2	3	4	5
0	30	12	8	8	2	0	0
0-0.005	10	6	2	1	0	1	0
0.005-0.02	6	1	1	3	1	0	0
0.02-0.12	3	0	2	0	1	0	0
89.3	1	0	0	0	1	0	0

Table 2.1.7: Summary of *B. cinerea* detection in strawberry crown tissue of 50 sample bulks (10 bulks of 5 plants per grower) by isolation plating and a PCR assay

PCR assay	Isolation onto agar		Sample bulks
	Negative	Positive	
Negative	12	18	30
Positive	7	13	20

Table 2.1.8: Occurrence of *Colletotrichum* sp. after incubation of crown and petiole tissues of strawberry plants from five grower supplied samples.

	% plants with *Colletotrichum* in:	
Sample	Crown	Petioles
BX08/47	6	4
BX08/52	0	0
BX08/55	10	0
BX08/62	0*	0
BX08/66	0	0

* Zero crowns showed infection after 7 days incubation, but 56% after 7 weeks

Conclusions

- The results confirm there is a risk of both *B. cinerea* and *C. acutatum* being introduced into new plantings as symptomless infections in young plants.
- There was no correlation between the proportion of the five crowns per bulk having *B. cinerea* according to isolation and the quantity of DNA extracted from the same five crowns.
- The recovery of both *B. cinerea* and *C. acutatum* from within surface disinfected crown tissue suggests it will not be easy to eliminate these infections by a pre-plant treatment.

Task 2.2: Evaluate the efficacy of a biocontrol product vectored by bees on control of botrytis fruit rot

Introduction

In work overseas there is evidence that *Trichoderma* spp. vectored into flowers by bees during pollination can reduce fruit rot in strawberry. Latent flower infection, rather than direct fruit infection, is the main cause of strawberry fruit rot. The objective of the current research was to investigate whether *Trichoderma* spp. (a mixture of *Trichoderma atroviride* IMI 206040, previously known as *Trichoderma harzianum,* and *Trichoderma polysporum* IMI 206039) formulated as Binab TF WP and Binab T-Vector reduced botrytis infection of the strawberry flowers. The biological control treatment was compared with a grower-standard fungicide spray programme for the control of botrytis during flowering and an untreated control. Additionally, any interaction between the fungicides and Binab products (to produce better or worse control than either alone) was investigated.

Methods

The diary of main events is given in Table 2.2.1.

Crop production

This work was conducted by ADAS in conjunction with the Red Beehive Company Ltd. (honey bees), and Biobest Biological Systems and their UK distributors of bumble bees, Agralan Ltd. The objective was to provide protection of flowers against infection by *B. cinerea* using bumble bees and honey bees to disperse the biocontrol product Binab T-Vector into the flowers. Bees pick up the powder by static charge and it is released as the bees land. The work with the *Trichoderma* species was carried out under an Administrative Experimental Approval from the Pesticide Safety Directorate, number COP 2008/00473. The products Binab T-Vector and Binab TF WP were obtained from Sweden from BINAB Bio-Innovation AB and stored in a freezer.

Two Spanish-tunnels (19 m x 6.4 m) (spaced with two tunnels in between) were newly erected at ADAS Boxworth and covered with Luminance THB polythene. The sides and doors at either end were netted to allow ventilation while confining the bees. Peat grow-bags on a two-high

stack of slatted produce trays were laid out in four rows, with five 1 m x 0.28 m bags per plot. Strawberry runners of Elsanta A+ F2 12-14 mm crowns from C. R. Melton and Sons, Wisbech ex-cold store (60-day plants) were planted ten to a grow-bag on 17 June 2008 and given overhead sprinkling (6 minutes, fourteen times a day) to facilitate establishment. At introduction of the bees, the overhead nozzles were changed on 18 July to give a finer spray mist and set to give a 15 second burst hourly between 08.00h and 16.00h. Plant feeding was via drippers with automatic dosing, and a change from starter to fruiting ratios.

The biological control agent *Amblyseius cucumeris* (Ambsure sachets) was used against thrips and *Aphidius colemani* (Aphisure) and *Aphidius ervi* were used against aphids. These were introduced regularly to both tunnels as a preventative measure.

Table 2.2.1: 2008 Crop diary, ADAS Boxworth Tunnels 1 and 4

Date	Event
WEEKS 25-28:	
17 June	Crop planted
4 July	First flower open
8 July	Binab TF WP spray applied (500 g / 400 L)
11 July	Aliette drench to all plots (3.75 kg / 1000 L)
WEEK 29:	
14 July	Fortress fungicide applied to all plots (0.25 L/ha)
15 July	Sporulating *B. cinerea* plates first introduced
16 July	First flower pick for incubation
17 July	Signum fungicide applied (1.8 kg/ha)
17 + 18 July	Bees installed & Binab T-Vector first added to hives
18 July	Botrytis inoculated infector plants introduced
WEEKS 30-31:	
23 July	Second flower pick for incubation
24 July	Frupica fungicide applied (0.9 kg/ha)
25 July	Larger bumble bee hives swapped for mini-hives
31 July	Switch fungicide applied (1.0 kg/ha)
1 August	Third flower pick for incubation
WEEK 32-33:	
4 August	Pick 1 of fruit for incubation. First pick for grade & yield
	Fourth flower pick for incubation
6 August	Signum fungicide (1.8 kg/ha)
14 August	Systhane 20 EW fungicide on all plots (450 mL/ 500 L)
15 August	Pick 2 of fruit for incubation
	Binab T-Vector removed from hives
WEEK 34:	
19 August	Pick 3 of fruit for incubation
WEEK 35:	
27 August	Pick 4 of fruit for incubation
28 Aug – 2 Sept	Bees removed from tunnels
WEEK 36:	
5 September	Final (12^{th}) fruit pick

Treatments

Details of treatments are shown in Table 2.2.2.

Table 2.2.2: Details of biocontrol and fungicide treatments for control of botrytis – ADAS Boxworth, 2008

Treat-ments	Tunnel	Bees present	Binab TF WP and Binab T-Vector	Botrytis fungicide programme 7 day intervals 1000 L water/ha
T1	4	Yes	No	Nil
T2	4	Yes	No	Signum, Frupica, Switch, Signum
T3	1	Yes	Yes	Nil
T4	1	Yes	Yes	Signum, Frupica, Switch, Signum

Application of Binab products and introduction of bees

A single spray of Binab TF WP using the Swedish label rate of 500 g in 400 L water/ha plus 1 kg granulated sugar was applied once to the plants throughout tunnel 1 on 8 July when there were a few scattered flowers. Sugar solution alone (1 kg in 400 L water) was sprayed over the plants in Tunnel 4 just before treating Tunnel 1.

The tunnels were orientated north-south with a hive of bumblebees and a hive of honey bees on plinths at the same height as the grow-bags either side of the southern door in each of the tunnels (Fig.1). The bees were introduced to both the tunnels once a number of flowers were open across the tunnels. The honey bees were in the crop from 17 July, and the bumble bees from 18 July. The bumble bees and cardboard hive were as commercially supplied to growers by Biobest, but the honey bee hive was constructed from plywood for the project. A bumble bee mini-hive was substituted for the larger hive in each tunnel after a week to reduce the competition by bees for flowers.

Each hive had a dispenser drawer which the bees moved through before exiting the hive. The dispenser used by the bumble bees was supplied by Biobest (as used in Sweden for the “Flying

Doctors" programme) attached by one-way tubes to the hive. The dispensers in Tunnel 4 were left empty, but in Tunnel 1 a layer 0.5 cm deep of Binab T-Vector powder was maintained in each dispenser (Table 2.2.2). The dispensers were checked every two days and the Binab T-Vector removed and replenished.

All work was carried out first in Tunnel 4, without the Binab products. Boot dips and hand wipes were used before exit from Tunnel 1. After assessment, all produce from Tunnel 1 was bagged before removal from the tunnel, and autoclaved.

To ensure inoculum of *B. cinerea* spores was present in both tunnels, pots of cv. Elsanta were inoculated with a solution of *B. cinerea* spores taken from cultures obtained from five different strawberry sources (fruit and plants). The inoculated plants were placed between the rows. In addition, plates of *B. cinerea* were made weekly from the same isolates and incubated to produce sporulation. Fifteen of these were placed in the pathways throughout each tunnel, with the isolates rotated through the various positions when they were replaced. The plates were placed individually (with their lids removed) in the rim of a plant pot filled with gravel, and sheltered from the overhead misting by a plant saucer fixed inverted 10 cm above the plate. The plates were first put out on 15 July, and removed on 28 August at the end of flowering.

The timing of flower opening for pollination, botrytis infection and fungicide application was monitored by using coloured wool to tag a flower bud in each replicate block that was approaching flower opening. The wool colour was changed each week. When flowers or fruit was harvested the wool colour was recorded. The crop was examined weekly and the maturation timing of the fruit and any deformation or disease noted. Tagged fruit was not picked during harvesting.

Figure 1: View of crop production with the two bee hives at the far end of the tunnel.

Fungicide applications

Routine applications to all plots in both tunnels were made at standard rates to control diseases other than botrytis. Aliette 80 WG was applied on 11 July, and preventative applications were made against powdery mildew using Fortress on 14 July and Systhane 20 EW on 14 August.

In addition to the routine fungicide applications, fungicides with activity against botrytis were made to half of the plots in each tunnel (T2 in Tunnel 4 and T4 in Tunnel 1). The other four plots in each tunnel received no botrytis fungicides (Table 2.2.2). Fungicide applications were made at 7 day intervals using 1000 L water /ha and standard rates.

Flower and fruit sampling

Flowers were sampled from each plot on four occasions, 16 and 23 July, 1 and 6 August. The first sample took 20 flowers per plot, but this was subsequently halved to avoid depleting fruit numbers. The first sample was after the application of the Binab TF WP spray in Tunnel 1, and subsequent samples were after the bees and dispensers were in position. The flowers were incubated in damp trays for 14 days in the light and examined for *B. cinerea*, *Trichoderma* spp. and other sporulation.

Fruit was picked every two days, collecting all the fully ripe berries separately from each plot and separating them into Class 1 (good shape and no rot), Class 2 (mis-shapes and no rot), Unmarketable (less than thumb-nail size, or rotted other than by botrytis) and Botrytis (pale brown marks). The number of fruit and the weight in each category was recorded for each plot. Results were combined into weekly yields for analysis. The twelfth and final pick was taken on the 5 September when there were only a few fruit left to pick.

Fruit were also picked on four occasions and incubated in multicell trays sealed in transparent polythene bags, with one fruit per cell. There were few fruit at the first pick on 4 August and so marketable fruit were taken from across the plots of each treatment to make up a 30 fruit sample. On subsequent picks, on 15, 19, 27 August, 30 fruit were sampled from each plot and assessed after 7 to 10 days for *B. cinerea*, *Rhizopus* spp., *Trichoderma* spp., *Penicillium* spp. and other non-sporulating fungi.

Results and discussion

Results are summarised in Tables 2.2.3 to 2.2.14.

Dispersal of Trichoderma

Incubation tests of flowers showed that *Trichoderma* did not occur on the flowers in Tunnel 1 before the bees were added (Table 2.2.3). Tunnel 1 plants had received a spray of Binab TF WP 8 days before, when these flowers were tight buds.

Flowers picked and incubated after the introduction of the bees did not have any *Trichoderma*

on them where Binab T-Vector was not added to the hives (Tunnel 4), but a high proportion (>80%) had *Trichoderma* spp. on them in the tunnel where Binab TF WP was sprayed (pre-flowering) and where Binab T-Vector had been inserted into the bee hives (Table 2.2.4 – 2.2.6).

Trichoderma developed on some ripe fruit after incubation for eight days. It was recorded in each pick from Tunnel 1 where Binab T-Vector was used. It was not initially found in Tunnel 4, where Binab T-Vector was not used, but towards the end of cropping it was also detected in this tunnel (Table 2.2.12). There was no *Trichoderma* on flowers picked at intervals throughout flowering in the non-Binab product tunnel, so it is possible that fruit which became infected by *Trichoderma* in the latter picks in the Binab-free tunnel may have received spores directly onto the fruit. This may have been from cross-contamination by staff. Where bees were carrying *Trichoderma* to flowers, because fruit was developing while flowers were still being produced, it is possible that fruit in the same truss received some Binab T-Vector. No *Trichoderma* mould was visible on fruit at picking.

Occurrence of B. cinerea in flowers

B. cinerea was detected on flowers at levels ranging from 8 to 90%. There was no significant difference between the tunnels after the application of Binab TF WP (sample 1) (Table 2.2.3). Development of the fungus was consistently at a lower incidence on flowers sampled from the tunnel where Binab T-Vector was used (Tables 2.2.3 – 2.2.6), with significant differences between treatments in samples 2, 3 and 4. Fungicide sprays applied during flowering did not consistently reduce the incidence of latent *B. cinerea* infection in them (Tables 2.2.3 - 2.2.6).

Table 2.2.3: Occurrence of *B. cinerea* and *Trichoderma* spp. on incubated strawberry flowers sampled on 16 July 2008 prior to the introduction of bees

Treat-ments	Botrytis control treatments (with + or without – products)	Mean % flowers[a] infected by: *B. cinerea*	*Trichoderma* spp.	Other
T1	- Fungicide / - Binab	25.0	0	13.8
T2	+ Fungicide / - Binab	22.5	0	7.5
T3	- Fungicide / + Binab	7.5	0	7.5
T4	+ Fungicide / + Binab	15.0	0	7.5
	Significance	ns	-	ns
	LSD (12 df)	20.38	-	10.84

[a] 20 flowers sampled per plot; assessed after 14 days incubation. This sample was taken before Binab T-Vector was used in Tunnel 1.

Table 2.2.4: Occurrence of *B. cinerea* and *Trichoderma* spp. on incubated strawberry flowers sampled on 23 July 2008 after the introduction of bees

Treat-ments	Botrytis control treatments (with + or without – products)	Mean % flowers[a] infected by: *B. cinerea*	*Trichoderma* spp.	Other
T1	- Fungicide / - Binab	55.0	0	55.0
T2	+ Fungicide / - Binab	52.5	0	60.0
T3	- Fungicide / + Binab	17.5	85.0	0
T4	+ Fungicide / + Binab	12.5	85.0	5.0
	Significance	$P = 0.009$	$P < 0.001$	$P = 0.001$
	LSD (12 df)	27.86	16.64	30.16

[a] 10 flowers sampled per plot; assessed after 14 days incubation.

Table 2.2.5: Occurrence of *B. cinerea* and *Trichoderma* spp. on incubated strawberry flowers sampled on 1 August 2008 after the introduction of bees

Treat-ments	Botrytis control treatments (with + or without – products)	Mean % flowers[a] infected by: *B. cinerea*	*Trichoderma* spp.	Other
T1	- Fungicide / - Binab	82.5	0	50.8
T2	+ Fungicide / - Binab	72.5	0	62.5
T3	- Fungicide / + Binab	35.0	92.5	37.5
T4	+ Fungicide / + Binab	12.5	92.5	28.9
	Significance	$P = 0.006$	$P < 0.001$	ns
	LSD (12 df)	38.58	10.43	35.28

[a] 10 flowers sampled per plot; assessed after 14 days incubation.

Table 2.2.6: Occurrence of *B. cinerea* and *Trichoderma spp.* on incubated strawberry flowers sampled on 6 August 2008 after the introduction of bees

Treat-ments	Botrytis control treatments (with + or without – products)	Mean % flowers[a] infected by: *B. cinerea*	*Trichoderma* spp.	Other
T1	- Fungicide / - Binab	90.0	0	2.5
T2	+ Fungicide / - Binab	85.0	0	10.0
T3	- Fungicide / + Binab	75.0	40.0	12.5
T4	+ Fungicide / + Binab	46.7	30.3	13.1
	Significance	$P < 0.05$	$P = 0.002$	ns
	LSD (12 df)	24.90	21.59	17.01

[a] 10 flowers sampled per plot, assessed after 14 days incubation.

Fruit losses to botrytis

Total fruit losses to visible botrytis (by weight) ranged from 2.9% to 4.8% with no significant differences between treatments (Table 2.2.7). Conditions produced in the tunnels to assist the development of botrytis in the crop (sporulating plates of *B. cinerea* and overhead misting throughout the day during flowering and fruiting) would have increased the probability of infection compared with commercial tunnels.

In fruit picked during week 35, a significantly lower weight of fruit was lost to botrytis from the fungicide-treated plants (2.3%) and the Binab T-Vector treated plants (2.6%) than on the untreated (5.6%). Surprisingly, losses in the combined fungicide and Binab T-Vector treatment (3.7%) were not significantly reduced compared with the untreated (Table 2.2.7).

The total number of fruit affected by botrytis over the season (around 60 in each plot) was not significantly reduced by any treatment (Table 2.2.9).

The incidence of latent botrytis within or on fruit as detected by an incubation test differed significantly between treatments in Picks 1 and 2, but not Picks 3 and 4 (Table 2.2.11). In the first pick, the combined fungicide and Binab TF WP treatment had significantly fewer fruit affected than other treatments (although the freshly open flowers probably were too early to receive the fungicide); in the second pick, there was least botrytis in the fungicide-treated (no Binab) fruit.

It is possible that some botrytis spores may have dispersed directly onto the fruit from the *B. cinerea* plates. The fungus may not have developed until the fruit was mature and incubated in polythene bags. If this occurred, it is unlikely that the *Trichoderma* spp. would have provided control as the dispersal by bees is primarily into flowers.

Other diseases

No treatment significantly affected occurrence of *Rhizopus* on incubated fruit (Table 2.2.13).

The incidence of *Penicillium* was greatest on untreated (receiving neither botrytis fungicides nor Binab products) incubated fruit in Picks 1-3, and fungicide-treated fruit (receiving no Binab products) of Pick 4 (Table 2.2.14).

Yield and quality

Total yield (around 15 kg per plot) was not significantly affected by treatment. There appeared to be greater yield in Tunnel 4 than Tunnel 1 (p=0.06) (Table 2.2.8).

Over the five weeks that fruit yields were recorded, treatment had no significant effect on the proportion of Class 1 fruit, Class 2 fruit or unmarketable fruit, or on the time to 50% yield (Tables 2.2.8 and 2.2.10).

Over the five weeks that fruit yields were recorded, the *Trichoderma*-only treated Class 1 fruit were significantly heavier, but not those from plots with fungicide plus *Trichoderma* (Table 2.2.10).

Fruit development in relation to fungicide application during flowering

The wool tags showed that Pick 1 Week 32 fruit would have been open flowers between 4 to 11 July and so were produced by the few flowers present when the Binab TF WP was sprayed, pre-bee introduction and pre-application of botrytis fungicides. All later fruit picks would have had bees working the flowers, although cool conditions reduced bee activity in Week 33. Pick 2 Week 33 fruit were flowers between 16 to 22 July and received Signum, Pick 3 Week 34 fruit were flowers between 22 to 25 July and received Frupica, and Pick 4 Week 35 were flowers between 25 to 28 July and received Switch only on the latest flowers.

Table 2.2.7: Effect of a fungicide programme and bee-vectored *Trichoderma* (Binab T-Vector) on the weekly proportion (by weight) of strawberry fruit with *Botrytis* fruit rot and the total loss to botrytis over the whole cropping period

Treatment	% weekly fruit pick affected by weight:				Total loss to *Botrytis* (g/plot)	% of total weight los to *Botrytis*
	Wks 32/33*	Wk 34	Wk 35	Wk 36		
T1 Untreated	0.9	4.8	5.6	8.6	666	4.0
T2 Fungicide	0.6	4.2	2.3	8.3	468	2.9
T3 Binab	2.2	7.4	2.6	10.5	661	4.7
T4 Fungicide + Binab	1.4	7	3.7	14.6	659	4.8
Significance	ns	ns	*P* <0.06	ns	ns	ns
LSD (12 df)	1.65	9.96	2.51	10.49	660.3	4.66

* First full week and part of week preceding.

Table 2.2.8: Effect of a fungicide programme and bee-vectored *Trichoderma* (Binab T-Vector) on quality including weight of fruit with *Botrytis*, and total fruit yield

Treatment	Accumulated fruit yield (g / plot):				
	Class 1	Class 2	Unmarketable	*Botrytis*	Total
T1 Untreated	4931	8160	2340	666	16,097
T2 Fungicide	5139	8493	2304	468	16,404
T3 Binab	4505	7064	1804	661	14,034
T4 Fungicide + Binab	3145	7912	2368	659	14,083
Significance	ns	ns	ns	ns	*P* <0.06
LSD (12 df)	1721.2	1477.0	697.2	660.3	2125.1

Table 2.2.9: Effect of a fungicide programme and bee-vectored *Trichoderma* (Binab T-Vector) on the mean number of fruit per plot, and the % with *Botrytis* in the field

Treatment	Mean total fruit per plot	Mean % with botrytis per plot
T1 Untreated	1116	5.4
T2 Fungicide	1081	3.7
T3 Binab	882	6.8
T4 Fungicide + Binab	955	5.5
Significance	*P* <0.06	ns
LSD (12 df)	183.4	5.34

Table 2.2.10: Effect of a fungicide programme and bee-vectored *Trichoderma* (Binab T-Vector) on mean fruit weight and days to achieve 50% yield

Treatment	Mean fruit weight (g):		Days to 50% yield by weight:	
	Class 1	Class 2	Total	*Botrytis*
T1 Untreated	14.01	16.61	15	23
T2 Fungicide	14.56	16.47	15	23
T3 Binab	16.72	18.41	14	18
T4 Fungicide + Binab	14.69	17.52	14	23
Significance	*P*<0.001	ns	ns	ns
LSD (12 df)	1.026	3.489	2.2	6

Table 2.2.11: Effect of a fungicide programme and bee-vectored *Trichoderma* (Binab T-Vector) on % incubated fruit with latent *Botrytis*

Treatment	Pick 1 Wk 32	Pick 2 Wk 33	Pick 3 Wk 34	Pick 4 Wk 35
T1 Untreated	100 (0.0)	87 (4.7)	65 (13.0)	68 (12.2)
T2 Fungicide	100 (0.0)	78 (5.7)	53 (13.6)	33 (12.3)
T3 Binab	97 (2.0)	97 (2.5)	61 (13.4)	38 (12.8)
T4 Fungicide + Binab	75 (5.0)	92 (3.8)	75 (11.9)	42 (12.9)
Significance:	*P* <0.05	P=0.05	ns	ns

( ) = Standard error. 30 fruit picked in Wk 32, 120 picked in other weeks.

Table 2.2.12: Effect of a fungicide programme and bee-vectored *Trichoderma* on % of incubated fruit with latent *Trichoderma*

Treatment	Pick 1 Wk 32	Pick 2 Wk 33	Pick 3 Wk 34	Pick 4 Wk 35
T1 Untreated	0 (0.0)	0 (0.0)	9 (7.1)	2 (2.7)
T2 Fungicide	0 (0.0)	0 (0.0)	27 (10.9)	8 (5.9)
T3 Binab	20 (6.2)	18 (4.0)	50 (12.3)	11 (6.8)
T4 Fungicide + Binab	18 (6.1)	38 (5.1)	38 (12.0)	15 (7.7)
Significance	*P*=0.051	*P*<0.001	ns	ns

( ) = Standard error. 30 fruit picked in Wk 32, 120 picked in other weeks.

Table 2.2.13: Effect of a fungicide programme and bee-vectored *Trichoderma* on % of incubated fruit with latent *Rhizopus*

Treatment	Pick 1 Wk 32	Pick 2 Wk 33	Pick 3 Wk 34	Pick 4 Wk 35
T1 Untreated	100 (0.0)	25 (7.3)	32 (10.4)	67 (12.8)
T2 Fungicide	100 (0.0)	23 (6.9)	50 (11.2)	79 (11.0)
T3 Binab	100 (0.0)	7.5 (4.4)	31 (10.3)	86 (9.6)
T4 Fungicide + Binab	100 (0.0)	9.2 (4.8)	17 (8.3)	73 (12.1)
Significance:	ns	ns	ns	ns

( ) = Standard error. 30 fruit picked in Wk 32, 120 picked in other weeks.

Table 2.2.14: Effect of a fungicide programme and bee-vectored *Trichoderma* on % incubated fruit with latent *Penicillium*

Treatment	Pick 1 Wk 32	Pick 2 Wk 33	Pick 3 Wk 34	Pick 4 Wk 35
T1 Untreated	48 (9.7)	19 (4.7)	5 (2.2)	6 (4.1)
T2 Fungicide	31 (8.0)	6 (2.8)	1 (0.9)	22 (7.2)
T3 Binab	33 (2.9)	2 (1.5)	2 (1.7)	0 (0.0)
T4 Fungicide + Binab	11 (5.2)	1 (1.2)	0 (0.0)	2 (2.2)
Significance	$P<0.05$	$P=0.005$	$P=0.098$	$P<0.05$

( ) = Standard error. 30 fruit picked in Wk 32, 120 picked in other weeks.

Conclusions

Trichoderma dispersal

- *Trichoderma* was successfully introduced to the flowers of strawberry plants using honey and bumble bees. The bees picked up Binab T-Vector powder from dispensers mounted on the hive exits and both species were observed landing on flowers.
- Incubated flowers had a lower incidence of *B. cinerea* in the tunnel where *Trichoderma* was used.

Control of B. cinerea by Trichoderma

- There was little effect of *Trichoderma* use on fruit botrytis, either of latent infection or visible rot.

Fungicide efficacy

- Four fungicide sprays applied during flowering against botrytis had no consistent effect on the incidence of *B. cinerea* on incubated flowers.
- Botrytis resulted in 3-5% loss of fruit by weight. This was not significantly reduced by the *Trichoderma* spp., fungicides or a combination of the two.

Trichoderma and fungicide interaction

- Infection of flowers and fruit by botrytis was similar when *Trichoderma* was used with or without fungicide application. This suggests that there was neither positive nor negative interaction between the activities of the fungicide and biological control agent products.

References

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Task 2.3: Validate and use the strawberry botrytis disease forecasting model (BOTEM) in a protected environment (EMR, Yr 1-3)

We need to determine whether the current EMR forecasting system (Botem), developed for open-field crops, can give reliable/accurate prediction of flower infections under protection.

Methods

To validate the model, we have regularly sampled flowers to determine the incidence of latent flower infections by botrytis. Sampling was done in the unsprayed tunnel of raspberry plants of cv. Elsanta (April-May) and an everbearer variety (September – October) at Manor Farm, near Borough Green, Kent, every Monday, Wednesday and Friday during flowering. On each day, 100 old flowers were randomly be sampled across the whole length of the tunnel.

These flowers were collected individually into 25 ml universal bottles and surface sterilized with 10 ml sodium hypochlorite (0.025% available chlorine [w/v]) (5% of Domestos) for 15 min to remove any spores on the surface on a shaker. The flowers were then rinsed with distilled water and placed separately on a piece of filter papers thoroughly wetted with distilled water in small sterile Petri dishes. The dishes were incubated in a glasshouse compartment (C10 or C14) at approximately 20°C for 1-2 weeks after which the flowers are examined for conidiophores of *B. cinerea*. Any flower on which conidiophores are detected was classified as infected.

Weather data (temperature and relative humidity) was collected at intervals of 30 minutes using USB-502 loggers inside the tunnel.

Results

We have obtained two sets of data (Table 1) on the percentage of latent infection of flowers. One set was collected in April-May on Elsanta and an everbearer cultivar. For the Elsanta set, there was a very low level of botrytis infection except one sample. However, the level of botrytis latent infection of flowers on everbearers was very high and varied considerably from sample to sample. The quality of flowers on everbearers was much lower than on Elsanta; petals in most flowers on the former had brown marks.

In addition, during the period of September and October, weather is generally much cooler and moister than in April-May. However, the lack of botrytis in Elsanta is unlikely to be due to a lack of inoculum since rotten fruit/due to botrytis was frequently observed in the open field planting immediately next to the unsprayed tunnel used for sampling.

We shall repeat the flower sampling in year 2 and then use the four data sets to evaluate the accuracy of Botem predictions. In addition to flower sampling, we shall conduct field experiments to determine where control programme based on the new model could achieve satisfactory disease control with reduced input.

Table 2.3.1, Percentage of latent infection of strawberry flowers by *Botrytis cinerea* in 2008 in Kent

Elsanta			Everbearer	
Sampling date	Infection		Sampling date	Infection
16/04/2008	3		05/09/2008	38
18/04/2008	2		08/09/2008	58
21/04/2008	2		10/09/2008	75
23/04/2008	5		12/09/2008	77
25/04/2008	4		15/09/2008	85
28/04/2008	51		17/09/2008	93
30/04/2008	4		19/09/2008	77
02/05/2008	3		22/09/2008	66
06/05/2008	3		24/09/2008	88
08/05/2008	2		26/09/2008	50
12/05/2008	4		29/09/2008	86
14/05/2008	0		01/10/2008	55
16/05/2008	0		03/10/2008	81
19/05/2008	2			

Objective 3. To establish the importance of alternative hosts as sources of inoculum of *Colletotrichum acutatum* for strawberries in order to develop a sustainable IPM system for blackspot

Task 3.1: Use molecular methods to compare the population of C. acutatum from alternative hosts with that from strawberry (EMR, years 1-2)

Objective: To compare populations of *C. acutatum* from alternative hosts with that from strawberry

Method

Isolation of C. acutatum

Strawberry fruits cv. Elsanta were harvested from a three year old protected crop at EMR on six dates. Harvested fruit was damp incubated in trays for 7 days after which fruit with symptoms typical of blackspot were collected for isolation. *C. acutatum* was isolated from blackspot lesions on fruit on to Potato Dextrose Agar (PDA) and incubated at 20^{o}C. One hundred strawberry petioles were collected from the strawberry crop in May and again in July. The petioles were surface sterilised in 5% bleach, rinsed, dipped in paraquat for 1 min., rinsed and then damp incubated under UV light for 7-14 days. The petioles were then examined for sporing colonies of *C. acutatum*. Any present were transferred to PDA and incubated at 20^{o}C. Weeds were collected from the strawberry crop from within the polytunnel and from the windbreak surrounding the polytunnel. These were damp incubated under UV light. Any likely colonies of *C. acutatum* sporing on the weeds were isolated on to PDA. Isolates of *C. acutatum* were similarly obtained from other fruit crops, including stored apples from orchards at EMR and cherry fruits from a commercial orchard in Kent.

Once cultures were free of contamination isolates were grown on sterile cellophane on PDA and once growth was established the mycelium was scraped off and stored in Eppendorf tubes at -80^{o}C prior to molecular analysis. The number and source of the isolates obtained are shown in Table 3.1.1. Isolates from the culture collection at EMR have also been included. These are the first 11 isolates in Table 3.1.1.

To date *C. acutatum* has been observed sporing on incubated weed samples but it has not been possible to obtain uncontaminated cultures. The weeds in the strawberry plot DM180 will be resampled.

Table 3.1.1. Isolates of *Colletotrichum acutatum* obtained for the population study October 2008

Host	Source	No. of isolates
Liriodendron	?	1
Cyclamen	?	1
Rose	?	1
Apple	Chile	1
Bramley	Kent	1
Strawberry	EMR	1
Strawberry	Isle of Wight	1
Strawberry	Kent	1
Strawberry	Suffolk	1
Strawberry	Nottinghamshire	1
Strawberry	Cornwall	1
Strawberry fruit	DM180 EMR 12/6/08*	4
Strawberry fruit	DM180 EMR 17/6/08	7
Strawberry fruit	DM180 EMR 18/6/08	3
Strawberry fruit	DM180 EMR 24/6/08	6
Strawberry fruit	DM180 EMR 27/6/08	12
Strawberry fruit	DM180 EMR 3/7/08	5
Strawberry green fruit	DM180 EMR 25/6/08	4
Strawberry petioles	DM180 EMR May 08	0
Strawberry petioles	DM180 EMR July 08	0
Cherry fruit EMR	WE plots EMR	0
Cherry fruit	Kent 2008	6
Mayweed	DM180 EMR	0
Groundsel	DM180 EMR	2
Thistle	DM180 EMR	0
Sowthistle	DM180EMR	0
Willow herb	DM180EMR	4
Black nightshade	DM180 EMR	0
Lupin	Cambs	1
Raspberry fruit	Cambs	1
Apple cv. Gala	TL161 EMR	21
Apple cv. Gala	WM plots EMR	2
Apple cv. Cox	TL109 EMR	15
Apple cv. Cox	CW109 EMR	1

* Harvest date

Molecular analysis

We have developed six SSR molecular markers. Preliminary screening of several selected isolates showed that there was much polymorphism for five of the six markers. We have obtained 123 isolates in total and extracted DNA for PCR. These PCR products will be analysed for each SSR markers in March 2009. In addition, we have also obtained additional number of isolates from weeds; these isolates are being processed for molecular characterisation.

Task 3.2: Use artificial inoculation to confirm the molecular findings (EMR)

Task 3.3: Evaluation of biofumigants to eliminate Colletotrichum-infested debris in soil

Work on this task has not yet started. The work is planned for Autumn / Winter 2008/09.

Task 3.4: Development of simple guidelines for blackspot management

Work on this task has not yet started. The work is planned for Autumn / Winter 2008/09

Objective 4: To develop an IPM system for European tarnished plant bug on strawberry using a trap crop, a semiochemical female repellant and tractor mounted vacuuming.

Task 4.1. Quantify the relative attractancy of candidate herbaceous flowering plants and cover crops to L. rugulipennis (EMR Year 1)

Summary

Chenopodium album (fathen), *Matricaria recutita* (mayweed) were sown to attract *Lygus rugulipennis*; these plants then acted as a source in experiments to compare the attractiveness of other plant species to the pest. Samples of arthropods were collected from the chosen trap plants *Artemisia vulgaris* (mugwort), *Matricaria recutita* (scented mayweed), *Medicago sativa* (lucerne- alfalfa), *Sinapsis arvensis* (charlock-mustard) and *Vicia sativa* (common vetch). Results showed that, overall, the highest numbers of *L. rugulipennis* nymphs were collected from mustard and mayweed. Lower numbers of *L. rugulipennis* adults were collected from lucerne than from the other three plant species.

A mark-recapture experiment was undertaken to quantify the attractiveness of the trap plants to *L. rugulipennis*. Source plants were sprayed with a protein marker and the trap plants sampled for *L. rugulipennis* adults 2 and 5 days after treatment. The ELISA analysis of these collected individuals is currently in progress.

Methods

Choice of plants: 'source' plants

Previous experience at EMR and elsewhere has shown that large numbers of *Lygus rugulipennis* are found on naturally occurring weed plants, in particular on *Chenopodium album* (fathen), *Matricaria recutita* (mayweed) and *Senecio vulgaris* (groundsel). Fathen and mayweed were thus chosen as the 'source' plants for the experiment; it was expected that *L. rugulipennis* populations would develop on these weeds and then individuals would disperse onto other plants as these weeds senesced. Source seeds were purchased as an 'uncleaned' mix to reduce costs.

Choice of plants: 'trap' plants

An extensive literature search was made to identify candidate plant species that have been shown to be attractive to capsids in other countries and cropping systems. Species with reported attractiveness to capsids are given in Table 4.1.1.

Table 4.1.1. Species with reported attractiveness to capsids from published literature

Family	Species	Common name
Leguminosae	*Medicago sativa*	Lucerne
	Trifolium pratense	Red clover
	Melilotus officinalis	Ribbed melilot
	Vicia sativa	Common vetch
Compositae	*Artemisia vulgaris*	Mugwort
	Matricaria recutita	Scented mayweed
	Senecio vulgaris	Groundsel
Chenopodiaceae	*Chenopodium album*	Fat hen
Cruciferae	*Sinapsis arvensis*	Charlock (mustard)
	Lobularia maritima	Alyssum or Alison

Table 4.1.2. Species chosen for the experiments

Species	Common name	Sowing rate (g/m^2)
Artemisia vulgaris	Mugwort	1
Matricaria recutita	Scented mayweed	0.4
Medicago sativa	Lucerne (alfalfa)	6
Sinapsis arvensis	Charlock	6
Vicia sativa	Common vetch	8

The choice of plants and the sowing rates used for the trap crop experiments are given in Table 4.1.2. Species were taken from 3 of the 4 plant families in Table 4.1.1; fat hen was not selected as it was considered that due to its prolific self seeding it would be difficult to manage in a commercial situation.

Management of the planting

The source plants were sown at 2.5 kg fat hen and 2.8 kg mayweed /ha on 19 May from a tractor mounted seed box. The seed was mixed with sawdust to enable an even distribution, and four parallel strips, each of 3 m wide, were sown in two pairs. The first pair of strips was separated from the second pair by c. 30 m. Within each pair the strips were 22 m apart. The trap plants were sown by hand on 4 July. These were sown between the pair of source plant strips such that each plot of trap plants was 10 m away from each source strip. The trap plants were sown in 2 x 5 m plots in a replicated blocks design with plots 2 m apart. The seeds were watered from a tractor mounted bowser after sowing.

There were 6 treatments (5 sown plant species as in Table 2 plus strawberry as a control) and each treatment was replicated 4 times. At the time the trap plants were sown the source plants were beginning to flower. Strawberry plants variety ‘Eros’ were purchased as 60 day plants on 29 July. They were planted into growbags, with 3 plants in each bag, and kept in a glasshouse until required for the experiment. They were taken to the field on 26 August at which time they were beginning to produce flowers. Sixteen bags (total of 48 plants) were used per experimental plot. The non-sown area of the field was treated with a contact herbicide on 14 August. A

Pyrethrum application (short persistence insecticide) was made to the trap plants on 27 August in an attempt to reduce the numbers of *L. rugulipennis* that had dispersed onto the trap plants before the mark-recapture experiment had begun.

Sampling: population development

The source plants were sampled on 15 August by using a sweep net; a sample consisted of 10 sweeps of the vegetation. As the plants were too small on 19 August to be able to do a sweep sample, the trap crops were sampled by tapping the foliage over a white bowl (23 cm diameter) and counting the numbers of *L. rugulipennis* present. On 20 August a comparison was made of catches from sweep net and suction sampling to identify the optimal sampling strategy from the trap plants; samples were 20 'sucks' and 10 sweeps of the foliage/flowers in both source and trap plants. In addition, sweep samples were taken on 26 August and 13 and 29 September in source and trap plants to monitor the population development of *L. rugulipennis* on the different plants.

Sampling: mark- recapture experiment

A protein based mark and recapture method with an attendant monoclonal antibody (MAb) assay is being used to assess the rate of invasion of plots of attractive trap plants by adult *L. rugulipennis*. The protein marker used was milk; a 20% milk solution containing 0.3 g/l EDTA was applied to the source plants on 27 August with an Autoglide arable sprayer at 1000 l/ha. On 29 August (2 days after treatment with the milk marker-DAT) tap samples were taken on all of the trap plant plots. A 30 tap sample was taken over a white bowl from each sown plot. Any *L. rugulipennis* adults collected from these samples were placed individually into specimen tubes. After each catch of *L. rugulipennis* the collecting bowl was washed with water to reduce possible cross contamination of any non-marked individuals with protein derived from marked individuals. Strawberry plants were sampled by visual inspection and tapping of insects onto the grow bag surface. The collected insects were returned to the laboratory and held in a freezer at -20ºC until assayed. A second tap sample and collection of individuals was made on 1 September (5 DAT). There was no rainfall within the 5 days duration of this experiment. Samples were also taken of individual *L. rugulipennis* from the source plants on both sample dates; these are being used as positive controls in the ELISA assay. Individuals collected from another area of the farm in October 2008 are being used as negative controls.

ELISA assay

This assay is currently in progress. The protocol being used is based on that described in Jones et al., 2006; this assay is an indirect ELISA. Individual insects are washed in buffer to remove the protein marker. Each sample consisting of the buffer containing the marker from an individual insect washing (plus positive and negative controls) is added to an individual well in a 96 well microplate and incubated for 2 hours at 37ºC, where the marker becomes adsorbed onto the plate. The plate is then washed, blocker (20% bovine serum) added to the wells, and then incubated for 1 hour at 37ºC. The plate is washed again and the primary antibody, which is specific to the protein marker (sheep anti casein antibody), is added to the wells and the plate is incubated for 30 mins. After another wash the secondary antibody (rabbit anti sheep) is added to the wells and incubated for 2 hours. The plate is then washed again and substrate added. After stopping the reaction with sulphuric acid the absorbance of the samples is read on a plate reader at 450 nm.

Results

Growth of the sown plants

The source plants germinated well and were beginning to flower at the time the trap crops were sown. Plants within the source strips continued to flower throughout the summer and there were still some flowering plants remaining on 29 September. An overview of the growth characteristics of the trap plants is given in Table 4.1.3.

Table 4.1.3. Growth characteristics of the trap plants used in the experiment

date	mugwort		mayweed		vetch		mustard		lucerne		strawberry	
	g	f	g	f	g	f	g	f	g	f	g	f
15 Aug	x	-	y	x	y	x	y	y	y	x	y	x
20 Aug	x	-	y	y	y	x	y	y	y	x	y	x
26 Aug	x	-	y	y	y	x	y	y	y	x	y	y
13 Sept	x	-	y	y	y	x	y	s	y	x	y	y
29 Sept	x	-	y	y	y	x	y	s	y	x	y	x

g=germinated; f=flowers present; s=senescing

All the sown seeds with the exception of the mugwort germinated successfully, although there was pressure from other weed species within the field. The mustard, mayweed and strawberry flowered but the other plants did not. By 13 September the mustard was going to seed but there were some flowers remaining in the mayweed plots. By this time the vetch plots had become overgrown with other weeds and could not be sampled. Fat hen overtook several of the experimental plots and was sampled to compare its attractiveness with the other sown plants.

Population dynamics of L. rugulipennis

In the first sample taken from the source plants on 15 August a total of 13 1-2 instar *L. rugulipennis* nymphs, 24 3-5 instar nymphs and 15 adults were caught in 30 sweeps, indicating that *L. rugulipennis* was present and reproducing on these plants. Some of the source plants were beginning to senesce at this time.

In the sample taken on 18 August *L. rugulipennis* were found to be present on the trap plants; the highest number caught was 3 adults per tap in one of the plots of mayweed.

In the comparison of sampling methods for *L. rugulipennis* done on 20 August similar numbers of nymphs were caught with both techniques (square root transformed means from all samples were 2.34 for sweep and 2.62 for suction sampling respectively). However significantly higher numbers of adult *L. rugulipennis* were caught in sweep samples than in suction samples (square root transformed means from all samples were 3.46 for sweep and 1.85 for suction sampling respectively; $P<0.001$). These results show that sweep sampling is the most effective method for assessing *L. rugulipennis* populations on flowering plants. However, because of their size and growth habits it is not possible to use sweep sampling on strawberry plants.

Numbers of predators and Hymenopteran parasitoids caught were also recorded in the sweep and suction sampling comparison. Numbers were low and only numbers of parasitoids were sufficient for statistical analysis. Significantly higher numbers were caught in suction samples compared to sweep samples ($P<0.001$). Overall, square root transformed means were 2.97 for sweep and 5.41 for suction samples.

In the population monitoring programme both *L. rugulipennis* and predatory arthropods were found on the plants. All developmental stages of *L. rugulipennis* were present indicating that *L.*

rugulipennis was reproducing and developing on all the trap plants tested and on the source plants. There was no consistent relationship between numbers of nymphs or adults of *L. rugulipennis* on any trap plant compared with the source plants over the sampling period. Adults and all stages of nymphs were present on the senescing source plants in the last sample taken on 29 September, although 1-2 instar nymphs were significantly lower on the source plants and mustard plots compared with the other plants on this date ($P<0.001$); square root transformed means were 4.24, 3.87, 1.31, 2.24, and 0.25 for mayweed, vetch, mustard, lucerne and source plants, respectively. These results indicate that adult female *L. rugulipennis* were still ovipositing into late September. There was no difference in numbers of *L. rugulipennis* adults on any trap or source plant on 29 September; square root transformed means were 3.30, 2.45, 3.89, 2.26 and 4.45 for mayweed, vetch, mustard, lucerne and source plants, respectively. Thus *L. rugulipennis* were still developing on the plants until late September.

There was a significant effect of plant type on total numbers of *L. rugulipennis* nymphs caught ($P<0.05$). There was a significant effect of time of sampling ($P<0.001$), with higher numbers caught in September than August (Tables 4.1.4 & 4.1.5); this was a result of the overall population development from adults (higher numbers caught in August-see below) to eggs to nymphs. There was also a treatment and time interaction ($P=0.005$), with the effect of plant type on numbers caught differing over time as the plants developed and senesced. Overall, significantly higher numbers of nymphs of *L. rugulipennis* were caught on the mustard and mayweed plots compared with the vetch and lucerne; there was no significant difference in catches on mayweed and lucerne ($P=0.05$) (Tables 4.1.4 - 4.1.5). Highest numbers of *L. rugulipennis* nymphs were caught on mustard in the first three samples, but by 29 September these plants were senescing and numbers of nymphs caught were lower (Tables 4.1.4 & 4.1.5).

Table 4.1.4. Means (square root transformed data) of *L. rugulipennis* nymphs caught in sweep samples of potential trap crops

Treatment	20 Aug	26 Aug	13 Sept	29 Sept	Overall mean
Mayweed	2.36	3.89	6.85	6.54	4.91
Vetch	2.07	2.60	5.65	6.17	4.12
Mustard	3.65	7.28	9.28	3.58	5.95
Lucerne	1.76	3.57	5.06	4.10	3.62
	LSD comparing means between treatments (21 df) 2.16				LSD (8 df) 1.35

Table 4.1.5. Means (back-transformed data) of *L. rugulipennis* nymphs caught in sweep samples of potential trap crops

Treatment	20 Aug	26 Aug	13 Sept	29 Sept	Overall mean
Mayweed	5.6	15.1	46.9	42.8	24.1
Vetch	4.3	6.8	31.9	38.1	17.0
Mustard	13.3	53.0	86.1	12.8	35.4
Lucerne	3.1	12.7	25.6	16.8	13.1

There was also a significant effect of plant type on numbers of *L. rugulipennis* adults caught ($P<0.01$) (Tables 4.1.6 & 4.1.7), with lower numbers caught on the lucerne plots. This may in part be the result of the relative growth patterns of the different trap crop plants (see Table 4.1.3). There was a significant effect of time of sampling ($P<0.001$) with higher numbers of adults caught in August than in September. There was also a plant type and time interaction ($P<0.01$) with the effect of plant type on numbers caught differing over time as the plants developed and senesced. This is in line with the results obtained for nymphs.

The fact that there was no significant difference in numbers of adults caught on mayweed, vetch

or mustard, but there were higher numbers of nymphs caught on mustard and mayweed compared with the other potential trap plants may imply that these plants are better hosts for *L. rugulipennis*, possibly increasing fecundity of adults and/or subsequent survival of nymphs.

Table 4.1.6. Means (square root transformed data) of *L. rugulipennis* adults caught in sweep samples of potential trap crops

Treatment	20 Aug	26 Aug	13 Sept	29 Sept	Overall mean
Mayweed	4.534	4.751	2.212	3.299	3.699
Vetch	4.745	3.501	2.038	2.487	3.193
Mustard	3.359	3.043	2.870	3.885	3.289
Lucerne	1.694	1.992	1.250	2.258	1.799
	LSD comparing means between treatments (24 df)				LSD (8 df)
			1.315		0.960

Table 4.1.7. Means (back-transformed data) of *L. rugulipennis* adults caught in sweep samples of potential trap crops

Treatment	20 Aug	26 Aug	13 Sept	29 Sept	Overall mean
Mayweed	20.6	22.6	4.9	10.9	13.7
Vetch	23.5	12.3	4.2	6.2	10.2
Mustard	11.3	9.3	8.2	15.1	10.1
Lucerne	2.9	4.0	1.7	5.1	3.2

Numbers of beneficials caught in the different treatments were lower than numbers of *L. rugulipennis*; highest numbers of beneficial species were found on the mayweed plots on 13 September (Table 4.1.8).

Table 4.1.8. Mean number of predators in sweep samples from trap and source plants on 13 September 2008.

Trap plants	Nabids	Orius	Anthocorids	Spiders	Chrysopid larvae
Matricaria recutita	0.3	21.5	3.5	0.5	1.0
Medicago sativa	0.5	0	0.5	1.8	1.8
Sinapsis arvensis	0	4	1.5	0.5	0
Vicia sativa +	0	1	0	0	1
Source plants	2.8	2.5	0.5	1.0	1.0

+ 1 plot only

Mark- recapture experiment

There was no apparent effect of the Pyrethrum application made on 27 August on numbers of adults or nymphs on the trap plants (Tables 4.1.7 – 4.1.7). Numbers of adult *L. rugulipennis* collected from the different plant species 2 and 5 days after application of the protein marker are shown in Table 4.1.9. These individuals are currently being analysed using the ELISA technique. Assays will be completed before the end of March and results will be presented at the following consortium meeting.

Table 4.1.9. Mean number of adult *L. rugulipennis* caught in tap samples 2 and 5 days after application of a protein marker

Trap plants	29 August	1 September
Matricaria recutita	4.0	3.7
Medicago sativa	0.5	0.3
Sinapsis arvensis	4.8	4.7
Vicia sativa	2.3	0
Strawberry	3.3	0
Source plants	11.0	8.3

Task 4.2. Evaluate the use of hexyl butyrate as a repellant of L. rugulipennis females (EMR, NRI Yrs 1-3)

Release rate measurements for hexyl butyrate are in progress at NRI in a laboratory wind tunnel at 27°C and 8 km/hr wind speed. Release from polyethylene sachets 2.5 cm x 2.5 cm x 120 µm thick was rapid with a linear release rate of 36.9 mg per day (Figure 4.2.1). Release from a range of polyethylene vials is being measured; the release rate from a vial 20 mm x 8 mm x 1.5 mm thick was 2.6 mg per day (Figure 4.2.1). If this vial was loaded with 200 mg hexyl butyrate this dispenser would last for > 77d in the field.

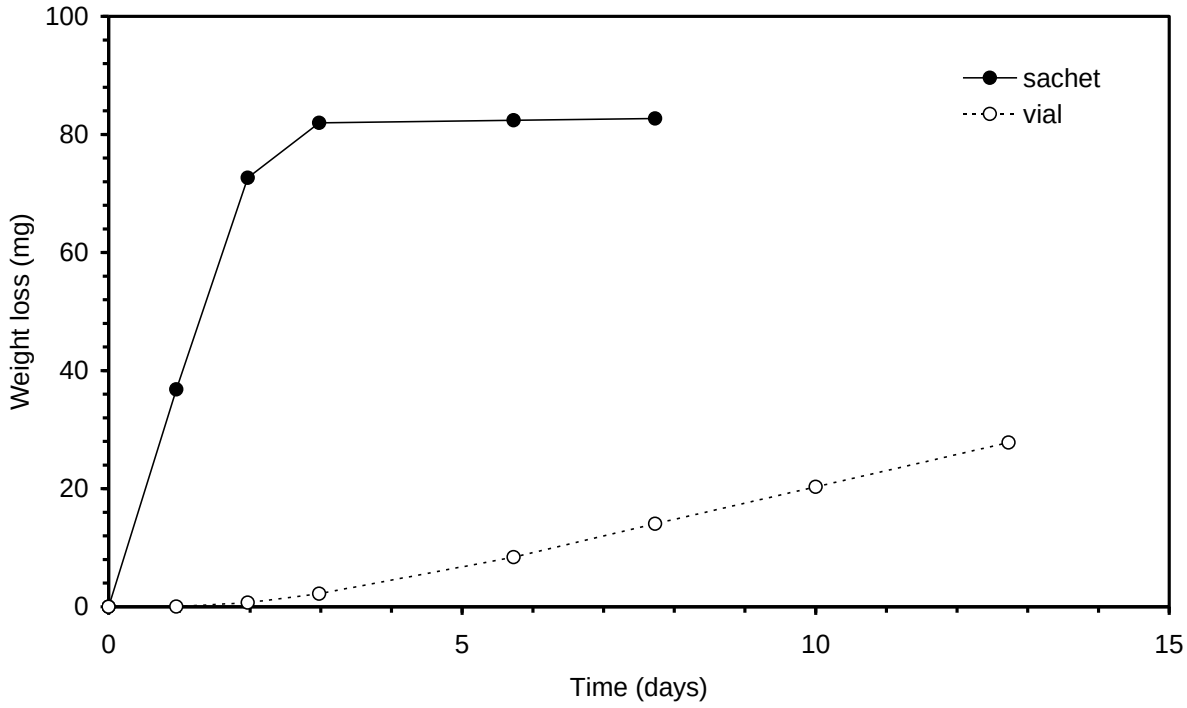


Figure 4.2.1. Release of hexyl butyrate from polyethylene sachets and polyethylene vials in a laboratory wind tunnel (mean of duplicate samples).

Task 4.3. Evaluate the use of regularly vacuumed trap crops in an integrated management system in commercial strawberry (EMR, Yrs 2, 3).

No work was due for this task in year 1.

Objective 5: To develop an IPM system for aphids which combines the provision of flowering herbage as sources of aphid natural enemies, semiochemical attractants to attract them into strawberry crops, introductions of biocontrol agents and end of season clean up sprays with selective insecticides.

Task 5.1. Evaluate the effectiveness of flowering plants to attract aphid predators and parasitoids (EMR, Yrs 1, 2, 3)

Summary

Medicago sativa, (lucerne), *Silene dioica* (red campion) *Galium verum* (lady's bedstraw), *Leucanthemum vulgare* (ox eye daisy), and a mixture of *Centaurea cyanus* (cornflower) + *Chrysanthemum segetum* (corn marigold) + *Anthemis arvensis* (corn chamomile) were sown in a replicated block design around a commercial strawberry plantation. Unfortunately many of the plots became overgrown with thistles and it was only possible to sample the flower mix plots. Both predatory arthropods and the pest *L. rugulipennis* were found on these plots.

Methods

Choice of plant species

An extensive literature search was undertaken to derive a short list of plant species attractive to beneficial species. Four species were selected and compared with a mix of three species that have been shown to be attractive to beneficials in orchards at EMR. The species and sowing rates are given in Table 5.1.1.

Table 5.1.1. Species chosen for assessment of their attractiveness to beneficial arthropods

Species	Common name	Sowing rate (g/m^2)
Medicago sativa	Lucerne	3
Silene dioica	Red campion	3
Galium verum	Lady's bedstraw	0.5
Leucanthemum vulgare	Ox eye daisy	0.5
Centaurea cyanus + *Chrysanthemum segetum* + *Anthemis arvensis*	Cornflower + corn marigold + corn chamomile	3 + 1.5 + 0.4

Experimental design

The experiment was set up around a newly planted strawberry crop at Robert Boucher & Son of Newlands Farm, Teynham, Sittingbourne, Kent, ME9 9JQ. The seeds were sown into a prepared bed 2 m wide. Each plot was 4 x 2 m. There were 5 treatments replicated 4 times in a randomised blocks design. There was no gap between the different plots. The seeds were sown by hand on 16 May and soil was raked over to cover them.

In addition the grower had sown a 'bee mix' alongside another strawberry planting on 14 May. This mix contained 36% *Trifolium pratense* (red clover), 20% pink clover, 20% *Onobrychis viciifolia* (sainfoin), 20% *Lotus corniculatus* (birds foot trefoil), 2% *Malva moschata* (musk mallow) and 2% *Centaurea nigra* (lesser knapweed). This planting was managed by the grower.

Within the commercial strawberry crop the grower agreed to sacrifice some beds and lucerne seeds were sown within the planting hole in the raised beds.

Assessment of seed germination

The plots were inspected on 25 June, 28 July, 14 August and 18 September and the state of growth of the plants was assessed.

Arthropod sampling

Only 2 samples were taken at this site, both on the flower mix, on 14 August and 18 September. The lucerne growing within the strawberry crop was also sampled on these dates. Each sample consisted of 10 sweeps with a sweep net over the foliage. A sample was taken from the 'bee mix' on 18 September. Samples were stored at -20ºC until sorted.

Results

Assessment of seed germination

On 25 June seedlings were emerging on most plots. However, some nettles were also germinating. No weeding was done at this time. By 28 July the corn chamomile in the flower mix was beginning to flower. However thistles were overgrowing the northern part of the planting. The plots were weeded by the grower on 14 August. At this time the seed mixture was flowering (all species), but very little growth of the other species was apparent. On 18 September the flower mix was still flowering with the dominant species being corn marigold. Red campion was apparent in the plots where it was sown but only a very few plants were flowering. Lucerne was also visible but was very short and was not flowering. There was no evidence that the ox eye daisy had germinated.

Components of the 'bee mix' germinated well giving good ground cover.

The lucerne sown within the raised beds of the crop were irrigated and grew more vigorously than the seeds sown around the strawberry field. However germination of the seeds was very patchy.

Arthropod sampling

In the samples taken from the flowering mix both predators and the pest, *L. rugulipennis,* were found. There was a mean of 5.7 parasitoids, 4 *Orius* sp., 1 Anthocorid and 10.3 *L. rugulipennis* per sweep sample on 14 August.

Task 5.2. Evaluate the effectiveness of plant derived semiochemicals to attract aphid predators and parasitoids (EMR/NRI, Yrs 1, 2, 3)

Summary

Laboratory experiments are assessing the effect of different release rates of four plant volatiles on *Orius laevigatus*, a predator that is often abundant in selectively sprayed plantations. One of the volatiles is methyl salicylate which has been shown to be attractive to beneficial species in other crops. This work is currently in progress in olfactometry choice test experiments. Release rates of methyl salicylate have been determined from two potential types of dispenser. Release of the compound was rapid; all the material (100 mg) was released within 10 days from both dispenser types.

Methods

Olfactometry. Laboratory experiments are assessing the effects of different release rates of the plant volatiles methyl salicylate, caryophyllene, hexenyl acetate and ocimene on a model biocontrol agent found in strawberry, *Orius laevigatus* (supplied by BCP). These volatiles have been shown to be attractive to various beneficial species in other crops.

Initial experiments used a glass y-tube olfactometer in choice test experiments. The olfactometer had a 12 mm internal diameter, 100 mm stem and 100 mm arms at a 60° angle. Air was pumped through Teflon tubing by a Wisa pump through an activated charcoal filter. The airflow was split by a brass T-junction (Swagelock, OH, USA), each flow of either 100 or 400 ml/min then passing into an airtight glass chamber (T.J. Adams – Glassblower, Kidlington, Oxfordshire, UK) into which the volatile source was placed. From the two glass chambers air flowed into the arms of the olfactometer via modified glass quick fit sockets. Diffused lighting was provided by a light source held 200 mm above and 100 mm in front of the branches of the olfactometer.

Insects were starved at 16°C in 14h:10h Light:Dark conditions for at least 12 hours before each assay. On the day of the assay they were allowed to acclimatise in the experimental room for 1 hour prior to the experiment. A single female was introduced into the stem of the olfactometer and then given 5 minutes to make a choice. If the insect failed to move more than 50 mm up the stem of the olfactometer it was excluded from the results. Insects were recorded as having selected an odour if they moved more than 50 mm up one of the arms and remained beyond this point for more than 30 seconds. Insects that moved more than 50 mm up the stem but did not select an arm were recorded as not having selected an odour. After each individual was tested the direction of the odour sources was swapped to account for any directional bias by the insects. Between bioassay sets, glassware was washed with acetone and distilled water and air dried on a hotplate at 50°C.

Pure volatile compounds were prepared as either 1 mg/ml or 0.2 mg/ml solutions in hexane. Test solutions were presented in 50 µl aliquots applied to a piece of filter paper (Whatnam No. 1) which was changed after every 5th individual test. Pure hexane was used in the control arm. To maintain humidity a piece of filter paper with 200 µl of distilled water was placed into the glass chamber.

Development of dispensers. Suitable dispensers for methyl salicylate and one other attractive volatile are being developed during the winter of 2008/09. Dispensers are being designed that can release two rates of volatile, and the release rates of the volatiles from these dispensers are being determined.

Results

Olfactometry. Individuals have been presented with methyl salicylate, caryophyllene, hexenyl acetate and ocimene singly at the highest dose and flow rate. There appears to be no clear preference for any volatile at this rate and experiments are ongoing with the chemicals being presented at the lower rate.

Development of dispensers. Initial work has focussed on determining release rates of methyl salicylate from two types of polyethylene sachet. Results are shown in Figure 5.2.1. Release of the compound was rapid; all the material (100 mg) was released within 10 days.

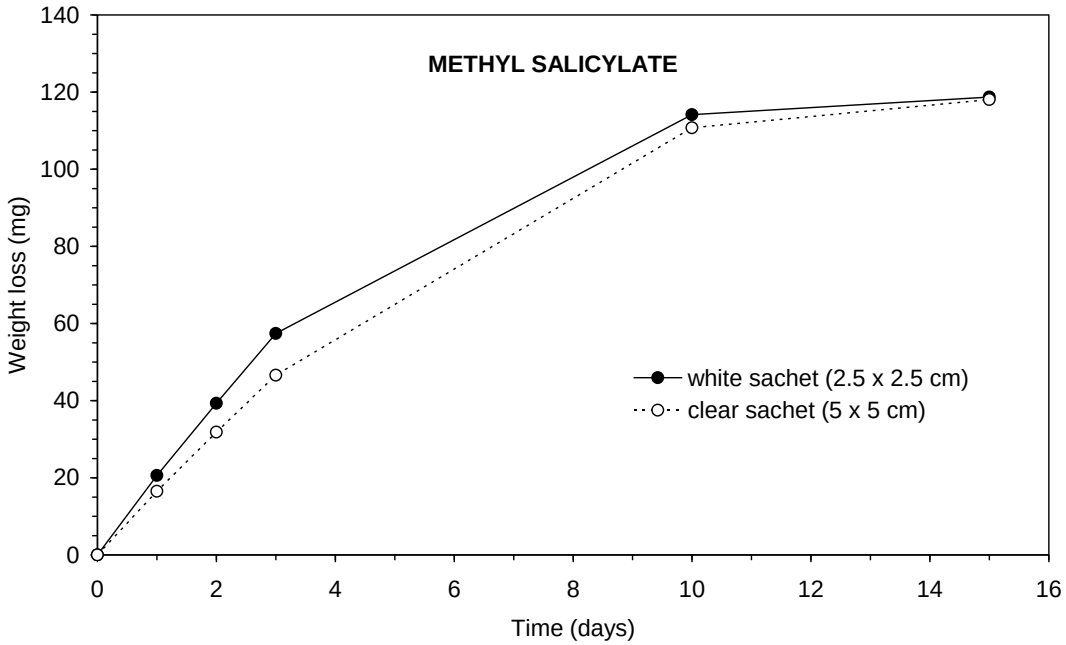


Figure 5.2.1. Release rates of methyl salicylate from sachet dispensers at 20-22°C

Task 5.3. Evaluate the efficacy of post harvest applications of selective insecticides to reduce populations of C. fragaefolii in the subsequent season (EMR Yrs 1, 2)

An experiment has been established to investigate autumn control of aphids on strawberry. The Experiment protocol ORETO GEP No ORETO 08/015 is as follows:

Objective: *To use end-of-season clean up sprays with selective insecticides to control* C. fragaefolii *in commercial strawberry production. Large scale experiments will evalaute late season sprays of aphicides to reduce populations of aphids in the following spring.*

Site

The trial is being done in the June-bearer strawberry plantation in 'Churchfield' at Langdon Manor Farm, Goodnestone, Faversham, Kent ME13 9DA by agreement of the proprietor Alastair Brook, Location: NGR TR 047 615

The strawberry field is 3.35 ha in area with the southern half of the variety Elsanta and the northern half of the variety Sonata. The crop is grown on the usual raised polythene mulched beds which run approximately E-W in direction. The field slopes gently to the west.

There are 66 beds of the variety Sonata in the northern half of the plantation and roughly the same number of the variety Elsanta in the southern half of the plantation. 60 adjacent beds of each variety are being used for the trial, the 60 northern most of Sonata and the 60 southernmost of Elsanta.

The crop was planted in May 2008. The beds are spaced 1.65 m apart and the plants are 0.35 m apart in the beds. They are planted alternately as a single and then a double plant along the bed (pattern like the 5 on a dice). The beds are approximately 1m wide and roughly 140 m long.

Treatments

Treatments were single sprays of Calypso (thiacloprid) applied in the autumn at 3 different timings at 2 week intervals, as given in Table 5.3.1.

Table 5.3.1. Treatments

Trt no	Colour code	Product	Active ingredient	Dose rate (/ha)	Timing of application
1	Red	Calypso	480 g/l thiacloprid SC	250 ml	4th week Sept
2	Blue	Calypso	480 g/l thiacloprid SC	250 ml	2nd week Oct
3	Yellow	Calypso	480 g/l thiacloprid SC	250 ml	4th week Oct
4	Green	Untreated	-	-	-

Spray application

Sprays were applied with the growers Berthoud Puma air assisted sprayer, operated by the growers spray operator (Jim) at the farms normal spray volume of 400 l/ha. The sprayer covers 5 beds with 2 air spouts (jets) per bed. Each air spout is furnished with 2 air-shear nozzles. The spray operations were supervised by a member of this study team. The sprayer calibration was checked

Experimental design and layout

A randomised block design with 6 replicates was used, with three replicates in the Sonata and 3 replicates in the Elsanta varieties. Plots were each 5 beds wide and ran the full length of the plantation and were side by side (with plot 101 northernmost). The 60 northern most beds of Sonata and the 60 southernmost of Elsanta were used.

Table 5.3.2. Randomisation of treatments to plots

Sonata (60 northern most beds)								
Block 1			Block 2			Block 3		
Plot no.	Trt no.	Col	Plot no.	Trt no.	Col	Plot no.	Trt no.	Col
101	2	B	201	1	R	301	3	Y
102	1	R	202	3	Y	302	4	G
103	3	Y	203	2	B	303	1	R
104	4	G	204	4	G	304	2	B
Elsanta (60 southern most beds)								
Block 4			Block 5			Block 6		
Plot no.	Trt no.	Col	Plot no.	Trt no.	Col	Plot no.	Trt no.	Col
401	2	B	501	1	R	601	4	G
402	3	Y	502	2	B	602	2	B
403	1	R	503	4	G	603	1	R
404	4	G	504	3	Y	604	3	Y

Approval

Calypso has a SOLA for use on protected strawberry (1497 of 2004). The maximum individual dose is 250 ml product /ha, the maximum dose per season 500 ml/ha and the harvest interval is 3 days.

Calypso has a SOLA for use on outdoor strawberry (2727 of 2003). The maximum individual dose is 250 ml product /ha, the maximum dose per season 500 ml/ha and the harvest interval is 3 days.

Meteorological records

Wet and dry bulb temperature with aspirated psychrometer, wind speed and direction before and after spraying were recorded. Full records are available from EMR met station.

Assessments

Numbers of *C. fragaefolii* present on plants treated with aphicide and untreated control plants will be recorded in May/June 2009 and effects of the treatments compared. Records will also be taken of any other species of aphid present including *Aphis gossypii* and *Myzus ascalonicus* which are both important pests of strawberry. The sample size will be adjusted according to populations on untreated plots.

Crop Destruction

No crop destruction is required

Task 5.4. Evaluate possibility of using the parasitoid Aphidius eglanteriae to control C. fragaefolii in early season introductions (BCP, EMR, Yrs 1, 2, 3)

Summary

Several hundred parasitised *C. fragaefolii* (mummies) were collected from field grown strawberry plants at EMR. The mummies, together with *C. fragaefolii*-infested strawberry plants were sent to BCP to enable a culture of the parasitoid to be set up. However, very few parasitoids successfully emerged.

Details of a possible alternative host for *Aphidius eglanteriae* was obtained from the literature and sent to BCP for information.

Objective 6: To develop a highly attractive 'super' trap for strawberry blossom weevil that combines visual, host plant volatile and sex aggregation pheromone attractants and to develop methods of using the trap for monitoring and control

Task 6.1. Optimise visual component (EMR, Yr 1)

Task 6.2. Adjust design to minimise the capture of non-target arthropods (EMR, Yrs 1, 2)

Task 6.3. Optimise choice of host plant volatile(s) and blend for synergising the sex aggregation pheromone (EMR, Yrs 1, 2)

Summary

Three replicated field experiments were conducted in 2008 at the start of a study to develop a highly attractive 'super trap' for strawberry blossom weevil based on synergising the weevil's aggregation pheromone with host plant volatiles and utilising the weevils' attraction to white flowers. The three experiments were as follows:

Exp 1. Two volatiles from strawberry flowers were tested as pheromone synergists

Exp 2. Two volatiles from raspberry flowers and the same 2 volatiles from strawberry flowers, one at a low, medium and high release rate, were tested as pheromone synergists

Exp 3. Different designs of funnel trap with different colours and sizes of cross vane were compared with the original sticky stake trap design used when the pheromone was originally developed.

Regrettably, numbers of strawberry blossom weevil captured in all 3 experiments were very small. The data contained numerous zero values and was too scanty for statistical analyses. For this reason it is not possible to draw firm conclusions from the data. The following general conclusions may be drawn:

1. In the experiment testing the strawberry flower volatiles as synergists, numbers captured in the pheromone alone treatment were as great as those captured in the pheromone + PV1 + PV2 treatment. If this high value were ignored, then the results would be in line with those from the experiment in Norway in 2007 where synergistic effects of PV1, and especially PV2, were demonstrated.

2. In the second experiment where the synergistic effect of both raspberry and strawberry flower volatiles was tested, no synergistic effect from any of the added volatiles was apparent.

3. Inspection of the total catches in the trap design experiment does indicate some broad treatment effects. The Agrisense funnel trap with no cross vanes or with 7.5 cm tall cross vanes and the sticky stake traps (with or without the added PV2) caught few weevils and so appeared less effective. The Agralan funnel traps with white or yellow cross vanes and the Agrisense funnel traps with 15 and 30 cm tall white cross vanes all preformed similarly. Incidentally, large numbers of mirids including *Lygus rugulipennis* and *L pratensis*, were captured in the Agrisense funnel traps with 30 cm tall white cross vanes indicating that this design might be very effective for these pests.

Methods and materials

Three experiments to develop the strawberry blossom weevil (SBW) super trap were conducted in 2008 as follows:

Exp 1. 2 volatiles from strawberry flowers were tested as pheromone synergists
Exp 2. 2 volatiles from raspberry flowers were tested as pheromone synergists
Exp 3. Trap design comparison

Standard SBW polythene sachet aggregation pheromone lures containing 100 µl of Grandlure I, Grandlure II and Lavandulol in a 1:4:1 ratio were used throughout. Initially commercial lures supplied by International Pheromone Systems were used but these were replaced by sachets made at NRI as in the original work by Cross et al. (2005).

Sites

Exp 1: The trial was initially set up in Peaches strawberry field at Mansfield's, Middle Pett Farm, Bridge, Canterbury, Kent by kind agreement of David Stanbury (farm manager) and Paul Keutenius (KG Growers consultant). The plantation was located at NGR TR 165 542. The plantation was usually sprayed with chlorpyrifos and/or thiacloprid (Calypso) for strawberry blossom weevil at flower stem extension but first flowers were often removed. The variety was Albion (everbearer), planted 2007, total area =18 ha, plant density = 42,500 plants/ha, bed spacing = 1.5 m, plant spacing in bed = 30 cm in zig-zag row, 4 rows/tunnel. The westerly edge of plantation adjacent to mixed deciduous woodland was used.

Because numbers of SBW were very low, the trial was terminated at Middle Pett Farm on 9 June 2008 and moved to an organic strawberry plantation at Hall-Hunter Farms, Tuesley Farm, Milford, near Godalming, Surrey on 11 June 2008 by kind agreement of Anna Costa (Fast advisor Rob Cook). The field, named SF1c, was adjacent to the railway station in Milford. The distance between beds was 1.6 m and between plants was 30 cm. Plants in SF1c were planted in 2007; surrounding fields were planted in 2008. Flowers are sown around the field on the north of the field. There is a small forest patch between the hospital and the strawberry field, a hedgerow to the west of the field (total area 9 acres).

Exp 2: The trial was started at Parkwood Farm, Brishing Lane, Boughton Monchelsea, Maidstone, Kent by kind agreement of John Worley. Three different fields, all > 1 year old, were used as follows:

1. 'Farm entrance' field. Total area = 1.8 ha, but 1 ha to the north covered with fleece. 0.8 ha not covered with fleece, cv. Symphony. Western edge adjacent to road hedgerow was used
2. 'Bramleys' field. Area = 1.2 ha, cv. Symphony. Eastern edge adjacent to windbreak to be used for deployment of traps
3. 'Marlpit' field. Area 1.2 ha, cv. Symphony. Windbreaks on western and eastern edges of field to be used for trap deployment

Because numbers of SBW captured at Parkwood Farm were very low, the trial was terminated on 9 June 2008 and moved to an organic strawberry plantation at Hall-Hunter Farms, Tuesley Farm, Milford, near Godalming, Surrey on 11 June 2008. The trial was located in field SF2a. Plants in this field were planted at different times in 2007. The oldest part was in blossom in early June, the rest at the end of June.

Exp 3. Organic strawberry plantation at Hall-Hunter Farms, Tuesley Farm, Milford. The trial was located in field SF2a. Plants in this field were planted at different times in 2007. The oldest part was in blossom in early June, the rest at the end of June.

Treatments

Treatments tested in the three experiments are given in Tables 6.1, 6.2 and 6.3 for the three trials, respectively. Details of the lures used are given in Table 6.4. Figures 6.1. and 6.2 show the dispensers and the traps used.

Table 6.1. Treatments tested in Experiment 1

Treat	Treat Code.	Trap	Company	Vane colour	Cross vane	Orifice	Killing material	Phero	Plant volatiles	Release rate
A	A1-A3	Funnel	AgriSense	White	16 cm (stand.)	Standard	liquid	-	None	-
B	B1-B3	Funnel	AgriSense	White	16 cm (stand.)	Standard	liquid	-	PV1	standard
C	C1-C3	Funnel	AgriSense	White	16 cm (stand.)	Standard	liquid	-	PV2	standard
D	D1-D3	Funnel	AgriSense	White	16 cm (stand.)	Standard	liquid	1:4:1	None	standard
E	E1-E3	Funnel	AgriSense	White	16 cm (stand.)	Standard	liquid	-	PV1+PV2	standard
F	F1-F3	Funnel	AgriSense	White	16 cm (stand.)	Standard	liquid	1:4:1	PV1	standard
G	G1-G3	Funnel	AgriSense	White	16 cm (stand.)	Standard	liquid	1:4:1	PV2	standard
H	H1-H3	Funnel	AgriSense	White	16 cm (stand.)	Standard	liquid	1:4:1	PV1+PV2	standard

Table 6.2. Treatments tested in experiment 2

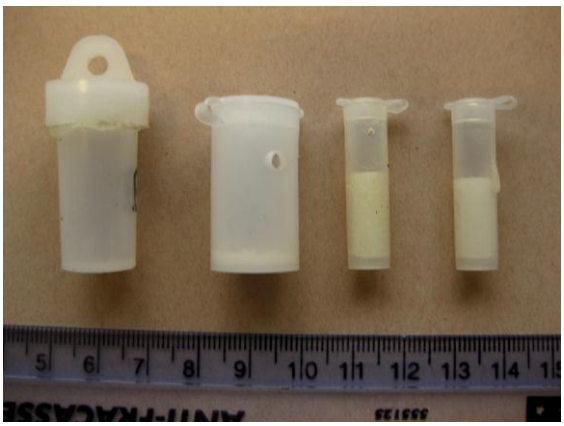
Treat	Treat Code.	Trap	Company	Vane colour	Cross vane	Orifice	Killing material	Phero	Plant volatiles	Release rate
1	G	Funnel	Agralan	White	Standard	Standard	liquid	1:4:1	-	-
2	R	Funnel	Agralan	White	Standard	Standard	liquid	1:4:1	PV2	2 holes
3	RY	Funnel	Agralan	White	Standard	Standard	liquid	1:4:1	PV2	0 holes
4	RR	Funnel	Agralan	White	Standard	Standard	liquid	1:4:1	PV2	3 lures x 2 holes
5	B	Funnel	Agralan	White	Standard	Standard	liquid	1:4:1	PV3	
6	W	Funnel	Agralan	White	Standard	Standard	liquid	1:4:1	PV4	
7	RB	Funnel	Agralan	White	Standard	Standard	liquid	1:4:1	PV3+PV2	
8	RW	Funnel	Agralan	White	Standard	Standard	liquid	1:4:1	PV4+PV2	

Table 6.3. Treatments evaluated in experiment 3

Treat	Treat Code.	Trap	Company	Vane colour	Cross vane	Orifice	Killing material	Phero	Plant volatiles	Release rate
1	G	Stake	Adrian	-	-	-	glue	1:4:1	-	-
2	YY	Stake	Adrian	-	-	-	glue	1:4:1	PV2	standard
3	RY	Funnel	AgriSense	White	No vein	Standard	liquid	1:4:1	PV2	standard
4	Y	Funnel	AgriSense	White	7.5 cm	Standard	liquid	1:4:1	PV2	standard
5	RB	Funnel	AgriSense	White	15 cm	Standard	liquid	1:4:1	PV2	standard
6	YB	Funnel	AgriSense	White	30 cm	Standard	liquid	1:4:1	PV2	standard
7	R	Funnel	Agralan	White	standard	Standard	liquid	1:4:1	PV2	standard
8	RR	Funnel	Agralan	Yellow	standard	Standard	liquid	1:4:1	PV2	standard

Table 6.4. Plant volatile synergists and their rates of application in the 3 experiments

Exp	Lure (s)	Active substance	Loading	Dispenser	Side holes
1	PV1	Germacrene D†	0,5 ml	Closed polyethylene vial 32 mm x 8mm	
1	PV2	Coded	0,5 g	Closed polyethylene vial 31 mm long, diameter 17 mm	
2	PV1	Germacrene D†	0,5 ml	Closed polyethylene vial 32 mm x 8mm	
2	PV2	Coded (standard)	0,5 g	Closed polyethylene vial 31 mm long, diameter 17 mm	2 holes
2	PV2	Coded (low release)	0,5 g	Closed polyethylene vial 31 mm long, diameter 17 mm	0 holes
2	3 x PV2	Coded (high release)	0,5 g	Closed polyethylene vial 31 mm long, diameter 17 mm	2 holes
2	PV3	Rasp 1, [p]	2,5 ml	Closed, sealed Agrisense polyethylene vial 35 mm long, diameter 14 mm base	
2	PV4	Rasp 2, [b]	2,5 ml	Closed, sealed Agrisense polyethylene vial 35 mm long, diameter 14 mm base	
3	PV2	Coded	0,5 g	Closed polyethylene vial 31 mm long, diameter 17 mm	
† Lure actually contained a mix of several volatiles of which Germacrene D was one major component					



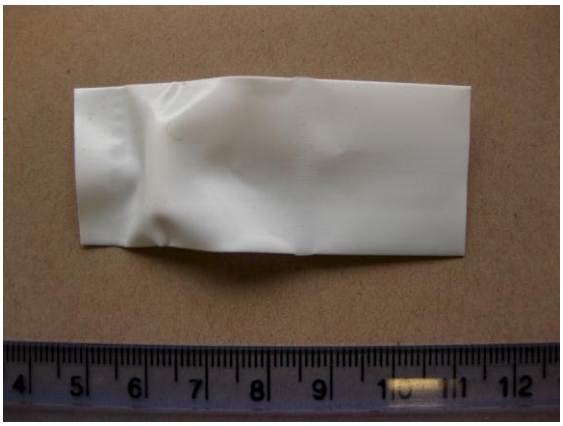


Fig 6.1. Dispensers. Left photo: Agrisense vial for PV3 & PV4 (1st left), standard vial for PV2 (2 holes) (2nd left), vial for PV1 (germacrene D) with 2 holes (3rd from left), vial for PV1 (germacrene D) with no holes (4th from left). Right photo. NRI Polythene sachet dispenser for aggregation pheromone

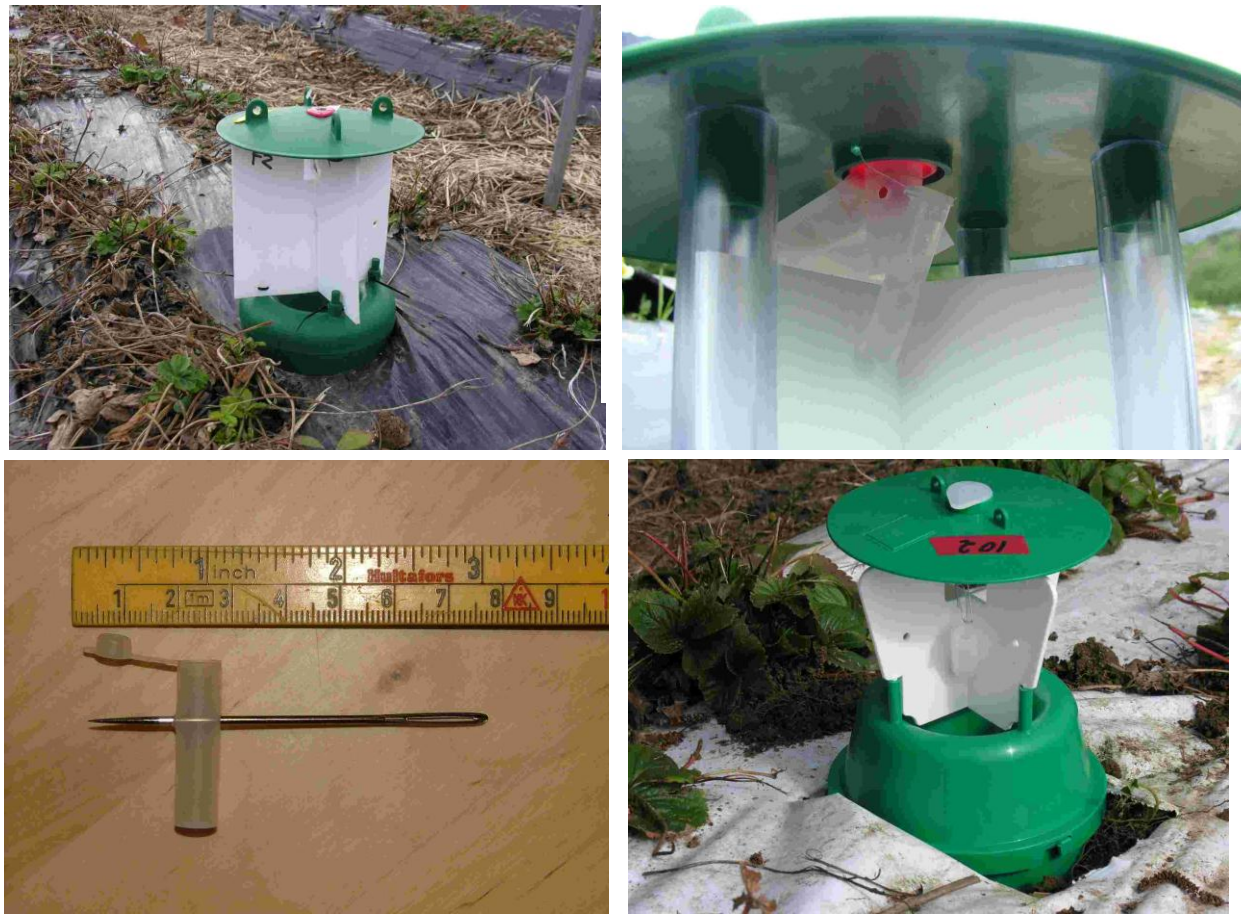


Figure 6.2. Top left) Agrisense funnel traps with white Correx cross vanes; top right) close up of aggregation pheromone sachet and polythene vial for PV2; bottom left) polythene vial showing method of making holes with needle to give different release rates; bottom right) Agralan funnel trap with white cross vanes and polythene vials used for PV3 and PV4

Experimental designs

For experiment 1, a fully randomised design with 3 replicates was used (as specified by A Wibe the coordinator for the trials for this experiment in Norway, Denmark and the UK). In all the trials, each trap represented one plot. In experiment 1, traps were spaced ~5m apart in an 8 x 3 grid. In the other experiments, randomised block designs were used with plots (traps) in each block spaced > 10 m apart and blocks spaced widely apart in different parts of field. A temperature logger (USB 500) was placed in the middle of the field.

Assessments

Catches of SBW and other arthropods were recorded at 1-2 week intervals. Arthropods were identified in the laboratory and the sex of SBW determined. Records of damage were done by counting number of damaged buds on the 4 plants closest to each trap.

Results

Release rate of PV2

Release rates of PV2 from the polyethylene vials were measured in the laboratory wind tunnel at NRI at 27°C, 8 km/h wind speed (Figure 2, Table 6.5).

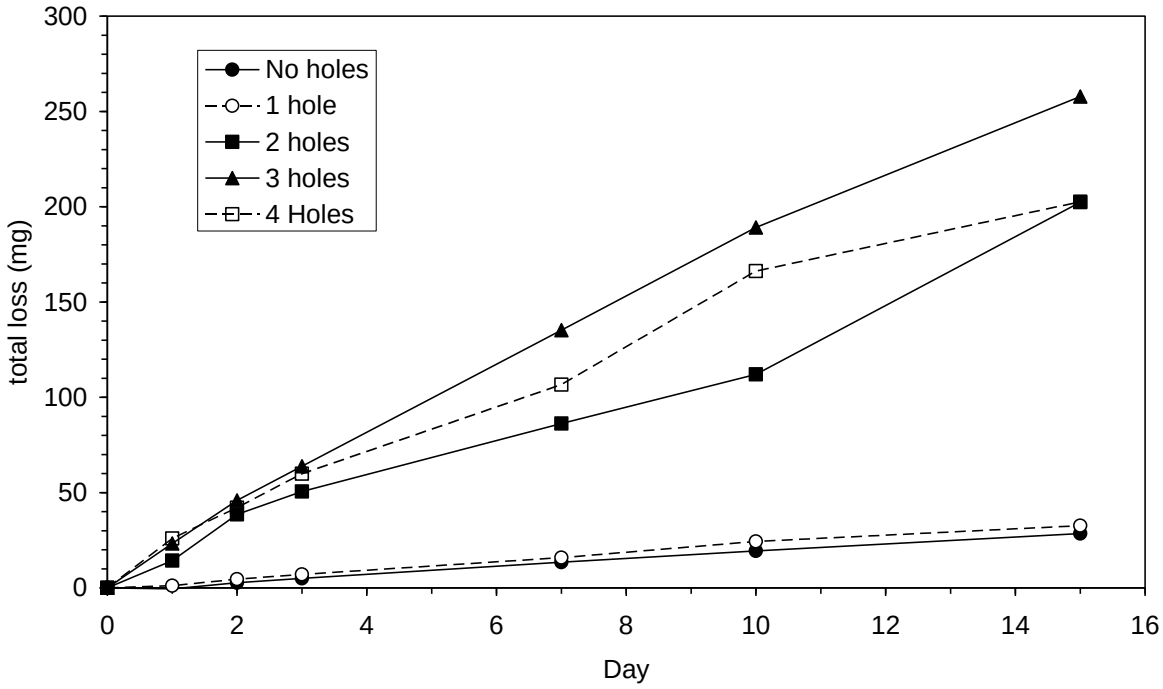


Figure 6.2. Release rate of PV2 from polyethylene vials measured in the lab wind tunnel at NRI (27°C, 8 km/h wind speed)

The vials released PV2 at a steady rate for the 16 days that measurements were done. Release rates were similar with no or one hole (~ 2 mg/day) but much higher with 2-4 holes (~15 mg/day).

Table 6.5. Release rates of PV2 from polyethylene vials measured in the lab wind tunnel at NRI

	Release rate (mg/d)
No hole	1.9
1 hole	2.2
2 holes	13.5
3 hole	17.2
4 hole	13.5

Release rates of aggregation pheromone from sachets

Records of the weight loss by NRI produced aggregation pheromone sachets deployed in the field at Tuesley Farm on 3 July 2008 were erratic (Table 6.6). The reason for this is unclear, especially the negative values obtained on 9 July.

Table 6.6. Weight loss by SBW pheromone sachets (mg) at intervals after their deployment in the field at Tuesley Farm on 3 July 2008

9.07	15.07	23.07	30.07	6.08
0.1	4.4	2.2	27.8	5.7
2.4	1.1	1.8		5.8
-33.8	2.2	2.7		
-4.8	1.5			

Experiment 1

A total of 37 SBW adults (14♀; 23♂) were captured over the entire course of the experiment from 9 April to 27 July 2008 (Table 6.7). Most individuals were captured in July when the experiment was located in the organic strawberry field at Tuesley Farm. Numbers captured in the pheromone alone treatment were as great as those captured in the pheromone + PV1 + PV2 treatment (Figure 6.2). If this high value were ignored, then the results would be in line with those from the experiment in Norway in 2007. Numbers of flower buds severed were small and erratic and there was no obvious association of higher of lower levels of damage with any particular treatment (Table 6.10).

Experiment 2

A total of 127 SBW adults (57♀; 66♂) were captured over the entire course of the experiment from 9 April to 27 July 2008 (Table 6.8). Peak catches occurred on 7 May (Parkwood Farm) and mid July (Tuesley Farm). However, the numbers captured by any one individual treatment were small (≤ 18 weevils from all three replicate traps for the whole season) (Figure 6.3). The data was inadequate for statistical analysis. A total of 13 weevils was captured in the untreated control (= aggregation pheromone only) treatment and none of the other treatments had markedly greater catches. No synergistic effect from any of the added volatiles was apparent. Numbers of flower buds severed were small and erratic and there was no obvious association of higher of lower levels of damage with any particular treatment (Table 6.11).

Experiment 3

Numbers of weevils captured were, again, small and the data unsuitable for statistical analysis (Table 6.9, Figure 6.4). However, inspection of the total catches does indicate some broad treatments effects. The Agrisense funnel trap with no cross vanes or with 7.5 cm tall cross vanes and the sticky stake traps (with or without the added PV2) caught few weevils and so appeared less effective. The Agralan funnel traps with white or yellow cross vanes and the Agrisense funnel traps with 15 and 30 cm tall white cross vanes all preformed similarly. Incidentally, large numbers of mirids including *Lygus rugulipennis* and *L pratensis*, were captured in the Agrisense funnel traps with 30 cm tall white cross vanes indicating that this design might be very effective for these pests (Figure 6.5).

Table 6.7. Total numbers of strawberry blossom weevil females and males captured in experiment 1.

	16.04	23.04	30.04	7.05	14.05	21.05	28.05	4.06	9.06†	17.06	25.06	3.07	8.07	15.07	22.07	Total
Female SBW																
control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PV1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
PV2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
phero	0	0	0	1	0	1	1	0	0	0	0	1	0	0	0	4
PV1+PV2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
phero+PV1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
phero+PV2	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	4
phero+PV1+PV2	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	4
Total	0	0	0	1	1	2	2	1	1	1	0	3	1	0	1	14
Male SBW																
control	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
PV1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
PV2	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	4
phero	0	0	0	0	0	0	0	0	0	0	1	3	0	2	1	7
PV1+PV2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1

phero+PV1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
phero+PV2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
phero+PV1+PV2	0	0	0	2	0	0	0	0	0	0	0	1	3	0	1	7
Total	0	0	0	2	0	0	1	0	0	0	3	6	4	4	3	23

†Traps were removed from the conventional strawberry field at Middle Pett Farm, Canterbury after records had been taken on 9 June and were redeployed in the organic strawberry field at Tuesley Farm, Godalming on 11 June 2008

Table 6.8. Total numbers of strawberry blossom weevil females and males captured in experiment 2

	16.04	23.04	30.04	7.05	14.05	21.05	28.05	4.06	9.06†	17.06	25.6	3.7	8.7	15.07	22.07	Total
Female SBW																
control	0	0	0	1	4	1	0	0	0	0	1	0	0	0	0	7
PV2	0	0	1	1	2	1	2	1	0	0	0	0	0	0	1	9
PV2+PV3	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	3
PV2+PV4	0	0	1	4	0	0	2	0	0	0	0	1	0	0	0	8
PV20hole	0	0	0	0	1	0	2	0	1	1	0	0	2	1	0	8
PV2x3	0	0	0	2	0	0	0	1	1	0	1	0	0	1	1	7
PV3	0	0	0	4	0	1	1	1	0	1	0	0	0	1	0	9
PV4	0	0	0	1	1	1	1	1	0	1	0	0	0	0	0	6
Total	0	0	2	14	9	4	8	4	2	3	2	1	2	3	3	57

Male SBW																
control	0	0	0	2	3	0	0	0	1	0	0	0	0	0	0	6
PV2	0	0	0	2	1	0	0	1	0	0	0	0	0	1	4	9
PV2+PV3	0	0	0	1	0	1	0	0	2	0	0	0	0	4	2	10
PV2+PV4	0	0	1	1	2	0	2	0	0	0	0	1	0	2	1	10
PV20hole	0	1	0	2	0	1	0	0	1	0	0	0	0	2	3	10
PV2x3	0	1	0	1	3	0	0	0	1	0	1	0	0	1	1	9
PV3	1	1	0	1	2	0	0	0	0	1	0	0	0	0	1	7
PV4	0	0	0	0	0	1	0	0	2	0	0	0	0	0	2	5
Total	1	3	1	10	11	3	2	1	7	1	1	1	0	10	14	66

†Traps were removed from the conventional strawberry field at Middle Pett Farm, Canterbury after records had been taken on 9 June and were redeployed in the organic strawberry field at Tuesley Farm, Godalming on 11 June 2008

Table 6.9. Total numbers of strawberry blossom weevil females and males captured in experiment 3

	3.07	8.07	15.07	22.07	Total
Female SBW					
Agralan-white	1	0	1	0	2
Agralan-yellow	1	0	1	1	3
funnel-30cm	3	1	1	0	5
funnel-15cm	5	0	1	0	6
funnel-7.5cm	0	0	0	0	0
funnel-no-vein	0	0	0	0	0
stake-phero only	1	0	0	0	1
stake-phero+PV2	1	0	0	0	1
Total	12	1	4	1	18
Male SBW					
agralan-white	7	2	1	2	12
agralan-yellow	5	1	3	0	9
funnel-30cm	3	1	0	0	4
funnel-15cm	1	2	2	1	6
funnel-7.5cm	1	0	0	0	1
funnel-no-vein	0	0	0	0	0
stake-phero only	0	2	0	1	3
stake-phero+PV2	2	1	1	0	4
Total	19	9	7	4	39

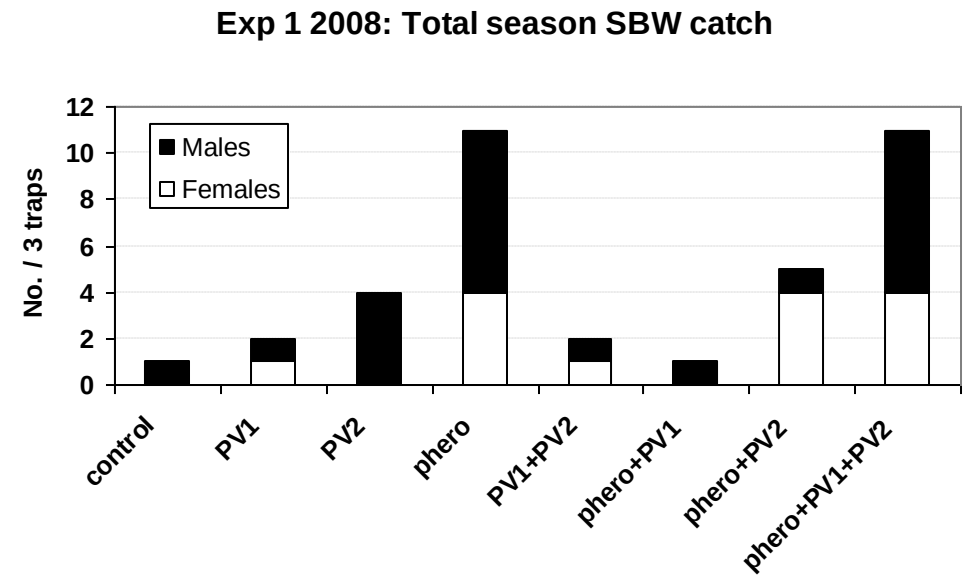


Figure 6.2. Total seasons catch by treatment (3 traps) of strawberry blossom weevil adults in experiment 1

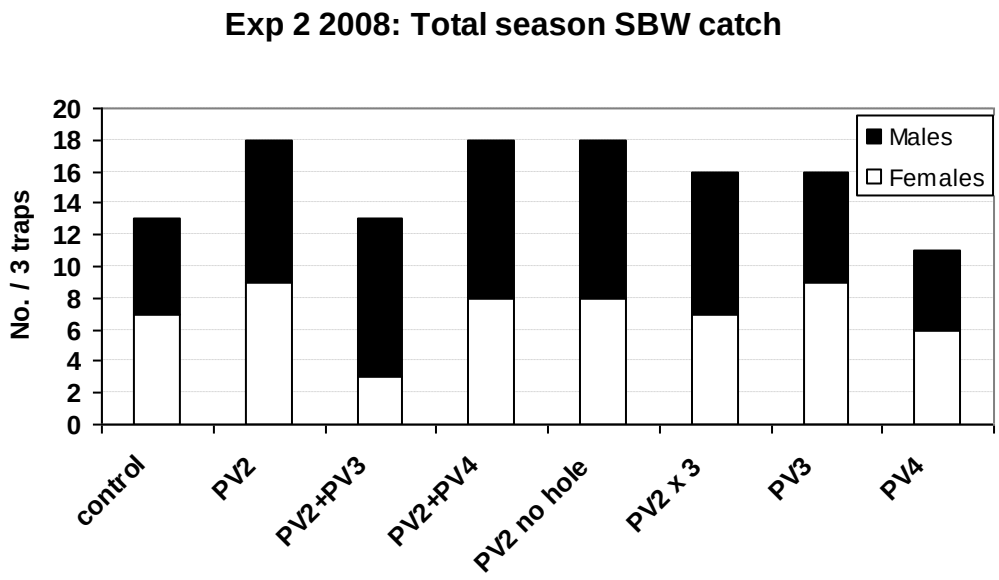


Figure 6.3. Total seasons catch by treatment (3 traps) of strawberry blossom weevil adults in experiment 2. Note, all traps were provided with a standard aggregation pheromone sachet lure

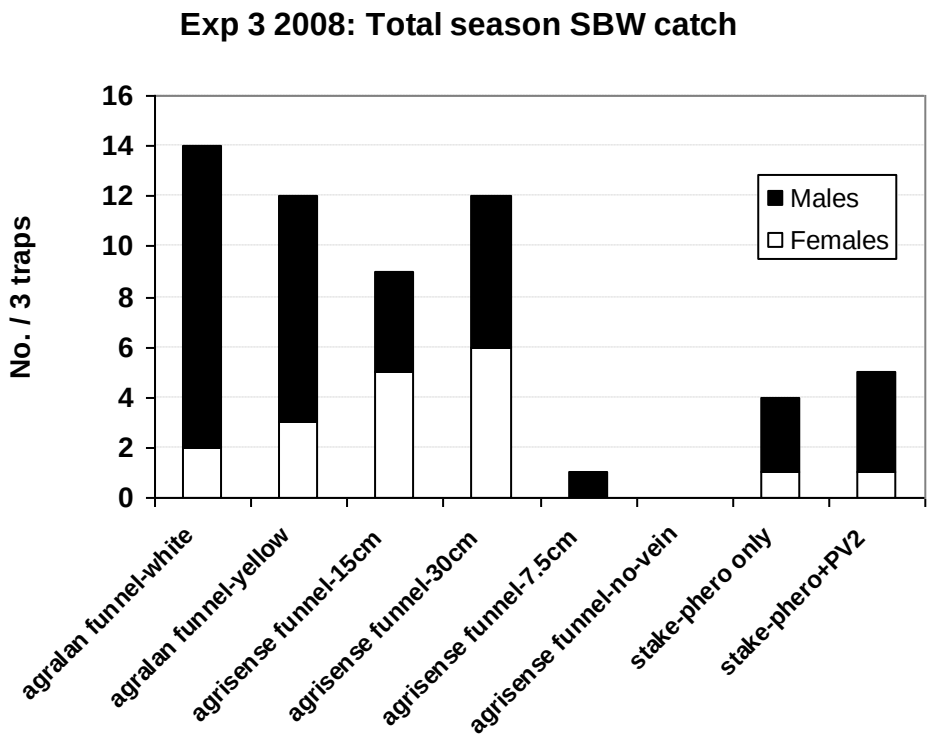


Figure 6.4. Total seasons catch by treatment (3 traps) of strawberry blossom weevil adults in experiment 3

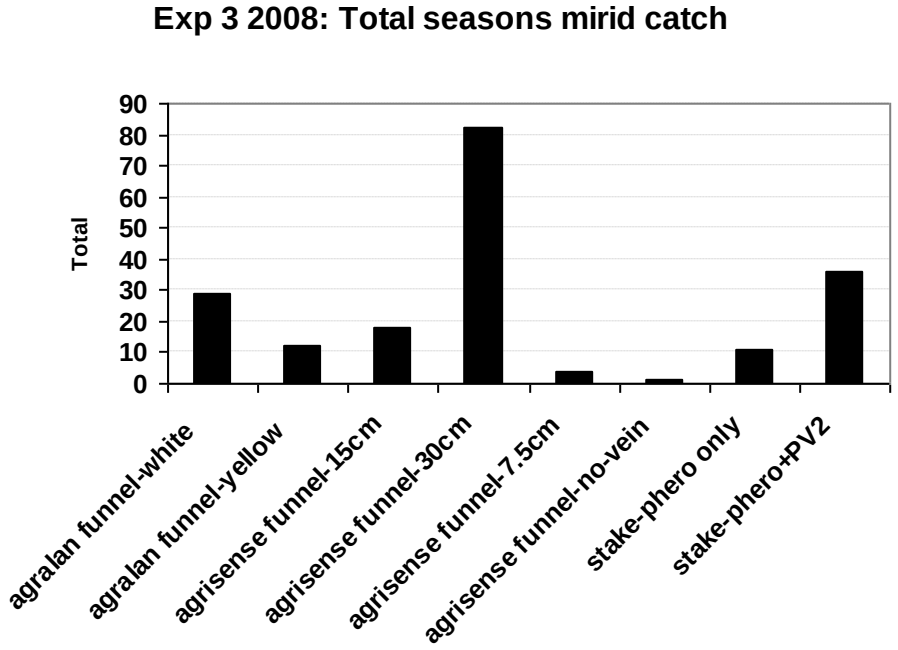


Figure 6.5. Total seasons catch by treatment (3 traps) of mirid adults in experiment 3

Table 6.10. Mean numbers of severed flower buds per plant on 4 June in **experiment 1**

control	1.3
PV1	0.3
PV2	0.3
phero	1.0
PV1+PV2	0.7
phero+PV1	2.3
phero+PV2	0.3
phero+PV1+PV2	0.3

Table 6.11. Mean numbers of severed flower buds per plant on 4 June in experiment 2

control	6.5
PV2	7.5
PV2+PV3	11.5
PV2+PV4	13.8
PV2 0hole	11.0
PV2x3	8.0
PV3	8.5
PV4	6.3

Conclusions

Regrettably, numbers of strawberry blossom weevil captured in all 3 experiments were very small. The data contained numerous zero values and was too scant for statistical analyses. For this reason it is not possible to draw firm conclusions from the data. The following general conclusions may be drawn:

1. In the experiment testing the strawberry flower volatiles as synergists, numbers captured in the pheromone alone treatment were as great as those captured in the pheromone + PV1 + PV2 treatment. If this high value were ignored, then the results would be in line with those from the experiment in Norway in 2007 where synergistic effects of PV1 and especially PV2 were demonstrated.

2. In the second experiment testing the synergistic effect of both raspberry and strawberry flower volatiles, no synergistic effect from any of the added volatiles was apparent.

3. Inspection of the total catches in the trap design experiment does indicate some broad treatment effects. The Agrisense funnel trap with no cross vanes or with 7.5 cm tall cross vanes and the sticky stake traps (with or without the added PV2) caught few weevils and so appeared less effective. The Agralan funnel traps with white or yellow cross vanes and the Agrisense funnel traps with 15 and 30 cm tall white cross vanes all preformed similarly. Incidentally, large numbers of mirids including *Lygus rugulipennis* and *L pratensis*, were captured in the Agrisense funnel traps with 30 cm tall white cross vanes indicating that this design might be very effective for these pests.

No work addressing the following three tasks was done in 2008:

Task 6.4. Examine the effect of reducing the amount of Grandlure I in the sex aggregation pheromone lure (EMR, Yr 3)

Task 6.5. Calibrate the super trap for pest monitoring purposes (EMR, Grower partners Yrs 3-5)

Task 6.6. Determine the efficacy of the super trap for control of strawberry blossom weevil by mass trapping (EMR, ADAS, Grower partners Yrs 4-5)

Objective 7: To develop and evaluate an Integrated Pest and Disease Management strategy, determining how components interact, its economic performance, effects on other pests, diseases and beneficials and the incidence of pesticide residues.

No work addressing the following tasks was due in year 1.

Task 7.1 - Devise an IPM programme (years 4-5, all partners).

Task 7.2. - Test IPM in commercial crops (years 4-5; all partners)

Task 7.3. - Prepare best practice guidelines (year 5; all partners)

Primary milestones

Milestone	Target month	Title	
P3.1	11	Blackspot isolates obtained for molecular analysis.	Y
P5.2.1	12	Olfactometry choice test experiments completed and suitable dispensers for methyl salicylate plus one other plant volatile to attract aphid natural enemies developed.	N
P6.1	12	Visual component of blossom weevil super trap optimised.	Y
P5.4.1	12	Lab culturing method for *Aphidius eglanteriae* developed.	N
P5.1.1	12	First year experiment to evaluate the effectiveness of flowering plants to attract aphid predators and parasitoids completed.	Y
P5.3.1	14	First year trial evaluating the efficacy of post harvest aphicide treatment completed.	
P2.2	22	Validation of the Botem model for protected crop completed.	
P1.4	24	Efficacy of Serenade against mildew determined.	
P2.4	24	Suitability of bees for dispersing BCAs evaluated.	
P4.2.1	24	Feasibility of use of hexyl butyrate as a repellant of *L. rugulipennis* females determined.	
P5.4.2	24	Preliminary biocontrol trials with *Aphidius eglanteriae* completed.	
P6.3	24	Optimum choice of host plant volatile(s) and blend for synergising the sex aggregation pheromone of blossom weevil established.	
P3.2	29	Population structure of blackspot determined.	
P1.6	33	Fungicide dissipation dynamics determined.	
P2.5	33	Model-based control strategies evaluated for botrytis.	
P3.4	36	An overall risk assessment scheme developed for blackspot.	
P4.3	36	System for regularly vacuuming trap crops for control of European tarnished plant bug developed.	
P5.4.3	36	Feasibility of using *Aphidius eglanteriae* as a biocontrol agent for strawberry aphid determined and release methods and rates for testing in the IPM trials in years 4 and 5 decided.	

P7.1	36	IPDM programme for testing in final two years of the project established and sites for conduct identified.	
P2.7	43	Efficacy of bee-vectored BCA against botrytis determined.	
P3.5	43	Possibility of eliminating blackspot inoculum using biofumigation determined.	
P1.8	48	Effects of nitrogen on mildew susceptibility determined.	
P1.9	48	Mildew control strategy (ies) devised.	
P1.10	48	Selected products against mildew evaluated.	
P7.2.1	48	First years experiments evaluating IPDM programme in commercial crops completed. Changes to the programme decided.	
P6.5	60	Blossom weevil super trap calibrated for pest monitoring purposes.	
P6.6	60	Efficacy of the super trap for control of strawberry blossom weevil by mass trapping quantified.	
P7.2.2	60	Second years experiments evaluating IPDM programme in commercial crops completed. Programme finalised and economic appraisal completed.	
P7.3	60	Best practice guidelines prepared.	

Secondary milestones

Milestone	Target month	Title	
S2.1	1	Site selected for botrytis.	Y
S1.1	2	Products selected for trial.	Y
S1.2	11	Site selected for mildew risk trial.	Y
S1.3	20	Mildew risk system coded as a computer program with Botem.	
S2.3	24	Incidence of botrytis on planting materials determined	
S5.1.2	24	Second year experiment to evaluate the effectiveness of flowering plants to attract aphid predators and parasitoids complete.	
S5.2.2	24	Field experiment testing the release rate of each plant volatile to attract aphid natural enemies completed and the most effective lure identified.	
S5.3.2	24	Second year trial evaluating the efficacy of post harvest aphicide treatment completed, feasibility determined and best treatment identified.	
S6.2	24	Design of super trap for blossom weevil adjusted to minimise the capture of non-target arthropods.	
S3.3	29	Cross-inoculation of selected blackspot isolates completed.	
S1.5	33	Alternative products selected for the larger trial against mildew.	
S2.6	36	Methods for reducing botrytis in planting materials determined.	
S1.7	36	Methods for reducing mildew in planting materials determined.	
S4.2.2	36	System for using hexyl butyrate as a repellant of *L. rugulipennis* females developed ready for testing in IPM programme in final 2 years.	
S5.1.3	36	Third year experiment to evaluate the effectiveness of flowering plants to attract aphid predators and parasitoids completed	
S5.2.3	36	Replicated field experiments evaluating the efficacy of the	

		most effective dispenser of the host volatiles deployed in lattice through the crop completed and the feasibility of using them for attracting aphid natural enemies determined.	
S6.4	36	The effect of reducing the amount of Grandlure I in the sex aggregation pheromone lure for blossom weevil established and optimum amount established.	

Technology transfer activities

A workshop was organised at East Malling Research to demonstrate the predictive models for strawberry grey mould and powdery mildew. In addition to researchers from EMR and Hatfield University, leading growers and consultants attended the meeting. We have discussed how to make the model predictions more accessible to growers (presentation style), field evaluation protocols and selection of evaluation site. Following the workshop, the software has been modified, evaluation protocols developed and sites selected for model evaluation in 2009.

Publications

10 Jul 2008. J Cross delivered a 15 minute lecture 'Development of zero pesticide residue Integrated Pest & Disease Management for UK fruit crops' at International Congress of Entomology, Durban, SA

11 Nov 2008. J Cross gave a 40 minute lecture at the EMRA soft fruit day about the zero residue soft fruit projects

Cross, J. V., Berrie, A. M. Xu, X., O'Neill, T., Wedgewood, E., Allen, J., Hall, D. R., Farman, D., Birch, N., Mitchell, C., Jorna, C., Shepherd, T., Boonham, N., & Spence, N. 2009. Free of pests, diseases and residues. HDC News No. 150 February 2009, 22-24[Update on HortLINK projects HL0175 and HL191].