Project title:	Integrated pest and disease management for high quality raspberry production
Project number:	SF 74
Project co-ordinator:	Mr John Place
Project administrator:	Dr Colin Gutteridge Chief Executive Officer East Malling Research New Road East Malling, Kent ME19 6BJ
Researchers:	Prof. Jerry Cross (EMR) Dr Angela Berrie (EMR) Dr Xiangming Xu (EMR) Mrs Gillian Arnold (EMR) Dr Tim O'Neill (ADAS) Mrs Janet Allen (ADAS) Dr Erika Wedgwood (ADAS) Mr Chris Dyer (ADAS) Dr Nick Birch (SCRI) Dr Tom Shepherd (SCRI) Dr Carolyn Mitchell (SCRI) Prof. David Hall (NRI)
Collaborators:	Agrisense BSC Ltd Assured Food Standards 2003 Ltd Bayer Crop Science Ltd Berryworld Ltd Co-operative Group (CWS) Ltd East Malling Ltd Horticultural Development Company KG Growers Ltd LEAF Marks & Spencer plc The East Malling Trust for Horticultural Research The Summerfruit Company The Worshipful Company of Fruiterers Waitrose
Report:	Year 3 Annual Report, 2009
Previous reports:	Year 2 Annual Report, 2008 Year 1 Annual Report, 2007
Date commenced:	1 April 2006
Expected completion date:	31 March 2011
Key words:	*Botrytis cinerea*, powdery mildew, raspberry beetle, raspberry cane midge, midge blight, aphids, protected raspberry crops

While reports issued under the auspices of the HDC are prepared from the best available information, neither the authors nor the HDC can accept any responsibility for inaccuracy or liability for loss, damage or injury from the application of any concept or procedure discussed.

Disclaimer

Whilst reports issued under the auspices of the HDC are prepared from the best available information, neither the authors nor the HDC can accept any responsibility for inaccuracy or liability for loss, damage or injury from the application of any concept or procedure discussed.

The results and conclusions in this report may be based on an investigation conducted over one year. Therefore, care must be taken with the interpretation of the results.

Use of pesticides

Only officially approved pesticides may be used in the UK. Approvals are normally granted only in relation to individual products and for specified uses. It is an offence to use non-approved products or to use approved products in a manner that does not comply with the statutory conditions of use, except where the crop or situation is the subject of an off-label extension of use.

Before using all pesticides check the approval status and conditions of use.

Read the label before use: use pesticides safely.

Further information

If you would like a copy of the full report, please email the HDC office (hdc@hdc.org.uk), quoting your HDC number, alternatively contact the HDC at the address below.

Horticultural Development Company
Tithe Barn
Bradbourne House
East Malling
Kent
ME19 6DZ

Tel: 01732 848 383
Fax: 01732 848 498

CONTENTS

GROWER SUMMARY

Headline

A Minimal Pesticide Residues Integrated Pest and Disease Management programme has been devised for raspberries, ready for testing in large scale grower trials in years 4 and 5 of the project.

Background and expected deliverables

Raspberries are very susceptible to *Botrytis*, powdery mildew, raspberry beetle, raspberry cane midge and aphids. Pesticides are currently relied on for control and are applied close to harvest. Intensive use of pesticides, including the organophosphate (OP) chlorpyrifos, which is used to control raspberry beetle and cane midge, is undesirable and unsustainable. Raspberry aphids, and the viruses they spread, are becoming more important. Indeed, some aphid populations have overcome the natural plant resistance.

Botrytis is the major cause of post-harvest fruit rotting and causes serious yield losses. Poor shelf-life reduces repeat buying. Retail surveillance has demonstrated that more than 50% of UK produced fruit contains fungicide residues and 22% contains chlorpyrifos residues. The major multiple retail customers are challenging raspberry producers to significantly reduce this incidence of residues.

The future registration of chlorpyrifos on raspberry is in doubt. Screening trials by East Malling Research have so far failed to identify any alternative insecticides with significant activity for cane midge control, though many different materials of a wide range of types have been tested. Loss of chlorpyrifos would have serious adverse consequences for the UK raspberry industry as there is no alternative control measure for the midge.

Raspberries suffer from rain damage and, to meet the requirements of major multiple retailers, the crop now has to be grown under protection. Recent observations indicate that this increases the risk of powdery mildew infection in protected crops. Plant protection methods have not been adapted for this new growing environment, which provides opportunities to reduce reliance on pesticides.

The strong market demand to reduce, or ideally to eliminate the occurrence of residues prompted this 5-year HortLINK project which officially started in April 2006, following considerable initial work in 2005. It aims to develop sustainable methods of integrated management of *Botrytis*, powdery mildew, raspberry beetle, raspberry cane midge (with associated disorder ‘midge blight’) and aphids on protected raspberry crops. Such methods would not rely on sprays of fungicides and insecticides during flowering or fruit development so that quality fruit can be produced with minimal risk of occurrence of detectable pesticide residues at harvest.

Summary of project and main conclusions

Progress on each objective of the project is summarised below:

Botrytis

Symptomless systemic infection in canes

Examination of floricane buds and cane base tissue from visibly healthy canes in February 2008, produced no evidence of symptomless systemic infection. By contrast, on floricanes

with visible *Botrytis* lesions or sclerotia, *B. cinerea* was recovered from most lateral buds. This result indicates that *B. cinerea* may develop from a localised point on a cane and infect other areas of the cane before any symptoms develop at these distant points. Experiments that test for symptomless systemic infection in primocanes were inconclusive. The occurrence of external inoculum as a common source of *B. cinerea* on primocanes was confirmed.

Sporulation of sclerotia

No sporulation was observed from sclerotia on fruiting canes immediately before or during flowering in an early crop covered by a Spanish tunnel in February. This result was in contrast to that in a covered crop at another site, in 2007, where sporulation from sclerotia occurred from early flowering and was still occurring when harvest ceased at the end of July. Sclerotia overwintering on fruiting canes are normally considered an important source of *Botrytis* inoculum in spring. The results suggest that sclerotia overwintering on canes may not be a major source of inoculum in crops covered early in the year, possibly because sclerotia are insufficiently wetted by winter rain.

Sources of B. cinerea *on tunnel crops*

Isolates of *B. cinerea* were collected from raspberry cane debris (15), primocanes (4), raspberry flowers (44), weeds in a tunnel crop of raspberry (1) and the air outside the crop (4) between April and October 2008. The isolates will be characterised by sequencing part of their DNA in order to determine the population structure of *B. cinerea* associated with raspberry.

Fruit infection

The incidence of flower infection was determined three times a week for 5 weeks in commercial crops in Kent and Cambridgeshire. Levels of infection varied greatly between sampling occasions, from 4% to 50% at the Kent site and from 2% to 39% at the Cambridgeshire site. Associated data on air temperature and relative humidity was also collected. The data will be used to refine the regression model developed in 2007 for prediction of flower infection from weather data.

Control by canopy manipulation

Primocane and leaf removal in a dense tunnel crop of cv. Glen Ample in 2007 reduced humidity around the canes and subsequent cane *Botrytis*. Examination of this crop in 2008 found no consequent effect on levels of fruit *Botrytis*. Lateral bud development from floricanes was generally better in the thinned areas of crop than in the areas with a dense canopy.

Suppression of sporulation from Botrytis scelotia by fungicides and natural products

Six fungicides (Folicur, Rovral WG, Signum, Switch, Teldor, UK3846) and two natural products (urea and potassium bicarbonate) were compared for their ability to suppress sporulation of *B. cinerea* from sclerotia on naturally-infected raspberry canes. None of the products worked except 5% urea which gave almost complete sporulation suppression. Any possible phytotoxic effects of this treatment on canes are currently being investigated.

Control of Botrytis fruit rot by fungicides and biocontrol agents

A field experiment in 2008 on Glen Ample evaluated programmes of new fungicides (Coded product HDCF 5, Switch) applied during flowering, with a natural product Chitoplant (chitosan from crushed crab shells) and two biocontrol agents – Serenade (*Bacillus subtilis*) and Shemer (*Metschnikowia fructicola)*. These biocontrol agents were also included in programmes with Switch. Teldor, as the standard fungicide, and an untreated control were included. The incidence of *Botrytis* on fruit at harvest was negligible at all four picking dates. In post-harvest tests the incidence of *Botrytis* fruit rot ranged from 8 to almost 70% and varied considerably in most of the picking dates. Fungicide treatments were most effective but only gave partial control. Shemer gave a limited degree of control but the other products were ineffective.

Management of fruit Botrytis by cooling

The incidence of raspberry fruit infected by latent *B. cinerea* at harvest varied between picks and crop source. Levels ranged from 70-89% in an outdoor crop untreated with *Botrytis* fungicides, from 18-79% in a covered untreated crop, and from 8-69% in a covered crop treated with *Botrytis* fungicides.

Development of infection causing visible damage within 9 days of harvest was greatly reduced in fruit given a specific experimental temperature regime. This was 1 day of field heat removal in air cooled to 2 ºC aiming to bring fruit temperature down to 6ºC, followed by 3 days cold storage at 3ºC and then 3 days at 17ºC to mimic transport and display followed by 2 days storage at 23ºC.

Incidence of visible *Botrytis* in weekly samples from a covered, unsprayed crop subjected to this cooling treatment were consecutively 16%, 1%, 3% and 49% at 9 days after harvest, compared with 37, 8, 40 and 78% in fruit stored at ambient (23ºC). Botrytis incidence was generally greater in fruit that was placed in 3ºC rather than the field heat removal area on the first day. It was also greater in fruit that was kept at at 23ºC rather than 17ºC to mimic the transport and display stage.

Fruit from all three crops had zero or near zero levels of visible *Botrytis* when assessed immediately after a storage period comprising 1 day in 2ºC, 3 days at 3ºC and 3 days at 17ºC.

Powdery mildew

Host-specificity

Electron microscopy of powdery mildews from raspberry and strawberry revealed no morphological difference between them. Previous work in this project, in which the DNA of isolates from raspberry and strawberry was analysed, suggests that they are two distinct groups.

Raspberry beetle

Flower volatile monitoring traps for raspberry beetle

The lure and trap system was tested at two sites in Scotland, but not England, since suitable raspberry beetle sites (sufficient pest pressure) were not available. In Scotland a combination of insecticide use (Calypso) and previous trapping resulted in very few beetles being caught and no fruit damage, even in control areas where insecticides had not been sprayed. Some bees were caught in the traps but this was largely due to very high local populations. The numbers of bees caught had no impact on bee populations or pollination. Final modifications (2009) will include a coarse mesh to prevent bees falling into the bucket

trap. Generally the grid system (50 traps / ha placed within tunnels) was more effective than perimeter trapping, especially if RB populations are low to moderate.

In parallel studies using the traps and lures in Norway (mainly organic), Switzerland (organic and conventional) and France (mainly conventional), the trap and lure system produced good results. Where pest populations were very high (organic sites surrounded by wild hosts) some fruit damage still occurred, but the addition of extra traps (lattice within crop + crop perimeter , with additional traps near wild host reservoirs) and the use of 2 attractants (A+B) are proving to be beneficial in such extreme conditions.

Interestingly, in Switzerland and Norway the improved bucket traps continued to catch raspberry beetles well into the flowering period (not seen with sticky traps in the same experiments) and thus helped to reduce subsequent pest populations in following seasons. Trials will continue in 2009 with the finalised trap and lure system (easy snap fit and bee proof) with the aim of commercialising the monitoring system in 2009, using an action threshold of about 5-10 raspberry beetles / trap week (as previously developed in EU CRAFT 'RACER' project). This guideline threshold is likely to be conservative, since it was developed using sticky traps without the improved lures currently used. Growers should adjust the threshold to suit their local conditions and fruit quality requirements.

Raspberry cane midge

Sex pheromone monitoring traps for raspberry cane midge

Raspberry cane midge and sex pheromone traps for pest monitoring are now available from Agralan. UK raspberry growers should be using the trap to monitor populations and improve the timing of sprays for control of the pest. A trap should be suspended at a height of 0.5 m in the centre of each of the main cropping plantations on the farm and monitored weekly though the season from 1 April – 30 September. A treatment threshold of a total of 30 midges per trap is proposed. Sprays should be targeted against the first generation (in May outdoors, but much earlier on early protected crops) and applied a few days after a threshold is exceeded. Chlorpyrifos is the only effective insecticide available. Sprays should be directed at the base of the canes.

Control of cane midge with sex pheromone Mating Disruption and Attract and Kill

One large-scale experiment was conducted on three farms in Kent from April - October 2008 to evaluate a mating disruption (MD) and an attract-and-kill (A&K) method of using the raspberry cane midge sex pheromone for control. The MD treatment used 3 kg of Ethyl Vinyl Acetate granules (~50,000 granules/kg) containing 10 g pheromone/ha, broadcast to the surface of the soil in the alleyways. The A&K treatment used 2000 lambda cyhalothrin treated cards (~ 7 × 7 cm) each bated with a rubber septum lure containing 200 µg of the pheromone. Untreated control plots were provided for comparison. One 1 ha plot of each treatment was provided on each of three farms in Kent. The sites had varying populations of raspberry cane midge, season totals of 2670, 5505 and 6569 midges being captured per trap in the untreated control plots at the three sites. The efficacy of the treatments was assessed by determining the numbers of midges caught in a sex pheromone trap in the centre of each plot together with counts of midge larvae that developed in artificial splits in the primocane.

The MD treatment failed to reduce total season catches at one site, but reduced catches by 94.2% and 85.1% at the other sites. Better and longer lasting trap suppression was achieved with the A&K treatment (91.6%, 99.2% and 98.2% respectively). Where the lowest degree of pheromone trap suppression occurred, total numbers of larvae recorded in splits in the treated plots were as great, or greater, than in the untreated controls. However, numbers of

larvae were reduced by 99.7% and 97.4% and by 68.1% and 86.0%, by the MD and A&K treatments at the other two farms.

These results are encouraging because it is the second time that control of the raspberry cane midge using the sex pheromone has been demonstrated. The most likely reason for the variation in results are differences in cane midge populations. MD and A&K treatments are known to perform poorly when populations are high. The high degree of trap shut down that is necessary for good control probably only occurs at low population densities. This indicates that the MD and A&K treatments perform well at low population densities, but not at high. The overall conclusion is that where populations are moderate to high, then MD and A&K treatments have to be used in combination with chemical control methods initially.

Because of efficacy and registration considerations, it has been decided to develop a practical A&K formulation for testing in the large scale IPM trails in the final two years of the project.

Identification of volatile substances from cane splits to attract female cane midge

Good progress was made with identifying a female attractant for raspberry cane midge. Mated females are known to be strongly attracted to odours from recently split raspberry primocanes. Fresh splits are preferred over old ones. Using SPME microfibres to sample the volatiles in situ followed by GC-EAG (gas chromatography coupled to an electro-antennogram), a number of volatile substances produced in larger amounts from wounded canes were identified. Most of these are produced by other plants when damaged, but two are more unusual and might be responsible for the specific attraction of female midges to raspberry canes. Experiments with several raspberry cvs showed consistency in the patterns of volatile emission from cane splits, indicating that development of an attractant lure will be feasible based on a few, consistent compounds now identified. Synthetic lures (polyethylene sachets containing 100 µl of a mixture of compounds) were developed that emulate the bouquet from cane splits and preliminary testing of their attractiveness to females was started, showing promising results in a small pilot study against 3rd generation female midges. The females are the damaging sex that lay eggs and control is likely to be more effective if we can target them rather than just the males as is the case if the sex pheromone is used only. Further tests to optimise the trap and lure system for female midges will be continued in 2009 at suitable field sites.

Aphids

In an experiment to evaluate three different timings of single sprays of pirimicarb (Phantom), thiacloprid (Calypso) and pymetrozine (Plenum) for the control of small and large raspberry aphids, in commercial raspberry production, Calypso sprayed on 19 October was the most effective treatment reducing numbers of aphids by 99% the following spring.

Minimal Pesticide Residue Integrated Pest and Disease Management (MPR-IPDM) programme

Based on the research conducted in the first 3 years of the project, a Minimal Pesticide Residue Integrated Pest and Disease Management programme has been devised ready for testing in years 4 and 5 of the project.

The key features of this programme are:

1. Good crop hygiene and cane management together with rapid fruit cooling and high quality cool chain marketing to avoid the need for fungicide sprays for *Botrytis.*

2. Apply 1-2 sprays of a powdery mildew fungicide in the spring as soon as the tunnel is covered then spray potassium bicarbonate subsequently for eradication of powdery mildew if the disease is observed.

3. Use raspberry beetle host volatile funnel traps with white cross vanes at a rate of >50/ha. Localised treatment with Calypso can be justified when trap catches exceed economic damage thresholds.

4. For raspberry cane midge, use a sex pheromone attract and kill treatment. Additional trapping of female cane midges using identified female cane midge attractants are under development and should complement the sex pheromone based traps in future.

5. Apply an autumn spray of thiacloprid (Calypso) for aphid control supplemented with introductions of predators and parasites for biocontrol in summer.

Financial benefits

In 2003, 8,000 tonnes of raspberries, worth £28.4M were produced from 1,260 ha grown in Britain. A further 4,800t, worth £18.2M, were imported. The UK fresh market is under-supplied outside of the main season. New varieties are now being utilised to spread the cropping season and it is expected that production will increase substantially, perhaps by three-fold. Surveillance of pesticide residues in soft fruit identifies raspberries as having a high occurrence of detectable residues. For example, the 2003 ACP survey found 50% of imported raspberries and 75% of home-grown raspberries had detectable residues. This greatly damages the consumer acceptability of raspberries and their image as a healthy food.

Control of powdery mildew and *Botrytis* in raspberry crops is already difficult. Anecdotal evidence suggests that 25-30% of bud loss is due to *Botrytis* and, as a result, the UK crop is not producing optimum yields. There is a limited range of pesticides that can be used and other means of crop protection (*e.g.* biological control) are not available. The knowledge and techniques developed in this project will define an integrated pest and disease management (IPDM) system for growing raspberries in protected environments. This will reduce or remove the incidence of detectable residues in fresh raspberries and give UK raspberry growers a competitive advantage.

Annual value in area of impact

Botrytis, powdery mildew, cane midge and raspberry beetle are problems wherever and however raspberry is grown in the UK. ADAS estimate that, at any one time, 60% of raspberry plantations are infected by these pests and diseases. Assuming 25% of the crop is forgone as a result of these infestations, this is equivalent to 2,000 tonnes of raspberries, worth £7M.

Expected annual added value

We make the following assumptions that arise from a successful project:

1. Losses in the current crop will be reduced by 10%, yielding an additional £2M of UK sales.

2. Enhanced competitiveness of UK raspberry growing will reduce imports by 50%, yielding an additional £10M of sales.

3. Increased consumer confidence in raspberries will grow the overall market by 20%, yielding a further £5M of sales.

Grower capital investment and cost recovery

It is not anticipated that this project will result in additional capital investments for growers. Pesticides typically cost £690/ha per annum. It is unlikely that costs of crop protection will be reduced and they may even increase if biological control systems are used extensively. However, this increase would be small in relation to the value of the crop.

Action points for growers

- The work has demonstrated that the need for fungicide sprays for *Botrytis* can be greatly reduced/avoided if good crop hygiene and cane management are combined with rapid fruit cooling and high quality cool chain marketing.

- Mildew can be controlled with 1-2 sprays of a powdery mildew fungicide in the spring as soon as the tunnel is covered, supplemented with subsequent sprays of potassium bicarbonate to eradicate powdery mildew if the disease is observed.

- Raspberry beetle traps (a green funnel trap with white cross vanes bated with a host volatile sachet lure lasting 6 weeks) may become commercially available in 2009. They should be used to monitor populations and direct local application of insecticide sprays.

- Raspberry cane midge sex pheromone traps should be deployed in the main raspberry plantations on each farm and used for monitoring cane midge and determining the timing and need for insecticide sprays.

- A spray of an aphicide such as thiacloprid (Claypso) or pirimicarb (Aphox) applied in early – mid October will reduce spring populations of large raspberry aphid by > 90% and should be considered as part of normal practice.

SCIENCE SECTION

Objective 1. *Botrytis*

1.1 *Inoculum sources*

Task 1.1.1 – Investigate the infection and subsequent development of *Botrytis* in relation to leaf ages and cane infection by conducting controlled inoculation experiments in a glasshouse compartment using potted raspberries cv. Glen Ample (years 1-2, EMR)

Reported in Year 2? It would be very helpful to make a reference to where each task is reported –other wise difficult to gauge if everything is on track

Task 1.1.2 – Identify the timing of infection and development of *Botrytis* in leaves and petioles on the primocane, and when invasion of the cane occurs, by frequent monitoring in a protected commercial unsprayed crop of cv. Glen Ample (year 2; EMR, ADAS)

Reported in Year 2?

Task 1.1.3 – Identify the start and duration of *Botrytis* sporulation on *Botrytis* cane lesions and other likely sources of *Botrytis* (weeds, crop debris) (years 1-3; ADAS, EMR)

Two experiments were done to investigate the possibility of *B. cinerea* overwintering within cane buds or the crown.

Task 1.1.3.1 Examine buds and cane base tissue on fruiting canes to determine whether they are a potential source of *Botrytis* in a crop

Materials and methods

In February 2008, before lateral bud break, samples of floricanes (15-20 per site) were taken from a crop of raspberry cv. Glen Ample in Cambridgeshire and Kent. The crop was covered and untreated with fungicides for *B. cinerea* in 2007. Six canes were each cut into three sections to obtain samples from low, medium and high positions. The underground base of each cane was also sampled. A bud was removed from each of the cane sections, and four slices of tissue were taken from each stem base. Tissues were surface sterilised and incubated on nutrient agar. At Kent, only canes with visible cane *Botrytis* were selected and furthermore buds were incubated intact on paraquat agar.

Results

From the Cambridgeshire crop, *B. cinerea* was not recovered from any of the 18 buds per height, or from the 18 cane base samples. *Fusarium* (species not identified) was commonly isolated. This result indicates that *B. cinerea* was not surviving over winter in the buds of floricanes or in the crown in this crop.

However, in the Kent crop, most buds above the ground had *Botrytis*, often coexisting with *Fusarium*. *Botrytis* was found on below ground buds at a much lower incidence than those above ground.

Previous studies in this project showed that localised cane infection can occur via petioles of attached leaves, at leaf scar wound sites and by direct infection of internode areas. These experiments were done to determine if there is any evidence for symptomless systemic infection in canes. No *Botrytis* was found on floricane buds or cane base tissue taken from visibly healthy canes. However, on floricanes with *Botrytis* lesions or sclerotia, most lateral buds contained *B. cinerea.*

Conclusions

These results indicate that symptomless infection can occur in buds of floricanes visibly affected by *Botrytis.* As most buds on a cane were usually infected, the infection may be systemic.

Task 1.1.3.2 Examine young primocane shoots to determine whether they are a potential source of *Botrytis* in the crop

Experiments were established in commercial crops in Cambridgeshire and Kent to determine if preventing air-dispersed conidia settling on primocanes, by covering developing shoots with a polythene bag from emergence, eliminates occurrence of *B. cinerea* on these shoots.

Materials and methods

Cambridgeshire

The same crop was used as in Task 1.1.3.1. After the first primocane flush had been burnt off, samples of the next emerging shoots were tagged on 15 May 2008. Two shoots within 5 m of each other at 10 locations were tagged. One of each pair was covered with a transparent bag held on a tripod frame and pinned to the ground, the other was left uncovered. Leaf samples were taken once the shoots had nearly reached the top of the bag on 29 August. Leaves were sampled from each of three heights per cane up to the highest fully expanded leaf (43-52 cm). Any senescent lower leaves were also sampled. Leaves were surface sterilised, dipped in paraquat and then incubated in damp trays covered with polythene to encouraged *Botrytis* sporulation.

Kent

In the experimental polytunnel at Salman's Farm, Penshurst, in April 2008, 20 developing primocanes were covered in clear sterile plastic sleeves and lightly secured in place by stapling the open end around the base of the cane to allow the canes to grow with the bags in place. The canes were located five in each of plots 1-4 of the cane manipulation trial, *i.e.* 10 in the thinned plots and 10 in unthinned plots. The covers were left in place for 1-2 weeks. The leaves inside the bags were then collected and placed in clean polythene bags. A similar number of leaves were also collected from 20 uncovered primocanes from the same plots. Both sets of samples were taken back to the laboratory. Leaves were incubated under UV light to check for latent *Botrytis* following the standard protocol. After incubation the number of *Botrytis* infected leaves were recorded.

Results

In the Cambridgeshire crop, *Botrytis* was present whether or not the primocane had been covered from emergence, with twice as many leaves infected when they had not been covered. None of the youngest (highest) bag-covered leaves had *Botrytis*, and none of the senescent leaves produced *Botrytis* from either source (Table 1.1.3.1).

Table 1.1.3.1: Recovery of *B. cinerea* from leaves of primocanes covered and uncovered during growth – Cambridge 2008

Treatment of primocane	Leaf height	No. of leaves: Tested	With *B. cinerea*
Covered	Low (dead)	2	0
	Low	5	1
	Medium	5	2
	High	5	0
Uncovered	Low (dead)	3	0
	Low	5	2
	Medium	5	1
	High	5	4

This observation suggests that *B. cinerea* may possibly arise on canes from overwintering in the crown as well as from deposition of conidia in the air. Further work is needed to confirm that *B. cinerea* may overwinter in the crown as this was not found by direct examination (Task 1.1.3.1). Recovery of *B. cinerea* from leaves of covered primocanes may have resulted from infection pathways other than systemic infection from the crown, for example insect dispersal, or entry through air ventilation holes or at the base of the polythene cover.

The leaf samples in Kent were collected on 13 May, by which time a lot of the lower leaves in the bagged spawn were dead. No *Botrytis* was found on either the dead leaves or the green leaves sampled from the bagged spawn. In both cases, the incubated leaves were rapidly colonised by another fungus which probably suppressed any *Botrytis* development. Most leaves collected from the unbagged spawn sporulated *Botrytis* after incubation.

Task 1.1.3.3 *Botrytis* sclerotia on fruiting canes as a source of inoculum

Objective

To investigate whether *B cinerea* sclerotia present on raspberry fruiting canes are sources of inoculum in raspberries grown under polytunnels.

Method

In April 2008, in the experimental polytunnel at Salman's Farm, Penshurst, Kent, 20 fruiting canes with visible *Botrytis* cane lesions and sclerotia present were tagged, and inspected with the aid of a hand lens for sporulating *Botrytis*. Inspections were repeated every 2-3 weeks until the end of flowering.

Results

The polythene cover was placed on the tunnel and the ends closed by 20 February 2008. The raspberry canes within the tunnel were also covered with fleece. The sclerotia on the tagged canes were checked for sporulation on 11 April and 13 May. As the tunnel was covered there was no opportunity for the sclerotia on the canes to become wetted. Therefore, on both inspections the *Botrytis* sclerotia remained dry and shrivelled with no sign of sporulation.

Conclusions

Sclerotia overwintering on fruiting canes are normally considered an important source of *Botrytis* inoculum in spring. However, the method of culture on this farm where the crop is covered very early means there is no opportunity for sclerotia to be wetted and initiate sporulation when temperatures rise in spring. Therefore, sclerotia overwintering on canes are not a major source of inoculum for the crop on Salman's Farm.

Task 1.1.3.4 Molecular comparison of populations of *Botrytis cinerea* from different sources related to raspberry crops to determine relationships and likely sources of *Botrytis* inoculum

Objective

To identify sources of *B. cinerea* inoculum in raspberries grown under polytunnels by molecular comparison of populations of *B. cinerea* from different sources related to raspberries.

Method

In April 2008, weeds and raspberry cane debris were collected from the polytunnel at Salman's Farm, Penshurst, washed and damp incubated under UV light to encourage any latent *Botrytis* present to sporulate. Sporing colonies of *B cinerea* were then plated onto PDA amended with rifamycin.

Once cultures were free of contamination, isolates were grown on sterile cellophane on PDA. Once growth was established, the mycelium was scraped off and stored in Eppendorf tubes at -80^{o}C prior to molecular analysis. Isolates were similarly collected from flowers, leaves, fruits and other sources relevant to raspberry.

Results

By October 2008 a total of 68 isolates had been collected (Table 1.1.3.4.1). These were predominantly from raspberry. So far, only one isolate has been collected from weeds and two from air samples. Further isolates will be collected in November 2008, concentrating on weeds inside and outside the tunnel.

DNA was extracted for 64 samples and screened for six SSR molecular markers. There are another 10-20 isolates, mainly from weeds, in the process of culturing, DNA extraction and molecular screening. Once all samples have been screened, statistical analysis will be carried out to determine whether there are significant differences among fungal populations from different sources (canes, flowers, air and weeds).

Table 1.1.3.4.1 Isolates of *Botrytis cinerea* collected in 2008 for the population study

Source	Date collected	Number of isolates
Cane debris (tunnel)	**10 April**	**15**
Air (outside tunnel)	**2 June**	**2**
Air (inside tunnel)	**7 May**	**2**
Primocane	**13 May**	**1**
Primocane green leaves	**13 May**	**3**
Flowers	**7 May**	**0**
Flowers	**14 May**	**3**
Flowers	**16 May**	**3**
Flowers	**19 May**	**3**

Flowers	**21 May**	**3**
Flowers	**23 May**	**4**
Flowers	**27 May**	**5**
Flowers	**29 May**	**6**
Flowers	**2 June**	**5**
Flowers	**4 June**	**5**
Flowers	**6 June**	**3**
Flowers	**10 June**	**4**
Dandelion flowers (tunnel)	**10 April**	**1**
Dandelion flowers (tunnel)	**13 November**	**2**
Willow herb (tunnel)	**13 November**	**1**
Buttercup (tunnel)	**13 May**	**0**
Plantain (tunnel)	**13 November**	**2**
Sowthistle (outside tunnel)	**13 November**	**5**
Groundsel	**13 May**	**0**
Willow herb (outside tunnel)	**13 November**	**2**
Hedge parsley	**13 May**	**0**
Forget-me-not (outside tunnel)	**13 November**	**9**

Task 1.1.4 – Identify the factors and conditions that initiate and influence the sporulation of *Botrytis* sclerotia overwintering on cane lesions (years1-2; EMR)

Task 1.1.5 – Seasonal variation in airborne inoculum (years 1-2; EMR, ADAS, CSL)

Objectives

In 2007, the consortium agreed to redirect most of the time allocated to powdery mildew to investigating infection of raspberry flowers by *Botrytis.* The objective was to develop mathematical models that relate the incidence of flower infection to inoculum concentration and weather conditions in the field, from which a disease forecasting system could be developed. We have obtained a further set of flower infection data and now are modelling the two data sets obtained in 2007 and 2008.

Materials and methods

The incidence of flower infection was determined in 2008 as in 2007. Flowers were sampled every two or three days during flowering at the Cambridge and Kent sites. On each sampling day, 100 fully-opened flowers with all petals still attached (and no necrosis on them) were randomly collected from the two sites at around 10 am. The flowers, with their petals then? removed, were placed individually into 25 ml universal bottles. At each site, about 15 batches of flowers were sampled over the flowering period.

The flowers collected on each sampling date were surface sterilized with sodium hypochlorite (0.025% available chlorine (w/v)) for 15 minutes to remove any spores on the surface, and then rinsed with distilled water. The flowers were placed separately on filter paper thoroughly wetted with distilled water in small sterile Petri dishes. The dishes were incubated in a glasshouse compartment or close to a window in a laboratory at approximately 20°C for 7 or 8 days, after which the flowers were examined for conidiophores of *B. cinerea*. Any flower on which conidiophores were detected was classified as infected.

Results from 2007 suggested that conidia are available throughout the flowering period and that the spore concentration in the air cannot be easily predicted from weather conditions. Furthermore, the incidence of flower infection was not directly related quantitatively to the

spore concentration. Thus, in 2008, it was decided not sample the air for *Botrytis* spores to estimate spore concentrations.

A USB temperature and humidity Duo logger was used to record temperature and humidity in the tunnel. Values of vapour pressure deficit (VPD, mmHg) were derived from temperature and relative humidity using the following empirical equation:

$$vpd = 4.6698\mathrm{e}^{0.06241\ \mathrm{temp}}\left(1 - rh/100\right).$$

In data analysis, we assumed that sampled flowers were exposed (and susceptible) to *Botrytis* for the previous 48 hours. Two approaches were used to analyse the data. First, we used a model developed for predicting infection of strawberry flowers by *Botrytis* to estimate potential infections of raspberry flowers. This strawberry model uses daytime (9 a.m. to 9 p.m.) average VPD and night time temperature (9 p.m. to 9 a.m.) to predict incidence of flower infection. Second, we developed a new model using the raspberry data only.

For the second approach, a straight regression of the incidence of flower infection in each 48 h period on corresponding averages of weather variables and conidia number was not appropriate for two reasons: (1) this simple regression analysis assumed that the effects of weather on day t and t+1 on the infection of flowers by conidia on day t and t+1 were the same, which is untrue, *e.g.*, weather variables on day t had no direct effects on the infection by conidia on day t+1; (2) this simple analysis ignores potential re-infections of the same flowers in two days. To overcome these shortcomings, a more complicated method was used to model the effects of daily weather variables on the incidence of daily flower infection, based on an approach previously used for strawberry. Details of this modelling approach are not described here and can be found in the published paper describing the strawberry model (Xu *et al.*, 2000, *Phytopathology* **90**: 1367-1374).

Results

Both weather and disease data have been summarised and will be used to model infection conditions in the near future. Table 1.1.5.1 presents the summary of flowering sampling in 2008. At the Kent site, the incidence ranged from 4% on 09/05 to nearly 50% on 02/06. At the Cambridge site, it ranged from 2% on 16/05 to 39% on 23/05.

Table 1.1.5.1 Summary of flower sampling data for both sites: date and incidence of flower infection with *Botrytis*

Cambridge		Kent	
Date	**Incidence (%)**	**Date**	**Incidence (%)**
12/05/2008	9	07/05/2008	7
14/05/2008	14	09/05/2008	4
16/05/2008	2	12/05/2008	10
19/05/2008	3	14/05/2008	10
21/05/2008	6	16/05/2008	11
23/05/2008	39	19/05/2008	7
27/05/2008	12	21/05/2008	12
28/05/2008	29	23/05/2008	11
30/05/2008	16	27/05/2008	16
02/06/2008	9	29/05/2008	27
04/06/2008	18	02/06/2008	50
06/06/2008	13	04/06/2008	45
09/06/2008	24	06/06/2008	11
11/06/2008	14	10/06/2008	14

Table 1.1.5.2 presents the summary of modelling results. A greater proportion of variance in the flower infection is explained by climatic variables in 2008 than in 2007 for both sites. Flower infection in 2007 appeared to be more influenced by temperature; in contrast, it was more affected by moisture conditions in 2008. However, there were significant differences in the relationship of flower infection with climatic variables between the Kent and Cambridge sites. Thus, the combined model for 2008 only accounted for 39% of the total variance in the observed data.

Table 1.1.5.2 Summary of modelling data of raspberry flower infection by *B. cinerea* in 2007 and 2008 at the Cambridge and Kent sites

Data sets	Climatic variables included	% variances accounted for
All site/year data	$DT + NT + DT^2 + DT^{½}$	20.0%
Cambridge/2007	$ADT + ADT^2 + ADT^{½}$	19.6%
Kent/2007	$ADV^2 + ADT^{½} + ADV * ADT$	37.4%
Cambridge/2008	NV + NV * NT + NT * NRH	54.3%
Kent/2008	ADV + ADRH + ADT * ADRH	72.0%
Kent/2008	$DV + NT + DV^{½}$	69.4%
2008	$ADRH + ADV^{½} + ADT * ADRH$	39.7%
2008	$NT + DRH + DV^{½}$	37.2%

DT, DV, DRH, NT, NV, NRH, ADT, ADV, ADRH: day average temperature, vapour pressure deficit and relative humidity; night average temperature, vapour pressure deficit and relative humidity; daily average temperature, vapour pressure deficit and relative humidity.

1.2 *Environmental manipulation*

Task 1.2.1 - Occurrence of disease in commercial tunnel crops of raspberry (years 1-3; ADAS and growers)

Task 1.2.2 – Assessment of latent *Botrytis* infection of green fruit as a measure of likely fruit rot at harvest. (year 1; ADAS, EMR, CSL)

Task 1.2.3 and Task 4.2.2 – Effect of tunnel environment manipulation on humidity and disease incidence (years 1-3; ADAS, EMR)

Assessments of cane *Botrytis* on fruiting canes in manipulated and control plots in January 2008 at the Kent site showed a higher incidence of cane *Botrytis* in the control plots. Assessments were conducted later in 2008 to ascertain whether the higher incidence of cane *Botrytis* in the control plots had any significant effect on bud development or the incidence of fruit rot at harvest.

Materials and methods

- need to include the thinning treatments applied
- are there any other environmental manipulations applied apart from thinning?

Bud development

In April 2008, bud development was assessed on 10-20 canes per plot to examine the effect of *Botrytis* on lateral bud development. The canes were divided into 3 zones (Zone 1 = bottom 0.75m of cane; Zone 2 = mid 0.75m cane; Zone 3 = top section - See Table 1.2.3.1) and the total numbers of buds and their state of development recorded. The assessment was repeated 4 weeks later.

Fruit rot

A random sample of 50 unripe (green/yellow) fruit was taken from the centre 9 m of the middle row of each plot on 25 June. Unripe fruit were surface sterilised together by immersing in a 0.5% Domestos® solution for 15 min and then immersed in sterile distilled water for 15 min. Unripe fruit were then placed on paraquat chloramphenicol agar (PCA) media under UV light at 20°C to induce sporulation for 10-14 days before assessment.

A random sample of ripe (red) fruit was taken from the centre 9 m of the middle row of each plot on one occasion on 25 June. Ripe fruit were placed in multicell plant propagation trays and incubated at ambient temperature within a polythene bag. The incidence of *Botrytis* fruit rot was assessed after 7 days.

Results

Bud development

The percentage of buds that had developed or failed to grow is shown in Table 1.2.3.1. Most buds developed in zone 3 (the top section) and least in zone 1 (the base). The differences between the buds in the control or manipulated plots were small, especially in zone 3. Differences were greatest in the bottom zones where the reduced light in the control plots probably had the greatest effect. Most *Botrytis* lesions were recorded in zones 2 and 3. In general a higher percentage of buds developed in manipulated plots in zone 2 than in the

control plots and this may have been due to the higher incidence of *Botrytis* cane infection in this zone in the control plots.

Table 1.2.3.1 Percentage of developed or non developed buds on raspberry fruiting canes in plots that were canopy thinned (manipulated) or left unthinned (control) in 2007 – Kent 2008

Date assessed	Treatment	Zone*	% buds well developed	% buds no growth
11 April	Control	1	3.8	94.3
	Manipulated	1	10.1	78.2
	Control	2	29.6	54.5
	Manipulated	2	44.2	38.9
	Control	3	57.6	24.6
	Manipulated	3	46.6	28.9
13 May	Control	1	0.9	99.1
	Manipulated	1	10.2	87.3
	Control	2	19.4	76.7
	Manipulated	2	27.5	64.7
	Control	3	39.3	47.2
	Manipulated	3	43.1	42.7

*Zone 1 = bottom 0.75m of cane, *Zone 2 = mid 0.75m cane, *Zone 3 = top section

Fruit rot

There were no obvious differences in *Botrytis* rot incidence on green fruit from either manipulated or control plots. *Botrytis* developed on 100% of the fruit from each treatment. The incidence of *Botrytis* fruit rot in ripe fruit from manipulated plots was slightly lower (47%) than that from control plots (59.5%), but not significantly different.

Conclusions

In general, bud development was better in all cane zones in manipulated plots compared to control plots. The higher incidence of cane *Botrytis* recorded in control plots in 2007 may have affected bud development in the control plots.

1.3 Control agents

Task 1.3.1 – Laboratory evaluation of fungicides and other treatments to suppress sclerotia sporulation. (year 1-2; EMR)

Objective

To evaluate chemicals for suppression of sporulation of *Botrytis* sclerotia on raspberry canes.

Method

Raspberry canes with *Botrytis* lesions and sclerotia were collected from a raspberry plantation in summer 2007 and stored dry at 4ºC until needed. The canes were cut into 10

cm lengths, soaked in water for a minimum of 15 mins and then dried on filter paper. The wetted canes were then divided into lots of 5 (representing 1 plot). The canes were treated with the following chemicals (Table 1.3.1.1) - fenhexamid (Teldor), iprodione (Rovral), tebuconazole (Folicur), pyraclostrobin + boscalid (Signum), cyprodonil +fludioxonil (Switch) urea and potassium bicarbonate, which were applied by putting cane pieces into a container of the chemical and agitating to ensure all of the cane was covered. The chemical was allowed to drain off and cane pieces placed in sandwich boxes. The canes were damp incubated in the light to encourage the sclerotia to sporulate. The numbers of sclerotia sporulating were recorded after 1 week, 2 weeks and 4 weeks.

Each treatment was replicated four times in a randomised block design and compared to an untreated control.

Table 1.3.1.1 Treatments applied to *Botrytis* sclerotia on raspberry cane pieces

Treatment	Product	Active ingredient	Rate / litre
1	untreated	water	-
2	Signum	pyraclostrobin + boscalid	1.8g
3	Teldor	fenhexamid	1.5g
4	Rovral WG	iprodione (750g/kg)	1g
5	Folicur	tebuconazole	0.8ml
6	Urea + wetter*	urea	50g
7	potassium bicarbonate + wetter*	potassium bicarbonate	20g
8	Coded product HDCF 5	experimental	0.83ml
9	Switch	cyprodonil + fludioxonil	1g

Wetter = Silwet at 0.1% concentration = 1ml/L

Results

Numbers of sclerotia present on the cane pieces were variable and ranged from 6 to 60, but as 5 or 6 cane pieces were included per plot total numbers of sclerotia per plot were similar. On average, over 50% of sclerotia were sporulating on untreated sclerotia at the first two assessments (Table 1.3.1.2). By the final assessment sporulation on the sclerotia was declining naturally and secondary fungi were beginning to develop on some sclerotia in some treatments. Only urea (Treatment 6) consistently reduced sporulation on the sclerotia. Numbers of sporing sclerotia were significantly less in urea-treated plots compared to untreated plots at all assessment times. None of the other treatments had any significant effect on sporulation apart from Teldor (Treatment 3) which by the final assessment date had significantly fewer sporing sclerotia compared to the untreated. No reduction in sporulation was noted at the first two assessments.

Table 1.3.1.2 Mean % (angular transformed) of *Botrytis* sclerotia on raspberry cane pieces sporulating after dipping in various chemical treatments assessed 7, 14 and 28 days after treatment. Figures in brackets are back-transformed means

Treatment	Mean % sclerotia with *Botrytis* sporing		
	7 days after treatment	14 days after treatment	28 days after treatment
1.Untreated	45.3 (50.6)	45.3 (50.4)	38.0 (38.0)
2. Signum	35.1 (33.1)	35.5 (33.7)	24.6 (17.3)
3. Teldor	38.8 (39.3)	35.9 (34.3)	14.0 (5.9)

4. Rovral WG	47.8 (54.8)	46.9 (53.3)	38.1 (38.2)
5. Folicur	44.3 (48.8)	45.4 (50.7)	39.0 (39.7)
6. Urea + wetter	11.4 (3.9)	22.1 (14.1)	17.3 (8.8)
7. Potassium bicarbonate + wetter	36.1 (34.7)	36.6 (35.6)	29.3 (23.9)
8. Coded product HDCF 5	39.4 (40.3)	44.6 (49.4)	37.7 (37.4)
9. Switch	45.5 (50.8)	43.5 (47.4)	37.2 (36.5)
F Probability	<0.001	0.017	0.012
SED (24 dof)	6.18	6.49	7.71
LSD (p= 0.05)	12.75	13.40	15.91

Conclusion

Only urea consistently reduced *Botrytis* sporulation on sclerotia at all assessment dates.

Task 1.3.2 – Field evaluation of suppression treatments. (year 2; EMR)
The rate of urea used in the experiment was high (50 kg/ha). In apples this rate is used post-harvest and just prior to leaf fall to encourage leaf rotting. Leaves may be scorched. There may be potential phytotoxic effects of urea on raspberry canes and buds. There is therefore a need to conduct trials with urea on dormant raspberry canes. A trial is planned for February 2009.

Task 1.3.3 and Task 4.3.1 – Glasshouse and field evaluation of natural products and commodity substances for control of *Botrytis* and powdery mildew. (years 1-3; ADAS, EMR)

Objective

To determine the relative efficacy of a range of fungicides and natural products for control of *Botrytis* on raspberry.

Method

A field experiment was conducted in 2008 at East Malling Research, Kent, in an open-field plantation of raspberry cv. Glen Ample planted as long canes in 2005.

Each plot consisted of a single row, 8 m long, separated from adjacent plots by an unsprayed guard row. In 2008, programmes of new fungicides (Coded product HDCF 5, Switch) with a natural product Chitoplant (chitosan from crushed crab shells) and two biocontrol agents – Serenade (*Bacillus subtilis*) and Shemer (*Metschnikowia fructicola*) were compared (Table 1.3.3.1). These biocontrol agents were also included in programmes with Switch. Teldor was included as the standard fungicide and an untreated control was included. The treatments were applied to plots using a Solo self propelled small plot mini sprayer at 1000 L/ha on three occasions (22 May, 6 June, 18 June). All treatments were replicated four times in a randomised block design. Crop development was again very variable. Plants at early flower at the time of the first spray were labelled and picking began when the labelled fruit were red. Prior to this, the plots were cleared of all ripe fruit.

Plots were regularly inspected for *Botrytis*. At harvest, a random sample of two punnets (approximately 100 fruit) of red fruit were picked from the central section of each plot and assessed for *Botrytis*, powdery mildew and any other diseases. The fruit was similarly picked and assessed on three further occasions coinciding with the spray timings. At each harvest a sample of 100 healthy red fruit were taken for post-harvest pathogen tests. The fruit were placed in individual modules in trays, covered in polythene and damp incubated. Rot incidence was assessed after seven days incubation at ambient temperature (20-25ºC) for all harvest dates.

A sample of green fruit was taken from each plot in July, surface sterilised in 5% by volume 'Domestos' bleach and incubated on agar containing paraquat and chloramphenicol (PCA) under lights to check for latent *B. cinerea* infection in the fruit. The incidence of cane diseases in the plots will be assessed in March 2009.

Results and discussion

Botrytis

The weather conditions in 2008 during most of the flowering period were very wet and favourable for *Botrytis* infection of flowers. Despite this, the incidence of *Botrytis* on fruit at harvest was negligible at all four dates. In post-harvest tests the incidence of *Botrytis* fruit rot varied from 8 to almost 70% (Table 1.3.3.2). In most of the fruit picks the incidence of *Botrytis* varied considerably. The lowest incidence of *Botrytis* was generally recorded in the fruit from fungicide-treated plots. The highest incidence of *Botrytis* was usually recorded in fruit from plots treated with Chitoplant or Serenade, except at Pick 4. No significant effects of treatments on *Botrytis* incidence were recorded at Pick 1 or at Pick 2 (treatment 4 - Switch almost significant compared to the untreated control). At Pick 3 the incidence of *Botrytis* compared to the untreated control was significantly lower in fruit from all fungicide-treated plots (Treatments 2, 3 and 4) and from plots treated with Shemer (Treatment 8) or Switch and Serenade (Treatment 9). At Pick 4 only fruit from Treatment 3 (Coded product HDCF 5) had significantly less *Botrytis* than the untreated control.

The incidence of *B. cinerea* in green fruit samples (Table 1.3.3.5) varied from around 30 to almost 60% infected fruit. The lowest incidence of *Botrytis* was recorded in fruit from plots treated with Teldor or Switch but there were no significant differences between treatments.

Other rots

In general the incidence of penicillium rot was low and varied from 1 to 8% (Table 1.3.3.3). There was no consistent effect of any of the treatments on the incidence of penicillium rot except at Pick 2 where the incidence of penicillium rot in fruit from untreated plots was significantly lower than in most other treated fruit.

The effect of the treatments on the incidence of mucor (including rhizopus) rot is shown in Table 1.3.3.4. The rot incidence was high, probably due to the wet conditions and ranged from 24 to 80%. There was no consistent effect of treatments on rot incidence, but at Pick 3 the incidence of mucor rot was significantly lower on fruit from Chitoplant-treated plots compared to untreated plots.

Table 1.3.3.1. Treatments applied to open-field raspberries in 2008, East Malling Research, Kent. All sprays were at applied three times at 10 day intervals from flowering

Treatment	Active ingredient	Product rate	No. of sprays applied
1.Untreated	-	-	0
2. Teldor	fenhexamid	1.5kg / ha	3
3. Coded product HDCF 5	Experimental	O.83L/ha	3
4. Switch	cyprodonil + fludioxonil	1.0kg/ha	3
5. Chitoplant	chitosan	0.5g/L	3
6. Serenade	Bacillus subtilis	10L/ha	3
7. Shemer	Metschnikowia fructicola	0.2%	3
8. Switch at first flower then 2 sprays Serenade	cyprodonil +fludioxonil + Bacillus subtilis	1.0kg/ha + 10L/ha	3
9. Switch at first flower then 2 sprays Shemer	cyprodonil +fludioxonil + *Metschnikowia*	1.0kg/ha + 0.2%	3

Table 1.3.3.2. Mean % incidence (angular transformed) of *Botrytis*-rotted fruit in post-harvest tests (7 days incubation at ambient temperature) on raspberries harvested from plots treated in 2008 with various chemicals or biocontrol agents at East Malling Research, Kent. Figures in brackets are back transformed means

Treatment	Pick 1 1 July	Pick 2 8 July	Pick 3 15 July	Pick 4 22 July
1.Untreated	20.8 (12.6)	34.4 (31.9)	46.6 (52.8)	40.3 (41.9)
2. Teldor	17.7 (9.2)	31.4 (27.1)	33.5 (30.5)	47.6 (54.6)
3. Coded product HDCF 5	16.9 (8.4)	34.8 (32.5)	34.1 (31.4)	25.0 (17.9)
4. Switch	19.4 (11.0)	21.0 (12.8)	33.1 (29.8)	41.0 (43.1)
5. Chitoplant	25.3 (18.3)	40.2 (41.7)	54.5 (66.3)	40.0 (41.2)
6. Serenade	25.2 (18.2)	46.0 (51.7)	48.5 (56.7)	34.4 (32.0)
7. Shemer	18.0 (9.6)	34.2 (31.5)	38.3 (38.4)	49.1 (57.2)
8. Switch at first flower then 2 sprays Serenade	16.4 (7.9)	37.5 (37.1)	37.2 (36.6)	31.2 (26.9)
9. Switch at first flower then 2 sprays Shemer	22.3 (14.4)	48.2 (55.5)	42.4 (45.5)	42.7 (46.0)
F Probability	0.306	0.041	<0.001	0.069
SED (24 dof)	4.26	7.24	4.16	7.41
LSD (p= 0.05)	8.88	14.95	8.58	15.3

Table 1.3.3.3 Mean % incidence (angular transformed) of penicillium-rotted fruit in post-harvest tests (7 days incubation at ambient temperature) on raspberries harvested from plots treated in 2008 with various chemicals at East Malling Research, Kent. Figures in brackets are back transformed means

Treatment	Pick 1 1 July	Pick 2 8 July	Pick 3 15 July	Pick 4 22 July
1.Untreated	11.9	4.9	14.1	16.7
	(4.3)	(0.7)	(5.9)	(8.3)
2. Teldor	13.3	11.0	13.7	16.5
	(5.3)	(3.6)	(5.6)	(8.0)
3. Coded product HDCF 5	9.6	17.9	13.1	14.9
	(2.8)	(9.5)	(5.1)	(6.6)
4. Switch	7.8	9.0	12.9	13.3
	(1.8)	(2.4)	(5.0)	(5.3)
5. Chitoplant	14.3	12.1	14.1	12.1
	(6.1)	(4.4)	(5.9)	(4.4)
6. Serenade	13.2	13.0	12.7	15.5
	(5.2)	(5.1)	(4.8)	(7.1)
7. Shemer	12.8	10.2	9.0	16.8
	(4.9)	(3.1)	(2.5)	(8.3)
8. Switch at first flower then 2 sprays Serenade	7.3	14.3	14.3	10.0
	(1.6)	(6.1)	(6.1)	(3.0)
9. Switch at first flower then 2 sprays Shemer	9.6	14.4	13.0	15.1
	(2.8)	(6.2)	(5.0)	(6.8)
F Probability	0.248	0.022	0.900	0.740
SED (24 dof)	3.05	3.11	3.49	4.09
LSD (p= 0.05)	6.37	6.41	7.21	8.44

Table 1.3.3.4. Mean % incidence (angular transformed) of mucor-rotted fruit in post-harvest tests (7 days incubation at ambient temperature) on raspberries harvested from plots treated in 2008 with various chemicals at East Malling Research, Kent. Figures in brackets are back-transformed means

Treatment	Pick 1 1 July	Pick 2 8 July	Pick 3 15 July	Pick 4 22 July
1.Untreated	52.0	56.2	40.7	36.1
	(62.2)	(69.0)	(42.5)	(34.8)
2. Teldor	46.6	50.4	45.6	34.2
	(52.8)	(59.4)	(51.0)	(31.6)
3. Coded product HDCF 5	45.9	47.3	41.3	34.6
	(51.6)	(54.0)	(43.5)	(32.2)
4. Switch	46.3	42.2	49.3	34.8
	(52.2)	(45.1)	(57.4)	(32.7)
5. Chitoplant	54.1	48.3	29.6	35.9
	(65.6)	(55.8)	(24.4)	(34.4)
6. Serenade	63.8	43.1	37.3	37.5
	(80.6)	(46.8)	(36.7)	(37.0)
7. Shemer	51.7	47.4	46.8	33.5
	(61.5)	(54.2)	(53.1)	(30.4)
8. Switch at first flower then 2	53.6	47.0	43.7	34.1
sprays Serenade	(64.8)	(53.4)	(47.8)	(31.4)
9. Switch at first flower then 2	57.5	39.7	40.8	31.3
sprays Shemer	(71.2)	(40.8)	(42.7)	(26.9)
F Probability	0.314	0.770	0.005	0.984
SED (24 dof)	7.36	8.89	4.23	5.39
LSD (p= 0.05)	15.36	18.35	8.72	11.12

Table 1.3.3.5. Mean % incidence (angular transformed) of *Botrytis*-rotted fruit in green fruit incubated on PCA harvested 1n July from raspberry plots treated in 2008 with various chemicals at East Malling Research, Kent. Figures in brackets are back-transformed means

Treatment	***Botrytis***
1. Untreated	50.3 (59.2)
2. Teldor	36.4 (35.2)
3. Coded product HDCF 5	48.2 (55.5)
4. Switch	34.2 (31.7)
5. Chitoplant	46.4 (52.4)
6. Serenade	45.7 (51.3)
7. Shemer	42.3 (45.3)
8. Switch at first flower then 2 sprays Serenade	51.1 (60.6)
9. Switch at first flower then 2 sprays Shemer	47.8 (54.8)
F Probability	0.937
SED (dof)	14.24
LSD (p= 0.05)	29.40

Conclusions

- The incidence of *Botrytis* on fruit at harvest was negligible at all four picking dates
- In post-harvest tests the incidence of *Botrytis* fruit rot ranged from 8 to almost 70% and varied considerably in most of the picking dates
- The lowest incidence of *Botrytis* was generally recorded in the fruit from fungicide-treated plots
- The highest incidence of *Botrytis* was usually recorded in fruit from plots treated with Chitoplant or Serenade
- The incidence of *Botrytis* on fruit from plots treated with Shemer (Treatment 8) was significantly less than that in untreated plots at Pick 3. There was no effect of this treatment on *Botrytis* incidence at the other pick dates
- In general the incidence of penicillium rot was low and varied from 1 to 8%. There was no consistent effect of any of the treatments on the incidence of penicillium rot except at Pick 2 where the incidence of penicillium rot in fruit from untreated plots was significantly lower than in most other treated fruit
- The incidence of mucor (including rhizopus) rot was high and ranged from 24 to 80%. There was no consistent effect of treatments on rot incidence, but at Pick 3 the incidence of mucor rot was significantly lower on fruit from Chitoplant-treated plots compared to untreated plots

Task 1.3.4 and Task 4.3.3 – Field evaluation of combined fungicide and other product programmes for control of raspberry diseases. (year 3; ADAS, EMR)

Task 1.3.5: Evaluation of post-harvest cold-storage treatments on development of fruit *Botrytis*

This task is additional to those listed in the proposal. It was devised in 2007 following an experiment that showed there were high levels of latent *Botrytis* in raspberry fruit from tunnel-covered crops, irrespective of whether *Botrytis* fungicides were applied during flowering or not. Further, it was demonstrated that rapid cooling to remove field heat and subsequent cool-chain management effectively controlled development of visible *Botrytis* up to 7 days post-harvest. This task was done by ADAS instead of a field trial (Task 1.3.3) with the agreement of the consortium. The objective of Task 1.3.5 was to test the effect of four post-harvest fruit-cooling regimes, compared with fruit retained at ambient, on the incidence of *Botrytis* in fruit from covered crops treated and untreated with *Botrytis* fungicides during flowering.

Materials and methods

One half of a well established tunnel crop of raspberries cv. Glen Ample in Cambridgeshire was treated with Teldor (fenhexamid) for control of *Botrytis*. Sprays were applied tractor-mounted sprayer at first open flowers and two weeks later (50% flowering). The other half of the tunnel was left unsprayed. A length of 20 m of a second tunnel was left both uncovered and unsprayed. Each of the three tunnel areas (termed crops A, B and C) was divided into three replicate blocks (three rows each) and fruit sampled from each area according to the details in Tables 1.3.5.1 and 1.3.5.2. Flowers were tagged with coloured wool three times a

week just as they were starting to open, and the relevant colour noted when the fruit was picked.

Twenty-five marketable ripe fruit were picked into each of two punnets per replicate (to give a single layer of fruit) and taken to their storage areas within two hours of picking. Punnets for each storage treatment were kept in cardboard trays and moved between conditions and assessed for fruit rots at the intervals shown in Table 1.3.5.3. Lids were put on fruit once on a shelf in either the packhouse area (to mimic transport and shop display storage areas) or in an office at ADAS Boxworth (mimicking home storage). A logger was kept in each storage area to monitor temperature and relative humidity. Each logger was kept in an empty punnet, and was moved into a fruit tray with the punnets when they were moved into that storage area. Disease assessments were carried out at intervals by looking through the clear transparent plastic sides of the punnet and over the top of the fruit without handling. The number of fruit by *Botrytis* and other fungi (including *Fusarium*, *Penicillium* and *Cladosporium*) were counted. Fruit was assessed for fungal growth at 2, 4, 7, and 9 days after picking. Where visible infection was still low at 9 days a further assessment was made at 11 days.

Table 1.3.5.1: Details of Glen Ample crops sampled for fruit cooling treatments, Cambridgeshire - summer 2008

Crop	Fungicide application	Tunnel covering	Fruit sampling frequency
A.	Unsprayed	Uncovered	Picks 1 and 3
B.	Unsprayed	Covered	Picks 1, 2, 3 and 4
C.	2 sprays of Teldor	Covered	Picks 1, 2, 3 and 4

Table 1.3.5.2: Crop diary of Glen Ample sampled for fruit cooling treatments, Cambridgeshire – summer 2008

Date	Week	Activity in crop
06.05.08	19	Tunnel covered with new Luminance THB polythene
12.05.08	30	1st flower pick
14.05.08	20	Teldor spray 1
28.05.08	22	Teldor spray 2
11.06.08	24	14th (final) flower pick
23.06.08	26	1st Fruit pick (flowers would have received 1st spray)
30.06.08	27	2nd Fruit pick (flowers open within 7 days of 2nd spray)
07.07.08	28	3rd Fruit pick (flowers open 7-14 days after 2nd spray)
14.07.08	29	4th Fruit pick

Table 1.3.5.3: Storage treatments evaluated and the number of days fruit was held in each location, Cambridgeshire - summer 2008

Treatment	Rapid field heat removal (2°C)*	Cold storage (3°C)**	Transport + final display (12°C)**	Home storage (20°C)**
1. Untreated control	0	0	0	9 d
2. `Good practice`	0	2 d	2 d	5 d

3. `Better practice` (rapid field heat removal)	1 d	1 d	2 d	5 d
4. No cool chain	1 d	1 d	0	7 d
5. Prolonged cold storage	1 d	3 d	3 d	2 d

* Room temperature during forced air cooling, mean tray temperature 6°C
** Mean tray temperatures

Results and discussion

Fruit storage temperatures

The temperature in the tray stack in the field heat removal area was a minimum 4.8°C, mean 6.5°C and maximum 7.5°C, although within the forced air stream the air temperature was 2°C. Humidity was always above 80% RH. The shop cold store was between 1.6°C and 3.9°C, with a mean temperature of 3.3°C. The humidity ranged between 25% and 70% RH over the month, probably according to the type and amount of farm produce present. The shelf life room was between 16°C and 18°C with a mean of 17°C (higher than the 12°C planned), and between 50% to 70% RH. Final storage was at a mean room temperature of 22.7°C with a range of 18°C to 28°C, and 50% to 80% RH.

Effect of treatments on Botrytis

The incidence of fruit affected by *B. cinerea* for picks 1 to 4 is shown in Tables 1.3.5.4 to 1.3.5.7. Where there was either zero or a trace of *Botrytis* in the early assessments this has not been tabulated here, but is shown in combination with other fungi in the tables of healthy fruit incidence (Tables 1.3.5.8 to 1.3.5.11). It is not always possible to identify the fungal species present in the initial stages of mould growth.

Cold-storage treatment had a significant effect on *Botrytis* levels at pick 1 (after 2, 7 and 9 days), pick 2 (after 9 days), pick 3 (after 7 and 9 days) and pick 4 (after 7 and 9 days), with the greatest *Botrytis* in ambient stored fruit and least in fruit given prolonged cold storage.

Crop source had a significant effect on the incidence of fruit *Botrytis* at picks 1 (after 9 days), 2 (after 11 days), 3 (after 7 and 9 days) and 4 (after 9 days), with greater levels in the uncovered + unsprayed crop (A), and in the covered and unsprayed crop (B), than in the covered and sprayed crop (C) in one or more of the storage treatments.

Teldor treatment appeared to reduce *Botrytis* in covered crops but only on fruit assessed at least 9 days after picking. Fruit pick 1, which corresponded to fungicide treatment at first open flowers, did not show a greater reduction in fruit *Botrytis* than in picks 2, 3 and 4, where fruit corresponded to flowers that were not fully open at the time of the fungicide application.

The “prolonged cold storage” treatment 5 (1 day of field heat removal, 3 days in 3ºC cold storage, then 3 days at 17ºC) resulted in zero fruit *Botrytis* at the 7 days assessments (*i.e.* after 2 days at 23ºC) at picks 2, 3 and 4, even in uncovered unsprayed and covered unsprayed crops. Pick 1 from the uncovered and unsprayed crop had 1% of fruit with *Botrytis*, but the covered sources had zero.

At pick 1, there was a significant storage × sources treatment interaction at the 2 and 7 day assessments. At the 7 day assessment, there were high *Botrytis* levels in T3 ('rapid field heat removal') and T4 ('no cool chain') from the uncovered unsprayed crop (A), and T1 ('ambient') from the covered sprayed crop (C), and zero or near-zero levels from T5 ('prolonged cold storage') from all three sources. There was no storage × source interaction at picks 2 to 4.

Table 1.3.5.4a: Effect of fungicide and cold-storage treatment on incidence of fruit *Botrytis* - first pick (flowers open at first Teldor spray)

Days before assessment under each storage regime	% fruit with visible *Botrytis cinerea*		
	Uncovered unsprayed	Covered unsprayed	Covered sprayed
After 2 days			
1. Untreated (ambient)	1.3	2.0	0.0
2. `Good practice`	0.7	0.0	1.3
3. `Better practice`	0.0	0.0	0.0
4. No cool chain	0.0	0.7	0.0
5. Prolonged cold storage	0.0	0.0	0.7
After 4 days			
1. Untreated (ambient)	4.7	1.3	0.7
2. `Good practice`	2.0	2.7	3.3
3. `Better practice`	1.3	3.3	1.3
4. No cool chain	2.0	0.7	1.3
5. Prolonged cold storage	0.7	0.7	0.7
After 7 days			
1. Untreated (ambient)	8.0	11.3	22.0
2. `Good practice`	0.7	4.7	10.7
3. `Better practice`	17.3	14.0	11.3
4. No cool chain	18.7	8.0	6.7
5. Prolonged cold storage	1.3	0.0	0.0
After 9 days			
1. Untreated (ambient)	69.3	37.3	26.0
2. `Good practice`	52.7	32.0	14.7
3. `Better practice`	67.3	42.7	34.7
4. No cool chain	42.0	25.3	19.3
5. Prolonged cold storage	18.0	16.0	10.7

Table 1.3.5.4b: Analysis of variance of effect of fungicide and cold-storage treatments on fruit *Botrytis* - first pick

Factor	Df	% fruit with visible *Botrytis cinerea*							
		2 days		4 days		7 days		9 days	
		F pr.	Lsd	F pr.	LSD	F pr.	LSD	F pr	LSD
Storage	4	P<0.05	0.74	n.s.	1.83	P<0.001	5.22	P<0.001	10.79
Sources	2	n.s.	0.58	n.s.	1.42	n.s.	4.04	P<0.001	8.36
Storage × sources	8	P<0.05	1.29	n.s.	3.16	P<0.01	9.04	n.s.	18.70
Residual	58								

Table 1.3.5.5a: Effect of fungicide and cold-storage treatment on incidence of fruit *Botrytis* - second pick (flowers open within 7 days of second Teldor spray)

Days before assessment under each storage regime	% fruit with visible *Botrytis cinerea*	
	Covered unsprayed	Covered sprayed
After 7 days		
1. Untreated (ambient)	4.0	1.3
2. `Good practice`	1.3	0.0
3. `Better practice`	0.0	0.0
4. No cool chain	0.7	0.0
5. Prolonged cold storage	0.0	0.0
After 9 days		
1. Untreated (ambient)	8.0	5.3
2. `Good practice`	2.7	2.0
3. `Better practice`	2.0	1.3
4. No cool chain	2.7	2.0
5. Prolonged cold storage	0.7	0.7
After 11 days		
1. Untreated (ambient)	18.7	8.0
2. `Good practice`	11.3	10.0
3. `Better practice`	8.7	7.3
4. No cool chain	8.0	6.0
5. Prolonged cold storage	10.7	4.7

Table 1.3.5.5b. Analysis of variance of effect of fungicide and cold-storage treatments on fruit *Botrytis* - second pick

Factor	Df	% fruit with visible *Botrytis cinerea*					
		7 days		9 days		11 days	
		F pr.	Lsd	F pr.	Lsd	F pr.	Lsd
Storage	4	n.s.	2.06	P<0.001	2.52	n.s.	5.58
Sources	1	n.s.	1.30	n.s.	1.59	P<0.05	3.53
Storage × sources	4	n.s.	2.91	n.s.	3.56	n.s.	7.89
Residual	38						

Table 1.3.5.6a: Effect of fungicide and cold-storage treatment on incidence of fruit *Botrytis* - third pick (flowers open 7-14 days after second spray)

Days before assessment under each storage regime	% fruit with visible *Botrytis cinerea*		
	Uncovered unsprayed	**Covered unsprayed**	**Covered sprayed**
After 4 days			
1. Untreated (ambient)	6.0	0.7	0.0
2. `Good practice`	0.0	0.0	0.0
3. `Better practice`	0.0	0.0	0.0
4. No cool chain	0.0	0.0	0.0
5. Prolonged cold storage	0.0	0.0	0.0
After 7 days			
1. Untreated (ambient)	29.3	6.0	8.0
2. `Good practice`	14.0	1.3	2.0
3. `Better practice`	19.3	1.3	2.7
4. No cool chain	36.0	10.0	4.0
5. Prolonged cold storage	0.0	0.0	0.0
After 9 days			
1. Untreated (ambient)	89.3	40.0	36.7
2. `Good practice`	67.3	28.0	17.3
3. `Better practice`	50.7	23.3	28.7
4. No cool chain	78.0	58.7	57.3
5. Prolonged cold storage	16.0	2.7	2.7

Table 1.3.5.6b: Analysis of variance of effect of fungicide and cold-storage treatments on fruit *Botrytis* - third pick

Factor	Df	% fruit with visible *Botrytis cinerea*					
		4 days		7 days		9 days	
		F pr.	Lsd	F pr.	Lsd	F pr.	Lsd
Storage	4	n.s.	1.88	P<0.05	10.28	P<0.001	12.35
Sources	2	n.s.	1.46	P<0.001	7.96	P<0.001	9.57
Storage × sources	8	n.s.	3.26	n.s.	17.80	n.s.	21.40
Residual	58						

Table 1.3.5.7a: Effect of fungicide and cold-storage treatments on fruit *Botrytis*, 2008 - fourth pick (flowers open 14-21 days after second Teldor spray)

Days before assessment under each storage regime	% fruit with visible *Botrytis cinerea*	
	Covered unsprayed	Covered sprayed
After 7 days*		
1. Untreated (ambient)	24.7	26.0
2. `Good practice`	5.3	4.7
3. `Better practice`	5.3	4.0
4. No cool chain	2.7	4.0
5. Prolonged cold storage	0.0	0.0
After 9 days		
1. Untreated (ambient)	78.0	68.7
2. `Good practice`	44.7	28.7
3. `Better practice`	63.3	38.7
4. No cool chain	30.7	38.0
5. Prolonged cold storage	49.3	18.0

* There were very few fruit with fungal growth at 4 days (see % healthy fruit, Table 1.3.5.11)

Table 1.3.5.7b: Analysis of variance of effect of fungicide and cold-storage treatments on fruit *Botrytis* - fourth pick

Factor	Df	% fruit with visible *Botrytis cinerea*			
		7 days		9 days	
		F pr.	Lsd	F.pr	Lsd
Storage	4	P<0.001	5.13	P<0.001	16.10
Sources	1	n.s.	3.25	P<0.01	10.18
Storage × sources	4	n.s.	7.26	n.s.	22.77
Residual	38				

Effects of treatments on healthy fruit

The incidence of fruit without any growth of *B. cinerea* or other fungal species is given in Tables 1.3.5.8 to 1.3.5.11. Storage treatment had a significant effect on % healthy fruit at pick 1 (2, 7 and 9 day assessments), pick 2 (9 day assessment), pick 3 (4, 7 and 9 day assessments) and pick 4 (4, 7 and 9 day assessments). All fruit in storage treatments 2 to 5 remained healthy, irrespective of source, after 7 days (pick 2), 4 days (pick 3) and 4 days (pick 4).

The source of raspberries had a significant effect on % healthy fruit at pick 1 (9 day assessment), pick 2 (11 day assessment), pick 3 (9 day assessment) and pick 4 (9 day assessment); *i.e.* the effect of raspberry source shows up relatively late, whereas the effect of storage treatment (above) can show after 2 days.

Table 1.3.5.8a: Effect of fungicide and cold-storage treatment on incidence of healthy fruit - first pick (flowers open at first Teldor spray)

Days before assessment under each storage regime	% healthy fruit		
	Uncovered unsprayed	Covered unsprayed	Covered sprayed
After 2 days			
1. Untreated (ambient)	97.3	97.3	98.0
2. `Good practice`	97.3	99.3	98.7
3. `Better practice`	100.0	100.0	100.0
4. No cool chain	100.0	99.3	100.0
5. Prolonged cold storage	100.0	100.0	99.3
After 4 days			
1. Untreated (ambient)	94.7	98.7	98.7
2. `Good practice`	98.0	97.3	96.7
3. `Better practice`	98.7	96.7	98.7
4. No cool chain	98.0	98.0	98.7
5. Prolonged cold storage	99.3	99.3	99.3
After 7 days			
1. Untreated (ambient)	90.7	87.3	74.7
2. `Good practice`	98.7	94.7	88.7
3. `Better practice`	80.0	85.3	87.3
4. No cool chain	80.7	91.3	92.7
5. Prolonged cold storage	98.0	100.0	100.0
After 9 days			
1. Untreated (ambient)	26.0	58.7	69.3
2. `Good practice`	44.0	66.7	82.7
3. `Better practice`	30.0	56.0	64.7
4. No cool chain	53.3	73.3	78.0
5. Prolonged cold storage	76.0	82.0	86.0

Table 1.3.5.8b: Analysis of variance of effect of fungicide and cold-storage treatments on healthy fruit - first pick

Factor	Df	% healthy fruit							
		2 days		4 days		7 days		9 days	
		F pr.	Lsd	F pr.	LSD	F pr.	LSD	F pr	LSD
Storage	4	P<0.01	1.48	n.s.	1.95	P<0.001	5.70	P<0.001	10.92
Sources	2	n.s.	1.14	n.s.	1.51	n.s.	4.42	P<0.001	8.46
Storage × sources	8	n.s.	2.56	n.s.	3.37	P<0.01	9.87	n.s.	18.92
Residual	58								

Table 1.3.5.9a: Effect of fungicide and cold-storage treatment on incidence of healthy fruit - second pick (flowers open within 7 days of second Teldor spray). Most fruit were still healthy by the second day

Days before assessment under each storage regime	% healthy fruit	
	Covered unsprayed	Covered sprayed
After 4 days		
1. Untreated (ambient)	100.0	98.7
2. `Good practice`	99.3	100.0
3. `Better practice`	99.3	100.0
4. No cool chain	100.0	100.0
5. Prolonged cold storage	100.0	100.0
After 7 days		
1. Untreated (ambient)	96.0	98.0
2. `Good practice`	98.0	100.0
3. `Better practice`	99.3	100.0
4. No cool chain	99.3	100.0
5. Prolonged cold storage	100.0	100.0
After 9 days		
1. Untreated (ambient)	92.0	93.3
2. `Good practice`	96.0	97.3
3. `Better practice`	97.3	97.3
4. No cool chain	96.7	98.0
5. Prolonged cold storage	99.3	99.3
After 11 days		
1. Untreated (ambient)	81.3	90.7
2. `Good practice`	88.7	90.0
3. `Better practice`	90.0	92.7
4. No cool chain	92.0	94.0
5. Prolonged cold storage	89.3	94.7

Table 1.3.5.9b: Analysis of variance of effect of fungicide and cold-storage treatments on healthy fruit - second pick

Factor	Df	% healthy fruit							
		4 days		7 days		9 days		11 days	
		F pr.	Lsd	F pr.	LSD	F pr.	LSD	F pr	LSD
Storage	4	n.s.	0.71	n.s	2.20	P<0.001	2.91	n.s.	5.29
Sources	1	n.s.	0.45	n.s	1.39	n.s.	1.84	P<0.05	3.35
Storage × sources	4	P<0.05	1.01	n.s.	3.11	n.s.	4.12	n.s.	7.49
Residual	38								

Table 1.3.5.10a: Effect of fungicide and cold-storage treatment on incidence of healthy fruit - third pick (flowers open 7-14 days after second spray)

Days before assessment under each storage regime	% healthy fruit		
	Uncovered unsprayed	Covered unsprayed	Covered sprayed
After 4 days			
1. Untreated (ambient)	94.0	99.3	100.0
2. `Good practice`	99.3	100.0	100.0
3. `Better practice`	100.0	100.0	100.0
4. No cool chain	100.0	100.0	100.0
5. Prolonged cold storage	100.0	100.0	100.0
After 7 days			
1. Untreated (ambient)	70.7	94.0	90.7
2. `Good practice`	86.0	98.7	98.0
3. `Better practice`	78.0	98.0	97.3
4. No cool chain	64.0	90.0	94.7
5. Prolonged cold storage	100.0	100.0	100.0
After 9 days			
1. Untreated (ambient)	10.7	55.3	57.3
2. `Good practice`	32.7	72.0	82.0
3. `Better practice`	48.0	75.3	69.3
4. No cool chain	22.0	40.7	41.3
5. Prolonged cold storage	84.0	97.3	96.7

Table 1.3.5.10b: Analysis of variance of effect of fungicide and cold-storage treatments on healthy fruit - third pick

Factor	Df	% healthy fruit					
		4 days		7 days		9 days	
		F pr.	Lsd	F pr.	Lsd	F pr.	Lsd
Storage	4	n.s.	1.89	P<0.05	10.29	P<0.001	12.79
Sources	2	n.s.	1.47	P<0.001	7.97	P<0.001	9.91
Storage × sources	8	n.s.	3.28	n.s.	17.83	n.s.	22.15
Residual	58						

Table 1.3.5.11a: Effect of fungicide and cold-storage treatment on incidence of healthy fruit - fourth pick (flowers open 14-21 days after second spray)

Days before assessment under each storage regime	% healthy fruit	
	Covered unsprayed	Covered sprayed
After 4 days		
1. Untreated (ambient)	98.0	97.3
2. `Good practice`	100.0	99.3
3. `Better practice`	100.0	98.7
4. No cool chain	100.0	100.0
5. Prolonged cold storage	100.0	100.0
After 7 days		
1. Untreated (ambient)	74.7	73.3
2. `Good practice`	94.7	95.3
3. `Better practice`	94.7	96.0
4. No cool chain	97.3	96.0
5. Prolonged cold storage	100.0	100.0
After 9 days		
1. Untreated (ambient)	22.0	25.3
2. `Good practice`	54.0	67.3
3. `Better practice`	35.3	60.7
4. No cool chain	69.3	60.0
5. Prolonged cold storage	50.0	82.0

Table 1.3.5.11b: Analysis of variance of effect of fungicide and cold-storage treatments on healthy fruit - fourth pick

Factor	Df	% healthy fruit					
		4 days		7 days		9 days	
		F pr.	Lsd	F pr.	Lsd	F pr.	Lsd
Storage	4	P<0.01	1.24	P<0.001	5.20	P<0.001	15.81
Sources	1	n.s.	0.79	n.s.	3.22	P<0.05	10.00
Storage × sources	4	n.s.	1.76	n.s.	7.21	n.s.	22.36
Residual	38						

Effect of treatments on other fungi

The incidence of fruit with *Penicillium* in pick 1, and *Fusarium* for picks 1 and 3, is given in Tables 1.3.5.12 to 1.3.5.14. Neither fungus was obvious before the 9 day assessment. There was little of either fungus and no significant differences from the other picks (data not presented). Neither *Mucor* spp. nor *Rhizopus* spp. was seen in 2008 on fruit from either the covered or uncovered crops.

Levels of *Penicillium* spp. were low (0-4.7%) and largely confined to fruit given ambient storage (no cooling). Source of raspberry had no statistically significant effect, although the disease appeared slightly more common in fruit from the Teldor-sprayed than the unsprayed crops.

Levels of *Fusarium* spp. were low (0-3.3%). There was no statistically significant effect of storage treatment. There was a significant effect of source, with least disease in the first pick of the covered areas, and least disease in the third pick from the covered unsprayed crop.

Table 1.3.5.12a: Effect of fungicide and cold-storage treatment on incidence of Penicillium - third pick (flowers open 7-14 days after second spray)

Days before assessment under each storage regime	% fruit with *Penicillium*		
	Uncovered unsprayed	Covered unsprayed	Covered sprayed
After 9 days			
1. Untreated (ambient)	1.3	4.7	3.3
2. `Good practice`	0.0	0.0	0.0
3. `Better practice`	0.0	0.7	2.0
4. No cool chain	0.0	0.7	2.0
5. Prolonged cold storage	1.3	0.0	0.0

Little *Penicillium*, and no significant differences were recorded on other pick dates.

Table 1.3.5.12b: Analysis of variance of effect of fungicide and cold-storage treatments on incidence of *Penicillium* - third pick

Factor	Df	% fruit with *Penicillium*	
		9 days	
		F pr.	Lsd
Storage	4	P<0.05	2.06
Sources	2	n.s.	1.60
Storage × sources	8	n.s.	3.57
Residual	58		

Table 1.3.5.13a: Effect of fungicide and cold-storage treatment on incidence of *Fusarium* - first pick (flowers open 7-14 days after second spray)

Days before assessment under each storage regime	% fruit with *Fusarium*		
	Uncovered unsprayed	Covered unsprayed	Covered sprayed
After 9 days			
1. Untreated (ambient)	1.3	1.3	0.7
2. `Good practice`	3.3	1.3	0.7
3. `Better practice`	1.3	1.3	0.7
4. No cool chain	3.3	0.7	0.0
5. Prolonged cold storage	4.0	1.3	1.3

Table 1.3.5.13b: Analysis of variance of effect of fungicide and cold-storage treatments on incidence of *Fusarium* - third pick

Factor	Df	% fruit with *Fusarium*	
		9 days	
		F pr.	Lsd

Storage	4	n.s	1.74
Sources	2	P<0.05	1.35
Storage × sources	8	n.s	3.01
Residual	58		

Table 1.3.5.14a: Effect of fungicide and cold-storage treatment on incidence of *Fusarium* - third pick (flowers open 7-14 days after second spray)

Days before assessment under each storage regime	% fruit with *Fusarium*		
	Uncovered unsprayed	Covered unsprayed	Covered sprayed
After 9 days			
1. Untreated (ambient)	2.7	0.0	2.7
2. `Good practice`	2.0	0.0	1.3
3. `Better practice`	0.7	0.0	0.7
4. No cool chain	3.3	1.3	0.0
5. Prolonged cold storage	0.0	0.0	0.0

Little *Fusarium*, and no significant differences, were shown on other pick dates.

Table 1.3.5.14b: Analysis of variance of effect of fungicide and cold-storage treatments on incidence of *Fusarium* - third pick

Factor	Df	% fruit with *Fusarium*	
		9 days	
		F pr.	Lsd
Storage	4	n.s.	1.51
Sources	2	P<0.05	1.17
Storage × sources	8	n.s.	2.62
Residual	58		

Conclusions

Botrytis occurred in fruit from all crops at significant levels. These varied between the picks, with 70% to 89% from an outdoor untreated crop, 18% to 79% from a covered untreated crop and 8% to 69% from covered treated crop following ambient incubation of up to 11 days.

Fruit, picked from a covered crop that was treated during flowering with *Botrytis* fungicides, stored at 3°C for 3 days, moved to display, and then into ambient 7 days from picking ("prolonged cold storage") had least visible *B. cinerea* at the final assessment (9-11 days after picking), ranging from 3% to 18% infected. Fruit from all sources coming out of "prolonged cold storage" after 7 days principally had zero *B. cinerea.*

There was little *Fusarium* spp. or *Penicillium* spp. and no Mucor spp. or *Rhizopus* spp. in any of the treatments.

Objective 2. Raspberry beetle

2.1. Conduct field experiments to develop a monitoring method and an economic threshold for raspberry beetle in crops grown in tunnels

Task 2.1.1. - Experimental sites (years 1 –5; SCRI, EMR)

Task 2.1.2. - Development and production of lures for laboratory and field-testing; development and supply of traps of different designs: (all years; AgriSense)

Task 2.1.3 - Initial comparison of trap designs (year 1; SCRI)

Task 2.1.4. - Calibrate traps for pest monitoring (years 2,3; EMR, SCRI, Grower Organisations, AgriSense)

2.2 *Optimise Lure for control*

Task 2.2.1. - Evaluate blends and dispensers (years 1,2; SCRI)

2.3 Choose appropriate control approach and develop suitable device

Task 2.3.1. - Identify suitable device for lure and kill or mass trapping (year 2; SCRI)

2.4 Deployment strategy for control device

Task 2.4.1. - Deployment strategy for control device (years 2-4; SCRI, EMR)

2.5 Generate further efficacy data for registration

Summary

The overall aim of the work was to determine whether the raspberry beetle mass trapping device could be exploited for control of the pest in commercial protected raspberry plantations by perimeter trapping (traps are deployed round the perimeter of the treated area), or by deployment of traps in a regularly spaced grid throughout the crop.

Materials and methods

Duration of study

April - July 2008

Sites

Most conventional farms spray routinely for raspberry beetle so populations were likely to be low.

Farm 1. Euan McIntyre, Wester Essendy, Blairgowrie, Perthshire, Scotland (NO 135 435) Mob: 07770933022 Email: eaunmcintyre@btconnect.com (Marketing Desk, Berry Garden)

Farm 2. Jock McFarlane, Easter Rattray Farm, Blairgowrie, PH10 7HQ Mob: 07703 330 724 Email: McFarlane@sol.co.uk (Marketing Desk, Berry Garden)

Farm 3. Peter Thomson. Thomas Thomson Ltd, Haugh Road, Blairgowrie, UK, PH10 7BJ Mob: 0784347961 Email: pthomson@tthomson.co.uk (marketing Desk, Berry Garden)

Insecticide use at Scottish trial sites (2007-2008)

Insecticide treatments applied to the three Scottish sites in 2007

Site number	treatment	Chemical	date applied	pest treated
1	control	Calypso	12-Jun	RB
		Calypso	28-Jun	RB
		Dynamec	16-Aug	mite
		Masai	18-Sep	mite
	lattice	Calypso	12-Jun	RB
		Calypso	28-Jun	RB
		Dynamec	16-Aug	mite
	perimeter	Calypso	12-Jun	RB
		Calypso	28-Jun	RB
		Dynamec	16-Aug	mite
		Masai	18-Sep	mite
2	control	Talstar	24-Apr	weevil
		Aphox	02-Jul	aphid
		Masai	09-Jul	RB
		Desis	17-Jul	RB
	lattice	Talstar	24-Apr	weevil
		Aphox	02-Jul	aphid
		Masai	09-Jul	RB
		Desis	17-Jul	RB
	perimeter	Talstar	24-Apr	weevil
		Aphox	02-Jul	aphid
		Masai	09-Jul	RB
		Desis	17-Jul	RB
3	control	Masai	15-May	leaf and bud mite
		Calypso	30-May	raspberry beetle
	lattice	Masai	24-May	leaf and bud mite
		Calypso	25-May	raspberry beetle
	perimeter	Masai	14-May	leaf and bud mite
		Calypso	25-May	raspberry beetle

Insecticide treatments applied to the three Scottish sites in 2008				
Site number	**treatment**	**Chemical**	**date applied**	**pest treated**
1	control	Dynamec	26-Jun	mite
		Calypso	28-Jun	RB
		Dynamec	18-Aug	mite
	lattice	Dynamec	26-Jun	mite
		Calypso	28-Jun	RB
		Dynamec	18-Aug	mite
	perimeter	Calypso	28-Jun	RB
2	control	Apollo + Masai	13-Jun	RB and spider mite
		Calypso	04-Jul	RB
		Calypso	17-Apr	RB
	lattice	Apollo + Masai	13-Jun	RB and spider mite
		Calypso	04-Jul	RB
		Calypso	17-Apr	RB
	Perimeter	Apollo + Masai	13-Jun	RB and spider mite
		Calypso	04-Jul	RB
		Calypso	17-Apr	RB
3	control	Calypso	12-May	clay coloured weevil
		Calypso+Dynamec	02-Jun	RB and RBLM
		Dynamec	16-May	RLBM
	lattice	Dynamec	16-May	RLBM
		Calypso+Dynamec	01-Jun	RB and RBLM
	perimeter	Dynamec	16-May	RLBM
		Calypso+Dynamec	02-Jun	RB and RBLM

Treatments are given in Table 2.5.1. Devices were modified AgriSense funnel traps with white Correx cross vanes and a slow release sachet (Suttera prototype) containing initially 2.5 ml of compound B. The funnel traps contained 3 cm of 1% detergent solution (see Fig. 2.5.2).

Table 2.5.1. Treatments

Code	Control approach	No. of devices /ha	Spatial arrangement of device
C	Untreated control	None	
P	Perimeter trapping	50	Spaced 8m apart round the entire perimeter of the plot, suspended from the top wire at a height of ~ 1m on outer edge of crop
L	Uniform lattice	50	Spaced in a ~ 14 × 14 m grid if possible throughout the plot

Treatment application

Treatments were deployed 2-3 weeks before 1% flowering (refer to Swiss phenology code, *i.e.* traps were in place before/during GS 51 (first flower buds visible). Devices were handled with disposable rubber gloves and were removed at the end of the growing season at the end of July. Note that compound B is highly flammable.

Experimental design

A large scale dispersed, randomised block experimental design was used with 3 replicates of the 3 treatments (Table 2.5.1). Plots were approx 1 ha of commercial protected raspberry

plantation, 3 plots on each of 3 farms. See sites above and Fig. 2.5.1. For allocation of plots to treatments see Table 2.5.3.

Treatment with insecticide for raspberry beetle

It was not practical to leave the entire 3 ha trial area untreated with insecticide for raspberry beetle. In each plot, 3 rows (in one tunnel span) was left untreated with insecticide. See Fig. 2.5.1. The rest of the area was treated immediately after flower, by the grower as per normal practice.

Assessments

Trap catches of adults

Weekly monitoring

The numbers of raspberry beetles captured in each trap as shown in Table 2.5.2 was recorded weekly. Numbers of non-target insects (differentiating bumble bees, honey bees, wasps, other main insect types) were recorded weekly as per raspberry beetle.

Table 2.5.2. Number and position of weekly monitoring traps

Treatment	Weekly monitoring traps (lure)		Weekly monitoring traps (sticky and no lure)	
	No.	**Position**	**No.**	**Position**
Untreated	0	-	8	Positioned in 2 rows with 4 traps at the edge and 4 traps in the middle
Perimeter	4	Around the edge of the plot	8	As above
Lattice	4	Around the edge of the plot	8	As above

Pre- and post- 1% flowering

The total numbers of beetles captured in every device up to 1% flowering and after 1% flowering was recorded. Numbers of non-target species was recorded into broad taxa.

Larvae and damage to fruit

In the middle of the harvesting period, a sample of 400 ripening fruit was sampled from three sampling points inside the insecticide free tunnel and from three sampling points from the treated areas. Each sampling point was a 10 × 10 metre grid.

Table 2.5.3. Site and treatment details

Field name	Farm name	Raspberry Cultivar	Treatment	Plot area (ha)	Row spacing (m)	Rows/ tunnel	No of traps	
							Density (no./ha)	Required
E1	Wester Essendy, Blairgowrie	Ample	C	1.31	1.8	3	0	0
E2	Wester Essendy, Blairgowrie	Ample	P	0.39	2.2	3	50	20(20)
E3	Wester Essendy, Blairgowrie	Ample	L	0.65	2.2	3	50	33(32)
J1	Easter Rattray Farm, B.gowrie	Octavia	C	0.64	2.2	3	0	0
J2	Easter Rattray Farm, B.gowrie	Octavia	P	0.64	2.2	3	50	32(28)
J3	Easter Rattray Farm, B.gowrie	Octavia	L	0.64	2.2	3	50	32(32)
P1	T Thomson, Blairgowrie	Ample	C	0.97	2.2	3	0	0
P2	T Thomson, Blairgowrie	Ample	P	0.73	2.2	3	50	37(36)
P3	T Thomson, Blairgowrie	Ample	L	0.73	2.2	3	50	37(36)

C = control, P = Perimeter, L = Lattice

Table 2.5.4. Dates of experimental set-up and sampling

	Site 1	Site 2	Site 3
Treatment set-up	30 April	29 April	28 April
Bucket traps emptied	16 June	26 June	12 June
Berry sampling	10-11 July	5-6 August	9-10 July

Crop growth stage

The growth stage of the raspberries was recorded on each sampling occasion, using the Swiss phenological key (00-99). A record of the range at each site and week and approximate % of plants in each growth stage (GS) was made. If the GS was variable a record of the range (en GS 51-55) and approx % of each GS was done (*e.g.* GS51=25% Gs 52 = 50%, GS 53= 25%).

Additional wild host study

At site 3 (Peter Thomson), 6 sticky traps were sited near to the closest wild hosts (*e.g.* brambles, wild raspberries, hawthorn). These were changed weekly and raspberry beetle and other insects recorded.

Data collation and statistical analysis

Data was collated into Excel spreadsheets and statistically analysed with appropriate tests.

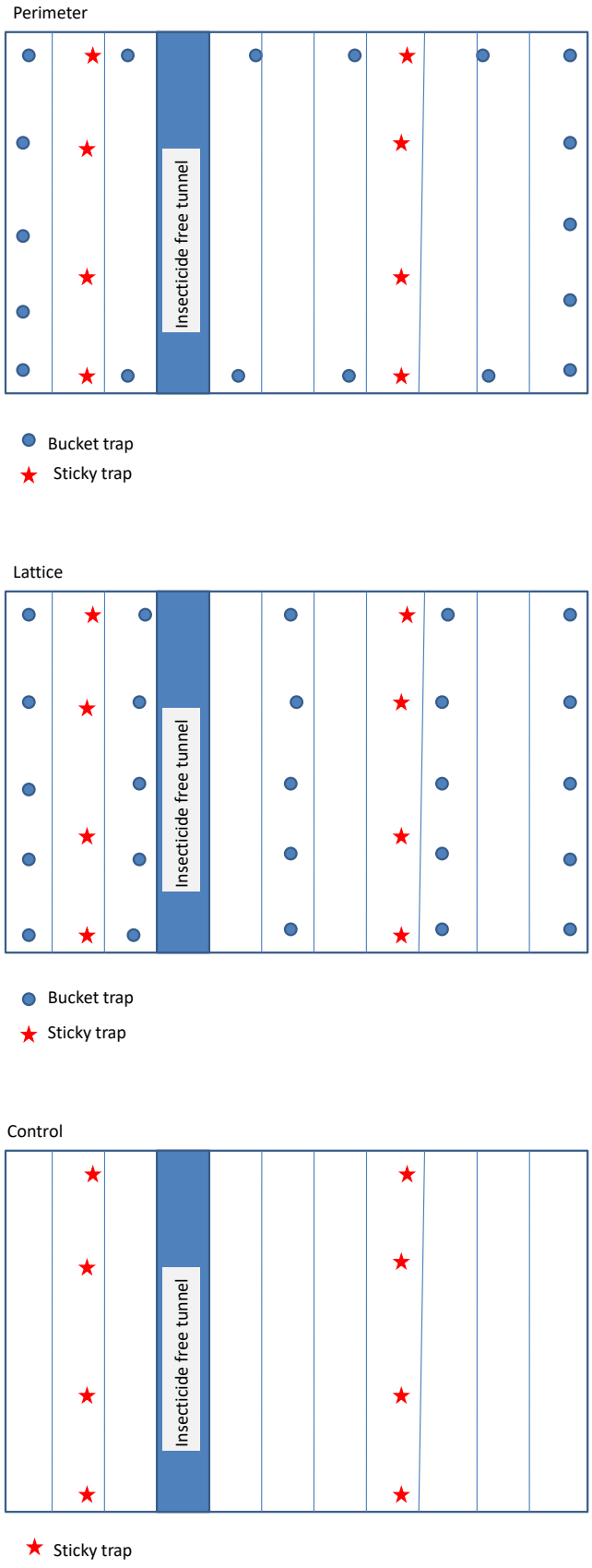


Figure 2.5.1. Position of bucket and sticky traps within the three treatments

Figure 2.5.2. Devices were modified AgriSense funnel traps with white Correx cross vanes and a slow release sachet (Suttera prototype) containing initially 2.5 ml of compound B attached to the top of the trap. The funnel traps contained 3 cm of 1% detergent solution

Results

Table 2.5.5. The number of raspberry beetle caught on the sticky traps

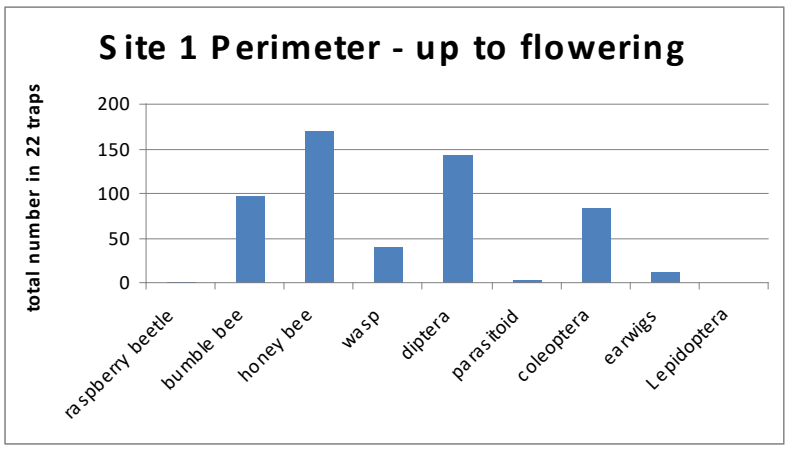
Sampling date	site	treatment	no. of raspberry beetle caught
28/04/2008	3	control	0
		Lattice	0
		Perimeter	0
30/04/2008	1	control	0
		Lattice	0
		Perimeter	0
29/04/2008	2	control	0
		lattice	0
		Perimeter	0
07/05/2008	3	control	0
		Lattice	0
		Perimeter	0
07/05/2008	1	control	0
		Lattice	0
		Perimeter	0
07/05/2008	2	control	0
		Lattice	0
		Perimeter	0
15/05/2008	3	control	0
		Lattice	0
		Perimeter	0
15/05/2008	1	control	1
		Lattice	0
		Perimeter	0
15/05/2008	2	control	1
		Lattice	1
		Perimeter	0
22/05/2008	3	control	1
		Lattice	0
		Perimeter	0
22/05/2008	1	control	0
		Lattice	0
		Perimeter	0

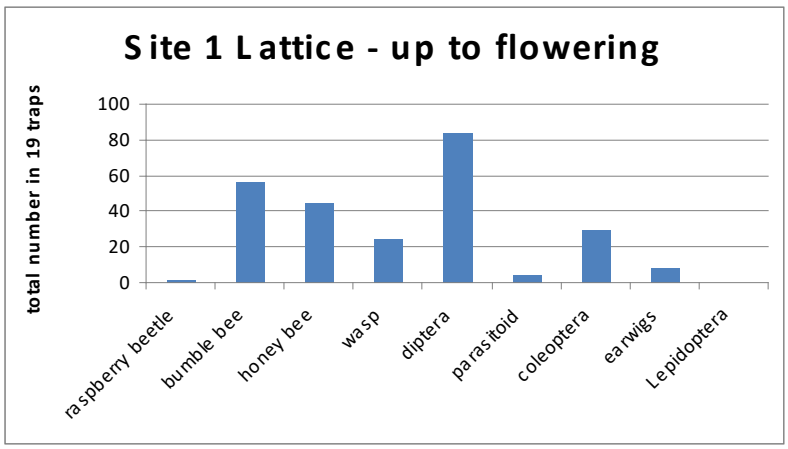
Table 2.5.5 continued

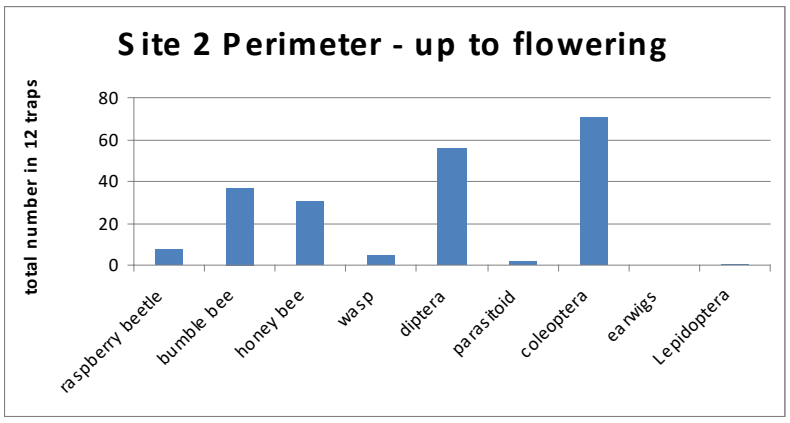
Sampling date	site	treatment	no. of raspberry beetle caught
22/05/2008	2	control	0
		Lattice	0
		Perimeter	0
29/05/2008	3	control	0
		Lattice	0
		Perimeter	1
29/05/2008	1	control	0
		Lattice	0
		Perimeter	1
29/05/2008	2	control	4
		Lattice	0
		Perimeter	0
05/06/2008	3	control	0
		Lattice	0
		Perimeter	0
05/06/2008	1	control	0
		Lattice	0
		Perimeter	0
	2	control	
		Lattice	not sampled
		Perimeter	

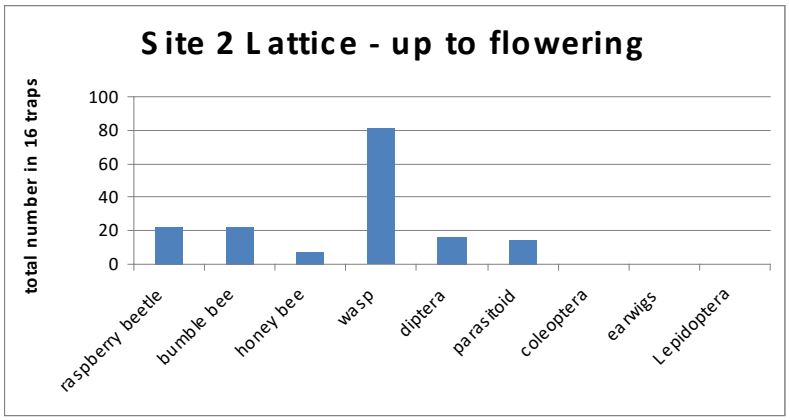
Table 2.5.6. The number of beetles caught between set-up and flowering

Site	Perimeter	Lattice
1	2	2
2	8	23
3	8	4









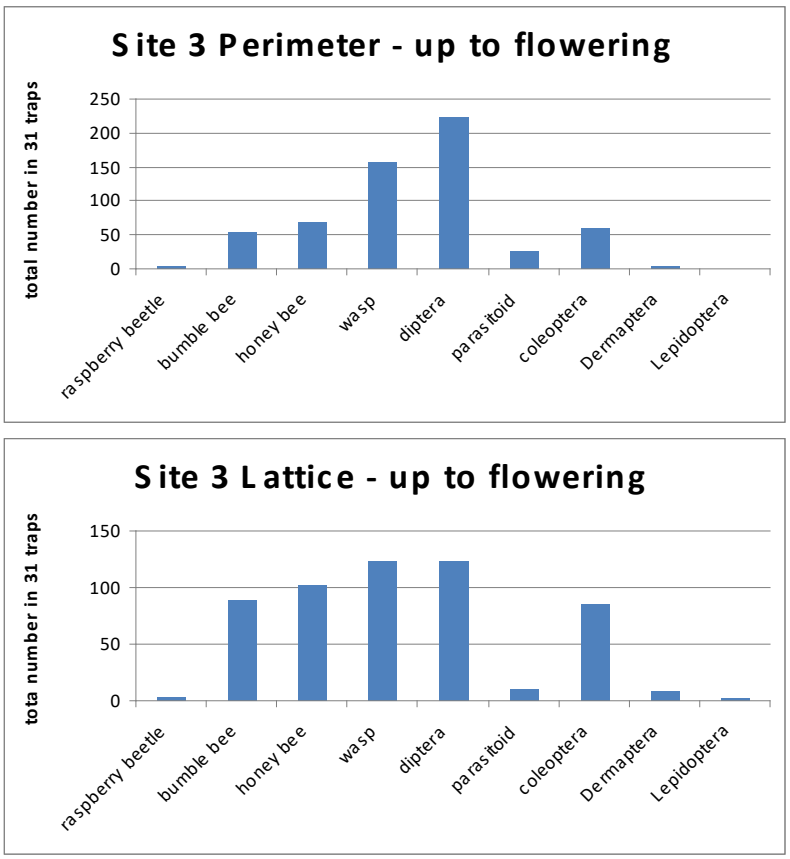


Figure 2.5.3. Total number of insects caught in the bucket traps between set-up and flowering

Table 2.5.7. The number of beetles caught on 6 sticky traps positioned in alternative hosts at site 3

sampling date	**no. of raspberry beetle caught**
28/04/2008	0
07/05/2008	0
15/05/2008	3
22/05/2008	0
29/05/2008	0

Table 2.5.8. The total number of larvae and damaged fruit in each treatment per 1200 berries. 1200 berries sampled in the insecticide free and the treated areas of each treatment

site	treatment	area	no. damaged (berry and hask)	no. of larvae
site 1	lattice	insecticide - free	1	0
		treated	1	0
	perimeter	insecticide - free	0	0
		treated	0	0
	control	insecticide - free	0	0
		treated	2	0
site 2	lattice	insecticide - free	0	0
		treated	0	0
	perimeter	insecticide - free	0	0
		treated	0	0
	control	insecticide - free	0	0
		treated	1	0
site 3	lattice	insecticide - free	4	3
		treated	2	1
	perimeter	insecticide - free	1	1
		treated	5	2
	control	insecticide - free	0	0
		treated	0	0

Progress: Working with Agrisense and PSD, we have agreed to launch the raspberry beetle trap and lure as a monitoring tool. As a result of results and grower feedback (UK, Norway, France, Switzerland) we have modified the design so that the trap is easier to assemble and will exclude bees. We have already received advance orders for the trap and lure system from several countries and have written technical leaflets and PR articles (using Agrisense's PR agency) to promote the IPM tools for raspberry beetle developed in the project Due to use of Calypso at the 2008 trial sites the consortium has agreed to move sites to another grower (outside the consortium) for 2009. He uses some Calypso, but only as part of IPM as required, rather than routinely, so it is hoped we will get more useful results on his farm.

Objective 3. Raspberry cane midge

3.1. Develop effective sex pheromone lure and trap for raspberry cane midge males

Task 3.1.1. - Make sex pheromone lures for testing: (all years; NRI)

Task 3.1.2. - Evaluate blends: (years 1,2; EMR)

Task 3.1.3. - Evaluate effects of release rates (years 1 ,2; EMR)

Task 3.1.4. - Evaluate trap designs (years 1,2; EMR)

3.2. *Investigate use of sex pheromone trap for monitoring raspberry cane midge males*

Task 3.2.1. - Pest monitoring in tunnels versus field crops (years 3-5; SCRI, EMR, Grower partners)

Task 3.2.2. - Establish thresholds (years 3-5; SCRI, EMR, Grower partners)

3.3. *Identify host plant wound attractant of females*

Task 3.3.1. - Prepare sites and develop rearing methods (years 1-3; SCRI, EMR)

Identify and prepare site/s (artificially wound primocanes early in the season) to develop a large and reliable population/s of raspberry cane midges (RCM) for experimental work. Develop rearing method to ensure insect supply outside of the normal field season.

Progress: Small tunnels have been set up at SCRI and are being inoculated with RCM supplied by EMR and ADAS. We will continue to inoculate them in Spring 2009 (again, using RCM supplied from England).

Task 3.3.2. - Behavioural analysis. (year 2; SCRI)

Conduct detailed behavioural analysis of male and female cane midge in response to natural and artificial wounds in primocanes using video-based behavioural analysis.

Progress: Using RCM supplied by EMR and ADAS, we have established laboratory cultures using modified rearing methods developed for the project. We have tested a four-arm olfactometer (supplied by Rothamsted) but females did not display normal host seeking behaviour. Further experiments have established an improved method using flight cages and raspberry plants (+/- wounds). Responsive female midges flew directly to the wound site and quickly laid eggs, indicating an important role for cane wound volatiles in pre-oviposition behaviour. Further video analysis using Ethovision software is in progress, but we will need to purchase a higher resolution video camera (1080p resolution) to record and analyse the micro-behaviour occurring after landing near the wound site on the plant. Observations to date suggest that the antennae and ovipositor receptors are used after landing to accept the split for egg laying. Further experiments using plants +/- physical and chemical signals are planned to investigate this in more detail, once we obtain more RCM females from our cultures and spring field infestations.

Task 3.3.3. - Collect wound volatiles (year 1; SCRI, NRI)

Collect volatile emanations from split and unsplit raspberry canes and prepare samples for GC-EAG analysis

Progress: Successfully completed. Used to identify bio-active volatiles which stimulate female antennae and attract females to splits in bioassays.

Task 3.3.4. - Identify wound volatile components.(years 1, 2, 3; SCRI, NRI)

INVESTIGATION OF ATTRACTION OF FEMALES OF RASPBERRY CANE MIDGE, *Resseliella theobaldii,* TO WOUNDED RASPBERRY CANES

INTRODUCTION

In the raspberry cane midge, *Resseliella theobaldii*, adult males emerge shortly before the females. Males are attracted to the females by a powerful sex pheromone (Hall *et al.*, in press) and mating takes place within a few hours. The mated females oviposit within 24 hr. There is a strong preference for the splits in primocanes (Gordon and Williamson, 1991) and fresh splits are preferred over old ones or ones already occupied with larvae (Pitcher, 1952). Nijveldt *et al*. (1963) observed in the laboratory that the scent from wounds and splits is an important stimulus for oviposition. They showed that spraying willow twigs with sap from young raspberry canes resulted in immediate egg-laying by gravid female *R. theobaldii*, whereas they did not lay on unsprayed twigs. This could be due to volatile and/or volatile chemicals in the sap, but the fact that older splits are less favoured suggests that ephemeral, volatile chemicals may be responsible, at least in part.

This work was carried out to identify chemicals produced on wounding raspberry primocanes that might be involved in attraction of gravid female *R. theobaldii*. Previous work at SCRI had shown that volatiles from raspberry canes could be collected by solid phase microextraction (SPME) and some of the compounds present had been characterised by GC-MS.

MATERIALS AND METHODS

Collection of Volatiles

NRI
Potted raspberry plants, var. Glen Moy, were supplied by EMR and maintained in a greenhouse at NRI. All collections were carried out in the laboratory at 20-22°C. A section of stem of the undamaged plant was isolated with a small wire cage (5 cm × 4 cm dia.) covered with transparent PET sheet (12.5 µm thick; Multi-purpose cooking bags, Sainsbury's plc) (Stewart-Jones and Poppy, 2006) (Fig. 3.3.4.1a).

SCRI
Several raspberry varieties maintained within glasshouses at SCRI, including Glen Prosen, Glen Ample, Malling Promise and Malling Delight were used for collection of volatiles. Sampling enclosures constructed from copper wire and covered with PET sheeting (as above) were used for collection of volatiles from specific sections of stem. Details of the sampling apparatus were given in last year's report. This enclosure system has been used for simultaneous collection of volatiles using two SPME fibres with different analyte specificites (Fig 3.3.4.1b).

NRI

Volatiles were collected by solid phase microextraction (SPME; Supelco). Blue (65 μm polydimethylsiloxane (PDMS)–divinylbenzene) and red fibres (100 μm PDMS) were evaluated with sampling times of 15 min, 30 min and 60 min. Most samples were taken with the blue fibre for 30 min. A small hole was made in the PET sheet round the stem, the needle inserted and the fibre exposed.

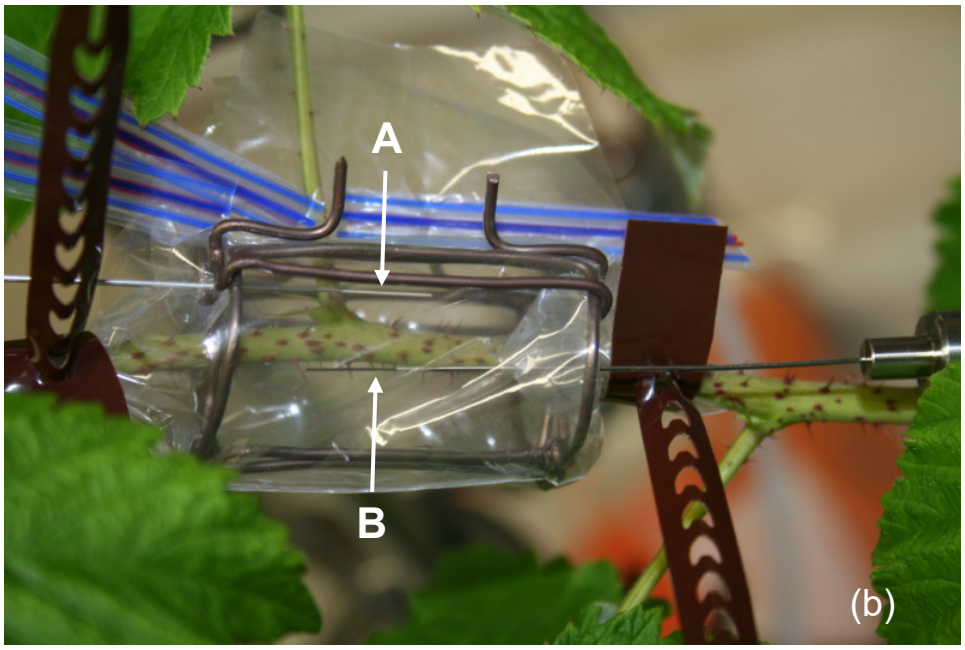


Fig. 3.3.4.1. Collection of volatiles by SPME from raspberry stem enclosed in PET sheet: (a) system used at NRI; (b) system used at SCRI showing use of two different SPME fibres within the sampling enclosure to sample volatiles from Malliing Promise. Fibre (A): carbowax-divinylbenzene, fibre (B): 85 μm carboxen - Polydimetyhylsiloxane

SCRI
Three different SPME fibre (Supelco) chemistries were evaluated. Yellow-green (70 μm carbowax—divinylbenzene (DVB)), light blue (85 μm carboxen (CAR)-PDMS) and light grey (50/30 μm DVB-CAR-PDMS) fibres with 23-gague protective sheaths were used for operation with the CTC analytics GC-MS autosampler SPME holder. Sampling times used in experiments ranged from 30 min. – 90 min. Most samples were collected over 90 minutes. Further details of sampling procedures and analysis of samples by GC-MS used at SCRI, in were included in last year's report.

NRI
Artificial splits were made with the tip of a dissection needle by splitting the surface for about 40 cm and then gently lifting the epidermis approx. 5 mm to one side of the split. This is the procedure used to measure oviposition by *R. theobaldii* in field experiments (Michelle Fountain, EMR, *pers. comm.*). The split was isolated with the PET sheet and collection started within 2 min. and collections were also made at 60 min and 120 min after making the split.

Gas Chromatography linked to Mass Spectrometry (GC-MS)

NRI
Volatiles collected on SPME fibres were analysed by GC-MS after thermal desorption in the injector of the GC. Analyses were carried out with a CP 3800 gas chromatograph linked to a Saturn 2200 ion trap mass spectrometer (Varian, Oxford, UK) operated in electron impact mode. Samples were analysed on a fused capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) coated with polar phase (Supelcowax-10, Supelco, Gillingham, Dorset, UK). The injection temperature was 220°C and the split was closed for 1 min. Oven temperature was held at 50°C for 2 min, then programmed at 6°C/min to 250°C and held for 5 min. Helium was used as the carrier gas (1.0 ml/min). Retention times were converted to retention indices relative to the retention times of *n*-hydrocarbons or acetates. Compounds were identified by comparison of their mass spectra with those in the spectral library (NIST) and assignments confirmed by comparison of retention times and mass spectra of authentic standards.

SCRI
Volatiles were analysed using a Trace gas chromatograph coupled to a Tempus time of flight (TOF) mass spectrometer (ThermoFisher, UK). Samples were analysed on a fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) coated with a polar phase (DB1701, Agilent Technologies, UK). Volatiles were desorbed from the SPME fibre for 2 min at 280°C (CAR-PDMS), 260°C (DVB-CAR-PDMS) or 200°C (Carbowax-DVB) within a programmable temperature vapourising (PTV) injector in splitless mode. Helium was used as carrier gas at a flow rate of 1.5 ml/min. After an initial hold for 2 min at 40°C, the GC oven was programmed to increase 10°C /min up to 240°C with a further 20 min hold at that temperature. The GC-MS interface temperature was 250°C and the MS was used in electron impact mode at 70 eV over a mass range of 25-650 amu, with a source temperature of 200°C. Data was acquired at 3 spectra/sec. The Xcalibur software package (V. 1.4) (thermFisher, UK) was used for data acquisition and analysis. Compounds were identified by comparison of their mass spectra with entries in MS spectral libraries (NIST, Wiley and Pal600K), and by comparison of mass spectral data and retention behavious with authentic standards.

Enantioselective Gas Chromatography

For analytical separation of enantiomers, a HP6850 gas chromatograph was used fitted with a fused silica capillary column (30 m × 0.32 mm i.d.) coated with CP-Chirasil-DexCB (Varian) and flame ionization detector (250°C). Carrier gas was helium (2.4 ml/min), injection was splitless (200°C) and the oven temperature was held at 60°C for 2 min then programmed at 6°C/min to 200°C.

Analysis by Gas Chromatography linked to Electroantennography (GC-EAG)

GC-EAG analyses were carried out with an HP 6890 GC (Agilent) fitted with capillary GC columns (30 mm × 0.32 mm i.d.) coated with polar (Wax10; Supelco) and non-polar (SPB1; Supelco) phases. Injection was splitless (220°C) and detection was by FID (250°). The oven temperature was programmed from 50°C for 2 min, then at 10°C/min to 250°C. The GC column effluent was split (1:1) between the FID and a silanized glass T-piece in the column oven. Nitrogen (200 ml/min) was blown continuously over the EAG preparation and every 17 sec this was diverted through the T-piece for 3 sec, blowing the contents over the EAG preparation, as described by Cork *et al*. (1990).

Following experience working with other midge species, the EAG preparation was set up by suspending the whole insect between glass electrodes containing electrolyte (0.1 M potassium chloride with 10% polyvinylpyrrolidone added to reduce evaporation). The ends of both antennae were inserted into the recording electrode and the body into the reference electrode. The electrodes were inserted onto silver/silver chloride electrodes held in micromanipulators on the portable EAG device developed by Syntech (INR-02; Syntech, Hilversum, The Netherlands). Recordings were made only from female midges from a mixed collection that were assumed to be mated.

Measurement of Release Rates

Dispensers were sealed white polyethylene sachets (2.5 cm a 2.5 cm × 120 µm thick containing the mixture of compounds (100 µl) adsorbed onto a cigarette filter. Two sachets were maintained in the laboratory fume hood (20-22°C) and weighed at intervals to determine the amount of material lost. The composition of the blend released was determined by placing both sachets in a clean jar and sampling by SPME for 1 min, followed by GC-MS analysis as above.

RESULTS

Collection and Analysis of Volatiles

Analyses of volatiles collected by SPME at both NRI and SCRI showed clear and reproducible differences between those from an intact raspberry cane and those with an artificially-made split (Fig. 3.3.4.2).

The compounds were identified (Table 3.3.4.1) and relative amounts determined (Fig. 3.3.4.3). Intact stems produced primarily 6-methyl-5-hepten-2-one, nonanal, decanal geranyl acetone (Fig. 3.3.4.4) and camphene. These compounds were present after making the split, but larger amounts were produced of (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol, 6-methyl-5-hepten-2-ol, linalool, myrtenal, geranial, citronellol, myrtenol, geraniol and benzyl alcohol (Fig. 3.3.4.4). Small amounts of octanal, hexan-1-ol, copaene and caryophyllene were present in volatiles from both intact and split stems. Traces of methyl salicylate and nerol were detected only in volatiles from split stems. There were some indications that the patterns of enhanced volatiles production may show some inter-varietal differences since a number of additional volatiles showing enhanced production were identified at SCR using raspberry varieties different to those used at NRI.

There was little difference in the results obtained with the red or blue SPME fibres used at NRI and the blue was adopted as standard. In an unreplicated test, sampling for 30 min gave appreciably larger GC peaks than sampling for 15 min, although increasing the sampling period for 1 hr made little difference. Generally amounts of volatiles sampled from the splits declined at 1 hr and 2 hr after making the split. The procedure was standardised by starting sampling as soon as possible after making the split (< 2 min) and sampling for 30 min.

There was greater variability in the range of volatiles entrained by the yellow-green and light blue fibres at SCRI, although there was a fair degree of overlap. A third fibre (grey) with which has some fibre chemistry common to those of the yellow-green and light blue fibres, produces results similar to a combination of the others. Overall, taken together, these fibres give very similar results to those obtained at NRI. Very similar patterns of enhanced volatiles production were found for four different cultivars, Glen Prosen, Glen Ample, Malling Promise and Malling Delight. Taken with the similar results obtained using Glen Moy at NRI, these results suggest that the response to wounding by enhance production of a common suite of volatiles is general across the cultivars studied.

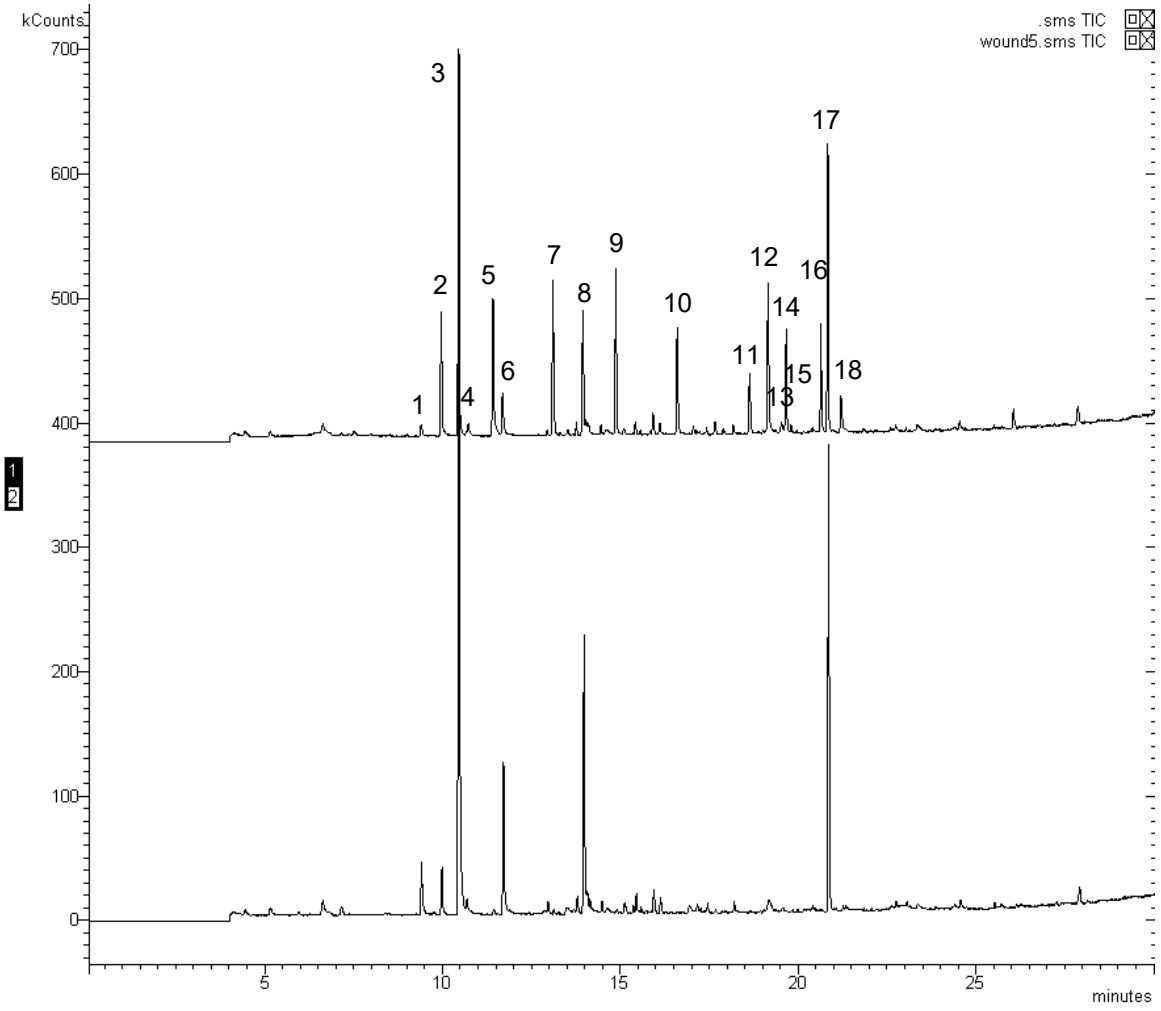


Figure 3.3.4.2. GCMS analysis of SPME collections from raspberry cane var. Glen Moy – upper after split, lower undamaged (PDMS/DVB fibre; 30 min sample time; polar GC column; see Table 3.3.4.1 for assignments)

Table 3.3.4.1a. Compounds identified in volatiles collected from undamaged and split raspberry canes by SPME and analysis by GC-MS. (peak numbers refer to those shown in Figure 3.3.4.2; compounds present only after splitting in bold)

No.	RT min	KI	Compound	Undamaged	Split
1	9.40	1293	octanal	X	X
2	9.98	1319	(*Z*)-3-hexenyl acetate	X	XX
3	10.46	1341	6-methyl-5-hepten-2-one	XXX	XXX
4	10.60	1348	1-hexanol	X	X
5	**11.50**	**1389**	**(*Z*)-3-hexenol**		**XX**
6	11.60	1394	nonanal	XX	X
7	**13.12**	**1465**	**6-methyl-5-hepten-2-ol**		**XX**
8	13.96	1505	decanal	XXX?	XX
9	**14.89**	**1549**	**linalool**		**XX**
10	**16.62**	**1634**	**myrtenal**		**XX**
11	**18.70**	**1742**	**geranial**		**X**
12	**19.16**	**1765**	**citronellol**		**XX**
13	**19.44**	**1780**	**methyl salicylate**		**x**
14	**19.66**	**1791**	**myrtenol**		**XX**
15	**19.80**	**1798**	**nerol**		**x**
16	**20.64**	**1846**	**geraniol**		**XX**
17	20.84	1857	geranyl acetone	XXX	XXX
18	**21.20**	**1878**	**benzyl alcohol**		**X**

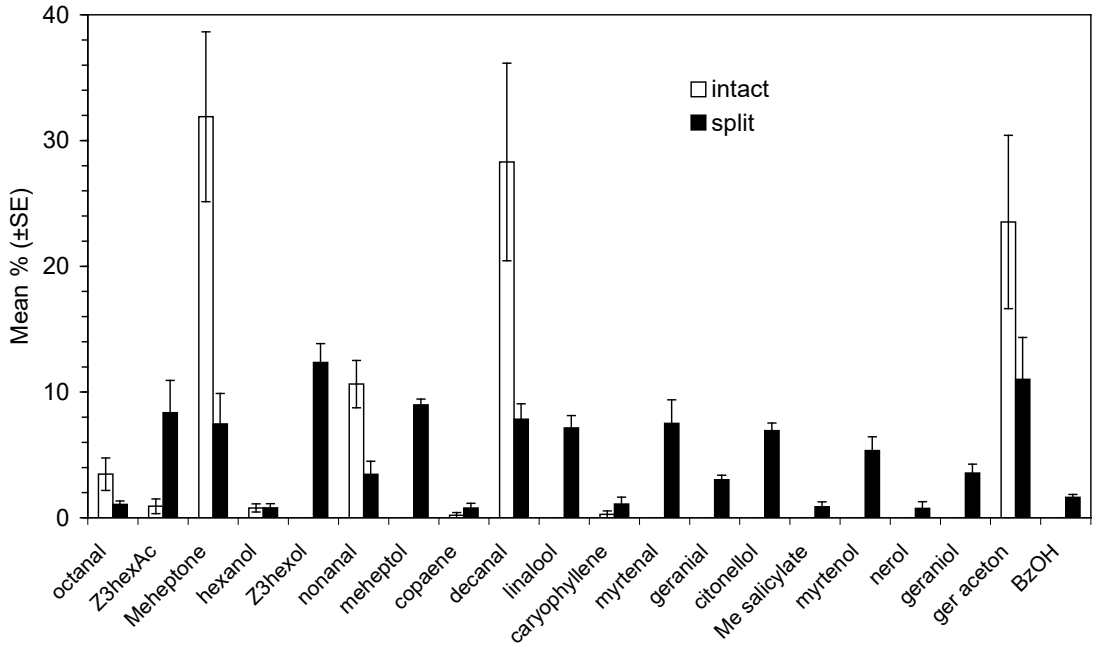


Figure 3.3.4.3. Relative amounts of compounds in volatiles from intact and split raspberry canes sampled by SPME followed by GC-MS analysis (*N* = 4 for intact and split stems)

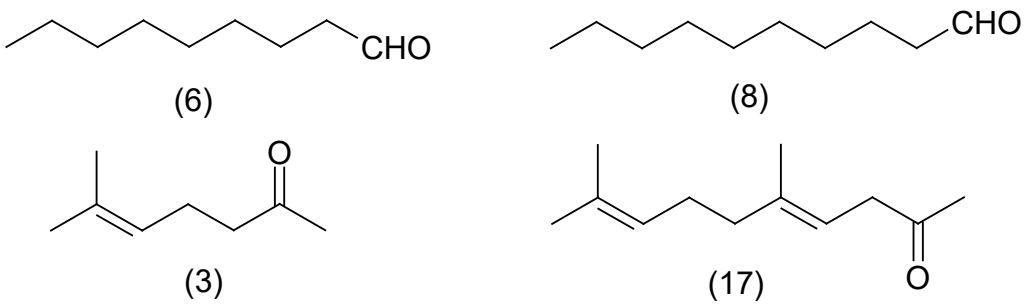


Figure 3.3.4.4. Structures of the main compounds found in volatiles from intact raspberry canes (6-methyl-5-hepten-2-one (3), nonanal (6), decanal (8), geranyl acetone (17))

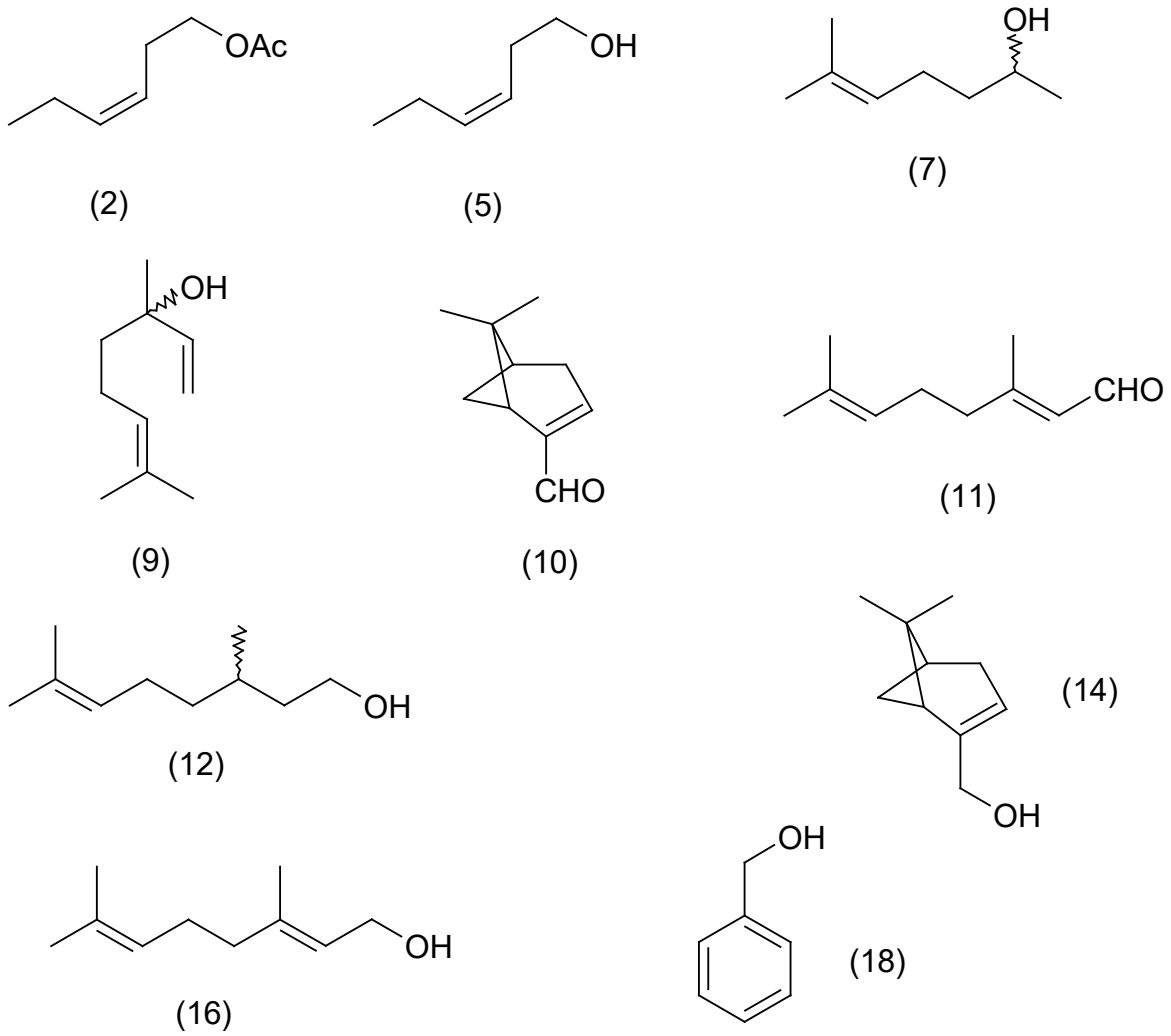


Figure 3.3.4.5 Structures of the main additional compounds produced by raspberry cane upon wounding ((*Z*)-3-hexenyl acetate (2), (*Z*)-3-hexenol (5), 6-methyl-5-hepten-2-ol (7), linalool (9), (1*R*)-myrtenal (10), geranial (11), citronellol (12), (1*R*)-myrtenol (14), geraniol (16) and benzyl alcohol (18))

Table 3.3.4.1b List of volatiles collected from cv. Malling Promise at SCRI showing enhanced abundance following mechanical damage to canes. Two different fibre chemistries were used: Fibre A: 70□m Carbowax-DVB.; Fibre B: 85□m Carboxen -PDMS. (tr: present in trace amounts:+: present in increasing quantities; M+: molecular weight of volatile).

No	Compound	M^+	Formula	Fibre B undamaged	Fibre A undamaged	Fibre B split	Fibre A split
5	(Z)-3-Hexen-1-ol	100	$C_6H_{12}O$	+	tr	+++++++	++
19	Camphene	136	$C^{10}H_{16}$		+++		+
20	2-Heptanol	116	$C_7H_{16}O$	+	+	++++++	++
21	2,4-hexadi	96	C_6H_8O	tr	tr	+++	tr
2	(Z)-3-Hexenyl acetate	142	$C_8H_{14}O_2$	++++++++	++++++++	++++++++	++++++++
3	6-Methyl-5-hepten	126	$C_8H_{14}O$	++	++	++	++
22	6-Methyl-5-hepten	128	$C_8H_{16}O$	+	+	++++	++
23	5-Ethyl-(5H)-furanone or 5-Methyl-4-	112	$C_6H_8O_2$ or $C_7H_{12}O$	tr	tr	++	+
9	Linalool	154	$C_{10}H_{18}O$	+	+	+++++	++++++
6	Nonanal	142	$C_9H_{18}O$	++	++++	++	++++
24	Citronellal	154	$C_{10}H_{18}O$	tr	tr	++	++
25	t-Pinocarveol or t-Verbenol)	152	$C_{10}H_{16}O$	tr	tr	++	++
26	5-Ethyl-(5H)-furanone or 5-Methyl-4-hexen	112	$C_6H_8O_2$ or $C_7H_{12}O$	tr	tr	++	tr
8	Decanal	156	$C_{10}H_{20}O$	+	++++	+	++++
13	Methyl salicyl	152	$C_8H_8O_3$	++++	+	+	+
14	Myrtenol	152	$C_{10}H_{16}O$	+	+	+++++	++++
12	citronellol	156	$C_{10}H_{20}O$	tr	tr	tr	+++++
10	Myrtenal	150	$C_{10}H_{14}O$	+	+	++++++	++++++
15	Nerol	154	$C_{10}H_{18}O$	tr	tr	tr	++
16	Geraniol	154	$C_{10}H_{18}O$	tr	tr	+	++++++
27	Z-Citral (Neral)	152	$C_{10}H_{16}O$	tr	tr	+	+++++
11	E-Citral (Gerani	152	$C_{10}H_{16}O$	tr	tr	+	+++++++

Chirality of Components

Volatiles from a split were collected by SPME and analysed by GC on an enantioselective cyclodextrin column.

(1*R*)-(-)-Myrtenal and (1*R*)-(-)-myrtenol were only available as single enantiomers and presumably derived from natural sources. The compounds in volatiles from split stems co-chromatographed with these on the cyclodextrin column and are assumed to have the same configuration.

The linalool was shown to be a 65:35 mixture of *R*- and *S*-enantiomers. Assignments were made by comparison of retention times with those of the linalool in lavender oil and sweet basil oil (known to be *R*) and that in sweet orange oil (known to be *S*).

The 6-methyl-5-hepten-2-ol was a 60:40 mixture of *S*- and *R*-enantiomers. Assignments were made by reacting the racemic material with vinyl acetate in petroleum ether in the presence of lipase from *Candida antarctica* or Amano K (SigmaAldrich). These enzymes catalyse acetylation of the *R*-enantiomer (*e.g.* Xiao and Kitazume, 1997; Gries *et al*., 2006).

The enantiomers of citronellol or the corresponding acetate could not be resolved on the GC column used.

Analysis by Gas Chromatography linked to Electroantennography (GC-EAG)

Analyses by GC-EAG were carried out on both collections of volatiles from split raspberry stems made by SPME and on synthetic compounds using a mated female *R. theobaldii* midge for the EAG preparation. Significant responses were observed, but it was subsequently found that there was a fault in the GC-EAG link so that sensitivity was not optimum (to be repeated in 2009).

In analyses of volatiles from split raspberry stems, the only consistent response was obtained to decanal (Fig. 3.3.4.6), and it was shown that the synthetic compound also gave a response (Fig. 3.3.4.7).

When larger amounts of material were used (100 ng, *i.e.* 50 ng to insect), EAG responses were also obtained to synthetic 6-methyl-5-hepten-2-ol, myrtenal and myrtenol.

When a 10-component blend of all the compounds produced only after splitting the stem was analysed, *i.e.* (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol, 6-methyl-5-hepten-2-ol, linalool, myrtenal, geranial, citronellol, myrtenol, geraniol and benzyl alcohol, responses were only observed to the 6-methyl-5-hepten-2-ol, myrtenal and myrtenol (Fig. 3.3.4.8).

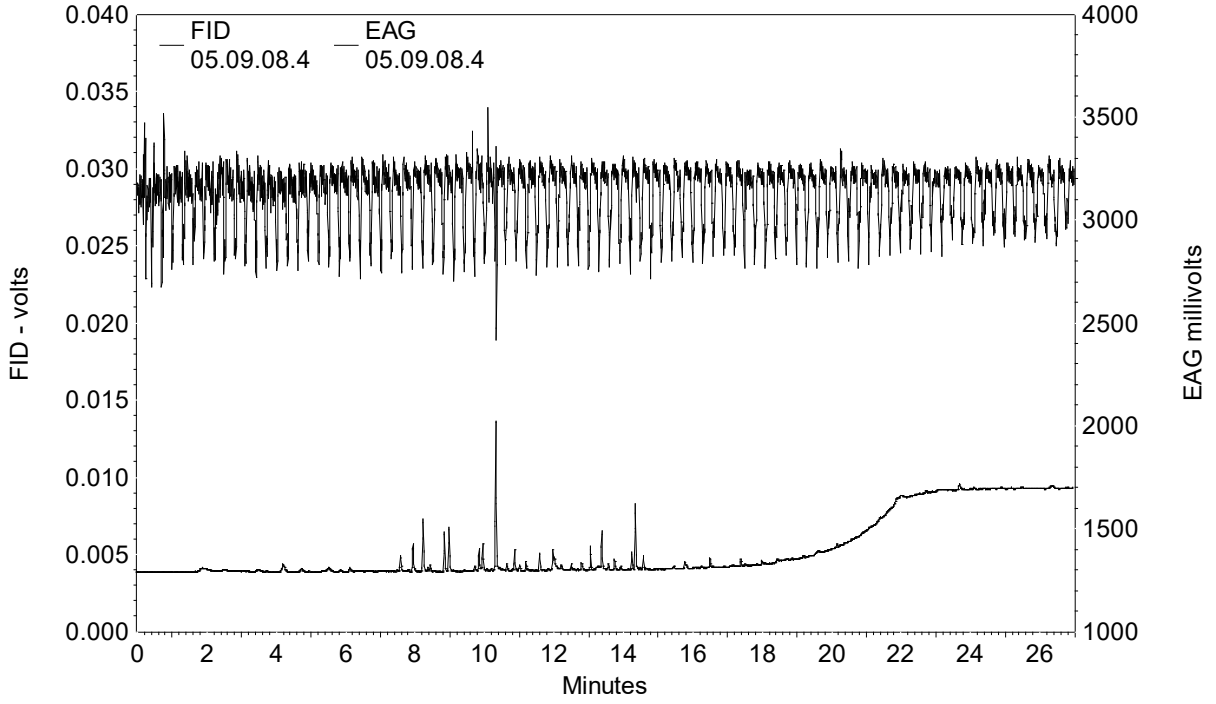


Figure 3.3.4.6. GC-EAG analysis of volatiles from split raspberry cane collected by SPME showing response to decanal at 10.36 min (upper EAG, lower FID)

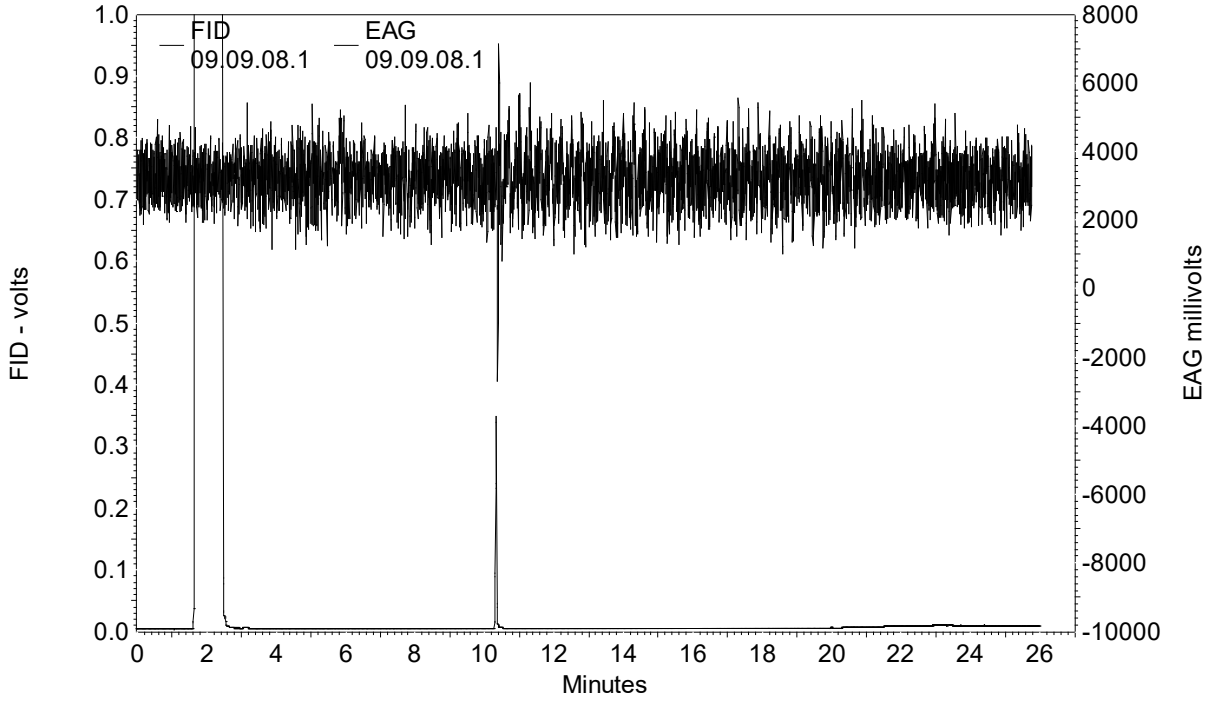


Figure 3.3.4.7. GC-EAG analysis of synthetic decanal at 10.36 min (upper EAG, lower FID)

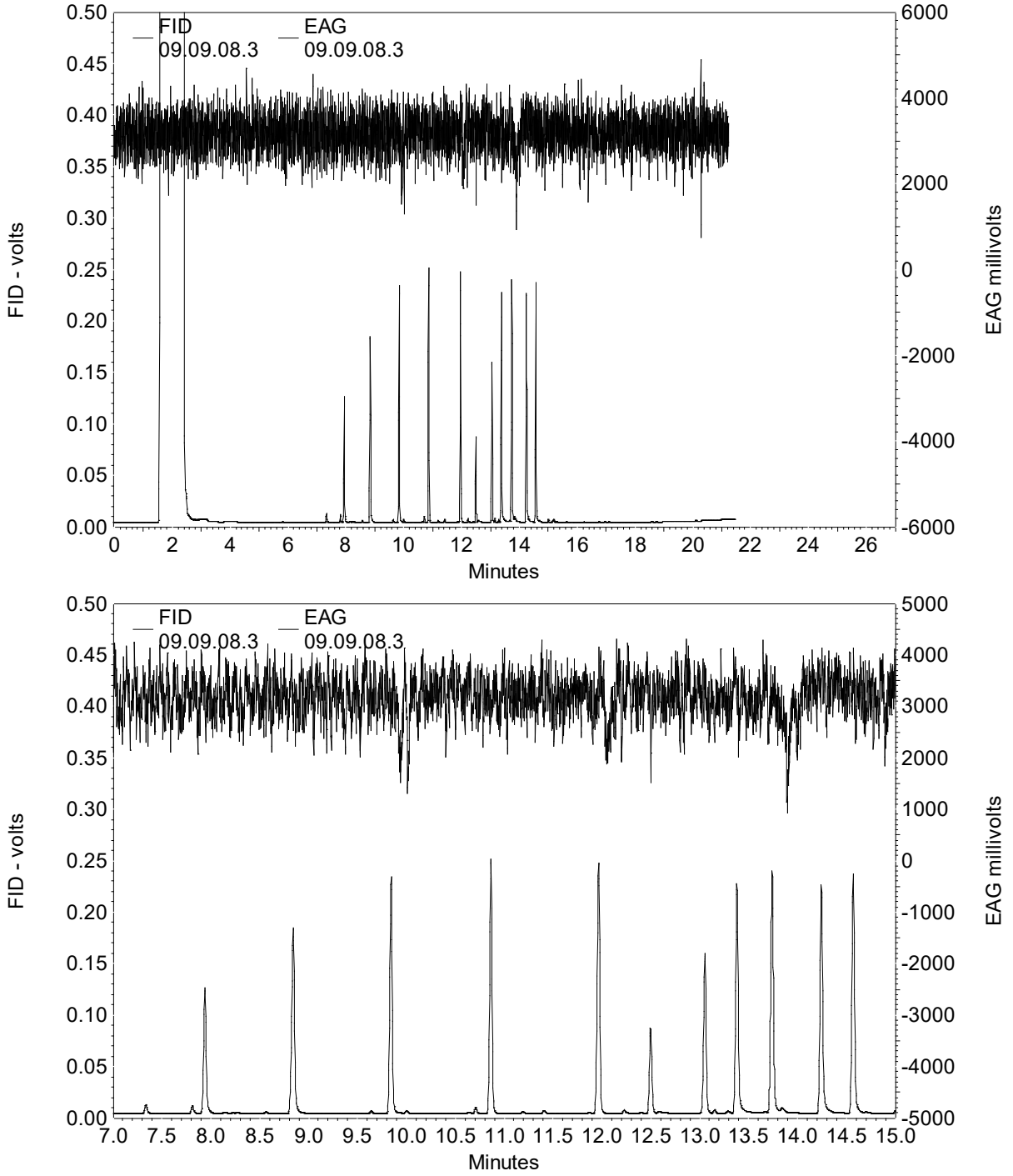


Figure 3.3.4.8. GC-EAG analysis of blend of 10 compounds produced by raspberry cane after splitting, *i.e.* (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol, 6-methyl-5-hepten-2-ol, linalool, myrtenal, geranial, citronellol, myrtenol, geraniol and benzyl alcohol (also includes citral as impurity in geranial at 12.5 min). Lower is expansion of upper. EAG responses to 6-methyl-5-hepten-2-ol (9.89 min), myrtenal (12.01 min) and myrtenol (13.79 min)

Measurement of Release Rates

For field tests a blend of equal amounts of the 10 components produced by raspberry stems after splitting was prepared in polythene sachet dispensers. In the laboratory, at 20-22°C, approximately 50 mg was released over 3 weeks (Fig. 3.3.4.9).

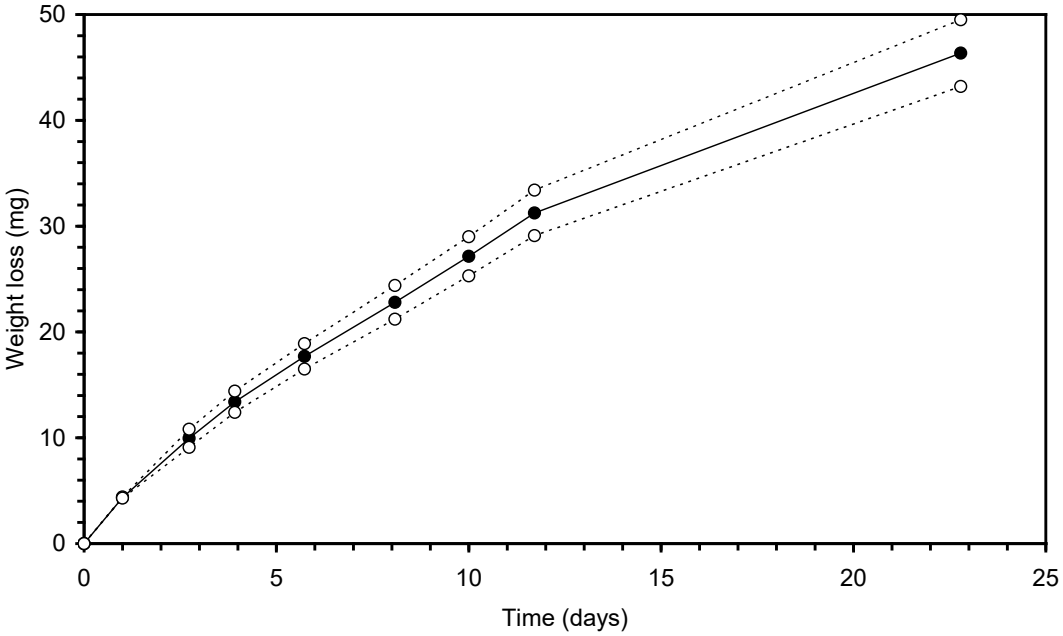


Figure 3.3.4.9. Release of 10-component blend from polythene sachet dispensers (2.5 cm × 2.5 cm × 120 µm thick) at 20-22°C

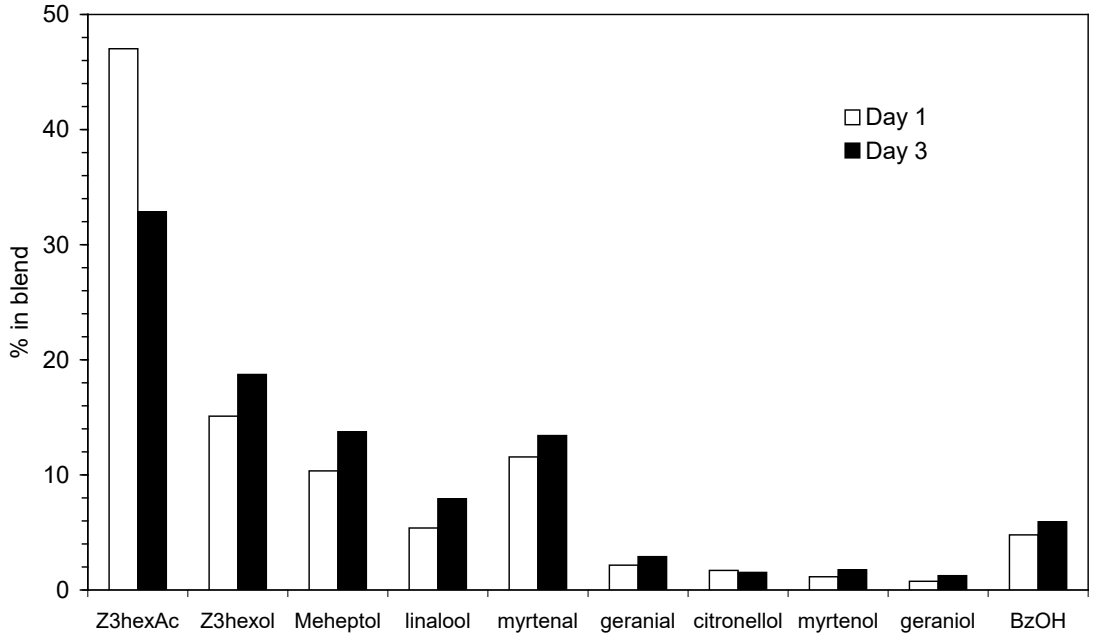


Figure 3.3.4.10. Release of 10-component blend from polythene sachet dispensers (2.5 cm × 2.5 cm × 120 µm thick) at 20-22°C as measured by SPME

Analysis of the blend released by SPME showed that, as expected, the lower-molecular weight compounds were released much faster than those of higher molecular weight, and the proportions changed with time (Fig. 3.3.4.10).

Discussion

As found at SCRI and NRI, volatiles can be collected conveniently from the stems of whole raspberry plants before and after wounding using SPME. Sampling for 15 min gave adequate peaks for analysis by GC-MS, although peaks seemed to be larger after sampling for 30 min and this was adopted as standard. Sampling was started as soon as possible after wounding. Sampling at 60 min and 120 min suggested the amount of material collected declined and very little material was obtained; on one occasion sampling was done 180 min after wounding.

There were very clear and reproducible differences in the composition of volatiles sampled from the intact stem and after wounding, and the compounds identified were essentially the same from both sites. Compounds produced by the intact stem were mainly the two aldehydes nonanal and decanal, with 6-methyl-5-hepten-2-one and the homologous geranyl acetone. After wounding, similar if not greater amounts of 10 other compounds were also produced: (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol, 6-methyl-5-hepten-2-ol, linalool, myrtenal, geranial, citronellol, myrtenol, geraniol and benzyl alcohol. Several of these have antibiotic properties and are probably produced by the plant to prevent fungal infection.

It would seem that production of these chemicals on wounding is extremely rapid, which might possibly explain why the linalool and 6-methyl-5-hepten-2-ol were produced as mixtures of enantiomers. The myrtenal and myrtenol were probably the pure *R*-enantiomers.

The (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol, linalool, geranial, citronellol, geraniol and benzyl alcohol are widely-occurring plant volatiles (www.pherobase.net). 6-Methyl-5-hepten-2-ol is also produced by a range of plants. Along with 6-methyl-5-hepten-2-one it is part of a blend of chemicals produced by wheat seedlings infested with the aphid *Rhopalosiphum padi* that causes dispersion of the aphid. However, it was not clear whether this compound was produced by the wheat or the insect (Quiroz *et al.*, 1997). Myrtenal and myrtenol are also found in a number of species of plant but have not been reported from raspberry and the plant-produced compounds have not been reported to have effects on insect behaviour.

Despite the GC-EAG system used in these studies having a fault that reduced sensitivity, consistent EAG responses were obtained from the antennae of female *R. theobaldii* midges to decanal in the volatiles from wounded raspberry canes. Responses were also found to larger quantities of synthetic 6-methyl-5-hepten-2-ol, myrtenal and myrtenol, but not to (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol, linalool, geranial, citronellol, geraniol and benzyl alcohol. The fault in the GC-EAG system has now been repaired and these analyses should be repeated to determine whether additional compounds elicit an EAG response.

A mixture of equal quantities of the 10 compounds produced by raspberry canes only after splitting was released from a polythene sachet for at least three weeks. However, the blend released differed widely from that in the sachet due to the wide range of volatilities of the components. Further work is required to develop a dispensing system to give similar release rates of the different compounds. This could be achieved by manipulation of the relative amounts loaded into the sachet, but the composition would change markedly in time and it will probably be necessary to use at least two different types of dispensers for compounds of differing volatilities.

References

Cork, A., Beevor, P. S., Gough, J. E., and Hall, D. R. 1990. Gas chromatography linked to electroantennography: a versatile technique for identifying insect semiochemicals, pp. 271–279, *In* A. R. McCaffery and I. D. Wilson (*Eds.*). Chromatography and Isolation of Insect Hormones and Pheromones, Plenum Press, London.

Gordon, S.C. and Williamson, B. (1991). Raspberry cane midge. *In* Ellis, M.A., Converse, R.H., Williams, R.N. and Williamson, B. (*Eds*) Compendium of raspberry and blackberry diseases and insects. APS Press, St Paul, Minnesota, pp 75-76.

Gries, R., Khaskin, G., Daroogheh, H., Mart, C., Karadag, S., Kubilay Er, M., Britton, R., and Gries, G. 2006. (2*S,*12*Z*)-2-Acetoxy-12-heptadecene: major sex pheromone component of pistachio twig borer, *Kermania pistaciella. Journal of Chemical Ecology,* 32:2667–2677.

Hall, D.R., Farman, D.I., Cross, J.V., Pope, T.W., Ando, T. and Yamamoto, M. (in press) (*S*)-2-Acetoxy-5-undecanone, Female Sex Pheromone of Raspberry Cane Midge, *Resseliella theobaldi* (Barnes). *Journal of Chemical Ecology*.

Nijveldt, W., Labruyere, R.E. and Engels, G.M.M.T. (1963). The stem disease problem of the raspberry. *Netherlands journal of Plant Pathology*, 69: 221-257.

Pitcher, R.S. (1952). Observations on the raspberry cane midge (*Thomasiiniana theobaldi* Barnes). I. Biology. *Journal of Horticultural Science*, 27: 71-94.

Quiroz, A., Pettersson, J., Pickett, J.A., Wadhams, L.J. and Niemeyer, H.M. (1997). Semiochemicals mediating spacing behaviour of bird cherry-oat aphid, *Rhopalosiphum padi*, feeding on cereals. *Journal of Chemical Ecology*, 23: 2599-2607.

Stewart-Jones, A. and Poppy, G. (2006). Comparison of glass vessels and plastic bags for enclosing living plant parts for headspace analysis. *Journal of Chemical Ecology*, 32: 845-864.

Xiao, L., and Kitazume, T. 1997. Optically active propargylic and allylic alcohols with a difluoromethyl group at the terminal carbon. *Tetrahedron Asymmetry*. 21:3597–3601.

Task 3.3.5. - Determine effective blend. (years 1, 2; 3; SCRI)

Once active components have been identified from the wound induced host plant volatiles, conduct behavioural analysis of midge in response to volatiles to determine an effective blend.

Progress: An initial pilot experiment was conducting in autumn 2008 by EMR and NRI, demonstrating that the prototype lure does attract female RCM under field conditions. This will need to be continued in 2009 (ideally in England where there are reasonably high RCM populations to show clear results) in order to optimise the lure release rate for most active attractant components.

3.4. Develop effective host volatile lure and trap for monitoring raspberry cane midge females

Task 3.4.1. - Make lures for testing: (years 3-5; NRI)

Produce lures with appropriate release rates required for field testing experiments in tasks 3.4. It is anticipated that higher release rates will be required.

Action: To be discussed with NRI and EMR at next meeting, so we plan experiments for 2009 and 2010.

Task 3.4.2. - Test lures and evaluate effects of release rates (year 3; SCRI, EMR)

Conduct two field experiments to test the lures in the field and to investigate the effects of lure release rate on trap catches. This will enable an appropriate release rate for midge female monitoring to be chosen.

Action: To be discussed with NRI and EMR at next meeting, so we plan experiments for 2009 and 2010.

Task 3.4.3. - Evaluate trap designs (years 3; SCRI, EMR)

Conduct two field experiments to evaluate trap designs, including delta traps, bottle traps and water traps. Experience with the apple leaf midge pheromone has shown that ease for use by growers with adequate sensitivity is the key requirement. These experiments will enable a suitable trap design for monitoring purposes to be chosen

Action: To be discussed with NRI and EMR at next meeting, so we plan experiments for 2009 and 2010.

3.5. ***Investigate use of the host plant volatile lure and trap system for monitoring***

Task 3.5.1. - Pest monitoring in tunnels versus field crops (years 3-5; SCRI, EMR, Grower partners)

Compare the temporal patterns of catches of males in the chosen sex pheromone lure and trap system and catches of females in the host volatile lure and trap system with direct observation (oviposition in artificial splits), and catches in water traps in a tunnel crop versus a field crop at two commercial raspberry production sites, one in Kent and one in Scotland (see also Task 3.2.1).

Action: Need to discuss best sites to get robust results (no/low RCM in Scotland in 2007-8). Suggest RCM trials are conducted at known infestation sites in England and RB trials conducted at suitable sites in Scotland (assuming RB populations are still very low in England).

Task 3.5.2. - Establish threshold (years 3-5 SCRI, EMR, Grower partners)

Examine the relationships between trap catches and population levels in commercial tunnel crops at 4 different sites, two in Kent and two in Scotland, to determine sensitivity and establish thresholds (see also Task 3.2.2).

Action: Need to discuss best sites to get robust results (no/low RCM in Scotland in 2007-8).

3.6. ***Investigate use of the sex pheromone, initially alone, then in conjunction with the host volatile attractant for control by disruption, mass trapping or lure and kill***

Task 3.6.1. - Obtain experimental approval (EMR, Agrisense)

Task 3.6.2. - Midge control by sex pheromone mating disruption (years 2-3, EMR, SCRI)

Task 3.6.3. - Identify best device for control by mass trapping or lure and kill (years 1; EMR)

Task 3.6.4. - Efficacy of midge control by mass trapping or lure and kill (years 3-5; EMR, SCRI)

Objectives

The overall aim of the work was to determine whether the raspberry cane midge sex pheromone could exploited for control of the pest in commercial protected raspberry plantations by mating disruption, where the pheromone is deployed alone, or by attract-and-kill where the pheromone is deployed in conjunction with insecticide treated card.

The work is primarily for scientific purposes, *i.e.* to determine whether the raspberry cane midge pheromone can be exploited for control of raspberry cane midge. The data may, however, be used in support of an application for registration in future.

Methods and materials

One large scale randomised dispersed block experiment was done from April - October 2008 to evaluate a mating disruption (MD) and an attract-and-kill (A&K) method of using the sex pheromone of the raspberry cane midge for control. The MD treatment used 3 kg of EVA granules containing 10 g/ha of the pheromone racemate broadcast to the surface of the soil in the alleyways. The A&K treatment used 2000 lambda cyhalothrin treated cards each bated with a rubber septum lure containing 200 µg of the pheromone racemate and suspended at a height of 15 cm from the crop wires. Untreated control plots were provided for comparison (Table 3.6.4.1). There were three replicate blocks of each of three treatments, the blocks being three farms in Kent and plots were ~1 ha (Figs. 3.6.4.1-3). The pheromone treated plots were well separated from the untreated control plots. The sites had varying populations of raspberry cane midge.

Table 3.6.4.1. Site and plot information site 1

Farm	Plot	Location	Variety	Ha	Details	Treat
1 John Myatt, Decoy Farm. High Halstow, Rochester ME3 8SR. Tel: 07771 846 345	1. Swigshole East, Decoy Farm	TQ 788 775	Joan Squire	0.64	5-6 yrs. Rectangular field of 21 rows (7 tunnels), 3 row beds. Distance between tunnel centres: 6.0 m, number of posts: 9. Distance between posts 19 m, length of rows: 152 m. Tunnelled mid-late Aug/early Sep.	MD
	2. Swigshole West, Decoy Farm	TQ 788 775	Joan Squire	0.62	5-6 yrs. L-shaped field of 15 rows with 7 posts and (5 tunnels) and 9 rows with 9 posts (3 tunnels). Distance between tunnel centres: 6.0 m. Distance between posts 19 m. Length of rows: 114 or 152 m. Tunnelled mid-late Aug/early Sep.	A&K
	3. Rye Street Farm, Cooling	TQ 748 762	Mixed	0.5	3-4 yrs. Long rectangular field of 15 rows (7.5 tunnels), 2 row beds. Distance between tunnel centres: 4.25 m, number of posts: 11, distance between posts 15.5 m, length of rows: 155 m. Tunnelled mid-late Aug/early Sep. Tunnels include (double cane row, Caroline, Squire, Polka, Brice and 2 tunnels of Joan Irene)	Untr
2 Tim Chambers, W B Chambers & Son, Belks Farm, Otham, Kent ME15 8RL. Tel: 01622 861 264 Mob: 07768 867 231 timchambers@farming.co.uk belksfarm@btinternet.com Berry World; Tim Newton Tel: 01992 471 833 Mob: 07850 264 908	4. SV1, Sutton Valence	TQ 811 509	Octavia	1.0	2 yrs. Rectangular field first 8 tunnels next to track, 3 row beds. Distance between tunnel centres: 7.5 m, number of posts: 25, distance between posts 7.0 m, length of rows: 168 m. Tunnelled mid-late June.	MD
	5. SV3, Sutton Valence	TQ 811 509	Octavia	0.87	3 yrs. Rectangular field 7 tunnels in alignment opposite office, 3 row beds. Distance between tunnel centres: 7.3 m, number of posts: 21, distance between posts 8.5 m, length of rows: 170 m. Tunnelled mid-late June.	A&K
	6. ST1, Stoneacre Farm	TQ 805 833	Octavia	0.96	4 yrs. Rectangular field 10 tunnels next to gateway, 3 row beds, distance between tunnel centres: 7.6 m, number of posts: 15, distance between posts 9.0 m, length of rows: 126 m. Tunnelled mid-late June. Trap in 5th tunnel.	Untr
3 Adam Shorter, Salmans Limited, Home Farm,	7. New Field, Top	TQ 51	Octavia	1.5	2-3 yrs. Square of 13 tunnels, 3 row beds, distance between tunnel centres: 7.5 m, number of posts: 16, distance between posts 9.4 m, length of rows: 141 m. Tunnelled in	MD

Penshurst Road, Bidborough, Tunbridge Wells, Kent TN3 0XH Tel: 01892 619 178 Mob: 07770 475 810 ashorter@salmans.co.uk Thomas Beldowski Mob: 07887 948 216		**2 4 3 7**			**May. NB: 1st 5 rows of plot not included. A road runs between the top and bottom of New Field.**	
	8. New Field, Bottom	**TQ 5 1 2 4 3 7**	**Octavia**	**1.3**	**2-3 yrs. Square of 14 tunnels, 3 row beds, distance between tunnel centres: 7.5 m, number of posts: 14, distance between posts 9.4 m, length of rows: 122 m. Tunnelled in May. NB: 1st 4 rows of plot not included (no canes). A road runs between the top and bottom of New Field.**	**A&K**
	9. Lower	**TQ 5 1 7 4 3 7**	**Octavia**	**2.0**	**5-6 yrs. Rectangle of 18 tunnels, 3 row beds, distance between tunnel centres: 7.5 m, number of posts: 20, distance between posts 8.0 m, length of rows: 152 m. Tunnelled in May.**	**Untr**

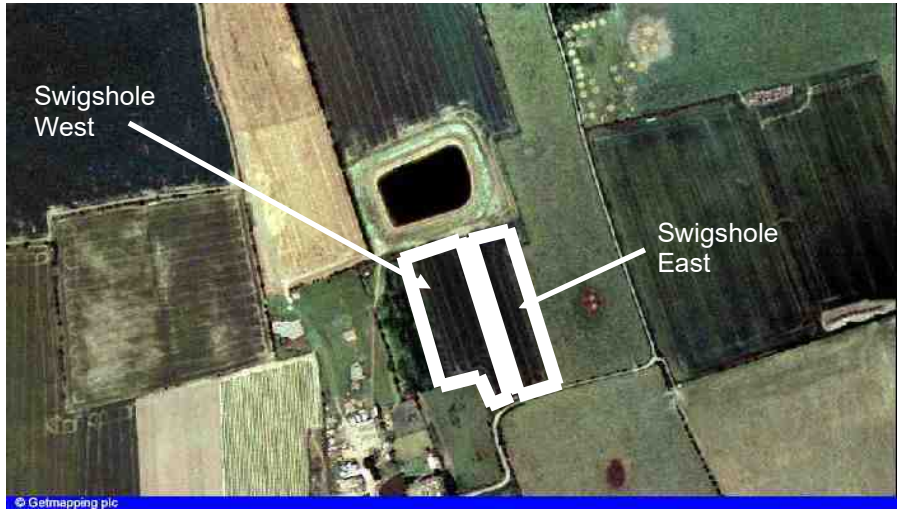


Figure 3.6.4.1. Decoy Farm plots

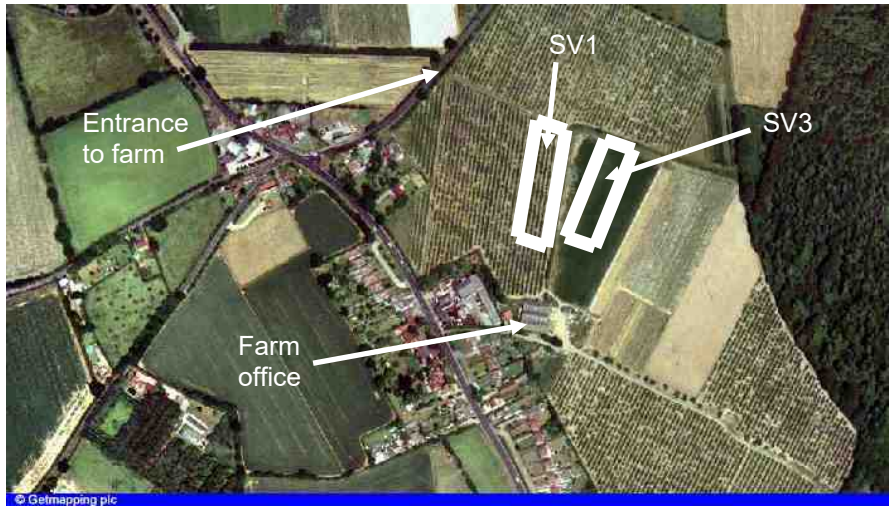


Figure 3.6.4.2. Belks Farm plots

Figure 3.6.4.3. Salmans Farm plot locations

Treatments

Mating Disruption (MD) dispensers were EVA (Ethyl Vinyl Acetate) granules containing 0.33% w/w of raspberry cane midge sex pheromone racemate prepared at NRI (Table 3.6.4.2, Fig. 3.6.4.4). In laboratory measurements at 27 ˚C, 8 km/h airspeed, 0.1% granules released 60% of their pheromone over a period of 31 days (Figure 3.6.4.5). The granules were dispersed on the soil surface at 3 kg granules/ha giving a pheromone dose of 10 g/ha. There were ~50,000 granules per kg of formulation and at a row spacing of 1.6 m, 6250 m of row per ha. Hence, 24 granules per m of row were applied.

Table 3.6.4.2. Treatments

Control approach		Pheromone dispenser	Insecticide target device‡ (size)	No. devices /ha	Dose /ha	Release rate
U	Untreated control	None	None	None	0	0
MD	Mating disruption	EVA granule loaded with 0.5% w/w pheromone†	None	150,000= 3 kg	10 g	160,000 ng/granule /hr
A&K	Attract-and-kill	200 µg rubber septum	3.3 × 3.3 cm lambda cyhalothrin card	2000	0.4 g	60 ng/lure/hr

†Granules Ethyl Vinyl Acetate

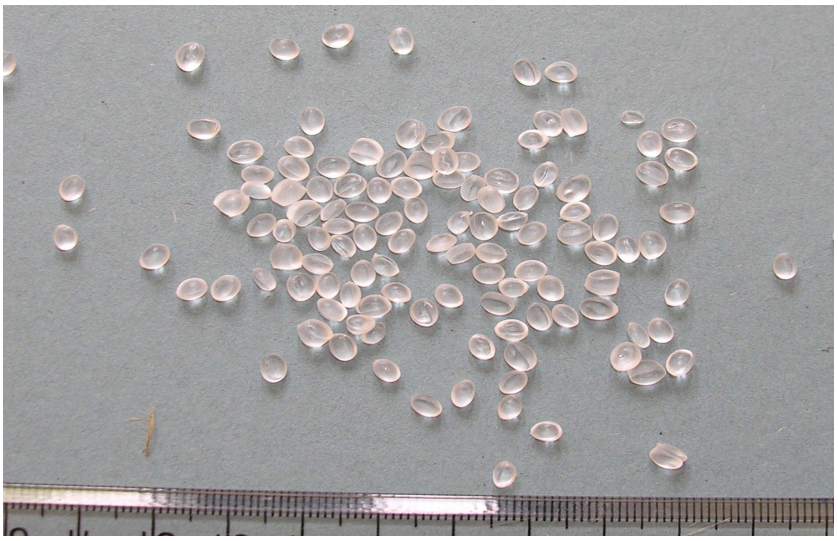


Figure 3.6.4.4. EVA granules used for the MD treatment

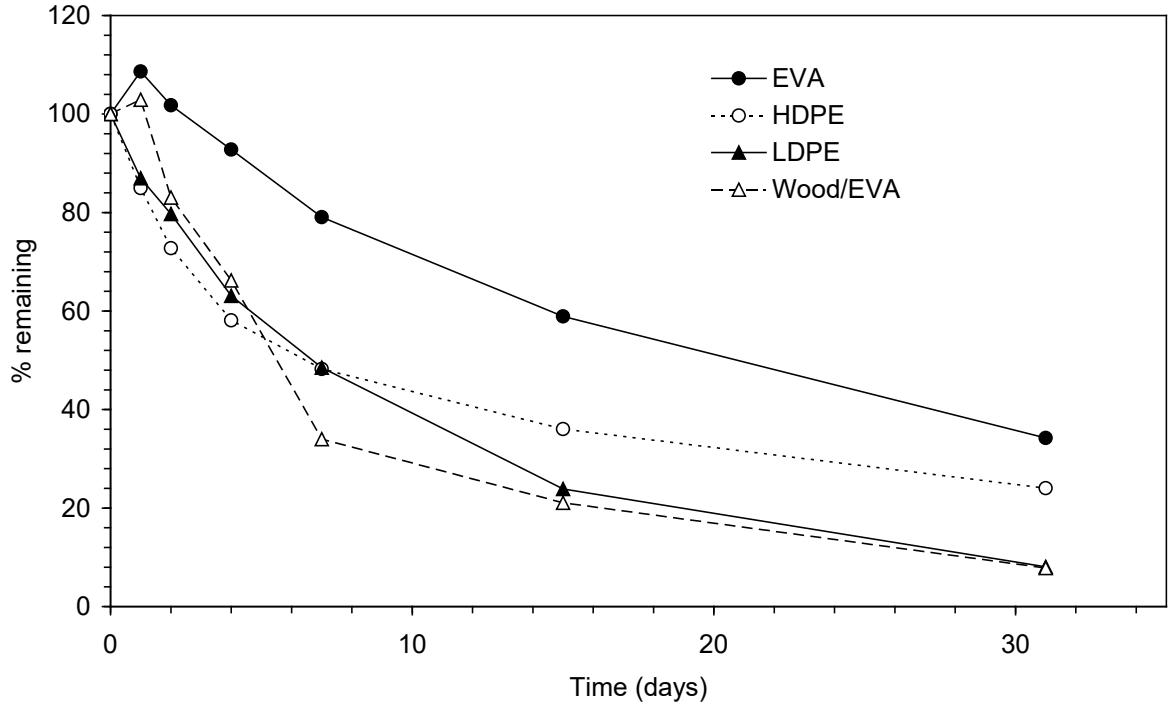


Figure 3.6.4.5. Release of raspberry midge pheromone from polymeric granules (0.1% pheromone; 27°C, 8 km/h windspeed; residual pheromone extracted and assayed by GC)

A&K devices were 3.3 cm × 3.3 cm squares of lambda cyhalothrin treated card (Agrisense Oliver fly target device card) each baited with a rubber septum lure initially loaded with 200 µg of the pheromone racemate. The release rate of pheromone was ~60 ng/hr/lure. Treatments were applied in early May 2008 by EMR science staff. The A&K devices were deployed at 2000/ha (Table 3.6.4.3) on every raspberry cane row, at a height of approximately 15 cm above the ground by attachment to the raspberry canes.

MD treatments were broadcast using site specific calibrated measuring spoons, to distribute a set amount of granules per bay onto the soil surface at 3 kg/ha (Table 3.6.4.4). At each site there were the rows were inside tunnels and each tunnel consisted of three rows of raspberries with two accessible alleyways. Therefore, the affective area for a single spoon of devices was the length of the bay multiplied by half the width of the tunnel.

Table 3.6.4.3. Attract-and-kill treatment applications

Plot	Area (ha)	Devices	No of rows	Length of row on whole plot (m)	Spacing of device (m)	Space between posts (m)	No of devices between each post
SV3	0.87	1740	21	3570	2.1	8.5	4.0
New Field	1.3	2600	42	5134	2.0	9.4	4.7
Bottom Swigshole	0.62	1240	15+9	1710+1368	2.5	19.0	7.6

West Total	2.79	5580

Table 3.6.4.4. Mating Disruption treatment applications

Plot	Area (ha)	No of Devices	No of alley-ways	Length of row (m)	Tunnel width (m)	Post spacing (m)	No of devices/half tunnel bay	Area of bay (m^2)	Wt of devices/half tunnel bay (g)
Swigshole East	0.64	96000	14	152	6.0	19	855	57	17.1
SV1	1.0	150000	16	168	7.5	7.0	394	26	7.875
New Field Top	1.5	208928	26	141	7.5	9.4	529	35	10.58
Total	3.14	454928							

Assessments

Populations of males

Two sex pheromone traps, one bated with a standard lure loaded with 10 µg of the sex pheromone racemate (the standard adopted for monitoring purposes) and one bated with a treatment lure (either MD granule or A&K lure), were hung in the centre of each plot at a height of 0.5 m above the ground level. The number of male midges captured each week was recorded.

Larval populations in splits in canes

Artificial splits in primocane: Fortnightly, throughout the growing season, 10 cm long, artificial splits were made in each of 20 primocanes in the central, untreated area of each plot. This was done by drawing a hooked needle vertically along the cane, making a slit through the periderm. Care was taken not to injure the cambium below. The needle tip was angled sideways (tangentially to the circumference of the cane) so that the periderm was separated from the cambium tissue, making a flap under which ovipositing cane midge females could lay their eggs. Making this flap was critical, otherwise the wound would have healed and oviposition would have been impossible. The canes in which artificial splits were made were marked with coloured tape so that they could easily be re-located.

Counts of eggs and larvae in splits: Fortnightly, the 20 split primocanes were collected from each area in each plantation and the number of eggs and larvae in each split counted under a binocular microscope in the laboratory. L1 and L2 larvae, which are small and translucent, turning slightly pinkish, were counted separately form L3 and L4 larvae which are larger, opaque and salmon pink or orange/yellow. The length of each split was recorded so that the number of larvae per unit length of split could be calculated.

Sexing of larvae in the splits: In order to determine whether larvae were monogenous, larvae from and the cane splits were transferred into ventilated plastic boxes moist filter paper, labelled with the number of larvae contained, site, plot number, date and split number, and reared through to adult. The number of adults emerging of each sex in each tube was determined.

Amounts of pheromone remaining in lures

Fortnightly, from each site:

A. Two replicate rubber septa were removed from the A&K devices and held in individual labelled tubes in freezer conditions, and transferred to NRI where the amount of pheromone remaining is to be estimated.
B. 20 granules contained within a mesh bag (for easy location) were removed from the field and held in individual labelled tubes in freezer conditions, and transferred to NRI where the amount of pheromone remaining is to be estimated.

Estimation of the number of primocanes per metre row length

On 2-3 occasions during the season, the number of primocanes on each of three 2m lengths of row was counted to gain an estimate of the numbers primocanes per metre of row length.

Data collation and statistical analysis

Data was collated into Excel spreadsheets and statistically analysed by ANOVA as appropriate.

Experimental permit

A 3-year consumer assessed experimental permit was issued by PSD so the work could be done without crop destruction. The maximum area that can be treated with the MD and A&K treatments, under the conditions of a consumer assessed or extrapolated experimental permit is 10 ha for each treatment per annum.

Results

Central sex pheromone monitoring traps

Season totals of 2670, 5505 and 6569 midges were captured per trap in the untreated control plots at the three sites respectively (Table 3.6.4.5). The MD treatment failed to reduce total season catches at Decoy Farm, but reduced catches by 94.2% at Belks Farm and by 85.1% at Salman's Farm. However, for the first generation (29 Apr-3 Jun), reductions were 89.0, 99.0 and 94.7% for the three sites respectively though the degree of suppression had already declined to zero at Decoy Farm by the second generation (10 Jun-22 Jul). It remained high at the other two sites (97.6% and 91.3%, respectively).

Better and longer lasting trap suppression was achieved with the A&K treatment. Total season reductions were 91.6%, 99.2% and 98.2% for the three sites respectively. For the first generation (29 Apr-3 Jun), reductions were 97.6%, 99.2% and 97.7%, for the second generation (10 Jun-22 Jul) they were 91.6%, 99.2% and 98.2% and for the rest of the season they were 85.8%, 99.4% and 98.7%. Thus, there was some evidence of a decline in the latter part of the season at Decoy Farm, but not at the other two sites.

Although monitoring traps containing high dose (200 µg) rubber septa lures were not placed in the untreated control plots (to avoid bias results), total season catches of males in the high dose lure traps in the MD and A&K plots confirmed the relative performance of these treatments in suppressing pheromone trap catches (Table 3.6.4.6 and Fig. 3.4.5.6). Catches in the MD plots were 11.4, 6.8 and 3.3 times greater in the high dose traps in the MD treated plots than in the corresponding A&K plots at the three sites.

The degree of trap shut down achieved at the three sites was not consistent with much poorer results at Decoy Farm, even though the populations in the untreated control plots were rather lower at this site. It should be noted that different plantations were used for the untreated plots and the pheromone treated plots at each farm and there was no within site replication. There was evidence that the MD treatment was loosing it's efficacy as the season progressed with better results for the first generation. The lab measurements of release rate indicated that the EVA granules used for the MD treatment released 60% of their pheromone in the first 31 days at 27°C. One explanation of the decline in trap catch reduction may be that the pheromone release rate from the EVA granules declined steeply through the season. Another possible explanation is that the granules progressively worked themselves into the soil surface, some being trampled by pickers as they walked through the tunnels. The trap catch reductions achieved by the A&K treatments remained consistent through the season.

Numbers of larvae in splits

At Decoy Farm, total numbers of larvae recorded in splits in the treated plots were as great, or greater, than in the untreated controls (Table 3.6.4.7 and Fig. 3.4.5.7). However, numbers of larvae were reduced by 99.7% and 97.4% by the MD and A&K treatments at Belks Farm and they were moderately reduced by 68.1% and 86.0%, respectively, at Salmans farm.

The failure in control at Decoy Farm is not surprising as the treatments did not give a high degree of suppression of the central sex pheromone traps. Excellent control of larvae was apparent from both treatments at Belks Farm and moderately good control was obtained at Salmans Farm, with the A&K treatment performing better than the MD treatment. The reason for the very good performance at Belks Farm and the moderate performance at Salmans Farm is not clear.

Sex ratio of larvae from splits

The Male: Female ratio of midges that emerged for larvae collected from the splits were 3:0, 2:5 and 2:8 for the untreated plots at the three sites (Table 3.6.4.8). They were 1:3 and 2:8 for the MD treatments at Decoy farm and Salmans Farm (note very few larvae were collected from the MD treatment at Belks Farm and none emerged). They were 9:0 and 6:3 for Decoy farm and Salmans Farm respectively. (Note very few larvae were collected from the A&K treatment at Belks Farm and none emerged.) Thus, much higher, male dominant sex ratios were found in the two A&K treated plots.

Conclusions

These results are encouraging because it is the second time that control of the raspberry cane midge using the sex pheromone has been demonstrated. Very good control was also achieved with MD and A&K treatments at one of three sites in 2006, although the treatments failed at two others. The reasons for failure were not clear. As reported here, good results were achieved in 2008 at one site, partial control at a second site and no control at a third. The most likely reason for the variation in results is differences in cane midge populations. MD and A&K treatments are known to perform poorly when populations are high. In the experimental methodology reported here, the control plots, though on the same farm, were in separate plantations and probably did not reflect the population levels in the pheromone treated plots on the same farm. The high degree of trap shut down that is necessary for good control probably only occurs at low population densities. This indicates that the MD and A&K treatments perform well at low

population densities, but not at high. The overall conclusion is that where populations are moderate to high, then MD and A&K treatments have to be used in combination with chemical control methods initially.

EU pesticide registration rules may make it comparatively difficult or costly to register an MD treatment. The raspberry cane midge pheromone, a C11 keto acetate, does not fall into the chemical structures of Lepidopteran pheromones that are automatically allowed onto Annex 1 of 94/414 (straight chain C10-C20 acetate, alcohol or aldehyde). For this reason, it may be easier to register an A&K treatment where the pheromone is classed as a co-formulant of an already registered insecticide (*e.g.* lambda cyhalothrin). However, such a course would require the cooperation of the parent agrochemical company of the insecticide chosen. Advice will be sought from PSD. The A&K treatment appears to be more effective and uses much less pheromone. However, the treatment tested (2000 cards with rubber septa/ha) is completely impractical for use by growers. More practical formulations need to be investigated, *e.g.*, a SPLAT treatments, where a paste containing the pheromone and a pyrethroid insecticide is splatted onto the polythene or the base of the plant. It is hoped to test such a treatment in 2009.

Table 3.6.4.5. Catches of males in standard sex pheromone traps (White 20 × 20 cm delta traps with 10 µg rubber septa) in centres of plots in 2008

Farm	Decoy Farm			Belks Farm			Salmans Farm		
Treatment	Control	MD	A&K	Control	MD	A&K	Control	MD	A&K
Trap	Rye	Swigshole East	Swigshole West	ST1	SV1	SV3	Lower	New Field top	New Field bottom
15-Apr*	*	*	*	0	0	0	*	*	*
22-Apr	*	*	*	*	*	*	*	*	*
29-Apr	32	2	9	3	3	7	52	0	1
06-May	32	0	0	234	13	2	13	9	0
13-May*	336	3	1	1512	1	3	110	6	3
20-May	118	1	1	232	2	2	118	1	4
27-May	102	6	2	11	1	2	140	7	0
03-Jun	16	58	2	9	0	0	0	0	2
10-Jun	17	33	7	11	0	0	7	0	1
17-Jun*	160	31	2	219	0	6	52	8	18
24-Jun	560	118	9	144	1	0	292	69	10
01-Jul	336	560	38	260	7	3	395	8	3
08-Jul	46	640	31	300	15	1	442	10	5
16-Jul	17	228	9	74	1	0	128	20	2
22-Jul*	164	185	8	194	4	3	360	296	10
29-Jul	240	255	59	590	11	2	320	8	7
05-Aug	10	320	36	524	25	4	380	5	6
12-Aug	39	272	7	568	31	3	552	58	3
19-Aug	65	74	2	620	97	0	520	87	6
26-Aug	380	1	0	*	64	2	640	59	17
02-Sep	*	*	*	*	21	1	520	210	11
09-Sep		*	*	*	21	1	1100	16	6
16-Sep*	*	*	*	*	*	*	320	48	1
22-Sep	*	*	*	*	*	*	79	36	0
01-Oct	*	*	*	*	*	*	23	12	0
09-Oct	*	*	*	*	*	*	6	3	2
Total	2670	2787	223	5505	318	42	6569	976	118

*** changed lures**

Table 3.6.4.6. Catches of males in pheromone traps (white 20 × 20 cm delta) baited with the treatment lure (one 200 µg rubber septum for A&K, one EVA granule for MD) in centre of plots

	Decoy Farm		Belks Farm		Salmans Farm	
	A&K (EVA lure)	MD (septa lure)	A&K (EVA lure)	MD (septa lure)	A&K (EVA lure)	MD (septa lure)
	Swigshole West	Swigshole East	SV3	SV1	New Field bottom	New Field top
13-May	1	0	2	4	0	4
20-May	2	1	1	3	3	3
27-May	5	*	1	1	0	2
03-Jun	1	5	0	0	1	2
10-Jun	2	24	0	1	14	0
17-Jun	5	11	5	2	15	5
24-Jun	8	19	0	3	7	11
01-Jul	21	150	0	8	14	6
08-Jul	8	224	4	9	15	6
16-Jul	2	96	2	0	11	17
22-Jul	7	30	0	7	5	86
29-Jul	1	58	2	11	12	4
05-Aug	3	118	0	18	7	6
12-Aug	2	16	3	10	2	43
19-Aug	2	41	0	20	0	22
26-Aug	0	6	0	19	2	16
02-Sep			0	10	1	73
09-Sep			0	9	3	30
16-Sep					0	21
22-Sep					0	12
01-Oct					0	6
Total	70	799	20	135	112	375

Table 3.6.4.8. Sex ratio of larvae successfully pupating from cane splits. No adults emerged from the larvae on SV1 and SV3. There were also fewer larvae on these plots

Plot/treatment	Date canes collected	Date emerged	No. male	No. female	Male:female sex ratio
Lower	16 Jul	6 Aug	14	1	
Untreated	16 Jul	11 Aug	44	10	
	16 Jul	13 Aug	2	5	
	16 Jul	15 Aug	12	8	
	16 Jul	19 Aug	4	2	
	16 Jul	22 Aug	2	0	
	12 Aug	9 Sep	1	0	
	12 Aug	3 Sep	0	2	
Total			79	28	2.8
NF Bottom	3 Jun	17 Jul	1	0	
A&K	16 Jul	6 Aug	4	0	
	16 Jul	11 Aug	5	0	
	16 Jul	13 Aug	2	1	
	16 Jul	15 Aug	2	0	
	12 Aug	9 Sep	3	1	
	12 Aug	3 Sep	2	1	
Total			19	3	6.3
NF Top	17 Jun	17 Jul	1	0	
MD	16 Jul	6 Aug	9	2	
	16 Jul	11 Aug	16	4	
	16 Jul	13 Aug	2	1	
	16 Jul	15 Aug	1	3	
	16 Jul	19 Aug	7	3	
Total			36	13	2.8
Rye Street	1 Jul	23 Jul	2	0	
Untreated	1 Jul	25 Jul	3	1	
	1 Jul	30 Jul	19	8	
	1 Jul	1 Aug	1	1	
	16 Jul	11 Aug	8	6	
	16 Jul	13 Aug	4	2	
	16 Jul	15 Aug	1	1	
	16 Jul	19 Aug	1	0	
	29 Jul.	29 Aug	1	0	
	12 Aug	1 Sep	0	2	
	29 Jul	1 Sep	1	0	
Total			14	9	3.0
SH East	3 Jun	17 Jul	2	0	
MD	17 Jun	17 Jul	1	0	
	1 Jul	25 Jul	4	0	
	1 Jul	30 Jul	2	3	
	1 Jul	1 Aug	0	1	
	16 Jul	11 Aug	5	5	
	16 Jul	13 Aug	2	3	
	16 Jul	19 Aug	3	0	
	12 Aug	1 Sep	1	0	
	12 Aug	9 Sep	8	4	
	12 Aug	3 Sep	7	6	
	12 Aug	22 Sep	1	0	

Total			**16**	**12**	**1.3**
Plot/treatment	**Date canes collected**	**Date emerged**	**No. male**	**No. female**	**Male:female sex ratio**
SH West	**3 Jun**	**17 Jul**	**4**	**1**	
A&K	**17 Jun**	**17 Jul**	**2**	**0**	
	17 Jun	**23 Jul**	**1**	**0**	
	1 Jul	**6 Aug**	**0**	**1**	
	16 Jul	**6 Aug**	**3**	**0**	
	16 Jul	**11 Aug.**	**23**	**3**	
	16 Jul	**13 Aug**	**7**	**0**	
	12 Aug	**1 Sep**	**1**	**0**	
	12 Aug	**9 Sep**	**1**	**1**	
	12 Aug	**3 Sep**	**3**	**2**	
Total			**36**	**4**	**9.0**
ST1	**1 Jul**	**23 Jul**	**1**	**0**	
Unt	**1 Jul**	**1 Aug**	**1**	**1**	
	1 Jul	**6 Aug**	**0**	**1**	
	16 Jul	**11 Aug**	**27**	**7**	
	16 Jul	**13 Aug**	**9**	**7**	
	29 Jul	**29 Aug**	**3**	**2**	
	16 Jul	**22 Aug**	**3**	**0**	
	29 Aug	**1 Sep**	**3**	**3**	
	29 Jul	**2 Sep**	**0**	**2**	
Total			**42**	**17**	**2.5**

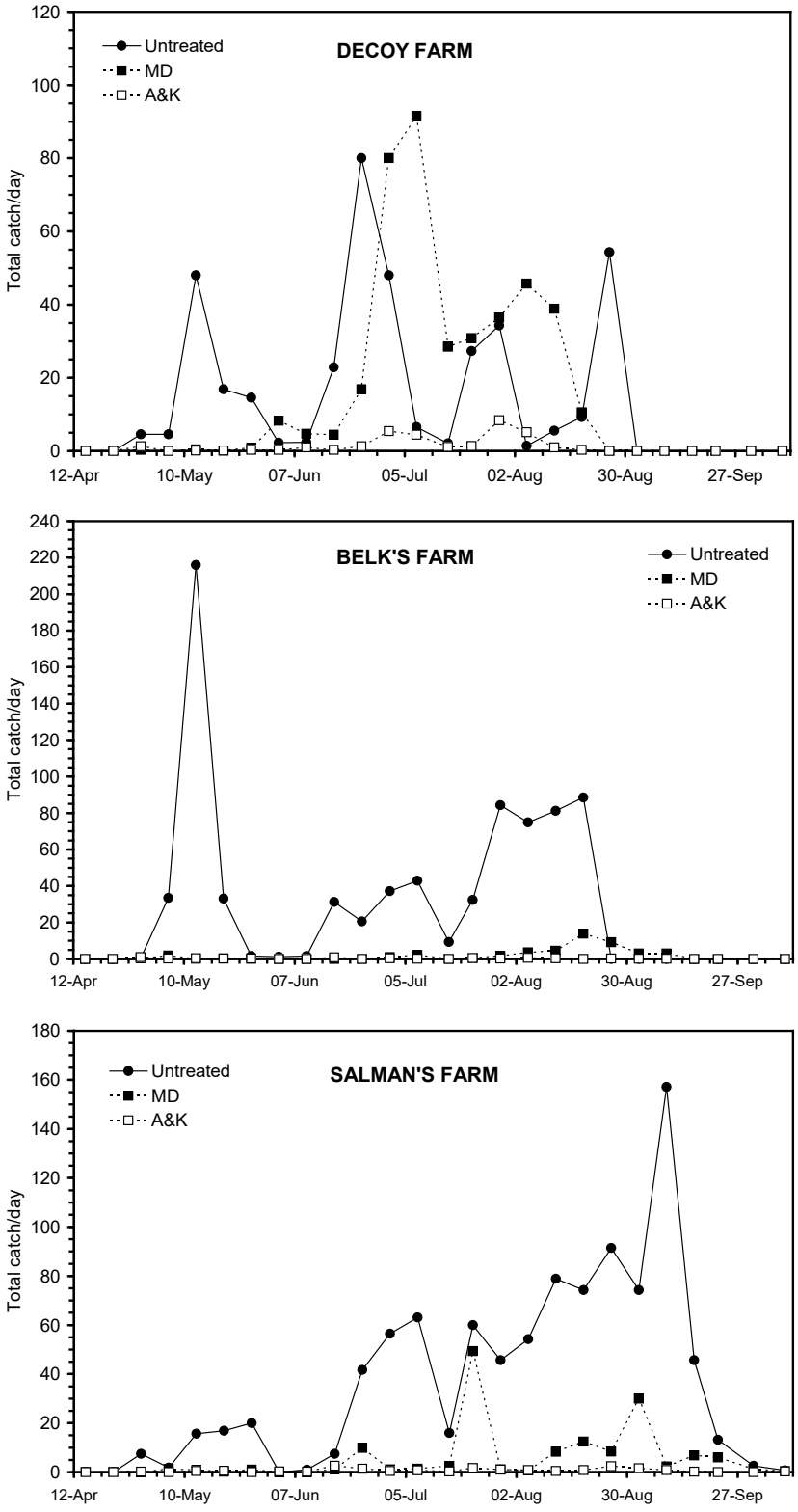


Fig 3.6.4.6. Catches of males in standard sex pheromone traps (White 20 × 20 cm delta with 10 µg rubber septa) in centres of field plots in 2008

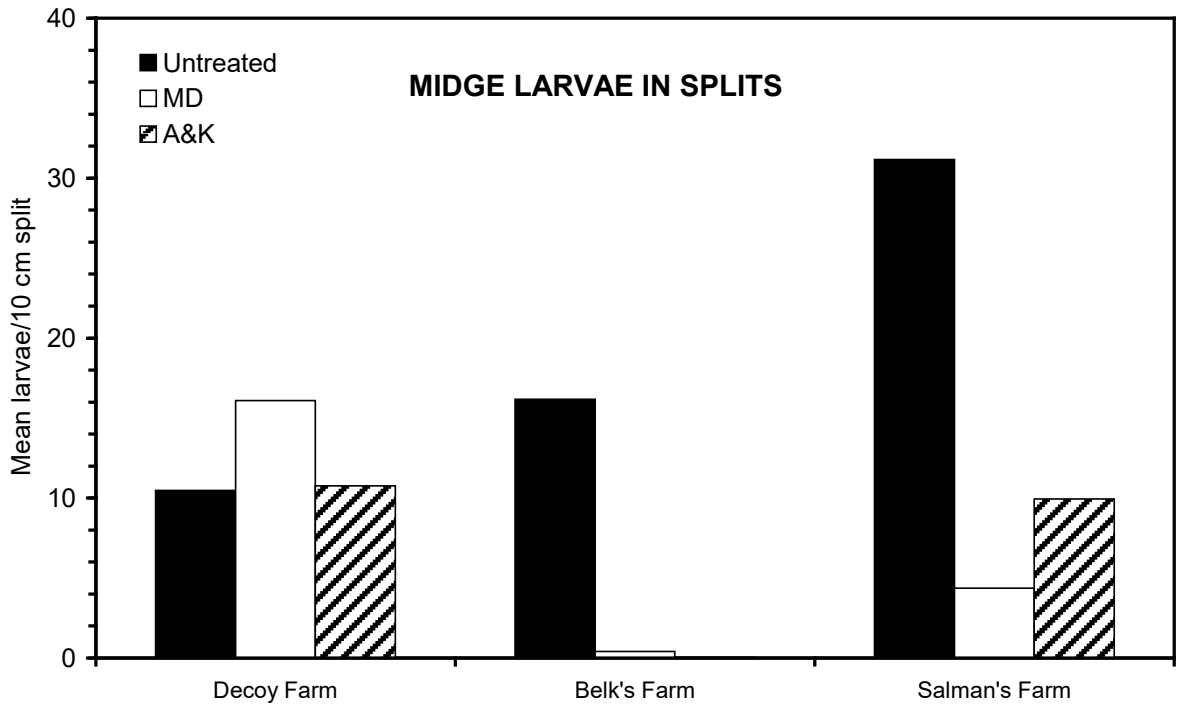


Figure 3.6.4.7. Mean number of larvae recorded per 10 cm of split

Objective 4. Mildew

4.1. *Inoculum sources*

Task 4.1.1. - Field monitoring of cleistothecia/ascospore development and disease development (years 1-3, EMR, CSL)

As agreed by the consortium in 2007, we have directed the research to assessing whether powdery mildew from strawberry could infect raspberry and vice versa. In 2008, we completed this study by focusing on morphological characteristics between the two pathogens. In addition, we have continued to monitor powdery mildew on those plants had primary mildew in 2007 in a tunnel at EMR.

Materials and methods

Powdery mildew from strawberry (cv. Elsanta) and raspberry (a breeding line from EMR) plants were taken to Wellesbourne for electromicroscopic work.

Results

Electromicroscopy showed that there were no apparent morphological differences between the mildew on the two fruits (Fig. 4.1.1.1).

All the shoots with primary mildew in 2007 died and did not re-grow in 2008. All new shoots did not show any mildew symptoms, suggesting that powdery mildew did not manage to overwinter on the buds of new shoots in 2007. This observation supports our previous observations that powdery mildew on mature raspberry plants appeared not to spread readily among leaves.

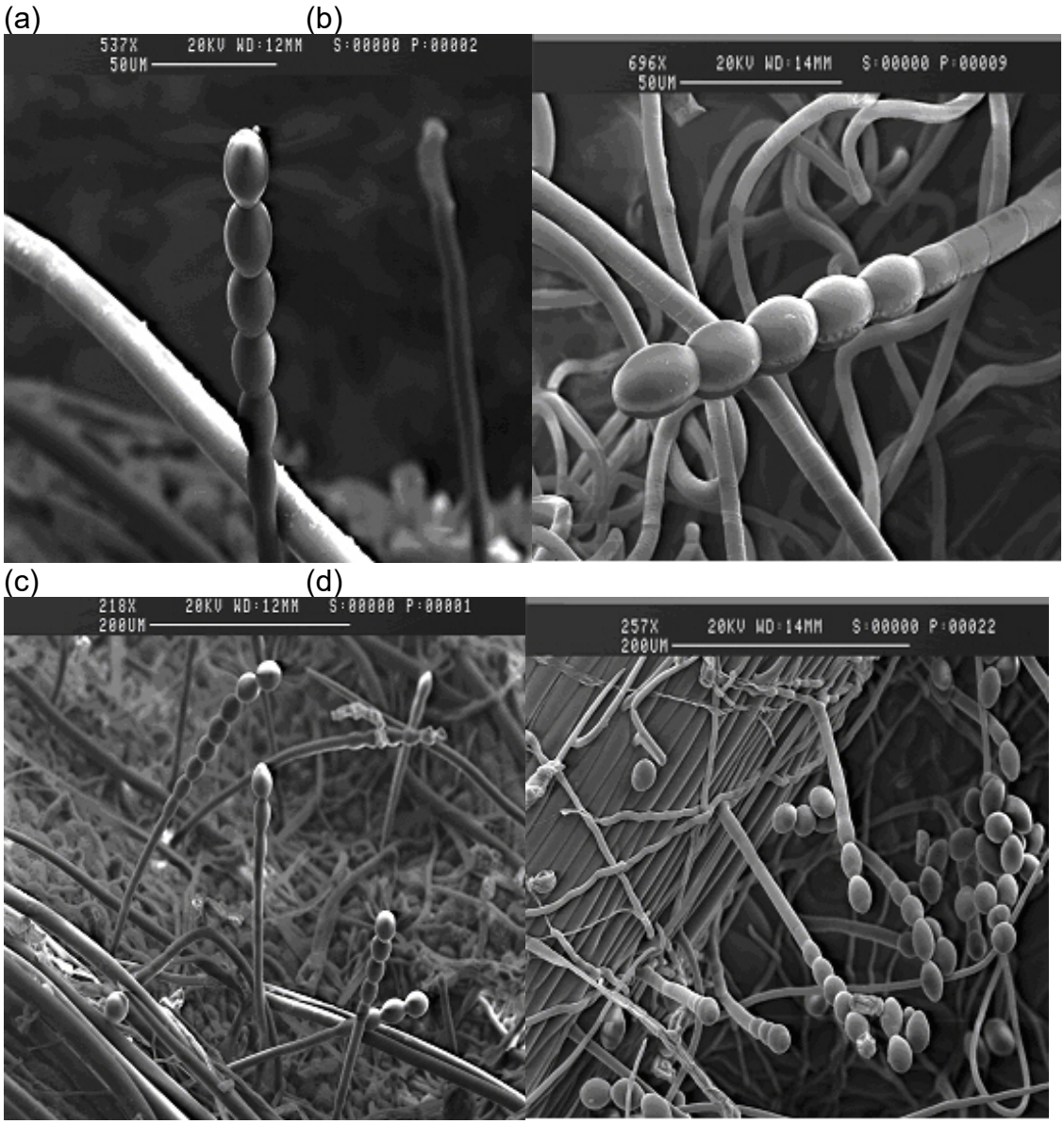


Figure 4.1.1.1. Scanning electron microscope images of raspberry (a,c) and strawberry (b,d) powdery mildew conidiophores

Task 4.1.2 – Comparison of mildew population in autumn and spring (year 2-3; EMR)

4.2. *Environmental manipulation*

As for *Botrytis* Task 1.2.3 (Years 1-3; ADAS, EMR)

4.3. *Control agents*

Task 4.3.1 See *Botrytis* Task 1.3.3– Glasshouse and field evaluation of natural products and commodity substances for control of *Botrytis* and powdery mildew. (Years 1-3), ADAS, EMR

Task 4.3.2 – Field evaluation of methods of mildew inoculum elimination. (years 2-3; EMR)

Task 4.3.3 See *Botrytis* Task 1.3.4 - Field evaluation of combined fungicide other product programmes for control of raspberry diseases. (Year 3), ADAS, EMR.

Objective 5. Aphids

5.1. *Autumn control aphids*

Task 5.1.1. - Evaluate autumn control strategy and identify moist effective products and timings . (years 1-3; EMR)

Autumn control of aphids on raspberry 2007 – 08

Objective

The objective of this experiment is to evaluate 3 different timings of single sprays of pirimicarb (Phantom), thiacloprid (Calypso) and pymetrozine (Plenum) for the control of small and large raspberry aphids, in commercial raspberry production.

Site

The edge rows of two Glen Ample plantations owned by Clockhouse Farm, Coxheath (courtesy of Robert Pascal).

Treatments

Treatments were single sprays of Calypso, Phantom or Pelnum applied at different timings, as given in Table 5.1.1.1.

Table 5.1.1.1. Treatments

Trt No.	Colour code	Product	Active ingredient	Dose rate (/ha)	Timing(s)†
1	Red	Calypso	480 g/l thiacloprid SC	250 ml	3rd week Sept
2	Red Blue	"	"	"	1st week Oct
3	Red Yellow	"	"	"	3rd week Oct
4	Black	Aphox	50% w/w pirimicarb WG	280 g	3rd week Sept
5	Black Blue	"	"		1st week Oct
6	Black Yellow	"	"	"	3rd week Oct
7	Blue	Plenum WG	50% w/w pymetrozine WG	400 g	3rd week Sept
8	Blue Blue	"	"	"	1st week Oct
9	Blue Yellow	"	"	"	3rd week Oct
10	Green	Untreated	-	-	-

Treatment application

Sprays were applied at 500 l/ha with a Birchmier motorised air assisted sprayer back pack sprayer by EMR staff. Spray application were made one-sided to each side of the pair of rows in the bed.

Experimental design and layout

The eastern and western outside rows of both of the two plantations were used, with one replicate on each edge. A randomised complete block experimental design with four replicates of the 10 treatments (= 40 plots) was done. Plots consisted of two bays.

Approval

Calypso has a SOLA for use on outdoor raspberry (1494/2004). The maximum individual dose is 250 ml product /ha, the maximum dose per season 750 ml/ha and the harvest interval is 3 days. Phantom is approved for used on raspberry and Plenum WG has a SOLA (1702/2006) for use on outdoor raspberries. The SOLA specifies a maximum of three treatments per crop and a harvest interval of 12 weeks.

Assessments

Small raspberry aphid (*Aphis idaei*) was not detected. Large raspberry aphid (*Amphorophora idaei*) occurred in adequate numbers for assessment. These were distinguished from the potato aphid, also present in small numbers on the plots.

Winter eggs: The number of over wintering eggs on a sample 96 canes per plot (one cane per stool on each of 16 stools in each of six 8 m lengths of row per plot) on 19 January 2007.

Summer breeding stages: The assessments were done on the 25 April and involved counting the number of adult, nymphs and mummified aphids per plot. A record was made of the position of each plot down the row so that this could be taken into account in the analyses.

Statistical analysis

Data for the total number of large raspberry aphids and numbers of aphid eggs per cane were Log_{10} transformed and subjected to ANOVA.

Results and conclusions

Calypso sprayed on the 19 October was the only treatment that was significantly effective at reducing the numbers of aphid eggs and large raspberry aphids on the raspberry canes (Table 5.1.1.2 and Figs 5.1.1.1 and 5.1.1.2).

Table 5.1.1.2. Mean number of aphid eggs and large raspberry aphids on canes and plots, respectively. Actual means are shown, data was analysed using Log^{10} transformed means. Treatments with different letters were significantly different

Treatment	Aphid eggs/cane			Aphids/plot		
	Actual mean	log^{10} mean		Actual mean	log^{10} mean	
Calypso 21/09/07	0.802	0.072	a	73	0.95	ab
Calypso 05/10/07	1.590	0.114	a	40	0.80	ab
Calypso 19/10/07	0.346	0.035	b	2	0.12	b
Aphox 21/09/07	2.107	0.171	a	336	1.17	a

Aphox 05/10/07	2.261	0.162	a	61	1.07	a
Aphox 19/10/07	0.460	0.047	a	87	1.04	ab
Plenum 21/09/07	0.201	0.021	a	192	1.10	a
Plenum 05/10/07	5.510	0.244	a	109	0.83	ab
Plenum 19/10/07	0.793	0.072	a	148	1.09	a
Untreated	5.715	0.283	a	499	1.46	a
F prob		0.277			0.335	
s.e.d (d.f. 27)		0.111			0.452	
l.s.d (d.f. 27)		0.228			0.928	

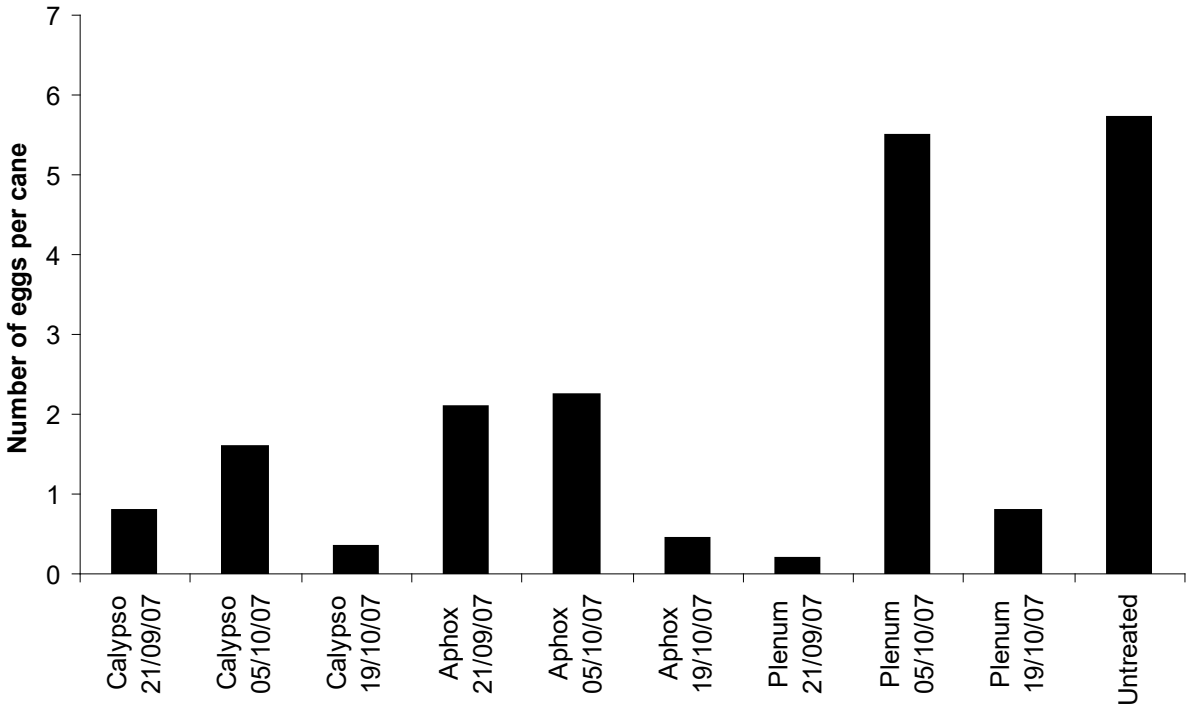


Figure 5.1.1.1. Number of aphid eggs per cane (mean of 384 canes)

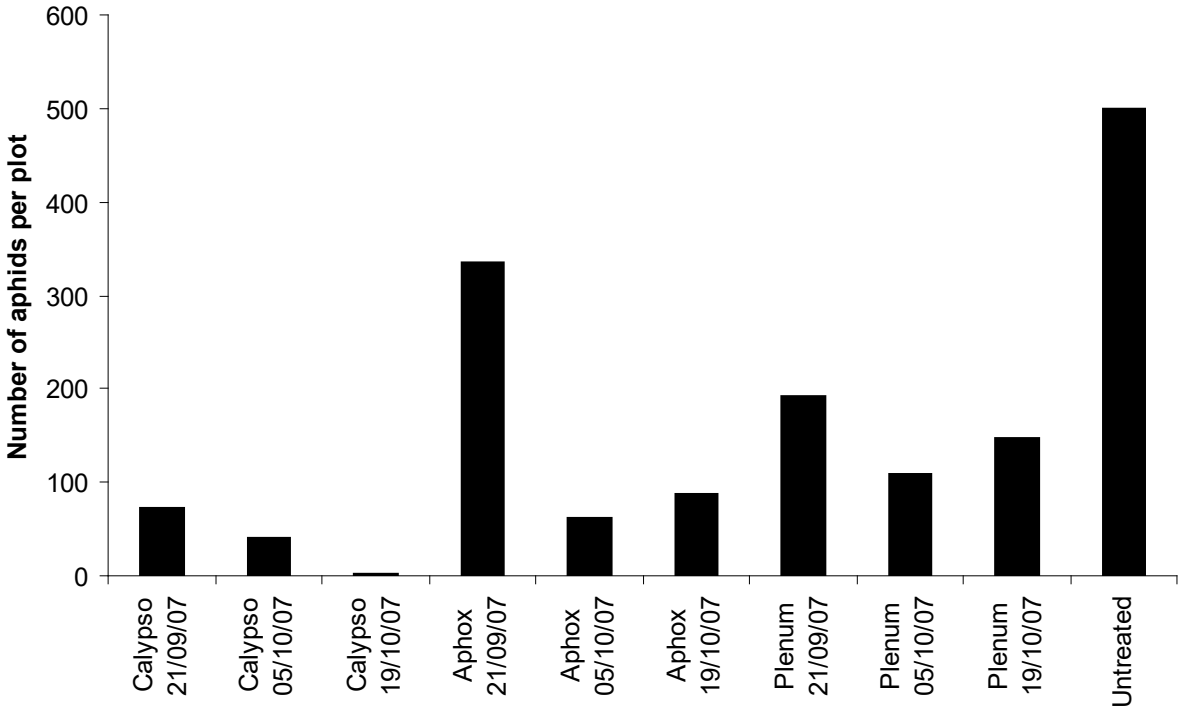


Figure 5.1.1.2. Mean number of large raspberry aphids (mean of 4 plots)

5.2. *Integrated aphid management*

Task 5.2.1. - Evaluate integrated aphid management based on biocontrol during the growing season and aphicides in the autumn (years 4-5; EMR, ADAS, Growers)

See work to be done on objective 6 below.

Objective 6. Integrated system

Task 6.1 - Devise an IPM strategy (years 4-5, all partners)

Task 6.1.1. Integrated disease management (IDM)

The following strategy is proposed based on results obtained in this project. Priority has been given to non-chemical methods of disease management in order to produce quality fruit with minimal risk of detectable fungicide residues at harvest. Consideration has also been given to control of other diseases (*e.g.*, cane blight, cane spot, rust) not investigated in this project. It is based on integration of five components (1) tunnel hygiene, (2) crop management, (3) fruit cooling, (4) natural products and (5) targeted use of fungicides. A sixth component (use of tunnel humidity to predict *Botrytis* risk during flowering) will be considered for the field trials in 2010 if this initial IDM strategy is insufficiently effective.

1. Tunnel hygiene

Remove old floricane and the primocane thinnings from alleyways and out of the tunnel (*i.e.* do not pulverise in situ) within 2 weeks of the end of harvest. Cut out canes visibly affected by *Botrytis* lesions or sclerotia at cane thinning in the autumn. Remove fruit that is unmarketable at each pick (*i.e.* do not leave it on the crop).

2. Crop Management

Thin canes after harvest to no more that 8/metre and maintain at this density (note: this will be done in 2009 for 2010 crop only).

Seek to minimise damage to lateral leaves during cane thinning (Increased risk of cane infection where leaves are broken off).

Cut-off cane tops around 20 cm above top wire in order to lower apical dominance and help avoid a dense leaf canopy near the cane top.

3. Fruit cooling

Place fruit within cool store within 2 hours of harvest (first berry picked in a batch) and cool to 2°C as quickly as possible. Maintain at 3 - 4°C during storage (up to 4 days after picking) and at ≤ 12°C during transport/display (for a maximum of 3 days) (ie the `prolonged cold store` treatment used in 2008 experiments). (Note: actual temperatures achieved will be recorded on 4 occasions during picking - see Task 6.2).

4. Targeted use of fungicides

(a) Do not apply any fungicides from the start of flowering to the end of harvest (Project experiments in Kent indicated most leaf infection by *Botrytis* occurs post-harvest; and 2x Teldor during flowering had little effect on incidence of latent fruit *Botrytis* in tunnel crops at Cambs site).

(b) Apply one spray of a powdery mildew fungicide (*e.g.* Systhane 20EW) in the spring as soon as the tunnel is covered (if powdery mildew is present, apply 2 sprays within 10 days).
(c) Apply three sprays before the start of flowering for control of cane spot and suppression of *Botrytis* development on canes. To comprise Thiram when buds at 5 cm, Signum 2 weeks later (while crop is still uncovered) and Teldor or Scala after a further 2 weeks.

(d) Apply 3 fungicides at 10-14 d intervals starting within 2-3 weeks post-harvest for control of *Botrytis*, powdery mildew, rust and cane blight (as soon as possible after fruiting cane is removed). Include some measure of cane spot control. Select product according to the diseases present and the anticipated disease risk (based on variety, site etc) from:

Fungicides should be used alternately, with no more than two sprays of any product. (Note: these fungicide treatments can only be applied in 2009 for the 2010 harvest; there will be no difference in autumn fungicide use between the 'Grower Standard' and the 'IDM Programme' for the 2009 fruit harvest.)

Task 6.1.1. Integrated pest management (IPM)

The following strategy is proposed based on results obtained in this project. Priority has been given to non-chemical methods of pest management in order to produce quality fruit with minimal risk of detectable insecticide residues at harvest. Consideration has also been given to control of other pests (*e.g.*, two-spotted spider mite) not investigated in this project.

1. Use of 50 raspberry beetle host volatile funnel traps with white cross vanes/ha to direct sprays of Calypso only where exceedence of threshold traps catches indicate where local treatment is necessary. The traps are to be deployed 3-4 weeks before flowering in a grid through the crop with extra traps deployed in areas that are likely to be sources of beetles (flowering hawthorn, wild blackberry, untreated raspberry or blackberry plantations). Each tunnel to have a minimum of 3 traps deployed, one at each end and one in the middle. If > 5 beetles have been caught by the start of flowering (= ~5% flowering) in any trap in that tunnel, the whole tunnel is to be treated with a spray of Calypso for raspberry beetle control. Note that although the traps will reduce the populations of raspberry beetle, they are not being used to control the beetle directly per se. This would require pesticide registration. They are being used as a monitoring device to direct local sprays of thiacloprid (Calypso) and reduce applications in general using an action threshold. The traps also are useful to show growers 'hot spots' in raspberry fields and surrounding vegetation so that control measures can be much more targeted over space and time.

2. Apply a sex pheromone attract and kill treatment for raspberry cane midge. A SPLAT raspberry cane midge 'attract and kill' formulation is being developed. The exact formulation and dose and numbers of treatment points are being investigated in preliminary laboratory investigations. The treatment will be applied to the polythene or the base of the canes at the first midge catch in sex pheromone traps in late April/early may, probably using mastic guns. It is hoped that season long control will be obtained.

3. A mid-October spray of thiacloprid Calypso) will be applied for aphid control supplemented with regular introductions of *Aphidius ervi* and *Aphidoletes aphidomyza* for biocontrol in summer.

4. Two spotted spider mite will be controlled by introduction of the predatory mite *Phytoseiulus persimilis* according to normal commercial protocols.

The proposed programme for evaluation in years 4 and 5 is set out in more detail below.

Proposed IPDM programme for evaluation in years 4 and 5

Sites

The IPDM strategy will be tested in 2009 at two sites:

Region	Managed by	Grower/Farm	Producer Organisation
East	ADAS (Erika Wedgwood)	Paul Harrold	Berry World
South East	EMR (Jerry Cross, Angela Berrie)		KG Growers

The same sites and crops will be used in 2009 and 2010.

The crops used must be:

- Glen Ample, Octavia or Tulameen
- Fully established (*i.e.* after at least one cropping season)
- Covered with the same type of tunnel structure (hoops)
- Covered with the same type and age of polythene on the 'IPM' and 'grower standard' areas (polythene type may differ between the three sites).

Additionally, a pest management trial will be done in Scotland managed by SCRI (Nick Birch; awaiting site confirmation from KG Ltd).

Treatments

At each site, the host farmer will apply the specified full IPDM programme (Table 1) to an area of at least 1 tunnel with pheromone (beetle traps + midge A&K) treatment to a surrounding area totalling 1 ha, and to an area of similar size and on the same variety in the same field, where he will apply his standard pest and disease control programme.

All pest and disease records and environment monitoring will be done by Consortium staff.

All crop work, fruit picking and yield records will be done by the host farmer.

Proposed IPDM programme for evaluation in years 4 and 5

Table 1. Grower actions

Target	Control method	Timing	Action	Monitoring/records by grower†
Botrytis	Tunnel hygiene	During picking	Remove fruit that is unmarketable at each pick (*i.e.* do not leave it on the crop).	
		Within 2 wks of harvest	Remove old floricane and the primocane thinnings from alleyways and out of the tunnel (*i.e.* do not pulverise in situ). Primocane debris should not be left in the tunnel.	
		Autumn	Cut out canes visibly affected by *Botrytis* lesions or sclerotia at cane thinning in the autumn.	
	Crop management	Within 2 wks of harvest	Cut old floricanes into sections before removing from the row in order to minimise damage to primocanes. Thin canes after harvest to no more that 8/metre and maintain at this density (note: this will be done in 2009 for 2010 crop only). Seek to minimise damage to lateral leaves during cane thinning (Increased risk of cane infection where leaves are broken off) by cutting long primocanes into sections before removal. Cut-off cane tops around 20 cm above top wire in order to lower apical dominance and help avoid a dense leaf canopy near the cane top.	
	Fruit cooling	At harvest	At 2 h after the start of fruit picking, at weekly intervals on 4 occasions, pick fruit following the normal commercial standards, into the usual punnets, from the IPM tunnel for the specified storage treatment (see Table 1a below). At the same time, pick fruit from the standard tunnel and cool and store according to the	Insert a datalogger with the batch of fruit from each tunnel to record actual temperatures achieved from picking up to the point of

			grower standard regime for the site.	disease assessment (7 days after picking) by ADAS/EMR staff.
Mildew	Spray fungicide	In spring as soon as infection seen	Apply one spray of a powdery mildew fungicide (*e.g.* Systhane 20EW) in the spring as soon as the tunnel is covered (if powdery mildew is present, apply 2 sprays within 10 days).	
Cane spot	Inspection	February	Check for symptoms. If the disease is found, apply spray programme (below)	
	Spray fungicide	Start 6 wks pre-flowering	Apply three sprays before the start of flowering for control of cane spot and suppression of *Botrytis* development on canes. To comprise Thiram from primocane emergence (approx 10 cm tall), Signum 2 weeks later (while crop is still uncovered) and Teldor or Scala after a further 2 weeks.	
Cane diseases	Spray fungicide	From 2-3 weeks post harvest	Apply 3 fungicides at 10-14 d intervals starting within 3 weeks post-harvest for control of *Botrytis*, powdery mildew, rust and cane blight (start spray programme 2 weeks after harvest even if the old fruiting cane is removed). Include some measure of cane spot control. Select product according to the diseases present and the anticipated disease risk (based on variety, site etc) from Table 2	
Phytophthora root rot	Fungicide to stem base		Drenching spray to stem base if the disease is known to be present on the site. Paraat (SOLA 2777/07) with 90 d HI; or Shirlan (SOLA 2168/05) before 31 March in year of harvest.	
Raspberry beetle	Agrisense white cross vane funnel traps with flower volatile sachet lures	3-4 weeks before flowering,, continued during flowering if trap catches remain	Grid of 50 white cross vane funnel traps with floral volatile sachet lure deployed per ha within tunnels, additional external traps adjacent to main beetle sources (hawthorn etc).Deploy one at each end of each tunnel + 1-2 in between in each tunnel,	Record of number of raspberry beetles in each trap at first flower

		above threshold.	depending on tunnel length. The lures last up to 4-6 weeks, depending on local weather conditions. Replace lures as needed. Spray Calypso (pyrethrum in organic crops) to any tunnel where > 5 beetles captured per trap	
Raspberry cane midge	Mating Disruption using SPLAT	At first midge catch in sex pheromone trap	Apply 2.5 kg of SPLAT containing 10g pheromone in 5000 0.5 g spots of per ha to polythene mulch or irrigation pipe to base of canes/polythene at first catch of midges in traps	Monitor midge sex pheromone traps weekly
	Directed spray of Decis	A few days after SPLAT application	Apply a spray of Decis (maximum dose in 200 L water per ha) directed at the polythene mulch, irrigation pipe and base of canes a few days after SPLAT application	
Aphids	Aphicide	Spring year 1, mid October year 1 for year 2	Spray in spring of year 1 necessary as no spray applied previous autumn	
	Aphidius ervi and *Aphidoletes aphidomyza*.		Normal programme of biocontrol agent introductions as advised by Syngenta Bioline	
Two-spotted spider mite	*Phytoseiulus persimilis*		Normal programme of predatory mite introductions. Re-introduction necessary after Calypso sprays	

† Normal crop scouting to be done at least fortnightly by grower

Table 1a. Cooling treatments for Class I fruit from IPDM tunnel and Grower tunnel (applied on 4 occasions at fortnightly intervals) to be applied for shelf life test

Store regime	Day 1 Wed	Day 2 Thur	Day 3 Fri	Day 4 Sat	Day 5 Sun	Day 6 Mon	Day 7 Tue	Day 8 Wed
Assessment for rots						Look over		Tip out
X (cold regime)	Pick and to field heat removal 1-2ºC. Fit lids	Move from 1-2ºC to 2-3ºC	Leave in cold store 2-3ºC	Move from 2-3ºC to 16ºC	Leave in pack-house 16ºC	Move from 16ºC to 20ºC	Leave in 20 ºC	Move from 20 ºC
Y (grower's standard regime)	Pick and to field heat removal to 4-5ºC. Fit lids.	Move to main cold store 4-5ºC	Leave in main cold store 4-5ºC	Move from 4-5ºC to 16ºC	Leave in pack-house 16ºC	Move from 16ºC to 20ºC	Leave in 20 ºC	Move from 20 ºC

Table 2a. **Fungicides for general disease control.** Fungicides should be used alternately, with no more than two sprays of any product. (Note: these fungicide treatments can only be applied in 2009 for the 2010 harvest; there will be no difference in autumn fungicide use between the `Grower Standard` and the `IDM Programme` for the 2009 fruit harvest).

Product	Approval	HI (d)	Protected or Outdoor	Diseases controlled (or partially controlled)					
				Botrytis	Cane blight	Mildew	Rust	Cane spot	Spur blight
Thiram	Label	7	O+P	(Yes)	No	No	??	Yes??	Yes
Folicur*	2160/08	14	P	Yes	Yes	Yes	Yes	??	??
Rovral WG**	Approved	7	O	Yes	No	No	No	No	No
Signum	0992/08	3	O	Yes	Yes	Yes	??	??	??
Switch	Provisional	14	O+P	Yes	No	Yes	??	??	??
Teldor*	Approved	1	O+P	Yes	No	No***	No	No	No

*And equivalent products approved on raspberry.
**Maximum of 1 spray per season to reduce risk of selecting resistant strains of *B. cinerea*
***No activity against strawberry powdery mildew; some activity recorded on gerbera and verbena powdery mildews

Table 3a. General records

	Item	Person responsible
1.	Highly visible notices at both ends of both the IPM and the Grower standard tunnels, with reminder of what can and cannot be done (*e.g.* fruit weighing, sprays, thinning).	Site manager (ADAS, EMR or SCRI)
2.	Total marketable yield per tunnel	Host farmer
	Total non-marketable yield per tunnel (record if possible; if not, an estimate)	Host farmer
3.	Crop diary of key events (spray dates, cane thinning, removal of floricane); spray application method including spray volume and pressure	Host farmer
4.	General records from fortnightly crop inspections for pests and diseases. Site manager to be kept informed of findings by host grower.	Host farmer
5.	Pesticide residue analysis at around 5% and 75% fruit pick; analysis for all fungicides and all insecticides approved on raspberry.	Fruit marketing group (KG, BW or SF)
6.	Cane density at start of flowering (*e.g.* count canes in 3 ×10 m lengths per tunnel)	Site manager (ADAS, EMR or SCRI)
7.	Targeted visits (x 4) to check the IPM programme is being followed and to detail the 'grower standard' programme being used.	Site manager (March/May/July/Sep)
8.	Temperature and humidity records in the two areas from April – Sept (2 loggers/area at mid canopy height). Download monthly. Send data to X. Xu at EMR by 31 October.	Site manager

Table 4a. Disease records. Protocols for disease assessments will be written by the consortium pathology group and followed by the ADAS/EMR/SCRI staff responsible for the site. All items will be carried out in each of the two tunnels per site

Item		Timing
1.	Shelf-life test 6 punnets of marketable fruit/tunnel filled to the normal level. Punnets should be from different pickers to ensure samples come from different tunnel locations. Place together in a clearly identifiable tray labelled with the tunnel, date and trial identifier. Follow temperature transfers shown in Table 1a. On Day 6, without touching the fruit, count the number with any mould. On Day 8, tip the fruit out and count the total and the number healthy, with *Botrytis*, mildew and other diseases. Record temperature from collection in the field to end of shelf-life test by use of a data logger placed with fruit; add a coloured string from the logger to the picker's label at the outside of the tray to facilitate finding logger again.	Weekly intervals for 4 weeks starting on a Wed (start 1 week after first pick)
2.	Incidence of floricanes with visible *Botrytis* lesions and/or sclerotia. Assess 100 canes at 1 m intervals spaced around the whole crop.	End February/early March
3.	Incidence of other cane diseases (cane blight, spur blight, cane spot). Assessed as above.	End February/early March
4.	Severity of powdery mildew and rust on floricane leaves. Assess 30 floricanes per tunnel spaced at least 1 m apart..	Late May/early June
5.	Incidence of ripe fruit with *Botrytis* and powdery mildew. Examine all row faces to estimate the % of fruit affected per tunnel.	Weekly June/July, 4 × prior to picking (close to fruit cold store picks)
6.	Severity of powdery mildew and rust on floricane and primocane leaves. Assess 30 floricanes and 30 primocanes per tunnel spaced at least 1 m apart.	Within 2 weeks of final fruit pick

Table 5a. Pest records. Protocols for pest assessments to be prepared by the consortium entomology group and carried out by ADAS/EMR/SCRI staff responsible for the site. All items will be carried out in each of the two tunnels per site.

Item		Timing
1.	Counts of raspberry beetle adults in each trap. The count at first (= ~5%) flower is to be done by the grower	Weekly
2	Counts of raspberry beetle/infested damaged fruit weekly during picking. Reject-grade or Class 2 fruit to be collected and assessed for beetle damage. In addition to the berry, examine the plug of each berry carefully as these are most likely to show signs of raspberry beetle damage/infestation.	Harvest
3	30 ~10 cm long artificial splits made fortnightly towards the base of the primocane, 10 in the centre, and 10 towards each end of each central plot tunnel. Fortnightly counts of eggs/larvae in 30 splits. Length of splits to be measures so the numbers of larvae/cm of split can be calculated	Fortnightly
4	Counts of aphids including records of the percentage parasitized. The sampling size may have to be adjusted to suit the level of infestation but a sample of 50 leaves per tunnel could be inspected as the norm.	Monthly
5	Counts of mites on leaf samples if damaging populations develop	Once or twice if necessary at appropriate time

Payment responsibilities of Fruit Marketing Groups

1. Payment to host growers for loss of marketable produce over and above that in the grower-standard treatment, to be determined by the fruit marketing group and host grower.
2. Payment for pesticide residue analyses (x samples for y residues each year).

Task 6.3. - Prepare best practice guidelines (year 5; all partners)

Progress on milestones

Primary milestones

Milestone	Target month	Title	Acheived?	On time?
P1.1	24	The time period when canes become infected by *Botrytis* via leaf infection identified	Y	y
P1.2	24	Fungicides or other treatments demonstrated to suppress sporulation of *B. cinerea* sclerotia in field trials	N[1]	N[1]
P1.3	36	Tunnel environmental manipulated by crop canopy management such that the risk of *Botrytis* is significantly reduced compared with standard practice	Y	Y
P2.1	12	Raspberry beetle lures developed and tested in the laboratory, and efficiency of trap types compared	Y	Y
P2.2	24	Raspberry beetle flower volatile dispenser and lure blend, using data from 2.3 in year 1 optimised	Y	Y
P2.4	48	The efficiency of beetle control/monitoring using improved (optimized) lure with standard trap at research plots and 'on-farm' locations re-examined	Y	Y
P2.5	48	Trials at grower protected raspberry plantations to integrate optimal raspberry beetle lures for enhanced monitoring and control undertaken.	Y	Y
P3.1	20	Attractive sex pheromone lure and trap for male raspberry cane midge monitoring developed	Y	Y
P3.2	56	Sex pheromone trap thresholds for male raspberry cane midge determined		
P3.3	34	Host plant volatile wound attractant of raspberry cane midge females identified	P	N
P3.4	40	Attractive host volatile lure and trap for female raspberry cane midge monitoring developed	Y	Y
P3.5	56	Host volatile trap thresholds for female raspberry cane midge determined		
P3.6	12	Experimental approval for raspberry cane midge semiochemical control trials obtained	Y	Y
P3.7	48	Feasibility of control of raspberry midge by sex pheromone mating disruption determined		
P3.8	24	Most promising device for control of raspberry cane midge by mass trapping or lure and kill identified	Y	Y
P3.9	60	Efficacy of midge control by mass trapping or lure and kill determined		
P4.2	36	Methods for eliminating mildew inoculum identified	N[2]	N[2]
P4.3	36	Programmes of fungicides and natural products demonstrated to provide control of mildew in field trials	Y	Y
P5.1	36	Effectiveness of autumn treatment strategy for aphids determined	Y	Y
P6.1	48	Integrated pest and disease management strategy tested on commercial nurseries and shown to result		

Milestone	Target month	Title	Acheived?	On time?
		in nil or minimal detectable pesticide residues at harvest		
P6.2	60	Best practice guidelines for IPM in protected raspberry written		
P6.3	60	Occurrence of pesticide residues in crops grown to IPM standard compared with conventional crops		
[1] Behind schedule but progressing [2] Work reassigned to *Botrytis* because disease didn't appear				

Secondary milestones

Milestone	Target month	Title	Acheived?	On time?
S1.1	12	Potted raspberry inoculated on leaf with *Botrytis* and occurrence of stem *Botrytis* at associated nodes recorded	Y	Y
S1.2	6	Unsprayed tunnel crops of Glen Ample in E and SE, to be used for disease monitoring, agreed with growers	Y	Y
S1.3	15	Start and duration of *B. cinerea* sporulation on cane lesions and sclerotia established, year 1 data	Y	Y
S1.4	27	Start and duration of *B. cinerea* sporulation on cane lesions and sclerotia, year 2 data	Y	Y
S1.5	24	Factors that initiate sporulation from overwintered *Botrytis* sclerotia identified	Y	Y
S1.6	24	Data collected on seasonal variation in airborne inoculum of *B. cinerea* (and *S. macularis*) in tunnel raspberry crops	Y	Y
S1.7	6	Questionnaire devised for growers to record disease occurrence and severity and crop production features	Y	Y
S1.8	24	Two years grower data on disease occurrence and crop production factors summarised	Y	Y
S1.9	12	Comparison of bulk and individual testing of 100 green raspberry fruit for latent *Botrytis*; determination if either or both relate to incidence of post-harvest *Botrytis* rots	Y	Y
S1.10	12	Crop canopy treatments to manipulate tunnel RH devised	Y	Y
S1.11	36	Effect of tunnel environment manipulation on humidity close to flowers/fruit established	Y	Y
S1.12	12	Natural products and commodity substances screened for control of *Botrytis* on pot plants	Y	Y
S1.13	36	Natural products and commodity substances screened for control of *Botrytis* in small field	Y	Y

Milestone	Target month	Title	Acheived?	On time?
		experiments		
S1.14	36	Programmes of fungicides and other products evaluated for control of *Botrytis* in field trials	N	N
S2.1	12	Prepare raspberry beetle experimental sites, both at research stations and identify grower sites for 'on-farm' trials	Y	Y
S2.2	12	Develop and test lures in the laboratory	Y	Y
S2.3	12	Undertake preliminary trials to obtain data on the blends of compounds in open-field trial sites undertaken	Y	Y
S2.4	24	Maintain experimental sites, both at research stations and at identified grower sites for 'on-farm' trials	Y	Y
S2.5	24	Flower volatile dispenser and lure blend, using data from 2.3 in year 1 optimised	Y	Y
S2.6	36	Using selected trap type from 2.2 in year 1 and standard lure, efficiency of placement of raspberry beetle traps within, at perimeter and out with the crop at sites in England and Scotland compared	Y	Y
S2.7	24	Experiments to obtain data on the effectiveness of lure and kill and/or mass trapping of raspberry beetle initiated	Y	Y
S2.8	36	Experimental sites as for 2.4 maintained	Y	Y
S2.9	40	The efficiency of beetle control/monitoring using improved (optimized) lure with standard trap at research plots and 'on-farm' locations re-examined		
S2.10	48	Trials at grower protected raspberry plantations to integrate optimal raspberry beetle lures for enhanced monitoring and control (method will depend on outcomes of year 3 trials) undertaken		
S2.11	57	Trials at grower protected raspberry plantations to integrate optimal raspberry beetle lures for enhanced monitoring and control to confirm efficacy of trialling repeated.		
S2.12	60	Raspberry beetle recommendations for the industry prepared		
S3.1	24	Best cane midge sex pheromone blend, including enantiomeric requirements, determined	Y	Y
S3.2	24	Appropriate lure type and release rate for midge sex pheromone determined	Y	Y
S3.3	24	Suitable sex pheromone trap design for cane midge monitoring determined	Y	Y
S3.4	24	Behavioural analysis for cane midge females in response to wounds complete	Y	Y
S3.5	24	Key wound volatile components identified	Y	Y
S3.6	36	Appropriate lure type and release rate for midge host volatile lure determined	N[3]	N[3]
S3.7	36	Suitable trap design for cane midge female	N[3]	N[3]

Milestone	Target month	Title	Acheived?	On time?
		monitoring determined		
S4.1	18	First year's data collected on spatial and temporal occurrence of powdery mildew		
S4.2	12	Powdery mildew detected by real-time PCR on cyclone spore trap collections		
S4.3	36	Powdery mildew populations in autumn and spring compared using SSR primers	N[4]	N[4]
S4.4	36	Effect of tunnel environment manipulation on powdery mildew determined	N[4]	N[4]
S4.5	18	Natural products and commodity substances screened for mildew control on pot plants	N[4]	N[4]
S4.6	36	Programmes of fungicides and other products evaluated for control of mildew in field trials	N[4]	N[4]
S5.1	18	First autumn aphid control experiment completed. Treatments for evaluation in second experiment identified.	Y	Y
S5.2	30	Second autumn aphid control experiment completed. Treatments for evaluation in third experiment identified	Y	Y
S5.3	36	IPM strategy for aphid control for evaluation in final 2 years of project identified	Y	Y
S6.1	39	Integrated pest and disease management strategy devised		
S6.2	39	Information on the effect of some environmental factors on rate of pesticide disappearance from leaves and fruits assembled		
S6.3	39	Sites for testing IPM agreed for SE, E, WM and Scotland		
S6.4	54	Results of all IPM trials collated		
S6.5	60	Interaction of IPM components, economic performance and effects on other pests, diseases and beneficials assessed		

[3] Work in progress but behind schedule
[4] Work reassigned to cold chain work on *Botrytis*

Technology transfer activities

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

11 November 2008. Jerry Cross gave a 40-minute lecture at the EMRA soft fruit day about the zero residue soft fruit projects

11 November 2008. Xiangming Xu gave a presentation at the EMRA soft fruit day about the epidemiology and raspberry *Botrytis*

Article for *HDC News* February 2009. Cross *et al*: Progress towards zero residue Integrated Pest and Disease Management (IPDM) for raspberry and strawberry: Update on Horticulture LINK projects HL0175 and HL191

Publications

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

