Project title:	Minimising pesticide residues in strawberry through integrated pest, disease and environmental crop management
Project number:	SF 094
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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

Progress is being made with developing non-pesticidal methods for control of the major pests and diseases of strawberry.

Background and expected deliverables

The overall aim of the proposed 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 in 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

In Year 2, the experiments on determining the possibility (and the amount) of powdery mildew in initial planting materials were repeated. As in the first year, the work failed to observe any mildew development from two batches of commercial Elsanta runners and module (tray) plants of Albion and Elsanta.

Alternative products

Several alternative products were evaluated together with a commercial fungicide on Albion under tunnels. There were a few alternatives that showed good controlling effects against mildew and these will be further evaluated next year.

Powdery mildew forecasting

The development of a prototype computerised system for predicting risks of mildew infections was completed. This model has been evaluated at one Kent site between June 2009 and September 2009. During this period, the model-managed tunnel received only three sprays, compared to many more in the conventional tunnel. However, mildew failed to establish on all plots despite the fact that Elsanta is said to be highly susceptible. It is therefore not possible to attribute whether the lack of powdery mildew is due to the lack of inoculum, control success, or cultivar resistance.

Botrytis

Latent infection in planting material

Ten samples of 50 cold-stored cv. Elsanta plants (six of A+ and four of medium waiting bed), from six propagators were assessed for the incidence of latent *Botrytis cinerea*. Internal infection by *B. cinerea* was detected in the crowns of nine samples, at levels ranging from 2% to 8%. Eight out of ten samples had *B. cinerea* in trimmed petioles, with infection in 5% to 50% of plants having a petiole stub. Incubation of plants without disinfection showed that *B. cinerea* was present in between 7% to 78% of plants within a sample. Overall, a mean 27% of A+ plants were infected with latent *B. cinerea* and 11% of waiting bed plants. However, samples with an average of 15% plants infected occurred in material from both propagation methods.

Fungicide applications to both A+ and waiting bed plants were principally directed at powdery mildew control, but some products also had activity against botrytis. Rovral, a fungicide used against Botrytis in strawberries, was also used by three propagators. There was no relationship between the use of fungicides and Botrytis incidence.

Control of fruit infection using Binab (Trichoderma spp.)

Work was not carried out on this task in 2009.

The control of latent infection in planting material

Plants of cold-stored A+ cv. Elsanta were given hot water treatment (35°C for 7 minutes, followed by 50°C for 2 minutes) pre-planting. *B. cinerea* in crowns and leaves was not controlled by this treatment; 36% of hot water treated and the same incidence of untreated plants had latent *B. cinerea.*

Five fungicides (Cercobin WG, Rovral WG, Scala, Signum and Switch) and one biocontrol agent (Serenade ASO) were used as a single application to plants with newly emerged leaves. After 21 days there was no difference in the proportion of these leaves with *B. cinerea* between the control (65%) and treated, although there was an indication of a reduction following the Cercobin WG drench (10% of leaves affected). Hot water treatment caused plant stunting, but no phytotoxicity was seen in leaves, flowers or fruit following the chemical applications.

Latent flower infection by Botrytis

A further batch of flower samples were assessed on Elsanta to determine latent flower infections, which is used to validate predictions given by Botem. In contrast to the last year, a considerable level of Botrytis infections were observed, which were consistent with the predictions given by Botem. Furthermore, a trial was conducted to determine whether latent flower/fruit infection differed significantly among three treatments on Elsanta: untreated, model-managed and conventional. Only a single round of Botrytis specific fungicide was applied in both model-managed and conventional tunnels. There were few differences in the incidence of latent fruit rots among the three treatments: the incidence of latent rots is less than 3% for all three treatments. This suggests that the incidence of latent fruit rots is too low under protection to justify fungicide applications that are specifically targeted at Botrytis.

Biocontrol of Botrytis

Experiments have been conducted to investigate where combinations of commercially available biological control agents (BCAs) might control *B. cinerea* on strawberry leaves more effectively than individual BCAs, focusing on the persistence of biocontrol activities, spread of BCAs among leaves and biocontrol efficacy in relation to application regimes: mixed versus single BCA, pre- versus post-inoculation application and sequential versus simultaneous application. [This was jointly funded by this Hort LINK project and a CRD project]. Three BCA products (Sentinel, Serenade and Trianum) were used for this study. Overall, Serenade did not significantly reduce sporulation of *B. cinerea* on strawberry leaf discs whereas Sentinel and Trianum gave a similar and significant biocontrol efficacy.

Biocontrol efficacy remained almost unchanged ten days after application at 20/20°C (day/night) or 24/16°C temperature regimes. In contrast, reduced biocontrol efficacy at 26/14°C suggests BCA survival was reduced under these conditions. Incidence of *B. cinerea* sporulation on leaf discs was c. 60% higher on leaves that emerged after the BCA application than on leaves directly exposed to BCA, indicating insufficient amount of the BCA had managed to spread to new leaves. Combinations of BCAs, whether applied simultaneously or sequentially (48 h apart), did not improve disease control over the most effective BCA within the combination applied alone. This indicated possible antagonism or interference between the BCAs. Results suggested that there was significant antagonism for most combinations of the three BCAs tested and the degree of antagonism increased as the time from BCA application to pathogen introduction lengthened.

Pesticide dissipation

An experiment was carried out to determine whether fungicide dissipation differs between protected and open-field conditions, and hence their persistence under protection. Systhane EW20 (myclobutanil) and Rovral FLO (iprodione) were applied at the full rate and sprayed until run-off with a hand-held sprayer. Leaves were sampled to estimate residues on two occasions: immediately and eight days after the spray. Results suggested that:

1. Iprodione is more persistent on strawberry leaves than myclobutanil.
2. Even without rainfall, fungicide dissipation is greater for those plants in open field than those under protection, especially for myclobutanil.
3. Only rainfall close to the time of fungicide application may lead to considerable wash-off of residues.

Black spot

Molecular comparison of black spot isolates

DNA was extracted from 186 isolates, most from apple (102), strawberry (52) and cherry (23), and a few (5) from weeds. All 186 isolates were screened for the six SRR primers, developed in Year 1 at EMR. Preliminary analysis of molecular data suggested 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. Overall, the isolate differences are more related to site isolates rather than to host differences.

European tarnished plant bug

Experiments were conducted to quantify the relative attractiveness of candidate herbaceous flowering plants and cover crops to the European tarnished plant bug (*Lygus rugulipennis*). Numbers of *L. ruguipennis* peaked in August and September in the trials and had begun to decline by October when overwintering adults were emerging. The capsid was attracted to mayweed, sweet alyssum and common vetch compared to strawberry and lucerne in an unprotected trap crop trial on a commercial site. *Lygus* nymphs were highly attracted to sweet alyssum. Earwigs were more likely to be present on strawberry than the trap crop species. In an experiment at EMR the three plant/plant mixes used, flowered at different times, so it was not possible to make a direct comparison of relative attractiveness on particular days. However, in season totals, lower numbers of *Lygus* adults were recorded on the plant mix of corn chamomile, corn marigold and cornflower than on mayweed and vetch.

In the lucerne experiment under protection at the commercial site, *Lygocoris pabulinus* was the most common capsid sampled on strawberry and lucerne. Although there were not high enough numbers of invertebrates to analyse statistically, it was evident that there was very little difference between the numbers of invertebrate taxa on lucerne compared to strawberry, including *L. pabulinus* .

At the protected strawberry bug vac site at least double the numbers of *L. rugulipennis*, spiders and earwigs were found in strawberry compared to lucerne. There was a significant reduction in male *L. rugulipennis* and *Lygus* nymphs on strawberry after bug vaccing. *Lygus* numbers were reduced by 39-61% on strawberry and 2-56% on lucerne. Beneficial insects were not significantly affected by the bug vac.

Four experiments were conducted at EMR to investigate the reported repellancy of hexyl butyrate to *L. rugulipennis*. Dispensers with hexyl butyrate release rates ranging from 0.2-37 mg/day were used in these experiments. There was no significant effect of hexyl butyrate on *L. rugulipennis* distribution at the rates emitted from these dispensers when compared with the controls. There was also no effect on the proportions of adult male and female *L. rugulipennis* in samples from treated plots.

Aphids

In the second year of an experiment in a commercial plantation to assess the attractiveness of different plants to predators and parasitoids of aphids, very low numbers of arthropods were collected from red campion and ox eye daisy. There was therefore no evidence of attractiveness of these plants to predators and parasitoids.

In an experiment sown in 2009 at EMR, significantly higher season totals of anthocorid and coccinellid adults (ladybirds) were found on vetch, while highest numbers of coccinellid larvae were found on the flower mix of corn chamomile, corn marigold and cornflower. These results may in part be due to a combination of plant flowering period and the developmental stage of the predator present at that time. Highest numbers of chrysopid larvae (lacewing) were found on mayweed. There were no significant differences in numbers of anthocorid nymphs or Orius nymphs and adults on the three treatments.

In experiments to assess the attractiveness of plant volatiles to predators and parasitoids, low numbers of beneficial species were caught in water traps containing lures of various plant volatiles in May and June. There was no evidence to suggest that any of the volatiles were attractive at the rates used at this time of year. In an experiment undertaken in August, higher numbers of hoverflies were caught in traps containing lures of germacrene or phenyl ethanol, but there was no effect of any of the volatiles tested at the rates of release used on any other beneficial species.

A large scale, randomised block experiment to test autumn sprays of Calypso to control *Chaetosiphon fragaefolii* (strawberry aphid) the following spring was undertaken. Autumn sprays (October-November 2008) of Calypso significantly reduced the numbers of aphids present on the strawberry leaves the following spring (April 2009). A spray at the beginning of October was more effective than an application in mid October or early November.

In order to determine the potential of a parasitoid for control of *C. fragaefolii*, parasitised aphids were collected from organic strawberry crops in Herefordshire in May 2009. Cultures are in place at BCP Certis and EMR. At 17ºC min: 27 ºC max mummies were observed nine days after parasitoid oviposition and adults emerged after a further six days (egg to adult 15 days). Initial tests show that *Aphidius. eglantariae* is fairly specific to *C. fragaefolii* and does not attack the other main aphid species commonly found on strawberry.

Strawberry blossom weevil super trap

Two replicated field experiments to test PV2 with other synomones (TMTT and caryophylene) and different trap designs were conducted.
Numbers of strawberry blossom weevil captured in the UK experiments were very small and not suitable for statistical analyses. General trends in the data were;

- Increasing catch of strawberry blossom weevil with increasing PV2 release rate.
- No increased attraction of strawberry blossom weevil with caryophylene nor TMTT synergism with the aggregation pheromone lure.
- Greater numbers of non-target arthropods being captured with increasing release rate of PV2.
- The traps with cross vanes coated with fluon captured more strawberry blossom weevil than the other designs.
- The addition of an excluder grid reduced the number of non-target arthropods captured.
- Green and yellow cross vane traps captured fewer non-target arthropods.

This work, together with the previous years work, suggests that the 'supertrap' to be evaluated in the remaining years of the project should incorporate a white cross vane funnel trap (coated with fluon), with an excluder grid, a standard aggregation pheromone lure and a high release rate PV2 lure.

Financial benefits

Botrytis, mildew, black spot, aphids, blossom weevil and capsid bugs are very common problems wherever and however strawberries are grown in the UK. A very high percentage of strawberry plantations are infected by these pests and diseases. No quantitative data on losses is available but conservatively assuming 10% of the crop is lost as a result of these infestations, this is equivalent to 5,074 tonnes of strawberries, worth £21 million.

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

Action points for growers

- The Botem model gives accurate predictions of Botrytis risk under tunnels and could be used in future for management of this disease.

- Monitor crops for the development of visible symptoms from latent Botrytis which can be introduced into new plantings by both A+ and Waiting Bed cold stored strawberry runners.

- A clean up spray of an aphicide in October or early November, after cropping has ceased, greatly reduces aphid populations the following spring and may obviate the need for spring treatment with aphicides and reduce the risk of residues.

- Use of tractor mounted bug vaccing shows promise for control of European tarnished plant bug (ETPB). Each pass of the bug vac results in a 50% reduction in ETPB numbers, several passes being needed during the period of risks (July – September) for good control. Correct setting of the height of the bug vac is important for good results.

- Potassium bicarbonate plus the adjuvant Silwet showed promise for control of strawberry mildew.

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

Methods

In order to examine whether there are viable mildew on cold stored runners that could act as initial inoculum after planting, we ordered two batches of Elsanta runners in May-June 2009, 80 from each of two producers. Then we potted them up and maintained each batch in separate controlled environment (CE) cabinets at 15°C. These plants were regularly examined for the presence of powdery mildew on young leaves.

Results

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

1.1.2 Module plants

Methods

As in 2008, we obtained two batches of module (tray) plants, 80 for Elsanta and 80 for Albion, to examine whether there was viable mildew in module plants that could act as initial inoculum after planting. Upon delivery, these plants were immediately placed into a CE cabinet at 15°C for one month for monitoring.

Results

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

1.1.3 Survival of mildew on runners in a cold store

Methods

In the autumn-winter 2008, we obtained ~ 350 runners infected with powdery mildew; these runners were harvested from plants maintained in a glasshouse compartment at EMR. These runners were stored at -2°C on 20 October 2008. Unfortunately, the runners were dead in February, probably because they were not as dormant as those runners lifted in the field. So this year, we have potted many runners (with mildew) in the glasshouse and will move these potted runners outside before lifting them into cold-store. In this way, we hope to maintain runners' viability until early spring when they will be potted out to examine whether mildew has survived on these plants in cold-store.

Results

This study is still ongoing.

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

This task will start in Year 3.

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

Methods

Experiments were conducted on strawberry plants under protection (DM182 EMR): three beds (each with double rows of plants) of cv. Elsanta and three beds of cv. Albion; total area is 40 m x 12 m. Runners were planted out on 27 April 2008 and covered three weeks later. Each bed has 230 plants (i.e. 115 in each double row of the bed). These plants were mown off in mid-July to ensure that mildew was well established on the re-growth before the first spray was applied on 24 August. A further two sprays were applied on 3 and 11 September 2009.

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 (9 ml/L) and Systhane EW20 (115 □l/L) and

untreated. Thus for each treatment, there were three replicate plots for each cultivar. 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 the five youngest fully unrolled leaves on each of nine plants in the central plot on 2 October 2009. Mildew was only assessed on cv. Albion because little mildew developed on cv. Elsanta.

A generalised non-linear mixed model was used to analyse data, where block is treated as a random factor and products treated as fixed. In this analysis the number of leaflets with mildew per plot is assumed to be binomially distributed. Comparison of individual treatments was based on pairwise standard errors of differences estimated from the mixed model analysis. In the second analysis, all the data was analysed together via analysis of variance of repeated measurement; logit of infected fruit per plot is used in this analysis.

Results

Overall results are given in Fig 1.3.1. Severe mildew epidemics developed on cv. Albion. Nearly 81% of leaflets had powdery mildew symptoms on the untreated plants. The mixed model analysis showed the significant ($P < 0.001$) differences among the 11 treatments. The incidence on leaflets in the ten treatments ranged from 30% of Potassium bicarbonate (+Silwet) to Serenade (74%). Three products did not result in significant reductions in the mildew incidence: Serenade, Garshield and Chitoplant. Of the remaining products, Potassium bicarbonate (+ silwet) had the lowest incidence (30%), significantly less than all other treatments. The remaining six treatments (apart from Farmfos – 66%), including Systhane, had a similar level of mildew control efficacy.

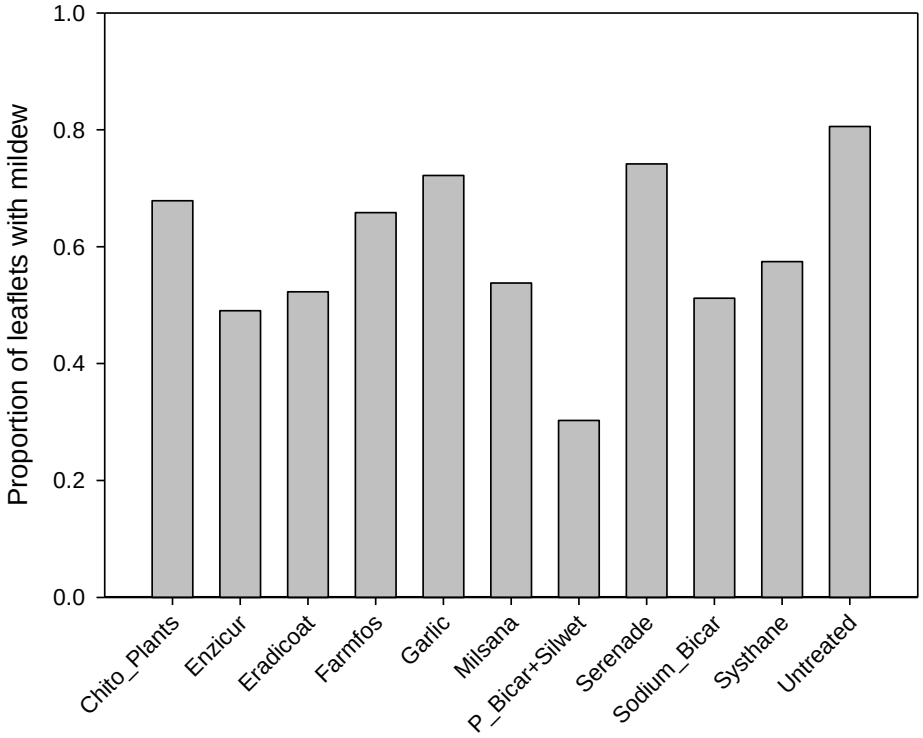


Figure 1.3.1 Overall incidence of leaflets with powdery mildew on cv. Albion for all eleven treatments.

1.3.2 Survival of biocontrol agents

We have conducted a larger trial than previously planned in conjunction with a CRD-funded project at EMR to investigate the persistence (survival) and spread of biocontrol agents (BCA) on strawberry leaves. Here we only give the summary of the work since the detailed results have just been accepted for publication by Biocontrol Science & Technology (Using combinations of biocontrol agents to control *Botrytis cinerea* on strawberry leaves under fluctuating temperatures by Xiangming Xu, Joyce Robinson, Mike Jeger, Peter Jeffries).

Experiments were conducted with *Botrytis cinerea* on strawberry leaves to investigate where combinations of commercially available products might control *B. cinerea* more effectively than individual BCAs. Specifically, we studied the persistence of biocontrol activities, spread of BCAs among leaves, and biocontrol efficacy in relation to application regimes: mixed versus single BCA, pre- versus post-inoculation application, and sequential versus simultaneous application. Three BCA products (Sentinel, Serenade and Trianum) were used for this study. Overall, Serenade did not significantly reduce sporulation of *B. cinerea* on strawberry leaf discs whereas Sentinel and Trianum gave a similar and significant biocontrol efficacy (average *c.* 60-70%). Biocontrol efficacy remained almost unchanged 10 days after

application at 20/20°C (day/night) or 24/16°C temperature regimes. In contrast, reduced biocontrol efficacy at 26/14°C suggests BCA survival was reduced under these conditions. Incidence of *B. cinerea* sporulation on leaf discs was c. 60% higher on leaves that emerged after the BCA application than on leaves directly exposed to BCA, indicating insufficient amount of the BCA had managed to spread to new leaves.

Combinations of BCAs, whether applied simultaneously or sequentially (48 h apart), did not improve disease control over the most effective BCA within the combination when applied alone. This indicated possible antagonism or interference between the BCAs. Results suggested that there was significant antagonism for most combinations of the three BCAs tested and the degree of antagonism increased as the time from BCA application to pathogen introduction lengthened.

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 carried out to determine whether fungicide dissipation differs 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 until run-off with a hand-held sprayer to 20 cv. Elsanta plants kept in a glasshouse compartment. Ten were then randomly chosen and placed inside a polytunnel and the other 10 placed outside. Plants were regularly watered from the pot base.

On the day of spraying (23/09/2009), a single fully unrolled leaf was randomly cut off from each plant, giving five composite samples [each with two leaves] for each condition [outside + tunnel]. Total leaf area was measured for each sample before being sent to QTS for quantification of residues. A second batch of samples was similarly taken on 01/10/2009 and sent for quantification of residues. Since there had been no rainfall since 23/09/09 (spray date), outside plants were sprayed to runoff with a hand-held sprayer after the second samples were taken. When leaves had dried, a further five samples were taken from the outside 10 plants and sent for quantification of residues.

Results

There was no rain between the two sampling times; for most days it was sunny. Figure 1.3.2 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 for iprodione (8.26 □g cm^{-2}) was more than 45 times of that for myclobutanil (0.18 □g cm^{-2}).

Overall, there were significant reductions in iprodione residues on leaves between day 0 and 8: 10.62 □g cm^{-2} versus 6.54 □g cm^{-2}, a reduction of 38%. Overall iprodione residues on leaves of strawberry plants inside the tunnel (9.53 □g cm^{-2}) were higher than that of the outside plants (7.63□□g cm^{-2}); this difference was nearly statistically significant (*P* = 0.075). This difference resulted primarily from the greater reduction in residues for outside plants than for inside (Fig. 1.3.2): 49% versus 29%.

Overall, there were significant reductions in myclobutanil residues on leaves between day 0 and 8: 0.32 □g cm^{-2} versus 0.08 □g cm^{-2}, a reduction of 75%. Overall myclobutanil residues on leaves of strawberry plants inside the tunnel (0.22 □g cm^{-2}) were higher than that of the outside plants (0.18□□g cm^{-2}); this difference was just statistically significant (*P* = 0.049). This difference resulted primarily from the greater reduction in residues for outside plants than for inside (Fig. 1.3.2): 87% versus 66%.

Spraying leaves with water to mimic rainfall until run-off on day 8 did not significantly affect the level of both iprodine and myclobutanil.

Conclusions

Several preliminary conclusions can be drawn regarding the dissipation of fungicides:

4. Iprodione is more persistent on strawberry leaves than myclobutanil;
5. Even without rainfall, fungicide dissipation is greater for those plants in open field than those under protection, especially for myclobutanil;
6. Rainfall that occurred 8 days after the application did not lead to appreciable wash-off of of residues.

Further experiments will be conducted in Year 3 to confirm whether these conclusions were true.

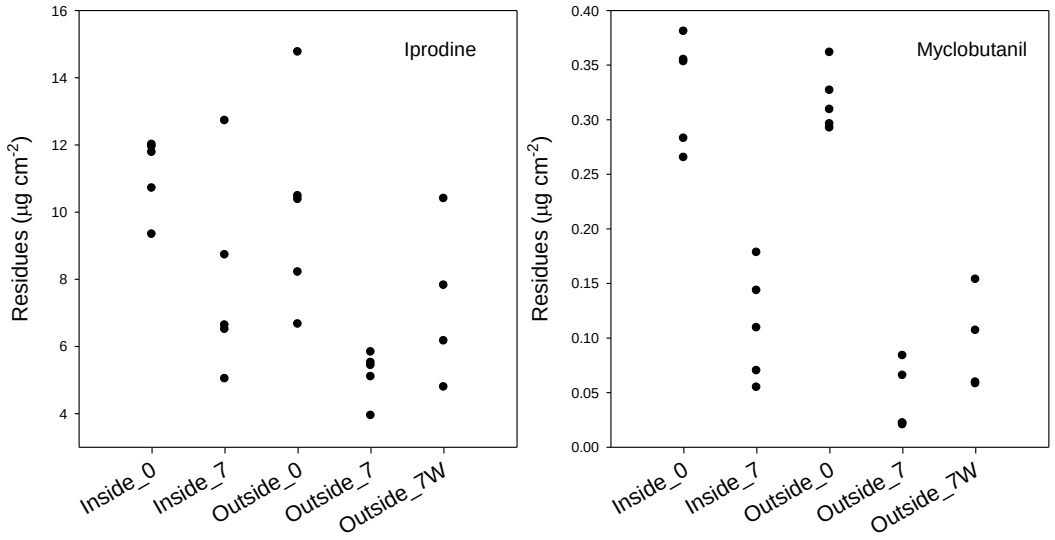


Figure 1.3.2 Scatter plot of residues of iprodione and myclobutanil on strawberry leaves sampled immediately after spray deposits were dried (day 0) and on day 7. X-axis label: Inside – plants were placed inside a polythene tunnel, Outside - plants were placed outside, 0 (7) – sampling day 0 or 7, W – on day 7 leaves were sprayed with water to run-off and leaves were sampled after water dried.

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

Methods

We have computerised the CSL-UOH mildew model that predicts mildew infection and subsequent development prior to visual symptoms. This system was evaluated at Goose Farm, Shalloak Rd, Broad Oak, Canterbury, Kent CT2 on tabletop everbearer strawberries (cv. Elsinore) under protection. There were four tunnels allocated to this study: two for conventional spray and two for model-based management. It was not possible to use unsprayed tunnels since the farm manager feared that this may create an unacceptable risk of powdery mildew in other commercial tunnels. The farm manager informed us that this cultivar is highly susceptible to mildew. Three data (temperature & humidity) loggers were installed in the tunnels to monitor in-tunnel temperature and humidity. Data were regularly downloaded to generate model predictions. There were two treatments

1. **Model managed:** sprays for mildew only applied when a predicted infection event occurred (EMR was responsible for running the model and interpreting the model predictions). **Tunnels 1-2 were allocated for this treatment.**

2. **Conventional programme: s**tandard farm programme as applied to the rest of the strawberry crop. **Tunnels 3-4 were allocated for this treatment.**

Non-mildew fungicides/nutrients were applied as normal in all four tunnels. The experiments started from 1 June onwards and prior to this time all tunnels were subjected to the same current management programme. Fungicides for mildew control were applied as normal for the Conventional Treatment tunnels. Mildew development on leaves and fruit was assessed every two weeks.

Results

This study lasted from early June to late September. During the entire period, the model managed tunnels only received three sprays – late June, mid July and early August. The conventional programme also received three sprays, albeit at different times. Although an infection risk was predicted in September, we decided against spraying because of the late season and lack of mildew symptoms in the tunnels.

Unfortunately, there was no mildew development in all tunnels. Hence it is difficult to establish whether the model management was successful in controlling mildew or there was no inoculum at the site. But it is certain that plants were over-sprayed in conventional tunnels at this site.

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

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 *Botrytis 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*. Current information on the incidence of latent *B. cinerea* in strawberry planting material received by UK strawberry growers is based on five samples of A+ of the cv. Elsanta assessed from

different propagators in 2008 as part of SF 94.

The objective of this work was to further determine the occurrence of *B. cinerea* in strawberry runners as the first stage in seeking to reduce initial botrytis inoculum at planting. In addition, information on the incidence of latent *B. cinerea* found in samples was examined in relation to production techniques and husbandry in propagation fields, including fungicide use. Ensuring that *B. cinerea*-free strawberry runners are planted could reduce fungicide requirements in the crop.

In 2008, *Colletotrichum* spp. were recorded by isolation from crowns of three of the samples, although *Colletotrichum acutatum* (black spot) was not confirmed by PCR testing of tissue from the same crowns. Material from propagators was checked for *Colletotrichum* spp. in 2009 to confirm whether this previously notifiable pathogen can occur as a latent infection in strawberry crowns.

Methods

Sample details

Samples of 60 cold-stored strawberry plants cv. Elsanta were either obtained from growers, or purchased directly from propagators (Table 2.1.1.1). Plants were transported by courier and cold-stored overnight at 4°C so that processing could be completed in a single day. Six samples of A+ and five samples of waiting bed material were obtained from six propagators, with four of the propagators supplying both types of plant. Sub-samples of 50 plants were taken for testing. The name of each propagator was recorded, but plant material and information was supplied with the agreement that the source would not be detailed in this report. Although there was replication of plants within each propagator's sample, material was obtained from a single batch per source.

Results were examined by analysis of variance to determine if mean levels in A+ runners and waiting bed plants differed significantly at $P<0.05$.

Table 2.1.1.1: Sampling details of cv. Elsanta cold-stored strawberry plants, A+ or waiting bed grown in 2008 for planting in 2009.

Sample number	Plant material	Propagator code	Received direct	Date processed
BX09/42	A+	PG4	No	20.05.09
BX09/47	A+	PG6	No	02.06.09
BX09/57	A+	PG7	Yes	18.06.09
BX09/58	Waiting bed	PG7	Yes	19.06.09
BX09/65	A+	PG8	Yes	25.06.09
BX09/66	Waiting bed	PG8	Yes	25.06.09
BX09/68	A+	PG9	Yes	26.06.09
BX09/69	Waiting bed	PG9	Yes	26.06.09
BX09/74	Waiting bed	PG4	No	13.06.09
BX09/99	A+	PG5	No	06.04.09

Tests for latent *B. cinerea* and *C. acutatum*

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 disinfection 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 folded young leaves and root bases, green leaves, a sample of roots, and senescing or rotting leaves and petioles.

Some plants had green petiole stubs from which the leaf blade had been trimmed prior to cold-storage. Up to six of these stubs were sampled per plant. The petiole ends were removed and surface disinfected in 10% by volume “Domestos” (to give approximately 0.5% active chlorine) for 5 minutes and rinsed before plating onto agar with the plant identification recorded. The ends were not re-trimmed before plating, except where there was rotted tissue which could have contained secondary infection, and this was removed to leave a leading edge of the rot. Plates were incubated at 20°C, with exposure to near-UV light to encourage sporulation. Totally necrotic petioles were incubated in the damp chamber.

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 disinfected (as above), rinsed in sterile distilled water and placed onto Potato Dextrose Agar (PDA).

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* and *Colletotrichum* spp. 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. Petiole and crown plates and damp chamber results were collated for each plant to determine the total number of plants which had botrytis recorded from any tissue source.

No PCR testing for the presence of *B. cinerea* DNA was carried out in 2009. Lateral flow devices were utilised to confirm the presence of botrytis on a selection of colony types, particularly where there was no sporulation. 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. Subcultures on PDA were taken from some samples to confirm the presence of *B. cinerea.*

Results

Botrytis in petiole stubs

The number of plants per sample with a petiole stub present varied from 11 to 28, usually with just one stub per plant. A+ and waiting bed plants had similar mean numbers of plants with petiole stubs per sample, 22 and 20 respectively (Table 2.1.1.2). Some stubs were mainly green while others had started to develop a brown rot down to about 10 mm from the cut end. Results from the petioles do not include any that were totally rotted - these were included in the unsterilised damp chamber results.

The percentage of plants with *B. cinerea* infected petioles was calculated as both the percentage of the total plants in the sample (i.e. the risk to the grower of introducing botrytis to the field) and as the percentage of plants with petiole stubs present. Where petiole botrytis occurred, it was present in between 2% and 16% of the total plant sample. Expressed as a proportion of the plants with petiole stubs present, samples BX09/42 and BX09/99 had 31% and 50% infection, respectively. These were two of four samples received via growers rather

than directly from propagators. For the other eight samples, their ranking of petiole infection did not differ between either method of showing the incidence of petiole infection.

The A+ plants had a mean 7.3% of total plants with petiole botrytis, nearly three times that (2.5%) of the waiting bed plants, although not significantly different. Three of the samples (BX09/65 waiting bed, BX09/68 A+ and BX09/74 A+) had no botrytis inside their petiole ends, although each had a smaller proportion of plants with petioles. Of the other A+ samples, BX09/42 and BX09/99 had the highest proportion (mean 14% and 16%) of total plants with petiole botrytis, while BX09/47 and BX09/57 had lower levels (6% and 8%). Of the other waiting bed samples, BX09/58, BX09/66 and BX09/69 each had around 3% of plants with petiole botrytis.

Table 2.1.1.2: Recovery of *Botrytis cinerea* from within trimmed petiole stubs of 50 strawberry plants ex cold-store. The % infection of petiole stubs is shown both as a proportion of total number of plants tested and of the number of plants from which petiole stubs were available.

Sample and type	No. of plants with petiole botrytis after 14 days	No. of plants with petiole botrytis after 21 days	Total no. plants (of 50) with petiole botrytis	% total plants with botrytis in petiole	No. of plants with petiole stubs present	% of plants with botrytis of those with petiole stubs
A+ runners						
BX09/42	8	0	8	16	26	31
BX09/47	2	4	4	8	28	14
BX09/57	3	3	3	6	24	13
BX09/65	0	0	0	0	17	0
BX09/68	0	0	0	0	21	0
BX09/99	7	*	7	14	14	50
Mean	3.3	1.4	3.7	7.3	21.7	18.0
Waiting bed runners						
BX09/58	2	2	2	4	22	9
BX09/66	1	2	2	4	28	7
BX09/69	1	1	1	2	20	5
BX09/74	0	0	0	0	11	0
Mean	1.0	1.3	1.3	2.5	20.3	5.3
Anova						
Lsd	4.12	2.44	4.08	8.16	9.02	23.10
P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

* overgrown and not assessed. Anova of means of A+ and waiting bed runners

Latent botrytis in crowns

For the majority of plants where *B. cinerea* was detected in crowns, only one section of the six pieces was found infected. This suggests a highly localised presence of mycelium within the crowns (within a diameter range from about 15 to 25 mm across).

B. cinerea was detected in the crowns of all samples except one. The A+ samples had a mean of 7.7% of crowns infected by *B. cinerea*, compared with 4.5% in waiting bed plants (no significant difference) (Table 2.1.1.3). The worst infected A+ material (BX09/42) had 18% of crowns infected, and the best (BX09/68) had 2% of crowns infected. Of the waiting bed samples, BX09/74 was the worst infected (10% infection) while BX09/58 had zero infection.

Table 2.1.1.3: Recovery of latent *B. cinerea* from within 50 crowns per sample.

Sample and type	No. of crowns with botrytis at 14 days	No. of crowns with botrytis at 21 days	Total no. crowns with botrytis	% crowns with botrytis
A+ runners				
BX09/42	8	7	9	18
BX09/47	3	2	3	6
BX09/57	4	1	4	8
BX09/65	0	3	3	6
BX09/68	0	1	1	2
BX09/99	3	*	3	6
Mean	3.0	2.8	3.8	7.7
Waiting bed runners				
BX09/58	0	0	0	0
BX09/66	1	2	2	4
BX09/69	1	2	2	4
BX09/74	5	3	5	10
Mean	1.8	1.8	2.3	4.5
Anova				
Lsd	4.03	3.14	3.71	7.41
P	n.s.	n.s.	n.s.	n.s.

* Crowns not assessed at 21 days. Anova of means of A+ and waiting bed runners

Botrytis in damp chambered plants

B. cinerea in damp chambers (Table 2.1.1.4) could have developed from mycelium or ungerminated spores or sclerotia on the tissue surface or mycelium inside the plants. A greater proportion of A+ samples were infected (mean 18%) than waiting bed plants (mean

4%), but this was not significantly different. The majority of samples had between one and three plants with botrytis out of the 50. The exceptions to this were in the A+ samples with the absence of botrytis in BX09/47, and infection levels of 18% and 78% of damp chambered plants (in BX09/99 and BX09/42, respectively).

Some roots, crowns with unfolded leaves, and mature leaves were found to contain botrytis (Table 2.1.1.5), although the rotted leaves and petioles were most frequently infected, and two A+ samples (BX09/99 and BX09/42) had a high percentage of plants infected because of botrytis infection of this tissue. However, there was no significant difference between the mean proportion of plants with rotted leaves for A+ (8.5%) and waiting bed (1.0%) runners.

Table 2.1.1.4: Recovery of *B. cinerea* in damp chambers and proportion of plants with *B. cinerea*

Sample and type	No. of damp chamber plants with botrytis *	Proportion of plants infected in any test (damp chamber, petiole or crown)	
		Number (of 50)	%
A+ runners			
BX09/42	39	42	84
BX09/47	0	7	14
BX09/57	1	8	16
BX09/65	3	6	12
BX09/68	2	3	6
BX09/99	9	15	30
Mean	9.0	13.5	27.0
Waiting bed runners			
BX09/58	1	3	6
BX09/66	3	7	14
BX09/69	1	4	8
BX09/74	3	8	16
Mean	2.0	5.5	11.0
Anova			
Lsd	17.72	17.22	34.44
P	n.s.	n.s.	n.s.

* Damp chambers assessed at 21 days only. Anova of means of A+ and waiting bed runners

Table 2.1.1.5: Number of strawberry plants (out of 50) infected by *Botrytis cinerea* and the tissue locations where *B. cinerea* was found after 21 days damp incubation of unsterilised tissue

	% of plants with *B. cinerea* developing from:			
Sample	Roots	Crown	Fresh mature leaves	Rotten Leaves/ petioles
A+ runners				
BX09/42	4	3	1	38
BX09/47	0	0	0	0
BX09/57	0	0	0	1
BX09/65	1	0	0	2
BX09/68	1	0	0	1
BX09/99	0	0	2	9
Mean	1	0.5	0.5	8.5
Waiting bed runners				
BX09/58	0	0	0	1
BX09/66	0	0	2	1
BX09/69	1	0	0	1
BX09/74	1	0	0	1
Mean	0.5	0.0	0.5	1.0
Anova				
Lsd	1.9	1.4	1.3	17.4
P	n.s.	n.s.	n.s.	n.s.

Anova of means of A+ and waiting bed runners

Incidence of total botrytis in plants

Data were examined to determine the total number of plants with *B. cinerea* in any of the three tests (crown, petiole or the remainder of the plant in the damp chamber). The sample with the highest incidence of botrytis in plants, 84%, (Table 2.1.1.4), and consistently the highest percentages across the tissue types, was BX09/42, an A+ sample from the Netherlands. The next highest (BX09/99) was 30%. Five samples from both A+ and waiting bed samples had 12-16% of plants with botrytis. Overall, there was no significant difference between A+ and waiting bed runners in the mean percentage of plants with botrytis. The lowest incidences of botrytis were from different propagators, with A+ BX09/68 and waiting bed BX09/58 both having 6% of plants infected.

Comparison of A+ and waiting bed material from the same propagators

Four propagators (coded PG4, PG7, PG8 and PG9) (Figure 2.1.1.1) provided both A+ and waiting bed material of cv. Elsanta from runners that would have been produced in spring 2008 and lifted in the winter before cold-storage until summer 2009.

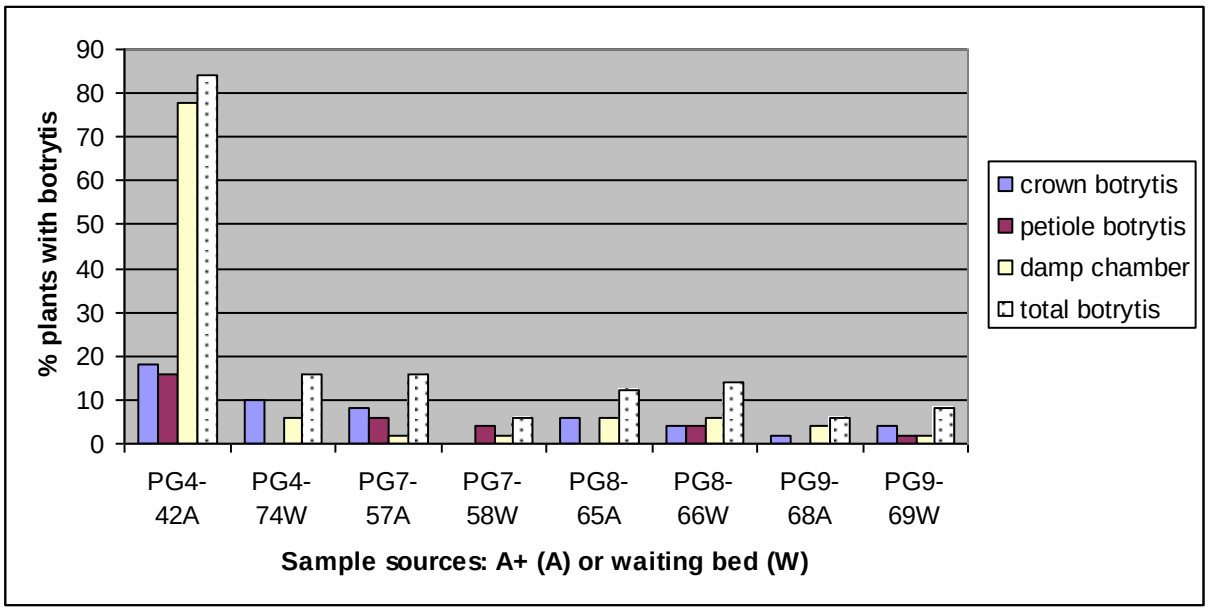


Figure 2.1.1.1: Cold-stored strawberry planting material 2009 from four propagators (PG4, PG7, PG8 and PG9). % of A+ or waiting bed plants having crown or petiole botrytis, % of plants with botrytis in damp chambers, and the % of plants with botrytis within any tissue.

From two suppliers, overall levels of infection in A+ and waiting bed plants were similar. From suppliers PG4 and PG7, levels were much greater and slightly greater, respectively, in A+ than waiting bed plants. Considering crown infection, material from propagator code PG4 had the highest percentages containing latent botrytis for A+ (A) and waiting bed (W) plants, with 18% and 10% respectively. Their A+ plants had one of highest proportions of plants with botrytis in petioles (16%), but the waiting bed plants had zero petiole botrytis. Damp chambered material contributed a high proportion of the positive botrytis records for A+ plants from PG4, but little in their waiting bed plants. Overall, there were more A+ PG4 plants infected by botrytis than waiting bed (84% and 16%, respectively). Information on fungicide application was provided by the propagator for their waiting beds, and fungicides giving control of botrytis were applied soon after transplanting and then again just before lifting. Propagator code PG7 had zero botrytis in the crowns of the waiting bed sample, but 8% of A+ crowns had botrytis. Petioles were similarly infected in the waiting bed (4%) and A+ material (6%), with 2% of plants having botrytis in each of the damp chambers. Overall, there were more A+ plants with botrytis (16%) than waiting bed plants (6%) from this propagator.

Propagator codes PG8 and PG9 each had similar botrytis incidences in their A+ and waiting bed material crowns and damp chambers (between 2% and 6%). Both had low incidences of petiole botrytis in the waiting bed material (4% in PG8 and 2% in PG9) and zero botrytis for

this tissue in the A+ planting material. Overall, % plant infection was similar for both A+ and waiting bed plants for these propagators (a mean of 13% for PG8 and 7% for PG9).

Infection of crowns by *C. acutatum* and other fungi

Colletotrichum spp. was not isolated from crowns or petioles of any sample, although an unidentified fungus with a similar appearance on the agar (salmon pink spore bodies) was found. This fungus had pycnidia rather than the acervuli produced by *Colletotrichum* spp., and the spore shape differed from that of *Colletotrichum* spp. A wide range of fungi were isolated from the surface sterilised crown sections including *Fusarium* spp. *Trichoderma* spp., a pycnidial fungus, and other unidentified species. All of the crowns in all of the samples contained one or more of these fungi. The majority of petioles were also infected by other fungi in addition to any infection by *B. cinerea*.

Information on crop husbandry and fungicide use

An example of a UK crop of A+ cv. Elsanta in early August 2009 is shown in Figure 2.1.1.2. Mother plant rows were 91 cm apart with 46 cm spacing in the row. The crop had good runner production, with about 13 stolons per mother and already five plants growing per stolon. The youngest runners were rooting a metre from the mother plant. Runner production varies between years depending on the growing conditions. In 2008, with low light levels and cool summer temperatures, only about nine runners were produced per mother plant.

Figure 2.1.1.2: Cv. Elsanta mother plant (centre) with stolons and runners in early

August 2009, UK. Leaf production had been reduced by poor weather.

Plants for waiting beds are grown on mother plants (Figure 2.1.1.3) until the three leaf stage and then lifted in late July or early August for transplanting at low density (Figure 2.1.1.4) for growing-on until November/December. Plants may need to be covered with fleece to protect them if a hard frost is forecast. The thinner canopy of waiting bed plants makes them more susceptible to frost than the A+ crop.

Figure 2.1.1.3: Source of runners for waiting beds (photo S. Walpole)

Figure 2.1.1.4: **Waiting beds in Germany in August 2009 (photo S. Walpole)**

Plants from the propagator (PG4) of waiting bed sample BX08/74 (16% of plants with botrytis) were planted in late July 2008 and given three applications of Paraat (dimethomorph) against *Phytophthora* spp. After about 6 weeks from planting the use of Rovral (iprodione) in a tank-mix with Captan (captan) would control *B. cinerea,* with the Captan able to control powdery mildew (Table 2.1.1.6). Monthly applications of Aliette (fosetyl-aluminium) were made, possibly to protect against red core, *Phytophthora fragariae*. Stroby (kresoxim-methyl) was used in late August and Nimrod (bupirimate) three times in September, to control powdery mildew; Stroby is also known to be active against *B. cinerea*.

After late September, no more products with activity against powdery mildew were applied. Teldor (fenhexamid) is only recommended against *B. cinerea*, as a protectant, and so an application given in early October would probably have been used to control botrytis on the leaves. Rovral was applied at the end of November 2008, probably not long before lifting of

the runners in December. Rovral is a protectant fungicide with some eradicant activity with approval for use on strawberries against botrytis. Thiram is a protectant product with activity against fungi including botrytis and was tank-mixed with the Rovral as the final application.

The propagator (PG8) of samples BX09/65 (12% of plants with botrytis) and BX09/66 (14% of plants with botrytis) applied a similar crop protection programme to both their outdoor A+ and waiting bed plants. The A+ plants were planted following carrots lifted in 2007, and the waiting bed plants were transplanted in August following a green manure. Both strawberry material types had a low incidence of botrytis. The A+ were grown at 400,000 plants per hectare, and the waiting bed at 140,000.

The A+ plants were given two applications of Signum (boscalid + pyraclostrobin) then a tank mix of Nimrod and Stroby in June 2008. Subsequently, three applications were made between mid-July and late August of the tank mix Nimrod, Captan and Stroby (Table 6). The use of Signum in June would have controlled botrytis at the time the runners were starting to be produced by the mother plants. Stroby would then have given some further botrytis control. As for sample BX09/74, there was a final application of Rovral tank mixed with thiram, not long before the A+ and waiting bed plants were lifted in December. The propagator did not name any disease problems that existed in the crops nor reasons for the fungicide applications.

Table 2.1.1.6: Summary of foliar fungicide products used on strawberry during propagation in 2008 by propagators PG4 and PG8, active ingredients and probable target diseases.

Propagator and crop type	Application period	Products used	Active ingredient(s)	Target disease
1. A+ runners	May-Sept	Signum	boscalid + pyraclostrobin	Powdery mildew, botrytis
		Nimrod	bupirimate	Powdery mildew
		Stroby	kresoxim-methyl	Powdery mildew
		Captan	captan	Powdery mildew
	Oct-Dec	Rovral	iprodione	Botrytis
		Thiram	thiram	Botrytis
2. Waiting bed	May-Sept	Signum	boscalid + pyraclostrobin	Powdery mildew, botrytis
		Nimrod	bupirimate	Powdery mildew
		Stroby	kresoxim-methyl	Powdery mildew
		Captan	captan	Powdery mildew
	Oct-Dec	Teldor	fenhexamid	Botrytis
		Rovral	iprodione	Botrytis
		Thiram	thiram	Botrytis

The propagator of BX09/99 (PG5) grew the A+ plants at 110,000 per hectare and saw no diseases in the field. No fungicide records were supplied. The crop was lifted in normal dry conditions at the end of November.

The propagator of BX09/47 (PG6) (14% of total plants with botrytis) does not routinely use fungicides on propagating material against botrytis. The crop in 2008 followed barley. Powdery mildew control products are applied if necessary in the summer, principally Systhane EW (myclobutanil) and Corbel (fenpropimorph), but alternatively Fortress (quinoxyfen) or Stroby. Only Stroby provides any direct protection against botrytis. No fungicides are applied within three weeks of lifting. The A+ plants in 2008 were grown at 160,000 per hectare and lifted in good conditions in December.

The A+ plants of BX09/57 (16% of total plants with botrytis) were grown at 350-400,000 per hectare following maize in 2007. Four fungicide products active against powdery mildew were used, with Signum also active against botrytis. Rovral was also applied, probably

against botrytis, before the plants were lifted in dry conditions in January 2009 in temperatures just above freezing. The same propagator (PG7) grew the waiting bed plants of BX09/58 (with 6% of total plants with botrytis) at a much lower density of 90,000 per hectare, also following maize. Two of the three powdery mildew fungicide products applied were also used on the A+ crop and none would have had activity against botrytis. The only disease recorded in crop was some slight powdery mildew in early October. The plants were lifted in December 2008 in dry conditions at 4°C. There was no use of fungicide by propagators on lifted plants going into store.

Discussion

Comparison between sample years

In general, the incidence of *B. cinerea* infection detected was greater in 2008 (SF 74) than 2009 (this project) (Table 2.1.1.7). Only one of the ten 2009 samples (BX09/42) had over 50% of plants with botrytis and none had over 20% with crown infection, but in 2008 four out of the samples were this highly infected. Similarly, in 2009 only two out of 10 samples had over 10% of all plants with botrytis in petiole stubs, whereas in 2008 three out of five samples had over 10% of plants with petiole infection. In the majority of damp chambers in 2009 botrytis developed in less than 10% of plants, but all but one sample in 2008 had at least this infection incidence. Further work is required to determine the relative importance of season, source, fungicide use and possibly other factors on the differing levels of latent *B. cinerea* in strawberry plants at planting.

Table 2.1.1.7: Sample details of cv. Elsanta strawberry planting material ex-cold storage tested in 2008 and 2009 and incidence of botrytis.

Sample code	Type and propagator code		Test date	% crowns with botrytis	% total plants with petiole botrytis	% plants with botrytis in damp chambers	% total plants with botrytis (any test)
BX08/47	A+	PG1	08.05.08	38	6	12	52
BX08/52	A+	PG2	12.05.08	22	16	32	56
BX08/55	A+	PG3	16.05.08	22	42	62	80
BX08/62	A+	PG4	23.05.08	24	6	88	90
BX08/66	A+	PG5	03.06.08	6	16	2	24
BX09/99	A+	PG5	06.04.09	6	14	18	30
BX09/42	A+	PG4	20.05.09	18	16	78	84
BX09/47	A+	PG6	02.06.09	6	8	0	14
BX09/57	A+	PG7	18.06.09	8	6	2	16
BX09/58	WB	PG7	19.06.09	0	4	2	6
BX09/65	A+	PG8	25.06.09	6	0	6	12
BX09/66	WB	PG8	25.06.09	4	4	6	14
BX09/68	A+	PG9	26.06.09	2	0	4	6
BX09/69	WB	PG9	26.06.09	4	2	2	8
BX09/74	WB	PG4	13.07.09	10	0	6	16

PG codes - propagators supplying strawberry plants lifted in 2007 and/or 2008.

Development of *B. cinerea* on and in plants

It is not known how *B. cinerea* enters the crowns of runners. There may be direct entry in the field from spores germinating in the humid centre of the plant. Alternatively, or in addition, there could be systemic infection by mycelium moving into the crown from either infected leaves via the petiole, via stolons from the mother plant, or through the roots. Also, symptomless infection in crowns may not develop in the field, but from mycelium or spores on external surfaces which penetrate the tissue in the humid conditions of the plastic-lined runner storage crate. The use of green fluorescent protein technology has shown that *B. cinerea* can live as an epiphyte on leaves with mycelium growing over the surface and penetrating the tissue when the conditions are right (USDA, 2005).

The more time the runners spend post-lifting in the sorting house before being cold-stored the greater the opportunity for fungal growth to progress in the runners. Cold-stored runners often have moribund petioles and leaf debris that would be susceptible to infection by botrytis. *B. cinerea* spores can germinate slowly at 1°C, the resting bodies (sclerotia) germinate above 2°C, and mycelium has been shown to grow (at least on agar) at 0°C

(Jarvis, 1977). One UK grower aims to have the runners lifted, size-graded and put into cold-storage the same day. A grower in Continental Europe washes the soil off the plants and transports them to and from a sorting house in Eastern Europe and this must allow a greater opportunity for fungal infection to establish.

Further fungal development can occur if the temperature is not kept sufficiently low or if there is a period of thawing during cold-storage. Storage to keep the runners should be about -1.7°C inside the crates and so this may require an actual store temperature of -2.0°C. Further opportunities for fungal growth can occur after plant dispatch from the cold-store and while plants are left to thaw in the crates just before planting. The use of misting after planting for about two weeks until the roots establish is likely to provide a further ideal opportunity for both any botrytis in moribund tissue to sporulate and infect new tissue and for any epiphytic botrytis growth to penetrate into the plants. Higher *B. cinerea* levels were shown in samples BX09/42 and BX09/99. This material, which did not come directly from the propagators' storage, had a longer period in which decay could have progressed after thawing on the holding before sending it to ADAS.

Further research is required to establish how the crown becomes infected and the most important period for the infection of the crowns. This would enable control measures to be better targeted. It is possible that latent infection may reduce plant vigour and have an effect on either establishment or flower production. It is not known whether there is systemic spread of infection from inside the crowns to some or all adjacent flower initials causing a latent flower infection distinct from that caused by spore infection of the open flower. Increased flower infection would likely lead to a greater incidence of botrytis grey mould on the fruit.

Fungicide use

Information was received from propagators for five crops which had on average 15% of plants with botrytis. Three of the crops had received regular applications of products with activity against botrytis; the other two crops only one or two applications. A sixth crop, with 6% botrytis incidence, received no botrytis control products. It was not known what the infection would have been in the crops which received multiple applications. However, of the three samples with zero petiole infection, two are known to have received fungicides active against botrytis throughout the summer during runner production and then again before lifting and petiole trimming. Use of fungicides to control powdery mildew may have an indirect effect on botrytis by reducing the amount of necrotic and senescent tissue, points at which *B. cinerea* can establish more readily than on healthy green tissue.

Conclusions

- The results confirm that there is a risk of *B. cinerea* being introduced into new plantings as symptomless infections in young plants.
- The presence of *C. acutatum* in the crowns or other tissues of planting material was not shown in 2009.
- Moribund leaves were sometimes an important source of *B. cinerea*. Botrytis on roots added to the incidence of botrytis.
- The recovery of *B. cinerea* from within surface disinfected crown tissue suggests it will not be easy to eliminate this infection totally by a pre-plant treatment.
- Waiting bed plant samples had a lower mean incidence of botrytis than A+, but this was because two samples of A+ had very high levels of botrytis.
- Fungicides with activity against botrytis (Rovral, Signum, Stroby, Thiram and Teldor) were used on A+ and waiting bed plants, some receiving several applications, but latent botrytis was still recorded after cold-storage.

References

Jarvis, W.R. (1977). Botryotinia and Botrytis species: taxonomy, physiology, and pathogenicity. A guide to the literature. Monograph No. 15, Canada Department of Agriculture.

USDA (2005). Uncovering the mysteries of gray mold. Work by W.F. Mahaffee at the United States Department of Agriculture, Agricultural Research Service Laboratory, Corvallis, Oregon. http://www.ars.usda.gov/is/AR/archive/sep01/mold0901.htm

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

This work was a development from Task 2.1.1 and the determination that latent botrytis was present in all the samples of commercial strawberry propagation material examined in 2008. The objective of this work was to determine whether treatment of the runners either pre-planting or on recently established plants would reduce the level of *B. cinerea* in the plants. Post planting treatments included fungicides more usually applied at flowering. Cercobin WG

is used as a systemic drench against *Verticillium,* but activity against botrytis has been reported. Rovral WG, Scala, Signum and Switch are all approved for the control of botrytis and are from different fungicide activity groups. Serenade ASO is a bio-control agent with label recommendations for botrytis control on strawberry. Achieving lower levels of botrytis in the field pre-flowering is likely decrease botrytis flower and fruit infection. *Colletotrichum acutatum* (black spot) is known to be transmitted on runners. Hot water treatment (35 °C for 7 minutes, then 50 °C for 2 minutes) has been found to reduce *C. acutatum* in strawberry runner cuttings (Johnson *et. al*) and its effectiveness against *B. cinerea* pre-planting will be recorded in this project.

Methods

The experiment was carried out in a commercial tunnel crop of strawberries cv. Elsanta near Cambridge. Plants were either taken out of cold-storage and treated immediately pre-planting or planted in the field and allowed to establish before receiving drench or spray application of commercial products. Botrytis incidence was assessed in plant crowns and other parts following hot water treatment. The crop was monitored for phytotoxicity and samples of leaves taken from the field to assess the levels of botrytis post-treatment. The dates of tasks carried out are shown in Table 2.1.2.1.

Table 2.1.2.1: Tasks carried out within the commercial strawberry crop, Cambridge 2009.

Date completed	Task
01/04/09	180 plants A+ extra cv. Elsanta taken from grower for hot/cold water treatment
02/04/09	Hot and cold water treatment of plants in the laboratory, followed by incubation
03/04/09	Crop planted by grower, and some bags replanted with hot water treated plants
15/04/09	Hot water treated plots in the crop assessed for phytotoxicity and vigour
15/04/09	Paraat drench applied to crop by grower against *Phytophthora* spp,
17/04/09	Plants established / new growth showing - grower stopped misting crop
21/04/09	Plant protection products applied by ADAS to pre-flowering crop
28/04/09	Leaf samples collected 7 days after plot treatment applications. Leaves frozen prior to incubation in damp chambers
12/05/09	Plots assessed for phytotoxicity and botrytis Leaf samples collected 21 days after plot treatments applications. Leaves surface sterilised, frozen and then incubated in damp chambers
23/06/09	Final crop assessment of flowers and fruit for phytotoxicity and disease

Sample details

A sample of 180 A+ extra (18 mm crown) cold-stored strawberry plants cv. Elsanta from the Netherlands was obtained from the grower. Plants were taken from two crates in a stack which had been left to thaw in the field preceding planting. It had not been possible to confirm a planting site earlier in the year and so a prior assessment of the latent botrytis incidence in the plants had not been possible. Plant samples were cold-stored overnight at 4°C so that laboratory treatment and processing could be completed in a single day. Sub-samples of 50 plants were taken for testing.

Hot water treatment of plants

Soil adhering to the crown and roots was washed off with tap water. Plants were treated in batches of ten, being fully immersed for the required period. The 130 plants for hot water treatment received 7 minutes at 35 °C and then moved directly to 50 °C for 2 minutes. The plants were then plunged into water at 8 °C for 10 minutes before being taken out and blotted dry. The control plants did not receive hot water treatment but were immersed for 10 minutes in cold water. Plants were blotted dry, wrapped in paper towel and stored at 4 °C until either planting or dissection for incubation for botrytis.

Eighty of the hot water treated plants (from across the different water-bath sessions) were kept back from the incubation for disease and planted the next day. Each replicate block received 20 plants from two of the treatment batches. Cold water treated plants were only used for laboratory assessment of latent botrytis.

Tests for latent *B. cinerea* and fungal assessments

All 50 of the cold water dipped control plants and 50 of the 130 hot water treated plant were taken for determining the incidence of latent botrytis. Crowns were sectioned and petiole stubs sampled and given surface disinfection before plating onto agar. Plant parts were incubated in damp chambers. All tissue was then examined for each plant and the incidence of *B. cinerea* and other fungi recorded. The techniques were described fully in the Methods under Task 2.2.1

Determination of latent botrytis in leaves post-planting

At 7 days after application of fungicide treatments, the oldest newly emerged/expanded leaf (i.e. not leaves that were open during cold storage) was cut off at the petiole base from alternate plants (i.e.10 leaves/plot) into one clean plastic bag. Wool tags were placed around the same-aged leaf of an unsampled plant at the start of each replicate to indicate the leaf age to be sampled on the next occasion. These leaves emerged under overhead misting.

In the laboratory, the leaves were laid upper-side uppermost, without touching, in a single layer on paper towel. Each 10 leaf sample was then sealed flat in a transparent polythene bag. The stack of bags was frozen (-20ºC) overnight. The frozen leaves and paper towel were then carefully taken out of the bag directly into a tray of the same size. The trays were sealed shut and incubated at room temperature in diurnal light. The number of leaf blades and petioles developing botrytis sporulation was recorded after 14 days.

At 21 days after application of fungicide treatments, the sampling was repeated, selecting the first leaf to have expanded in the field (as before) from the alternate plant (i.e. the plant not sampled previously). The protocol was modified to surface disinfect the leaves before freezing to determine the incidence of latent botrytis inside the leaves (i.e. not including mycelium or spores on the leaf surface). The leaves from each plot were immersed in 25 ml of 8% a.i. sodium hypochlorite in 975 ml tap water for 4 minutes, replacing the bleach solution between plots. Leaves were rinsed in tap water, excess water shaken off, and the leaves laid on paper towel for freezing, before being incubated in trays and assessed as previously.

Field experiment

The hot water treated plants were planted on 3 April 2009 in the same tunnel as other material planted the same day with runners from the same delivery from the propagator. The experiment was conducted within a commercial polytunnel crop at Chivers Fruit Farms, near Cambridge. Each plot comprised two 10-hole peat grow-bags on ridged plastic mats placed on woven groundcover material covered soil ridges (with bare soil between the ridges). One planted grow-bag was used as a guard between each plot. The treatments were randomised within four replicate blocks along a whole leg-row, with six planted bags as discard at either end of the row. The crop received overhead misting for two minutes every 10 minutes for two weeks until the roots had established, and then drip irrigation. The grower did not apply any fungicide sprays to the area of the experiment until flowering.

Fungicide treatments

The plants were treated when two new leaves had emerged and overhead misting of the crop for establishment had been completed. Only one application per treatment was given.

1. Untreated control
2. Hot-water-treatment to runners (7 mins 35°C then 2 mins 50°C before cooling
3. Cercobin WG drench at 1 g/L (0.25 g / plant in 250 ml water) (SOLA 2008/1381)
4. Rovral WG spray at 1 kg/ha
5. Scala spray at 2 L/ha
6. Signum spray at 1.8 kg/ha
7. Switch spray at 1 kg/ha
8. Serenade ASO spray at 10 L/ha

A beaker was used to pour the fungicide drench (T3) over the plant crown and into each grow-bag planting hole. Spray applications (T4 – T6) were applied using a single nozzle (02F110LD) lance pressure-assisted knapsack sprayer operating at 2 bar, to deliver 1000 L / ha of water.

Field assessments

Plants which had been hot water treated and planted were assessed for phytotoxicity and vigour two weeks after hot water immersion and compared with untreated plots. The number of plants per plot with any discolouration, necrosis, distortion or stunting was counted together with the proportion with one, two or three new leaves emerged. Plots were assessed for visible botrytis and phytotoxicity pre-flowering, 21 days after the fungicide applications. The number of plants with leaf markings or stunting was recorded. A final crop assessment of botrytis, and phytotoxicity to flowers and fruit, was carried out mid-fruiting, 11 weeks after planting, when there were still flowers present. The incidence of symptoms per plot was assessed using a 0 to 4 index.

Results

Botrytis in surface disinfected petiole stubs and crowns

The number of plants per sample with a petiole stub present was similar for each of the treatments, about a third of the plants (Table 2.1.2.2). Some stubs were mainly green, but others were developing a brown rot down to about 10 mm from the cut end. Results from the petioles do not include any that were totally rotted - these were included in the un-sterilised damp chamber results. The percentage of plants with *B. cinerea* infected petioles was calculated as both the percentage of the total plants in the sample (i.e. the risk to the grower of introducing botrytis to the field), and as the percentage of plants with petiole stubs present.

Petiole botrytis was present in 14% of cold water treated plants, but only 2% of hot water treated (Table 2.1.2.2). Expressed as a proportion of the plants with petiole stubs present, 50% and 6% of samples were infected with botrytis, respectively. Although the latter percentages look significantly different, the information is based on only 8 plants showing petiole botrytis. *B. cinerea* was found in 6% of crowns, with no difference between treatments (Table 2.1.2.3).

Table 2.1.2.2: Effect of hot water treatment pre-planting on the recovery of *B. cinerea* from within trimmed petiole stubs of strawberry plants. The % infection of petiole stubs is shown both as a proportion of total number of plants tested and of the number of plants from which petiole stubs were available

Treatment	No. plants in sample	No. plants with petiole stubs	No. plants with petiole botrytis	% of total plants with petiole botrytis	% of plants with petioles with petiole botrytis
Cold water	50	14	7	14.0	50.0
Hot water	55	18	1	1.8	5.5

Table 2.1.2.3: Effect of hot water treatment pre-planting on the recovery of *B. cinerea* from within crowns, and the total number of plants with *B. cinerea* from all tests (petiole or crown incubation for 14 days, or damp incubation for up to 35 days)

Treatment	No. plants in sample	No. plants with crown botrytis	% of plants with crown botrytis	Total number of plants with botrytis (all tests)	% of plants with botrytis (all tests)
Cold water	50	3	6.0	19	38.0
Hot water	55	3	5.5	19	34.5

Table 2.1.2.4: Effect of hot water treatment pre-planting on the recovery of *B. cinerea* and the tissue locations where *B. cinerea* was found after 21 and 35 days incubation

Treatment	Incub-ation period	Plant parts or total number of plants with botrytis in damp chambers						
		No. plants	Roots	Crown	Green leaves	Rotten leaves / petioles	Total no. of plants	% of plants
Cold water	21 days	50	0	0	2	9	9	18.0
Cold water	35 days	50	0	0	1	12	13	26.0
Hot water	21 days	55	0	0	1	4	4	7.3
Hot water	35 days	55	0	0	0	15	15	27.3

Botrytis detected by humid incubation of plant parts

B. cinerea in damp chambers (Table 2.1.2.4) could have developed from mycelium or ungerminated spores or sclerotia on the tissue surface or mycelium inside the plants. Results exclude the infection detected in petiole and crown sections by isolation on agar. Green leaves contained botrytis (Table 2.1.2.4), although the rotted leaves and petioles were more frequently infected. No botrytis was recovered from either the roots or the crown remnants in either sample.

Incidence of total botrytis in plants

The total number of plants with *B. cinerea* in any of the three tests; crown and petiole sections (Tables 2.1.2.2 & 2.1.2.3), or the remainder of the plant in the damp chamber incubated for 35 days (Table 2.1.2.4) was calculated (Table 2.1.2.3). There was no difference between the hot water and cold water treated plants, with around 36% affected by *B. cinerea* in both treatments.

Field assessments

Assessment of the plots on 15 April, 12 days after planting, showed that there was no difference in growth stage between the hot water treated plants and the untreated controls, with means of 73% and 76%, respectively of plants having only one new leaf emerging. The remaining plants had two new leaves. There was a low incidence of leaf distortion in most plots examined, and this was not attributed to the hot water treatment with 8% and 9% of leaves distorted in the hot water and untreated plots, respectively. Plants were drenched or sprayed on 21 April 2009 when two new leaves had unfolded, 18 days after planting.

The first sample of ten first-unfolded leaves was collected 7 days later. Hot water treated plants were noticeably stunted, with smaller leaves by this date. All leaf samples had 100% of leaves with patches of sporulating *B. cinerea* after damp incubation. None of the leaves had visible symptoms of botrytis at picking. Pre-planting assessments had shown only 36% of plants to have latent botrytis (internal and external). There were no botrytis symptoms on plants in the field at 21 days after fungicide applications. A few plots had small leaf markings on one or two plants, but there was no significant difference between treatments (Table 2.1.2.5). However, a highly significant proportion of hot water treated plants (mean 79%) were stunted across all the replicates (Table 2.1.2.5).

Table 2.1.2.5: Effect of pre-planting hot water treatment and post-planting fungicide treatment applications on phytotoxicity symptoms (12 May 2009) at 21 days after fungicide applications

Treatment	Mean % of plants with phytotoxicity	Standard error
T1 Untreated	3.8	2.74
T2 Hot water treated	78.8	5.72
T3 Cercobin WG	2.5	2.25
T4 Rovral WG	6.3	3.47
T5 Scala	3.8	2.74
T6 Signum	5.0	3.13
T7 Switch	6.3	3.47
T8 Serenade ASO	5.0	3.13
Significance	<0.001	
d.f.	21	

The second pick of ten oldest newly expanded leaves (from the ten plants not sampled at the first pick), at 21 days after the application of treatments in the field, showed that there were fewer leaves with latent *B. cinerea* than at the earlier sampling when external as well as internal botrytis had been recorded. Leaf blades were more commonly infected than petioles (Table 6). No leaves had botrytis symptoms at sampling. The highest incidence of botrytis was in the leaves of untreated plants (T1 + T2 combined), with a mean 61.2% of leaves with botrytis compared with the sprayed treatments which ranged from 10% to 33% (P = 0.067).

The fungicide spray treated plants had a third of their leaves infected, but the Cercobin WG drench had only 10% of leaves with latent botrytis, and Serenade ASO 18% (Table 2.1.2.6). Only two of the four plot samples for both Cercobin WG and Serenade ASO had botrytis in either the leaf blade or petiole. One of four Switch treated plots had no leaf botrytis; all other treatments had botrytis recorded from all four replicates.

Table 2.1.2.6: Proportion of petioles and leaves with botrytis - Pick 2 on 12 May 2009, 21 days after treatment applications. Assessment 14 days after incubation

Treatment	Mean % of petioles with botrytis	Standard error	Mean % of leaf blades with botrytis	Standard error
T1 Untreated	2.5	1.81	65.0	13.37
T2 Hot water treated	5.0	2.49	57.5	13.88
T3 Cercobin WG	0.0	0.01	10.0	8.37
T4 Rovral WG	2.5	1.81	32.5	13.14
T5 Scala	0.0	0.01	32.5	13.14
T6 Signum	5.0	2.49	32.5	13.14
T7 Switch	0.0	0.01	32.5	13.15
T8 Serenade ASO	2.5	1.81	17.5	10.63
Significance	n.s. 0.107		n.s. 0.111	
d.f.	21		21	

The crop was assessed in the middle of fruit production on 24 June (Table 2.1.2.7). There were no differences between treatments in the presence of botrytis browning on the plants, with only one fruit seen to be affected, on two plots. Any phytotoxicity was due to be measured by distortion of the flowers and fruit; however there had been severe powdery mildew infection across the whole crop and this probably caused the distortion visible on up to 25% of plants in some plots. There were no significant differences in distortion between treatments. Some plots had up to 50% of leaf area covered by powdery mildew lesions, but there was no difference between the untreated and treated plots.

Table 2.1.2.7: Effect of pre-planting and post-planting fungicide treatments on phytotoxicity and botrytis in the field (24 June 2009). All plots assessed using a severity index

Treatment	Mean index score			
	Plants with Botrytis	Flower distortion	Fruit distortion	Powdery mildew
T1 UT	0	0.5	0	1.5
T2 HWT	0	1.3	0	1
T3 Cercobin WG	0.3	0.5	0.3	1.3
T4 Rovral WG	0	0.5	0.3	1.5
T5 Scala	0	0.5	0.3	1.5
T6 Signum	0.3	0.8	0.5	1.3
T7 Switch	0	0.5	0.3	1.3
T8 Serenade ASO	0	0.5	0.3	1.3
Significance	ns	ns	ns	ns
l.s.d. (21d.f.)	0.38	1.27	0.67	0.69

Severity index: 0 = None 1 = Slight 1-25% 2 = Moderate >25-50%
3 = Above average >50-75% 4 = >75-100%

Infection by *Colletotrichum acutatum*

Colletotrichum spp. was not isolated from crowns or petioles of any sample of the hot water and cold water treated plants, although a fungus with a similar appearance on the agar (salmon pink spore bodies) was found on two petiole samples. This fungus had pycnidia rather than the acervuli produced by *Colletotrichum* spp., and the spore shape differed from that of *Colletotrichum* spp.

Information on crop husbandry and fungicide use

Only Paraat drench was applied to the experiment by the grower before June (pre-flowering). The grower applied foliar fungicides to the trial area from 5 June. Both botrytis and powdery mildew control products were applied (Table 2.1.2.8).

Table 2.1.2.8: Fungicides applied by the grower to the A+ cv. Elsanta strawberry crop in 2009

Field	Application month	Summary of Products	Active ingredient(s)	Target disease
323	April	Paraat	dimethomorph	Phytophthora
	May	Teldor	fenhexamid	Botrytis
		Fortress	quinoxyfen	Powdery mildew
		Amistar	azoxystrobin	Black spot
		Systhane 20EW	myclobutanil	Powdery mildew
	June	Fortress	quinoxyfen	Powdery mildew
		Teldor	fenhexamid	Botrytis
		Systhane 20EW	myclobutanil	Powdery mildew
		Nimrod	bupirimate	Powdery mildew
	July	Nimrod	bupirimate	Powdery mildew
		Corbel	fenpropimorph	Powdery mildew
	August	Stroby	kresoxim-methyl	Powdery mildew
		Corbel	fenpropimorph	Powdery mildew
		Fortress	quinoxyfen	Powdery mildew
	September	Corbel	fenpropimorph	Powdery mildew
		Stroby	kresoxim-methyl	Powdery mildew

Discussion

Fungicide use

Only a single fungicide application was given to the plants in the experiment. Growers apply several fungicides to the crop throughout the season for powdery mildew control. A few products such as Signum (boscalid + pyraclostrobin) also have activity against botrytis and their use would reduce the amount of botrytis surviving in the crop from spores arriving during the growing season.

Significant differences were not shown between the incidences of botrytis at 21 days after fungicide treatment, probably because of the variation between plots of the same treatment. It is possible that with at least a third of plants having latent botrytis (as seen from the cold water treatment incubations), the random allocation of plants with or without infection in various tissues to each plot gave different control challenges within each plot.

Leaf testing indicated more plants were infected by botrytis at 5 weeks after planting than immediately ex-cold-storage (61% and 36%, respectively). Further latent botrytis infection of leaves post-planting by internal movement of mycelium may have occurred. All sampled

leaves had botrytis in or on the leaves 7 days after fungicide application and some of this may have penetrated inside the leaves. Fungicide control by 21 days after treatment may have been gained by killing the fungal mycelium in the plant, preventing penetration of externally colonising mycelium and/or or inhibiting spore germination on the leaf surface.

The hot water treatment at 50 °C for 2 minutes was ineffective against *B. cinerea* on the 18 mm crown diameter plants tested in this experiment, (although only six crowns in total were infected). Published work on black spot control was carried out on smaller cuttings and these would have achieved a quicker increase in internal temperature. The lower proportion of plants with petiole botrytis in hot water treated plants compared with cold water treated plants may indicate some control of botrytis in peripheral or thinner tissues. Stunting was caused by the hot water, but a cultivar other than Elsanta may have been less susceptible.

Conclusions

- Crown tissue, petiole stubs and rotted leaves of cv. Elsanta A+ strawberry runners post cold-storage were infected by *B. cinerea*, confirming there is a risk of *B. cinerea* introduction into new plantings as symptomless infections. Hot water treatment at 35°C for 7 minutes followed by 2 minutes at 50°C was ineffective, plants having a similar incidence of botrytis to control plants (36%). Hot water treatment damaged plants, causing stunting.
- Neither hot water treatment of runners pre-planting, nor a single application of various fungicides 18 days after planting significantly reduced botrytis levels in newly emerged leaves.
- There was an indication that a Cercobin WG drench reduced latent botrytis (P=0.06).

References

Johnson, A.W, Simpson, D.W. & Berrie, A. (2006). Hot water treatment to eliminate *Colletotrichum acutatum* from strawberry runner cuttings. Acta Horticulture 708 255-258.

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

Evaluation of the biocontrol product Binab T Vector was carried out in 2008, but complications in the UK registration of this product as a pesticide meant that no trial was possible on a commercial crop in 2009.

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

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

Methods

Flower infection

To validate the model, we have regularly sampled flowers 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 to remove any spores on the surface in a shaker. The flowers were then rinsed with distilled water and placed separately on a piece of filter papers thoroughly wetted with distilled water in small sterile Petri dishes. The dishes were incubated in a glasshouse compartment (C10 or C14) at approximately 20°C for 1-2 weeks after which the flowers are examined for conidiophores of *B. cinerea*. Any flower on which conidiophores are detected was classified as infected.

Management trial

The usefulness of Botem predictions in practical botrytis management was evaluated on the main season protected cv. Elsanta crops at Manor Farm, Near Borough Green, Kent. Each of three tunnels [ca. 100 m length with 3 double beds] 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, either Rovral (iprodione) or pyrimethanil (it 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 (temperature & humidity) 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 on 14, 21 and 28 May and 4 June. 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 placed onto seed trays, well separated from each other and covered with polythene bag for incubation. Fruit were assessed for botrytis and other rots 1-2 weeks after incubation.

Results

Flower infection

We have obtained one set of data (Table 2.3.1) on the percentage of latent infection of flowers on cv. Elsanta. In contrast to the previous year, the level of botrytis infection varied greatly, ranging from 2% to 35%. The overall pattern of observed flower infection followed closed with the predicted (Fig. 2.3.1): severe infection occurred in mid April.

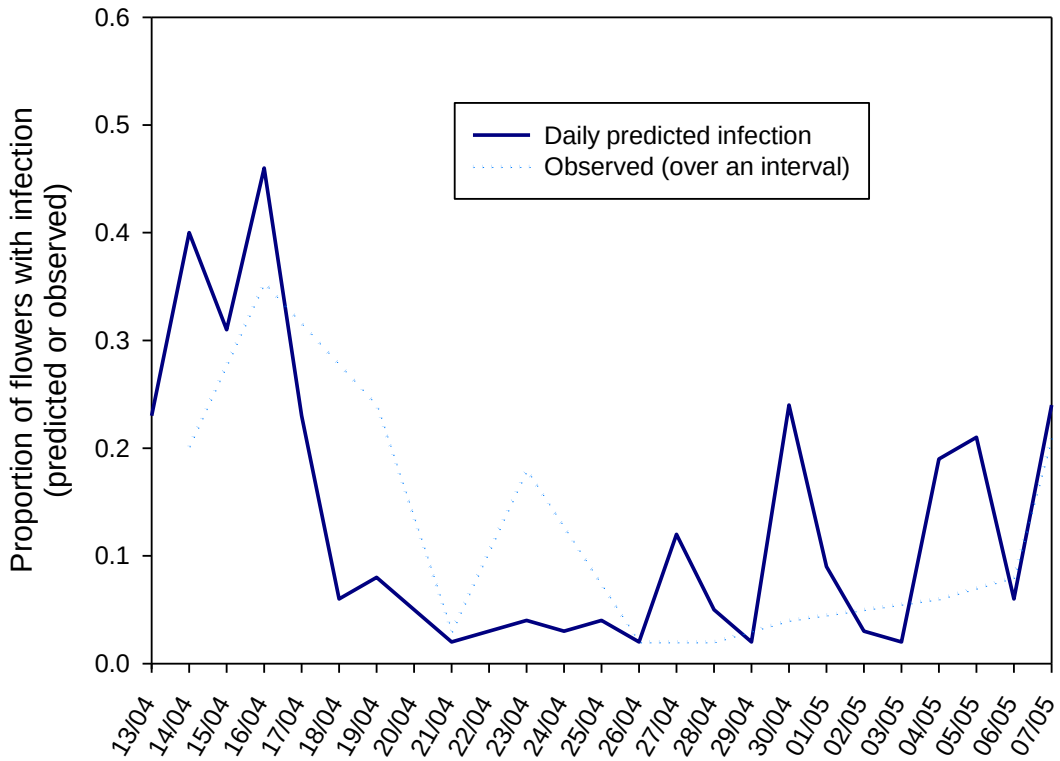
Table 2.3.1, Percentage of latent infection of strawberry flowers of Elsanta by *Botrytis cinerea* in 2009 in Kent

Sampling date	Infection
16/04/2009	20
18/04/2009	35
21/04/2009	24
23/04/2009	3
24/04/2009	18
27/04/2009	2
29/04/2009	2
01/05/2009	4
05/05/2009	6
07/05/2009	8
19/05/2009	21

Management trial

The threshold for spray was set to a daily predicted infection of fruit more than 10%. This was satisfied only between 14/04-17/4 (Fig. 2.3.1) and hence Rovral was applied on 16/04. A single spray was also applied in the conventional plot on 24 April for botrytis. There was a very low level of latent botrytis rot on green fruit. Even for the untreated fruit, only about 2.4% of fruit had latent botrytis, compared to 0.5% and 1.4% for the conventional and model-based treatments, respectively. Interestingly, most botrytis rots originated from the first sample, 4.5% and 7.7% for the model-based and untreated treatments, respectively. This agrees well with the model predictions for flower infections [14-17/4] (Fig. 2.3.1). However, there was a considerable amount of other rots. In total there were 9.4% of rotten fruit (including botrytis) for the untreated, compared to 5.0% and 6.8% for the conventional and model-based treatments, respectively. We hope to repeat this trial next year but with more tunnels such that we can analyse data statistically.

Fig. 2.3.1. Predicted and observed proportion of flowers with botrytis infection



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)

Isolation of *C. acutatum*

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

Once cultures were free of contamination isolates were grown on sterile cellophane on PDA and once growth was established the mycelium was scraped off and stored in Eppendorf tubes at -80^{o}C prior to molecular analysis.

As reported last year, we have already obtained > 100 isolates, primarily from strawberry and apple. We had encountered difficulties in isolating pure isolates from weeds due to frequent contaminations. We are currently in process of isolating many isolates from weeds.

Molecular analysis

We have developed six SSR molecular markers. We have extracted DNA from 186 isolates, most from apple (102), strawberry (52) and cherry (23), and a few (5) from weeds. A few isolates from unknown hosts were also included. Most isolates were collected from three fields at East Malling Research: apple (cv. Cox – TL109: 33, cv. Gala – TL161: 33) and strawberry (DM180): 52 (strawberry) + 5 (weeds). Isolates from cherry were from two orchards, about 200 miles apart (Kent, and Herefordshire)
There were also 20 isolates from cv. Bramley in a Kent orchard.

All 186 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 hence it was excluded from subsequent analysis. Fig 3.1.1 shows an example of SSR peaks for one single isolate. 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. Preliminary analysis of molecular data suggested 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, and (3) the isolate differences are more related to site isolates rather than to host differences. In the coming year, we shall complete screening of all collected isolates (with more from weeds).

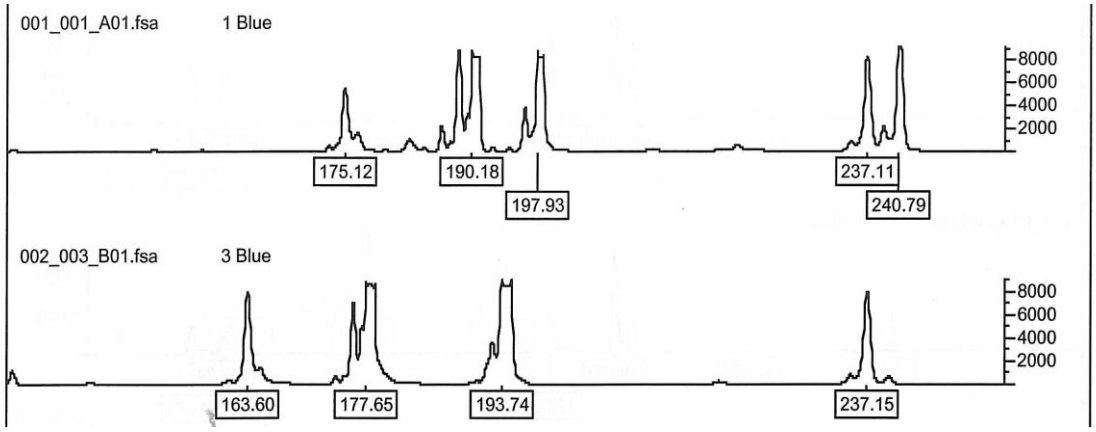


Fig. 3.1.1 SSR peaks for two isolates for illustration purpose only.

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

We aim to do molecular characterisation by March next year. Furthermore, we have attended a meeting (3-4 March 2009) in Belgium on black spot. Several groups of researchers from Denmark, Finland, UK, Belgium and Norway that are currently actively engaged in black spot research exchanged their recent findings and discussed future collaborations. The general conclusions from these large European research projects agree with our current understanding of black spot:

(1) This fungi can infect many hosts, including cherry and apple

(2) 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 on other isolates.

(3) Elimination of initial inoculum is critical

(4) Disease development needs wet-moist conditions

(5) Disease spread can be very rapid in field

We have obtained 20 samples of DNA from Europe for inclusion in SSR screening and hence we may be able to better interpret fungal diversity in relation to the findings from other European countries. Based on molecular data, we shall select up to 20 isolates for inoculation studies on green fruit.

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

A small pilot trial is in progress to determine whether several biofumigants as determined in the strawberry biofumigation LINK project could also reduce viability of *Colletotrichum* on infested debris. Results are not expected to be available until autumn 2010.

Task 3.4: Development of simple guidelines for blackspot management

Work on this task has not yet started. The work is planned for Autumn/Winter 2009

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

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

Summary

Numbers of *L. ruguipennis* peaked in August and September in the trials on a commercial site and had begun to decline by October when overwintering adults were emerging. The capsid was attracted to mayweed, sweet alyssum and common vetch compared to strawberry and lucerne in the unprotected trap crop trial. *Lygus* nymphs were highly attracted to sweet alyssum. Earwigs were more likely to be present on strawberry than the trap crop species. In the experiment at EMR the three plant/plant mixes used flowered at different times so it was not possible to make a direct comparison of relative attractiveness on particular days. However, in season totals, lower numbers of *Lygus* adults were recorded on the plant mix than on mayweed and vetch.

In the lucerne experiment under protection on the commercial site, *Lygocoris pabulinus* was the most common capsid sampled on strawberry and lucerne. Although there were not high enough numbers of invertebrates to analyse statistically it was evident that there was very little difference between the numbers of invertebrate taxa on lucerne compared to strawberry, including *L. pabulinus*

At the protected strawberry bug vac site at least double the numbers of *L. rugulipennis*, spiders and earwigs were found in strawberry compared to in lucerne. There was a significant reduction in male *L. rugulipennis* and *Lygus* nymphs on strawberry after bug vaccing. *Lygus* numbers were reduced by 39-61% on strawberry and 2-56% on lucerne. Beneficial insects were not significantly affected by the bug vac.

Experiment 1. Assessment of potential trap crops for *Lygus rugulipennis*

Materials and methods

In 2009 a small scale field trial was established in a western edge bed of 'Park West' strawberry plantation (cv. Albion) at Robert Boucher and Son, Newlands Farm, Teynham, Sittingbourne, Kent ME9 9JQ (NGR TQ 956 622, Fig. 4.1.1), by kind agreement of Hugh Boucher. The bed was 69 m long and surrounded by cv. Elsanta (adjacent crop) and cv. Flamenco strawberry plantations. The row spacing was 1.9 m. The crop remained unprotected during the trial. The plots were marked out on 26 March.

Figure 4.1.1. Map of the experimental plot location (trap crop plot 2009)

Treatments were four species of herbs plus strawberry as a control (Table 4.1.1). A randomised block design with four replicates was used (Table 4.1.2). Plots were 3.4 m lengths of row end to end. Seeds were sown by hand into the planting holes on 7 April 2009 by the grower and additional plants were sown in the glasshouses at EMR on 31 March to act as additional plug plants to fill in gaps where germination failed in the field. The planting holes in the strawberry beds were 20 cm apart in two rows. A 'pinch' of seeds (~10) was placed into each hole and covered lightly with soil.

Trickle irrigation and feed was provided as per the strawberries and initially a covering of fleece to prevent grazing of newly emerged shoots by rabbits was provided. The plots were hand weeded and gaps filled with glasshouse grown plants on each visit as necessary. The experimental area and adjacent strawberry bed remained unsprayed with insecticides for the duration of the trial.

Table 4.1.1. 5 species of plant sown in the trial on 7 April. Strawberry was planted on 20 May

Trt no	Colour code	Common name	Species name
1	Red	lucerne	*Medicago sativa*
2	Blue	common vetch	*Vicia sativa*
3	Yellow	sweet alyssum	*Lobularia (=Alyssum) maritima*
4	White	mayweed	*Matricaria recutita*
5	Green	strawberry	*Fragaria ananassa*

A USB-500 temperature and relative humidity data logger was placed in the crop. Full records are available from the EMR meteorological station.

Table 4.1.2. Randomisation of treatments

Plot No.	Plant	Plot No.	Plant
101	*Lobularia*	301	*Lobularia*
102	*Vicia*	302	*Fragaria*
103	*Medicago*	303	*Vicia*
104	*Fragaria*	304	*Matricaria*
105	*Matricaria*	305	*Medicago*
201	*Medicago*	401	*Vicia*
202	*Lobularia*	402	*Medicago*
203	*Fragaria*	403	*Lobularia*
204	*Vicia*	404	*Matricaria*
205	*Matricaria*	405	*Fragaria*

The plots were assessed fortnightly and a record made of the growth stage of each species (photographs, Figs. 4.1.3a-e). A 2 m section in the centre of each plot was tap sampled (x 6) into a bowl with one side cut out. Records were made of beneficial species (honey bees, bumble bees and predators). *Lygus* bugs were pooted into tubes for subsequent identification and sexing in the laboratory under a microscope.

Results

Sampling began on 30 April, but no insects were captured using tap sampling until 28 May when one female *L. rugulipennis* was present on *Matricaria recutita* (Fig. 4.1.2). Small numbers of females found at this time were overwintered females from autumn 2008. The first nymphs were observed 2 weeks later, in the middle of June. Female numbers then peaked at the beginning of July and mid August with very large numbers of nymphs present from the beginning of August (Fig 4.1.2). The last sampling date was 5 October when nymph numbers began to fall and remaining adults were overwintering forms (darker in colour). The phenology data suggested that *Lobularia maritima* was the most attractive plant to the *L. rugulipennis,* especially the nymphs. Strawberry was less attractive (Figs. 4.1.2 - 4.1.4a-d).

$Log_{10(+1)}$ transformed data of total numbers of taxa over the growing season showed there were no significant differences in plant species preference in the numbers of most beneficial taxa (Fig. 4.1.5). However, earwigs were more likely to be found on strawberry (*Fragaria ananassa*) plants than the other four plant species (ANOVA; p=0.004, df 12, sed 0.0773, lsd 0.1683). The majority of the mirids found in the trap crops and strawberry plants were *Lygus rugulipennis*.

Significantly more male *L. rugulipennis* (ANOVA $log_{10(+1)}$ transformed data; p=0.008, df 12, sed 0.2336, lsd 0.5089) occurred on *M. recutita, L. maritime* and *M. sativa* than *V. sativa* or *F. ananassa*. Female *L. rugulipennis* were also more abundant (ANOVA $log_{10(+1)}$ transformed data; p=<0.001, df 12, sed 0.1889, lsd 0.4116) in *M. recutita*, *L. maritime* and *M. sativa* than *V. sativa* or *F. ananassa* (there was no significant difference between numbers of females in *V. sativa* and *M. sativa*).

Numbers of *Lygus* nymphs (ANOVA $log_{10(+1)}$ transformed data; p=<0.001, df 12, sed 0.1743, lsd 0.3798) were significantly higher in *L. maritima* than any other plant species, followed by *M. sativa, M. recutita* and *V. sativa* which had higher numbers of *Lygus* nymphs than *F. ananassa* over the season.

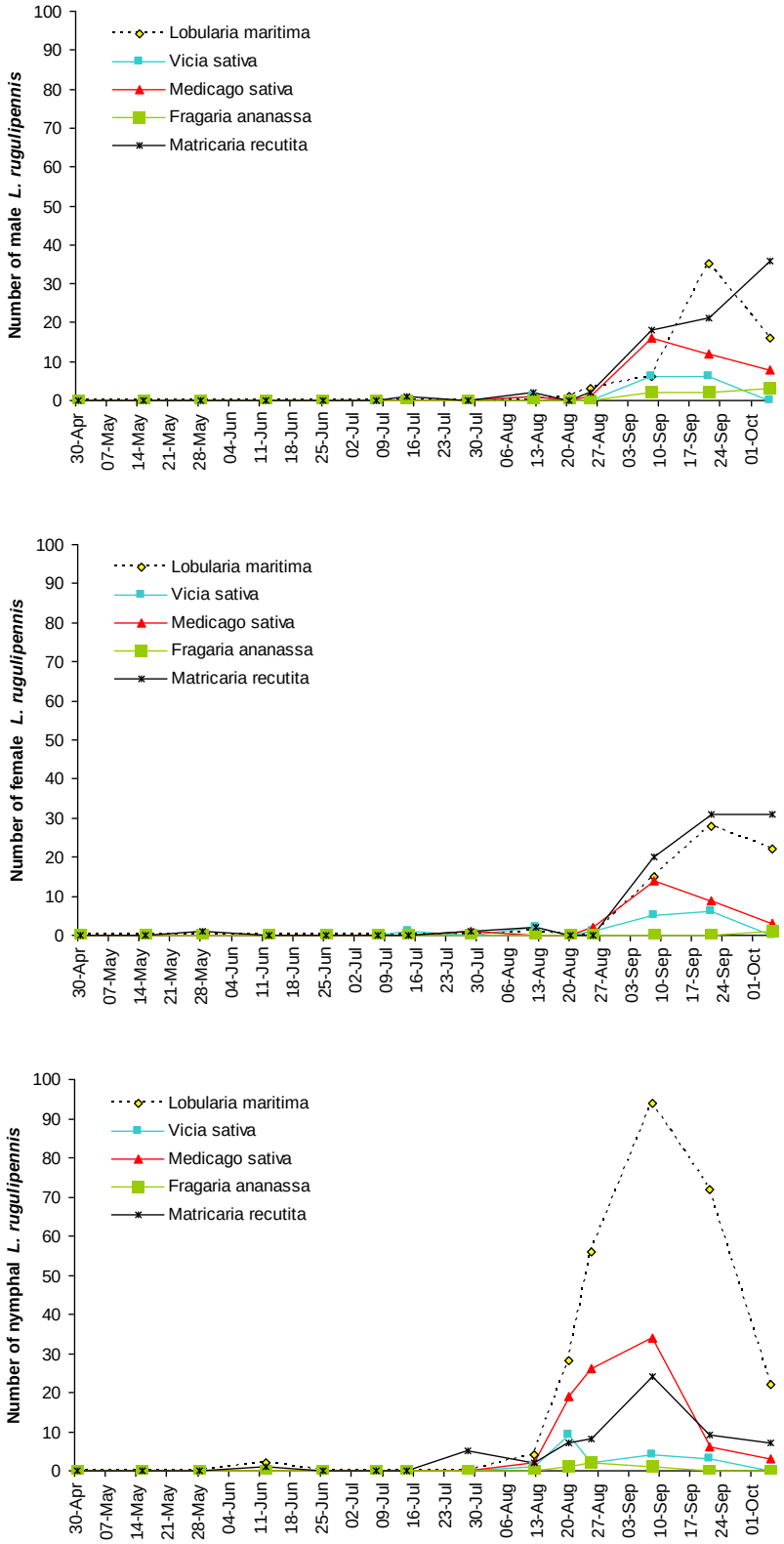


Figure 4.1.2. Numbers of male (top), female (middle) and nymph (bottom) *L. rugulipennis* in the 5 plant species throughout the season

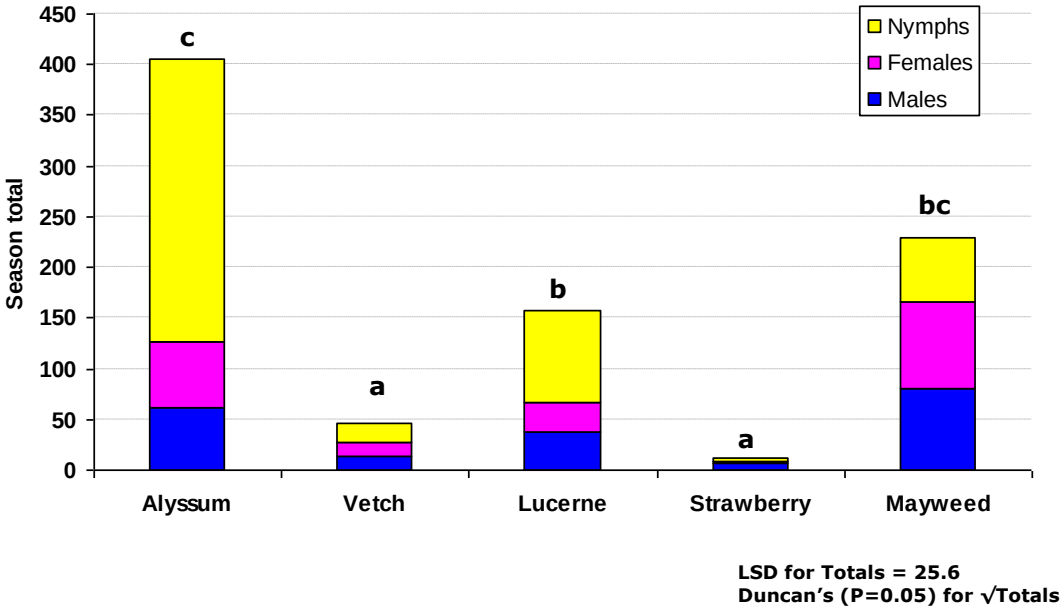


Figure 4.1.3. Seasons total numbers *L. rugulipennis* in the 5 plant species. Bars marked with the same letter do not differ significantly in a Duncan's multiple range test (P=0.05) of the square root transformed valued. The LSD (P=0.05) for the untransformed values is 25.6.

30 April

15 June

28 July

11 August

21 September

Figure 4.1.4a. Photographs of *Lobularia (=Alyssum) maritime* throughout the season

30 April

15 June

28 July

11 Aug

21 September

Figure 4.1.4b. Photographs of Vicia sativa throughout the season

30 April

15 June

28 July

11 August

21 September

Figure 4.1.4c. Photographs of Medicago sativa throughout the season

30 April

15 June

28 July

August 11

21 September

Figure 4.1.4d. Photographs of Matricaria recutita throughout the season

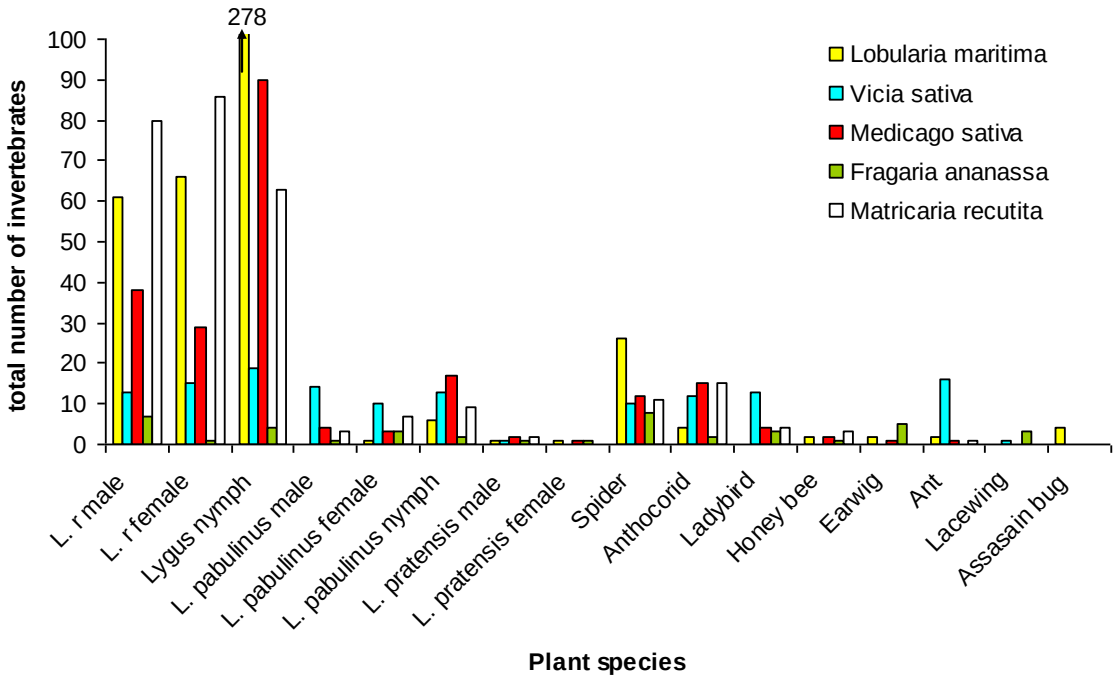


Figure 4.1.5. Total numbers of invertebrates on 4 potential trap crop plant species and strawberry. L.r = *Lygus rugulipennis*, L. pabulinus = *Lygocoris pabulinus*, L. pratensis = *Lygus pratensis*

Conclusions

- Numbers of *L. ruguipennis* peaked in August and September
- By 5 October numbers of *Lygus* had begun to decline
- The only beneficial invertebrate affected by plant species in this trial was earwigs. Higher numbers were found on strawberry plants (*Fragaria ananassa*) than any of the other four plant species
- *Lygus rugulipennis* was the dominant mirid found in the crop
- Significantly more male and female *L. rugulipennis* occurred on *M. recutita, L. maritime* and *M. sativa* than *V. sativa* or strawberry
- *Lygus* nymphs were much higher in *L. maritima* than any other plant species. They were also more abundant on *M. sativa, M. recutita* and *V. sativa* than strawberry

Experiment 2. Assessment of potential trap crops for *Lygus rugulipennis*

Materials and Methods

Experimental design

In 2009 a replicated experiment was set up at EMR to investigate the attractiveness of different plants to both *L. rugulipennis* and to beneficial species. Thus plant species selected were described in the literature as either attractive to capsids or to beneficial species. The plants used and sowing rates are shown in Table 4.1.3. Five of the treatments were annual plants and four were perennials. Because the perennials were unlikely to flower in the first year these four treatments were blocked separately from the annuals.

The plants were sown along the north/south boundaries of a strawberry planting at EMR, with annuals on the eastern boundary and perennials on the west. Each treatment plot was 4 x 2 m and there were no gaps between the treatment plots. There were five replicates of each treatment. Seeds were sown on 7 April 2009, and raked in; they were not irrigated. Plots were checked for germination on 13 May and 3 June, and for stage of growth on 1 and 22 July. The plots were weeded by hand on 16 and 17 June.

Table 4.1.3. Plants sown in experiment at EMR

Species	Common name	Reported as attractive to:	Sowing rate (g/m^2)	Type
Medicago sativa	Lucerne	capsids	6	P
Leucanthemum vulgare	Oxeye daisy	capsids	1	P
Silene dioecia	Red campion	beneficials	4	P
Echium vulgare	Vipers bugloss	beneficials	2	P
Borago officinalis	Borage	beneficials	5	A
Matricaria recutita	Mayweed	capsids	0.3	A
Vicia sativa	Vetch	capsids	4	A
Lobularia maritime	Alyssum (white)	capsids	0.1	A
Centaurea cyanus + *Anthemis arvensis* + *Chrysanthemum segetum*	Cornflower + Corn marigold + Corn chamomile	beneficials	3 + 1.5 + 0.4	A

Sampling of arthropods

Due to the slow growth of the perennial plants only the annual plants were sampled in 2009. Samples were 10 sweeps per plot taken on 16 June, 1, 22 and 31 July and 24 August. Arthropods were taken to the laboratory and sorted and identified.

Results

Germination of seeds

Naturally occurring weed seeds germinated in all plots making it difficult to be certain about germination of all of the sown seeds early in the experiment. However, by 13 May it was apparent that the mayweed, campion, lucerne and the flower mix were germinating well. The oxeye daisy, borage, and allysum did not germinate at all. The plots that had been sown with these seeds were subsequently herbicide treated to reduce weed pressure on the other plots. By 3 June the mayweed was beginning to flower. In the seed mixture only the corn chamomile was close to flowering on 13 May. By 1 July the mayweed was beginning to senesce, but the seed mix was still flowering well (mostly corn chamomile), and the vetch was beginning to flower. By 27 July the mayweed was senescing and the mix and vetch were flowering. At the time of the last sample on 24 August plants in all the plots were senescing.

Arthropod sampling

Numbers of *L. rugulipennis* and other capsids on the different plants are given in this section and numbers of beneficials in section 5.1. The three plant/plant mixes used flowered at different times so it was not possible to make a direct comparison of relative attractiveness on particular sample days. However, in season totals, lower numbers of *L. rugulipennis* adults were recorded on the plant mix than on mayweed and vetch. By the time the vetch began to flower, there were very low numbers of *C. norvegicus* in all the plots. However, in June and July significantly higher numbers were found on the mayweed compared with the mixture (Table 4.1.4) ($P<0.01$ for nymphs and $P<0.001$ for adults).

Table 4.1.4. Totals of different capsid species found on the sown plants in 50 sweeps on each sample date.

Date	Treatment	*L. rugulipennis*		*C. norvegicus*		Other capsids
		nymphs	adults	nymphs	adults	
16 June	mix	53	3	27	138	1
	mayweed	35	10	65	274	1
1 July	mix	7	7	0	46	26
	mayweed	18	54	0	85	15
22 July	mix	16	12	0	7	16
	mayweed	10	25	0	5	8
	vetch	20	56	0	16	29
31 July	mix	28	4	0	0	2
	mayweed	8	4	0	1	4
	vetch	20	18	0	1	3
24 Aug	mix	3	26	0	1	0
	mayweed	5	35	0	0	1
	vetch	35	61	0	0	3

Figure 4.1.5. Flower mix and mayweed plot at EMR on 17 July 2009.

Conclusions

- The three plant or plant mixes used flowered at different times so it was not possible to make a direct comparison of relative attractiveness on particular days.
- In season totals, lower numbers of *Lygus* adults were recorded on the plant mix than on mayweed and vetch.
- In June and July significantly higher numbers of *C. norvegicus* were found on the mayweed compared with the sown mixture

Experiment 3. Assessment of lucerne as a trap crop for *Lygus rugulipennis* (planted 2008)

A small scale experiment was done on lucerne planted in strawberry tunnels. The plots were in the western edge bed of 'Park West' strawberry plantation (cv. Albion) at Robert Boucher and Son, Newlands Farm, Teynham, Sittingbourne, Kent ME9 9JQ (NGR TQ 956 622, Fig. 4.1.1. *Lucerne plots 2008*), by kind agreement of Hugh Boucher. The rows were 80 m long and surrounded by cvs. Elsanta and Flamenco strawberry. The row spacing was 1.5 m. The crop was not protected when visited on 26 March, but had been covered with polythene tunnels by 20 May. The tunnels were still covered by the end of the trial (21 September). The plots were located in the first three tunnels (12 beds) on the south side of the plantation (Fig. 4.1.6).

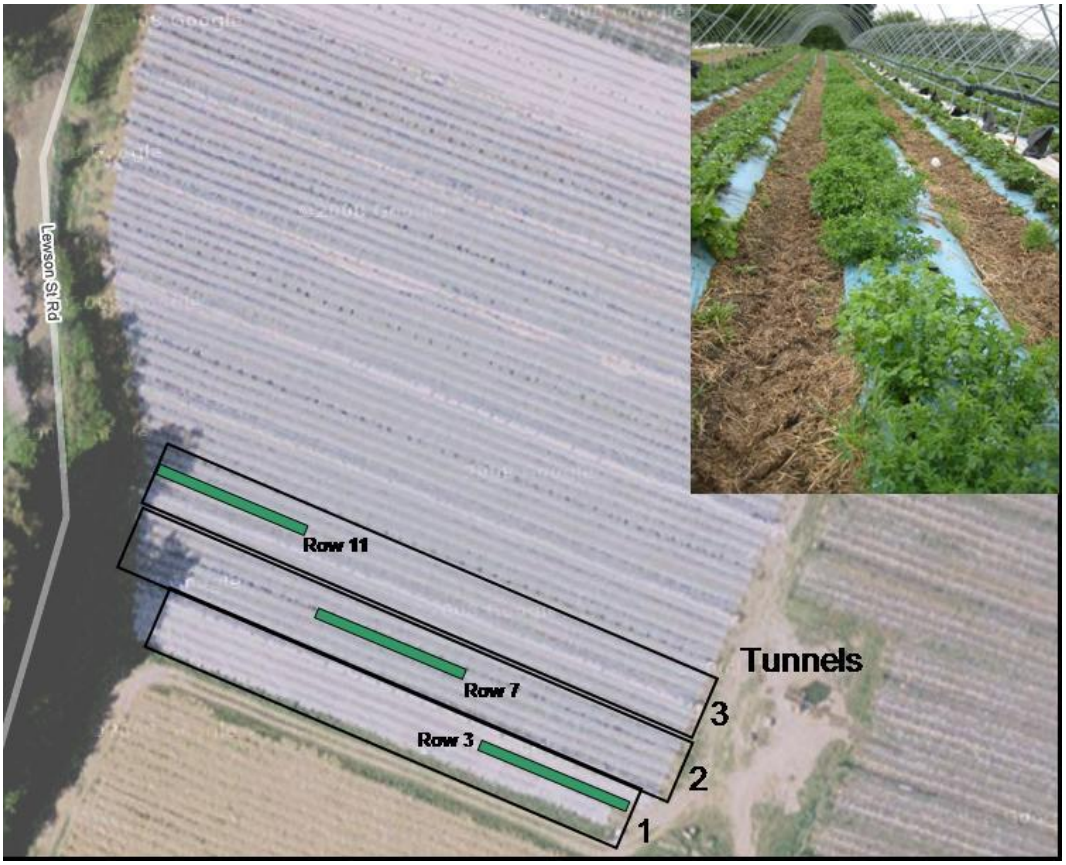


Figure 4.1.6. Location of the lucerne strips (green bars) sown within the strawberry plantation in 2008. Picture of plots on 30 April 2009 (top right)

The Lucerne was sown in 2008 in 20 m strips in the centre row of the first three tunnels (4 rows/tunnel, i.e. lucerne in row 3, 7, 11) (Fig. 4.1.6). Backup plants for gapping-up were sown in plugs in a glasshouse at EMR. The experimental area and adjacent strawberry beds remained unsprayed with insecticides until after 8 September when there was an overall spray of insecticides to control the capsids. The plots were assessed fortnightly and a record made of the growth stage of lucerne (photographs, Fig. 4.1.10). Twenty-five tap samples were taken from the centre of each plot and the adjacent strawberry beds into a bowl with one side cut out. Records were made of beneficial species (honey bees, bumble bees and predators). *Lygus* bugs were pooted into tubes for subsequent identification and sexing in the laboratory under a microscope. Between 30 April and 28 July, and 8-21 September the sampling regime in Fig. 4.1.7a was followed. When the mirids were at their peak the sampling was intensified (Fig. 4.1.7b).

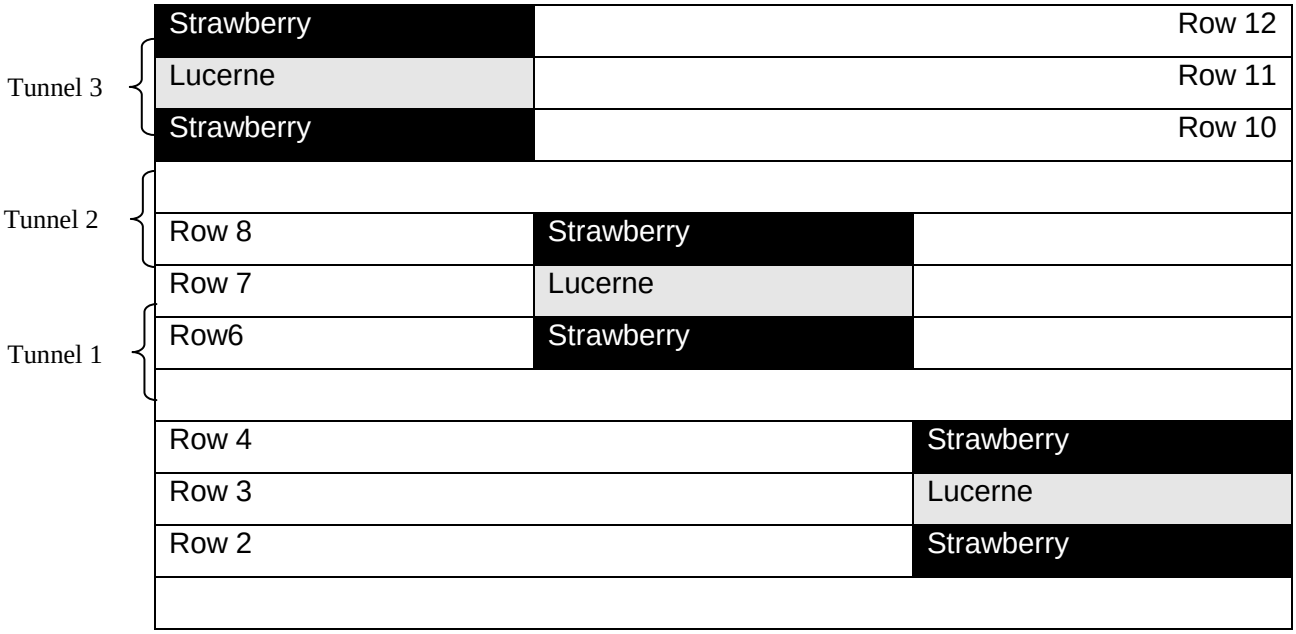


Figure 4.1.7a. Areas sampled between 30 April and 28 July, and 8-21 September

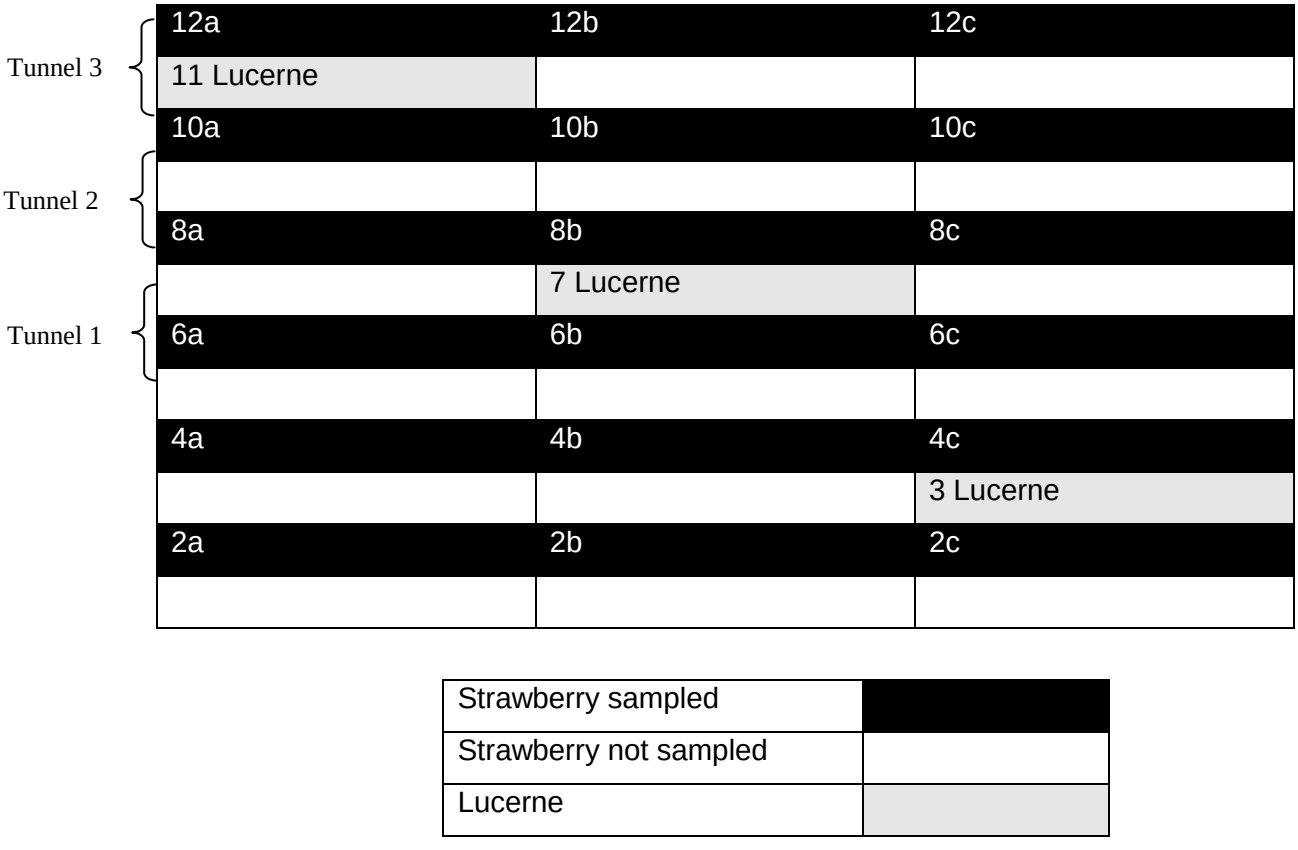


Figure 4.1.7b. Areas sampled on 12, 20 and 25 August

Results

The most abundant beneficial insects from the first sampling regime (Fig. 4.1.7a) were earwigs. As with the the previous studyon this site (Experiment 1, Fig. 4.1.4 & 4.1.7) more earwigs were found in strawberry than lucerne (ratio 1:13 in lucerne:strawberry). Very few *L. rugulipennis* were sampled from the plots through the season. Only 8 individuals were found by tap sampling.

The most common capsid was *Lygocoris pabulinus* (Table 4.1.5 & Fig. 4.1.9). There was very little difference between the numbers of other invertebrates on lucerne compared to strawberry, including *L. pabulinus.* The data was not considered extensive enough for statistical analysis, but is summarised below.

Table 4.1.5. Total numbers of beneficial invertebrates and *Lygocoris pabulinus* from tap samples (x25/plot), between 30 April - 28 July, and 8-21 September

Row	Crop	Spider	Anthocorid	Earwig	*L. pabulinus* male	*L. pabulinus* female	*L. pabulinus* nymphs
2	Strawberry	3	0	2	3	0	0
3	Lucerne	0	1	0	0	0	0
4	Strawberry	2	0	5	1	0	0
6	Strawberry	0	1	3	0	1	0
7	Lucerne	0	1	0	0	0	0
8	Strawberry	1	0	3	0	0	0
10	Strawberry	1	1	2	0	2	0
11	Lucerne	2	2	1	0	0	0
12	Strawberry	0	0	3	2	0	1

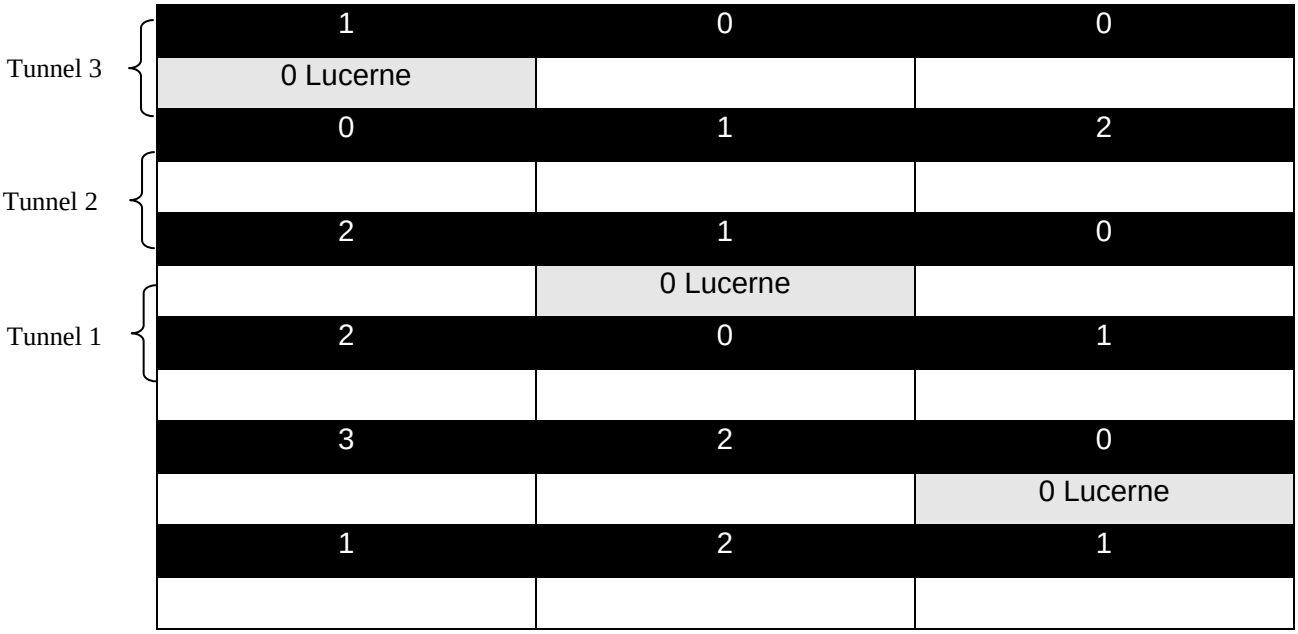


Figure 4.1.8. Numbers of earwigs sampled from the strips of lucerne and adjacent strawberry plants (12, 20 and 25 August)

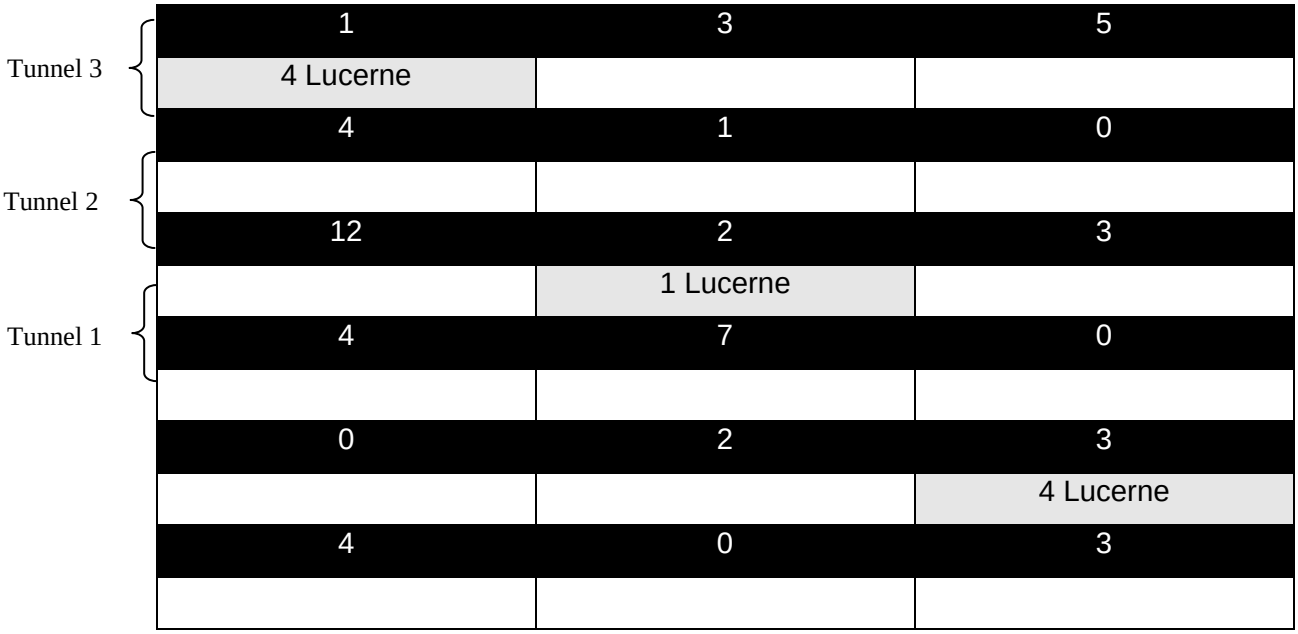


Figure 4.1.9. Total numbers of *Lygocoris pabulinus* sampled from the strips of lucerne and adjacent strawberry plants (12, 20 and 25 August)

15 May

12 June

7 July

12 September

Figure 4.1.10. Photographs of *Medicago sativa* throughout the season

Conclusions

- More earwigs were found in strawberry than lucerne (ratio 1:13 lucerne:strawberry)
- Very few *L. rugulipennis* were sampled from the plots through the season
- The most common capsid was *Lygocoris pabulinus*
- There was very little difference between the numbers of other invertebrates on lucerne compared to strawberry, including *L. pabulinus*

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

Summary

Four experiments were done at EMR; two were on a purpose sown plot of a mixture of mayweed and fat hen and the remaining two were on a strawberry planting. Dispensers with hexyl butyrate release rates ranging from 0.2-37 mg/day were used in these experiments. There was no significant effect of hexyl butyrate on *L. rugulipennis* distribution at the rates emitted from these dispensers when compared with the controls and thus no evidence of repellancy of this compound. There was also no effect of the hexyl butyrate treatment on the proportion of males and females present compared with the controls.

General methods

Four experiments were done to assess the effects of hexyl butyrate on *L. rugulipennis*; this chemical has been reported to repel capsids in other research. The first two experiments were done in a purpose sown plot of a mixture of mayweed and fat hen at EMR, and the third and fourth were done in a strawberry planting, also at EMR. Regular sweep samples were taken from the mayweed/fat hen plot and tap samples from strawberries in the strawberry planting through the summer to determine the optimal timing for this experiment, i.e. when numbers of *L. rugulipennis* adults were high on the plants. This occurred in August and September 2009. Different types of dispensers were used to obtain different release rates of hexyl butyrate. The rates of release were determined in a wind tunnel at 27°C by NRI.

Experiment 1

Methods

Two rates of release of hexyl butyrate were tested using a 1 ml pipette dispenser and a 0.2 ml pipette dispenser, both filled with 100 µl hexyl butyrate. The experiment was set up on 4 August 2009 in a randomised block design with five replicates of the two release rate treatments and a blank (no dispenser). The dispensers were held on 1 m canes secured in the ground, and treatments were at least 10 m apart. Around each dispenser or blank treatment, sampling was done within a 1m circle close to each supporting cane (centre) and also over a similar area 3 m away from the cane. For the outer sampling areas plants were sampled 3 m to the north, east, south and west of each dispenser separately. Plants were tapped over a washing up bowl of 26 cm in diameter to assess the numbers of capsids

present. For each tap plants were tapped five times over the bowl to ensure that all arthropods were dislodged. Six taps were done in each of the central areas and five taps were done in each of the outer areas. Tap samples were done on 4 August (pre-treatment), 6 and 12 August, and numbers of *Lygus rugulipennis* adults and nymphs were recorded. Each nymphal instar was recorded separately, but instars were combined prior to analysis. Other capsid species and beneficial insects were also recorded.

Results

Release rates of hexyl butyrate from the different dispensers in shown in Table 4.2.1. The release rates for dispensers used in experiment 1 were 0.9 and 0.2 mg/day for the 1 ml and 0.2 ml pipette respectively.

Overall comparisons by ANOVA on $\log_{10}(n+1)$ transformed data from the arthropod sampling showed that there was no difference in numbers of *L. rugulipennis* adults or nymphs in any of the areas sampled on either of the two post treatment sample dates. Numbers recorded in pre and post treatment sample dates are shown in Tables 4.2.2 and 4.2.3.

Table 4.2.1. Release rates of hexyl butyrate from different dispenser types.

Dispenser	Size	Amount	Temp	Mg/day	Ref
Hexyl butyrate					
standard vial		100ul	27°C	2.5	2008/78
white sachet	2.5 x 2.5	100ul	27°C	36.9	2008/78
1 ml pipette		100ul	27°C	0.9	2009/81
0.2 ml pipette		100ul	27°C	0.17	2009/81

Table 4.2.2. Total numbers of *Lygus rugulipennis* adults recorded at different distances from hexyl butyrate dispensers and controls in a plot of mayweed and fat hen in experiment 1 in 2009. (DAT = days after treatment)

	Pre-treatment 4 Aug Dispenser rate			6 Aug (2 DAT) Dispenser rate			12 Aug (8 DAT) Dispenser rate		
Position	control	0.9 mg/d	0.2 mg/d	control	0.9 mg/d	0.2 mg/d	control	0.9 mg/d	0.2 mg/d
Centre	-	-	-	0	0	0	3	1	5
East	2	0	2	0	2	1	2	0	3
North	4	1	3	1	0	1	0	0	0
West	2	1	2	2	1	0	2	2	5
South	2	1	1	1	2	2	1	0	1

Table 4.2.3. Total numbers of *Lygus rugulipennis* nymphs recorded at different distances from hexyl butyrate dispensers and controls in a plot of mayweed and fat hen in experiment 1 in 2009.

	Pre-treatment 4 Aug Dispenser rate			6 Aug (2 DAT) Dispenser rate			12 Aug (8 DAT) Dispenser rate		
Position	control	0.9 mg/d	0.2 mg/d	control	0.9 mg/d	0.2 mg/d	control	0.9 mg/d	0.2 mg/d
Centre	-	-	-	20	19	20	26	18	65
East	16	17	16	11	9	11	22	26	73
North	20	15	17	9	4	13	14	24	49
West	13	15	21	13	7	8	22	17	66
South	13	12	17	21	8	10	17	22	63

Experiment 2

Methods

Experiment 2 was done using the same plot and design as in Experiment 1, and was set up on 28 August 2009. The 1ml pipette dispensers were replaced with new 1ml pipette dispensers and the 0.2 ml pipette dispensers were replaced with a higher rate vial which was shown to release hexyl butyrate at 2.5 mg/day at 27°C (Table 4.2.1). Post-treatment tap samples were done on 1 and 7 September using the same sample areas as used in Experiment 1. *Lygus rugulipennis* adults and nymphs were recorded, as were other capsid species and beneficial insects.

Results

The dispensers used in this experiment released hexyl butyrate at 0.9 and 2.5 mg/day (Table 4.2.1). As there were two sampling occasions a split plot analysis was done; no transformation of the data was required. Results of treatments on adult *L. rugulipennis* are given in Table 4.2.4 and on nymphs in Table 4.2.5. There was no effect of treatment on numbers of *L. rugulipennis* collected. Mean numbers of adults overall were 1.74 in the control treatment, 1.78 in the 2.5 mg/d treatment and 1.46 in the 0.9 mg/d treatment (P=0.685); for nymphs the respective means were 1.26, 1.50 and 1.24 (P=0.829). There was no evidence of any reduction in numbers of adults or nymphs close to the dispensers compared with those 3 m away in any direction. For adults there was a slight suggestion that numbers were actually higher close to the dispensers (P=0.092).

Table 4.2.4. Mean numbers of *Lygus rugulipennis* adults collected at different distances from hexyl butyrate dispensers and controls in a plot of mayweed and fat hen in experiment 2.

	Dispenser type		
Position	control	0.9 mg/d	2.5 mg/d
Centre	1.5	2.0	2.6
East	1.8	1.3	1.2
North	1.2	1.4	1.4
West	2.1	1.0	2.1
South	2.1	1.6	1.6

Table 4.2.5. Mean numbers of *Lygus rugulipennis* nymphs collected at different distances from hexyl butyrate dispensers and controls in a plot of mayweed and fat hen in experiment 2.

	Dispenser type		
Position	control	0.9 mg/d	2.5 mg/d
Centre	1.4	1.8	1.7
East	0.6	1.2	0.7
North	2.0	1.0	1.7
West	1.3	1.1	2.0
South	1.0	1.1	1.4

Experiment 3

Methods

This experiment was set-up on 28 August 2009 on a first year everbearer strawberry planting of variety Evie 2 (plot EMR DM 183). 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. Beds were 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. The experimental design was a randomised block design with 6 replicates of both a high rate vial dispenser and a blank. Each replicate used one 40 plant strawberry plot. Alternate beds were used. The vial dispensers or blanks were held on dowels at 10 cm above the crop canopy and were placed in the middle of the plot. Sampling for *L. rugulipennis* was done over a white plastic saucer of 24 cm diameter by placing the saucer under each plant and tapping four or five times. Tap samples were done on eight plants in the centre of each plot, within 1 m of the dispenser, and on the eight plants at either end of the plot, at approximately 3m away from the dispenser (Figure 4.2.1). Tap samples were done on 28 August 2009 (pre-treatment) and on 1, 8 & 14 September. *Lygus rugulipennis* adults and nymphs were counted. Nymphs were recorded in two categories; 1 + 2 or 3 + 4 + 5 instars. Other capsid species and beneficial insects were also recorded.

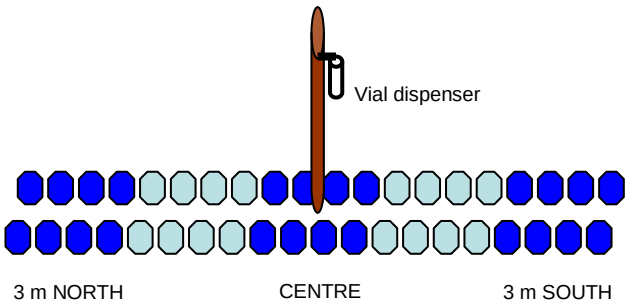


Figure 4.2.1. Sampling protocol in the strawberry planting.

Results

The dispensers used in this experiment released hexyl butyrate at 2.5 mg/d. Counts were compared with a repeated measures ANOVA. For adults and nymphs there was no significant difference between samples taken close to the dispensers compared to those at either 3 m north or south within the strawberry bed. Mean overall numbers of adults were 5.11 in the control and 5.44 in the hexyl butyrate treatment (P=0.624) and for nymphs the respective numbers were 3.70 and 3.69 (P=0.973). Numbers of *L. rugulipennis* adults and nymphs in samples taken from the different areas are shown in Tables 4.2.6 and 4.2.7. There was some evidence that numbers were higher in the southern samples than the north or central ones (P=0.068 for adults and 0.062 for nymphs); it is not clear why this should be the case.

Table 4.2.6. Mean numbers of *Lygus rugulipennis* adults collected close to and 3 m north or south of hexyl butyrate dispensers and controls in a strawberry planting in experiment 3.

	Dispenser type	
Position	control	2.5 mg/d
Centre	5.39	5.83
North + south	4.97	5.25

Table 4.2.7. Mean numbers of *Lygus rugulipennis* nymphs collected close to and 3 m north or south of hexyl butyrate dispensers and controls in a strawberry planting in experiment 3.

	Dispenser type	
Position	control	2.5 mg/d
Centre	2.89	3.44
North + south	4.11	3.81

Experiment 4

Methods

A second experiment was set up on EMR DM 183. Different plots were used for the treatments, but again with a randomised block design with 6 replicates. The same tap sampling strategy was used. Treatments were either high release clear sachets set 20 cm above the crop canopy (Fig 4.2.2), or a control.

The pre-treatment sample was done on 21 September after which the dispensers were attached. A post-treatment tap sample was done on 22 September. *Lygus rugulipennis* adults and nymphs were counted. Nymphs were recorded in two categories; 1 + 2 or 3 + 4 + 5 instars. A further tap sample was done on 25 September and an assessment of the proportion of male and female capsids present was made from this sample.

Figure 4.2.2. A high release sachet loaded with hexyl butyrate.

Results

The release rate of hexyl butyrate from the clear sachets was 36.9 mg/d (Table 4.2.1). Data were analysed as a split plot experiment, with date as the split plot. Overall mean numbers of *L. rugulipennis* adults were 4.89 in the control and 5.37 in the hexyl butyrate treatments; numbers for nymphs were 0.47 and 0.35. There was thus no effect of treatment on overall numbers of adults (P=0.598) or nymphs (P=0.625). There was no evidence that hexyl butyrate was repelling adults or nymphs from the area around the dispensers (P=0.540 and 0.476 for adults and nymphs respectively). Mean numbers in the different treatments are shown in Tables 4.2.8 and 4.2.9.

Table 4.2.8. Mean numbers of *Lygus rugulipennis* adults collected close to and 3 m north or south of hexyl butyrate dispensers and controls in a strawberry planting in experiment 4.

	Dispenser type	
Position	control	36.9 mg/d
Centre	4.75	5.83
North + south	4.96	5.14

Table 4.2.9. Mean numbers of *Lygus rugulipennis* nymphs collected close to and 3 m north or south of hexyl butyrate dispensers and controls in a strawberry planting in experiment 4.

	Dispenser type	
Position	control	36.9 mg/d
Centre	0.417	0.262
North + south	0.500	0.393

An alternative mechanism for the reported repellancy of hexyl butyrate is that the compound affects the behaviour of females such that they do not produce pheromone; this in turn could reduce the number of males in the area and thus in the samples. Proportions of male and female *L. rugulipennis* present in the last sample in 2009 were analysed using an angular transformation. Overall means of proportions of males present were 33.3 in the control and 44.2 in the hexyl butyrate treatment; this was not significantly different (P=0.198). There was also no effect of distance from the dispenser on proportions of male *L. rugulipennis* caught (P=0.247); proportions are given in Table 4.2.10.

Table 4.2.10. Proportion of male *Lygus rugulipennis* collected close to and 3 m north or south of hexyl butyrate dispensers and controls in a strawberry planting in experiment 4.

	Dispenser type	
Position	control	36.9 mg/d
Centre	24.8	45.8
North + south	37.6	43.4

The results from this experiment do not give any evidence to support the suggestion that hexyl butyrate affects the pheromone production of females.

Conclusions

- Dispensers were successfully designed to release different rates of hexyl butyrate
- There was no significant repellent effect of hexyl butyrate on *L. rugulipennis* at the rates emitted from these dispensers when compared with the controls.
- There was no significant difference in proportions of adult female and male *L. rugulipennis* in the hexyl butyrate treatment compared with the untreated control.

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

Material and methods

The trial was set up in 'Owens 3' everbearer strawberry plantation (cv. Elsinor) at Langdon Manor Farm, Goodnestone, Faversham, Kent ME13 9DA (Fig. 4.1.9, NGR TQ 024 593) by kind agreement of Alastair Brooks and his farm manager Andrew Reeve. The plots were marked out on 26 March and the grower planted the lucerne in a zig-zag pattern in the polythene mulched raised beds on 20 April.

Approximately 10 seeds were sown into each planting hole and lightly covered with soil. Back-up plants for plugging in gaps were sown at EMR in a glasshouse. The plots were weeded and gaps filled as necessary on each visit to the site.

The strawberry plants (everbearer, cv. Elsinor) were planted in April. The plantation was 88 x 90 m and consisted of 11 tunnels with 5 row beds. Three centre beds were used for the lucerne (44 x 1 m, i.e., half the length of bed). The beds were located in tunnels 1, 5 and 11 (Fig. 4.3.1 & 4.3.2). The polytunnel protection had been erected by 12 June. Trickle irrigation and feed was provided as per the strawberries.

Lucerne doesn't normally require nitrogen but does respond well to phosphate and potash fertiliser. In this experiment it was not necessary to apply granular fertiliser to the lucerne plots. The lucerne was cut to the ground on 24 August to encourage flowering and to attract capsids.

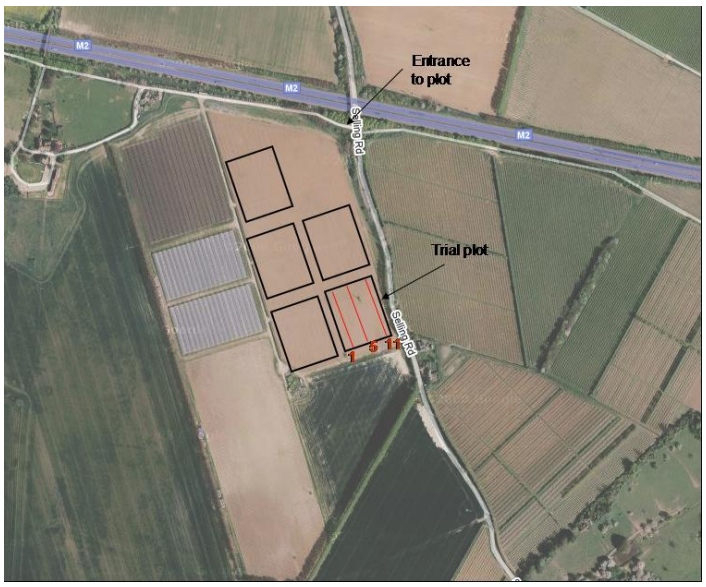


Figure 4.3.1. Location of plot used for trial at Langdon Manor Farm

The site was visited fortnightly by EMR staff and a record made of the growth stage of the lucerne and the strawberries. Plots (Fig. 4.3.2) were sampled from 30 April to 5 October. Three bug vac treatments were applied to the centre three lucerne and adjacent strawberry beds in each tunnel on 21 and 25 August and 4 September (Fig. 4.3.3). It was not possible to manoeuvre the machinery close to the tunnel edges, i.e. the first and fifth bed in each tunnel. An assessment of *L. rugulipennis* and beneficial invertebrates was made immediately before and after bug vaccing to determine the effect of the treatment.

Each of the three lucerne beds (Fig. 4.3.2 - grey) and the strawberry beds adjacent to the lucerne (yellow) were sampled. Half of strawberry beds (pink) in-between were also sampled. The sampling was done by sweeping using a washing up bowl with one side cut out (25 sweeps per plot).

The numbers of beneficial insects was recorded whilst on the plots (e.g., coccinellids, syrphids, earwigs, etc.). The samples of *L. rugulipennis* were pooted into tubes and identified under a microscope in the laboratory to check the species and sex of the insects.

Bed		Tunnel
1		
2		
3		1
4		
5		
6		
7		
8		2
9		
10		
11		
12		
13		3
14		
15		
16		
17		
18		4
19		
20		
21		
22		
23		5
24		
25		
26		
27		
28		6
29		
30		

31		
32		
33		7
34		
35		
36		
37		
38		8
39		
40		
41		
42		
43		9
44		
45		
46		
47		
48		10
49		
50		
51		
52		
53		11
54		
55		

Figure 4.3.2. Diagram of sampling plan. Beds planted with lucerne are highlighted in grey. The plot (11 tunnels) was not treated with insecticides

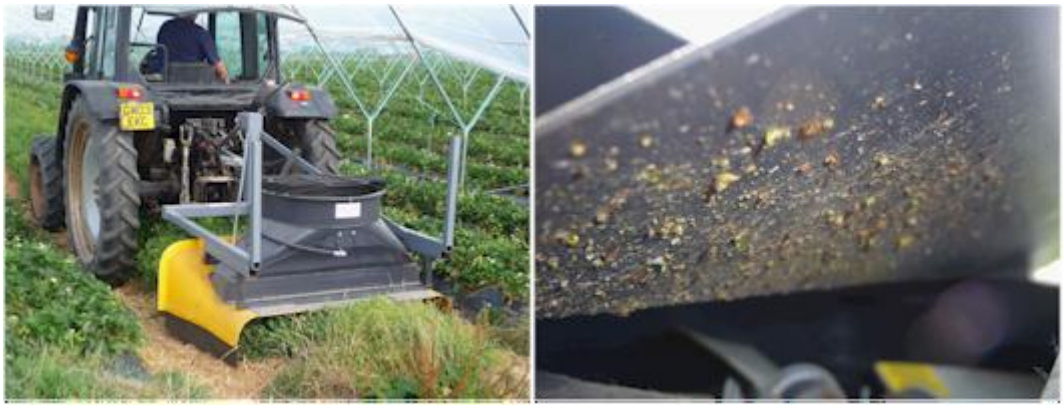


Figure 4.3.3. Bug vac travelling over the lucerne and damaged *Lygus* on the blades after the treatment

Results

At least double the numbers of *L. rugulipennis*, spiders and earwigs were found in strawberry compared to lucerne (Table 4.3.1). Anthocorids appeared to be more abundant in lucerne. There was no significant difference between the numbers of anthocorids, earwigs, lacewings, ladybirds, spiders or female *L. rugulipennis*.

Numbers of *Lygus* per 75 tap sample began to increase from the second week of July (Fig. 4.3.4). Numbers of nymphs reached a peak after mid August and then declined by late September. The adults sampled at this time were overwintering forms.

Numbers of nymphs on lucerne was much higher than on strawberry plants at the peak of the population, but because the lucerne was cut this sampling could not be replicated at the following assessment, two weeks later (Fig 4.3.5). In general adults were more abundant on the strawberry plants (Figs. 4.3.4. and 4.3.5.).

There was a significant reduction in numbers of male *L. rugulipennis* and *Lygus* nymphs on strawberry after bug vaccing (ANOVA $\log_{10(+1)}$ transformed data; p=0.043, df 2, sed 0.0635, lsd 0.2733; p=0.042, df 2, sed 0.0829, lsd 0.3566, respectively).

Beneficial insects were not significantly affected by the bug vac, but numbers were much lower than *Lygus*. Lygus were reduced by 39-61% on strawberry and 2-56% on lucerne (Table 4.3.2).

Table 4.3.1. Comparison of the numbers of the most abundant invertebrates in strawberry and lucerne (30 April – 5 October, data from post bug vaccing not included)

Row	Crop	L. r male	L. r female	Lygus nymph	Spider	Anthocorid	Earwig	Lacewing
2	Strawberry	20	18	36	11	10	0	4
3	Lucerne	6	12	26	13	14	0	8
4	Strawberry	16	10	58	21	15	0	8
9	Strawberry	10	18	59	17	6	2	8
17	Strawberry	5	13	39	26	5	1	7
22	Strawberry	14	29	34	18	4	2	3
23	Lucerne	10	11	25	5	3	0	0
24	Strawberry	14	13	61	16	2	0	8
29	Strawberry	4	8	35	10	2	0	6
34	Strawberry	14	10	40	7	0	1	1
42	Strawberry	22	16	29	13	3	0	8
47	Strawberry	8	14	25	9	7	2	6
52	Strawberry	24	18	42	7	13	12	8
53	Lucerne	6	3	27	1	9	1	5
54	Strawberry	10	6	55	11	8	8	4
	Total in strawberry	161	173	513	166	75	28	71
	Total in lucerne	**22**	**26**	**78**	**19**	**26**	**1**	**13**
	Total in strawberry/4	**40.25**	**43.25**	**128.25**	**41.5**	**18.75**	**7**	**17.75**

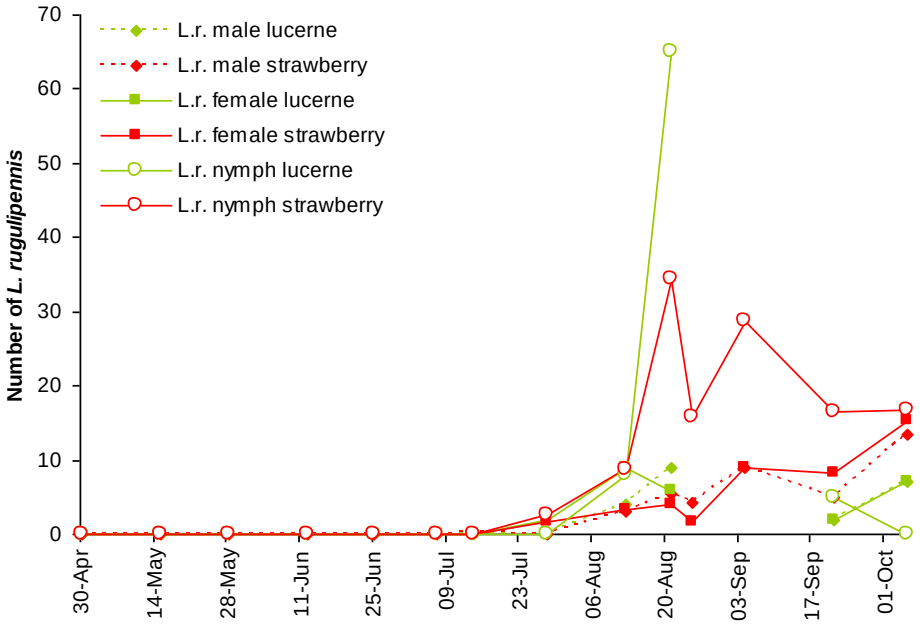


Figure 4.3.4. Numbers of male, female and nymph *L. rugulipennis* on lucerne and strawberry under polytunnel. NB: Number of capsids on strawberry divided by 4 for comparison with lucerne plots (3 x lucerne, 12 strawberry plots sampled). The lucerne was not sampled on two occasions in August and September, because it had been cut and was too short to tap sample

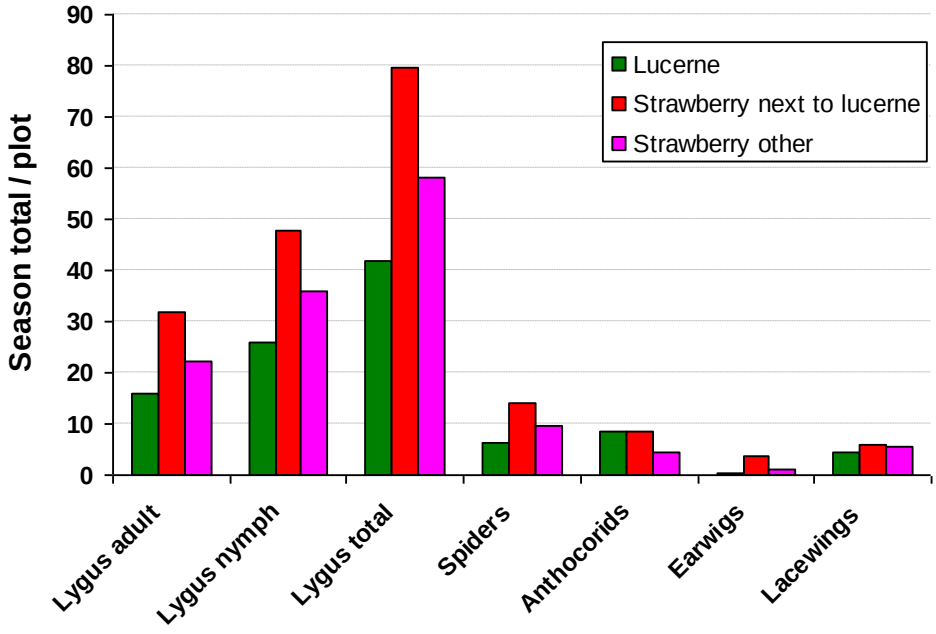


Figure 4.3.5. Comparison of the numbers of the most abundant invertebrates in strawberry and lucerne (30 April – 5 October, data from post bug vaccing not included)

Table 4.3.2. Total numbers of the most abundant invertebrates in strawberry and lucerne on 3 dates pre and post bug vaccing. The percentage of invertebrates remaining post bug vaccing is included. Lucerne was not sampled on 25 August and 4 September because it was too short to tap sample

Date	Sample	Crop	L. r male	L. r female	Lygus nymphs	Spider	Anthocorid	Ladybird	Earwig	Lacewing
21-Aug	pre	Strawberry	22	16	138	45	39	2	8	14
		Lucerne	9	6	65	7	21	0	1	2
21-Aug	post	Strawberry	10	12	62	16	17	1	1	7
		Lucerne	4	4	64	32	24	4	0	3
25-Aug	pre	Strawberry	17	7	63	15	6	1	7	24
25-Aug	post	Strawberry	11	16	33	14	12	0	4	6
04-Sep	pre	Strawberry	36	36	134	30	11	0	3	18
04-Sep	post	Strawberry	14	8	37	20	15	0	1	1
All dates	pre	Strawberry	75	59	335	90	56	3	18	56
	post	Strawberry	35	36	132	50	44	1	6	14
All dates	**% remaining**	**Strawberry**	**47**	**61**	**39**	**56**	**79**	**33**	**33**	**25**
21-Aug	**% remaining**	**Lucerne**	**44**	**67**	**98**	**457**	**114**	**400**	**0**	**150**

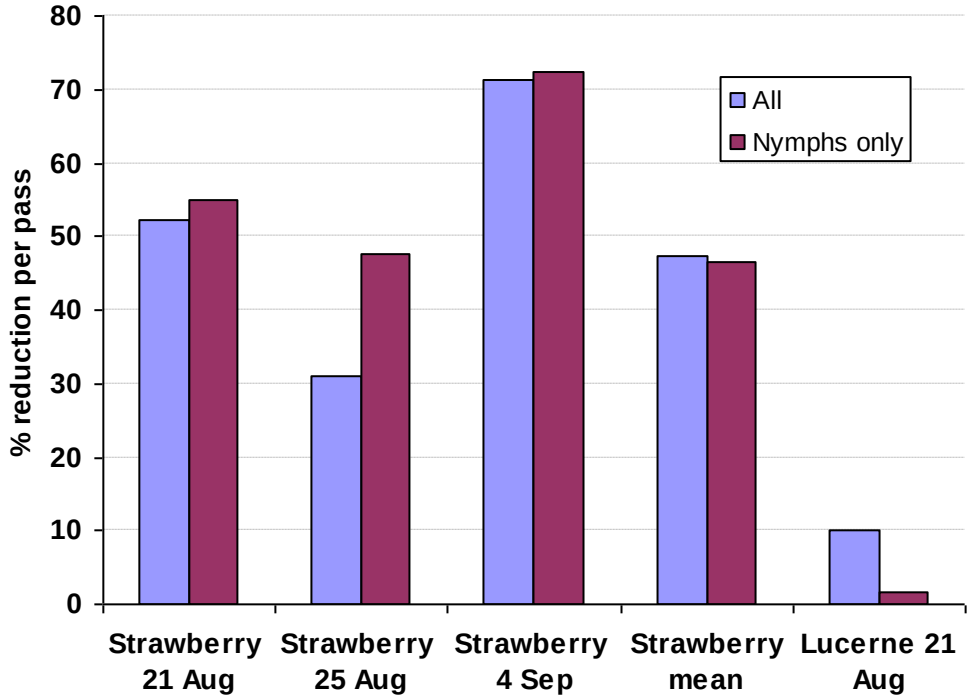


Figure 4.3.6. % reductions per pass in Lygus sp (mainly L. rubgulipennis) numbers as a result of bug vaccing in strawberry and lucerne

15 May

12 June

28 July

11 August

9 September – post cutting

Figure 4.3.7. Photographs of Medicago sativa throughout the season

Conclusions

- At least double the numbers of *L. rugulipennis*, spiders and earwigs were found in strawberry compared to lucerne
- Numbers of *Lygus* began to increase from the second week of July. Numbers of nymphs reached a peak after mid August and then declined by late September
- In general, adult *Lygus* were more abundant on the strawberry plants
- There was a significant reduction in male *L. rugulipennis* and *Lygus* nymphs on strawberry after bug vaccing
- Beneficial insects were not significantly affected by the bug vac
- *Lygus* were reduced by 39-61% on strawberry and 2-56% on lucerne (Table 4.3.2)

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

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

Summary

In the second year of an experiment on a commercial plantation very low numbers of arthropods were collected from red campion and ox eye daisy. There was thus no evidence of attractiveness of these plants to predators and parasitoids of aphids. In the experiment sown in 2009, the three annual plant/plant mixes that germinated flowered at different times, so it was not possible to make a direct comparison of relative attractiveness of the three treatments on particular sample days. However, in season totals there were no significant differences in numbers of anthocorid nymphs or Orius nymphs and adults on the three treatments. Higher season totals of anthocorid and coccinellid adults were found on vetch, while highest numbers of coccinellid larvae were found on the flower mix. These results may be due to a combination of plant flowering period and the developmental stage of the predator present at that time. Highest numbers of chrysopid larvae were found on mayweed.

Methods

2008 experiment continuation

Medicago sativa, (lucerne), *Silene dioica* (red campion) *Galium verum* (lady's bedstraw), *Leucanthemum vulgare* (ox eye daisy), and a mixture of *Centaurea cyanus* (cornflower) + *Chrysanthemum segetum* (corn marigold) + *Anthemis arvensis* (corn chamomile) were sown in a replicated block design around a commercial strawberry plantation in 2008 (see report for 2008).

Experimental design

The experiment was set up around a newly planted strawberry crop at Robert Boucher & Son of Newlands Farm, Teynham, Sittingbourne, Kent, ME9 9JQ. The seeds were sown into a prepared bed 2 m wide. Each plot was 4 x 2 m. There were five treatments replicated four times in a randomised blocks design. There was no gap between the different plots. The seeds were sown by hand on 16 May 2008 and soil was raked over to cover them. The two perennial plant species, ox eye daisy and red campion, did not flower in 2008, and were retained for sampling in 2009 (see Table 5.1.1). The remaining plots were herbicide treated early in 2009. Thus in 2009 there were two treatments replicated four times.

Table 5.1.1. Species retained from 2008 for assessment of their attractiveness to beneficial arthropods

Species	Common name	Sowing rate (g/m^2)
Silene dioica	Red campion	3
Leucanthemum vulgare	Ox eye daisy	0.5

Arthropod sampling

Samples were taken on 30 April, 29 May, 13 June and 6 July from each treatment plot. The first and last samples were taken with a sweep net (10 sweeps per plot) and the other two by tap sampling over a bowl (10 taps per plot). Arthropods were returned to the laboratory and identified under a stereomicroscope. On 30 April and 6 July samples were also taken from nettle beds close to the experimental planting. No samples were taken after 6 July as the campion was senescing and producing seed and other weeds had invaded the treatment plots.

Results

Very low numbers of predators were found on the perennial plants or on the nettles (Table 5.1.2). There was no evidence of attractiveness of these plants to predators and parasitoids of aphids.

2009 experimental design

In 2009 a replicated experiment was set up at EMR to investigate attractiveness of different plants to both *L. rugulipennis* and to beneficial species. Details of experimental design and sampling protocols are given in section 4.1, with details of attractiveness to predators and parasitoids reported in this section.

Results

The three annual plant/plant mixes that germinated all flowered at different times so it was not possible to make a direct comparison of relative attractiveness of the three treatments on particular sample days for the whole experiment. Season totals of each species were compared by ANOVA after a $\log_{10n+1}$ transformation.

There were no significant differences in numbers of anthocorid nymphs or Orius nymphs and adults on the three treatments over the whole season. Significantly higher season totals of anthocorid adults ($P<0.01$) and coccinellid adults ($P=0.005$) were found on vetch, while significantly higher numbers of coccinellid larvae ($P<0.01$) were found on the flower mix. These results may be due to a combination of plant flowering period and the developmental stage of the predator present at that time. Significantly higher numbers of chrysopid larvae ($P<0.05$) were found on mayweed.

During mid-late July the mayweed was beginning to senesce, but the seed mix was still flowering well (mostly corn chamomile), and the vetch was beginning to flower. The 22 July sample date is the only one where all three treatments were flowering. On this date there were significantly higher numbers of anthocorid and coccinellid adults on the vetch (Table 5.1.3).

Table 5.1.2. Total numbers of arthropods collected at Newlands Farm from red campion and ox eye daisy in 40 sweeps (30 April and 6 July) or 30 taps 29 May and 13 June). Nettle sample was 20 sweeps on 30 April and 30 on 6 July

Treatment	Date	Anthocorid		Orius spp	Coccinellid		Chrysopid	Spiders	Parasitoids
		nymphs	adults	adults	larvae	adults	larvae		
Red campion	30.04.09	0	1	0	0	1	0	1	2
	29.05.09	5	1	0	1	0	0	2	0
	13.06.09	0	0	0	0	0	0	1	2
	06.07.09	0	0	0	0	0	0	0	0
Oxeye daisy	30.04.09	0	0	0	0	0	0	0	4
	29.05.09	1	1	4	1	0	0	0	3
	13.06.09	7	6	5	0	0	1	0	3
	06.07.09	0	0	0	0	0	0	1	0
Nettle	30.04.09	0	0	1	0	1	0	1	1
	06.07.09	0	0	0	0	1	0	1	3

Table 5.1.3. Total numbers of most abundant beneficial species collected from annual plants in 50 sweeps on each date										
Date	Treatment	Anthocorid		Orius spp		Coccinellids		Chrysopid	Spiders	Parasitoids
		nymphs	adults	nymphs	adults	larvae	adults	larvae		
16 June	mix	0	0	0	26	63	5	0	1	18
	mayweed	0	1	0	22	25	0	4	1	20
1 July	mix	0	0	0	22	3	7	1	4	60
	mayweed	0	0	2	10	3	11	6	6	127
22 July	mix	2	1	14	29	0	7	1	4	32
	mayweed	1	4	8	34	0	9	2	9	74
	vetch	1	24	6	18	0	43	0	6	55
31 July	mix	5	0	13	6	0	4	0	4	13
	mayweed	0	3	1	20	0	1	2	4	50
	vetch	1	15	2	35	0	35	0	3	121
24 Aug	mix	1	0	3	9	0	0	1	14	7
	mayweed	2	1	7	7	0	0	0	5	9
	vetch	9	20	14	39	0	7	0	17	86

Conclusions

- In the second year of an experiment on a commercial plantation very low numbers of arthropods were collected from red campion and ox eye daisy. There was thus no evidence of attractiveness of these plants to predators and parasitoids of aphids.
- In the experiment sown in 2009 the three annual plant/plant mixes that germinated flowered at different times so it was not possible to make a direct comparison of relative attractiveness of the three treatments on particular sample days.
- There were no significant differences in total numbers of anthocorid nymphs or Orius nymphs and adults on the three treatments throughout the season.
- Higher season totals of anthocorid and coccinellid adults were found on vetch.
- Highest numbers of coccinellid larvae were found on the flower mix.
- Highest numbers of chrysopid larvae were found on mayweed.
- These results may in part be due to a combination of plant flowering period and the developmental stage of the predator present at that time.

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

Summary

Low numbers of beneficial species were caught in water traps containing lures of different plant volatiles in May and June in strawberry or outside the cropping area. There was no evidence to suggest that any of the volatiles were attractive at the rates used at this time of year. It is possible that few beneficial species were present at this time. In an experiment undertaken in August, higher numbers of hoverflies were caught in traps containing lures of germacrene or phenyl ethanol, but there was no effect of any of the volatiles tested at the rates of release used on any other beneficial species.

Materials and methods

Experimental design

To determine if plant volatile lures could be used to increase numbers of beneficial species in strawberry plantations a series of experiments were undertaken to assess the effects of these lures on catches of aphid predators and parasitoids in water traps. The traps were 14 cm diameter Petri dish bases, 2 cm deep, with a raised central unit to allow a volatile dispenser to be attached above the water. The inside of the dish was painted yellow and the outside matt black (see figure).

Where the assessments were made in strawberry plantings traps were held above the crop canopy on 60 cm dowels placed in the raised beds. Where the experiment was set up outside strawberry plantings the traps were approx 60 cm above the ground surface. Each trap contained water with a small amount of detergent to make any arthropods caught sink to the bottom of the trap. Arthropods caught were removed from the water on collection dates and held in 70% ethanol until they were identified in the laboratory.

Figure 5.2.1. Water trap used in the plant volatile experiments

Experiment 1:

Methods

The experiment was set up in two separate unsprayed strawberry plantings and in two non-cropping areas with natural weed growth at EMR. One replicate of each of treatments 1 to 15 (Table 5.2.1) were placed in the strawberry plantings and 1 replicate of each of treatments 1 to 9 in each weed area. Lures were loaded with 100µl of the relevant volatile. Traps were put out on 20 May, spaced at least 10 m apart, and arthropods were collected on 26, 27, 29 May and 1, 5 June.

Table 5.2.1. Volatile treatments used for experiment 1

Treatment number	Volatile	Dispenser	Release rate
1	Blank	-	-
2	methyl salicylate	Sachet	High
3	methyl salicylate	Vial	Low
4	ocimene	Sachet	High
5	ocimene	Vial	Low
6	germacrene	Sachet	High
7	germacrene	Vial	Low
8	caryophyllene	Sachet	High
9	caryophyllene	Vial	Low
10	(*E,Z*)-2,4-nonadienal	Sachet	High
11	(*E,Z*)-2,4-nonadienal	Vial	Low
12	cis-jasmone	Sachet	High
13	cis-jasmone	Vial	Low
14	farnesene	Sachet	High
15	farnesene	Vial	Low

Release rates of volatiles from different dispensers were measured in a wind tunnel at NRI.

Results

Release rates of volatiles are given in Table, showing the effectiveness of using vials and sachets to provide low and high release rates.

Table 5.2.2. Release rates of volatiles from different dispensers at constant temperatures.

Dispenser	Size	Amount	Temp	mg/day	Ref
methyl salicylate					
standard vial		100ul	22°C	1.3	2008/39
clear sachet	5x5	100ul	22°C	17	2008/39
white sachet	2.5x2.5	100ul	22°C	14	2009/006
cis-jasmone					
clear sachet	2.5x5	100ul	22°C	2.3	2009/006
white sachet	2.5x2.5	100ul	22°C	1.9	2009/006
(*E,Z*)-2,4-nonadienal					
clear sachet	2.5x5	100ul	22°C	11.6	2009/006
white sachet	2.5x2.5	100ul	22°C	7.7	2009/006
farnesene					
clear sachet	5x5	100ul	22°C	2.1	2008/039
white sachet	2.5x2.5	100ul	27°C	4	
vial		100ul	22°C	0.55	2008/039
phenylethanol					
clear sachet	5x5	100ul	22°C	1.8	2009/066
caryophyllene					
White sachet	2.5x2.5	100ul	22°C	4.6	2008/005

Chrysopids, coccinelids, staphylinids, spiders, predatory bugs and syrphids were recorded in low numbers throughout this experiment in traps either in strawberry or outside the cropping area. Numbers were too low for formal statistical analysis. For example, coccinelids were caught but there was a maximum of two per trap on any collection date and only 12 were caught across all traps for the duration of the experiment. It is possible that few beneficial species were present at this time in the season.

Experiment 2.

Methods

A second experiment to assess the attractiveness of plant volatiles to beneficial insects was set up in a grass/weed plot in the organic area at EMR, using the same style of water traps as were used in experiment 1. Four replicates of 10 treatments (Table 5.2.3), all at high release rates, were set up on 7 August 2009. Traps were placed 10 m apart. The dispensers were changed on 26 August 2009. The traps were emptied on 10, 14, 17, 21 & 28 August and 1, 4 & 7 September.

Figure 5.2.2. Experiment 2 was set up in a grass/weed plot

Table 5.2.3. Volatile treatments for Experiment 2			
Treatment number	**Volatile**	**Dispenser**	**Release rate**
1	blank	-	-
2	farnesene	Sachet	High
3	methyl salicylate	Sachet	High
4	phenyl ethanol	Sachet	High
5	cis-jasmone	Sachet	High
6	(*E,Z*)-2,4-Nnnadienal	Sachet	High
7	ocimene	Sachet	High
8	germacrene	Sachet	High
9	caryophyllene	Sachet	High
10	Blank	-	-

Results

A range of beneficial species were caught in the traps, but generally in low numbers. Only one predatory anthocorid adult was caught during this experiment and coccinellids, Orius spp, spiders and chrysopids were caught in low numbers (Table 5.2.4). Numbers were too low for formal statistical analysis. Parasitoids were caught in all traps, including those without synomone lures, indicating that there was no effect of any lure on parasitoid behaviour. However, there was an effect of lure on numbers of hoverflies caught, with significantly higher numbers in the phenyl ethanol and germacrene treatments (Table 5.2.5).

Table 5.2.4. Total numbers of beneficial species caught in water traps baited with plant volatiles (total of 8 sampling dates)						
Treatment	coccinellid	Orius spp	lacewing	hoverfly	parasitoid	spider
Blank	2	1	3	12	34	4
farnesene	2	1	0	12	38	4
methyl salicylate	4	1	1	14	47	5
phenyl ethanol	5	0	2	27	42	7
cis-jasmone	5	0	2	11	58	5
(*E,Z*)-2,4-nonadienal	2	2	2	1	45	13
ocimene	1	1	1	7	33	6
germacrene	5	1	5	32	45	4
caryophyllene	4	0	3	6	34	9
Blank	1	0	1	9	64	3

Table 5.2.5. Mean numbers of hoverflies caught in water traps baited with plant volatiles		
	Transformed mean numbers of hoverflies	Actual mean numbers of hoverflies
Blank	0.454	2.62
farnesene	0.476	3.00
methyl salicylate	0.540	3.50
phenyl ethanol	0.838	6.75
cis-jasmone	0.496	2.75
(*E,Z*)-2,4-nonadienal	0.075	0.25
ocimene	0.369	1.75
germacrene	0.762	8.00
caryophyllene	0.270	1.50
F prob	0.032	
sed	0.1785	
lsd (28 df)	0.3656	

Conclusions

- In these field experiments the only beneficial species that was attracted to any of the plant volatiles tested was adult hoverflies. It is not clear if this lack of response is due to the rate of release of the volatiles.
- More work is needed to assess the effects of a range of release rates on selected 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)

Methods

A large scale, randomised block, experiment to test end-of-growing season sprays with selective insecticides to control *Chaetosiphon fragaefolii* was done in a commercial (June-bearer) strawberry plantation in 'Churchfield' at Langdon Manor Farm, Goodnestone, Faversham, Kent ME13 9DA (NGR TR 047 615) by agreement of the proprietor Alastair Brook (Fig. 5.3.1). The strawberry field was 3.35 ha and planted in May 2008. The crop was grown on the usual raised polythene mulched beds which ran approximately in an E-W direction. The field sloped gently to the west. There were 66 beds of the variety Sonata in the northern half of the plantation and roughly the same number of the cultivar Elsanta in the southern half of the plantation. Sixty adjacent beds (1 m wide, 140 m long) of each variety were used for the trial, the 60 northern most of cv. Sonata and the 60 southernmost of cv. Elsanta. The beds were 1.65 m apart and the plants 0.35 m apart in the beds (planting alternated; single, double, single, double.... and so on, along the bed).

Treatments were single sprays of Calypso (thiacloprid) applied in autumn 2008 at 3 different timings at 2 week intervals (Table 5.3.1). Sprays were applied with the grower's Berthoud Puma air assisted sprayer, operated by the grower's spray operator at the farm's normal spray volume (400 l/ha). The sprayer covered five beds with two air spouts (jets) per bed. Each air spout was furnished with two air-shear nozzles. The sprayer calibration was checked and spray operations were supervised by Adrian Harris (EMR).

Table 5.3.1. Treatments applied in autumn 2008

Trt no	Colour code	Product	Active ingredient	Dose rate (/ha)	Timing of application
1	Red	Calypso	480 g/l thiacloprid SC	250 ml	4 Oct 08
2	Blue	Calypso	480 g/l thiacloprid SC	250 ml	19 Oct 08
3	Yellow	Calypso	480 g/l thiacloprid SC	250 ml	4 Nov 08
4	Green	Untreated	-	-	-

A randomised block design with six replicates was used, with three replicates in the cv. Sonata and three replicates in the cv. Elsanta. Plots were five beds wide and ran the full length of the plantation and were adjacent (plot 101 northernmost). Calypso had a SOLA for use on protected strawberry (1497 of 2004) and outdoor strawberry (2727 of 2003). The maximum individual dose was 250 ml/ha, the maximum dose per season 500 ml/ha and the harvest interval was three days.

Table 5.3.2. Randomisation of treatments to plots

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

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

Total numbers of *C. fragaefolii* present on 25 strawberry leaves per plot were recorded on 7 April 2009 (Fig. 5.3.1). Each leaf sampled was 4 m apart in the plot, except block 6 which were 2 m apart. Samples were collected and examined under a microscope in the laboratory to confirm species. A note was made of any other species present, e.g., *Aphis gossypii* and *Myzus ascalonicus* are also important pests of strawberry. No crop destruction was required for this trial.

Figure 5.3.1. Plot on the assessment date (7 April 2009) and aphids on the underside of a strawberry leaf

Results

The actual volume of spray applied for each application was between 101-104% of the required amount. The weather conditions were suitable for the spray operations to be conducted (Table 5.3.3).

Table 5.3.3. Air temperature and humidity conditions at the time of spray application

Date	At beginning of spray applications					At end of spray applications				
	h	Temp (˚C)		RH%	Windspeed (km h^{-1})	h	Temp (˚C)		RH%	Windspeed (km h^{-1})
		Dry bulb	Wet bulb				Dry bulb	Wet bulb		
4 Oct 08	07:53	8	8	100	2	08:40	7	7	100	4
19 Oct 08	09:40	10	8.5	81	6 gust 9	10:05	10	8.5	81	gust 9
4 Nov 08	09:40	11	11	100	2	10:10	11	11	100	2

All of the Calypso application timings in autumn 2008 significantly reduced the numbers of aphids present on the strawberry plants in the following spring compared to the control (ANOVA Log_{10} (+1) transformed data, F pr. <001, sed 0.1873, lsd 0.3992) (7 April 09). In addition, a spray of Calypso in early October was more effective than an application in either mid October of the beginning of November (Fig. 5.3.2). Samples bought back to the laboratory confirmed that the majority of the aphids were *C. fragaefolii*, but there were a small number of *Myzus persicae var. nicotianae* present.

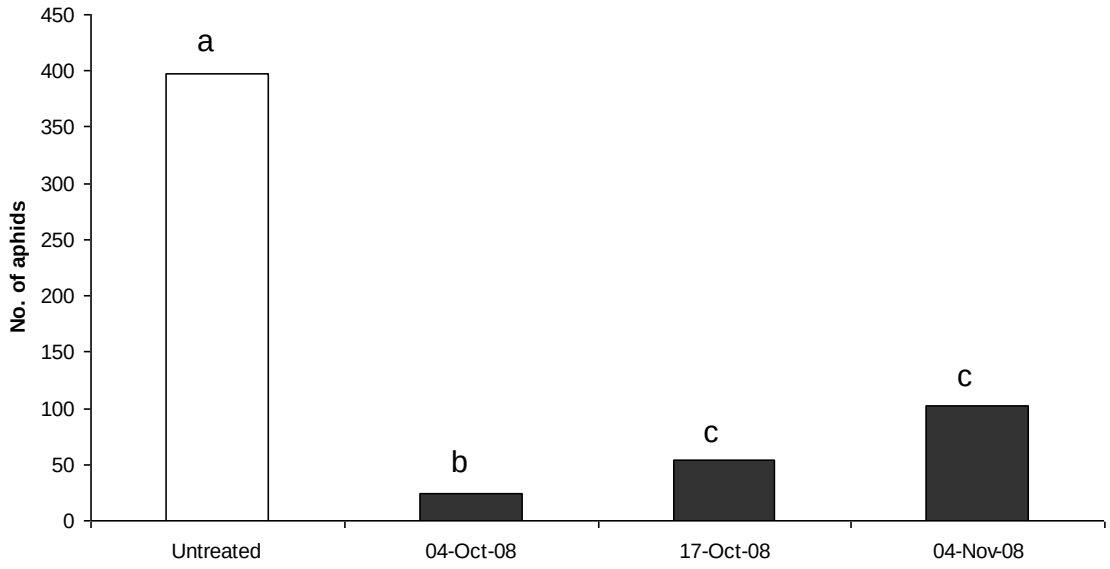


Figure 5.3.2. Mean number of aphids per treatment (25 leaves sampled per plot). Different letters mark significant differences between the treatments and the untreated control

Conclusions

- Autumn sprays of Calypso significantly reduced the numbers of aphids present on the strawberry leaves the following spring
- A spray at the beginning of October was more effective than an application in mid October or early November

2009/2010 experiment

A second trial was begun in 2009 for assessment in spring 2010 (spray application have been applied). The protocol is summarised below (ORETO No. 09/026)

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 four 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 'Caravan' strawberry plantation at Arnold Farm, Langley where the trial is to be 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 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 8 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	Hutchinson's (Paul Hamlyn, Graham Waters)	

Figure 1. Farm plan of plantations at Arnold Farm, showing 'Caravan' where the trial is being done in red surround

Figure 2. Google map aerial photo of Arnold Farm showing 'Caravan' plantation marked (red box)

Treatments

Treatments will be single sprays of Calypso (thiacloprid) applied in the autumn at four different timings at 2 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	4th week Sept
2	White	Calypso	480 g/l thiacloprid SC	250 ml	2nd week Oct
3	Blue	Calypso	480 g/l thiacloprid SC	250 ml	4th week Oct
4	Yellow	Calypso	480 g/l thiacloprid SC	250 ml	2nd week Nov
5	Green	Untreated	-	-	-

Spray application

Sprays to be applied with the growers 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). The spray operations are to be supervised by a member of this study team. The sprayer calibration is to be checked on first spraying occasion.

Experimental design and layout

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

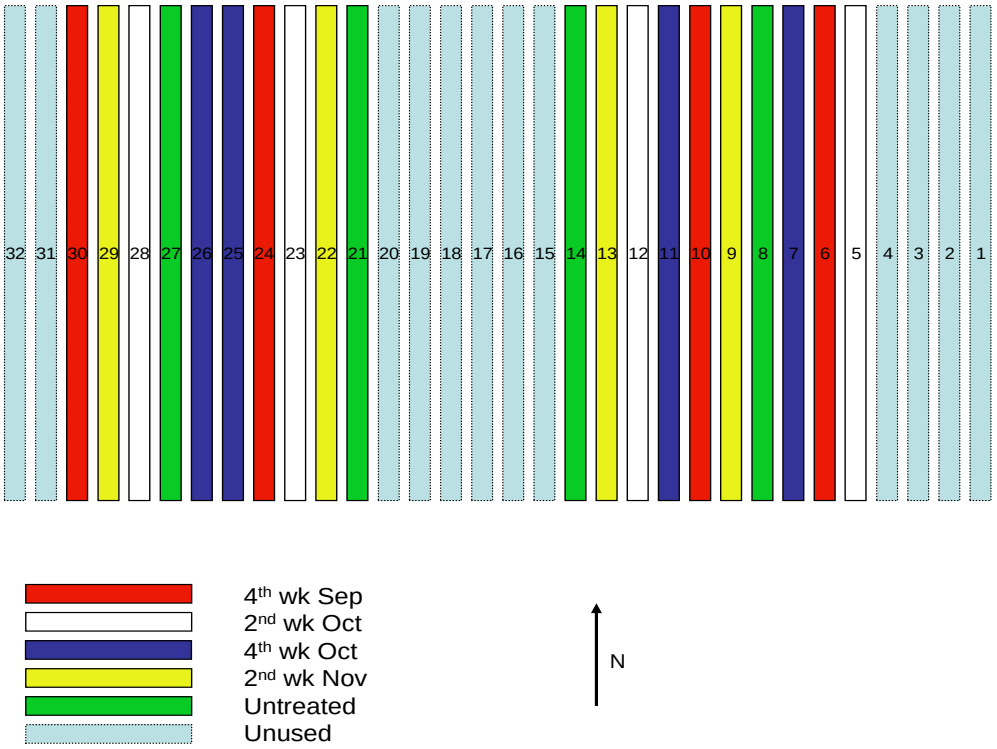


Figure 3. Layout of plots in tunnels in Caravan field. Note tunnels 1-4, 15-20, 31-32 are not used (tunnels 15-20 were planted at a different time)

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 3 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 3 days.

Meteorological records

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

Assessments

Two assessments to be done as follows:

1. Autumn 2009: In mid November 2009, 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 will 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)

Parasitised *C. fragaefolii* were collected from organic strawberry crops in Herefordshire in May 2009. Parasitised mummies were isolated and adult parasitoids were allowed to emerge in the laboratory. Fifty-five percent of the mummies were hyper-parasitised by *Alloxysta* sp. (Hymenoptera: Charipidae). Emerging *Aphidius* keyed out as *Aphidius eglanteriae* (Hymenoptera: Braconidae). Cultures are in place at BCP Certis.

Once the hyper-parasitoids had been screened out, *A. eglanteriae* were reared on *C. fragaefolii* on strawberry plants. At 17ºC min : 27 ºC max, parasitised mummies were observed nine days after oviposition and adults emerged after a further six days (egg to adult 15 days).

Adult *A. eglanteriae* were presented to a number of different aphid species that can occur on strawberries. These were *Aphis gossypii, Myzus persicae, Acyrtosiphon malvae* (rogersii), *Macrosiphum rosae* and *Macrosiphum euphorbiae.* The different aphid species were

placed in petri dishes (8 cm diameter), with about 100 aphids per dish. A single female *Aphidius eglanteriae* was placed in each dish and observed for up to five hours. The parasitoids were then removed and the aphids placed either on plants or on leaves (in petri dishes) and observed after two weeks. Oviposition was observed in *Myzus persicae* and *Macrosiphum euphorbiae*, but there was no successful parasitism by *A. eglanteriae* in any of the five aphid species. Aphid mortality was between 7 and 10%.

Aphidius eglanteriae mummies and adults were sent to Viridaxis S.A., a Belgian company using hydrogel beads as artificial media for rearing aphid parasitoids. *Aphidius eglanteriae* showed no interest in ovipositing in the beads without aphid hosts. With aphid hosts fed on artificial media, there was a very low level of parasitism, but insufficient to maintain the population. Viridaxis believe that modification of the diet would be required to achieve parasitism.

In addition, at EMR parasitised *C. fragaefolii* mummies from the original collection from Herefordshire were removed from the strawberry leaves and put in a cage and left to emerge. Hyperparasitoids, when seen, were removed from the cage. Emerged *Aphidius eglanteriae* were introduced to a culture of *C. fragaefolii* on potted strawberry plants in a Perspex culture box with side and front vents. The culture was maintained in a CT room at 20°C, and checked and watered weekly. Additional *C. fragaefolii*-infested plants were added as required, i.e. when high numbers of adult parasitoids were present; this was normally on a two-weekly cycle. Parasitoids were provided to BCP in early June, 11 August and 16 September 2009. Maintenance of the culture is ongoing as a backup for cultures at BCP Certis.

Conclusions

- Initial tests show that *A. eglanteriae* is fairly specific to *C. fragaefolii* and does not attack the other main aphid species commonly found on strawberry. The only other host species found in the literature is *Longicaudus trirhodus*, a species alternating between rose in winter and aquilegia / thalictrium in summer.
- Field collected *A. eglanteriae* were heavily hyper-parasitised in May and further investigation into this may be required.
- Mass rearing using artificial media is not currently possible.
-

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

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

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

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

Summary

Two further replicated field experiments were conducted in the UK in 2009 to develop a highly attractive 'super trap' for strawberry blossom weevil based on synergising the weevil's aggregation pheromone with host plant volatiles and utilising the weevils' attraction to white flowers. In experiment 1, the host plant volatile PV2 from wild strawberry flowers (at 3 release rates of 2, 15 and 126 mg/day) and the leaf volatiles TMTT and caryophylene were tested alone and in mixtures as synergists of the strawberry blossom weevil sex aggregation pheromone. In experiment 2, a range of designs of funnel traps with cross vanes were evaluated.

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

- The P+PV2 high lure captured more weevils the other P+PV2 lures and there was a trend to increasing catch with increasing PV2 release rate
- Neither caryophylene nor TMTT showed any noticeable synergism of the aggregation pheromone lure. There was a trend to greater numbers of non-target arthropods being captured with increasing release rate of PV2
- The trap design with the cross vanes coated with fluon captured more *A. rubi* than the other designs, suggesting that the fluon was beneficial and increased the numbers of weevils that fell into the trap.
- Traps with white cross veins captured similar numbers of non-target arthropods, except the new AgriSense *Byturus* trap which was furnished with an excluder grid over the top of the funnel. The green and yellow Agralan traps captured less non-target arthropods.

This work together with the previous years work suggests that the 'supertrap' to be evaluated in the remaining years of the project should incorporate a white cross vanes funnel trap (cross vanes coated with fluon and with grid to exclude non-target arthropods), a standard aggregation pheromone lure and a high release rate PV2 lure. The trap should be stood on the ground to maximise weevil catches.

Methods

Two experiments to develop the strawberry blossom weevil (SBW) super trap were conducted in 2009 as follows:

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

Standard SBW polythene sachet aggregation pheromone lures containing 100 µl of Grandlure I, Grandlure II and Lavandulol in a 1:4:1 ratio supplied by International Pheromone Systems were used throughout.

Sites

Site 1. Haygrove Ltd, Redbank Farm, Little Marcle Rd, Ledbury, Herefordshire HR8 2JL (Contact person: Graham Moor, FAST). Replicate 1 was in 'Sunflower' plantation (2 ha) at NGR SO 666 390. It was planted with Evie 2 everbearer strawberries in April 2009. Replicate 2 was in 'New Grass Field' plantation (2.5 ha) at NGR SO 676 385. It was planted with Evie 2 on 30 March 2009.

Site 2: Hall-Hunter Farms, organic site at Tuesley Farm, Milford, near Godalming. The fields were adjacent to railway station in Milford (kind agreement of Anna Costa. The FAST advisor was Rob Cook. Field SF2b planted with Florence in 2008 was used for replicates 3 and 4.

Treatments

Experiment 1 (synergists): The first experiment evaluated three strawberry host plant volatiles (PV2, TMTT and caryophylene) as potential synergists of the *A. rubi* aggregation pheromone. PV2, which had been shown to be a highly active synergist in the previous years work, was tested at three release rates (Table 6.3.1. and 6.3.2.). Modified AgriSense funnel traps with 16 cm tall Correx white cross vanes were baited with a standard strawberry blossom weevil aggregation pheromone lure supplied by International Phereone Systems (polythene sachet containing 100 µl of Grandlure I, Grandlure II and Lavandulol in a 1:4:1 ratio) and/or host plant volatiles, as shown in Tables 6.3.1. and 6.3.2. See figure 6.3.1. for photographs of lures and traps.

Table 6.3.1. Treatments in experiment 1 (synergists)

Trt.	*A rubi* aggregation pheromone lure	Plant volatile lure(s)
A	standard	none
B	standard	PV2 low
C	standard	PV2 standard
D	standard	PV2 high
E	none	TMTT
F	standard	TMTT
G	none	caryophylene
H	standard	caryophylene
I	standard	PV2 standard+TMTT
J	standard	PV2 standard+ caryophylene
K	standard	PV2 standard+TMTT+caryophylene

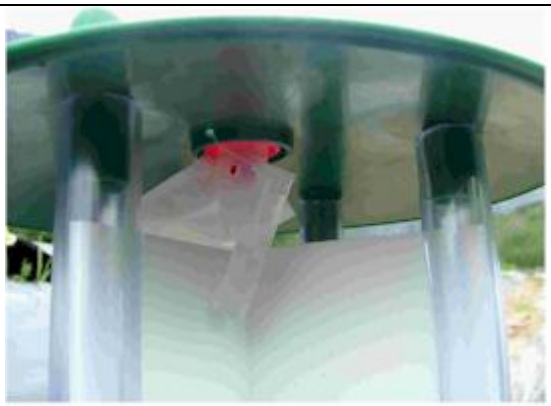
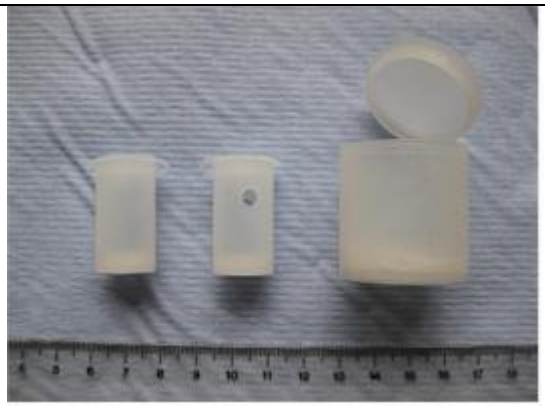
Table 6.3.2. Lures used in experiment 1 (synergists)

Lure name	Active substance	Loading	Dispenser	Release rate
PV2 low	Coded	0.5 g	Closed polyethylene vial 31 mm long, diameter 17 mm (no side holes)	2 mg/day
PV2 standard	Coded	0.5 g	Closed polyethylene vial 31 mm long, diameter 17 mm with two ~2mm diameter side holes	12 mg/day
PV2 high	Coded	3.0 g	Large open polythene vials (3 cm x 3 cm)	126 mg/day
TMTT	TMTT	50 µl	polythene vial (22 x 8 a 1 mm)	0.5 mg/day
Caryophyllene	caryophyllene	100 µl	Standard polythene vials 22mm x 9mm & a volume of 0.5ml. Cigarette filter	4.6 mg/day

Experiment 2 (trap designs): The attractiveness of different trap designs baited with standard aggregation pheromone+PV2 lures for strawberry blossom weevil adults as well as contamination of traps by non-target arthropods was evaluated. This experiment was done alongside experiment 1 above. Treatments are given in Table 6.3.3. and lure formulations in Table 6.3.4.

Table 6.3.3. Treatments in experiment 2 (trap designs)			
Trt.	Funnel trap design	Cross vanes	Trap surface
L	AgriSense	White Correx 16 cm tall	Standard
M	AgriSense	White Correx 16 cm tall	Roughened with sandpaper
N	AgriSense	White Correx 16 cm tall	Coated with fluon
O	Agralan	White moulded	Standard
P	Agralan	Yellow moulded	Standard
Q	Agralan	Green moulded	Standard
R*	AgriSense	White moulded cross veins	Standard

Table 6.3.4. Lures in experiment 2 (trap designs)				
Lure	Active substance	Loading	Dispenser	Release rate
PV2 standard	Coded	0.5 g	Closed polyethylene vial 31 mm long, diameter 17 mm with two ~2mm diameter side holes	12 mg/day
Aggregation pheromone	Lavandulol:Grandlure II:Grandlure I 1:4:1	100 µl	Polythene sachet (IPS)	1.8 mg/day

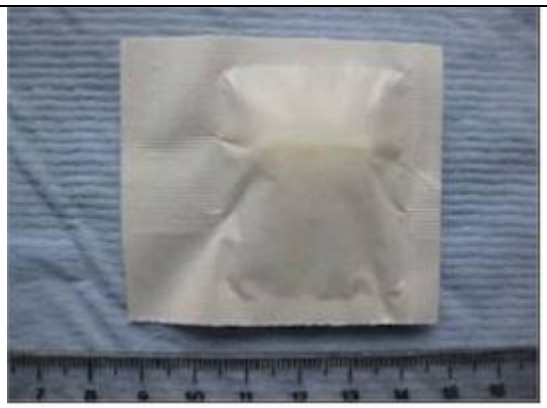


Figure 6.3.1. Top left) Agrisense funnel traps with white Correx cross vanes; top right) close up of aggregation pheromone sachet and polythene vial for PV2; middle left) polythene vials for low, medium and high release rate of PV2; middle right) Agralan funnel trap with white cross veins; bottom left) International Pheromone Systems A. rubi aggregation pheromone sachet

Experimental designs

Randomised complete block experimental designs with four replicates were used for each experiment. The two experiments were done simultaneously. Two replicates were located at site 1 and two at site 2. The traps, spaced at least 20 m, were placed at edges of the strawberry fields adjacent to hedgerows and other strawberry blossom weevil overwintering sites.

Assessments

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

Results

Release rate of PV2

Release rates of PV2 from the polyethylene vials were measured in the laboratory windtunnel at NRI at in 2008, 27°C, 8 km/h windspeed. The release rate from closed vials with no holes was ~2 mg/day (low release rate) and from standard release rate vials with 2 holes was ~15 mg/day (see year 1 project report). Measurements on the large open vials were not made.

Experiment 1 (synergists)

Only very small numbers of strawberry blossom weevil adults were captured over the whole season and almost none at the Milford site (Figure 6.3.2.). Numbers of males and females captured were similar. Most weevils were captured in the latter half of May and early June (Table 6.3.5.). Data were too small for meaningful statistical analysis. Season totals were calculated and examined for trends. The P+PV2 high lure captured markedly more weevils than the other P+PV2 lures and there appeared to be a strong trend towards an increased catch with increasing PV2 release rate. Neither caryophylene nor TMTT showed any noticeable synergism of the aggregation pheromone lure.

Much larger numbers of non-target arthropods were captured (Figure 6.3.3.). Diptera predominated, followed by Coleoptera. There were small numbers of honeybees and bumble bees. There was a trend towards greater numbers of non-target arthropods being captured with increasing release rates of PV2.

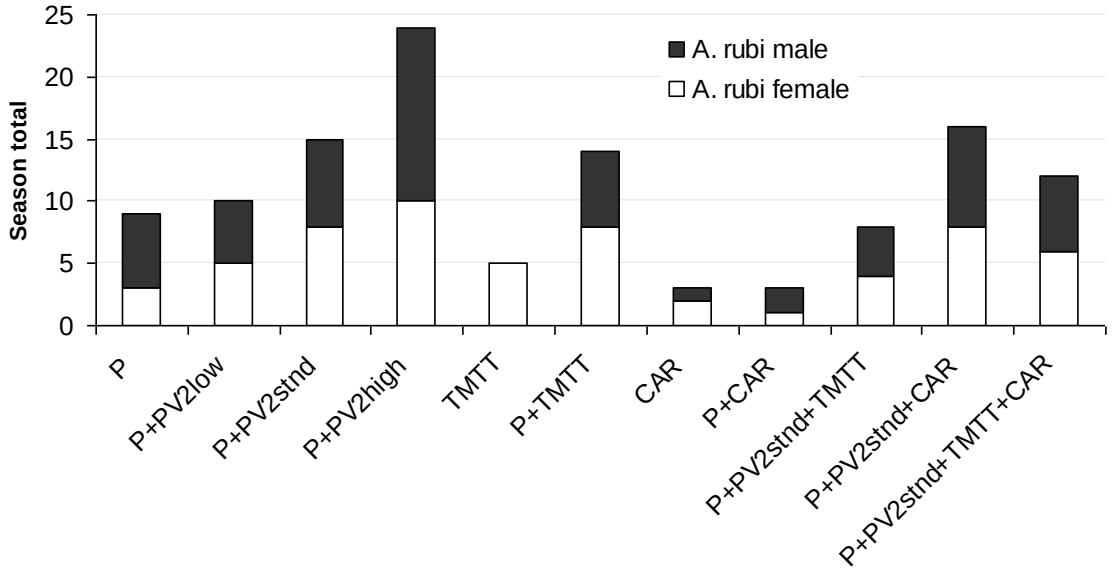


Figure 6.3.2. Total numbers of *A. rubi* adults captured between April and August 2009 in white cross vane funnel traps baited with different lures: P = aggregation pheromone, CAR = caryophylene

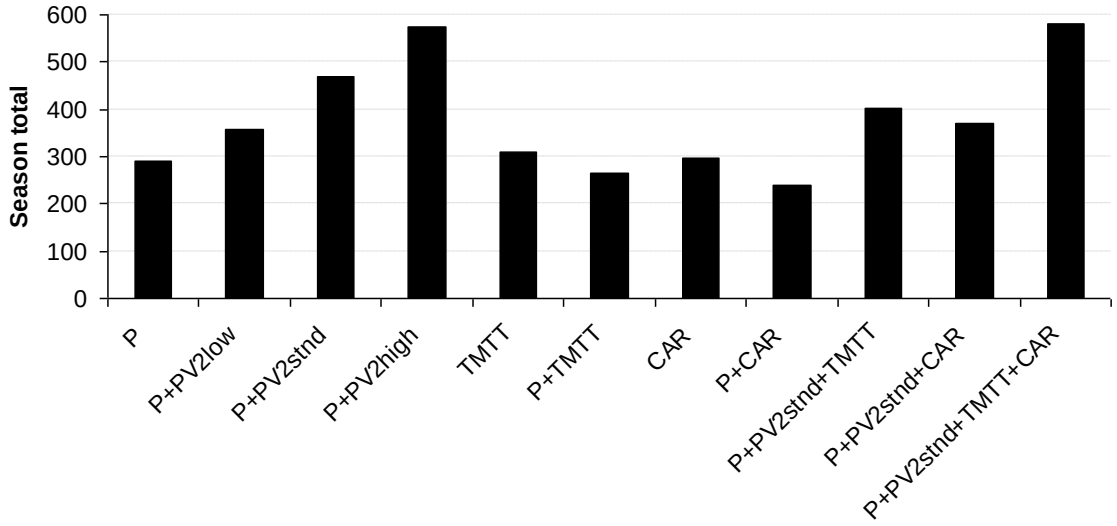


Figure 6.3.3. Total numbers non-target arthropods captured between April and August 2009 in white cross vane funnel traps baited with different lures: P = aggregation pheromone, CAR = caryophylene

Table 6.3.5. Total numbers of *A. rubi* captured by treatment and date at in Experiment 1 (synergists) at Milford and Hereford

Date	P	P+PV2low	P+PV2stnd	P+PV2high	TMTT	P+TMTT	CAR	P+CAR	P+PV2stnd+TMTT	P+PV2stnd+CAR	P+PV2stnd+TMTT+CA	P	Total
						Milford							
08 Apr	0	0	0	0	0	0	0	0	0	0	0		**0**
15 Apr	0	0	0	0	0	0	0	0	0	0	0		**0**
23 Apr	0	0	0	0	0	0	0	0	0	0	0		**0**
29 Apr	0	0	1	0	0	1	0	0	0	0	0		**2**
06 May	1	0	2	0	0	0	0	0	0	1	0		**4**
12 May	0	0	0	0	0	0	0	0	0	0	0		**0**
20 May	0	1	0	0	0	0	0	0	0	0	0		**1**
28 May	0	0	0	0	0	0	0	0	0	0	0		**0**
02 Jun	0	0	0	0	0	0	0	0	0	0	0		**0**
09 Jun	0	0	0	0	0	0	0	0	0	0	0		**0**
12 Jun	0	0	0	0	0	0	0	0	0	0	0		**0**
30 Jun	0	0	0	0	0	2	0	0	0	0	0		**2**
07 Aug	0	0	0	0	0	0	0	0	1	0	0		**1**
18 Aug	0	1	0	1	0	0	0	1	0	0	0		**3**
Total	**1**	**2**	**3**	**1**	**0**	**3**	**0**	**1**	**1**	**1**	**0**		
						Hereford							
16 Apr	0	0	0	2	0	1	0	1	0	0	0		**4**
23 Apr	1	0	0	1	0	2	0	0	1	0	1		**6**
29 Apr	1	0	0	0	0	1	0	0	0	0	0		**2**
07 May	1	0	1	0	0	1	1	0	2	1	0		**7**
13 May	3	0	2	3	1	1	1	0	0	2	0		**13**
22 May	0	1	1	2	0	0	0	0	0	0	0		**4**
29 May	1	2	5	6	1	1	0	1	0	6	2		**25**
05 Jun	0	1	0	4	0	0	0	0	1	3	2		**11**
12 Jun	0	1	0	0	0	0	0	0	1	0	0		**2**
19 Jun	0	0	0	2	0	2	0	0	0	0	2		**6**

25 Jun	0	0	0	0	0	0	0	0	0	0	0	**0**
02 Jul	0	1	2	2	1	0	0	0	0	1	2	**9**
09 Jul	0	1	1	1	1	1	0	0	1	1	1	**8**
16 Jul	1	0	0	0	0	0	0	0	0	0	1	**2**
24 Jul	0	0	0	0	0	1	1	0	1	0	0	**3**
29 Jul	0	1	0	0	1	0	0	0	0	1	0	**3**
04 Aug	0	0	0	0	0	0	0	0	0	0	1	**1**
12 Aug	0	0	0	0	0	0	0	0	0	0	0	**0**
Total	**8**	**8**	**12**	**23**	**5**	**11**	**3**	**2**	**7**	**15**	**12**	

Experiment 2 (trap designs)

Once again, numbers of *A. rubi* captured were very small and too low for meaningful statistical analyses (Figure 6.3.4.). Most weevils were captured in the latter half of May and early June (Table 6.3.6.). Season totals were calculated and examined for trends. Interestingly, the trap design with the cross vanes coated with fluon captured more *A. rubi* than the other designs.

The traps with white cross vanes captured similar numbers of non-target arthropods, except the new AgriSense *Byturus* trap which was furnished with an excluder grid over the top of the funnel. The green and yellow Agralan traps captured less non-target arthropods (Figure 6.3.5.).

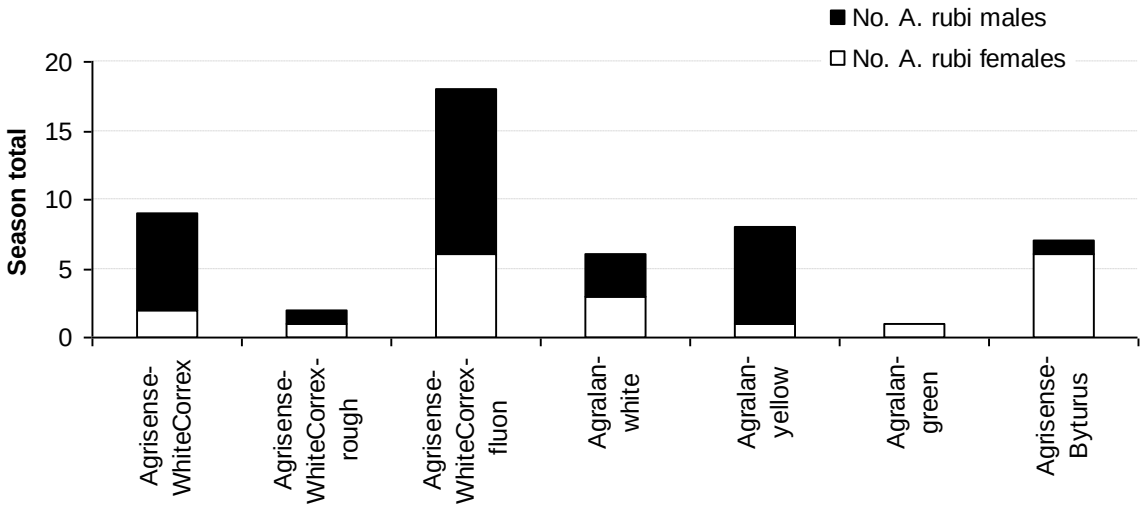


Figure 6.3.4. Total numbers of *A. rubi* adults captured between April and August 2009 in different designs of cross vane funnel traps baited with aggregation pheromone and PV2 standard lures

Table 6.3.6. Total numbers of *A. rubi* captured by treatment and date at in experiment 2 (trap designs) at Milford and Hereford

Date	Agrisense-WhiteCorrex	rough	fluon	Agralan-white	Agralan-yellow	Agralan-green	Agrisense-Byturus	Total
				Milford				
08 Apr	0	0	0	0	0	0		**0**
15 Apr	0	0	0	0	0	0		**0**
23 Apr	0	0	0	0	0	0		**0**
29 Apr	0	0	0	0	0	0	0	**0**
06 May	0	1	1	0	0	1	1	**4**
12 May	0	0	0	0	0	0	0	**0**
21 May	0	0	0	2	1	0	1	**4**
28 May	0	0	0	0	0	0	0	**0**
02 Jun	0	0	0	0	0	0	0	**0**
09 Jun	0	0	0	0	0	0	0	**0**
23 Jun	0	0	0	0	2	0	0	**2**
30 Jun	1	0	0	0	0	0	0	**1**
07 Aug	0	0	1	1	0	0	0	**2**
18 Aug	0	0	0	1	0	0	0	**1**
Total	**1**	**1**	**2**	**4**	**3**	**1**	**2**	
				Hereford				
08 Apr	0	0	0	0	0	0		**0**
16 Apr	0	0	1	0	0	0	1	**2**
23 Apr	1	0	0	0	0	0	0	**1**
29 Apr	0	0	2	0	0	2	0	**4**
07 May	0	0	0	0	0	0	0	**0**
13 May	1	0	3	0	0	0	0	**4**
22 May	1	0	2	0	0	0	0	**3**
29 May	2	0	1	0	0	0	0	**3**

05 Jun	1	0	2	0	1	1	1	**6**
12 Jun	0	0	2	0	0	1	2	**5**
19 Jun	0	0	0	0	0	0	0	**0**
25 Jun	0	0	0	0	0	0	0	**0**
02 Jul	2	0	0	0	2	0	0	**4**
09 Jul	0	0	3	2	2	0	1	**8**
16 Jul	0	1	0	0	0	0	0	**1**
24 Jul	0	0	0	0	0	0	0	**0**
29 Jul	0	0	0	0	0	0	0	**0**
04 Aug	0	0	0	0	0	0	0	**0**
12 Aug	0	0	0	0	0	0	0	**0**
Total	**8**	**1**	**16**	**2**	**5**	**4**	**5**	

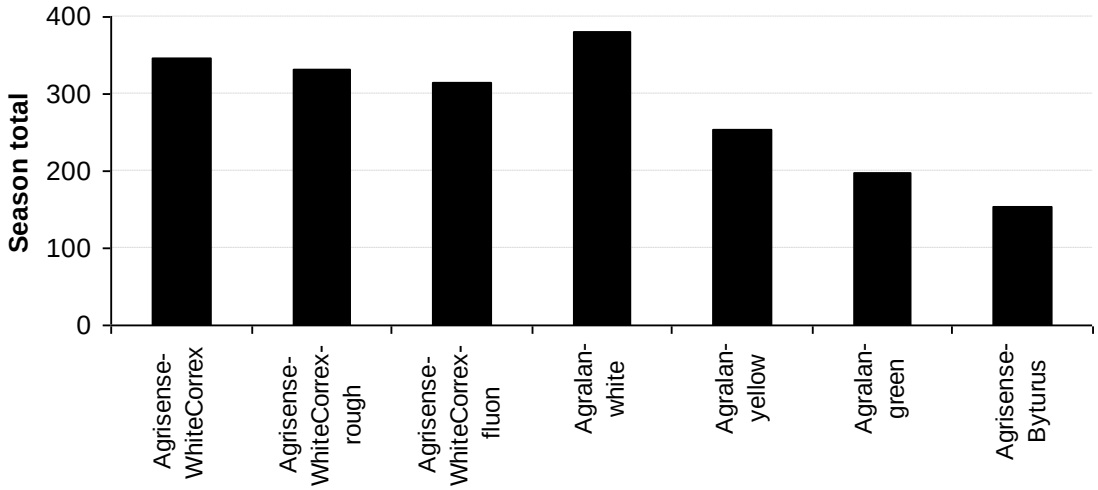


Figure 6.3.5. Total numbers of non-target arthropods captured between April and August 2009 in different designs of cross vane funnel traps baited with aggregation pheromone and PV2 standard lures

Conclusions

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

- The P+PV2high lure captured markedly more weevils the other P+PV2 lures and there was a strong trend to increasing catch with increasing PV2 release rate.
- Neither caryophylene nor TMTT showed any noticeable synergism of the aggregation pheromone lure.
- There was a trend to greater numbers of non-target arthropods being captured with increasing release rate of PV2.
- Interestingly, the trap design with the cross vanes coated with fluon captured more *A. rubi* than the other designs suggesting that the fluon was beneficial and increased the numbers of weevils that fell into the trap.
- The traps with white cross veins captured similar numbers of non-target arthropods, except the new AgriSense *Byturus* trap which was furnished with an excluder grid over the top of the funnel.
- The green and yellow Agralan traps captured less non-target arthropods.

This work, together with the previous years' work, suggests that the 'supertrap' to be evaluated in the remaining years of the project should incorporate a white cross vanes funnel trap (cross vanes coated with fluon and with grid to exclude non-target arthropods), a standard aggregation pheromone lure and a high release rate PV2 lure. The trap should be stood on the ground to maximise weevil catches.

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

Work to begin in year 3

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

Work to begin in year 3

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)

Work to begin in year 4

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

No work addressing the following tasks was due in year 2.

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

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

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

Primary milestones

Milestone	Target month	Title	
P3.1	11	Black spot 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 black spot determined.	
P1.6	33	Fungicide dissipation dynamics determined.	
P2.5	33	Model-based control strategies evaluated for botrytis.	
P3.4	36	An overall risk assessment scheme developed for black spot.	
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 black spot 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 year's 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 year's experiments evaluating IPDM programme in commercial crops completed. Programme finalised and economic appraisal completed.	
P7.3	60	Best practice guidelines prepared.	

Secondary milestones

Milestone	Target month	Title	
S2.1	1	Site selected for botrytis.	Y
S1.1	2	Products selected for trial.	Y
S1.2	11	Site selected for mildew risk trial.	Y
S1.3	20	Mildew risk system coded as a computer 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 black spot isolates completed.	
S1.5	33	Alternative products selected for the larger trial against mildew.	
S2.6	36	Methods for reducing botrytis in planting materials determined.	
S1.7	36	Methods for reducing mildew in planting materials determined.	
S4.2.2	36	System for using hexyl butyrate as a repellant of *L. rugulipennis* females developed ready for testing in IPM programme in final 2 years.	
S5.1.3	36	Third year experiment to evaluate the effectiveness of flowering plants to attract aphid predators and parasitoids completed.	
S5.2.3	36	Replicated field experiments evaluating the efficacy of the	

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

Explanation for non-achievement of milestones

The primary milestone P5.4.2: preliminary biocontrol trials with *Aphidius eglanteriae*, scheduled to be completed by month 24 has not been completed. Initial stocks of parasitised *Chaetosiphon fragaefolii* were found to be heavily hyper parasitised and this reduced the number of parasitoids that could be put into culture. Experiments were done to assess the possibility of mass production of the parasitoid. These experiments showed that *A. eglanteriae* could not parasitise the aphid species commonly used to mass rear other aphid parasitoids (those tested were *Aphis gossypii, Myzus persicae, Acyrtosiphon malvae* (rogersii)*, Macrosiphum rosae* and *Macrosiphum euphorbiae*). Therefore the culture had to be maintained on *Chaetosiphon fragaefolii*, which does not increase in numbers as rapidly as some other aphid species. The possibility of rearing the parasitoids in artificial media was also assessed. However, *A. eglanteriae* would not oviposit in the artificial media currently used for other parasitoid species. Therefore, it has not yet been possible to develop a technique to mass rear *A. eglanteriae*. The possibility of using another parasitoid in biocontrol studies is being discussed within the consortium.

The secondary milestone S5.2.2: field experiment testing the release rate of each plant volatile to attract aphid natural enemies completed and the most effective lure identified, scheduled to be completed by month 24 has not been fully completed. A range of plant volatiles were tested in the field but none was shown to be attractive to any beneficial species except hoverflies. Thus the most effective lure for use in strawberry has not yet been identified.

Technology transfer activities

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
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'.*
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
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

Publications

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

