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Headline

- Good progress is being made in developing integrated pest and disease management strategies in raspberries.

Background and expected deliverables

Raspberries are very susceptible to *Botrytis*, mildew, raspberry beetle, raspberry cane midge and aphids. Currently, pesticides are relied on for control and are applied close to harvest. Intensive use of pesticides, including the organo-phosphate (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 has 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 beyond 2008 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 and recent observations indicate that this increases the risk of mildew 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

Botrytis inoculum sources, effects of environmental manipulation and use of control agents

Inoculum sources

Cane infection by *Botrytis cinerea* is believed to arise at the leaf nodes *via* mycelial growth down the petiole. Information on the period when this occurs should help to devise a rational treatment to prevent cane infection. Artificial inoculation of tunnel-grown raspberry plants at different times of the year with a spore suspension of *B. cinerea* revealed no difference in the susceptibility of leaves of different age. However, whereas leaves on young primocanes inoculated in June were not susceptible, leaves of all ages on older primocanes inoculated in September were susceptible. Infection was predominantly symptomless.

- This result suggests that post-harvest control of *Botrytis* may be particularly important to prevent cane infection.

Tunnel-grown crops of Glen Ample in Cambridgeshire and Kent were monitored at intervals from June to October for occurrence of *Botrytis* on leaves and primocanes. *Botrytis* was present at a low incidence in visibly healthy leaves and petioles on primocanes from June to August. The fungus was found at a high incidence in attached old leaves of a dense crop in May. *Botrytis* lesions on primocanes were first seen in October.

- These results suggest that primocane leaf infection can occur throughout the growing season, although canes may not become infected until October. Further work is required to determine if canes may become infected before October.

Tunnel crops of raspberry in Cambridge and Kent were regularly monitored to identify the start and duration of *Botrytis* sporulation on cane lesions, and other likely sources of *Botrytis* (e.g. weeds, crop debris). Sporulation of *Botrytis* sclerotia on fruiting canes occurred from mid-May (when crops were at first open flower) through to August (when canes were cut out). High temperatures (>27°C) reduced sporulation. Weeds and crop debris in the tunnels examined at two sites in 2006 were not found to be a source of *B. cinerea* spores.

The effect of moisture and two temperatures (5 and 10°C) on sporulation of *Botrytis* sclerotia on raspberry canes was investigated. Sporulation occurred on sclerotia that were wetted, or incubated in high humidity for 2 weeks, at both temperatures. Sclerotia incubated in the field at this time did not sporulate.

- Treatments to prevent or reduce sporulation of sclerotia will be evaluated in 2007.

Seasonal variation in airborne inoculum of *B. cinerea* was investigated using a new spore trap. The spores are trapped in small vials prior to extraction of fungal DNA for quantification of *B. cinerea*. The test output is linearly related to the number of spores, indicating the method can be used to estimate the number of spores.

- Spore trapping will be undertaken in two commercial tunnel raspberry crops in 2007.

Environmental manipulation

Information was obtained on cropping practices and diseases of protected summer-fruiting raspberry from a survey of growers in England and Scotland. In 2005, five out of 11 growers reported problems with fruit *Botrytis* and all reported occurrence of cane *Botrytis*, usually at a low incidence. Two growers reported occurrence of powdery mildew on their crops.

Fruit samples from 20 crops were tested to examine whether information on the occurrence of latent *Botrytis* in unripe fruit can be used to help predict the risk of post-harvest *Botrytis* development. Latent *B. cinerea* was detected in unripe (yellow) fruit, with a large difference between crops; many samples had over 50% of fruit infected. No association was found between sampling date, interval since the last *Botrytis* fungicide spray, variety, and incidence of latent *Botrytis* in unripe fruit. The incidence of *Botrytis* in ripe fruit did not appear to relate to the incidence in unripe fruit. Levels of *Botrytis* were higher in ripe fruit than unripe fruit in 11 out of 20 samples. High levels of *Penicillium* developed on most samples of ripe fruit, except for samples from two organic crops.

The effect of canopy pruning on occurrence of *Botrytis* and powdery mildew was tested in tunnel crops in Cambridge and Kent. Although there was a high incidence of *Botrytis* fruit infection, there was no obvious treatment effect. The incidence of *Botrytis* on leaves and primocanes was negligible at both sites. At the Kent site, despite the removal of large numbers of leaves or primocanes, there was no impact on humidity except nearest the open end of the tunnel where there was more air circulation. No powdery mildew was observed at either site.

Control agents

A series of experiments were conducted to determine the relative efficacy of a range of fungicides and natural products for control of *Botrytis* on raspberry.

In the first experiment, conducted in a tunnel, significant reductions in cane *Botrytis* were observed following treatment with Frupica, Scala, Teldor and Rovral WP. Milsana and Talat were slightly less effective. Scala and potassium bicarbonate, at the rates and frequencies used, were phytotoxic to raspberry leaves.

In a second experiment (Cambridge, 2006), Amistar, Folicur, Scala and Talat applied to a tunnel-grown crop of Glen Ample were all more effective than several other products at reducing latent *Botrytis* in fruit. Scala and Folicur caused phytotoxicity on the leaves. The ripe fruit had particularly high levels of *Botrytis* at the second pick (early July), with 84% in untreated plots, reduced to 39% by Talat. Ripe fruit infection on samples picked in late June and late July had around 40% of untreated fruit infected by *Botrytis*, with the best treatments developing infection on about 20% of the fruit. In early July,

Botrytis infection was much greater on ripe fruit than on surface-sterilised yellow fruit picked three days earlier; possibly some infection developed from external contamination rather than latent internal *Botrytis* originating from flower infection.

In a third experiment (Kent, 2006), conducted in an open-field crop, the weather conditions during most of the flowering period were not favourable for *Botrytis* infection. The fungicides applied at the label recommended dose were most effective in reducing *Botrytis* rot in post-harvest tests. None of the other chemicals (natural products) evaluated were effective in reducing *Botrytis*, except possibly Hortiphyte Plus which requires further evaluation.

Powdery mildew

Mildew inoculum sources, effects of environmental manipulation and use of control agents

Inoculum sources

Mildew development was monitored in order to understand the mode of overwintering and subsequent spread during the growing season. Plants infected with mildew were then maintained in a polytunnel throughout the winter and the following growing season (2006). Preliminary results show that:

- mildew appears to overwinter in the buds (as mycelia and/or conidia)
- spread from these primary lesions to leaves of other canes is very rare under the tunnel conditions
- infection conditions appear to be very specific (mildew failed to transfer to healthy leaves in inoculation experiments).

Control agents

A range of fungicides and natural products were evaluated for control of powdery mildew and phytotoxicity on glasshouse crops of cvs. Glen Ample and Joan Squire. Mildew failed to develop, despite repeated artificial inoculation. Potassium bicarbonate with and without wetter caused leaf margin browning or necrotic spots. Spray residues

on the leaves were not obvious, except with the experimental product HDCF5 and, to a lesser degree, Frupica SC.

Raspberry beetle

Semiochemical-based monitoring and trapping systems for managing raspberry beetle

Two very active volatile attractants from raspberry flowers have been identified for raspberry beetle, compounds A and B. This work aimed to develop monitoring and trapping systems for managing raspberry beetle using these compounds.

The initial field trials were done in open-field sites at SCRI especially set up to provide large numbers of raspberry beetles. Three field trials, one in 2005 and two in 2006, tested different trap designs and different types of lures. Pervious research used non-UV reflectant white sticky traps but these can become contaminated by dust or by non-target insects, especially small flies. The prototype traps developed were based on standard funnel traps using white cross-vanes to visually attract adult raspberry beetles.

In 2005, two forms of the funnel traps were compared with the sticky trap. When used with a standard lure (thick-walled vials with compound B), both funnel traps were equally effective, and at least twice as effective as the sticky trap, in capturing adult raspberry beetles. When compared with other non-target insects, the funnel traps caught many fewer flies than the sticky traps and all traps caught very few pollinators. The cross-vaned funnel trap will be used as the standard for monitoring raspberry beetles in tunnels in 2007.

Also in 2005, two types of dispenser were tested in the laboratory. A thin-walled dispenser, thought to increase the evaporation of the attractant, was compared with the standard thick-walled dispenser. Although the evaporation rates were improved using the thin-walled vials, the design and reliability of the dispensers was problematic and further development ceased.

In 2006, a range of prototype sachet dispensers were manufactured using the attractant chemicals, compound A and compound B. Their efficiency was assessed in both the laboratory and in the field. Two field trials showed that single-sided sachets

did not differ significantly compared with the standard thick-walled vial dispenser in two-week exposures trials. However, in longer laboratory evaporation studies, the sachets evaporated at a higher rate than the standard dispenser and may not have sufficient attractant to last for the five to six weeks that may be required in field conditions. A new type of sachet with a more controlled vapour release has been found and will be tested in the laboratory and field in 2007.

To date, most of the research has concentrated on the use of compound B. To test the effect of a mixture of compounds A and B on trapping raspberry beetle, a single field trial was set up to compare compound A and compound B on their own versus a mixture of A and B. All three sets of attractants caught raspberry beetles, but compound B was the most effective and compound A the least effective. The mixture was approximately midway between the single compounds. This suggests that, at least with the standard thick-walled dispensers, there is no advantage in using a mixture of compounds A and B. The situation may change if the new type of sachets with variable evaporation rates proves successful.

Raspberry cane midge

Semiochemical-based systems of managing cane midge

Lure and trap optimisation

The raspberry cane midge sex pheromone was identified in previous research by EMR and NRI. The work reported here aimed to utilise the pheromone for managing cane midge.

To date, ten field studies have been conducted. Five experiments aimed at optimising the raspberry cane midge pheromone lure. Different pheromone dispensers and pheromone blends and release rates were evaluated. It was shown that the major component of the pheromone alone is highly attractive to raspberry cane midge males and that three minor components identified in previous work are not essential and did not significantly increase attractiveness. Rubber septa dispensers were shown to be satisfactory, with a life of at least one month in the field in the UK. The raspberry cane midge pheromone has two enantiomers (i.e. a left and right hand form). It was shown that the S enantiomer (left hand form) is the natural one but there was no evidence that the opposite enantiomer inhibits the attractancy of the natural enantiomer and,

crucially, a mixture of the two enantiomers, which is much less expensive to produce, is highly attractive. Lures containing 0.1 millionths of a gram of the pheromone were shown to be attractive but, interestingly, greatest catches occurred with lures containing 100 millionths of a gram. At higher doses the attractancy greatly decreased, an important finding which will be exploited in control strategies. For pest monitoring purposes, an optimal loading of 10 millionths of a gram was determined.

Based on work with the apple leaf midge, standard delta traps were considered to be most suitable for use by growers for pest monitoring. Standard 20 × 20 cm traps commonly used for monitoring codling and tortrix moths in orchards were adopted and proved satisfactory. However, an experiment was done comparing the attractancy of 6 different colours of trap (white, black, yellow, blue, red, green). The different colours caught similar numbers of raspberry cane midge, but the white, yellow and blue colours caught significantly greater numbers of non-target insects (flies, bees, etc.). Green and black traps are impractical.

- This finding suggests that red coloured traps would be preferable.

Monitoring raspberry cane midge

Using the standard lures and white delta traps, the seasonal temporal pattern and magnitudes of catches of cane midge in raspberry plantations subject to different management have been investigated. Traps were made available to 33 UK growers in a pre-commercial test in 2006. The traps have proved effective and easy to use for monitoring raspberry cane midge. Very large variation between plantations in total numbers caught has indicated plantations which are at risk with a high pest pressure. In polytunnels, first flight occurred much earlier than in field crops and much earlier than the date forecast by the ADAS temperature model. A nominal threshold of 30 midges per trap per week has been used but further analysis of the data is needed to determine if this needs adjusting.

Identifying host plant wound attractant of females

Work is just starting on this part of the project. A suitable site as reservoir of raspberry cane midge has been identified in Scotland and measures are in place to build up a population of midges for experimental work.

Control by disruption, mass trapping or lure and kill

A large-scale, multi-site field experiment was run from April-September 2006 in commercial raspberry plantations in SE and E England to evaluate the efficacy of a sex pheromone Mating Disruption (MD) treatment and an Attract and Kill (A&K) treatment in comparison with an untreated control for control of raspberry cane midge. For each treatment 2000 devices per ha were deployed. The MD and A&K treatments were effective outdoors but were ineffective in the polytunnel crops. Possible explanations for this difference in efficacy are that pheromone release was too rapid from the dispensers when they were deployed in the polytunnels where temperatures were much higher than outdoors and/or that the pheromone did not disperse so effectively in the enclosed polytunnel environment. A dispenser with a more uniform release rate over a longer period is being sought for further large scale field trials of these promising control methods in 2007.

Aphids

Raspberry aphid controlled by late season sprays of aphicides

A large-scale experiment was conducted in commercial plantations near Maidstone, Kent, to test different timings of autumn sprays of thiacloprid (Calypso) for the control of aphids, including small and large raspberry aphids. Single sprays of Calypso were applied to replicate plots of Glen Ample on 28 August, 8 September, 22 September, 6 October and 20 October 2005. Populations of aphids were assessed in winter (eggs) and spring, though this latter assessment could only be done on half of the trial plots as the others were lost due to the collapse of tunnels under a heavy fall of snow. All the Calypso spray timings greatly reduced populations of large raspberry aphids that developed the following spring but the best control was achieved with the spray on 6 October which reduced populations by 97%. Numbers of small raspberry aphid were too small to draw conclusions from the data. The trial is being repeated in autumn 2006.

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 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 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, 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.

A successful outcome to this project could potentially reduce losses in the current crop by 10%, yielding an additional £2M of UK sales. This will also enhance the competitiveness of UK raspberry growing. It could increase consumer confidence in raspberries. If the overall market grew by 20%, a further £5M of sales would result.

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

- It is too early to provide firm grower guidelines on practices to improve management of disease in raspberries; however, be aware that high volume sprays of potassium bicarbonate for mildew control may cause leaf margin browning and necrotic leaf spots on raspberry grown under protection.

- Raspberry cane midge sex pheromone traps are commercially available and should be used for pest monitoring and timing sprays.

- Good progress is being made in devising semiochemical control methods for raspberry beetle and cane midge.

- One trial showed that excellent control of raspberry aphids can be achieved by a post-harvest application of thiacloprid (Calypso), greatly reducing the need to spray for aphids in spring. The optimum time of application was late September – early October. Other products have not been tested but most good aphidicides are probably effective, though more persistent products are expected to give best results.

SCIENCE SECTION

Objective 1: *Botrytis* - to identify inoculum sources, examine the effects of environmental manipulation and the use of control agents

Task 1.1. Inoculum sources

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

Materials and methods

Experiments were conducted in a polythene tunnel at East Malling Research to determine the susceptibility of leaves to *Botrytis* infection in relation to leaf age and to identify the timing of infection of primocane leaves by *Botrytis*. The cultivar Glen Ample was used in this study. Plants were potted up in June 2005 and pruned in the 2005 winter. Leaves on a set of 36 canes were inoculated in June and a further 36 in September 2006, and subsequently sampled to assess the extent of infection by *Botrytis*. At the time of June and September inoculations, there were on average about 15 and 30 leaves on each cane, respectively. For a given inoculation experiment, the following experimental protocols were used:

1. All leaves on 36 randomly selected canes were inoculated with *Botrytis* conidial suspension in the tunnel (inoculation strength ca. 5×10^5 conidia per ml).

2. Following inoculation, overhead misters were switched on for 24 hours to maintain high humidity for infection to take place.

3. Four inoculated and three un-inoculated canes were sampled every 2 weeks to determine the *Botrytis* latent infections on leaves, petioles and primocanes.

4. Leaves and canes were paraquat-treated and incubated on wet blue paper in a gravel tray. The tray was covered with a wet polythene bag to prevent contamination. Individual leaf positions were marked with paper tag labels.

5. *Botrytis* development on individual leaflets, petioles and canes was then assessed 3-4 weeks later.

6. This sampling was done four times following each inoculation.

Incidence of *Botrytis* infection was summarised for leaves in the following four categories:

- young (top five fully unrolled leaves)
- mid-age (next five leaves)
- mature (next 10 leaves)
- old (remaining leaves)

Generalised linear modelling (GLM) was used to determine whether the incidence of infection of leaves by *Botrytis* was affected by leaf age.

Results

June inoculation

Leaves on 36 selected canes were inoculated on 15/06/06. Canes and leaves were sampled on 30/06, 14/07, 28/07, 11/08 and 25/08. No *Botrytis* lesions were observed on either leaves or canes for samples taken on the first four sample dates. For samples taken on 25/08, sporulating *Botrytis* colonies were seen only on 12 out of ca 180 inoculated leaflets. On the control samples taken on the same day, there were 10 leaflets with sporulating *Botrytis* out of ca. 135 un-inoculated leaflets. GLM analysis showed that the incidence of infection did not differ between the inoculated and the control samples, suggesting that the June inoculation did not result in an appreciable amount of infection.

September inoculation

Leaves on the 36 selected canes were inoculated on 01/09 and sampled on 15/09, 29/09, 13/10 and 27/10. Leaflets with sporulating lesions were frequently observed in both inoculated and the control samples. Overall the incidence of leaflets with *Botrytis* was significantly higher in the inoculated leaflets than in the control sample as shown by the GLM analysis (Table 1.1.1.1). Overall there were no significant differences in the incidence of leaflets with *Botrytis* lesions between the four leaf age groups. Disease incidence on samples taken on the 29/09 and 13/10 was significantly greater than on

the two earlier sampling dates (Table 1.1.1.1). Sclerotia were also observed but at a lower frequency except on the last sampling date for the control sample.

Table 1.1.1.1: Number of leaflets with sporulating colonies and sclerotia (number in the brackets) of *Botrytis cinerea*

Leaf age	Total leaflets	15/09/2006	29/09/2006	13/10/2006	27/10/2006
Control					
Young	45	0 (0)	15 (0)	17 (4)	13 (8)
Middle age	45	3 (0)	8 (0)	9 (5)	2 (10)
Mature	90	18 (7)	15 (0)	20 (5)	10 (23)
Old	90	18 (2)	25 (1)	11 (0)	6 (3)
Inoculated					
Young	60	18 (0)	33 (1)	20 (0)	21 (0)
Middle age	60	19 (1)	47 (1)	10 (1)	17 (3)
Mature	120	27 (0)	82 (1)	39 (2)	41 (6)
Old	120	18 (0)	99 (0)	19 (1)	52 (6)

Conclusions

Based on the data, we have drawn the following preliminary conclusions

1. Infection of leaves appeared not to be related to leaf age *per se*

2. Infection is critically influenced by the cane age

3. Infection was only possible on relatively old canes

Further inoculation experiments will be carried out in the coming season to confirm whether these conclusions are correct.

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 protected commercial unsprayed crops of cv. Glen Ample

Cane infection is believed to arise at the leaf nodes via mycelial growth down the petiole. Information on the period when this occurs should help to devise a rational treatment to prevent cane infection.

Materials and methods

Monitoring areas were marked out in tunnels in Cambridgeshire and Kent. At the Cambridge site these were 10 m lengths of row across all three rows of the tunnel. Fifteen whole leaves (usually with five leaflets per petiole) were sampled from each of top, middle and bottom positions from plants spaced throughout the tunnels across all three rows (and six faces) at each site. Sampling commenced in May in Kent and in June in Cambridge and continued at three-weekly intervals continued until August (Kent) or November (Cambridge).

The leaves were surface sterilised in sodium hypochlorite, rinsed in tap water, and paraquat-dipped (2.5% by volume of Gramoxone) for 1 minute before rinsing in tap water. The leaves were then spaced out, with the upper surface uppermost, on moistened paper in trays. The trays were covered in transparent polythene and incubated at room temperature in ambient light for 10 days. Senescent leaves collected at each sampling at the Kent site were damp incubated without paraquat treatment.

After incubation, fungal sporulation and non-sporulating mycelium were seen on the leaves and petioles; sclerotia also developed on some petioles sampled at the Kent site. Growth was identified after microscope examination where required. The presence of *Botrytis* was recorded separately for the leaflets and the petioles.

In addition, at both sites, on the same days as the leaf sampling, primocanes were examined for leaf and cane *Botrytis* on 30 tagged plants at positions throughout the tunnels. Leaves were examined for brown lesions *in situ*; samples were taken from non-tagged plants to check the cause of lesions. Once primocane lesions were seen, their height up the cane was recorded.

Results and discussion

Leaf and petiole infection

Neither site had a high incidence of *Botrytis* on the leaves and there did not appear to be any difference between infection at the different sample heights (Table 1.1.2.1). At the Cambridge site, the early season (June) samples had the most leaf blades with

Botrytis. The leaves did not have any lesions visible at picking. At the Kent site, there was a noticeable amount of petiole infection in late July, although it was absent from the topmost (youngest) leaves. Sclerotia did not develop on the leaf blades and petioles until late August. *Botrytis* was not found in leaves sampled in October. A high level of *Botrytis* was recorded in mature green leaves collected in May from the lowest position on the primocane.

Fusarium sp. was common on the leaves sampled at both sites, *Colletotrichum* sp. was recorded on leaves from Kent, and *Penicillium* sp. was sometimes common in samples from Cambs.

Table 1.1.2.1: Occurrence of *Botrytis* in primocane leaves according to height in the crop canopy, leaf colour and sample date - commercial crops, 2006

Sample date	No. green leaves (of 15) developing *Botrytis*						No. senescent leaves		
	Top		Mid		Bottom		Sample	Developing *Botrytis*	
	L	P	L	P	L	P	Total No.	L	P
Cambridge									
6 Jun	2	1	2	2	3	1	-	-	-
26 Jun	1	1	0	0	0	0	-	-	-
18 Jul	0	0	0	0	0	0	-	-	-
22 Aug	3	2	1	0	0	0	-	-	-
11 Sep	2	0	1	0	1	0	-	-	-
6 Oct	0	0	0	0	0	0	-	-	-
30 Oct	0	0	0	0	0	0	-	-	-
Kent									
22 May (46 leaves)	-	-	-	-	19	9			
4 Jul	0	0	1	0	0	0	37	0	1
24 Jul	0	0	0	8	0	5	83	4	6
25 Aug	0	1	0	3	3	3	45	2	4

L – lamina P - petiole

Primocane infection

At the Cambridge site, no brown lesions (which might be *Botrytis*) were seen on the leaves of the monitoring plants in the field. In mid-August a leaf, not on a monitored plant, which had snapped at the petiole base and remained attached, had a brown petiole with a brown lesion on the cane at the attachment point.

The first cane lesion seen, on 5 October 2006, was not on a monitored plant. The white "water-mark" lesion had black sclerotia under the epidermis. There were also ruptures in the epidermis which produced sporulation after incubation in a damp chamber.

Further sclerotia on primocanes were seen from 30 October on the monitored plants, within the whitened areas of “water-mark” lesions, which were up to 50 cm long. The lesions occurred at all heights (5 to 150 cm from the base), often radiating out from a leaf scar and so probably corresponding to the range of heights at which *Botrytis* occurred on the leaves. Possibly infection of canes, or the appearance of bleached cane symptoms, coincides with the period of epidermis maturation and browning.

Three primocane lesions were recorded on 30 October and a further three on 29 November. Primocane monitoring will be repeated in early March 2007, to record whether there is further manifestation of lesions after an extended period from leaf drop.

At the Kent site, no primocane lesions had occurred by February 2007.

Conclusions

- *Botrytis* was present at a low incidence in visibly healthy leaves and petioles on primocanes from June to August. The fungus was present at a higher incidence in attached senescent leaves in May

- *Botrytis* bleached lesions and sclerotia on primocanes were first seen in October

- These results suggest that primocane leaf infection can occur throughout the growing season, although canes may not become infected until October. Further work is required to determine if canes may become infected before October

1.1.3. Identify the start and duration of *Botrytis* sporulation on *Botrytis* cane lesions and other likely sources of *Botrytis* (weeds, crop debris)

Cane lesions

In early 2006, all of the plants in the monitoring areas of the unsprayed tunnel at both sites were examined closely for *Botrytis* sporulation, and tagged if either lesions or sclerotia were present. At the Kent site, a total of 14 fruiting canes with *Botrytis* were found and tagged, while at the Cambridge site nine affected canes were found and tagged out of about 200 plants assessed. At the Kent site, the sclerotia were behind a

large amount of leaf canopy on the laterals, whereas the grower at the Cambridge site maintained fewer canes.

At the Cambridge site, recording commenced in February before the crop was covered. It was only at the end of May, in the week after the polythene was put on, that sporulation was observed from the sclerotia (Table 1.1.3.1). The crop was at first open flower at this time and sclerotia on most canes sporulated. Sporulation was still occurring when recording ceased a fortnight after the end of harvest. For the 10 days leading up to 18 July, when no sporulation was observed, the tunnel air temperature had reached over 27°C, and this may have stopped the production of spores. Lesions, with the marks of concentric growth rings, were usually present on canes, but never sporulated, and became less distinct over time. A few canes had lesions without any sclerotia on or under the epidermis. Fruiting canes were cut off at the base on 8 August, and removed from the crop on 6 October.

At the Kent site, the sclerotia on only one cane sporulated, this was on 19 May. At this time, 25% of sclerotia in a nearby, uncovered, crop were observed to be sporulating. No sporulation was observed in two monitoring occasions in August.

Table 1.1.3.1: Monitoring of *Botrytis* sporulation on fruiting canes, Cambridge, 2006

Date	**Number of plants with *Botrytis* on a cane**	**Number of plants with sclerotia**	**Number of plants with sporulating sclerotia**	**Number of plants with lesions**	**Crop growth stage**	
20.02.06	7	4	0	6	Bare stems	
06.03.06	8	6	0	3		
21.03.06	8	5	0	4		
04.04.06	8	6	0	7	Leaf buds opening	
18.04.06	9	6	0	8	2-3 leaflets opening	Primocane 10 cm high
03.05.06	9	5	0	6	10cm laterals	
16.05.06	9	5	0	7	Flower buds	**Tunnel covered**
31.05.06	9	6	6	7	First open flowers	
06.06.06	9	4	1	5	30% open flower	
20.06.06	9	4	3	6		
03.07.06	9	4	4	5	50% fruit	
18.07.06	9	5	0	5		Very hot week
01.08.06	9	5	3	4		**Tunnel uncovered 26 July**

15.08.06	9	5	2	3	Old fruit picked off	Fruiting canes cut

Weeds and crop debris

Cane pruning debris, leaves and various species of weeds were examined for *Botrytis* sporulation. At the Cambridge site, samples were picked up at twenty positions along the length and between the rows of the tunnels. Samples were removed monthly for damp chambering to see if sporulation could be encouraged. The tunnel floor was bare earth with grass pathways between tunnels. There were no weeds to examine until mid April when thistle, groundsel, chickweed, dandelion, redshank, nightshade and bindweed started to appear. At the Kent site, dock, daisy, mayweed and willow herb were seen as well as groundsel and dandelion. No *Botrytis* sporulation was ever seen at either site on either weeds, leaf or cane debris examined between 20 Feb – 15 Aug (Cambridge) or 2 May – 25 Aug (Kent), either in the tunnel or after incubation of debris in the laboratory.

Conclusions

- Sporulation on *Botrytis* sclerotia on fruiting canes can occur from mid-May (when crops are usually at first open flower) through to at least mid-August

- High temperatures (>27ºC) appear to reduce the occurrence of sporulation

- Weeds and crop debris in the tunnels examined at two sites in 2006 were not a source of *B. cinerea* spores

1.1.4 Investigations on factors affecting sporulation of *Botrytis* sclerotia on raspberry canes

Materials and methods

Canes with sclerotia were obtained for the initial experiments from canes submitted from various plantations in the UK as part of the cane blight survey. These were randomised and divided amongst the treatments to avoid any effect of cane origin.

The canes were cut in to sections with about 4-5 sclerotia per section. The canes were surface sterilised by soaking in bleach (5 ml bleach in 45 ml water + wetter) for 4 mins, followed by rinsing in sterile water and blotting dry. A few sclerotia from the canes were picked off, cut in half and placed on Potato Dextrose Agar (PDA) to check viability after sterilising to ensure that the treatment has not affected viability. The remaining canes with sclerotia were divided into the following treatments:

1. Field incubation – The canes were placed in a net bag and hung outside on a post near the raspberry plantation.

2. 6 hrs wetting at 5°C. The canes were thoroughly wetted and then placed in a plastic lidded box on damp paper in an incubator at 5°C with lights. After 6 hrs the canes were removed from the incubator, dried and then returned to the damp plastic lidded box at 5°C.

3. 6 hrs wetting at 10°C. The canes were thoroughly wetted and then placed in a plastic lidded box on damp paper in an incubator at 10°C with lights. After 6hrs the canes were removed from the incubator, dried and then returned to the damp plastic lidded box at 10°C.

4. Damp incubation at 5°C. The canes were placed without prior wetting in a plastic lidded box on damp paper in incubator at 5°C.

The sclerotia were frequently inspected and assessed for signs of sporulation.

Results and discussion

The surface sterilisation treatment did not appear to have affected sclerotia viability as the treated sclerotia all produced colonies of *Botrytis* on PDA.
After 2 weeks incubation around 9-13% of sclerotia were sporulating on the sclerotia held at 5 or 10°C with little difference between treatments. Sporulation was particularly extensive on the sclerotia incubated at 10°C. There was no obvious sporulation on the sclerotia in field incubation. One month later the number of sclerotia sporulating in treatments 2 and 4 had fallen to 3-8% while that in treatment 3 had increased to more than 14%. Many shrivelled sclerotia were observed in all treatment. No sporulation was observed on the field-incubated sclerotia.

Conclusions

- It would appear that provided the sclerotia are wetted, or incubated in high humidity for 2 weeks, sporulation will occur
- The study will be repeated prior to setting up experiments to evaluate chemicals to suppress sclerotia sporulation.

1.1.5. Seasonal variation in airborne inoculum of *B. cinerea*

A new spore trap was tested during 2006. Instead of using conventional cellophane tape, this new spore trap uses small vials, which can be used directly for extracting fungal DNA for quantification.

The spore trap was installed in the field plot used for canopy manipulation trials to log number of spores. Several sample vials from the trap, together with several vials with known number of *Botrytis* spores, were sent to CSL to determine the feasibility of quantifying spores.

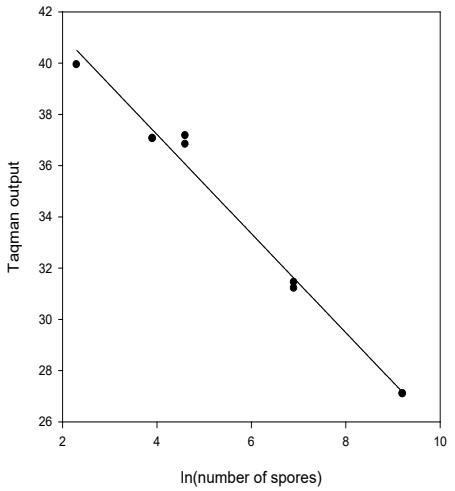


Fig. A Test results of new spore trap

The Taqman output is linearly related to natural logarithm of the number of spores (the figure on the right), indicating the method can be used to estimate the number of spores. In addition, we were able to extract fungal DNA from the field vials. Spore trapping will be undertaken in two commercial tunnel raspberry crops in 2007.

Task 1.2. Environmental manipulation

1.2.1. Occurrence of disease in commercial tunnel crops of raspberry

In 2004, a questionnaire was sent by HDC to levy-paying growers to record the occurrence and severity of *Botrytis* and other diseases on their holdings and details of their crop production system. Seven growers responded to questions about disease incidence and fungicide use in their 2004 crops. Rust was reported by five growers, cane *Botrytis* and fruit *Botrytis* was reported by four, but only two reported powdery mildew on the leaves. Three had seen virus symptoms. All growers said they had cane blight, but this may have been spur blight that was misdiagnosed. Five growers used

fungicides before covering their crops, and four of these also sprayed the covered crop. From two to four fungicide applications were made pre-harvest. Three growers made post-harvest applications, and these were additional to pre-harvest sprays.

In 2005, 11 growers from England and Scotland replied to the survey (Appendix 1) on protected summer fruiting raspberries, most growing both cvs Glen Ample and Tulameen, and three also growing Octavia. All crops were in Spanish tunnels, covered with clear polythene except at two sites where Luminance THB was preferred. Tunnels were 7 to 8 m wide, and varied between 100 to 200 m long, with a length of 250 m at one site. All growers adjusted ventilation in the tunnels by pushing the polythene up the sides of the tunnel, with six growers doing this based on temperature, and the remainder also considering relative humidity. There was bare soil in the pathways at six sites, the others having grass. Five growers left the soil within the rows bare; the others used black polythene mulch.

Plants were spaced 2 to 2.5 m apart between the rows. In-row spacing at planting varied widely, ranging from 0.3 m to 0.7 m. Primocane retention also varied, with 6 – 8 canes per metre being the most common, while four growers selected either 10 or 12 canes, and one grower kept up to 15 canes per metre of row. Growers removed their primocanes in either April/May or May/June, all but one grower selecting thick and/or medium canes, rather than thin. Only one grower removed the lower leaves of primocanes during the growing season.

Cutting and removing fruiting canes was carried out between September and October by four growers, with four others continuing to either December or January. Two growers started earlier, and had completed the work by September. All but one grower pulverised their prunings *in situ* rather than removing them from the tunnel. Growers differed in when they tied in their new fruiting canes. Three growers carried out the work from after harvest in July, to September, four others tied-in between October and December, and the other four between December and February. Most growers tipped their fruiting canes in either February or March, mainly to a final height of 1.8m (6 ft).

Some information was obtained on fungicide use, but not all details were supplied. Five of the growers used Elvaron Multi before covering, and the same number applied this chemical post-harvest. Rovral WP was used by three growers on the covered crop and eight used Teldor. Two growers used Potassium carbonate before covering, and another applied it twice after covering. All growers used an air-assisted sprayer. A

range of water volumes from 225 to 1200 L/ha was used, with around 400 L/ha being the most common.

Growers were asked about the disease problems in an established tunnel crop in 2005. Nine growers saw slight cane *Botrytis*, the others having a greater level. Five growers saw no fruit *Botrytis*, although three had slight and two had moderate fruit infection. There was no agreement as to whether cane *Botrytis* was worse in a covered or an uncovered crop, but fruit *Botrytis* was seen more in uncovered crops. Only two growers had slight powdery mildew on their fruit and leaves, most had none, and they had seen no difference between uncovered and covered crops. One grower commented that *Botrytis* and powdery mildew was only a problem on the leg rows as these do not get sprayed directly. Some growers used ventilation to reduce humidity and control disease. Fungicide spray timing and water volume were important.

All growers had rust on their crops, with seven having a slight infection and four a moderate infection. Some growers had seen more on their uncovered crops, but others had more on their covered. Five growers had slight cane blight, three had a moderate infection, but two had none on their crops. Slight virus infection was seen by five growers, one had a moderate infection, but four had none.

1.2.2. Assessments of latent *Botrytis* infection of unripe fruit as a measure of likely fruit rot at harvest

The objective of this task is to examine whether information on the occurrence of latent *Botrytis* in unripe fruit can be used to help predict the quality of ripe fruit, specifically the risk of post-harvest *Botrytis* development.

Materials and methods

Crop sampling

Sampling was carried out on growers' holdings, collecting from both protected and outdoor crops across the UK, with differing levels of pesticide inputs. Half of the crops were of cv. Glen Ample and the other half were of cv. Tulameen. Information on crop husbandry was gathered from each site. A total of 20 crops were sampled, half of each of the two cultivars being from the south-east of England (taken by EMR), and the other half from elsewhere in the UK (taken by ADAS). Each crop was sampled twice, initially

to obtain unripe fruit and then to collect ripe fruit. Part of each sample was tested for latent *Botrytis* immediately using an incubation test; part was frozen pending a molecular test to quantify *B. cinerea* DNA to be carried out by CSL.

Test for latent *Botrytis* in unripe fruit

At the first sampling, two weeks after the start of commercial harvesting, 100 lateral branches were cut from the canes in each crop. This was to obtain a colour range of unripe fruit (green, yellow and pink). Where possible, 100 fruit of each colour were cut off the branches and stored for molecular testing. Only yellow fruit were used in visual assessments of latent *Botrytis* using the incubation test, with 100 fruit cut off behind the calyx per crop sample. Berries for this test were surface sterilised and placed in transparent plastic lidded trays containing agar with paraquat and chloramphenicol incorporated. The trays were exposed to a diurnal light regime at 20°C for up to 10 days when the fungi present on each fruit were recorded.

Test for latent *Botrytis* in ripe fruit

The second samples were taken from the same crop rows two weeks after the first, selecting 100 firm red marketable fruit. The fruit was examined on arrival for *Botrytis*, but none was found. Fruit was transferred either to multicell trays (EMR) or new punnets (ADAS), leaving a space between each fruit. The fruit was then sealed in a transparent polythene bag and exposed to a diurnal light regime at 20°C for 7 days. At Day 7, as for the unripe fruit, the different fungi present on each fruit were counted, together with a count of the number of healthy fruit.

Results and discussion

Crops sampled

A summary of the 20 crops sampled is given in Table 1.2.2.1. Site details of the ADAS-sampled crops are given in Appendix 2.

Table 1.2.2.1: Summary of raspberry crops sampled for latent *Botrytis* in fruit 2005

Cropping season	Covering	Fungicide sprays	ADAS		EMR	
			GA	Tul	GA	Tul
Early	Covered	Minimal	1	0	0	0
Main	Covered	Standard	3	2	4	**3**
Main	Uncovered	Standard	1	1	3	**1**
Main	Uncovered	Organic	1*	1	1	**0**

*Glen Ample (GA), not available, Glen Magna substituted. Tulameen (Tul)

Occurrence of latent *Botrytis* in unripe and ripe fruit (ADAS sampled sites)

Botrytis grew from both the unripe and ripe fruit, without sporulating, and isolations were made to confirm its identity. There were large differences between crop samples in the number of fruit with *Botrytis*, particularly for the unripe fruit (Table 1.2.2.2). Many samples showed a high incidence (>50%) of infected fruit. Crop identification numbers relate to picking sequence. There was no correlation between the percentage of rotted fruit and the sampling date, although sampling for unripe fruit extended between 14 June and 13 July 2005, and ripe fruit between 21 June and 27 July 2005

Although there was a range of intervals, from 5 to 34 days, between the last fungicide application and the picking of the unripe fruit, there was no obvious correlation between harvest interval and latent *Botrytis* incidence. There was no indication of a difference in cultivar susceptibility. The highest proportion of unripe fruit affected by *Botrytis* was from an unsprayed crop, although other crops were as badly affected when ripe fruit were sampled. The *Botrytis* incidence did not differ according to whether or not the crop was covered. The Scottish fruit (Crop 9) had low *Botrytis* in both the unripe and ripe fruit. This crop had a similar number of fungicide applications to other crops, but Hortiphos (liquid calcium and phosphorus) was added at each application, with Hortiphyte also added at the penultimate spray before harvest.

Table 1.2.2.2: Details of site factors possibly associated with the occurrence of latent *Botrytis* in fruit

Crop No.	County	Site *	Path Cover	Pre-sample fungicide product	Unripe harvest interval	% *Botrytis* unripe Day 10	Ripe harvest interval	% *Botrytis* Ripe Day 7
1	Bucks	G	Soil	Rubigan	30	24	37	44
2	Cambridge	T	Soil	Teldor	23	51	30	65
3	Cambridge	T	Soil	Teldor	23	61	30	63
4	Norfolk	T	Soil	Teldor	34	17	41	94
5	Hereford	T	Grass	Teldor	16	21	23	68
6	Cambridge	O	Grass	None	n.a.	86	n.a.	74
7	Oxon	O	Grass	Teldor	7	57	15	84
8	Bucks	O	Soil	Thianosan	23	10	28	46
9	Scotland	T	Soil	Teldor	5	0	18	40
10	Devon	O	Grass	None	n.a.	4	n.a.	94

*Glasshouse, Tunnel or Open

Occurrence of other fungi (ADAS sampled sites)

Penicillium grew from the surface-sterilised unripe fruit of some crops, with the glasshouse crop having the highest proportion of fruit infected, and more tunnel than open-field crops having significant levels (Table 1.2.2.3). Most of the unripe fruit became covered in a non-sporulating brilliant white mycelium (not *Botrytis*).

None of the unripe fruit (Table 1.2.2.3) from the two organic crops (6 and 10) was fungus-free (without *Botrytis*, *Penicillium* and/or the unidentified fungus), but few ripe fruit had any *Penicillium* in contrast to over 50% of the fruit in most other (non-organic) crops (Table 1.2.2.4). *Mucor or Rhizopus* was not found in unripe fruit, but was a particular problem in the ripe (not surface sterilised) fruit of the glasshouse crop (Crop 1) and the outdoor sprayed crop (Crop 7). Crop 7 had a row base cover of green waste as mulch, rather than the black plastic or bare soil found in the other crops sampled. The ripe fruit from all the crops had hardly any healthy fruit after the 7 day shelf life test at 20°C, most fruit having at least one fungus on it (Table 1.2.2.4).

Table 1.2.2.3: Occurrence of fungi other than *Botrytis* on raspberry fruit harvested unripe (yellow fruit stage), and the number without any fungus

Variety	Crop No.	Number of fruit				
		Total Day 10-14	Healthy	Penicillium	Mucor/ Rhizopus	Other (not *Botrytis*)
Glen Ample	1	107	8	69	0	60
Glen Ample	2	99	1	45	0	37
Tulameen	3	99	0	28	0	32
Tulameen	4	99	31	0	0	57
Glen Ample	5	99	48	10	0	38
Tulameen	6	100	0	1	0	72
Tulameen	7	100	26	1	0	36
Glen Ample	8	100	37	35	0	29
Glen Ample	9	55	45	0	0	10
Glen Magna	10	100	0	2	0	99

Table 1.2.2.4: Occurrence of diseases other than *Botrytis* on raspberry fruit harvested ripe (firm red stage)

Variety	Crop No.	Number of fruit				
		Total Day 7	Healthy	Penicillium	Mucor /Rhizopus	Other (not *Botrytis*)
Glen Ample	1	72*	0	62	7+28	9
Glen Ample	2	100	4	52	0	7
Tulameen	3	100	7	62	0	5
Tulameen	4	100	0	89	5	2
Glen Ample	5	100	3	83	0	2

Tulameen	6	99	1	5	1	61
Tulameen	7	75	4	39	0+25	1
Glen Ample	8	92	0	91	4	8
Glen Ample	9	100	0	100	12	41
Glen Magna	10	100	0	5	0	48

*+ Fruit lost to Rhizopus before any other fungi could be assessed

Occurrence of latent *Botrytis* in unripe and ripe fruit (East Malling sampled sites)

Samples from Kent showed no difference in cultivar susceptibility. The unsprayed, open crop had the highest proportion of ripe fruit with *Botrytis* after 7 days incubation, with 90% infected compared with a range from 45% to 69% for the other four open field crops (Table 1.2.2.5). All but one of the covered, sprayed, crops had less *Botrytis*-infected ripe fruit than the open-field crops, with a range from 2% to 21%. The surface-sterilised yellow fruit had a higher *Botrytis* incidence than the subsequently sampled ripe fruit. The differences in incidence attributed to site factors for the ripe fruit, were not apparent for the yellow. The incidence of *Botrytis* in the yellow fruit was not consistently correlated with the incidence in the ripe fruit.

Table 1.2.2.5: Details of site factors possibly associated with the occurrence of latent *Botrytis* in green/yellow fruit, and post-harvest after 7 days incubation of ripe fruit. Kent plantations 2005

Crop Sample	Variety	Type	Spray	% *Botrytis* infected fruit	
				Latent	Post-harvest
R61/05	Tulameen	Covered - French tunnel	Spray	34	2
R62/05	Glen Ample	Covered - French tunnel	Spray	67	21
R63/05	Glen Ample	Open	Spray	54	56
R69/05	Tulameen	Covered - Rain sheets from late April	Spray	99	50
R70/05	Glen Ample	Covered - Rain sheets from late April	Spray	92	21
R71/05	Glen Ample	Open	Spray	99	64
R72/05	Tulameen	Covered - Rain sheets from late May	Spray	92	29
R73/05	Glen Ample	Open	Spray	76	45
R74/05	Glen Ample	Covered - from June just prior to harvest	Spray	46	4
R75/05	Glen Ample	Covered - from April pre-flowering	Spray	82	16
R76/05	Tulameen	Open	Spray	76	69
R96/05	Glen Ample	Open	None	84	90

Occurrence of other fungi (East Malling sampled sites)

All of the samples of the surface sterilised yellow fruit had *Penicillium*, with six of the crops having more than 25% of the fruit infected (Table 1.2.2.6). The open field,

sprayed crops had a lower *Penicillium* incidence than all but one of the covered crops. In contrast to the yellow fruit, only 8%, or less, of ripe fruit from the sprayed crops had *Penicillium*, and the fruit from the unsprayed crop had only 13% infection (Table 1.2.2.7).

Cladosporium occurred on half of the yellow fruit samples, but was absent from all the fruit harvested from open field crops. More crops were affected when ripe fruit was sampled, with a maximum incidence of 27%, with the unsprayed crop remaining free of *Cladosporium.*

Mucor was only found on the yellow fruit of the unsprayed crop, and was present on a few fruit in six of the red fruit samples.

Table 1.2.2.6: Occurrence of *Botrytis* and other fungi in yellow fruit from Kent crops, 2005

Variety	Crop No. R/05	Number of fruit						
		Total	Healthy	*Botrytis*	Peni-cillium	Mucor	Clado-sporium	Other
Tulameen	61	100	4	34	60	0	32	18
Glen Ample	62	100	0	67	44	0	35	25
Glen Ample	63	100	1	54	14	0	0	40
Tulameen	69	100	0	99	51	0	0	8
Glen Ample	70	100	2	92	11	0	0	10
Glen Ample	71	98	0	97	2	0	0	0
Tulameen	72	100	0	92	81	0	3	0
Glen Ample	73	100	2	76	10	0	0	40
Glen Ample	74	99	0	45	26	0	17	20
Glen Ample	75	100	1	82	15	0	2	44
Tulameen	76	100	0	76	23	0	0	22
Glen Ample	96	100	0	84	48	16	0	1

Table 1.2.2.7: Occurrence of *Botrytis* and other fungi in ripe fruit from Kent crops, 2005

Variety	Crop No. R/05	Number of fruit						
		Total	Healthy	*Botrytis*	Peni-cillium	Mucor	Clado-sporium	Other
Tulameen	61	106	98	3	2	1	0	0
Glen Ample	62	104	53	22	7	1	21	0
Glen Ample	63	100	33	56	1	0	6	0
Tulameen	69	104	32	52	1	0	11	0
Glen Ample	70	100	51	21	8	0	21	0
Glen Ample	71	100	25	64	2	0	5	0
Tulameen	72	100	42	29	1	2	27	0
Glen Ample	73	100	40	45	2	5	9	0
Glen Ample	74	100	95	4	1	0	0	0
Glen Ample	75	101	82	16	0	0	3	0
Tulameen	76	100	23	69	6	1	15	0
Glen Ample	96	104	0	94	13	9	0	2

Quantification of latent *Botrytis cinerea* by a molecular test (PCR)

Parts of the samples of unripe fruit collected in 2005 for detection of latent *Botrytis* by the paraquat treatment and incubation test, were frozen on receipt. Quantification of latent *B. cinerea* by a PCR test is in progress at CSL. Results will be compared with data on the incidence of *B. cinerea* recorded in unripe and ripe fruit from these crops.

Discussion

High levels of *Botrytis* were found in either or both the yellow and the red fruit from all crops - sprayed as well as unsprayed. There was no consistent correlation between the incidence of *Botrytis* in the yellow fruit and that in the fruit at the time of normal ripe fruit harvest. The samples of red fruit were probably seldom from the same flowering stage as the yellow (samples were taken a fortnight apart) and there may be different levels of inoculum and infection success during the flowering period.

There were no differences between cvs Glen Ample and Tulameen in susceptibility to *Botrytis*. Open-field sites in Kent had more ripe fruit with *Botrytis*, but this was not found in the ADAS-sampled plantations. Two of the three unsprayed crops showed higher levels of *Botrytis* than sprayed crops. *Penicillium*, *Cladosporium* and *Mucor* were found on most samples, and would have been more easily recorded where the fruit surface was not covered by *Botrytis* mycelium. Most crops had *Penicillium* infected fruit, with some having a high proportion of fruit affected. ADAS-samples showed infection of the ripe fruit as well as most of the yellow fruit samples, whereas in the Kent samples *Penicillium* mainly affected the yellow fruit. Open-field, sprayed crops tended to have less *Penicillium* on yellow fruit than tunnel crops.

Conclusions

- Latent *Botrytis* was detected in unripe (yellow) fruit, with large difference between crops and many samples having over 50% of fruit infected

- No association was found between sampling date, interval since the last *Botrytis* fungicide spray, variety, and incidence of latent *Botrytis* in unripe fruit

- The incidence of *Botrytis* in ripe fruit did not appear to relate to the incidence in unripe fruit. Levels of *Botrytis* were higher in ripe fruit than unripe fruit in 11/20 samples

- High levels of *Penicillium* developed on most samples of fruit, except for samples from two organic crops

1.2.3 and 4.2.2: Crop canopy pruning for control of *Botrytis* and powdery mildew

Materials and methods

The experiments were conducted on two commercial sites in protected crops of cv. Glen Ample. Both crops were covered by Spanish tunnels using the same type of polythene (Luminance THB), supplied by Visqueen, BPI Agri Ltd. The experimental procedure followed at the sites differed due to the nature of the plantation management at the two sites.

Site 1, Kent

Site 1 was located at Salmans Farm, Penshurst, Kent, on an established one-year-old crop of Glen Ample. The crop was covered in March and, in addition, the crop rows were covered with fleece within the tunnel. The tunnel contained three rows of raspberries, with almost a continuous row of fruiting cane, resulting in a thick canopy increasing in density as the crop progressed. Primocane was removed mechanically once in early May from all the rows. Plots, either canopy manipulated or standard were established on 8 May as 7.2 m lengths in all three rows. Blocks were separated from adjacent blocks by 7.2 m row length. Each treatment was replicated three times in a randomised block design (Fig 1). On 12 May up to four leaves were removed from the fruiting laterals on canes in the canopy manipulation plots, starting at the lowest leaf on the lateral and continuing up to the first leaf with a flower bud present in the axil. Temperature and RH sensors were hung in the centre row of each plot at three heights (low, mid and top) in the canopy. On 4 July, in the canopy manipulation plots, the primocanes were thinned in all three rows to three primocanes per stool or six to eight primocanes per 1m row length. No primocanes or leaves were removed from the standard plots.

Site 2, Cambridge

Site 2 was located at Sunclose Farm, Milton, Cambridgeshire, on an established mature plantation of Glen Ample. The tunnel contained three rows of raspberries which had been thinned to stools of six fruiting canes, well separated from adjacent stools, resulting in an open canopy in contrast to that at site 1. All raspberry rows were treated with herbicide in April to eliminate the first flush of primocane. Plots were established in April, either standard (T1) or canopy manipulated (T2), each treatment replicated three times in a randomised block design as at site 1. Leaves were not thinned on fruiting laterals at this site. On 1 May, 28 June, 21 July, and 5 October, primocanes were thinned to six primocanes per plant in T1 plots and to four primocanes per plant in T2, with 2.5 plants per metre. Temperature and RH sensors were placed in the plots as at site 1.

Assessments

At each visit, the primocanes in the centre row of each plot were assessed for incidence of *Botrytis* and powdery mildew. At approximately three-week intervals from the time manipulation treatments were first applied, random samples of 20 fully expanded leaves (or single leaflets from the Cambridge site) were taken from the bottom, middle and top tier of leaves from the centre 3 metres of the middle row of each plot. The incidence of latent *Botrytis* was assessed on the leaves following treatment with paraquat and damp incubation under lights.

In early June and July, a random sample of 50 green/yellow fruit were taken from the centre 3 metres of the middle row of each plot and the incidence of latent *Botrytis* assessed after two weeks incubation under lights on paraquat agar.

On three occasions in June, July and/or August 50 to 100 red fruit were collected at random from the centre 3 metres of the middle row of each plot. In addition a random sample of red fruit was also collected from a raspberry crop (conventional fungicide programme) in an adjacent tunnel and from the unsprayed uncovered crop at the tunnel end (Fig. 1). The fruit were incubated under high humidity and the incidence of *Botrytis* and other rots assessed after 7 days.

The incidence of *Botrytis* and other diseases on the canes was assessed post-harvest in October or November and in February.

Results and discussion

Site 1, Kent

After the leaf removal in May there were obvious visual differences between treated and untreated plots. However, a week after treatment the crop was covered in large mesh plastic netting used to support the fruiting laterals which compressed the crop such that there were no longer obvious differences in canopy density. No differences in relative humidity between the plots were noted on the RH sensors in the crop. The humidity in the treated plots was reduced following the spawn thinning in July, but only in plot 6 which was located near the open end of the tunnel (Fig. 1), suggesting that this was due to the improved air circulation at the tunnel end. No differences in humidity were noted in the plots located away from the tunnel end.

No *Botrytis* or powdery mildew was observed in the plots. No *Botrytis* was found in leaf samples taken in June and July. *Botrytis* was found in leaf samples taken in August (Table 1.2.3.1) but there was no obvious pattern relating *Botrytis* incidence to canopy manipulation treatment. No *Botrytis* was observed on primocanes in any of the plots. The incidence of *Botrytis* rot in fruit sampled from the plots varied from 12 to more than 80% (Table 1.2.3.2), but there was no obvious pattern relating *Botrytis* incidence to treatment. The highest incidence of *Botrytis* was in fruit from the uncovered crop (81-97%) and the lowest incidence in fruit from the sprayed covered crop (36.7%).

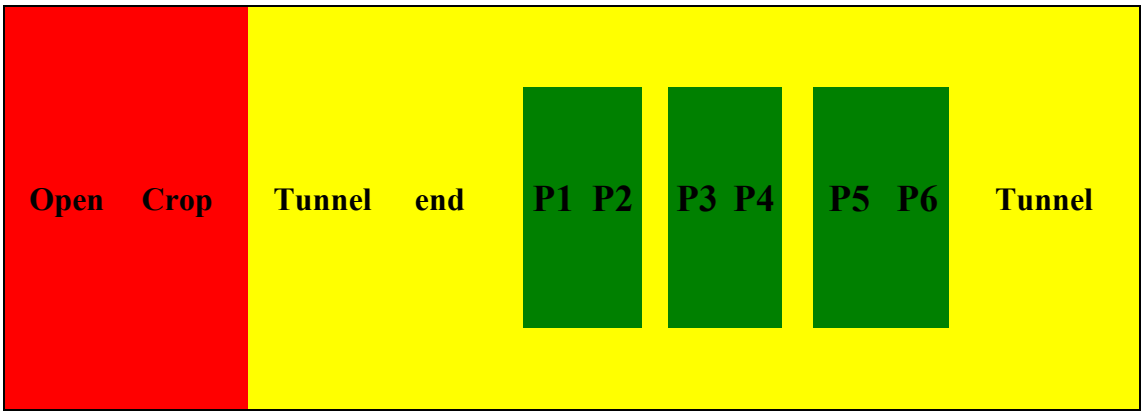


Figure 1: Plot layout at Site 1 – Salmans Farm

Site 2, Cambridge

The temperature and RH sensors failed to function correctly so no records of temperature and humidity are available for the site.

No powdery mildew was observed in the crop. No *Botrytis* was found in leaf samples taken in June and July. No *Botrytis* was observed on primocanes until the end of October when lesions were observed on a total of three canes in the control plots with

the higher cane density. The incidence of *Botrytis* rot in fruit sampled from the plots varied from below 30% to more than 90% infected fruit (Table 1.2.3.3). There were no obvious large differences between treatments in the incidence of *Botrytis* fruit rot. As at Site 1, the highest incidence of *Botrytis* fruit rot was in fruit from the unsprayed uncovered crop (97-100%) and the lowest in fruit from the covered sprayed crop (16-68%).

Conclusions

- At site 1, despite the removal of large numbers of leaves or primocanes from the plots there was no impact on humidity except in plots nearest the open end of the tunnel where there was more air circulation. Plot design will be reviewed and the experiment repeated in 2007
- Despite a high incidence of *Botrytis* fruit rot the incidence of the fungus on leaves and primocanes was negligible at both sites
- There was no obvious relationship between treatment and *Botrytis* incidence on the fruit
- *Botrytis* incidence in fruit was greatest in unsprayed uncovered crops and least in sprayed covered crops
- No powdery mildew was observed at either site

Table 1.2.3.1: Percentage leaf lamina and petioles with *Botrytis* in raspberry leaf samples taken from primocanes in a tunnel crop cv. Glen Ample at Penshurst, Kent, on 25 August 2006

Sample position	Treatment	*Botrytis* on lamina	*Botrytis* on petioles	Sclerotia on petioles
Bottom	Environment manipulated	0	0	3
	Normal	3	6	14
Middle	Environment manipulated	0	0	13
	Normal	0	0	11
Top	Environment manipulated	4	15	24
	Normal	0	0	28

Table 1.2.3.2: Percentage fruit rots in various raspberry samples taken from tunnelled crop cv. Glen Ample at Penshurst, Kent, (Site 1) in 2006. Green or yellow fruit samples were incubated on paraquat agar; red fruit samples were assessed after 7 days damp incubation at ambient temperature

Sample	Environment manipulated Unsprayed				Normal Unsprayed			
	Bot	Pen	Mucor	Clad	Bot	Pen	Mucor	Clad

Green fruit, 13 Jun	83	44	2	27	59	26	2	55
Red fruit, 28 June	53	4	33	17	51	7	30	12
Yellow fruit, 4 July	58	0	34	95	69	0	4	96
Red fruit, 14 July	13	0	40	0	19	0	32	0
Red fruit, 4 August	66	21	22	0	68	33	28	0

Table 1.2.3.3: Percentage fruit rots in various raspberry samples taken from tunnelled crop cv. Glen Ample at Milton, Cambridge, (Site 2) in 2006. Yellow fruit samples were incubated on paraquat agar; red fruit samples were assessed after 7 days damp incubation at ambient temperature

Sample	Environment Manipulated Unsprayed				Normal Unsprayed			
	Bot	**Pen**	**Other**	**Clad**	**Bot**	**Pen**	**Other**	**Clad**
Yellow fruit, 26 June	93	32	0	0	91	37	0	0
Yellow fruit, 11 July	68	24	6	0	68	27	6	0
Yellow fruit, 21 July	58	59	6	0	66	62	4	0
Red fruit, 27 June	89	22	8	0	93	17	17	0
Red fruit, 11 July	81	41	3	0	85	49	1	0
Red fruit, 24 July	27	23	13	0	36	27	12	0

Task 1.3. Control agents

The objective of this task is to determine the relative efficacy of a range of fungicides and natural products for control of *Botrytis* on raspberry.

1.3.3. Glasshouse and field evaluation of natural products and commodity substances for control of *Botrytis*

Experiment 1 - Cambridge, 2005

Materials and methods

Commencing on 3 October 2005, the efficacy of various products in controlling *Botrytis* on leaves and canes was evaluated in a replicated trial on potted raspberry plants, cv. Glen Ample, in a polytunnel in Cambs. There were two pots in each plot, each with one to four, non-woody, primocanes. Fungicide products with known efficacy against *Botrytis* (Teldor, Talat, Rovral and Scala) were selected for comparison, together with Signum (approved for protected strawberries, but not raspberries), Frupica SC (approved for strawberries, but not raspberries) and a new fungicide product (HDCF5). The commodity substance potassium bicarbonate plus wetter, permitted as a fungicide on raspberries (principally used against powdery mildew), and the natural products

Milsana plus wetter and Orosorb (which may increase plant resistance to *Botrytis*), were also tested. Sprays were applied at 1000 L/ha. All fungicide products were applied four times, spraying at 14 day intervals, and the commodity and natural products were applied weekly to give seven applications. Spray treatments commenced before inoculation, on 5 October 2005, of the four lowest leaves on each of the canes. Care was taken only to inoculate the leaf blades, (not the cane), so that any subsequent cane infection might be traced back through the petioles. *Botrytis cinerea* was applied at 10^6 spores/ml, using a mixture of isolates from raspberry cane infections collected from four different holdings. Any phytotoxicity on the whole plant was recorded and described, together with the number of inoculated leaves remaining and whether they had necrosis.

Plants were assessed for *Botrytis* at intervals. Canes sections were harvested in June 2006 (as floricanes) to include the axillary buds of all four inoculated leaves (about 18 cm long). Sections of the same length were also taken from higher up the canes (40 cm from the ground) to provide an uninoculated area for comparison with the inoculated. The cane sections were incubated in damp conditions and then assessed for the presence or absence of sporulating *Botrytis* or black *Botrytis sclerotia* on or under the epidermis.

Results and discussion

By the middle of November, the warm autumn had delayed leaf senescence and only three leaves in the trial had visible *Botrytis*. (Table 1.3.3.2). Phytotoxicity was only found with two products, with most of the leaves sprayed with Scala having chlorosis between the lateral veins and some down-rolling, and potassium bicarbonate with wetter caused leaf margins to become dark brown. Leaf drop of the inoculated leaves was increased to varying extents by some products. Although 87% of the inoculated leaves were present on the control plants in November, both Signum and potassium bicarbonate treated plants had lost almost half of the original number. There was no significant difference between treatments in the number of inoculated leaves with necrotic patches (without *Botrytis* sporulation and of unknown cause). Although Signum had the lowest proportion of attached inoculated leaves with necrosis, many leaves in this treatment had dropped already. All the fungicides left some spray deposit on the leaves, but neither potassium bicarbonate, Milsana nor Orosorb left any residues.

Fourteen days after the seventh fungicide application date, the same lower leaves were re-inoculated to try to improve the incidence of visible *Botrytis*. This re-inoculation on 22 November 2005 was followed by an application of all spray treatments on the 28 November and the 12 December. Products previously applied at 7-day intervals were also applied on the 7 and 19 December to give a total of eleven applications for these products with recommendations for frequent application. Most leaves were still present in early December, with a similar number of inoculated leaves present as in November, and some plants were still producing new leaves from the top of the canes.

By early December, a total of eight leaves in the whole trial had *Botrytis* sporulation, and this was insufficient for analysis. It is possible that *Botrytis* may enter the leaves without creating a lesion, and remain latent until it reaches the cane. Leaves dried on the plant and leaflets dropped in mid-January 2006 usually leaving the petiole in place on the cane.

Table 1.3.3.2: Proportion of inoculated lower leaves still present in November 2005 and showing necrotic areas and occurrence of phytotoxicity and residues on the whole plant

Product	Dose rate per ha at 1000 L water/ha	% of inoculated leaves remaining	% of inoculated leaves with necrosis	Mean Phytotoxicity index (0-5	Mean residue index (0-3)
Control	N/a	87	26	0.0	0
Teldor	1.5 kg/ha	90	39	0.1	0.8
Talat	3.0 kg/ha	64	36	0.2	1.5
Rovral WP	1.5 kg/ha	77	28	0.1	2.2
Scala	2.0 kg/ha	72	34	4.1	1.5
Signum	1.8 kg/ha	54	15	0.1	1.0
Frupica SC	0.68 L/ha	81	35	0.1	1.5
HDCF5	1.44 kg/ha	82	38	0.4	2.0
Pot.bicarb.	5.0 g/L	51	42	2.9	0.0
Milsana	5.0 ml/L	85	33	0.0	0.0
Orosorb	2.0 ml/L	79	48	0.6	0.0
Significance		P=0.004	P=0.583	P=0.001	P<0.001

By January, no visible *Botrytis* (either sporulation, sclerotia or bleached lesions) was seen on the leaf-inoculated canes, and once the epidermis of the canes ripened it was not possible to distinguish any diseased necrotic tissue from natural cane browning.

On the cane sections examined in June 2006 after damp incubation, several treatments gave a significant reduction in the incidence of cane *Botrytis* (Table 1.3.3.3). Frupica SC resulted in canes with no *Botrytis*, and Rovral WP, Scala and Teldor resulted in 10% of canes, or less, becoming infected. Signum had caused increased drop of the inoculated leaves, but did not seem to have stopped *Botrytis* entering the canes as there was no reduction in cane *Botrytis*. It is possible that prematurely senescing Signum treated leaves provided more opportunity for fungal colonisation. The natural product, Milsana, gave comparable control to the approved raspberry fungicides. Potassium bicarbonate gave a slight reduction in cane *Botrytis*, but there had been severe phytotoxicity in the crop with leaf margin blackening and premature leaf drop. Orosorb was ineffective and some leaves had margin browning.

Some of the upper canes had *Botrytis*, and this was not related to whether *Botrytis* was present lower down the same cane. Natural infection of the upper canes probably occurred. This may have arisen via the primocane leaves, which were still partially green and attached in December. There were no significant differences between treatments, but plants sprayed with Talat, Rovral WP, Frupica SC and Milsana had no upper cane *Botrytis*, compared with the 20% of canes infected in the unsprayed plots.

Conclusions

- Spray programme of Frupica, Scala, Teldor and Rovral WP reduced the incidence of cane *Botrytis* from 49% to less than 10%. Milsana and Talat were slightly less effective

- Spray programme of Orosorb and Signum did not reduce cane *Botrytis*

- Scala and potassium bicarbonate at the rates and frequencies used were phytotoxic to raspberry leaves

Table 1.3.3.3: Proportion of canes sampled in June 2006 which produced *Botrytis* sporulation (lower cane sections include the nodes from inoculated leaves)

Product	**% of lower cane sections (leaves inoculated) with *Botrytis***	**% of upper cane sections (leaves uninoculated) with *Botrytis***
Control	49	20
Teldor	6	11
Talat	13	0
Rovral WP	10	0
Scala	8	10
Signum	58	27
Frupica SC	0	0
HDCF5	34	49
Pot.bicarb.	24	15
Milsana	13	0
Orosorb	47	12
Significance	P=0.009	P=0.192

Experiment 2 - Cambridge, 2006

Materials and Methods

A polytunnel trial using cv. Glen Ample was set up in Cambridge to determine the efficacy of selected fungicides, natural products and commodity substances as programmes of three preventative sprays for the control of *Botrytis*. The control of *Botrytis* on the fruit is achieved by preventing infection of the flowers. The usual commercial spray timings of 5-15% flowering, 40-50% flowering and 70-75% flowering were used, with an additional earlier timing, at green bud, for one product (Talat). Control was assessed on the leaves, flowers and fruit of raspberry floricanes, including the sampling of yellow fruit for latent *Botrytis*. By tagging open flowers at the time of the fungicide applications and monitoring the development of the fruit, both the single unripe and three ripe fruit samples had a known fungicide application history. Any fungicidal effect on cane diseases on the developing primocanes will be recorded in 2007.

The tunnel was 72 m long and covered with Luminance TSB polythene within days of the first spray at green bud. All three rows were used in the trial, but only the inner faces of the outer rows and one face of the central row were treated. Plots were 5 m long and the central 3 m was assessed for disease and phytotoxicity. Chemical treatments had four replicates and there were two control treatments to give eight replicates. Other than the fungicide applications, the crop was maintained as normal commercial practice. Fruit not required for samples was picked by the grower (and destroyed) to prevent fruit rotting on the crop.

A number of products with full or specific off-label approval were selected to compare their efficacy, together with three natural products with potential efficacy against *Botrytis* (Table 1.3.3.4). All products were applied as fine droplets at 1000 L/ha, with a pressure-assisted knapsack sprayer using a vertically held 2-m boom. Applications were made on the 16 May, 30 May, 9 June and 19 June 2006.

Table 1.3.3.4: Fungicide and natural products evaluated on a tunnel crop of raspberry - Cambridge, 2006 (Experiment 2)

Product	Active ingredients	Rate /ha	Rate based on
1. Control	Water	-	-
2. Teldor	Fenhexamid (50% w/w)	1.5 kg/ha	Approval on raspberries.

			1 day harvest interval.
3. Talat	Fenhexamid (16.7% w/w) + Tolylfluanid (33.3% w/w)	3 kg/ha	Approval on raspberries. 14 day harvest interval.
4. Rovral WP	Iprodione (50% w/w)	1.5 kg/ha	Approval on raspberries. Minimum 10 day interval. 7 day harvest interval.
5. Scala	Pyrimethanil (400 g/L)	2 L/ha	SOLA 0544/04 on protected raspberries. 1 day harvest interval.
6. Folicur	Tebuconazole (250 g/L)	0.8 L/ha	SOLA 0897/05 on raspberries for cane blight. 14 day harvest interval.
7. Amistar	Azoxystrobin (250 g/L)	1 L/ha	SOLA 1194/05 on protected raspberries for powdery mildew. Max. 2 sprays. AEA for 3 applications, with fruit to be destroyed.
8. Talat (4 spray)	Fenhexamid (16.7% w/w) + Tolylfluanid (33.3% w/w)	3 kg/ha	Approval on raspberries. Maximum 4 sprays. 14 day harvest interval.
9. Horti-phyte Plus	Phosphorus, Citrus extract, 40 essential herbal oils	6 ml/L	Soft fruit fertiliser label rate of 3 - 6 ml/L
10. Calcium nitrate	Calcium nitrate	3.5 g/L	EMR strawberry rate at 500 L/ha.
11. Orosorb	Orange oil Surfactants Borax	2 ml / L	Label rate protected crops. 0 day harvest interval.

Red fruit was sampled on the 29 June, 7 July and 24 July 2006, taking 100 fruit per plot to assess for visible disease. Out of these, 50 apparently healthy fruit per plot were incubated in a multicell tray (one berry per cell) sealed in a polythene bag for 10 days. The numbers of fruit with *Botrytis* or other fungi including Penicillium, Mucor or Rhizopus were counted. Red fruit were taken corresponding to the flowers present at each of the three sprays given to all plots, however yellow fruit were only sampled on the 5 July and these would have been flowers at the 40-50% open flower stage. Yellow fruit was surface sterilised with 5% by volume 'Domestos' bleach, rinsed and then incubated in sealed transparent containers for 4 to 7 days on agar containing paraquat and chloramphenicol. Only 50 yellow fruit were taken per plot, to leave sufficient fruit of the same development stage to be picked as ripe fruit.

Results

No *Botrytis* leaf or flower infection was seen during the trial, and no visible *Botrytis* occurred on the fruit hanging in the crop. No other diseases developed in the crop up to the time of the final harvest.

Marketability

Most of the 100 ripe fruit picked to assess marketability prior to the shelf-life tests were satisfactory. The percentage marketable fruit at each of the three picks were 81-94%, 96-98% and 78-99% respectively. There were no significant differences between treatments in the incidence of marketable fruit, spotted fruit, fruit with shrunken drupes or crumbly fruit (fruit with loosely arranged oversized drupes) in any of the three picks. Possibly crumbly fruit and shrunken drupes might have been *Botrytis*-related, but because *Botrytis* often developed after incubation even in apparently healthy fruit, it was not possible to clarify this. There was an indication that Amistar and Orosorb application caused an increased number of crumbly fruit, because these treatments had a consistently high proportion of affected fruit. Occasionally, receptacle plugs had split in half causing the drupes to mature differently on either half, but this was not a treatment affect.

Latent *Botrytis* and other fungi on yellow and red fruit

Fruit samples from first spray applications to flowers

The red fruit developing from the flowers receiving the first fungicide applications were incubated indoors in four replicate stacks of trays arranged in plot order. At assessment, it was found that the upper layers of the stack had been affected by the extreme hot weather during their period of incubation. A covariate analysis was done to take account of the position of the plot sample in the stack, and there was shown to be a significant effect of position for all the recorded values. The adjusted values for each plot are shown in the table, and show significant differences between treatments for the *Botrytis* incidence and proportion of healthy fruit, whereas this was not shown using the unadjusted records.

Plots treated with Amistar, both Talat timings, and Scala, all had significantly fewer red fruit with *Botrytis* than the untreated (Table 1.3.3.5). When all fungi were included in the assessment, Amistar and Talat gave the cleanest fruit.

Table 1.3.3.5: Occurrence of *Botrytis* and other fungi in a shelf-life test on red fruit picked 29 June 2006, Cambridge (Experiment 2). Fruit sprayed at 5-15% flowering (1st spray) on 30 May 2006

Product	% with *Botrytis*	% with *Penicillium*	% with white fungus	% without fungus
1. Control water	40.6	20.3	9.7	40.4
2. Teldor	31.4	15.7	12.0	51.3
3. Talat x3	16.5	14.3	9.1	65.8
4. Rovral WP	33.3	23.4	20.3	41.2
5. Scala	25.4	28.5	11.6	47.3
6. Folicur	28.2	23.4	14.6	46.8
7. Amistar	22.4	13.1	6.8	63.3
8. Talat x4	18.8	11.6	7.6	67.3
9. Hortiphyte Plus	35.7	19.0	13.4	39.6
10. Calcium nitrate	45.0	15.1	26.4	34.8
11. Orosorb	45.3	24.9	9.1	43.1
Significance	P=0.003	P=0.205	P=0.373	P<0.001
LSD (d.f. 33)	13.64	11.51	14.06	14.35

Fruit samples from second spray applications to flowers

Over half of the yellow fruit had latent *Botrytis* in the untreated plots, and this was significantly reduced following Amistar, Folicur, Scala or the three applications of Talat (Table 1.3.3.6). In the best treatment (Amistar), only 15% of fruit were infected. Other fungi were also present in the fruit, and these were not affected significantly by the fungicide treatments. Only 18% of the untreated fruit were free from any fungal growth. The Amistar and two Talat treatments produced the highest proportion of healthy fruit (over 45%). However, although Scala treated plots had a low *Botrytis* incidence, the fruit became mouldy with other fungi. Less than 15% of fruit from flowers treated with either calcium nitrate or Orosorb were fungus-free, but more fruit from Horiphyte Plus treated plots were healthy.

When the fruit in the crop ripened 3 days later and were picked for incubation, there were still significant differences between the treatments in the proportions with *Botrytis* and without fungus (Table 1.3.3.7). However, a greater proportion of all the fruit had *Botrytis*, with seven of the treatments having similar levels to the untreated plots (84% affected). Talat (three applications) was still one of the best *Botrytis* reduction treatments, and Amistar treated plots were still less affected than the untreated, but the difference was much less than seen on yellow fruit. Four applications of Talat were no more effective than the three applications. Both Talat treatments were particularly effective in reducing the proportion of fruit affected by all fungi. Amistar was the only other product to have more healthy fruit than the untreated plots. The red fruit was not

surface sterilised and so air-borne spores on the fruit surface could have germinated during the incubation and increased the proportion of mouldy fruit.

Table 1.3.3.6: Occurrence of *Botrytis* and other fungi in a shelf-life test on yellow fruit picked 5 July 2006, Cambridge (Experiment 2). Fruit sprayed as flowers at 40-50% flowering (2nd spray) on 9 June 2006

Product	% with *Botrytis*	% with *Penicillium*	% with white fungus	% without fungus
1. Control water	55.3	20.1	14.9	18.1
2. Teldor	37.1	19.1	7.5	37.8
3. Talat x3	18.1	18.0	6.5	59.4
4. Rovral WP	34.4	22.8	10.2	35.2
5. Scala	29.0	35.5	16.5	24.5
6. Folicur	24.0	24.5	13.0	41.0
7. Amistar	15.0	28.0	11.5	46.5
8. Talat x4	37.0	16.0	4.5	48.0
9. Hortiphyte Plus	46.0	11.0	4.5	40.5
10. Calcium nitrate	56.5	24.5	13.5	12.0
11. Orosorb	64.2	28.2	12.1	7.0
Significance	P<0.001	P=0.178	P=0.091	P=0.01
LSD (d.f. 34)	20.21	13.68	7.97	24.73

Table 1.3.3.7: Occurrence of *Botrytis* and other fungi in a shelf-life test on red fruit picked 5 July 2006, Cambridge (Experiment 2). Fruit sprayed as flowers at 40-50% flowering (2nd spray) on 9 June 2006

Product	% with *Botrytis*	% with *Penicillium*	% with white fungus	% without fungus
1. Control water	83.5	15.6	6.8	7.7
2. Teldor	80.0	18.5	8.0	10.0
3. Talat x3	38.5	15.0	12.5	34.5
4. Rovral WP	74.7	16.4	11.8	9.9
5. Scala	77.5	30.5	10.0	7.0
6. Folicur	84.5	18.0	9.0	7.5
7. Amistar	67.5	15.0	5.0	18.5
8. Talat x4	50.5	23.0	12.5	27.0
9. Hortiphyte Plus	84.0	12.0	9.0	7.5
10. Calcium nitrate	81.0	14.5	10.0	6.0
11. Orosorb	89.0	17.5	7.9	4.0
Significance	P<0.001	P=0.205	P=0.204	P<0.001
LSD (d.f. 34)	10.68	10.59	5.18	9.11

Fruit samples from third spray applications to flowers

At the third assessment, there was found to be a decrease in the % of fruit per plot with *Botrytis* depending on row position (Table 1.3.3.8). The western row had a mean of 53% of fruit affected, the centre row (sprayed and assessed on the western face) had 38%, and the eastern row had only a mean 20% affected. It was possible that there was less *Botrytis* where the plants received a longer period of bright sunlight, the

western-most row being more shaded. During the week before picking it was very sunny with exceptionally high temperatures (up to 43°C inside the tunnel).

A GLM analysis was used which produced predicted means that were adjusted to allow for the different distribution of the four replicates of each treatment across each row, followed by an accumulated analysis of variance. Taking out the effect of the rows halved the unexplained variability and produced significant differences between treatments. Amistar and Folicur treatments gave least fruit with *Botrytis*. They also had the least *Penicillium*, which contributed to their overall greater proportion of 56% healthy fruit. The Talat and Scala treatments were not as effective as they had been for previous samples in controlling *Botrytis* on the fruit.

Table 1.3.3.8: Occurrence of *Botrytis* and other fungi in a shelf-life test on red fruit picked 24 July 2006, Cambridge (Experiment 2). Fruit sprayed as flowers at 70-75% flowering (3rd spray) on 19 June 2006

Product	**% with *Botrytis***	**% with *Penicillium***	**% with white fungus**	**% without fungus**
1. Control water	36.4	18.4	12.2	42.2
2. Teldor	45.7	27.3	11.5	30.3
3. Talat x3	33.5	17.0	13.9	44.8
4. Rovral WP	46.8	22.2	7.9	34.7
5. Scala	32.9	17.0	16.0	38.5
6. Folicur	21.2	9.5	15.6	56.1
7. Amistar	29.0	10.0	11.9	55.8
8. Talat x4	34.9	31.0	15.5	31.5
9. Hortiphyte Plus	49.9	17.0	12.0	35.0
10. Calcium nitrate	35.7	17.6	10.0	44.1
11. Orosorb	43.4	23.0	17.0	30.5
Significance	P=0.051	P=0.059	P=0.683	P=0.036

Phytotoxicity

No phytotoxicity was seen after the first spray applications on 30 May. After the second applications, some interveinal chlorosis and margin yellowing was seen on the leaves. There was both marked treatment-related chlorosis, and a less distinct, interveinal chlorosis throughout the tunnel; the latter was considered to be caused by a mineral deficiency.

At the first assessment on 19 June (Table 1.3.3.9), plants treated with Scala had very distinct yellowing across the leaf surfaces; by the end of July only the tissue along the veins was green. Plants treated with Folicur had less marked interveinal chlorosis, but

also yellow areas around the leaf margins, especially towards the apex, which became necrotic in places.

At the second assessment on 3 July (Table 1.3.3.10) in addition to the leaf browning and/or chlorosis recorded after Scala and Folicur treatments, three of the Hortiphyte Plus treated plots had brown or yellow leaf margins. At this time, some of the yellow fruit across the trial was mis-shaped and the incidence of this on the plots was recorded as a potentially phytotoxicity (Table 1.3.3.10). Some Folicur treated plots had fruit with shrunken drupes, and the Scala treated plots had fruit with the two halves at a different maturity (as a result of a split receptacle plug). Some Orosorb treated fruit had large drupes. The plots were, however, variable.

Only some treatments left spray residue. It was particularly noticeable in the Rovral WP treated plots, where many leaves were spattered with a white deposit (index 3), although both Teldor and Talat left specks of chemical on the leaves.

Table 1.3.3.9: Occurrence of leaf phytotoxicity and residues in Experiment 2, assessed 16 June 2006 after spray applications on 30 May and 9 June

Product	Leaf phytotoxicity index (0-5)	Residue (0-3)
1.Control water	0.1	0.0
2. Teldor	0.8	1.5
3. Talat x3	0.2	1.0
4. Rovral WP	0.2	3.0
5. Scala	2.8	0.2
6. Folicur	1.8	0.0
7. Amistar	0.2	0.0
8. Talat x4	0.0	1.0
9. Hortiphyte Plus	0.0	0.0
10. Calcium nitrate	0.8	0.0
11. Orosorb	0.2	0.2
Significance	P<0.001	P<0.001
LSD (d.f. 34)	1.03	0.44

Table 1.3.3.10: Occurrence of leaf phytotoxicity and residues in Experiment 2, assessed 3 July 2006 after last spray application on 19 June

Product	Leaf Browning (0-5)	Inter-veinal chlorosis (0-3)	Residue (0-3)	Immature fruit deformity (0-5)
1. Control water	0.1	1.1	0.0	0.4
2. Teldor	0.2	1.2	0.5	0.8
3. Talat x3	0.0	1.2	1.0	1.8
4. Rovral WP	0.0	0.5	2.0	0.0
5. Scala	0.0	3.0	0.0	1.0
6. Folicur	1.8	1.5	0.0	1.5
7. Amistar	0.2	1.2	0.2	0.0

8. Talat x4	0.5	1.2	0.8	0.0
9. Hortiphyte Plus	1.2	1.0	0.2	0.0
10. Calcium nitrate	0.8	1.5	0.2	0.2
11. Orosorb	0.0	1.2	0.0	1.0
Significance	P<0.001	P<0.001	P<0.001	P=0.053
LSD (d.f. 34)	0.71	0.59	0.58	1.10

Conclusions

- Amistar, Folicur, Scala and Talat were all more effective than the other products at reducing latent *Botrytis*, although the results varied between the samples. The remaining products gave no significant control. Both Scala and Folicur caused phytotoxicity on the leaves

- The ripe fruit had particularly high levels of *Botrytis* at the second pick, in early July, with 84% in untreated plots, reduced to 39% by Talat. Ripe fruit infection on samples picked in late June and late July had around 40% of untreated fruit infected by *Botrytis*, with the best treatments still developing infection on about 20% of the fruit

- In early July, the *Botrytis* infection was much greater on ripe fruit than on surface sterilised yellow fruit picked three days earlier, and it is possible some infection may have developed from external contamination rather than latent internal *Botrytis* originating from flower infection

Experiment 3 - Kent, 2006

Materials and methods

The trial was established in an open-field plantation of raspberry cv Glen Ample planted as long canes in 2005 and left unsprayed and cropped in 2005 to establish *Botrytis* inoculum in the crop. In 2006, the treatments (Table 1.3.3.11) were applied to plots using CP15 knapsack sprayer at 1000L/ha on three occasions (9 June, 16 June and 28 June). All treatments were replicated four times in a randomised block design. Crop development was very variable. At start of spraying the growth varied from early flower to early green fruit on different plants within the same row. Plants at early flower at the time of the first spray were labelled and picking started when the labelled fruit were red. Prior to this the plots were cleared of all ripe fruit.

Plots were regularly inspected for *Botrytis* and powdery mildew and any other diseases. At harvest a random sample of two punnets 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 tests. The fruit were placed in individual modules in trays and damp incubated. Rot incidence was assessed after seven days incubation at ambient temperature.

A sample of green fruit was taken from each plot in July and incubated on paraquat agar under lights to check for latent *Botrytis* infection in the fruit.

The incidence of cane diseases in the plots will be assessed in March 2007.

Table 1.3.3.11: Treatments applied to open-field raspberries in 2006, East Malling Research, Kent, 2006 (Experiment 3)

Product	Active ingredient	Product rate
1. Untreated	-	-
2. Wetcit	Alcohol ethoxylate	200 ml / 100 L spray
3. Farmfos	Potassium phosphite	6 ml / L
4. Hortiphyte Plus	Potassium phosphite + other nutrients	6 ml / L
5. Farmfos + Wetcit	Potassium phosphate + Alcohol ethoxylate	6 ml / L + 200 ml / 100 L spray
6. Calcium chloride flake	Calcium chloride	8 g / L
7. UKA 379	Experimental	1.44 kg/ha
8. UKA 374	Experimental	0.4 kg/ ha
9. Talat	Tolylfluanid + fenhexamid	3 kg/ha
10. Signum	Pyraclostrobin + boscalid	1.8 kg / ha
11. Switch	Cyprodonil + fludioxonil	1.0 kg / ha
12. Talat (50%) + Wetcit	Tolylfluanid + fenhexamid + Alcohol ethoxylate	1.5 kg / ha + 200 ml/100 L spray

Results and discussion

The incidence of *Botrytis* in fruit at harvest was negligible (<1%) (Table 1.3.3.12). There was a low incidence of cane spot infection on leaves and fruit (Table 1.3.3.13), but no obvious differences between treatments. No powdery mildew was observed on any of the plots or guard rows

In post-harvest tests, more than 50% of the fruit from untreated plots at the first pick (Table 1.3.3.13) developed *Botrytis*. All fungicide treatments (UKA379, UKA374, Talat,

Signum and Switch) reduced the incidence of *Botrytis* by 75%. None of the other chemicals evaluated, including Talat at half-label recommended dose with Wetcit, reduced the incidence of *Botrytis*. The incidence of *Botrytis* in fruit from picks 2 and 3 was too low for meaningful comparisons to be made, most likely because weather conditions at flowering were dry and not favourable for *Botrytis* infection. In fruit from pick 4, the lowest incidence of *Botrytis* was recorded in fruit from plots treated with Hortiphyte plus or Talat. However, the incidence of *Botrytis* varied considerably between plots within treatments so it is unlikely that these differences are significant.

The incidence of *Botrytis* in green fruit samples (Table 1.3.3.12) varied from 14 to 36% infected fruit. The incidence between plots varied considerably and there were no consistent differences between treatments.

Table 1.3.3.12: Percentage raspberry fruit infected with *Botrytis* and possible cane spot at harvest following treatment with various chemicals in open-field Glen Ample at East Malling in 2006 (Experiment 3)

Treatment	**% *Botrytis* rotted fruit**			**% possible cane spot infected fruit**		
	12 July	**19 July**	**26 July**	**12 July**	**19 July**	**26 July**
1. Untreated	0	0.2	1.6	3.6	2.5	2.0
2. Wetcit	0	0.2	0.4	0.9	1.5	1.4
3. Farmfos	0.2	0.3	0.2	2.8	2.4	2.6
4. Hortiphyte	0.5	0.3	0.3	2.3	2.9	2.1
5. Farmfos + Wetcit	0.4	0.2	0.3	1.5	2.6	1.8
6. Calcium chloride	0.1	0.5	0.4	1.5	3.2	2.2
7. UKA379	0.2	0.3	0.4	1.9	2.6	2.8
8. UKA374	0	0.4	0.6	1.6	2.2	2.1
9. Talat	0	0.1	0.3	2.8	2.8	2.2
10. Signum	0	0	0	1.4	1.1	1.4
11. Switch	0.5	0.6	0.6	1.2	2.1	1.9
12. Talat (50%) +Wetcit	0	0.2	0.4	2.1	2.1	2.2

Table 1.3.3.13: Percentage *Botrytis*-rotted fruit in post-harvest tests on raspberries harvested from plots treated in 2006 with various chemicals at East Malling Research, Kent (Experiment 3)

Treatment	% *Botrytis* rotted fruit				
	Pick 1 13 Jul	Pick 2 19 Jul	Pick 3 26 Jul	Pick 4 31 Jul	Green fruit on PCA, 26 Jul
1. Untreated	56.5	3.0	3.6	15.1	30.4
2. Wetcit	66.0	4.8	1.4	22.2	26.9
3. Farmfos	58.8	3.5	6.8	18.1	29.3
4. Hortiphyte	51.8	1.8	2.0	5.4	14.9
5. Farmfos + Wetcit	64.0	5.8	10.2	21.1	37.1
6. Calcium chloride	47.5	1.8	5.5	30.0	31.6
7. UKA379	12.0	1.8	1.2	11.1	21.9
8. UKA374	9.0	2.5	1.8	10.9	14.7
9. Talat	11.3	3.3	3.1	7.7	18.8
10. Signum	7.5	3.3	6.0	21.4	28.4
11. Switch	9.3	3.5	9.6	24.2	34.9
12. Talat (50%) +Wetcit	44.5	3.5	8.5	30.1	35.8

Conclusions

- The weather conditions during most of the flowering period were not favourable for *Botrytis* infection
- The fungicides applied at the label recommended dose were most effective in reducing *Botrytis* rot in post-harvest tests
- None of the other chemicals evaluated were effective in reducing *Botrytis* except possibly Hortiphyte Plus, which requires further evaluation
- The trial will be repeated in 2007 paying particular attention to evaluating fungicide programmes in combination with nutrient products such as Hortiphyte Plus

Objective 2. Raspberry beetle

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

Tasks 2.1.1- 2.1.3. – Establish experimental sites, develop and produce lures and traps and compare trap designs in laboratory and field-testing

Introduction

The objective of these tasks was to prepare sites for future raspberry beetle trials at both SCRI and at EMR and conduct trials to optimise raspberry beetle trap design and lure efficiency. Much of this can be done under open-field conditions prior to subsequent testing in commercial raspberry tunnels. Previous work at SCRI, both as core-funded and EU-CRAFT 'RACER-project' used white sticky traps developed in Switzerland to monitor raspberry beetle numbers in open-field plantations in the UK, Switzerland and Finland. Concurrently, the active flower volatiles were developed at SCRI as attractants using simple prototype lures under SEERAD funding and subsequently developed under HDC Studentship (CP14).

Methods and materials

Sites:

Three sites were prepared for use at SCRI, Invergowrie, Dundee for raspberry beetle research in 2005 and 2006. Two were established open-field sites (F4 & F6 (OS ref: NO 337 297)) and a small experimental tunnel area established (E8 (OS ref: NO 334 297)) was planted in 2005. Sites F4 and F6 are multi-cultivar replicated experimental sites that have been used for previous entomological research, including raspberry beetle and raspberry aphid epidemiology and maintained without insecticide use for several years. They were maintained without insecticide use to provide large numbers of raspberry beetles for assessing the efficiency of traps and lures. Site E8 was planted in 2005 using two high health raspberry cultivars, Glen Ample and Glen Rosa under Haygrove Spanish tunnels and have been maintained using insecticides and irrigation in their establishment phase. Because the plants originated as plant modules, their first fruiting year will be in 2007.

Meteorological data:

Meteorological data was collected from the SCRI AgroMet site located ca. 600 m north east of field observation sites. Weekly temperature mean, maximum, minimum temperature, precipitation and solar radiation for the duration of the experiment were recorded.

Statistical analysis and advice:

All field experimental design and analysis is done in consultation with staff from BioSS (Dundee). Data was analysed using 'GenStat Release 8.2 (PC/Windows XP)' and ANOVA done. Where required the data may be transformed before analysis, usually by a log transformation. Where this is done it will be referred to in the text.

With the laboratory trials, the data is collected in an Excel spreadsheet and mean evaporation rates are calculated. The evaporation rate is then calculated as the daily loss of product over a variable number of days (mg^{-d}).

2005

Comparison of trap types

Location:
F4, SCRI, Invergowrie, Dundee DD2 5DA

Raspberry Cultivar:
Glen Clova

Start date and duration: 1
8 May 2005 for 4 weeks

Trap Type:
Two types of trap were tested in 2005, the standard vertical white sticky trap (AgriSense BCS Ltd Product BC2245 (http://www.agrisense.co.uk/_attachments/Documents/CDTDBC2245En(vertical_white_sticky_trap).pdf.)) and two prototypes based on AgriSense Green Funnel Trap (Product BC255501 (http://www.agrisense.co.uk/_attachments/Documents/CDMSBC25501En(green_funnel_trap).pdf.) The first was green funnel trap with white Correx cross-vanes and the second was a similar design, but with 12 mm PTFE strips (DuPont Teflon PTFE Threadseal Tape 12mm × 0.075mm ×12m) added to the intersection points to reduce the grip so adult beetles would drop more readily into the funnel.

Figure 2: Prototype raspberry beetle funnel trap with Correx cross-vanes and standard thick-walled vial

Lure Type:

Thick-walled plastic vials containing Compound B was used on all three trap types.

Data collected:

The numbers of raspberry beetles, other coleopteran, bees and other insects were recorded in each trap over the 4-week experimental period.

Evaporation rate tests (2005) for different dispenser (lure) types (Laboratory)

Location:

Laboratory CG05

Laboratory fume cupboard at 15 to 18ºC

Start and duration:

25 May 2005 for 15 weeks

Lure type:

Standard thick-walled dispenser and experimental thin-walled dispenser, both with Compound B. Five replicated were hung in a fume cupboard in a constant air-flow.

Weight loss was recorded at 3, 6 and 9 weeks to four decimal places using a calibrated Mettler AE100 balance.

Experimental design:
Fully randomised design with five replicates per treatment.

2006

After the problems with the thin-walled lures in 2005, further discussions with AgriSense resulted in lures based on heat-sealed sachets. Details of these and other lures used in field and laboratory studies at SCRI in 2006 are shown in Table 1.

Table 2.1.1.1: Lures were supplied by AgriSense for experiments in 2006 at SCRI

Active Ingredient	Dispenser Type	Volume	Expt. number
Compound B	Thick-walled	2.5 ml	2, 3, 4, 5
Compound A	Thick-walled	2.5 ml	2, 4, 5
Compound B	Sachet (rpw-rpw*) (Double-sided)	2.0 ml	5
Compound A	Sachet (rpw-rpw*) (Double-sided)	2.0 ml	5
Compound B	Sachet (rpw-foil**) (Single-sided)	2.0 ml	2, 3, 5
Compound A	Sachet (rpw-foil**) (Single-sided)	2.0 ml	5
Empty	Thick-walled	-	Controls
Empty	Sachet (rpw-rpw*) (Double-sided)	-	Controls
Empty	Sachet (rpw-foil*) (single-sided)	-	Controls

*RPW-RPW – permeable membrane on both sides - faster releasing
**RPW-foil – permeable membrane on one side, non-permeable foil on second side – slower release

Comparison of vial and sachet dispensers for Compound B using white sticky traps

Location:

F4, SCRI

Raspberry cultivar:

Glen Clova

Start date and duration:

16 May for 2 weeks

Trap Type:

Standard white sticky traps (AgriSense BCS Ltd Product BC2245) were the only trap type used in this assessment

Experimental design:

Randomised block design with eight replicates per treatment

Data recorded:

Numbers of raspberry beetles, flies (Diptera), beetles other than raspberry beetle (Coleoptera) and other insects.

Lure Type:
Two types of lure were tested, a thick-walled plastic vial and an experimental thin-walled vial (both supplied by AgriSense) using compound Compounds A or B (four treatments in total).

Comparison of dispenser type (lure) using Compound B and funnels traps

Location:
F4, SCRI

Raspberry Cultivar:
Glen Ample

Start date and duration:
5 June for 2 weeks

Trap Type:
Prototype based on AgriSense Green Funnel Trap (Product BC255501) with white Correx cross-vanes

Lure Types:
Thick walled dispenser and single-sided slow release sachet (rpw-foil) both with Compound B

Experimental design:
Randomised block design with six replicates per treatment

Data recorded:
The numbers of raspberry beetles caught each week were recorded over the two week test period.

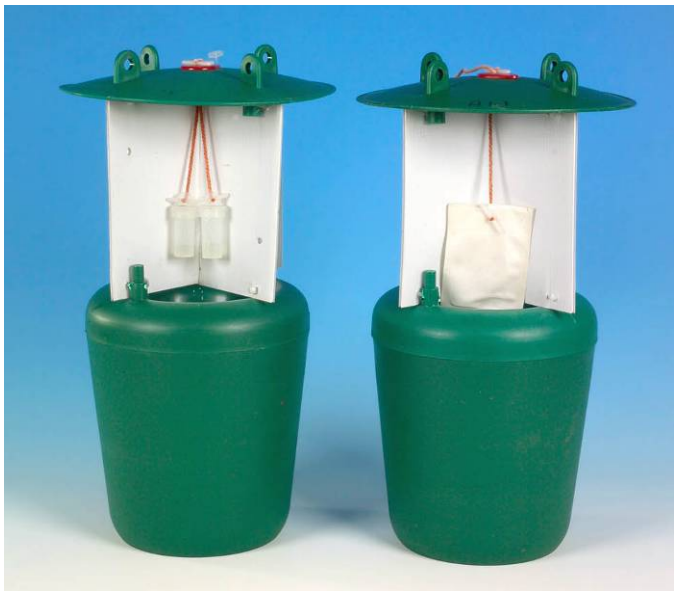


Figure 3: Prototype raspberry beetle funnel traps showing the two types of dispenser, standard thick-walled vial (left) and an experimental sachet (right)

Comparison of single or combined attractants using Compounds A and B

Location:

F4, SCRI

Raspberry Cultivar:

Malling Jewel

Start date and duration:

25 May for 2 weeks

Trap Type:

Standard white sticky traps (AgriSense BCS Ltd Product BC2245)

Lure Type:

Thick walled dispenser with either two dispensers of Compound A (AA), two dispensers of Compound B (BB) or one of each of Compound A and B (AB)

Experimental design:

Randomised block design with eight replicates per treatment

Data recorded:

The numbers of raspberry beetles caught in each sticky trap was recorded over the 2 week experimental period.

Evaporation rate tests (2006) for different dispenser (lure) types

Location:

Laboratory CG05, Laboratory fume cupboard at 15 to 22ºC

Start and duration:

25 May 2005 for 15 weeks

Lure Type:

Standard thick-walled dispenser, single-side sachet and double-sided sachet (see Table 1); both with Compound A and Compound B. Six replicated were hung in a fume cupboard in a constant air-flow. Weight loss was recorded weekly to four decimal places using a calibrated Mettler AE100 balance.

Experimental design:

Fully randomised design with six replicates per treatment

Data collected:

Lure weights were recorded weekly and evaporation rates (mg/day) calculated.

Results

Task 2.1. Developing sites

Existing open-field sites at SCRI (F4 and F6) were identified and assessed for use in 2005 and 2006. In 2005, a small tunnel site was prepared and planted (E8) using industry standard Spanish tunnels (funded by SCRI). This comprises six 20m × 3 row tunnels separated by additional polyethylene skirts of two raspberry cultivars, Glen Ample and Glen Rosa and will be available for use in 2007.

Task 2.1.2. Development and production of lures and traps

In both 2005 and 2006, a range of dispensers (lures) were provided by AgriSense (See experiment 1A/2005, experiment 1B/2005 and Table 1) for testing both in the field and laboratory.

In 2005, the two prototype traps based on AgriSense Green Funnel traps were tested against the standard white sticky trap in an open-field site (Experiment 1A/2005). The numbers of different insect in each of the categories is shown in Figure 4.

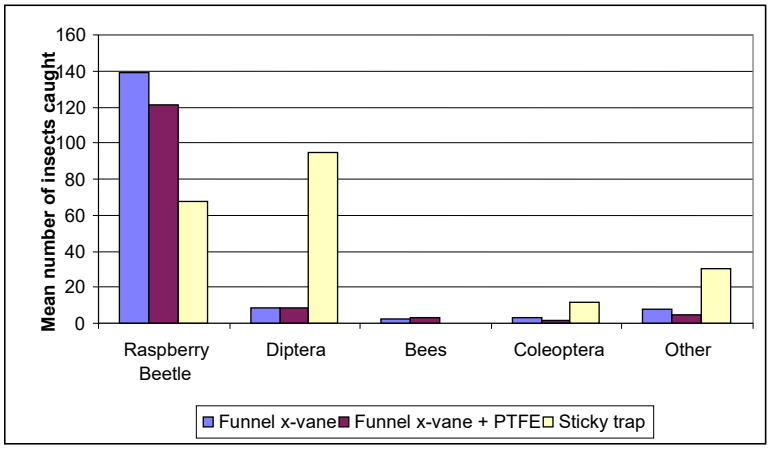


Figure 4: Mean number of insects caught using the three trap types, funnel cross-vane, funnel cross-vane + PTFE and white non-UV reflective sticky trap (Experiment 1A/2005)

There were significant differences ($P<0.001$) in the mean numbers of raspberry beetle trapped using the three types of trap. The mean number of beetles caught on the sticky traps was significantly lower than for both the funnel traps treatments. There were no significant differences in the mean numbers of raspberry beetle caught using the two types of funnel traps. There were no significant differences in the mean numbers of Coleoptera, other than raspberry beetle, trapped in the different types of traps. Diptera numbers required log transformation prior to analysis. There were significant differences ($P<0.001$) in the mean numbers of Diptera caught in the different types of trap with significantly greater numbers caught using sticky traps when compared with both types of funnel traps. The numbers of bees trapped was very low in all treatments and was therefore removed from the analysis. There were no significant differences in the means for 'others'. Examination of the maximum temperature during the period of the experiment showed that the maximum temperature exceeded the theoretical raspberry beetle flight temperature of 15°C on 20 out of 28 days.

In 2006, three field-trials assessed the relative efficacy of different types of dispensers and mixtures of attractants (Compounds A and B singly, and a mixture of Compounds A & B).

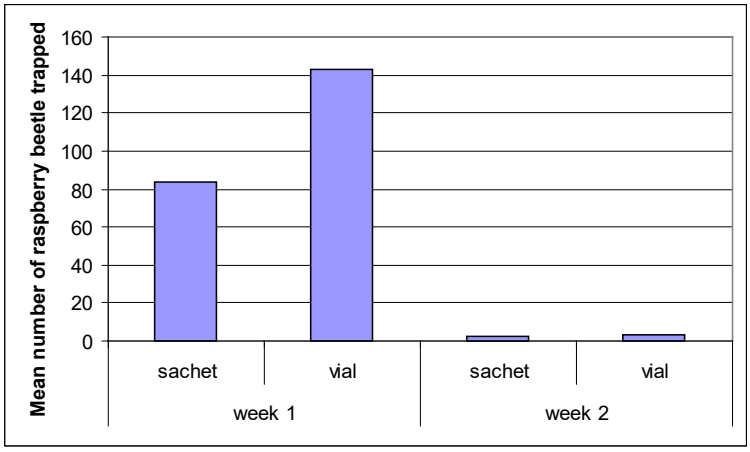


Figure 5: Mean number of raspberry beetles trapped using a white non-UV reflective sticky trap in conjunction with a sachet containing Compound B or a vial containing Compound B (Experiment 2/2006)

Figure 5 shows the numbers or raspberry beetles caught over the 2-week period of experiment 2/2006. Although there was a significant difference ($P<0.001$) in the number of raspberry beetles caught between weeks but there were no significant differences found between treatments. Very low numbers were caught in the second week due to a period of cold, but relatively sunny weather. There were 4 days when the temperature exceeded 15°C in week 1, but only one in week 2. The mean temperature in week 1 was 11.2°C, whereas it was only 8.9°C in week 2. In week 1, the average sun hours were 3.5 hr^{-d} whereas in week 2 the figure was 9.0 hr^{-d}.

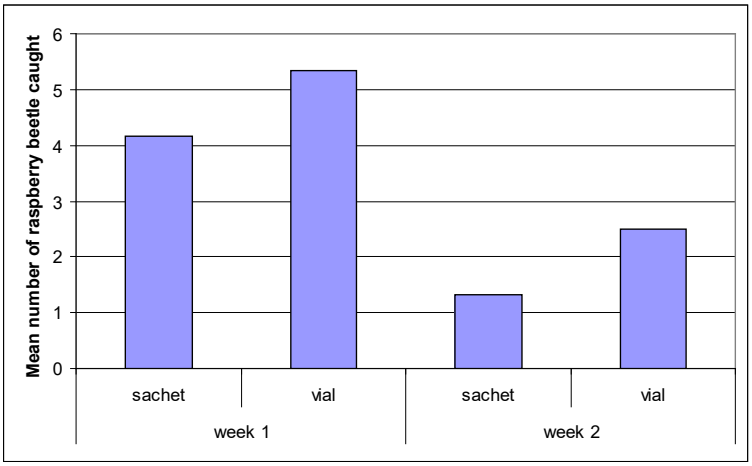


Figure 6: Mean number of raspberry beetles caught using funnel traps in conjunction with a sachet containing Compound B or a vial containing Compound B (Experiment 3/2006)

There was a significant difference (P=0.004) in the number of raspberry beetles caught between weeks in experiment 3/2006, but the there were no significant differences found between treatments. In this experiment the numbers of beetles were much less than in experiment 2. The temperatures in both weeks were much higher and the mean temperatures were 14.4 and 15.8°C respectively and in each of the 14 days for the experiment temperatures were close to the theoretical flight temperature of exceeded it by up to 10°C. These high temperatures also probably induced adjacent early flowering raspberries to begin to flower, thus compete with the traps by releasing their own natural attractants.

To test the efficacy of mixtures of the Compound A and Compound B, a simple evaluation was done in experiment 4/2006 using sticky traps with two vials of each compound and a set with one vial of Compound A and one of Compound B. Each sticky trap has two vials. Compound B was consistently the most effective in attracting raspberry beetles. The results are shown in Figure 7.

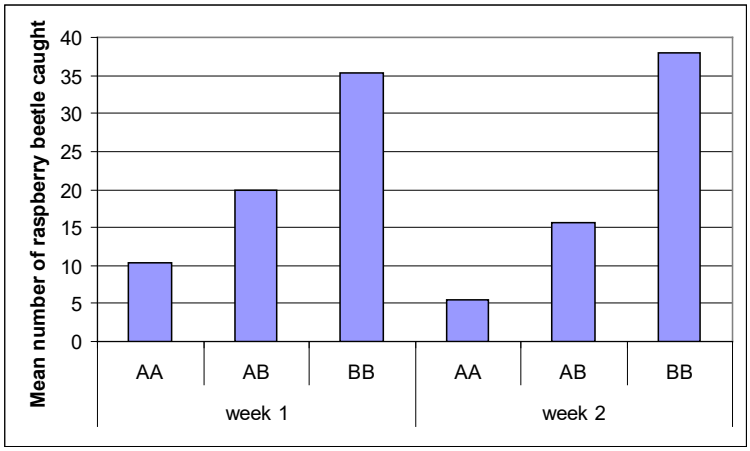


Figure 7: Mean number of raspberry beetles caught using vials containing AA (Twin vials of Compound A only), AB (single vial of each of Compound A & B) or BB (Twin vials of Compound B only) (Experiment 4/2006)

There was no significant difference in the number of beetles caught between weeks, but in all cases there was a significant difference (P<0.001) in the number of beetles caught between treatments. Treatment BB (Compound B) consistently caught the

highest number of raspberry beetles whilst treatment AA (Compound A) caught the least number. A mixture of AB increased the number of beetles caught compared to AA, but the overall increase was 50% or less than that of BB.

Laboratory Studies

Evaporation rate tests for different dispenser (lure) types were done in the laboratory in 2005 (experiment 1A/2005) and in 2006 (experiment 5/2006). In 2005, evaporation from the standard thick-walled vial was recorded on two occasions, 3 and 9 weeks and the mean evaporation was calculated and shown in Table 2.1.1.2.

Table 2.1.1.2: Mean loss of chemicals A and B from thick and thin-walled vials over a 3 and 9 week period in a fume cupboard on 2005. (Data from week 6 not shown)

Treatment	Mean loss (g) 3 wks	Mean loss (g) 9 wks	Evaporation rate mg $^{-d}$ 9 wks	Number of reps
A: thick vial – Compound A	0.4044	0.9024	14.32	5
B: thin vial – Compound A	0.3520	0.9570	15.19	3
C: thick vial – Compound B	0.3998	1.0607	16.83	5
D: thin vial – Compound B	0.4849	1.4604	23.18	3

Considerable problems in sealing the lids of the thin-walled vials resulting in reduced numbers of replicates. Increased evaporation was observed with the thin-walled vials with both Compound A and Compound B. The unreliability of the thin-walled vials in this experiment and the difficulties experienced in the corresponding field trial resulted in the abandonment of the thin-walled vials for future development.

After further discussions with AgriSense in early 2006 a range of prototype sachet-based lures were manufactured for field (experiment 3/2006, above) and laboratory testing (experiment 5/2006). The laboratory evaporation tests are shown below in Table 2.1.1.3.

Table 2.1.1.3: The number of days and evaporation rate of each chemical and dispenser type combination lasted (*experiment finished but chemical still remained in vials)

Chemical	Dispenser type	No of days dispenser lasted**	Evaporation rate mg $^{-d}$
Compound B	sachet	13	104.6
Compound B	foil sachet	20	72.4
Compound B	vial	36*	16.5
Compound A	sachet	24	55.7
Compound A	foil sachet	27	53.8
Compound A	vial	36*	11.6

* experiment finished but chemical remaining in vial

* some sachets may have very low residual volumes of volatile, but evaporation rate judged to be insufficient to be effective

The single-sided (foil) and double-sided sachets gave very similar rates of evaporation (range 40.0 to 54.0 mg^{-d}) whereas with the standard thick-walled vial treatments, the rates were considerably lower (16 and 12 mg^{-d} respectively for Compound B and Compound A), but very similar to the evaporation rates observed in 2005.

Conclusions

These experiments have compared the lures, the dispensers and the traps as set out in milestone P2.1.

- The funnel trap is significantly more effective than the white non-UV reflective sticky trap at catching adult raspberry beetles. The numbers of non-target insects is less than the sticky white traps and their efficiency does not appear to be reduced due to the traps becoming saturated. These traps are likely to be more acceptable to growers as they do not have any adhesive and are generally quicker to change. The traps are more durable and may last for several seasons.

- There was no significant difference in the number of raspberry beetles caught using the standard thick-walled Compound B vial or Compound B sachet. This was seen when used in conjunction with both the sticky trap and the funnel trap although there was a repeatable trend for the vials to be more effective.

- Comparison of the chemicals showed that Compound B attracted significantly more raspberry beetles than Compound A or a combination of both chemicals when released from standard thick-walled vials. The combination of chemicals attracted significantly more raspberry beetles than Compound A on its own.

- In laboratory test in 2005, the thin-walled vials allowed more evaporation than the corresponding thick-walled vials after 9 weeks. However, further development was suspended due to difficulties in transporting and handling of the thin-walled vials and their propensity to leak, probably due to the design of the lids and lid restraining strap. In 2006, the rate of evaporation of both Compound A and Compound B is slowest from the standard vial. The sachets slightly increased the evaporation rate of the chemicals. However, under field conditions the sachets only lasted a few days and require further modification in 2007.

Objective 3. Raspberry cane midge

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

Task 3.1.1 – 3.1.3. Make lures, evaluate blends and release rates

Comparison of pheromone dispensers and effect of minor components

Introduction

The objective of this experiment was to test the attractiveness of rubber septa or polythene vial dispensers loaded with 100 µg of racemic major component of the raspberry cane midge sex pheromone, 2-acetoxy-5 undecanone, alone or in admixture with three minor components at their naturally-occurring levels. A replicated field experiment was conducted in May and June 2005 in commercial raspberry plantations in Kent, UK. Release rates were measured in a laboratory wind tunnel under constant temperature and wind speed.

Methods and materials

Sites

Belks Farm, Otham, Kent (by kind permission of Tim Chambers)

Plantation 1: Glen Ample plantation unprotected until about 10 June 2005

Plantation 2: Glen Lion plantation already protected

Plantation 3: Glen Ample plantation unprotected until about 10 June 2005

Plantation 4: Glen Lion plantation already protected

Beech Farm, West Peckham, Kent (by kind permission of Harry Wooldridge)

Plantation 5: Joan Squire primocane plantation (outdoor)

Plantation 6: Autumn Bliss primocane plantation (outdoor)

Plantation 7: Glen Ample plantation protected

Plantation 8: Glen Ample plantation (outdoor)

East Malling Research (Farm Manager Nigel Osborne)

Plantation 9: RF171 three rows of mixed varieties and adjacent plantings which were scheduled for grubbing

Plantation 10: DF158 – four rows of mixed raspberry varieties

Treatments

Five different treatments as specified in Table 1 below:

Table 3.1.1.1: Treatments

Treatment no. and name	Dispenser	Pheromone blend‡	Components in blend
1. Septa A	Rubber septum	A	100 µg Racemic major component
2. Vial A	Polythene vial †	A	100 µg Racemic major component
3. Septa B	Rubber septum	B	100 µg Racemic major component + 3 C11 minor components
4. Vial B	Polythene vial †	B	100 µg Racemic major component + 3 C11 minor components
5. Untreat	None	None	None

† Low density polythene vials from Just Plastics – deployed closed
‡ Treatment B contains 2-undecanone, racemic 2-undecanol and racemic 2-undecyl acetate, all at 30% of the major component. Both treatments also contain BHT at 20% of the major component

Lures were suspended inside standard white 20 × 20 cm delta trap with white sticky base. Lures were changed at 1 week intervals. Traps were suspended at a height of 30-50 cm above the ground.

Experimental design

Randomised complete block with 10 replicates was used. Each replicate sited in one of the 10 raspberry plantations above. Plots were individual delta traps each containing a single lure (no lure in untreated). Traps are spaced at least 10 m apart.

Assessments

Traps were deployed on 11 May 2005. Weekly counts of the numbers of raspberry cane midge males caught in each trap were made until 7 June 2005.

Statistical analysis

Analysis of variance with log10(n+1) transformation to stabilise variances was done, including and excluding the untreated control, which contained mostly very low or zero values, was done. Means were separated by pairwise LSD testing. This was done firstly at the P=0.05 level, but this showed small differences to be statistically significant which were not consistent from week to week. The pairwise comparisons were than repeated at the P=0.01 level.

Measurement of release rates

Rubber septum and polyethylene vial dispensers loaded with pheromone blend B (100 µg major component) were maintained in a laboratory wind tunnel at 27°C and 8 km/hr wind speed. At intervals volatiles released from individual dispensers during 2-4 hrs were collected on Porapak resin, eluted with dichloromethane and analysed by gas chromatography. Results are the mean of two separate samples.

Results

Trapping experiments

The baited traps caught an average of 280.4 male raspberry cane midges per trap compared to an average of 1.5 in similar traps not baited with a lure (Table 2). Analysis of variance of the $\log_{10}$ (n+1) transformed counts followed by least significant difference (P=0.01) pairwise comparisons of means showed that all the lures caught significantly more midges than the untreated control. However, there were no consistent, statistically significant differences between the other treatments. In the first weeks catches (17 May), Septa B caught significantly more than Vial B, but this difference

was not apparent in subsequent weeks nor in the analysis of the total catches (Figure 8).

Table 3.1.1.2: Mean and mean $log_{10}(n+1)$ transformed numbers of raspberry cane midge males captured per trap in the first raspberry cane midge dispenser/blend experiment in 11 May-14 June 2005

	17 May	24-May	31-May	07-Jun	14-Jun	Total
SeptaA	57.5	21.1	93.2	45.8	17.6	235.2
VialA	67.5	20	98.1	44.4	34.8	264.8
SeptaB	121	26.5	120.3	64.9	23.5	356.2
VialB	32.7	10.4	97.3	81.8	43.2	265.4
Untreated	0.1	0.4	0.3	0.4	0.3	1.5
$Log_{10}(n+1)$ transformed data, ANOVA including untreated controls						
SeptaA	1.316bc	1.032b	1.488b	1.175b	0.924b	2.012b
VialA	1.407bc	1.040b	1.540b	1.233b	1.098b	2.096b
SeptaB	1.848c	1.116b	1.590b	1.287b	0.908b	2.310b
VialB	1.197b	0.686b	1.424b	1.349b	1.336b	2.148b
Untreated	0.030a	0.108a	0.078a	0.095a	0.060a	0.290a
Fprob	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SED (36 d.f.)	0.1998	0.1669	0.1807	0.1860	0.1926	0.1352
LSD (P=0.05)	0.4053	0.3384	0.3665	0.3772	0.3906	0.2743
LSD (P=0.01)	0.5434	0.4538	0.4914	0.5058	0.5237	0.3768

Note: Means followed by the same letter do not differ significantly at the P=0.01 level

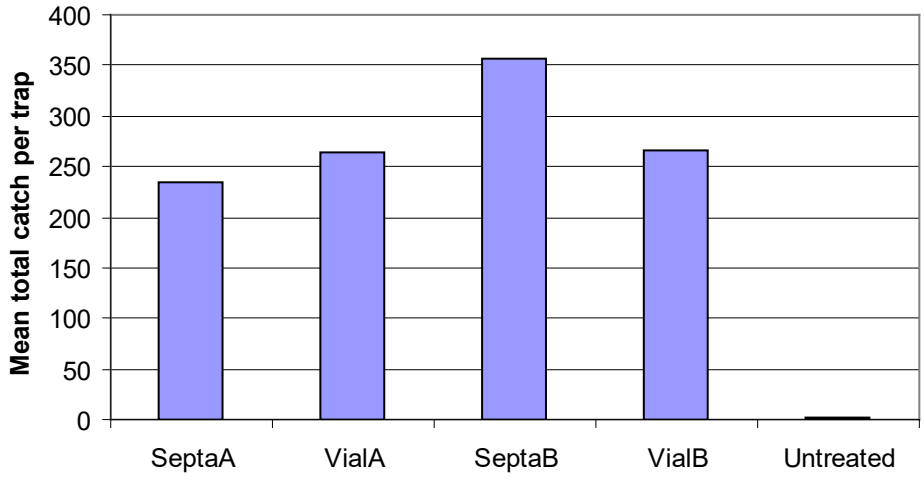


Figure 8: Mean total catch of midges per trap 11 May – 7 June 2005

Release rate measurements

Release rate data are shown in Figure 9. The rubber septa showed a roughly exponential decline in release rate of the major component. The minor components were released at approximately 30% of the rate of the major component. Release of the major component from the polyethylene vial increased during the first 10 days, then remained relatively constant for the next 15 days and then declined. Release of the minor components seemed to decline relatively evenly throughout. Apart from the changes in overall release rate, the ratio of major to minor components varied dramatically from approximately 1:1 to 3:1 and back to 1:1 during the experiment. For both dispensers the release rate of the major component had dropped to approximately 10% of the maximum after 33 days.

Conclusions

- Racemic 2-acetoxy-5-undecanone is highly attractive to raspberry cane midge males alone

- The three minor components are not essential and did not significantly (P=0.01) increase attractiveness at the lure loadings tested

- There was no consistent difference between the rubber septa and vial dispensers. However, the higher catches in the vials in week 1 may have been due to a slower initial release rate

- The release rate of pheromone from rubber septa showed a more predictable exponential decline compared with the release from polyethylene vials. Furthermore, the ratio of release rates of major to minor components remained reasonably constant from the septa. The release rate from both dispensers had declined to 10% of the maximum after 33 days at 27°C, and hence lures should remain active for at least one month in the field in the UK

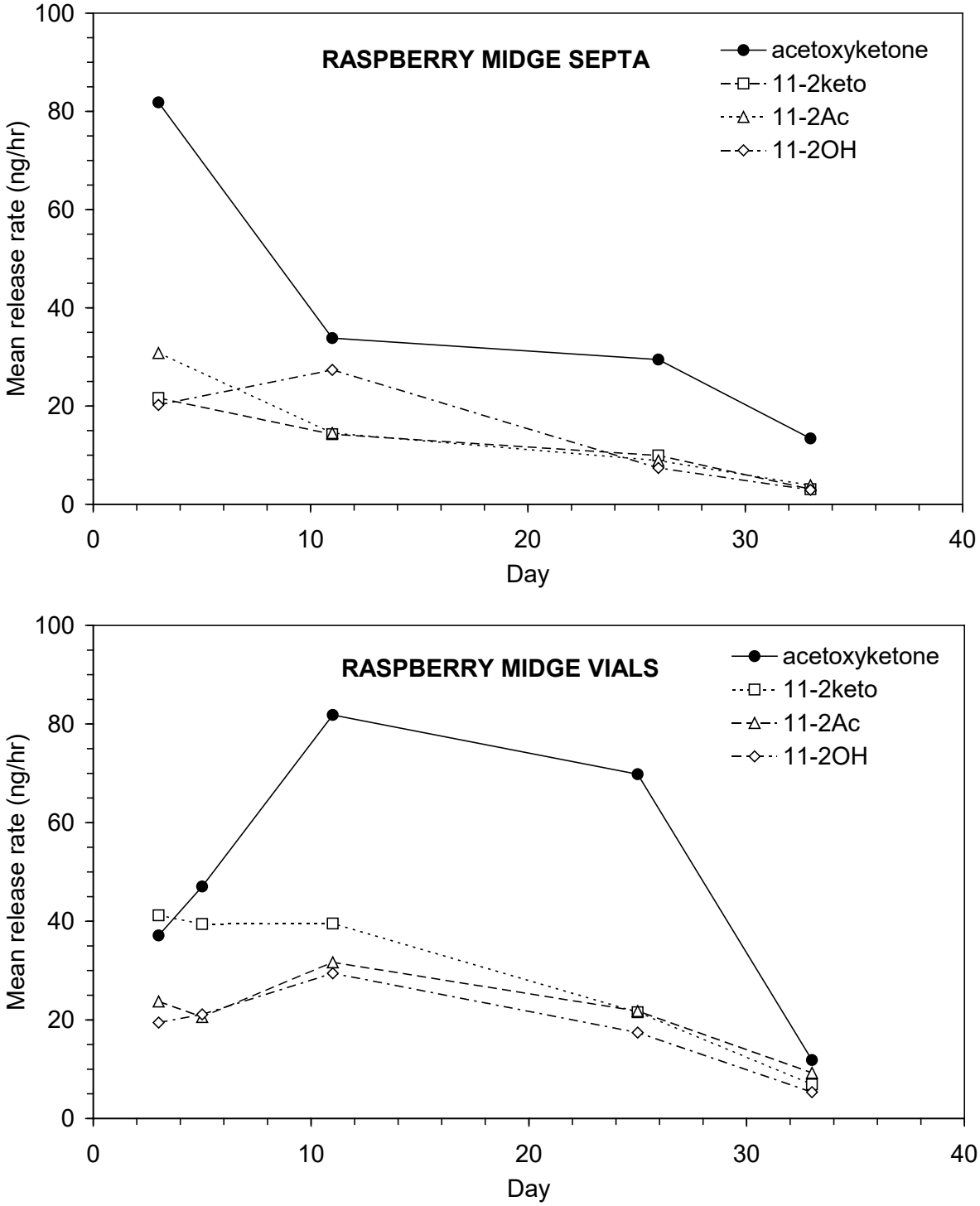


Figure 9: Release rates of pheromone components from rubber septa (upper) and polyethylene vial (lower) dispensers in a laboratory wind tunnel at 27°C and 8 km/hr wind speed

Comparison of attractiveness of enantiomers of major pheromone component

Introduction

The objective of this experiment was to determine which of the raspberry cane midge pheromone enantiomers is attractive and the relative attractiveness of the racemic blend compared to the singe enantiomers alone.

Methods and materials

Sites

Two experiments were conducted in two adjacent cane midge infested primocane plantations at Beech Farm, West Peckham, Kent (by kind permission of Harry Wooldridge). NGR TQ 646 527.

Plantation 1: Joan Squire primocane plantation (outdoor)
Plantation 2: Autumn Bliss primocane plantation (outdoor)

Treatments

Four different treatments as specified in Table 3.1.1.3 below were used on rubber septa dispensers:

Table 3.1.1.3: Treatments

Treatment	Amount	Component
A. Racemic	20 µg	Racemic 2-acetoxy-5-undecanone
B. Enantiomer 1†	10 µg	2*R*-acetoxy-5-undecanone
C. Enantiomer 2‡	10 µg	2*S*-acetoxy-5-undecanone
D. Untreated	none	None

† Contains 3% enantiomer 2
‡ Contains 1% enantiomer 1

Racemic pheromone was prepared at NRI and the enantiomers were separated by chiral HPLC by Prof Tsetse Ando at the Tokyo University of Agriculture and Technology. The enantiomers are designated according to their order of elution on the HPLC column. They were analysed by GC on a chiral Cyclodextrin column at NRI, on which the order of elution is reversed. The naturally-produced pheromone corresponds

to Enantiomer 2 above. This was subsequently shown to be the 2*S* enantiomer by synthesis of both enantiomers.

Lures were rubber septa suspended inside standard white 20 x 20 cm delta trap with white sticky base. Lures were changed at 1 week intervals. Traps were suspended at a height of 30-50 cm above the ground. Traps were initially deployed on 5 July 2005.

Experimental design

Randomised complete blocks with five replicates. Plots are individual delta traps each containing a single lure (no lure in untreated). Traps are spaced at least 10 m apart.

Assessments

The numbers of raspberry cane midge males in each trap were counted on 12, 19 and 26 July 2005.

Statistical analysis

Analysis of variance was done on the total trap catches after $\log_{10}(n+1)$ transformation for each experiment separately and on the two experiments combined, with and without inclusion of the untreated control.

Results

Actual mean numbers were greatest with enantiomer 2, and smallest with enantiomer 1, the racemic giving intermediate values. Treatment effects in the ANOVA of the $\log_{10}(n+1)$ transformed data were highly significant (Table 4). All the lures caught much higher numbers of midges than the untreated. In experiment 1, there was no statistically significant difference between enantiomer 2 and the racemic, which both caught more than enantiomer 1. However, the differences between these treatments were not large. In experiment 2 and in the analysis of both experiments combined, enantiomer 2 caught significantly more than the racemic or enantiomer 1, which did not differ significantly.

This experiment provides evidence that enantiomer 2 (2*S*-acetoxy-5-undecanone), the naturally-produced enantiomer, is the attractive enantiomer. It is not possible to rule out

enantiomer 1 being attractive although the catches may have been due to the presence of 3% of enantiomer 2 in the sample of enantiomer 1 used.

Conclusions

- The racemic 2-acetoxy-5-undecanone is highly attractive. There is no evidence that the opposite enantiomer to the natural one inhibits the attractancy of the natural enantiomer

- Enantiomer 2, the natural 2*S* enantiomer, caught significantly more midges than enantiomer 1

- It is not possible to rule out enantiomer 1 being attractive although the catches may have been due to the presence of 3% of enantiomer 2 in the sample of enantiomer 1 used

Table 3.1.1.4: Mean and mean $log_{10}(n+1)$ numbers of raspberry cane midge captured per trap 6-12 July 2005 in two replicated (n=5) experiments at in 'Joan Squire' and 'Autumn Bliss' primocane raspberry plantations Beech Farm, West Peckham, Kent. In column 4 the data for the two experiments have been combined into a single analysis

Treatment	Exp 1	Exp 2	Exps 1 and 2 combined
Untransformed			
A. Racemic 20µg	54.6	82.6	68.6
B. Enantiomer 1 10µg	32.4	62.6	47.5
C. Enantiomer 2 10µg	68.2	126.4	97.3
D. Untreated	3.8	0.6	2.2
$Log_{10}(n+1)$ transformed			
A. Racemic 20µg	1.727c	1.792b	1.759b
B. Enantiomer 1 10µg	1.418b	1.741b	1.579b
C. Enantiomer 2 10µg	1.779c	2.061c	1.920c
D. Untreated	0.667a	0.181a	0.424a
Full ANOVA			
Fprob	<0.001	<0.001	<0.001
SED†	0.1288	0.1347	0.1285
LSD (P=0.05)	0.2806	0.2935	0.2618
ANOVA excluding untreated			
Fprob			0.020
SED†			0.118
LSD (P=0.05)			0.2313

† 12 d.f. for exp 1 or 2 individually, 32 d.f. where exps 1 and 2 are combined, 23 d.f. where experiments are combined but untreated excluded from ANOVA
Note: mean values followed by the same letter do not differ significantly using simple LSD test (P=0.05)

Re-examination of effect of minor pheromone components and attractiveness of enantiomers of major component

Introduction

A replicated experiment was conducted in two adjacent commercial raspberry plantations to test the attractiveness at a low and very low release rate of the single enantiomers of the major component of the raspberry cane midge sex pheromone, 2-acetoxy-5-undecanone, in comparison with the 50-50 racemic mix of the enantiomers with or without the 3 minor components. The experiment aimed to determine which of the enantiomers is attractive, the relative attractiveness of the racemic blend and the effects of addition of the minor components.

Methods and materials

Sites

Two adjacent cane midge infested primocane plantations at Beech Farm, West Peckham, Kent (by kind permission of Harry Wooldridge). NGR TQ 646 527

Plantation 1: Joan Squire primocane plantation (outdoor)
Plantation 2: Autumn Bliss primocane plantation (outdoor)

Treatments

Treatments were rubber septa dispensers loaded with the different raspberry cane midge candidate pheromone components, as shown in Table 7.

Lures were suspended inside standard white 20 × 20 cm delta trap with white sticky base. Lures to be changed at 1 week intervals. Traps suspended at a height of 30-50 cm above the ground.

Table 3.1.1.5: Treatments

Treatment no. and name	Amount, component
A. Rac 0.1	0.1 µg racemic 2 acetoxy 5 undecanone
B. Rac 0.01	0.01 µg racemic 2 acetoxy 5 undecanone
C. Rac 0.1+minor	0.1 µg racemic 2 acetoxy 5 undecanone + 30% of each minor components*
D. Rac 0.01+minor	0.01 µg racemic 2 acetoxy 5 undecanone + 30% of each minor components*
E. Enant1 0.1	0.1 µg fraction 1 (2*R* acetoxy 5 undecanone)
F. Enant1 0.01	0.01 µg fraction 1 (2*R* acetoxy 5 undecanone)
G. Enant2 0.1	0.1 µg fraction 2 (2*S* acetoxy 5 undecanone)
H. Enant2 0.01	0.01 µg fraction 2 (2*S* acetoxy 5 undecanone)
I. Control	untreated control (no lure)

*Minor components are 2-undecanone, racemic 2-undecanol, racemic 2-undecyl acetate

Experimental design

A randomised complete block with six replicates of nine treatments was used. Three blocks were located in each plantation Plots were individual delta traps each containing a single lure (no lure in untreated). Traps are spaced at least 10 m apart.

Assessments

Weekly counts of the numbers of raspberry cane midge males caught in each trap over a 3-week period until the flight period ended.

Statistical analysis

Two types of analyses of variance were done, the first with no partitioning of treatment factors (a simple two-way ANOVA), the second where treatment factors for control versus treated, racemic versus enantiomer, dose, +/- minor component were included in the analysis which subdivided the eight degrees of freedom for treatments into single degrees of freedom to assess various dose and other effects explicitly.

The total counts were analysed as recorded, with a square root and with a log transformation [$\log_{10}$(count+1)]. The latter of these was the most effective in giving reasonable variance homogeneity so was used in the subsequent analysis.

Results

Total numbers caught were rather small but treatment effects were clear. Analysis of variance of the log transformed data without inclusion of treatment factors gave highly significant treatment effects (Table 3.1.1.6). Enantiomer 1 did not catch significantly more midges than the control. The racemic 0.1, racemic 0.1 + minor and enantiomer 2 0.1 treatments (A, C and G) all caught the largest and similar numbers of midges. The low dose treatments racemic 0.01, racemic 0.01 + minor and enantiomer 2 0.01 treatments (B, D and H) caught lower, intermediate numbers, though the enantiomer 2 0.01 treatment (H) caught significantly less.

Table 3.1.1.6: Mean total numbers and mean $\log_{10}$(n+1) transformed total numbers of raspberry cane midge caught

Treatment	Mean total numbers of male midges captured per trap			
	n	n'	$\log_{10}$(n+1)†	
A. Rac 0.1	22.2	19.1	1.302	ef
B. Rac 0.01	9.8	8.6	0.983	de
C. Rac 0.1+minor	20.2	17.0	1.255	ef
D. Rac 0.01+minor	5.8	3.8	0.680	cd
E. Enant1 0.1	0.7	0.4	0.159	ab
F. Enant1 0.01	1.0	0.6	0.201	ab
G. Enant2 0.1	24.7	22.2	1.366	f
H. Enant2 0.01	3.3	2.3	0.520	bc
I. Control	0.2	0.1	0.050	a

Fprob	<0.001
SED (40 d.f.)	0.1734
LSD (P=0.05)	0.3504

n' Back-transformed means calculated from the analysis of variance of the log $_{10}$(n+1) data
† Means followed by the same letter do not differ significantly Duncan's multiple range test (P=0.05)

Figure 10: Back-transformed mean total numbers of midges caught per trap

The factorial analysis gave the following Fprob values:

Control versus treated		<0.001
Rac versus Enant		<0.001
Within Rac:	Dose	<0.001
	+/- minor 0.161	
	Dose.+/-minor	0.304
Within Enant:	Number	<0.001
	Dose	0.002
	Number.Dose	<0.001

Racemic: There is a strong overall dose effect, with 0.1 > 0.01 > control (all differences highly significant). There was no statistically significant evidence of an effect of adding the minor (p=0.161), nor any interaction with dose (p=0.304). Indeed, the mean without minor is higher than that with minor (1.142 compared to 0.967 on the log scale).

Enantiomer: There was a strong overall difference between Enantiomers and a dose effect, but also an interaction. This is explained by there being no dose effect for the

inactive enantiomer 1, but a strong one for the active enantiomer 2 where 0.1 > 0.01 > Control (again all significant).

The means for comparison of enantiomer 2 with the racemate are given in Table 3.1.1.7 below (those for racemate (Rac) are without the addition of the minor as it was inactive). It is clear that at the higher (most effective) dose of 0.1, there was no statistically significant difference in effect between enantiomer 2 and Rac; at the lower dose of 0.01. However, it appears that enantiomer 2 is more effective than Rac.

Table 3.1.1.7: Grand mean $\log_{10}(n+1)$ total numbers of midges captured per taps

	Lure loading†	
	High	**Low**
Enantiomer 2	1.366	0.520
Racemate	1.302	0.983
SED (40 d.f.)	0.1734	
LSD (P=0.05)	0.350	

† High = 0.1 µg of racemate, 0.05 µg of enantiomer
Low = 0.01 µg of racemate, 0.005 µg of enantiomer

Conclusions

- The naturally-occurring Enantiomer 2 (2*S*-acetoxy-5-undecanone) is attractive to male raspberry cane midges and Enantiomer 1 is unattractive. Slightly increased catches with enantiomer 1 compared to the control (not statistically significant) were due to the presence of traces of enantiomer 2 in the enantiomer 1 preparation

- Enantiomer 1 is neutral in its activity. It is neither attractive nor does it inhibit the activity of enantiomer 2. Hence the racemate is equally attractive as enantiomer 2 (the latter at half the dose)

- There was no increased attraction form addition of the minor components

- The higher dose caught significantly more than the lower dose

Task 3.1.3. Evaluate the effects of release rate

Effect of pheromone loading on attractiveness

Introduction

The objective of this experiment was to determine the effect of loading/release rate of racemic 2-acetoxy-5-undecanone (major component of the raspberry cane midge sex pheromone) on trap catch of male raspberry cane midge.

Materials and methods

A large scale 6 × 6 Latin square field experiment comparing weekly catches of midges in delta traps bated with rubber septa lures loaded with 1000, 100, 10, 1, 0.1, 0 µg racemic 2-acetoxy-5-undecanone.

Sites

Beech Farm, West Peckham, Kent (by kind permission of Harry Wooldridge)

Plantation 5: Joan Squire primocane plantation (outdoor)
Plantation 6: Autumn Bliss primocane plantation (outdoor)

Treatments

Six different treatments as specified in table 3.1.3.1 below:

Table 3.1.3.1: Treatments

Treatment name	**Lure loading (µg racemic 2-acetoxy-5-undecanone/lure)**
A	1000
B	100
C	10
D	1
E	0.1
F	0

Lures were rubber septa suspended inside standard white 20 x 20 cm delta trap with white sticky base. Traps were first deployed on 2 August 2005 and suspended at a height of 30-50 cm above the ground.

Experimental design

A 6 × 6 Latin square with six replicates of the six treatments was used. Three replicates were located in Joan Squire, three replicates in the Autumn Bliss plantation. Plots were individual delta traps each containing a single lure (no lure in untreated). Traps were spaced approximately 10 m apart.

Assessments

Counts of the numbers of raspberry cane midge males caught in each trap were made on 8, 18, 24 and 30 August and on 8 September 2005. Fresh sticky bases were deployed on each occasion. Lures were not changed.

Statistical analysis

Analysis of variance with square root transformation of data gave best results in respect of variance homogeneity. Linear, quadratic and logistic curves were fitted to the data. Means were separated by LSD test at 5% level.

Results

The ANOVA of square root transformed data showed that Dose is highly significant (P<0.001) (Table 3.1.3.2). In respect of individual means, there were no differences of sufficient size to be statistically significant from dose 1000 down to dose 1 (and even dose 0.1 is only significantly lower than the dose with highest mean, 100).

Table 3.1.3.2: Mean square root transformed total numbers of raspberry cane midge caught per trap and back transformed mean values

Lure loading (dose) (µg/lure)	√n	Back-transformed means
1000	16.44	270.3
100	21.23	450.7
10	20.03	401.2
1	18.45	340.4
0.1	13.79	190.2
0	1.83	3.4
SED (20 d.f.)	3.061	
LSD (P=0.05)	6.39	

However, rather than look at individual differences, there are clear strong trends in the data, which are adequately explained by a linear and quadratic trend (both p<0.001),

with lack of fit not significant (p=0.795). The linear and quadratic trends that were fitted in the ANOVA assume equal spacing between the doses (essentially equivalent to using a log scale for dose). This is adequate as an approximation but there was a problem of where to fit the zero dose on this scale (the default used above would set this as equivalent to a dose of 0.01; putting it at 0.001 gave very similar results with the Fprob for lack of fit being 0.824). On this second scale the maximum value is estimated as being obtained at dose 21.2 (95% confidence interval of [3.3, 134.6] – the asymmetry is due to calculating the CI on the $\log_{10}$ scale and then back-transforming the values); the maximum value was estimated as 407 back-transformed to the original count measurement (Figure 11). Thus, without more information on the response between the doses used in this experiment, it is difficult to draw conclusions as to the underlying nature of that response. Clearly a polynomial (quadratic) is of limited use as it does not behave in a meaningful way outside the dose range, although here it does indicate the presence of a maximum response between a dose of 3 and of 135. To enable a logistic to be tested against an alternative of high dose reduction, considerably more doses would be required, both above 1000 to see if the reduction was real and also lower than 1 to give more form to the curve at the lower end.

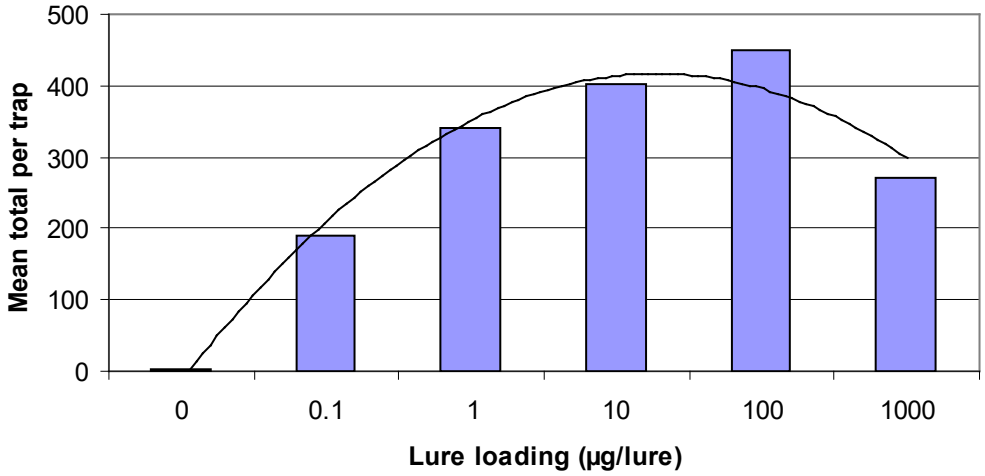


Figure 11: Mean total catches at the different doses (lure loadings) and fitted polynomial (quadratic) showing maximum catch at approximately 20 µg/lure

Conclusions

- A strong positive dose response was demonstrated
- There was a high degree of attractancy even at the lowest dose of 0.1 µg/lure

- The greatest catches occurred with the 100 µg/lure dose but the mean catch at this dose did not differ significantly from the catches at 1, 10 or 100 µg

- Fitting a quadratic curve to the data indicated the presence of a maximum response between a dose of 3 and of 135 µg/lure

- The existence of a maximum was not proven by this experiment, which needs repeating with a wider range of doses

Raspberry cane midge lure loading experiment 2006

Introduction

The objective of this experiment was to determine the effect of the release rate of raspberry cane midge pheromone from lures on attractiveness to male raspberry cane midge.

Methods and materials

Site

The experiment was done in adjacent Joan Squire and Autumn Bliss everbearer plantations at Beach Farm, West Peckham, Kent(NGR TQ 646 528) by kind permission of Harry Wooldridge

Treatments

Treatments were rubber septa dispensers loaded with increasing amount of raspberry cane midge pheromone racemate, and having correspondingly increasing release rates of the pheromone, as shown in Table 3.1.3.3.

Table 3.1.3.3: Treatments. Note that treatment J was added assessment on 31 August 2006

Treatment	Lure load (racemate/lure))	Estimated release rate‡ (/hr)
A	0	0

B	1 ng	600 fg
C	10 ng	6 pg
D	100 ng	60 pg
E	1 µg	600 pg
F	10 µg	6 ng
G	100 µg	60 ng
H	1000 µg	600 ng
I	10 mg	6 µg
J	No lure	0

‡ Estimated from measurements made on rubber septa loaded with 100 µg in the laboratory windtunnel at NRI at 27°C and 8 km/hr wind speed in 2005. See experiment 1

Note that release rates have been estimated form those measured in the laboratory windtunnel at NRI at 27°C and 8 km/hr. The mean release rate from two replicate 100 µg rubber septa was 80 ng/hr and 40 ng/hr after 10 days. The mean of these values is given in Table 3.1.3.3.

Experimental design

A randomised block experimental design with five replicates was used. Freshly made lures were used and the experiment was re-randomised on 31 August 2006, after the first count was done. Plots were single standard white (20 × 20 cm) delta traps deployed at a height of 0.5 m. The re-randomised design took into account the new treatment J and rotated the direction of the blocks so that blocks 1, 2 and 3 were in the northern plantation of Joan Squire (polytunnel protected) and blocks 4 and 5 were in the Autumn Bliss plantation (open field).

Assessments

The number of midges captured in each trap was counted on 31 August and 8 September 2006 weekly and the bases renewed until sufficient midges have been captured to show a clear dose response.

Statistical analysis

No statistical analysis has been done on the data as yet.

Results

The two assessments, which because the plots were re-randomised and fresh lures deployed were essentially two repeats of the experiment, gave remarkably similar results (Table 2, Figure 1). The very low dose treatments B and C did not catch significantly more midges than the zero (treatment A) or the no lure treatments (treatment J). Treatments D-F showed strongly increasing catches with increasing dose but treatment G, at which maximum catches occurred, only had a small increase in catches over treatment F. Further increases in dose resulted in large decreases in catch.

Table 3.1.3.4: Mean numbers of male raspberry cane midge captured per trap

Treatment	Loading (μg)	31 August 2006	8 September 2006	Total
A	0	2	4	6
B	0.001	2	0	2
C	0.01	2	1	3
D	0.1	3	16	19
E	1	25	23	48
F	10	151	122	273
G	100	166	158	324
H	1000	34	24	58
I	10000	13	15	28
J	no lure	0	8	8

Conclusions

The dose-response showed a clear maximum at a dose of 100 μg. The greatest catches per unit dose was at a lure loading of 0.1 μg. These results clearly corroborate the results of experiment 3 in 2005, when the dose response showed a clear maximum and the 100 μg lure load also gave maximal catches. This interesting result has an important implication for the development of Mating Disruption and Attract and Kill control approaches.

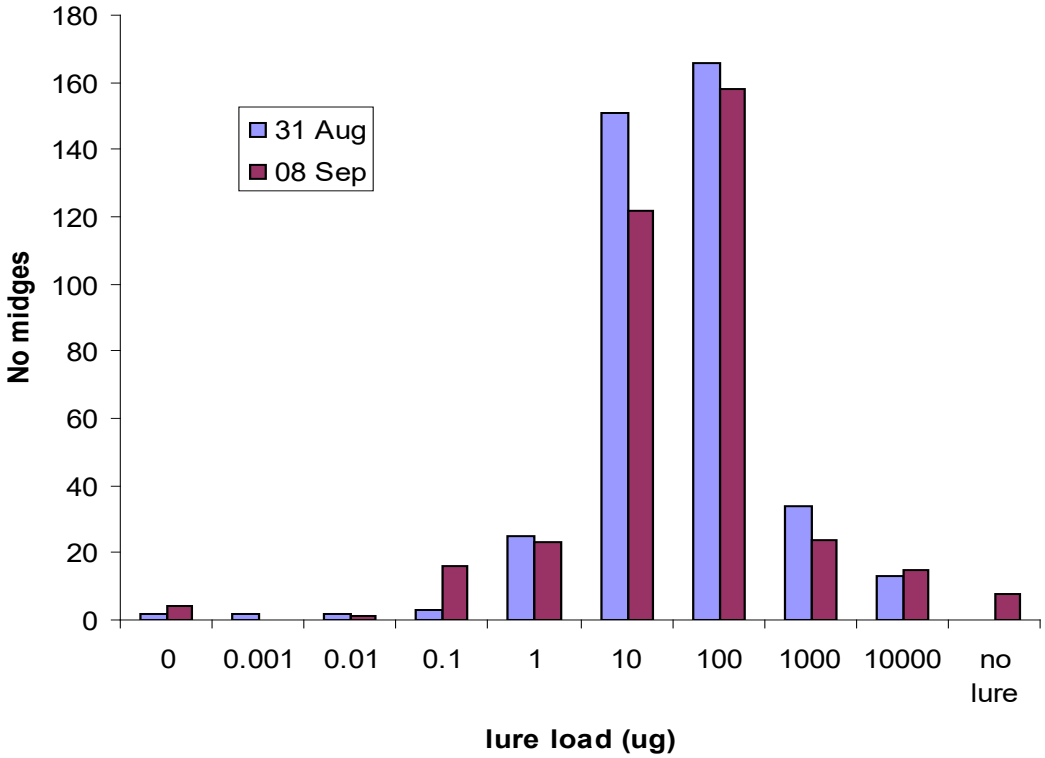


Figure 12: Mean number of midges captured per trap

Task 3.1.4. – Evaluate trap designs

Experiment 6. Comparison of the attractiveness of different coloured raspberry cane midge pheromone traps 2006

Introduction

The overall aim of the work was to determine whether the raspberry cane midge responds differently to different coloured delta traps. Six different coloured traps were compared red, yellow, blue, white, black and, green.

Methods and materials

A replicated field experiment was conducted twice with re-randomisation of plots at EMR in 2006.

Site

The experiments were done in CW129 raspberry plantation at EMR (field opposite Great East). The plantation contains a large number of different varieties summer fruiting raspberries. The plantation comprised 16 rows spaced 2.45m apart, each row having ten posts spaced 13.4m apart. For the first experiment, traps are deployed in rows 5, 8 and 11 on posts 3 to 8. For the second experiment, the traps are deployed in rows 4, 7 and 10 on posts 3 to 8. The plantation was 0.52 hectares in area.

Treatments

Proposed treatments were delta traps made from black, white, red, blue, yellow and green Correx. Sticky inserts were not used. The coloured base of the trap was coated with Ecotack. Traps were deployed at 0.5m and were baited with rubber septum lures loaded +with 10µg of pheromone racemate. Traps were deployed for experiment 1 on 3 July 2006 and for experiment 2 on 10 August 2006.

Experimental design

A randomised block experimental design was used with three replicates of the six different coloured treatments. Plots were single pheromone traps.

Assessments

The number of midges captured in each coloured trap were checked regularly until sufficient midges for statistical analysis had been captured when a count was made of the numbers caught in each trap. This was done on 8 August 2006 for experiment 1 and on 23 August 2006 for experiment 2. Additionally, the number of non-target arthropods, identified to broad taxa (bumble bees, flies, thrips, parasitic hymenoptera, beetles, aphids, lacewings, syrphids, moths, bugs, spiders, grasshoppers), in each trap was counted

Data collation and statistical analysis

Data were statistically analysed by ANOVA after square root transformation to stabilise variances .

Results and conclusions

The analyses of variance of square root transformed data showed that there were no significant effects of treatment on the numbers of raspberry cane midge captured in either experiment (Tables 3.1.4.1 and 3.1.4.2). However, in both experiments treatment did greatly affect numbers of non-target arthropods captured. In both experiments, the white and blue traps captured significantly greater numbers of on-target arthropods than the other trap colours. The predominant non-target taxa were thrips and flies. These results suggest that blue and white traps should be avoided because they become more heavily contaminated with non-target arthropods. Green or black traps are impractical. Similar work with apple leaf midge gave large catches of non-target arthropods in yellow traps. In conclusion, red traps are probably best for practical purposes.

Table 3.1.4.1: Mean numbers and mean square root transformed numbers of raspberry cane midge males and non-target arthropods caught in experiment 1

Trap colour	Raspberry cane midge males		Non-target insects	
	n	√n	n	√n ‡
Green	152	10.5	132	11.4 b
Yellow	88	9.3	124	11.1 b
Black	27	4.7	32	5.7 b
White	66	7.6	1004	30.5 a
Blue	48	6.8	893	27.9 a
Red	161	11.1	156	12.4 b
Fprob		0.511		0.003
SED (10 d.f.)		3.59		5.00
LSD (P = 0.05)		8.00		11.14

‡ Means followed by the same letter do not differ significantly (P = 0.05) in a Duncan's multiple range test

Table 3.1.4.2: Mean numbers and mean square root transformed numbers of raspberry cane midge males and non-target arthropods caught in experiment 2

Trap colour	Raspberry cane midge males		Non-target insects	
	n	√n	n	√n ‡
Green	36	5.54	52	7.19 b

Yellow	36	5.86	88	9.36 b
Black	19	4.33	28	5.16 b
White	31	5.49	352	18.28 a
Blue	33	5.34	298	16.90 a
Red	61	7.76	49	6.93 b
Fprob		0.248		0.001
SED (10 d.f.)		1.261		2.444
LSD (P = 0.05)		2.811		5.445

‡ Means followed by the same letter do not differ significantly (P = 0.05) in a Duncan's multiple range test

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

Sex pheromone trap monitoring of raspberry cane midge in raspberry plantations 2005

Introduction

The objective of this work was to compare the seasonal temporal pattern of catches of male raspberry cane midge in raspberry plantations subject to different management.

Methods and materials

Single white delta traps bated with polythene vial dispensers containing 100 μg of the racemic major component of the pheromone plus 30% of each of the minor components, were deployed at a height of 50 cm in the centres of 10 raspberry plantations in Kent and monitored weekly from 10 May to end of September 2005. Plantations comprised a range of varieties grown under protection or in the open field (Table 10). Two plantations at East Malling Research contained a very wide range of varieties (variety collections) and had not received any pesticide sprays. The other plantations were sprayed with pesticides including chlorpyrifos for cane midge control. Regrettably, the pheromone was not available for deployment until 10 May, 4 days after the forecast date of first emergence by ADAS on 6 May 2005 at East Malling Research.

Table 3.2.1.1: Plantations

Plantation no.	Location	Varieties	Protected/open
1	Belks Farm, Otham	G. Ample	open
2	Belks Farm, Otham	G. Lyon	protected
3	Belks Farm, Otham	G. Ample	open

4	Belks Farm, Otham	G. Lyon	protected
5	Beech Farm, W. Peckham	Joan Squire	open
6	Beech Farm, W. Peckham	Autumn Bliss	open
7	Beech Farm, W. Peckham	G. Ample	protected
8	Beech Farm, W. Peckham	Ample/Tulameen	open
9	East Malling Research	Mixed varieties	open
10	East Malling Research	Mixed varieties	open

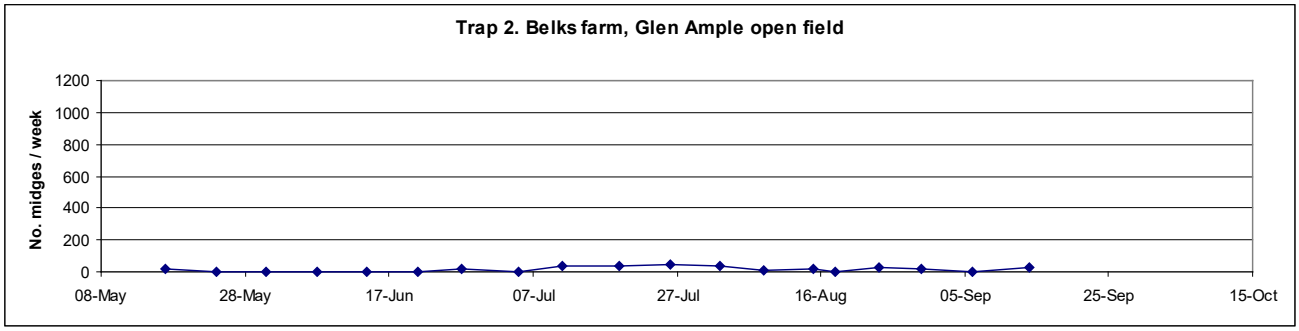
Results

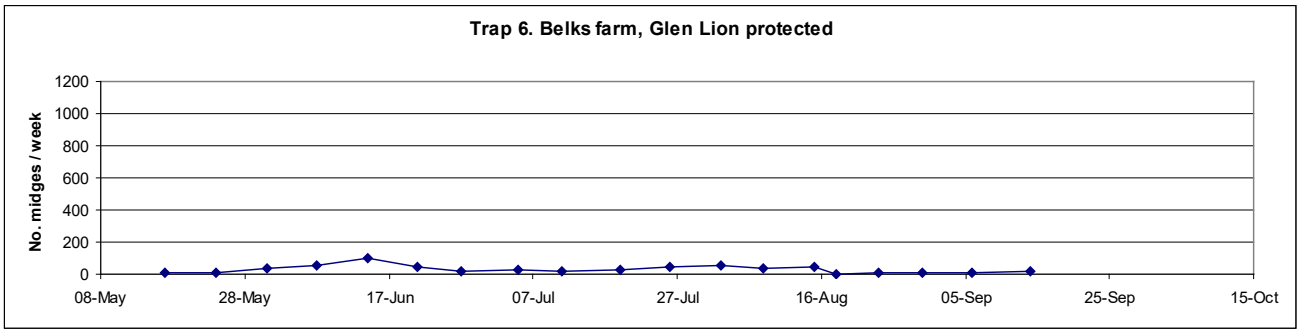
There was no clear pattern of midge emergence at the 10 sites making it difficult to discern distinct generations of midge emergence (Table 3.2.1.2, Figures 13(a) and 13(b). The first generation appears to have occurred in late May - June, the second in late July – early August, the third in the second half of August and September, but the generations were overlapping with no clear delineation between each successive generation. Small numbers of midges were captured in the first week the traps were deployed at all sites. There was evidence of the start of a first generation in May at approximately the time of the ADAS forecast but this was difficult to distinguish and numbers were small compared to numbers that emerged later in May or in June or July. There were large differences in the numbers of midges caught, very large numbers (>>1000 over the season) being caught in five of the plantations with small numbers (< 1000) in the five others. First catches from 10-17 May varied from 1 to 112 midges/trap and were not necessarily a good indication of the magnitude of subsequent total catches.

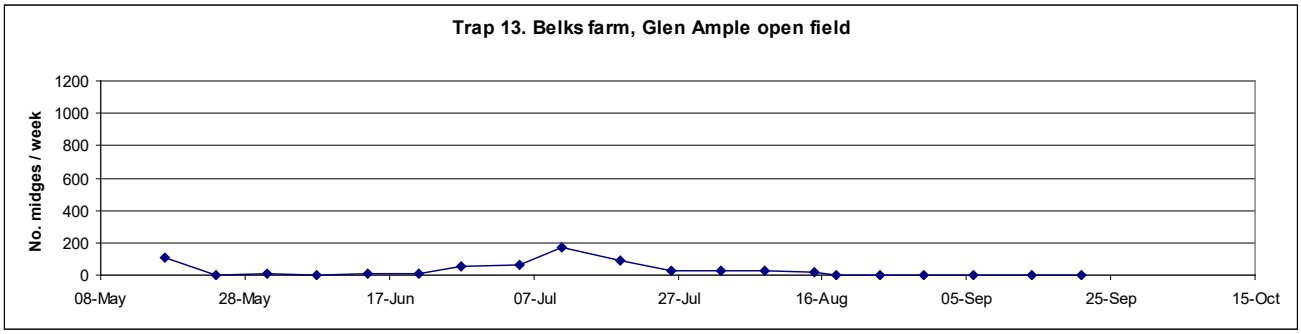
Table 3.2.1.2: Numbers of raspberry cane midge males captured

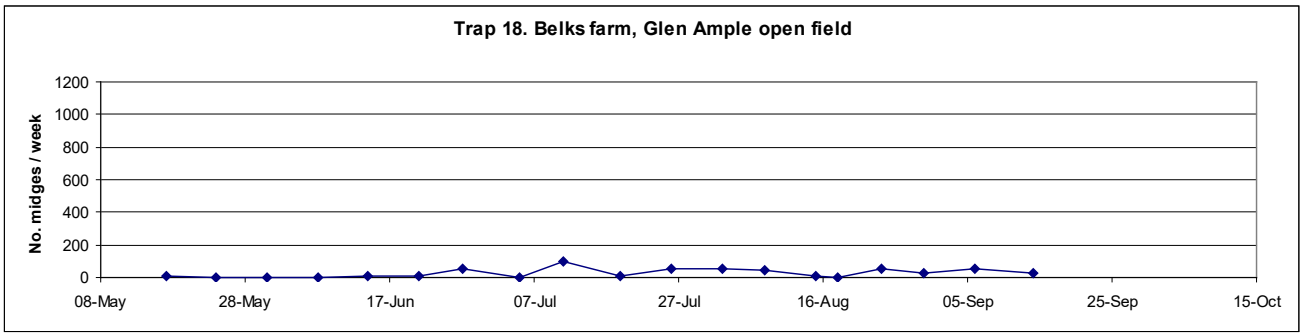
Trap	1	2	3	4	5	6	7	8	9	10
17 May	16	7	112	11	3	7	97	1	61	12
24 May	1	5	2	0	5	0	56	2	27	6
31 May	0	32	9	1	275	326	55	10	247	18
07 Jun	0	52	0	1	256	249	33	50	162	15
14 Jun	2	97	7	5	125	110	35	17	29	5
21 Jun	0	45	8	11	150	141	324	32	238	62
27 Jun	18	16	58	54	218	269	434	166	434	171
05 Jul	2	25	63	3	155	108	92	1	706	145
11 Jul	33	18	167	100	141	398	344	6	386	58
19 Jul	33	30	87	12	552	510	536	87	278	180
26 Jul	49	50	24	55	281	390	312	55	265	82
02 Aug	39	57	26	51	7	4	656	23	78	34
08 Aug	9	39	29	41	6	1	325	13	39	43
15 Aug	21	47	21	8	*	*	*	*	*	*
18 Aug	*	*	*	*	253	102	863	34	226	355
24 Aug	24	10	*	56	714	194	1060	76	151	309
30 Aug	19	13	*	29	744	69	1040	110	29	62
06 Sep	1	10	*	51	182	188	623	26	37	70
14 Sep	23	19	*	29	34	92	379	17	*	*

21 Sep			*		43	58	16	29	*	*
Total	290	572	613	518	4144	3216	7280	755	3393	1627









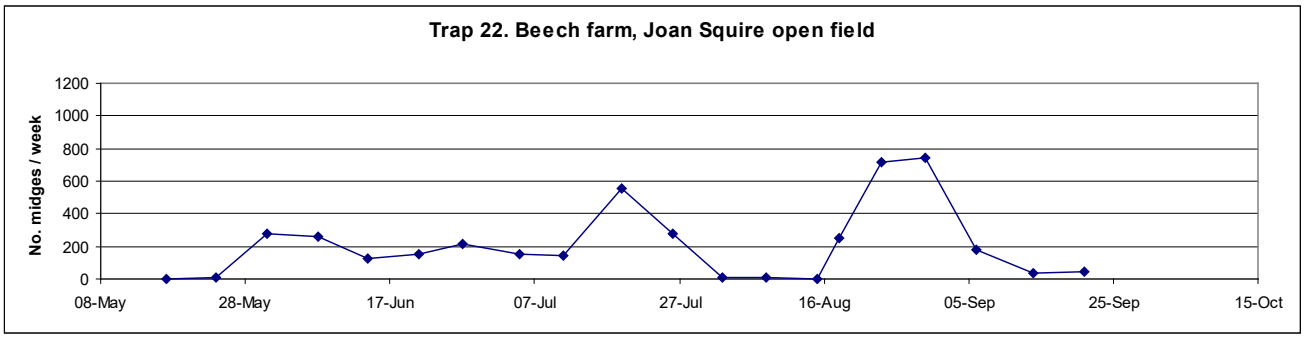
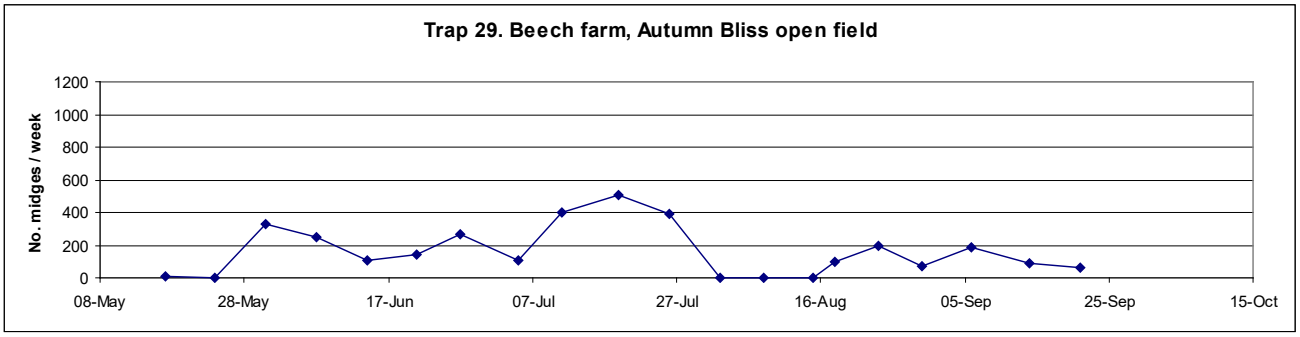
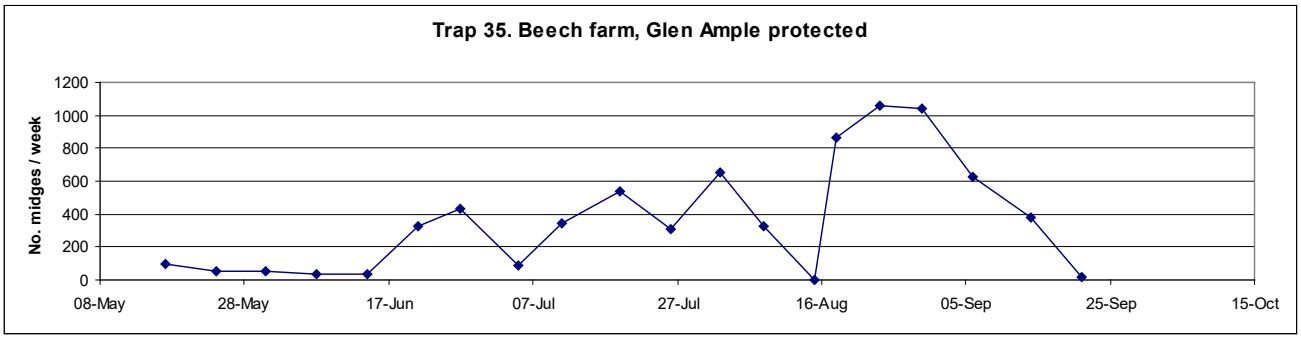
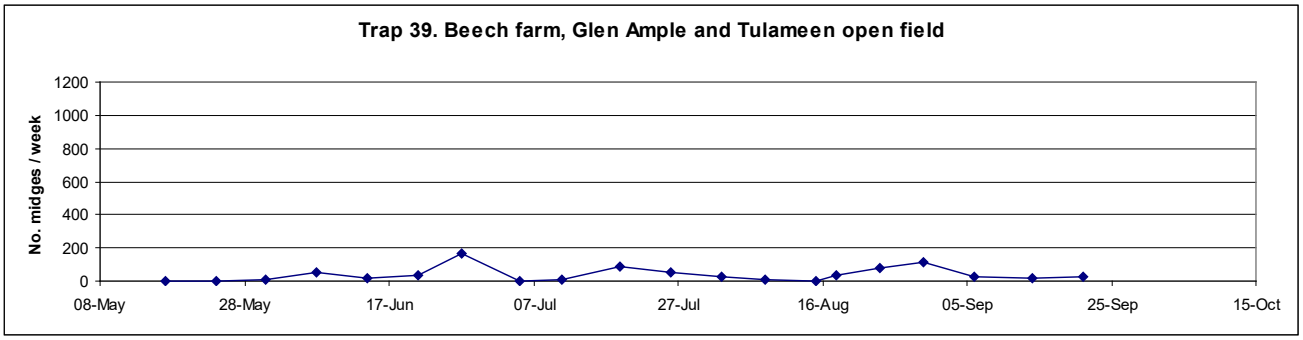
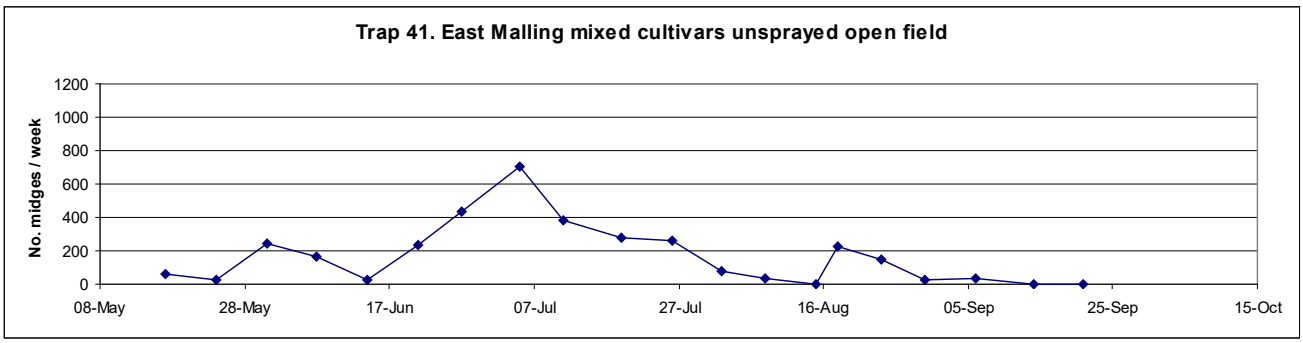


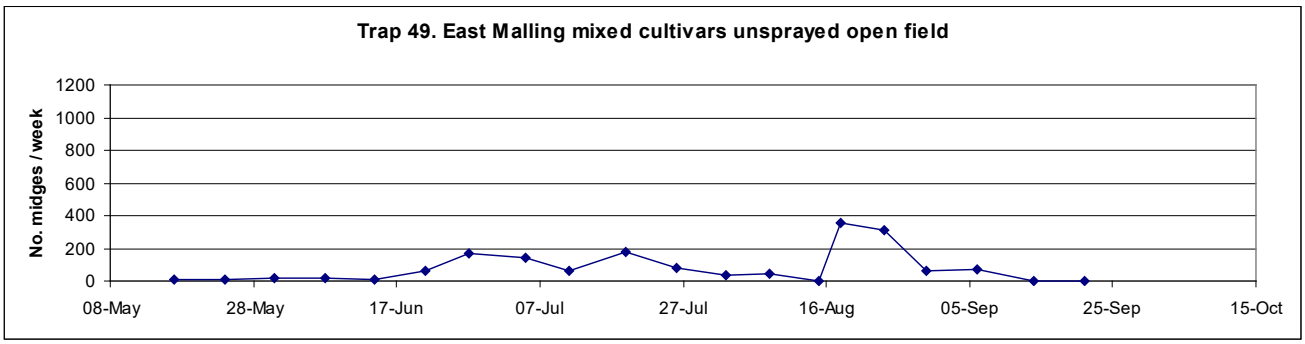
Figure 13(a): Seasonal catches of raspberry cane midge in plantations 1-5











Conclusions

- Sex pheromone traps effective and easy to use for monitoring raspberry cane midge
- Large variation between plantations in total numbers caught
- Difficult to discern generations
- Very small numbers captured in early-mid May (ADAS forecast first emergence 6 May at EMR)
- Much large numbers of midges were caught in late May/June and this may be a better time to spray
- Control of the midge post-harvest in the latter half of August and September needs to be investigated as the results indicate that there is a large 3rd generation flight at this time

Beta test of commercial use of raspberry cane midge sex pheromone trap for monitoring in commercial plantations

Raspberry cane midge sex pheromone traps were supplied to 30 growers in the UK for pre-commercial (Beta) testing in 2006. The results of these tests have not yet been obtained or collated.

EU collaborative ring test of use of raspberry cane midge sex pheromone trap for monitoring in commercial plantations in EU countries

Objectives

The overall objective of this multi-institute collaborative study was to establish an economic damage threshold for raspberry cane midge catches in sex pheromone traps and to gain information on the timing of different generations of the pest in different countries at different latitudes in Europe (Table 1). Thresholds may need to differ for different varieties and in different cropping systems.

Methods and materials

The flight activity of male raspberry cane midge was monitored through the season in raspberry plantations with different midge populations in different countries using sex pheromone traps. The numbers of midges caught was related to larval populations and crop damage.

Table 3.2.1.3: Countries collaborating in the ring test in 2006

Country	Collaborator(s)	No. of sites
UK	J Cross	3
Hungary	G Vetec	2
Italy	A Grassi	4
Norway	N Trandem	2
Sweden	T Neilsen	1
Poland	B Labanowska	2
Russia	M Shternshis	2
Serbia	S Milonkovic	?

Well separated, reasonably large (> 0.5 ha) protected and/or non-protected raspberry plantations of one or two (the dominant) cultivars were selected with different populations of raspberry cane midge in each participating country. In part of the plantation, preferably in the centre, an area not to be treated with insecticides for raspberry cane midge in 2006 was demarked. Ideally, this area was to be sufficiently wide for insecticide spray drift into the central 2-3 rows to be minimal. At each site, two standard (white) delta pheromone traps were deployed in the centre of each plantation, separated by at least 20-30m. They were suspended so that the base was at a height of 0.5 m above the ground exactly. One trap was oriented parallel to the rows, one at right angles. Each trap contained a 20 × 20 cm white sticky base (provided by EMR). Traps were baited with a standard rubber septum lure loaded with 10 µg of the raspberry cane midge sex pheromone major component racemate, which were replaced at approximately 1 month intervals. It was intended that traps were to be set out before the first flight of the midges in spring (early April in UK) and maintained and recorded weekly until the midge flight ceased at the end of the growing season (end of September in UK), though full seasons recording was not done at several sites The sticky bases were generally refreshed weekly, or at least each time a trap record was taken. Other species caught in interesting or consistent numbers were to be identified and counted.

Weekly, 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, taking care not to injure the cambium below. Each week, 10 primocanes with one week old and 10 primocanes with 2 week old splits were to be 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 from 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 can be calculated. The data was collated into spread sheets and sent to J Cross, East Malling Research.

Graphs of the mean numbers of eggs and larvae and the total of these per cm of split for each sampling occasion were plotted against the Julian date of sampling (day number from 1 January, counting 1 January as 1). Summary statistics were then extracted for each data set. These included the Julian days of the 5%, 50% and peak catches of males in the pheromone traps, the total and peak numbers caught for each generation, the Julian dates of the 5% and peak larval numbers for each generation and the total and peak numbers of eggs and larvae for each generation

Results and conclusions

To date, data from all countries except Serbia has been received, but the quality of the data varies considerably. At several sites, the pheromone trap records were not started early enough in the season and in many data sets, records of numbers of eggs and larvae in splits have not been comprehensively taken.

The Julian dates of the 5% and peak catches of midges of each of four successive generations can be ascertained from the data (e.g. Figure 14). In Italy, there was an average of 40 days between generations. It is expected that the effects of latitude on the timing of the generations will be ascertained by further analysis of the data.

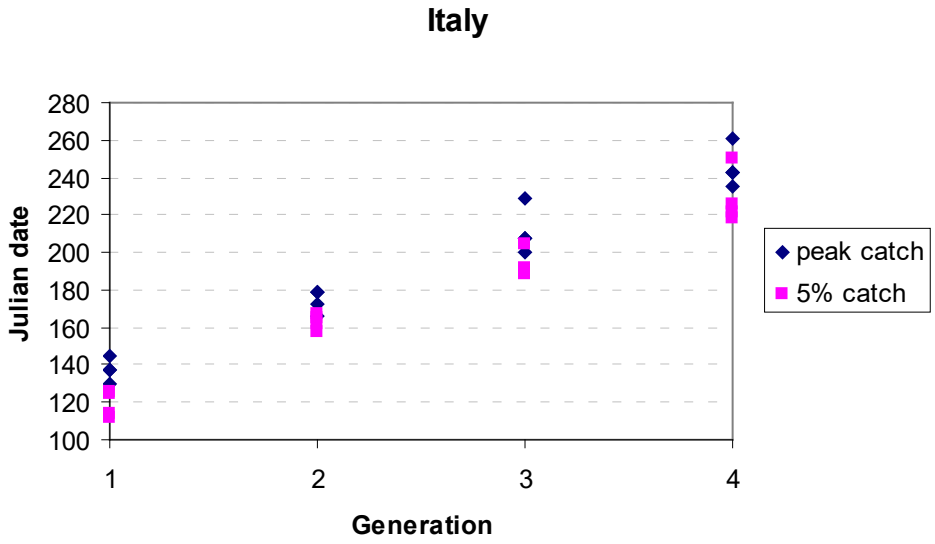


Figure 14: Julian date of the first and peak catches of four successive generations of male raspberry cane midges in four plantations in Italy in 2006

Graphs of total numbers of eggs and larvae were plotted against peak and total trap catch per generation have been plotted for the UK, Italy and Hungary to date (Figure 2). Linear regression lines through the origin had R^2 values of approximately 0.4. The following general conclusions can be derived from the data:

- A peak catch of 500 midges/generation lead to an average of approximately 20 larvae/cm of split and to a maximum of 100 larvae/cm of split

- A total catch of 1000 midges/generation lead to an average of 20 larvae per split and to a maximum of 70 larvae/cm of split

- A tolerance threshold for larvae is unknown but it is probably very low, e.g. 0.1 larvae/cm

- Therefore, the trap catch damage threshold is likely to be low. A nominal threshold of 30/trap to trigger treatment reasonable

These are preliminary conclusions which may be modified subject to inclusion of further data from 2006. It is intended that the ring test is repeated in 2007.

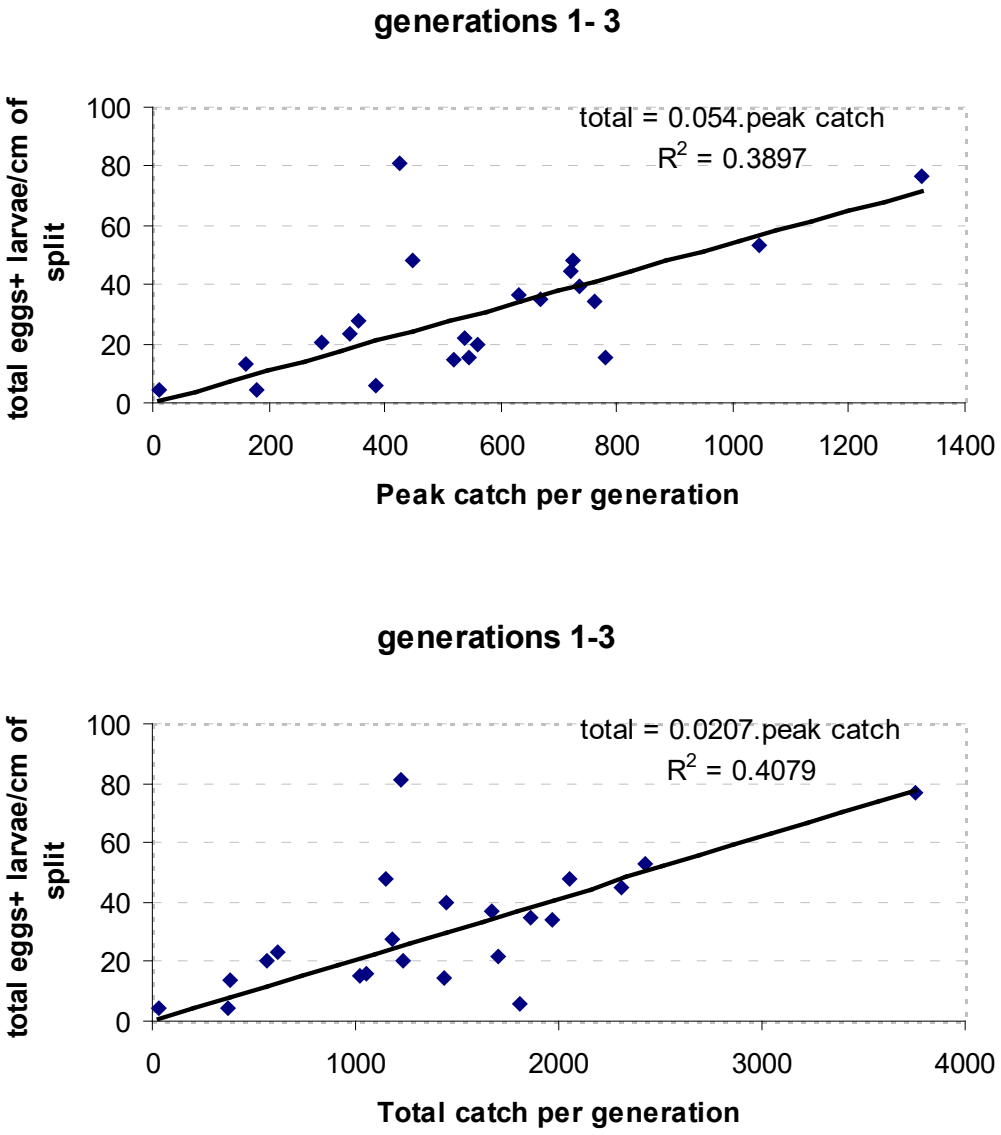


Figure 15: Relationships between peak numbers of midges (above) and total numbers of midges (below) captured per generation and the mean total numbers of eggs and larvae recorded in artificial splits for that generation. Trend lines through the origin are plotted

Objective 3.3. Identify host plant wound attractant of females

Introduction

The size of raspberry cane midge populations in the field can be quite variable depending on location and raspberry cultivar. Midges are more prevalent in southern Britain where they can cause considerable losses due to the interaction between the midges and a range of pathogenic fungi that colonise midge larvae feeding sites. In northern Britain, nearer the limit of raspberry cane midge range, there tends to be fewer midges, although in some years damage can be high.

Material and methods

Site selection and preparation

A site at SCRI (F6) has been identified as a potential source of raspberry cane midge larvae and cocoons for establishing and maintaining a reservoir of insects for research purposes. Ten over wintered raspberry canes from a single block of eight rows of raspberry cultivars Glen Clova and Malling Leo were cut in March 2006 and the bark of the lower 300mm of canes was removed to expose the presence or absence of raspberry cane midge/ midge blight 'patch lesions'. The number of canes with 'patch lesions' and the relative level of cane damage were recorded. Canes were subsequently damaged to provide oviposition sites on 08 May 2006. Canes from the same block were sampled on 14 March 2007 and levels of raspberry cane midge/midge blight damage assessed.

Results

The levels of raspberry cane midge and midge blight damage in 2006 and again in 2007 are shown in table 1 (RCM). The increase in both damaged canes and level of damage intensity indicate that with appropriate cane management adequate raspberry cane midge cocoons should be available for experimental work in 2007.

Table 3.3.1.1: (RCM): Levels of raspberry cane midge/ midge blight damage in raspberry cane midge reservoir block at SCRI

2006			**2007**		
Cultivar	No canes midge blight	Damage intensity	Cultivar	No canes midge blight	Damage intensity

Glen Clova	5	low	Glen Clova	6	moderate
Malling Leo	5	low	Malling Leo	7	moderate

Objective 3.6. Investigate the 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

Raspberry cane midge: Field evaluation of the efficacy of sex pheromone mating disruption and attract and kill treatments 2006

Summary

A large scale, dispersed randomised block field experiment was done from April-September 2006 in commercial raspberry plantations in SE and E England to evaluate the efficacy of a sex pheromone Mating Disruption (MD) treatment and an Attract and Kill (A&K) treatment in comparison with an untreated control for control of raspberry cane midge. The MD treatment comprised 2000 polythene cap dispensers per ha, each initially containing 1 mg of raspberry cane midge sex pheromone racemate, deployed in a regular lattice through the crop at a height of 15 cm. Similarly, the A&K treatment comprised 2000 devices per ha deployed in a similar way. Each device was a 10 × 6.7 cm oblong of plastic laminated card which was surface coated with a microencapsulated formulation of the SP insecticide lambda cyhalothrin (1/6 of an AgriSense Olive Fly Target Device) and which had a polythene cap dispenser, initially loaded with 100 μg of the pheromone, fixed to the centre. Nine plots (each ~1.0 ha area), three on each of three farms, were used for the experiment, each farm acting as a block. At two farms the raspberries were grown in polythene clad French tunnels. At the other farm, the raspberries were grown outdoors. The effectiveness of the treatments was assessed by deployment of a single raspberry cane midge delta pheromone trap (standard 10 μg rubber septum lure) in the centre of each plot which was monitored at 2-3 week intervals throughout the experiment, and by counting the number of raspberry cane midge larvae that developed in artificial splits made fortnightly in the primocane in the centre of each plot.

There were large differences in the levels of midge infestation at the three farms, as indicated by the total seasons midge catch in the untreated control plots (Table below). In the polytunnel crops where midge populations were low and high respectively, the

MD and A&K treatments suppressed catches by 71-85%, well short of the near complete shut down probably required for successful control. However, trap catches were suppressed by 99% in the outdoor crops where population levels were intermediate. In the polytunnel crops, there was no evidence that either the MD or A&K treatments gave any control of larvae in artificial splits. Indeed, at one site numbers of larvae were markedly lower on the untreated control plot than the MD or A&K treated plots and at the other numbers were similar. However, in the outdoor crops, the A&K treatment appeared to give complete control of larvae and the MD treatment 98% control.

There was substantial (~50%) loss of pheromone when the MD polythene cap dispensers were initially dosed in the laboratory. Rapid release of pheromone occurred in the field, especially in the polytunnel crops where only approximately 20% of the initial amount applied remained after 1 month. The rate of release was lower in the outdoor crop, where 40% remained after 1 month.

These results suggest that the MD and A&K treatments were effective outdoors but were ineffective in the polytunnel crops. One possible explanation for this difference in efficacy suggested by the data is that pheromone release was too rapid from the dispensers when they were deployed in the polytunnels where temperatures were much higher than outdoors. Another possible explanation is that the pheromone did not disperse effectively in the enclosed polytunnel environment. However, caution must be exercised in drawing conclusions from these results. The effectiveness of the MD and A&K treatment in the outdoor crops was based on a single replicate. A dispenser with a more uniform release rate over a longer period should be sought before further large scale field trials are done.

Table 3.6.1.1:

Farm, raspberry cropping system		Pheromone trap catch of males			Larval numbers in artificial splits		
		Seasons total in untreated	% trap suppression		Seasons total in untreated	% control of larvae	
			MD	A&K		MD	A&K
1	Polytunnel	41	85	71	6	0	0
2	Polytunnel	4867	85	80	317	0	0
3	Outdoor	445	99	99	40	98	100

Introduction

The overall aim of the work is to determine whether the raspberry cane midge sex pheromone can be exploited for control of the midge in commercial raspberry plantations by mating disruption, where the pheromone is deployed alone, or by attract and kill where the pheromone is deployed in conjunction with lambda cyhalothrin coated target devices.

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

Methods and materials

One large scale, dispersed randomised block field experiment was done from April - October 2006 to evaluate the efficacy of a Mating Disruption (MD) treatment and an Attract and Kill (A&K) treatment in comparison with an untreated control for control of raspberry cane midge in commercial raspberry plantations in SE England.

Sites

Nine raspberry plots (cv. Gen Ample) each of approximately 1.0 ha area were selected on three farms in SE and E England for the experiment (Table 3.6.1.1). At two of the farms, Belks Farm and Salmans Farm, the raspberries were grown in polythene tunnels. At Barn Farm, the crop was in the open field with no protection.

Treatments

Treatments are given in Table 3.6.1.3. MD devices were low density polythene caps which were each initially loaded with 1 mg of the midge sex pheromone racemate. The A&K devices were 10 x 6.7 cm oblongs of white plastic laminated card which were surface treated with a microencapsulated formulation of the SP insecticide lambda cyhalothrin (1/6 of an AgriSense Olive Fly Attract and Kill Target Device) with a polythene cap dispenser initially loaded with 100µg of the pheromone fixed to the centre with a drawing pin. The polythene cap dispensers were loaded with the pheromone in a fume cupboard in the laboratory. Solutions of the raspberry cane midge pheromone racemate in dichloromethane (10 g/l for the MD dispensers, 1 g/l for the A&K devices) were prepared. 100 µl of solution was dispensed into the well of each

cap using a micropipette and the solvent allowed to evaporate, a process which took less than ½ an hour. The caps were stored in the freezer until use.

Treatment application

Treatments were deployed by the farm staff on 26, 27 and 28 April 2006 at Belks Farm, Salmans Farm and Barn Farm respectively. The devices for treatments 2 and 3 were fixed to the horizontal wire or to the primocane with a twist tie at height of approximately 15 cm above the ground in a regularly spaced lattice e.g. at Barn Farm, where the row spacing was 3.0 m, devices were deployed every 1.75 m along each row.

Experimental design

A large-scale dispersed, randomised block experimental design to be used with three replicates of the three treatments (Table 3.6.1.2). Plots were 1 ha areas of commercial protected raspberry plantation, three plots on each of 3 farms. Each farm is regarded as a separate block in the experimental design. Untreated plots were well separated from the MD and A&K treated plots, which were themselves adjacent (see above). For allocation of plots to treatments see Table 3.6.1.3.

Insecticide treatments

A sub-plot comprising one central tunnel of three rows per plot, or six rows in the centre of each plot in the outdoor raspberries at Barn Farm, was left untreated with insecticides for the duration of the experiments. No insecticide treatments for cane midge (chlorpyrifos) were applied to the rest of the plantations at Belks Farm.

Assessments

Release rate of pheromone from dispensers in field: At approximately fortnightly intervals, two replicate high dose and two replicate low dose polythene cap dispensers were collected from each MD and A&K plot respectively. The pheromone remaining in the each cap was extracted in solvent and the amount present measured by GC analysis.

Populations of males: A single sex pheromone trap, bated with a standard rubber septa lure loaded with 10 µg of the sex pheromone racemate (the standard adopted for monitoring purposes) was deployed in the centre of each plot at a height of 0.5 m above the ground. The number of midges captured was counted at intervals throughout the experiment. Lures were renewed monthly.

Larval populations in splits in canes: Approximately 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 to avoid injury 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 can lay their eggs. The canes in which artificial splits were made were marked with coloured tape so that they can easily be re-located.

Approximately fortnightly, 20 primocanes with 2 week old splits, 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. The length of each split was be recorded so that the number of larvae per unit length of split could be calculated.

Data collation and statistical analysis

Data was collated into Excel spreadsheets and means calculated. Further statistical analysis of the data was considered unnecessary.

Table 3.6.1.2: Locations of plots

Plot	Plantation	Polytunnel?
Farm 1. W B Chambers & Son, Belks farm, Otham, Kent ME15 8RL		
1	Field 20 at Belks Farm Otham. NGR TQ 8052 0191 Glen Ample, 1.53 ha. 16 tunnels 107 m long	Yes
2	South half of field 29 at Belks farm, Otham. NGR TQ 8052 2489. Glen Ample, 0.82 ha. 11 tunnels 165 m long	Yes
3	North half of field 29 at Belks farm, Otham. NGR TQ 8052 2489. Glen Ample, 0.82 ha. 11 tunnels 165 m long	Yes
Farm 2. Salmans Ltd, Home Farm, Penshurst Road, Bidborough, Tunbridge Wells, Kent TN3 0XH		
4	Eastern part of Field 14, New Field Ample at Penshurst. NGR TQ 517 440. 12 tunnels 150 m long. Area 1.4 ha.	Yes
5	Eastern half of Field 2, Lower Ample at Penshurst. NGR TQ 517 440. 0.9 ha area comprising 10 tunnels 120 m long. Divided from western half by windbreak. Rows run N-S approx.	Yes
6	Western half of field 2, Lower Ample at Penshurst. NGR TQ 517 440. 0.9 ha area comprising 11 tunnels 120 m long. Divided from eastern half by windbreak. Rows run N-S approx.	Yes
Farm 3. Barn Farm, Wix Road, Bradfield, Manningtree, Essex CO11 2UX		
7	Pheasant Run. Glen Ample, NGR TM 148 300 3 m row spacing. Area approximately 1.5 ha. Rows run ESE-WSW.	No
8	Eastern half of Churchfield. Glen Ample. NGR TM 148 300 1.1 ha area consisting of 26 rows spaced 3 m apart and 140 m long. Rows run East –West. Cross alley at 140 m from West end.	No
9	Western part of Churchfield. Glen Ample. NGR TM 148 300 1.5 ha area consisting of 26 rows spaced 3 m apart and 200 m long. Rows run East –West. Cross alley at 140 m from West end.	No

Table 3.6.1.3: Treatments and allocation to plots

No.	Control approach	Pheromone lure	Insecticide target device‡ (size)	No. of devices /ha	Dose /ha	Plot numbers
1	Untreated control	None	None	None †	0	1, 4, 7
2	Mating disruption	1 mg cap	None	2000	2 g	2, 5, 8
3	Attract and kill	100 µg cap	6.7 x 10 cm	2000	0.2g	3, 6, 9

‡ Olive Fly Attract and Kill (A&K) Target Device are squares of plastic coated cardboard surface coated with 0.05% lambda cyhalothrin

Figure 16: (a) MD device fixed to primocane (left) (b) A&K device fixed to primocane

Results

Effect of treatments on pheromone trap catches

There were large differences in the levels of midge infestation at the three farms. Populations were low at Belks Farm (total of 41 midges captured in untreated), moderate at Barn Farm (total 445 midges caught in untreated) and at Salmans Farm (total of 4867 midges caught in untreated) (Table 3.6.1.4, Figure 17). The MD and A&K treatments suppressed catches by 85% and 81% on average, respectively. The degree of trap suppression (99%) at Barn Farm (outdoor) was greater than at the other two sites.

Table 3.6.1.4: Catches in standard (white delta with10 μg rubber septa) sex pheromone traps in centre of each plot 2006

Treatment	Plot (Fm)	1st gen Apr-May	2nd gen Jun-Jul	3rd gen Aug-Sep	Total
MD	2 (1)	3	3	*	6
	5 (2)	206	6	542	754
	8 (3)	1	3	0	4
	Mean	70	4	271	345
A&K	3 (1)	8	4	*	12
	6 (2)	361	26	570	957
	9(3)	6	0	0	6
	Mean	125	10	285	420
Untreated	1 (1)	8	33	*	41
	4 (2)	546	1822	2499	4867
	7 (3)	325	17	103	445
	Mean	293	624	1301	2218

Control of larval infestations

There was no evidence that either the MD or A&K treatments had any suppressive effect on numbers of larvae in artificial splits at Belks Farm or Salmans Farm (Table 3.6.1.5, Figure 18). Indeed, at Belks Farm, numbers of larvae were markedly lower on the untreated control plot than the MD or A&K treated plots. At Salmans Farm, numbers were very similar. However, at Barn Farm, larval numbers were consistently and substantially lower on the MD and A&K treated plots than the untreated control. The A&K treatment appeared to give complete control of larvae and the MD treatment 98% control.

Release rate of pheromone from dispensers

Measurements of the amounts of pheromone in dispensers on Day 0, i.e. after dosing in the lab but before deployment in the field, indicate that approximately 50% of the pheromone racemate was lost during the solvent drying process (Table 3.6.1.6). The MD dispensers were dosed with 1 mg of pheromone racemate but only 525 µg was measured at Day 0. The A&K dispensers were dosed with 100 µg of which 91 µg remained, so little loss appears to have occurred at the lower dose.

Rapid release of the pheromone occurred subsequently in the field. In the polytunnel crops, only 20-30% of the Day 0 pheromone amount remained after 1 month. This equates to an average release rate of ~500 ng pheromone per dispenser per hour from the MD dispensers and ~100 ng pheromone per dispenser per hour for the A&K dispensers. In the outdoor crops, release rate were lower, 40% of the day 0 amount remaining after 1 month.

Table 3.6.1.5: Total number of cane midge eggs plus larvae per 10 cm of split

Treatment	Plot	1st generation May-mid Jun	2nd generation Mid Jun-mid Jul	3rd generation Mid Jul-Aug	Total
MD	2	31.6	7.8	35.6	75.0
	5	45.2	137.5	148.5	331.2
	8	0.9	0.0	0.0	0.9
	Mean	25.9	48.4	61.4	135.7
A&K	3	48.6	23.1	29.2	100.9
	6	60.4	213.7	85.0	359.1
	9	0.0	0.0	0.0	0.0
	Mean	36.3	78.9	38.1	153.3
Untreated	1	0.0	1.8	4.3	6.1
	4	18.8	123.3	174.8	316.9
	7	25.7	13.7	0.5	39.9
	Mean	14.8	46.3	59.9	121.0

Table 3.6.1.6: Mean and % amounts of pheromone (µg racemate) remaining in dispensers at intervals after deployment in the field. Note that the % amounts are expressed as a percentage of the amounts on Day 0 (after dosing in the lab and before deployment). Means are of two replicates, except the Day 0 samples which are of six replicates

Farm	Date	Interval (days)	MD lures ‡		A&K lures ‡	
			µg racemate	% remaining	µg racemate	% remaining
	Day 0	0	583	100	90	100
Belks Farm	25 May	29	168	29	18	20
(polytunnel)	7 Jun	42	119	20	18	20
	23 Jun	58	84	14	14	16
	7 Aug	103	23	4	6	6
	21 Aug	117	15	3	2	2
Salmans Farm	4 May	7	297	51	46	51
(polytunnel)	19 May	22	176	30	29	32
	26 May	29	277	53	38	42
	2 Jun	36	126	22	22	25
	15 Jun	49	78	13	14	16
	11 Aug	106	19	3	2	2
	25 Aug	120	4	1	19	21
	25 Sep	151	6	1	0	0
Barn Farm	11 May	13	235	40	35	38
(outdoor)	7 Jun	40	226	39	39	43
	23 Jun	56	147	25	7	8
	3 Aug	97	32	6	*	*
	17 Aug	111	37	6	5	6

‡ MD and A&K lures were dosed with 1mg and 100 µg of pheromone racemate in 100 µl of dichloromethane respectively, but approximately 50% was lost as the solvent evaporated.

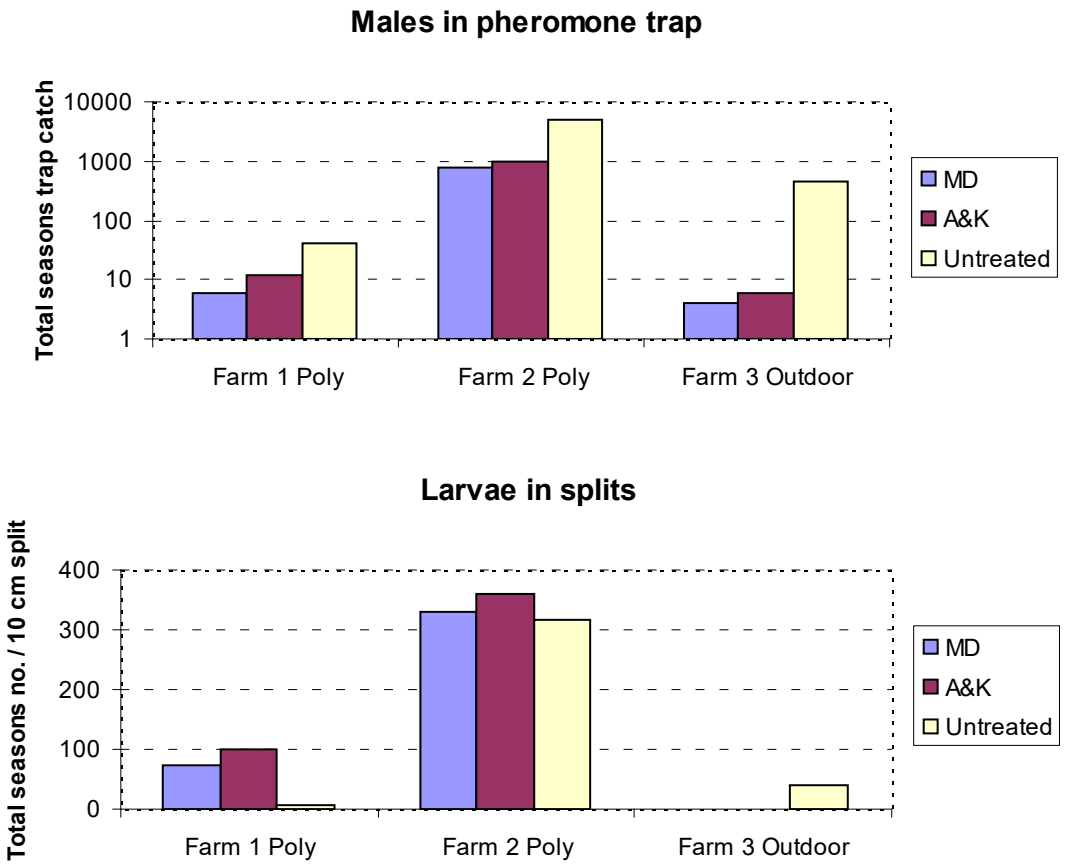


Figure 18: Total seasons catch in delta pheromone trap in centre of each plot (upper histogram, note Log_{10} scale) and total seasons number of larvae recorded per 10 cm of artificial split (lower histogram)

Conclusions

- The MD and A&K treatments suppressed pheromone trap catches by 71-85% in the polytunnel crops where the populations were low and high respectively. This degree of suppression fell well short of the near complete shut down probably required for successful control

- Trap catches were suppressed by 99% in the outdoor crops where population levels were intermediate

- In the polytunnel crops, there was no evidence that either the MD or A&K treatments had any suppressive effect on numbers of larvae in artificial splits

- In the outdoor crops, the A&K treatment appeared to give complete control of larvae and the MD treatment 98% control

- There was substantial (~50%) loss of pheromone when the polythene cap dispensers were initially dosed in the laboratory

- Rapid release of pheromone occurred in the field, especially in the polytunnel crops where only approximately 20% of the initial amount applied remained after 1 month

- The rate of release was lower in the outdoor crop, where 40% remained after 1 month

- These results suggest that the MD and A&K treatments were effective outdoors but were ineffective in the polytunnel crops

- One possible explanation for this difference in efficacy suggested by the data is that pheromone release was too rapid from the dispensers when they were deployed in the polytunnels where temperatures were much higher than outdoors

- Caution must be exercised in drawing conclusions from these results. The effectiveness of the MD and A&K treatment in the outdoor crops was based on a single replicate

- A dispenser with a more uniform release rate over a longer period should be sought before further large scale field trials are done

Objective 4: Mildew

Task 4.1 Inoculum sources

4.1.1. Field monitoring of cleistothecia/ascospore development and disease development

Mildew development was monitored in order to understand the mode of overwintering and subsequent spread during the growing season. Several plants from EMR breeding programme infected with mildew in 2005 were dug out and potted up in late 2005. These plants were then maintained in a polytunnel throughout the winter and the following growing season (2006). Mildew development was regularly monitored.

Preliminary results from the monitoring can be summarised as:

- Mildew appeared to over winter in the buds (as mycelia and/or conidia) because all leaves on several shoots were completely covered with mildew colonies at the time of their emergence, just like apple powdery mildew
- Unlike other mildews, the spread of mildew from these primary lesions to leaves of other canes (even of the same plant) is very rare under the tunnel conditions. In contrast, powdery mildews on blackcurrant and apple in the same tunnel spread very rapidly
- Several artificial inoculation experiments were carried out but these failed to transfer mildew to healthy leaves

We have tested whether molecular markers (SSR) developed for strawberry mildew could be used for raspberry mildew and confirm that this is the case. Based on a limited number of mildew samples, SSR results suggested that raspberry mildew is different from strawberry mildew. This will be further tested in the coming season using other genes. Currently CSL is testing whether the Taqman method developed for quantification of strawberry mildew could be used for raspberry mildew as well.

Task 4.3 Control agents

4.3.1 Glasshouse and field evaluation of natural products and commodity substances for control of powdery mildew

Experiment 1 - Cambridge, 2005

Materials and methods

A number of raspberry crops were visited throughout the summer and early autumn 2005 to collect this obligate pathogen in order to allow multiplication on culture plants prior to trial inoculation. However, no natural infection was found until October and disease did not develop when spreader plants were inoculated. The trial was thus run in order to gather information on any possible phytotoxicity of the products which did not have recommendations for use on protected raspberries.

The phytotoxicity of various products was evaluated in a replicated trial on potted raspberry plants, cv. Glen Ample, in a glasshouse in Cambs. Fungicide products with known efficacy against powdery mildew (Amistar, Systhane 20E and Rubigan) were selected for comparison, together with a new fungicide product (UKA379a). The commodity substance potassium bicarbonate, permitted as a fungicide on raspberries (principally used against powdery mildew), and the natural compound pest control products Majestic and Agri-50E which produce a physical barrier were also applied. The plant extract, Milsana, and the microbial product Companion (containing *Bacillus subtilis*) were also tested. All the products were applied at 7 day intervals, at a volume of 1000 L/ha, with six application dates from 7 October 2005.

Results and discussion

Distinctively phytotoxic symptoms (with the whole plant affected) were leaf margin browning, mosaic and leaf thickening was caused by potassium bicarbonate plus wetter. Majestic sprayed leaves had brown, curled-up margins. Companion treated plants had mosaic leaves with rolling and brown margins. Systhane 20 EW had leaf down-rolling, areas of chlorosis and some brown margins. It was noted that the Milsana treated leaves were a healthy-looking darker green than the control plants. There was no significant difference in the phytotoxicity index between the treatments, possibly because many plots were recorded as affected when some symptoms may have resulted from other causes, such as earlier high glasshouse temperatures.

Spray residues on the leaves were not obvious, except with the experimental product HDCF5, although Frupica SC produced some deposit.

Table 4.3.1.1: Phytotoxicity and spray residues following application to glasshouse grown raspberry plants

Product	Dose rate per ha at 1000 L water / ha	Mean phytotoxicity index (0-5)	Mean residue index (0-3)
Control	-	0.8	0
Amistar	1 L/ha	1.7	0.3
Systhane 20 EW	45 ml / 100L	2.1	0
Rubigan	330 ml/ha	1.0	0.3
Frupica SC	0.68 L/ha	0.9	1.0
Majestic	25 ml/L	2.5	0
Agri-50E	3 ml/L	1.4	0
HDCF5	1.44 kg/ha	0.5	3.0
Pot. Bicarbonate	5 g/L	3.0	0
Milsana	5 ml/L	0.8	0
Companion	1 ml/L	2.1	0
Significance		P=0.224	P<0.001

Experiment 2 - Cambridge, 2006

Material and methods

In 2006, the powdery mildew control trial was repeated, but using cv. Joan Squire because of its high susceptibility to the disease. Two canes were planted per 10 L pot in February 2006, and placed in a glasshouse at 18°C with two pots per plot raised off the floor in four replicate blocks, with two water control treatments.

In May, June and July 2006, `spreader` plants of cv. Glen Ample were inoculated with spores from young leaves affected by powdery mildew. Systemically infected plants obtained from EMR variety trials were also positioned amongst the plants. Both methods of inoculation were unsuccessful. On 17 August, heavily infected leaves from SCRI were used to inoculate both the spreader plants and the plots of cv. Joan Squire. Both dry spores and a spore spray were applied and the plants covered by misted bags for 72 hours. No powdery mildew developed during the trial.

Ten products were selected (Table 4.3.1.2) and applied three times at 1000 L/ha at 7 day intervals, commencing 25 August 2006.

Table 4.3.1.2: Products applied in Cambridgeshire glasshouse trial against powdery mildew (Experiment 2)

Product	Active ingredients	Rate /ha	Rate based on
1. Control	Water	-	
2. Amistar	Azoxystrobin (250 g/L)	1 L/ha (1 ml/L)	SOLA 1194/05 for raspberry (AEA 3 appl.)
3. Systhane 20EW	Myclobutanil (20% w/w)	45 ml/100 L (0.45 ml/L)	SOLA 1189/05 for protected raspberry
4. Rubigan	Fenarimol (120 g/L)	330 ml/ha (0.33 ml/L)	Approval on raspberry
5. Signum	Boscalid (26.7% w/w) Pyraclostrobin (6.7% w/w)	1.8 kg/ha (1.8 g/L)	Extension of use 1673/04 protected strawberry (AEA for raspberry)
6. Folicur	Tebuconazole (250 g/L)	0.8 L/ha (0.8 ml/L)	SOLA 0897/05 for raspberry
7. UKA379a	Experimental product	1.44 kg/ha (1.44 g/L)	Supplier's trials on protected strawberry
8. Potassium bicarbonate	Potassium hydrogen carbonate (99.9% w/w) commodity substance	5 g/L (no wetter)	A commercially used rate as a fungicide on raspberry
9. Milsana + wetter	Extract of giant knotweed, *Reynoutria sachalinensis*	5 ml/L + 2.5 ml/L wetter	Supplier's test rate of 0.5% + 0.25% T/S Forte wetter
10. Farm-Fos 44	Potassium phosphite	5 L/ha (5 ml/L)	Raspberry foliar feed 2.5 – 10 L/ha (max 1% concentration)
11. NF 149	Experimental product	125 ml/100L (1.25 ml/L)	Supplier's trials on strawberry powdery mildew

Results and discussion

Plants sprayed with potassium bicarbonate developed necrotic spots on the leaf blades within five days of the first application. No other products caused phytotoxicity, and no spray residues were seen.

In both years, the potassium bicarbonate caused leaf scorch with or without wetter, so this commodity product does not appear to be as crop safe as commercially formulated fungicides.

The conditions for successful powdery mildew inoculation were not achieved. These might be either poor plant tissue susceptibility (possibly influenced by nutrition or tissue maturity) or failure to provide specific temperature or humidity levels for spore germination and germ-tube penetration. In both 2005 and 2006, powdery mildew was rarely seen on commercial crops – it normally spreads from the hottest area of a tunnel. In the past, it was routinely found in glasshouses, where it may over-winter on the November emerging primocane or on buds. It is possible that with both the adoption of telescopic tunnels, and the increasing use of fleece protection, that the resulting raised humidity levels may favour a resurgence of powdery mildew.

Objective 5. To determine whether raspberry aphids can be adequately controlled by early or late season sprays of aphicides supplemented with introductions of biocontrol agents in spring and summer

Task 5.1.1. Evaluate autumn control strategy and identify most effective product and timings

Autumn control of aphids on raspberry 2005 – 06

Introduction

The objective of this experiment is to test different timings of autumn sprays of thiacloprid (Calypso) for the control of aphids, including small and large raspberry aphids, in commercial raspberry production.

Methods and materials

A large scale replicated experiment comparing 5 different timings of single applications of the aphicide thiacloprid (Calypso) with an untreated control was done in commercial plantations at a farm in Kent in autumn 2005-spring 2006.

Sites

Two adjacent Glen Ample plantations at Clockhouse Farm, Linton, Kent, were used.

Old Platt (No. 212) NGR TQ745 505: Consisting of 54 rows 118.5 m long. Rows are spaced 9' (=2.74 m) apart. Total area of plantation = 1.75 ha. Tunnels cover pairs of rows, but are not covered with polythene. The rows run NNE SSW. The plantation was pruned and trained in mid-August 2005.

Shaw Field (No. 211) NGR TQ 744 505: Consisting of 48 rows 158.9 m long. Rows are spaced 9' (=2.74 m) apart. Total cropping area of plantation = 2.09 ha. Tunnels cover pairs of rows, but are not covered with polythene. The rows run NNE SSW. The plantation was pruned and trained in mid-August 2005.

Treatments

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

Table 5.1.1.1: Treatments

Treatment no.	Product	Active ingredient	Dose rate (/ha)	Dates of application 2005
1	Calypso	480 g/l thiacloprid SC	250 ml	28 Aug
2	"	"	"	8 Sept
3		"	"	22 Sept
4	"	"	"	6 Oct
5	"	"	"	20 Oct
6	Untreated	-	-	-

Treatment application

Calypso has a SOLA for use on outdoor raspberry (1494 of 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. Because of the presence of the tunnels, sprayer access was only possible in every other row, the legs of the tunnels blocking alternate rows. In effect, the crop was therefore regarded as consisting of two row beds on a double (5.48 m) row spacing. Sprays were applied at 500 l/ha with a modified Hardi mini variant air assisted sprayer by farm spray operator under the supervision of EMR staff. The sprayer had four air/spray jets per side. The forward speed was 6 kmh. Spray application was made one-sided to each side of the pair of rows in the bed. Blue Albuz ATR nozzles at a pressure of 9.5 bar gave the appropriate flow rate of 3.425 l/nozzle/minute.

Experimental design and layout

A randomised complete block experimental design with four replicates of the six treatments (= 24 plots) was used. Blocks 1 and 2 were in Old Platt plantation, blocks 3 and 4 were in Shaw Field plantation, the entire areas of both plantations being used for the experiment. Plots were two rows wide and the full length of the plantation (~100 m) long, with two guard rows between plots. The central 80m in each plot was assessed.

Assessments

Small raspberry aphid (*Aphis idaei*) was not detected. Large raspberry aphid (*Amphorophora idaei*):occurred in adequate numbers for assessment.

Winter eggs: The number of overwintering eggs on a sample 96 canes per plot (one cane per stool on each of 16 stools in each of six 8m lengths of row per plot) on 19-24 January 2006.

Summer breeding stages: Unfortunately, a heavy overnight fall of snow in mid-April 2006 caused the tunnels in Shaw Field plantation (blocks 2 and 3) to collapse and this part of the experiment was lost. The assessment on blocks 1 and 2 was delayed until 20 April 2006, as late as possible before first insecticide spray. Numbers of growing shoots infested on a sample of 60 canes (10 in each of 6 8m lengths of row per plot) were counted.

Statistical analysis

Analysis of variance was done on the data, followed by separation of means using Duncan's multiple range test (P = 0.05).

Results and conclusions

All the Calypso spray timings greatly reduced populations of large raspberry aphid eggs that were found in the dormant period in January (Table 2). The different spray timings did not differ significantly when means were compared in a Duncan's multiple range test, but trends in the data suggest that the best control was achieved with the spray on 6 October, which reduced populations by 97%. There were no significant

treatment differences in the numbers of adults and nymphs in April due to variability in the data and the fact that only two replicates of data were available. However, all the spray treatments had smaller mean numbers of aphids than the control and the 20 October application date had the smallest numbers of aphids. Numbers of small raspberry aphid were too small to draw conclusions from the data. The experiment is being repeated in autumn 2006 to spring 2007.

Table 5.1.1.2: Mean numbers and square root transformed numbers of large raspberry aphid eggs found per 96 canes on 19-24 January 2006 and mean numbers of large raspberry aphid adults and nymphs per 60 canes on 20 April 2006

Date of Calypso application 2005	Eggs/96 canes 19-24 Jan 06		Aphids/60 canes 20 April 06		
	n	√n †	Adults	Nymphs	Total
28 Aug	23.25	4.24 b	5.5	6.5	12.0
8 Sept	12.5	3.49 b	5.0	3.5	8.5
22 Sept	7.5	2.44 b	3.5	13.5	17.0
6 Oct	5.25	1.78 b	3.0	5.5	8.5
20 Oct	12.25	3.18 b	0.5	0	0.5
Untreated	145.75	10.73 a	5.1	30.9	36.0
Fprob		<0.001			
SED (15 d.f.)		1.658			
LSD (P = 0.05)		3.533			

† Means followed by the same letter do not differ significantly in a Duncan's multiple range test (P = 0.05)

Technology transfer

Presentations

A 30-minute lecture was given by Jerry Cross to the ADAS/EMR Soft fruit conference entitled 'Addressing Pesticide Residues in Raspberries through a New HortLINK Project.' A summary of the presentation was published in the conference proceedings (see below).

Publications

Jerry Cross, Angela Berrie, Xiangming Xu Colin Gutteridge, Tim O'Neill, Erika Wedgwood, Janet Allen, David Hall, Dudley Farman, Stuart Gordon, Nick Birch, Enzo Casagrande. *2006.* Addressing Pesticide Residues in Raspberries through a New HortLINK Project. Proceeding of the 2006 ADAS/EMR Soft Fruit Conference, Sutton Coldfield, 5pp

K Sugden, 2006. New Horticulture LINK project to develop integrated pest and disease management programme for protected raspberries. *Grower* Sept 2006

Jerry Cross, David Hall & Lakmali Amarawardana, 2006. Pheromones of midge pests of fruit crops. National Fruit Show Handbook 2006

K Sugden, 2006. New Soft Fruit Horticulture LINK projects get the go-ahead. *Grower* October 2006

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Craig Williamson, Barn Farm, Bradfield, Essex
Harry Wooldridge, Beech farm, W. Peckham, Kent
Tim Newton, Berry World

Richard Harnden, KG Fruits
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Appendix 1: Questionnaire either posted and e-mailed to growers

Occurrence of fungal diseases on tunnel-covered summer-fruiting raspberry – 05

Background information
(please enter information, or tick the relevant box, as required)

1. Farm address (optional)	2. County (if you do not wish to give full address)

3.. In which county(ies) do you grow protected raspberries?

4. Which variety/ varieties do you grow and protect by polythene clad tunnels from immediately pre-flowering until the end of harvest? (tick 1 or more boxes). Please give approximate area of each.

Glen Ample	Tulameen	Other(s)(please specify):

For the following questions, please answer with reference to ONE established (2 or more years old) crop of Glen Ample or Tulameen that was covered in 2005, and how you managed that crop and tunnel in 2005. Choose your main (largest area) crop of Glen Ample or Tulameen that was covered.

Tunnel type, cover and its management

4. Variety chosen (please tick)

Glen Ample	Tulameen

5. Year the chosen crop was planted (please tick)

2003	2002	2001	2000	1999	1998	1997

6. Type of tunnel

Spanish	Other (please specify)

7. Type of cover

Clear	Luminance THB	ADR	Solotrol	Other (please specify)

8. Size of tunnels (approximate)

Length (metres)	Width (metres)	Nº of rows planted:

9. Are you able to increase the tunnel ventilation?

Yes	No	How do you increase ventilation?

10. How do you decide when to ventilate the tunnels

Decision based on:			
Temperature	RH	Temp and RH	Other (please specify)

Your raspberry production system

11. Planting system

On the flat	Raised bed	Other

12. Floor cover across alleyways

Bare soil	Tumbledown	Sown grass sward	Mypex – type	Other (name)

13. Floor cover within crop rows

Bare soil	Organic mulch	Polymulch (what type/colour)	Other (please specify)

14. Plant spacing

Row centre to row centre	Outside row to tunnel leg	In row spacing at planting	How many primocanes/m of row do you aim to retain to fruit each year?

Your method of crop production (fruiting canes)

15. Do you tip the fruiting canes?

Yes	No

16. If yes, when?

Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec

17. What is the final cane height after tip removal? (please state if the height given is in feet or metres)

Glen Ample	Tulameen	Other (please specify)

18. What type of cane are you retaining?

Thick	Medium	Thin

19. When do you tie-in fruiting cane?

Jan	Feb	Mar	July	Aug	Sep	Oct	Nov	Dec

20.When do you remove surplus primocanes? (Please select more than one if necessary)

Mar	Apr	May	June	July	Aug	Sep

21. Do you remove the lower leaves of primocanes during the growing season?

Yes	No

22. If yes, when? (Please select more than one if necessary)

June	July	Aug	Sep	Oct

Removal of old canes

23. When are the old fruited canes cut out and removed from the rows?

July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar

24. How do you dispose of prunings?

Remove and burn	Pulverise *in situ*	Other (please explain)

25. Are primocanes supported in the rows, after pruning out old fruit cane and before tying-in?

Yes	No

26. Fungicide sprays used last year and their timing (one tick per spray, e.g. Teldor ✓✓ = 2 sprays of Teldor). *This is a key table: please complete as well as you can.*

Product	Before covering (end winter/early spring	Once covered up to first fruit pick	During harvest	Post-harvest when uncovered	Rate/ha (kg or Litres)
Amistar					
Bavistin DF					
Chlorothalonil (e.g. Repulse)					
Cleancrop Curve					
Corbel					
Croptex Fungex					
Cuprokylt FL					
Delsene 50 Flo					
Elvaron Multi					
Folicur					
Frupica					
Nimrod					
Potassium hydrogen carbonate					
Rovral WP					
Rubigan					
Scala					
Shirlan or Shirlan Programme					
SL567A					
Talat					
Teldor					
Unicrop Thianosan					
Other (please specify)					

Spray application

27. What type of sprayer do you use? (please tick)

Tractor mounted hydraulic:	Air-assisted:	If air-assisted, what is the air speed?

28. What spray volume (Litres/ha) do you usually use? (please express your answer as a range if you alter the volume with crop growth)

29. What spray pressure do you usually use? (please express your answer as a range if you alter the pressure with crop growth)

30. Which of the following occurred **in your established tunnel crop in 2005**?
(please see below for guide to severity estimates)

	Nil	**Slight**	Moderate	Severe
Cane *Botrytis*				
Fruit *Botrytis*				
Fruit rot (unknown)				
Powdery mildew on fruit				
Powdery mildew on leaves				
Rust				
Cane blight				
Virus				
Other:				

Nil - Not seen
Slight - Less than 5% of canes, fruit or leaf area affected
Moderate - 6% - 25% of canes, fruit or leaf area affected
Severe - more than 25% canes, fruit or leaf area affected

Comparison with your established outdoor crops in 2005
(choose the same variety, Glen Ample or Tulameen, when comparing covered and uncovered crops)

31. Did you see more disease in the covered or the uncovered crops? (please tick)

Problem:	Covered crop	**Uncovered crop**	No difference	Don't know
Cane *Botrytis* was greater on:				
Fruit *Botrytis* was greater on:				
Powdery mildew was greater on:				
Rust was greater on:				
Other:				

Your opportunity to comment

32. What do you feel is the most important feature or action to achieve effective control of *Botrytis* and powdery mildew in your covered crops?

***Botrytis*:** **Powdery mildew:**

33. Please add any comment you feel relevant to obtaining better control of fungal diseases in covered crops grown with minimal use of fungicides

Please return the competed form to:

Claire Baker
ADAS Arthur Rickwood
Mepal
Ely
Cambridge
CB6 2BA

or email to: Claire.Baker@adas.co.uk

Appendix 2. Details of crop husbandry and disease incidence on ten crops sampled by ADAS in 2005

Crop No.	Cover	Row base cover	Pathway cover	Debris	Ripe fruit *Botrytis* on picking dates	Cane *Botrytis* on picking dates	Other disease in crop on picking dates	2005 Fungicides + most recent to sampling
1	Glass	Bare soil	Bare soil	Not obvious	None 14/6 None 21/6	Some 14/6 Some 21/6	None 14/6 None 21/6	Shirlan 25/2 Rubigan 16/5
2	Tunnel	Black plastic	Bare soil. Grass by legs	Obvious	None 24/6 None 1/7	Some 24/6 Some 1/7	Virus. Frost 1/7 Dying leaves 1/7	5 sprays + Pot bicarb & Sulphur 10/5Teldor 2/6
3	Tunnel	Black plastic	Bare soil. Grass by legs	Obvious	None 24/6 None 1/7	A little 24/6 None 1/7	None 24/6 Dying leaves 1/7	2 sprays + Rovral WP 11/5 Teldor 2/6
4	Tunnel	Bare soil	Bare soil	Not obvious	None 27/6 None 4/7	A little 27/6 A little 4/7	None 27/6	Thiram × 2 Teldor 25/5
5	Tunnel	Bare soil	Grass/clover	Obvious	None 29/6 None 6/7	None 29/6 None 6/7	None 29/6 None 6/7	8 sprays from 24/3 + Teldor 14/6
6	Open	Grass	Grass	Not pruned	Yes 04/7 Yes 8/7	A little 04/7 Yes 8/7	None 04/7 None 8/7	Not sprayed at all
7	Open	Green waste mulch	Grass	Not obvious	None 04/7 Yes 11/7	Present 04/7 Present 11/7	Spur blight Phytophthora	Thianozan 11 & 22/4 Elvaron 6 and 17/5 Folicur 31/5
8	Open	Bare soil	Bare soil	Obvious	None 06/7 None 11/7	Present 06/7 Present 11/7	Spur blight Phytophthora	7 sprays from 11/3 + Thianosan & Nimrod 7 and 14/6
9	Tunnel	Bare soil	Bare soil	2004 debris	None 09/7 None 22/7	None 09/7 None 22/7	Spur blight	3 sprays from 11/6 + Teldor 4/7
10	Open	Black plastic	Grass/clover	Not obvious	A little 13/7 Some 27/7	Low 13/7 Low 13/7	Cane spot	Not sprayed at all