Project title:	Minimising pesticide residues in strawberry through integrated pest, disease and environmental crop management
Project number:	SF 94 (HL0191)
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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

- New control approaches have been developed for the main pests and diseases of strawberry and these have been combined into a new Integrated Pest and Disease Management programme which will reduce pesticide use and greatly reduce the incidence of pesticide residues.

Background and expected deliverables

The overall aim of the project is to develop alternative, sustainable, non-pesticidal methods for managing Botrytis, mildew, black spot, aphids, blossom weevil and capsid bugs on strawberry so greatly reducing (by >50%) 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 2 seasons.

Summary of project and main conclusions

Progress on each objective of the project is summarised below

Powdery mildew

Inoculum in planting material

Over the last three years, we did not find a significant level of latent powdery lesions on planting materials. Furthermore, symptomatic mildew lesions (spores) on green leaves did not appear to survive in cold store if the green leaves become senescent, but can survive over the winter on green leaves.

Alternative products

None of alternative products tested showed any significant control effects against powdery mildew. This is primarily due the fact that the trial was conducted on a two-year old Albion plantation where it had a very high level of inoculum.

Mildew forecasting

On early covered ever-bearers, the model-managed plots had similar levels of powdery mildew as the conventional managed plots. However, the managed plots only received only 4 sprays compared to more than 20 sprays in the conventional plots. However, on a late 60-day Sonata crop, the evaluation trial failed to give any result because the initial mildew level was so high that a routine control programme failed to reduce the level before the trial could commence.

Botrytis

Occurrence of latent Botrytis cinerea

Fifty ex-cold store strawberry Elsanta plants, supplied by the grower on the day of planting, were examined for latent infection by *B. cinerea*. *B. cinerea* was detected in 8% of the plants sampled indicating localised infection.

Fungicide efficacy

Fungicide sprays and drenches were applied 3 weeks after planting. The fungicide treatments were: Untreated control, Cercobin WG drench at 1 g/L (0.25 g per plant), Teldor spray at 1.5 kg/ha, Scala spray at 2 L/ha, Signum spray (old label) at 1.8 kg/ha, Switch spray at 1 kg/ha and Serenade ASO spray at 10 L/ha. In crop assessments were carried out 2 weeks after treatment. 20 tagged leaves per plot were sampled, surface disinfected and placed into humid incubation and assessed for *B. cinerea*. Overall within this crop *B. cinerea* was at a low level. No clear consistent differences were shown between the fungicide treatments, but Signum showed some initial promise in the leaf humid incubation assessments.

Control of fruit infection using Binab (Trichoderma spp.)

A grower standard spray programme of 4 fungicides at weekly intervals was compared with three biocontrol treatments; Prestop (*Gliocladium* spp.) and Serenade ASO (*Bacillus subtilis*) applied as weekly sprays and Binab T-Vector (*Trichoderma* spp.) vectored by bees.

Assessments were carried out on leaves, flowers and fruit to assess levels of botrytis. A high level of latent infection by *B. cinerea* was present in flowers and leaves of strawberries in the two experimental tunnels. Both bumble bees and honey bees effectively transferred the biocontrol product from the hives to the flowers. None of the treatments significantly reduced the incidence of latent infection by *B. cinerea* in strawberry flowers or fruit, or the incidence of botrytis fruit rot.

BOTEM forecasting of botrytis

Validation results in 2010 again confirms those of previous years: botrytis risk on June-bearers (Elsanta) covered early in the early spring is very low. The level of fruit with latent botrytis infection is very low in both conventional and unsprayed plots. The results from all three years (2008-2010) suggested that for early-covered June-bearers fungicide application is not necessary to manage grey mould.

Pesticide dissipation

Fungicide residues are very persistent on leaves of strawberry plants grown under protection: residues virtually did not reduce 10 days after applications. In contrast, much of fungicide residue was washed off those plants in open conditions due to the rain one day after the application. Thus, it is critically important to establish harvest intervals for strawberry grown under protection for each pesticide; using the data from open-field conditions may result in significant amount of residues on fruit under protection.

Black spot

Molecular comparison of black spot isolates

Molecular analysis of isolates from different hosts at several sites suggested that significant differentiation among isolates only occurred between different sites but not between hosts at the same site. Thus, it does not appear that there is significant host-pathogen association for this pathogen yet.

Using artificial inoculation to confirm the molecular findings

Thirteen isolates of *C. acutatum* , previously isolated from strawberry, apple, weeds, primula and alder were inoculated onto strawberry fruits of the variety Red Glory. All isolates caused lesions on the fruit but there were differences in lesion size and sporulation of *C. acutatum*. The highest lesion scores were on fruit inoculated with isolates from strawberry, apple and alder. The lowest scores were on isolates from weeds. The results indicate that weeds and other non-strawberry hosts could act as a source of inoculum for *C. acutatum* in strawberry plantations. Further tests will be conducted in 2011. Similar tests on plants are still in progress.

Evaluation of biofumigants to eliminate Colletotrichum-infested debris in soil

In the Hortlink biofumigation project (HL0177 – SF 77) biofumigants to control verticillium on strawberry were investigated. The project identified lavender waste and some brassica products, including Biofence as potential biofumigants. Soil fumigation is an important part of the integrated approach to control blackspot in strawberry production. The purpose of this study is to evaluate the efficacy of these products against *C. acutatum* in the laboratory, based on the protocol developed for *Verticillium dahliae* testing. Protocols for evaluating the efficacy of the biofumigants have been established. The tests will be set up later in 2011, once blackspot-infected strawberry debris has been collected.

Development of simple guidelines for blackspot management

Simple guidelines will be developed to assist growers in making decisions regarding the need for management measures against blackspot, based on published data and newly available information on blackspot from this project. These guidelines will assess the relevance of various inoculum sources (runner origin, site history, alternative hosts etc), available control methods (fungicide efficacy, BCAs and biofumigation), production systems and local environmental conditions. Draft guidelines have been produced. These will be used in the IPDM trials established in 2011 and discussed and amended as necessary.

European tarnished plant bug

A large scale field experiment was done to evaluate the use of the bug vac for control of *L. rugulipennis* in strawberry. Weekly bug vacs at the peak of *L. rugulipennis* populations (from the beginning of July, peaking at the end of August) were applied to half of the plots. Both the non-bug vacced and bug vacced plots were sampled before and after each bug vac operation. Overall the numbers of most invertebrates including *L. rugulipennis* adults and nymphs were reduced by 10 - 40%. The reduction of fruit damage in the bug vacced plots was lower, but not significantly so. A number of recommendations for the bug vac operations have been made; 1) the bug vac to be front mounted to prevent bugs flying away as the tractor passes over the beds, 2) begin bug vaccing as soon as the rise in populations is detected with the pheromone traps (~4 weeks before detection in field using traditional sampling methods), 3) more frequent passes over crop – at least 3 times per week.

In an experiment to test the neccessary growing conditions of alyssum (attractant of *L. rugulipennis*) in strawberry crops, alyssum seed sown directly into soil did not establish well and seedlings were subject to competition from weeds and drying out. Plug plants sown

directly into the soil were also vulnerable to competition from weeds. Plants grown in grow bags with drip irrigation developed best. Trials with alyssum varieties are showing that the varietyClear Crystal produces more vigorous growth and more flowers than Snow Crytal>Snow Drift>Easter Bonnet>Gold Ball.

Hexyl butyrate dispensers were used in combination with live female *L. rugulipennis* and artificial sex pheromone in field experiments to determine the mechanism of reported population reductions. Results were not consistent, but in general a lower % of males were found in samples when hexyl butyrate was present than when it was absent.

Aphids

Small plot experiments were done to assess the effects of sowing flowering plants alongside strawberry plantings on the numbers of aphid predators and parasitoids in the crop. The plants used were lucerne (*Medicago sativa*)*, red campion* (*Silene dioecia*)*, viper's bugloss* (*Echium vulgare*) and a mixture of annual species, cornflower (*Centaurea cyanus*)*,* corn marigold (*Anthemis arvensis*) and corn chamomile (*Chrysanthemum segetum*). There was no apparent effect of these flowering plants on the numbers of beneficials found in adjacent strawberry plants when compared with a bare soil control.

Earlier work has demonstrated that various plant volatiles are attractive to a range of insect predators. However, work within this project both in laboratory olfactometry and field trapping experiments has failed to identify an attractive volatile for any predators of strawberry pests, with the exception of hoverflies. Further experiments with mass releases of a commercially available predator, *Orius laevigatus*, failed to show any response of this predator to lures containing farnesene, methyl salicylate or a mixture of farnesene, methyl salicylate, phenylethanol and caryophyllene.

In a field scale field trial using 4 different timings of Calypso between the end of September and beginning of November, all applications reduced the numbers of aphids (*Macrosiphum euphoriae*) present on the crop the following spring compared to the untreated control (less than 50 aphids/100 leaves compared to more than 400 aphids/100 leaves).

The parasitoid *Aphidius eglanteriae* has proved to be a difficult species to mass produce so an alternative species, *Ephedrus cerasicola* was assessed for its effectiveness in reducing *C. fragaefolii* populations in a potted plant experiment. A mix of six parasitoids was used and compared with *E. cerasicola* alone and an untreated control; this mix has been designed to

contain species that attack all the main aphid pests of strawberry. Results showed that releasing parasitoids onto aphid-infested plants significantly reduced the populations of both *C. fragaefolii* and *M. euphorbiae*.

Strawberry blossom weevil super trap

Three field trials in Kent and Hereford were set up to determine if the supertrap could be used as a mass trapping (MT) device for *A. rubi*. Supertraps were found to be a sensitive indicator of the presence of *A. rubi* populations, but it was not clear if the catches were related to the population density. Further work is needed to establish the relationship between monitoring traps (in small numbers in crops) and weevil populations and where best to site the traps in crops for monitoring purposes. The 2010 data suggest that the MT treatment (grid 36 supertraps per ha) performed well at one site where the *A. rubi* populations were low, but at the two organic sites, where *A. rubi* populations were higher, they only captured < 30% of the weevils and did not reduce severing damage in the crop. The results do suggest that the density of deployment of 36 traps in a 1 ha plot (= 25 traps per ha in large plots) is insufficient where populations are moderate or high and the density needs to be increased, or the traps used in conjunction with chemical treatments. Ideally, a smaller, low cost trap should be developed which can be deployed economically at higher densities for MT. It is likely that the supertraps will perform better at very low populations densities, in crops which come into flower later and if they are deployed continuously through the season.

A small scale field trial was done to test combinations of trap designs for *L. rugulipennis* and *A. rubi*. White cross vanes on the bucket traps were a repellent to *L. rugulipennis* males. The *A. rubi* lures did not interfere with catches of *L. rugulipennis*. In previous experiments *L. rugulipennis* catches were impeded by the grids used as bee excluders. Numbers of *A. rubi* were too small to draw conclusions from. Any future combined monitoring trap should not have white cross vanes or a grid. The ideal trap would be a green cross vane that attracts *L. rugulipennis* and *A. rubi*.

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.

If the incidence of *B. cinerea* in propagation material can be reduced, and if this is shown to reduce risk of fruit rot, then fungicide application during flowering to control fruit *B. cinerea* could be reduced. A secondary benefit for growers would be an end to the picking disruption entailed by delaying harvesting (or picking and destroying) fruit developing within fungicide sprayed flowering crops.

Ultimately if the use of a biological product can be shown to decrease levels of *B. cinerea* developing from flowers to fruit, the reduction in spray costs and the disruption of harvest intervals will produce financial benefits.

Action points for growers

- The risk of Botrytis on early covered June-bearer strawberries is very low so spraying with fungicides against Botrytis may not be necessary. For everbearers later in the season, the EMR Botem computer-based forecasting model (available from Prof Xiangming Xu at EMR, xiangming.xu@emr.ac.uk) can be used to time sprays of fungicides or biocontrol agents and may result in a substantial reduction in fungicide use.
- Effective early control of powdery mildew is essential to minimise the risk later in the crop and if such good early control is achieved then a computer based forecasting model available from EMR can be used to time sprays and may result in a substantial reduction in fungicide use.

- A preliminary simple management system for blackspot has been devised and is summarised in Tables 3.4.1.-3.4.4 in the science section of this report. This updates the current HDC factsheet (Factsheet 14/02). A copy of the document can be obtained from Dr Angela Berrie at EMR (Angela.Berrie@emr.ac.uk) or the HDC.
- Sex pheromone traps for monitoring European tarnished plant bug, a serious pest of late season strawberry, have been developed and are available to growers who wish to cooperate in a pre-release testing programme in 2011. For further information contact Dr Michelle Fountain (Michelle.Fountain@emr.ac.uk).
- Application of a late season spray of an aphicide (e.g. Claypso) in late October or November will greatly reduce populations of several of the most damaging and common aphid pests of strawberry and result in greatly reduced aphid populations the following spring, possibly obviating the need to spray. New formulations of mixtures of aphid parasitoid species are available from biocontrol suppliers and can be introduced in spring and will help prevent low spring populations from increasing.
- A new Integrated Pest and Disease Management programme which should reduce the use of pesticides and greatly reduce the incidence of residues on fruits at harvest has been devised and is being tested on a large scale on three commercial farms in 2011-12. The programme to be tested on everbearers is given in Table 7.1 on pages 93-95 of this report. An electronic copy of the documents can be obtained by HDC members from Prof Jerry Cross at EMR (Jerry.Cross@emr.ac.uk).

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)

1.1.1 Cold stored runners

Work completed

1.1.2 Module plants

Work completed

1.1.3 Survival of mildew on runners in a cold store

Methods

In October 2009 many runners (with mildew) were potted in the glasshouse and these runners were maintained over the winter in a polytunnel at EMR. About 20 runners with mildew were lifted and moved to cold-store at -2°C in December and January. In addition, runners from heavily-infected cv. Albion from an experimental plot were harvested in December 2009 and stored at -2°C. These three batches of runners, together with those remained in the polytunnel over the entire winter, were potted up (or re-potted) on 19 March 2010 and placed in CE cabinets: one batch in a CE cabinet (15°C and 75% rh).

Mildew was assessed on 9 April 2010 on the plants in CE cabinets as well as those plants that and remained in the polytunnel.

This experiment is being repeated in the 2010-11 winter. Currently, runners with mildew are being potted up.

Results

The day and night average temperatures from 1 December 2009 to 28 February 2010 are shown in Fig. 1.1.3.1. The 2009-10 winter was cold; on 22 occasions, average night temperature was below 0°C and on three occasions average day temperature was below 0°C.

Of those plants remaining in the polytunnel but re-potted and incubated in a CE cabinet, all 13 plants had mildew. None of plants stored from December (9 survived out of 17) and January (15 out of 17 alive) showed any mildew symptoms. For the Albion runners, again there were no mildew lesions on 12 surviving plants (out of 15). For those plants remaining in the polytunnel, 20 out of the 39 plants had fresh lesions on young leaves that just emerged in the spring.

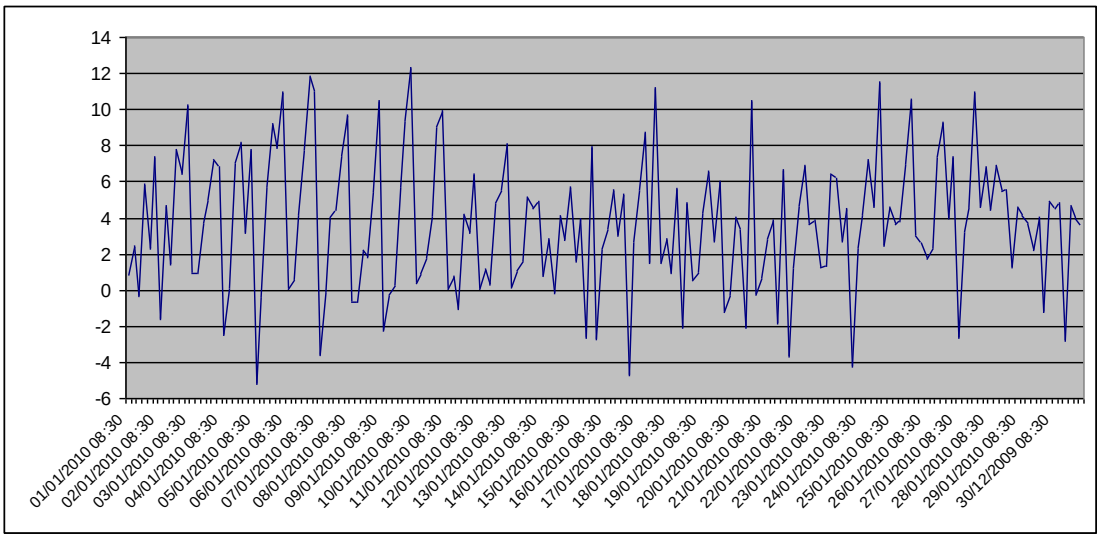


Figure 1.1.3.1 Average day and night temperatures from 01/1209 to 28/02/2010 in a polytunnel where strawberry plants were kept over the winter.

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

Methods

Two treatments were tested: normal and high nitrogen input during the fruiting period. Peat growing bags were used; 10 cold-stored runners of cv. Elsanta were planted in each bag. Initially all plants from both treatments were subjected to the same fertigation regime before flowering; the master concentration of fertigation was: N – 120 mg/l, P – 45 mg/l, K – 176 mg/l, Ca – 71 mg/l, Mg - 20 mg/l, Na - 37.4 mg/l, CI – 48 mg/l and S – 18 mg/l. After

blossom, plants in the high nitrogen treatment received fertigation with the master concentration of N – 197 mg/l, P – 45 mg/l, K – 203 mg/l, Ca – 71 mg/l, Mg - 42 mg/l, Na - 37.4 mg/l, CI – 48 mg/l and S – 18 mg/l; whereas plants in the normal nitrogen regime received fertigation with the master concentration of N – 128 mg/l, P – 45 mg/l, K – 248 mg/l, Ca – 71 mg/l, Mg - 42 mg/l, Na - 37.4 mg/l, CI – 48 mg/l and S – 63 mg/l.

In total, 16 growing bags (i.e. 160 plants) were used in the experiments, which were conducted in the confined GroDome compartment (day temperature – 22°C and night 16°C). Fig. 1.2.1 shows the exact experimental set-up of the 16 grow bags):

N H N H
H N H N
N H N H
H N H N

Figure 1.2.1. Experimental set up of 16 grow bags allocated to normal (N) and high (H) nitrogen treatment.

Irrigation and fertigation were delivered to plants via four irrigation lines, each with separate controls; two of these were randomly allocated to each treatment. Twenty drippers were attached to each irrigation line at a regular interval; five of 20 drippers were then inserted to each grow bag. The amount of water used to irrigate plants was such that the moisture content inside the grow bag was around the maximum holding capacity (determined in preliminary experiments using the same type of the bags) but without excess leaking of water from the bag. Moisture content in the growing media was checked daily. If necessary, the amount of irrigation water was adjusted. In general, apart from the first few days of the blossom period, each bag received about 2 L water per day. The master fertigation solution was mixed with irrigation water at a ratio of 1:100 via a control and delivered to each bag.

Two weeks after the onset of flowering, 20 mildewed plants were systematically placed between grow bags. These mildewed plants were placed about 10 cm higher than the plants in grow bags in order to facilitate spore dispersal to experimental plants. Mildew was assessed three weeks later.

This experiment was conducted twice in 2009: the first from late May to early August, and the second from mid August to mid October. In the first experiment, because of severe mildew, percentage area of mildew was estimated on each leaflet of top three fully unrolled leaves on each plant. In the second experiment, the number of mildew lesions was counted on each leaflet of top two fully unrolled leaves on each plant. Thus, we had 80 plants for

each treatment. Data were subjected to generalised linear model analysis to assess treatment effects.

Results

Severe mildew developed in the first trial. On average, about 65% of leaflets were infected. Although the incidence of leaflets infected was significantly ($P < 0.05$) greater in the high nitrogen treatment (67%) than in the normal nitrogen treatment (64%), the difference was very small. Similarly, there were highly significantly ($P< 0.001$) differences in the percentage of area infected between the two treatments: 10% and 7% infected for the high and normal nitrogen treatment, respectively.

The same pattern of the results was obtained in the second trial where the mildew was less severe. About 71% of leaflets were infected in the high nitrogen treatment, compared to the 63% in the normal nitrogen treatment; but this difference was not significant. In contrast, the number of mildew lesions was significantly greater ($P < 0.05$) in the high nitrogen treatment (3.2 per leaf) than in the normal nitrogen treatment (2.5 per leaf).

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

Methods

Experiments were conducted on strawberry plants under protection (plot DM182 EMR): 3 beds of cv. Albion. Runners were planted out on 27 April 2009 and covered three weeks later. Each bed had 230 plants (each with a double row of 115 plants per row). The first spray was applied late May and a further three sprays were applied at an interval of 10 days.

Each bed was designated as a block, containing all 11 treatments (see below). Each plot was treated with an appropriate product. In total there were 11 treatments: Serenade (5 ml/L), Garshield (5 ml/L), sodium bicarbonate (3g /L), Chitoplant (0.5 g/L), potassium bicarbonate (10 g/L) together with Silwet (0.5 ml/L), Enzicur (2 ml/L) + Addit (2.5 ml/L), Milsana (12 ml/L), Farmfos (10 ml/L), Eradicoat (15 ml/L) and Systhane EW20 (115 □l/L) and untreated. Thus, for each treatment, there were three replicate plots. In total, the spray was timed to dispense 600 ml to each plot with a knapsack sprayer – equivalent to 1000 L/ha. The number of mildewed leaflets was recorded on up to the five youngest fully unrolled leaves on each of 10 plants in the central plot on 15 July 2010.

Generalised non-linear mixed model will be used to analysis data, where block is treated as a random factor and products treated as fixed. In this analysis, number of leaflets with mildew per plot is assumed to be binomially distributed. Furthermore, logit of infected fruit per plot was subjected to a linear mixed model analysis where spatial position of each treatment plot was considered as well.

Results

The proportion of mildewed leaflets varied greatly from plot to plot (Table 1.3.1). Although there were large differences among treatments, these differences were not statistically significant once the spatial location of each plot was taken into account in statistical analysis. The lack of differences among treatments may be due to the fact that this plantation had much high inoculum potential before of severe mildew epidemics in the previous season.

Table 1.3.1 Proportion of infected leaflets (five leaves up to 10 plants) in each plot

	Block 1	Block 2	Block 3	Overall
Chito_Plants	0.39	0.43	0.30	0.38
Enzicur	0.30	0.47	0.24	0.34
Eradicoat	0.61	0.32	0.22	0.39
Farmfos	0.49	0.21	0.21	0.30
Garshield	0.51	0.57	0.19	0.42
Milsana	0.35	0.61	0.21	0.39
Potassium bicarbonate + Silwet	0.19	0.64	0.23	0.34
Serenade	0.67	0.24	0.11	0.36
Sodium bicarbonate	0.19	0.26	0.15	0.20
Systhane	0.61	0.62	0.16	0.49
Untreated	0.44	0.30	0.09	0.28
Mean	0.43	0.43	0.19	0.35

1.3.2 Survival of biocontrol agents

Completed.

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)

Methods

An experiment was done to determine whether fungicide dissipation differed between under protection and open-field conditions, and hence their persistence under protection. Systhane EW20 and Rovral FLO were applied at the full rate and sprayed with a farm sprayer (cv. Albion plants (EMR plot DM182)) on 30 September 2010. Half of the plants were unprotected and half protected. There were heavy rainfalls on 1 October 2010 following the application.

On the day of spray application, a single fully unrolled leaf was randomly cut off from each of the 10 plants randomly selected, giving five composite samples (each with two leaves) for each condition - protected and unprotected. Total leaf area was measured for each sample before being sent to QTS for quantification of residues. A second batch of samples were similarly taken on 8 October 2010 and sent for quantification of residues.

Results

There was heavy rain the day after the application. Figure 1.4.1 shows residues for all individual samples. As expected from previous research studies on many crops, there were large differences in residues among individual samples for both chemicals. Overall, the residue immediately after application for iprodione (1.63 μg cm^{-2}) was more than 20 times of that for myclobutanil (0.08 μg cm^{-2}).

Overall, there were significant reductions in iprodione residues on leaves between day 0 and 8. There was no reduction in iprodione residues on leaves of strawberry plants inside the tunnel seven days after application; in contrast more than 60% reduction in the iprodione residues was observed on the outside plants (Fig. 1.4.1). Overall, there were significant reductions in myclobutanil residues on leaves between day 0 and 8. Reduction in the myclobutanil residues was much greater on the outside strawberry plants (ca. 85%) than inside the tunnel (ca. 43%).

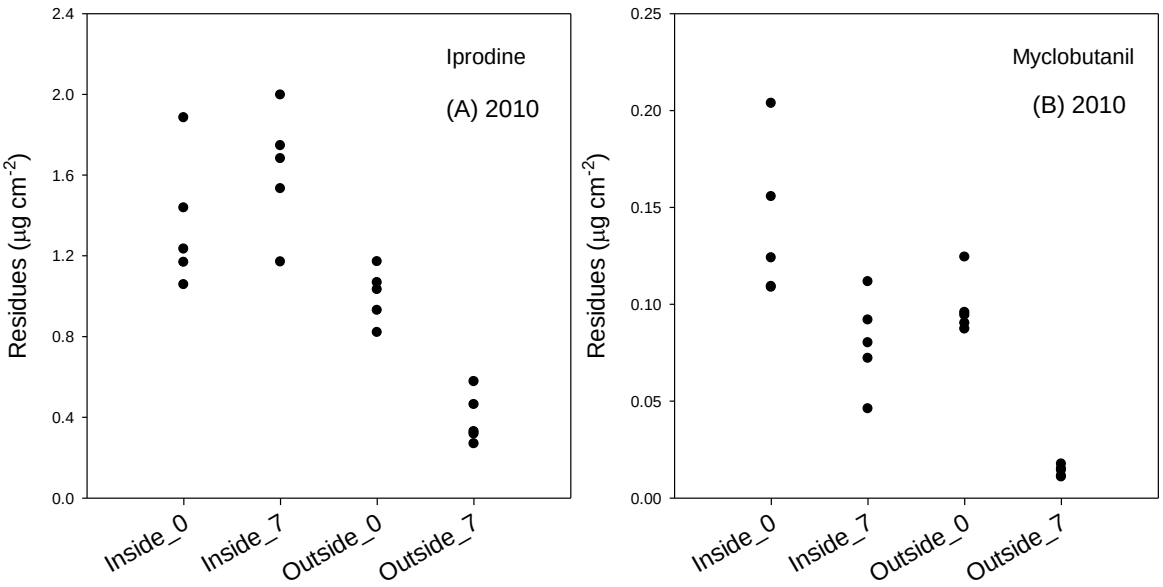


Figure 1.4.1 Scatter plot of residues of iprodione and myclobutanil on strawberry leaves sampled immediately after spray deposits were dried (day 0) and on day 7. X-axis label: Inside – plants grown under protection, Outside - plants in open conditions, 0 (7) – sampling day 0 or 7.

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

Methods

The mildew warning system was evaluated at two farms in Kent. However, mildew failed to develop (as in 2009) on tabletop everbearer strawberries (c.v. Elsinore) at Goose Farm, Shalloak Rd, Broad Oak, Canterbury. This experiment was terminated in July. An experimental setup, similar to 2009, was also conducted at Goodings Field at Gaskains, Norham Farm, Selling, Kent on 2nd season tabletop everbearer strawberries (cv. Red Glory). There were four tunnels allocated to this study: two (tunnels 2 and 4) for conventional spray and two (tunnels 1 and 3) for model-based management. The conventional programme was a standard farm programme. Non-mildew fungicides/nutrients were applied as normal in all four tunnels. The experiments started from 27 May onwards and ended in early August. In tunnel 1 a small section (10 m long) in the end of the tunnel was allocated as an unsprayed area.

Three data loggers were installed in the tunnels to monitor in-tunnel temperature and

humidity. Data were regularly downloaded to generate model predictions. Mildew was assessed on several occasions, and the final one on 13 August. On each date, five leaves on each of randomly selected 20 plants were assessed in each plot.
At the Selling farm, the same trial was also conducted on a newly planted 60 day strawberry cv. Sonata. The crop was planted in late July 2010.

Results

On cv. Red Glory, during the entire period the model-managed tunnels only received four sprays – late June, mid and late July and early August. The conventional programme received more than 20 sprays.

Mildew was monitored weekly from 27 May onwards. Powdery mildew lesions were first seen on untreated plants on 21 June, but were not seen in both conventional and managed plots on the same date. Significant numbers of new mildew infections were seen on untreated plants on 14 July. The grower was advised to spray the untreated plot as soon as possible in order to reduce spread to the model-managed section in the same tunnel. A low level of mildew was also seen in managed and conventional plots on 14 July. On the final assessment date (13 August), the untreated plot had nearly 67% of leaflets with mildew, compared to 39% (36.7% and 41.3%) for the managed plots, and 45% (46% and 43.3%) for the conventional plots.

On the 60-day Sonata crop, severe mildew was observed one week after planting in all plots. Despite an intensive control programme, it was not possible to control mildew successfully on these plots. It was decided to terminate the trial four weeks later. However, weather data from the tunnel was collated. Running the model indicated that weather conditions during late July to early September were very favourable for mildew development.

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

and

Task 2.1.2: Determine whether pre-planting or post-establishment treatments are able to reduce the incidence of botrytis in ex-cold stored strawberry plants

Introduction

Strawberry runners kept in cold-store until required for planting often have moribund petioles and leaf debris at planting that are susceptible to infection by *B. cinerea*. *B. cinerea* spore germination and mycelial growth can occur on plants at the low temperatures found in cold-storage. In some other crop species (e.g. lettuce, primula), it has been found that some plants which appear healthy have symptomless (latent) infection by *B. cinerea*. Work as part of this project and elsewhere has shown that *B. cinerea* can occur as a latent infection of strawberry crowns and petioles and be present without symptoms on fresh leaves and roots.

The objective of this work was to determine whether treatment of recently established plants would reduce the level of *B. cinerea* in them. Post-planting treatments included fungicides more usually applied at flowering. Cercobin WG is used as a systemic drench against *Verticillium,* but activity against *B. cinerea* has been reported.

Teldor, Scala, Signum, and Switch are all approved for the control of *B. cinerea* and are from different fungicide activity groups. Serenade ASO is a bio-control agent with label recommendations for *B. cinerea* control on strawberry. Achieving lower levels of sporulating *B. cinerea* in the field pre-flowering is considered likely to decrease *B. cinerea* flower and fruit infection.

Methods

The experiment was carried out in a commercial crop of strawberries cv. Elsanta at Place UK near Norwich. The dates on which tasks were carried out are shown in Table 2.1.1. Plants were planted by the grower in raised beds and allowed to establish before receiving a single drench or spray application of the products shown in Table 2.1.2 on 1 July 2010. There were four replicates of each treatment arranged in randomised blocks. *B. cinerea* incidence was assessed in plant crowns and other parts. The crop was monitored for phytotoxicity and samples of leaves were taken from the field to assess the levels of *B. cinerea* post-treatment.

Table 2.1.1: Details of work to determine latent *B.cinerea* in a commercial strawberry crop, Place UK Norwich 2010

Date	Task
07/06/2010	Trial planted with cv. Elsanta by grower
08/06/2010	Trial laid out as per plan, grower was currently misting the plants this will continue for 2 to 3 weeks, 50 plants sampled, to check for *B.cinerea*
17/06/2010	Examination of laboratory sample for *B.cinerea*
22/03/2010	Examination of laboratory sample for *B.cinerea*
29/06/2010	Examination of laboratory sample for *B.cinerea*
01/07/2010	Trial sprayed with all treatments. The newest fully expanded leaf was tagged. The plants were very well established 2 to 3 true leaves and some flowers opening. Fortress applied by grower for powdery mildew and quite a bit of powdery mildew observed on the plots
15/07/2010	In situ leaf assessments carried out and leaf samples taken.
16/07/2010	Leaves surface sterilised and frozen over night
26/07/2010	Leaves assessed for *B. cinerea* day 7. Isolations taken - *B. cinerea* confirmed 10/8/10
10/08/2010	Leaves assessed for *B. cinerea* 21 days after incubation
12/08/2010	Assessment carried out of just actively sporulating *B. cinerea* on leaves and petioles
14/09/2010	In a separate trial *B. cinerea* was observed coming out of runners and petioles from 6 out of 40 tissues sampled showed clear *B. cinerea* growth after surface sterilisation

Sample details

A sample of fifty (18mm crown) cold-stored strawberry plants cv. Elsanta were obtained from the grower at planting. Plants were taken from a spare crate which had been left to one side for us to thaw in cold store post planting.

Tests for latent B. cinerea on plants at planting

Soil adhering to the crown and roots was washed off with tap water. Each plant was given an identification number which was used with each set of incubated material. The roots were cut off and mature leaves were taken off at the petiole base. The material removed from the plant crown was placed without surface sterilisation into a damp chamber comprising a tray lined with moist paper towel inside a sealed transparent polythene bag. The composite parts of each plant were laid out in a set position and assessed individually for fungal growth after 21 days incubation at room temperature under diurnal lighting. The plant parts assessed in damp chambers were: crowns with emerging young leaves and root bases, green leaves, a sample of roots, and senescing or rotting leaves and petioles.

Each crown (one per plant) was quartered to be able to sample internal tissue. Six cubes of about 5 x 5 x 5 mm were cut from inside each crown, three from the upper half and three from the lower half. All sections from one plant at a time were surface sterilised (10% by volume “Domestos” for five minutes, approximately 0.5% active chlorine), rinsed in sterile distilled water and placed onto Potato Dextrose Agar (PDA). Plates were incubated at 20°C, with exposure to near-UV light to encourage sporulation.

Fungal assessments

Tissue sections and damp chambers were examined for *B. cinerea* and *Colletotrichum* spp. The number of sections per plate with each fungus was recorded. The number of sections per plant free of any other fungi and clean of all fungi was also recorded. Agar plate assessments were made at both 14 days and 21 days to allow sufficient time for *B. cinerea* to grow out of the sections. The second assessment was made without reference to the first assessment. In some cases, colonies of *B. cinerea* became overgrown by other fungi between the two assessments and the *B. cinerea* was not visible, however, the plant was recorded as having infected tissue in the final total. Crown plates and damp chamber results were collated for each plant to determine the total number of plants which had *B. cinerea* recorded from any tissue source.

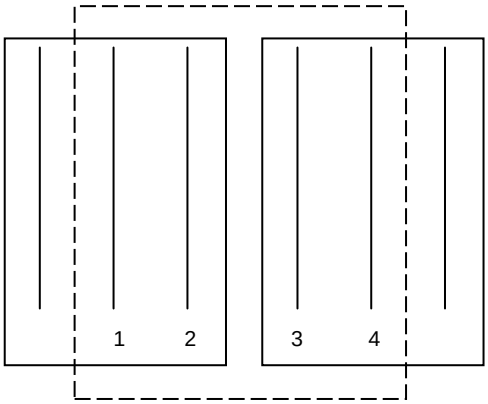
All samples were examined under low power magnification throughout assessment to look for *B. cinerea* conidiophores. The identities of samples of spores were confirmed under higher power magnifications. Where there was no sporulation present, subcultures on PDA were taken from some samples to confirm the presence of *B. cinerea.*

Fungicide applications

The runners were planted and grown to commercial standards by Place UK. Iit was ensured that no fungicides were applied by the grower with activity against *B. cinerea* but those to control powdery mildew were used.

The beds were left un-tunnelled until very close to fruiting and due to adverse weather conditions the fungicide applications were delayed until three weeks after planting, so some flowers were already present. During this time (three weeks) the grower was misting the crop to aid establishment. Single applications of fungicides (Table 2.1.2) were applied using a pressure assisted single nozzle sprayer to each 4 m plot with a guard length of ten plants between plots (Figure 2.1.1). The drench was applied using a beaker pouring the solution over the foliage and into the planting hole. On the application date (01/07/10) several leaves per plot were tagged with wool on the newest fully expanded leaf so it could be identified for sampling after 14 days.

Block	Plot	Treatment	**Block**	Plot	Treatment	**Block**	Plot	Treatment	**Block**	Plot	Treatment
1	7	T5	**2**	14	T4	**3**	21	T2	**4**	28	T3
1	6	T7	**2**	13	T3	**3**	20	T3	**4**	27	T4
1	5	T4	**2**	12	T5	**3**	19	T7	**4**	26	T1
1	4	T3	**2**	11	T1	**3**	18	T6	**4**	25	T7
1	3	T1	**2**	10	T7	**3**	17	T1	**4**	24	T2
1	2	T2	**2**	9	T6	**3**	16	T4	**4**	23	T6
1	1	T6	**2**	8	T2	**3**	15	T5	**4**	22	T5



Crop was planted into polythene covered raised beds. Plots were 4 m long with a guard length of ten plants between plots. The trial area was split over two tunnels.

Figure 2.1.1. Trial plan task 2.1, Norfolk 2010

Table 2.1.2: Treatments applied soon after planting for control of *B. cinerea* – Norfolk 2010

Treatment	Active ingredient	Application rate
1 Untreated	Water	-
2 Cercobin WG (drench)	Thiophanate-methyl	1 g/L (0.25 g per plant)
3 Teldor	Fenhexamid	1.5 kg/ha
4 Scala	Pyrimethanil	2.0 L/ha
5 Signum	Boscalid + pyraclostrobin	1.8 kg/ha (old label)
6 Switch	Cyprodinil + fludioxonil	1.0 kg/ha
7 Serenade ASO	*Bacillus subtilis*	10.0 L/ha

Post-spray assessments

At 14 days after the spray application, an *in situ* assessment was carried out. The crop was first examined for any phytotoxic effects and any dead or dying plants. For each plant the number of green leaves with any obvious brown lesions likely to be *B. cinerea* was recorded. The number of leaves with *B. cinerea* sporulation visible and the number with both symptoms were also recorded. The average number of unfolded leaves per plant per plot was estimated. Finally, twenty of the previously tagged leaves per plot were sampled and placed into clean labelled plastic bags. These were returned to the laboratory for incubation.

The sampled leaves were surface sterilised (2.5% by volume sodium hypochlorite for 5 minutes, approximately 8% active chlorine) rinsed in tap water and drained. Five leaves were spaced on to folded paper towel and placed into plastic bags and frozen (-20ºC) overnight. The trays were then incubated at 20ºC with diurnal lighting. The leaves and petioles were scored separately and together for *B. cinerea* seven and 21 days later, a hand

lens was used to identify sporulation. The reason that the leaves and petioles were scored separately was because latent botrytis moving from the crown out to leaves should show itself at the petiole stubs, whereas leaves could be infected by external *B. cinerea*.

Results

Latent *B. cinerea* was confirmed at a low incidence (2-4%) in roots, petioles and leaves and at a slightly higher incidence (8%) in the crowns of young plants at the time of planting (Table 2.1.3). In total 12 % of the 50 plants sampled showed botrytis, with only one plant showing symptoms in both damp and plate isolations.

At five weeks after planting, 11 – 20% of plants had leaf necrotic lesions suggestive of botrytis, with sporulating *B. cinerea* visible on a few of them (Table 2.1.4). None of the fungicide treatments applied 14 days earlier significantly reduced occurrence of the symptoms.

When samples of leaves were tested for latent *B. cinerea*, a high incidence of white to grey floccose mycelium, provisionally identified as *B. cinerea*, developed on the leaf blades and petioles after seven days incubation (Table 2.1.5). There was no *B. cinerea* sporulation by this date. The incidence of leaflet blades affected by this fungal growth was reduced by Signum from 98% to 70%, and not by other treatments. Overall the occurrence of leaflet blade infection was consistently greater than petiole infection and in most instances those leaves infected on the petioles were also infected on the blades.

When the same leaves were re-assessed 14 days later, over 80% of leaflet blades in all treatments showed fungal growth. There were no significant differences between treatments in this or other assessment categories. The second assessment was carried out independently of the first. In some cases, colonies of suspected *B. cinerea* became overgrown by other fungi between the two assessments and the *B. cinerea* was not visible. More commonly the mycelial growth was identified as *Trichoderma* spp. or other fungi and not *B. cinerea*, as *B. cinerea* elsewhere on the leaves was sporulating. A final assessment was therefore carried out only recording sporulating colonies of *B. cinerea* confirmed using low power magnification. *B. cinerea* was confirmed on 27-43% of leaflet blades and 0-10% of leaflet petioles (Table 2.1.6). There were no significant differences between treatments.

Table 2.1.3: Recovery of *B. cinerea* from strawberry plants cv. Elsanta ex-cold store from two tissue incubation tests (crown isolations and plant damp incubation), Norfolk 2010

% *B. cinerea* on	Roots	Petioles	Dead leaves	Crowns	Leaves	Total no. of plants affected /50
Damp chambers	2	2	4	0	2	4
PDA+s plates	-	-	-	8	-	4

Table 2.1.4: Occurrence in the field of *B.cinerea* symptoms on leaf tissues, Norfolk 2010.

Treatment	Average leaves unfurled/plant	Mean number plants with Bot. lesions	% Plants Bot. lesions	% Plants sporulating Bot.
1.Untreated	12.8	2.8	13.8	0.0
2.Cercobin WG	10.8	3.3	16.2	0.0
3.Teldor	10.8	2.5	12.5	2.3
4.Scala	10.3	3.3	16.2	0.0
5.Signum	9.8	3.8	18.8	0.0
6.Switch	12.0	4.0	20.0	2.1
7.Serenade	12.5	2.3	11.2	0.0
Significance	ns	ns	ns	ns
LSD (18 df)	2.53	12.32	2.71	0.36

Bot. – *B. cinerea* *ns – not significant*

Table 2.1.5: Leaf damp chamber seven day assessment (leaves picked 14 days after fungicide application Norfolk 2010)

Treatment	% Clean leaflet blades	% Clean petioles	% leaflet blade with Bot.	% petiole with Bot.	% of entire leaf with Bot*.
1.Untreated	2.5	56.2	97.5	43.8	100.0
2.Cercobin WG	2.5	40.0	97.5	58.8	98.8
3.Teldor	2.5	56.2	97.5	43.8	98.8
4.Scala	8.8	52.5	91.2	47.5	100.0
5.Signum	30.0	68.8	70.0	30.0	100.0
6.Switch	10.0	52.5	90.0	47.5	97.5
7.Serenade	6.3	66.2	93.8	33.8	98.8
Significance	<0.001	ns	<0.001	ns	ns
LSD (18 df)	11.28	21.11	11.28	20.96	2.29

Bot. – *B. cinerea; ns – not significant*
* leaflet blade and petiole

Table 2.1.6: Occurrence of sporulating *B. cinerea* on leaves picked 14 days after fungicide application (21 day assessment) - Norfolk 2010

Treatment	% Leaflet blades with sporulating *B. cinerea*	% petioles with sporulating *B. cinerea*	% entire leaves with botrytis
1.Untreated control	42.5	1.3	42.5
2.Cercobin WG	32.5	8.8	32.5
3.Teldor	27.5	0.0	27.5
4.Scala	36.2	5.0	36.3
5.Signum	40.0	10.0	40.0
6.Switch	33.8	5.0	33.8
7.Serenade	33.8	5.0	33.8
Significance	ns	ns (0.061)	ns
LSD (18 df)	20.18	6.77	20.18

Bot. – *B. cinerea* *ns – not significant*

Figure 2.1.2:

Sporulating *B. cinerea* on strawberry leaflet after 21 days incubation

Discussion

This experiment confirmed the results of earlier work and showed that young strawberry plants can be symptomlessly infected by *B. cinerea* at the time of planting. A single application of fungicides with known activity against *B. cinerea* at 21 days after planting failed to reduce the incidence of *B. cinerea* symptoms in the field, or latent infection within leaves 14 days later. The level of infection confirmed in the leaves at this time (28-43%) was much greater than the incidence detected in plants at planting (up to 8%). Possibly infection by *B. cinerea* in young plants at planting was localised and /or at low quantities, and consequently underestimated by the isolation and incubation tests. Alternatively, the incidence of infected plants may have increased between planting and sampling five weeks later due to external inoculum. As plants were misted for three weeks, to aid establishment, conditions were likely to be conducive to further infection of leaves by *B. cinerea* from conidia in the air.

The failure of treatments to reduce incidence of latent *B. cinerea* within plants was disappointing. Products from a range of different fungicide groups were used, so the lack of efficacy is very unlikely to be due to fungicide resistance. Possibly treatments were insufficiently able to penetrate leaves to eradicate *B. cinerea* mycelium, and are better suited as treatments to prevent establishment of new infections arising from external inoculum. This would suggest that efforts to reduce latent *B. cinerea* in young strawberry plants at planting would be better directed at preventing infections during the plant propagation stage.

Conclusions

- There is increasing evidence that young strawberry plants at planting are symptomlessly infected by *B. cinerea*
- Treatment of young strawberry plants soon after planting with a single treatment of fungicides or biofungicides currently available for control of *B. cinerea* did not reduce the incidence of latent infection in a crop

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 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 could reduce *B. cinerea* infection of the strawberry flowers.

In 2008 the efficacy of Binab, applied once as a spray (Binab TF WP) and subsequently as a dry powder (Binab T-Vector) transmitted to flowers by honey bees and bumble bees, was evaluated for control of flower infection, fruit infection and fruit rot in a replicated experiment at ADAS Boxworth. *Trichoderma* spp. was 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 *B. cinerea*.

In 2010 work was done in a commercial crop. The Binab product containing *Trichoderma spp* was compared with two other biological control products, a grower standard fungicide spray programme (for the control of *B. cinerea* during flowering) and an untreated control. The other biologicals to be assessed were; Serenade ASO (*Bacillus subtilis)* and a new product developed in Finland called Prestop (*Gliocladium catenulatum*) applied as sprays. This latter product has been shown to reduce *B. cinerea* in various edibles including tomatoes and strawberries as well as ornamental plants.

Methods

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 and carried out at Sunclose Farm near Cambridge. Below is the timetable of when tasks were carried out (Table 2.2.1)

Table 2.2.1: Trial diary task 2.2, Sunclose Farm Cambs. 2010

Date ***Wks 18-23***	Task ***Treatment application and flower sampling***
5/5/10	Hives set up and trial marked out
6/5/10	Pre trial leaf and flower samples taken for incubation BINAB WF spray applied 500g/ha +1kg sugar in 400L water to tunnel B.
11/5/10	Flowers tagged (blue wool) and 20 flowers sampled/plot (pick1). 1st Sprays carried out in tunnel A and BINAB vector introduced into tunnel B
19/5/10	Flowers tagged (red wool) and 20 flowers sampled/plot (pick 2). 2nd Sprays carried out in tunnel A and BINAB vector replaced in tunnel B
26/5/10	Flowers tagged (pale blue wool) and 10 flowers sampled/plot (pick 3). 3rd Sprays carried out in tunnel A and BINAB vector replaced in tunnel B
24/5/10	Pre trial flower and leaf samples assessed
1/6/10	Flowers tagged (pink/blue wool) and 20 flowers sampled from leg rows (pick 4). 4th Sprays carried out in tunnel A and BINAB vector replaced in tunnel B.
1/6/10	Flower pick 1 (11/5) assessed.
8/6/10	BINAB product removed from hives.
9/6/10	Final flower pick (5) 10 flowers from leg rows sampled. Flower pick 2 (19/5) damp chambered flowers assessed.
Wks 24-27	***Fruit sampling and assessments***
14/6/10	In situ fruit assessment 1. Fruit pick 1 - corresponding to leaf tag 1 - blue wool
16/6/10	Flower pick 3 (26/5) flowers assessed.
21/6/10	In situ fruit assessment 2. Fruit pick 2 - corresponding to leaf tag 2 - red wool Damp chambered fruit assessed from pick 1 (14/6)
22/6/10	Flower pick 4 (1/6) flowers assessed.
25/6/10	Fruit from pick 1 (14/6) assessed
28/6/10	In situ fruit assessment 3, corresponding to leaf tag 3 - pale blue wool
28/6/10	Fruit assessed from pick 2 (21/6)
29/6/10	Flower pick 5 (9/6) flowers assessed.
2/7/10	Fruit assessed from pick 2 (21/6)
5/7/10	In situ fruit assessment 4, corresponding to leaf tag 4 – pink/blue wool. Trial cleared up and temperature loggers downloaded

Hive set-up and spray application

Two commercial 150 m, 4-bed Spanish tunnels were used for the trial. The crop of strawberry cv. Evie 2 was planted into raised beds in 2009. The use of a crop in its second year was to ensure there was a natural inoculum of *B. cinerea* in the crop. The fungicide spray programme was carried out in Tunnel A and the bee dispersal programme in Tunnel B situated 8 tunnels apart to limit bees moving between tunnels. There were four replicate blocks within the centre two rows of each tunnel. Plots were 6 m long with 1 m guard plots between and the trial was situated in the front 75 m of the tunnels (Figure 2.2.1). The grower was fully briefed on fungicides which do not have activity against *B. cinerea* and could be applied for the control of other fungal diseases such as powdery mildew.

At the onset of flowering 50 leaves and 40 flowers were sampled from both tunnels to assess the background levels of *B. cinerea*. One week before the Binab T-Vector was introduced and the spray programme initiated the honey and bumble bee hives were set up on 5 May 2010 and the bees allowed to acclimatise with the dispensers empty but attached. One honey bee hive was set up at the front right of each tunnel with a water-proof shelter to protect against runoff from the polytunnel (Figure 2.2.2). The bumble bee hives were placed in the middle of the tunnels on crates to raise the hives to canopy level (Figure 2.2.2). The bee densities were advised by Robin Dean (Red Bee Company for honey bees) and Mike Abel (Agralan Ltd, agents for Biobest bumble bees). At this time 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 B.

By 11 May 2010 both honey and bumble bees were settled in and showing activity and the Binab T-Vector was added to the dispensers in Tunnel B. The bees walk through the dispenser and pick up the BINAB powder by static attraction, which is then broken when the bees “earth” themselves as they land on a flower. The dispensers were changed weekly to determine how much of the product had been dispersed.

Before each spray application and weekly in tunnel B, flowers that were just opening (and receptive to pollinators) were tagged. A minimum of three flowers per replicate block in each tunnel were tagged with different colour wool each week. This was necessary to trace their development through the next weeks to ripe fruit.

In Tunnel A the fungicide and Prestop applications commenced on the same day the Binab T-Vector was introduced to Tunnel B (11/5/10). The sprays were applied 4 times at 7 day

intervals using a pressure assisted knapsack sprayer at the rates shown in Table 2.2.2, with a separate sprayer reserved for use with the Prestop. The Binab T-Vector remained in the dispensers till one week after the final spray.

Tunnel A, 4th tunnel in field
standard spray regime

Bumble bee hive

DISCARD		Block	Plot	Treatment		Block	Plot	Treatment		DISCARD
		1	1	1		3	9	4		
		1	2	4		3	10	2		
		1	3	2		3	11	3		
		1	4	3		3	12	1		
		2	5	2		4	13	3		
		2	6	4		4	14	1		
		2	7	1		4	15	4		
		2	8	3		4	16	2		

Trial carried out on raised beds in the two centre rows of a four row tunnel- Plots 6m long with 1 m guard plot between.

Honey bee hive

Tunnel B, 12th tunnel in field
Binab vector tunnel

Bumble bee hive

DISCARD		Plot	Treatment		Plot	Treatment		DISCARD
					4	5		
		2	5					
					3	5		
		1	5					

Trial carried out on raised beds in the two centre rows of a four row tunnel- Plots 6m long with 1 m guard plot between.

Honey bee hive

Figure 2.2.1: Task 2.2 Trial plan Sunclose Farm, Cambs. 2010

Table 2.2.2. Details of biocontrol products and a fungicide programme evaluated for control of strawberry fruit botrytis – Cambs, 2010

Tunnel	Treatment	Active ingredient + Harvest interval	Rate	Timing
A	1. Untreated (water spray)	-	1000 L / ha	During flowering, 4 times at 7 day intervals
A	2. Serenade ASO	*Bacillus subtilis* 0 days	10 L/ha in 1000 L water	During flowering, 4 times at 7 day intervals
A	3. Prestop	*Gliocladium catenulatum* 0 days	6 kg in 1000 L water / ha	During flowering, 4 times at 7 day intervals
A	4. Signum (old label)	Boscalid + pyraclostrobin 3 days	1.8 kg/ha in 1000 L water	During flowering, 1st spray. 7 day interval before next product
	Frupica	Mepanipyrim 3 days	0.9 L /ha in 1000 L water	2nd spray
	Teldor	Fenhexamid 3 days	1.5 kg/ha in 1000 L water	3rd spray
	Switch	Cyprodinil + fludioxonil 1 day	1.0 kg/ha In 1000 L water	4th Spray
B	BINAB TF WP Spray	*Trichoderma spp* 0 day	500 g/ha plus 1kg sugar in 400 L water	7 days pre-flowering or early flowering
B	BINAB T-Vector powder carried on bee bodies*	*Trichoderma spp* 0 day	Refill tray to 5 mm once a week**	Throughout and continuing until 7 days after sprays in Tunnel A

N.B. Tunnel B was always entered after Tunnel A, to prevent movement of Binab from Tunnel B. A bee suit or leggings was worn in Tunnel B and taken off before exiting.

*Bees supplied as hives of honey bees and bumble bees.

** Honey bee 2010 tray 17 g BINAB T-Vector powder, Bumble bee tray 30g BINAB T-Vector 2010.

Figure 2.2. **2.2 A -** Honey bee hive with Binab T-Vector dispenser on the front and **B** - Biobest bumble bee hive with dispenser on top.

Assessments

The leaves and flowers sampled prior to the introduction of the vector and the spray applications were incubated in a damp chamber; leaves were frozen overnight (-20ºC) and incubated in diurnal light for 21 days before being assessed for *B. cinerea*. The flowers were also damp chambered but without freezing and assessed after 21 days for *B. cinerea, Trichoderma, Gliocladium* or other fungi.

Figure 2.2.3 Sporulating *B. cinerea* on leaf, flower and fruit samples. Example of tagged fruit clockwise from top left

In the four plots of tunnel B and all plots in tunnel A, 20 freshly-opened flowers/plot were sampled before the spray applications each week. These were spaced out evenly into a double thickness of tissue and damp chambered (without surface sterilisation) for 21 days in diurnal light with petals attached. These were assessed for *B. cinerea, Trichoderma, Gliocladium* or other fungi and the number of clean flowers (i.e. no fungal infection of any sort). This was carried out for flower picks 1 and 2, however, due to the nature of the variety the third pick coincided with a marked reduction in new flowers as fruit started to develop. Due to this the third pick consisted of 10 flowers/plot and the fourth and fifth flower picks were sampled from the leg rows rather than plots. In Tunnel B these would have been visited by bees so, in effect, were comparable. In Tunnel A the leg rows were sprayed with a different spray programme using the excess from the trial application (LHS leg row T6 - Signum, Serenade, Teldor, Serenade - Fungicide programme B. RHS leg low T7. - Serenade, Frupica, Serenade, Switch - Fungicide programme C) and were sampled to gauge the level of *B. cinerea* infection within the tunnel to compare with fruit assessments.

Fruit assessments for visible *B. cinerea* in field were carried out four times at seven day intervals starting two weeks after the last spray application and one week after the Binab T-Vector powder was removed (14/6/10). The assessments corresponded roughly to when the flowers tagged at each of the four sprays were ready for picking. The fruit was assessed for any brown lesions and actively sporulating *B. cinerea* and also the approximate number of healthy fruit per plot.

Fruit was sampled twice from the corresponding flowers tagged in the first two spray applications. 25 fruit were picked per plot at random taking fruit from all classes. The fruit were incubated at ambient temperature in multicell trays. At day 7 and 11 the fruits were assessed for the percent of fruit showing *B. cinerea, Trichoderma, Gliocladium* or other fungi and the number of marketable i.e. clean fruit.

Results and discussion

Dispersal of Binab T-Vector

Binab T-Vector was first introduced into the hives in week 19. Over the following four weeks, bumble bees dispersed 28 g of the product and honey bees dispersed 21 g (Table 2.2.3). There were large differences in the quantity dispersed each week, with a notably high amount (13 g) by bumble bees in week 20-21 and a low amount (0.4 g) by honey bees in week 22-23. This could be attributed to the average day time temperature in week 20-21 being up to 5ºC warmer (Table 2.2.3 and Figure 2.2.4).

Table 2.2.3: Comparison of Binab T-Vector usage by bumble bees and honey bees from hive dispensers and mean day time temperature – Cambs. 2010

	Weight of product dispersed (g)		
Week	**Bumble bees**	**Honey bees**	**Mean day temperature ºC**
19-20 (11/5/10)	6.0	9.0	18.8
20-21 (17/5/10)	12.6	7.4	23.4
22-23 (31/5/10)	5.5	4.1	18.6
Total	24.0	20.5	

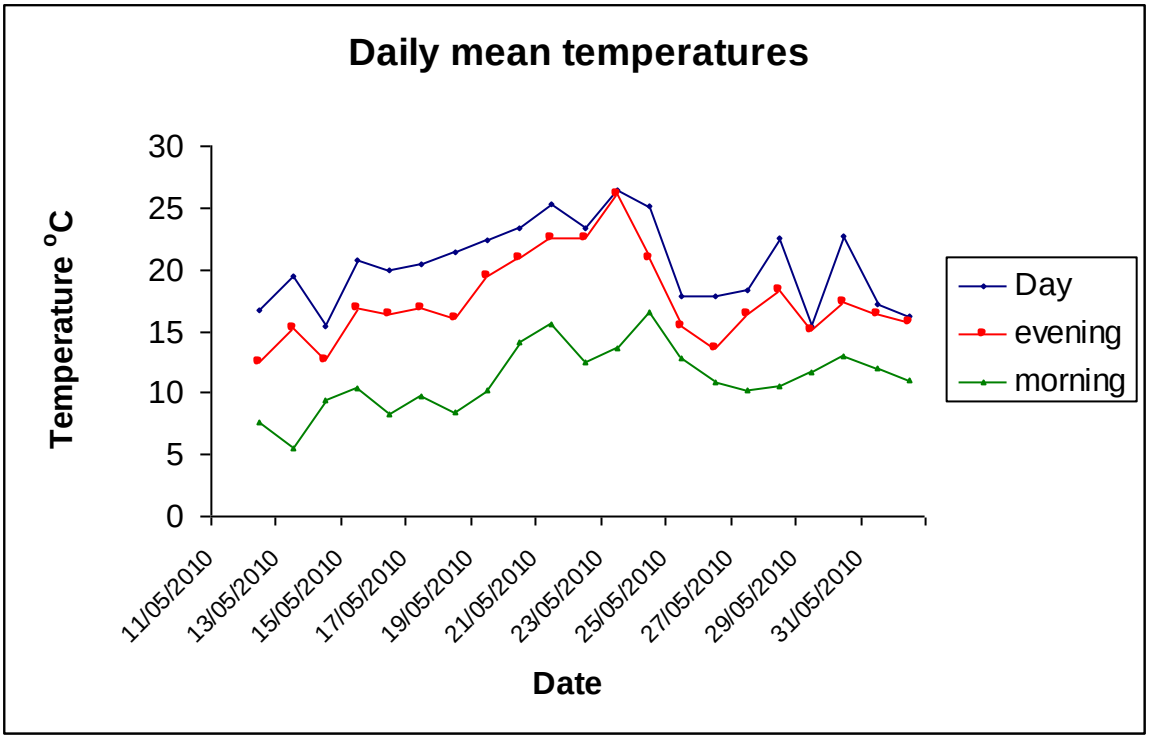


Figure 2.2.4: Daily temperature means in Tunnel B – Binab tunnel: Morning 6am-8am, Day 8am-6pm and evening 6pm-9pm – Cambs, 2010

Table: 2.2.4. Occurrence of latent *B. cinerea* in two tunnels of strawberry plants before the start of control treatments – Cambs, 5th May 2010

	% tissues affected by *B. cinerea*	
	Flowers	**Leaves**
Tunnel A	52.5	100
Tunnel B	90	100

Background levels of latent B. cinerea

A high incidence of latent *B. cinerea* (over 50%) was detected in the leaves and flowers of strawberry plants in both tunnels in early May, before treatments for control of *B. cinerea* had commenced (Table 2.2.4). No *Trichoderma* spp. was detected on plants at this time.

Occurrence of *Trichoderma* spp and *Gliocladium* sp. on flowers

On flowers sampled on 11 May, one week after application of Binab WF spray (pick 1). *Trichoderma* spp were detected on 30% of flowers from treated plots and 0-3% of flowers from untreated plots. The failure of a Binab WF spray application to result in the occurrence of *Trichoderma* spp. on most flowers may have been due to shading of some flowers by leaves (the canopy of these 2nd year plants was quite dense) and/or the inclusion in the samples of flowers which had developed in the week after applications.

After the addition of Binab T-Vector to hives and application of Prestop as a spray, the active ingredients of these products (*Trichoderma* spp. and *Gliocladium* sp., respectively), were readily detected on flowers. At samples taken on 19 and 26 May (picks 2 and 3), *Trichoderma* spp. alone were detected on 23% of flowers in the bees tunnel and at low levels (2-12%) in the tunnel containing treatments 1 to 4. This result indicates that bees moved from the tunnel containing their hives to adjacent tunnels. *Gliocladium* sp. was detected almost exclusively on plants treated with Prestop (Tables 2.2.5 and 2.2.6), with up to 50% of flowers colonised by the fungus, significantly greater than on plants not treated with Prestop.

Tables 2.2.5 to 2.2.8 show that proportions of flowers had both *Botrytis* and either *Trichoderma* spp. or *Gliocladium* sp. (with these flowers also counted under the separate heading for each fungus to give the total % of flowers affected by each fungus).

Effect of treatments on flower infection by *B. cinerea*

At flower picks 2 to 5, a moderate to high incidence of infection by *B. cinerea* (50-60%) was found in untreated flowers (Tables 2.2.5-2.2.8). In the tunnel with Binab T-Vector dispensers, around half of the flowers with *Trichoderma* spp. present also had *B. cinerea*. *B. cinerea* was also present on a proportion of the flowers with *Gliocladium* sp. in plots which had received Prestop application.

None of the treatments significantly reduced this level of infection at any of the picks. As

discussed earlier, possibly this is due to shading of flowers from spray and/or the development of flowers between spray applications (i.e. a spray application at weekly intervals is insufficient to protect all flowers). In this latter respect daily vectoring of a biocontrol product by bees would seem preferable, but this also failed to reduce flower infection. Better results may be possible using a biocontrol product other than Binab T-Vector.

Table 2.2.5: Occurrence of *B. cinerea* and *Trichoderma* spp. on incubated strawberry flowers sampled 19/05/10 (pick 2) – Cambs, 2010

Treatments	**Mean % flowers[a] infected by:**					
	Clean	**Bot.**	**Tri.**	**Bot. and Tri.**	**Gli.**	**Bot. and Gli.**
1. Untreated	12.5	60.0	6.2	1.2	0.0	0.0
2. Serenade ASO	6.2	83.8	3.8	3.8	1.2	0.0
3. Prestop	7.5	71.2	2.5	1.2	16.2	7.5
4. Fungicide programme A	17.5	60.0	10.0	7.5	0.0	0.0
5. Binab T-Vector	0.0	77.5	22.5	13.8	0.0	0.0
Significance	ns	ns	0.022	0.038	0.048	0.007
Lsd (15 df)	11.63	22.40	12.23	8.70	12.23	4.35

[a] 20 flowers sampled per plot, assessed after 21 days incubation.
Bot. – *B. cinerea,* Tri. - *Trichoderma* spp, Gli. – *Gliocladium* spp.
Treatment 4. Fungicide programme A - Signum, Frupica, Teldor, Switch.

Table 2.2.6: Occurrence of *B. cinerea* and *Trichoderma* spp. on incubated strawberry flowers sampled 26/05/10 (pick 3) – Cambs, 2010

Treatments	Mean % flowers[a] infected by:					
	Clean	Bot.	Tri.	Bot. and Tri.	Gli.	Bot. and Gli.
1. Untreated	25.0	52.5	12.5	7.5	5.0	2.5
2. Serenade ASO	15.0	57.5	7.5	5.0	7.5	5.0
3. Prestop	10.0	50.0	0.0	0.0	50.0	10.0
4.Fungicide programme A	22.5	50.0	5.0	0.0	12.5	2.5
5. Binab T-Vector	22.5	47.5	22.5	10.0	2.5	0.0
Significance	ns	ns	0.023	ns	<0.001	ns
Lsd (15 df)	23.83	33.98	13.05	9.33	13.05	8.26

[a] 10 flowers sampled per plot, assessed after 21 days incubation.
Bot. – *B. cinerea,* Tri. - *Trichoderma* spp, Gli. – *Gliocladium* spp.
Treatment 4. Fungicide programme A - Signum, Frupica, Teldor, Switch.

Table 2.2.7: Occurrence of *B. cinerea* and *Trichoderma spp.* on incubated strawberry flowers sampled 01/06/10. (Pick 4) – Cambs, 2010

Treatments	Mean % flowers[a] infected by:					
	Clean	Bot.	Tri.	Bot. and Tri.	Gli.	Bot. and Gli.
5. Binab T-Vector	25.0	52.5	5.0	1.3	1.3	5.0
6. Fungicide programme B	25.0	45.0	7.5	0.0	0.0	0.0
7. Fungicide programme C	30.0	35.0	10.0	0.0	0.0	0.0
Significance	ns	ns	ns	ns	ns	ns
Lsd (15 df)	33.52	33.02	9.09	4.98	4.98	19.91

[a] 20 flowers sampled from the front and back of the leg rows of both tunnels, assessed after 21 days incubation.
Bot. – *B. cinerea,* Tri. - *Trichoderma* spp, Gli. – *Gliocladium* spp.
Treatment 6. Fungicide programme B - Signum, Serenade, Teldor, Serenade.
Treatment 7. Fungicide programme C - Serenade, Frupica, Serenade, Switch.

Table 2.2.8: Occurrence of *B. cinerea* and *Trichoderma spp.* on incubated strawberry flowers sampled taken 10/6/10. (Pick 5) – Cambs. 2010

Treatments	Mean % flowers[a] infected by:					
	Clean	Bot.	Tri.	Bot. and Tri.	Gli.	Bot. and Gli.
5. Binab T-Vector	5.0	50.0	12.5	10.0	5.0	5.0
6. Fungicide programme B	25.0	35.0	0.0	0.0	0.0	0.0
7. Fungicide programme C	10.0	55.0	0.0	0.0	0.0	0.0
Significance	ns	ns	ns	ns	ns	ns
Lsd (15 df)	45.26	50.11	29.87	22.99	19.91	19.91

[a] 10 flowers sampled from the front and back of the leg rows of both tunnels, assessed after 21 days incubation.
Bot. – *B. cinerea,* Tri - *Trichoderm*a spp, Gli. – *Gliocladium* spp.
Treatment 6. Fungicide programme B - Signum, Serenade, Teldor, Serenade.
Treatment 7. Fungicide programme C - Serenade, Frupica, Serenade, Switch.

Effect of treatments on fruit infection by *B. cinerea*

The incidence of fruit with latent infection by *B. cinerea* was assessed twice; on samples collected on 14 June (pick 1) and 21 June (pick 2). At pick 1, latent infection was greatest in untreated fruit (27%), and appeared lower following treatment with Prestop, Serenade ASO and the fungicide programme, although differences were not statistically significant (Table 2.2.9). At pick 2, high levels of latent *B. cinerea* (57-69%) were recorded in fruit irrespective of the control treatments applied (Table 2.2.11).

Trichoderma spp. also developed on some fruit after humid incubation for 7 days at ambient temperature. Levels ranged from 0-4% (pick 1) and 2-11% (pick 2) and were not significantly affected by treatment. *B. cinerea* was present on most of the fruit with *Trichoderma* spp. (fruit with both *B. cinerea* and *Trichoderma* spp. were also counted in Tables 2.2.9 – 2.2.11 under their separate headings to enable recording of the % of fruit with each fungus).

When fruit were re-assessed after incubation for 11 days, levels of *B. cinerea* detected had increased from around 20% to around 40% (pick 1) and from around 60% to around 70% (pick 2), still with no significant differences between treatments (Table 2.2.10 and 2.2.12).
The proportion of fruit that developed symptoms of *B. cinerea* in the crop was initially very low (0.2-3.5%), with no significant treatment effect (Table 2.2.13). At assessments done 1

and 2 weeks later, the proportion of fruit affected was around 10%, again with no significant treatment effects (Tables 2.2.14 - 15). At a final assessment on 5 July, levels of symptoms ranged from 7 to 21% with significantly more on plants in the beehive tunnel (21%) than on untreated plants in the other tunnel (8%). Possibly this is due to a difference in tunnel environments rather than a true treatment effect; bees had to be located in a tunnel separate from other treatments in order to minimise inter plot interference.

Table 2.2.9: Occurrence of *B. cinerea* and *Trichoderma* spp. on incubated strawberry fruit sampled 14/06/10 after 7 days incubation (pick 1) – Cambs, 2010

Treatments	**Mean % fruit[a] infected by:**			
	Clean	**Botrytis**	**Trichoderma**	**Both**
1. Untreated	73.0	27.0	4.0	4.0
2. Serenade ASO	86.0	14.0	0.0	0.0
3. Prestop	82.0	18.0	1.0	1.0
4. Fungicide programme A	*81.0	18.0	1.0	1.0
5. Binab T-Vector	*78.0	21.0	0.0	0.0
Significance	ns	ns	ns	ns (0.059)
Lsd (15 df)	16.21	16.36	2.58	2.91

[a] 25 fruit sampled per plot.
*Where the total % is <100% fruit showed other fungal growth such as *Mucor* and *Cladosporium*.

Table 2.2.10: Occurrence of *B. cinerea* and *Trichoderma* spp. on incubated strawberry fruit sampled 14/06/10 after 11days incubation (pick 1) – Cambs, 2010

Treatments	**Mean % fruit[a] infected by:**			
	Clean	**Botrytis**	**Trichoderma**	**Both**
1. Untreated	*56.0	43.0	4.0	4.0
2. Serenade ASO	58.0	42.0	0.0	0.0
3. Prestop	71.0	39.0	1.0	1.0
4. Fungicide programme A	62.0	38.0	1.0	1.0
5. Binab T-Vector	52.0	48.0	0.0	0.0
Significance	ns	ns	ns(0.059)	ns(0.059)
Lsd (15 df)	21.83	23.48	2.91	2.91

[a] 25 fruit sampled per plot.
*Where the total %is <100% fruit showed other fungal growth such as *Mucor* and *Cladosporium*.

Table 2.2.11: Occurrence of *B. cinerea* and *Trichoderma* spp. on incubated strawberry fruit sampled 21/06/10 after 7 days incubation (pick 2) – Cambs, 2010

Treatments	Mean % fruit[a] infected by:			
	Clean	Botrytis	Trichoderma	Both
1. Untreated	*42.0	57.0	4.0	4.0
2. Serenade ASO	*40.0	58.0	11.0	11.0
3. Prestop	*36.0	62.0	2.0	2.0
4.Fungicide programme A	*29.0	69.0	10.0	10.0
5. BINAB T-Vector	37.0	63.0	6.0	6.0
Significance	ns	ns	ns	ns
Lsd (15 df)	21.82	22.25	10.98	1.35

[a] 25 fruit sampled per plot.
*Where the total %is <100% fruit showed other fungal growth such as *Mucor* and *Cladosporium*.

Table 2.2.12: Occurrence of *B. cinerea* and *Trichoderma* spp. on incubated strawberry fruit sampled 14/06/10 after 11 days incubation (pick 2) – Cambs, 2010

Treatments	Mean % fruit[a] infected by:			
	Clean	Botrytis	Trichoderma	Both
1. Untreated	28.0	72.0	4.0	4.0
2. Serenade ASO	*26.0	72.0	6.0	6.0
3. Prestop	26.0	73.0	3.0	2.0
4. Fungicide programme A	10.0	90.0	5.0	5.0
5. Binab T-Vector	*18.0	81.0	9.0	9.0
Significance	ns	ns	ns	ns
Lsd (15 df)	10.98	18.45	9.50	9.27

[a] 25 fruit sampled per plot.
*Where the total % is <100% fruit showed other fungal growth such as *Mucor* and *Cladosporium*.

Table 2.2.13: Occurrence of visible *B. cinerea* damage on fruit in the crop (14/6/10) – Cambs, 2010

Treatments	Mean total fruit per plot	% clean fruit per plot	% fruit with Botrytis
1. Untreated	112.7	99.8	0.2
2. Serenade ASO	100.7	99.3	0.7
3. Prestop	62.0	98.3	1.7
4. Fungicide programme A	97.2	98.5	1.5
5. Binab T-Vector	109.2	96.5	3.5
Significance	ns	ns	ns
Lsd (15 df)	41.12	2.55	2.55

Table 2.2.14: Occurrence of visible *B. cinerea* damage on fruit in the crop (21/6/10) – Cambs. 2010

Treatments	Mean total fruit per plot	% clean fruit per plot	% fruit with Botrytis
1. Untreated	62.8	90.3	9.7
2. Serenade ASO	65.3	89.4	10.6
3. Prestop	52.8	89.6	10.4
4. Fungicide programme	78.8	91.4	8.6
5. Binab T-Vector	54.0	91.7	8.3
Significance	ns	ns	ns
Lsd (15 df)	24.93	5.08	5.08

Table 2.2.15: Occurrence of visible *B. cinerea* damage on fruit in the crop (28/6/10) – Cambs. 2010

Treatments	Mean total fruit per plot	% clean fruit per plot	% fruit with Botrytis
1. Untreated	50.8	92.0	8.0
2. Serenade ASO	49.3	94.8	5.2
3. Prestop	30.0	91.2	8.8
4. Fungicide programme	38.5	91.3	8.7
5. Binab T-Vector	31.0	87.4	12.6
Significance	ns (0.067)	ns	ns
Lsd (15 df)	17.80	7.29	7.29

Table 2.2.16: Occurrence of visible *B. cinerea* damage on fruit in the crop (05/07/10) – Cambs. 2010

Treatments	Mean total fruit per plot	% clean fruit per plot	% fruit with Botrytis
1. Untreated	28.5	92.2	7.8
2. Serenade ASO	29.3	92.1	7.9
3. Prestop	22.3	93.1	6.9
4. Fungicide programme	24.5	87.5	12.5
5. Binab T-Vector	21.5	79.4	20.6
Significance	ns	0.017	0.017
Lsd (15 df)	8.37	8.42	8.42

Conclusions

- A high level of latent infection by *B. cinerea* was present in flowers and leaves of strawberries in both of the experimental tunnels.

- Both bumble bees and honey bees dispersed Binab T-Vector from the hives. The detection of *Trichoderma* spp. on strawberry flowers at a significantly higher incidence in the bee tunnel than in an adjacent tunnel indicates they transferred the biocontrol product to the flowers.

- *Trichoderma* spp. was detected on only around 5-22% of flowers sampled, suggesting the biocontrol product was not transferred to, or did not establish on, all flowers.

- *Gliocladium* spp. was detected on around 16-50% of flowers a week after the spray application of Prestop, suggesting either that the fungus did not establish on all flowers, and/or that the interval between spray applications (7 d) was too long to treat all flowers at an appropriate stage.

- None of the treatments tested significantly reduced the incidence of *B. cinerea* (latent or visible in the field) in flowers or fruit.

- None of the treatments tested significantly reduced the incidence of visible *B. cinerea* symptoms in the crop.

- Development of *Trichoderma* on fruit incubated for 7 days at ambient temperature was relatively low (0-11% of fruit) compared with the development of *B. cinerea* (14-69%).

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

Methods

Flower infection

To validate the model, flowers were regularly sampled to determine the incidence of latent flower infections by botrytis. Sampling was done in an unsprayed tunnel of strawberry plants of cv. Elsanta (April-May) at Manor Farm, near Borough Green, Kent, every Monday, Wednesday and Friday during flowering. On each day, 100 old flowers (with all petals attached) were randomly 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 on a shaker, to remove any spores on the surface. 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.

Management trial

The usefulness of Botem predictions in practical botrytis management was evaluated on the main season protected Elsanta crops at Manor Farm, Near Borough Green, Kent. There were six plots, ~ 50 m long (two plots in each tunnel); each plot was randomly allocated to one of the following three treatments:

1 – Untreated: no Botrytis sprays, normal sprays for mildew, pests and nutrients.

2 – BOTEM managed: sprays for Botrytis were only applied when the fruit infection predicted by BOTEM had reached a threshold of 10%. The plot was then sprayed with a fungicide with curative action, which was either Rovral (iprodione) or pyrimethanil (may have some curative effects). These products were alternated to minimise the risk of fungicide resistance. Treatments for mildew, pests and nutrients were sprayed as normal (Table 1).

3 – Conventional programme: standard farm programme as applied to the rest of the strawberry crop.

Non-botrytis fungicides/nutrients were applied as normal in all three tunnels. Three data loggers were installed in the tunnels to monitor in-tunnel temperature and humidity. Data were regularly downloaded to generate model predictions. During harvest, about 100 green/yellow fruit were sampled from each tunnel weekly; in total four batches of fruit were sampled. The fruit were surface sterilised with sodium hypochlorite (0.025% available chlorine (w/v)) (5% of Domestos) for 15 min and then rinsed with distilled water. Fruit were then placed onto seed trays, well separated from each other, and covered with a polythene bag for incubation. Fruit were assessed for botrytis and other rots one-two weeks after incubation.

Results

Flower infection

The percentage of latent infection of flowers on Elsanta was very low, ranging from 0% to 6% (Table 2.3.1).

Table 2.3.1, Percentage of latent infection of strawberry flowers of Elsanta by *Botrytis cinerea* in 2010 in Kent

Sampling date	Infection
12-Apr	1
14-Apr	6
16-Apr	4
19-Apr	4
21-Apr	1
23-Apr	0
26-Apr	0
28-Apr	1
30-Apr	0
04-May	0
06-May	0

Management trial

The threshold for spray application was set to a daily predicted infection of fruit more than 10%. This was not met throughout the trial period. Thus, the model-managed plots did not receive any spray against *B. cinerea.* This low level of flower infection was consistent with the low level of latent botrytis rot on green fruit. Overall, about 2.2% green fruit were found to have latent botrytis, compared to 0.8% for the conventional treatment. The difference was very small and not statistically significant.

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)

Molecular analysis of sampled isolates

All collated isolates were screened for the six SRR primers. Of the six primers, only one failed (most likely due to some problems in primer quality since this primer revealed considerable polymorphism in preliminary screens). For another primer pair, there was hardly any polymorphism among 186 isolates, and so it was excluded from subsequent analysis. Analysis of molecular variance (AMOVA) was used to determine whether groups of isolates from different hosts are genetically distinct, and whether host species affects isolate genetic differences. Results suggest that:

(1) there were no overall significant differences in isolates from apple, strawberry, cherry and weeds;

(2) within the same host species, there are significant differences in groups of isolates from different sites/cultivars;

(3) the isolate differences appeared to be more related to site isolates rather than to host differences.

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

Several research groups in Europe and other parts of the world are currently actively engaged in black spot research. The general conclusions from the large European research projects are that *Colletotrichum acutatum* can infect many different plant species, including cherry and apple. Cross-infection among hosts is common although there is some evidence to suggest that one specific group of isolates from strawberry is more aggressive on strawberry than isolates from other hosts. The purpose of this study was to test the pathogenicity of the *Colletotrichum* isolates obtained from various plant species in England and screened molecularly in Task 3.1, on strawberry.

Methods

Two mycelial plugs of isolates of *Colletotrichum* spp. from strawberry, cherry, apple, rose, willowherb, black nightshade and alder were placed onto Potato Dextrose medium (PDA) and the plates incubated at 25^{o}C in the dark for 8 days. A conidial suspension was prepared by flooding the cultures with sterile distilled water (SDW), rubbing with a glass rod and filtering the suspension through two layers of cheese cloth. Spore concentration was determined using a haemocytometer and diluted to 10^5 conidia per ml for each isolate.

Fruit

Unripe strawberry fruits cv. Red Glory were surface sterilised in 0.5% sodium hypochlorite and rinsed in SDW and allowed to dry. The fruit was then inoculated with two separate 5 microlitre drops of a conidial suspension of *Colletotrichum* spp. Four replicates were prepared per isolate and each replicate consisted of ten fruits.

After inoculation the fruits were placed in a moist chamber and incubated at 25^{o}C. Fruits were inspected daily for symptoms. Symptoms of *C. acutatum* were assessed using a scoring system based on lesion size and sporulation (Table 3.2.1).

Plants

Potted strawberry plants cv. Elsanta in a glasshouse isolation compartment at EMR were inoculated by applying a 5 second spray of a conidial suspension of *Colletotrichum* using a hand-held sprayer. The plants were then placed in plastic bags for 48 hours to allow spore germination and infection. The bags were then removed. High humidity in the compartment was maintained using a humidifier. Four replicates were prepared per isolate and each replicate consisted of 5 plants.

After inoculation the plants were inspected weekly for signs of infection. After three months numbers of lesions on each plant was recorded. Symptomless plant parts were also collected and checked for *Colletotrichum* following treatment with paraquat and incubation at high humidity under UV light.

Results and discussion

Fruit

The fruit were inoculated on 30 November and assessed for blackspot on 15 December. The results are shown in Table 3.2.1. All isolates caused lesions on the fruit but there were differences in lesion size and sporulation of *C. acutatum*. The highest scores were on fruit inoculated with isolates from strawberry, apple and alder. The lowest scores were on isolates from weeds. The results indicate that weeds and other non-strawberry hosts could act as a source of inoculum for *C. acutatum* in strawberry plantations.

The tests will be repeated in 2011, including additional isolates from apple and strawberry.

Table 3.2.1, Pathogenicity of 13 isolates of *Colletotrichum* sp. from various hosts on strawberry fruit cv. Red Glory. Inoculated 30 November, assessed 15 December m, 2010

Isolate number	Host origin	Mean pathogenicity score
1	Strawberry Isle of Wight	2.2
2	Strawberry Suffolk	2.3
3	Weed EMR	1.4
4	Willowherb EMR	1.8
5	Willowherb EMR	1.8
6	Willowherb EMR	1.9
7	Alder EMR	3.0
8	Alder EMR	2.2
9	Alder EMR	2.3
10	Apple cv. Bramley Chartham	3.1
11	Apple cv. Bramley Kent	2.5
12	Apple cv. Bramley Ightham	2.7
13	Primula	2.0

Score

0 = No infection

1 = Small lesion

2 = Large lesion no sporulation

3 = Large lesion sporulation

4 = Large lesion sporulation + mycelium growth

5 = Completely rotted fruit

Plants

Strawberry plants cv. Elsanta were inoculated with 13 different isolates of *C. acutatum* (Table 3.2.1) on 20 December 2010. After one month lesions, possibly due to *C. acutatum*, were present on the petioles of some plants. The plants will be scored for blackspot. The trial will be repeated in 2011.

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

In the Hortlink biofumigation project HL0177 (HDC SF 77) biofumigants to control verticillium wilt on strawberry were investigated. The project identified lavender waste and some brassica products, including Biofence as potential biofumigants. Soil sterilisation is an important part of the integrated approach to control of blackspot in strawberry production. The purpose of this study to be done in 2011 is to evaluate the efficacy of these products against *C. acutatum* in the laboratory based on the protocol developed for *Verticillium dahliae* testing.

Proposed methods

Soil preparation

Approximately 50 L of soil will be obtained from the plot adjacent to DM 180 at EMR. The moisture content will be measured using a moisture probe and the moisture content adjusted to 22 % (field capacity). The soil will then be divided up into three lots. One lot will be left untreated. Lavender waste will be added and combined with the second lot and Biofence added and combined with the third soil lot. The untreated soil will then be used to part fill three 15 kg sterile grey crates. Similarly the treated soil will also be placed in each of three crates.

Colletotrichum inoculum preparation

Strawberry fruits, infected with *C. acutatum* will be cut into halves and ten halves sealed into green mesh bags (wind break green mesh). These will be placed in soil in the grey crates, 3 bags per crate, 5-10 cm below the soil surface. The crates will be covered to prevent the soil drying out. The boxes will be left to incubate at ambient temperature in a cool place.

Detection of colletotrichum

At the start of the incubation in soil five lots of ten dried strawberry fruit halves will be taken from the same batch as used in the soil tests. These will be washed well under running tap water, then surface sterilised in 3% sodium hypochlorite for 2 min, rinsed in sterile water for 1 min and then dried in a laminar flow hood and chopped into smaller pieces using a sterile scalpel and tweezers. The pieces will then be plated onto a semi-selective medium for colletotrichum (either modified Mathur's medium or modified Dextrose Peptone Yeast Extract Agar). Plates will be incubated at 25^{o}C in the dark for 5-7 days. Percent survival of colletotrichum will be determined by calculating the number of infected fruit that yielded colletotrichum growth on the medium out of the total number plated.

One bag will be sampled from each crate after two weeks, four weeks and eight weeks and the strawberry pieces processed as above.

The tests will be set up later in 2011 once sufficient blackspot- infected strawberry debris has been obtained.

Task 3.4: Development of simple guidelines for blackspot management

Simple guidelines will be developed to assist growers in making decisions regarding the need for management measures against blackspot, based on published data and newly available information on blackspot from this project. These guidelines will cover the relevance of various inoculum sources (runner origin, site history, alternative hosts etc), available control methods (fungicide efficacy, BCAs and biofumigation), production systems and local environmental conditions. Draft guidelines have been produced and are detailed below in Tables 3.4.1-3.4.4. These will be discussed and amended in 2011.

Table 3.4.1 Risk assessment – Factors likely to affect the incidence of blackspot in a strawberry crop

Item	Options	Disease risk
Site	• Virgin site	Low
	• Strawberry land with adjacent crops	High
	• Strawberry in crop history, no adjacent crops	moderate
Source of planting material	• UK origin	Low risk
	• Home produced non-certified	High risk
	• Non UK origin	High risk
Cropping system	• Open field	High risk depending on weather
	• Glasshouse	Low risk depending on irrigation method
	• Polytunnel early cover	Low-moderate risk
	• Polytunnel pre-flowering cover	Moderate risk
Crop age	• Annual or first year	Low risk
	• 2 years or older	Moderate-high
Cultivar type	• June-bearer	Low-moderate risk
	• Everbearer	Low-high risk
Irrigation	• Overhead	High risk
	• Trickle or drip	Low risk
Nutrition	• High nitrogen inputs	High risk
Adjacent crops / weeds	• Apples, cherries	Moderate-high risk
	• Weed cover	Moderate-high risk
Herbicide use	• glufosinate ammonium	Increase risk if weeds of runners infected
	• glyphosate	
	• diquat	

Table 3.4.2 Weather factors likely to affect blackspot incidence

Item	Optimum	Range
Temperature	20-25^{o}C	?
Humidity	> 80%	?
Rainfall	Moderate rainfall	?

Table 3.4.3 Efficacy of various fungicides registered for use on strawberry in the UK against strawberry blackspot

Active ingredient	Product name	Efficacy
azoxystrobin	Amistar	+++
Bacillus subtilus	Serenade	?
bupirimate	Nimrod	0
captan	Alpha Captan	++
chlorothalonil	Various eg Bravo 500	+
cyprodonil + fludioxonil	Switch	+++
dimethomorph	Paraat	0
fenhexamid	Teldor	0
fenpropimorph	Corbel	++
fosetyl-Al	Aliette	0
Gliocladium catenulatum	Prestop	?
iprodione	Rovral	0
Kresoxim-methyl	Stroby	++
mepanipyram	Frupica	+
myclobutanil	Systhane	+
potassium bicarbonate	Potassium bicarbonate	?
pyraclostrobin + boscalid	Signum	+++
pyrimethanil	Scala	0
quinoxyfen	Fortress	?
sulphur	Various e.g. Headland Sulphur	0
thiophanate-methyl	Cercobin WG	++ (sensitive isolates)
thiram	Thianosan	++

0 = No activity, + = some efficacy, ++ = moderate efficacy, +++ = Good efficacy, ? = Not known

Table 3.4.4 Non- chemical control options and importance for blackspot control

Method	Importance
Certified disease-free plants	++++
Avoid overhead irrigation	++++
Sanitation	++++
Resistant cultivars	+
Straw mulching	+ (protected) +++ (outdoor)
Frequent harvesting	+++
Removing all ripe and damaged fruit at harvest	+++
Location	+
Rotation	+++
Soil-less culture	+

+ = Low importance, ++++ = High importance

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 Years 1-3)

The aim of the study was to determine the success of different methods of planting and establishing a trap crop of alyssum in the leg rows of protected strawberry crops and to determine how effective the alyssum acts as a trap crop for European tarnished plant bug. Direct seeding of alyssum to soil, transplanting modules to the soil and growing modules in old versus new peat bags were compared. The success of establishment and growth of the alyssum grown by the four methods was compared, as well as the attractiveness to *Lygus rugulipennis,* other pests and important natural enemies.

Methods

One replicated small plot experiment compared five treatments: 1) alyssum seeded in soil; 2) alyssum modules transplanted to the soil; 3) alyssum modules in used (ex strawberry) peat

bags; 4) alyssum in new peat bags; 5) no alyssum. Records of the establishment and growth of alyssum, and *L. rugulipennis* in alyssum and adjacent strawberry plots were taken fortnightly through the season, as well as records of other predators and pests.

The site was at Park East strawberry plantation (Cv Elsanta) at Robert Boucher and Son, Newlands Farm, Teynham, Sittingbourne, Kent ME9 9JQ by kind agreement of Hugh Boucher. The plantation was located at NGR TQ 956 622 (Fig. 4.1.1). The rows were 69 m long. The row spacing was 1.9 m. The crop was covered when visited on 4 March 2010. The plot was located on the second bed from the west side of the plantation (Fig. 4.1.1).

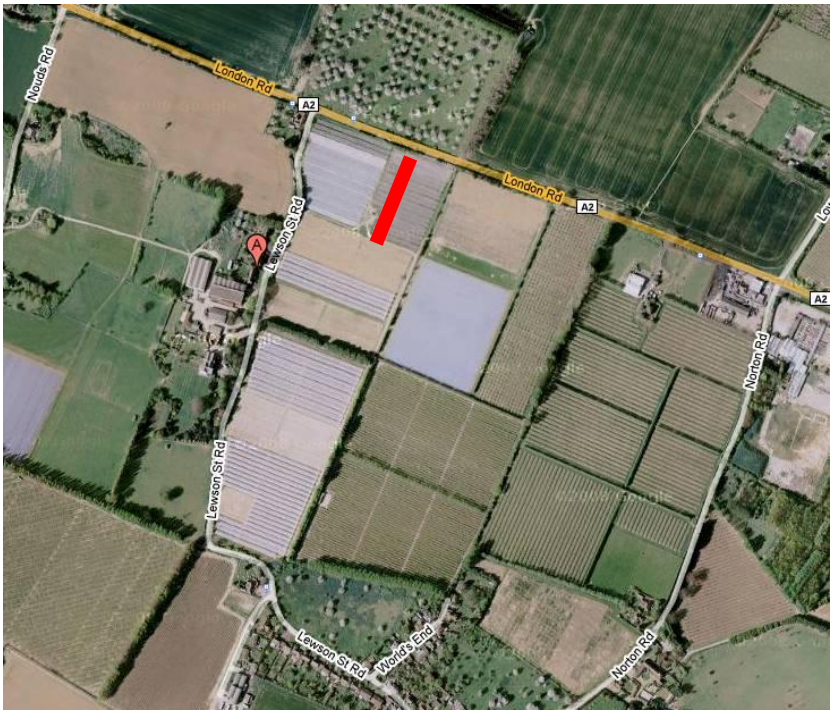


Figure 4.1.1. Map of the experimental plot location (red bar) at Newlands Farm.

Treatments were different methods of growing alyssum and an untreated (no alyssum) control (Table 4.1.1). A randomised block design with three replicates was used. Plots were 4.4 m long (two hoops spaced 2.2 m apart) with two replicates end to end on either side of one tunnel (Fig. 4.1.2).

Table 1.1.1. Treatments of alyssum

Treatment No.	Medium	Descriptor
1	Leg row soil	Seed sown directly into soil in a double row in late March 2010, then thinned to one plant every 10 cm
2.	Leg row soil	Pre sown in modules and then planted out into soil in a double row
3.	Old strawberry peat bag	As 2, but planted into old used peat bags, 20 plants per 1 m bag in two rows ~ 10 cm apart
4.	New strawberry peat bag	As 3, planted into new peat bags.
5.	Untreated	No alyssum

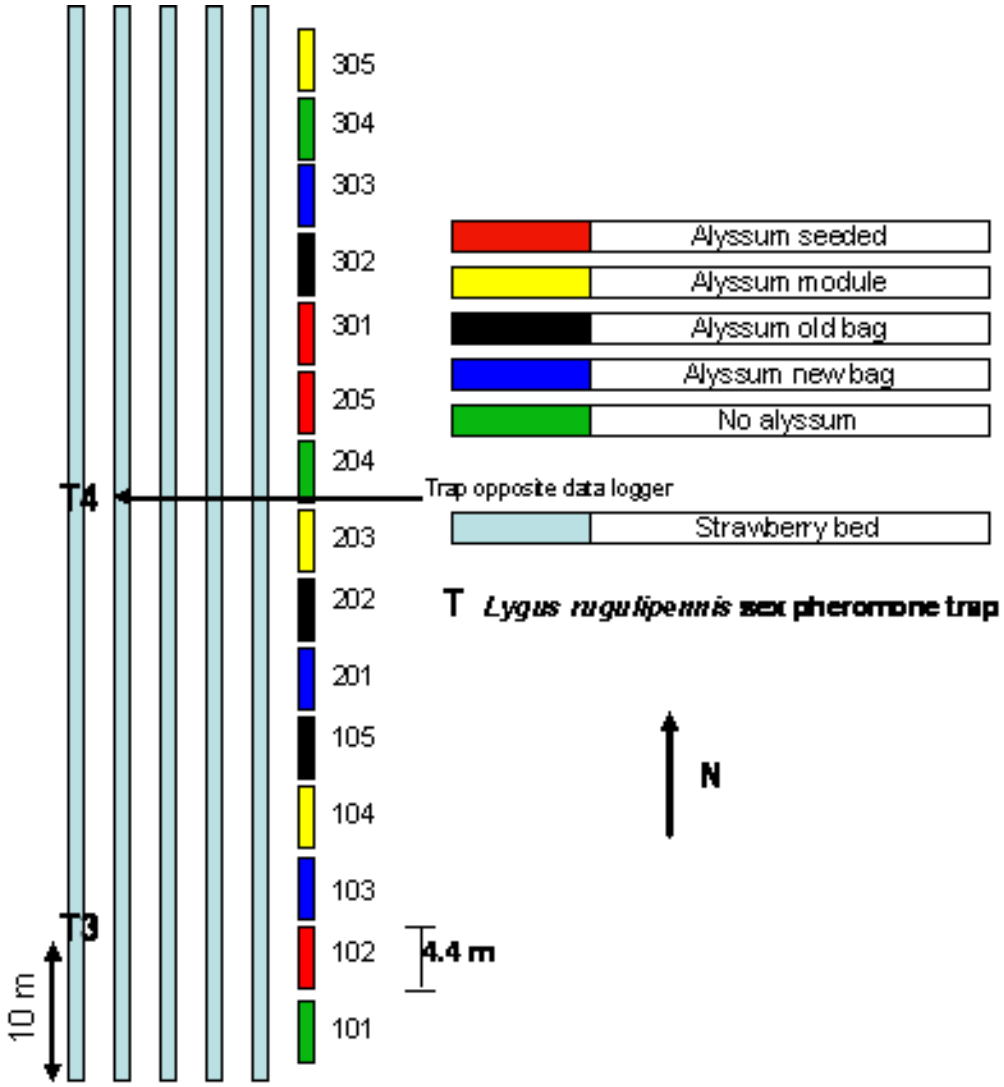


Figure 4.1.2. Layout of the plots in Tunnel 2 (from west edge) of Park West Field

The sweet alyssum (*Lobularia maritima*) for treatments 2-4 was pre-sown in a greenhouse in modules by EMR (750 plug plants on 25 Mar 2010, 10-20 seeds per plug). Plants were thinned to one plant per module on 7 Apr 2010. Seed was obtained from Ball Colegrave Ltd,

Banbury ('snowdrift' Ball. 517348 Lot: 2003217501). When plants were of adequate size for handling, they were planted into new 1 m peat bags *in situ*, 20 plants per bag in two rows of 10 (29 Apr 2010). Each alyssum peat bag plot comprised four 1 m peat bags, grown end to end, and provided with trickle irrigation. 200 seeds were sown directly into the soil, in two rows spaced 10 cm apart by Hugh Boucher's staff. Each alyssum plot contained 80 plants in a double row with 10 cm between plants in the row and 10 cm between rows. Old peat bags were acquired from Mark Young of Driscoll's.
The strawberry beds in the tunnel where the trial was located were to remain untreated with any insecticides which would affect *L. rugulipennis*. A Stevenson's screen with a data logger temperature and humidity recorder was deployed in the centre of the experimental area in early April and remained for the duration of the experiment.

The experiment was visited fortnightly and a record made of the growth stage of the alyssum and the strawberries. Two *L. rugulipennis* sex pheromone traps were deployed in the edge strawberry row, and populations checked fortnightly. Each alyssum plot (four bags per plot) was sampled fortnightly from May by sweeping using a washing up bowl with one side cut out. Eight sweeps were done per plot, two from each of the bags. The total number of *L. rugulipennis* adults and nymphs were recorded as well as numbers of taxa to species where possible. Eight tap samples were also done on the strawberry next to each plot.

In Aug 2010 six varieties of alyssum were sown into modules in a glasshouse at EMR to assess their flowering and vigour. Varieties trialled were Clear Crystal 736187, Gold Ball 358992, Snow Crystals 482249, Easter Bonnet 482244 and Snowdrift 517348. The number of flowers and the height of 10 plants in the middle of each tray were measured.

Results

Sampling began on 8 June and the final assessment was done on 8 July. Although there were significant numbers of pollen beetles on strawberry and alyssum (Table 4.1.2) by 22 July the alyssum plants had finished flowering and were beginning to die back. In addition, very few *L. rugulipennis* were captured in the pheromene trap around that date (Fig. 4.1.3). A rise in temperature was related to a consiquent rise in male *L. rugulipennis* in the pheromone traps (Fig. 4.1.4).

Alyssum seed sown directly into soil did not establish well and seedlings were subject to competition from weeds and drying out. Plants grown in the grow bags grew best (Fig. 4.1.5). Trials with alyssum varieties are underway to examine vigour and flowering (Fig. 4.1.6, 4.1.7).

Table 4.1.2. Numbers of invertebrates found on the strawberry and alyssum plants using tap sampling. Zero's are omitted for clarity (L.r. = *L. rugulipennis*)

Strawberry						Alyssum		
L.r. male	L.r. nymph	Spider	Pentat-omid	Aphid	Pollen beetle	Spider	Pollen beetle	Bumble-bee
1	1	3	3	22	5	2	57	2

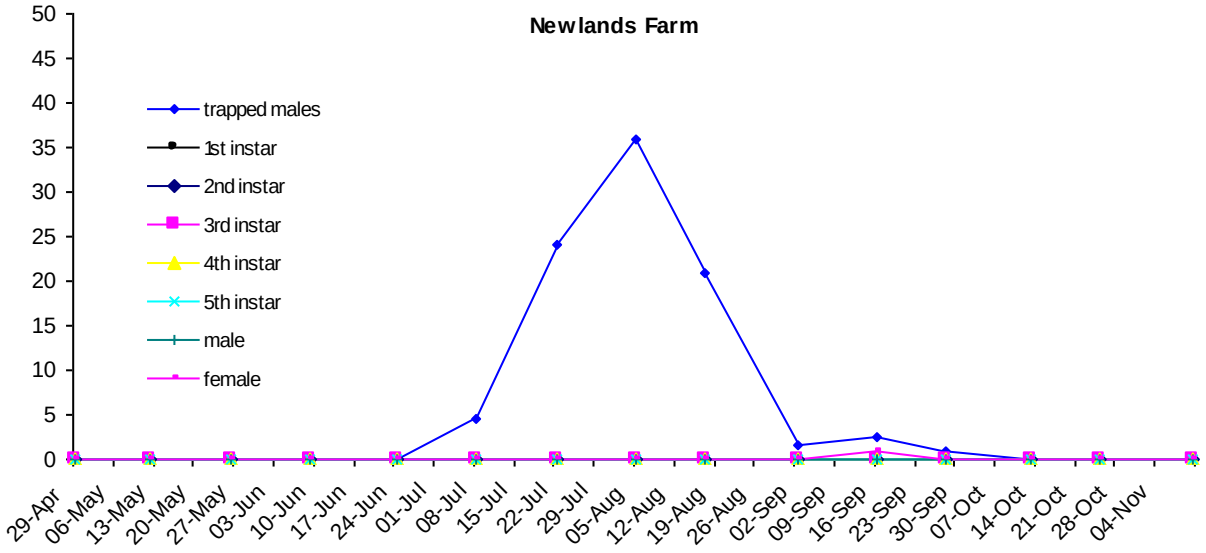


Figure 4.1.3. Phenology of *L. rugulipennis* throughout the 2010 growing season at Newlands Farm, comparing pheromone trap catches and tap sampling.

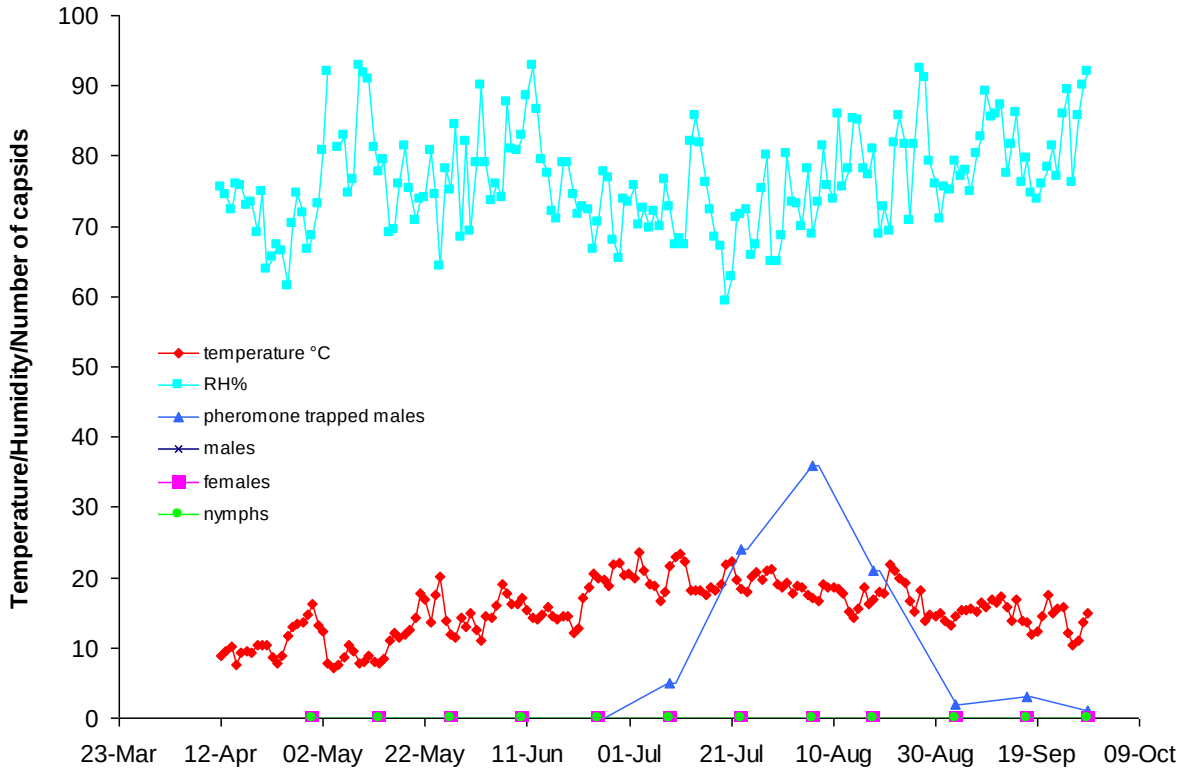


Figure 4.1.4. Phenology of *L. rugulipennis* throughout the 2010 growing season at Newlands Farm, in relation to temperature and humidity data.

Control plot

Alyssum in old peat bag

Alyssum in new peat bag

Alyssum planted into soil

Alyssum seed directly sown

Figure 4.1.5. Growth of the alyssum plants in the different media and growing conditions (8 Jul 2010).

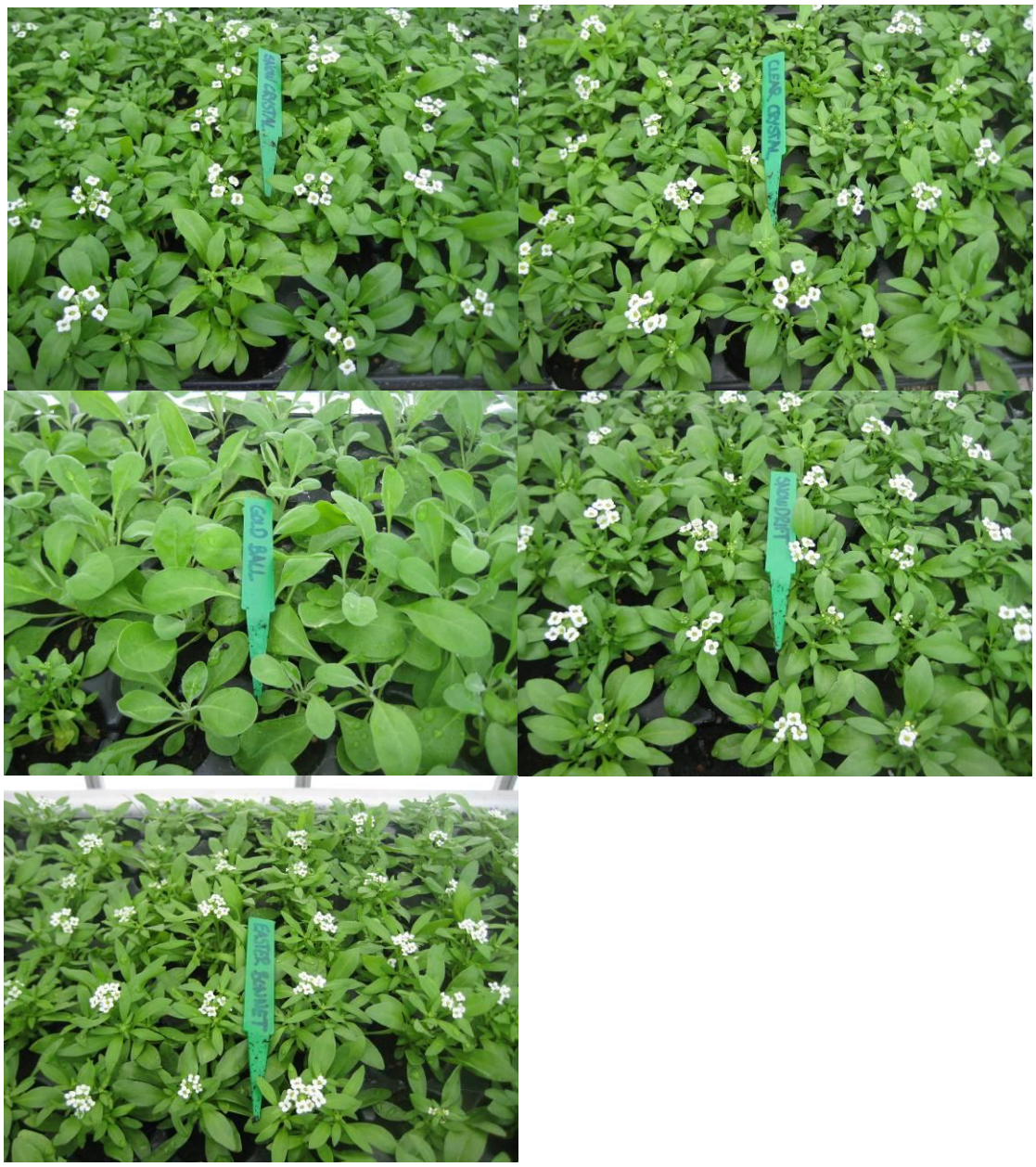


Figure 4.1.6. Photographs of different Alyssum varieties growing as plug plants taken on 4 October 2010.

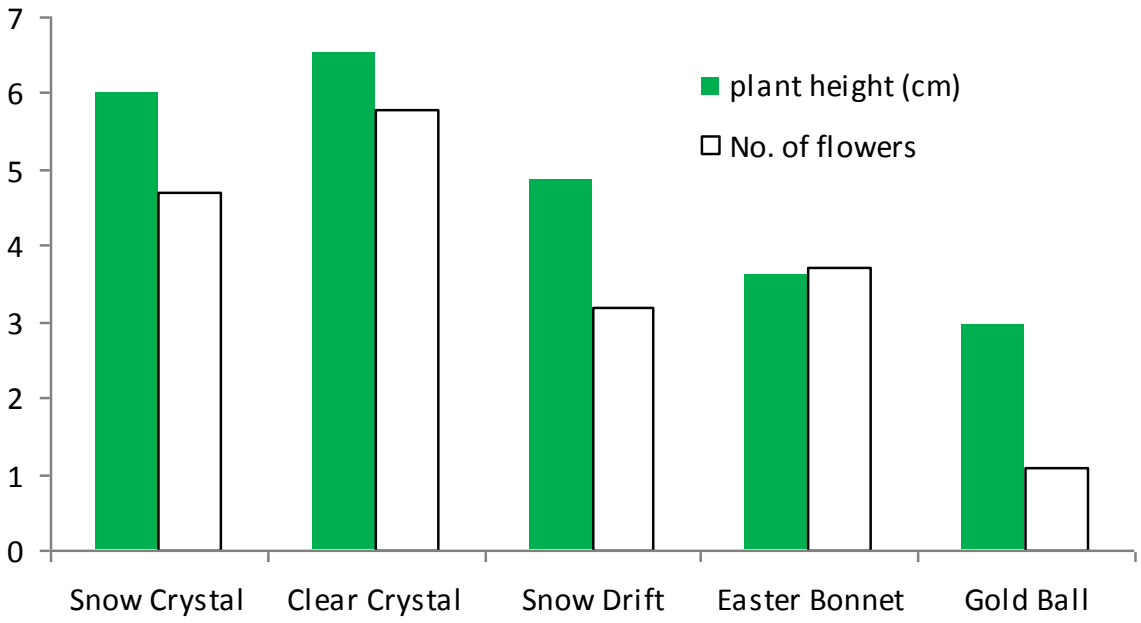


Figure 4.1.7. Growing habit of Alyssum varieties measured on 5 October 2010.

Conclusions

- Numbers of *L. ruguipennis* peaked in August in the pheromone traps, but very few other capsids were found in the crop
- The trial failed because the variety of alyssum used the previous year had been changed to make it more suitable to rockery gardens, hence, less vigorous. The plant flowered early and then died off

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

4.2. Evaluation of the use of hexyl butyrate as a repellent for Lygus rugulipennis

Two different mechanisms have been proposed for the reduction in numbers of *L. rugulipennis* reported from earlier experiments on the use of hexyl butyrate. The first is that the compound is a repellent for all stages of the pest. The second is that the compound causes females to stop producing sex pheromone and thus reduces the number of males and subsequent population development within the treated areas. In 2008, dispensers were identified that released different rates of hexyl butyrate. These dispensers were used in field experiments in purpose sown weed plots and in a strawberry planting at EMR in 2009. There was no effect of hexyl butyrate on numbers of *L. rugulipennis* adults close to or circa 3 m away from the dispensers at any of the rates used. In the final experiment there was no difference in proportions of males and females around the dispensers compared to the untreated controls. However, this was late in the season and it is possible that females were not calling for males at this time. Thus it is still unclear what, if any, effect hexyl butyrate is having on *L. rugulipennis* populations. These experiments aimed to clarify the mechanism of potential population reduction.

Methods

Hexyl butyrate dispensers were used in combination with live female *L. rugulipennis* and artificial sex pheromone in field experiments to determine the mechanism of reported population reductions. The experiments were done on a purpose sown weed field at EMR. The species used were *Matricaria perforata* and *Cheopodium album* and they had been sown on 29 April 2010.

Experiment 1

There were six treatments:

1. Hexyl butyrate
2. Hexyl butyrate + virgin female *L. rugulipennis*
3. Hexyl butyrate + artificial sex pheromone of *L. rugulipennis*
4. Virgin female *L. rugulipennis*
5. Artificial sex pheromone of *L. rugulipennis*
6. Untreated control

The hexyl butyrate dispenser was a colourless sachet loaded with 250 µl of hexyl butyrate.

These have a release rate of 18 mg/day at 20°C in the laboratory. The artificial sex pheromone lure of *L. rugulipennis* was a 1 ml pipette tip loaded with 100 µl of the pheromone with a release rate of approximately 40 µg/day. Lures and dispensers were provided by NRI. The virgin female *L. rugulipennis* were reared in the laboratory. They were collected as nymphs from a weed planting and reared on green beans at 20°C. Adult females were held individually and provided with green beans, bee pollen and frozen Dipteran larvae. When they were at least six days old they were considered sexually mature. Mature females were placed individually into containers consisting of a hair curler covered with fine mesh, into which cut green beans and damp tissue was placed.

The treatments were secured in green bucket traps that had been identified as the most attractive trap for *L. rugulipennis* in HL0184. The base of the bucket traps were at least 20 cm above the ground to prevent slugs entering the traps, and the traps were no higher than canopy height. They were hung on shepherd's crooks using flexible plastic coated wire to adjust the heights.

Females in the dispensers were replaced weekly (or immediately if dead/escaped). Hexyl butyrate dispensers and *L. rugulipennis* pheromone lures were also replaced weekly. At the first collection time they were placed in a sealed unit in a freezer at -20°C to be taken to NRI to assess the amounts of pheromone and hexyl butyrate remaining.

A randomised block design was used. Traps were placed at least 10 m apart and within the plant canopy on 24 August 2010. There were four replicates of each treatment.

Traps were inspected every two-three days. Numbers of *L. rugulipennis* in the traps were counted and sexed. Every two-three days the weeds at 1 m from each trap were tap sampled to assess *L. rugulipennis* populations in the vicinity of the dispensers. Two sample areas were tapped; one upwind and one downwind from the trap, with four taps in each area. Insects were counted and the numbers in each instar and the sex of the adults were recorded. The insects were then returned to the plants. The experiment was continued until 14 September 2010.

A temperature logger was placed in a Stevensons screen to record temperatures each hour throughout the experiment.

Experiment 2

There were two treatments:

1. A 3 x 3 grid of nine hexyl butyrate dispensers in colourless sachets in a 10 x 10 m area with a virgin female *L. rugulipennis* in a green bucket trap at the central dispenser
2. A virgin female *L. rugulipennis* in a green bucket trap at the centre of a 10 m x 10 m area

The hexyl butyrate dispensers were attached to bamboo canes at 40 cm above the ground. *L. rugulipennis* were reared in the laboratory to provide virgin females for the experiment as in experiment 1. The hexyl butyrate dispensers were provided by NRI as in experiment 1. Females and dispensers were suspended in bucket traps as in Experiment 1.

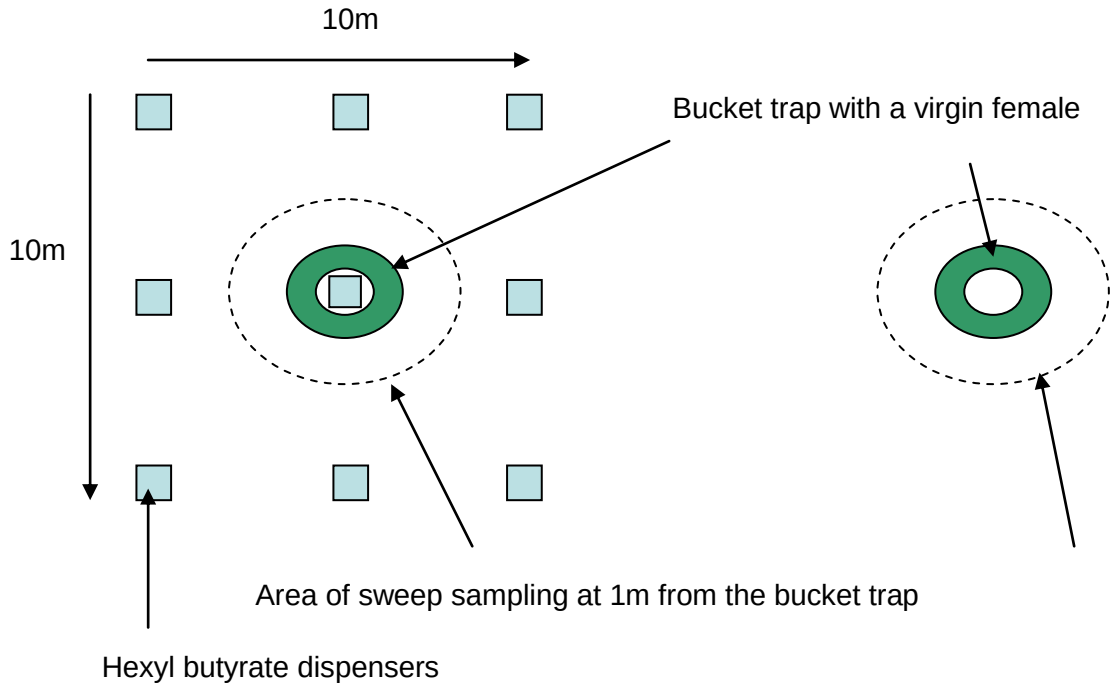


Figure 4.2.1. Experimental design for Experiment 2

A randomised block design was used, with five replicates of each treatment.

The experiment was set up on 1 September 2010. A pre-treatment sweep sample (10 sweeps) of the weeds at 1 m from the bucket trap was done as in Fig. 4.2.1. Traps were inspected every two-three days and numbers of *L. rugulipennis* in the traps were counted and sexed. Females in the dispensers were replaced weekly. Every three-four days the weeds at 1 m from each trap were sweep sampled to assess *L. rugulipennis* populations in

the vicinity of the dispensers. Insects were placed in a freezer at -20°C and the instars and sex of the adults were recorded. The experiment was continued until 16 September 2010.

Results

Experiment 1

Bucket trap catches were low and there was no significant difference between treatments (Table 4.2.1.).

Table 4.2.1. *Lygus rugulipennis* adults caught in bucket traps

Treatment	Total males	Total females
1. Hexyl butyrate	2	2
2. Hexyl butyrate + virgin female *L. rugulipennis*	2	5
3. Hexyl butyrate + artificial sex pheromone of *L. rugulipennis*	3	1
4. Virgin female *L. rugulipennis*	4	4
5. Artificial sex pheromone of *L. rugulipennis*	7	2
6. Untreated control	4	2

Tap sample data were analysed to assess the numbers of males in the samples and the % males were also analysed on the angular scale. A factorial treatment structure was used to assess the different components of the treatments. Dates 1-5 were analysed separately and then using a repeated measures analysis.

(i) Numbers of males

For dates 3, 4 & 5 there was a slight indication of overall lure differences (p=0.045, 0.065 and 0.078 respectively). However the results were different on the different dates, as reflected in the significance of the time:lure interaction (p=0.041) in the repeated measures analysis. For time 1 numbers were higher in the artificial sex pheromone treatment than where no lures were used. For time 4 numbers were higher in the virgin female treatment than in the artificial sex pheromone treatment. For time 5 numbers in the virgin female treatment were higher than where no lure was used. Thus there were no consistent differences between treatments over the different times.

(ii) % males (on angular scale)

There were some slight differences for different dates, mainly with hexyl butyrate as a factor

and the wind direction as a factor, but not with the lure as a factor. In the repeated measures analysis there was an overall difference between +/- hexyl butyrate, with a lower % of males when hexyl butyrate was present than when it was absent.

Experiment 2

Bucket trap catches were low. There was one female *L. rugulipennis* in each treatment over the period, and four and six males caught in the hexyl butyrate and control treatments respectively, with no significant difference between treatments.

Samples collected in the sweep sampling showed no significant effect of hexyl butyrate as a repellent, with no difference in either the number of adults or the proportion of males.

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

The aim of this study is to determine the efficacy of trap cropping with sweet alyssum (*Lobularia maritima*) grown in the strawberry tunnel leg rows in combination with bug vaccing of strawberry for control of the European tarnished plant bug, *Lygus rugulipennis*.

Methods

The site was 'Owens 3' everbearer strawberry plantation (cv. Elsinor) at Langdon Manor Farm, Goodnestone, Faversham, Kent ME13 9DA NGR TQ 024 593 (Fig. 4.3.1) by kind agreement of Alastair Brooks and his manager Andrew Reeve. It was planted in April 2009. The area of the plantation used was approximately 200 x 90 m and consisted of 22 tunnels with five row beds.

Figure 4.3.1. Approximate location of the two blocks of 11 Elsinor tunnels (marked in green with red surround)

The experiment was a two way factorial comparison in split plot design. The main effect factor had two levels, ±bug vaccing, i.e. a programme of bug vaccing applications vs. no bug vaccing. The split plot factor had three levels, alyssum sprayed with insecticide, alyssum not sprayed with insecticide and no alyssum, as follows;

Main effect treatment factor (±bug vaccing)

1. Bug vacced
2. Not bug vacced (untreated)

Split plot effect factor (Alyssum)

1. Alyssum sprayed with insecticide
2. Alyssum not sprayed with insecticide
3. No alyssum

A randomised block design with three replicates of the main effect treatment factor and one replicate of the split plot factor was used (Fig. 4.3.2). Each main effect plot was one tunnel (approximately 90 m long) and guarded on each side by two unused (bug vacced) tunnels. Each main plot was divided into three sub plots, each 30 m long (= twelve 2.5 m hoops) (Fig. 4.3.3).

The alyssum (*Lobularia maritima*) was pre-sown in a greenhouse in modules by B R Brooks and Son staff. EMR provided the seed, obtained from Ball Colegrave Ltd, Banbury. When plants were of adequate size for handling, they were planted in new 1 m peat bags *in situ*, 20 plants per bag in two rows of 10. Each alyssum sub-plot comprised two 1 m peat bags, grown end to end (Fig. 4.3.2), and provided with trickle irrigation. There were six main effect plots each requiring 8 x 1 m bags planted with alyssum. Therefore 48 bags were needed, each with 20 plants.

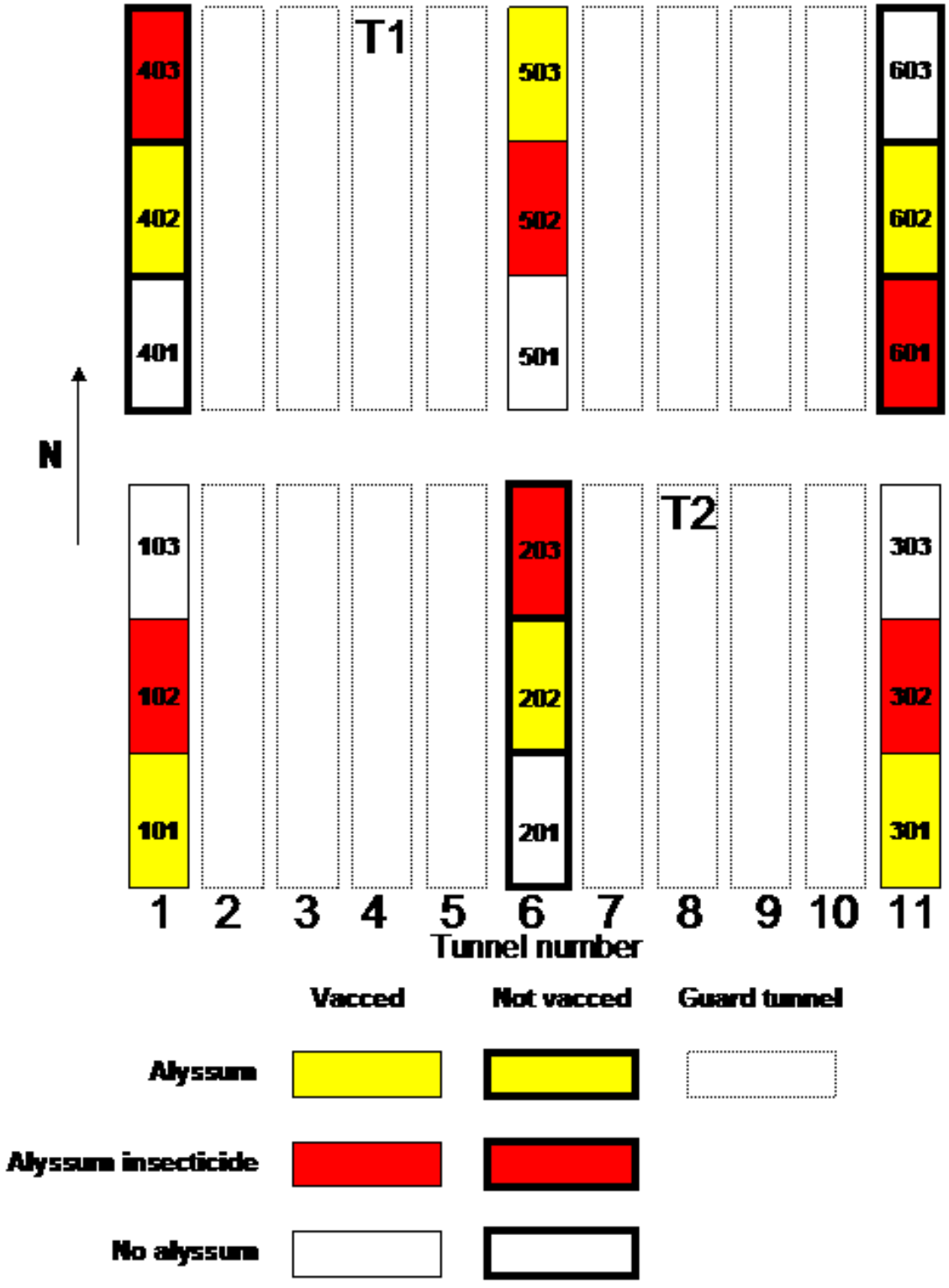


Figure 4.3.2. Diagram of layout of main effect plots and sub-plots. T = *L. rugulipennis* sex pheromone trap

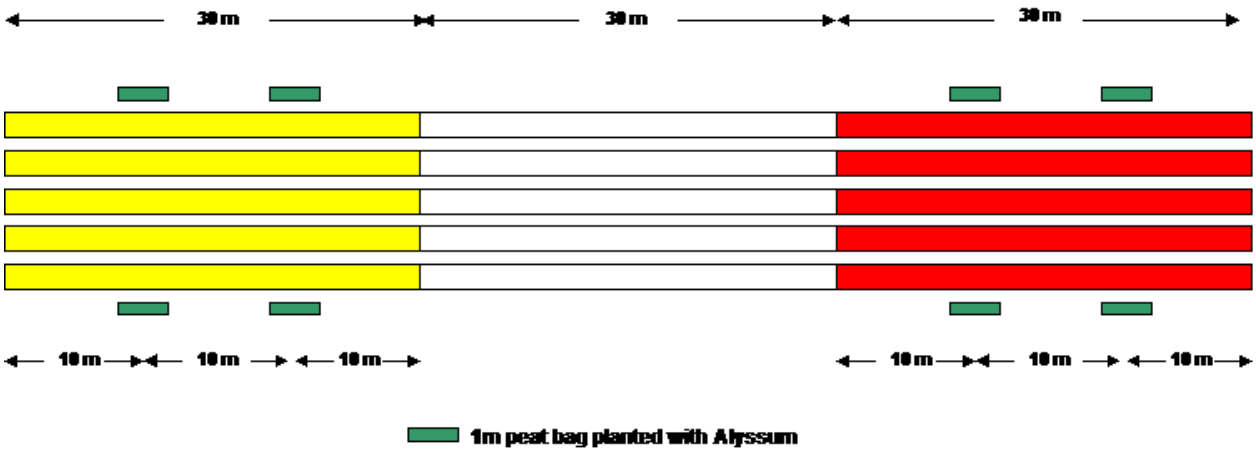


Figure 4.3.3. Diagram of layout of peat bags (in pairs, end to end) in a typical tunnel

Bug vaccing was done weekly by B R Brooks farm staff (liaison with Andrew Reeve) during periods when above threshold (> 1/40 plants) numbers of *L. rugulipennis* were present in the bug vacced plots.

The alyssum in the (red plots) (see Fig. 4.3.3) was to be sprayed by the grower's staff with the insecticide chlorpyrifos 480 g/l EC when populations of *L. rugulipennis* started to build up. The spray was to be applied with a hand lance at a concentration of 1 ml/l water and a volume of 50-200 ml/bag to give good wetting of the alyssum foliage. The strawberry beds were to remain untreated with any insecticides which would affect *L. rugulipennis*. Aphox was used for aphid control. A Stevenson's screen with a data logger temperature and humidity recorder was deployed in the centre of the experimental area in early April and remained for the duration of the experiment. Two *L. rugulipennis* sex pheromone traps were deployed (Fig. 4.3.2).

Sampling and assessments were done by EMR staff. The experiment was visited fortnightly (weekly when the populations increased) and a record made of the growth stage of the alyssum and the strawberries (digital photograph of the alyssum and adjacent strawberry bed).

The strawberries in each plot were sampled fortnightly from May to September by sweeping using a washing up bowl with one side cut out. Ten plants were sweep sampled per plot. The total number of *L. rugulipennis* adults and nymphs (recorded separately by instar) were recorded per plot. Numbers of other major pest and predator groups were recorded into broad taxa (coccinellids, syrphids, earwigs, heteroptera etc.).

Each alyssum plot (two pairs of bags per sub-plot) was to be sampled fortnightly. 16 sweeps were to be done per plot, four from each of the two pairs of bags. The total number of *L. rugulipennis* adults and nymphs (recorded separately by instar) and numbers of other taxa were also recorded.

On 19 August (all fruit) and 2 September (green fruit only) over 100 fruits in the centre of each main plot were assessed for capsid damage. The percentage fruit damage was recorded.

Results

The numbers of *L. rugulipennis* sampled from the plots rose steadily from the beginning of July, peaking at the end of August (Fig. 4.3.4).

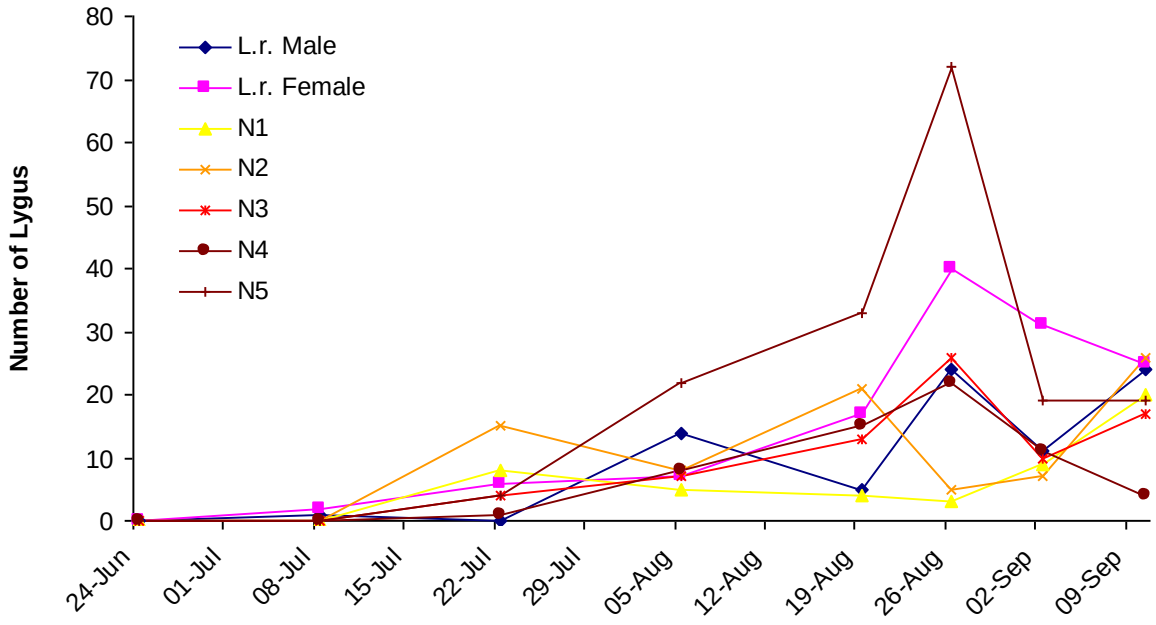


Figure 4.3.4. Total number of different life stages of *L. rugulipennis* nymphs on the plots at Langdon Manor Farm

This year, both the non-bug vacced and bug vacced plots were sampled before and after each bug vac operation. Overall, the numbers of most invertebrates, including *L. rugulipennis* adults and nymphs, were reduced by 10 - 40% (Fig. 4.3.5). However, there was no significant difference except for earwig numbers, which were reduced by more than 40% in the bugvac plots (ANOVA P= 0.008). The estimates for lygus populations were lower than the percentage reduction recorded in the previous year (up to 50%), which only sampled the bug vacced plots, with no comparison to control plots. It is likely that the disturbance of the

first sampling effort meant that many insects moved away from the strawberry foliage.

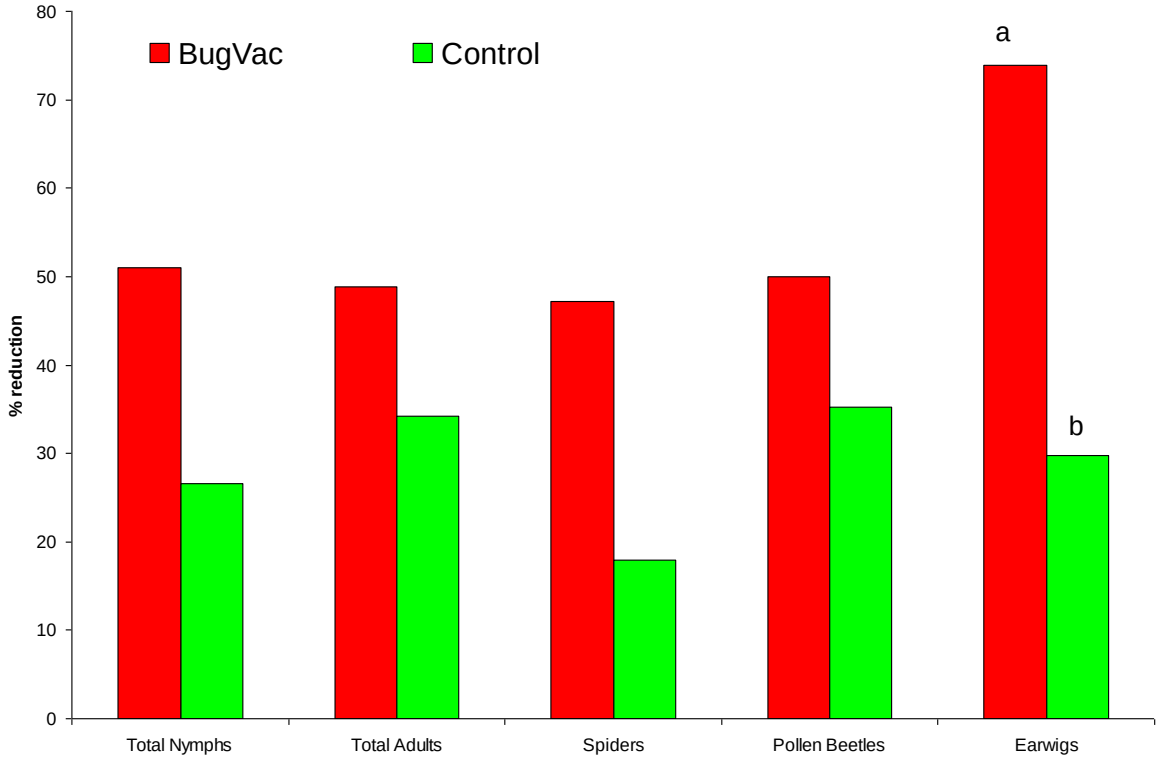


Figure 4.3.5. Percentage reduction in invertebrates sweep sampled from strawberry plants pre and post bug vaccing on the bug vacced and non bug vacced (control) plots. Total nymphs = *L. rugulipennis*, Total adults = *L. rugulipennis*.

The bug vac operator noticed that many insects – probably lygus adults – were flying away as the tractor passed over the crop. The tractor was venting air before the vac passed over (rear mounted), warning of approach. It is suggested that the bug vac be front mounted and that the frequency of passes is increased to at least 3 per week. The plots were small compared to a normal strawberry plantation and allowed for immigration into the plots from the surrounding area, so it is probable that treating larger areas would be more effective. In addition, the number of bug vacs on the front of the tractor could be increased to at least three to reduce operator time.

Assessments of fruit damage between the treated plots was not significant, however, there was a tendency for there to be more damaged fruit on the plots which were not bug vacced (Fig 4.3.6 and 4.3.7). The assessments would have been biased to damaged fruit as the pickers would not have picked damaged fruit for market.

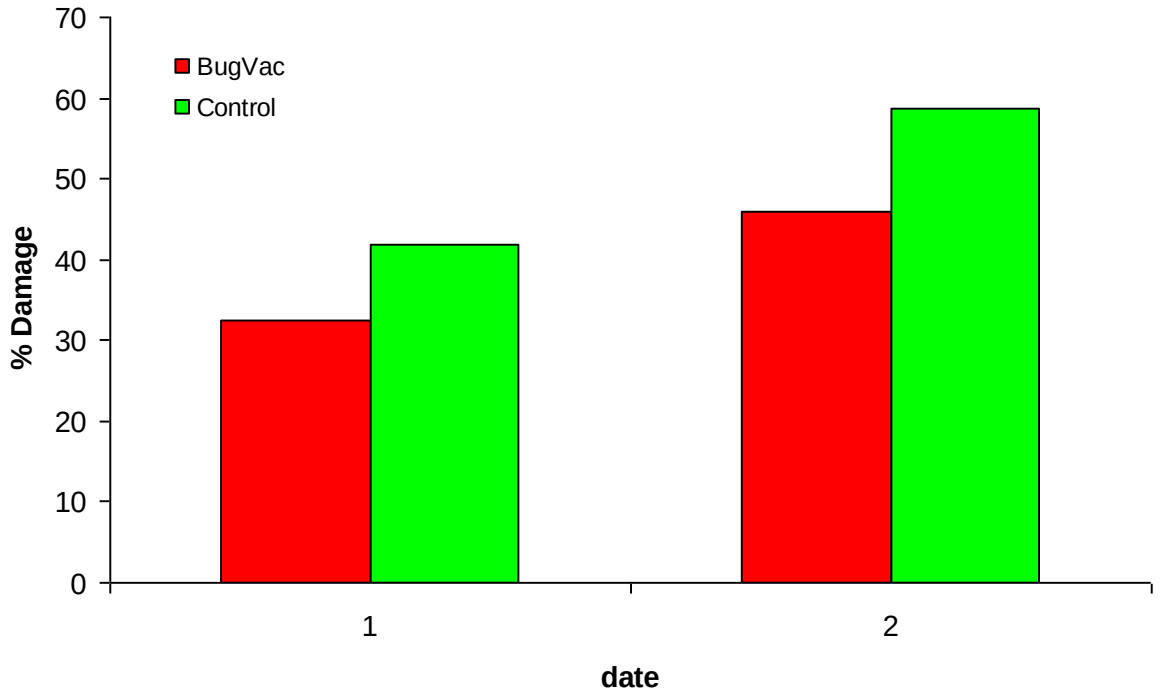


Figure 4.3.6. Fruit damage assessment of over 100 fruit from the centre of the main plots on 19 August (all fruit) and 2 September (green fruit only).

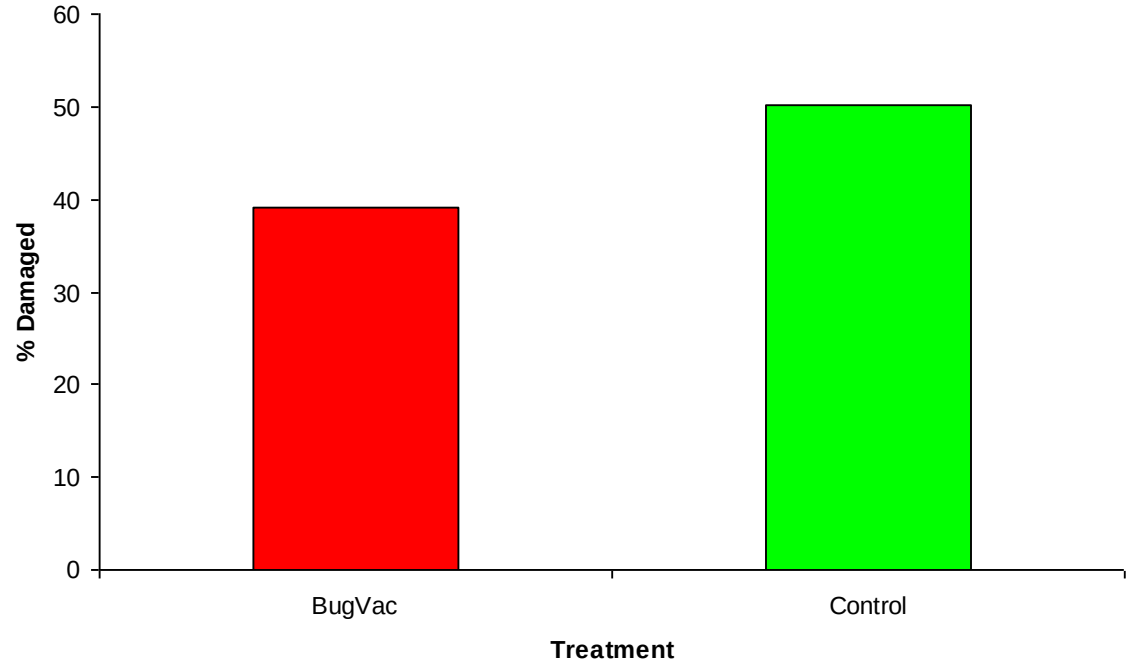


Figure 4.3.7. Total fruit damage of over 100 fruit from the centre of the main plots on.

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 1-3)

Earlier work has demonstrated that some flowering plant species are attractive to insect predators and, if used around crops, may increase biocontrol of pests. In 2009 nine species or species mixes were sown at EMR. Four of the five annual treatments grew well and numbers of beneficials were recorded during the growing season. Three of the four perennial plants germinated but did not flower in 2009. The aim of the experiment in 2010 was to assess the attractiveness of the perennial plants to predators and parasitoids of aphids and to compare their attractiveness with that of the annual species. The effect of the flowering plants on numbers of predators in adjacent strawberry plants was also assessed.

Methods

The experiment was set up on a strawberry planting at EMR (DM183). The strawberry planting was an everbearer strawberry planting of cv. Evie 2. This planting had a double row of plants grown on raised beds through polythene mulch. The plants were 40 cm apart in the rows and 35 cm apart between the rows. There were five raised beds and each of the beds was 3 m apart. Beds were aligned north/south. In 2009 beds had been divided into plots of 8.6 m long (40 plants). Each plot was separated in the bed from other plots by an unplanted area 3 m long. In 2010 these unplanted areas were planted up with plants of the same strawberry variety to enable samples from plants adjacent to each flower plot to be made.

There were five replicates of each perennial plant treatment in a randomised block design along the western side of the strawberry planting. Since numbers of beneficials on strawberry plants were also assessed, a bare soil treatment was included as a control. The treatments are shown in Table 5.1.1.

Table 5.1.1. Treatments to assess the attractiveness of perennial plants around a strawberry planting

Species	Common name	Flower colour	Sowing rate (g/m^2)
Medicago sativa	Lucerne	Yellow	6
Silene dioecia	Red campion	Pink	4
Echium vulgare	Vipers bugloss	Blue and pink	2
Bare soil	-	-	-

A mixture of annual species, cornflower (*Centaurea cyanus*), corn marigold (*Anthemis arvensis*) and corn chamomile (*Chrysanthemum segetum*), were resown at a rate of 3 + 1.5 + 0.4 g/m^2 in seven replicate plots along the eastern edge of the strawberry planting. Unsown areas were included as controls.

Each treatment plot was 4 x 2 m and there were no gaps between the treatment plots. Seeds were assessed for germination and the plots weeded as necessary.

Assessments began once the plants begin to flower. Sweep or tap samples were taken from each flower plot as dictated by the height and accessibility of the plant cover. Each sample was either 10 sweeps with a standard sweep net or 10 areas were tapped (with five taps per area). Tap samples were taken from the strawberries in the bed adjacent to each plant treatment, with six plants tapped per plot (with each plant tapped five times). All beneficial insects were identified to insect order. Samples were taken approximately every two weeks until the plants senesced; tap samples were taken from the strawberries before the sweep or tap samples of the flowers.

Results

Assessments began on the 14 May through to August 2010. The different flowering plant species were assessed though the main part of their flowering period, which differed from species to species. The perennial plantings were assessed in May and June. However, on 14 and 25 May only the red campion was in flower. On 25 May the horticultural fleece covers were removed from the lucerne, which was still low to the ground. By 3 June both the viper's bugloss and the red campion were flowering and sweep samples were taken. These sweep samples are awaiting assessment.

The height of the red campion and viper's bugloss, and the attractiveness of the latter to bees made sweep sampling difficult in June, therefore on 7 and 24 June all of the perennial plots were tap sampled. Table 5.1.2 shows the beneficial insects found in the tap samples. This table also shows the mirids sampled, and although the majority of the mirids were *Calocoris norvegicus*, the pest species *Lygus rugulipennis* (1 adult and 2 nymphs) was found in the lucerne on 7 June. The lucerne also had high numbers of flea beetles and aphids, and the viper's bugloss was attractive to bees (both bumble and honey bees). The results of the tap samples on the strawberries adjacent to the perennial flower plots are shown in Table 5.1.3.

The annual plantings were assessed in July and August on three occasions. The sweep samples of the flowers are awaiting assessment. The beneficial and pest species of arthropods found in tap samples of strawberry plants (six plants per replicate) adjacent to plantings of a flowering annual plants mix are shown in Table 5.1.4. In addition to the results shown, there were also numerous pollen beetles (42 in untreated plots and 22 in the flower mix plots) and a leaf beetle in the samples of 7 July.

Conclusions

From the results shown here there was no apparent effect of flowering plants on the numbers of beneficials found in adjacent strawberry plants.

Table 5.1.2. Arthropods (beneficial and pest species) found in tap samples of perennial flowers in plots around a strawberry planting (total of five plots with 10 tapping areas per plot).

Date	Species	Colour	Stage	Spider	Harvestman	Orius sp. A	Mirid A	Mirid N	Coccinelid A	Coccinelid L and P	Syrphid L	Soldier beetle
07-Jun	Red Campion	Pink	Flowering	3	0	0	1	2	0	0	0	2
	Viper's bugloss	Blue/pink	Flowering	3	1	3	5	4	1	0	0	0
	Lucerne	Yellow	Flowering	1	0	0	2	5	2	1	0	1
24-Jun	Red Campion	Pink	Flowering + seed set	0	0	0	0	37	0	1	0	0
	Viper's bugloss	Blue/pink	Flowering	3	0	11	5	2	0	1	2	0
	Lucerne	Yellow	Flowering	2	2	3	3	2	0	0	0	1

Table 5.1.3. Arthropods found in tap samples of the strawberries (6 plants per plot, 5 plots) adjacent to plantings of flowering perennial plants

Date	Species	Spider	Harvest-man	Anthocorid A	Orius A	Mirid A	Mirid N	Earwig	Coccinelid L	Parasitoid (ichneumonid)	Flea beetle	Shield bug N	Strawberry blossom weevil
07-Jun	Red campion	4	2	0	0	0	4	0	0	0	12	0	1
	Viper's bugloss	3	1	0	1	0	6	1	2	0	25	0	0
	Lucerne	0	1	0	0	0	5	0	1	1	8	1	0
	Bare soil	1	2	1	0	0	2	1	1	0	16	0	1
23-Jun	Red campion	3	6	0	0	1	0	3	2	0	1	0	2
	Viper's bugloss	1	8	0	0	1	0	0	0	0	1	0	2
	Lucerne	1	0	1	0	1	1	0	1	0	0	0	2
	Bare soil	2	3	0	0	0	0	0	0	0	1	0	1

Table 5.1.4. Arthropods (beneficial and pest species) found in tap samples of strawberry plants (six plants per replicate) adjacent to plantings of a flowering annual mix

Date	Treatment	Replicates	Spider	Harvestman	Anthocorid A	Anthocorid N	Orius sp. A	Orius sp. N	Mirid A	Mirid N	Earwig	Coccinelid A	Coccinelid L and P	Parasitoid (ichneumonidae)	Lacewing L	Syrphid L	Soldier beetles	Lygus rugulipennis A	L rugulipennis N	Caterpillars	Strawberry blossom weevils
07-Jul	untreated	6	2	9	0	0	0	0	1	1	0	1	4	0	0	0	2	0	0	1	1
	flower mix	6	3	5	0	0	0	0	0	0	2	1	2	0	1	0	1	0	0	3	2
20-Jul	untreated	7	4	2	1	0	6	0	2	2	0	8	6	1	2	0	0	1	0	0	0
	flower mix	7	0	1	0	0	8	0	2	0	1	12	14	1	0	0	0	0	1	0	0
11-Aug	untreated	7	1	1	0	1	12	1	0	0	0	8	0	4	1	7	1	0	28	0	0
	flower mix	7	5	0	0	0	7	0	0	0	0	8	0	0	1	3	1	1	23	0	0

A = adults, L=larvae, P = pupae, N = nymphs

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

Earlier work has demonstrated that various plant volatiles are attractive to a range of insect predators. However, work within this project, both in laboratory olfactometry and field trapping experiments, has failed to identify an attractive volatile for any predators of strawberry pests, with the exception of hoverflies. This experiment aimed to determine if a predator could perceive particular volatiles and if their behaviour was affected by them. Field experiments were done with released adult predators to ensure that high numbers were present at the start of the experiment.

Methods

The field experiment was done in an organic strawberry planting. A randomised block design was used with four replicates of each treatment (Fig. 5.2.1.). Treatments were:

1. Farnesene
2. Methyl salicylate
3. Farnesene, methyl salicylate, phenylethanol + caryophyllene
4. Untreated control

The volatiles were provided by NRI and were in colourless sachets with 200 µl of each of methyl salicylate, farnesene, phenylethanol and caryophyllene in the combined dispenser, and with 250 µl of each of the individual compounds in the dispensers. Dispensers were put out on 9 September 2010 and were changed on 15 September.

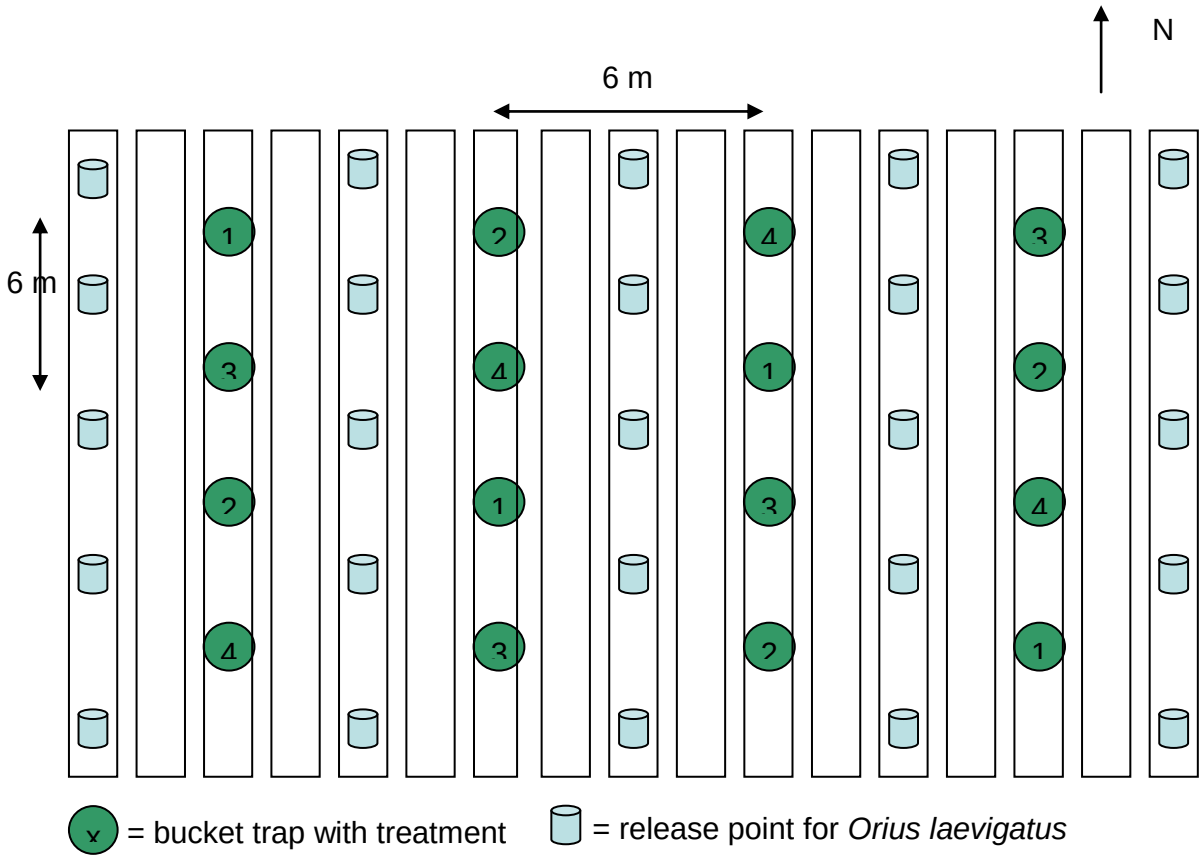


Figure 5.2.1. The experimental design used to evaluate the effectiveness of plant derived semiochemicals to attract aphid predators and parasitoids, in particular *Orius laevigatus*

Dispensers were suspended in green bucket traps with white cross vanes, with the traps just above the height of the crop canopy and placed at least 6 m apart. *Orius laevigatus* was used as the model predator species; this species is commonly found naturally occurring in strawberry fields and is also commercially reared for release. Tap samples of two areas within the crop were done to ensure that this predator was not already abundant in the crop. After setting up, *O. laevigatus* adults obtained from BCP Certis were released into the planting. Approx 4000 adults were released between the dispensers at 25 release points on 9 September and this was repeated on 15 September.

Bucket traps were examined and any predators or aphid parasitoids present identified. Ten strawberry plants close to the traps were tap sampled one day after predator release and on two further occasions. Any predators were identified and counted and returned to the plants from which they were collected. This experiment was set up on 9 September and the last assessment was on 23 September.

Results

Two areas were tap sampled before setting up the experiment but no *Orius laevigatus* were found. One release point was sampled one day after the first and second release dates and this sample had 10 and 23 *O. laevigatus* on the two dates. There were only five *O. laevigatus* caught five days after the second release date. Despite the high numbers of *O. laevigatus* released, few were caught at the release points and this was reflected in the tap and bucket catches (Table 5.2.1). The methyl salicylate treatment had the highest number of *O. laevigatus* in both tap and bucket catches. However since 8000 individuals were released in this experiment there was no evidence to suggest that using a lure containing any of these compounds at the release rates used would attract large numbers of *Orius laevigatus* into the crop.

Table 5.2.1. Total catches of *Orius laevigatus* adults in bucket traps and tap samples over 14 days (with 5 sample times).

Treatment	Bucket catches	Tap samples
Farnesene	2	1
Methyl salicylate	4	3
Volatile mix	0	0
Control	1	1

Data are being analysed to assess any potential effect of the treatments on other beneficial species.

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)

Treatments for a second autumn aphicides trial were applied in autumn 2009 and assessed in spring 2010 (spray application have been applied). The objective was evaluate the use of end-of-season clean up sprays with selective insecticides to control aphids on strawberry including *C. fragaefolii*, *Myzus ascalonicus*, *Aphis gossypii* etc., in commercial strawberry production. A large scale experiment evaluated four timings of late season sprays of the aphicide Calypso to reduce populations of aphids the following spring.

Methods

The experiment was done on 'Caravan' strawberry plantation at Arnold Farm, Langley, Kent. Details are shown in Table 5.3.1 and the location of the field Fig. 5.3.1.

Table 5.3.1. Site details

Grower name	Sean Charlton
Business name	G Charlton & Sons
Address	Rumwood Farm, Langley, Maidstone ME17 3ND
Contact persons	James Weeks Simon Beasley
Email	James: james.weeks09@virgin.net
Mobile phone	James: 07721450737
Plantation location (NGR)	At Arnold Farm, Langley ME17 1TF NGR TQ 811 527
Plantation name (s)	Caravan
Area (ha)	0.5 ha used (whole field 1 ha). The plantation has 32 tunnels running N-S
Variety	Elsinore (everbearer)
Growing system/media	Table top:1 m peat bags with eight plants per bag
Planting date	July 2009
Table spacing	1.3 m
Plot width (rows)	1 tunnel containing 5 table tops
Plot length (m)	~ 30 m
Protection	Polythene will be removed in mid November 2009 and replaced in mid March 2010
Marketing desk	Summer Fruit Company
Liaison	Lindrea Latham
Advisors	Hutchinsons (Paul Hamlyn, Graham Waters)

Figure 5.3.1. Google map aerial photo of Arnold Farm showing 'Caravan' plantation (red box)

Treatments were single sprays of Calypso (thiacloprid) applied in the autumn at four different timings at two week intervals (Table 5.3.2).

Table 5.3.2. Treatments

Product	Active ingredient	Dose /ha	Date of application
Calypso	480 g/l thiacloprid SC	250 ml	22 Sep 09
Calypso	480 g/l thiacloprid SC	250 ml	12 Oct 09
Calypso	480 g/l thiacloprid SC	250 ml	27 Oct 09
Calypso	480 g/l thiacloprid SC	250 ml	16 Nov 09
Untreated	-	-	-

Sprays were applied with the grower's purpose constructed air assisted sprayer, operated by the growers spray operator at the farms normal spray volume of 450 l /ha. The sprayer covered five table tops with seven Albuz hydraulic nozzles (mixed colours). The spray

operations were supervised by A. Harris (EMR). The sprayer calibration was checked on first spray occasion.

A randomised block design with four replicates was used. Plots were one tunnel of five table top beds and ran the full length of the tunnel and were side by side (Fig. 5.3.2).

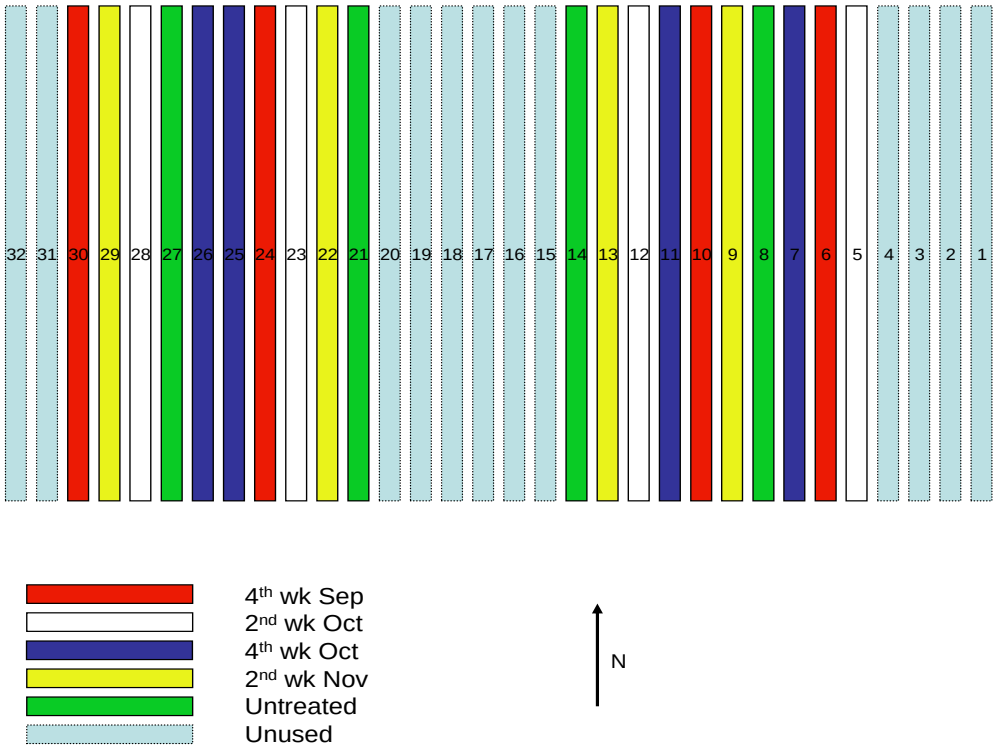


Figure 5.3.2. Layout of plots in tunnels in Caravan field. Note tunnels 1-4, 15-20, 31-32 are not used (tunnels 15-20 were planted at a different time)

Calypso has a SOLA for use on protected strawberry (0334 of 2006 Expires 31 December 2014) and outdoor strawberry (0333 of 2006 Expires 31 December 2014). The maximum individual dose is 250 ml product /ha, the maximum dose per season 500 ml/ha and the harvest interval is three days.

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

Two assessments were done (20 November 2009 and 9 March 2009). For the first assessment five whole plants from each plot were removed to the laboratory and examined for aphids. For the second assessment 100 leaves sampled from the central three rows of

each plot and the numbers of aphids counted. Specimens were returned to the laboratory for species identification.

Results

Very few aphids were found in the autumn on the strawberry plants and there was no significant difference between the numbers on the treated or control plot (Fig 5.3.3, ANOVA on $\log_{10}(n+1)$ transformed data). 95% of the aphids were *Macrosiphum euphoriae.*

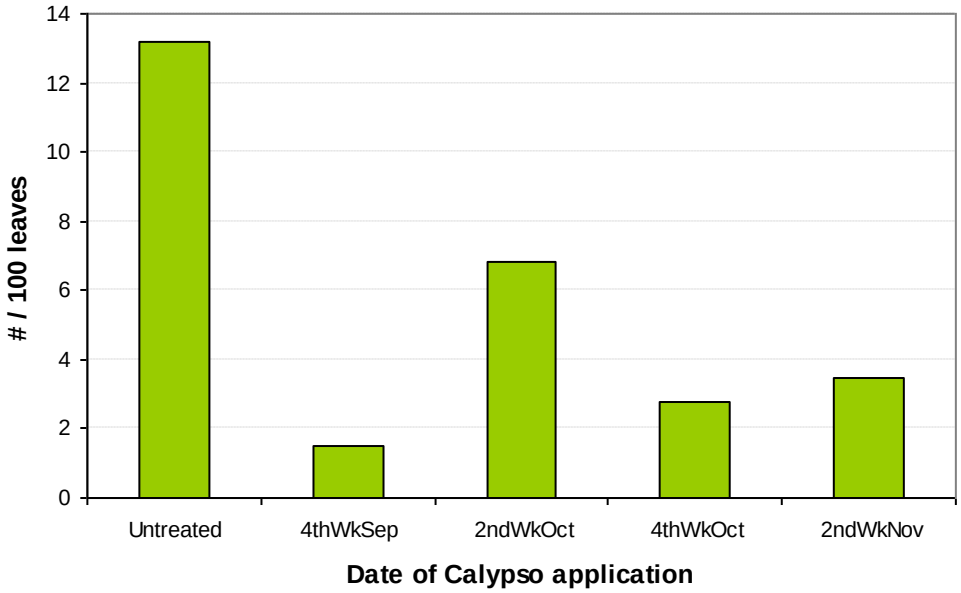


Figure 5.3.3. Numbers of aphids found on the strawberry plants on 20 November 2009.

However, by the second assessment on 9 March 2010 numbers had increased dramatically on the control plots and were significantly higher than on any of the treated Calypso treated plots (Fig 5.3.4, ANOVA on $\log_{10}(n+1)$ transformed data, $P<0.001$). Again, the main species of aphid present on the plants was *Macrosiphum euphoriae.*

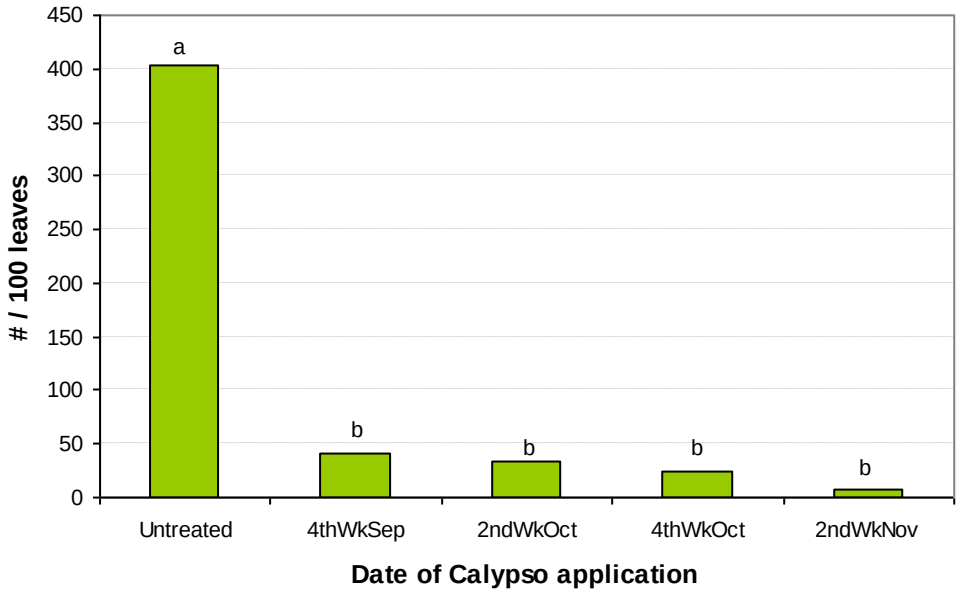


Figure 5.3.3. Numbers of aphids found on 100 strawberry plant leaves on 9 March 2010.

2010/2011 experiment

Autumn control of aphids on strawberry 2010 – 11

Experiment protocol ORETO GEP No ORETO 10/028

Objective

To use end-of-season clean up sprays with selective insecticides to control aphids on strawberry, including *C. fragaefolii*, *Myzus ascalonicus*, *Aphis gossypii* etc, in commercial strawberry production. A large scale experiment will evaluate two timings of late season sprays of the aphicide Calypso to reduce populations of aphids the following spring.

Site

There is one site for this experiment, as shown in Table 1. The location of 'MOB' strawberry plantation at Arnold Farm, Langley where the trial is being done, is shown in Figures 1 and 2.

Table 1. Site details

Grower name	Sean Charlton
Business name	G Charlton & Sons
Address	Rumwood Farm, Langley, Maidstone ME17 3ND
Contact persons	James Weeks
Email	James: james@rumwoodgreenfarm.co.uk
Mobile phone	James: 07721450737
Plantation location (NGR)	At Arnold Farm, Langley ME17 1TF NGR TQ 811 527
Plantation name (s)	Mob
Area (ha)	1.3 ha. The plantation has 16 tunnels running N-S
Variety	Elsinore (everbearer)
Growing system/media	Table top:1 m peat bags with eight plants per bag
Planting date	
Table spacing	1.3 m Tunnels 7.8 m
Plot width (rows)	One tunnel containing five table tops
Plot length (m)	107.5 m
Protection	Polythene removed in mid November 2009 and replaced in mid March 2010
Marketing desk	Summer Fruit Company
Liaison	Lindrea Latham
Advisors	Hutchinsons (Paul Hamlyn, Graham Waters)

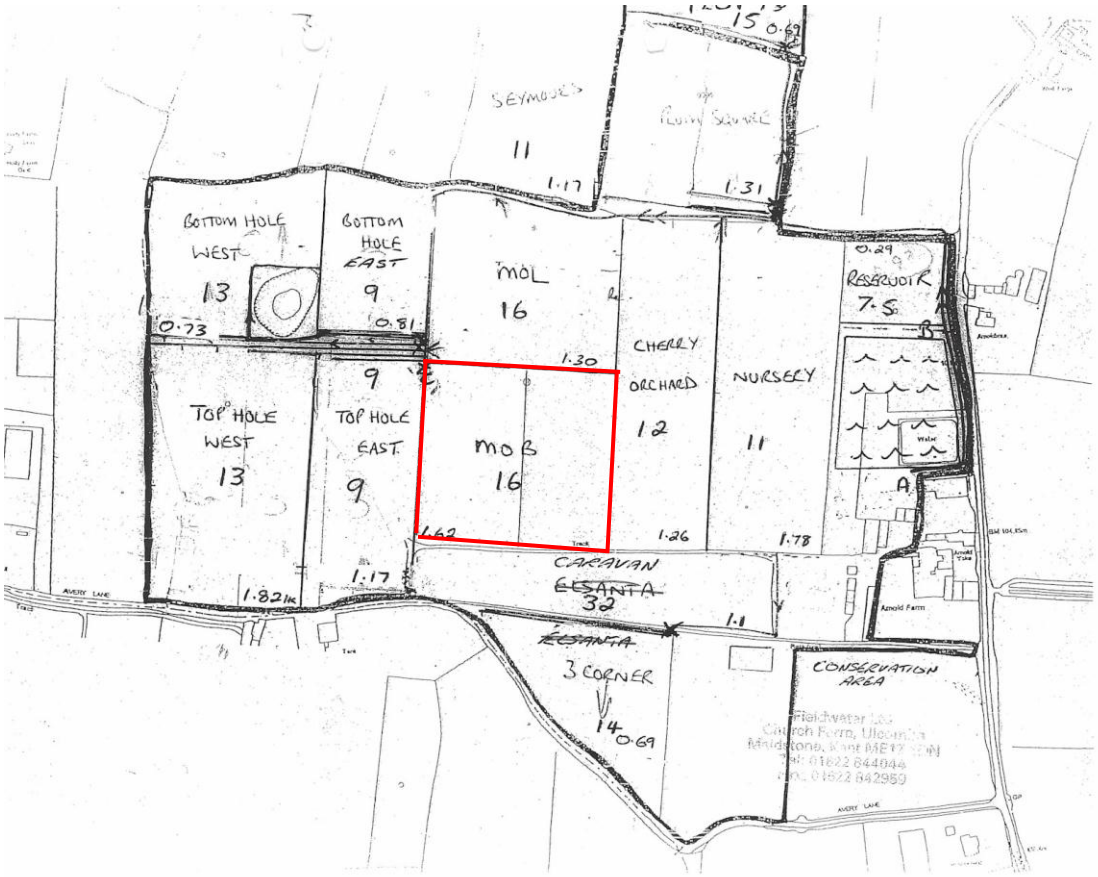


Figure 1. Farm plan of plantations at Arnold Farm, showing MOB plantation in red surround

Figure 2. Google map aerial photo of Arnold farm shown MOB plantation marked in red surround (Note this aerial photo is no longer current).

Treatments

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

Table 2. Treatments

Trt no	Colour code	Product	Active ingredient	Dose rate (/ha)	Timing of application
1	Red	Calypso	480 g/l thiacloprid SC	250 ml	1st week Nov
2	White	Calypso	480 g/l thiacloprid SC	250 ml	4th week Nov
3	Red White	Calypso	480 g/l thiacloprid SC	250 ml	1st and 4th week Nov
4	Green	Untreated			

Spray application

Sprays were applied with the grower's purpose constructed air assisted sprayer, operated by the growers spray operator at the farms normal spray volume of 450 l /ha. The sprayer covers five table tops with seven Albuz hydraulic nozzles (mixed colours).

Experimental design and layout

A randomised block design with four replicates was to be used. Plots are each one tunnel of five table top beds and ran the full length of the tunnel and are to be side by side. See figure 3 for diagram of layout of plots.

Table 3. Randomisation of treatments to plots

Block 1 Caravan tunnels 5-9			Block 2 Caravan tunnels 10-14		
Plot no.	***Trt no.***	***Col***	***Plot no.***	***Trt no.***	***Col***
101	2	W	201	4	G
102	1	R	202	3	RW
103	3	RW	203	1	R
104	4	G	204	2	W

Block 3 Caravan tunnels 21-25			Block 4 Caravan tunnels 26-30		
Plot no.	***Trt no.***	***Col***	***Plot no.***	***Trt no.***	***Col***
301	3	RW	401	1	R
302	2	W	402	4	G
303	4	G	403	2	W
304	1	R	404	3	RW

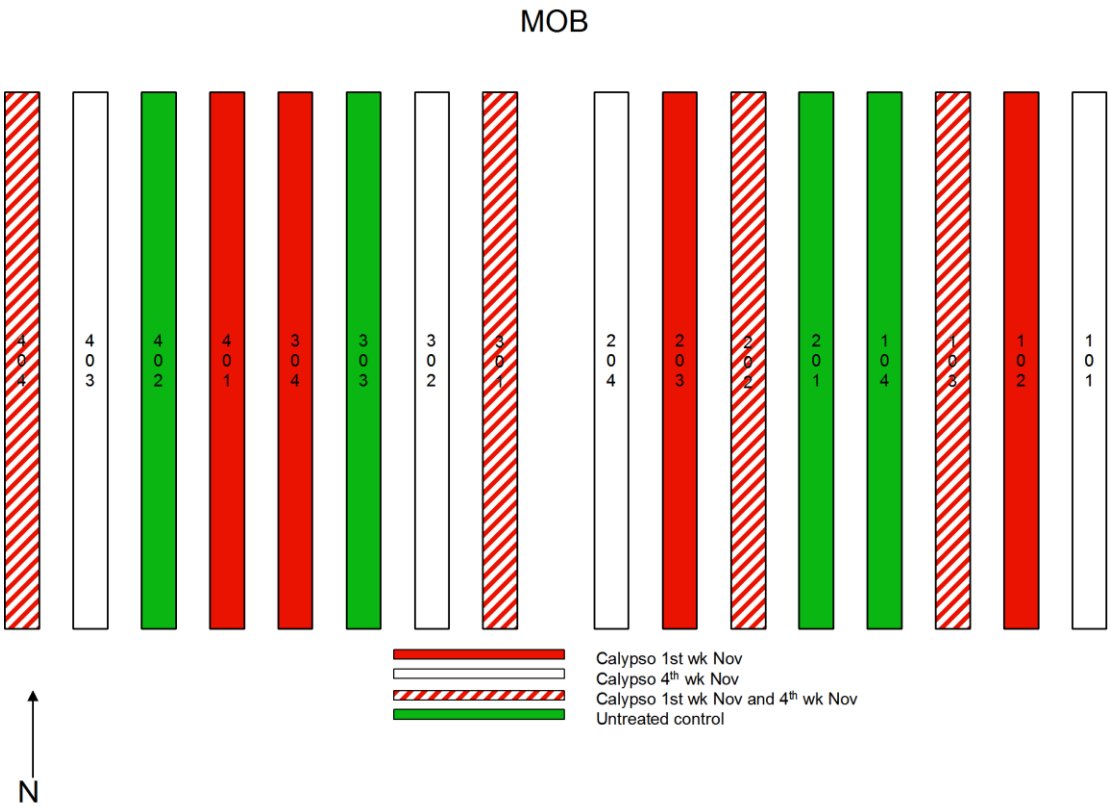


Figure 3. Layout of plots in tunnels in Mob field.

Approval

Calypso has a SOLA for use on protected strawberry (0334 of 2006 Expires 31 December 2014). The maximum individual dose is 250 ml product /ha, the maximum dose per season 500 ml/ha and the harvest interval is three days.

Calypso has a SOLA for use on outdoor strawberry (0333 of 2006 Expires 31 December 2014). The maximum individual dose is 250 ml product /ha, the maximum dose per season 500 ml/ha and the harvest interval is three days.

Meteorological records

Wet and dry bulb temperature with aspirated psychrometer, wind speed and direction were taken before and after spraying. Full records available from HRI-EM met station.

Assessments

Two assessments were done as follows:

1. Autumn 2010: In mid December 2010, at least three days after the last Calypso spray has been applied but before polythene covers are removed if possible.
2. Spring 2010. In mid March, 2-3 weeks after the polythene covers have been restored in spring

Numbers of aphids of each species present per plant in each plot will be assessed. The sample size (number of plants examined) will be adjusted according to populations on untreated plots to get good data for statistical analyses. Growing points and undersides of mature and semi-mature leaves to be assessed separately.

Crop Destruction

No crop destruction is required

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

Aphidius eglanteriae has proved to be a difficult species to mass produce. No alternative host species have been identified. Also the rate of population increase of the parasitoid within *C. fragaefolii* was very slow. Thus this species is unlikely to be a good candidate for production as a biocontrol agent. Therefore an alternative species, *Ephedrus cerasicola*, which has been shown to parasitise *C. fragaefolii* and has been recorded as a native UK species, was assessed for its effectiveness in a potted plant experiment. Since *Macrosiphum euphorbiae* has been reported to be becoming difficult to control with insecticides during the summer, parasitoids were tested against this species also. A mix of six parasitoids was used; this mix has been designed to contain species that attack all the main aphid pests of strawberry. If the mix proves successful this should make it more straightforward for growers to use this biocontrol strategy.

Methods

The experiment was done in a polytunnel at EMR.

A randomised block design was used with four replicates of each treatment. Treatments were:

1. Releases of *E. cerasicola*
2. Releases of a mix of six parasitoid species currently being trialled in Europe by BCP Certis. Species in the mix are *Aphidius colemani*, *A. matricariae*, *A. ervi*, *Praon volucre*, *E. cerasicola* and *Aphelinus abdominalis*. These attack a range of aphid species.
3. Untreated control

Potted strawberry plants infested with *C. fragaefolii* and *M*. *euphorbiae* were used in the experiment. An experimental plot consisted of four plants. Plants were arranged on a Mypex surface within the polytunnel and were drip irrigated. Each plot of four plants was covered with horticultural fleece suspended over a frame to minimise the movement of released parasitoids. A pre-release count of aphids was made on two leaves, one old and one recently unfurled, on each plant. These leaves were marked to enable future assessments to be made of the same leaves. Parasitoids were obtained from BCP Certis; each species was supplied as mummies in separate tubes. Mummies were held at 20°C until adult emergence and left to mate. Mated females were then used in the experiments. Parasitoids were released at a rate of four females per plant (i.e. 24 *E. cerasicola* per plot for treatment 1 and

4 of each of the six species in treatment 2). The first release was made on 3 August 2010. Two further introductions of parasitoids were made at the same rates on 16 and 27 August 2010.

One plant from each plot was assessed on 8 September 2010 and numbers of aphids (*C. fragaefolii* and *M. euphorbiae*) and any mummies present were counted. Initial sampling indicated that many of the leaves assessed before the parasitoid releases had died. Thus the sampling procedure was amended and all the leaves and flowering/fruiting clusters were assessed on each plant. A second plant was assessed on 14 September and a third on 18 September. Thus there were three sampling times after the first parasitoid release. For analysis aphid numbers per leaf or cluster were calculated and square root transformed. Two single degree of freedom contrasts were used within the treatment factor; the first assessed control versus treated and the second compared single species releases with the mix of six species. A plant factor was also included to assess the effect of time of sampling on the numbers present.

Results

Very few parasitised aphids were found during the post-release assessments; aphids often respond to parasitism by becoming more active and moving off the leaves. The number of aphids of both species significantly decreased with time of sampling. However, this may have been partly due to the deteriorating quality of the plants over time.

In the overall analysis of numbers per leaf *C. fragaefolii* were significantly lower in the treated compared to the control plots (P=0.04), although neither treatment individually was significantly lower than the control (P=0.8). In the analysis of numbers per cluster, aphids were again lower in the treated than in the control plots (P=0.03), but individually only numbers in the single parasitoid treatment were significantly lower than the control. In the comparison of single versus mix of parasitoids there was an indication that numbers in the single treatment were significantly lower than in the mixed species treatment (P=0.08) (Table 5.4.1). Of the parasitoids in the mix treatment only *E. cerasicola* will parasitise *C. fragaefolii* (Table 5.4.3), however, other parasitoid species may feed on the aphids and so contribute to biocontrol.

Table 5.4.1. Effect of parasitoid releases on C. fragaefolii numbers on strawberry

Parasitoid treatment	Numbers per leaf		Numbers per cluster	
	Square root trans numbers	Back trans numbers	Square root trans numbers	Back trans numbers
Single	3.52	12.38	2.81	7.91
Mix	3.79	14.37	4.00	16.00
Untreated	5.91	34.91	4.77	22.74
LSD (6 df) P=0.05	2.453		1.361	

For *M. euphorbiae* numbers per leaf were significantly lower in the treated than in the control plots (P=0.009) and numbers in the mixed species treatment were lower than in the single treatment (P=0.03). On clusters the same result was obtained with numbers in the treated plots being lower than the control (P=0.03), with only the mixed species treatment being significantly lower than the control. There was slight evidence of numbers being lower in the mixed species treatment than the single treatment (P=0.095) (Table 5.4.2). *Ephedrus cerasicola* does not parasitise *M. euphorbiae* (Table 5.4.3), so the single species treatment was not expected to reduce numbers of this aphid species.

Table 5.4.2. Effect of parasitoid releases on *M. euphorbiae* numbers on strawberry

Parasitoid treatment	Numbers per leaf		Numbers per cluster	
	Square root trans numbers	Back trans numbers	Square root trans numbers	Back trans numbers
Single	0.730	0.534	0.86	0.734
Mix	0.148	0.022	0.14	0.020
Untreated	1.122	1.258	1.38	1.900
LSD (6 df) P=0.05	0.5053		0.884	

Table 5.4.3. Specificity of parasitoids used in the mix treatment to aphids occurring on strawberry

	Aphidius ervi	***Aphidius matricariae***	***Ephedrus cerasicola***	***Praon volucre***	***Aphidius colemani***
Aphis gossypii		√	√	√	√
Aulacorthum solani	√	√	√	√	
Chaetosiphon fragaefolii			√		
Macrosiphum euphorbiae	√			√	
Myzus ascalonicus		√	√	√	
Myzus persicae	√	√	√	√	√

Conclusions

Numbers of aphids of both species were not completely eliminated in these experiments. This is likely to be due to the high populations present at the time of the first release.

However, these results show that releasing parasitoids onto aphid-infested plants can significantly reduce the populations of both *C. fragaefolii* and *M. euphorbiae* on the plants.

Releasing parasitoids when initial aphid populations are lower is likely to be a more effective strategy for the control of aphids in strawberry crops.

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)

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)
And

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)

In previous work, a supertrap for strawberry blossom weevil, *Anthonomus rubi,* was developed. It comprises an Agralan white cross vane funnel trap baited with an aggregation pheromone sachet lure which, in addition to the 100 µl of the 1:4:1 blend of Grandlure 1:Grandlure II: lavandulol, also contains 200 mg of the host volatile PV2 isolated from wild strawberry flowers. The objective of this study was to evaluate the efficacy of a grid of 36 *A. rubi* supertraps per ha (large scale density = 25 per ha) for monitoring strawberry blossom weevil to direct a localised spray of the insecticide chlorpyrifos if the nominal trap threshold was exceeded.

Large strawberry fields on three farms with a known history of strawberry blossom weevil infestation were selected for the work. Two well separated plots were marked out in each

field. One plot in each strawberry field, of approximately 1 ha area, received the monitoring trap treatment, the other was an untreated control. Records of catches of *A. rubi* and flower severing damage to the crop were taken at early, mid and end of flower. Where very high catches of weevils were recorded (> 25 per trap/week) a localised spray of thiacloprid (Calypso) or, for organic farms, pyrethrum, was to have been applied.

Methods

At each site there were two plots, one treated and one untreated. The three sites selected for the trail were as follows:

Field 1: Peaches Valley strawberry field at Mansfield's, Middle Pett Farm, Bridge, Canterbury, Kent by kind agreement of David Stockbury. The plantation was located at NGR TR 165 542 and was usually sprayed with chlorpyrifos and/or thiacloprid (Calypso) for strawberry blossom weevil at flower stem extension, but first flowers were often removed. The cultivar was Albion (everbearer) planted in September 2008. The whole field was 8.4 ha. The plant density was 42,500 plants/ha (bed spacing = 1.5 m, plant spacing in bed 30 cm in zigzag row, four rows/tunnel). The edge of plantation adjacent to hedgerow was used. The site was covered with polythene by mid-March. The start of flower stem extension occurred in April.

Field 2: Field 2B, cv. Florence (June bearer) in second year. At Hall-Hunter Farms' organic site at Tuesley Farm, Milford, near Godalming by kind agreement of Andrzej Zygora. The plantation consisted of 115 beds (five bed tunnels). The 40 most north-westerly beds in the control site were covered with deep straw. The MT devices were deployed in rows 1, 15, 30, 40, 50, 60.

Field 3: In Southfield (treated) and Baeza (control) organic plantations at Haygrove Ltd., Redbank Farm, Little Marcle Rd, Ledbury, Hereford HR8 2JL by kind agreement of Angus Davison. The crops were similar and were reported to have low to moderate levels of blossom weevil. They were replanted with potted Evie 2 (everbearer) in March 2010. Southfield (MT treated plot) consisted of 12 tunnels (9-20 from North) of 7.4 m width. Each tunnel contained four beds (each containing three rows of strawberries). The plot was surrounded by hedgerows on three sides. Baeza (control plot) consisted of 12 tunnels of 8 m width, containing five beds of strawberries each. There were hedgerows on three sides of the field.

There were two treatments as follows:

1. Mass Trapping: Monitoring with a grid of supertraps to direct local sprays of thiacloprid (Calypso) or pyrethrum (organic crops)

A 6 x 6 grid of 36 Agralan white cross vane funnel traps per ha each baited with a standard A. *rubi* aggregation pheromone lure (polythene sachet containing 100 µl of Grandlure I, Grandlure II and Lavandulol in a 1:4:1 ratio) containing 200 mg of additional PV2 host plant volatile prepared and supplied by International Pheromone Systems and deployed at the start of flower stem extension. Details of the lure are given in Table 1. If >25 weevils were captured in any one trap, a localised spray, of thiacloprid (Calypso) in conventional or pyrethrum in organic crops, was be applied. The white cross vanes of the traps were coated with fluon. The buckets contained water with a thin film (2 mm) of cooking oil on top to prevent evaporation. Ferric phosphate slug pellets were broadcast round each trap. Traps were held upright with purpose made bent metal rods.

2. Untreated control: No treatment for strawberry blossom weevil

Table 6.1. Lures supplied by David Hartley, International Pheromone Systems

Lure	Active substance	Loading	Dispenser	Release rate
Aggregation+ PV2	Lavandulol:Grandlure II:Grandlure 1 1:4:1 PV2	100 µl 200 mg	Polythene sachet with	~1.2 mg/day ~ 1.2 mg/day

Trap catches: To assess the attraction and efficiency of traps to both strawberry blossom weevil and other arthropods, the traps were emptied at least three times, once at the start of flowering, once at the end of flowering, once at the end of the experiment. The numbers of male and female strawberry blossom weevil in each trap were recorded.

Severing damage: The numbers of severed flower buds and total number of flowers (buds, flowers, fruitlets) per plant were recorded on at least two occasions, once at the start of flowering and once at the end of flowering. Recording of damage was done in a grid of fixed locations by counting the number of damaged buds on a fixed number of plants at each location. Samples were taken from 1) each edge of the field 2) two intersecting transects across the field 3) the centre of the field.

Lure release rate and life

Three lures to be kept in freezer (-20 ˚C) at outset. 10 extra lures were deployed at Tuesley

Farm, Milford, well away from trial area. One was collected in every two weeks and stored temporarily in the freezer, then sent to D. Farman, NRI, for determination of the amount of each substance remaining in each sachet.

A temperature and humidity logger was deployed in a Stevenson's screen at each site to take hourly records.

Results

Catches of A. rubi *adults in supertraps*

Totals of 43, 63 and 223 strawberry blossom weevil adults were captured at the three sites (Table 6.2). At sites 1 and 2, the sex ratio was strongly female biased, but at site 3 the sex ratio did not differ significantly from 1:1. The numbers of weevils captured reflected the previous history of blossom weevil control on the three sites, with the greatest numbers been found in the two organic sites and the least in the conventionally managed site.

Flower bud severing damage

No severing damage was recorded at site 1 (Middle Pett Farm).

At site 2 (Tuesley Farm), a single severed bud was recorded in the visual inspection of the untreated control plot in a sample of 200 at first flower on 12 May (Table 6.3). None were recorded in the treated plots. None were recorded in the beat samples. Note that at this time small numbers of *A. rubi* adults had been captured in the traps (Table 6.2). Flower severing remained at very low levels on 25 May but increased slightly on 8 June when a grand mean of 3.3% of buds were severed in the MT plot versus 1.3% in the untreated. Note that the % severing in the centres of the plots was very similar (1.5 and 1.3%, respectively), the high average level in the in the MT plot being due to a high level of infestation at the edges of the field - apparent in both the visual inspection and the beat sampling.

At site 3 (Redbank Farm), the later planted everbearer (potted Evie 2) did not start to flower until June. Because of the higher catches of *A. rubi* adults in the supertraps, a more comprehensive assessment with large samples was done. On 3 June, at the start of flowering, overall means of 0.8% and 1.6% buds were severed in the MT and untreated plots, respectively (Table 6.4). The means in the centre of the plots and the edges of the plots were 0.6 and 1.3%, and 0.9 and 1.7%, respectively. By 22 June, the % severing had increased to 3.2 and 3.5% in for the MT and untreated plots, respectively. Severing damage was higher at the edge of the untreated plot and lower in the centre of the untreated plot, than the MT plot, but visually the levels of damage were very similar and there was no evidence that the MT treatment had greatly reduced the incidence of severing damage.

Adult A rubi *in beat samples*

No *A. rubi* adults were found in beat samples at site 1.

At site 2, no adult *A. rubi* were found in beat samples on 12 May (Table 6.3). Small numbers were found on 25 May with two adults found on 920 plants sampled on the MT plot and seven adults out of 320 sampled on the untreated plot, all from the field edge. On 8 June, three adults were found fro 460 plants sampled on the MT plot, but none were found in 250 plants sampled in the untreated plot.

At site 3, on 3 June 13 adults were found by beat sampling 1000 plants on the MT plots, versus 16 adults out of 500 plants on the untreated plot (Table 6.4). The higher numbers of weevils present in the untreated plot suggested that the MT treatment was working at this stage, but on 22 June, 17 and five weevils were collected from 700 plants on the MT and untreated plots, respectively. Most of these were found at the edge of the field.

Efficacy of the MT treatment

Comparisons of the numbers of *A. rubi* adults captured in the supertraps with the numbers found by beat sampling in the crop (Table 6.5) indicated that at site 1, 100% of weevils were captured in the traps (none were found in the crop) with 31% and 24% of individual captured in the traps at sites 2 and 3, respectively. Comparing the numbers of *A. rubi* adults in the crop in the MT and untreated plots indicated a 71% reduction at site 3, but no reduction at site 3. However, there was no indication that the MT treatment reduced the incidence of severing damage. Indeed, the data indicates that there was more severing damage in the MT plot than the untreated at site 2 and only a 9% reduction at site 3.

Discussion

Using the trap for population monitoring

The results indicate that the supertraps are a sensitive indicator of the presence of *A. rubi* populations, but that catches may not be linearly related to the population density. At site 1, the traps caught a mean of 1.2 weevils (~ 0.3 weevils/trap/week) when none could be found in the crop by beat sampling or inspection for severing damage. At site 2 (Tuesley Farm) where there were intermediate populations levels, 1.8 weevils were captured per trap (0.4/trap/week) from a population of 138 weevils in the crop per ha estimated by beat sampling. At site 3 (Redbank), 6.2 weevils were captured per trap (1.2 per trap per week) where a population of 689 weevils per ha was estimated by beat sampling. Although these data do indicate an increasing trend in catches with increasing population density, far more data will be required before a valid graphical, correlation or regression analysis between

density and catches can be made. There is a high probability that the supertraps in grids of 36 were interfering with each other. Further work is needed to establish the relationship between monitoring traps (in small numbers in crops) and weevil populations. This raises the question of where to put monitoring traps. Deploying them at the edges of fields, next to hedges where weevils are likely to have overwintered, will give the most sensitive early warning of the start of crop invasion. But to get a better representation of the average population density more traps need to be sited in other parts of the field.

Efficacy of control

Taken at face value, the data gathered in 2010 suggest that the MT treatment (grid 36 supertraps) performed exceeding well at site 1 where the *A. rubi* populations were low, but at the two organic sites, where *A. rubi* populations were higher, they only captured < 30% of the weevils and did not prevent severing damage in the crop. Clearly, it is premature to draw such conclusions generally and more data is needed. The results do suggest that the density of deployment of 36 traps in a 1 ha plot (= 25 traps per ha in large plots) is insufficient where populations are moderate or high.

It is likely that the supertraps will perform best at very low population densities because in this situation they will not be competing with the natural *A. rub*i populations. It is also likely that they will perform better on crops which come into flower later (e.g. later spring planted, or everbearers that are de-blossomed early) because weevils will be trapped out before damage can be done. Furthermore, the traps are likely to give best results if they are deployed continuously through the season. They are known to trap the newly emerged adults (that are in reproductive diapause) in July-August and will deplete populations throughout the growing season if deployed continuously. The current life of lures is about 120 days.

Conclusions

The following conclusions can be tentatively drawn for the 2010 results, but further work is needed to validate findings:

- Supertraps are a sensitive indicator of the presence of *A. rubi* populations but catches may not be linearly related to the population density. Further work is needed to establish the relationship between monitoring traps (in small numbers in crops) and weevil populations and where best to site the traps in crops for monitoring purposes
- The 2010 results suggest that the MT treatment (grid 36 supertraps per ha) performed well at one site where the *A. rubi* populations were low but at the two organic sites, where *A. rubi* populations were higher, they only captured < 30% of the weevils and did not reduce severing damage in the crop

- The results do suggest that the density of deployment of 36 traps in a 1 ha plot (= 25 traps per ha in large plots) is insufficient where populations are moderate or high and the density needs to be increased, or the traps used in conjunction with chemical treatments
- Ideally, a smaller, low cost trap should be developed which can be deployed economically at higher densities for MT
- It is likely that the supertraps will perform better at very low populations densities, in crops which come into flower later and if they are deployed continuously through the season.

Table 6.2. Total numbers of *A. rubi* males and females captured in 36 supertraps in the 1 ha Mass Trapping plots

Middle Pett Fm, Canterbury, Kent				**Tuesley Farm, Milford, Surrey**				**Redbank Farm, Ledbury, Herefordshire**			
Date	***A. rubi*** ♂	***A. rubi*** ♀	**Total**	**Date**	***A. rubi*** ♂	***A. rubi*** ♀	**Total**	**Date**	***A. rubi*** ♂	***A. rubi*** ♀	**Total**
30-Apr	0	4	4	12-May	1	1	2	12-May	61	22	83
26-May	7	32	39	25-May	8	31	39	03-Jun	30	91	121
				08-Jun	10	12	22	22-Jun	21	8	29
Total	**7**	**36**	**43**	**Total**	**19**	**44**	**63**	**Total**	**112**	**121**	**223**

Table 6.3. No. of flower buds severed by *A. rubi* and no. of *A. rubi* adults recorded by beat sampling at Tuesley Farm, Milford, Surrey in 2010

Date Growth stage	**Treatment**	**Sampling transect**	**Severed flowers on plant**			**Severed flowers in beat sample**			**Adult *A rubi* in beat sample**		
			No buds assessed	No. buds severed by A rubi	% buds severed	No. of plants beat sampled	No. severed buds	No. severed buds per plant	No. of plants beat sampled	No. *A. rubi* adults	No. *A. rubi* per plant
12 May 1st flower	MT	NE-SW transect	500	0	0	50	0	0	50	0	0
		SE-NW transect	500	0	0	50	0	0	50	0	0
		Mean			**0**			**0**			**0**
	Untr	N-S transect	200	1	0.5	20	0	0	20	0	0
		E-W transect	†			†			†		
		Mean			**0.5**			**0**			**0**
25 May 95% flower	MT	N-S transect	500	0	0	100	3	0.030	100	1	0.01
		E-W transect	500	0	0	100	0	0.000	100	0	0
		Edge	1900	0	0	380	64	0.168	380	1	0.003
		Centre	1700	0	0	340	1	0.003	340	0	0
		Mean			0			0.050			0.003
	Untr	N-S transect	200	0	0	40	0	0	40	0	0
		E-W transect	500	0	0	100	7	0.07	100	7	0.07
		Edge	2400	0	0	120	7	0.06	120	0	0
		Centre	1200	0	0	60	0	0	60	0	0
		Mean			**0**			**0.033**			**0.018**
8 June	MT	N-S transect	500	3	0.6	50	13	0.260	50	0	0
		E-W transect	500	16	3.2	50	12	0.240	50	0	0
		Edge	1900	148	7.8	190	150	0.789	190	2	0.011
		Centre	1700	26	1.5	170	17	0.100	170	1	0.006
		Mean			**3.3**			**0.347**			**0.004**
	Untr	N-S transect	200	0	0.0	20	0	0	20	0	0
		E-W transect	500	15	3.0	50	7	0.140	50	0	0
		Edge	2400	22	0.9	120	29	0.242	120	0	0
		Centre	1200	15	1.3	60	7	0.117	60	0	0
		Mean			**1.3**			**0.125**			**0**

†No flowers present due to straw

Table 6.4. No. of flower buds severed by *A rubi* and no. of *A rubi* adults recorded by beat sampling at Ledbury 2010

			No. flower buds severed by *A. rubi*					**No. A rubi adults**		
Date (2010)	Treatment	Sampling zone	No. plants	No. trusses	No. severed buds	No. trusses/plant	No. severed buds/plant	No. plants sampled	No. *A rubi*	No. *A rubi*/plant
03 Jun	MT	East edge	30	108	47	3.6	1.6	200	1	0.005
		West edge	30	132	10	4.4	0.3	200	3	0.015
		South edge	30	110	27	3.7	0.9	200	4	0.002
		North edge	30	103	18	3.4	0.6	200	5	0.025
		Centre	30	137	19	4.6	0.6	200	0	0.000
		Mean				**3.9**	**0.8**			**0.009**
		Mean centre				**4.6**	**0.6**			**0.000**
		Mean edge				**3.8**	**0.9**			**0.012**
03 Jun	Untr	East edge	30	153	76	5.1	2.5	100	2	0.020
		West edge	30	132	45	4.4	1.5	100	5	0.050
		South edge	30	131	13	4.4	0.4	100	1	0.010
		North edge	30	182	72	6.1	2.4	100	5	0.050
		Centre	30	103	38	3.4	1.3	100	3	0.030
		Mean				**4.7**	**1.6**			**0.032**
		Mean centre				**3.4**	**1.3**			**0.030**
		Mean edge				**5.0**	**1.7**			**0.033**
22 Jun	MT	East edge	10	55	49	5.5	4.9	100	8	0.080
		West edge	10	63	18	6.3	1.8	100	1	0.010
		South edge	10	51	20	5.1	2.0	100	1	0.010
		North edge	10	52	27	5.2	2.7	100	4	0.040
		Centre	10	44	22	4.4	2.2	100	1	0.010
		N-S transect	10	54	43	5.4	4.3	100	1	0.010
		E-W transect	10	68	45	6.8	4.5	100	1	0.010
		Mean				**5.5**	**3.2**			**0.024**
		Mean centre				**5.5**	**3.7**			**0.010**
		Mean edge				**5.5**	**2.9**			**0.035**
22 Jun	Untr	East edge	10	33	38	3.3	3.8	100	1	0.010
		West edge	10	41	12	4.1	1.2	100	0	0.000
		South edge	10	57	35	5.7	3.5	100	0	0.000
		North edge	10	66	91	6.6	9.1	100	4	0.040
		Centre	10	47	13	4.7	1.3	100	0	0.000
		N-S transect	10	67	27	6.7	2.7	100	0	0.000
		E-W transect	10	71	31	7.1	3.1	100	0	0.000
		Mean				**5.5**	**3.5**			**0.007**
		Mean centre				**6.2**	**2.4**			**0.000**
		Mean edge				**4.9**	**4.4**			**0.013**

Table 6.5. Effect of Mass Trapping with a grid of 36 *A. rubi* supertraps in a 1 ha plot in 2010

Site (Farm)	Plants/ha	*A. rubi* in 1 ha plot			No. *A rubi* adults/ha in plot			severed buds		
		In 36 traps	In Crop	% captured	MT	Untr	% reduction	MT	Untr	% reduction
Middle Pett	42500	43	0	100	0	0	-	0	0	0
Tuesley	38162	63	138	31	138	469	71%	3.3%	1.3%	-
Redbank	39063	223	689	24	689	683	-	3.2/plant	3.5/plant	9

Combining the *A. rubi* trap and the *L. rugulipennis* trap to make a united monitoring devise for both pests.

Methods

The trial was in 'Southfield' organic plantation at Haygrove Ltd, Redbank Farm, Little Marcle Rd, Ledbury, Hereford HR8 2JL by kind agreement of Alastair Davidson and Graham Moor. This plantation had moderate levels of blossom weevil, and was replanted with potted Evie 2 (everbearer) in March 2010. The experimental plot consisted of 12 tunnels (9-20 from the west; tunnels 1-4 are planted with raspberries). The tunnels were 7.4 m width. In each tunnel there were four beds (each containing three rows of strawberries).

Traps were deployed on 13 July. The treatments were a factorial comparison of trap design (two levels), and lure composition (three levels) (Table 6.6). A Latin square design comprising six replicates of the six treatments was used.

Table 6.6. Treatments

Treatment no.	Factor 1: Trap design	Factor 2 Lure(s)
1. GA	Green cross vane no grid	*A. rubi*
2. GL	Green cross vane no grid	*L. rugulipennis*
3. GLA	Green cross vane no grid	*A. rubi* + *L. rugulipennis*
4. WA	White cross vane with grid	*A. rubi*
5. WL	White cross vane with grid	*L. rugulipennis*
6. WLA	White cross vane with grid	*A. rubi* + *L. rugulipennis*

Traps were Agrisense funnel traps with either white or green cross vanes. The white cross vane traps were deployed with a bee excluder grid over the funnel. This is because the white cross vane traps attract non-target insects, such as, honeybees and bumblebees. This was not necessary with the green cross vane traps because they do not attract bees. Lures were either the standard *Anthonomus rubi* sachet containing 100 µl of the normal 1:4:1 blend of Grandlure I: Grandlure 2: lavandulol plus 1 g of the strawberry flower volatile 2, 4-dimethoxybenzene, provided by International Pheromone Systems Ltd or *Lygus rugulipennis* pipette tips containing 100 µl of the standard blend of hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal (10% in sunflower oil). Plots were single traps deployed in a square grid, spaced two tunnels (= 14.8 m) apart in the leg rows of the Spanish tunnel protected strawberry field.

The traps were stood on the ground and held in place with a wire hoop, and contained water plus a few drops of detergent to break the surface tension. *Lygus rugulipennis* lures were renewed on each visit.

The grower was requested to avoid spraying the field for the two target pests for as long as possible. A temperature/humidity data logger was deployed in a Stevenson's screen in the field to take half hourly records.

Counts of the number of male *Lygus rugulipennis* and *Anthonomus rubi* in each trap were made.

Results

Significantly more *L. rugulipennis* males were trapped into green cross vane traps than white cross vane traps (ANOVA P<.001) and more were caught in traps baited with *L. rugulipennis* pheromone than *A. rubi* pheromone baited traps (ANOVA, P=0.009). The *A. rubi* lures did not interfere with catches of *L. rugulipennis*. In previous experiments *L. rugulipennis* was less attracted to white cross vane traps and, in addition, impeded by the grids used as bee excluders. *A. rubi* was observed in all traps regardless of whether there were *Anthonomus* baits or not. However, the numbers were very low and probably not high enough for differences to be seen. Any future combined monitoring trap should not have white cross vanes or a grid. The ideal trap would be a green cross vane that attracts *L. rugulipennis* and *A. rubi*.

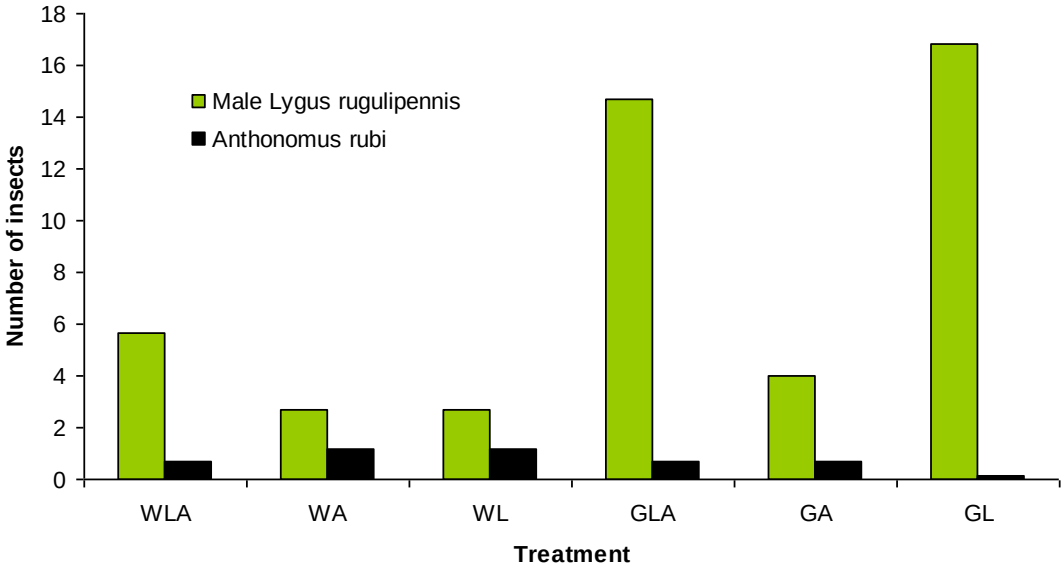


Figure 6.1. Mean number of male *L. rugulipennis* and *Anthonomus rubi* trapped in green (G) or white (W) cross vane traps with *Lygus* (L) and/or *Anthonomus* (A) lures.

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.

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

IPDM programmes for June-bearer and ever-bearer strawberries incorporating the findings of the first 3 years of this project with existing best practice have been devised for testing at three commercial farms (two Junebearers, one everbearer) in 2011-12. The programme to be tested on everbearers at B R Brooks and Son, Kent is given in Table 7.1..

Table 7.1. Treatment programme, monitoring and assessments for IPDM strawberry trial B R Brooks, Goodnestone, nr Faversham in 2011

Ever-bearer crop. New planting (8 tunnels = 0.4 ha) of Driscoll Amesti in Homestall Field, 4 tunnels IPDM, 4 tunnels growers.

Week number/date	**Growth stage**	**Target**	**EMR tasks**	**Grower applied treatments / tasks**
Wk 12 21 March	Planting		Place two loggers in crop to monitor temperature and humidity for mildew model and Botem. Deploy monitoring traps for thrips, European tarnished plant bug, tortrix moths and slugs	Broadcast Ferramol or Slugx (ferric phosphate) baits at planting
Wk 14 4 April			Start fortnightly pest and natural enemy monitoring (see separate protocol)	Deploy polythene covers
		Mildew	Start weekly crop inspections for powdery mildew, check mildew risk from model and report to grower	
		Red core / crown rot		Routine spray application of Aliette
		Blossom weevil		Deploy grid of 50 SBW supertraps per ha in leg rows.
		European tarnished plant bug	Provide peat bags and alyssum Clear Crystal White seed	Sow alyssum Clear Crystal seed in heated greenhouse to produce plugs plants for 72 1 m bags
		Aphids		Introduce APHIDSURE fragaria of six parasitoids. Start releases two weeks after planting with 1 tube/200m^2. Three releases at three week intervals, with further introductions as necessary.
Wk 16 18 April	Early flower	Mildew and Botrytis		Routine spray application of Signum
		Tarsonemid mite and western flower		Introduce *Amblyseius cucumeris* AMBSURE ABS sachets at 1 per 2m length of bed before flowering when temperatures are >12 ˚C. Repeat

		thrips		every six-eight weeks.
Wk 18 2 May		Husbandry		De-blossom plants
		European tarnished plant bug		Plant out alyssum in drip irrigated peat bags in leg rows from early April onwards, two bags end to end every 10 m in every leg row of the IPDM plot. If plot consists of three tunnels 85 m long, then, 18 bags will be needed for each of four leg rows = 72 bags. Plant with plug plants
		Mildew	Continue weekly crop inspections for powdery mildew, check mildew risk from model and report to grower	Apply Fortress for mildew if risk identified or mildew found. **Routinely vent tunnels every morning throughout cropping period**
Wk 20 16 May	Just pre-flowering	Botrytis, blackspot		Apply straw cover to alleys and polythene beds to reduce disease risk
		Two spotted spider mite		Introduce *Phytoseilus persimilis* predatory mites at 10/m^2 at first sight of any spider mites (or earlier if spider mite always seen). Monitor. Repeat after two weeks if necessary.
		Blackspot		Routine application of Signum**,** especially if planting material non-UK origin. Will also give some control of Botrytis and powdery mildew
		Slugs and snails		Broadcast Ferramol or Slugx (ferric phosphate) baits before strawing if slug risk
		Botrytis	Place bumblebee colonies in tunnel with Prestop mix for botrytis control. Use for first four weeks of flowering	
Wk 21 23 May	Start of flowering	Western Flower Thrips		Introduce *Orius* predators at 0.25/m^2, repeat after two weeks. If high risk, use a rate of 0.5/m^2.
		Mildew	Continue weekly inspections for mildew. Check mildew risk from model.	Apply potassium bicarbonate if risk identified by model, alternating with Sulphur. Don't tank mix potassium bicarbonate and Sulphur
		Botrytis	Continue checking Botrytis risk using Botem	
		European tarnished plant		Spray alyssum with pyrethrum with hand lance if ETPB adults occur.

		bug		Bug vac crop if ETPB found at > 1/40 plants in crop
Wk 25 20 June	Before fruiting	Botrytis	Check botrytis risk using Botem Remove bees.	If risk detected after first four weeks when bees finished use Serenade as recommended
		Mildew	Assess incidence of mildew as leaf area mildewed on three leaves on each of 35 plants	
Wk 29 18 Jul		European tarnished plant bug		Start weekly bug vaccing for ETPB. Continue to end of September, depending on traps and monitoring results.
Wk 26-40 27 Jun – 2 Oct	Harvest		On four occasions i.e. wks 27, 31, 35 and 39. Assess incidence of rots (Botrytis, mildew, blackspot etc) and pest damage (WFT, ETPB, slug etc) at harvest in three areas of the tunnel on 4 metre row lengths. Take a random sample of 8 x 50 fruits. 4 x 50 fruits to be incubated at ambient temperature in multicell trays in high humidity and assessed for rots after 5-7 days. 4 x 50 fruits to be rapid cooled to remove field heat, then held at 4^{o}C for 3 days followed by three days at ambient temperature and assessed for rots	○ Grower to harvest fruit as normal and keep records of marketable yield and waste **from the IPDM and the Standard tunnels** ○ Samples required from each plot for **residue testing** in weeks 26, 30, 34, 38 ○ At each pick grower must ensure all discard fruit is removed from the plantation and **kept in a cold store for assessment by EMR.** The removal is an essential requirement for the IPDM programme and the fruit needs to be examined by EMR to determine the causes of downgrading
Post harvest	October 2011	Mildew	Assess mildew incidence as leaf area mildewed on three leaves on each of 35 plants	
Post harvest	October 2011	Aphids		Spray with Calypso to clean up aphids

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

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

Appendix 1

SIX MONTHLY REPORT TO HORTICULTURE LINK PROGRAMME MANAGEMENT COMMITTEE

Project Number:	SF94
Project Title:	Minimising pesticide residues in strawberry through integrated pest, disease and environmental crop management
Project Partners:	EMR, ADAS, Fera, NRI, Berry Gardens, Berry World Ltd, TotalBerry, Mack Multiples Division, Marks & Spencer plc, Sainsbury's plc, International Pheromone Systems Ltd, Horticultural Development Company, East Malling Trust for Horticultural Research, East Malling Ltd, Jane & Paul Mansfield Soft Fruit Ltd, Agralan Ltd, Robert Boucher and Son, Red Beehive Company Ltd, Biological Crop Protection Ltd, Koppert UK Ltd
Report Written by:	Scientific consortium members
Project Start/Completion Dates:	1 April 2008 – 31 March 2013
Reporting Period:	30-36 month
Number of Months Since Commencement:	36 months
Date of Last Management Meeting:	26 January 2011
1. Project objectives:	(from project proposal, or other more recently approved planning document)

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.
Objective 2: To develop an IPM system for botrytis through reducing initial inoculum levels in planting material, accurate prediction of risk of flower infection, and the use of BCAs vectored by bees.
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.
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.
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.
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.
Objective 7: To develop and evaluate an Integrated Pest and Disease Management programme for strawberries, determining how components interact, economic performance, effects on other pests, diseases and beneficials and the incidence of pesticide residues.

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

Primary milestones

Milestone	Target month	Title	Achieved ?
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.	Y
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.	Y
P2.2	22	Validation of the Botem model for protected crop completed.	Y
P1.4	24	Efficacy of Serenade against mildew determined.	Y
P2.4	24	Suitability of bees for dispersing BCAs evaluated.	Y
P4.2.1	24	Feasibility of use of hexyl butyrate as a repellant of *L. rugulipennis* females determined.	Y
P5.4.2	24	Preliminary biocontrol trials with *Aphidius eglanteriae* completed. (see below)	N
P6.3	24	Optimum choice of host plant volatile(s) and blend for synergising the sex aggregation pheromone of blossom weevil established.	Y
P3.2	29	Population structure of blackspot determined.	Y
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	Achieved ?
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 programme with Botem.	Y
S2.3	24	Incidence of botrytis on planting materials determined	Y
S5.1.2	24	Second year experiment to evaluate the effectiveness of flowering plants to attract aphid predators and parasitoids completed.	Y
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. (see below)	Part Y
S5.3.2	24	Second year trial evaluating the efficacy of post harvest aphicide treatment completed, feasibility determined and best treatment identified.	Y
S6.2	24	Design of super trap for blossom weevil adjusted to minimise the capture of non-target arthropods.	Y
S3.3	29	Cross-inoculation of selected blackspot isolates completed.	Part Y
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.	

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

There is only one primary milestone for this reporting period (P3.2 Population structure of blackspot determined), which has been achieved on time.

One secondary milestone (S3.3 Cross-inoculation of selected blackspot isolates completed) has been slightly delayed but this should not affect the overall project progress.

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

Powdery mildew: Over the last three years, we did not find a significant level of latent powdery lesions on planting materials. Furthermore, symptomatic mildew lesions (spores) on green appeared not to survive in cold store if the green leaves become senescent but can survive over the winter on green leaves. None of alternative products tested showed any significant control effects against powdery mildew. This is primarily due the fact that the trial was conducted on a two-year old Albion plantation where it had a very high level of inoculum.

On early covered ever-bearers, the model-managed plots had similar level of powdery mildew as the conventional managed plots. However, the managed plots only received only 4 sprays compared to more than 20 sprays in the conventional plots. But on a late 60 days Sonata crops, the evaluation trial failed to give any result because that the initial mildew level is so high that routine control programme failed to reduce the level before the trial could commence.

Botrytis: Fifty ex-cold store strawberry plants, cv. Elsanta supplied by the grower on day of planting, were examined for latent infection by *B. cinerea*. *B. cinerea* was detected in 8% of the plants sampled indicating localised infection. Fungicide sprays and drenches were applied 3 weeks after planting. The fungicide treatments were: Untreated control, Cercobin WG drench at 1 g/L (0.25 g per plant), Teldor spray at 1.5 kg/ha, Scala spray at 2 L/ha, Signum spray (old label) at 1.8 kg/ha, Switch spray at 1 kg/ha and Serenade ASO spray at 10 L/ha. In crop assessments were carried out 2 weeks after treatment, 20 tagged leaves per plot were sampled, surface disinfected and placed into humid incubation and assessed for *B. cinerea*. Overall within this crop *B. cinerea* was at a low level. No clear consistent differences were shown between the fungicide treatments, but Signum showed some initial promise in the leaf humid incubation assessments. A grower standard spray programme of 4 fungicides at weekly intervals was compared with three biocontrol treatments; Prestop (*Gliocladium* spp.) and Serenade ASO (*Bacillus subtilis*) applied as weekly sprays and Binab T-Vector (*Trichoderma* spp.) vectored by bees. Assessments were carried out on leaves, flowers and fruit to assess levels of botrytis. A high level of latent infection by *B. cinerea* was present in flowers and leaves of strawberries in the two experimental tunnels. Both bumble bees and honey bees effectively transferred the biocontrol product from the hives to the flowers. None of the treatments significantly reduced the incidence of latent infection by *B. cinerea* in strawberry flowers or fruit, or the incidence of botrytis fruit rot.

BOTEM forecasting of botrytis: Validation results in 2010 again confirms those of previous years: botrytis risk on June-bearers (Elsanta) covered early it the early spring is very low. The level of fruit with latent botrytis infection is very low in both conventional and unsprayed plots. The results from all three years (2008-2010) suggested that for early-covered June-bearers fungicide application is not necessary to manage grey mould.

Pesticide dissipation: Fungicide residues are very persistent on leaves of strawberry plants grown under protection: residues virtually did not reduce 10 days after applications. In contrast, much of fungicide residues was washed off on those plants in open conditions due to the rain one day after the application. Thus, it is critically important to establish harvest intervals for strawberry grown under protection for each pesticide; using the data from open-field conditions may result in significant amount of residues on fruit under protection.

Black spot: Molecular analysis of isolates from different hosts at several sites suggested that significant differentiation among isolates only occurred between different sites but not between hosts at the same site. Thus, it does not appear that there is significant host-pathogen association for this pathogen yet.

European tarnished plant bug: A large scale field experiment was done to evaluate the use of the bug vac for control of *L. rugulipennis* in strawberry. Weekly bug vacs at the peak of *L. rugulipennis* populations (from the beginning of July, peaking at the end of August) were applied to half of the plots. Both the non-bug vacced and bug vacced plots were sampled before and after each bug vac operation. Overall the numbers of most invertebrates including *L. rugulipennis* adults and nymphs were reduced by 10 - 40%. The reduction of fruit damage in the bug vacced plots was lower, but not significantly so. A number of recommendations for the bug vac operations have been made; 1) the bug vac to be front mounted to prevent bugs flying away as the tractor passes over the beds, 2) begin bug vaccing as soon as the rise in populations is detected with the pheromone traps (~4 weeks before detection in field using traditional sampling methods), 3) more frequent passes over crop – at least 3 times per week. In an experiment to test the neccessary growing conditions of alyssum (attractant of *L. rugulipennis*) in strawberry crops alyssum seed sown directly into soil did not establish well and seedlings were subject to competition from weeds and drying out. Plug plants sown directly into the soil were also vunerable to competition from weeds. Plants grown in grow bags with drip irrigation developed best. Trials with alyssum varieties are showing that the cultivar Clear Crystal has more vigorous growth and more flowers than Snow Crytal>Snow

Drift>Easter Bonnet>Gold Ball.
Hexyl butyrate dispensers were used in combination with live female *L. rugulipennis* and artificial sex pheromone in field experiments to determine the mechanism of reported population reductions. Results were not consistent, but in general a lower % of males were found in samples when hexyl butyrate was present than when it was absent.

Aphids : Small plot experiments were done to assess the effects of sowing flowering plants alongside strawberry plantings on numbers of aphid predators and parasitoids in the crop. The plants used were *Medicago sativa, Silene dioecia, Echium vulgare* and a mixture of annual species, cornflower (*Centaurea cyanus*), corn marigold (*Anthemis arvensis*) and corn chamomile (*Chrysanthemum segetum*. There was no apparent effect of these flowering plants on the numbers of beneficials found in adjacent strawberry plants when compared with a bare soil control.
Earlier work has demonstrated that various plant volatiles are attractive to a range of insect predators. However, work within this project both in laboratory olfactometry and field trapping experiments has failed to identify an attractive volatile for any predators of strawberry pests, with the exception of hoverflies. Further experiments with mass releases of a commercially available predator, *Orius laevigatus*, failed to show any response of this predator to lures containing farnesene, methyl salicylate or a mixture of farnesene, methyl salicylate, phenylethanol and caryophyllene.

In a field scale field trial using 4 different timings of Calypso between the end of September and beginning of November, all applications reduced the numbers of aphids (*Macrosiphum euphoriae*) present on the crop the following spring compared to the untreated control (less than 50 aphids/100 leaves compared to more than 400 aphids/100 leaves).
Aphidius eglanteriae has proved to be a difficult species to mass produce so an alternative species, *Ephedrus cerasicola* was assessed for its effectiveness in reducing *C. fragaefolii* populations in a potted plant experiment. A mix of six parasitoids was used and compared with *E. cerasicola* alone and an untreated control; this mix has been designed to contain species that attack all the main aphid pests of strawberry. Results showed that releasing parasitoids onto aphid-infested plants significantly reduced the populations of both *C. fragaefolii* and *M. euphorbiae* on the plants.

Strawberry blossom weevil super trap: Three field trials in Kent and Hereford were set up to determine if the supertrap could be used as a mass trapping device for *A. rubi*. Supertraps were found to be a sensitive indicator of the presence of *A. rubi* populations, but it was not clear if the catches were related to the population density. Further work is needed to establish the relationship between monitoring traps (in small numbers in crops) and weevil populations and where best to site the traps in crops for monitoring purposes. The 2010 data suggest that the MT treatment (grid 36 supertraps per ha) performed well at one site where the *A. rubi* populations were low, but at the two organic sites, where *A. rubi* populations were higher, they only captured < 30% of the weevils and did not reduce severing damage in the crop. The results do suggest that the density of deployment of 36 traps in a 1 ha plot (= 25 traps per ha in large plots) is insufficient where populations are moderate or high and the density needs to be increased, or the traps used in conjunction with chemical treatments. Ideally, a smaller, low cost trap should be developed which can be deployed economically at higher densities for MT. It is likely that the supertraps will perform better at very low populations densities, in crops which come into flower later and if they are deployed continuously through the season. A small scale field trial was done to test combinations of trap designs for *L. rugulipennis* and *A. rubi*. White cross vanes on the bucket traps were a repellent to *L. rugulipennis* males. The *A. rubi* lures did not interfere with catches of *L. rugulipennis*. In previous experiments *L. rugulipennis* catches were, impeded by the grids used as bee excluders. Numbers of *A. rubi* were too small to draw conclusion from. Any future combined monitoring trap should not have white cross vanes or a grid. The ideal trap would be a green cross vane that attracts *L. rugulipennis* and *A. rubi*.

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

None

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

Technology transfer activities

(1) 27 October 2009. The project was in part reported by J Cross in his ½ hour lecture 'Reducing pesticides and pesticide residues' at the EMRA Food and Waste conference at EMR.
(2) 10 November 2009. J Cross gave a 40 minute invited plenary lecture at the Nordo Baltic Soft Fruit conference entitled 'UK research into monitoring and control of European Tarnished Plant Bug, *Lygus rugulipennis'.*
(3) 17 November 2009. An overview of the project was reported to KG Growers as part of their members training day at EMR by J Cross.
(4) 11 February 2010. Work on the strawberry blossom weevil super trap was briefly described by J Cross as part of his inaugural professorial lecture at the University of Greenwich, as well as being overviewed in the booklet that accompanied the lecture.
(5) Cross, JV, Fountain, M.T., Hall, D.R. (2010) Management of European tarnished plant bug in late season strawberries. "Integrated Plant Protection in Fruit Crops" Subgroup "Soft Fruits". "Workshop on Integrated Soft Fruit Production" 7th Meeting in Budapest, Hungary, Monday 20th – Thursday 23rd September 2010.
(6) Michelle Fountain 29 June 2010. Talk to the Strawberry Growers Club at East Malling Research on the use of the trap for monitoring capsids in strawberry. HDC/EMRA meeting.
(7) EMRA/HDC Soft Fruit Day, Technical Up-Date on Soft Fruit Research, East Malling Research, Kent.

Publications

(1) Cross J V. 2010. To spray, or not to spray: That is the question. Horticultural Entomology in the 21st century. Inaugural professorial lecture 11 February 2010, P 42-43 and p66-67
(2) N. Harvey and X.-M. Xu (2010). Powdery mildew on raspberry is genetically different from strawberry powdery mildew. *Journal of Plant Pathology* (In press)
(3) X.-M. Xu, E. Wedgwood, A.M. Berrie, J. Allen and T.M. O'Neill (2010) Epidemiology of strawberry and raspberry grey mould in open field and under protection. Agronomy for Sustainable Development (submitted)

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

Most of the exploitation of the project by growers will come in the final years of the project. However, the use of autumn sprays against aphids the following season has been very successful and could be implemented now. It is hoped that SBW and Lygus traps will soon be available as monitoring tool for the pests in strawberry plantations.

GUIDANCE NOTES

- All sections should be written by the project research co-ordinator in consultation with the project partners.

- The report should normally be 2-4 pages long, excluding the list of publications.

- The approved project proposal document should be used as a reference when describing research progress in sections 2 and 4 and for reporting on how the exploitation opportunities have developed during the course of the research in section 7.

- Proposed and actual dates of completion for objectives and milestones should be given in sections 2 and 3.

- The research report in section 4 needs to contain enough detail to give a clear idea of the state of the project, highlights and any critical issues. Simply stating that the project is on schedule and meeting its objectives is not sufficient.

- When commenting in section 4 on any delay or non-achievement (or indeed early achievement) of project milestones an assessment should be given of the reasons for this, the likely impact on the project overall. This must be related to any proposals to change the project plan in section 5.

- Any changes in project staffing and their impacts should be included in section 5.

- Only publications and presentations arising as a direct result of the LINK project should be listed in section 6.

- The report, once approved by the consortium, should be submitted to the joint LINK programmes Secretariat who will pass to the relevant sponsor Project Monitoring Officer and the Programme Management Committee

- Please note that as well as referring to technical issues and objectives the sponsor and PMC will consider the performance of the consortium (is good collaboration evident? Are in-kind contributions from companies being received as planned? Are future plans realistic? etc).

- Submission of reports will be on a rolling six monthly basis, deadline dates to be agreed project by project.

Reports should be sent to:

hortlink@defra.gsi.gov.uk

Joint LINK Programmes Secretariat

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