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Horticultural Development Company

Grower summary

SF 74

Integrated pest and disease management for high quality protected raspberry production

Annual Report, Year 2

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Use of pesticides

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

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

Read the label before use: use pesticides safely.

Further information

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

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Headline

- Further progress has been made in 2007 to develop improved integrated pest and disease management systems to reduce the incidence of pesticide residues occurring in raspberries.

Background and expected deliverables

Raspberries are very susceptible to *Botrytis*, powdery mildew, raspberry beetle, raspberry cane midge and aphids. Pesticides are currently relied on for control and are applied close to harvest. Intensive use of pesticides, including the 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 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. Recent observations indicate that this increases the risk of powdery mildew infection in protected crops. Plant protection methods have not been adapted for this new growing environment, which provides opportunities to reduce reliance on pesticides.

The strong market demand to reduce, or ideally to eliminate the occurrence of residues prompted this 5-year HortLINK project which officially started in April 2006,

following considerable initial work in 2005. It aims to develop sustainable methods of integrated management of *Botrytis*, powdery mildew, raspberry beetle, raspberry cane midge (with associated disorder 'midge blight') and aphids on protected raspberry crops. Such methods would not rely on sprays of fungicides and insecticides during flowering or fruit development so that quality fruit can be produced with minimal risk of occurrence of detectable pesticide residues at harvest.

Summary of project and main conclusions

Progress on each objective of the project is summarised below

Botrytis

Cane infection

Inoculation experiments showed for a second year that cane age rather than leaf age significantly influences infection of primocane leaves; leaf infection was only possible on relatively old canes. Three infection routes to cane infection were identified:

i) via petioles of attached leaves.
ii) at leaf scar wound sites on the cane.
iii) direct infection of internode areas, especially on relatively old canes (after fruiting).

Both inoculation studies and crop observations indicate the latter is the major infection route. Botrytis lesions and sclerotia on primocanes were rarely found before October and usually not until late winter.

Fruit infection

Commercial tunnel grown crops were monitored in Cambridge and Kent. Before or during the flowering period, *B. cinerea* spores were only found at low levels on lesions on floricanes (fruiting canes) or on sporulating sclerotia. No other sources of spores were identified. Sporulation was more evident after fruiting, on over-ripe fruit and occasional weeds. Despite the sparsity of *B. cinerea* sources in tunnels during flowering, inoculum of *B. cinerea* was detected in the air on many days during this period. The incidence of flower infection at one of the two sites was accurately predicted (0.7 correlation) by a regression model using daytime temperature and

inoculum of *B. cinerea* in the air. Incubation tests to relate the mean quantity of *B. cinerea* DNA in fruit with the incidence of fruit developing botrytis rot were inconclusive.

Control by canopy manipulation

Removal of primocanes and leaves in a dense tunnel crop of cv. Glen Ample reduced humidity around the canes and subsequent leaf and cane botrytis infection. However, it had no significant effect on fruit botrytis infection. Primocane thinning in a less dense crop had no effect on humidity or disease incidence.

Control with fungicides and natural products

The incidence of latent *Botrytis* in fruit from an outdoor crop of cv. Glen Ample was significantly reduced (by up to 58%), by three sprays of Teldor during flowering; sprays of Hortiphyte Plus had little or no effect. However, use of Teldor increased the incidence of *Penicillium* and *Mucor* fruit rot in post-harvest tests, compared with untreated plants.

Management of fruit botrytis by cooling

A high incidence of raspberry fruit (>50%) were infected by latent *B. cinerea* at harvest, including those from covered crops. Development of infection to create visible damage within 9 days of harvest was largely prevented (over 98%) in fruits that were stored cold (4.5°C) for 4 days immediately after harvest, or stored cold for 2 days then cool (12°C) for 2 days. Fruit stored at ambient after harvest (even those from fungicide-treated covered crops), suffered over 50% rots within 7 days. Rapid cooling and effective cool-chain management may be sufficient to prevent botrytis fruit rot without the need for fungicide sprays during flowering. After storage for four days at 4.5°C, over 98% fruits from an unsprayed covered crop were still visibly healthy after a further 3 days at ambient temperature.

Powdery mildew

Host specificity

A DNA analysis of eight isolates of powdery mildew from raspberry and 27 from strawberry suggest that raspberry and strawberry isolates of *Podosphaera aphanis*

are two distinct groups. Cross-inoculation studies were inconclusive due to the failure of raspberry isolates to infect raspberry; a strawberry isolate infected strawberry and not raspberry.

Raspberry beetle

Semiochemical-based monitoring and trapping systems for managing raspberry beetle

- The new sachet slow release system was more effective in the field than the previous vial system for attracting raspberry beetles and lasted 4 weeks. Compound B was more attractive than A under field conditions (confirming previous SCRI studies).

- The surface area of the white cross vanes is positively associated with increased raspberry beetle catches under field conditions at SCRI.

- In Kent, lattice deployment was more effective at one site than perimeter trapping, whilst at the second site they were equally effective.

- In Eastern Scotland, lattice deployment was more effective than perimeter trapping in the pre-flowering period at one site, whilst at the second site both deployment systems were similarly effective.

- Beetle catches in traps were higher before crop flowering (up to green fruit stage) in Eastern Scotland but this effect was less obvious in Kent.

- Both fruit damage and the numbers of raspberry beetle eggs found in flowers were very low at all sites (Kent and Eastern Scotland) monitored in 2007. Although pesticide-treated areas were not monitored in 2007, it is likely that sprayed areas were also not economically damaged by this pest. Climate (relatively cool, wet summer) is likely to have affected pest numbers.

- Although some beneficial non-target organisms (e.g. honey and bumble bees) were trapped, especially after flowering, the numbers caught were likely to be low as a proportion of local populations and therefore unlikely to

affect local pollination success. Modifications to the trap are being considered to further reduce this risk.

Raspberry cane midge

Semiochemical-based systems of managing cane midge

Lure and trap optimisation:

The height of trap deployment was shown to have a strong affect on the numbers of midges caught in sex pheromone traps. Traps at ground level caught approximately three times as many midges as traps at 0.5 m above the ground and approximately six times as many as at a height of 1m. Only small numbers of midges were caught at greater heights. A standard height of trap deployment of 0.5 m is recommended for pest monitoring purposes. Work on female traps is pending identification of behaviourally-active cane wound attractants. This is in progress but has been delayed due to very low pest numbers in 2007.

Identifying host plant wound attractant of females:

- Methods have been developed to monitor *in situ* production of wound volatiles released from artificially split canes. Several plant compounds have been selected for further studies.

- Due to low incidence of raspberry cane midge in 2007, insufficient numbers were collected to establish a laboratory population for behavioural studies. EMR and ADAS will assist SCRI in 2008 to obtain suitable numbers for bioassays and GC-EAG at SCRI and NRI.

Control by disruption, mass trapping or lure and kill:

A large scale field experiment comparing prototype lure and trap for raspberry cane midge is pending successful completion of the above stages (reliant on sufficient pest numbers to test identified plant wound chemicals).

Aphids

Raspberry aphid controlled by late season sprays of aphicides

A large scale replicated field experiment compared the efficacy of single sprays of Calypso on 7 and 21 September, 5 19 October and 6 November 2006 for control of large raspberry aphid. Each Calypso treatment reduced numbers of eggs in winter and numbers of aphids in spring. However, the 19 October timing clearly gave the best results reducing aphid numbers by >95%.

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 integrated pest and disease management (IPDM) system for growing raspberries in protected environments. This will reduce or remove the incidence of detectable residues in fresh raspberries and give UK raspberry growers a competitive advantage.

Annual value in area of impact

Botrytis, powdery mildew, cane midge and raspberry beetle are problems wherever and however raspberry is grown in the UK. ADAS estimate that, at any one time, 60% of raspberry plantations are infected by these pests and diseases. Assuming

25% of the crop is forgone as a result of these infestations, this is equivalent to 2,000 tonnes of raspberries, worth £7M.

Expected annual added value

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

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

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

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

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

- Cane density critically influences the risk of cane Botrytis, a high density of canes making crops prone to the disease. Ensure that an open canopy structure with adequate numbers of canes for optimal yield is maintained by thorough thinning.

- The work has shown that pre-harvest sprays are not warranted for cane Botrytis control on protected crops. Note that this assumes that spur blight is not significant and the need for sprays for other diseases at this time has not been investigated.

- Programmes of three sprays of Teldor or Switch (and some experimental fungicides) applied during flowering gave a significant reduction in latent Botrytis infection of fruits.

- Rapid removal of field heat and efficient cool chain marketing greatly slows the development of Botrytis in harvested fruit and extends shelf life.

- Preliminary results indicate raspberry and strawberry mildew are different diseases and don't cross-infect.

- Sex pheromone monitoring traps are commercially available for raspberry cane midge and should be used by all growers for determining the prevalence of the pest in their plantations and for timing sprays. The trap has been calibrated and an economic threshold determined.

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 leaves and canes in relation to their age by conducting controlled inoculation experiments in a glasshouse compartment using potted raspberries cv. Glen Ample

Introduction

Based on the data obtained in 2006, we have drawn the following preliminary conclusions:

1. Infection of leaves appears not to be related to leaf age *per se*
2. Infection is critically influenced by the cane age
3. Infection is only possible on relatively old canes

Further experiments were carried out in 2007 to confirm whether these conclusions are correct.

Materials and methods

Experiments were conducted in a polythene tunnel at East Malling Research to determine the susceptibility of leaves and canes to *Botrytis* in relation to leaf and cane age and to identify the timing of infection of primocanes and leaves by *Botrytis*. Cultivar Glen Ample was used in this study. Plants were potted in June 2005 and pruned in the 2005 and 2006 winter. In 2007, leaves were inoculated several times from May to September but only sampled once following each inoculation for disease assessment. Inoculation was conducted on 15/05, 30/05, 27/06, 12/07, 02/08, 23/08 and 14/09. The following procedures were adopted on each inoculation date:

1. All leaves on four randomly selected canes were inoculated with a *B. cinerea* conidial suspension using a hand-held sprayer 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 about a week after inoculation to determine *Botrytis* infections on leaves, petioles and primocanes.
4. Leaves and canes were surface sterilised with sodium hypochlorite, paraquat-treated and incubated on wet paper towel in a gravel tray. Canes were cut into 3-7 pieces. 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 assessed 3-4 weeks later.

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 was used to determine whether the incidence of infection of leaves by *Botrytis* was affected by leaf age and sampling time, assuming that proportion of infected leaves per treatment is binomially distributed.

Results

Only a few inoculated leaves were infected by *Botrytis* on the first two inoculation dates and thereafter the incidence increased steadily with inoculation time, irrespective of leaf ages (Fig. 1.1.1.1). The overall disease incidence of inoculated leaves was 6%, 3%, 25%, 49%, 82% and 89% for leaves inoculated on 15/05, 30/05, 27/06, 12/07, 23/08 and 14/09, respectively; the corresponding incidence for un-inoculated leaves was 0, 0, 0, 6%, 94% and 89% (Fig. 1.1.1.1).

There were no old leaves in the sample until the inoculation on 02/08. The overall incidence was 40%, 41%, 49% and 75% for young, mid-age, mature and old leaves, respectively. The overall frequency of sporing lesions was much higher than that of

sclerotia – 369 and 44 out of the total 786 leaves, respectively; this trend was consistent over different leaf age groups or inoculated/un-inoculated leaves.

Sporing lesions were found on many pieces of canes, particularly on canes inoculated on 23/8 and 14/9. These were respective 12 (out of 18) and 10 (out of 26) pieces of canes with sporing lesions covering large surface areas for canes inoculated on 23/8 and 14/9, compared to the corresponding 2 (out of 21) and 1 (14) pieces of un-inoculated canes. *Botrytis* was found on 201 out of 784 petioles (all as sporing lesions), there were 0, 0, 1, 9, 80 and 111 infected petioles on 15/05, 30/05, 27/06, 12/07, 23/08 and 14/09, respectively.

As shown in Fig. 1.1.1.1, most variability in the disease incidence was attributable to variation between inoculation dates, which accounted for 81% of the total deviance. The next most important source of variability was the comparison between inoculated and un-inoculated, accounting for ca. 10% of the total deviance: 3% and 7% were due to its main effect and its interactions with time, respectively. Overall, the inoculated leaves had significantly ($P < 0.001$) higher disease incidence (52%) than un-inoculated leaves (39%); however, for the last two inoculation dates, disease incidences were not significantly different between inoculated and un-inoculated samples (Fig. 1.1.1.1). The incidence (50%) of inoculated canes with botrytis on the last two inoculation dates was significantly greater ($P < 0.001$) than that of un-inoculated canes (9%).

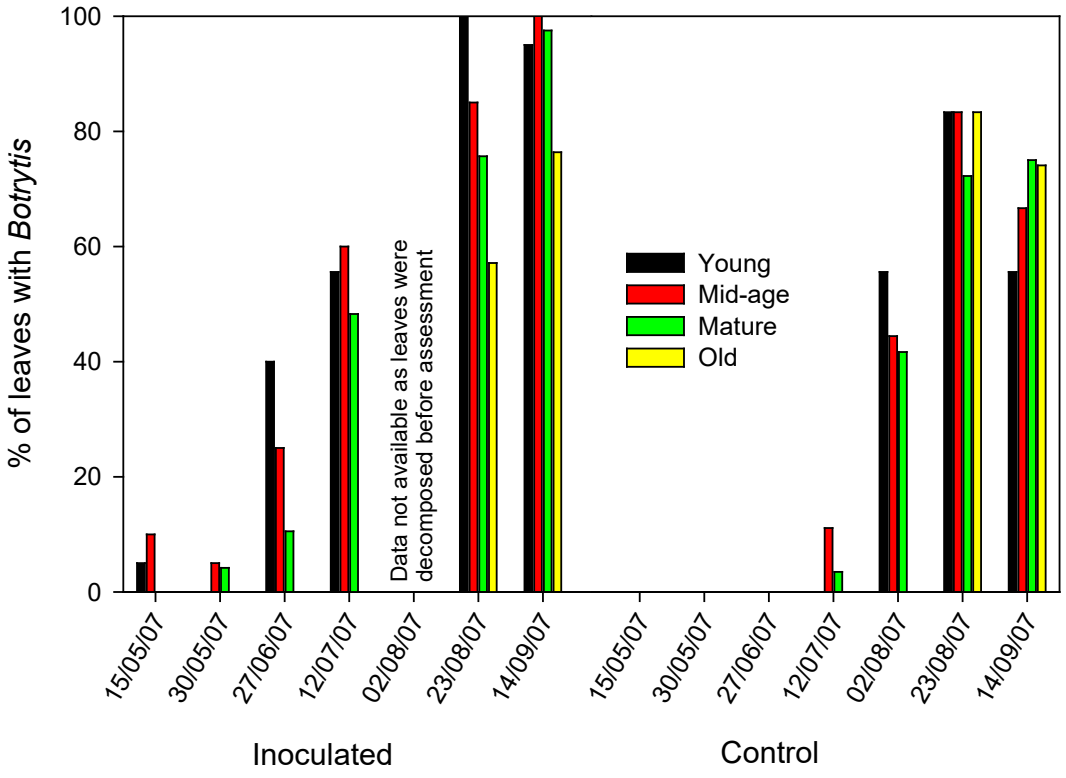

Figure 1.1.1.1. Overall incidence of raspberry leaves of cv. Glen Ample with latent *Botrytis cinerea*. Leaves and canes were inoculated on seven occasions between 15 May and 23 August; canes were assessed a week after inoculation. Leaves were divided into four categories: Young (top five youngest fully unrolled eaves), Mid-age (next five leaves), Mature (next 10 leaves), and Old (all others). For first few inoculations, mature and old leaves may not be present or only a few were present. At each sampling time, three un-inoculated canes were also sampled.

The main effect of leaf age was statistically significantly ($P < 0.05$) but accounted for only 3% of the total deviance. Higher incidence on old leaves may have resulted from the fact that old leaves were not present when canes were relative young (i.e. in May and June) and hence less susceptible to the pathogen. Thus, summarised over all inoculations, incidence of disease on younger leaves is expected to be less than on the old leaves. Indeed, when data were separately analysed for the last two inoculations (23/08 and 14/09), there were no significant differences in disease incidences between the four leaf age groups.

Conclusions

Based on the data obtained in 2006 and 2007, we have drawn the following conclusions:

- Infection of leaves is not critically related to leaf age *per se;*
- But it is critically influenced by the cane age;
- Infection of leaves is most likely to take place on relatively old canes;
- Infection of canes is also possible when the cane is relatively old; furthermore, nearly all infection on canes appeared to result from direct infection of canes by the pathogen, and was not associated with leaf scars (i.e. not spread from infections on leaves);
- A high incidence of uninoculated leaves were found to be infected by *B. cinerea* by August.

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

Introduction

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. The objective was to determine the period when leaves become infected.

Materials and methods

Monitoring areas were marked out in tunnels in Cambridgeshire and Kent. The density of canes was much greater at the Kent site than the Cambridge site (see section 1.2.3). 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. Samples of leaves were taken on 02/07, 23/07, 22/08 and 13/09 at the Cambridge site. At the Kent site, only old leaves (at the bottom of the cane) were sampled on 15/05, 12/06, 09/07 and 09/08. Canes from which leaves were sampled on 15/05 at the Kent site were subsequently mechanically removed before the next sampling.

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 2-3 weeks.

After incubation, sporulating and non-sporulating mycelia 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, commencing within the period of the leaf sampling, and again in 2008, 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.

Generalised linear modelling was used to determine whether the incidence of infection of leaves by *Botrytis* was affected by leaf age and sampling time, assuming that proportion of infected leaves per age group on each sampling time is binomially distributed.

Results

Leaf and petiole infection

At the Cambridge site, disease incidence was much higher in 2007 than in 2006, by nearly 30%. The overall incidence of leaves infected with *Botrytis* differed significantly ($P < 0.01$) among the four sampling times: there was much less on 22/08 (13%) than on the other three dates (Fig. 1.1.2.1a). The incidence on leaves from the bottom position (13%) was lower ($P < 0.05$) than from the upper (43%) and the middle (32%) positions.

At the Kent site, a high level (53%) of *Botrytis* was recorded in mature green leaves collected on 15/05 from the bottom position on the primocane (Fig. 1.1.2.1b). All leaves collected on 12/06 developed *Botrytis* sclerotia on them. On 09/07, a third of

leaves sampled developed *Botrytis* (all as sporing lesions). Nearly 60% leaves collected on 09/08 were infected.

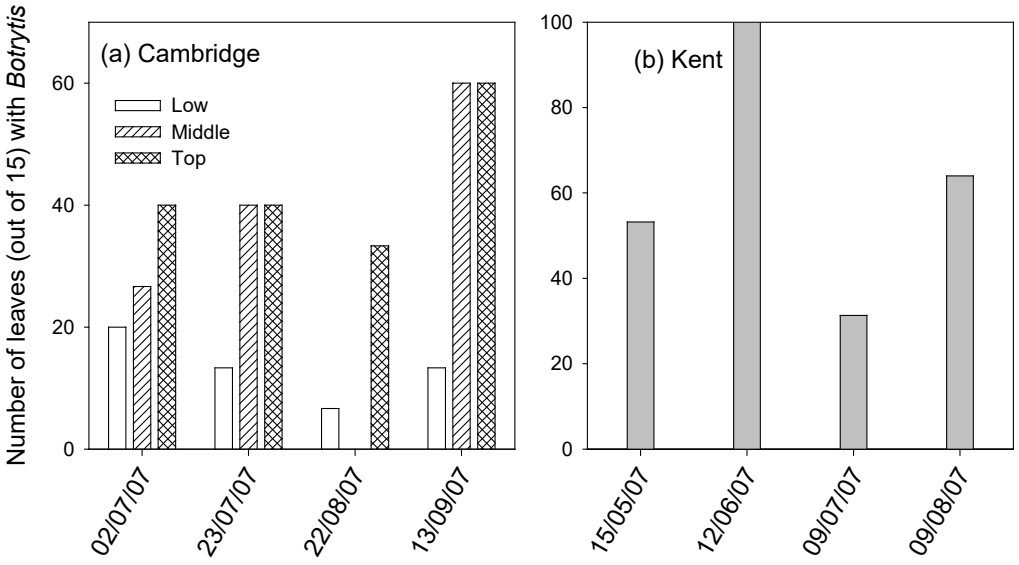

Figure 1.1.2.1. Overall incidence of raspberry leaves of cv. Glen Ample with latent *B. cinerea*; leaves were sampled on several occasions from plants grown under protection in Cambridge and Kent. Leaves were divided into three categories: around the top, middle and bottom of the cane; but in Kent leaves were sampled only from the bottom of the cane.

Primocane infection

At the Cambridge site, in 2007 dark brown lesions (which might be *Botrytis*) were first seen on the leaves of the monitored plants on 24/08. The tunnel had been uncovered. At this time the majority of leaves were still green, only the lowest leaves had dropped. Canes ranged from 1 to over 2 m high, with 14 to 30 leaf nodes. Frequently, the leaf blade and midrib had abscised, leaving the petiole attached. Fifteen stools (each comprising about seven canes) from two rows were examined in detail, selecting three canes with browning per stool to record the position of suspected *Botrytis* damage (Table 1.1.2.1). Six stools had brown areas on the leaf blades, all stools had some brown petioles and all but two stools had browning on the canes. Cane browning was usually associated with a brown petiole. All but eleven scored areas out of 104 were below 0.5 m from the cane base. There was a mean of two brown petioles per primocane, canes having an average of 17 leaf nodes. Leaf blades seldom had lesions, and those that did tended to cling to the petiole, rather than falling.

By 14/09 there had been little change in browning incidence, with only six more petioles brown and two more cane lesions (results not presented). One spur blight lesion was seen. No further records were taken in 2007 as the natural winter browning of the cane epidermis made observations difficult.

In 2008, on 24/01, all 30 marked canes were examined and neither white *Botrytis* lesions nor sclerotia were found, nor evidence of *Botrytis* on any other canes in the tunnel. This was in contrast to the situation in 2006 in the same tunnel, when white *Botrytis* lesions with concentric ring marks together with sclerotia were recorded on 30/10, with more on 29/11 and the number then remaining constant in 2007 after 16/3. Further recording will be carried out at the Cambridge site in 2008.

Table 1.1.2.1: Suspected *Botrytis* browning on leaf blades, petioles and primocanes on three canes of 15 raspberry stools, Cambridge – August 2007

No. of suspect botrytis lesions per stool			**Approx height above stem base of infected area (cm)**				
Leaf	**Petiole**	**Cane**	**0-50**	**51-100**	**100-150**	**150-200**	**>200**
2	4	3	5	2	0	0	0
0	2	3	3	0	0	0	0
4	4	4	6	2	0	0	0
1	6	1	7	0	0	0	0
1	1	3	5	0	0	0	0
0	4	5	5	0	0	0	0
0	4	6	5	1	1	0	0
0	11	9	12	0	0	0	0
0	7	3	5	0	0	0	0
0	10	3	6	1	0	0	0
0	11	1	11	0	0	0	0
0	4	0	3	0	0	0	0
3	6	2	7	4	0	0	0
1	4	1	5	0	0	0	0
0	8	0	8	0	0	0	0

In Kent, a high mean of 42% of leaves per stool had died in the crop by 09/08, but these were not associated with lesions (Table 1.1.2.2). Only 1.5% of leaves had lesions, with 119 suspected cane lesions. By 19/09, before winter leaf drop, 6.7% of leaves had lesions, but over half of the potential cane lesions had been discounted as botrytis lesions. By 2008, on 07/02, 34 of the suspected cane lesions had developed into white *Botrytis* lesions, many with sclerotia, with a significant proportion (17 out of the 30 stools) showing infection.

Conclusions

- At the Cambridge site, occurrence of cane browning in autumn 2007 was associated with petiole browning, most occurring at the cane base. None had developed into typical cane botrytis lesions (watermark symptoms or sclerotia) by late January 2008.
- At the Kent site, cane browning at the leaf attachment point suspected to be botrytis was seen in August 2007. 34 out of 119 suspect lesions had developed into cane lesions by 7 February 2008.

Table 1.1.2.2: Occurrence of botrytis lesions on primocanes on 30 raspberry stools, Kent – August 2007 to February 2008

Stool	Total suspect cane lesions per 2 canes		Actual botrytis lesions per 2 canes	% leaves dead		% leaves with lesions		% leaves green	
	09/08	19/09	07/02	09/08	19/09	09/08	19/09	09/08	19/09
1	3	7	1	30	55	2	8	53	38
2	3	1	1	53	48	0	6	47	46
3	2	1	0	50	47	0	4	50	49
4	9	7	3	56	57	0	7	41	37
5	2	0	0	38	39	5	8	62	53
6	2	0	0	29	41	1	8	71	52
7	2	3	0	29	38	2	3	71	60
8	7	0	1	41	35	0	8	60	57
9	3	2	1	42	36	0	11	58	58
10	5	1	2	49	50	0	7	62	43
11	3	2	1	37	37	0	5	63	58
12	3	1	2	34	41	2	9	66	50
13	5	0	1	44	53	0	3	56	44
14	5	1	1	42	49	0	6	58	45
15	4	0	0	40	40	1	14	60	47
16	4	1	3	33	35	5	3	67	62
17	5	1	0	52	50	0	10	51	41
18	9	2	4	77	81	10	5	23	14
19	4	2	0	64	54	0	8	36	38
20	7	3	1	61	62	0	10	39	29
21	6	2	1	50	51	3	8	44	41
22	7	2	4	41	42	0	5	60	52
23	3	2	4	63	64	3	3	37	33
24	3	1	3	26	38	0	2	74	61
25	7	0	0	45	36	0	16	55	48
26	2	1	0	21	35	0	10	79	55
27	1	0	0	30	42	7	3	70	55
28	0	0	0	17	13	2	5	83	82
29	1	0	0	9	30	0	0	91	70
30	2	0	0	44	42	2	6	56	52
Total	**119**	**43**	**34**						
Mean				**42**	**45**	**2**	**7**	**58**	**49**

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

Introduction

Investigations were conducted at two sites – Salman's Farm, Penshurst, Kent and Sunclose Farm, Milton, Cambridge. At both sites an established plantation of raspberry cv. Glen Ample grown under polythene tunnel protection was used for the study. The objective was to identify sources of *B. cinerea* conidia and the periods of spore production.

Materials and methods

Cane lesions

In early 2007, all of the plants in the monitoring areas of an unsprayed tunnel at both sites were examined closely for sporulation of *B. cinerea* on fruiting canes, and tagged if either lesions or sclerotia were present. Tagged canes were then inspected every 2-3 weeks for signs of sporulation.

Weeds and crop debris

Monitoring of the crop for sporulation of *B. cinerea* on weeds and crop debris commenced in March / April at each site and continued at monthly intervals. On each visit, pruning debris, leaves and various species of weeds were examined for *B. cinerea* sporulation. Samples were taken at twenty positions along the length and between the rows of the tunnels and also from weeds outside the tunnel. Samples were taken at each visit for damp incubation in the laboratory to check for botrytis if none was visible during the visit.

Results

Cane lesions

At the Kent site, the incidence of cane botrytis was very low. A total of 7 fruiting canes in three 100 m rows of raspberry plants were found with botrytis and tagged in February. Sclerotia ranging in number from 1 to more than 40 were present on the tagged canes. The polythene was put on the tunnel at the end of February and in addition the crop was also covered in fleece. Conditions within the tunnel were warm and very dry, particularly in April, which in 2007 was unusually warm and dry.

Consequently many of the sclerotia being monitored shrivelled and failed to sporulate. In early April, a few botrytis conidiophores were observed on two sclerotia on two of the monitored canes. No other sporulation was observed.

At the Cambridge site, 13 affected canes were found and tagged out of about 200 plants assessed. Sclerotia were present on most of these canes but on some only the 'water mark' lesions typical of cane botrytis were present. Recording commenced in mid March before the crop was covered but it was not until 22 May after the polythene was put on, that sporulation was observed from the sclerotia (Table 1.1.3.1). The crop was at early flower at this time and sclerotia on two of the 13 canes sporulated. Sporulation was still occurring when recording ceased at the end of harvest in July.

Table 1.1.3.1: *Botrytis* sporulation on raspberry fruiting canes, weeds and crop debris in a crop of raspberry cv. Glen Ample, at Cambridge, 2007

	No of canes with (of c. 200)				Visible *Botrytis*	
Sample date	**botrytis**	**sclerotia**	**sporing sclerotia**	**water mark lesions**	**crop debris**	**on weeds**
15 March	14	13	0	5	No	No weeds
30 March	13	12	0	5	-	
11 April	13	12	0	11	No	No
9 May	13	12	0	13	No	No
22 May	13	-	2	-		
13 June	13	9	3	13	No	No
2 July	13	11	7	13	fruit, leaves	willow herb

Weeds and crop debris

At the Kent site, the tunnel floor was a mixture of grass with weeds and crop debris. Weed growth was also present outside the tunnel. Sampling commenced in early April and continued at roughly monthly intervals until mid July (Table 1.1.3.2). At the first sampling in April, the polythene cover was present and the ends sealed. Weed growth was present but the debris on the ground was very dry. No botrytis sporulation was observed in the tunnel and after incubation botrytis was seen only on one piece of cane debris (Table 1.1.3.2). No botrytis sporulation was observed on any of the weeds or crop debris within the tunnel at any of the observation dates. Similarly no botrytis sporulation was observed on weeds growing outside the tunnel. However, once the collected samples were damp-incubated, botrytis was observed sporulating after 14 days, especially on dandelion flower heads, dead leaves and

cane debris (Table 1.1.3.2). No significant sporing botrytis was noted in the tunnel until harvest where it was mainly present on over ripe fruit.

Table 1.1.3.2: Incidence of botrytis in weeds and crop debris at various sampling dates in tunnelled crop of raspberry cv. Glen Ample at Penshurst 2007

Sample date	Item	Botrytis sporulation visible on site	% Botrytis sporulation after lab incubation
2 April	Cane debris tunnel alley	no	1.1 (on sclerotia)
	Weed - groundsel	no	0
	Weed - dandelion	no	0
	Weed – willow herb	no	0
15 May	Cane debris tunnel alley	no	13.3 (on canes)
	Grass	no	0
	Weeds - dandelion	no	100 (flower heads)
	Other weeds (dock, willow herb, clover, chickweed)	no	0
	Dead leaves on base fruit laterals	no	100
	Weed outside tunnel – hedge parsley	no	100
12 June	Cane debris tunnel alley	no	2.6 (on canes)
	Tunnel weeds	no	Eaten by flies
	Outside weeds	no	0
	Dead leaves on base fruiting laterals	no	100
17 July	Leaf debris on ground at cane base	no	24.4
	Grubbed spawn in tunnel alley	no	54.1 lesions 13.1 sclerotia

At the Cambridge site, in contrast to Kent, 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. No *B. cinerea* sporulation was seen in the tunnel or after incubation of debris in the laboratory until early July, when it occurred on fallen fruit and leaf debris and willowherb.

Given the very low level of visible botrytis before harvest in the tunnels at both sites, it is difficult to understand the source of inoculum for flower infection. It is possible that buds on the fruiting cane become systemically infected with botrytis during the late summer / autumn when botrytis inoculum is plentiful, and act as inoculum sources. This will be explored in 2008.

Conclusions

- Sporulation of *B. cinerea* sclerotia on fruiting canes can occur from mid-May (when crops are usually at first open flower) through to at least mid-August. However, at both sites the incidence of cane botrytis and sclerotia was very low.

- Weeds and crop debris in tunnels examined at two sites in 2007 appear not to be a source of *B. cinerea* spores for flower infection as no obvious botrytis was observed on them until after damp incubation in the laboratory.

Task 1.1.5 Seasonal variation in airborne inoculum of B. cinerea and flower infection

Objectives

Based on results obtained in 2006, the consortium agreed to redirect most of the time allocated to powdery mildew to investigating infection of raspberry flowers by *Botrytis.* The objective was to develop mathematical models that relate the incidence of flower infection to inoculum concentration and weather conditions in the field from which a disease forecasting system could be developed.

Materials and methods

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

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

A new volumetric spore trap (Burkard Manufacturing Co Ltd, Rickmansworth, Hertfordshire, UK) was used to sample air continuously within the plot at approximately 10 L/min throughout the experimental periods. Instead of using conventional cellophane tape, this new spore trap uses small vials, which can be used directly for extracting fungal DNA for molecular quantification. Spores were sampled daily from 10 am to 10 am and vials were sent to CSL for quantification of *Botrytis cinerea* DNA by a TaqMan PCR test.

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

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

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

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

Results

Weather conditions

As expected, there was much more variability in daytime conditions than in the night time. Thus, only average daytime temperature, relative humidity and VPD are shown in Fig. 1.1.5.1. During the peak flower period, there were two very dry periods between 20/05 and 02/06. Overall weather patterns were similar between the two sites: average day temperature ranged from 10-29°C, humidity from 40 to 90% and VPD from 1 to 18 mmHg.

Inoculum of *B. cinerea*

At the Kent site, the spore trap failed on several days because of a flat battery despite the fact the battery was fully charged and replaced every week and the trap was fitted with a large solar panel for recharging the battery. A considerable number of spores were trapped as early as late April when the trap was set up at the Kent site. At both sites, the level of inoculum varied greatly among days (Fig. 1.1.5.2). For example, the maximum daily number of spores caught was 4,800 on 16/05 at the Kent site. There were many days at both sites where no spores were trapped as determined by the molecular quantification method.

Flower infection

Similarly, the incidence of flower infection varied greatly among sampling occasions for both sites (Fig. 1.1.5.2). At the Kent site, the incidence ranged from 2% on 23/05 to nearly 56% on 30/05. At the Cambridge site, it ranged from 1% on 14/05 to 51% on 18/06. There were no apparent relationships between spore trapping data and incidence of flower infections (Fig. 1.1.5.2). Incidence of flower infection, particularly the peak of infection, at the Kent site appeared to be associated with the periods of high moisture (Fig. 1.1.5.1-2).

Modelling

The strawberry model consistently over-estimated the incidence of flower infection for both sites (data not shown). This is likely due to the fact that the strawberry model was developed from data collected on an open-field crop. Thus, the model identified night temperature and day vapour pressure deficit as two limiting factors. However,

for tunnel crops night time temperature is no longer expected to be a limiting factor for *Botrytis* development.

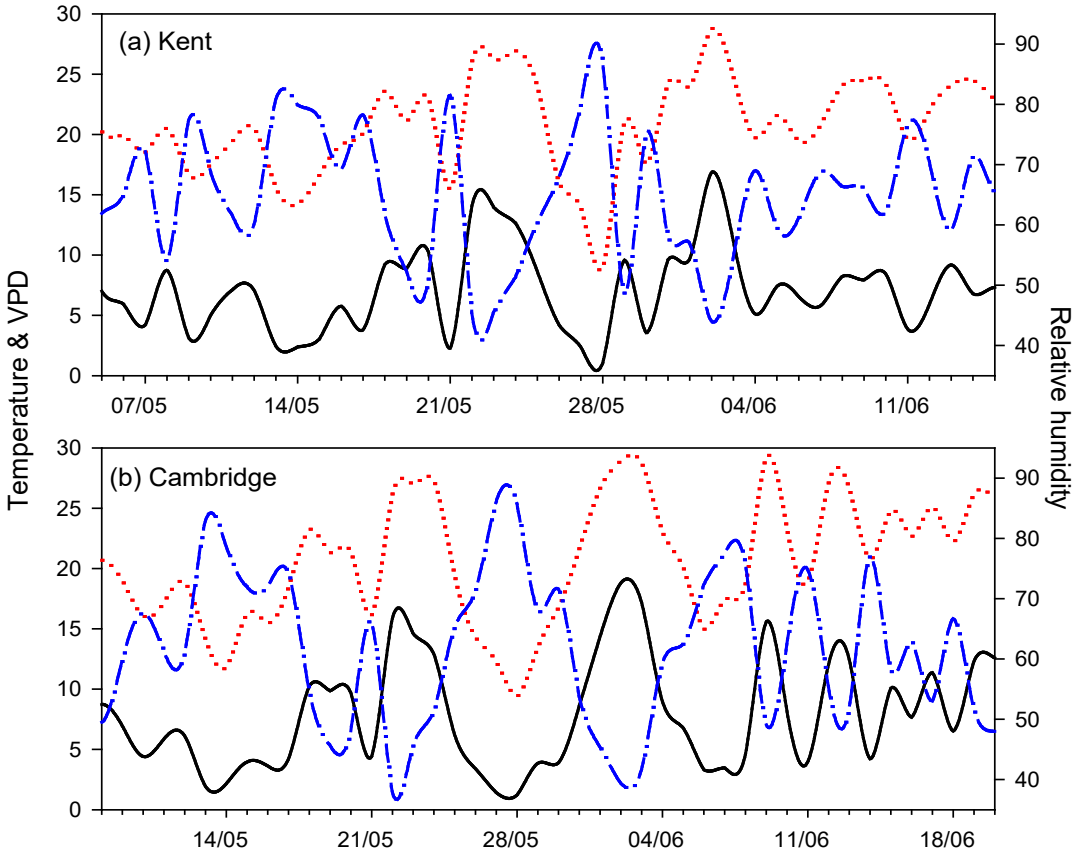

Fig. 1.1.5.1. Day time average vapour pressure deficit (solid), temperature (dotted) and relative humidity (dash-dotted-dash) in 2007 at Kent (a) and Cambridge (b) sites

Incidence of flower infection at the Cambridge site appeared not to be related to any weather variables recorded. This was confirmed by the fact that statistical analysis failed to derive a model relating incidence of flower infection at the Cambridge site to weather variables. In contrast, a regression model was developed for the data collected at the Kent site. This model only needs daytime temperature. Correlation between the predicted and the observed was about 0.7.

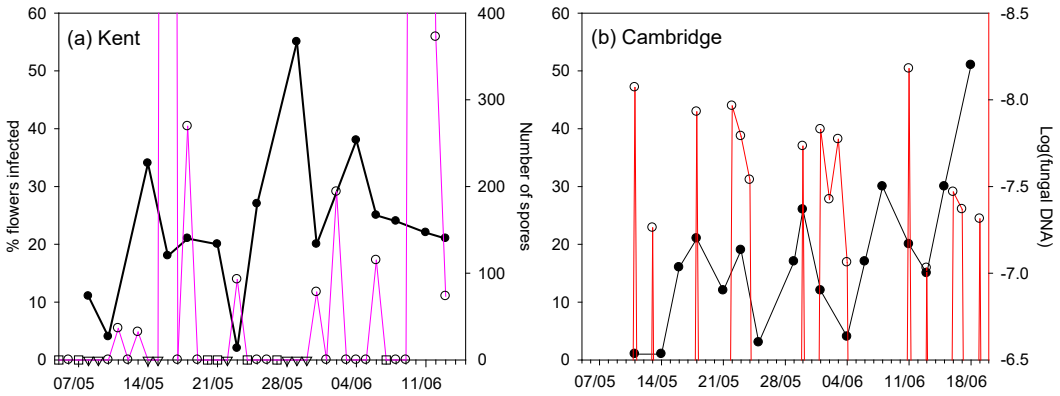

Fig. 1.1.5.2. Daily number or amount of *B. cinerea* spores (open circles) and incidence of flower infection (filled circles) on each sampling day in 2007 at Kent (a) and Cambridge (b) sites

Conclusions

Based on the 2007 data, the following preliminary conclusions can be drawn

- *Botrytis* inoculum is present in air during the flowering period;
- Inoculum levels varied greatly day-to-day but this variability is not related to any weather variables recorded in the tunnel;
- Infection of flowers occurred frequently under protection, but did not appear to relate to the level of inoculum;
- The strawberry *Botrytis* model over-estimated the extent of flower infection under protection;
- Incidence of flower infection was well predicted at one site but not at a second site by a new model.

Further data on flower infection are necessary to confirm these findings and to develop a new model for predicting infection of raspberry flowers.

Task 1.2. Environmental manipulation

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

Introduction

Results of samples taken in 2005 from commercial plantations were presented in the Year 1 report. In incubation tests, there was no consistent relationship across the sites between the percentage of yellow fruit sampled from a crop and the percentage of red fruit with latent botrytis sampled a fortnight later. The objective here was to determine if the quantity of *B. cinerea* DNA in green fruit related to the development of botrytis fruit rot after incubation.

Materials and methods

Samples of green fruit (50 per site) were taken in 2005 from 22 sites for DNA quantification using TaqMan PCR at the same time as fruit (yellow and red) were incubated for visual assessment. Samples were frozen until testing. The PCR results became available in 2007.

Results

For the ADAS sampled fruit, with one exception, the crops with less than 25% of yellow fruit with botrytis after incubation had less than 1 mg of *B. cinerea* DNA /g of green fruit (Table 1.2.2.1). However, where there was a higher proportion of fruit with botrytis after incubation there was no proportional correlation with the botrytis content assayed at up to 35 mg per g of green fruit. There may have been growth of the fungus during the prolonged storage period before PCR, particularly as during this period thawing occurred in the freezer following a power-cut. The DNA results for green fruit did not correlate with the relative levels of infection of the red fruit. The red fruit was not sent for PCR analysis.

Table 1.2.2.1: Mean *B. cinerea* DNA content in samples of green fruit and incidence of yellow and red fruit sampled at the same time with botrytis – ADAS samples

ADAS sample code	*Botrytis* (mg per g)	*Botrytis* (% infected)		Crop details		
	Green	Yellow	Red	Variety	Situation	Fungicides
05/101	little DNA	24	44	Glen Ample	Covered	Minimal
05/109	2.178	51	65	Glen Ample	Covered	Sprayed
05/110	1.935	61	63	Tulameen	Covered	Sprayed
05/112	35.284	17	94	Tulameen	Covered	Sprayed
05/113	0.002	21	68	Glen Ample	Covered	Sprayed
05/118	6.590	86	74	Tulameen	Open	None
05/120	14.444	57	84	Tulameen	Open	Sprayed
05/123	0.224	10	46	Glen Ample	Open	Sprayed
05/132	0.012	0	40	Glen Ample	Covered	Sprayed
05/134	0.006	4	94	Glen Magna	Open	None

For the EMR sampled fruit, visually assessed yellow fruit did not show any correlation with the PCR results obtained from green fruit sampled at the same time (Table 1.2.2.2). The green fruit would have been between about two to five days younger than the yellow fruit, and it is possible that conditions for botrytis infection differed when each of these were flowers. The red fruit sent for PCR was sub-sampled from the fruit incubated for visual assessment, but there was still no correlation between the PCR and visual results. If there is a large variation in the level of latent *B. cinerea* between fruit in a batch, one or a few fruit could greatly alter the mean value of a batch, and confound any possible relation with disease incidence. The DNA botrytis content of the yellow fruit was not consistently lower than that of the red fruit from the same site, although this might have been expected if the red fruit became contaminated externally with botrytis while ripening in the crop. The red fruit, was probably not, however, from the same flower (and latent infection) timing as the yellow fruit.

Across all the samples, there were no relationships between *B. cinerea* DNA content and variety, whether or not covered, or if fungicides had been applied at flowering.

Table 1.2.2.2: Mean *B. cinerea* DNA content in samples of green and red fruit and incidence of yellow and red fruit, sampled at the same time, with botrytis – EMR samples

EMR sample code	*Botrytis* (mg per g)	*Botrytis* infected	*Botrytis* (% infected)	*Botrytis* (mg per g)	Crop details		
	Green	Yellow	Red	Red	Variety	Situation	Fungicides
R61/05	0.083	34	2	0.028	Tulameen	Covered	Sprayed
R62/05	0.063	67	21	0.019	Glen Ample	Covered	Sprayed
R63/05	0.022	54	56	0.060	Glen Ample	Open	Sprayed
R69/05	0.044	99	50	little DNA	Tulameen	Covered	Sprayed
R70/05	0.040	92	21	0.074	Glen Ample	Covered	Sprayed
R71/05	little DNA	99	64	0.245	Glen Ample	Open	Sprayed
R72/05	No DNA	92	29	little DNA	Tulameen	Covered	Sprayed
R73/05	0.096	76	45	0.028	Glen Ample	Open	Sprayed
R74/05	0.028	46	4	0.024	Glen Ample	Covered	Sprayed
R75/05	0.250	82	16	0.015	Glen Ample	Covered	Sprayed
R76/05	0.021	76	69	0.479	Tulameen	Open	Sprayed
R96/05	no sample	84	90	0.034	Glen Ample	Open	None

Conclusions

- There was some evidence that the mean quantity of latent *B. cinerea* DNA in green fruit correlated with the incidence of yellow fruit, picked at the same time, that developed botrytis after incubation. Further testing is required to confirm or refute this observation.
- There was no evidence that the mean quantity of latent *B. cinerea* DNA in a batch of red fruit related to the incidence of these fruit that developed botrytis after incubation.

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

Introduction

Further experiments were carried out to investigate whether thinning crops by cane and leaf removal could lead to a reduction in *Botrytis* on fruit, leaves and canes.

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.

Kent site

Plot layout. 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 May from all of the rows. Two treatments were imposed: canopy subjected to standard (T1) or additional thinning (T2). Two blocks, each with a pair of neighbouring plots (one randomly assigned to canopy manipulated and the other to the standard) were established in early May in all three rows; each plot was about 14 m in length. Adjacent blocks were separated by 7 m row length. Thus, each treatment was replicated twice in a randomised block design. All the plots were at least 8 m away from the tunnel opening.

Treatments. On 23/05, lateral leaves were thinned, as in 2006, and spawns were also thinned in the canopy manipulation plots. On 04/07, the primocanes were thinned in the canopy manipulation plots as in 2006. A USB temperature-humidity duo logger was hung in the canopy in the centre row of each plot at each of the three heights (40 cm, 80 cm, and 150 cm above the ground) several days before leaf removal.

Disease assessment. Random samples of 20 fully expanded leaves were taken from the bottom, middle and top tier of leaves from the centre 3 metres of the middle row of each plot on 12/06 and 27/06. Leaves were first surface-sterilised by immersing them in a 0.5% Domestos® solution (0.025% wt/vol chlorine available) for 4 min, rinsed with tap water, immersed in 0.5% paraquat solution for 1 min, and finally rinsed with tap water; leaves were then placed on a piece of wet paper towel in a gravel tray. The tray was covered with a polythene bag to prevent contamination. *Botrytis* development on individual leaflets and petioles was assessed 3-4 weeks later. Cane *Botrytis* was assessed in early February 2008 by counting number of canes with *Botrytis* in each plot at three heights, corresponding to the heights of data loggers.

A random sample of 50-100 unripe (green/yellow) or ripe (red) fruit was taken from the centre 9 metres of the middle row of each plot on several occasions. Unripe fruit were surface sterilised together by immersing in a 0.5% Domestos® solution for 15 min and then immersed in sterile distilled water for 15 min. Unripe fruit were then placed on paraquat chloramphenicol agar (PCA) media to induce sporulation for 10-14 days before assessment. Ripe fruit were placed in multicell plant propagation trays and incubated at ambient temperature within a polythene bag. Samples of fruit were taken on 15/06 (green and red), 27/06 (green, red), and 09/07 (green and red).

Cambridge site

This site was located at Sunclose Farm, Milton, near Cambridge, on an established mature plantation of cv. Glen Ample. The tunnel contained three rows of raspberries with a more open canopy than the Kent site. All raspberry rows were treated with herbicide in April to eliminate the first flush of primocane. Plots were established in April; each treatment was replicated three times in a randomised block design.

Leaves were not thinned on fruiting laterals at this site. Primocanes were thinned to either eight (T1, control) or four per stool (T2) on 03/05 and 20/06. Each plot was three rows wide and 5 m long, with each of the pairs of plots separated by 10 m, pruned to the plantation's usual density of six canes per stool. Duo temperature and humidity sensors were placed in one pair of plots in one block at three heights 0.55 m, 1.1 m and 1.75 m) in the centre row of each plot.

Twenty leaflets were randomly sampled from each of the three height ranges in each plot on 27/06, 11/07 and 25/07. Random samples of 60 yellow and 50 red marketable fruit were taken from all heights in the centre 3 metres of the middle row of each plot on 25/06, 09/07 and 23/07. Leaflets and fruit were similarly treated and incubated as material from the Kent site. Cane botrytis was similarly assessed in January 2008.

Data analysis

Generalised linear modelling was used to determine whether the incidence of infection of leaves, fruit or canes by *Botrytis* was affected by canopy treatments over all sampling times, assuming that proportion of infected leaves, fruit or canes per sample or per plot is binomially distributed. For fruit *Botrytis*, logistic regression was

first applied to samples of fruit of the same age taken on the same day to determine whether the treatment had influenced the *Botrytis* development on fruit. Then, ANOVA of repeated measurements was applied to all the fruit sampled to assess the overall treatment effects. Percentage of fruit with *Botrytis* was first arcsin-transformed before ANOVA. Only when the overall effect of a treatment factor is statistically significant based on a deviance test or F-test, was the significance of the difference between individual levels of the treatment factor established.

Results

Kent site

Removal of leaves and spawns in late May resulted in not only obvious visual differences between treated and untreated plots but also marked increases in daily average VPD (mmHg) in the manipulated plots relative to the control plots at all three canopy heights (Fig. 1.2.3.1). Similarly, thinning primocanes in early July also resulted in a reduction in VPD in the treated plots compared with the control plots (Fig. 1.2.3.1).

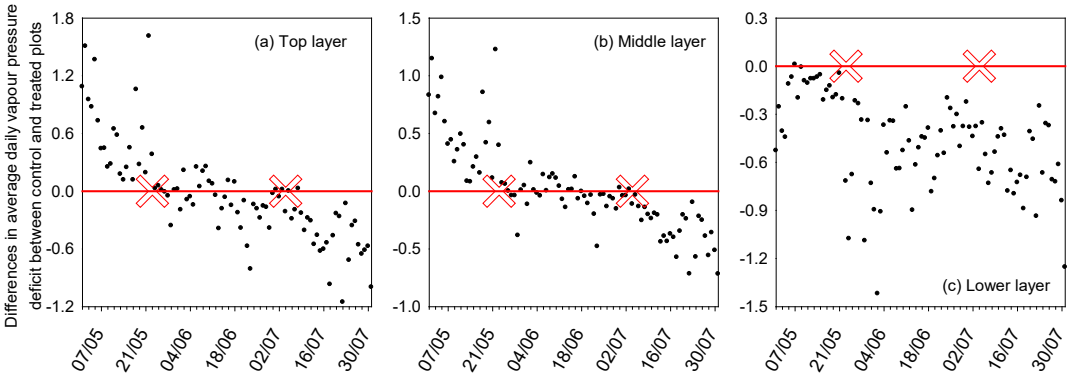

Figure 1.2.3.1. The differences in 24 h (9 pm – 9 pm) average vapour pressure deficits between the control and the manipulated plots of a raspberry crop of cv. Glen Ample over time in 2007 under protection at the Kent site. The 'x' signs indicate the date on which lateral leaves or primocanes were manually removed in the manipulated plots.

Incidence of leaves with *Botrytis* was very high (> 68%) on 12/06 in all plots irrespective of leaf positions (Fig. 1.2.3.2). There were no significant differences in the incidences between the two treatments, and between leaf positions. The overall incidence of leaves with *Botrytis* was 86%. On 27/06, the incidence of leaves with *Botrytis* differed significantly ($P < 0.01$) among three leaf positions (Fig. 1.2.3.2): 87%, 87% and 37% for leaves at the bottom, middle and top, respectively. There

were significant interactions between canopy manipulation and leaf positions in affecting disease incidence (Fig. 1.2.3.2). Incidence was much less on the top leaves in manipulated plots (14%) than the control (61%). On all three sampling occasions in 2007, there were no significant differences in the incidence of green or red fruit with *Botrytis* between the manipulated (88%) and control (89%) plots (Table 1.2.3.1).

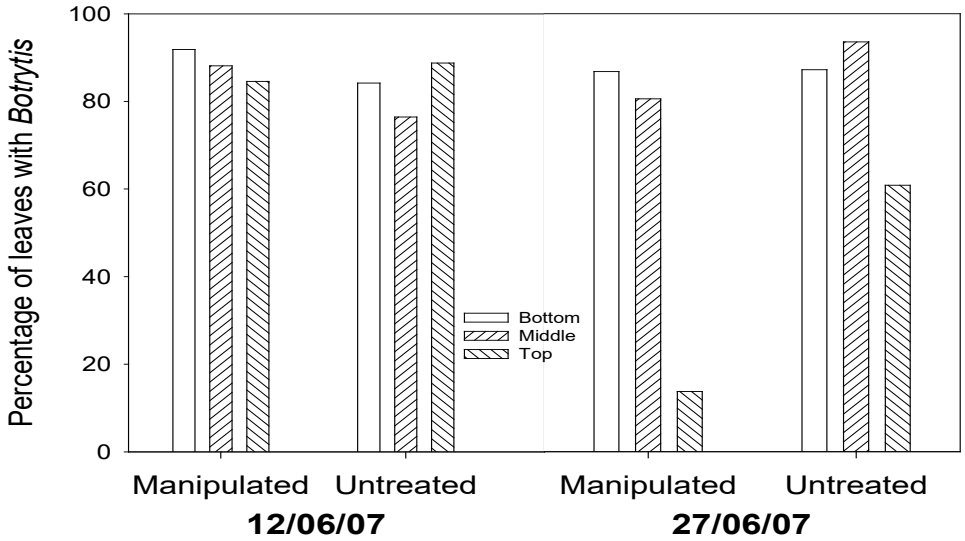

Figure 1.2.3.2. Overall incidence of raspberry leaves with latent *Botrytis cinerea* at three heights between the control and the manipulated plots of cv. Glen Ample under protection at the Kent site.

Table 1.2.3.1: Percentage fruit rots in various raspberry samples taken from tunnelled crop cv. Glen Ample at two sites (Kent and Cambridge); plots of crops were subjected to current agronomy practices (control) or to additional canopy thinning (manipulated)

Kent						
	Unripe fruit			Ripe fruit		
	15/06/07	27/06/07	09/07/07	15/06/07	27/06/07	09/07/07
Manipulated	100	89	100	73	79	93
Control	89	87	100	90	84	83
Cambridge						
	Unripe fruit			Ripe fruit		
	25/06/07	09/07/07	23/07/07	25/06/07	09/07/07	23/07/07
Manipulated	98	89	65	95	71	85
Control	98	83	67	97	67	83

Botrytis lesions on canes were visible in July 2007 in the two manipulated plots; these lesions were all associated with damage created when lateral leaves were manually removed in late May. In February 2008, cane *Botrytis* lesions were frequently seen in all plots irrespective of the treatment. But the incidence of canes with *Botrytis* was significantly ($P < 0.01$) greater in the control (24%) than in the manipulated (12%) plots; this difference accounted for nearly 90% of the total deviances in the observed incidences. Furthermore, incidence of cane *Botrytis* differed ($P < 0.01$) among three heights – 11%, 14% and 9% for the bottom, middle and top, respectively (Fig. 1.2.3.3). Most of the cane botrytis lesions were not associated with leaf infection.

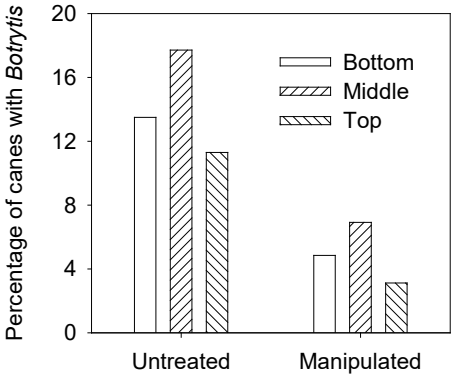

Figure 1.2.3.3. Incidence of canes with *B. cinerea* at the Kent site, assessed in February 2008

Cambridge site

Additional removal of spawns and primocanes did not lead to a large visual difference between treated and untreated plots. Canopy manipulation did not result in any appreciable increase in vapour pressure deficit in the treated plots relative to the control plots over time (Fig. 1.2.3.4).

Very few of the 60 leaflets were found to be infected by *Botrytis* in all plots on all sampling occasions, ranging from 0 to 3% per treatment. Only in six out of the 24 combinations of sampling time, treatment and leaf position, were there any leaves infected; of these six cases, five were for young leaves (top positions). No cane *Botrytis* lesions were observed on 24/1 in 2008 in any plot.

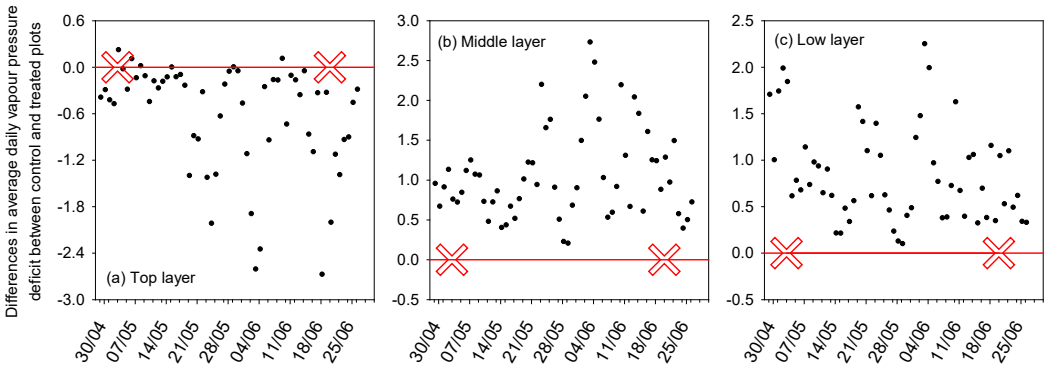

Figure 1.2.3.4. The differences in 24 h (9 pm – 9 pm) average vapour pressure deficits between the control and the manipulated plots of raspberry crops of cv. Glen Ample over time under protection at the Cambridge site. The 'x' signs indicate the date on which lateral leaves or primocanes were manually removed in the manipulated plots.

There were no significant differences in the incidences of *Botrytis* on either yellow or red fruits between the treated and untreated plots (Table 1.2.3.1). The incidence of *Botrytis* was 84% and 83% for the treated and control, respectively, for both fruit and colours across all sample dates. Disease incidence differed significantly ($P < 0.01$) among the sampling dates (Table 1.2.3.1).

Conclusions

- Canopy manipulation resulted in microclimate change (drier conditions) in a dense crop but not in a thin crop. Such changes in microclimate did not result in any appreciable reductions in the infection of fruit by *Botrytis.* Several explanations are possible: i) flower infection may not depend so critically on high ambient humidity since moisture in the stigma surface may be sufficient; ii) extreme low levels of inoculum may be sufficient to cause flower infection.
- Much higher incidences of leaf and cane *Botrytis* was observed in the control than in the thinned plots in a dense crop.
- Botrytis can easily invade canes at wounds caused by leaf removal.
- Most cane lesions in a dense crop were not associated with leaf infection.
- Cane lesions generally develop several months after fruit harvest and it is possible that infection also occurs post-harvest.

Task 1.3 Control agents

1.3.1 – Laboratory evaluation of fungicides and other treatments to suppress sclerotia sporulation.

Introduction

The objective of this task is to evaluate chemicals for suppression of sporulation of botrytis sclerotia on raspberry canes. A reduction in sporulation of sclerotia could result in a reduction in fruit botrytis if sclerotia are a major source of *B. cinerea* conidia.

Materials and methods

Raspberry canes with botrytis lesions and sclerotia were collected from a raspberry plantation in summer 2007 and stored at 4°C until needed. The canes were cut into 10 cm lengths and soaked in water for approximately 15 min and then dried. The canes were divided into lots of 5 (representing 1 plot) and treated with the following chemicals: azoxystrobin (Amistar), fenhexamid (Teldor), iprodione (Rovral), tebuconazole (Folicur), urea and potassium bicarbonate. Treated cane pieces were then incubated in damp chambers in the light to encourage the sclerotia to sporulate. The numbers of sclerotia sporulating was assessed after 1 week, 2 weeks and 4 weeks. Each treatment was replicated four times in a randomised block design and compared with an untreated control.

Results

The experiment is currently in progress and the results will be reported in the next annual report.

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

Introduction

The objective was to determine the relative efficacy of a range of fungicides and natural products applied before and during flowering for control of fruit botrytis on raspberry.

Materials and methods

A field experiment was conducted on in 2007 at East Malling Research, Kent, in an open-field plantation of raspberry cv. Glen Ample planted as long canes in 2005. Each plot consisted of a double row 8 m long separated from adjacent plots by an unsprayed guard row. In 2007, the treatments (Table 1.3.3.1) applied were based on the results obtained in 2006 and consisted of comparisons of programmes of Teldor or Hortiphyte Plus alone or in combination. The treatments were applied to plots using a Solo self-propelled small plot mini-sprayer at 1000 L/ha on five occasions (25 May, 4 June, 15 June, 26 June and 6 July). All treatments were replicated four times in a randomised block design. Crop development was again very variable. Plants at early flower at the time of the first spray were labelled and picking started when the labelled fruit were red. Prior to this the plots were cleared of all ripe fruit.

Plots were regularly inspected for botrytis. At harvest, a random sample of two punnets (approximately 200 fruit) of red fruit were picked from the central section of each plot and assessed for botrytis, powdery mildew and any other diseases. The fruit was similarly picked and assessed on three further occasions coinciding with the spray timings. At each harvest, a sample of 100 healthy red fruit was taken for latent infection by *B. cinerea*. The fruit were placed in individual modules in trays, covered in polythene and damp incubated. Rot incidence was assessed after seven days incubation at ambient temperature (20-25^{o}C) for all harvest dates. In addition, for picks 1 and 4, an assessment of rots was made after 3 days.

A sample of green fruit was taken from each plot in July, surface sterilised in 5% by volume ‘Domestos’ bleach and incubated on agar containing paraquat and chloramphenicol (PCA) under lights to check for latent *B. cinerea* infection in the fruit. The incidence of cane diseases in the plots will be assessed in March 2008.

Results and discussion

Botrytis

The weather conditions in 2007 during most of the flowering period were very wet and favourable for botrytis infection of flowers. Despite this, the incidence of botrytis on fruit at harvest was very low. In post-harvest tests the incidence of botrytis fruit rot varied from 40-80% (7 days incubation) (Table 1.3.3.2). As expected, rotting in the post-harvest tests was significantly greater after 7 days incubation compared with 3 days (Table 1.3.3.2) but in general did not significantly alter the order of efficacy of the treatments. In most of the fruit picks the highest incidence of botrytis (7 day incubation) was recorded in the fruit from untreated plots. At most of the harvest dates, Teldor alone or mixed with Hortiphyte Plus (treatments 2, 4 and 5) significantly reduced botrytis rot incidence compared with the untreated control. The programmes based on Hortiphyte Plus alone (treatments 3 and 6) did not reduce the incidence of botrytis compared with the untreated control except on one occasion at pick 2. Overall there was no significant difference in botrytis rot incidence in fruit treated with Teldor alone or in mixture with Hortiphyte plus indicating that Teldor was most likely responsible for the reductions in botrytis incidence in the mixed treatments. At pick 2 however, there was an overall significant (P=0.042) effect of Hortiphyte Plus in reducing the incidence of botrytis suggesting that this product may have some small inconsistent effect in reducing rot incidence.

The incidence of *B. cinerea* in green fruit samples (Table 1.3.3.5) varied from around 60% to more than 90% infected fruit. Teldor alone or in mixture with Hortiphyte Plus (treatments 2 and 4) significantly reduced the incidence of botrytis rotted fruit. There was no significant effect of Hortiphyte Plus on botrytis.

Table 1.3.3.1: Details of fungicides and natural products applied to open-field raspberries in 2007, East Malling Research, Kent

Treatment		Active ingredient	Product rate	Spray timing	Number of sprays applied
1.	Untreated	-	-	-	0
2.	Teldor	fenhexamid	1.5 kg / ha	3 sprays at 10 day intervals from flowering	3
3.	Hortiphyte Plus	potassium phosphite + other nutrients	6 ml / L	3 sprays at 10 day intervals from flowering	3
4.	Teldor +Hortiphyte Plus	fenhexamid + potassium phosphite + other nutrients	1.5 kg/ha + 6 ml/L	3 sprays at 10 day intervals from flowering	3
5.	Teldor + Hortiphyte Plus	fenhexamid + potassium phosphite + other nutrients	1.5kg/ha + 6 ml/L	2 sprays at 10 day intervals from flowering, then Hortiphyte plus only at 10 day intervals	2+3
6.	Hortiphyte plus	potassium phosphite + other nutrients	6 ml / L	5sprays at 10 day intervals from flowering	5

Table 1.3.3.2: Incidence of botrytis-rotted fruit in post-harvest tests (3 or 7 days incubation at ambient temperature) on raspberries harvested from plots treated in 2007 with various chemicals at East Malling Research, Kent. Data presented are angular transformed with back transformed means in parenthesis

Treatment		% botrytis rotted fruit					
		Pick1 3 July (3 days)	**Pick 1 3 July (7 days)**	**Pick 2 10 July (7 days)**	**Pick 3 17 July (7 days)**	**Pick 4 23 July (3 days)**	**Pick 4 23 July (7 days)**
1.	Untreated	19.8 (11.4)	49.1 (57.2)	65.2 (82.4)	60.6 (76.0)	41.6 (44.2)	65.8 (83.2)
2.	Teldor (3 sprays)	8.4 (2.1)	38.8 (39.2)	58.6 (72.8)	36.0 (34.5)	23.6 (16.0)	47.1 (53.7)
3.	Hortiphyte Plus (3 sprays)	21.3 (13.2)	49.6 (58.1)	61.7 (77.5)	50.2 (59.0)	29.9 (24.8)	52.5 (62.9)
4.	Teldor + Hortiphyte Plus (3 sprays)	6.3 (1.2)	27.8 (21.7)	40.6 (42.3)	36.1 (34.8)	22.9 (15.1)	42.4 (45.5)
5.	Teldor (2 sprays) + Hortiphyte Plus (5 sprays)	10.6 (3.4)	30.8 (26.3)	52.7 (63.3)	30.4 (25.7)	29.9 (24.8)	43.6 (47.5)
6.	Hortiphyte Plus (5 sprays)	23.9 (16.4)	61.7 (77.6)	49.4 (57.7)	57.0 (70.4)	37.5 (37.1)	57.2 (70.7)
F Probability		<0.001	0.003	0.028	0.002	0.001	0.079
SED (15 dof)		3.70	7.44	6.86	6.94	3.99	8.06
LSD (p= 0.05)		7.89	15.85	14.63	14.80	8.49	17.19

Table 1.3.3.3: Incidence of *Penicillium*-rotted fruit in post-harvest tests (7 days incubation at ambient temperature) on raspberries harvested from plots treated in 2007 with various chemicals at East Malling Research, Kent. Data presented are angular transformed with back transformed means in parenthesis

Treatment		% *Penicillium* rotted fruit			
		Pick 1 3 July	**Pick 2 10 July**	**Pick 3 17 July**	**Pick 4 23 July**
1.	Untreated	5.1 (0.8)	7.8 (1.8)	13.8 (5.7)	8.9 (2.4)
2.	Teldor (3 sprays)	15.2 (6.8)	10.5 (3.3)	17.5 (9.0)	14.3 (6.1)
3.	Hortiphyte Plus (3 sprays)	8.4 (2.1)	7.9 (1.9)	9.6 (2.8)	8.7 (2.3)
4.	Teldor + Hortiphyte Plus (3 sprays)	4.9 (0.7)	12.6 (4.7)	10.6 (3.4)	12.5 (4.7)
5.	Teldor (2 sprays) + Hortiphyte Plus (5 sprays)	5.1 (0.8)	12.3 (4.5)	11.4 (3.9)	7.8 (1.8)
6.	Hortiphyte Plus (5 sprays)	8.2 (2.0)	5.8 (1.0)	7.8 (1.8)	7.8 (1.8)
F Probability		0.050	0.246	0.132	0.287
SED (15 dof)		3.26	3.15	3.39	3.30
LSD (p= 0.05)		6.95	3.31	7.23	7.03

Other rots

In general the incidence of *Penicillium* rot was greater in Teldor-treated fruit with the percentage of rotted fruit significantly greater than the untreated at pick 1 (Table 1.3.3.3). There was some evidence to suggest that overall Hortiphyte Plus reduced the incidence of *Penicillium* rot in fruit (Pick 3, P=0.037) but none of the individual treatments were significant compared with the untreated.

The treatments had a similar effect on the incidence of *Penicillium* rot in the green fruit samples (Table 1.3.3.5). Overall there was significantly more I rot in Teldor-treated fruit compared to other treatments (P=0.077). Individually treatments 2 and 4 had significantly more rot than the untreated (Table 5),

The effect of the treatments on the incidence of *Mucor* (including rhizopus) rot is shown in Table 1.3.3.4. The rot incidence ranged from <1% to more than 15%. As with *Penicillium* rot overall there was a significantly higher (P=0.007) incidence of *Mucor* rot in plots treated with Teldor compared to the other treatments. Individually there was significantly more *Mucor* rot in plots treated with Teldor alone (Treatment 2) at Pick 1 than in untreated plots.

Table 1.3.3.4: Incidence of *Mucor*-rotted fruit in post-harvest tests (7 days incubation at ambient temperature) on raspberries harvested from plots treated in 2007 with various chemicals at East Malling Research, Kent. Data presented are angular transformed with back transformed means in parenthesis

Treatment		% *Mucor* rotted fruit			
		Pick 1 3 July	**Pick 2 10 July**	**Pick 3 17 July**	**Pick 4 23 July**
1.	Untreated	11.7 (4.1)	13.5 (5.5)	13.1 (5.2)	6.4 (1.2)
2.	Teldor (3 sprays)	23.0 (15.3)	14.3 (6.1)	18.4 (10.0)	4.3 (0.6)
3.	Hortiphyte Plus (3 sprays)	15.3 (6.9)	11.3 (3.8)	13.7 (5.6)	3.0 (0.3)
4.	Teldor+Hortiphyte Plus (3 sprays)	16.8 (8.3)	19.9 (11.5)	15.5 (7.1)	7.8 (1.8)
5.	Teldor(2)+Hortiphyte Plus (5 sprays)	12.2 (4.4)	17.8 (9.3)	13.6 (5.6)	8.6 (2.2)
6.	Hortiphyte Plus (5 sprays)	16.3 (7.9)	5.5 (0.9)	15.1 (6.8)	9.8 (2.9)
F Probability		0.164	0.125	0.830	0.413
SED (15 df)		4.26	4.96	4.27	3.55
LSD (p= 0.05)		9.07	10.56	9.10	7.56

Table 1.3.3.5: Incidence of botrytis and penicillium-rotted fruit in green fruit incubated on PCA harvested on 2 July from raspberry plots treated in 2007 with various chemicals at East Malling Research, Kent. Data presented are angular transformed with back transformed means in parenthesis

Treatment		% rotted fruit	
		Botrytis	**Penicillium**
1.	Untreated	70.9 (89.3)	13.0 (5.1)
2.	Teldor (3 sprays)	55.4 (67.7)	24.5 (17.2)
3.	Hortiphyte Plus (3 sprays)	74.6 (92.9)	22.1 (14.1)
4.	Teldor + Hortiphyte Plus (3 sprays)	52.3 (62.7)	23.3 (15.7)
5.	Teldor (2 sprays) + Hortiphyte Plus (5 sprays)	71.9 (90.4)	21.9 (14.0)
6.	Hortiphyte Plus (5 sprays)	64.8 (81.9)	14.9 (6.7)
F Probability		0.010	0.134
SED (15 df)		6.10	4.75
LSD (p= 0.05)		13.01	10.13

Teldor and treatments containing Teldor were most consistently effective in controlling *Botrytis* rot. However, this treatment also resulted in a higher incidence of *Penicillium* and *Mucor* rots. Previous research has shown that use of certain groups of fungicides such as Rovral (iprodione) for control of *Botrytis* has resulted in an increase in incidence of pythiaceous fungi including *Mucor* and *Rhizopus* (Maas,

1998) mainly due to the fungicide being ineffective against these fungi and eliminating other potentially antagonistic fungi. This may also be true for Teldor.

There was a small inconsistent effect of Hortiphyte Plus in reducing rotting due to *Penicillium* and *Mucor* and occasionally *Botrytis* which might justify its use in a programme especially if there was also a nutritional benefit to plant growth. However, Hortiphyte Plus is not sufficiently active in reducing rotting to substitute for the use of fungicides. Hortiphyte Plus (Hortifeeds) is a plant feed containing phosphite fertiliser and bio-stimulants (citrus and herbal oils). Phosphites are known to have effects on plant disease by inducing resistance in the plant (Ribeiro Junior *et al.*, 2006). It is possible that a full-season programme of treatments would be more effective in reducing rotting. This may be explored in trials in 2008.

Conclusions

- The best control of botrytis rot was achieved by Teldor alone or in combination with Hortiphyte Plus;
- There was no additional benefit in botrytis control by the addition of Hortiphyte Plus to Teldor;
- Use of Teldor significantly increased the incidence of *Penicillium* and *Mucor* rots compared with the untreated control.
- Use of Hortiphyte Plus resulted in small inconsistent reductions in fruit rot, but the reductions were not sufficient for Hortiphyte Plus to be considered as a substitute for fungicides.

References

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Ribeiro Junior, P M; Resende, M L V. De; Pereira, R B; Cavalcanti, F R; Amaral, D R & Padua, M A. De 2006. Effect of potassium phosphite on the induction of resistance in cocoa seedlings (*Theobroma cacao*) against *Verticillium dahliae*. *Ciencia e Agrotecnologia* 30 (4) 629-636

1.3.5. The effect of cold storage on the incidence of botrytis and other fungi on fruit post-harvest

Introduction

This experiment was carried out to determine the effect of either cold storage, or cold then cool storage, on the incidence of visible botrytis on fruit seven days after picking, compared with fruit storage at ambient temperatures. The incidence of visible botrytis on fruit from unsprayed covered, unsprayed open and commercially sprayed covered crops was also compared. Elsewhere in this project, fruit storage at ambient temperature was the standard incubation method for determination of botrytis incidence.

Materials and methods

Firm ripe fruits of cv. Glen Ample were picked from three 3 m long plots spaced along the central row of an unsprayed tunnel at Milton, Cambridge, and also from the same row which had remained uncovered at one end of the tunnel. Fruit was taken at the same time from similarly positioned plots in an adjacent, commercially-sprayed tunnel of the same variety. Fruit was taken from all heights, with each sample receiving half its fruit from each face of the row. 150 marketable fruit were picked from each plot directly into punnets, with 25 fruit in a single layer per punnet. Each plot supplied fruit for one of three replicates. Fruit was picked in the morning and batches requiring cold storage were in place within two hours of picking.

Two punnets (a total of 50 fruit) from each plot were allocated at random to each of three storage regimes. The first regime gave four days in a grower's cold store at 4.5

°C (rising temporarily to 7 °C when pallets of fruit were loaded into the store). The second regime gave two days in the same cold store before removal to a cool shelf at around 12 °C in the packing area. Ventilated punnet lids were fitted to the punnets at the start of storage. The pairs of punnets from the same replicate of each of the three crop sources were placed together in a produce tray, with the three replicates of trays being stacked. The remaining 150 fruit for the third storage regime were returned to the laboratory for incubation in the same conditions as used in standard fruit incubation tests to determine latent botrytis within this project; the fruit were transferred to multicell trays (one fruit per cell) and then sealed in a transparent plastic bag. The bags were left in a room without air conditioning, near a window but out of direct sunlight. Temperatures ranged from a mean daily minimum of 17 °C to a mean daily maximum of 20°C. After four days the punnets were collected from the cold and cool storage areas and placed in the same room as the multicell trays for a further five days.

The fruit was assessed at two, four, seven and nine days without touching. Fungal growth characteristic of *Botrytis*, *Penicillium* and *Mucor* was recorded. Some fruit were colonised by more than one fungus: *Botrytis* spread rapidly across the fruit and could conceal *Penicillium* and so fruit with a high *Botrytis* incidence probably underestimated the proportion of fruit with *Penicillium*.

The fruit was picked for storage on 16 July 2007, about ten days before the last fruit in the crop ripened. It was probable that fungicide sprays were not applied directly to the flowers which produced the fruit for the trial in order to comply with the harvest interval of the first fruit ripening in the crop at that time (only one fungicide was applied by the grower during flowering). The flowers would have been open for pollination between the 6 and 11 June 2007.

Results

There was no visible fungal growth on fruit two days after harvest following storage in either cold or ambient conditions. After four days, 28% of fruit had *Botrytis* when sourced from outdoor untreated plots and stored at ambient (Table 1.3.5.1). However, even after seven days from harvest, there was still no visible *Botrytis* on tunnel-sourced fruit which had been cold-stored for four days, and only 2% of outdoor fruit from this storage regime had *Botrytis*. At seven days, there was a trace of

Botrytis on tunnel-sourced fruit which had been on a cool shelf. Outdoor fruit stored at ambient throughout was almost all affected by *Botrytis* after seven days.

After nine days from harvest, tunnel-sourced fruit which had been stored below ambient temperature for four days, before five days at ambient, was still virtually free of *Botrytis*. Fruit from the fungicide treated tunnel remained free of visible *Botrytis* for nine days. Outdoor fruit given below ambient regimes showed a rapid increase in *Botrytis* between seven and nine days storage, reaching 54% in the cool stored fruit.

Table 1.3.5.1: Effect of fruit source and storage conditions on occurrence of *Botrytis* on raspberry fruit

Storage treatment		**Mean % fruit affected by *B. cinerea***		
		Outdoor unsprayed	**Covered unsprayed**	**Covered sprayed**
After 4 days				
1.	Cold, cold	2.7	0	0
2.	Cold, cool	3.3	0.7	0
3.	Ambient	28.0	2.7	0.7
After 7 days				
1.	Cold, cold	2.0	0	0
2.	Cold, cool	15.3	0.7	1.3
3.	Ambient	94.0	52.0	58.0
After 9 days				
1.	Cold, cold	41.3	0.7	0
2.	Cold, cool	54.0	0.7	1.3
3.	Ambient	98.7	78.7	78.7

All fruit were stored at ambient after day 4 following harvest

The similarity in mean *Botrytis* incidence for treatments 1 and 2 at each scoring interval (fruit after four days cold storage and fruit moved to cool conditions from cold storage) may indicate it is the initial cold storage that is important, although the regime of cool storage for four days was not tested. There was a significant interaction between storage regime and the source of the fruit (Table 1.3.5.2).

Table 1.3.5.2: Significant differences in % fruit with visible *Botrytis* under different storage regimes

Factor	**Df**	**% *Botrytis* after:**

		4 days		7 days		9 days	
		F pr.	Lsd	F pr.	Lsd	F pr.	Lsd
Storage	2	P<0.05	6.95	P<0.001	4.81	P<0.001	5.84
Sources	2	P<0.01	6.95	P<0.001	4.81	P<0.001	5.84
Storage x sources	4	P<0.05	12.03	P<0.001	8.33	P<0.001	10.12
Residual	18						

Penicillium was not seen until after the fruit had been harvested for seven days (Table 1.3.5.3). This may relate to the time interval needed for the fungal growth to become visible, rather than the change to ambient because fruit at ambient throughout initially appeared healthy even at day 4. There was no difference in the incidence of *Penicillium* between the fruit sources (p=0.14), with a mean 5% of fruit affected. Storage treatment significantly influenced the incidence of affected fruit (p<0.01), with more covered fruit showing *Penicillium* after ambient storage throughout. The incidence of *Penicillium* on outdoor fruit stored at ambient was probably under-recorded because most fruit had *Botrytis* which could obscure *Penicillium*. There was no significant difference at day 7 between cold storage for either two or four days.

Table 1.3.5.3: Effect of fruit source and storage conditions on occurrence of *Penicillium* on raspberry

Storage treatment		Mean % fruit affected by *Penicillium* after 7 days		
		Outdoor unsprayed	Covered unsprayed	Covered sprayed
1.	Cold, cold	3.3	0.7	0.7
2.	Cold, cool	1.3	6.7	2.0
3.	Ambient	2.7	12.0	14.0

Mucor was also first seen following seven days storage (Table 1.3.5.4). It was at a low incidence, but was able to spread to neighbouring fruit in the punnet. Cold storage for four days appeared to have totally inhibited the appearance of *Mucor* even after a further three days at ambient. Transfer after two days to cool storage allowed significantly more fruit (p<0.01) to become affected. Outdoor fruit which received two days cool storage did not differ significantly (p=0.70) from the tunnel cropped fruit, with a mean 5% of fruit with *Mucor*. Fruit incubated throughout at ambient was too badly affected by *Botrytis* to distinguish the *Mucor* clearly, leading to under-recording.

Table 1.3.5.4: Effect of fruit source and storage conditions on occurrence of *Mucor* on raspberry

Storage	Mean % fruit affected by *Mucor* after 7 days

treatment		Outdoor unsprayed	Covered unsprayed	Covered sprayed
1.	Cold, cold	0	0	0
2.	Cold, cool	7.3	4.0	4.0
3.	Ambient	0.7	1.3	0

All fruit remained visibly healthy at two days after harvest. Four days after harvest, the proportion of fruit visibly healthy was consistently the smallest for the ambient stored outdoor fruit, with only half the fruit looking healthy (1.3.5.5), and only 1% after nine days. The tunnel fruit (both sprayed and unsprayed) kept at ambient throughout showed a marked reduction in visibly healthy fruit from seven days after harvest, whereas the initially cold and cool stored fruit in punnets were much less affected. At 9 days after harvest, 82% of fruit stored cold for 4 days and 61% of those cold and cool stored (4 days in total), had no visible fungal growth, compared with 10% or those stored at ambient throughout. There was a significant statistical interaction between the source of the fruit and the storage regime ($p<0.01$) for the mean percentage fruit infected.

Table 1.3.5.5: Effect of fruit source and storage conditions on occurrence of fruit unaffected by rots

Storage treatment		Mean % fruit visibly healthy		
		Outdoor unsprayed	Covered unsprayed	Covered sprayed
After 4 days				
1.	Cold, cold	97.3	100.0	100.0
2.	Cold, cool	96.7	99.3	100.0
3.	Ambient	50.5	94.0	97.3
After 7 days				
1.	Cold, cold	92.7	98.0	99.3
2.	Cold, cool	67.3	87.3	94.0
3.	Ambient	4.0	29.3	28.0
After 9 days				
1.	Cold, cold	58.0	94.0	94.0
2.	Cold, cool	20.0	80.7	81.3
3.	Ambient	1.3	13.3	14.7

All fruit stored at ambient from day 4 after harvest.

Conclusions

- A high proportion of fruit from rain-covered raspberry crops are infected by latent botrytis; covering reduces the level of infection only slightly compared with uncovered crops.

- Cold storage (4d) or cold (2d) plus cool (2d) storage immediately after harvest reduces the incidence of visible botrytis rot almost to zero, compared with ambient storage for 7-9 days.
- *Penicillium* and *Mucor* developed on fruit more slowly than *Botrytis*.

Objective 2. Raspberry beetle

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

2.1.4 – Calibrate traps for pest monitoring

Introduction

Based on the results from the 12-month report and in addition to the large-scale grower-based field trials, two small-scale trials were undertaken at SCRI (field site F4) to further refine the volatile dispensers and to investigate alternative, less costly traps.

Methods and materials

2.1.4.1 – Experimental sites:

Small-scale trials at SCRI

Two sites were used for trials at SCRI, Invergowrie, Dundee for raspberry beetle research in 2007. Both were established open-field sites (F4 & F6 (OS ref: NO 337 297)). These are multi-cultivar replicated experimental sites that have been used for previous entomological research, including raspberry beetle and raspberry aphid epidemiology. The small tunnel E8 was not used in 2007. Due to an unusually dry spring, both small-scale trials were irrigated on 20 or 23 April using an overhead irrigator applying water at a rate of 20 mm ha^{-1}.

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. In addition, individual temperature and humidity data was collected at the tunnel sites using Battery Powered Remote Temperature and Humidity Logger (USB-502, Adept Scientific, Letchworth Garden City, Herts).

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

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

Task 2.2 Optimise lure for control

Task 2.2.1. Evaluate blends and dispensers

Materials and Methods

Site: F6, SCRI

Trap type: Standard AgriSense Ltd funnel traps the white Correx vanes as per specification in year 1 were used. Twenty-five percent antifreeze solution (Smith's Blucol) was added to the traps to reduce evaporation of the liquid in the trap base.

Dispensers: Two types of dispenser containing butanone (Compound A) or pentanone (Compound B) were compared. The standard lure was the thick-walled vials used in 2006 and they were compared with experimental sachets lures based on Suterra Biolure membrane dispenser (Suterra LLC, Bend Oregon, USA provided by AgriSense Ltd) (Fig. 2.2.1.1). The pentanone sachets had a 7 mm diameter hole punched in the non-permeable outer membrane whilst butanone sachets had a larger 9 mm diameter hole. The thick-walled plastic vials were attached to the traps by 76mm long, plastic ended Treasury Tags (QConnect ref: KF04572) inserted through a hole in the lid and through a hole drilled in the lid of the vial. The sachets were attached as for the vials, but the lower end of the Treasury Tag was attached to 19 mm foldback clips (Rapesco Group plc, Sevenoaks, England) holding the sachet.

Figure 2.2.1.1. Examples of Suterra 'Biolure' membrane dispensers used at SCRI in 2007 to compare evaporation rate and performance of pentanone and butanone lures. Note the hole punched in the outer (white) impervious membrane exposing the dark semi-permeable membrane and the pads to retain the volatile in the top right sachets. (The diameter of the punched hole can be adjusted to alter the evaporation rate.)

Experimental layout: Randomised block design with two blocks of raspberry cultivar Glen Clova (blocks 1 and 2) and four block of cultivar Malling Leo (blocks 3 to 6). Experiment set up on 11 May 2007 and observations continued for seven weeks.

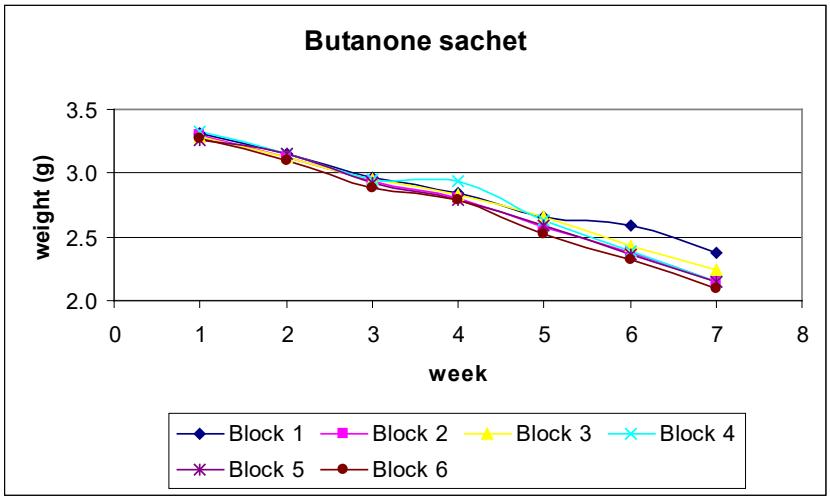
Assessment of evaporation rate: Individual vials and sachets were allocated to specific traps. Each vial or lure was weighed using a calibrated laboratory balance (Ohaus, Explorer Pro model EP214 in laboratory VG16 at SCRI). The lures were collected from the field at weekly intervals. Each vial/sachet was placed into a sealed aluminium dish to prevent undue handling and disturbance. After weighing, the lures were returned to their specific trap in the field.

Assessment of raspberry beetle numbers: The number of insects caught in each individual trap was counted weekly.

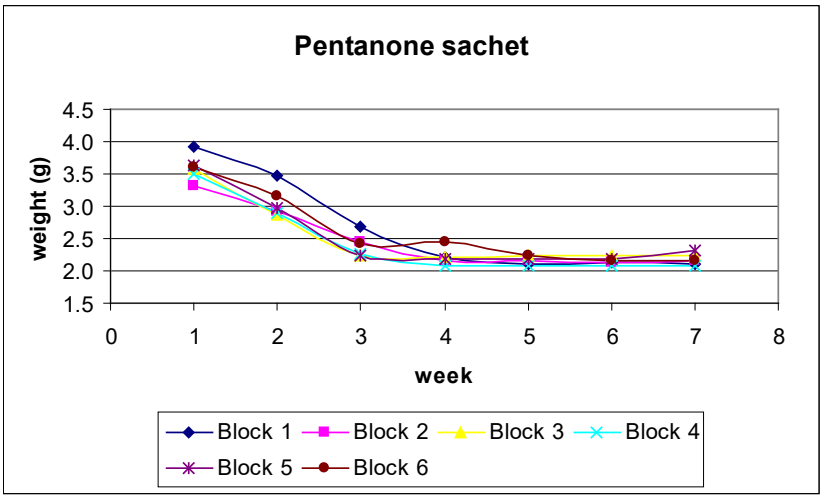
Results

The loss of volatile due to evaporation for each block is shown in figure 2.2.1.2 below.

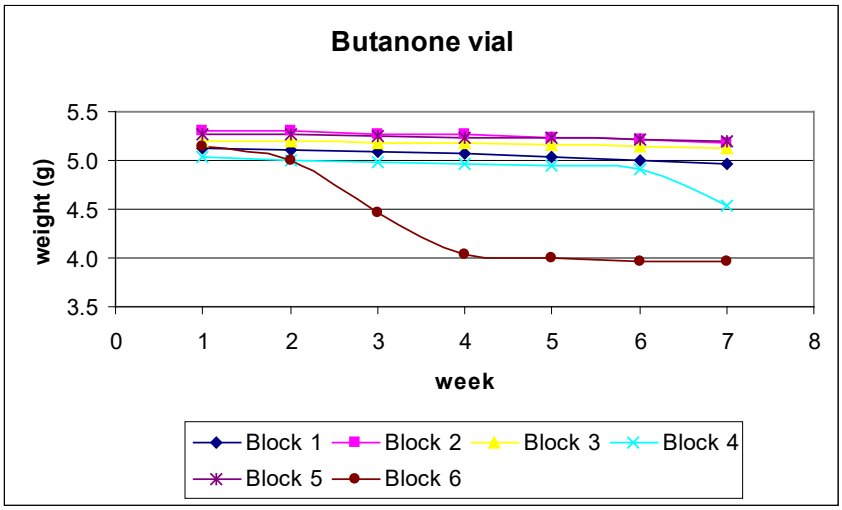
(a)



(b)



(c)



(d)

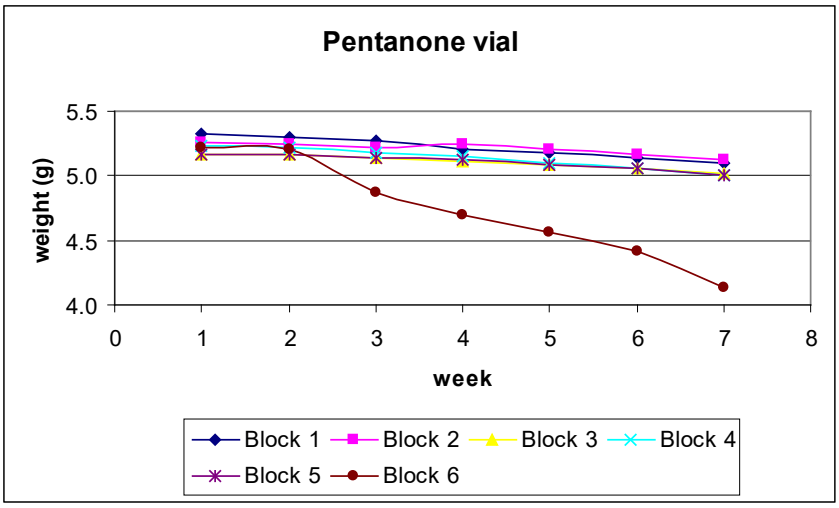


Figure 2.2.1.2 Individual weekly weights (g) for (a) butanone sachets (b) pentanone sachets (c) butanone vials (d) pentanone vials. (Block 6 vials – both leaked and were removed from analysis)

Table 2.2.1.1 Mean daily evaporation rate for the four dispensers tested. (Block 6 butanone vial and pentanone vial calculations removed as they were leaking)

Dispenser	Number of replicates	Evaporation rate (mg/day)
Butanone sachet	6	25.5
Pentanone sachet	6	87.3
Butanone vial	5	2.1
Pentanone vial	5	3.0

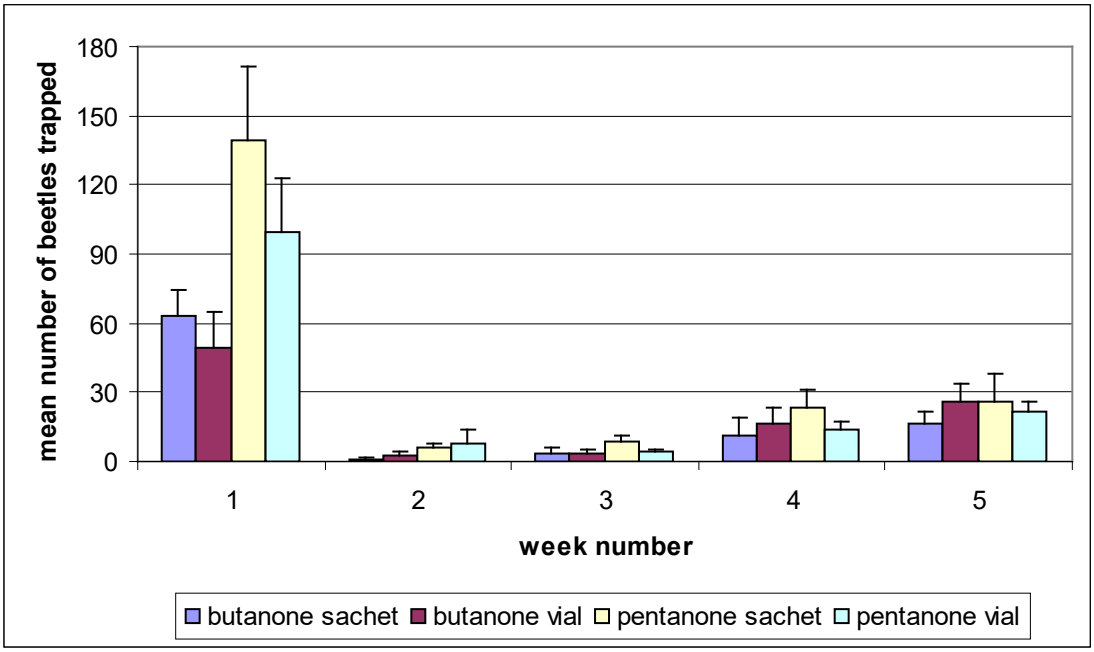


Figure 2.2.1.3 Mean weekly number of beetles trapped using the four dispensers

The evaporation rate (Table 2.2.1.1) from both types of sachets, pentanone (87.3 mg^{-d}) and butanone (25.5 mg^{-d}), was much greater than from the vials, pentanone (3.0 mg^{-d}) and butanone (2.1 mg^{-d}). In the experimental conditions experienced, the pentanone sachet was empty after four weeks. Most beetles were caught in the first seven days of the trial which represented the period when there was the least number of open raspberry flowers (Fig. 2.2.1.3). Overall, the greatest number beetles were caught in traps combined with pentanone with the sachet catching more than the vial in most weeks. In one replicate of the butanone sachet and also one replicate of the pentanone sachet leaked. The leaks were aggravated by having to remove them from the traps in the field so that they could be weighed in the laboratory. Glen Clova, the earlier of the two cultivars used, was in full flower by 31 May whilst Malling Leo has not started to flower.

Discussion

The evaporation rate from the sachets was much higher than the vials and the greatest evaporation rate (pentanone sachet) attracted the most beetles, especially in week one.

- Although the pentanone sachet only lasted 4 weeks, this may be the length of time required. If longer release times are required, modification to the evaporation rate may be possible by using smaller holes in the outer impervious membrane. The 7 mm diameter hole probably can be reduced to 4 or 5 mm and the volume of volatile reduced as a consequence.
- Although two vials leaked, modification is not required to the design as they were subjected to more than normal handling as they had to be removed from the trap to assess their weights on a weekly basis. In normal use they do not need to be removed during the trapping period.
- Pentanone is more attractive to raspberry beetle than butanone, as previously reported in Year 1 HortLINK report.

Task 2.2.1 Identify suitable device for lure and kill or mass trapping

Comparison of trap types

Materials and Methods

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

Raspberry Cultivar:
Glen Clova

Start date and duration:

10 May 2007, observed for 14 days (samples on day 7 and 14).

Trap Type:

Figure 2.3.1.1 Large 3-vane prototype trap (tested at SCRI, 2007) showing position of dispenser and polythene collection bag containing 25% antifreeze solution

The standard trap was the prototypes based on AgriSense Green Funnel Trap (Product BC255501) developed in 2005 and used in 2006. This was a green funnel trap with white Correx cross-vanes with a surface area of 1024 cm^2. The other three prototype traps were all based on trap supplied by AgriSense. These traps which use clear polythene tubing to catch the insects had provision for three equal sized vanes that joined in the centre (Fig. 2.3.1.1). The size of the white Correx vanes in each trap was adjusted to give the surface areas between 840 cm^2 and 420 cm^2. The four traps, standard, prototype 1 (large - 840 cm^2), prototype 2 (medium – 560 cm^2) and prototype 3 (small – 420 cm^2).

Results

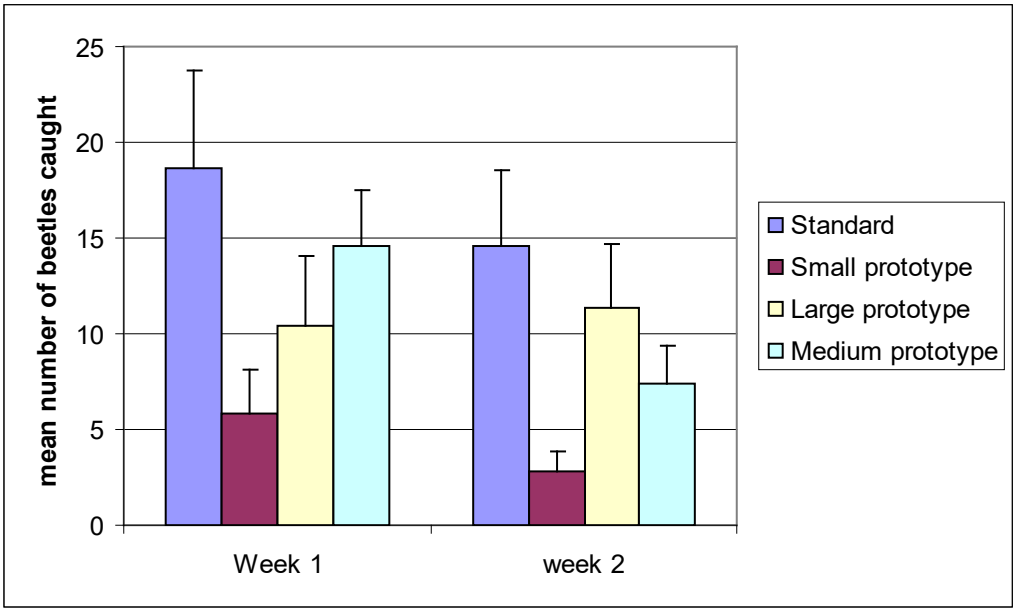


Figure 2.3.1.2 Mean number of raspberry beetle caught at SCRI (F4) using a standard and three different prototype trap types. Error bars represent standard error

Analysis of variance showed that there was a significant difference in the number of beetles caught between treatments (d.f. 3; v.r. 4.75; F = 0.007) but there was no significant difference between weeks (Fig. 2.3.1.2). The standard trap design caught the greatest number of beetles in week 1 (18.6) and week 2 (14.6). The small prototype trap caught the least number of beetles in both weeks (week 1 = 5.8; week 2 = 2.8) and the medium prototype trap caught more beetles in the first week (14.6) than the large prototype trap (10.4) but less beetles in the second week (7.4) when compared to the large prototype trap (11.4).

Discussion

- The traps were tested in a raspberry plantation at SCRI (F4) starting at white bud stage just before and during early flowering, so the observations were restricted to a two week period when the traps were known to be most effective (previously reported results).
- The standard cross-vane trap (SCRI/AgriSense) is the most effective, having the largest surface area to attract beetles.
- In week one at the SCRI site there is a suggested relationship between the white surface area of the vanes in the prototype traps but this was less obvious in week two.

- The prototype traps were not sufficiently robust to withstand Scottish climatic conditions and would at best only last one season of use, whereas the standard bucket trap can be re-used for several seasons.

Task 2.4.1 Deployment strategy for control device

Objectives

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

Materials and Methods

Duration of experiment

Kent - 24 April – 10 July 2007

Eastern Scotland – 1 May – 11 July 2007

Sites

Four commercial sites were used. Three Glen Ample plantations were used (with one exception where Tulameen was used) on each site (Table 2.4.1.1). Each protected raspberry plantation was approximately 1 hectare in size. These sites were chosen as they were thought to have low to moderate populations of raspberry beetle.

Table 2.4.1.1 Details of the location, cultivar and age of raspberry plantations used in large

Site 1 details ***John Myatt, Decoy Farm. High Halstow, Rochester, Kent ME3 8SR. Tel 07771846345***			
Field name	**NGR**	**Cultivar**	**Age (years)**
Bungalow	TQ 786 768	Glen Ample	3
Fullers	TQ 786 768	Glen Ample	3
Rye Street	TQ 748 762	Tulameen	9
Site 2 details ***Tim Chambers, W B Chambers & Son, Belks farm, Otham, Kent ME15 8RL Mob: 07768 867231Email: belksfarm@btinternet.com (Marketing Desk Berry World)***			
Field name	**NGR**	**Cultivar**	**Age (years)**
B29	TQ 8052 2489	Glen Ample	6
L1	TQ 813 527	Glen Ample	3
L14	TQ 813 527	Glen Ample	3
Site 3 details ***Euan McIntyre, Wester Essendy, Blairgowrie, Perthshire, Scotland Mob: 07770933022 Email: eaunmcintyre@btconnect.com (Marketing Desk, Berry Garden)***			
Field name	**NGR**	**Cultivar**	**Age (years)**
E1	NO 135 435	Glen Ample	2
E2	NO 135 435	Glen Ample	5
E3	NO 135 435	Glen Ample	3
Site 4 details ***Jock McFarlane, Easter Rattray Farm, Blairgowrie, PH10 7HQ Mob: 07703 330 724 Email: McFarlane@sol.co.uk (Marketing Desk, Berry Garden)***			
Field name	**NGR**	**Cultivar**	**Age (years)**
J1	NO 216 462	Glen Ample	8
J2	NO 216 462	Glen Ample	8
J3	NO 216 462	Glen Ample	8

Treatments

Devices were modified AgriSense funnel traps with white Correx cross-vanes and with a polythene vial dispenser containing initially 2.5 ml of pentanone (compound B). The funnel traps contained 3 cm of 25% antifreeze (ethylene glycol) in water with a drop of Teepol detergent to reduce surface tension (50% antifreeze at EMR). The treatments were applied at least 2 weeks before flowering commenced.

Three treatments were used: perimeter, lattice and control. Perimeter trapping consisted of the traps being suspended from the top wire at the outer most position in the plantation at 8 metre spacing around the entire perimeter of the plantation. The traps in the lattice trapping were positioned regularly through out the plantation,

suspended from the top wire, at a density of 50 traps per hectare. The control treatment contained no buckets traps but did contain sticky traps for monitoring (Fig. 2.4.1.1). The distribution of the traps within the 1 hectare sites are shown in figure 2.4.1.2.

Assessments

Eight bucket traps were checked weekly and the number of beetles recorded. Sampling of all the bucket traps was done at flowering and at the end of the trial. The number of non target insects was also recorded. One middle tunnel and one end tunnel in all plantations were left untreated by insecticides throughout the duration of the trial.

Standard non-UV reflective white sticky traps were positioned in all three treatments to allow weekly monitoring of the beetle population. See table 2.4.1.2 and figure 2.4.1.2.

Table 2.4.1.2 Location of weekly monitoring traps

	Weekly monitoring traps (AgriSense funnel plus pentanone - compound B lure)		**Weekly monitoring traps (sticky and no lure)**	
Treatment	**No.**	**Position**	**No.**	**Position**
Untreated	0		5	Centre of plot and middle edges of plot
Perimeter	8	Centre of plot, corner and middle edges of plot	5	As above
Lattice	8	Centre of plot, corner and middle edges of plot	5	As above

Flowering commenced at site 1 (24 April 2007), site 2 (15 May 2007), site 3 (14 May 2007) and site 4 (17 May 2007). During the flowering period (Table 2.4.1.3), before insecticide sprays were applied for raspberry beetle control, a bulk sample of 200 flowers was sampled from each of 9 sampling points in a regular grid through each plot (Fig. 2.4.1.2). The sampling points were at least 5m from the nearest device. At each point, 10 flowers were taken from each of 20 plants in 3 rows in a square around the central point. The flowers were frozen and counts of eggs were done in the lab subsequently.

During the harvesting period (Table 2.4.1.3), a sample of 200 ripening fruit were sampled using the same protocol as for flowers, but only from sampling points in the two untreated tunnels (Fig. 2.4.1.2).

Table 2.4.1.3 Dates of flower samples and berry samples taken the four sites for egg counts and damage assessment

Site	Date of flower sample	Date of berry sample
1	5 June 2007	26 June 2007
2	5 June 2007	3 July 2007
3	11 June 2007	11 July 2007
4	19 June 2007	10 July 2007

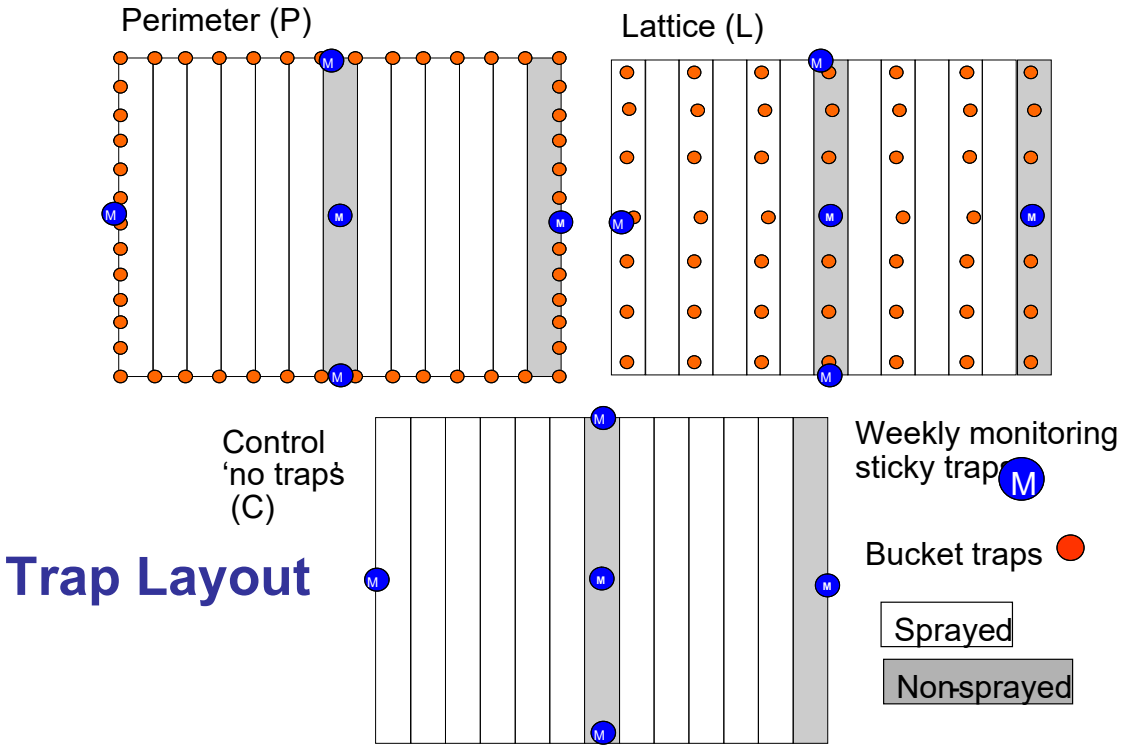


Figure 2.4.1.1 Arrangement of control traps and monitoring traps within the three 1 hectare plots. The bucket traps used were the standard bucket trap with a pentanone plastic vial lure. The bucket traps were positioned at approx 50 traps per hectare

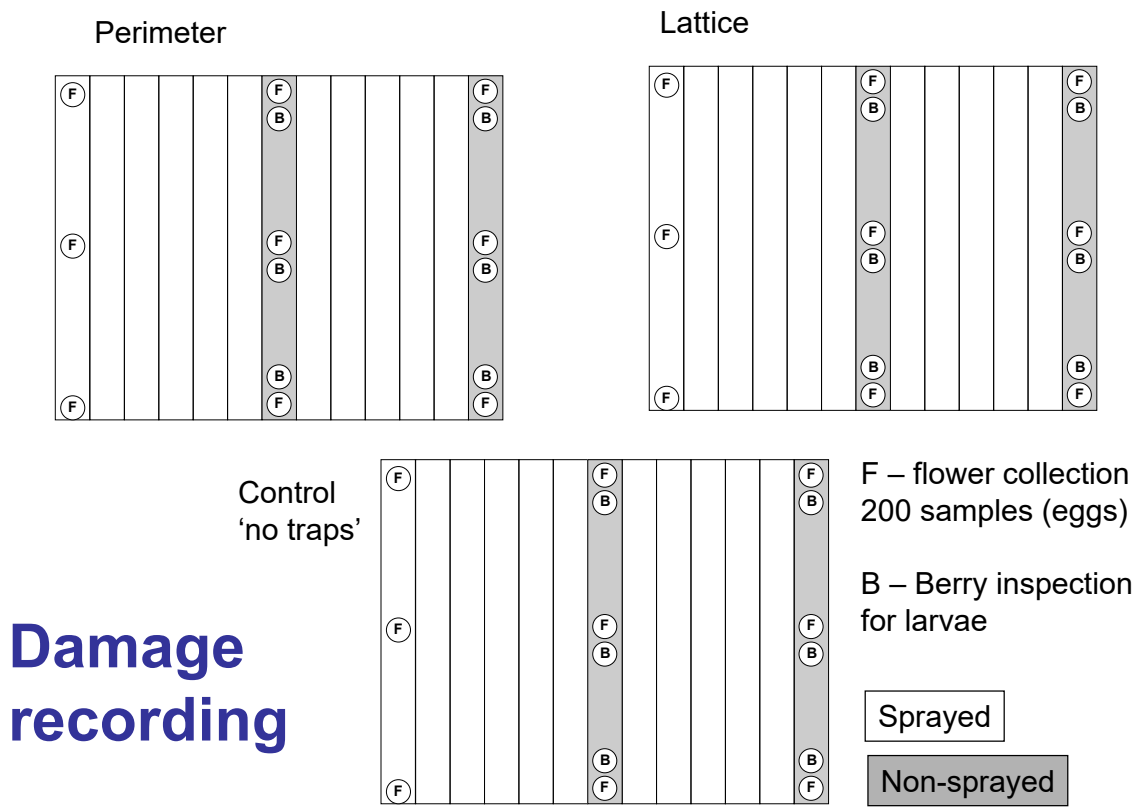


Figure 2.4.1.2 Location of untreated tunnels and sampling points for sampling raspberry beetle eggs (from flowers) and larvae (from fruit)

Table 2.4.1.4 Information about the 12 plantations used in the experiment and the number of traps used in each plantation

Plot no.	Field name	Farm name	Treatment	Plot area (ha)	Row spacing (m)	Rows/ tunnel	No of devices	
							Density (no. /ha)	Required (actual)
1	Bungalow	Decoy Farm, High Halstow	C	0.6	1.8, 2.43	2	0	0
2	Fullers	Decoy Farm, High Halstow	P	0.4	1.8, 2.43	2	50	20(20)
3	Rye Street	Rye Street farm, Cooling	L	0.7	2.43	2	50	30(30)
4	B29	Belks Farm, Otham	C	1.64	2.43	3	0	0
5	L1	Ledian farm, Otham	P	1.55	2.43	3	50	76(60)
6	L4	Ledian farm, Otham	L	0.85	2.43	3	50	46(40)
7	E2	Wester Essendy, Blairgowrie	C	0.93	2.2	3	0	0
8	E1	Wester Essendy, Blairgowrie	P	1.3	1.9	3	50	56(56)
9	E3	Wester Essendy, Blairgowrie	L	0.8	2.2	3	50	40(39)
10	J2	Easter Rattray Farm, B.gowrie	C	0.83	2.2	3	0	0
11	J3	Easter Rattray Farm, B.gowrie	P	0.8	2.2	3	50	40 (40)
12	J1	Easter Rattray Farm, B.gowrie	L	0.76	2.2	3	50	40 (36)

Results

Monitoring traps

Kent 2007

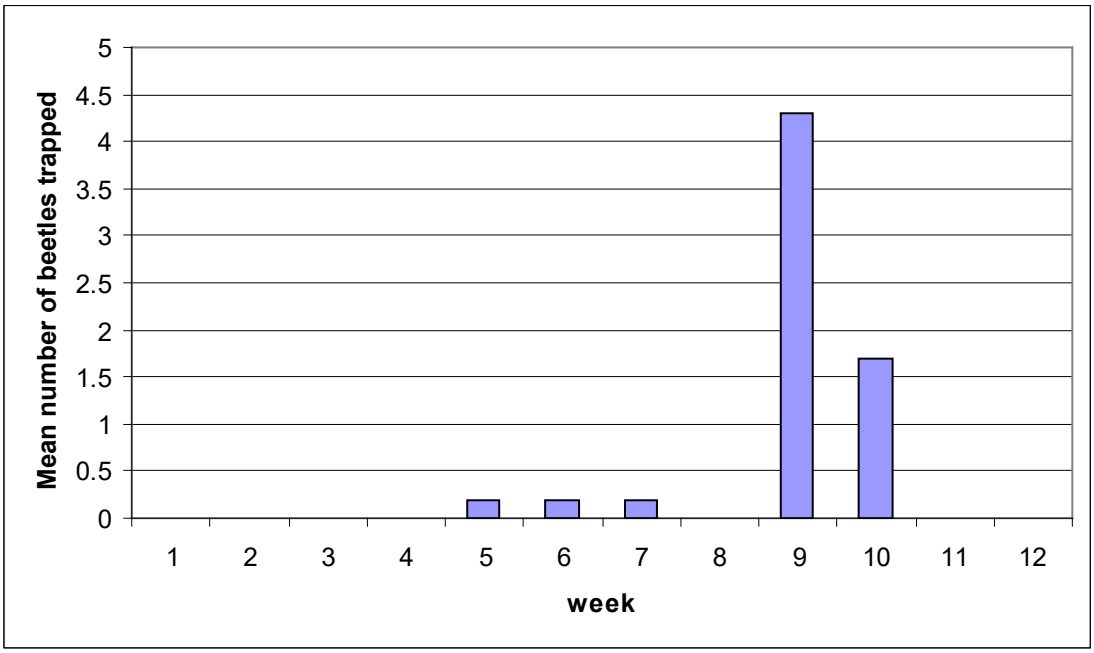
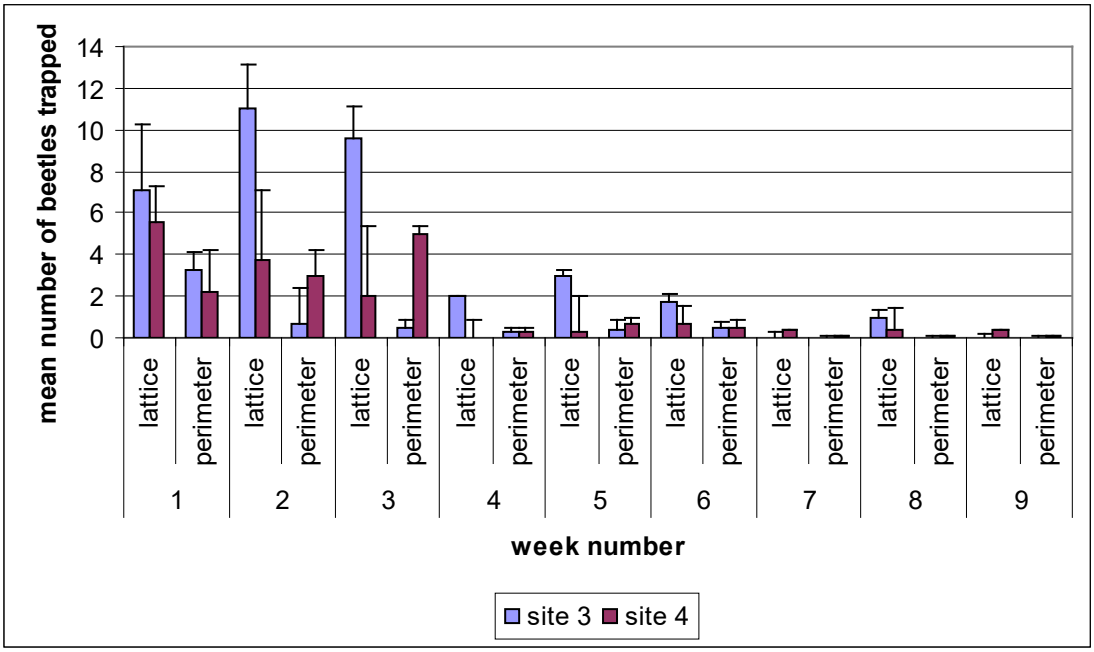


Figure 2.4.1.3 Mean number of beetles trapped at sites 1 and 2 (Kent) on the sticky monitoring traps (24 April - 10 July 2007)

The data from the three treatments (perimeter, lattice and control) at both sites in Kent were grouped together as the numbers caught were very low. Even with the data grouped it was not possible to analyse. The number of beetles captured in the white sticky monitoring traps in Kent (Fig. 2.4.1.3) was quite low, only 87 over the whole monitoring period on the 6 plots (21 from site 1 and 66 from site 2). The first beetles were captured in the white sticky monitoring traps on 8 May at site 2. The peak beetle capture was on 5 June at site 2 and 12 June at site 1, a total of 27 and 5 beetles on all five sticky traps, respectively. The numbers declined after this date and no beetles were found at either site after 26 June.

Eastern Scotland 2007

(a)



(b)

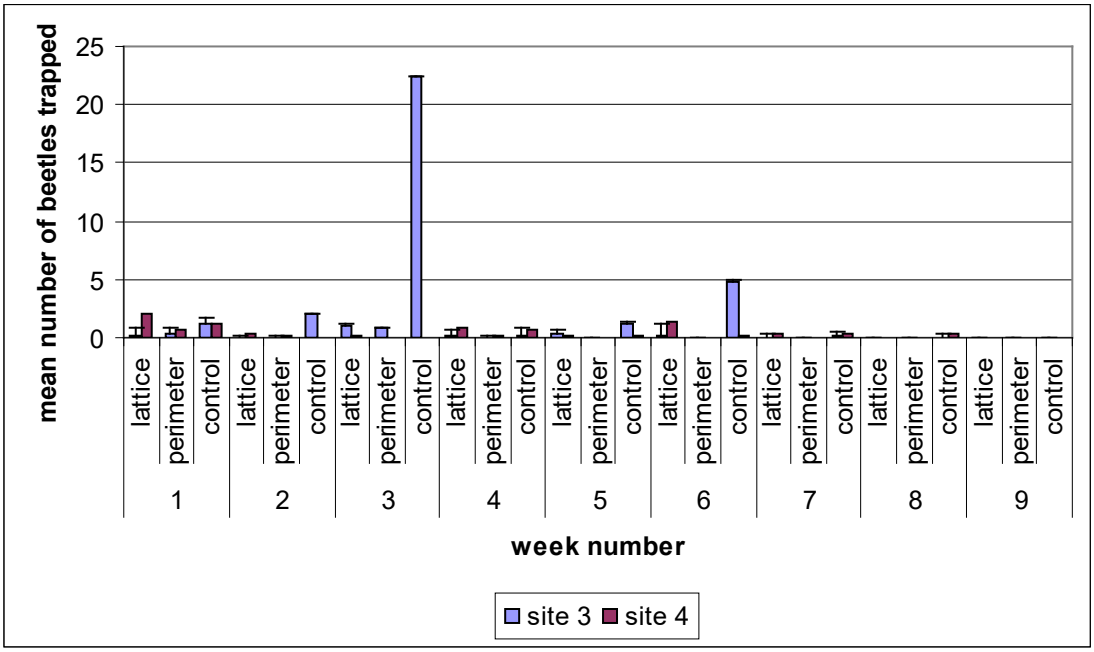


Figure 2.4.1.4 Mean number of raspberry beetles caught in the weekly monitoring (a) funnel traps and (b) sticky traps in eastern Scotland. Error bars represent standard error

Analysis of variance showed that there was a significant difference in the number of raspberry beetles caught between weeks at each site (site 3: d.f 8; v.r.7.14; $F < 0.001$, site 4: d.f. 8; v.r. 3.43; $F < 0.001$). There was a great reduction in the numbers of beetles caught after flowering at both sites (week 3) (Fig. 2.4.1.4a). There were significantly more beetles caught in the perimeter trapping at site 3 (d.f. 1; v.r. 21.19; $F < 0.001$) but no significant difference at site 4. Due to the low numbers of beetles

caught on the sticky traps no analysis was done. However, in weeks 3 and 6 at site 3, sticky traps caught more raspberry beetles in control plots than lattice or perimeter protected plots (Fig. 2.4.1.4b).

Funnel traps

Kent 2007

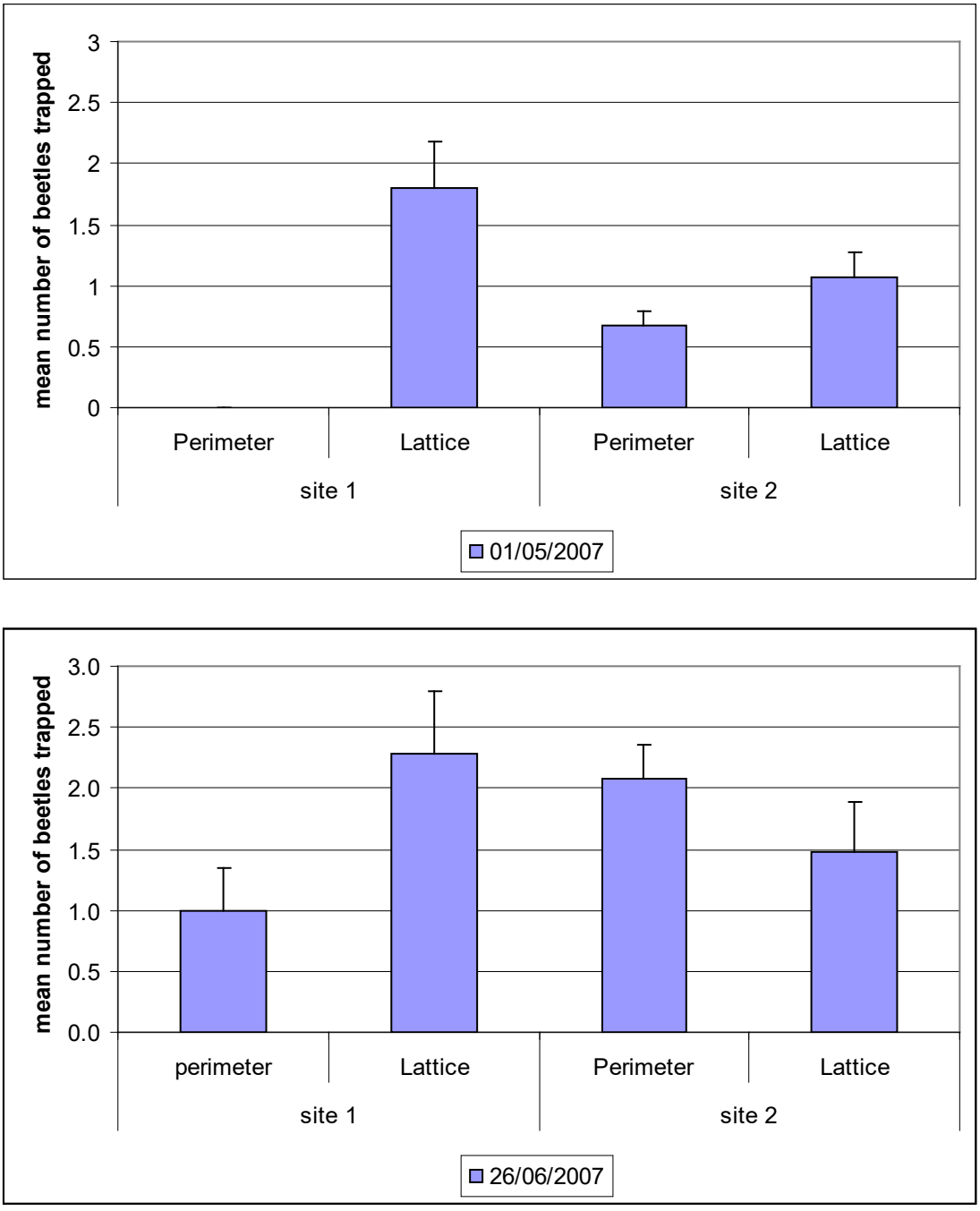
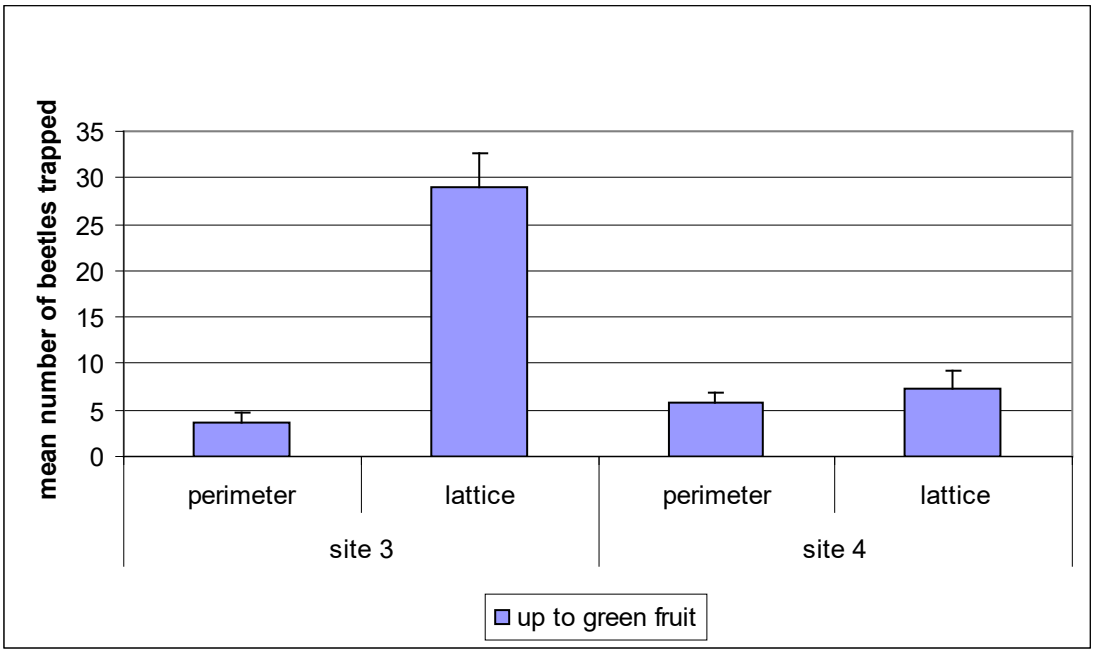


Figure 2.4.1.5 Mean number of raspberry beetles caught in the bucket traps (a) 01/05/2007 (b) 26/06/2007. Error bars represent standard error

The numbers caught in Kent (Fig. 2.4.1.5 a&b) were too low to perform any statistics. The number of raspberry beetles trapped in the funnel traps ranged from 0-12 on each sampling occasion. However, the average number of beetles captured was low, with many traps containing no beetles.

Eastern Scotland

(a)



(b)

Figure 2.4.1.6 Mean number of beetles caught in the bucket traps (a) before green fruit (1 May -12 June 2007) and (b) after green fruit (12 June -11 July 2007). Error bars represent standard error

Analysis of variance showed that for both sites there was a significantly greater number of raspberry beetles caught before green fruit (1 May -12 June 2007) (Fig. 2.4.1.6a) when compared with after green fruit (12 June -11 July 2007) (Fig. 2.4.1.6b) (site 3: d.f 1; v.r.65.7; F < 0.001, site 4: d.f. 1; v.r. 33.58; F < 0.001). At site 3, there were a significantly greater number of beetles caught in the lattice trapping compared with perimeter trapping (d.f. 1; v.r. 48.85; F < 0.001) but there was no significant difference at site 4.

Number of beetles trapped in the edge versus the centre

Table 2.4.1.5 Number of raspberry beetle adults captured on white sticky monitoring traps in Scotland and Kent during the assessment period (EMR 24 April – 10 July 2007; SCRI 8 May – 28 June 2007)

Site	Centre -1 trap				Edge - total of 4 traps (mean)		
	Control	Lattice	Perimeter		Control	Lattice	Perimeter
1	1	0	0		8 (2)	11(2.75)	1(0.25)
2	7	0	1		29 (7.25)	11(2.75)	18(4.5)
3	3	0	23		7(1.25)	6(1.5)	137(34.25)
4	9	2	1		18(4.5)	3(0.75)	14(3.5)
Total	**20**	**2**	**25**		**60(15)**	**31(7.75)**	**170(42.5)**

With only one sticky trap positioned in the middle of the plantation and four traps positioned in the outer corners of the plantation, comparisons of the numbers of catches was difficult (Table 2.4.1.5). There was great variation in the number of beetles caught between sites but combining the data from the four sites showed that in the control plantation the number of beetles caught around the edge was lower than in the middle. The number of beetles caught in the perimeter and lattice plantation was higher around the edge than in the middle.

In the lattice plots, there was very little difference in the number of beetles caught in the bucket traps at the edge of the plantation when compared to the middle of the plantation (Table 2.4.1.6). At sites 1, 2 and 3 there was little difference between the numbers caught in the edge and middle of the lattice plot and the numbers caught in the perimeter plot. At site 4, the number of beetles caught in the perimeter plot was much lower than in the edge and middle traps of the lattice plot.

Table 2.4.1.6 Number of raspberry beetle adults caught (over 2 sampling times) in bucket traps. (EMR; Site 1 - 1 May and 26 June 2007, Site 2 - 1 May and 26 June 2007, SCRI; Site 3 - 12 June and 10 July 2007, Site 4 - 19 June and 11 July 2007)

Site	Treatment	Position	Total number of beetles	Number of traps	Mean number of beetles
1	lattice	edge middle	82 36	20 12	**4.10** **3.00**
	perimeter		20	20	**1.00**
2	lattice	edge middle	74 28	23 17	**3.22** **1.65**
	perimeter		165	60	**2.75**
3	lattice	edge middle	165 99	20 16	**8.25** **6.19**
	perimeter		232	40	**5.80**
4	lattice	edge middle	682 419	24 16	**28.42** **26.19**
	perimeter		210	56	**3.75**

Raspberry beetle eggs and fruit damage

Kent 2007

Egg samples

The flower samples were taken on the 5 June at sites 1 and 2. Despite examining 10,800 raspberry flowers under a microscope, no raspberry beetle eggs were found on any of the sites in Kent, including the untreated controls. A total of 4 adults were found in the flowers. These were at the control and lattice plots at site 1. Because of the low numbers no conclusions can be made about the treatment affects on controlling females laying eggs.

Fruit samples

Table 2.4.1.7 Number of berries and husks with raspberry beetle larvae or evidence of feeding at sites 1 and 2. Fruit samples taken on 26 June (site 1) and 3 July (site 2).

Treatment	Site	Number of larvae	Number of fruit with damage to		Total
			receptacle	flesh	
Control	1	5	3	14	30
	2	4	0	4	
Lattice	1	3	13	19	35
	2	0	0	0	
Perimeter	1	0	0	2	6
	2	1	1	2	

The fruit samples were taken on 26 June (site 1) and 3 July (site 2) (Table 2.4.1.7). The numbers of berries and husks with larvae or evidence of feeding was very low. The numbers were higher in the lattice (35) and control (30) than in the perimeter (6).

Eastern Scotland 2007

Egg samples

Table 2.4.1.8 Number of flowers and berries with the presence of eggs, larvae and adult raspberry beetle. Samples taken on 11 June (site 3) and 19 June (site 4). All samples were taken before insecticides were applied

Site	Treatment	Sample area	Development stage	Eggs	Larvae	Adult
3	perimeter	3	early green	1 - stamen		
	perimeter	9	early green	1 - stigma		
	lattice	1	open flower			1
	lattice	1	early green			1
	lattice	2	early green			1
	lattice	5	green	1- stigma		
	control	1	open flower	1- stamen		
	control	4	early green	1 -stamen		
4	perimeter	2	early green	2 - stigma		
	perimeter	5	early green	1 - stamen		
	perimeter	5	early green	1 - stamen		
	perimeter	6	early green	1 - stamen		
	perimeter	9	early green	2 - stigma		
	perimeter	9	green	1 - stigma		
	control	2	early green	1 - stamen		
	control	2	early green	1 - stamen		
	control	2	early green	1 - stamen		
	control	2	green	1 - stamen		
	control	9	green	1 - stamen		
	lattice	5	open flower	1 - stamen		
	lattice	5	early green	1 - stamen		
	lattice	5	early green	1 - stamen		
	lattice	6	early green	1 - stamen		
	lattice	7	early green	1 - stamen		

Fruit samples

Table 2.4.1.9 Number of berries and husks with raspberry beetle larvae or evidence of feeding. Samples taken on the 11 July (site 3) and 10 July (site 4)

Site	Treatment	No of larvae	Number of fruit with damage to husk	Number of fruit with damage to berry
3	lattice	0	0	1
	perimeter	0	0	0
	control	0	1	1
4	lattice	0	1	1
	perimeter	0	2	0
	control	0	0	0

Overall the number of raspberry beetle eggs found at both sites in eastern Scotland and in all treatments was very low (Table 2.4.1.8). Although not statistically analysed it is noticeable that there was no difference between the three treatments. There were 17 eggs found on the stamens and 7 eggs found on the stigmas. There were no larvae found at either site with only very occasional evidence of feeding damage to both the husks and the berries (Table 2.4.1.9).

Number eggs and amount of damage to fruit at the edge versus the centre

The sampling technique used resulted in a bias towards samples taken at the edge of the plantation and therefore it is hard to make any conclusions about the results obtained (Table 2.4.1.10 & 2.4.1.11). No eggs were found at sites 1 and 2. At site 3, six eggs were found at the edge and two eggs were found in the centre. At site 4, nine eggs were found at the edge and seven were found in the centre. At site 2, there were more infested fruit at the edge than in the centre. At the other sites, the numbers were very low.

Table 2.4.1.10 Number of eggs and larvae found during sampling of flowers and green fruit at Site 3 (11 June 2007) and Site 4 (19 June 2007). No eggs were found at Sites 1 and 2 (sampled 5 June)

Site	Position	Treatment	Development stage	Number of eggs	Number of larvae	Number of adults
3	edge	control	open flower	1- stamen	0	0
	middle	control	early green	1 -stamen	0	0
	edge	lattice	open flower		0	1
	edge	lattice	early green		0	1
	edge	lattice	early green		0	1
	middle	lattice	green	1- stigma	0	0
	edge	perimeter	early green	1 - stamen	0	0
	edge	perimeter	early green	1 - stigma	0	0
4	edge	control	early green	1 - stamen	0	0
	edge	control	early green	1 - stamen	0	0
	edge	control	early green	1 - stamen	0	0
	edge	control	green	1 - stamen	0	0
	edge	control	green	1 - stamen	0	0
	edge	lattice	early green	1 - stamen	0	0
	middle	lattice	open flower	1 - stamen	0	0
	middle	lattice	early green	1 - stamen	0	0
	middle	lattice	early green	1 - stamen	0	0
	middle	lattice	early green	1 - stamen	0	0
	edge	perimeter	early green	2 - stigma	0	0
	edge	perimeter	early green	2 - stigma	0	0
	edge	perimeter	green	1 - stigma	0	0
	middle	perimeter	early green	1 - stamen	0	0
	middle	perimeter	early green	1 - stamen	0	0
	middle	perimeter	early green	1 - stamen	0	0

Table 2.4.1.11 Number of damaged berries found during sampling at Site 1 (26 June 2007), site 2 (3 July 2007), site 3 (11 July 2007) and Site 4 (10 July 2007). Within each treatment, 600 berries were sampled at 3 different positions within the plantation

Site	Position	Treatment	Number of damaged husks	Number of damaged berries	Number of larvae	Total number of fruit affected
1	edge	control	0	1	1	2
	middle	control	0	3	3	6
	edge	lattice	0	0	0	0
	middle	lattice	0	0	0	0
	edge	perimeter	0	0	0	0
	middle	perimeter	1	2	1	4
2	edge	control	3	11	4	18
	middle	control	0	3	1	4
	edge	lattice	11	17	3	31
	middle	lattice	2	2	0	4
	edge	perimeter	0	2	0	2
	middle	perimeter	0	0	0	0
3	edge	control	1	1	0	2
	middle	control	0	0	0	0
	edge	lattice	0	0	0	0
	middle	lattice	0	1	0	1
	edge	perimeter	0	0	0	0
	middle	perimeter	0	0	0	0
4	edge	control	0	0	0	0
	middle	control	0	0	0	0
	edge	lattice	0	1	0	1
	middle	lattice	1	0	0	1
	edge	perimeter	0	0	0	0
	middle	perimeter	2	0	0	2

Non-Target Organisms

Kent 2007

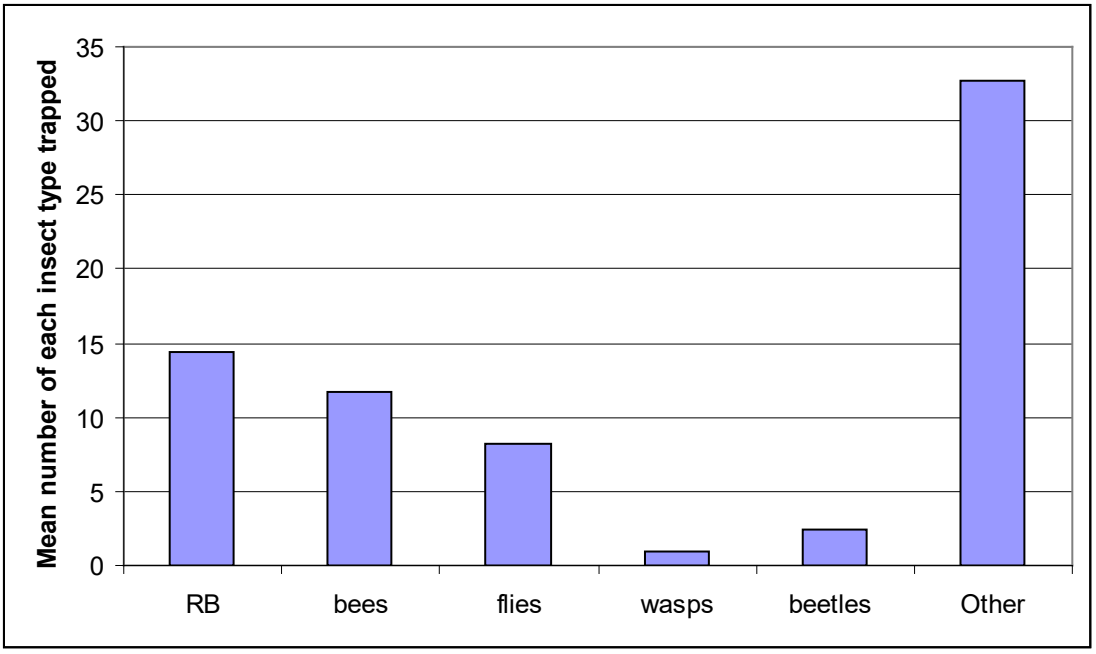
Significantly more honey bees and solitary bees captured in the lattice compared to the perimeter trap treatments when they were emptied on 1 May. No other significant differences occurred between the treatments in the Kent samples (Table 2.4.1.12). However, large numbers of non-target and often beneficial invertebrates were trapped in the funnel traps by 26 June, including bumblebees and honey bees.

Table 2.4.1.12 Mean number of non-target invertebrates captured in raspberry beetle funnel traps in Kent.
P = perimeter traps, L = Lattice traps. Analysis of unbalanced design using regression was used in GenStat.
NA = not analysed because numbers were too low, NS = not significant

		01 May 2007															
Site	**Treat.**	**Bumble bee**	**Honey bee**	**Solitary bee**	**Slug**	**Lepid -optera**	**Coleop -tera**	**Shield bug**	**Dip -tera**	**Syr -phid**	**Chryso -pid**	**Capsid**	**Dermap -tera**	**Spider**	**Wasp**	**Centi -pede**	**Ant**	**Total non-target**
Fullers	P	0.00	0.13	0.00	0.00	0.00	0.38	0.00	0.25	0.00	0.00	0.13	0.13	0.13	0.00	0.00	0.00	1.13
L1	P	0.00	4.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.13
L4	L	0.29	3.00	0.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.86
Rye	L	0.55	7.82	3.91	0.09	0.09	0.09	0.09	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.73
F-Prob		**NS**	**<0.001**	**<0.001**	**NS**	**NS**	**NS**	**NS**	**NS**	**NA**	**NA**	**NS**	**NS**	**NS**	**NA**	**NA**	**NA**	
LSD			**0.273**	**0.197**														
		26 June 2007																
Fullers	P	11.50	13.75	2.13	0.00	0.13	6.13	0.00	4.25	0.00	0.00	0.75	6.63	2.25	1.00	0.00	1.00	49.50
L1	P	7.88	21.63	1.63	0.00	0.38	26.00	0.38	5.13	0.13	0.13	0.00	6.00	0.38	0.38	0.13	0.00	70.13
L4	L	12.40	8.00	1.00	0.00	0.00	6.60	0.20	4.40	0.00	0.00	0.00	4.20	0.20	0.20	0.00	0.00	37.20
Rye	L	12.20	14.40	7.20	0.20	0.00	7.60	0.60	8.80	0.00	0.00	0.00	8.00	0.60	1.00	0.00	0.00	60.60
	No significant differences between treatments																	

Eastern Scotland

(a)



(b)

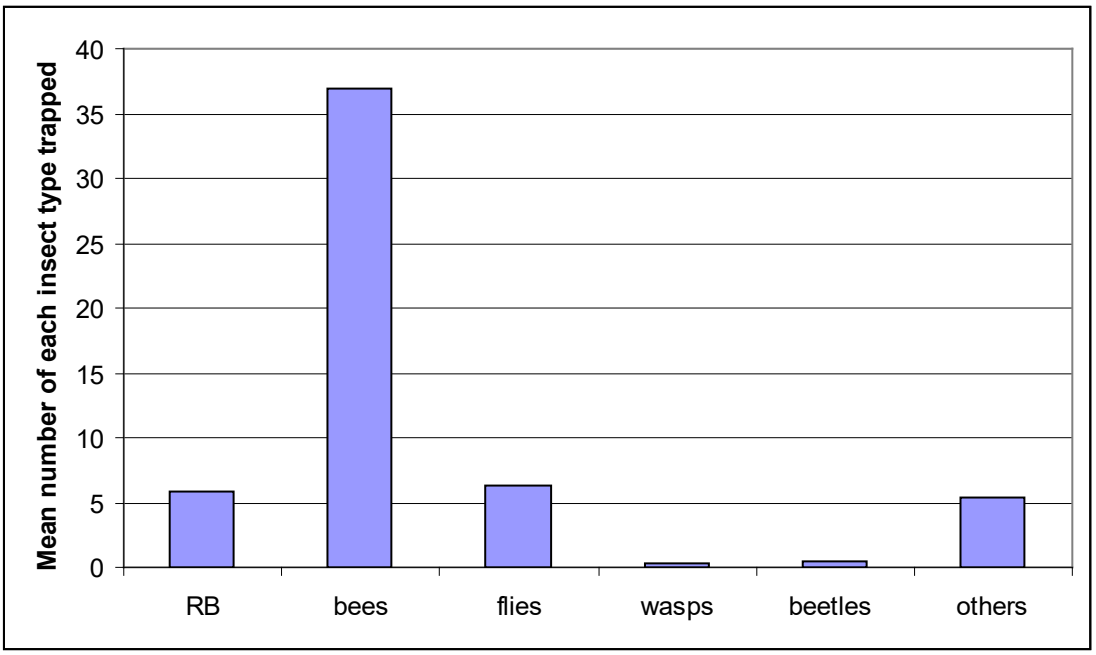


Figure 2.4.1.7 Mean number of each insect type trapped in the bucket traps before green fruit at (a) site 3 and (b) site 4 in Scotland, 2007

Overall the number of non-target insects caught with the exception of bees (honey and bumble) is quite low (fig. 2.4.1.7 a&b). This number varied between sites with a large number (38) caught at site 3 in Scotland. Neither Scottish grower used

introduced bees for pollination but both sites had honey bee hives situated within 1.5 km.

Discussion

- Across all sites there were no consistent differences between numbers of raspberry beetle caught between lattice and perimeter design. Overall, numbers were low, as expected from plantations that had received regular applications of insecticides in previous years and climatic conditions during the trapping period (colder and wetter than previous years).
- Very few raspberry beetle eggs and larvae were found in all three treatments, which resulted in no correlation between trap catches and damage with exception of one site in Kent where control and lattice design had more raspberry beetle larvae and damaged fruit than the perimeter plot.
- The number of bees caught at two sites (L1 in Kent and Site 4 in eastern Scotland) may be an anomaly due to local commercial honey bee hives. The number of bees trapped probably only represents a tiny part of the local population. However, should this cause concern, the trap design may require modification.
- Date of flower opening had a large effect on trapping efficiency, due to competition effects with opened raspberry flowers, as previously reported. This means that the window for effective raspberry beetle trapping is from raspberry beetle emergence to first flowering for any particular cultivar/site combination.

Objective 3. Raspberry cane midge

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

Raspberry cane midge trap height experiment

Objectives

The overall aim of the work was to determine the most appropriate height for capturing raspberry cane midge in delta traps. Six different height traps were deployed; ground level, 0.5, 1.0, 1.5, 2.0 and 2.5m from the ground. The work will be used to determine the best positioning for raspberry midge monitoring traps for use in future studies.

Sites

The plot (CW131, 0.52 ha) was at East Malling Research and was comprised of 16 rows spaced 2.5m apart. Each row had 11 posts spaced 13m apart.

Treatments

The experiment was set up on 4 June. Treatments comprised of white delta traps with sticky bases labelled with the trap height from the ground (Table 3.1.1). Traps were deployed on posts and measured so that the base of the trap was the appropriate height from the ground. They had one lure with 10µg of raspberry cane midge pheromone.

Experimental design

A small scale randomised block design was used with 4 replicates of the 6 different heights (Table 8).

Table 3.1.1. Plan of trap positioning, trap height (m) and plot number

0m 101	1.0m 201	2.0m 301	1.5m 401
0.5m 102	2.5m 202	1.5m 302	2.0m 402
1.0m 103	2.0m 203	2.5m 303	0.5m 403
1.5m 104	0.5m 204	1.0m 304	0m 404
2.0m 105	0m 205	0.5m 305	2.5m 405
2.5m 106	1.5m 206	0m 306	1.0m 406

Assessments

Populations of males

The sticky bases were changed and midges counted on 4 occasions (11, 18, 25 June and 2 July).

Data collation and statistical analysis

Data was collated into Excel spreadsheets, $\log_{10}$ transformed and statistically analysed by ANOVA.

Results and discussion

Numbers of midges captured in traps placed on the ground was significantly higher than any other trap height (ANOVA on $\log_{10}$ transformed data, Fprob = <0.001, sed = 0.204, lsd = 0.614) (Fig. 3.1.1). However, these traps were contaminated with soil and plat debris making this option for raspberry midge monitoring impractical. At the next height, 0.5m, significantly more midges were captured compared to traps >1 m. The number decreased with height, except at 2.5m which had significantly more midges than traps at 2 m (Fig. 3.1.1). The reason for the higher values at 2.5 m is unclear. It is recommended to growers that raspberry cane midge monitoring traps are placed in plots at a standard height of 0.5 m.

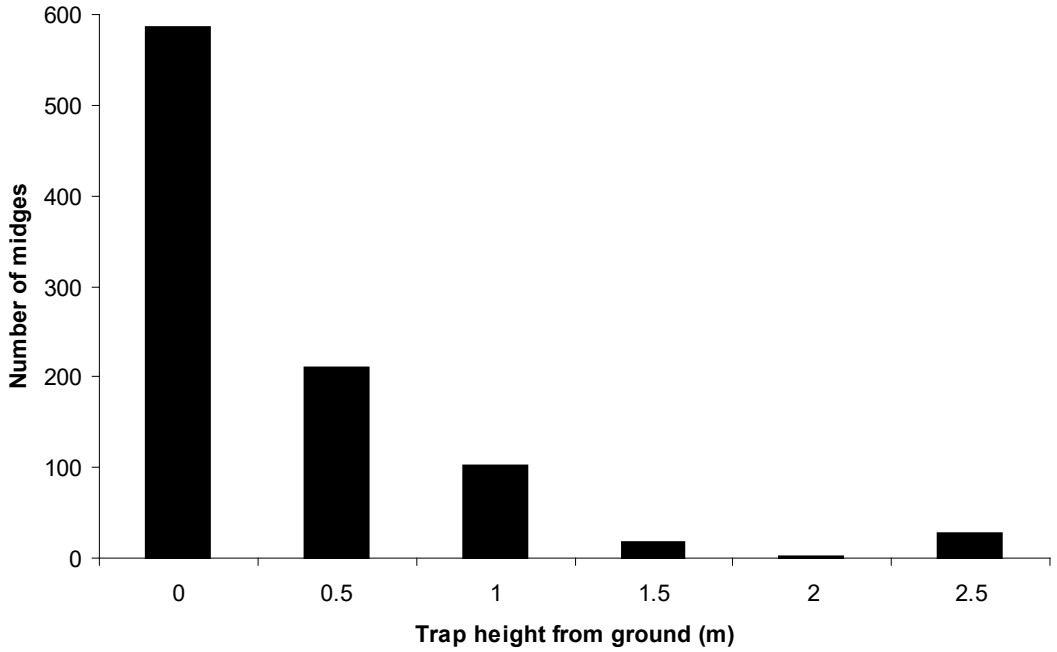


Figure 3.1.1. Number of male midge captured in white delta traps with sticky bases at different height in a raspberry plot

Task 3.3. Identify host plant wound attractant of females

Entrainment of cane volatiles *in situ*

Introduction

A system is being developed for entrainment of volatiles from localised regions of raspberry stems (immature plants) and canes (mature plants). The approach involves the use of a metal framed enclosure (Figure 3.3.1) covered in plastic (PET) film (Figure 3.3.2) to isolate a region of air above the plant stem. Volatiles present in the vicinity of the stem are entrained using a solid phase microextraction (SPME) fibre which is inserted into the enclosure and exposed to the air (Figures 3.3.5. 3.3.6) 4). Experiments conducted with a prototype system have demonstrated that changes in the chemical environment within the enclosure can be reproducibly detected following artificial wounding of the stem.

Entrainment of volatiles

Preliminary experiments were conducted using the raspberry cultivar Malling Delight, with entrainment of volatiles being effected using a 70 µm Carbowax/Divinylbenzene SPME fibre.

Figure 3.3.1 Copper support frames for sampling enclosure and Figure 3.3.2 PET film attached to frame

The copper frames were pre-washed with solvents and baked out at 120-140 °C. Precut films from the Multi-Purpose Cooking Bags ([poly(ethyleneterephthalate)], Sainsbury's Supermarkets) were also similarly baked out then attached to the frames in the open position using shaped copper clips (Figure 3.3.2) This makes it much easier to attach the entrainment enclosure to the plant.

Once positioned on the plants (Figure 3.3.3) the enclosures were partially sealed, using reusable adjustable ties which were provided with the cooking bags to wrap the film around the stem above and below the support frame. In addition, sealing strips from freezer bags were used to seal the enclosures along the long axis. The enclosed space was flushed out with filtered air at about 200 ml/minute for 10 minutes (Figure 3.3.4). The enclosures have an internal volume of about 30 ml which gives about 66 air changes. The airline is removed and the enclosures sealed.

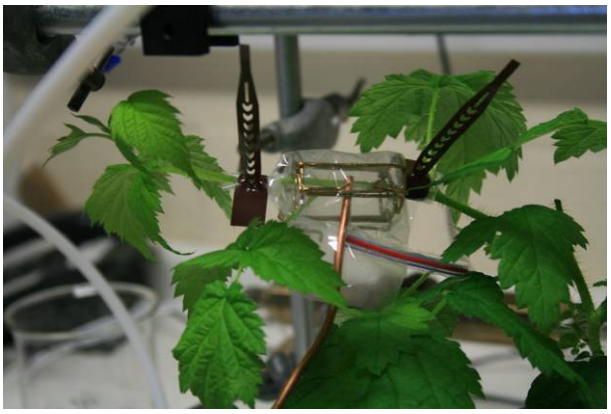


Figure 3.3.3. Copper support frame/ film close around plant stem but not yet sealed
Figure 3.3.4. The partially sealed enclosure is flushed out with filtered air.

A fibre holder needle was pushed though a small perforation in the film and the fibre was exposed as close to and parallel with the stem or wound as possible. Entrainment times could be variable, but ware initially set to 3 hours. Entrainments were carried out in pairs, one being an unwounded control plant and the other having a 2-3 cm manually created wound. (Figures 3.3.5; 3.3.6)

On completion of entrainment, the SPME fibre was withdrawn into its protective sheath, and the fibre holder assembly was removed and fitted to the autosampler of the GC-MS system.

Figure 3.3.5 SPME fibre exposed close to stem within sampling enclosure
Figure 3.3.6 Sampling of a pair of plants, unwounded on left hand side, wounded on right hand side

The sampling system shown above was designed in this project for use with small plants grown in a glasshouse. Individual plants were moved from the glasshouse to the laboratory for the duration of sampling. Subsequently they were returned to the

glasshouse. A larger version of the sampling enclosure was designed for use with more mature raspberry canes, and this has undergone a successful trial in a field plot. (Figures 3.3.7, 3.3.8). The SPME fibre and fibre holder assembly is bound to the plants stem using the re-useable plastic ties.

Figures 3.3.7 and 3.3.8 Sampling enclosure and exposed SPME fibre with field-grown raspberry cane

Chemical analysis of entrained volatiles

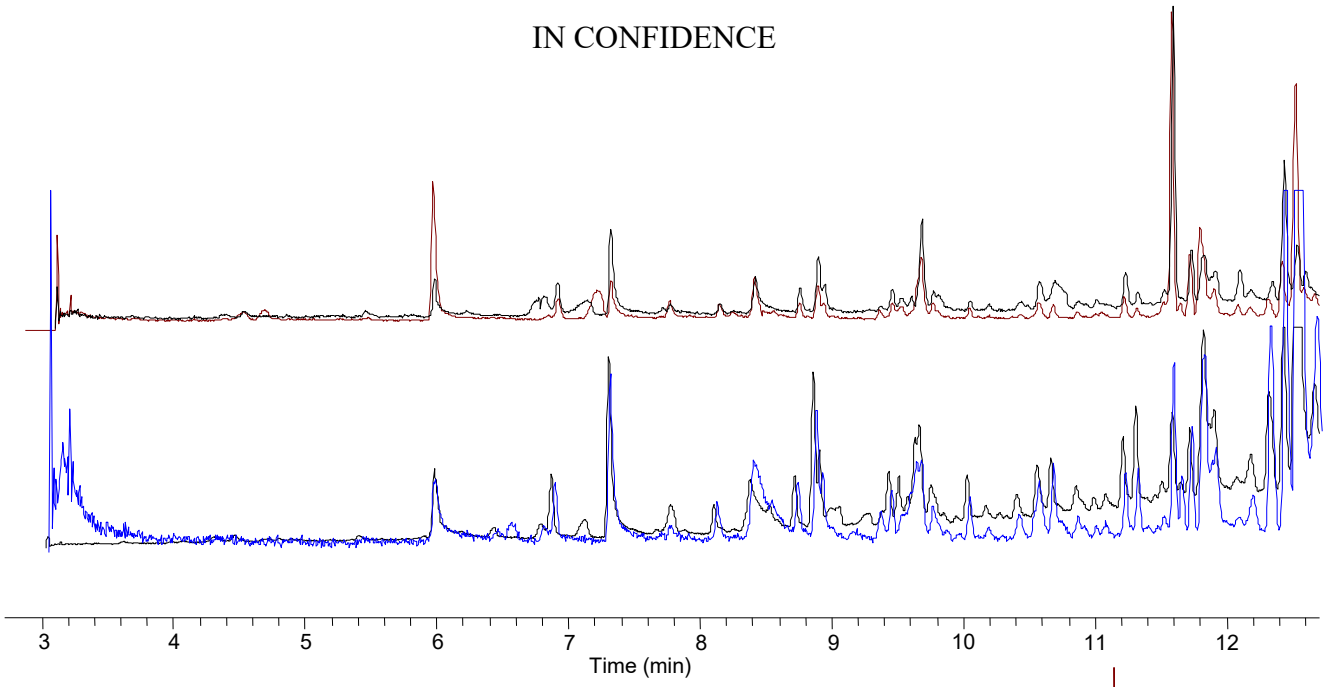
Methodology

Separation of volatiles was effected on a DB 1701 GC column (30m x 0.32mm x 1.0 µm) using helium carrier gas at a flow rate of 1.2 ml/min. The GC -MS consisted of a ThermoFinnigan Tempus Time-of-flight (TOF) system operating at a data acquisition rate of 3 spectra/second. Data was acquired using the Xcalibur software package. Samples were desorbed for 2 minutes into a PTV injector assembly at 200 °C, operating in splitless mode.

Results of pilot experiments

The results from two replicate analyses of wounded and undamaged plants are shown in Figure 3.3.9. The profile of volatile compounds emitted from artificially wounded canes entrained on the SPME fibre are broadly similar for all four plants

over the initial 13 minutes of the chromatographic separation. There are, however, clear difference between the wounded and undamaged plants over the period 13-17 minutes in the chromatogram. The main difference is that there is an increase in the abundance of a suite of monoterpenes and also in methyl salicylate in the volatiles entrained from wounded plants, in comparison with unwounded controls. These terpenes appear to constitute a group of structurally related components, many of which are known to have behavioural effects on insects and plants (e.g. attract natural enemies) and/or are produced in response to insect herbivory. The identities of the compounds listed in Figure 3.3.9 are provisional, based on matches with entries in mass spectral libraries, and have yet to be confirmed (currently in progress; SCRI + NRI).



(1) Monoterpene alcohol M^+ 154 (like linalool?)
(1a) Monoterpene aldehyde M^+ 154 (like citronallall?)
(2) Methyl salicylate
(3) Monoterpene alcohol M^+ 156 (like citronellol?)
(3a) Monoterpene M^+ 150
(4) Monoterpene alcohol M^+ 154
(5) Monoterpene aldehyde M^+ 152 (like citral?)
(6) Monoterpene. .aldehyde M^+ 152 (citral ?)

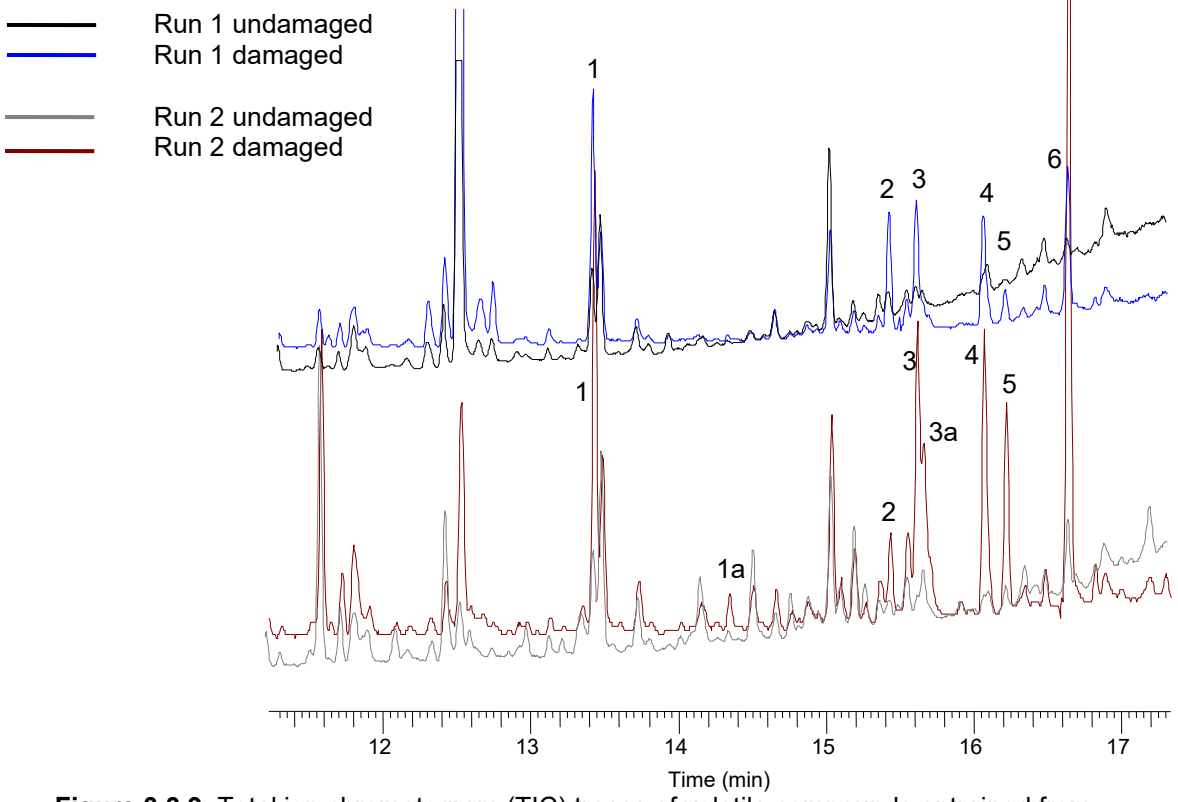


Figure 3.3.9 Total ion chromatogram (TIC) traces of volatile compounds entrained from damaged and undamaged plants using SPME fibres.

Future work

Having demonstrated the feasibility of the sampling methodology, future work will focus of further optimisation of various experimental parameters, ultimately leading to experimentation using different raspberry cultivars and sampling periods.

These include

Sampling time – Clearly, sampling times of 3 hours are sufficient to entrain sufficient volatiles for MS analysis. However, we intend to test a number of sampling times, coupled with time course studies, to examine the temporal dynamics of the plant's response to damage.

Choice of fibre – A range of different SPME fibre chemistries are available which have different analysis specificities. Some of these will be tested and compared to identify the optimal choice of fibre. The experimental design may accommodate the simultaneous use of two or possibly three different types of fibre for collection of samples.

Field sampling – Optimisation of sampling conditions for field work will be required. This will probably require the use of a battery powered field entrainment to provide the controlled filtered airflow needed to flush out the sampling enclosure prior to sample collection.

Use with naturally split plants – This will follow on once the base experimental conditions have been verified. Initially we will use glasshouse-grown raspberry plants of different genotype and growth stage and then move on to field sampling.

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

No work was done on this task; pending successful identification of behaviorally-active wound volatile components once sufficient raspberry cane midges are collected and reared (in progress, SCRI, ADAS, EMR and NRI).

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

No work was done on this task; pending successful identification of behaviorally-active wound volatile components (in progress, SCRI and NRI (GC + GC-EAG, ADAS, EMR (supply of live cane midges for bioassays).

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

Field evaluation of the efficacy of sex pheromone mating disruption and mass trapping 2007

Objectives

The overall aim of the work was to determine whether the raspberry cane midge sex pheromone could be exploited for control of the pest in commercial protected raspberry plantations by mating disruption (high dose of pheromone deployed alone), or by mass trapping (pheromone deployed with killing device).
The work was primarily to determine whether the raspberry cane midge pheromone could be exploited for control of raspberry cane midge. The data may, however, be used in support of an application for registration in future.

Parallel study

The study was run in parallel to a similar study evaluating mass trapping treatments for raspberry beetle. The same plots were used for the two studies, but with a different randomisation of treatments. It was considered that the semiochemical treatments for the two pests were completely independent.

Methods and materials

Sites

One large scale, dispersed randomised block experiment was conducted for one season (April – October 2007). Twelve, approximately 1 ha areas of commercial protected raspberry plantation, 3 on each of 4 farms, were selected (Tables 3.6.1 and

3.6.2). These were expected to have low to moderate populations of raspberry cane midge.

Table 3.6.1. Sites managed by EMR. U = untreated control plots, MD = mating disruption traps, MT = mass trapping

Site and Owner	Address	Plot	Location	Area (ha)	Variety	Description	Treatment (Table 3)
1. John Myatt	Decoy Farm. High Halstow, Rochester ME3 8SR	Bungalow field, Decoy Fm	NGR TQ 786 768	0.6	Glen Ample	3 years old, 15 French tunnels each containing 2 rows. Rows spaced 1.8m apart inside tunnels, 2.43m between rows between tunnels. Rows ~90m long. Post spacing 9m.	MT
		Fullers field, Decoy Fm	NGR TQ 786 768	0.6	Glen Ample	5 years old, 10 French tunnels each containing 2 rows. Rows spaced 1.8m apart inside tunnels, 2.43m between rows between tunnels. Rows ~100 m long. Post spacing 9m.	MD
		Rye Street field, Rye Street Fm, Cooling	NGR TQ 748 762	0.7	Tulameen	8 years old, 12 French tunnels each containing 2 rows. Rows spaced 2.43m apart throughout. Rows ~ 120m long. Post spacing 9m.	U
2. Tim Chambers	WB Chambers & Son, Belks Farm, Otham, Kent ME15 8RL	Field B29, Belks Fm	NGR TQ 8052 2489	1.64	Glen Ample	6 years old, 11 Spanish tunnels (3 rows/tunnel) 165m long. Row spacing 2.43m.	MD
		Field L1, Ledian Fm	NGRTQ 813 527	1.55	Glen Ample	3 years old, 14 Spanish tunnels (3 rows/tunnel) ~150m long. Row spacing 2.43 m	MT
		Field L4, Ledian Fm	NGRTQ 813 527	0.85	Glen Ample	4 years old, 10 Spanish tunnels (3 rows/tunnel) wide ~130m long. Row spacing 2.43m.	U

Table 3.6.2. Sites managed by SCRI. U = untreated control plots, MD = mating disruption traps, MT = mass trapping.

Site and Owner	Address	Plot	Location	Area (ha)	Variety	Description	Treatment (Table 3)
3. Euan McIntyre	Wester Essendy, Blairgowrie, Perthshire, Scotland	Field E1	NO 135 435	1.30	Glen Ample	3 years old, 13 Spanish tunnels (3 rows/tunnel ~ 150 m long). Row spacing 1.9 m.	MT
		Field E2	NO 135 435	0.93	Glen Ample	5 years old, 11 Spanish tunnels (3 rows/tunnel ~ 125 m long). Row spacing 2.2 m.	MD
		Field E3	NO 135 435	1.00	Glen Ample	2 years old, 10 Spanish tunnels (3 rows/tunnel ~ 145 m long). Row spacing 2.2 m.	U
4. Jock McFarlane	Easter Rattray Farm, Blairgowrie, PH10 7HQ	Field J1		0.76	Glen Ample	8 years old, 12 Spanish tunnels (3 rows/tunnel ~ 98 m long). Row spacing 2.2 m.	MT
		Field J2		0.83	Glen Ample	8 years old, 12 Spanish tunnels (3 rows/tunnel ~ 94 m long). Row spacing 2.2 m.	MD
		Field J3		0.80	Glen Ample	8 years old, 12 Spanish tunnels (3 rows/tunnel ~ 89 m long). Row spacing 2.2 m.	U

Treatments

Mating disruption (MD) devices were polythene sachets each initially loaded with 50 mg of the midge sex pheromone racemate (Table 3.6.3, Fig. 3.6.1). These released the pheromone racemate at rates of approximately 0.5 mg/day at 28 ºC in the laboratory. The mass trapping (MT) devices were Lynfield type traps each baited with a rubber septa lure initially loaded with 200 µg of the pheromone racemate (Table 3). The traps contained 50 ml of water + 50% glycol, and were suspended at a height of 15 cm from the ground by attachment to the existing post by a wire attached to a nail hammered into the post at a 90 degree angle (Fig. 2).

Table 3.6.3. Treatments

Code	Control approach	Pheromone lure	Insecticide target device‡ (size)	No. devices /ha	Dose /ha	Release rate
U	Untreated control	None	None	None	0	0
MD	Mating disruption	50 mg sachet	None	200	10 g	0.5 mg/day
MT	Mass trapping	200 µg septum	Lynfield trap	200	0.2g	60 ng/hr
‡ Lynfield type traps were food pots Insulpak uk LTD (C97-250), 85 mm diameter x 55 mm depth. 4 holes cut into the sides were 25mm diameter and were 25 mm from the bottom of the pot. Total pot volume was 250 ml. 50 ml liquid filled to a depth of 10 mm.						

Figure 3.6.1. Mating Disruption sachet

Figure 3.6.2. Mass trapping device

Treatment application

The treatments were applied at the Kent sites between 12-13 April and at the Scottish sites at site 3 on 7 May and at site 4 on 8 May 2007. The devices for both treatments were deployed on every post in the central row of each tunnel a height of approximately 15 cm above the ground in a regularly spaced lattice. The devices were handled with disposable rubber gloves and were removed at the end of the growing season.

Experimental design

A large scale dispersed, randomised block experimental design was used with 4 replicates of the 3 treatments (Table 3.6.3). Blocks were the 4 separate farms. Plots were approximately 1 ha of commercial protected raspberry plantation, 3 plots on each of 4 farms (Tables 3.6.1 and 3.6.2).

Assessments

Populations of males

A white delta trap, bated with a standard lure (10 µg) of the raspberry cane midge sex pheromone racemate was placed in the centre of each plot. In addition, a trap with a high dose sachet (50 mg) was placed in the centre of each MD plot to see if male midges were attracted to or repelled by the high sex pheromone dose. These traps were at a height of 0.5 m above the ground. The number of male midges captured each week was recorded.

The numbers of male raspberry midges in the MT devices were counted monthly. A sample of 25 traps in a 5x5 grid on each plot was assessed on 26 June, 27 July and 5 September in Kent and 25 June and 23 July in Scotland.

Larval populations in splits in canes

Artificial splits in primocane: Fortnightly, throughout the growing season, 20 artificial cane splits were made, 1 in each of 20 primocanes. The splits were distributed in 4 groups of 5 spaced equally along the central, unsprayed row of each plot. Each split was 10cm long approximately and was made by drawing a hooked needle vertically

down the cane, making a slit through the periderm. Care was taken not to injure the cambium below. The needle tip was angled sideways (tangentially to the circumference of the cane) so that the periderm was raised from the cambium tissue, making a flap under which ovipositing cane midge females could lay their eggs. The first cane splits were made on 17 April.

The primocanes used were marked with coloured tape so that they could be easily re-located.

Counts of eggs and larvae in splits: Fortnightly, the 20 artificially split primocanes were collected from each area in each plantation and the number of eggs and larvae in each split counted under a binocular microscope in the laboratory. The length of each split was recorded so that the number of larvae per unit length of split could be calculated.

Amounts of pheromone remaining in lures

24 sachets at each site were individually numbered and pre-weighed to the nearest 0.1 mg before deployment at each site and were fixed to the post with a drawing pin. Fortnightly, two of the pre-weighed sachets at each site were sampled and replaced with fresh ones. The sachets were reweighed to determine the amount of pheromone lost.

Also fortnightly, from each MT site, two rubber septa were removed and replaced from the Lynfield traps and held in individual labelled tubes at -20^{o}C, these were subsequently transferred to NRI where the amount of pheromone remaining was analysed. The traps with new septa were marked to prevent them being reanalysed during the season.

Crop growth stage

The growth stage of the raspberries was recorded on each sampling occasion.

Instructions to host grower

The host grower was made fully aware of the requirements of the trial. It was most critical to make sure that the untreated areas were not inadvertently over-sprayed

with insecticides that were likely to interfere with the conduct of the trial (e.g. chlorpyrifos, deltamethrin, thiacloprid). Each tunnel which was not to be treated was clearly signed to this affect and bays where the primocane were to be artificially split were taped and clearly labelled so as not to be pruned. Overall sprays of fungicide treatments were permissible.

Data collation and statistical analysis

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

Crop destruction

Experimental Approvals allowing this work to proceed without crop destruction were obtained from PSD. The maximum area that could be treated with the MD and MT treatments, under the conditions of a consumer assessed or extrapolated experimental permit was 10 ha for each treatment.

Results

Catches of males in monitoring traps in centres of plots

The number of males caught in the white delta sticky monitoring traps was much lower in the MT and MD treatments compared to the control in Kent (Fig. 3.6.3). This demonstrates that both treatments caused near trap shutdown in this trial. Only 8 male raspberry cane midge were captured at the Scotland sites in all treatments combined over the whole fruit growing season (Table 3.6.4).

Table 3.6.4. The total number of male raspberry midges trapped in the white sticky delta traps with a pheromone lure.

Site	1. Decoy, Kent			2. Belks, Kent			3. Euan, Scotland			4. Jock, Scotland		
Treatment	**U**	**MD**	**MT**	**U**	**MD**	**MT**	**U**	**MD**	**MT**	**U**	**MD**	**MT**
Total number of male midges	626	7	78	1322	43	69	2	0	0	4	0	2

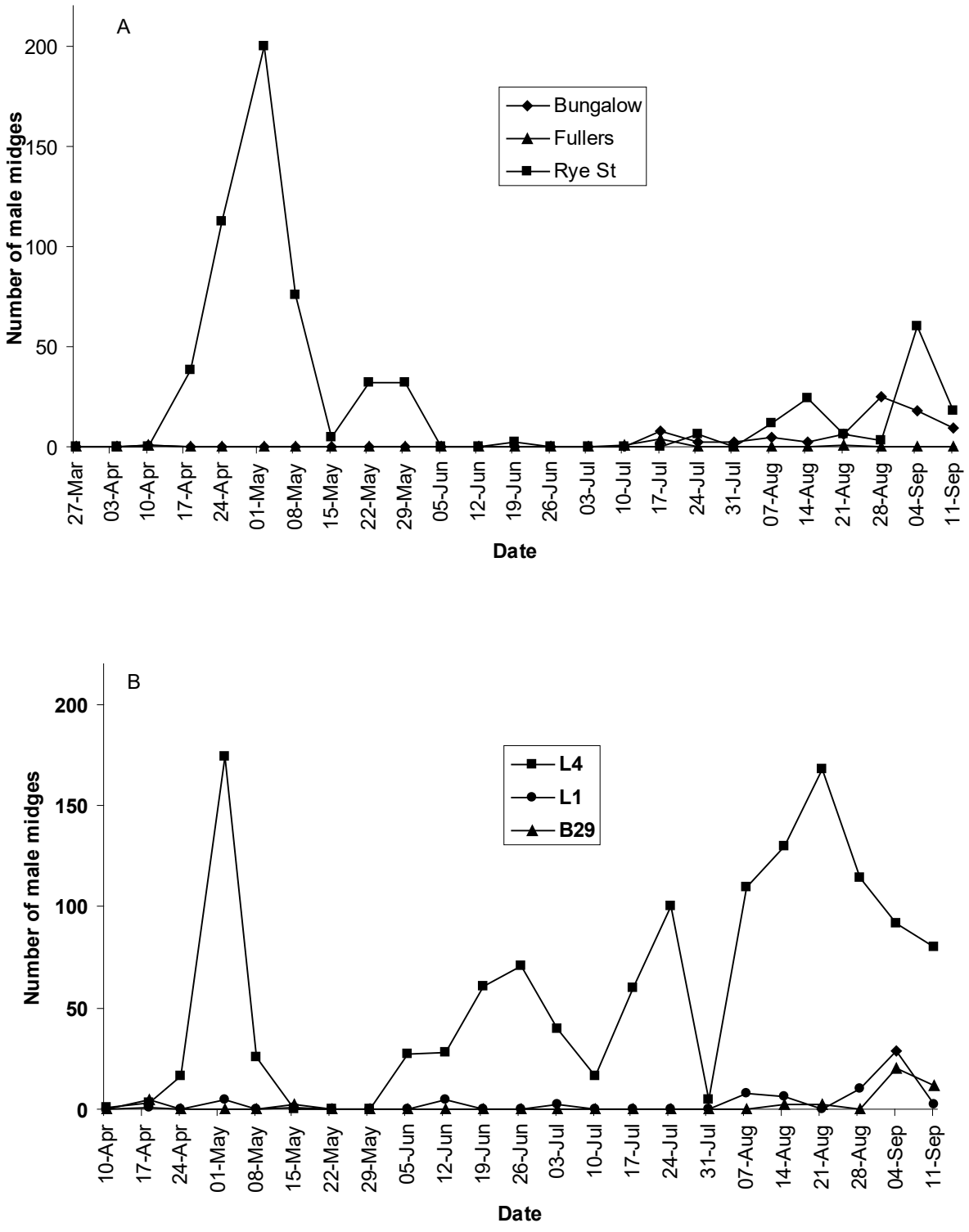


Figure 3.6.3. The number of male raspberry cane midge trapped in the sticky monitoring traps with female sex pheromone lures. A = Decoy Fm. B = Belks Fm, ■ = untreated control, ◆ = mating disruption (MD), ▲ = mass trapping (MT) treatments.

The high dose sachets in white sticky delta traps (on MD plots) did capture male midges, but numbers were very low and never exceeded 10 midges per week (Fig. 3.6.4).

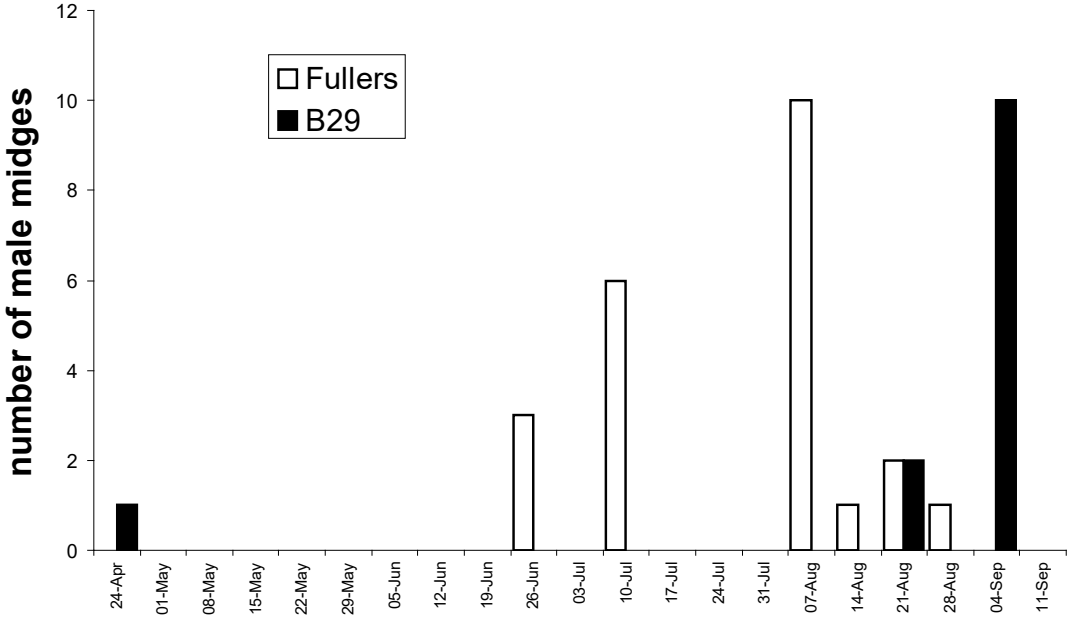


Figure 3.6.4. The number of male raspberry cane midge trapped in the sticky monitoring traps with the high dose pheromone sachets at the Kent sites.

Equally the number of midges trapped in the Lynfield mass trapping (MT) devices was very low. At the Bungalow plot (Site 1) midges were only trapped on one of the sampling occasions in one of the 25 traps (24 midges on 26 June). In plot L1 (Site 2), there were a total of 13 midges in 3 traps on 26 June and 2 midges in one trap on 5 September. No midges were found in any of the traps at Sites 1 and 2 on 27 July (data not shown). No midges were found on either sampling date (25 June and 23 July) at the two Scottish sites (Sites 3 and 4) (Table 3.6.5).

Table 3.6.5. The total number of male raspberry midges trapped in the Lynfield mass trapping devices over the season. 25 traps on each plot checked twice on the season at each site.

Site	1. Decoy, Kent	2. Belks, Kent	3. Euan, Scotland	4. Jock, Scotland
Total number of male midges	24	15	0	0

Egg and larval populations in splits in canes

No raspberry cane midge eggs or larvae were found in any of the artificially splits in the plots at Sites 3 and 4 (Scotland). There was no clear evidence that the number of eggs laid in cane splits was reduced by the mass trapping and mating disruption techniques in the Kent trials. Indeed, the numbers of eggs present in the treated plots was higher than in the untreated control (Table 3.6.6).

No larvae were found in the cane splits from the Scotland trials (Site 3 and 4). There was more than double the number of larvae in the mass trapping treatment compared to the control and the untreated plots. However, with only 2 of the 4 replicate sites having significant numbers of midges it is difficult to conclude if this is a general trend (Table 3.6.7).

Table 3.6.6. Total number of raspberry cane midge eggs/10cm of artificial cane split. U = untreated control plots, MD = mating disruption traps, MT = mass trapping.

	MD		MT		U	
	B29	**Fullers**	**L1**	**Bung-alow**	**L4**	**Rye**
15 May	0.0	0.0	0.0	0.0	0.0	0.0
29 May	0.6	0.0	0.0	0.0	0.0	0.4
12 June	0.6	0.1	1.0	0.0	0.0	0.0
27 June	0.0	0.0	0.0	0.0	0.0	0.0
11 July	0.0	1.5	0.0	0.1	0.1	0.0
24 July	0.0	0.1	0.2	1.0	0.6	0.0
7 August	0.0	0.0	0.0	0.0	0.0	0.0
20 August	0.0	5.9	0.3	0.0	0.0	0.0
5 September	0.0	0.0	0.0	0.0	0.0	0.0
Total number	1.2	7.6	1.6	1.1	0.7	0.4
	MD		**MT**		**U**	
Total number	**8.8**		**2.7**		**1.1**	

Table 3.6.7. Total number of raspberry cane midge larvae/10cm of artificial cane split. U = untreated control plots, MD = mating disruption traps, MT = mass trapping.

	MD		MT		U	
	B29	**Fullers**	**L1**	**Bung-alow**	**L4**	**Rye**
15 May	2.7	0.0	0.6	0.0	0.0	7.5
29 May	0.0	0.0	0.1	0.0	0.0	1.9
12 June	0.0	0.8	0.0	0.0	0.0	0.0
27 June	6.9	0.1	5.6	4.2	2.1	1.6
11 July	0.4	0.1	4.2	0.4	0.1	1.4
24 July	0.0	2.9	3.9	4.5	8.0	1.2
7 August	0.4	0.6	11.0	3.3	5.3	0.0
20 August	1.3	6.8	12.9	15.9	0.0	2.7
5 September	9.8	4.1	8.0	2.1	0.0	0.0
Total number	21.4	15.4	46.3	30.3	15.5	16.4
	MD		**MT**		**U**	
Total number	**36.8**		**76.6**		**31.7**	

Amounts of pheromone remaining in lures

The mating disruption sachets lost weight linearly over the period of the study (Figs. 3.6.5 and 6). Of the initial 50 mg pheromone placed into the sachet the sachets in Scotland lost up to 21 mg (in 113 days, Fig. 6), whilst the sachets in Kent lost up to 44 mg (in 146 days, Fig. 5). In the laboratory wind tunnel sachets lost 31 mg in 28 days (Fig. 3.6.7).

The mass trapping lures lost a similar amount of pheromone in both Kent plots (L1 and Bungalow) over time (initial volume 200 µg). The pheromone remaining in the lures towards the end of the study period was reduced to approximately 20% (Fig. 3.6.8).

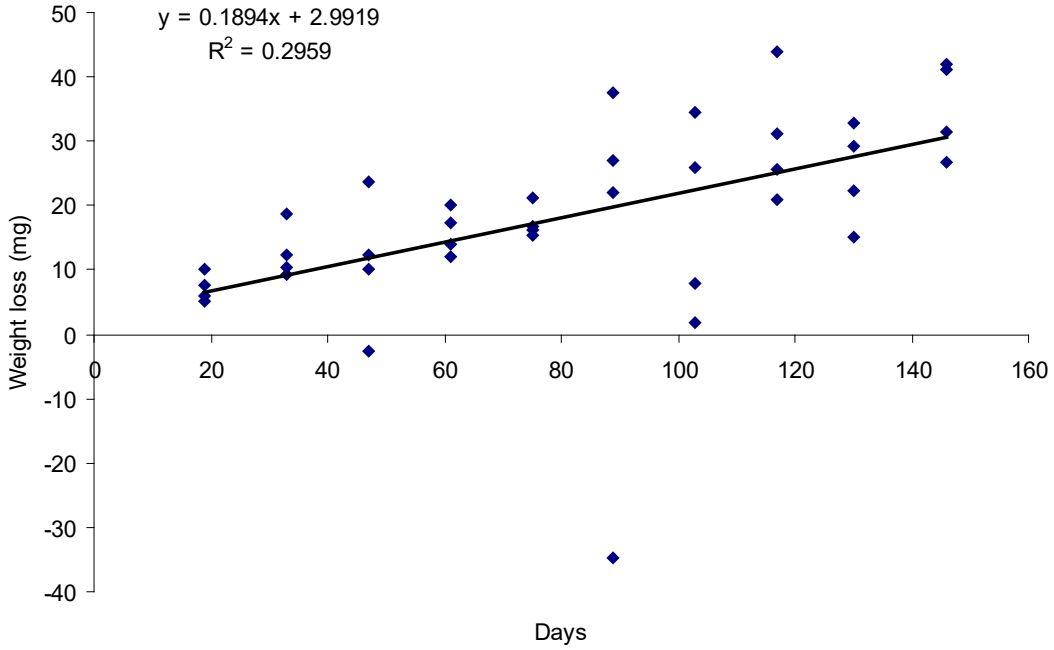


Figure 3.6.5. Weight loss of the mating disruption sachets (Fig. 1) at Sites 1 and 2 (Kent, data combined) over time (extreme minus values omitted)

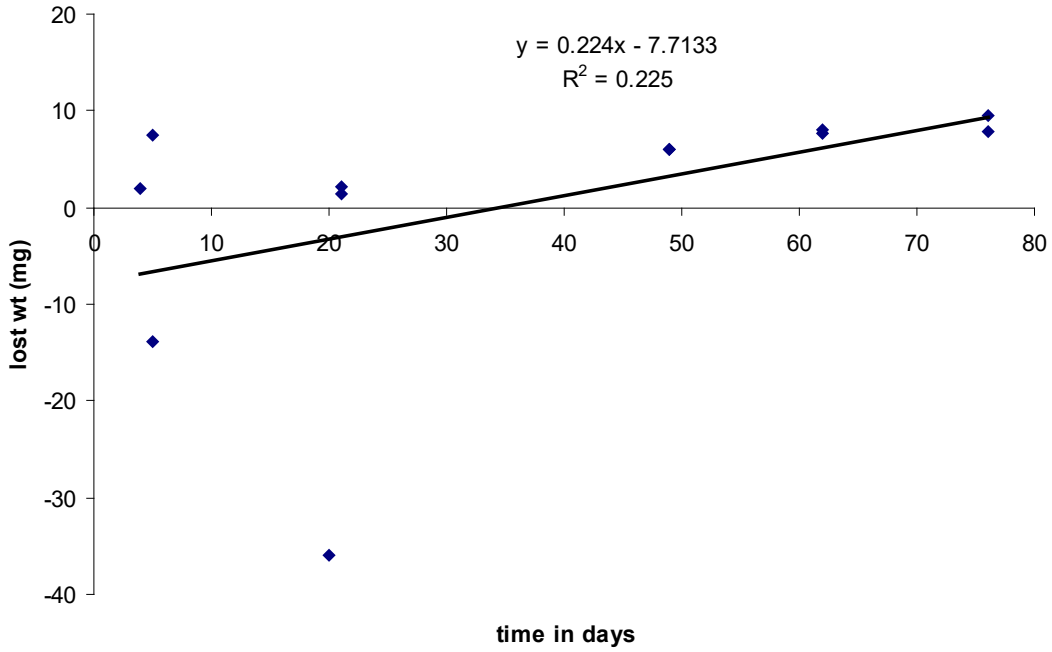


Figure 3.6.6. Weight loss of the mating disruption sachets (Fig. 1) at Sites 3 and 4 (Scotland, data combined) over time.

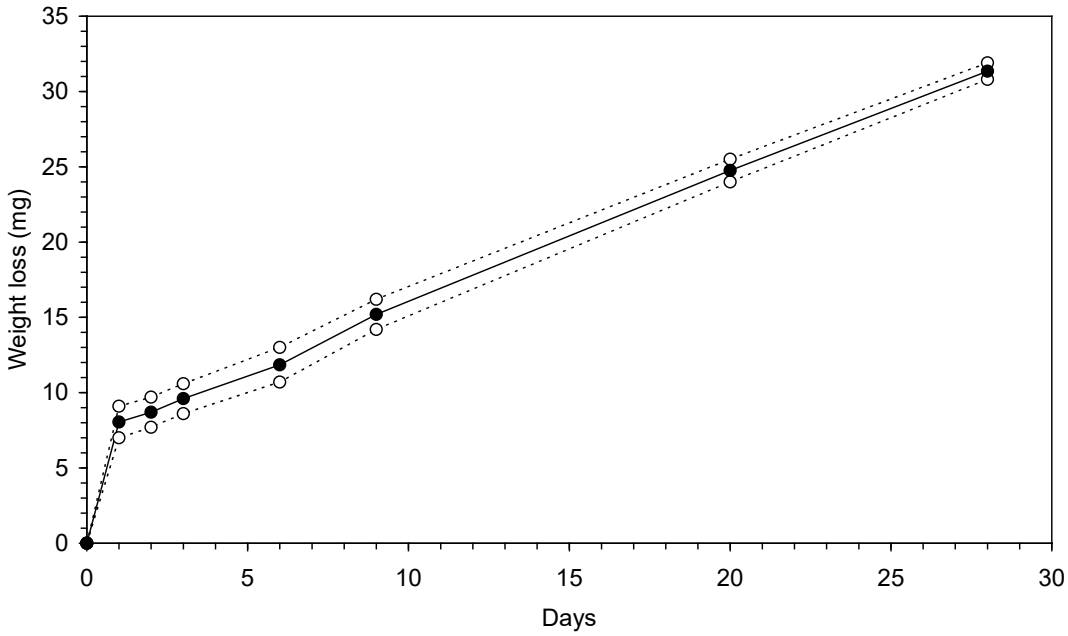


Figure 3.6.7. Weight loss of the mating disruption sachets placed in a wind tunnel.

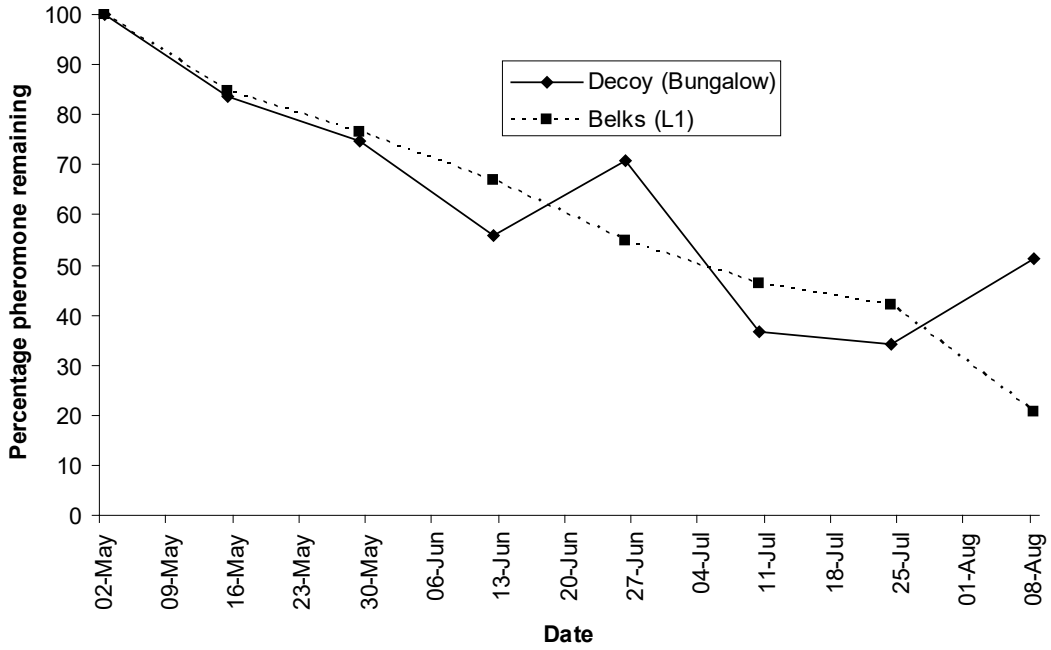


Figure 3.6.8. Percentage loss of pheromone lost from the initial 200 µg in the lures in the mass trapping devices at Decoy Farm and Belks Farm

Objective 4: Mildew

Task 4.1. Inoculum sources

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

Introduction

As agreed by the consortium in 2007, we have directed the research to assessing whether powdery mildew from strawberry could infect raspberry and vice versa. Two types of experiments were conducted. First, strawberry and raspberry plants were inoculated with mildew from raspberry and strawberry, respectively. Second, we have conducted molecular analysis of mildew strains collected from raspberry and strawberry to determine whether they are likely to come from different species.

Materials and methods

Strawberry (cv. Elsanta) and raspberry (cv. Joan Squire) plants were inoculated in controlled environmental cabinets with mildew from raspberry and strawberry, respectively. Inoculated plants were maintained in the cabinets and regularly assessed for powdery mildew. Young leaves were frequently inoculated with mildew conidia using a paintbrush.

For molecular work, many samples of strawberry and raspberry mildew were collected from different areas. Raspberry mildew samples were obtained from SCRI, field crops in Cambridge, glasshouse plants at EMR in early spring and field plants in autumn. Strawberry mildew samples were obtained from several regions in the UK, and from a few other countries (Italy, China, USA and Israel). In order to increase the probability of extracting mildew DNA from leaves, a leaf disc (diameter 0.5 cm) with a single lesion was cut out immediately in the field and placed into a centrifuge vial containing 1-2 ml of 95% ethanol. The vials were then shaken manually for 10-20 seconds and placed in the laboratory under ambient conditions. After 24 h, the disc was removed from each vial in the flow cabinet and the vial was then left open to dry inside the cabinet. Once dried, these vials were then stored in a fridge until DNA extraction. For a few samples from Italy and Israel, leaves with lesions were taken

and dried under ambient conditions; discs with a lesion were then cut out and extracted for mildew DNA.

These mildew samples were screened for their genotypes of several SSR primers developed for strawberry mildew at EMR. Furthermore, samples of strawberry (27 samples) and raspberry (8 samples) mildew were selected for sequencing their ITS region. The resulting sequences were analysed with cluster analysis using MEGA software to quantify their genetic relationships.

Results

Inoculation of strawberry plants with spores powdery mildew from strawberry resulted in powdery mildew development. Inoculation of raspberry plants with spores of powdery mildew from raspberry did not result in powdery mildew development.

Cross-inoculation of strawberry and raspberry plants with raspberry and strawberry mildew conidia did not result in visible lesions on either strawberry or raspberry. The reason for failure to establish powdery mildew or raspberry is unclear; possibly a very narrow range of environmental conditions facilitate infection on raspberry. Several inoculation methods and incubation conditions were previously tested without success (year 1 report).

SSR results suggested that raspberry mildew is different from strawberry mildew. This was further supported by the ITS sequences (Fig. 4.1.1.1), where strawberry mildew samples were clearly separated from the raspberry mildew samples.

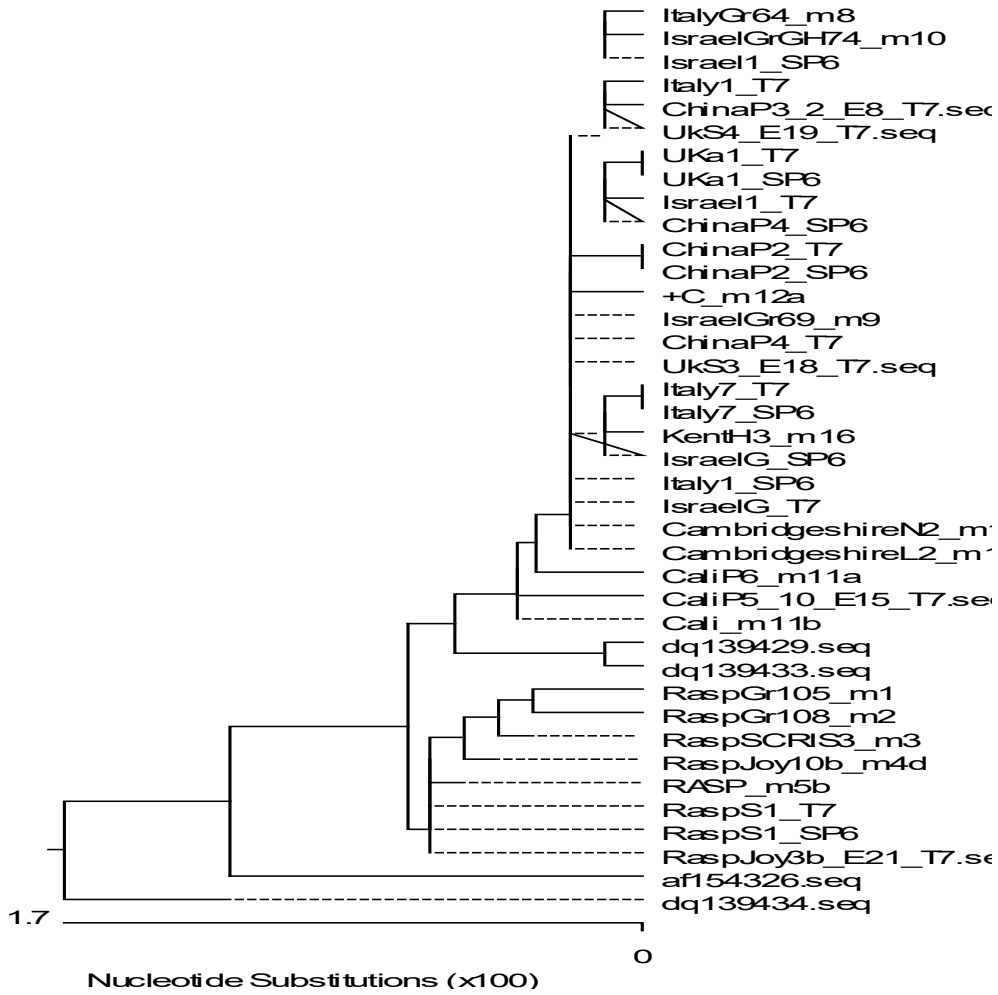


Figure 4.1.1.1. Phylogenetic trees depicting the genetic relationships between a selected number of isolates of strawberry and raspberry mildew based on ITS sequences. Names for strawberry mildew isolates began with their country origins - UK, Italy, Israel, China and USA (California). All raspberry mildew isolates started with 'Rasp'.

Conclusions

- Powdery mildew from strawberry and raspberry failed to infect raspberry;
- Molecular data based on SSR and ITS sequences suggested that strawberry and raspberry mildews are two distinct groups;
- Further molecular work will be carried out in 2008 using functional genes to compare mildews from strawberry and raspberry.

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 2006 – 07

Objective

The objective of this experiment was 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 2006-spring 2007.

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 were spaced 9' (=2.74 m) apart. Total area of plantation = 1.75 ha. Tunnels covered pairs of rows, but were not covered with polythene. The rows ran NNE SSW.

Shaw Field (No. 211) NGR TQ 744 505: Consisting of 48 rows 158.9 m long. Rows were spaced 9' (=2.74 m) apart. Total cropping area of plantation = 2.09 ha. Tunnels covered pairs of rows, but were not covered with polythene. The rows ran NNE SSW.

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

Treat. no.	Colour code	Product	Active ingredient	Dose rate (/ha)	Timing(s)
1	Red	Calypso	480 g/l thiacloprid SC	250 ml	7 Sept
2	Blue		"	"	21 Sept
3	Yellow		"	"	5 Oct
4	Black		"	"	19 Oct
5	White				6 Nov
6	Green	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 kmph. 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 (Table 5.1.1.2). 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.

Table 5.1.1.2. Randomisation of treatments to plots

Block 1		Block 2		Block 3		Block 4	
Plot no.	**Treat no.**	**Plot no.**	**Treat no.**	**Plot no.**	**Treat no.**	**Plot no.**	**Treat no.**
101	6	201	5	301	1	401	2
102	5	202	3	302	2	402	3
103	4	203	4	303	3	403	4
104	3	204	1	304	4	404	1
105	1	205	6	305	5	405	6
106	2	206	2	306	6	406	5

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 over wintering 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 January 2007.

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

Statistical analysis

Analysis of variance was done on the data after square root and log10(n+1) transformation to stabilise variances.

Results and conclusions

All the timings of Calypso treatment reduced the numbers of eggs in winter and the numbers of aphids in spring. However, the 19 October timing clearly gave the best results reducing aphid numbers by >95%. The winter egg data showed a smooth time/response curve with a clear minimum at 19 October. The spring aphid data was more erratic.

Treatment	Eggs	Adults	Nymphs	Mummies	Total aphids
Untreated control	86	6.8	13.8	0.4	21.1
Calypso 7 Sep	17	5.5	25.9	1.0	32.4
Calypso 21 Sep	12	1.0	9.5	0.5	11.0
Calypso 5 Oct	6	6.3	27.1	0.4	33.8
Calypso 19 Oct	3	0.5	2.1	0.3	2.9
Calypso 6 Nov	49	16.6	59.7	0.8	77.0

Autumn control of aphids on raspberry 2007 – 08

Experiment ORETO GEP No 07/03

Objective

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

Site

The edge rows of one of two Glen Ample plantations owned by Clockhouse Farm, Coxheath (courtesy of Robert Pascal Tel office: 01622 743955, Mob: 07973 490379). Deputy farm managers James Dearing (Mob: 07973 539165) Nick Deppe (Mob: 07976 555036). One plantation is at Clockhouse farm, the other at Teston

Note: Because of the presence of the tunnels, sprayer access is only possible in every other row, the legs of the tunnels blocking alternate rows. In effect, the crop should therefore be regarded as consisting of two row beds on a double (5.48 m) row spacing.

Treatments

Treatments are single sprays of Calypso, Aphox or Plenum applied at different timings, as given in Table 5.1.1.5 overleaf.

Table 5.1.1.5. Treatments

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

Treatment application

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

Experimental design and layout

The eastern and western outside rows of one of the two plantations are being used, with two replicates in each of the rows. A randomised complete block experimental design with 4 replicates of the 10 treatments (= 40 plots) to be used. Plots are 2 rows wide and 1/20 of the plantation length long. Randomisation is given in Table 5.1.1.6.

Table 5.1.1.6. Randomisation of treatments to plots

Block 1			Block 2			Block 3			Block 4		
Plot no.	**Trt no.**	**Col**	**Plot no.**	**Trt no.**	**Col**	**Plot no.**	**Trt no.**	**Col**	**Plot no.**	**Trt no.**	**Col**
101	5	Bk B	201	9	B Y	301	8	B B	401	7	B
102	2	R B	202	1	R	302	3	R Y	402	4	Bk
103	9	B Y	203	3	R Y	303	5	Bk B	403	6	Bk Y
104	10	G	204	8	B B	304	4	Bk	404	2	R B
105	1	R	205	2	R B	305	6	Bk Y	405	9	B Y
106	6	Bk Y	206	5	Bk B	306	7	B	406	10	G
107	3	R Y	207	10	G	307	9	B Y	407	8	B B
108	7	B	208	4	Bk	308	1	R	408	3	R Y
109	4	Bk	209	6	Bk Y	309	2	R B	409	5	Bk B
110	8	B B	210	7	B	310	10	G	410	1	R

Progress on milestones

Primary milestones

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

Milestone	Target month	Title	Acheived?	On time?
P6.1	48	Integrated pest and disease management strategy tested on commercial nurseries and shown to result in nil or minimal detectable pesticide residues at harvest		
P6.2	60	Best practice guidelines for IPM in protected raspberry written		
P6.3	60	Occurrence of pesticide residues in crops grown to IPM standard compared with conventional crops		

Secondary milestones

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

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

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

Technology transfer activities

27 June 2007. Consortium visit to Belks Farm Otham and Salmans farm, Penshurst attended by consortium members and host growers and advisors.

21 September 2007. Talk about zero residues IPM methods given by J Cross at workshop

24-27 Septmebr 2007. IOBC IPP soft fruit crops workshop held at EMR. Several papers about the project presented to 70 international delegates.

28 Nov 2007 HortLINK 2007, Lewis Media Centre, Millbank Towers. Poster presented on project.

6 December 2007. EMRA zero residues day. 20 minute talk given about zero residues soft fruit production by J Cross. Attended by ~ 100 growers, technical experts etc

18 March 2008. Sainsbury's Biopesticide/IPM Confernce, Sainsbury's Headquarters, Holbourne, London. Presentation and 1 our discussion session on non-pesticidal methods for controlling UK fruit pests lead by J Cross. The session was attended by approximately 40 persons including growers, technical experts and Sainsbury's fruit suppliers

2007. Articles featuring Hortlink research in HDC News No. 129, 130, 1332 (SCRI & EMR).

1 June 2007. LEAF Open Farm Sunday event at SCRI (1000 attendees)by Nick Birch.

14 Sept 2007, Arlnap University. Presentation by Nick Birch to IOBC Working Group on Semiochemicals and IPM (c. 50 delegates).

24 Sept 2007. EMR. Presentation by Nick Birch to IOBC Working Group on Integrated Soft fruit production.

7 Feb 2008. Planning meeting at Bioforsk As. Nick Birch met with Nina Trandem to coordinate testing oh raspberry beetle traps and lures in Norway during 2008.

Publications

Jerry Cross and Angela Berrie. 2008. Development of zero pesidice residue Integrated Pest and Disease Management programmes for UK fruit crops. ICE 2008 Abstract.

Jerry Cross, David Hall, Michelle Fountain, Adrian Harris and Dudley Farman. 2008.Utilising sex pheromones of raspberry cane midge, *Resseliella theobaldi*, and apple leaf midge, *Dasineura mali*, for pest monitoring and control. ICE 2008 Abstract.

Jerry Cross, Catherine Baroffio, Alberto Grassi, David Hall, Barbara Łabanowska, Slobodan Milenković, Thilda Nilsson, Margarita Shternshis, Christer Tornéus, Nina Trandem, Gábor Vétek Monitoring raspberry cane midge, *Resseliella theobaldi*, with sex pheromone traps: results from 2006. IOBC-wprs Bulletin

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

Angela Berrie, Tim O'Neill, Erika Wedgwood, Barbara Ellerker. Evaluation of alternative chemicals for control of botrytis in raspberries. IOBC Bulletin (in press)

Birch, A.N.E., Gordon, S.C., Mitchell, C., Shepherd, T., Griffiths, D.W., Robertson, G. & Brennan, R. 2007. Development of semiochemical attractants, lures and traps for raspberry beetle, *Byturus tomentosus* at SCRI; from fundamental chemical ecology to testing IPM tools with growers. *IOBC Bulletin,* Lund, Sweden, September 2007.

Birch, A.N.E., Gordon, S.C., Mitchell, C., Shepherd, T., Griffiths, D.W., Robertson, G. & Brennan, R. 2007. Development of semiochemical attractants, lures and traps for raspberry beetle, *Byturus tomentosus* at SCRI; from fundamental chemical ecology to testing IPM tools with growers. *IOBC Bulletin 'Integrated Crop Protection in Fruit Crops',* East Malling, Kent, September 2007.

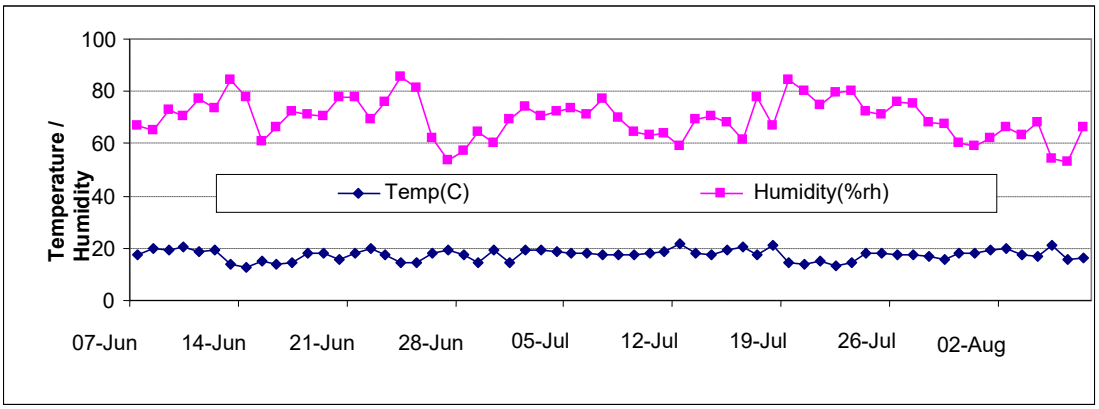
Birch, A.N.E. Gordon, S.C. Article in HDC News No. 130, promoting Hortlink 0175.

Birch, A.N.E., Gordon, S.C., Brennan, R.M., Jennings, S.N. & Mitchell, C. 2007. Breeding for durable resistance to the large raspberry aphid, *Amphorophora ideai*, in field and protected raspberry plantations: Co-evolution and IPM. *IOBC Working Group: Integrated Plant Protection in Fruit Crops, subgroup, 'Soft Fruits',* East Malling Research, 24-25 September 2007.

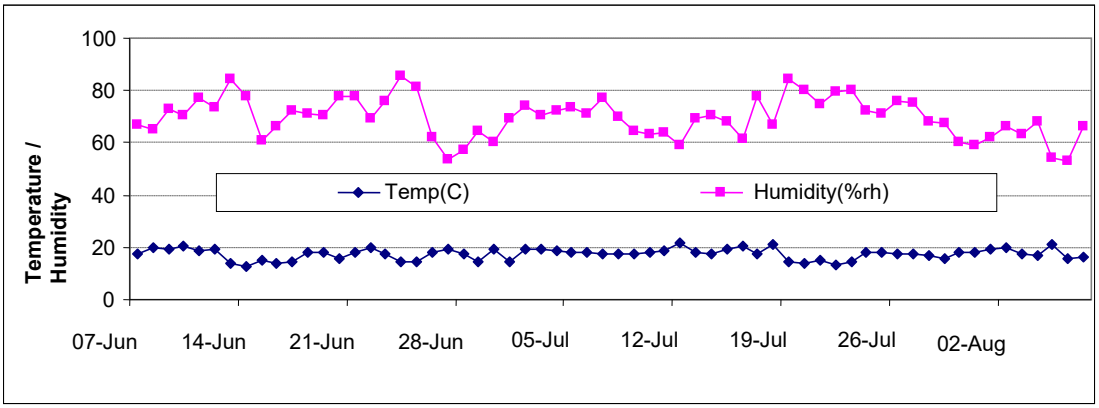
Birch, A.N.E., Gordon, S.C., Mitchell, C., Shepherd, T., Griffiths, D.W., Robertson, G. & Brennan, R.M. 2007. Development of semiochemical attractants, lures and traps for raspberry beetle*, Byturus tomentosus* at SCRI – from fundamental chemical ecology to testing IPM tools with growers. *IOBC Working Group: Integrated Plant Protection in Fruit Crops, Subgroup, 'Soft Fruits',* East Malling Research, 24-25 September 2007.

Trandem, N., Gordon, S.C., Birch, A.N.E., Ekeland, M. & Heiberg, N. 2008. Mass trapping of raspberry beetle as a possible control method - pilot trials in Norway. *IOBC (Bulletin Series) Oral presentation and ongoing collaboration with Bioforsk,* Ghent University Press, Ghent.

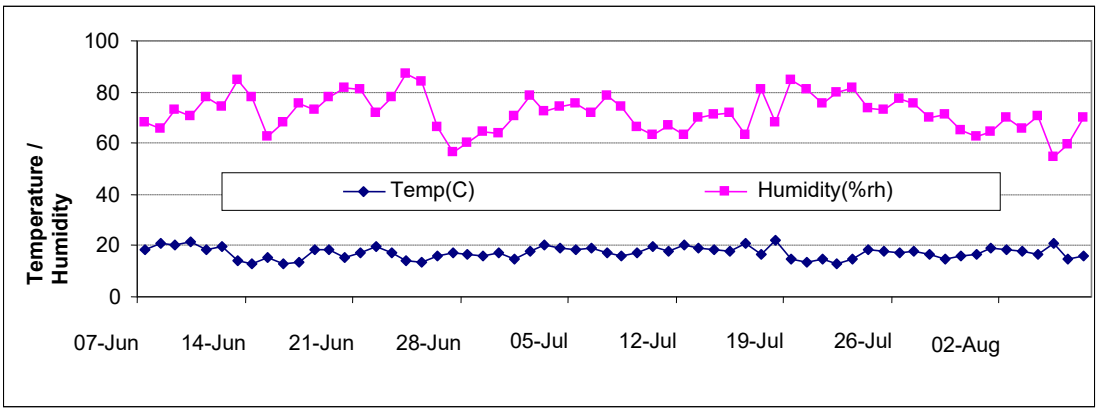
Appendix 1



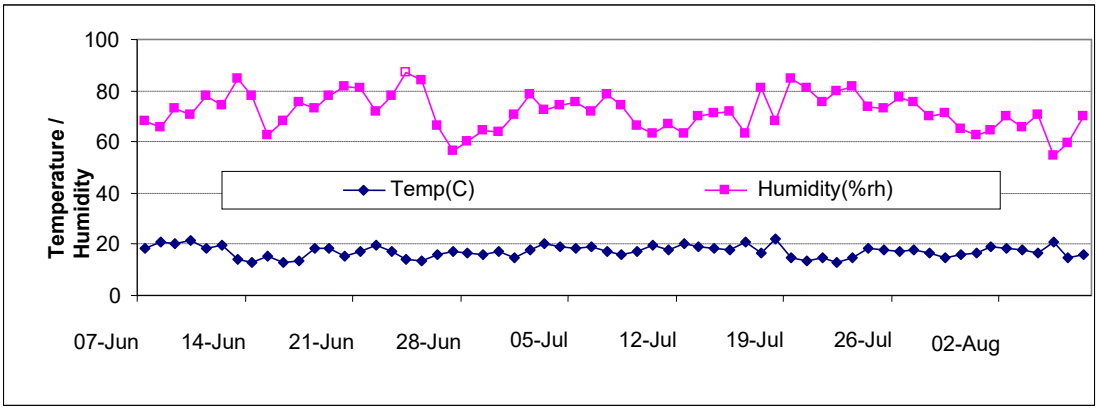
Site 4 perimeter



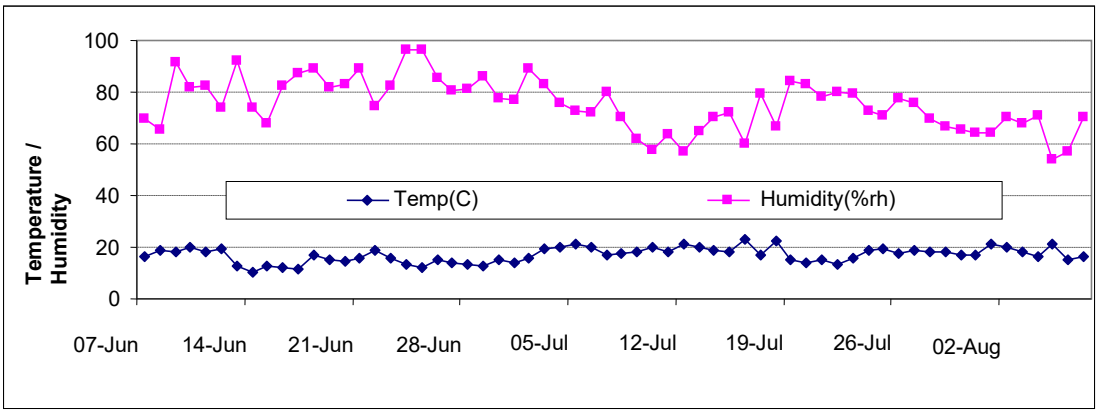
Site 4 Lattice



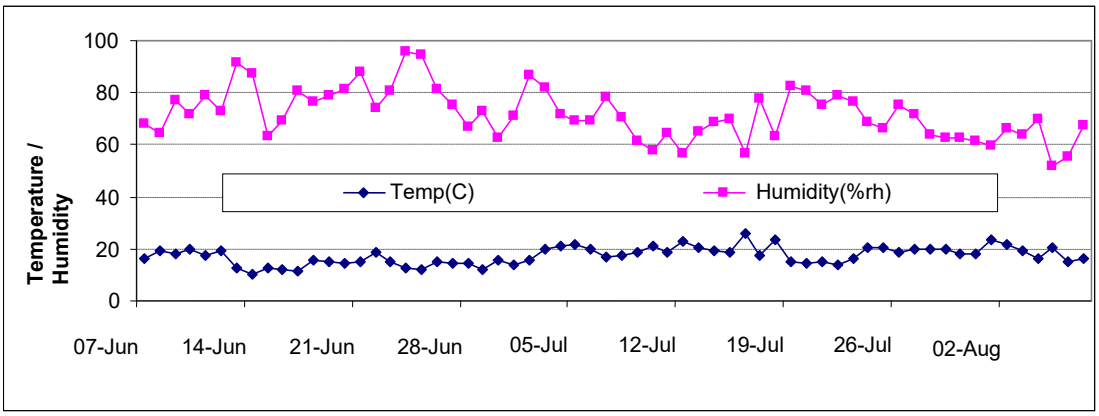
Site 4 Control



Site 3 perimeter



Site 3 lattice



Site 3 Control

Appendix 2

Appendix 1, Table 1. dates on which the Poly tunnels were put up and removed for each site

Address	Plot	Treatment		Uncovered 2006	Covered 2007	Uncovered 2007	1st Flower	50% Flower	1st Fruit Set	1st Pink fruit	1st Red Fruit	First Pick
		Midge	Beetle									
Decoy Farm. High Halstow, Rochester ME3 8SR	Bungalow field, Decoy Fm	MT	U		24/04/07		24/04/07	01/05/07	08/05/07	29/05/07	05/06/07	12/06/07
	Fullers field, Decoy Fm	MD	P		24/04/07		24/04/07	01/05/07	08/05/07	29/05/07	5/06/07	12/06/07
	Rye Street field, Rye Street Fm, Cooling	U	L		29/05/07		01/05/07	08/05/07	15/05/07	05/06/07	12/06/07	19/06/07
WB Chambers & Son, Belks Farm, Otham, Kent ME15 8RL	Field B29, Belks Fm	MT	U	All tunnels removed between 31/07/06 and 04/08/06	All tunnels erected between 24/04/07 and 15/05/07	All tunnels removed between 30/07/07 and 05/08/07	15/05/07	22/05/07	29/05/07	05/06/07	12/06/07	19/06/07
	Field L1, Ledian Fm	MD	P				15/05/07	22/05/07	29/05/07	05/06/07	12/06/07	19/06/07
	Field L4, Ledian Fm	U	L				15/05/07	22/05/07	29/05/07	05/06/07	12/06/07	19/06/07
Wester Essendy,	Field E1	MT	U		All tunnels							
	Field E2	MD	P									

Blairgowrie,	Field E3	U	L		erected between 01/06/07 and 05/06/07							
Easter Rattray Farm, Blairgowrie	Field J1	MT	U									
	Field J2	MD	P									
	Field J3	U	L									

MT = Mass Trapping, MD = Mating Disruption, P = Perimeter, L = Lattice, U = Untreated

Appendix 3

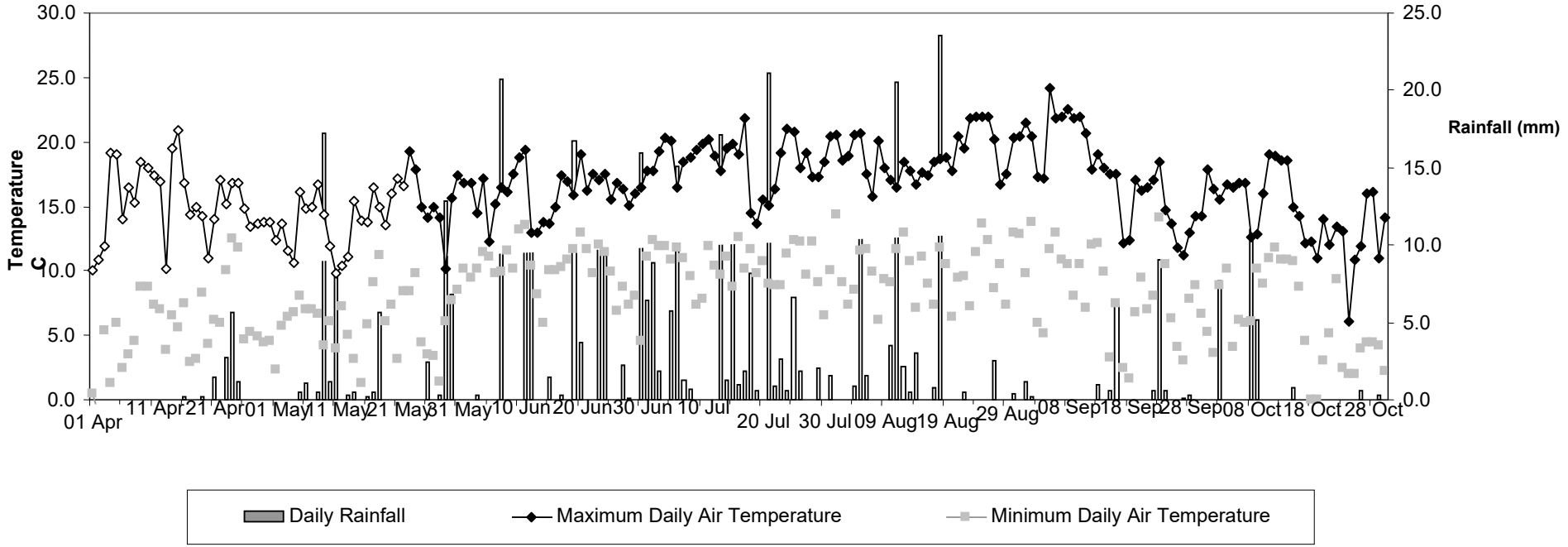
Appendix 3, Table 1. Dates on which insecticides were applied to the English sites 2006 and 2007

Address		Decoy Farm. High Halstow, Rochester ME3 8SR			WB Chambers & Son, Belks Farm, Otham, Kent ME15 8RL		
Plot		Bungalow field, Decoy Fm	Fullers field, Decoy Fm	Rye Street field, Rye Street Fm, Cooling	Field B29 Belks Fm	Field L1 Ledian Fm	Field L4, Ledian Fm
Treat-ment	**Midge**	MT	MD	U	MT	MD	U
	Beetle	U	P	L	U	P	L
Date							
24/05/2006							Chlorpyrifos 1 l/ha
25/05/2006					Chlorpyrifos 1 l/ha		
08/06/2006							Bifenthrin 0.298 l/ha
16/06/2006						Pirimicarb 0.241 kg/ha	
19/06/2006							Pirimicarb 0.239 kg/ha
23/06/2006					Pirimicarb 0.238 kg/ha		
20/09/2006							Pirimicarb 0.282 kg/ha
21/09/2006						Pirimicarb 0.278 kg/ha	
25/04/2007					Pirimicarb 0.278 kg/ha		
03/05/2007					Chlorpyrifos 1 l/ha	Chlorpyrifos 1 l/ha	Chlorpyrifos 1 l/ha
10/05/2007						Chlorpyrifos 1 l/ha	Chlorpyrifos 1 l/ha
11/05/2007					Chlorpyrifos 1 l/ha		
31/05/2007						Pirimicarb 0.278 kg/ha	
06/06/2007							Pirimicarb 0.238 kg/ha
27/08/2007					Pirimicarb 0.278 kg/ha		
07/09/2007						Pirimicarb 0.278 kg/ha	

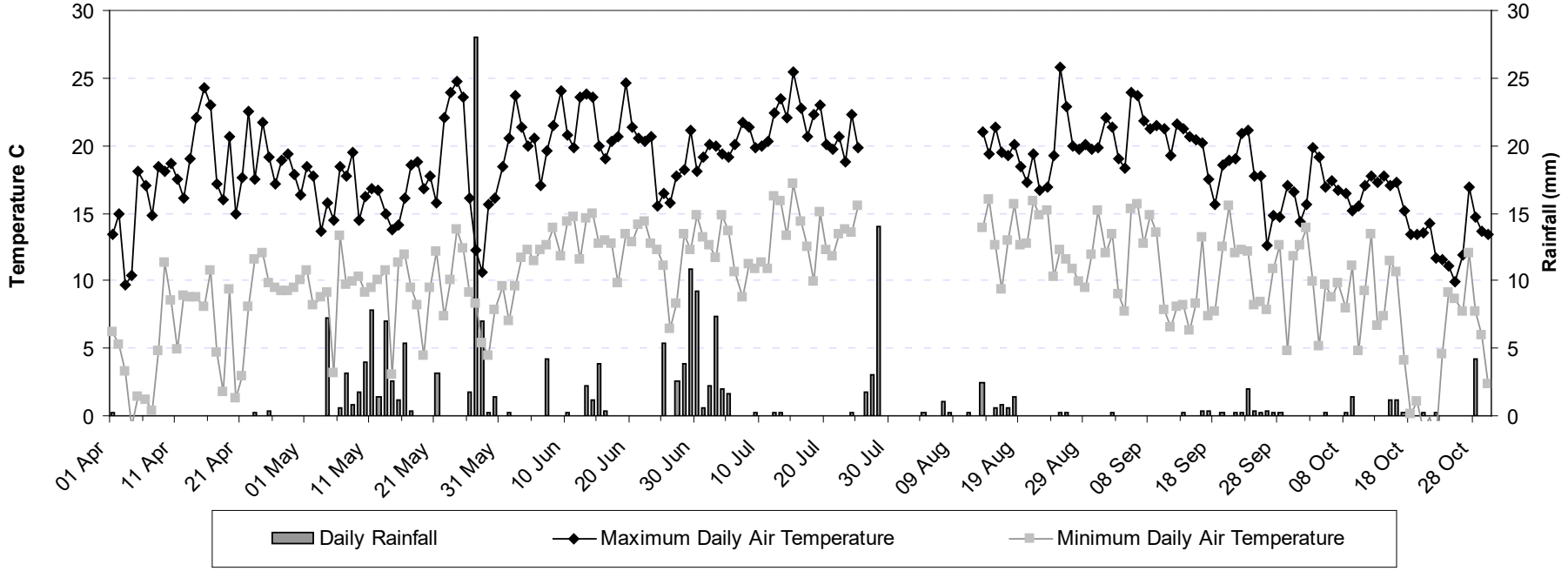
Appendix 3, Table 2. dates on which insecticides were applied to the Scottish sites 2006 and 2007

Address		Wester Essendy, Blairgowrie,			Easter Rattray Farm, Blairgowrie		
Plot		Field E1	Field E2	Field E3	Field J1	Field J2	Field J3
Treat-ment	**Midge**	MT	MD	U	MT	MD	U
	Beetle	U	P	L	U	P	L
Date							
Pending: Data currently not available							

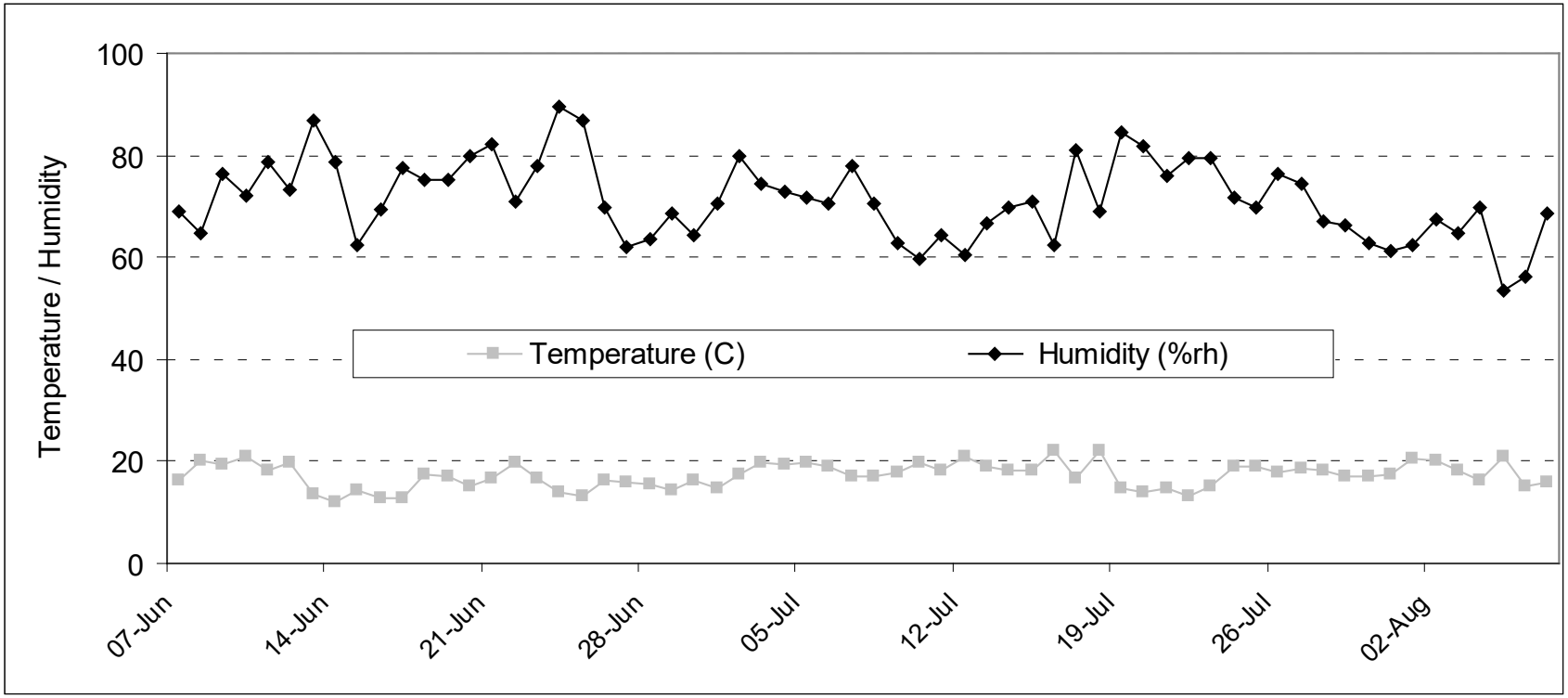
Appendix 4. Meteorological records



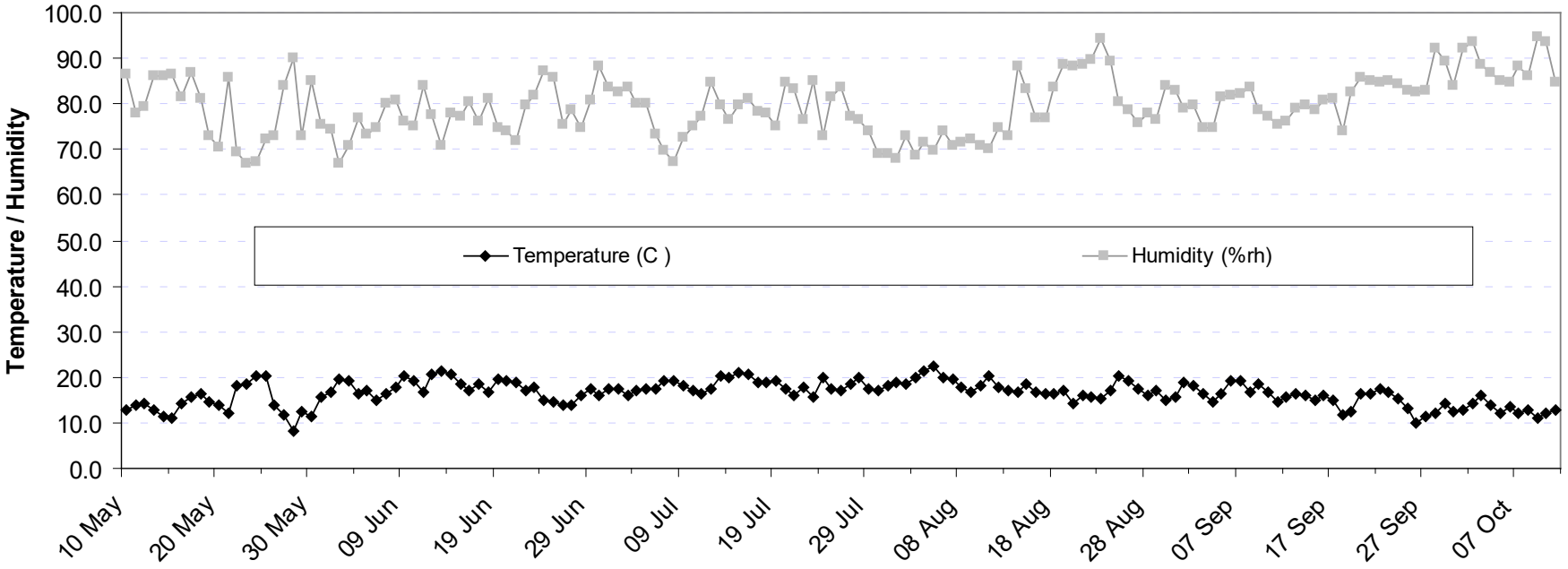
Appendix 4, Figure 1. Daily air temperature and rainfall at SCRI Dundee in 2007. Records from SCRI Meteorological Station



Appendix 4 Figure 2. Daily air temperature and rainfall in Kent in 2007. Records from East Malling Research Meteorological Station.



Appendix 4, Figure 3. Mean daily air temperature and humidity from the six polytunnels in Scotland in 2007. Data recorded with USB-502 data logger.



Appendix 4, Figure 4. Daily air temperature and humidity from a Spanish polytunnel at East Malling Research in Kent in 2007. Data recorded with USB-502 data logger.