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Project co-ordinator:	Mr John Place
Project administrator:	Dr Mike Solomon (replaced Dr Colin Gutteridge) Chief Executive Officer East Malling Research New Road East Malling, Kent ME19 6BJ
Researchers:	Prof. Jerry Cross (EMR) Dr Angela Berrie (EMR) Prof Xiangming Xu (EMR) Mrs Gillian Arnold (EMR) Dr Tim O'Neill (ADAS) Mrs Janet Allen (ADAS) Dr Erika Wedgwood (ADAS) Mr Chris Dyer (ADAS) Dr Nick Birch (SCRI) Dr Tom Shepherd (SCRI) Chris Jorna (SCRI) Prof. David Hall (NRI)
Collaborators:	Agrisense BSC Ltd Assured Food Standards 2003 Ltd Bayer Crop Science Ltd Berryworld Ltd Co-operative Group (CWS) Ltd East Malling Ltd Agriculture and Horticulture Development Board KG Growers Ltd LEAF Marks and Spencer plc The East Malling Trust for Horticultural Research The Summerfruit Company The Worshipful Company of Fruiterers Waitrose Ltd
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The following are members of the Consortium for LINK project HL 0175:

East Malling Research
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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

[Researcher A Name]
[Position]
[Organisation]
Signature ..... Date .....

[Researcher B Name]
[Position]
[Organisation]
Signature ..... Date .....

Report authorised by:
[Organisation A's authority Name]
[Position]
[Organisation]
Signature ..... Date .....

[Organisation B's authority Name]
[Position]
[Organisation]
Signature ..... Date .....

CONTENTS

GROWER SUMMARY

Headline

A successful Integrated Pest and Disease Management (IPDM) programme has been developed for raspberries which will greatly reduce and possibly eliminate pesticide residues. The programme will give good results but is likely to cost on average between £25 and £45 extra per tonne to implement compared to a conventional grower management and routine pesticide based programme.

Background and expected deliverables

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

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

The future registration of chlorpyrifos on raspberry is in doubt. Earlier screening trials by East Malling Research (EMR) failed to identify any alternative insecticides with significant activity for cane midge control, though in a 2009 HDC funded trial (SF 101), sprays of neonicotinoid insecticides (e.g. Calypso, Gazelle) gave good curative control of cane midge larvae in splits. Immediate loss of chlorpyrifos however would have serious adverse consequences for the UK raspberry industry as there is currently 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.

In the first three years of the project (2006-08) individual new technologies were developed along with control methods for the major pests and diseases of raspberry which do not rely on pesticide use that is likely to lead to residues in fruits at harvest. At the end of the three years, these were combined with existing methods into a minimal residues IPDM programme which was tested in years 4 (2009) and 5 (2010) of the project. This report summarises the results of all the work which took place over the 5 years of the project.

Summary of project and main conclusions

Objective 1. Botrytis

Task 1.1. Inoculum source

Previous field observations led to formulation of the hypothesis that infection of canes arose from invasion by mycelium from the petioles of infected leaves and that only mature and old leaves are susceptible. The research results obtained did not fully support this previous hypothesis about cane infection; cane age rather than the leaf age *per se* influences leaf susceptibility with leaves on young canes less susceptible than those on old canes. On older canes, leaves of all ages, ranging from young expanding to old senescent, were equally susceptible. Furthermore, both controlled inoculation and field monitoring suggested that most cane infection resulted from the direct infection of canes by the pathogen rather than through invasion via the petioles of infected leaves although infection of cane occurs readily following wounding around the petioles.

Provided the sclerotia are wetted, or incubated in high humidity for 2 weeks, sclerotia on canes will sporulate. Although sclerotia overwintering on fruiting canes are normally considered an important source of botrytis inoculum in spring, the incidence of cane botrytis and sclerotia was very low. Sporulation of botrytis sclerotia on fruiting canes can occur from mid-May (when crops are usually at first open flower) through to at least mid-August, especially so for the Cambridge site in the trial where the crop was covered late in the spring. In contrast, when the crop was covered very early in the spring, there appears to be no opportunity for sclerotia to be wetted and hence to initiate sporulation when temperatures rise in spring. Therefore, sclerotia overwintering on canes are not a major source of inoculum for early covered crops. Weeds and crop debris do not appear to be a main source of *Botrytis cinerea* spores for flower infection.

B. cinerea may possibly arise on canes from overwintering in the crown as well as from deposition of conidia in the air. Symptomless infection can occur in buds of floricanes visibly affected by botrytis; as most buds on such a cane were usually infected, the infection may be systemic.

Task 1.2. Environment manipulation

Botrytis cinerea causes disease on both the fruit and cane of raspberry. The incidence of latent and post-harvest fruit botrytis was examined in 19 commercial open-field and protected crops. Many samples showed a high incidence of infected fruit (>50%), even on protected crops sprayed with fungicides. Differences between open-field and protected crops, between sprayed and unsprayed crops and between two varieties (Glen Ample and Tulameen) were not statistically significant. The incidence of latent infection by *B. cinerea* in unripe fruit did not correlate with the incidence of botrytis fruit rot developing on ripe fruit.

Experiments were conducted in two commercial crops to investigate whether the removal of lateral leaves and thinning of primocanes during the flowering and fruiting period could reduce the incidence of fruit and cane infection by *B. cinerea.* Canopy manipulation resulted in considerable decreases in humidity inside the canopy at one site, where the original cane density was very high, (20 canes/m) and not at the second site where cane density was lower (10 canes/m). Canopy thinning did not significantly reduce the incidence of fruit botrytis at either site but reduced the incidence of leaf and cane infection in the dense crop. Results suggest that a significant reduction of cane infection by canopy manipulation can be realised for situations where cane density and disease pressure are high. The present studies suggest that in dense canopies in a protected crop, cane lesions are more likely to

result from direct infection of canes by the pathogen, although the pathogen can readily invade wounds on canes, including de-leafing wounds.

Tasks1.3-1.4 Individual strategies for management of Botrytis cinerea

Only urea appeared to consistently suppress sporulation of sclerotia on canes. Application of urea at 50 kg/ha did not result in any obvious sign of phytotoxicity on raspberry plants when applied in winter.

Fungicides, in particular UKA379, UKA374, Talat (tolylfluanid + fenhexamid), Signum (pyraclostrobin + boscalid), Switch (cyprodonil + fludioxonil), Scala (pyrimethanil), Amistar (azoxystrobin) and Folicur (tebuconazole,) gave the most consistent control of botrytis in post-harvest tests. None of the alternative chemicals evaluated had any effect on botrytis incidence apart from Hortiphyte Plus which showed some reduction in rotting at one pick date at the Kent site. However, further trials showed that Hortiphyte Plus applied alone did not significantly reduce botrytis incidence.

Task 1.3.5. Management of fruit botrytis by cooling

The incidence of botrytis at harvest was very low, but rapidly developed in fruit samples post-harvest, suggesting that post-harvest cool chain treatments may delay the onset of fungal rotting. Latent *B. cinerea* occurred in fruit from all crops at significant levels, with the incidence varying greatly between picks. The outdoor untreated crop had the highest incidence; spraying protected crops only led to a very small reduction in the incidence of latent infection. Most importantly, there was virtually no fruit with visual grey mould at harvest; fungal rots (including those by *B. cinerea*) usually only appear after being stored for 8 days.

Compared with ambient storage, initial cool storage of the fruit significantly delayed the onset of fungal rotting. Furthermore, these results suggested that the rapid cooling (within 1-h of the pick) is critically important to delay the onset of fruit rotting.

Objective 2. Raspberry Beetle

Task 2.1. Conduct field experiments to develop monitoring methods

On station experiments (Years 1 and 2) at SCRI were used to test different designs of the trap and lure. The non-sticky impact (funnel) traps had clear advantages over the original Swiss designed white sticky traps. The latter were difficult to handle, needed changing twice

a week, caught many more non-target insects (becoming saturated quickly) and were more expensive in the long term (disposable and labour intensive).

Task 2.2. Optimise lure for control

On station trials were conducted at SCRI in Year 1, testing single (compound B) versus double (A+B, A+A, B+B) attractants. In the UK , attractant B was found to suffice, enhancing RB catches of just the visual component of the trap (white, non UV reflectant cross vanes) by x10-x50 times. In areas of very high pest pressure (e.g. organic smallholdings in Norway and alpine Switzerland) some advantage was seen in adding the second attractant (A).

Task 2.3. Choose appropriate control approach and optimise device

In discussion with ADAS, Agrisense, Suterra, MRS Ltd and registration agencies (HSE, PSD) it was agreed that it was not currently economical to register the RB trap and lure for 'lure and kill'. Given this consensus, it was agreed that the most appropriate approach to control for the main U.K. raspberry growing sector was to choose precision monitoring (i.e. detection of 'hot spots') within the crop, using combined action thresholds (currently 5/trap/week when used at a rate of 50/ha) for spraying approved insecticides.

In other countries where organic production is more profitable (e.g. Norway), collaborative trials were run by Bioforsk (Dr N. Trandem). These showed that the trap and lure system at 50/ha were insufficient to reduce fruit damage to low (UK) levels, although 40-50% reduction in fruit damage was achieved over 2-3 years. Instead, the control approach for these Norwegian organic growers was modified so that extra traps were placed outside the cropped area (nearby woodlands with wild hosts) so that more RB were caught before they entered the crop and interception fences could be erected to interfere with RB flight paths. The bee excluder mesh was designed by SCRI and Agrisense Ltd half way through the HortLINK project. This proved to be very effective in stopping bees from entering the traps.

Trials in the UK, France (Ctifl) and Norway (Bioforsk) showed that the traps were most effective when deployed 4-6 weeks before first flowering of the crop. However, additional trials in Switzerland and Norway also showed continued catches throughout the season, with a second RB flight peak in late July-early August (alpine Switzerland, 2 sites). Again, the traps provided a route to regionally fine-tuned monitoring and control strategies. This requires more knowledge transfer and training of growers in IPDM, so is a medium (2+ years) to long term strategy. In both Scotland and Norway, growers became familiar and

confident with the traps and lures in 1-2 years of on farm trials and had customised deployment strategies for their own fields within 2-3 years.

Task 2.4. Deployment strategies

In the on-farm trials in the UK, complemented by additional on-farm trials in Norway and Switzerland, a lattice design (within crop) was compared with perimeter trapping, both at 50 traps/ha. In several on farm trials over 2-3 years the lattice deployment design was shown to be more effective than perimeter trapping. This in part reflects the finding during HL0175 that at several UK study sites, RB occurs mainly as resident populations within tunnels, with few immigrating from surrounding vegetation (hedgerows, wild bramble etc) outside the crop. The opposite was observed in parts of Norway, where huge reservoirs of RB were detected in surrounding woodlands using the traps; these flew into organic smallholdings over a more extended period than in the UK.

Thus, the precision monitoring system enabled optimal deployment strategies to be designed and deployed for differing geographical, climatic and agronomic conditions. It is recommended that the traps are used for at least a year at each site to monitor pest movement before optimising the spatial and temporal deployment of the traps for subsequent years. Besides raspberry, the trap and lure system was shown to be very effective when deployed in blackberry crops (UK and Switzerland).

Given the high cost of investing in traps (which should last for 5+ years, with annual investment in new lures), some UK growers may prefer to monitor at lower precision, using 5-10 traps/ha rather than 50/ha (the current recommendation for precision monitoring). This has the advantages of a) reducing initial grower costs, b) enabling growers to become familiar with the IPDM technology before making a bigger investment, c) growers can move traps around their farms, allowing them to monitor crops with differing flowering and fruiting periods, d) they can also monitor the efficacy of applied insecticides (post application monitoring) in different parts of the farm easily (the traps are easily moved between sites).

Objective 3: Semiochemical-based systems of managing cane midge

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

The female sex pheromone of the raspberry cane midge (*Resseliella theobaldi*) (Cecidomyiidae) was identified as (*S*)-2-acetoxy-5-undecanone and the synthetic

compound was shown to be highly attractive to male midges in the field. The *R*-enantiomer was unattractive but the racemic mixture containing equal amounts of the *R*- and *S*-enantiomers was as attractive as the *S*-enantiomer. This is an important result as the racemic material is much easier and cheaper to synthesise than either of the pure enantiomers. In field trapping trials, increasing the loading of pheromone in the lure gave increased catches of midges up to 1 mg, but further increase in loading decreased catches, indicating an optimum loading of 0.1 mg – 1 mg per rubber septum lure. Colour of the trap did not have any effect on catches of midges, but greater numbers of non-target arthropods were caught in white and blue traps. Red traps are recommended for practical use. The height of the trap above the ground had a very significant effect on catches of midges. Traps positioned on the ground caught most midges with catches dropping dramatically at higher positions. In practice it is not feasible to place traps on the ground for long periods and a trap height of 0.5 m is recommended.

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

A ring test was conducted by fruit entomologists in nine EU countries and Russia in 2006 and a strong linear relationship between sex pheromone trap catches of raspberry cane midge and numbers of larvae found subsequently in splits in raspberry canes was established. The relationship has not been used directly for setting trap thresholds because the relationship between larval infestations and crop damage has not been established. However, a low 'nominal threshold of 30 midges per trap per week was set for timing of sprays of insecticide.

Task 3.3. Investigation of attraction of raspberry cane midge to volatiles from wounded raspberry primocanes

Mated females of raspberry cane midge are known to be strongly attracted to odours from recently split raspberry primocanes. Fresh splits are preferred over old ones suggesting the attraction is due, at least in part, to volatile chemicals produced. Using solid-phase microextraction (SPME) to sample the volatiles *in situ* it was shown that a characteristic suite of chemicals was produced after splitting, and these were similar for five varieties of raspberry. The components were identified and the 18 most abundant were selected for further study, including six produced by intact stems and 12 produced after splitting. Of these, four elicited EAG responses from the antenna of a female *R. theobaldi* midge, including three from the group produced only after splitting. For field studies exclusion of the least abundant compounds gave a reduced set of 13 compounds and it was shown that dispensing four of these from a polyethylene vial and the other nine from a polyethylene

sachet gave a reasonable approximation to the blend observed from raspberry canes after splitting.

Field trapping studies were carried out in Hungary and the UK during 2009 and 2010 and these have given variable results. In general, numbers of female *R. theobaldi* trapped were very low, although significant numbers were caught in the test in Hungary during 2010. At two sites in Hungary and one in the UK during 2009, more males were caught in traps baited with the synthetic cane volatiles than in unbaited traps. At one of these sites numbers caught with the cane volatiles were similar to those caught with the sex pheromone. At two other sites in the UK, numbers of male *R. theobaldi* caught with the cane volatiles were significantly less than those caught in unbaited traps. The former three sites were all open-field while the latter two were covered and it was thought that this factor might be affecting the performance of the synthetic lures. However, these results could not be repeated in 2010. Numbers of male *R. theobaldi* caught in traps baited with the total volatile mixture were not greater than those caught in unbaited traps in either Hungary or the UK, although a reduced blend of the four most volatile compounds showed some attraction to males in the UK. Although considerable progress has been made, further work in both laboratory and field is required. The development of lures attractive to gravid female *R. theobaldi* would provide powerful new tools for monitoring and control of this pest.

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

Preliminary work towards this objective is described above in section 3.3. As a female attractant was not developed no other work was done on this sub-objective.

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

As an attractive host volatile lure was not fully developed (still at prototype stage) in 3.3 and 3.4. above, no further work was done on this sub-objective other than testing different blends, release rates and dispenser designs in small scale experiments (UK and Hungary).

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

Between 2006 and 2010, the efficacies of 5 Mating Disruption (MD), 2 Attract and Kill (A&K) and 1 Mass Trapping (MT) raspberry cane midge sex pheromone treatments were evaluated in large-scale, unreplicated field experiments for control of raspberry cane midge in commercial raspberry plantations in SE and E England in comparison with untreated controls.

The treatments evaluated comprised a wide range of dispenser/device types and dose rates of pheromone per ha, the upper dose limit being restricted to 10 g per ha by the terms of the experimental permit for the work. The efficacy of the treatments was evaluated in terms of how effectively they suppressed catches of male midges in single standard sex pheromone traps deployed in the centres of each plot, and in terms of the degree to which they reduced larval infestations in artificial splits in the primocanes through the season.

None of the 8 pheromone treatments performed consistently well, and none appeared satisfactory for control in commercial plantations. The sex pheromone trap catch was suppressed compared to its untreated control in all but one of the 21 different ~ 1 ha plot trials in which the 8 different treatments were evaluated, but good control of larvae only occurred in those trials where a high (>90%) or very high (>98%) degree of trap shut down resulted, though not necessarily so as poor control resulted in two trials where there was a very high degree of trap shut down. One of the main problems encountered with the different formulations was sustaining an adequate release of pheromone through the season.

Of the treatments evaluated, a treatment with 5000 0.4 g dollops of SPLAT (Specialized Pheromone & Lure Application Technology) containing 0.5% sex pheromone racemate per ha (10 g pheromone racemate/ha) was the best for ease of application and steady release rate and the most promising for further development. SPLAT is a proprietary (ISCA technologies, CA, USA) wax emulsion formulation used to control the release of semiochemicals. This treatment gave good control of first generation larvae in one trial in 2010, though control broke down for the second generation despite a second application. The SPLAT formulation and method of use (size and number of dollops) allows the release rate to be adjusted to a considerable extent and the amorphous and flowable quality of this formulation means that its application can be mechanized making application of large numbers of dollops per ha economically feasible. Further trials exploring a range of pheromone doses in SPLAT dollops of varying size and with higher numbers of dollops per ha are needed to improve the treatment to obtain a reliable and acceptable degree of efficacy.

Objective 4. Powdery mildew

In Year 2, the consortium decided to omit the powdery mildew from this project. Instead the work focused more on *B. cinerea* for the following reasons:

1. Powdery mildew failed to establish in crop despite repeated efforts of artificial inoculation by EMR and ADAS
2. Noticeable amounts of powdery mildew were not observed in commercial crops.

Thus, for powdery mildew, research activities were only carried out with meaningful results on the genetic differences between powdery mildews on raspberry and strawberry. Both diseases are believed to be caused by the same fungal species (*P. aphanis*). However, this paper shows that the mildews on these two hosts are genetically distinct. Sequencing the ITS region of a number of selected samples from the two fungi clearly indicates that these two fungi are genetically different.

Objective 5. Aphids

The aphid species that are significant pests of tree and bush fruit crops in Europe are mostly host-alternating. They spend autumn, through winter, spring and early summer on their winter host, typically woody tree or bush fruit species. In mid summer they migrate to herbaceous hosts. In the autumn, there is a return migration to the winter woody host by males and pre-sexual females (gynoparae), the latter producing sexual females (oviparae) which mate with the males and lay overwintering eggs on the bark. The aim of autumn applications of aphicides is to control a very high proportion of the gynoparae, males and oviparae before overwintering eggs are laid. Logically, the best time to treat is immediately before egg-laying commences, catching the maximum proportion of the migrants i.e. when the autumn migration of gynoparae is near its end and at the start of the male migration, because oviparae cannot lay eggs unless they are mated. There is normally a 2-3 week delay between the migration of gynoparae and that of the males.

Large scale field trials were done in commercial raspberry plantations in Kent to test different timings of autumn sprays of pirimicarb (Phantom), thiacloprid (Calypso) and pymetrozine (Plenum) for the control of small raspberry aphid (*Aphis idaei*) and large raspberry aphid (*Amphorophora idaei*). Single sprays were applied to replicate plots of Glen Ample in the autumns of 2005, 2006, 2007 and 2008. Populations of aphids were assessed in the winter (numbers of eggs) and spring (numbers of adults and nymphs). Calypso sprays greatly reduced populations of large raspberry aphids that developed the following spring by up to 99% in most years. Aphox, Phantom and Plenum gave less consistent results. Early – mid October was the optimum time for a single application of Calypso to reduce spring populations of large raspberry aphid and should be considered as part of an Integrated Pest Management programme.

Objective 6. IPDM programme

Based on the research conducted in the first 3 years of the project, a Minimal Pesticide Residue Integrated Pest and Disease Management (IPDM) programme was devised and was tested and refined in years 4 (2009) and 5 (2010) of the project. The key features of this programme were:

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

2. Application of 1-2 sprays of a powdery mildew fungicide in the spring as soon as the tunnel was covered; then subsequent sprays of potassium bicarbonate for eradication of powdery mildew if the disease is observed.

3. Use of 50+ Agrisense raspberry beetle host volatile funnel traps with white cross vanes/ha. Sprays of Calypso are used only where trap catches exceed thresholds, indicating where local treatment is necessary (e.g. hot spots within tunnels, whole tunnels or field-grown crops in adjacent fields and whole farm level). Note that no Calypso sprays were applied in the trial in Kent (2009), even though the traps catch threshold was greatly exceeded.

4. Application of a sex pheromone attract and kill treatment (SPLAT) for raspberry cane midge.

5. Removal of spent floricane soon after harvest in August.

6. Application of post-harvest fungicide sprays to control cane diseases on new spawn, starting in August.

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

The IPDM programme was implemented by growers in large (~ 1 ha) plots at Hugh Lowe Farms, Mereworth, Kent and Sunclose farms, Cambridgeshire. Yields of waste and marketable fruit, the incidence of pest and disease damage, shelf life and the incidence of

pesticide residues were assessed in the IPDM managed plots in comparison with a similar plot where the growers standard pest and disease management programme was applied.

Yields and quality

The IPDM programme gave similar yields and quality to the conventional growers programme in both years of the project. The IPDM pest and disease control methods gave a high standard of control of the main pests and diseases of raspberry.

Residues

At the Cambridgeshire site in 2009, the IPDM programme had no residues of the botrytis fungicide fenhexamid compared to 0.02 mg/kg for the grower standard. Residues of pyrimethanil were reduced by 80-95%. In 2010 at this site, residues of azoxystrobin were zero on the IPDM treated plot compared to trace levels on the grower's plots. However, trace levels of cyprodinil and fludioxinil occurred in the IPDM treated plots whereas these fungicides were not detected in the growers plot. At the Kent site, trace residues of pyrimethanil on the grower's plot were not detected in the IPDM plot. Thus the IPDM programme greatly reduces residues but pre-flowering sprays of fungicides may still result in trace residues. It is anticipated that insecticide residues would be eliminated.

Pesticide use

We have estimated that on average, numbers of sprays would reduce from 9 per season for a typical growers pest and disease control programme to 5 sprays for the IPDM, a reduction of 44% (Table 6.2.13).

Variable costs of the IPDM versus the grower's standard programme

We have estimated that the IPDM programme developed will cost growers approximately £300-540 more per ha to implement than a typical routine chemical control programme. The major increased costs are due to labour required for removing debris from the tunnel twice per year to improve hygiene and the costs of raspberry beetle control using the Agrisense raspberry beetle monitoring traps. These increased costs are partially offset by the savings in pesticides. Assuming an average yield of 12 t/ha, then the increased cost is about £25-45/tonne.

Financial benefits

In 2009, 16,000 tonnes of raspberries, worth £111m were produced from 1,757 ha grown in Britain. The UK fresh market is under-supplied outside of the main season. New varieties are now being utilised to spread the cropping season and production has increased, by 45%

over the last decade, and continues to do so. 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.

Effects of the IPDM programme on yield and quality, pesticide use and costs

The large scale grower trials in the final years of this project indicated that the yield and quality expected from the IPDM programme are the same as those from the grower's standard programme, i.e. both programmes give good control of the range of pests and diseases on raspberry. It has been estimated that the IPDM programme developed will cost growers approximately £300-540 more per ha to implement than routine chemical control programmes, depending on the extent to which their cane management practices, which vary considerably, have to be improved. The major increased costs are due to labour required for improved cane management and removing debris from the tunnel to improve hygiene and the costs of raspberry beetle control using the Agrisense raspberry beetle monitoring traps. Assuming an average yield of 12 t /ha, then the increased cost is about £25-45/tonne.

Benefits of greatly reducing pesticide residues and pesticide dependence

The high incidence of pesticide residues on conventionally produced raspberries damages the consumer acceptability of raspberries and their image as a healthy food. This work has demonstrated that residues can be greatly reduced and if pre-blossom fungicide sprays were reduced, they could probably be eliminated completely. This is clearly a valuable benefit, which is hard to quantify, but is likely to cost £25-45/tonne.

Action points for growers

An HDC factsheet (13/11 – Residue reduction in commercial raspberry crops) has been prepared to give detailed grower recommendations arising from this project. The recommendations refer to summer-fruiting protected crops but equally will apply to double-fruited primocane crops (i.e. those that crop in the spring and the autumn). The key features of the minimal residues IPDM programme which growers should implement are listed below. The guidelines must be considered alongside features known to influence pest and disease risk. These include site, varietal susceptibility, age of plantation, duration of tunnel covering, tunnel height, number of rows per tunnel and crop management practices.

Fruit Botrytis

- Cover crops at least 2 weeks before flowering (ideally before spawn emergence), to keep canes dry and reduce germination of *Botrytis* sclerotia.

- On protected crops, do not apply sprays of fungicides for Botrytis during flowering and fruit development. These are of little benefit and as good or better control of Botrytis can be achieved by good crop hygiene and cane management to ensure the canopy does not become dense, so allowing good air circulation in the crop (see cane diseases, below).

- Ensure rapid cooling of fruit to 1-2 ºC immediately at harvest, followed by cool storage at 3-4ºC (but note that other fruit rots may not be fully controlled).

Powdery mildew

- On varieties susceptible to powdery mildew (e.g. Glen Ample), apply 1-2 preventative sprays of a powdery mildew fungicide in the spring as soon as the tunnel is covered, then subsequently apply sprays of potassium bicarbonate for eradication of powdery mildew if the disease is observed.

- Crops covered for a long period, in low tunnels, or also covered with fleece, are more at risk of mildew due to reduced air movement.

Cane diseases (Botrytis, cane blight, spur blight and cane spot)

- Where possible cut out and remove all spent floricane from the tunnel within 2-3 weeks of final harvest. Take care to minimise damage to primocanes by cutting the floricanes into sections prior to removal.

- Machinery exists in other industries to collect prunings, chop and dispose of them. Such machines could be investigated for use in raspberry crops. Where cane debris is left in the tunnel, pull it into the centre of alleyways and chop it thoroughly to speed decay.

- Apply a programme of 2-3 post-harvest fungicide sprays to control cane diseases on new spawn, starting from soon after removal of cladding and old floricanes.

- Work elsewhere suggests that cane blight is becoming more important due to the way crops are now grown; when tunnel covers are removed at the end of harvest the soft canes are susceptible. Therefore consider a cane blight protectant spray in the autumn.

- In the spring, a cane disease spray is less likely to be required for crops covered early. However, consider applying a spray treatment to canes (e.g. for cane spot) where tunnels are covered late. Where there are no tunnel gutters, leg rows are more at risk of cane diseases due to the greater water splash.

- In late autumn, after leaf fall, make a final check and remove diseased and damaged primocanes at or just before the final selection for tying in.

Raspberry beetle

- When planting a new raspberry plantation, where possible, avoid planting near wild blackberry, wild raspberry, hawthorn and other raspberry beetle hosts.

- Around 3-4 weeks before flowering, deploy 50+ Agrisense raspberry beetle host volatile funnel traps with white cross vanes per ha, in a regular grid through each plantation. Deploy additional traps near any remaining wild sources of raspberry beetle.

- If >5 beetles have been caught in any trap by the start of flowering, apply a spray of thiacloprid (e.g. Calypso) locally to those tunnels where the threshold has been exceeded. Insecticide treatment may be unnecessary, or may only be required in hot spots or at the edges of the crop.

Raspberry cane midge

- For varieties susceptible to cane midge, deploy two sex pheromone monitoring traps in each field in early spring (March in early protected crops, early April outdoors) and monitor weekly.

- Apply a spray of chlorpyrifos directed to the base of the primocane a few days after a threshold catch of 30 midges per traps is exceeded.

Aphids

- Apply a spray of thiacloprid (Calypso) or another suitable aphicide in early October for aphid control.

- Make a programme of introductions of predators and parasites for biocontrol in spring and summer to prevent aphid attacks, as advised by biocontrol suppliers.

SCIENCE SECTION

Objective 1. Botrytis

To develop sustainable management strategies to reduce overwintering inoculum and subsequent disease spread through an increased understanding of the dynamics of leaf, cane and fruit infection and how tunnel and crop canopy management affects the disease.

1.1. Inoculum sources

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

Introduction

Botrytis cinerea Pers, ex Fr. can cause diseases on both cane and fruit of raspberry, namely cane botrytis and grey mould. Cane botrytis has many features in common with spur blight, caused by the fungus *Didymella applanata* (Niessl.) Sacc; it is common to find both diseases in the same planting or on the same canes. Cane botrytis is generally considered to be the more damaging of the two diseases. It is believed that both fungi infect the nodal areas of young canes after invading senescent leaves; as a result the axillary buds at infected nodes are suppressed and many fail to produce fertile lateral shoots in spring (Williamson and Hargreaves, 1981). In open field plantings, the first lesions on primocanes often appear in July on nodes near the base; as the season progresses, they develop at nodes higher on the canes. Prominent sclerotia form beneath the epidermis during winter and erupt in spring. During periods of high humidity, these sclerotia become covered with the grey mycelium and conidia.

Inoculum of *B. cinerea* in raspberry crops probably originates from within the plantation and probably mainly from lesions on fruiting canes that result from infection the previous season (Jennings and Carmichael, 1975). Most stem lesions occur at nodes and were believed to follow infection of leaves (Hockey, 1952). Resistance to cane botrytis is believed to be relatively high in young canes (Williamson et al., 1979). Artificial inoculation of wounded leaves or canes showed that infections of *B. cinerea* occurring up to early September have the potential to cause nodal lesions, depress axillary growth and induce lateral shoot failure

the following spring (Williamson and Jennings, 1986). Thus, post-harvest fungicide sprays may be necessary to reduce incidence of cane botrytis and potential yield loss.

The disease is most severe inside a dense canopy, generally on the lower half of primocanes and this is believed to be because only mature to senescent leaves are susceptible. In Scotland, most severe outbreaks of cane botrytis in open field plantings can be found in high-density nursery plantings, but because such plants are dug, planted, and cut back before bud failure occurs in the fruiting year, the disease does not usually result in economic loss (Williamson et al., 1979). The removal of the first flush of primocanes to control cane vigour has reduced the severity of cane botrytis, probably because second-flush canes are susceptible to infection for a shorter period in late summer (Williamson et al., 1979).

We conducted inoculation studies in a polythene tunnel to determine whether and how (1) cane infection is related to leaf infection, and (2) infections of canes and leaves are affected by their age.

Materials and methods

Inoculation and disease assessment

Inoculation experiments were conducted in a polythene tunnel at East Malling Research, UK, to determine the susceptibility of leaves to *B. cinerea* in relation to leaf age and whether cane infection resulted from mycelial invasion via petioles of infected leaves. Experiments were conducted in 2005-2007 using cv. Glen Ample. Plants were potted into 5 L pots in June 2005, pruned annually in the winter and kept under protection in a plastic tunnel throughout the three years. Different plants were used for inoculation each year.

Table 1.1.1 gives a summary of experimental details for each inoculation. In 2005 and 2006, the main objective was to determine whether and when *B. cinerea* mycelia invade leaf petioles and subsequently infect canes following inoculations of leaves as hypothesised previously (Hockey, 1952; Labruyere and Engels, 1963; Williamson, 1991). Thus, several batches of inoculated canes were sampled weekly or bi-weekly following each inoculation, incubated and assessed for botrytis development. In 2007, on the basis of the results from 2005 and 2006, the main objective was to determine the susceptibility of tissues to *B. cinerea* in relation to cane and leaf age; thus, leaves on different plants were inoculated on

several occasions from May to September but canes and leaves were only sampled once for disease assessment following each inoculation.

Leaves and canes were inoculated without first being wounded. Up to 50 canes were randomly selected for each inoculation, depending on the particular experiment. Before inoculation, the youngest fully unrolled leaf on each selected cane was tagged with a label. Three *B. cinerea* isolates obtained from raspberry were grown on potato dextrose agar (PDA) for 10-14 days. Conidia were collected by rinsing the cultures with sterile distilled water, the three spore suspensions were mixed and the concentration adjusted to 5 x 10^5 conidia mL^{-1}. A hand-held sprayer (500 mL) was used to dispense inoculum onto both sides of all leaves until run off as well as the cane for all selected canes. Immediately after inoculation, overhead misters were switched on for 24 h to maintain high humidity/free water conditions that were favourable for infection.

Table 1.1.1. Summary of controlled inoculation experiments on infection of raspberry canes and leaves by *Botrytis cinerea*. In all experiments, cv. Glen Ample was used

Factors	Controlled inoculation experiments										
	2005		2006		2007						
Inoculation date	02/09	11/11	15/06	06/09	15/05	30/05	27/06	12/07	02/08[a]	23/08	14/09
Sampling date	09/09 16/09 23/09 14/10 21/10 28/10	18/11 25/11 07/12	30/06 14/07 28/07 11/08 25/08	15/09 29/09 13/10 27/10	18/05	08/06	06/07	20/07	10/08	31/08	21/09
No. of canes sampled	6(0)[c]	6(0)					4(3)				

[a]: samples were taken on this date but most inoculated leaves decomposed very quickly
[b]: x(y) → x number of inoculated canes and y number of non-inoculated canes sampled on each date.
[c]: sampled from the first flush of primocanes that were later mechanically removed before the next sampling date.

A number of inoculated canes were randomly taken to determine the latent *B. cinerea* infection on leaves, petioles and primocanes at several intervals after inoculation (Table 1). In addition to the inoculated canes, a number of un-inoculated canes were also randomly taken for comparison in 2006 and 2007. Each cane was cut into three-seven pieces with leaves attached; these were first surface-sterilised by immersing them in a 0.5% Domestos® solution in a 10 L bucket (sodium hypochlorite. 0.025% w/v available chlorine) for 4 min, rinsed with tap water, immersed in 0.5% paraquat (chloramphenicol) solution for 1 min, and finally rinsed with tap water. Surface sterilisation was done in order to prevent *B. cinerea* conidia that had not infected leaves at the time of detaching leaves from subsequently

infecting the leaves. Thus, any *B. cinerea* colonies that developed from tissues are most likely to have originated from established infections at the time of sampling. Paraquat was used to kill host tissues and induce botrytis sporulation (Biggs, 1996).

The relative position of each cane section was labelled; canes and attached leaves were then placed on wet paper towels in a gravel tray. The tray was covered with a polythene bag to prevent contamination. The trays were incubated in a glasshouse compartment with temperature set to 22°C in the daytime and 16°C at night (16:8 day/night). Development of *B. cinerea* on individual leaflets, petioles and canes was assessed 3-4 weeks later.

Data analysis

In calculating overall incidence of leaf infection, a leaf was considered to be infected if either its petiole or any of its leaflets was infected. Disease was then summarised for each category of leaf age: (1) young (top five fully unrolled leaves), (2) mid-age (next five leaves), (3) mature (next 10 leaves), and (4) old (all other leaves). Generalised linear modelling (GLM) was used to determine whether the incidence of leaf infection was affected by leaf age and inoculation (or sampling) time (Cox and Snell, 1989), assuming that proportion (*p*) of infected leaves per age group or per treatment is binomially distributed. Thus, the logit transformation of *p* ($\ln\left(p/(1-p)\right)$) was used to assess the effects of treatment factors on the incidence of leaves infected: $\ln\left(p/(1-p)\right) = Inoculation + Time + Age$, where *Inoculation*, *Time* and *Age* represent whether leaves were inoculated, sampling time, and leaf age, respectively.

For inoculations in 2005, the *Inoculation* factor was not needed as no control canes/leaves were sampled. For inoculations in 2005 and 2006, the model was fitted to data from each inoculation separately, namely *Time* represents different sampling times within each inoculation. But for 2007, *Time* represents different inoculations. In addition to the main effects, interactions between two factors were also included whenever necessary; interaction of leaf age with other factors were not included for 2007 data because mature and old leaves were not present for early inoculations.

Only when the overall effect of a treatment factor was statistically significant based on a deviance test, was the significance of the difference between individual levels of this treatment factor then established by comparing their parameter estimates against the respective standard error of the difference. Genstat (Payne, 2006) was used for statistical analysis.

Results

2005 - September inoculation

Botrytis cinerea was only found on a single mature leaf out of approximately 90 leaves sampled on 9 September. None of the leaves sampled on 16 September was infected. Thereafter, the overall incidence of leaves with *B. cinerea* increased; the incidence was 5%, 22%, 21%, 23% and 13% for samples taken on 23 and 30 September, and 14, 21 and 28 October, respectively. Differences in the incidence among leaf age groups varied with sampling times (Fig. 1.1.1); the overall incidence was 10%, 15% and 12% for young, mid-age and mature leaves, respectively. The overall frequency of *B. cinerea* sclerotia and sporulating lesions was similar: 46 and 41 out of the total 672 leaves, respectively. On only six occasions was *B. cinerea* observed on canes – all as sclerotia: one each on 30 September and 21 October, and two on 14 and 28 October. *Botrytis cinerea* was found on petioles on 10 occasions: one each on 9 and 16 September, and 14 October, three on 30 September and four on 21 October.

Logistic regression indicated that there were no significant differences in disease incidence among three leaf age groups. In contrast, disease incidence differed significantly ($P < 0.001$) among the seven sampling times. The incidence for samples taken on 9 and 16 September was significantly ($P < 0.01$) lower than that for all other sampling times; incidence for the sample taken on 23 September was also significantly ($P < 0.01$) lower than those samples taken on 30 September, and 14 and 21 October.

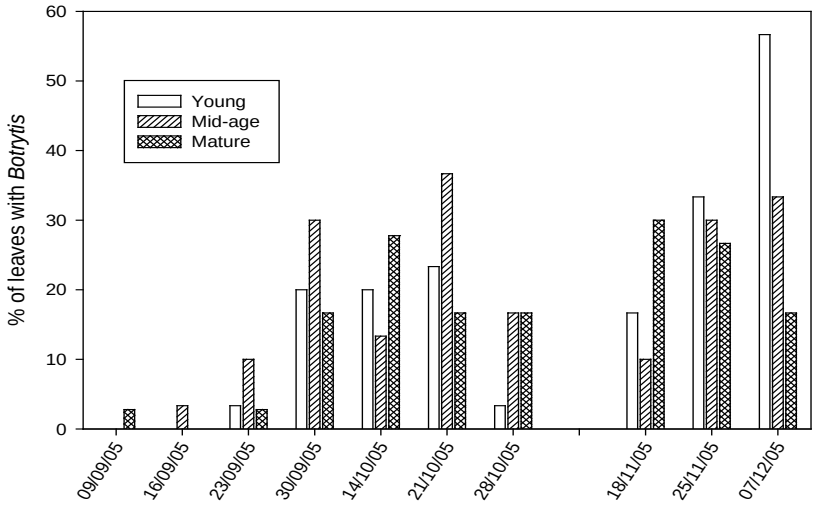


Figure 1.1.1 Overall incidence of raspberry leaves of cv. Glen Ample with latent *Botrytis.* Leaves and canes were inoculated on two occasions (02/09/05 and 11/11/05) and sampled on several dates. Leaves were divided into three categories: Young (top five youngest fully unrolled leaves), Mid-age (next five leaves), Mature (next 10 leaves), and Old (all others). For the first few inoculations, mature and old leaves may not be present or only a few were present. Un-inoculated canes were also sampled on each occasion.

2005 - November inoculation

A considerable amount of *B. cinerea* was observed in all three samples; the overall incidence was 19%, 30% and 36% for samples taken on 18 and 25 November, and 07 December, respectively. Differences in the incidence among leaf age groups varied with sampling times (Fig. 1.1.1); the overall incidence was 36%, 24% and 24% for young, mid-age and mature leaves, respectively. The overall frequency of *B. cinerea* sclerotia on leaves was much higher than that of sporulating lesions – 68 and 24 out of the total 270 leaves, respectively. On only one occasion was *B. cinerea* observed on a piece of cane (25 November). *Botrytis cinerea* was found on 43 out of 270 petioles (nearly all as sclerotia): nine, 11 and 23 in samples taken on 18 November and 25 November, and 07 December, respectively.

There were no significant differences in the incidence of *B. cinerea* among the three leaf age groups. In contrast, the incidence differed significantly ($P < 0.01$) among the three sampling times. The overall incidence for the sample taken on 18 November was significantly ($P < 0.05$) lower than that for other two samples.

2006 - June inoculation

No *B. cinerea* was observed on either leaves or canes for both inoculated and un-inoculated samples taken on 30 June, 14 and 28 July, and 11 August. For samples taken on 25 August, *B. cinerea* colonies were seen only on 12 out of 60 inoculated leaves. Out of 45 un-

inoculated leaves taken on the same day, there were 11 leaves with botrytis. There were five and two petioles with *B. cinerea* on the inoculated and un-inoculated leaves, respectively. GLM analysis showed that incidence did not differ between the inoculated (27%) and the control (29%), and among different leaf age groups.

2006 - September inoculation

Leaves with botrytis lesions were frequently observed in both inoculated and control samples after incubation. The overall incidence was 59%, 86%, 66% and 69% for inoculated leaves taken on 15 and 29 September, and 13 and 27 October, respectively; the corresponding incidence for un-inoculated leaves was 41%, 47%, 55% and 65% (Fig. 1.1.2). The overall incidence was 57%, 52%, 67% and 72% for young, mid-age, mature and old leaves, respectively. The overall frequency of lesions on leaves was much higher than that of sclerotia – 389 and 64 out of the total 664 leaves, respectively; this trend was consistent over different leaf age groups or inoculated/un-inoculated leaves. On only four occasions was *B. cinerea* observed on canes. *Botrytis cinerea* was found on 27 out of 664 petioles (all as sporulating lesions): 17 and eight of these lesions were observed in inoculated samples taken on 29 September and 27 October, respectively.

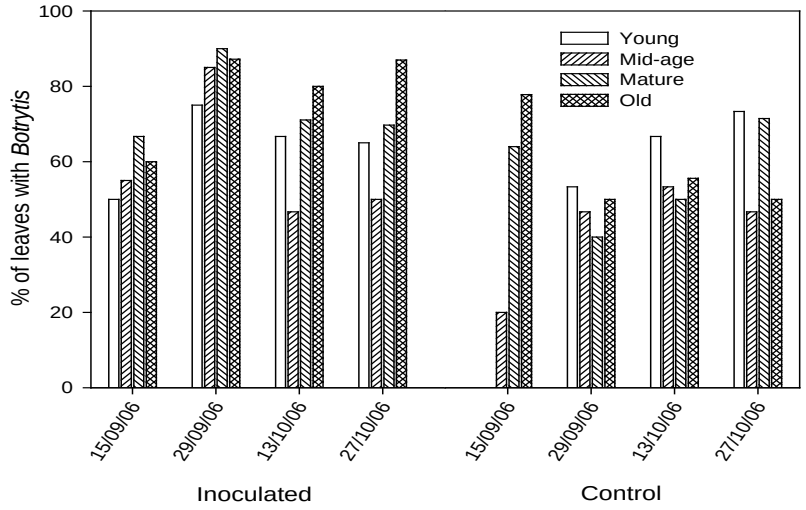


Figure 1.1.2. Overall incidence of raspberry leaves of cv. Glen Ample with latent *Botrytis.*. Leaves and canes were inoculated on two occasions (15/06/06 and 06/09/06) and sampled on several dates; only a few inoculated leaves were infected for the first inoculation and results are not shown. Leaves were divided into four categories: Young (top five youngest fully unrolled leaves), Mid-age (next five leaves), Mature (next 10 leaves), and Old (all others). On each sampling time, un-inoculated canes were also sampled.

Overall, the incidence of leaves with *B. cinerea* was significantly ($P < 0.001$) higher on the inoculated leaves (71%) than on the control sample (51%). The four leaf age groups also differed significantly ($P < 0.01$) in the disease incidence. Incidences on the mature and old

leaves were greater than those on the young and mid-age leaves. The incidence in samples taken on 29 September was significantly ($P < 0.05$) greater than on the other sampling dates. There were significant ($P < 0.001$) interactions between sampling dates and inoculated/un-inoculated; this is mainly due to the higher incidence on inoculated leaves taken on 29 September (Fig. 1.1.2).

2007 inoculation

Only a few leaves inoculated on the first two dates (15 and 30 May) were infected and thereafter the incidence increased steadily with inoculation time, irrespective of leaf ages (Fig. 1.1.3). The overall incidence of inoculated leaves was 6%, 3%, 25%, 49%, 82% and 89% for leaves inoculated on 15 and 30 May, 27 June, 12 July, 23 August and 14 September, respectively; the corresponding incidence for un-inoculated leaves was 0, 0, 0, 6%, 94% and 89% (Fig. 1.1.3). There were no old leaves on the canes until the inoculation on 2 August. The overall incidence was 40%, 41%, 49% and 75% for young, mid-age, mature and old leaves, respectively. The overall frequency of sporulating 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.

Sporulating lesions were found on many pieces of canes, particularly on canes inoculated on 23 August and 14 September. There were respectively 12 (out of 18) and 10 (out of 26) pieces of canes with sporulating lesions covering large surface areas for canes inoculated on 23 August and 14 September, compared with the corresponding two (out of 21) and one (14) pieces of un-inoculated canes. *Botrytis cinerea* was found on 201 out of 784 petioles (all as sporulating lesions), there were 0, 0, 1, 9, 80 and 111 infected petioles on 15 and 30 May, 27 June, 12 July, 23 August and 14 September, respectively.

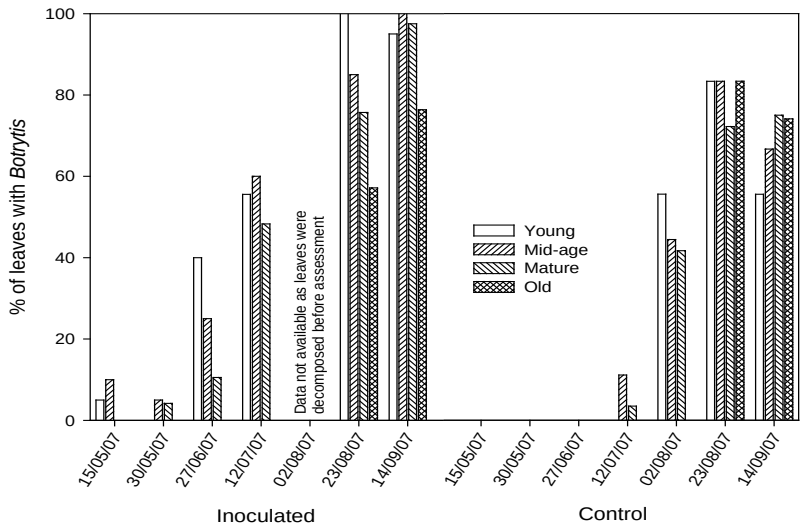


Fig. 1.1.3. Overall incidence of raspberry leaves of cv. Glen Ample with latent *Botrytis*. Leaves and canes were inoculated on seven occasions; several inoculated canes were taken a week after inoculation for assessment. Leaves were divided into four categories: Young (top five youngest fully unrolled leaves), Mid-age (next five leaves), Mature (next 10 leaves), and Old (all others). For the first few inoculations, mature and old leaves may not be present or only a few were present. Un-inoculated canes were also sampled on each occasion.

Most variability in the disease incidence was attributable to variation between inoculation dates (Fig. 1.1.3), which accounted for 81% of the total deviance. The next most important source of variability was between inoculated and un-inoculated leaves, 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 a significantly (P < 0.001) higher disease incidence (52%) than un-inoculated leaves (39%); however, for the last two inoculation dates, disease incidence was not significantly different between inoculated and un-inoculated samples (Fig. 1.1.3). Incidence of inoculated canes (50%) with *B. cinerea* on the last two inoculation dates was significantly greater (P < 0.001) than that of un-inoculated canes (9%). The main effect of leaf age was statistically significantly (P < 0.05) but accounted for only 3% of the total deviance. However, when data were separately analysed for the last two inoculations (23 August and 14 September), there were no significant differences in disease incidences between the four leaf age groups.

Discussion

Results from the present studies do not fully support previous conclusions, drawn from observations in open-field crops, regarding the nature of cane and leaf infection by *B. cinerea.* Previous observations led to the hypothesis that cane botrytis lesions mainly resulted from invasion of *B. cinerea* mycelium through petioles of infected leaves (Hockey, 1952; Labruyere and Engels, 1963). It was believed that only mature to senescent leaves

can be infected and hence most lesions were observed near the bottom of the cane (Williamson, 1991). However, the present inoculation study suggests that infection of leaves was not influenced by the leaf age *per se* but by the cane age, and that cane botrytis resulting from mycelial invasion via petioles of infected leaves is infrequent compared with possible direction infection of canes.

Inoculation experiments suggested that leaves on young canes were less susceptible to infection than on old canes, irrespective of leaf position on the cane. Thus, leaf age *per se* does not greatly affect its susceptibility to *B. cinerea*, in contrast to the previous conclusion that only mature and senescent leaves are susceptible (Williamson, 1991). On all inoculated plants, no visible lesions were observed on leaves that were still attached to the cane prior to sampling. *Botrytis cinerea* is known to be able to infect young leaves but remain latent until leaf senescence such as in strawberry (Sutton et al., 1990; Sutton et al., 1997) and primula (Barnes and Shaw, 2002).

Higher incidences in old leaves in 2007 may have resulted from the fact that old leaves were only present where canes were of an age such that all leaves were more or less susceptible to the pathogen. In contrast, young leaves were present throughout the season, including in the early season when they were on young canes and hence less susceptible. Thus, summarised over all of the inoculations, the incidence of *B. cinerea* on younger leaves is expected to be less than on the old leaves. By contrast, when data were separately analysed for the late inoculations in 2007, there were no significant differences between the four leaf age groups. If old leaves were more susceptible, then it would have been expected that old leaves in late control (uninoculated) samples would have much higher incidences than young leaves when sampled on the date because old leaves have been subjected to natural inoculum for a far longer period of time than young leaves.

The present data also suggest that infection of raspberry leaves occurs frequently irrespective of their ages. Compared with the un-inoculated control, more than 70% of infection in 2006 and 2007 likely resulted from infection due to natural inoculum. Therefore, it can be concluded that most infection on leaves artificially inoculated in 2005 were also likely to have resulted from infection by natural inoculum. Indeed, for the last few samples in 2006 and 2007, there were no significant differences in the incidence between the inoculated and control samples. It is not surprising that there is a certain level of background inoculum given the fact that *B. cinerea* is a ubiquitous pathogen. However, it should be noted that inoculation did lead to an overall higher incidence of leaf infection. In contrast to the higher incidence of

leaf infection, cane infection around the bud area was infrequent. This may suggest that most cane botrytis did not result from invasion through petioles from infected leaves, as suggested from previous field studies (Williamson, 1991).

The location of cane lesions observed in the present inoculation experiments also supports the argument that most cane botrytis did not result from mycelial invasion through petioles from infected leaves. Except in the last two inoculations in 2007, few botrytis lesions (sporulating or sclerotia) were observed on inoculated canes, despite the fact that many leaves and/or petioles were infected and they were incubated under conditions conducive for disease spread. Where lesions occurred on canes, the majority type was a discrete lesion not close to the bud/leaf scar area. For the last two inoculations in 2007, nearly 50% of pieces of canes were infected with *B. cinerea,* most covering the cane surface and not directly associated with buds or leaf scars.

From this limited number of canes infected, we hypothesise that *B. cinerea* is more likely to infect raspberry canes directly rather than via invasion from petioles as suggested by previous research observations. This hypothesis was supported by the field observations (see the next section). Histological observation confirmed that invasion of the epidermis, cortex and outermost phelloid cells of the cane occurred freely by *B. cinerea* and *D. applanata* mycelium from the abaxial side of the petiole and that fungi cannot grow from a saprophytic base, as in the petiole, to infect the axillary buds (Williamson, 1984). However, this does not necessarily exclude the possibility of direct infection of canes by *B. cinerea.*

Another possible avenue for infection of canes is through wounds created when leaves fall prematurely due to damage, although infection via this route may be less important with natural leaf drop since an abscission layer has already formed and may act as a barrier. Systemic infection of shoots/plants by *B. cinerea* could be another explanation. Systemic infection by *B. cinerea* was recently described in primula and lettuce (Barnes and Shaw, 2002; Barnes and Shaw, 2003; Shafia A and Shaw, 2007). Further research is needed to clarify this possibility.

In summary, conclusions drawn from the present studies differed considerably from previous observations. Firstly, our results indicate that it is cane age rather than leaf age that influences leaf susceptibility to *B. cinerea.* Secondly, differences in the susceptibility to *B. cinerea* are very small between leaves of different ages. Finally, cane botrytis is more likely

to result from direct infection of the cane than through mycelial invasion via the petioles of infected leaves.

These differences from previous observations may result from changes in cropping systems as well as differences in experimental methods. Previous conclusions regarding the infection of leaves, and subsequently canes, were mainly deduced from field observations and from open-field crops. The protected environment may affect not only microclimatic conditions but also possibly tissue susceptibility to the pathogen.

Further research is needed to confirm these findings, particularly histological studies of possible direct infection of canes, and to determine conditions conducive to infection of canes by *B. cinerea* during both the growing and dormant seasons, which will have important implications for control strategies.

Literature cited

Barnes SE, Shaw MW. (2002) Factors affecting symptom production by latent *Botrytis cinerea* in *Primula* × *polyantha*. *Plant Pathology* **51:**673-681.

Barnes SE, Shaw MW. (2003) Infection of commercial hybrid primula seed by *Botrytis cinerea* and latent disease spread through the plants. *Phytopathology* **93:**573-578.

Biggs AR. (1996) Detection of latent infections in apple fruit with paraquat. *Plant Disease* **80:**1062-1067.

Cox DR, Snell EJ. (1989) Analysis of binary data. Chapman and Hall, London.

Hockey JF. (1952) Grey mould wilt of raspberry. *Scientific Agriculture* **32:**150-152.

Jennings DL, Carmichael E. (1975) Resistance to grey mould (*Botrytis cinerea* Fr) in red raspberry fruits. *Horticultural Research* **14:**109-115.

Labruyere V, Engels G. (1963) Fungi as the cause of diseases of the raspberry cane and their connection with the occurrence of the raspberry cane midge. *Netherlands Journal of Plant Pathology* **69:**235-257.

Payne RW. (2006) The guide to GenStat® release 9 - Part 2: Statistics. VSN International, Hemel Hempstead, UK.

Shafia A, Shaw M. (2007) Behaviour of latent infection of *Botrytis cinerea* in lettuce (*Lactuca sativa*). Cape Town, SA.

Sutton JC, Dale A, Luby JJ. (1990) Alternative methods for managing grey mold of strawberry. Houston, Texas.

Sutton JC, Li D-W, Peng G, Yu H, Zhang P, Valdebenito-Sanhueza RM. (1997) A versatile adversary of *Botrytis cinerea* in crops. *Plant Disease* **81:**316-329.

Williamson B. (1984) Polyderm, a barrier to infection of red raspberry buds by *Didymella applanata* and *Botrytis cinerea*. *Annals of Botany* **53:**83-89.

Williamson B. (1991) Cane *Botrytis*. In: Ellis, M, R Converse, R Williams and B Williamson (eds), Compendium of raspberry and blackberry diseases and insects, pp. 10-11. American Phytopathological Society.

Williamson B, Hargreaves AJ. (1981) Effects of *Didymella applanata* and *Botrytis cinerea* on axillary buds, lateral shoots and yield of red raspberry. *Annals of Applied Biology* **97:**55-64.

Williamson B, Jennings DL. (1986) Common resistance in red raspberry to *Botrytis cinerea* and *Didymella applanata*, two pathogens occupying the same ecological niche. *Annals of Applied Biology* **109:**581-593.

Williamson B, Lawson H, Woodford J, Hargreaves AJ, Wiseman J, Gordon S. (1979) Vigour control, an integrated approach to cane, pest and disease management in red raspberry (*Rubus idaeus*). *Annals of Applied Biology* **92:**359-368.

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

Introduction

As we discussed in the previous section, cane infection is believed to arise at the leaf nodes via mycelial growth down the petiole. The previous section focused on controlled inoculation studies to examine this possibility, whereas this section deals with field monitoring of the time when leaves become infected in 2007 and 2008.

Materials and methods

Monitoring areas were marked out in the experimental tunnels in Cambridgeshire and Kent. The density of canes was much greater at the Kent site than the Cambridge site. 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. In 2006, sampling commenced in May in Kent and in June in Cambridge and continued at three-weekly intervals until August (Kent) or November (Cambridge). In 2007, samples of leaves were taken on 02/07, 23/07, 22/08 and 13/09 in 2007 at the Cambridge site, whereas only old leaves (at the bottom of the cane) were sampled on 15/05, 12/06, 09/07 and 09/08 at the Kent site. 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 before assessment for the presence of botrytis on the leaflets and the petioles.

In addition, at both sites, commencing within the period of the leaf sampling in 2006 and 2007, 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

In 2006, neither site of the leaves had a high incidence of botrytis and there did not appear to be any difference between infection at the different sample heights; about 10% of green leaves had botrytis. At the Cambridge site, the early season (June) samples had the most leaf blades with latent botrytis. At the Kent site, there was a noticeable amount of petiole infection in late July, although it was absent from the topmost (youngest) leaves. Sclerotia did not develop on the leaf blades and petioles until late August. A similar level of botrytis was recorded in mature green leaves collected in May from the lowest position on the primocane.

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. 1.1.4a). 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. 1.1.4b). All leaves collected on 12/06 developed botrytis sclerotia on them. On 09/07, a third of leaves sampled developed botrytis (all as sporulating lesions). Nearly 60% leaves collected on 09/08 were infected.

Primocane infection

At the Cambridge site, no brown lesions (which might be botrytis) were seen on the leaves of the monitoring plants in the field in 2006. The first cane lesion seen, on 5 October 2006, was not on a monitored plant. Further sclerotia on primocanes were seen from 30 October on the monitored plants, within the whitened areas of "water-mark" lesions, which were up to 50 cm long. The lesions occurred at all heights (5 to 150 cm from the base), often radiating out from a leaf scar and so probably corresponding to the range of heights at which botrytis occurred on the leaves. Possibly infection of canes, or the appearance of bleached cane symptoms, coincides with the period of epidermis maturation and browning. Only three primocane lesions were recorded on 30 October and a further three on 29 November. At the Kent site, no primocane lesions had occurred by February 2007.

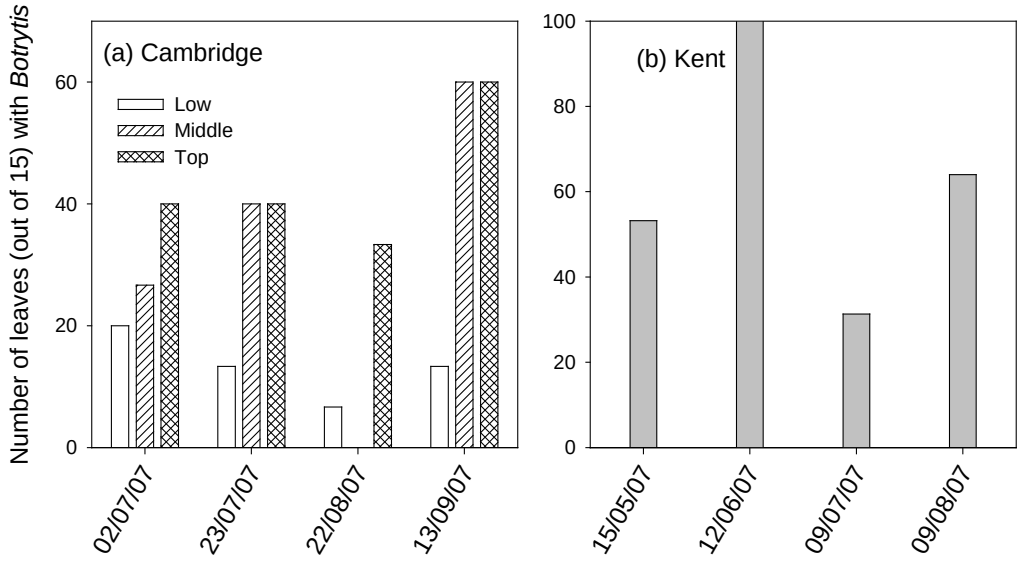


Figure 1.1.4. Overall incidence of raspberry leaves of cv. Glen Ample with latent *B. cinerea*; leaves were sampled on several occasions from plants grown under protection in Cambridge and Kent. Leaves were divided into three categories: around the top, middle and bottom of the cane; but in Kent leaves were sampled only from the bottom of the cane.

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 August. The tunnel had been uncovered. At this time the majority of leaves were still green, only the lowest leaves had dropped. Cane browning was usually associated with a brown petiole. 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. 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 11 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 September 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. On 24 January, 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 October, with more on 29 November and the number then remaining constant in 2007 after 16 March.

In Kent, a high mean of 42% of leaves per stool had died in the crop by 09 August 2007, but these were not associated with lesions. Only 1.5% of leaves had lesions, with 119 suspected cane lesions on 30 assessed canes. By 19 September2007, 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 07 February, only 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

Overall there was very low level of cane infection. Assessment of cane infection in autumn was usually not reliable; most suspect infections turned out not to be cane botrytis when assessed early the next year. Number of leaves infected varied between the two seasons but did not show any apparent relationships with cane lesions.

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

Materials and methods

It is important to identify important sources of inoculum and timing when sporulation occurred. For this purpose, various potential types of inoculum source were frequently sampled and monitored for the presence and sporulation of *B. cinerea*. 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 this study.

Cane lesions

In early 2006, all of the plants in the monitoring areas of the unsprayed tunnel at both sites were examined closely for botrytis sporulation and tagged if either lesions or sclerotia were present. At the Kent site, a total of 14 fruiting canes with botrytis were found and tagged, while at the Cambridge site nine affected canes were found and tagged out of about 200 plants assessed. At the Kent site, the sclerotia were behind a large amount of leaf canopy on the laterals, whereas the grower at the Cambridge site maintained fewer canes. These canes were then inspected every two-three weeks for signs of sporulation. Similar observations were made in the same tunnels in 2007. Usually, recording commenced in February before the crop was covered.

In April 2008, it was also investigated whether *B. cinerea* sclerotia present on raspberry fruiting canes are sources of inoculum in raspberries grown under polytunnels. At the Kent site, 20 fruiting canes with visible botrytis cane lesions and sclerotia present were tagged and inspected with the aid of a hand lens for sporulating botrytis. Inspections were repeated every two-three weeks until the end of flowering.

Weeds and crop debris

In 2006 and 2007, 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, cane pruning debris, leaves and various species of weeds were examined for botrytis sporulation. At the Cambridge site, samples were picked up at twenty positions along the length and between the rows of the tunnels. Samples were removed monthly for damp chambering in the laboratory to see if sporulation could be encouraged.

The tunnel floor was bare earth with grass pathways between tunnels. There were no weeds to examine until mid April when thistle, groundsel, chickweed, dandelion, redshank, nightshade and bindweed started to appear. At the Kent site, dock, daisy, mayweed and willow herb were seen as well as groundsel and dandelion.

Buds, leaves and young primocane shoots

Given the very low level of visible botrytis before harvest in the tunnels at both sites found in 2006 and 2007, it is difficult to understand the source of inoculum for flower infection. In 2008, the possibility was examined 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, i.e. the possibility of *B. cinerea* overwintering within cane buds or the crown.

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

Experiments were established in commercial crops in Cambridgeshire and Kent to determine if preventing air-dispersed conidia settling on primocanes, by covering developing shoots with a polythene bag from emergence, eliminates occurrence of *B. cinerea* on these shoots. The two tunnel crops were used. At the Cambridge site, after the first primocane flush had been burnt off, samples of the next emerging shoots were tagged on 15 May 2008. Two shoots within 5 m of each other at 10 locations were tagged. One of each pair was covered with a transparent bag, held on a tripod frame and pinned to the ground, the other was left uncovered. Leaf samples were taken once the shoots had nearly reached the top of the bag on 29 August. Leaves were sampled from each of three heights per cane up to the highest fully expanded leaf (43-52 cm). Any senescent lower leaves were also sampled. Leaves were surface sterilised, dipped in paraquat and then incubated in damp trays covered with polythene to encouraged botrytis sporulation.

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

Molecular comparison of populations of Botrytis cinerea from different sources

Experiments were conducted to identify sources of *B. cinerea* inoculum in raspberries grown under polytunnels by molecular comparison of populations of *B. cinerea* from different sources related to raspberries. In April 2008, weeds and raspberry cane debris were collected from the polytunnel at the Kent Site, washed and damp incubated under UV light to encourage any latent botrytis present to sporulate. Sporulating colonies of *B cinerea* were then plated onto PDA amended with rifamycin.

Once cultures were free of contamination, isolates were grown on sterile cellophane on PDA. Once growth was established, the mycelium was scraped off and stored in Eppendorf tubes at -80^{o}C prior to molecular analysis. Isolates were similarly collected from flowers, leaves, fruits and other sources relevant to raspberry. DNA from these samples were extracted and screened for the six published SSR primers (developed by a French research group). These data were then subjected to analysis of molecular variance to detect population differences.

Results

Cane lesions

At the Cambridge site, it was only at the end of May 2006, in the week after the polythene was put on, that sporulation was observed from the sclerotia; but only nine canes had sclerotia or lesions. The crop was at first open flower at this time and sclerotia on most canes sporulated. Sporulation was still occurring when recording ceased a fortnight after the end of harvest. For the 10 days leading up to 18 July, when no sporulation was observed, the tunnel air temperature had reached over 27°C, and this may have stopped the production of spores.

Lesions, with the marks of concentric growth rings, were usually present on canes but never sporulated and became less distinct over time. A few canes had lesions without any sclerotia on or under the epidermis. In 2007 only 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 2007 but it was not until 22 May after the polythene was put on that sporulation was observed from the sclerotia. 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.

At the Kent site, the sclerotia on only one cane sporulated, this was on 19 May 2006 (the crop was covered). At this time, 25% of sclerotia in a nearby, uncovered, crop were observed to be sporulating. No sporulation was observed in two monitoring occasions in August. In 2007, the incidence of cane botrytis was very low: only a total of seven fruiting canes in three 100 m rows of raspberry plants were found with botrytis in February. Sclerotia ranging in number from one to more than 40 were present on these seven tagged canes.

The polythene was put on the tunnel at the end of February 2007 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. Many of the sclerotia on the tagged canes shrivelled and failed to sporulate. In early April 2007, a few botrytis conidiophores were observed on two sclerotia on two of the monitored canes. No other sporulation was observed.

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

Weeds and crop debris

No *B. cinerea* sporulation was ever seen at either site on either weeds, leaf or cane debris examined between 20 Feb – 15 Aug 2006 at the Cambridge site or 2 May – 25 Aug 2006 at the Kent site either in the tunnel or after incubation of debris in the laboratory.

At the Kent site in 2007, 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. 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 *B. cinerea* sporulation was observed in the tunnel and after incubation; *B. cinerea* was seen only on one piece of cane debris. No *B. cinerea* sporulation was observed on any of the weeds or crop debris within the tunnel at any of the observation dates and similarly no botrytis sporulation was observed on weeds growing outside the tunnel. However, once the collected samples were damp-incubated, *B. cinerea* was observed sporulating after 14 days, especially on dandelion flower heads, dead leaves and cane debris. No significant sporulating *B. cinerea* was noted in the tunnel until harvest where it was mainly present on over ripe fruit.

At the Cambridge site in 2007, 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 willow herb.

Buds, leaves and young primocane shoots

From the Cambridgeshire crop, *B. cinerea* was not recovered from any of the 18 buds per height, or from the 18 cane base samples. Fusarium (species not identified) was commonly isolated. This result indicates that *B. cinerea* was not surviving overwinter in the buds of floricanes or in the crown in this crop. However, in the Kent crop, most buds above the ground had botrytis, often coexisting with fusarium. Botrytis was found on below ground buds at a much lower incidence than those above ground. No botrytis was found on floricane buds or cane base tissue taken from visibly healthy canes. However, on floricanes with botrytis lesions or sclerotia, most lateral buds contained *B. cinerea.*

In the Cambridgeshire crop, botrytis was present whether or not the primocane had been covered from emergence, with twice as many leaves infected when they had not been covered (seven out of 18 leaves, compared to three out of 17 leaves). None of the youngest (highest) bag-covered leaves had botrytis, and none of the senescent leaves produced botrytis from either source. The leaf samples in Kent were collected on 13 May, by which time a lot of the lower leaves in the bagged spawn were dead. No botrytis was found on

either the dead leaves or the green leaves sampled from the bagged spawn. In both cases, the incubated leaves were rapidly colonised by another fungus, which probably suppressed any botrytis development. Most leaves collected from the unbagged spawn sporulated botrytis after incubation.

Molecular comparison of populations of Botrytis cinerea from different sources

A total of 68 isolates had been collected. These were predominantly from raspberry. So far, only one isolate has been collected from weeds and two from air samples. DNA were extracted from 64 samples and screened for six SSR molecular markers. There were enough isolates from raspberry flowers (39) and cane debris inside the tunnel (19) that have good quality SSR data. Analysis of molecular variance suggested no significant differences between the two fungal populations.

Conclusions

Although, sclerotia overwintering on fruiting canes are normally considered an important source of botrytis inoculum in spring, the incidence of cane botrytis and sclerotia was very low. Sporulation on botrytis sclerotia on fruiting canes can occur from mid-May (when crops are usually at first open flower) through to at least mid-August, especially so for the Cambridge site where the crop was covered late in the spring. In contrast, when the crop is covered very early in the spring, there appears to be no opportunity for sclerotia to be wetted and hence to initiate sporulation when temperatures rise in spring. Therefore, sclerotia overwintering on canes are not a major source of inoculum for early covered crops.

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

The results suggest that *B. cinerea* may possibly arise on canes from overwintering in the crown as well as from deposition of conidia in the air. Symptomless infection can occur in buds of floricanes visibly affected by botrytis; as most buds on such a cane were usually infected, the infection may be systemic.

Fungal population comparison suggests that *B. cinerea* from cane debris and from raspberry flowers (fruit) can be effectively be considered as one population.

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

Conventionally, sclerotia overwintering on fruiting canes are considered an important source of botrytis inoculum in spring. Therefore, experiments were carried out to determine the conditions that may influence sporulation.

Materials and methods

Canes with sclerotia were obtained for the initial experiments from canes submitted from various plantations in the UK as part of the cane blight survey. These were randomised and divided amongst the treatments to avoid any effect of cane origin. The canes were cut in to sections with about four-five sclerotia per section. The canes were surface sterilised by soaking in bleach (5 ml bleach in 45 ml water + wetter) for four minutes, followed by rinsing in sterile water and blotting dry. A few sclerotia from the canes were picked off, cut in half and placed on PDA to check viability after sterilising to ensure that the treatment has not affected viability. The remaining canes with sclerotia were divided into the following treatments:

1. Field incubation – The canes were placed in a net bag and hung outside on a post near the raspberry plantation.
2. Six hours wetting at 5°C. The canes were thoroughly wetted and then placed in a plastic lidded box on damp paper in an incubator at 5°C with lights. After six hours the canes were removed from the incubator, dried and then returned to the damp plastic lidded box at 5°C.
3. Six hours wetting at 10°C. The canes were thoroughly wetted and then placed in a plastic lidded box on damp paper in an incubator at 10°C with lights. After Six hours the canes were removed from the incubator, dried and then returned to the damp plastic lidded box at 10°C.
4. Damp incubation at 5°C. The canes were placed without prior wetting in a plastic lidded box on damp paper in incubator at 5°C.

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

Results

The surface sterilisation treatment did not appear to have affected sclerotia viability as the treated sclerotia all produced colonies of botrytis on PDA. After two weeks incubation around 9-13% of sclerotia were sporulating on the sclerotia held at 5 or 10°C with little difference between treatments. Sporulation was particularly extensive on the sclerotia incubated at 10°C. There was no obvious sporulation on the sclerotia in field incubation. One month later

the number of sclerotia sporulating in treatments 2 and 4 (i.e. incubated under 5°C) had fallen to 3-8% while that in treatment 3 (i.e. incubated under 10°C) had increased to more than 14%. Many shrivelled sclerotia were observed in all treatments. No sporulation was observed on the field-incubated sclerotia.

Conclusions

It would appear that provided the sclerotia are wetted, or incubated in high humidity for two weeks, sporulation will occur.

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

Introduction

Raspberry fruit are very susceptible to *B. cinerea*, resulting in serious yield losses (McNicol *et al.*, 1985). The fungus can also cause disease on raspberry canes, namely cane botrytis, which may lead to nodal lesions, impaired axillary growth and lateral shoot failure in spring (Williamson and Jennings, 1986). Much research has been conducted on the epidemiology and management of strawberry grey mould. Although using bio-control agents to manage *B. cinerea* has received much attention in the last decade (Sutton and Peng, 1993; Elad, 1994; Sutton, 1994; Elad, 1996), current control of grey mould in commercial strawberry and raspberry production in the UK still relies heavily on the scheduled application of fungicides during the flowering and early fruiting periods. This is usually effective; however, under conditions particularly conducive to disease, control may still be less than satisfactory.

Nearly all published research studies on raspberry grey mould to date are for crops grown in open field. In the UK, raspberry is now mostly grown under protection, usually Spanish tunnels, to extend the production season. The risk of grey mould is greatly reduced under protection, compared with open-field (Xiao *et al.*, 2001), which is primarily due to the protection of crops from rainfall, thus reducing risks associated with flower and fruit infection. Nevertheless, control of grey mould on strawberry and raspberry grown under protection is still often based on the strategy developed for open-field crops, i.e. scheduled applications of fungicides during the flowering and early fruiting period. Research is needed to assess whether this approach for grey mould management on crops grown under protection is justified.

In this section, we report findings on infection of flowers and fruit by *B. cinerea* on raspberry grown under protection.

Materials and methods

Flower infection

The incidence of flower infection was determined in 2007 and 2008 in two unsprayed raspberry crops (one in Kent and the other in Cambridgeshire) of cv. Glen Ample, about 180 km apart. During the flowering period, flowers were sampled every few days. On each sampling day, 100 fully-opened flowers with all petals still attached (no necrosis on them) were randomly collected from each crop between 8:30-9:30 am. These flowers were collected individually into 25 ml universal bottles. Each season, 12-15 batches of flowers were sampled over the flowering period at each site.

The flowers sampled were surface sterilised with 10 ml sodium hypochlorite (0.025% available chlorine (w/v)) for 15 min on a shaker to remove any spores on the surface and then rinsed several times with distilled water. The flowers were placed separately on a piece of filter paper thoroughly wetted with distilled water in sterile Petri dishes (diameter: 5 cm). The dishes were incubated at ambient temperatures of approximately 20°C for 10-14 days before individual flowers were examined for the presence of *B. cinerea* conidiophores. Any flower on which conidiophores were detected was classified as infected. Similarly, the incidence of flower infection was determined over three years (2007-09) in one unsprayed strawberry crop of cv. Elsanta (June-bearer) in Kent. Sampling and assessment procedure was the same as for raspberry.

A new volumetric spore trap (Burkard Manufacturing Co Ltd, Rickmansworth, Hertfordshire, UK) was used to sample air continuously within the raspberry plots at each site 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 over 24 hours from 10 am to 10 am and vials were quantified for *B. cinerea* DNA by a TaqMan PCR test (Suarez *et al.*, 2005). Spore sampling was only done in 2007 because DNA results were found to be not very useful in explaining the observed incidence of flower infection.

A USB 502 logger (Measurement Computing, Norton, MA, USA) was installed at the canopy height (ca. 1 m for raspberry and ca. 20 cm for strawberry) to record temperature (°C) and rh in the tunnel at an interval of 30 min. Values of vapour pressure deficit (vpd, mmHg) were derived from temperature (T) and rh using the following empirical equation: $vpd = 4.6698\mathrm{e}^{0.06241\mathrm{T}}\left(1 - rh/100\right)$.

Data analysis

The observed percentage of infection of raspberry flowers was first compared to the predicted value given by a previously published model based on average daily (08:00 GMT to 07:59 GMT next day) temperature and vpd, i.e. model 5 as described by Xu, Harris et al. (Xu *et al.*, 2000). The model predicts daily incidence of infected flowers (PI_t) using the equation: $\ln\left(P_t/(1-P_t)\right) = -5.33 - 0.964 \bullet vpd + 5088 \bullet Temperature$. To compare the observed incidence with the predicted, we assumed that sampled flowers were exposed (and susceptible) to B. cinerea for the previous 48 hours prior to sampling. Thus the predicted incidence of flower infection (PBI_t) for the batch of flowers sampled on day t is

$$PBI_t = PI_{t-2} + (1 - PI_{t-2}) \bullet PI_{t-1} \qquad (1)$$

where PI_{t-1} and PI_{t-2} are the predicted daily infection on day t-1 and t-2, respectively. Pearson's and Spearman correlation coefficients were calculated to assess the correlation between the predicted and observed incidences of flower infections.

The development of a new model for infection of raspberry flowers, relating the incidence of flower infection to weather conditions, was attempted. As pointed out previously (Xu *et al.*, 2000), a straight regression of the incidence of flower infection in each 48 h period on corresponding averages of weather variables was not appropriate. The same modelling approach was adopted as used previously for developing strawberry flower infection models (Xu *et al.*, 2000) to determine the effects of weather variables on the incidence of daily flower infection. The logit of daily incidence of flower infection (DII_t) was first assumed be linearly related to (i.e. function of) daily weather variables, i.e. $\ln\left(DII_t/(1-DII_t)\right) = f(w_t)$, leading to $DII_t = \dfrac{\exp(f(w_t))}{1+\exp(f(w_t))}$. However, the daily incidence of flower infection (DII_t) was not available in the present data sets. Instead, under the assumption that sampled flowers were exposed to infection for the previous 48 hours prior to sampling, the incidence of flower infection (p_t) for each batch of flowers sampled on day t was related to DII_t:

$$p_t = \frac{\exp(f(w_{t-2}))}{1+\exp(f(w_{t-2}))} + \left(1 - \frac{\exp(f(w_{t-2}))}{1+\exp(f(w_{t-2}))}\right) \bullet \frac{\exp(f(w_{t-1}))}{1+\exp(if(w_{t-1}))} \qquad (2)$$

Equation (2) was fitted to the observed data using the FITNONLINEAR procedure, which estimates parameters using the maximum likelihood method, in Genstat™ (Payne, 2006). The following weather variables were included in the regression analysis: temperature, rh and vpd. For each variable, day (08:00 GMT to 19:59 GMT) and night (20:00 GMT to 07:59

GMT the next day) averages as well as daily (08:00 GMT to 07:59 GMT the next day) averages were used. Models involving all combinations of up to four weather variables were fitted to the data, and the best model was selected based on the percentage of variation of accounted for, number of variables, and analysis of residuals.

Results

Infections of flowers

As expected, there was greater variability in the daytime conditions than in the night time in both years. There were similarities in the overall weather patterns between the two sites despite some differences. As an example, Figure 1.1.5 plots out average daytime temperature, rh and vpd values in two raspberry crops where flower infection was monitored in 2007, indicating similar patterns between the two sites; average day temperature ranged from 10-29°C, rh from 40 to 90%, and vpd from 1 to 18 mmHg.

Daily number of estimated airborne conidia varied greatly (Fig. 1.1.6); for example it ranged from no spores to nearly 5,000 spores (average = ca. 169) at the Kent site. However, there were no discernible relationships between the estimated number of spores and the incidence of flower infection. For example, on several occasions a certain level of flower infection was observed but the Taqman qPCR failed to detect any fungal DNA. A similar pattern was also observed at the Cambridge site (data not shown). For this reason, spore trapping was not done in 2008.

Similarly, the incidence of flower infection varied greatly among sampling occasions for both sites within each year (Fig. 1.1.7). At the Kent site, the incidence in 2007 ranged from 2% on 23/05 to nearly 56% on 30/05 (average = 23%); at the Cambridge site, it ranged from 1% on 14/05 to 51% on 18/06 (average = 18%). In 2008, it ranged from 4% to 50% (average = 15%) at the Kent site and from 2% to 39% (average = 17%) at the Cambridge site.

The previously published model overestimated the level of infection of raspberry flowers. Moreover, the overall trend of predicted infection did not follow the observed pattern for all four data sets. For example, the average observed flower infection at the Kent site in 2007 was 23%, compared to the corresponding predicted average of 53%. At both sites in 2007, the model predicted high incidences of infection during the period of early to mid-June (Fig. 1.1.7a,b) because of high humidity (low vpd) (Fig. 1.1.5). However, only at the Cambridge site did the observed incidence increase during this period (Fig. 1.1.7b). Both Pearson's correlation and Spearman’s rank correlation coefficients between observed and predicted

incidences were not significantly different from zero for the 2007 Kent data set and the 2008 Cambridge data set. The positive correlation was close to the 5% significance level for the 2007 Cambridge data set and was significant ($P < 0.01$) for the 2008 Kent data set.

Table 1.1.2 presents a summary of the modelling results. A greater proportion of variance in the incidence of flower infection was explained by climatic variables in 2008 than in 2007 for both sites. Flower infection in 2007 appeared to be more influenced by temperature and in 2008 by moisture conditions. There were significant differences in the relationship between the incidence of flower infection and climatic variables between the Kent and Cambridge sites. Consequently, a single model derived from the four data sets only explained about 20% of the total variation in the observed incidences of flower infection. Incidence of flower infection at the Cambridge site appeared to be less related to any of the weather variables recorded than at the Kent site (Table 1.1.2).

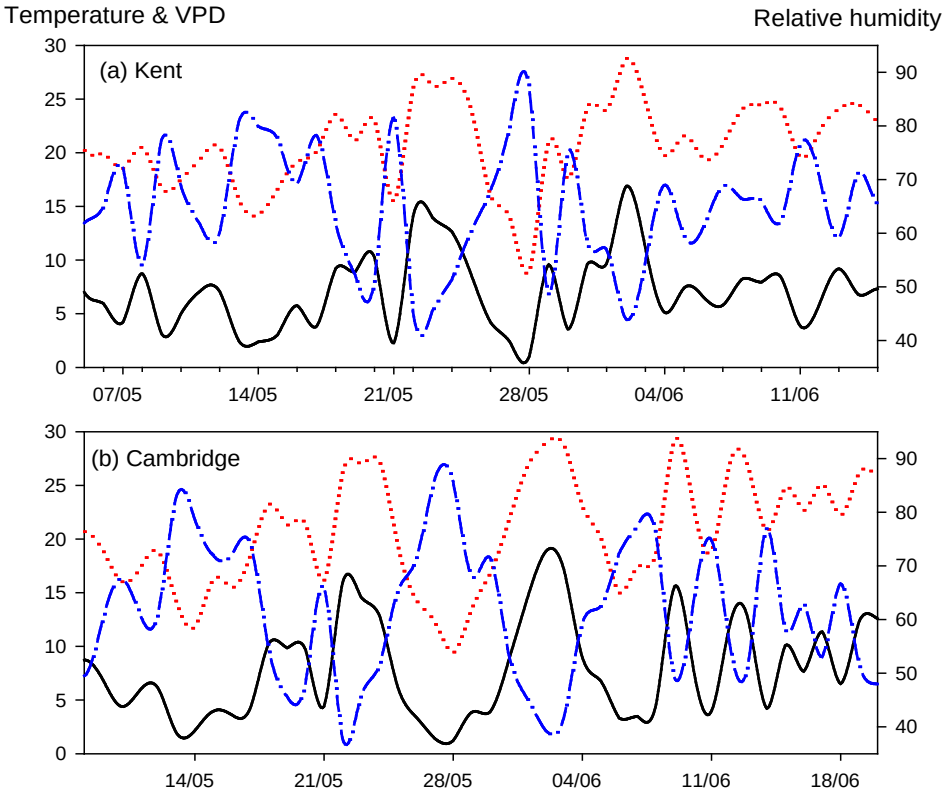


Fig. 1.1.5. Average day time (0800 GMT – 1959 GMT) vapour pressure deficit (solid), temperature (dotted) and relative humidity (dash-dotted-dash) inside the raspberry crop canopy under protection in 2007 at the Kent and Cambridge sites where flowers were sampled for assessment of *B. cinerea* infection

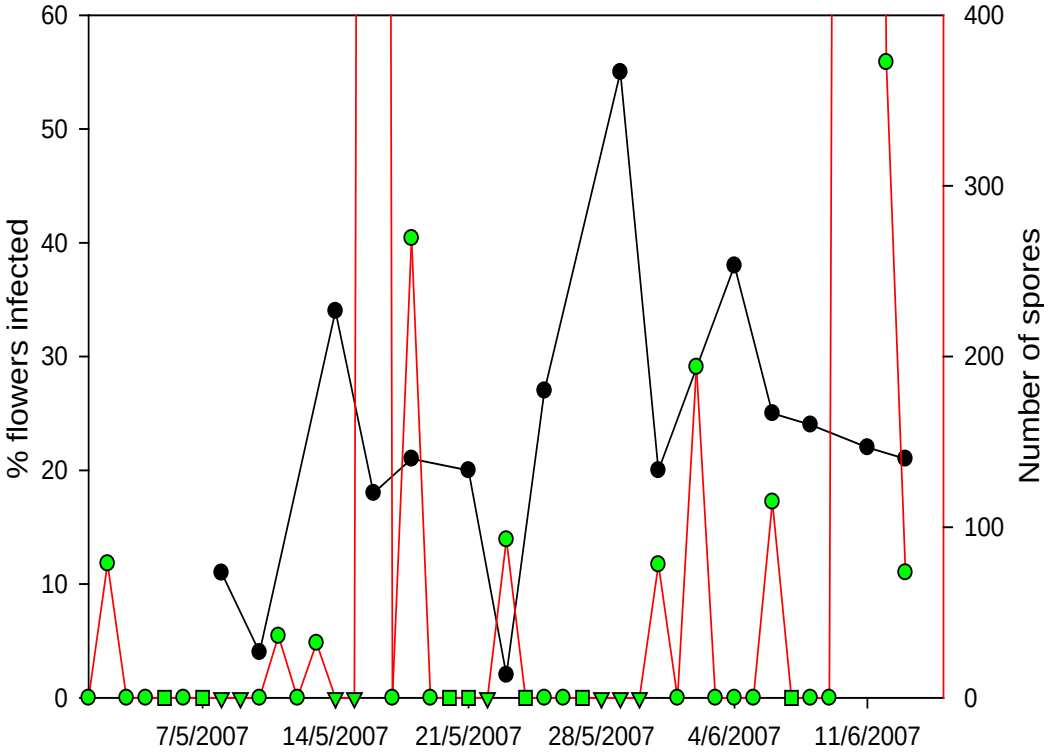


Fig. 1.1.6. Daily number of *B. cinerea* spores (green symbols) and incidence of flower infection (black circles) in 2007 at the Kent site; green square: greater than the limit of detection but less than the limit of quantification, green triangle: the trap stopped working.

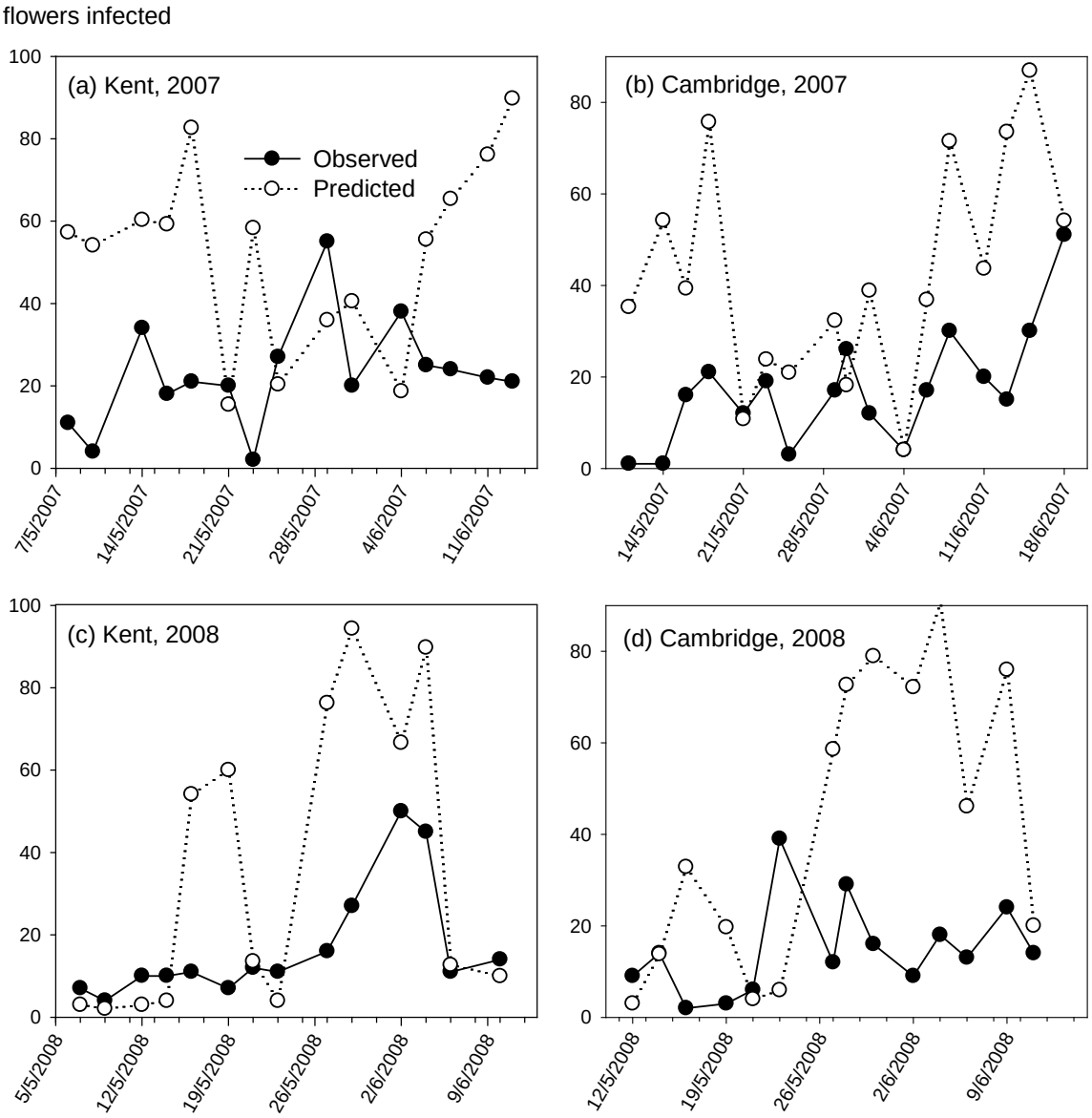


Fig. 1.1.7. The observed (filled circles) and predicted (open circles) incidence of infection by *B. cinerea* of raspberry flowers on each sampling day in 2007 and 2008 in an unsprayed tunnel at the Kent and Cambridge sampling sites

Table 1.1.2. Summary of modelling of infection of raspberry flower by *B. cinerea* in 2007 and 2008 in two unsprayed protected raspberry crops, cv. Glen Ample, in Cambridge and Kent

Data sets	Variables included[a]	% variances accounted for
Cambridge in 2007	$ADT + ADT^2 + ADT^{½}$	19.6
Kent in 2007	$ADV^2 + ADT^{½} + ADV * ADT$	37.4
Cambridge in 2008	NV + NV * NT + NT * NRH	54.3
Kent in 2008	$DV + NT + DV^{½}$	69.4
2008[b]	$NT + DRH + DV^{½}$	37.2
All four data sets	$DT + NT + DT^2 + DT^{½}$	20.0

[a]: DT - day average temperature,
DV – day average vapour pressure deficit,
DRH - day average relative humidity,
NT – night average temperature,
NV vapour pressure deficit,
NRH – night average relative humidity,
ADT - daily average temperature,
ADV - daily average vapour pressure deficit
ADRH - daily average relative humidity.
[b]: A combined model for 2007 data cannot be derived.

Discussion

The overall incidence of flower infection and latent infection of fruit on raspberry was higher than we observed on early covered Elsanta strawberry, which may be explained by differences in inoculum and weather conditions between raspberry and strawberry. Inoculum sources comprise sclerotia on plant debris, plant organs and weeds, and resting mycelia on several plant tissues (Jarvis, 1962; Braun and Sutton, 1987; Mertely *et al.*, 2000; Stromeng *et al.*, 2009). On strawberry inoculum level is likely to be lower than in raspberry because of covering planting beds very early in the season with polythene and the use of a straw mulch over pathways and soil. Moreover, strawberry crops usually only last for one-two years. In contrast, weeds and plant debris are much more abundant in raspberry crops because of their longer perennial nature and canopy structure. Raspberry crops under protection usually flower during the period from early May to mid-June, about a month earlier than the flowering time of June-bearer strawberry under protection sampled in this study. Thus, conditions for infection of flowers, especially the night temperature as identified for field-grown crops (Xu *et al.*, 2000), may not be as limiting in raspberry as in the June-bearer strawberry in this study. The high incidence of latent infection on raspberry might also be due to the possibility that *B. cinerea* grows in raspberry as an endophyte, as recently found on lettuce (Sowley *et al.*, 2010); further research is needed to understand this potential avenue of fruit rotting.

A previously published strawberry model (Xu *et al.*, 2000) did not consistently predict the incidence of flower infections accurately over several seasons on raspberry grown under protection. Its predictions also appeared to over-estimate the incidence of flower infection. The inconsistent performance of the model predictions may have resulted from the nature of data from which the model was developed. The model was developed from the data collected from an open-field strawberry crop for which flowering is usually in the period from late April to early June. It identified temperature (primarily night time) and vapour pressure deficit (primarily day time) as two limiting factors for infection of flowers during this period. But for crops under protection, night time temperature during flowering is no longer expected to be a limiting factor for infection. Another reason may be the difference in the level of inoculum between crops grown in open-field and under protection. In open-field, sporulation of *B. cinerea* on plant residues in spring is expected to be much greater than under protection because of the sheltering of colonised plant residues from rain.

It was not possible to develop a single model to relate the incidence of infection of raspberry flowers to weather factors. Not only the relationship of flower infection with climatic factors varied greatly among the four data sets, but the percentage of variance explained by each model also varied greatly among the data sets. These results suggest that factors other than temperature and humidity may need to be considered for some data sets. However, given the high level of latent infection in both sprayed and unsprayed fruit, it is doubtful that a predictive model for flower infection will be of any significant use in practical management of grey mould on raspberry under protection. No attempt was made to develop another model for infection of strawberry flowers under protection because of the low levels of disease observed over the three years. Furthermore, given the high level of latent infection in the sprayed commercial crops (as observed in our samples), the need for (or effectiveness of) the spray against *B. cinerea* on raspberry grown under protection was questioned.

Literature cited

Braun PG, Sutton JC, 1987. Inoculum sources of *Botrytis cinerea* in fruit rot of strawberries in Ontario. *Canadian Journal of Plant Pathology* **10**, 133-141.

Elad Y, 1994. Biological control of grape grey mould by *Trichoderma harzianum*. *Crop Protection* **13**, 35-38.

Elad Y, 1996. Mechanisms involved in the biological control of *Botrytis cinerea* incited diseases. *European Journal of Plant Pathology* **102**, 719-732.

Jarvis WR, 1962. The dispersal of spores of *Botrytis cinerea* Fr. in a raspberry plantation. *Transactions of the British Mycological Society* **45**, 549-559.

McNicol RJ, Williamson B, Dolan A, 1985. Infection of red raspberry styles and carpels by *Botrytis cinerea* and its possible role in post-harvest grey mould. *Annals of Applied Biology* **106**, 49-53.

Mertely JC, Chandler CK, Xiao CL, Legard DE, 2000. Comparison of sanitation and fungicides for management of *Botrytis* fruit rot of strawberry. *Plant Disease* **84**, 1197-1202.

Payne RW, ed 2006. *The guide to GenStat® release 9 - Part 2: Statistics*. Hemel Hempstead, UK: VSN International.

Sowley ENK, Dewey FM, Shaw MW, 2010. Persistent, symptomless, systemic, and seed-borne infection of lettuce by *Botrytis cinerea*. *European Journal of Plant Pathology* **126**, 61-71.

Stromeng GM, Hjeljord LG, Stensvand A, 2009. Relative contribution of various sources of *Botrytis cinerea* inoculum in strawberry fields in Norway. *Plant Disease* **93**, 1305-1310.

Suarez MB, Walsh K, Boonham N, O' Neill T, Pearson S, Barker I, 2005. Development of real-time PCR (TaqMan (R)) assays for the detection and quantification of *Botrytis cinerea in planta*. *Plant Physiology and Biochemistry* **43**, 890-899.

Sutton J, 1998. *Botrytis* fruit rot (gray mould) and blossom blight. 28-31 *in*: Maas J, ed. *Compendium of strawberry diseases*. APS Press, St Paul, Minnesota.

Sutton JC, 1994. Biological control of strawberry disease. *Advances in Strawberry Research* **13**, 1-12.

Sutton JC, Peng G, 1993. Biocontrol of *Botrytis cinerea* in strawberry leaves. *Phytopathology* **83**, 615-621.

Williamson B, Jennings DL, 1986. Common resistance in red raspberry to *Botrytis cinerea* and *Didymella applanata*, two pathogens occupying the same ecological niche. *Annals of Applied Biology* **109**, 581-593.

Xiao CL, Chandler CK, Price JF, Duval JR, Mertely JC, Legard DE, 2001. Comparison of epidemics of *Botrytis* fruit rot and powdery mildew of strawberry in large plastic tunnel and field production systems. *Plant Disease* **85**, 901-909.

Xu X-M, Harris DC, Berrie AM, 2000. Modelling infection of strawberry flowers by *Botrytis cinerea* using field data. *Phytopathology* **90**, 1367-1374.

Task 1.2. Environment manipulation

Introduction

Raspberry fruit are very susceptible to *Botrytis cinerea* Pers. ex Fr. resulting in serious yield losses (McNicol *et al.*, 1985). The fungus can also cause disease on raspberry canes, namely cane botrytis. The first lesions on primocanes often appear in July on nodes near the base; as the season progresses, they develop at nodes higher on the canes. Most stem lesions are believed to follow infection of leaves (Hockey, 1952; Williamson, 1991). Prominent sclerotia form beneath the epidermis of canes during winter and erupt in spring. During periods of high humidity, these sclerotia become covered with conidia, an important inoculum source for infection of flowers (Jennings and Carmichael, 1975).

In the UK, raspberry is now mostly grown in the UK under protection, usually Spanish tunnels, to extend the production season. It is believed that the risk of grey mould is greatly reduced under protection, compared with open-field. However, there have been no experimental data to substantiate this claim. In the absence of soundly based guidance, growers still apply fungicides to control *B. cinerea* during flowering and fruiting as well as to the canes post-harvest. Cane infection leads to nodal lesions, impaired axillary growth and lateral shoot failure in spring (Williamson and Jennings, 1986). There is a general lack of biological and epidemiological information on cane and fruit botrytis in protected raspberry, necessary to develop a rational integrated disease management strategy.

Development of botrytis in open-field raspberries is highly dependent on initial inoculum and specific environment conditions (Jarvis, 1962, 1964; Harrison and Williamson, 1986). Research on other crops has shown that botrytis diseases can be managed effectively by targeting these two factors (Xu *et al.*, 2000; O'Neill *et al.*, 2002). In field crops of raspberry, correlations were found between post-harvest fruit botrytis and high relative humidity in the five-day period before picking and rainfall during picking (Jarvis, 1964). Cane botrytis is most severe inside a dense canopy, generally on the lower half of primocanes (Williamson *et al.*, 1979), because it is believed that only mature to senescent leaves can be infected (Williamson, 1991). The removal of the first flush of primocanes to control cane vigour has reduced the severity of cane botrytis, probably because second-flush canes are susceptible to infection for a shorter period in late summer (Williamson *et al.*, 1979).

Manipulating canopy structure through cultural practices can significantly alter microclimatic conditions, particularly increasing air movement. Since for many fungal diseases infection and sporulation often need a prolonged period of high humidity, increased air movement within a crop plantation is expected to reduce the risk of disease development. This has been demonstrated in several pathosystems. Incidence and severity of grape bunch rot, caused by *B. cinerea,* were significantly reduced by leaf removal in the fruiting zone (Duncan *et al.*, 1995). Narrower spacing led to higher incidence of *B. cinerea* on strawberry fruit but higher yield (Legard *et al.*, 2000). For raspberry, an open canopy may have the additional benefit of minimising premature senescence of lower leaves due to shading. However, there has been no clear demonstration of beneficial effects of such cultural practices on cane and fruit botrytis on raspberry grown under protection.

A research programme was undertaken to obtain key knowledge necessary for development of an integrated management strategy for botrytis diseases on raspberry grown under

protection. This work reports results from field experiments, aiming to establish (1) the incidence of latent and post-harvest fruit botrytis in commercial crops grown either under protection or in open-field and (2) whether removal of lateral leaves and thinning of primocanes during flowering and fruiting period could reduce incidences of cane, leaf and fruit infection by *B. cinerea*.

Materials and methods

Occurrence of latent and post-harvest fruit botrytis in commercial crops

Samples of fruit were taken from 19 commercial crops. Fruit were collected from both protected and open field crops across the UK, and from crops with differing levels of pesticide inputs. Around half of the crops were of cv. Glen Ample and the others were of cv. Tulameen. Information on crop husbandry was gathered from each site. Each crop was sampled twice, initially to obtain unripe fruit and then to collect ripe fruit.

At the first sampling, two weeks after the start of commercial harvesting, 100 lateral branches were cut from the canes in each crop. Unripe yellow fruit were examined for latent *B. cinerea* using an incubation test. Berries were surface sterilised by immersing in 0.025% w/v sodium hypochlorite for 15 min, rinsed in sterile distilled water (15 min), and then placed on paraquat chloramphenicol agar (PCA). Paraquat was used to kill host tissue and induce *B. cinerea* sporulation (Biggs, 1996).

The second samples of red fruit were taken from the same crop rows two weeks after the first, selecting 100 firm red marketable fruit. Fruit were transferred either to multicell trays or new punnets, leaving a space between each fruit. The fruit was then sealed in a transparent polythene bag and exposed to a diurnal light regime at 20°C for 7 days prior to assessment for *B. cinerea.*

Canopy manipulation

Experiments were conducted in 2006 and 2007 in protected crops of cv. Glen Ample in Kent and Cambridgeshire. Both crops were covered by Spanish tunnels using the same type of polythene (Luminance THB). Experimental procedures followed at the two sites differed due to the nature of the plantation management at the two sites.

Kent site: The crop was covered in February 2006 and 2007; 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 canes (around 20/m length of row), resulting in a thick

canopy. Primocanes were removed mechanically in early May. Two treatments were imposed: standard canopy management (T1) or additional manipulation (T2). In 2006, three randomised blocks were established on 8 May. Plots were 7 m in length and blocks were separated by 7 m. In 2007, a similar design was used but with the plot size doubled to 14 m; only two blocks were possible. For both years all the plots were at least 8 m away from the tunnel opening.

On 14 May 2006, up to four leaves were removed from the fruiting laterals on canes in the T2 plots, starting at the lowest leaf on the lateral and continuing up to the first leaf with a flower bud present in the axil. On 4 July2006, primocanes were thinned in the T2 plots to three primocanes per stool or six to eight primocanes per 1m row length. All removed leaves or canes were taken out of the tunnel. On 23 May2007, lateral leaves were thinned as in 2006 and spawn was also thinned in the T2 plots. On 4 July2007, the primocanes were thinned in the T2 plots as in 2006. A USB temperature-RH duo logger was hung in the canopy in the central row of each plot at each of the three heights (40 cm, 80 cm and 150 cm above the ground).

Random samples of 20 fully expanded leaves were taken from the bottom, middle and top tier of leaves from the central three metres of the middle row of each plot on three occasions. Leaves were surface-sterilised by immersing them in 0.025% sodium hypochlorite for four minutes, rinsed with tap water, immersed in 0.5% paraquat solution for one minute, and finally rinsed with tap water; leaves were then placed on wet paper towel in a gravel tray and covered with a polythene bag to prevent contamination. Development of *B. cinerea* was assessed three-four weeks later. In 2007 leaf samples were taken twice. In calculating the incidence of leaf infection, a leaf was considered to be infected if either its petiole or any of its leaflets was infected. Cane botrytis was assessed in early February 2007 and 2008 by counting the number of canes with *B. cinerea* lesions or sclerotia in each plot at three heights, corresponding to the height of data loggers.

A random sample of 50-100 unripe (green/yellow) or ripe (red) fruit was taken from the central three metres of the middle row of each plot on several occasions. Unripe fruit were surface sterilised and placed on PCA media as described above. Red fruit were not surface-sterilised and were incubated in multicell trays or punnets seven days before assessment.

Cambridgeshire site: The tunnel contained three rows of raspberries which had been thinned to an open canopy of around 10 canes/m length of row. Herbicide was used 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 as at the Kent site.

Leaves were not thinned on fruiting laterals at this site. In 2006, primocanes were thinned to six primocanes per plant in T1 plots and to four primocanes per plant in T2 plots on 1 May28 June, 21 July and 5 October; the plant-to-plant distance within a row was about 40 cm. In 2007, primocanes were thinned twice (3 May and 20 June), leaving eight primocanes per plant in T1 plots and four in T2. USB temperature-RH duo sensors were placed in one pair of the plots in one block at three heights.

Twenty leaves were randomly sampled from each of the three positions in each plot three times in 2006, and four times in 2007. As at the Kent site, a random sample of 50-100 unripe and ripe fruit was taken from the central three metres of the middle row of each plot on several occasions within each year. Fruit were similarly treated and incubated before disease assessment. Cane botrytis was assessed in early February each year.

Data analysis

Generalised linear modelling (GLM) was used to determine whether the incidence of infection of leaves, fruit or canes by *B. cinerea* was affected by treatment factors (Cox and Snell, 1989), assuming that the proportion (*p*) of infected leaves, fruit or canes per sample is binomially distributed. Thus, for the canopy manipulation study, the logit transformation of *p* ($\ln\left(p/(1-p)\right)$) was used to assess the effects of treatment factors on the incidence:

$$\ln\left(p/(1-p)\right) = Treatment + Age + Age \times Treatment$$

where *Treatment* and *Age* represent whether the plot was subjected to additional leaf or spawn thinning and the position of leaves (or canes), respectively. Similar GLM was used to assess the differences in the incidence of latent and post-harvest fruit botrytis between crops grown in open-field and under protection, between sprayed and unsprayed, and between the two cultivars.

For fruit botrytis in the canopy study, logistic regression was first applied to samples of fruit of the same age taken on the same day to determine whether the treatment had affected the incidence of infected fruit. Then, ANOVA of repeated measurements was applied to all the fruit sampled within the same year at each site to assess the overall treatment effects. Percentage of fruit with infected with *B. cinerea* was first arcsin-transformed before ANOVA. Genstat (Payne, 2006) was used for statistical analysis.

Results

Latent B. cinerea and post-harvest rot

There were large differences between crop samples from commercial plantations in the incidence of latent *B. cinerea* in unripe fruit, ranging from 0 to 99%, and post-harvest botrytis fruit rot, ranging from 2% to 90% (Table 1.2.1). Many samples showed a high incidence (>50%) of infected fruit. On average, the incidence of latent and post-harvest infected fruit on sprayed (55%, 46%), covered (53%, 43%) and cv. Glen Ample (54%, 45%) were less than on unsprayed (85%, 82%), uncovered (65%, 59%) and cv. Tulameen (65%, 58%). However, because of large variation within each group, these differences were not statistically significant. There was no apparent correlation between incidences of latent and post-harvest fruit infection by *B. cinerea*: parametric and non-parametric (Spearman's and Kendall's Rank correlation) correlation coefficients were close to zero.

Canopy manipulation

Kent site 2006: After the leaf removal in May there were obvious visual differences between treated and untreated plots, which resulted in an overall increase in daily average vapour pressure deficit (VPD, mmHg) in the manipulated plots relative to the control plots (Figure 1.2.1) (i.e. increased temperature and reduced humidity). There were no longer obvious differences in canopy density several weeks later because the crop was covered in large mesh plastic netting in late May to support the fruiting laterals, which compressed the crop. Thinning primocanes in early July again appeared to have resulted in reduction in VPD in the treated plots compared with the control plots (Figure 1.2.1).

Table 1.2.1. Summary of crop details and incidence of latent *B. cinerea* in unripe raspberry fruit and post-harvest botrytis after seven-day incubation

Variety	Crop type	Fungicide sprays	Percentage with *B. cinerea*	
			Latent	Post-harvest
Glen Ample	Covered - just prior to harvest	Spray	46	4
Glen Ample	Covered - Rain sheets	Spray	92	21
Glen Ample	Glasshouse	Spray	24	44
Glen Ample	Open	None	84	90
Glen Ample	Open	Spray	10	46
Glen Ample	Open	Spray	54	56
Glen Ample	Open	Spray	99	64
Glen Ample	Open	Spray	76	45
Glen Ample	Tunnel	Spray	51	65
Glen Ample	Tunnel	Spray	21	68
Glen Ample	Tunnel	Spray	0	40
Glen Ample	Tunnel	Spray	67	21
Glen Ample	Tunnel	Spray	82	16

Table 1.2.1. Summary of crop details and incidence of latent *B. cinerea* in unripe raspberry fruit and post-harvest botrytis after seven-day incubation

Variety	Crop type	Fungicide sprays	Percentage with *B. cinerea*	
			Latent	Post-harvest
Tulameen	Covered - Rain sheets	Spray	99	50
Tulameen	Covered - Rain sheets	Spray	92	29
Tulameen	Open	None	86	74
Tulameen	Open	Spray	57	84
Tulameen	Open	Spray	76	69
Tulameen	Tunnel	Spray	61	63
Tulameen	Tunnel	Spray	17	94
Tulameen	Tunnel	Spray	34	2

No *B. cinerea* was found in leaf samples taken in June and July. The fungus was found in leaf samples taken in August but there were no significant differences in incidences between the control (19%) and manipulated (17%) plots. However, there were significant interactions ($P < 0.01$) between canopy manipulation and leaf positions in affecting the incidence of infection (Figure 1.2.2); incidence was much less on the bottom leaves in manipulated plots (3%) than in the control (21%). No cane botrytis lesions were observed in any plot in February 2007.

Incidence of red fruit with *B. cinerea* sampled on 13/06 was significantly ($P < 0.01$) less in the control (59%) than in the manipulated plots (83%). On all other sampling occasions, there were no significant differences between the treated and the control (Table 1.2.2). Except for the red fruit sample taken on 14/07 (16%), disease incidences all exceeded 50% (Table 1.2.2). Overall, there were no significant differences in the incidence of fruit with *B. cinerea* between the two treatments.

Kent site 2007: Removal of leaves and spawn in late May resulted in marked increases in daily average VPD in the manipulated plots relative to the control plots at all three heights (Figure 1.2.3). Similarly, thinning primocanes in early July also resulted in a reduction in VPD in the treated compared to the control plots.

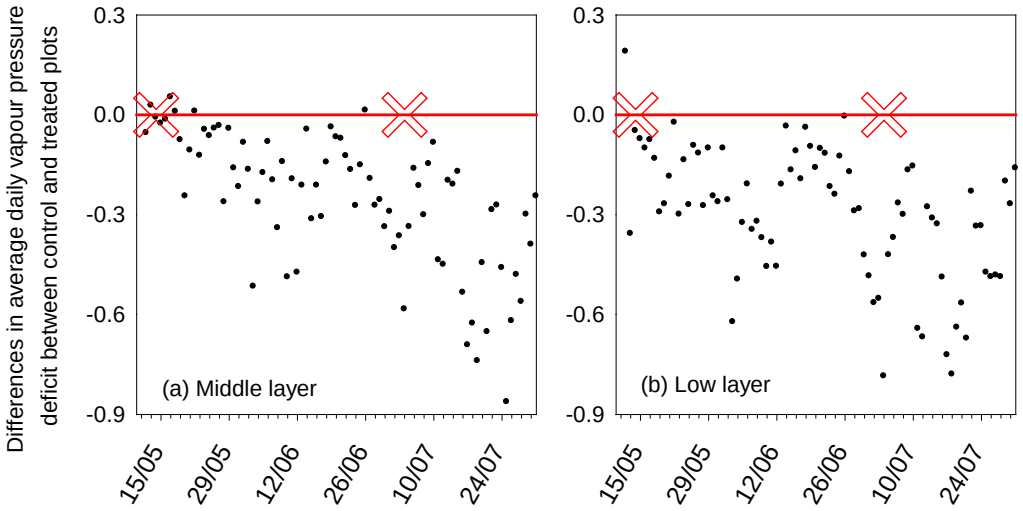


Figure 1.2.1 The differences in daily (9 pm – 9 pm) average vapour pressure deficits (mm Hg) between the control and the manipulated plots of raspberry cv. Glen Ample over time in 2006 under protection at the Kent site. Three out of the six loggers placed on the top layer of the canopy were stolen and hence data for the top position were not available. The 'x' signs indicate the date on which lateral leaves (May) or primocanes (July) were manually removed in the manipulated plots. Values less than zero indicate the VPD is greater in the manipulated than the control plots.

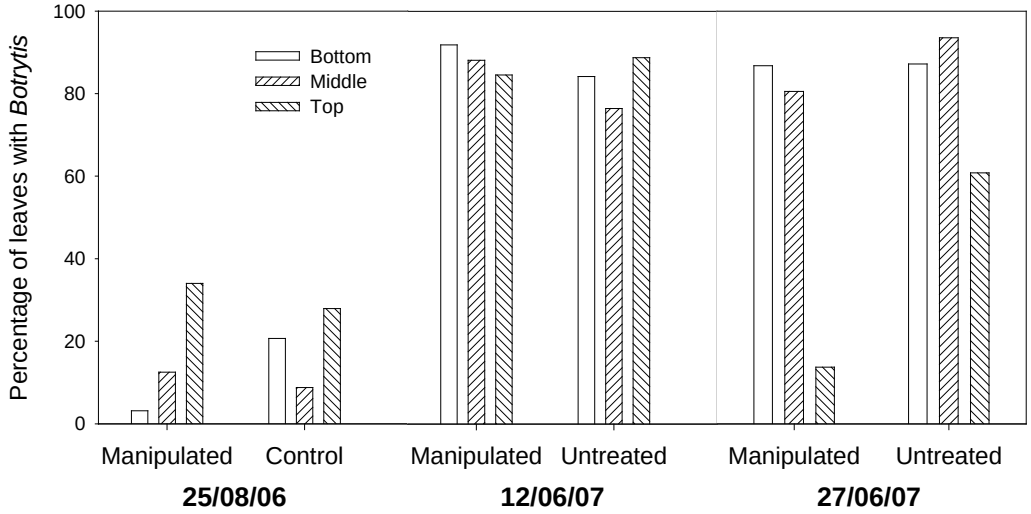


Figure 1.2.2. Overall incidence of raspberry leaves with *B. cinerea* at three heights between the control and the manipulated plots of cv. Glen Ample in 2006 and 2007 under protection at the Kent site.

The incidence of leaves infected with *B. cinerea* was very high (> 68%) on 12/06 in all plots irrespective of leaf position (Figure 1.2.2). There were no significant differences between the two treatments or between leaf positions. On 27/06, the incidences of leaves with *B. cinerea* differed significantly ($P < 0.01$) among three leaf positions (Figure 1.2.2): 87%, 87% and 37% for leaves at the bottom, middle and top, respectively. There were significant interactions between canopy manipulation and leaf positions (Figure 1.2.2). Incidence was

much less on the top leaves in manipulated plots (14%) than in the control (61%). On all three sampling occasions in 2007, there were no significant differences in the incidences of green or red fruit with *B. cinerea* between the manipulated (88%) and control (89%) plots (Table 1.2.2).

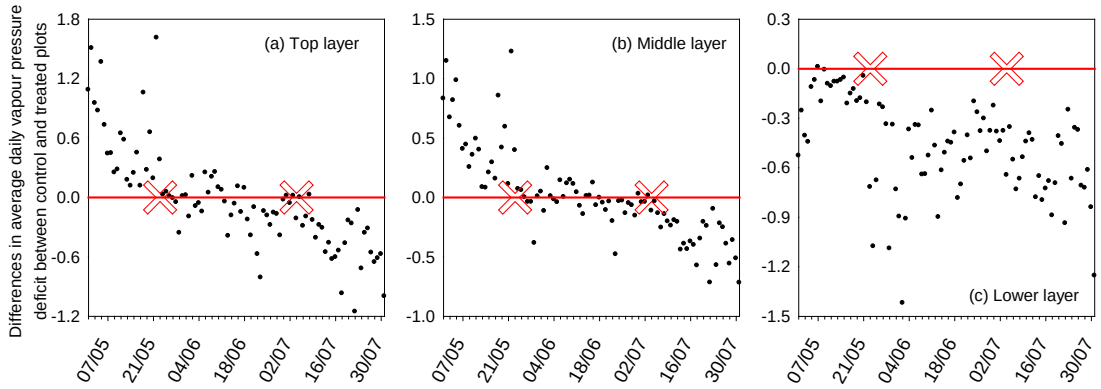


Figure 1.2.3. The differences in daily (9 pm – 9 pm) average vapour pressure deficits (mm Hg) between the control and the manipulated plots of raspberry crops of cv. Glen Ample over time in 2007 under protection at the Kent site. The 'x' signs indicate the date on which lateral leaves and spawns (May) or primocanes (July) were manually removed in the manipulated plots. Values below zero indicate the VPD is lower in the manipulated than the control plots.

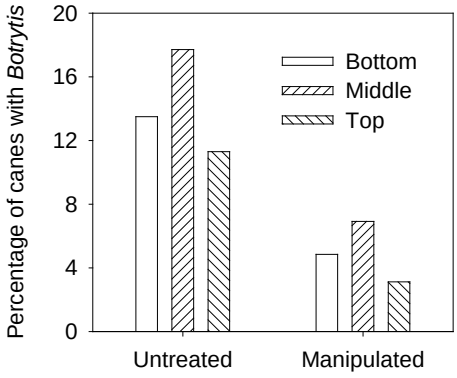


Figure 1.2.4. Overall incidence of raspberry canes with *B. cinerea* at three heights between the control and the manipulated plots of cv. Glen Ample grown under protection, assessed in February 2008 at the Kent site.

Cane botrytis lesions were visible in July 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 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 deviance in the observed incidence. Furthermore, the incidences of cane botrytis differed ($P < 0.01$) among three heights – 11%, 14% and 9% for the bottom, middle and top, respectively (Figure 1.2.4).

Cambridgeshire site: In both 2006 and 2007, additional removal of spawn and primocanes did not lead to much visual difference between treated and untreated plots. No data on humidity was obtained in 2006 due to failure of sensors. In 2007, canopy manipulation did not result in any appreciable increase in VPD in the treated relative to the control plot over time (Figure 1.2.5).

Only two out of 1,080 leaves sampled in 2006 developed *B. cinerea* on them after incubation. Similarly in 2007, very few leaves were found to be infected by *B. cinerea*. 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 in February 2007 and 2008 in any plot.

There were no significant differences in the incidence of *B. cinerea* on both green and red fruit between the treated and control plots on all sampling occasions in 2006 (Table 1.2.2). The overall incidence was 69% and 73% for the treated and control, respectively. Similarly in 2007, there were no significant differences in the incidence of *B. cinerea* on either yellow or red fruits between the treated and untreated plot. The overall incidence was 84% and 83% for the treated and control, respectively. In both years, disease incidence differed significantly ($P < 0.01$) among the sampling dates (Table 1.2.2).

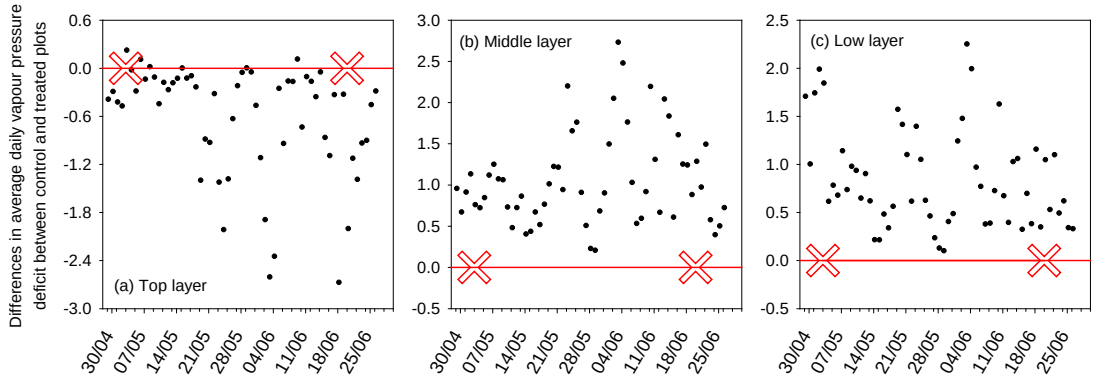


Figure 1.2.5. The differences in daily (9 pm – 9 pm) average vapour pressure deficits (mm Hg) between the control and the manipulated plots of raspberry crops of cv. Glen Ample over time in 2007 under protection at the Cambridgeshire site. The 'x' signs indicate the date on which primocanes were manually removed in the manipulated plots. Values below zero indicate the VPD is lower in the manipulated than the control plots.

Discussion

Incidence of latent *B. cinerea* in fruit was greatest in unsprayed uncovered crops and least in sprayed covered crops. However, the reduction in the incidence under protection was not statistically significant, indicating that establishment of latent infection in fruit, resulting

mainly from infection of flowers (McNicol *et al.*, 1985), does not need free water. There was no consistent correlation between the incidence of *B. cinerea* in yellow fruit and that in ripe fruit. The samples of red fruit were probably seldom from the same flowering stage as the yellow (samples were taken a fortnight apart) and there may be different levels of inoculum and infection success during the flowering period. Management of *B. cinerea* on crops under protection is, therefore, still critical if the fruit is to have a longer shelf life.

Canopy manipulation resulted in considerable microclimatic changes in the crop at the Kent site but not at the Cambridgeshire site. At the former, removal of leaves and thinning of primocanes led to increased air circulation in the plantation, and thus reduced relative humidity and increased vapour pressure deficit. However, such changes in microclimate, namely drier conditions, did not result in any appreciable reductions in the infection of fruit by *B. cinerea.* This is in contrast to results obtained on bunch rot of grape caused by *B. cinerea* where thinning the canopy led to a significant reduction of bunch rot (English *et al.*, 1993; Duncan *et al.*, 1995). Several explanations may account for this difference. *B. cinerea* in raspberry fruit, as in strawberry, is believed to result mainly from infection of flower parts, such as stigma (McNicol *et al.*, 1985), and remains latent until fruit ripen. Surface moisture on the stigma may be sufficient for infection to take place, which may also explain why the incidence of latent infection on raspberry grown under protection is still very high as observed in the present study. Therefore, infection of flowers may not depend so critically on high ambient humidity. In contrast, for grape bunch rot, secondary spread to neighbouring healthy fruit is an important pathway for disease spread; this spread may be significantly increased under moister conditions. Thus, removal of leaves around the fruit zone, given the nature of the tight fruit bunch in grape, may lead to increased air circulation, which is sufficient to reduce secondary spread. Raspberry flowers (and fruit) are spatially well separated, unlike grapes, and thus air circulation around them may be already high.

Despite the comparable incidences of latent *B. cinerea* on fruit at both sites, infection of leaves and canes at the Cambridgeshire site was much less than at the Kent site. In addition, the incidence at the Kent site was much higher in 2007 than in 2006. These differences are probably due to factors related to the level of inoculum and moisture in the tunnel. Inoculum level was expected to be lower at the Cambridge site because of its intensive management and the lower crop density than at the Kent site. Since no fungicides active against *B. cinerea* were used in 2006, the inoculum level was expected to be higher in 2007 than in 2006. Successful infection of leaves and canes may need a higher inoculum dose than infection of flowers. Infection of leaves and canes may also depend critically on

high humidity. Infection at the Kent site is, therefore, expected to be more severe because of the denser canopy, about twice that of the Cambridgeshire site. For the same reason (high humidity), canopy thinning reduced the incidence of leaf infection in both years and cane infection in 2007 at the Kent site. However, the exact effect of canopy thinning on leaf infection varied with year. Similarly, the effect of canopy manipulation on botrytis bunch rot of grape varied with seasonal weather patterns and with growing systems (English *et al.*, 1993).

At the Kent site, nearly all cane botrytis lesions observed in the manipulated plots resulted from infection of wounds created when lateral leaves were removed in the summer. These botrytis wound lesions became visible several weeks after leaf removal whereas cane lesions in control plots were not observed until the following February. In the control plots, nearly all cane botrytis lesions observed were not around the bud or leaf scar areas, and hence are not likely to be associated with leaf infection. This is contrary to the previous hypothesis, based on field observations, that cane botrytis resulted from invasion of mycelia via petioles of infected leaves (Hockey, 1952; Labruyere and Engels, 1963). The low incidence in the manipulated plots compared with the control plots suggests that direct cane infection is favoured by higher ambient humidity, confirming previous field observations that a dense canopy may lead to a high incidence of cane botrytis (Williamson *et al.*, 1979).

Conclusions

Covering raspberry crops during flowering and fruiting to protect them from rain had no significant effect on the incidence of fruit with latent infection by *B. cinerea* at harvest. A high proportion (>50%) of fruit at harvest from covered, fungicide-treated crops may still be infected. Post-harvest fruit management (e.g. rapid cooling) is likely to be important in preventing visible botrytis fruit rot from developing during the standard shelf-life period. Canopy thinning did not significantly affect development of fruit botrytis in either of two crops. However, it did reduce the incidence of leaf and cane infection by *B. cinerea* in a crop where canopy density and disease pressure were high. Contrary to previous reports, the present data suggest that cane botrytis is more likely to have resulted from direct infection of canes by the pathogen rather than from mycelial colonisation via petioles of infected leaves. Such direct infection may only take place under conditions of high moisture and a high level of inoculum in autumn. Further field studies are needed to confirm these conclusions and to develop control strategies based on the new knowledge.

Table 1.1.2. Percentage botrytis fruit rot in raspberry samples taken from tunnel crops of cv. Glen Ample at two sites; parts of crops were subjected to current agronomy practices (control) or to additional canopy thinning (manipulated). Unripe fruit samples were incubated on paraquat agar; red fruit were incubated at ambient temperature for seven days before assessment

Treatment	Kent											
	Unripe fruit			Ripe fruit			Unripe fruit			Ripe fruit		
	13/06/06	04/07/06	28/06/06	14/07/06	04/08/06		15/06/07	27/06/07	09/07/07	15/06/07	27/06/07	09/07/07
Manipulated	83	58	53	13	65		100	89	100	73	79	93
Control	59	69	51	19	68		89	87	100	90	84	83

	Cambridge											
	Unripe fruit			Ripe fruit			Unripe fruit			Ripe fruit		
	26/06/06	11/07/06	21/07/06	27/06/06	11/07/06	24/07/06	25/06/07	09/07/07	23/07/07	25/06/07	09/07/07	23/07/07
Manipulated	93	68	58	89	81	27	98	89	65	95	71	85
Control	91	68	66	93	85	36	98	83	67	97	67	83

1.3. Control agents

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

Materials and methods

Raspberry canes with *B. cinerea* lesions and sclerotia were collected from a raspberry plantation in summer 2007 and stored dry at 4C until needed. The canes were cut into 10cm lengths and soak in water for a minimum of 15mins and then dried on filter paper. The wetted canes were then divide into lots of five (representing one plot).The canes were then treated with the following chemicals (Table 1.3.1) - fenhexamid (Teldor), iprodione (Rovral), tebuconazole (Folicur), pyraclostrobin + boscalid (Signum), cyprodonil + fludioxonil (Switch), urea and potassium bicarbonate. The chemicals were applied by putting cane pieces into a container of chemical and agitating to ensure that all the cane was covered with chemical. The chemical was allowed to drain off and cane pieces were placed in plastic sandwich boxes. The canes were damp incubated in the light to encourage the sclerotia to sporulate. The numbers of sclerotia sporulating were recorded after one week, two weeks and four weeks. Each treatment was replicated four times in a randomised block design and compared to an untreated control. Analysis of variance was applied to the angular transformed percentage data.

Table 1.3.1 Treatments applied to botrytis sclerotia on raspberry cane pieces

Treatment	Product	Active ingredient	Rate / litre
1	untreated	water	-
2	Signum	pyraclostrobin + boscalid	1.8g
3	Teldor	fenhexamid	1.5g
4	Rovral WG	iprodione (750g/kg)	1g
5	Folicur	tebuconazole	0.8ml
6	Urea + wetter*	urea	50g
7	potassium bicarbonate + wetter*	potassium bicarbonate	20g
8	HDC F5	experimental	0.83ml
9	Switch	cyprodonil + fludioxonil	1g

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

Results

Numbers of sclerotia present on the cane pieces were variable and ranged from six to 60, but as five or six cane pieces were included per plot total numbers of sclerotia per plot were similar. On average over 50% of sclerotia were sporulating on untreated sclerotia at the first two assessments (Table 1.3.2). By the final assessment sporulation on the sclerotia was declining naturally and secondary fungi were beginning to develop on some sclerotia in some treatments. Only urea (Treatment 6) consistently reduced sporulation on the sclerotia.

Numbers of sporulating sclerotia were significantly less in urea-treated plots compared to untreated plots at all assessment times. None of the other treatments had any significant effect on sporulation apart from Teldor (Treatment 3), which by the final assessment date had significantly fewer sporulating sclerotia compared to the untreated. No reduction in sporulation was noted at the first two assessments.

Conclusion

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

Table 1.3.2. Mean % (angular transformed) of *B. cinerea* sclerotia on raspberry cane pieces sporulating after dipping in various chemical treatments assessed seven, 14 and 28 days after treatment. Figures in brackets are back transformed means

Treatment	Number of days after treatment		
	7	**14**	**28**
1.Untreated	45.3 (50.6)	45.3 (50.4)	38.0 (38.0)
2. Signum	35.1 (33.1)	35.5 (33.7)	24.6 (17.3)
3. Teldor	38.8 (39.3)	35.9 (34.3)	14.0 (5.9)
4. Rovral WG	47.8 (54.8)	46.9 (53.3)	38.1 (38.2)
5. Folicur	44.3 (48.8)	45.4 (50.7)	39.0 (39.7)
6. Urea + wetter	11.4 (3.9)	22.1 (14.1)	17.3 (8.8)
7. Potassium bicarbonate + wetter	36.1 (34.7)	36.6 (35.6)	29.3 (23.9)
8. HDC F5	39.4 (40.3)	44.6 (49.4)	37.7 (37.4)
9. Switch	45.5 (50.8)	43.5 (47.4)	37.2 (36.5)
F Probability	<0.001	0.017	0.012
SED (24 df)	6.18	6.49	7.71

Task 1.3.2 – Field evaluation of suppression treatments. (year 2; EMR)

The rate of urea used in the experiment (section 1.3.1) was high (50 kg/ha). In apples this rate is used post-harvest and just prior to leaf fall to encourage leaf rotting. Leaves may be scorched. There may be potential phytotoxic effects of urea on raspberry canes and buds. There is therefore a need to conduct trials with urea on dormant raspberry canes. A trial was conducted in February 2009 to evaluate the treatment for possible phytotoxic effects.

Methods and materials

An open field of raspberry cv Glen Ample located at EMR was used for this trial. The three treatments were applied to whole rows of raspberry using a tractor trailed air blast sprayer at 1000 L/ha so that canes were thoroughly wetted. Sprays were applied to full cane height before bud burst. The three treatments were (1) untreated, (2) urea at 50 kg/ha, and (3) urea (50 kg/ha) + wetter (1 ml/L). A plot was of the whole row length with guard row between plots. Each treatment was replicated twice. Canes were examined two and four weeks after treatment for signs of scorch damage and any other signs of phytotoxicity eg chlorotic leaves, fewer flowers etc.

Results

The trial was assessed on several occasions and finally on June 4 2009. There were no obvious signs of phytotoxicity in the plots sprayed with urea. There were also no obvious differences in bud development.

Task 1.3.3 and Task 4.3.1 – Glasshouse and field evaluation of natural products and commodity substances for control of botrytis and powdery mildew. (Years 1-3; ADAS, EMR); Task 1.3.4 and Task 4.3.3 – Field evaluation of combined fungicide and other product programmes for control of raspberry diseases. (Year 3; ADAS, EMR).

Because powdery mildew failed to establish in the crop, despite repeated efforts of artificial inoculation, this section only deals with product trials against *B. cinerea*.

Introduction

Botrytis fruit rot (*Botrytis cinerea*) is the most common and one of the most important diseases of raspberry. The fungus rots fruit in the field but mainly causes rapid and devastating losses of picked fruit after harvest. Fruit infection usually occurs via the flowers (McNicol *et al.*, 1985) where the fungus can remain latent until the fruit matures, when, under conditions of high humidity, rapid colonisation of the fruit occurs. The fungus also causes lesions on primocanes.

Currently fungicides are relied on for control and are applied close to harvest. Intensive use of fungicides in this way is undesireable and unsustainable. Retail surveillance has shown that >50% of UK produced fruit have fungicide residues, most of which are from fungicides targeted at control of botrytis. Much of the UK raspberry crop produced for supermarkets is now grown under protection, which may provide the opportunity to reduce the reliance on pesticides by developing alternative, non-pesticidal approaches for control of botrytis fruit rot on raspberry.

Materials and Methods

Three field experiments were conducted on two sites (Kent and Cambridgeshire) in 2006 and 2007.

Experiment 1, Kent, 2006

The experiment was established in an open-field plantation of raspberry cv. Glen Ample planted as long canes in 2005 and left unsprayed and cropped in 2005 to establish *B. cinerea* inoculum in the crop. In 2006, the treatments (Table 1.3.3) were applied to plots using a CP15 knapsack sprayer at 1000 L/ha on three occasions (9 June, 16 June and 28 June). All treatments were replicated four times in a randomised block design. Each plot was 8 m long. Blocks were separated from adjacent blocks by an unsprayed guard row of raspberries. Crop development was very variable: at the start of spraying the growth varied from early flower to early green fruit on different plants within the same row. Plants at early flower at the time of the first spray were labelled and picking started when the labelled fruit were red. Prior to this the plots were cleared of all ripe fruit.

Plots were regularly inspected for botrytis. 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 were taken for post-harvest tests. The fruit were placed in individual modules in trays, covered in polythene and damp incubated. Rot incidence was assessed after seven days incubation at ambient temperature (20-25^{o}C).

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 was assessed in March 2007.

Experiment 2, Cambridgeshire, 2006

The experiment was set up in a polytunnel containing three rows of established raspberries cv. Glen Ample. The tunnel was 72 m long and covered with Luminance TSB polythene before the first spray at green bud. All three rows were used in the experiment, but only the inner faces of the outer rows and one face of the central row were treated and sampled. Plots were 5 m long and the central 3 m was assessed for disease and phytotoxicity. The treatments in Table 1.3.4 were applied to plots as fine droplets at 1000 L/ha, with a pressure-assisted knapsack sprayer using a vertically held 2 m boom on three occasions on 30 May (5-15% flowering), 9 June (40-50% flowering) and 19 June (70-75% flowering). An additional spray was applied at green bud (16 May) for one product (Talat). All treatments were replicated four times in a randomised block design. Two untreated control treatments, each replicated four times were also included. Other treatments (other than botrytis fungicides) for pest and diseases control were applied to all plots as normal commercial practice. Fruit not required for samples was picked by the grower (and destroyed) to prevent fruit rotting on the crop.

Plots were regularly assessed for botrytis on the leaves, flowers and fruit of raspberry floricanes. Open flowers were tagged at the time of each fungicide application so that treatment efficacy could be related back to application timing.

Table 1.3.3. Treatments applied to open-field raspberries (Experiment 1) in 2006, East Malling Research, Kent

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

Table 1.3.4. Treatments applied to protected raspberries (Experiment 2) in 2006, Cambridgeshire

Product	Active ingredient	Product rate
1. Untreated	-	-
2. Teldor	fenhexamid	1.5 kg/ha
3. Talat (3 sprays)	fenhexamid + tolylfluanid	3 kg/ha
4. Rovral WP	iprodione	1.5 kg/ha
5. Scala	pyrimethanil	2 L/ha
6. Folicur	tebuconazole	0.8 L/ha
7. Amistar	azoxystrobin	1 L/ha
8. Talat (4 sprays)	fenhexamid + tolylfluanid	3 kg/ha
9. Hortiphyte Plus	potassium phosphite + other nutrients	6 ml/L
10. Calcium nitrate	calcium nitrate	3.5 g/L
11. Orosorb	orange oil + borax + surfactants	2 ml/L

One hundred red fruit per plot were sampled on 29 June, 7 July and 24 July and assessed for visible disease. At each harvest 50 healthy fruit per plot were incubated at ambient temperature (20-25^{o}C) in a multicell tray (one berry per cell) sealed in a polythene bag for 10 days. Rot incidence was assessed after seven days. A sample of yellow fruit was taken from each plot on 5 July and surface sterilised with 5% by volume 'Domestos' bleach, rinsed and then incubated under lights in sealed transparent containers for four to seven days on agar containing paraquat and chloramphenicol (PCA) to check for latent botrytis infection in the fruit.

Experiment 3, Kent, 2007

The trial was established in the open-field plantation of raspberry cv. Glen Ample used in 2006. Each plot consisted of a double row 8 m long separated from adjacent plots by an unsprayed guard row. In 2007, the treatments (Table 1.3.5) 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. Assessments for *B. cinerea* and powdery mildew were conducted as in 2006.

Table 1.3.5. Treatments applied to open-field raspberries (Experiment 3) in 2007, East Malling Research, Kent

Treatment	Active ingredient	Product rate	Spray timing	# of sprays applied
1.Untreated	-	-	-	0
2. Teldor	fenhexamid	1.5kg / ha	Three sprays from flowering at 10 day intervals	3
3. Hortiphyte Plus	potassium phosphite + other nutrients	6 ml / L	Three sprays at 10 day intervals from flowering	3
4. Teldor +Hortiphyte Plus	fenhexamid + potassium phosphite + other nutrients	1.5kg/ha + 6ml/L	Three sprays at 10 day intervals from flowering	3
5. Teldor + Hortiphyte Plus	fenhexamid + potassium phosphite + other nutrients	1.5kg/ha + 6ml/L	Two 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	Five sprays at 10 day intervals from flowering – NB same number of sprays as treatment 5	5

Results

Experiment 1, Kent, 2006

The incidence of *B. cinerea* in fruit at harvest was negligible (<1%). No powdery mildew was observed on any of the plots or guard rows. In post-harvest tests, more than 50% of the fruit from untreated plots at the first pick (Table 1.3.6) developed botrytis. All fungicide treatments (HDC F55, HDC F56, Talat, Signum and Switch) reduced the incidence of *B. cinerea* by at least 75%. None of the other chemicals evaluated, including Talat at half-label recommended dose with Wetcit, reduced the incidence of *B. cinerea*. The incidence of *B. cinerea* in fruit from picks 2 and 3 was too low for meaningful comparisons to be made, most likely because weather conditions at flowering were dry and not favourable for *B. cinerea* infection. In fruit from pick 4, the lowest incidence of *B. cinerea* was recorded in fruit from plots treated with Hortiphyte plus or Talat. However, the incidence of *B. cinerea* varied considerably between plots within treatments and these differences were not significant.

The incidence of *B. cinerea* in green fruit samples (Table 1.3.6) varied from 15 to 37% infected fruit. The incidence between plots varied considerably and there were no consistent differences between treatments. No botrytis lesions were observed on the canes in any of the treatments when assessed in March 2007.

Experiment 2, Cambridgeshire, 2006

No botrytis leaf or flower infection was seen during the experiment and no visible botrytis occurred on the fruit hanging in the crop. No other diseases developed in the crop up to the time of the final harvest. The marketability of the fruit did not differ between treatments.

In post-harvest tests, more than 40% of the fruit from untreated plots at the first pick (Table 1.3.7) developed botrytis. Plots treated with Amistar, Talat at both timings and Scala all had significantly fewer fruit with botrytis than the untreated. The incidence of *B. cinerea* in fruit harvested on 5 July was high, with more than 80% infected fruit in untreated plots. Plots treated with Amistar or Talat at both timings had significantly fewer fruit with botrytis than the untreated but the incidence of botrytis was still high. In the final red fruit sample (24 July) the lowest incidence of botrytis was in fruit sampled from plots treated with Amistar or Folicur. None of the alternative chemicals evaluated (calcium nitrate, Orosorb, Hortiphyte Plus) reduced the incidence of *B. cinerea* at any of the sample dates.

The incidence of latent *B. cinerea* in the yellow fruit samples (Table 1.3.7) varied from 15 to more than 60% infected fruit. The incidence of botrytis was significantly lower in fruit from plots treated with Amistar, Folicur, Scala or Talat (three sprays). Some phytotoxicity was noted, mainly leaf chlorosis, on plants treated with Scala and Folicur.

Table 1.3.6. Percentage botrytis-rotted fruit in post-harvest tests on raspberries harvested from plots treated in 2006 with various chemicals at East Malling Research, Kent (Experiment 1)

Treatment	Pick 1 13 July	Pick 2 19 July	Pick 3 26 July	Pick 4 31 July	Green fruit 26 July
1. Untreated	56.5	3.0	3.6	15.1	30.4
2. Wetcit	66.0	4.8	1.4	22.2	26.9
3. Farmfos	58.8	3.5	6.8	18.1	29.3
4. Hortiphyte	51.8	1.8	2.0	5.4	14.9
5. Farmfos + Wetcit	64.0	5.8	10.2	21.1	37.1
6. Calcium chloride	47.5	1.8	5.5	30.0	31.6
7. HDC F55	12.0	1.8	1.2	11.1	21.9
8. HDC F56	9.0	2.5	1.8	10.9	14.7
9. Talat	11.3	3.3	3.1	7.7	18.8
10. Signum	7.5	3.3	6.0	21.4	28.4
11. Switch	9.3	3.5	9.6	24.2	34.9
12. Talat (50%) +Wetcit	44.5	3.5	8.5	30.1	35.8

Experiment 3, Kent, 2007

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 30-80% (Table 1.3.8). In most of the fruit picks the highest incidence of botrytis was recorded in

the fruit from untreated plots. The programmes based on Hortiphyte Plus alone did not reduce the incidence of botrytis compared to the untreated control. At most of the harvest dates Teldor alone or mixed with Hortiphyte Plus (treatments 2, 4 and 5) was most effective in reducing botrytis rot.

The incidence of *B. cinerea* in green fruit samples (Table 1.3.8) varied from around 60 to more than 90% infected fruit. The lowest incidence of botrytis was recorded in fruit treated with Teldor alone or in mixture with Hortiphyte Plus (treatments 2 and 4).

Table 1.3.7. Percentage botrytis-rotted fruit in post-harvest tests on raspberries harvested from plots treated in 2006 with various chemicals (Experiment 2), Milton, Cambridgeshire

Treatment	Red fruit 29 June	Red fruit 5 July	Red fruit 24 July*	Yellow fruit 5 July
1. Untreated	40.6	83.5	36.4	55.3
2. Teldor	31.4	80.0	45.7	37.1
3. Talat (3 sprays)	16.5	38.5	33.5	18.1
4. Rovral WP	33.3	74.7	46.8	34.4
5. Scala	25.4	77.5	32.9	29.0
6. Folicur	28.2	84.5	21.2	24.0
7. Amistar	22.4	67.5	29.0	15.0
8. Talat (4 sprays)	18.8	50.5	34.9	37.0
9. Hortiphyte Plus	35.7	84.0	49.9	46.0
10. Calcium nitrate	45.0	81.0	35.7	56.5
11. Orosorb	45.3	89.0	43.4	64.2

*24 July, predicted means given from GLIM analysis to allow for a plot row effect.

Table 1.3.8. Percentage botrytis-rotted fruit in post-harvest tests on raspberries harvested from plots treated in 2007 with various chemicals at East Malling Research, Kent (Experiment 3)

Treatment	Pick 1 3 July	Pick 2 10 July	Pick 3 17 July	Pick 4 23 July	Green fruit 2 July
1. Untreated	55.0	79.5	75.6	81.9	86.0
2. Teldor (3 sprays)	39.5	71.7	36.1	53.6	66.0
3. Hortiphyte Plus (3 sprays)	57.5	75.3	58.9	61.1	90.6
4. Teldor + Hortiphyte Plus (3 sprays)	22.0	42.5	35.6	45.6	62.5
5. Teldor (2 sprays) + Hortiphyte Plus (5 sprays)	26.7	62.8	26.7	47.5	87.0
6. Hortiphyte Plus (5 sprays)	76.9	57.2	69.7	69.2	80.8

Discussion

In all three experiments, even in the open-field crop trial in Kent in 2007 when the weather conditions during flowering were wet and very favourable for botrytis infection, the incidence of botrytis on fruit at harvest was negligible. Samples of green or yellow fruit taken from each of the crops and incubated on PCA to encourage sporulation of latent infections showed that

fruit were infected with botrytis at quite a high incidence, which was reflected in the incidence of botrytis in the fruit samples after post-harvest incubation. In all three experiments the most consistent control was achieved with the fungicides. HDC F55, HDC F56, Amistar, Scala, Talat, Signum and Switch were the most effective fungicides. Of the alternative chemicals only Hortiphyte Plus showed any hint of an effect on botrytis in the 2006 Kent experiment. In the 2007 Kent experiment, programmes based solely on Hortiphyte Plus were ineffective, but where a fungicide (Teldor) was included in the programme, either with each treatment or only early in the programme, botrytis control was significantly better than that in the untreated.

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) and it is possible that this explains the reduction in disease observed here. However, the reduction in botrytis incidence was not present where Hortiphyte Plus was used alone, so it is possible that the reduction in botrytis rot was due entirely to the fungicide. The programme of sprays applied here was started at early flower. It is possible that there may be improved benefits of Hortiphyte Plus if application is started prior to flowering. This possibility was explored in experiments in 2008.

Literature cited

McNicol, R J; Williamson, B and Dolan, A 1985. Infections of red raspberry styles and carpels by *Botrytis cinerea* and its possible role in post-harvest grey mould. Ann. Appl. Biol. 106: 49-53

Ribeiro Junior, P M; Resende, M L V. De; Pereira, R B; Cavalcanti, F R; Amaral, D R and 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**:** 629-636

Task 1.3.5. Evaluation of post-harvest cold storage treatments on development of fruit botrytis

Introduction

The experiments described above indicated that there was a high incidence of latent botrytis infection in raspberry fruit grown under protection. Strategies to improve air circulation and reduce the botrytis risk were unsuccessful (Task 1.2). Similarly alternative chemicals evaluated were largely ineffective. Preliminary studies conducted by ADAS at the site in Cambridgeshire showed that rapid cooling of fruit at harvest to remove the field heat and subsequent cool chain management effectively controlled the development of visible botrytis within seven days of harvest. In 2008 trials were set up to compare the development of *B. cinerea* in various post-harvest cooling regimes compared to fruit kept at ambient temperature.

Materials and methods

Three experiments were carried out to determine the effect of rapid post-harvest cold/cool storage treatment on the incidence of visible botrytis on fruit. Two experiments were conducted in 2007 and 2008 at the same site near Cambridge, UK and the third experiment conducted at a commercial site in 2010 in Kent, UK.

2007 trial

Firm ripe fruits of cv. Glen Ample were picked from three 3 m long plots spaced along the central row of an unsprayed tunnel, 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. Thus, there were three crop types: (1) covered and unsprayed, open and unsprayed, and covered and sprayed. Fruit was taken from all heights, with each sample receiving half its fruit from each face of the row. At each sampling time, 150 marketable fruit were picked from each plot directly into punnets, with 25 fruit in a single layer per punnet. Fruit was picked in the morning of 16 July and punnets of fruit requiring cold storage were in place within two hours of picking.

Two punnets (a total of 50 fruit) from each plot were allocated randomly to each of the three storage regimes: (1) 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), (2) two days in the same cold store at 4.5 °C before removal to a cool shelf at around 12 °C in the packing area, and (3) in ambient conditions. Ventilated punnet lids were fitted to the punnets at the start of storage. The pairs of punnets from the same replicate (i.e. crop type) of each of the three crop sources were placed together in a produce tray, with the three replicates of trays being stacked. For the six punnets allocated to the ambient condition, the fruit were transferred to multicell trays (one fruit per cell), sealed in a transparent plastic bag and incubated in the lab out of direct sunlight, where 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/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.

2008 trial

One half of a well-established tunnel crop of raspberries cv. Glen Ample was treated with Teldor (a.s. fenhexamid) at first open flowers and two weeks later (50% flowering) to control

grey mould. A length of 20 m of a second tunnel was left both uncovered and unsprayed. Each of the three crop types (Table 1.3.9) was divided into three replicate blocks (three rows each) and fruit sampled from each area according to the details in Table 1.3.9. There were five post-harvest storage regimes (Table 1.3.10). Flowers were tagged with coloured wool three times a week just as they were starting to open, and the relevant colour noted when the fruit was picked.

At each pick, 25 marketable ripe fruit were picked into each of two punnets per replicate for each treatment (Area x Storage) and taken to their storage areas within two hours of picking. Punnets for each storage treatment were kept in cardboard trays and moved between conditions at the intervals shown in Table 1.3.10. Lids were put on once on a shelf in either the packhouse area (to mimic transport and shop display storage areas) or in an office (mimicking home storage). A logger was kept in each storage area to monitor temperature and relative humidity. Each logger was kept in an empty punnet, and was moved into a fruit tray with the punnets when they were moved into that storage area. Disease assessments were carried out at intervals by looking through the clear transparent plastic sides of the punnet and over the top of the fruit without handling. The number of fruit by botrytis and other fungi (including fusarium, penicillium and cladosporium) were counted. Fruit was assessed for fungal growth at 2, 4, 7, and 9 days after picking.

Table 1.3.9. Details of cv. Glen Ample crops sampled for fruit cooling treatments, Cambridgeshire - summer 2008

Crop	Fungicide application	Tunnel Covering	Fruit picking times, from flowers open at various intervals from spraying
A.	Unsprayed	Uncovered	At 1st and 2nd spray (Picks 1 and 3)
B.	Unsprayed	Covered	At 1st and 2nd spray and 7 days after each (Picks 1, 2, 3 and 4)
C.	2 sprays of Teldor	Covered	At 1st and 2nd spray and 7 days after each (Picks 1, 2, 3 and 4)

Table 1.3.10. Storage treatment cooling regimes evaluated (mean punnet temperature shown) Cambridgeshire- summer 2008

Treatment	Rapid field heat removal (6°C)	Cold storage (3°C)	Transport + final display (17°C)	Home storage (23°C)
1. Untreated control	0	0	0	9 d
2. `Good practice`	0	2 d	2 d	5 d
3. Rapid field heat removal	1 d	1 d	2 d	5 d
4. No cool chain	1 d	1 d	0	7 d
5. Prolonged cold storage	1 d	3 d	3 d	2 d

2010 trial

Only two storage regimes were used (Table 3). On each of four pick dates (28 July, 4 August, 11 August and 18 August), about 25-40 marketable ripe fruit were picked into each of 10 punnets per regime from an unsprayed cv. Octavia tunnel crop and taken to their storage areas within two hours of picking. Punnets for each storage treatment were kept in plastic trays and moved between conditions and assessed for fruit rots at day 6 and 8.

Table 1.3.11. Cooling treatments applied to fruit from commercial crops of raspberry at Cambridge and Kent, UK in trials to compare the effect of cold storage conditions on development of fruit rot.

	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**
Storage Regime	Rapid heat removal	Cold-storage on farm		Simulated transport and shop display		Simulated storage at home (not refrigerated)		
Cold	1-2ºC	2-3ºC	2-3ºC	16ºC	16ºC	20ºC (assess)	20 ºC	Assess
Normal	1-2ºC	4-5ºC	4-5ºC	16ºC	16ºC	20ºC (assess)	20 ºC	Assess

Statistical analysis

Linear mixed models were applied to the logit-transformed proportion of healthy fruit and fruit with grey mould at each assessment time. In both years, crop types, storage regimes and assessment time were treated as fixed effects, and a power model was used to model the temporal correlation among the four assessment times. In addition, different picks were treated as a random effect in 2008. For the 2010 trial, a simple generalised linear model was applied to the data with different picks treated as replicates.

Results

2007 trial

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. 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 that had been stored at cold/cool conditions before five days at ambient was still virtually free of botrytis. 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.

There were no significant interactions among the three treatment factors but their main effects were all significant ($P < 0.001$). Overall, fruit from the outside had the lowest (65.5%) proportion of healthy fruit than fruit from protected sprayed (84.1%) and unsprayed (82.9%); the opposite is true for proportion of grey fruit: protected sprayed (5.4%), protected unsprayed (5.7%), and outside (13.7%). The cold storage treatment had a significantly greater proportion of healthy fruit (94.5) than the standard storage (85.5%), which in turn was greater than the ambient storage (52.6%). However, the proportion of grey mould was similar for the two non-ambient regimes (ca. 2.4%), which was significantly less than under the ambient regime (20.0%). The proportion of healthy fruit decreased over time, 99.7% (2 day), 92.8% (4 day), 66.7% (7 day), and 50.8% (9 day). There was virtually no fruit with grey mould until the day 9 assessment when about 31.4% fruit developed grey mould.

Apart from *B. cinerea*, penicillium was another important rotting agent and not seen until after the fruit had been harvested for seven days. Storage treatment significantly influenced the incidence of affected fruit ($P < 0.01$), with more fruit showing penicillium after ambient storage throughout. Mucor was also first seen following seven days storage but at a low incidence. Cold storage for four days nearly completely inhibited the appearance of mucor even after a further three days at ambient.

2008 trial

The temperature in the tray stack in the field heat removal area ranged from 4.8°C to 7.5°C (mean 6.5°C), although within the forced air stream the air temperature was 2°C. Relative humidity was always above 80%. Temperature inside the shop cold store ranged from 1.6°C to 3.9°C (mean 3.3°C), whereas the shelf life room varied between 16°C and 18°C with a mean of 17°C (higher than the 12°C planned). Final storage was at a mean room temperature of 22.7°C with a range of 18°C to 28°C.

Overall, the proportion of fruit with grey mould was significantly affected by the storage regime, crop type and assessment time, as well as by the interactions of assessment time with cold storage regime and crop type (Fig. 1.3.1). The effect of crop type shows up relatively late, whereas the effect of storage treatment can show after two days. The treatment differences were consistent among the four picks. Levels of other fungi rot varied with treatments but generally were at a very low level.

Virtually no botrytis occurred in fruit from all crops on day 4 (< 1%), but infection increased to nearly 8% on day 7 and to nearly 34% on day 9. In general, the greatest botrytis level was

found in ambient stored fruit (16.6%) and least in fruit given prolonged cold storage (3.7%). Nearly all fruit in storage treatments 2 to 5 remained healthy, irrespective of source, after seven days. There were significant interactions between assessment times and cold store regime with the greatest rate of increase in grey mould with time for the ambient regime and the least with the prolonged cold regime. (Fig. 1.3.1). The “prolonged cold storage” treatment 5 (one day of field heat removal, three days in 3^{o}C cold storage, then three days at 17^{o}C) resulted in nearly zero incidence of grey mould at the seven day assessments (i.e. after two days at 23^{o}C), even in uncovered unsprayed and covered unsprayed crops.

A greater level of grey mould was found in the uncovered + unsprayed crop (19.1%) than on the two covered treatments (unsprayed 9.6% and sprayed 7.8%). Similarly, the rate of increase in grey mould with time is greatest for the uncovered crop than for the covered crop (Fig. 1.3.1).

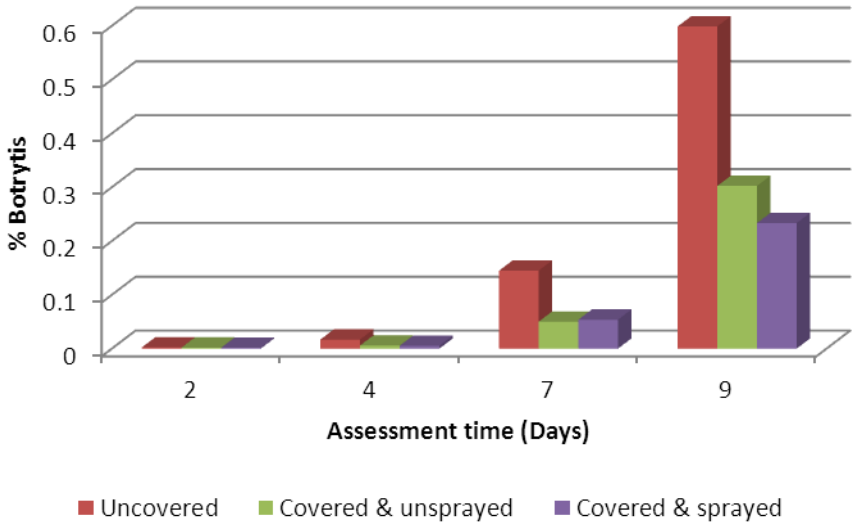


Fig. 1.3.1. Percentage of fruit with grey mould for three fruit sources when assessed 2, 4, 7 and 9 days after harvest.

Similarly, the proportion of healthy fruit, taking into account other fungal rots and damage, was significant by storage regime, crop type and assessment time as well as by the interactions of assessment time with cold storage regime and crop type. However, the trend is opposite to that for the percentage of grey mould (Fig. 2). Percentage of rots reduced from 99.7% on day 2 to 66.5% on day 9, with the greatest reduction found in ambient storage and fruit from the uncovered crop.

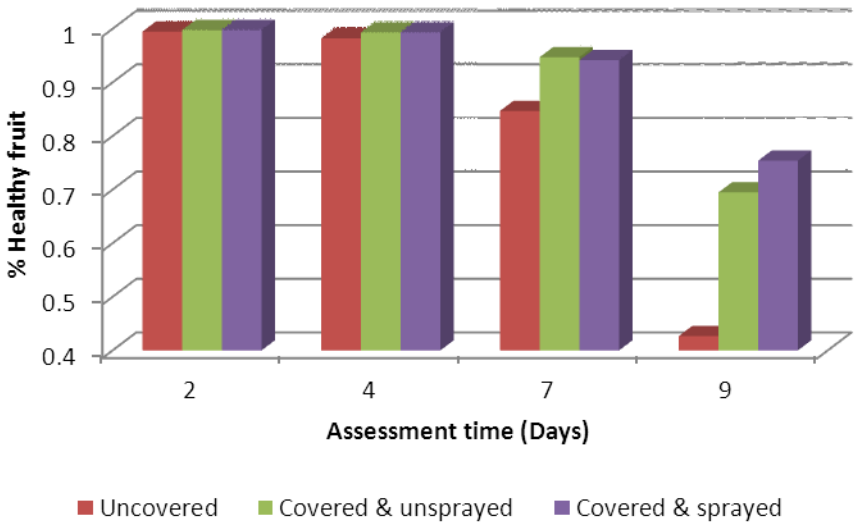


Fig. 1.3.2. Percentage of healthy fruit for three fruit sources when assessed 2, 4, 7 and 9 days after harvest.

2010 trial

Very little grey mould was observed on day 6 (about 1.2%). Thus statistical analysis was applied to data from day 8 assessment. There were no significant different differences between the two cold storage regimes. The average level of grey mould was 12.2% and 14.2% for the cold and standard storage regime respectively; the corresponding value for healthy fruit was 65.3% and 66.4%. There was a considerable amount of fruit rot due to cladosporium (12.5%) and mucor or rhizopus (6.2%), but this was not significantly affected by storage treatment.

Conclusions

Latent *B. cinerea* occurred in fruit from all crops at significant levels, with the incidence varying greatly between picks. The outdoor untreated crop had the highest incidence; spraying of protected crops only led to a very small reduction in the incidence of latent infection. Most importantly, there was virtually no fruit with visual grey mould at harvest; fungal rot (including that caused by *B. cinerea*) usually only appears after being stored for eight days.

Compared with ambient storage, initial cool storage of the fruit significantly delayed the onset of fungal rotting. Furthermore, these results suggested that the rapid cooling (within one hour of the pick) is critically important to delay the onset of fruiting rotting.

Objective 2. Raspberry beetle

To devise semiochemical-based monitoring and trapping systems for managing raspberry beetle by optimising the raspberry beetle floral attractants and exploiting them for control in and around areas of protected cropping.

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

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

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

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

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

2.2. Optimise lure for control

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

2.3. Choose appropriate control approach and develop suitable device

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

2.4. Deployment strategy for control device

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

2.5. Generate further efficacy data for registration - The development and on-farm testing of an optimised trap and attractant lure system for raspberry beetle (2006-2011).

Introduction

The larvae of the raspberry beetle, *B. tomentosus* (De Geer) in Europe, and the raspberry fruitworm, *Byturus unicolor* Say, in North America, generally cause more damage on raspberries than the adults. The tolerance level for fruit damage and larval contamination in the UK is effectively zero. The adult beetles are also capable of causing injury by feeding damage to unopened buds, developing leaves and open flowers. In North America, raspberry beetle prefers red and purple raspberries but in Europe they also infest blackberries (both wild and cultivated) and hybrid berries, such as tayberry and loganberry.

Life History and Description

Adult beetles emerge from the soil in late April and early May. In Europe, foliar damage is usually confined to young primocane leaves at the base of the plant. In North America they begin feeding along the midrib of partially folded leaves and are usually found on the midrib of young leaves. Later in the season the adults seek protection between the flower buds. They attack these buds and make large entrance holes to feed on the floral parts. In some parts of Europe adult beetles may migrate to adjacent rosaceous flowers such as apple, pear or hawthorn to feed before returning to red raspberry varieties for feeding, mating and ovipositing when these flowers open.

In the case of *B. tomentosus*, shiny creamy white eggs (1.5 mm long) are laid, attached to floral parts. Normally there is one egg laid per flower, unless most flowers in the area have one egg already attached. The young larvae initially feed on basal drupelets until the torus softens and then they begin to tunnel, feeding mainly on the plug. When infested fruit is picked the larvae often remain attached to the cuplike interior of the fruit and thus become a contaminant in harvested berries, causing crop rejection. The females of *B. unicolor* deposit greyish white eggs most commonly on swollen, unopened flower buds. However, at times they may be laid inside buds or on developing fruit. The greyish white eggs (approximately 1 mm long) hatch after a few days, and the larvae commonly bore through the bud and enter the receptacle torus, where they begin to tunnel. As the larvae increase in size, the tunnel is made larger and ultimately becomes a groove in the receptacle adjacent to the berry. The larvae remaining on the receptacle soon drop to the ground, where they pupate and remain in pupation cells in the soil over winter. The fully grown larvae are slender, 5.75 to 6 mm long and 0.53 mm wide. They are nearly cylindrical, tapering towards either end. Each body segment has sparse light-coloured, stiff hairs arranged in two transverse rows along the length of the body. The adult beetle of both species ranges in length from 3.7 to 4.5 mm. Beetles are oblong to oval, convex above, changing colour from dull yellow to pale brown to reddish brown as they mature and covered with tiny hairs. Antennae are 11-segmented and terminate in a three-segmented club.

Damage

Early season infestations are suspected if longitudinal holes in the foliage leave a tattered appearance. Such foliar injury is caused by adults feeding on unfolding leaves. If sufficient feeding occurs leaves may become skeletonised. As flower buds appear they are also attacked by the adult beetles, which feed on the insides; numerous beetles may destroy the entire flower cluster. Larvae attack raspberry receptacles, developing drupelets and, at times, the carpels of the berry. Later the larvae feed on the plug and on adjacent surfaces of

ripening drupelets. In tunnelling through the receptacles the larvae cause extensive damage, often loosening berries so that they fall off prior to harvest. Contamination of harvested fruit by larvae can be a serious problem, with very low or zero tolerance of fruit contamination by retailers.

Control options

When raspberry beetles are a problem in commercial plantings on conventionally managed (non organic) farms they are currently controlled by applications of an insecticidal spray, which in the UK is generally thiacloprid (Calypso). Pre-bloom sprays are applied with care to avoid harming bees as flower buds appear and again before flowers open. A single application at the late green fruit stage often gives adequate control but takes about 10 days before picked fruit are free of contaminating larvae. In some years in England and Scotland, autumn-fruiting cultivars growing under plastic tunnels may be heavily infested. Cultivation of the soil in late summer or early autumn to expose the pupae to predators and the elements helps reduce populations.

There is a general consensus across Europe to reduce pesticide usage and find environmentally benign solutions as part of IPM, which is required by all EU member states by 2014 (91/414 EEC). Pest trapping has been suggested as a control method which could reduce pesticide use and eliminate residues on fresh soft fruit grown using organic, conventional and emerging IPM production systems. Such a trap has been developed at SCRI, using 'biomimicry' to design a trap based on the key visual and olfactory features of the raspberry flower. The prototype trap and lure involved a less effective and non user friendly white, vertical sticky trap with a simple wick and vial attractant dispenser system. This sticky trap design was used at a recommended density of 1,700/ha by organic growers in Switzerland (Schmidt et al., 2006) and required changing at least once or twice a week, due to heavy non-target insect and dust contamination. The development and testing of an improved trap and lure system was performed in collaboration with commercial partners at Agrisense/Suterra Ltd (horticultural end-users) and Agralan Ltd (gardening end-users) together with testing partners at EMR, NRI and ADAS.

Development and prototype testing of trap and lure system on-station

Two very active volatile attractants from raspberry flowers were previously identified at SCRI for raspberry beetle using combined EAG and GC-MS. For commercial confidentiality the two most active compounds were coded A and B. These had already been shown to be active in small scale bioassays using a linear track olfactometer and wind tunnel (Birch *et al*., 2004; 2007). Initial field trials were done in open-field sites at SCRI especially set up to provide

large numbers of raspberry beetles. Three field trials, one in 2005 and two in 2006, tested different trap designs and different types of lures. Previous research used non-UV reflectant white sticky traps but these often become contaminated by dust or by non-target insects, especially small flies. Sticky traps are difficult to handle and needed changing one-two times per week to avoid saturation, thus quickly losing efficacy and being too labour intensive for most growers. The improved prototype non-sticky impact traps for the Hortlink project developed were based on standard bucket (funnel) traps (as used for forestry pests) but adapted for raspberry beetle by SCRI using white, non-sticky and non UV-reflective cross-vanes to visually attract adult raspberry beetles.

In 2005 two designs of the funnel traps were compared with the sticky trap. When used with a standard lure (thick-walled plastic 10 ml vials with compound B), both funnel traps were equally effective, and at least as twice as effective as the sticky trap, in capturing adult raspberry beetles. The funnel traps caught many fewer flies than the sticky traps and all traps caught very few pollinators (bees). The cross-vaned funnel trap was therefore selected as the standard design for monitoring raspberry beetles in on-station polytunnels in 2007.

Two types of attractant dispenser were tested in the laboratory in 2005. A thin-walled dispenser, thought to increase the evaporation of the attractant, was compared with the standard thick-walled dispenser. Although the evaporation rates were improved using the thin-walled vials, the design and reliability of the dispensers was problematic and further development ceased. In 2006, a range of prototype sachet dispensers were manufactured by Agrisense/Suterra using the attractant chemicals, compounds A and B. Their efficiency was assessed in both the laboratory and in the field. Two field trials showed that single-sided sachets did not differ significantly compared with the standard thick-walled vial dispenser in two-week exposures trials. However, in longer laboratory evaporation studies, the sachets evaporated at a higher rate than the standard dispenser and had insufficient attractant to last for the five to six weeks that may be required in field condition. A new type of sachet with a more controlled vapour release was sourced and subsequently tested in the laboratory and field in 2007.

Most research concentrated on the use of compound B, which is more attractive than compound A in single compound tests under laboratory and field plot tests. To test the effect of a mixture of compounds A and B on trapping raspberry beetle under more realistic conditions, a single field trial was set up to compare compound A and compound B on their own versus a mixture of A and B. All three sets of attractants caught raspberry beetles, but compound B was the most effective and compound A the least effective. The A+B mixture

was approximately midway between the single compounds in the Scottish trials. This suggested that, at least with the standard thick-walled plastic dispensers, there is no advantage in using a mixture of compounds A and B, particularly in Scotland where pest populations are not extremely high. Populations are typically high in organic raspberry plantations in Norway when surrounded by woodlands containing alternative host plants. Under high raspberry beetle population pressure subsequent Norwegian trials using improved sachet dispensers showed some advantage using attractants A+B combined to increase catches over extended seasons and high pest populations (Trandem *et al*., 2008)

Development of the deployment strategy on-station and on-farm

Improved slow release attractant system

The new sachet slow release system developed by Suterra Ltd with SCRI, and tested on-farm in 2008-9, was more effective in the field than the previous vial system for attracting raspberry beetles and lasted four-six weeks, depending on ambient temperatures which affect evaporation rates. Compound B was found to be more attractive than compound A under field conditions in Scotland, confirming previous SCRI studies. Further studies compared different surface areas of white cross vanes to determine the effectiveness of the visual component of the modified bucket trap. The surface area of the white cross vanes was positively associated with increased raspberry beetle catches under field conditions at SCRI.

Spatial deployment

Larger-scale on-farm experiments were also initiated in 2008. In Kent, lattice deployment was more effective at one site than perimeter trapping, whilst at the second site they were equally effective. In E. 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. This variability between sites became a regular feature of on-farm trials, where local vegetation, local climate, age of crop and surrounding plantations and crop management all appeared to influence trap catches. Similar patterns were also seen in Norway, Switzerland and France, highlighting that growers need at least one-two seasons to become familiar with the trapping system at each site and the localised behaviour of the pest. After two-three seasons scientists and participating growers became familiar with sources of the pest outside the crop as well as ‘hot spots’ within the crop which could be managed via precision applications of insecticides rather than prophylactic spraying. Grower confidence in weekly trap catches and action thresholds was also built up during this one-two year training period on farms, again facilitating reduced insecticide applications in fields with low and reducing pest populations.

Effect of host flower phenology on trap efficacy

Beetle catches in on-farm traps during 2007 were higher before crop flowering (up to green fruit stage) in E. Scotland but this effect was less obvious in Kent. Interestingly, in both Norway and Switzerland, where flowering and fruiting seasons are extended due to local climate, raspberry beetle was still caught in moderate numbers during flowering and fruiting, thus reducing future generations of the pest. Numbers of raspberry beetle eggs found on flowers and fruit damage were very low at all sites (Kent and E. 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. Local climate (a relatively cool, wet summer) is likely to have affected pest numbers in the 2007 trials in Scotland and England.

Non-target catches

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 were considered to further reduce this risk. In consultation with Agrisense Ltd, a bee-excluding plastic mesh was subsequently fitted to the funnel entrance all traps, which prevented catches of non-target pollinators (mainly honey and bumble bees) in future on-farm trials.

Continued on-farm IPM assessments (2008-10)

The lure and trap system was tested at two sites in Scotland, but not England since suitable raspberry beetle sites (sufficient pest pressure) were not available. In Scotland a combination of insecticide use (Calypso) and previous trapping resulted in very few beetles being caught and no fruit damage, even in control areas where insecticides had not been sprayed. Some bees were caught in the traps but this was largely due to very high local populations. The numbers caught had no impact on bee populations or pollination. However, final modifications (2009) included a coarse mesh to prevent bees falling into the bucket trap. Generally the grid system (50 traps/ha placed within tunnels) was more effective than perimeter trapping, especially if raspberry beetle populations are low to moderate (e.g. newly established plantations or mature plantations where insecticides have been routinely applied and sources of re-infestation are restricted in the area).

In parallel studies using the raspberry beetle traps and lures in Norway (mainly organic), Switzerland (organic and conventional) and France (mainly conventional) the trap and lure system produced good results in terms of season-long monitoring, detection of migrations, detection of ‘hot spots’ for localised treatments and wild host sources and population reduction over two-three years on trapping. Where pest populations were very high (organic

sites surrounded by wild hosts) some fruit damage still occurred, but the addition of extra traps (grid + perimeter + near wild host reservoirs) and the use of two attractants (A+B) are proving to be beneficial in such conditions. Interestingly, in Switzerland and Norway the improved bucket traps continued to catch raspberry beetles well into the flowering period (not seen with sticky traps in the same experiments) and thus helped to reduce subsequent pest populations in following seasons.

Trials continued with the finalised trap and lure system (easy snap fit and bee proof) with the aim to commercialise the monitoring system in 2009, using an action threshold of five raspberry beetles/trap week (as previously developed in EU CRAFT 'RACER' project). This threshold is likely to be conservative, since it was developed using sticky traps without the improved lures currently used. In 2009 the lure and improved trap (easy assembly with snap fittings) system was tested at two sites in Scotland, but not England, since suitable raspberry beetle sites (sufficient pest pressure) were not available. In Scotland a combination of insecticide use (Calypso) and previous trapping over the previous season resulted in very few beetles being caught in either the IPM or the grower's standard practice tunnels, and no fruit damage, even in control areas where insecticides had not been sprayed. Some bees were caught in the traps but this was largely due to very high local populations. Generally the grid system (50 traps/ha placed within tunnels) was more effective than perimeter trapping, especially if raspberry beetle populations are low to moderate.

In parallel studies using the traps and lures in Norway (mainly small organic farms), Switzerland (organic and conventional, higher altitude plantations) and France (mainly conventional) the trap and lure system produced good results. Where pest populations were very high (e.g. organic sites in Norway surrounded by wild hosts) some fruit damage still occurred, but the addition of extra traps (grid + perimeter + near wild host reservoirs) and the use of two attractants (A+B) proved to be to be beneficial in such conditions. Interestingly, in Switzerland and Norway the improved bucket traps again continued to catch raspberry beetles well into the flowering period (not seen with sticky traps in the same experiments) and thus helped to reduce subsequent pest populations in following seasons. Agrisense commercialise the optimised raspberry monitoring system in mid 2009, using an action threshold of five raspberry beetles/trap week (as previously developed in EU CRAFT 'RACER' project). This threshold is likely to be conservative, since it was developed using sticky traps without the improved lures currently used. Agralan Ltd also produced a smaller scale version of the trap with lure for gardeners, which was marketed from 2010. Positive feedback has been received from both companies and from end users. SCRI were involved in writing of the technical information for the monitoring system during 2009-10 and also with

ADAS to update technical advisory sheets for UK growers, based on Hortlink results and commercialised IPM products.

In 2009 UK trials, raspberry beetle traps successfully detected the pest in tunnels and to a varying extent in surrounding vegetation (mainly neighbouring hedgerows of hawthorn and bramble, alternative feeding hosts for this pest). Very high numbers were found in the surrounding vegetation at the Kent site. Hot spots were not always at tunnel entrances, indicating resident pest populations within tunnels. A threshold of 5 adults/trap/week was used for spraying insecticides, except at the Kent site, where no insecticides were applied in the IPDM tunnels. Traps inside tunnels greatly reduced pest numbers during the first three-four weeks of trapping and continued to catch raspberry beetles after flowering and during fruit harvest. The IPM treatment in Scotland, where Calypso insecticide was used after the 5/trap/week threshold was exceeded, was as successful as the conventional treatment (prophylactic spraying), with 0% raspberry beetle damage being recorded in both treatments. The IPM treatment in Kent, where Calypso insecticide was not used, gave slightly inferior control (0.26% berries damaged) compared with the conventional treatment (0.007% berries damaged). Raspberry beetle damage was zero in both the IPDM and conventional plots at the Cambridgeshire site, where only small numbers of raspberry beetles were captured in the traps during the flowering and fruiting season.

In 2010 similar trends to 2009 were observed in both Scotland and England. Catches of the pest within the crop were generally lower than the previous year in the trial sites, indicating that use of the traps and lures for monitoring over multiple seasons (two-three in this case) can help to reduce localised pest pressure. This could be via a combination reducing the resident populations inside tunnels, reducing populations outside tunnels (although these were typically low in most UK sites) and by treating detected 'hot spots' within tunnels, open field plantations and hedgerows. The site-specific variation in the distribution of raspberry beetle outside the cropped area (e.g. hedgerows, host patches in woodlands, old plantations, etc) was again a feature. As with most IPDM tools and approaches growers need to build up local knowledge of pest and disease distributions, patterns of movement and pest reservoirs to design and implement a successful control strategy.

R+D Highlights: 2005-10

Spatio-temporal monitoring of raspberry fields and surrounding habitats: Lattice, perimeter and surrounding habitat

In general, the within-crop lattice was more successful than the perimeter trapping, in terms of detecting the distribution of the pest and also allowing 'hot spots' (see below) to be

detected and treated, thus reducing insecticide usage. In situations with high raspberry beetle populations and multiple pest reservoirs in the local landscape (e.g. organic raspberry growers in Norway) the combination of lattice and perimeter trapping, together with additional traps near wild hosts and disused plantations is also recommended.

Optimal trap height and lure efficacy

In the UK the optimal height (pre-Hortlink trials on-station) was found to be between 1.0 and 1.6m above the ground, hanging from the top wire in a gap within the crop. Linked PhD studies in Norway confirmed this information, but interestingly found that the trap and lure was optimised close to the ground for another pest species, *Anthonomus rubi* (Blagogie, 2010). These studies used mark-release methods to confirm that the trap with the lure attracted x7 more raspberry beetle than the trap without the lure. In the UK the lure enhancement was typically x10-x50. In most cases lure B on its own sufficed (e.g. UK plantations where the raspberry beetle population is low to moderate). In Norway where raspberry beetle populations were much higher (traps typically catching 30-50 beetles/trap/week over several weeks), the addition of attractant A improved trap catches (i.e. A+B > B+B). This suggests that for organic growers facing very high raspberry beetle populations, a dual attractant (A+B) approach could be beneficial in a lure and kill system, if approved.

'Hot spot' detection within the crop

In the Scottish on-farm trials and in some English trials, 'hot spots' were detected within tunnels, typically towards the centre of the tunnel rather than near tunnel doors. This indicates that at least in the majority of tunnel grown raspberries where there is little immigration of raspberry beetle from outside, the pest prefers to feed towards the middle of the poly-tunnel rather than near the tunnel entrances. Further microclimate studies are needed to investigate this aspect in more detail (ongoing agro-ecology research at SCRI).

Monitoring efficacy of grower's standard pest control methods (Calypso) versus IPDM

Raspberry growers in the UK are typically risk averse. We set the action threshold at 5/trap/week based on previous EU studies but in most cases growers sprayed Calypso when several traps caught even one raspberry beetle, especially in the first year of the trials when grower confidence in the IPDM system was lowest. Where the suggested threshold was followed, fruit damage in the IPDM treatments was as good or nearly as good as the grower's standard regime (prophylactic spraying). In Norway on organic farms, fruit damage is much higher than in the UK (often >40%) and they rely on high prices and hand sorting to obtain clean fruit. Given this very high pest pressure, several Norwegian growers found at least a 50% reduction in fruit damage after two-three years. Some achieved much better

control by adding in extra traps in surrounding vegetation and by installing nets to intercept raspberry beetle flights previously determined using the raspberry beetle trap and lure system.

Potential for future monitoring and 'lure and kill' approach for growers and gardeners

Our main focus in the UK during this project was to develop a precision monitoring tool, to allow growers to decide if they need to take action and if so, if they want to spot treat localised 'hot spots'. This strategy evolved during the project and appeared to be the preferred option for participating growers. In the UK some growers also used extra traps to check the efficacy of their own insecticide treatments, since the damage threshold is effectively zero for this pest. In contrast, Norwegian organic growers were satisfied to learn more about raspberry beetle movement and to reduce local populations using a longer term strategy of pest reduction, year on year. They were also prepared to invest in additional traps outside the plantation and to install net fences and door covers to reduce immigration into their tunnels, presumably because the product value was high and many smaller organic growers sold directly from their farms to the public. In the UK the relatively high cost of registration for lure and kill and niche market size means that it is only currently economic for the companies to market the trap and lure for monitoring. However, the users indirectly benefit from reducing local pest populations by trapping (the beetle drowns within the trap when caught) and from spot treatments using approved insecticides, which reduces their risk and pesticide costs.

Implications for monitoring raspberry beetle: On-farm technology transfer and grower experiences

Although not yet officially surveyed, through communicating directly with participating growers in the on-farm trials, they seem keen to use the trap and lure. ADAS, in consultation with SCRI and EMR, are revising advisory leaflets, bearing in mind the costs and benefits of various options. The simplest and cheapest option for growers currently is to use c. 5-10 traps/ha for monitoring. This gets them familiar with the monitoring process without major expenditure. Growers can move the traps and lures around their farms to find out more about the effects of local habits, climates and fruiting times. If growers detect moderate to large numbers of the pest they may then be prepared to invest in more traps (lattice layout) to detect 'hot spots' for localised insecticide treatments and to monitor efficacy of wider insecticide applications. Organic growers who can achieve high returns (e.g. Norway, Switzerland) appear to be prepared to invest in a higher density of traps/ha and to reduce the pest population over multiple and extended growing seasons, in conjunction with other complementary IPDFM tools (e.g. flight interference mesh fences, mesh doors on tunnels, height adjustable trap holders, etc).

Field-landscape scale studies in UK, Norway, Switzerland and France: Working Group IOBC Integrated Plant protection in Fruit Crops – subgroup Soft Fruit

In the UK and France, the main raspberry beetle catch is generally four-six weeks before raspberry flowering, with trap efficacy dropping (but with continued catches) throughout the flowering and fruiting period. In contrast, in Switzerland (Baroffio et *al*., 2010) using open field plantations at higher altitudes (100-1300 m) a second flight activity peak was detected in late July-early August, which continued for several weeks. In Norway (Trandem et al., 2008), raspberry beetle catches in traps were very site dependent, probably affected by combinations of latitude, local climate, size of plantations relative to local habitat acting as reservoirs, crop protection history, crop management). Despite these regional and country specific variations in raspberry beetle ecology and behaviour, at each site patterns emerge after two-three years of monitoring, particularly when 50 traps/ha minimum were deployed. From these agro-ecology studies, useful information was recorded on number of raspberry beetle flight peaks, pre- versus post- flower initiation trap catches, flight directions, flight heights during the season, pest reservoirs outside crop, hot spots of resident pest populations within the crop, flight behaviour relative to flower phenology. Generally, raspberry beetle follows flowering of cultivars within plantations, being most attracted to newly opened buds and least attractive to green and ripe fruit.

References and relevant publications

AgriSense Ltd: Raspberry beetle monitoring kit: htt://www.agrisense.co.uk/Product_ag.php?id=177

Baroffio, C., Mittaz, C. 2010. Raspberry beetle: flight monitoring with semiochemical traps. IOBC/wprs Bulletin 54, pp. 397-400.

Blagogie, G.K.J. 2010. Mass trapping of the raspberry beetle and the strawberry blossom weevil. MSc Thesis. Norwegian Universities of Life Sciences, pp1-62.

Birch, A.N.E., Begg, G.S., Brennan, R.M., Fenton, B., Gordon, S.C., Griffiths, B.S., Griffiths, D.W., Hillier, J., Malloch, G., Squire, G.R. and Wheatley, R.E. 2002. Developing sustainable pest management strategies for a changing future. *Annual Report of the Scottish Crop Research Institute for 2001/2002*, pp.184-189.

Birch, A.N.E., Gordon, S.C., Shepherd, T., Griffiths, D.W., Robertson, G. and Brennan, R.M. 2008. 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,* East Malling Research, 24-27 September 2007. IOBC/wprs Bulletin 39, pp. 1-3.

Birch, A.N.E., Gordon, S.C., Fenton, B., Malloch, G., Mitchell, C., Jones, A.T., Griffiths, D.W., Brennan, R.M., Graham, J. and Woodford, J.A.T. 2004. Developing a sustainable IPM system for high value *Rubus* crops (raspberry, blackberry) for Europe. *Acta Horticulturae* 649, 289-292.

Birch, A.N.E., Gordon, S.C., Shepherd, T., Griffiths, D.W., Robertson, G. and 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,* Lund, Sweden, 9-14 September 2007. IOBC *Pheromones and other Semiochemicals 2009,* 41, pp. 75-78.

Schmid, A., Hoehn, H., Schmid, K., Weibel, F., and Daniel, C. 2006. Effectiveness and side effect of glue-traps to decrease damage caused by *Byturus tomentosus* in raspberry. *J. Pest Sci*. 79, 137-142.

Trandem, N., Gordon, S., Birch, A.N.E., Ekeland, M., Heiberg, N. 2008. Mass trapping of raspberry beetle as a possible control method – pilot trials in Norway. IOBC/wprs Bulletin 39, pp. 5-10.

Objective 3: Semiochemical-based systems of managing cane midge

To devise semiochemical-based systems of managing cane midge, thus controlling 'midge blight', by identifying host plant attractants (targeting females) and exploiting them together with the sex pheromone (targeting males) for pest monitoring and control.

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

Summary

The female sex pheromone of the raspberry cane midge was identified as (*S*)-2-acetoxy-5-undecanone and the synthetic compound was shown to be highly attractive to male midges in the field. The *R*-enantiomer was unattractive but the racemic mixture containing equal amounts of the *R*- and *S*-enantiomers was as attractive as the *S*-enantiomer. This is an important result as the racemic material is much easier and cheaper to synthesise than either of the pure enantiomers. In field trapping trials increasing the loading of pheromone in the lure gave increased catches of midges up to 1 mg, but further increase in loading decreased catches, indicating an optimum loading of 0.1 mg – 1 mg per rubber septum lure. Colour of the trap did not have any effect on catches of midges, but greater numbers of non-target arthropods were caught in white and blue traps. Red traps are recommended for practical use. The height of the trap above the ground had a very significant effect on catches of midges. Traps positioned on the ground caught most midges with catches dropping dramatically at higher positions. In practice it is not feasible to place traps on the ground for long periods and a trap height of 0.5 m is recommended.

Pheromone identification

Raspberry cane midge females produce a powerful sex pheromone to attract males for mating. The pheromone was identified as 2-acetoxy-5-undecanone by NRI and EMR in the 8 month Defra project HH3214SSF immediately before this LINK project. 2-Undecanone, 2-acetoxyundecane and 2-undecanol were present at approximately 30% of the major component.

In years 1 and 2 of this LINK project, (*S*)-2-acetoxy-5-undecanone was shown to be the natural, highly attractive enantiomer, the *R* enantiomer was shown to be unattractive and, crucially, the racemic compound to be just as attractive as the *S* enantiomer. Addition of the three minor components in racemic form at three different overall loadings did not affect catches. The pheromone could be dispensed from both rubber septa and polyethylene vials

for at least one month under field conditions, but the former was preferred as it gave a more uniform release. This work is comprehensively reported by Hall et al. (2009).

Effects of sex pheromone lure loading and release rate on attractiveness

In 2006, an experiment was done to determine the effect of lure loading and release rate of raspberry cane midge pheromone from lures on attractiveness to male raspberry cane midge in adjacent cvs Joan Squire and Autumn Bliss plantations at Beech Farm, West Peckham. Treatments were rubber septa dispensers loaded with increasing amounts of raspberry cane midge pheromone racemate (0, 1 ng, 10 ng, 100 ng, 1 µg, 10 µg, 100 µg, 1000 µg, 10 mg, no lure) and having correspondingly increasing release rates of the pheromone (0, 600 fg, 6 pg, 60 pg, 600 pg, 6 ng, 60 ng, 600 ng, 6 µg, 0 per hour, respectively). [(The release rates were estimated from laboratory windtunnel measurements at NRI at 27°C and 8 km/hr. The mean release rate from two replicate 100 µg rubber septa was 80 ng/hr and 40 ng/hr after 10 days)]. A randomised block experimental design with five replicates was used. Plots were single standard white (20 × 20 cm) delta traps deployed at a height of 0.5 m. The number of midges captured in each trap was counted on 31 August and 8 September 2006, plots being re-randomised and fresh lures deployed for the two assessments, i.e. essentially two repeats of the experiment.

The results (Figure 3.1.1) were surprising. Lures initially loaded with 1 µg of the pheromone racemate and which released 600 pg of pheromone/hr were significantly attractive. Maximum attractancy occurred at 600 ng/hr though 60 ng/hr (initial loading 10 µg) performed nearly as well. Attractancy was significantly reduced at the greater release rate. These results corroborated the results of an experiment in 2005, when the dose response showed a clear maximum and the 100 µg lure load also gave maximal catches. This interesting result has important implications for the development of Mating Disruption and Attract and Kill control approaches.

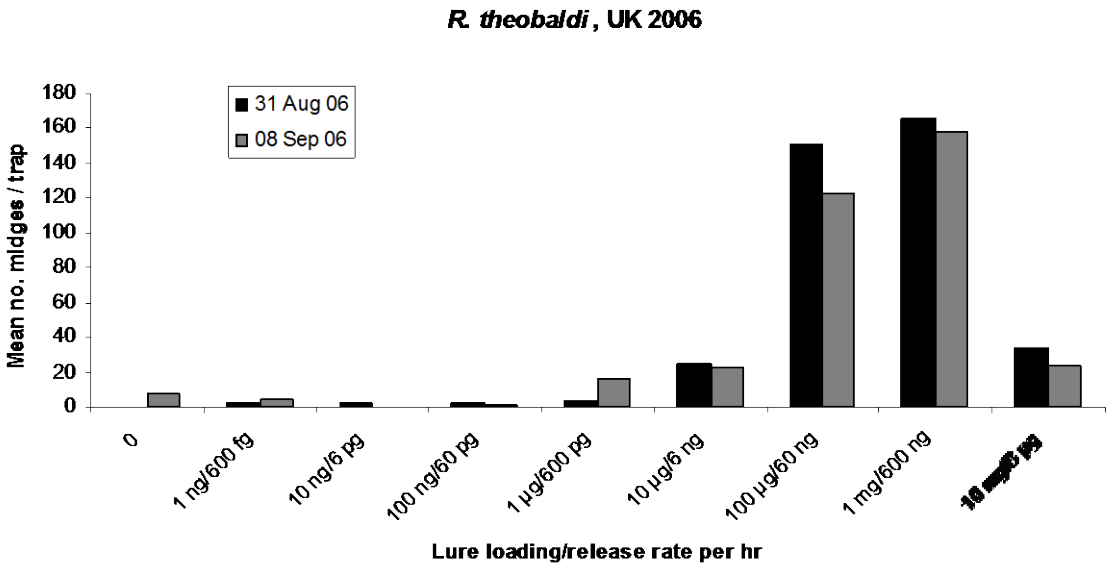


Figure 3.1.1. Effect of lure loading and release rate of sex pheromone racemate from rubber septum lures on catches of male *R. theobaldi* in 2006

Attractiveness of different coloured raspberry cane midge pheromone traps 2006

An experiment was carried out in 2006 to determine whether the raspberry cane midge responds differently to different coloured sex pheromone delta traps. A replicated field experiment was conducted twice with re-randomisation of plots in CW129 raspberry plantation at EMR. The plantation (0.052 ha) contained a large number of different varieties of summer fruiting raspberries in 16 rows spaced 2.45m apart and 140 m long. Treatments were delta traps made from black, white, red, blue, yellow or green Correx. Sticky inserts were not used. The coloured base of the trap was coated with Ecotack.

Traps were deployed at a height of 0.5m and were baited with rubber septum lures loaded with 10µg of pheromone racemate. Traps were deployed for experiment 1 on 3 July 2006 and for experiment 2 on 10 August 2006. A randomised block experimental design was used with three replicates of the six different coloured treatments. Plots were single pheromone traps. The numbers of midges captured in each coloured trap were checked regularly until sufficient midges for statistical analysis had been captured when a count was made of the numbers caught in each trap. This was done on 8 August 2006 for experiment 1 and on 23 August 2006 for experiment 2. Additionally, the number of non-target arthropods, identified to broad taxa (bumble bees, flies, thrips, parasitic hymenoptera, beetles, aphids, lacewings, syrphids, moths, bugs, spiders, grasshoppers), in each trap was counted. Data were statistically analysed by ANOVA after square root transformation to stabilise variances.

The analyses of variance of square root transformed data showed that there were no significant effects of treatment on the numbers of raspberry cane midge captured in either experiment (Tables 3.1.1). However, in both experiments treatment did greatly affect numbers of non-target arthropods captured. In both experiments, the white and blue traps captured significantly greater numbers of non-target arthropods than the other trap colours. The predominant non-target taxa were thrips and flies. These results suggest that blue and white traps should be avoided because they become more heavily contaminated with non-target arthropods. Green or black traps are impractical. Similar work with apple leaf midge gave large catches of non-target arthropods in yellow traps. In conclusion, red traps are probably best for practical purposes.

Table 3.1.1. Mean numbers and mean square root transformed numbers of raspberry cane midge males and non-target arthropods caught in experiment 1

Trap colour	Raspberry cane midge males		Non-target insects	
	n	√n	n	√n ‡
	Experiment 1			
Green	152	10.5	132	11.4 b
Yellow	88	9.3	124	11.1 b
Black	27	4.7	32	5.7 b
White	66	7.6	1004	30.5 a
Blue	48	6.8	893	27.9 a
Red	161	11.1	156	12.4 b
Fprob		0.511		0.003
SED (10 d.f.)		3.59		5.00
LSD (P = 0.05)		8.00		11.14
	Experiment 2			
Green	36	5.54	52	7.19 b
Yellow	36	5.86	88	9.36 b
Black	19	4.33	28	5.16 b
White	31	5.49	352	18.28 a
Blue	33	5.34	298	16.90 a
Red	61	7.76	49	6.93 b
Fprob		0.248		0.001
SED (10 d.f.)		1.261		2.444
LSD (P = 0.05)		2.811		5.445

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

Height of deployment of raspberry cane midge sex pheromone traps

The effect of height of deployment of raspberry cane midge sex pheromone delta traps was investigated in an experiment in CW129 plantation at EMR in 2007. Six different height traps were compared; ground level, 0.5, 1.0, 1.5, 2.0 and 2.5m from the ground. White delta traps

were used, baited with a lure with 10µg of raspberry cane midge pheromone racemate. A randomised block design with four replicates was used. The experiment was set up on 4 June and sticky bases were changed and midges counted on four occasions at weekly intervals. Data was $\log_{10}$ (n+1) transformed and statistically analysed by ANOVA.

Numbers of midges captured in traps placed on the ground were significantly higher than at any other trap height ($P < 0.001$). However, these traps were contaminated with soil and plant 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. 3.1.2). The reason for the higher values at 2.5 m is unclear. Based on this results and results of similar experiments on other midges, a standard height of trap deployment of 0.5 m was chosen.

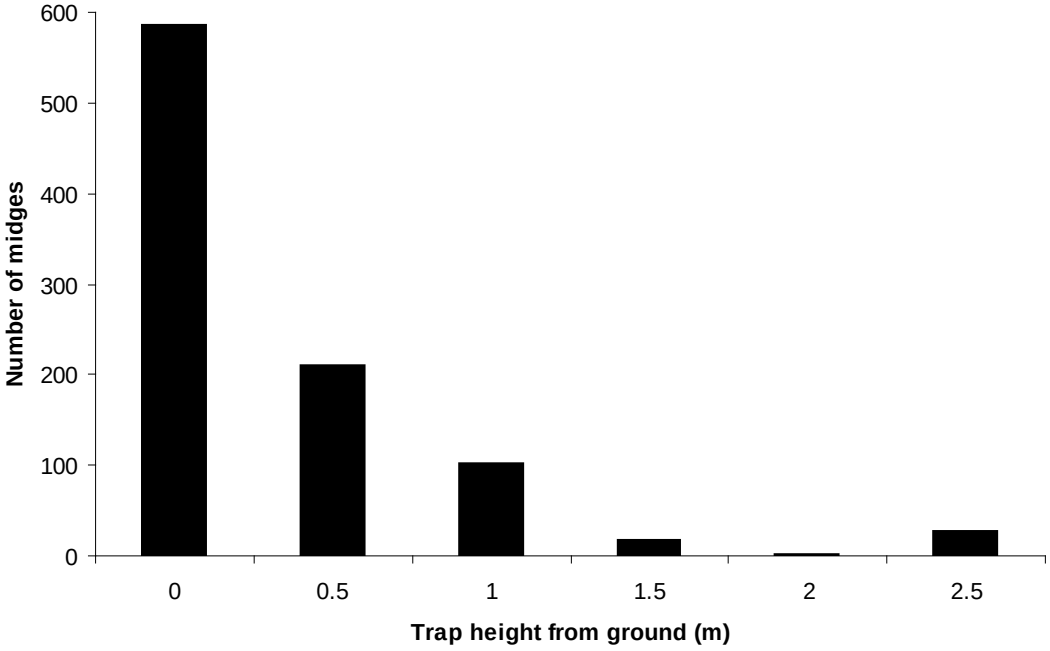


Figure 3.1.2. Number of male raspberry cane midges captured in white delta traps at different heights

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

A ring test was conducted by fruit entomologists in nine EU countries and Russia in 2006 and a strong linear relationship between sex pheromone trap catches of raspberry cane midge and numbers of larvae found subsequently in splits in raspberry canes was established (Cross et al., 2008). The relationship has not been used directly for setting trap thresholds because the relationship between larval infestations and crop damage has not been

established. However, a low 'nominal threshold of 30 midges per trap per week was set for timing of sprays of insecticide.

References

Cross, J., Baroffio, C., Grassi, A., Hall, D., Labanowska, B., Milenkovic, S., Nilsson, T., Shternshis, M., Torneus, C., Trandem, N. And Vetek, G. 2008. Monitoring raspberry cane midge, *Resseliella theobaldi*, with sex pheromone traps: results from 2006, pp. 11-17,*in* Ch. Linder and J.V. Cross (ed.). Integrated Plant Protection in Soft Fruits, IOBC/WPRS Bulletin 39, 11-17.

Hall, D.R., Farman, D.I., Cross, J.V., Pope, T.W., Ando, T. and Yamamoto, M. (2009) (S)-2-Acetoxy-5-undecanone, Female Sex Pheromone of Raspberry Cane Midge, *Resseliella theobaldi* (Barnes). *Journal of Chemical Ecology* 35, 230-242.

3.3. Investigation of attraction of raspberry cane midge, Resseliella theobaldi, to volatiles from wounded raspberry primocanes

Summary

Mated females of raspberry cane midge (*Resseliella theobaldi*) (Cecidomyiidae) are known to be strongly attracted to odours from recently split raspberry primocanes. Fresh splits are preferred over old ones suggesting the attraction is due, at least in part, to volatile chemicals produced. Using solid-phase microextraction (SPME) to sample the volatiles *in situ* it was shown that a characteristic suite of chemicals was produced after splitting, and these were similar for five varieties of raspberry. The components were identified and the 18 most abundant were selected for further study, including six produced by intact stems and 12 produced after splitting. Of these, four elicited EAG responses from the antenna of a female *R. theobaldi* midge, including three from the group produced only after splitting. For field studies, exclusion of the least abundant compounds gave a reduced set of 13 compounds and it was shown that dispensing four of these from a polyethylene vial and the other nine from a polyethylene sachet gave a reasonable approximation to the blend observed from raspberry canes after splitting.

Field trapping studies were carried out in Hungary and the UK during 2009 and 2010 and these have given variable results. In general, numbers of female *R. theobaldi* trapped were very low, although significant numbers were caught in the test in Hungary during 2010. At two sites in Hungary and one in the UK during 2009, more males were caught in traps baited with the synthetic cane volatiles than in unbaited traps. At one of these sites numbers caught with the cane volatiles were similar to those caught with the sex pheromone. At two other sites in the UK numbers of male *R. theobaldi* caught with the cane volatiles were significantly less than those caught in unbaited traps. The former three sites were all open-field while the latter two were covered and it was thought that this factor might have affected the performance of the synthetic lures. However, these results could not be repeated in 2010.

Numbers of male *R. theobaldi* caught in traps baited with the total volatile mixture were not greater than those caught in unbaited traps in either Hungary or the UK, although a reduced blend of the four most volatile compounds showed some attraction to males in the UK. Although considerable progress has been made, further work in both laboratory and field is required. The development of lures attractive to gravid female *R. theobaldi* would provide powerful new tools for monitoring and control of this pest.

Introduction

Raspberry cane midge, *Resseliella theobaldi*, adult males emerge shortly before the females. Males are attracted to the females by a powerful sex pheromone (Hall et al., 2009) and mating takes place within a few hours. The mated females oviposit within 24 hours. There is a strong preference for the splits in primocanes (Gordon and Williamson, 1991) and fresh splits are preferred over old ones or ones already occupied with larvae (Pitcher, 1952). Nijveldt (1963) observed in the laboratory that the scent from wounds and splits is an important stimulus for oviposition. They showed that spraying willow twigs with sap from young raspberry canes resulted in immediate egg-laying by gravid female *R. theobaldi*, whereas they did not lay eggs on unsprayed twigs. This could be due volatile chemicals in the sap, but the fact that older splits were less favoured suggests that ephemeral, volatile chemicals may be responsible, at least in part.

This work was carried out to identify chemicals produced on wounding raspberry primocanes that might be involved in attraction of gravid female *R. theobaldi* and to produce a synthetic lure that emulates the bouquet and is attractive to *R. theobaldi.*

Materials and methods

Collection and analysis of volatiles

In 2008, volatiles were collected by SCRI and NRI from damaged and undamaged potted raspberry plants of a range of varieties. The region of the stem used for sampling was enclosed in a polyester oven bag (Stewart-Jones and Poppy, 2006). Artificial splits were made with the tip of a dissection needle by splitting the surface for about 40 mm and then gently lifting the epidermis approximately 5 mm to one side of the split, as used to measure oviposition by *R. theobaldi* in field experiments. Volatiles were collected on solid-phase microextraction (SPME) fibres using a range of different fibre coatings and sampling times.

The collections were analysed by Gas Chromatography linked to Mass Spectrometry (GC-MS) and compounds were identified by their mass spectra, GC retention times and

comparison with authentic standards. Some of the collections were also then analysed by Gas Chromatography linked to Electroantennography (GC-EAG) using female midges from a mixed collection that were assumed to be mated.

Sap composition

Weighed samples of raspberry canes (approx 2 gm) were crushed in a pestle and mortar under solvent (1:1 diethyl ether and petroleum spirit; 10 ml). After 5 min the solvent was removed and dried with magnesium sulphate. For quantification dodecyl acetate (5 µg) was added as internal standard and the extracts analysed by gas chromatography.

Dispensers and measurement of release rates

The above work showed that, on splitting, raspberry canes produced a characteristic suite of at least 18 chemicals, including the six compounds emitted by undamaged canes. Systems to dispense the synthetic chemicals at appropriate rates for evaluation as attractants for female *R. theobaldi* were developed using sealed polyethylene vials (20 mm x 8 mm x 1 mm thick) for the more volatile compounds and sealed polyethylene sachets (5 cm x 5 cm x 120 µ thick) for the less volatile compounds.

Field trapping tests

Trapping tests with delta traps were carried out in UK and in Hungary in 2009 and 2010 to compare catches of *R. theobaldi* in traps baited with the synthetic cane volatiles, the sex pheromone and an unbaited control. Traps were standard white delta traps with 20 x 20 cm bases and were deployed in randomised complete block designs with three replicates. Traps were spaced 10 m apart and trap catches were counted and sticky inserts renewed approximately every week.

In 2009, the following treatments were evaluated:

1. A blend of 13 raspberry cane volatiles dispensed from a polythene vial and a sachet dispenser, used together
2. Standard sex pheromone rubber septum lure containing 10 µg of the sex pheromone racemate
3. Untreated control (no lure)

The tests in Hungary used separate vials and sachets for treatment 1. For those at EMR the vial was placed in the sachet.

Treatments evaluated in 2010 are shown in Table 1.

Table 3.3.1. Lures evaluated in 2010 field tests

Treatment	Description	Dispenser/No. of compounds		
		Vial	Sachet	Septum
A	Total volatiles	4	9	
B	Total minus intact cane compounds	3	7	
C	EAG-active compounds		3	
D	Total volatiles sachet		9	
E	Total volatiles vial	4		
F	Pheromone			1
G	Untreated (= no lure)			

For all the trapping tests, total catches were transformed to log(x+1) and subjected to ANOVA. Where significant differences between means were indicated, the differences were tested for significance by an LSD test ($P < 0.05$).

Results

Collection and analysis of volatiles

Analyses of volatiles collected by SPME at both NRI and SCRI showed clear and reproducible differences between those from an intact raspberry cane and those with an artificially-made split. Six main compounds were produced from intact stems. These were present after making the split, but significant amounts of 12 other compounds were also produced (Figure 3.3.1).

Generally amounts of volatiles sampled from the splits declined at 1 hr and 2 hr after making the split. Similar patterns of volatiles to those from Glen Moy were found for four other cultivars: Glen Prosen, Glen Ample, Malling Promise and Malling Delight. These results suggest that the response to wounding by enhance production of a common suite of volatiles is general across the cultivars studied.

In analyses of volatile collections from split canes by linked GC-EAG with a female midge EAG preparation, responses were observed to four of the compounds, three of which were among those produced only after splitting of the cane.

Sap composition

Solvent extraction of crushed raspberry cane gave a suite of chemicals similar to that produced by artificially split canes except for the complete absence of the two ketones which were significant components in the blends of volatiles from both intact and split stems.

Quantitatively, the higher molecular weight components were relatively more abundant in the solvent extract than in the volatiles sampled by SPME (Figure 3.3.1).

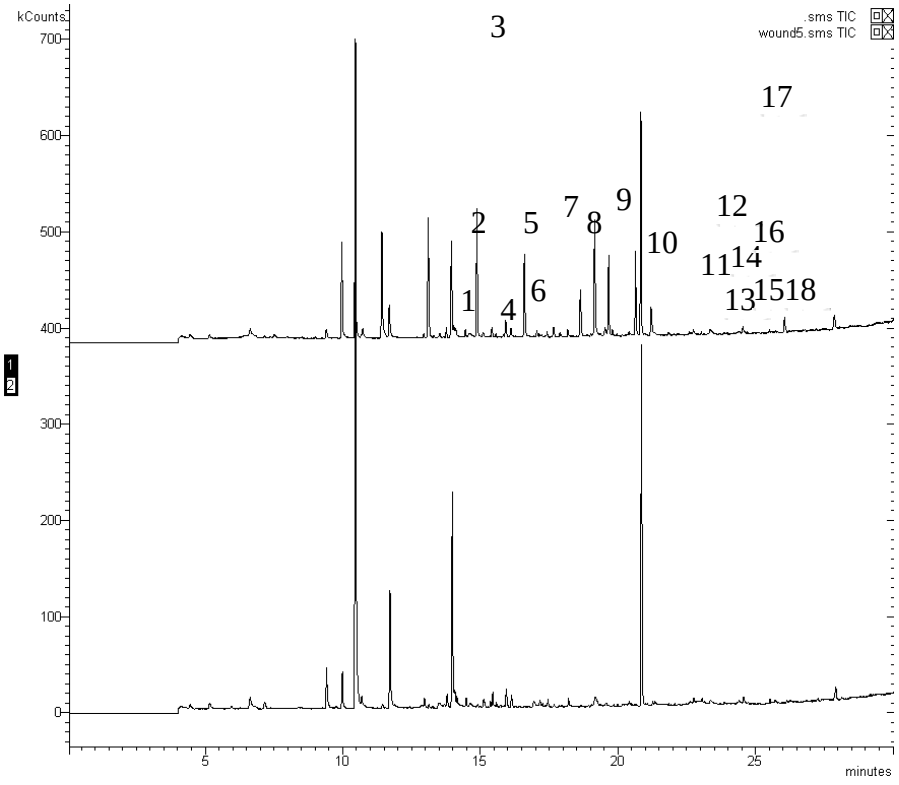


Figure 3.3.1. GCMS analysis of SPME collections from raspberry cane var. Glen Moy: lower undamaged, upper after splitting (PDMS/DVB fibre; 30 min sample time; polar GC column).

Dispenser design

The 13 main components of the 18 previously identified to be emitted from split raspberry canes were selected for use in lures. Compounds present in very small relative amounts were omitted. The more volatile of these were dispensed from a polyethylene vial and the less volatile from a polyethylene sachet. After some experimentation it was found that a combination of four compounds in the vial and nine in the sachet gave the best approximation to the blend produced from split raspberry canes, as measured by SPME sampling and GC-MS analysis. Placing the vial inside the sachet did not affect the release rates and gave a more convenient lure to use.

Field trapping tests

Field trapping tests were carried out at two open-field sites in Hungary in 2009. Catches of female *R. theobaldi* were very low at both sites. Catches of males were much higher and at both sites catches were significantly higher ($P < 0.05$) in traps baited with the synthetic cane volatiles than in unbaited traps (Figure 3.3.2). Traps baited with the synthetic female sex pheromone attracted more males than those baited with the cane volatiles, but this was only significant at one site, Nagyrede (Figure 3.3.2).

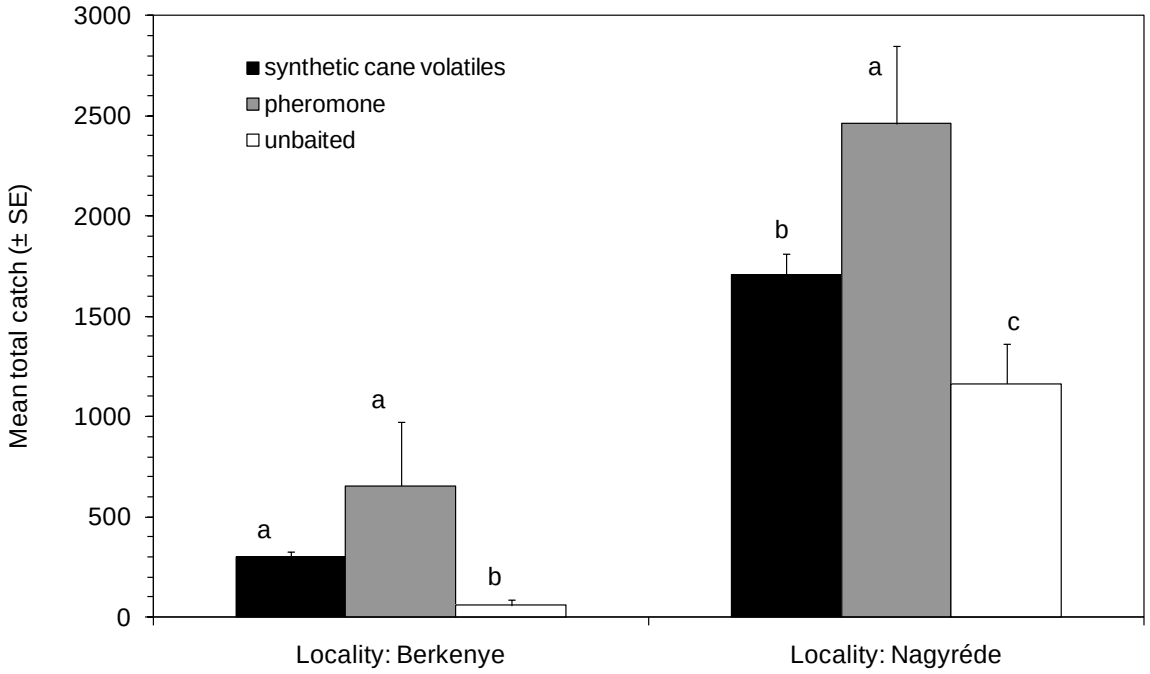


Fig. 3.3.2. Catches of male *R. theobaldi* in delta traps baited with synthetic cane volatiles in Hungary (Berkenye organic Autumn Bliss, n=3, total catches 11/6/09-2/7/09 with four readings, F=9.84, df=2,4, P=0.03; Nagyrede conventional Cv.: Fertődi zamatos, n=3, total catches 17/6-8/7/09 with four readings, F=18.87, df=2,4, P=0.009; for each site means with the same letter are not significantly different P>0.05).

Trapping trials were carried out at three sites in Kent during 2009 and the results were more variable (Figure 3.3.3). As in Hungary, very few females were caught in any of the traps. At East Malling, an open-field site, the result was similar to that in Hungary with significantly more *R. theobaldi* males caught in traps baited with the synthetic cane volatiles than in unbaited. Numbers of males caught in traps baited with the pheromone alone were higher but not significantly so.

Both the other two sites at Yalding and West Peckham were covered and significantly fewer male *R. theobaldi* were caught in the traps baited with synthetic cane volatiles. More males were caught in traps baited with the pheromone than in unbaited traps, but at West Peckham the difference was not significant. Results in Figure 3 are for the whole 20-day period. A similar pattern of results was observed after the first week of trapping indicating that the variable results were not due to loss of activity of the lures.

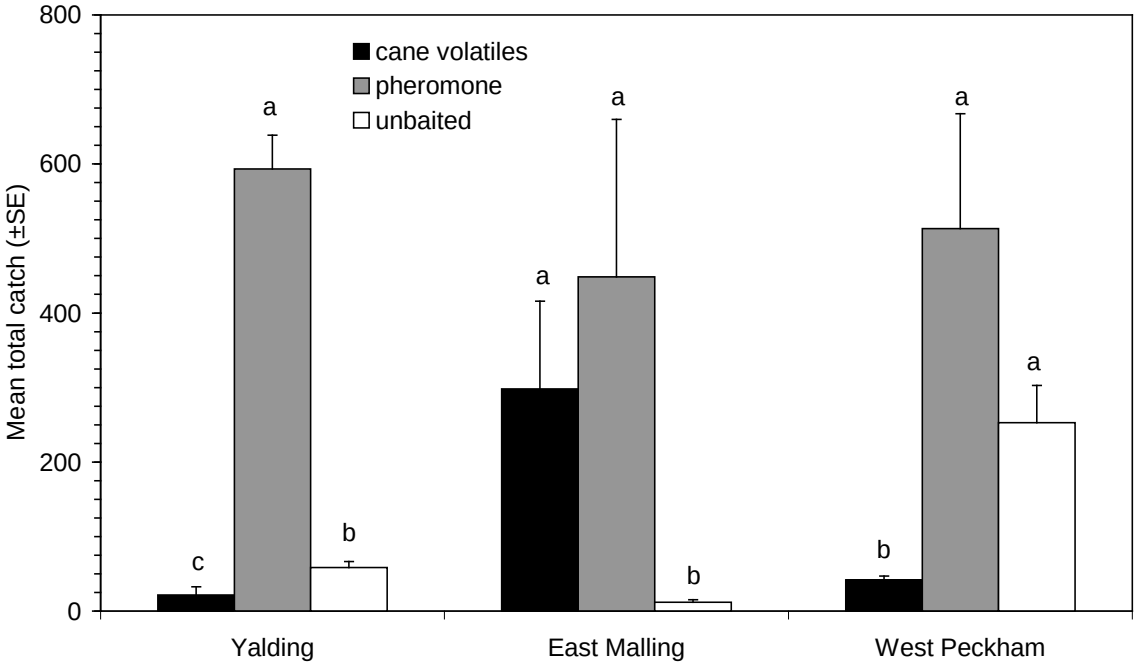


Fig. 3.3.3. Catches of male *R. theobaldi* in traps baited with synthetic cane volatiles, the pheromone and unbaited at three sites in Kent (3 reps at each site; total of three readings 6-26 May 2009; Yalding (cv Maravilla) F=35.91, df=2,4, P=0.003; East Malling (cv Tulameen) F=26.84, df=2,4, P=0.005; West Peckham (cv Tulameen), F=27.12, df=2,4, P=0.005; at each site means with the same letter are not significantly different P>0.05).

During 2010, very few female *R. theobaldi* were captured in the trapping experiments in the UK (Figure 3.3.4). Much larger numbers of males were captured and there were strong, statistically significant (P < 0.001) treatment effects. Both the sex pheromone (treatment F, 188 males per trap) and the host volatile vial containing four components (treatment E, 35 males/trap) captured significantly more males than the unbaited control (16 males per trap). Of the four compounds in Treatment E, only one was only produced from the raspberry cane after splitting, this being a common “green leaf volatile” found in most green plants.

In contrast, in an unreplicated experiment in Hungary in 2010, totals of 6 – 36 females were captured per trap for the different treatments (Table 3.3.2). The lowest numbers were captured in the unbaited trap, but the data were erratic and week to week none of the traps performed consistently the best. This variability, together with the lack of replication which makes statistical comparisons impossible, means that it is not possible to conclude that any of the lures showed significant attraction to females. Catches of males were greatest in the sex pheromone baited trap, followed by the untreated control. No attraction of males to any of the host volatiles lures was apparent.

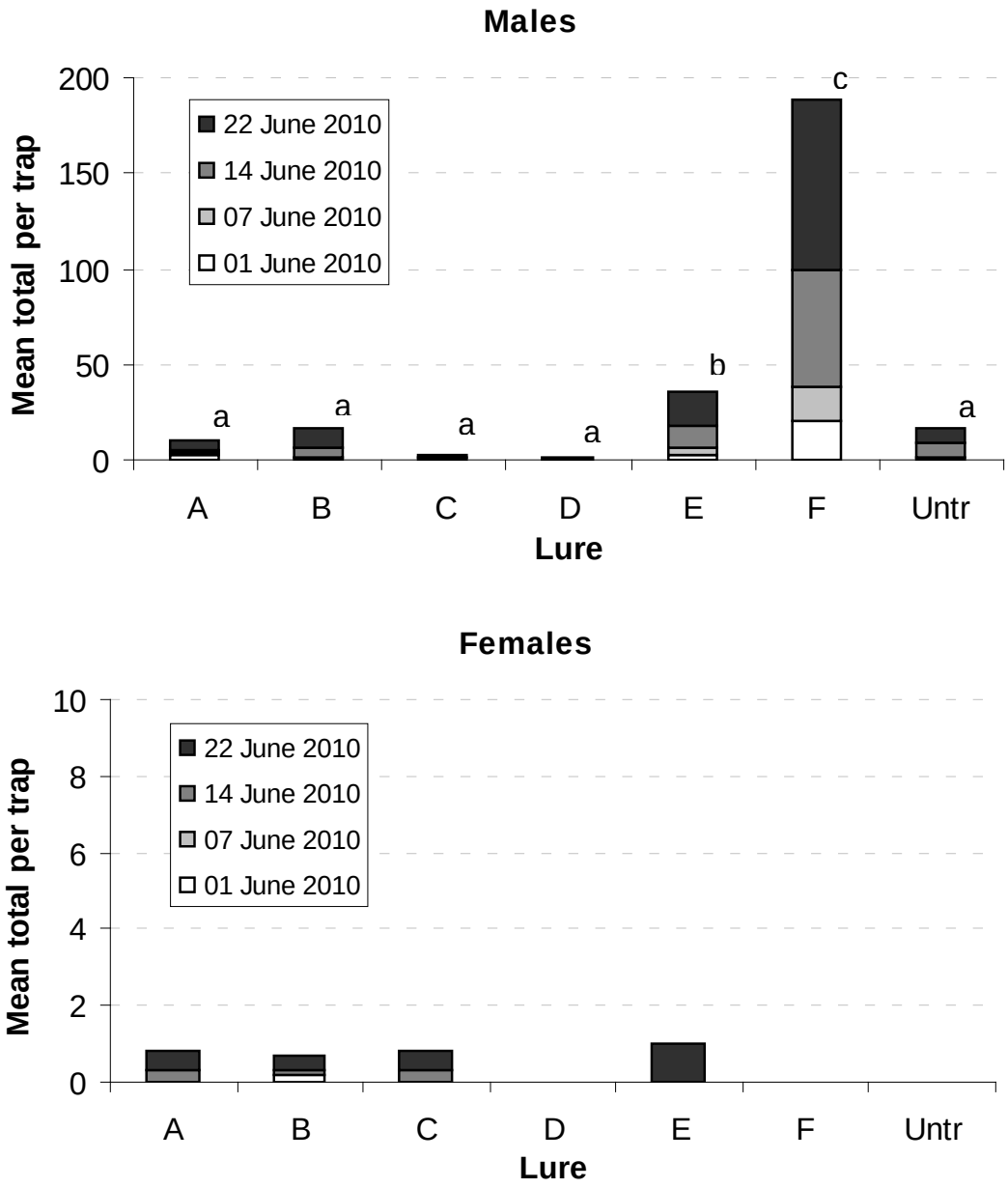


Fig. 3.3.4. Mean total catches of male (above) and female (below) *R. theobaldi* in delta traps baited with synthetic cane volatiles blends (A-E), the pheromone and unbaited at two sites in Kent 2010 (3 reps at each site; total of four readings 27 May – 22 June 2010. Bars with the same letter are not significantly different in an LSD test (P<0.05) on log10(n+1) transformed data. Data for females was not suitable for statistical analysis.

Table 3.3.2. Catches of *R. theobaldi* in Hungary in 2010

Treatment	10/06-16/06	17/06-23/06	24/06-30/06	01/07-08/07	Total
Males					
A	13	19	1	13	46
B	15	18	2	19	54
C	26	47	4	1	78
D	23	14	2	4	43
E	9	36	2	8	55
F	64	71	7	8	150
Unbaited	23	71	2	6	102
Females					
A	0	2	0	10	12
B	0	5	1	30	36
C	0	16	0	0	16
D	0	7	2	1	10
E	0	11	0	1	12
F	0	0	4	8	12
Unbaited	1	1	1	3	6

Discussion

Solid-phase microextraction (SPME) proved to be an excellent technique for sampling the rapid burst of compounds released by raspberry canes after splitting. Using this technique it was shown that a characteristic suite of chemicals was produced after splitting, and these were similar for five varieties of raspberry. The components were identified and the 18 most abundant were selected for further study, including six produced by intact stems and 12 produced after splitting. Of these, four elicited EAG responses from the antenna of a female *R. theobaldi* midge, including three from the group produced only after splitting.

The selected compounds included a wide range of chemical structures and volatilities which presented a considerable problem to dispense reliably for field studies. Exclusion of the least abundant compounds gave a reduced set of 13 compounds and it was shown that dispensing four of these from a polyethylene vial and the other nine from a polyethylene sachet gave a reasonable approximation to the blend observed from raspberry canes after splitting.

Field trapping studies with these compounds were carried out in Hungary and the UK during 2009 and 2010 and these gave variable results. In general, numbers of female *R. theobaldi* trapped were very low, although significant numbers were caught in the test in Hungary during 2010. Greater numbers of male *R. theobaldi* were caught. At two sites in Hungary and

one in the UK during 2009, more males were caught in traps baited with the synthetic cane volatiles than in unbaited traps. At one of these sites numbers caught with the cane volatiles were similar to those caught with the sex pheromone. At two other sites in the UK numbers of male *R. theobaldi* caught with the cane volatiles were significantly less than those caught in unbaited traps. The former three sites were all open-field while the latter two were covered and it was thought that this factor might be affecting the performance of the synthetic lures. However, these results could not be repeated in 2010. Numbers of male *R. theobaldi* caught in traps baited with the total volatile mixture were not greater than those caught in unbaited traps in either Hungary or the UK, although a reduced blend of the four most volatile compounds showed some attraction to males in the UK.

The development of lures attractive to gravid female *R. theobaldi* would provide powerful new tools for monitoring and control of this pest. Although considerable progress has been made, further work in both laboratory and field is required. This should involve more detailed EAG studies of all the compounds identified and development of a laboratory bioassay for more rapid screening of the behavioural effects of the compounds and their blends. Future field studies should be carried out under different growing conditions with careful monitoring of the midge populations and their behaviour.

References

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

Hall, D.R., Farman, D.I., Cross, J.V., Pope, T.W., Ando, T. and Yamamoto, M. (2009) (*S*)-2-Acetoxy-5-undecanone, Female Sex Pheromone of Raspberry Cane Midge, *Resseliella theobaldi* (Barnes). *Journal of Chemical Ecology* 35, 230-242.

Nijveldt, W. (1963). Biology, phenology and control of the raspberry cane midge (*Thomasiniana theobaldi*) which is closely associated with the fungal diseases or blight on raspberries in the Netherlands. *Netherlands Journal of Plant Pathology*, 69:222-234.

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

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

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

Preliminary work towards this objective is described above in section 3.3. As a female attractant was not developed no other work was done on this sub-objective.

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

As an attractive host volatile lure was not developed in 3.3 and 3.4. above, no work was done on this sub-objective.

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*

Summary

Between 2006 and 2010, the efficacies of five Mating Disruption (MD), two Attract and Kill (AandK) and one Mass Trapping (MT) raspberry cane midge sex pheromone treatment were evaluated in large-scale, unreplicated field experiments for control of raspberry cane midge in commercial raspberry plantations in S East and E England in comparison with untreated controls. The treatments evaluated comprised a wide range of dispenser/device types and dose rates of pheromone per ha, the upper dose limit being restricted to 10 g per ha by the terms of the experimental permit for the work. The efficacy of the treatments was evaluated in terms of how effectively they suppressed catches of male midges in single standard sex pheromone traps deployed in the centres of each plot, and in terms of the degree to which they reduced larval infestations in artificial splits in the primocanes through the season.

None of the eight pheromone treatments performed consistently well, and none appeared satisfactory for control in commercial plantations. The sex pheromone trap catch was suppressed compared to its untreated control in all but one of the 21 different ~ 1 ha plot trials in which the eight different treatments were evaluated. Good control of larvae only occurred in those trials where a high (.>90%) or very high (>98%) degree of trap shut down resulted, though not necessarily so, as poor control resulted in two trials where there was a very high degree of trap shut down. One of the main problems encountered with the different formulations was sustaining an adequate release of pheromone through the season.

Of the treatments evaluated, a treatment with 5000 0.4 g dollops of SPLAT containing 0.5% sex pheromone racemate per ha (10 g pheromone racemate/ha) was the best for ease of application and steady release rate and the most promising for further development. SPLAT (Specialized Pheromone and Lure Application Technology) is a proprietary (ISCA technologies, CA, USA) wax emulsion formulation used to control the release of semiochemicals. This treatment gave good control of first generation larvae in one trial in 2010, though control broke down for the second generation despite a second application. The

SPLAT formulation and method of use (size and number of dollops) allows the release rate to be adjusted to a considerable extent and the amorphous and flowable quality of this formulation means that its application can be mechanised making application of large numbers of dollops per ha economically feasible. Further trials, exploring a range of pheromone doses in SPLAT dollops of varying size and with higher numbers of dollops per ha, are needed to improve the treatment to obtain a reliable and acceptable degree of efficacy.

Introduction

The aim of the work reported here was to investigate the exploitation of the raspberry cane midge sex pheromone racemate for control of the pest by Mating Disruption (MD), Attract and Kill (A and K) or Mass Trapping (MT) approaches. The dose-attractancy relationship for raspberry cane midge (Figure 3.1.1) suggested two alternative main control strategies for exploiting the raspberry cane midge sex pheromone for control: 1) competitive MD, A and K or MT (false trail following) using large numbers of devices with release rates of ~60 ng/hr which give maximum attractancy; 2) non-competitive MD using high ambient pheromone concentrations sufficient to desensitise the males' pheromone reception and/or response or hide (camouflage) the trails of calling females using smaller numbers of devices with high release rates (> 6 µg/hr).

At the outset, no other information was available to suggest which strategy/approach might be best, nor which dispensers or devices would be effective. A largely arbitrary choice of treatments was therefore made for testing initially. The upper limit of the amount of pheromone that could be used per season was restricted to 10 g per ha by the terms of the non-crop destruction experimental permit granted by the UK registration authority (PSD, now CRD) and this, and the likely high cost of the pheromone, were taken into account. It was therefore decided to test a range of different approaches over the five years of the project in the hope that an effective, reliable and efficacious treatment could be found which could then be optimised. Testing in large plots was necessary to minimise immigration of males into the centres of plots.

Materials and methods

Each year for five years, large scale (~ 1 ha plots) field experiments were done in commercial raspberry plantations in SE and E England to evaluate the efficacy of a range of MD, A and K and MT treatments in comparison with an untreated control for control of raspberry cane midge.

Sites

The farms and commercial raspberry plantations where the trials were done are given in Table 3.6.1.

Table 3.6.1. Sites

Year	Site no.	Location (farm)	Details (P = protected; O = open)	
2006	1	W B Chambers and Son, Belks Farm, Otham, Kent ME15 8RL	Field 20 at TQ 805 019 Glen Ample, 1.53 ha.	P
	2		Field 29 (south half) at TQ052 2489. Glen Ample, 0.82 ha	P
	3		Field 29 (north half) at 02 2489. Glen Ample, 0.82 ha	P
	4	Salmans Limited, Home Farm, Penshurst Road, Bidborough, Tunbridge Wells, Kent TN3 0XH	Field 2, Lower Ample (east part) at Penshurst at TQ 517 440. 0.86 ha	P
	5		Field 2, Lower Ample (west part) at Penshurst at TQ 517 440. 0.86 ha	P
	6		Field 14. New field Ample at Penshurst at TQ 517 440. 1.4 ha.	P
	7	Barn Farm, Wix Road, Bradfield, Manningtree, Essex CO11 2UX.	Churchfield (east). Glen Ample. 1.1 ha	O
	8		Churchfield (west). Glen Ample. 1.1 ha	
	9		Pheasant Run. Glen Ample, 1.5 ha.	O
2007	10	Decoy Farm. High Halstow, Rochester ME3 8SR.	Bungalow field at TQ 786 768 Glen Ample 0.6 ha.	P
	11		Fullers field at TQ 786 768 Glen Ample 0.6 ha.	P
	12		Rye Street Field at Rye Street Farm, Cooling. at TQ 748 762 Tulameen 0.7 ha.	P
	13	W B Chambers and Son, Belks Farm, Otham, Kent ME15 8RL	Field B29 at Belks Farm, Otham. NGR TQ 8052 2489. Glen Ample 1.64 ha.	P
	14		Field L1 at Ledian Farm, Otham NGR TQ 813 527. Glen Ample 1.55 ha	P
	15		NW quarter of Field Ll4 at Ledian Farm, Otham at TQ 813 527. Gen Ample 0.85 ha	P
2008	16	Decoy Farm, High Halstow, Rochester ME3 8SR.	Swigshole East at TQ 788 775 Joan Squire 0.64 ha	P
	17		Swigshole West at TQ 788 775. Joan Squire 0.62 ha	P
	18		Rye Street Farm, Cooling at TQ 748 762 Tulameen mixed cvs 0.7 ha	P
	19	W B Chambers & Son, Belks Farm, Otham, Kent ME15 8RL.	SV1 at Sutton Valence at TQ 811 509 Octavia 1 ha	P
	20		SV3 at Sutton Valence at TQ 811 509 Octavia 1 ha	P
	21		ST1 at Stoneacre Farm at TQ 805 833 Octavia 1 ha	P
	22	Salmans Limited, Home Farm, Penshurst Road, Bidborough, Tunbridge	New Field, Top at TQ 512437Octavia 1.5 ha	P
	23		New Field, Top at TQ 512437Octavia 1.3 ha	P
	24		Lower at Octavia 2 haTQ 517437	P
2009	25	Hugh Lowe Farms, Barons Place, Mereworth, Maidstone, Kent ME18 5NF	Jubilee 1 at Bulls Farm, Mereworth. at TQ 665 548 1.0 ha	P
	26		Highwood's Bank at Bulls Farm, Mereworth. at TQ 665 548 5.5 ha	P

Table 3.6.1. Sites

Year	Site no.	Location (farm)	Details (P = protected; O = open)	
	27	W B Chambers and Son, Belks Farm, Otham ME15 8RL	ST3 at Holly Farm at TQ 804 534 primocane cv Polka 3 ha	P
	28		ST5 at Holly Farm at TQ 804 534 primocane cv Polka 2 ha	P
	29	P R and O N Harrold, Sunclose Farm, Milton Cambs CB24 6DQ	Xmas tree field Octavia. Tunnels 1-3	P
	30		Xmas tree field Octavia, Tunnels 4-6, 12-14	P
2010	31	Hugh Lowe Farms, Barons Place, Mereworth, Maidstone, Kent ME18 5NF	Jubilee 1 at Bulls Farm, Mereworth. at TQ 665 548 1.0 ha	P
	32		Highwood's Bank at Bulls Farm, Mereworth. at TQ 665 548 5.5 ha	P
	33	P R and O N Harrold, Sunclose Farm, Milton Cambs CB24 6DQ	Xmas tree field Octavia. Tunnels 1-3	P
	34		Xmas tree field Octavia, Tunnels 4-6, 12-14	P

Treatments

Treatments evaluated in the 5 years are listed in Table 3.6.2. and the dates when they were applied in Table 3.6.3. In 2006, the MD treatment comprised 2,000 polythene cap dispensers per ha, each initially loaded with 1 mg of raspberry cane midge sex pheromone racemate, deployed in a regular lattice through the crop at a height of 15 cm (Table 3.6.2; Figure 3.6.1a). The A and K treatment tested comprised 2,000 plastic laminated cards surface coated with a microencapsulated formulation of the SP insecticide lambda cyhalothrin and which had a polythene cap dispenser (initially loaded with 100 µg of the pheromone) fixed to the centre per ha) (Table 3.6.2; Figure 3.6.1b).

In 2007, we tested 200 polythene sachet MD devices and 200 Mass Trapping (MT) devices per ha (Table 3.6.2; Figure 3.6.1c). The sachets were each initially loaded with 50 mg of the midge sex pheromone racemate and 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 septum lure initially loaded with 200 µg of the pheromone racemate and released 60 ng pheromone/hr at 28 ºC in the laboratory. The traps contained 50 ml of water + 50% glycol, and were suspended at a height of 15 cm from the ground ((Table 3.6.2; Figure 3.6.1d). The MT rather than A and K devices were used because they gave records of whether or not attracted midges were killed (by drowning)

In 2008, a new MD treatment, comprising 3 kg/ha of 0.5% w/w pheromone racemate EVA granules (~ 150,000 granules/ha) (Table 3.6.2; Figure 3.6.1e) broadcast by hand to the soil surface between the rows, was tested in comparison with an A and K treatment. The latter comprised 2,000 plastic laminated lambda cyhalothrin cards (similar to those tested in 2006)

each with a rubber septum dispenser loaded with 200 µg of the pheromone racemate (Table 3.6.2; Figure 3.6.1f) and an untreated control.

In 2009, a new *R. theobaldi* sex pheromone MD/A and K method using SPLAT formulation technology was developed and tested. The SPLAT was more likely to be practical for use by growers, would not get lost in soil and would have a large number of pheromone sources per ha, so having a better chance of success. SPLAT (Specialized Pheromone and Lure Application Technology) is a proprietary base matrix formulation of biologically inert materials used to control the release of semiochemicals with or without pesticides. The SPLAT matrix emits semiochemicals for a time interval ranging from 2–16 weeks. Having a wide range of viscosities and application methods (e.g. applicator sprays, aerial applicator sprays, caulking gun type tubes, etc.), SPLAT increases productivity by mechanizing the application of pheromone dispensing points. The amorphous and flowable quality of this adaptable product allows for an easy transition from small-scale manual applications to large-scale mechanical applications.

The SPLAT was used in conjunction with a directed spray of the contact acting insecticide, deltamethrin. The aim was to use a competitive MD approach to attract the male midges with the sex pheromone to numerous artificial pheromone sources where they would then be killed by a surface deposit of insecticide, to be applied subsequently. The insecticide chosen was deltamethrin (Decis), a product already approved for use in raspberry. It is a light stable synthetic pyrethroid with excellent knock down properties and good persistence. It was used as a separate spray to avoid registration difficulties.

A new SPLAT formulation of the cane midge pheromone (Table 3.6.2; Figure 3.6.1g) was produced by mixing 4 g of the raspberry cane midge sex pheromone racemate per kg of SPLAT base formulation (supplied by ISCA Technologies [contact Agenor Mafra-Neto] California) in the laboratory at NRI. The formulation was then transferred to caulking guns. The IPDM plots were then treated with 2.5 kg of SPLAT containing 10 g of raspberry cane midge pheromone racemate per ha. The SPLAT was applied in 5,000 0.5 g, 7 cm long strings per ha to the polythene mulch or lay flat polythene irrigation pipe. Depending on the row spacing, approximately one SPLAT string was dispensed per metre of row (Table 3.6.2; Figure 3.6.1g). 1-3 days after SPLAT application, Decis was applied by the grower to the polythene at 600 ml of product in 200 l of water per ha to polythene mulch on which SPLAT has been applied.

In 2010 the SPLAT treatment was evaluated again, but with modifications. The product was formulated (at a slightly higher concentration) by ISCA Technologies and dispensed in 5,000 0.4 g dollops (= 2 kg/ha) from a caulking gun onto the polythene rather than in 0.5 g 7 cm long strings (Table 3.6.2; Figure 3.6.1h). This was done to slow the release rate of the pheromone to make the formulation last longer. Note that two applications were made at site 31, on 20 April and 30 July 2010, respectively (Table 3.6.3).

Table 3.6.2. Raspberry cane midge sex pheromone treatments evaluated in comparison with untreated controls in large scale (1 ha) plots

Year	Trt. no.	Figure 3.6.1	Approach	Dispenser	Loading (racemate/ dispenser)	No. /ha	Phero Dose /ha	Target device for A and K/MT	Deployment height	Sites (See table
2006	1	a	MD	polythene cap	1 mg	2000	2 g	none	15 cm	2, 4, 7
	2	b	A and K	polythene cap	100 µg	2000	0.2 g	6.6 x 6.6 cm plastic laminated card surface coated with microencapsulated lambda cyhalothrin	15 cm	3, 5, 8
	3		Untreated	-	-	-	-	-	-	1, 6, 9
2007	4	c	MD	polythene sachet	50 mg	200	10 g	none	15 cm	10, 12
	5	d	MT	rubber septum	200 µg	200	4 mg	Lynfield trap	15 cm	9, 13
	6		Untreated	-	-	-	-	-	-	11, 14
2008	7	e	MD	EVA granules	0.33% w/w	150,000 (= 3 kg)	10 g	none	on soil in alleys	15, 18, 21
	8	f	A and K	rubber septum	200 µg	2000	40 mg	6.6 x 6.6 cm plastic laminated card surface coated with microencapsulated lambda cyhalothrin	15 cm	16, 19, 22
	9		Untreated	-	-	-	-	-	-	17, 20, 23
2009	10	g	MD	0.5 g, 7 cm long SPLAT string	2 mg	5000	10 g	none	On polythene mulch or irrigation pipe	25, 27, 29
	11		Untreated	-	-	-	-		-	26, 28, 30
2010	12	h	MD	0.4 g SPLAT dollops	2 mg	5000	10 g	none	On polythene mulch or irrigation pipe	31, 33
	13		Untreated	-	-	-	-	-	-	32, 34

Figure 3.6.1. Devices evaluated (see Table 3.6.2: (a) polythene cap; (b) A and K card with polythene cap; (c) polythene sachet; (d) Lynfield trap; (e) EVA granules; (f) A and K card with rubber septum; (g) SPLAT string; (h) SPLAT dollop)

Table 3.6.3. Dates when treatments were applied

Year	Trt. no.	Site	Date	Site	Date	Site	Date
2006	1	2	26 April	4	27 April	7	28 April
	2	3	26 April	5	27 April	8	28 April
2007	4	10	12 April	12	12 April		
	5	9	13 April	13	13 April		
2008	7	15	24 May	18	24 May	21	24 May
	8	16	25 May	19	25 May	22	25 May
2009	10	25	9 April	27	27 May	29	28 April
2010	12	31	20 April, 30 July	33	21 May		

Assessments

Release rates of pheromone from dispensers

Release rate measurements were done on two or more replicate dispensers, either freshlymade and then held in a windtunnel at NRI at 27 °C and 8 km/hr wind speed or on those deployed in the field in the same way as the treatments and collected in from the field at intervals after deployment. The release rate of pheromone was estimated by weight loss or by extraction in solvent or by entrainment and the amount present measured by GC analysis.

Populations of males

A single sex pheromone trap, baited with a standard rubber septum lure loaded with 10 µg of the sex pheromone racemate (the standard adopted for monitoring purposes) was sited in the centre of each plot early in spring before the first generation emergence of midges had started. This trap was held at a height of 0.5 m above the ground. The number of males midges captured each week was recorded throughout the growing season

Larval populations in splits in canes

Fortnightly, throughout the growing season, 10 cm long, artificial splits were made in each of 10-20 primocanes in the central, untreated area of each plot. This was done by drawing a hooked needle vertically along the cane, making a slit through the periderm. Care was taken not to injure the cambium below. The needle tip was angled sideways (tangentially to the circumference of the cane) so that the periderm was separated from the cambium tissue, making a flap under which ovipositing cane midge females could lay their eggs. Making this flap was critical as otherwise the wound would heal making oviposition impossible. The sections of primocane with the splits were collect (cut out with secateurs) and transferred to the laboratory, where the numbers of eggs and larvae in each split were counted. The length

of each split was measured so that the number of larvae per unit length of split could be calculated.

Results

Release rates of pheromones from lures

The release rates of raspberry cane midge sex pheromone racemate from the various dispensers used in the trials, measured from dispensers held in a wind tunnel at 27°C and 8 km/hr wind speed at NRI and/or from dispensers deployed in the field during the trials, are shown in Figure 3.6.2.

In 2006, measurements of the amounts of pheromone in dispensers on Day 0, i.e. after dosing in the lab but before deployment in the field, indicated that approximately 50% of the pheromone racemate was lost during the solvent drying process (Figure 3.6.2). The MD dispensers were dosed with 1 mg of pheromone racemate but only 525 µg was measured at Day 0. The A and K dispensers were dosed with 100 µg of which 91 µg remained, so little loss appears to have occurred at the lower dose. Rapid release of the pheromone occurred subsequently in the field. In the polytunnel crops only 20-30% of the Day 0 pheromone amount remained after one month. This equates to an average release rate of ~500 ng pheromone per dispenser per hour from the MD dispensers and ~100 ng pheromone per dispenser per hour for the A and K dispensers. In the outdoor crops release rates were lower, with 40% of the day 0 amount remaining after one month.

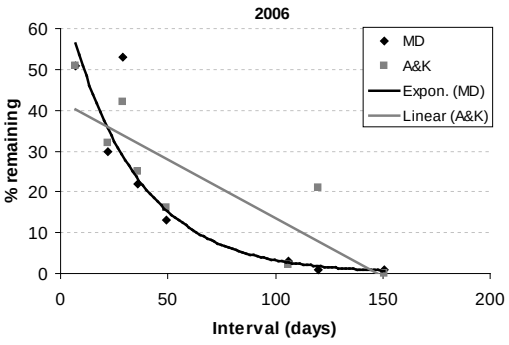
In 2007, the mating disruption sachets lost weight linearly over the period of the study (Figure 3.6.2). Of the initial 50 mg pheromone placed into the sachets, up to 44 mg was lost in 146 days. In the laboratory wind tunnel sachets lost 31 mg in 28 days. The mass trapping lures lost a similar amount of pheromone in both Kent plots over time (initial volume 200 µg). The pheromone remaining in the lures towards the end of the study period was reduced to approximately 20%.

In 2008, laboratory measurements at 27 ˚C, 8 km/h airspeed, showed that the 0.1% granules released 60% of their pheromone over a period of 31 days (Figure 3.6.2.)

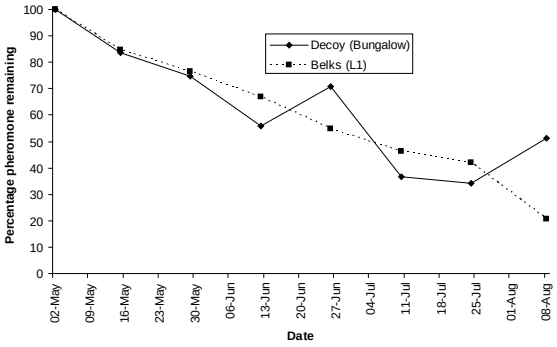
In 2009, dollops used in the determination of residual pheromone had mean original weights (SE, n) of 0.15 gm (0.006, 20), 0.53 gm (0.03, 20) and 0.98 gm (0.06, 20). It was noticed that droplets of SPLAT lost approximately half their weight within a few days of exposure as

water and cosolvents were lost (Figure 3.6.2). Amounts of residual pheromone are plotted as % of the original loading. Release seems to be faster from the smallest dollops but was still quite slow with 50% still remaining after 52 days exposure at 27 °C. Release rates measured by collection of volatiles emitted showed initial high release rates, which declined markedly over time. Results plotted as µg/day for 0.7 gm and 0.15 gm dollops show release is faster from the larger dollops containing more pheromone. The release rate expressed as the percentage of pheromone content released is faster from the smaller droplets. The release rate expressed as percentage of the amount in the dollop equates to 3.7%/day at Day 1, 1.0% at Day 31 and 0.3% at Day 92 for the larger dollop. For the smaller dollops release was 0.8% at Day 72 and 0.6% at Day 92.

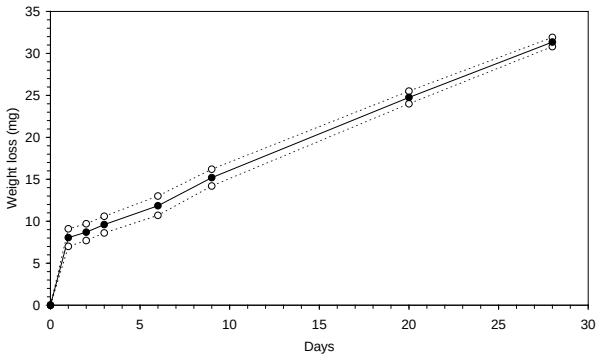
In 2010, approximately 55% of the pheromone was released from the SPLAT dollops in the field between 26 April when the treatment was applied and 2 August (Figure 3.6.2). The release rate was reasonably steady over this period, but thereafter the release from this initial application appeared to virtually stop. The second application made on 30 July showed a rapid loss of 30% of the pheromone in the first three days (it may be that there was a lower initial amount in the batch) and a steady gradual release of a further 10% over the following month until early September when measurements were terminated.



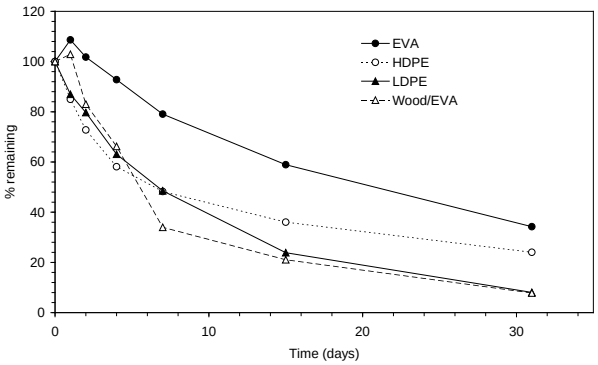
2006: Mean and % amounts of pheromone (µg racemate) remaining in dispensers at intervals after deployment in the field. Note that the % amounts are expressed as a percentage of the amounts on Day 0 (after dosing in the lab and before deployment). Means are of two replicates, except the Day 0 samples which are of six replicates



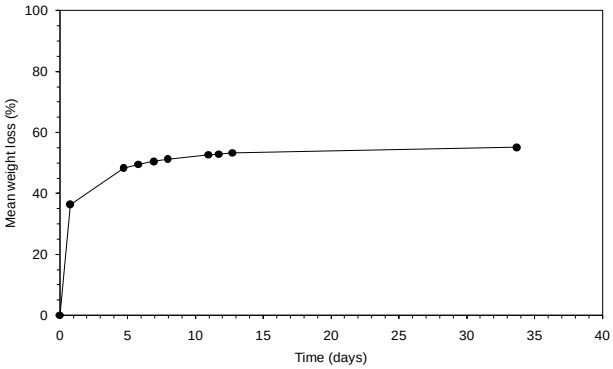
2007: Percentage loss of pheromone lost from the initial 200 µg in the lures in the mass trapping devices at Decoy Farm and Belks Farm



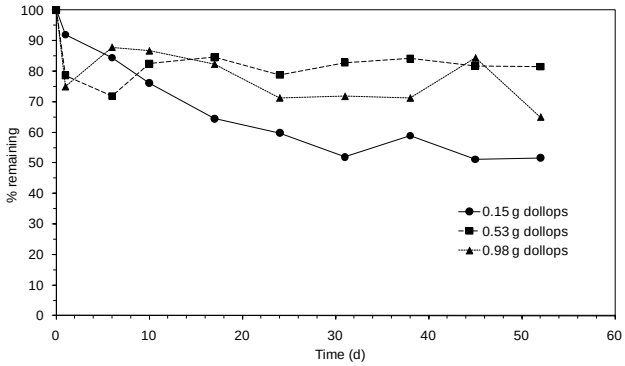
2007: Weight loss of the mating disruption sachets placed in a wind tunnel.



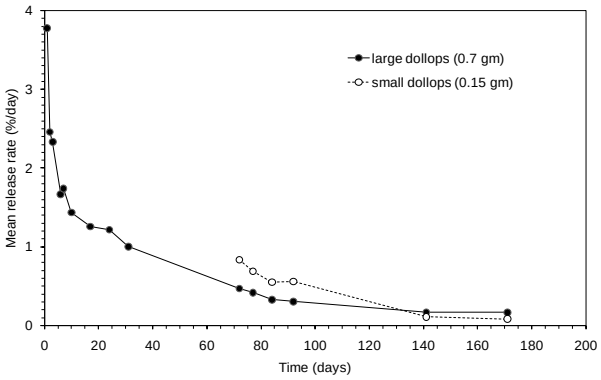
2008: Release of raspberry midge pheromone from polymeric granules (0.1% pheromone; 27°C, 8 km/h windspeed; residual pheromone extracted and assayed by GC).



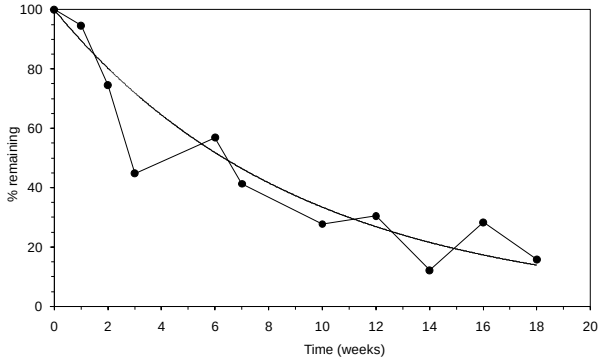
2009: Weight loss of SPLAT dollops (mean weight 0.36 gm) exposed in windtunnel at 27°C and 8 km/hr windspeed (results are means of four replicates)



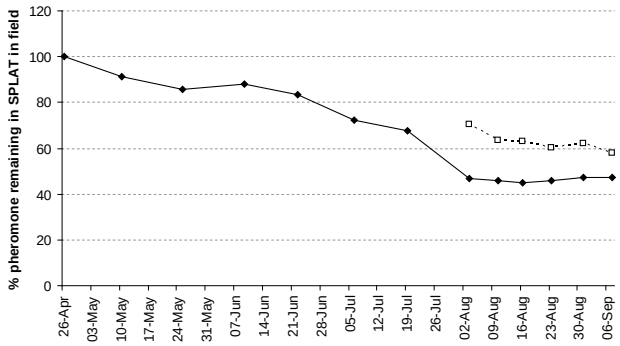
2009: Release of raspberry cane midge pheromone from SPLAT droplets of different sizes as measured by assay of residual pheromone (nominal 0.5%; windtunnel at 27°C and 8 km/hr windspeed)



2009: Release of raspberry cane midge pheromone (µg/day) from SPLAT droplets as measured by entrainment (nominal 0.5%; windtunnel at 27°C and 8 km/hr windspeed)



2009: Release of raspberry cane midge pheromone from SPLAT applied in the field (dotted line is exponential fit $y=100\exp(-0.11)x$; $R^2 = 0.81$)



2010: Percentage reduction in raspberry cane midge sex pheromone in SPLAT over time. Solid line = first application and dashed line = second application.

Figure 3.6.2. Release of raspberry cane midge sex pheromone racemate from the various dispensers used in the trials, measured from dispensers held in a windtunnel at 27°C and 8 km/hr windspeed at NRI, and/or from dispensers deployed in the field during the trials

Efficacy of MD, A and K and MT treatments

In 2006, the MD and A and K treatments were effective outdoors where a high degree of trap suppression was achieved but were ineffective in the polytunnel crops where trap suppression was less effective (Table 3.6.4). Possible explanations for this difference in efficacy are that pheromone release was too rapid from the dispensers when they were deployed in the polytunnels where temperatures were much higher than outdoors and/or is that the pheromone did not disperse so effectively in the enclosed polytunnel environment.

Results in 2007 were disappointing (Table 3.6.4). Although the MD treatment gave fairly good suppression of trap catches the MT treatment was less effective in this respect and neither treatment prevented larval attack in artificial splits in the canes. It was concluded that 200 devices per ha was probably too small a number.

In 2008, although both the MD and A and K treatments failed at one site (Decoy Farm), very good control was achieved at another (Belks Farm) and intermediate results at a third (Salmans Farm) (Table 3.6.4). The reasons for the different results at the different sites are unclear. There was evidence that the MD treatment was losing its efficacy as the season progressed, with better results for the first generation. Lab measurements of release rate indicated that the EVA granules used for the MD treatment released 60% of their pheromone in the first 31 days at 27°C. One explanation of the decline in trap catch reduction may be that the pheromone release rate from the EVA granules declined steeply through the season. Another possible explanation is that the granules progressively worked themselves into the soil surface, some being trampled by pickers as they walked through the tunnels.

The trap catch reductions achieved by the A and K treatments remained consistent through the season.

In 2009, regrettably, the SPLAT MD treatment was unsuccessful in suppressing pheromone trap catches of males and at the one site where they were found, the treatment was ineffective in controlling larvae (Table 3.6.4). The weekly pheromone trap catches did show that the SPLAT formulation gave a very high degree of trap shut down for the first two-three weeks, but thereafter the suppression of catches declined sharply. Measurements of the amount of pheromone remaining in the formulation showed a sharp decline in the first few weeks (Figure 3.6.2) which may be at least in part the explanation for the poor results.

In 2010, the MD (SPLAT) treatment at the site in Kent (31, 32) reduced the season total numbers of male midges in the sex pheromone traps by 97% but numbers of larvae were only 31% lower in the MD treated compared to the untreated plot (Table 3.6.4). However, examination of the catches through the season (Figure 3.6.3) shows that 11 of the 15 midges captured in the MD trap were in trap records on 19 and 26 April, probably all or mostly before the MD treatment was applied on 20 April (Table 3.6.3). If these individuals are excluded from the calculation, the suppression increases to 99.3% with only four individuals being caught in the MD plot between 4 May and 21 September compared to 582 in the untreated plot. Control of the first generation of larvae up to 29 June was good, but larval control broke down subsequently.

Table 3.6.4. Efficacy of the pheromone treatments in reducing catches in the sex pheromone traps deployed in plot centres and in reducing the numbers of larvae in artificial splits in the primocane

			Pheromone trap catch		Larvae in splits	
Year	Trt	Site	Seasons total in untreated	% reduction by treatment	Seasons total in untreated	% reduction by treatment
2006	1. MD	2	41	85	6	<0
		4	4867	85	317	<0
		7	445	99	40	98
	2. A and K	3	41	71	6	<0
		5	4867	80	317	<0
		8	445	99	40	100
2007	4. MD	10	1322	97	1.72	<0
		12	626	99	1.81	6
	5. MT	9	1322	95	1.72	<0
		13	626	88	1.81	<0
2008	7. MD	15	2670	<0	11	<0
		18	5505	94	16	99.7
		21	6569	85	31	68
	8. A and K	16	2670	92	11	<0
		19	5505	99	16	97
		22	6569	98	31	86
2009	10. MD	25	13	78	0	0
		27	4455	46	24	<0
		29	99	26	0	0
2010	12. MD	31	589	97	782	31
		33	41	44	1931	79

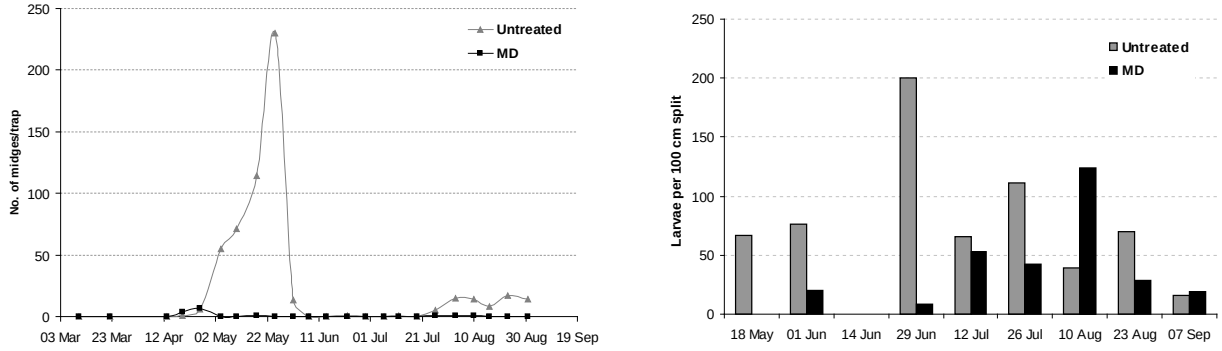


Figure 3.6.3. No. of males in sex pheromone traps and numbers of larvae per 100 cm of split at sites 31 (MD) and 32 (Untreated) in 2010

Discussion

None of the pheromone treatments performed consistently well, and none appeared satisfactory for control in commercial plantations. Of the treatments tested, treatment 12 (SPLAT dollops) appeared the most promising for further development and investigation. Looking at the results as a whole (Table 3.6.4), of the 8 different formulations tested in 21 different plots all but one (site 15) caused a suppression of the sex pheromone trap catch compared to the untreated control but only those formulations that gave a high (>90%) or very high (>98%) degree of trap shut down resulted in good control of larvae, though not necessarily so. The trials at sites 7, 8, 18, 19 and 22 gave good (>85% control) of larvae and had 99, 99, 94, 99 and 98% trap shut down, respectively. However, the trial at site 31 had 97% trap shut down (99.3% if pre-treatment catches are excluded, see above) but only a 31% reduction in larvae, though there was a good level of larval control for the first generation (Figure 3.6.3). The trial at site 9 had 95% trap shut down but larval numbers were not reduced. These results lead to the conclusion that a high degree of trap shut down is a necessary, though not a complete or sufficient, indicator of good performance.

The midge population levels in the test plantations varied widely. Taking the results at face value, good control appeared to be achieved with treatments 7 and 8 at sites 18 and 19, respectively, even though the sites were heavily infested, with >5,000 midges being captured in the monitoring traps in the control plots over the season. Pheromone-based control systems are normally considered to only be effective when populations are low.

The treatments evaluated comprised a very wide range of dose rates of pheromone per ha. The upper limit that could be used was restricted to 10 g per ha by the terms of the non-crop destruction experimental permit granted by the UK registration authority (PSD, now CRD).

The results of the attractancy experiments (Figure 3.1.1) suggest that release rates >6 µg/hr from individual dispensers are needed for non-competitive MD (sources camouflaged or males desensitised by over load of pheromone). The polythene sachets tested in 2007 initially contained 50 mg of pheromone and had a release rate of ~1 mg/day (= 42 µg/hr) but treatment 4 using 200 of these (= 10 g pheromone) per ha was ineffective at both sites 10 and 12 where it was tested, even though these sites had comparatively low population levels. This implies that the non-competitive approach is ineffective/inappropriate, or that a very much high dose/number of dispensers would be needed to affect control, which would be prohibitively costly.

The results of the attractancy experiments (Figure 3.1.1) indicate that devices releasing 600 ng/hr give maximum attractancy and should be optimal for competitive MD/A and K/MT (false trail following). To sustain this release rate over 150 days (~5 months), a dispenser would need to be initially loaded with 2.2 mg of pheromone racemate, assuming a perfect steady release rate without other loss or degradation. To dispense the maximum dose of 10 g/ha, 4,545 such idealised dispensers would be needed per ha. In reality, dispensers are far from ideal and do not have a uniform release rate, often releasing the pheromone rapidly at first with a decreasing release rate thereafter. On the other hand, a release rate of 60 ng/hr is almost as attractive as 600 ng/hr. These above calculations and deductions lead to the conclusion that competitive MD or A and K with large numbers (many thousands) of dispensers per ha is likely to be the most cost effective strategy for exploiting the pheromone for control. Dose rate and numbers of dispensers/ha are likely to be significant factors in the performance of the competitive MD or A and K treatment. It would be beneficial to evaluate higher dose rates, ideally with larger numbers (many thousands) of dispensers per ha.

Of the formulations tested, those used for treatments 1, 2, 5 and 8 were impractical for cost effective manufacture or use/deployment by growers. One of the main problems encountered with the different formulations was sustaining an adequate release of pheromone through the season. Of the formulations evaluated, only the EVA granules and the SPLAT were practical for use by growers. The EVA granule formulation performed poorly because the pheromone was released too rapidly and the granules got lost in the soil.

The SPLAT formulation and method of use (size and number of dollops) allows the release rate to be adjusted to a considerable extent and treatment 12 (5,000 0.4 g SPLAT dollops containing 0.5% w/w pheromone racemate) appeared to have a good release profile. The amorphous and flowable quality of this adaptable product means that SPLAT application can

be mechanized (e.g. applicator sprays, aerial applicator sprays, caulking gun type tubes, etc.). Treatment 12 (5,000 0.4 g SPLAT dollops per ha) was the most promising formulation for ease of application and steady release rate and is the most promising for further development.

Further trials exploring a range of pheromone doses in dollops of varying size and with higher numbers of dollops per ha are needed to optimise the treatment. Post treatment application of a synthetic pyrethroid insecticide (e.g. deltamethrin) to target the SPLAT dollops and surface coat them with a contact insecticide, so giving the treatment possible A and K properties, also needs to be investigated

References

Cross, J.V. 2010. To spray , or not to spray: That is the question. Horticultural entomology in the 21st century. Inaugural Professorial Lecture, Natural Resources Institute, University of Greenwich, 11 February 2010. 96 pp.

Hall, D. R., Amarawardana, L., Hilbur, Y., Boddum, T., Cross, J.V., 2011. The chemical ecology of plant-feeding midges (Diptera: Cecidomyiidae). Journal of Chemical Ecology

Objective 4. Mildew

To develop sustainable management strategies by identifying primary inoculum sources, especially the role of ascospores, and quantifying the effects of tunnel and crop canopy management on disease development.

4.1. Inoculum sources

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

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

4.2. Environmental manipulation

4.3. Control agents

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

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

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

Introduction

In Year 2, the consortium decided to omit the powdery mildew from this project, instead the focus was more on *B. cinerea* for the following reasons:

3. Powdery mildew failed to establish in crop despite repeated efforts of artificial inoculation by EMR and ADAS
4. Noticeable amounts of powdery mildew were not observed in commercial crops.

Thus, for powdery mildew, only research activities with meaningful results on the genetic differences between powdery mildews on raspberry and strawberry were carried out.

Powdery mildew on raspberry is genetically different from strawberry powdery mildew

Introduction

The powdery mildew fungi (*Erysiphales*) are common obligate plant pathogens that are easily recognised visually. However, species identification is often difficult or impossible (Glawe, 2008). Taxonomy and identification of the *Erysiphales* have traditionally been based on the morphology of the teleomorph and host range. Recently emphasis has moved to the anamorphic morphology (Braun, 1987; Cook et al., 1997) and the use of ribosomal DNA internal transcribed spacer (rDNA ITS) sequences (Saenz and Taylor, 1999; Hirose et al., 2005; Cook et al., 2006; Kiss et al., 2006; Inuma et al., 2007; Chen et al., 2008; Jankovics et al., 2008; Kiss et al., 2008).

Powdery mildew, caused by *Podosphaera aphanis* (Wallr.) U. Braun and S. Takamatu (known earlier as *Sphaerotheca macularis sensu auct.* NZ), can infect leaves, leaf petioles, flower trusses, flowers and fruit, and is a serious disease of cultivated strawberries (*Fragaria* x *ananassa*) (Miller et al., 2003; Blanco et al., 2004; Amsalem et al., 2006; Willocquet et al., 2008). This pathogen is also believed to cause powdery mildew on raspberry (Ellis et al., 1991). Although fungal isolates from strawberry have been shown not to infect raspberry and vice versa (Ellis et al., 1991), it is now being claimed that recent severe epidemics of powdery mildew on strawberry in some parts of the UK arose from inoculum originating from raspberry plantations nearby. If confirmed, it will have considerable implications on disease management on both crops. Preliminary investigations were thus carried out at East Malling Research, UK to compare powdery mildew on strawberry and raspberry. Scanning Electron microscopic examinations found no apparent morphological differences between mildews from the two hosts but cross-inoculation failed to produce successful infections in polythene tunnel, glasshouse compartment or controlled environmental conditions (15-20°C at 70-95% relative humidity) (Xu, unpublished).

The failure of numerous cross-inoculation efforts led to the investigation of whether strawberry and raspberry powdery mildews can be practically considered as two distinct species. Specifically, a number of samples were selected from each pathogen for sequencing their rDNA ITS region, since recent studies suggested that ITS sequencing can be used to distinguish powdery mildews that are morphologically indistinguishable (Jankovics et al., 2008).

Materials and methods

Individual leaves with sporulating mildew lesions were sampled from strawberry plants in the UK, USA, China, Italy and Israel, whereas mildew raspberry samples were sampled from

three sites in the UK only (Dundee, Cambridge and East Malling). A leaf disc (diameter of 0.5 cm) with a single lesion was cut in the field and placed immediately into a centrifuge vial containing 1-2 ml of 95% ethanol; the vials were then shaken manually for 10-20 seconds and kept under ambient conditions until DNA extraction. After at least 24 h, the disc was removed from each vial and the vial was then left open to dry. For Italian and Israeli samples of strawberry mildew, leaves with lesions were taken, dried under ambient conditions and shipped. Discs with a lesion were then cut out from these dried leaf samples and treated with ethanol, as above. A total 720 samples were obtained for strawberry mildew and 19 for raspberry mildew (11 from East Malling, 2 from Cambridge and 6 from Dundee [Scotland]).

To each dried sample vial, 600 μl cell lysis solution (5mM TRIS, 10mM EDTA, 0.5% SDS) and 5 μl of 20 mg/ml proteinase K was added. Tubes were incubated overnight at 55ºC and vortexed vigorously for 1 min. After samples were cooled to room temperature, 200 μl of ice-cold protein precipitation solution (3 M ammonium acetate) was added and tubes spun at 13,000 rpm for 10 min. The supernatant was decanted and mixed with 600 μl isopropanol, and precipitate spun down at 13,000 rpm for 10 min. The pellet was washed in 70% ethanol, followed by a final spin at 13,000 rpm (10 min). The pellet was air-dried and re-suspended in 50 μl water.

The universal primers EKITSF (CTTGGTCATTTAGAGGAAGTAA) and Ek28R (ATATGCTTAAGTTCAGCGGG) were used to PCR across the whole ITS1 and ITS2 regions (Anderson and Cairney, 2004). Bands were cut from gels and cloned; both strands were sequenced, and the sequences matched to the GenBank database using the BLASTN program (http://www.ncbi.nlm.nih.gov/blast). A total of 28 samples were sequenced for their ITS region: 20 strawberry mildew samples (seven from the UK, four from Israel, three from Italy, three from USA, and three from China) and eight raspberry mildew samples (two from Cambridge, three from Dundee [Scotland] and three from East Malling).

Relationships among the ITS sequences were analysed using MEGA4 (Tamura et al., 2007). Four sequences from GenBank were included in the analysis: DQ139429 (*P. pannosa* isolate P-M from host *Prunus laurocerasus*), DQ139433 (*P. pannosa* isolate R-P from host *Rosa* sp.), AB026136 (*P. aphanis var. aphanis* from host *F. grandiflora* - (Takamatsu et al., 2000)) and AF073355 (*P. aphanis* from host *F. x ananassa* (= *F. grandiflora*) - (Cunnington et al., 2003)). Preliminary analysis including two other *P. aphanis* sequences (AB000938 and AB026141 in *Agrimonia*) in GenBank revealed these sequences to be distantly related to *P. aphanis* isolated from strawberry and were therefore excluded from this study. Isolates with

the same ITS sequence from the same region were excluded from phylogenetic analysis. Both parsimony and neighbour joining analyses were performed with insertions and deletions included; bootstrap was based on 500 replicates**.**

Results

The ITS sequences are deposited in GenBank as GU942442 – GU942462 (Table 4.1). Because universal primers were used, a number of contaminating ascomycetes and basidiomycetes were present among the bands sequenced. However, all the samples produced a sequence of either 545 bp, 546 bp or 547 bp (when primers removed) which closely matched *Sphaerotheca* and *Podosphaera* in GenBank (sequences are available upon request). The topology produced based on 479 bp is the same for both distance and parsimony-based analyses and is shown in the neighbour joining tree in Fig. 4.1. The outgroup sequence is DQ139430 (*P. pannosa* from *Rosa*).

The samples from Eurasian (China, Japan, Italy, Israel and UK) strawberry and raspberry formed two distinct and well-supported clades, with bootstraps of 93% and 65%, respectively. However, the three strawberry-derived sequences from California and the GenBank sequence AF073355 remained unresolved outside these two well-supported clades. Nucleotide differences are greater between the three groups of samples (Eurasia strawberry, raspberry and California strawberry) than the within-group differences. As a group, the Eurasia group on average differed by 4.4 nucleotides with the raspberry group, and 3.3 nucleotides with the California group, whereas there are on average 2.4 nucleotide differences between raspberry and California groups.

Table 4.1. Powdery mildew samples used in this study and their sequence numbers as deposited in GenBank

Sample ID	Host	Country/Region	GenBank sequence ID
S_ChinaP3	Strawberry	China	GU942443
S_ChinaP4	Strawberry	China	GU942444
S_Italy1	Strawberry	Italy	GU942445
S_Italy2	Strawberry	Italy	GU942446
S_Italy3	Strawberry	Italy	GU942447
S_Israel1	Strawberry	Israel	GU942448
S_USA_Cali3	Strawberry	USA	GU942449
S_USA_Cali2	Strawberry	USA	GU942450
S_USA_Cali1	Strawberry	USA	GU942451
S_Eng_Kent1	Strawberry	England	GU942452
S_Eng_Her1	Strawberry	England	GU942453
S_Eng_Camb1	Strawberry	England	GU942454
R_Sco2	Raspberry	Scotland	GU942455
R_Eng_Kent4	Raspberry	England	GU942456
R_Eng_Camb1	Raspberry	England	GU942457
R_Eng_Camb2	Raspberry	England	GU942458
R_Sco1	Raspberry	Scotland	GU942459
R_Eng_Kent1	Raspberry	England	GU942460
R_Eng_Kent2	Raspberry	England	GU942461
R_Sco1b	Raspberry	Scotland	GU942462

Discussion

As claimed in literature (Ellis et al., 1991), there was no cross-infection between strawberry and raspberry mildew, which was confirmed by our recent unpublished results. Recent microsatellite-based studies indicated that there was no clear cut evidence for pathogen adaptation to particular hosts in strawberry mildew or particular geographic regions (Harvey and Xu, 20010). Furthermore, there is also the lack of strong race-specific interactions between strawberry cultivars and powdery mildew (Xu et al., 2008), unlike other mildews such as wheat (Wolfe and McDermott, 1994), cucurbits (del Pino et al., 2002), melon (McCreight, 2006) and groundsels (Clark, 1997). This is consistent with the finding of the importance of the additive component in strawberry resistance to powdery mildew (Hsu et al., 1969; McNicol and Gooding, 1979; Simpson, 1987; Nelson et al., 1995; Davik and Honne, 2005).

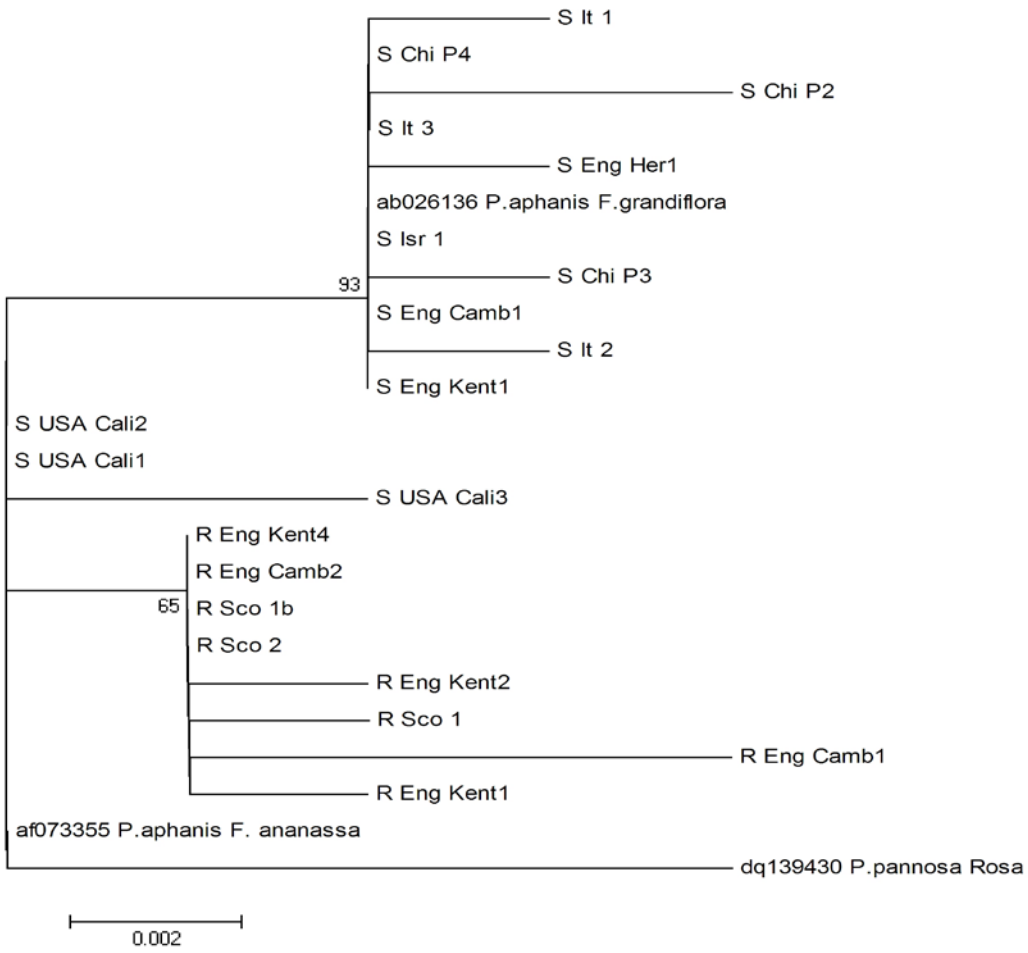


Fig. 4.1. Phylogenetic relationships among powdery mildews samples from strawberry and raspberry in different countries inferred using the Neighbour-Joining method (Saitou and Nei, 1987). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (Pairwise deletion option). There were a total of 479 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura et al., 2007). Sample details are given in Table 4.1.

In summary, powdery mildews on strawberry and raspberry are genetically distinct and might represent two cryptic species of the *Erysiphales*. The differences in the rDNA ITS region between Eurasian strawberry and raspberry mildew samples is about 4.4 bp, less than 1%. However, the average differences in nucleotides between *P. pannosa* sequences, and raspberry, Eurasia strawberry and California samples are only 4, 5 and 3 bp, respectively. Recent results from cross-inoculation, ITS sequencing and AFLP analyses have led to the conclusion that for morphologically indistinguishable powdery mildews 1-5 single nucleotide

positions in their ITS region and different host ranges are to be considered as different taxa with distinct host ranges (Jankovics et al., 2008) for powdery mildew fungi belonging to the genus *Erysiphe*.

There is also no clear evidence for cross-infection between the two pathogens, particularly given the fact that recent research suggested there is no strong evidence for race-specific interactions in strawberry. Therefore, raspberry mildew may be considered to be a separate species from *P. aphanis* on strawberry. Further research is needed to understand why mildew samples from strawberry in California do not fit into the strawberry clade as defined in this study. This work showed that the ITS sequences of strawberry powdery mildew samples determined in the present and previous studies are more variable than those of other powdery mildew species infecting the same host plant species. The same is true for the ITS sequences of raspberry powdery mildew samples.

Literature cited

Amsalem L., Freeman S., Rav-David D., Nitzani Y., Sztejnberg A., Pertot I., Elad Y., 2006. Effect of climatic factors on powdery mildew caused by *Sphaerotheca macularis* f. sp *fragariae* on strawberry. European Journal of Plant Pathology 114: 283-292.

Anderson I.C., Cairney J.W.G., 2004. Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. Environmental Microbiology 6: 769-779.

Blanco C., de los Santos B., Barrau C., Arroyo F.T., Porras M., Romero F., 2004. Relationship among concentrations of *Sphaerotheca macularis* conidia in the air, environmental conditions, and the incidence of powdery mildew in strawberry. Plant Disease 88: 878-881.

Braun U., 1987. A monograph of the Erysiphales (powdery mildews). Beihefte zur Nova Hedwigia 89.

Chen R.-S., Chu C., Cheng C.-W., Chen Y.-W., Tsay J.-G., 2008. Differentiation of two powdery mildews of sunflower (*Helianthus annus*) by a PCR-mediated method based on ITS sequences. European Journal of Plant Pathology 121: 1-8.

Clark D., 1997. The genetic structure of natural pathosystems. In: Crute I.R., Holub E.B. and Burdon J.J., (eds). The gene-for-gene relationship in plant-parasite interactions,'pp.' 231-243. CAB International, Wallingford, UK.

Cook R.T.A., Henricot B., Henrici A., Beales P., 2006. Morphological and phylogenetic comparisons amongst powdery mildews on *Catalpa* in the UK. Mycological Research 110: 672-685.

Cook R.T.A., Inman A.J., Billings C., 1997. Identification and classification of powdery mildew anamorphs using light and scanning electron microscopy and host range data. Mycological Research 101: 975-1002.

Cunnington J.H., Takamatsu S., Lawrie A.C., Pascoe I.G., 2003. Molecular identification of anamorphic powdery mildews (Erysiphales). Australasian Plant Pathology 32: 421-428.

Davik J., Honne B.I., 2005. Genetic variance and breeding values for resistance to a wind-borne disease [*Sphaerotheca macularis* (Wallr. ex Fr.)] in strawberry (*Fragaria x ananassa* Duch.) estimated by exploring mixed and spatial models and pedigree information. Theoretical and Applied Genetics 111: 256-264.

del Pino D., Olalla L., Perez-Garcia A., Rivera M.E., Garcia S., Moreno R., de Vicente A., Tores J.A., 2002. Occurrence of races and pathotypes of cucurbit powdery mildew in Southeastern Spain. Phytoparasitica 30: 459-466.

Ellis M.A., Converse R.H., Williams R.N., Williamson B., eds, 1991. *Compendium of raspberry and blackberry diseases and insects*. St. Paul, Minnesota, USA: APS Press, 100.

Felsenstein J., 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783-791.

Glawe D.A., 2008. The powdery mildews: A review of the world's most familiar (yet poorly known) plant pathogens. Annual Review of Phytopathology 46: 27-51.

Harvey N., Xu X.-M., 20010. Population variability of strawberry powdery mildew (*Podosphaera aphanis*) at the molecular level in different geographical regions. IOBC/WPRS Bulletin 54**:** 545-553.

Hirose S., Tanda S., Kiss L., Grigaliunaite B., Havrylenko M., Takamatsu S., 2005. Molecular phylogeny and evolution of the maple powdery mildew (*Sawadaea, Erysiphaceae*) inferred from nuclear rDNA sequences. Mycological Research 109: 912-922.

Hsu C.S., Watkins R., Bolton A.T., Spangelo L.P., 1969. Inheritance of resistance to powdery mildew in cultivated strawberry. Canadian Journal of Genetics and Cytology 11: 426-438.

Inuma T., Khodaparast S.A., Takamatsu S., 2007. Multilocus phylogenetic analyses within *Blumeria graminis*, a powdery mildew fungus of cereals. Molecular Phylogenetics and Evolution 44: 741-751.

Jankovics T., Bai Y., Kovács G.M., Bardin M., Nicot P.C., Toyoda H., Matsuda Y., Niks R., Kiss L., 2008. *Oidium neolycopersici*: intraspecific variability inferred from amplified fragment length polymorphism analysis and relationship with closely related powdery mildew fungi infecting various plant species. Phytopathology 98: 529-540.

Kiss L., Khosla K., Jankovics T., Niinomi S., Braun U., Takamatsu S., 2006. A morphologically ill-founded powdery mildew species, *Pleochaeta indica*, is recognized as a phylogenetic species based on the analysis of the nuclear ribosomal DNA sequences. Mycological Research 110: 1301-1308.

Kiss L., Kovács G.M., Daughtrey M.L., 2008. *Oidium longipes*, A New Powdery Mildew Fungus on Petunia in the USA: A Potential Threat to Ornamental and Vegetable Solanaceous Crops. Plant Disease 92: 818-825.

McCreight J.D., 2006. Melon-powdery mildew interactions reveal variation in melon cultigens and *Podosphaera xanthii* races 1 and 2. Journal of the American Society for Horticultural Science 131: 59-65.

McNicol R., Gooding H., 1979. Assessment of strawberry clones and seedlings for resistance to *Sphaerotheca macularis* (Wall *ex* Frier). Horticultural Research 19: 35-41.

Miller T.C., Gubler W.D., Geng S., Rizzo D.M., 2003. Effects of temperature and water vapor pressure on conidial germination and lesion expansion of *Sphaerotheca macularis* f. sp *fragariae*. Plant Disease 87: 484-492.

Nelson M.D., Gubler W.D., Shaw D.V., 1995. Inheritance of powdery mildew resistance in greenhouse-grown versus field-grown California strawberry progenies. Phytopathology 85: 421-424.

Saenz G.S., Taylor J.W., 1999. Phylogeny of the *Erysiphales* (powdery mildews) inferred from internal transcribed spacer ribosomal DNA sequences. Canadian Journal of Botany 77: 150-168.

Saitou N., Nei M., 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406-425.

Simpson D.W., 1987. The inheritance of mildew resistance in everbearing and day-neutral strawberry seedlings. Journal of Horticultural Science 62: 327-332.

Takamatsu S., Hirata T., Sato Y., 2000. A parasitic transition from trees to herbs occurred at least twice in tribe *Cystotheceae* (*Erysiphaceae*): evidence from nuclear ribosomal DNA. Mycological Research 104: 1304-1311.

Tamura K., Dudley J., Nei M., Kumar S., 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24: 1596-1599.

Tamura K., Nei M., Kumar S., 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA) 101: 11030-11035.

Willocquet L., Sombardier A., Blancard D., Jolivet J., Savary S., 2008. Spore dispersal and disease gradients in strawberry powdery mildew. Canadian Journal of Plant Pathology 30: 434-441.

Wolfe M.S., McDermott J.M., 1994. Population genetics of plant pathogen interactions: the example of the *Erysiphe graminis* - *Hordeum vulgare* pathosystem. Annual Review of Phytopathology 32: 89-113.

Xu X.-M., Robinson J., Simpson D., 2008. Interactions between isolates of powdery mildew (*Podosphaera aphanis*) and cultivars of strawberry, *Fragaria* x *ananassa*. IOBC Bulletin 39: 203-209

Objective 5. Aphids

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.

Summary

The aphid species that are significant pests of tree and bush fruit crops in Europe are mostly host-alternating. They spend autumn, through winter, spring and early summer on their winter host, typically woody tree or bush fruit species. In mid-summer they migrate to herbaceous hosts. In the autumn, there is a return migration to the winter woody host by males and pre-sexual females (gynoparae), the latter producing sexual females (oviparae) which mate with the males and lay overwintering eggs on the bark. The aim of autumn applications of aphicides is to control a very high proportion of the gynoparae, males and oviparae before overwintering eggs are laid. Logically, the best time to treat is immediately before egg laying commences, catching the maximum proportion of the migrants i.e. when the autumn migration of gynoparae is near its end and at the start of the male migration,

because oviparae cannot lay eggs unless they are mated. There is normally a 2-3 week delay between the migration of gynoparae and that of the males.

Large scale field trials were done in commercial raspberry plantations in Kent to test different timings of autumn sprays of pirimicarb (Phantom), thiacloprid (Calypso) and pymetrozine (Plenum) for the control of small raspberry aphid (*Aphis idaei*) and large raspberry aphid (*Amphorophora idaei*). Single sprays were applied to replicate plots of Glen Ample in the autumns of 2005, 2006, 2007 and 2008. Populations of aphids were assessed in the winter (numbers of eggs) and spring (numbers of adults and nymphs). Calypso sprays greatly reduced populations of large raspberry aphids that developed the following spring by up to 99% in most years. Aphox, Phantom and Plenum gave less consistent results. Early–mid October was the optimum time for a single application of Calypso to reduce spring populations of large raspberry aphid and should be considered as part of Integrated Pest Management programme.

Introduction

Together with other pests and diseases raspberries are also susceptible to aphids (Alford 2005; Gordon et al., 1997; Birch et al., 2002) including the small raspberry aphid (*Aphis idaei*) and large raspberry aphid (*Amphorophora idaei*). Currently pesticides are relied upon for control and are applied when infestations become evident in the growing season. Intensive use of pesticides, including pirimicarb, is undesirable and unsustainable. The incidence of pirimicarb residues in raspberries at harvest was found to be 10 % (surveillance of retail produce in 2002 by KG Fruits). The harvest interval for pirimicarb is 3 days. Aphids also transmit viruses to raspberries and are becoming more important because as aphid populations increase strains are developing that can overcome the plant's natural resistance (Birch et al. 2002; Jones 2003).

Work in Germany, the Netherlands and the UK has demonstrated that the rosy apple aphid (*Dysaphis plantaginea*) and apple grass aphid (*Rhopalosiphum insertum*) can be effectively controlled on apple by sprays of insecticides in the autumn (Wyss, 1997; Helsen, 2001; Helsen and Simonse, 2002; Wyss and Daniel, 2004; Cross et al., 2007). In the UK study very good control was achieved with one or two sprays of pirimicarb applied in October after the autumn migration has occurred but before significant numbers of over wintering eggs are laid. Autumn application has given better control than spring application, possibly because the aphids are more vulnerable and exposed in the autumn than in the spring.

In the first four years of the project, four, large scale, randomised complete block design field trials were conducted (2005-2008) in protected commercial raspberry crops. In the final two years of the project (2009-2010) autumn sprays were combined with increased monitoring of individual sites and the deployment of biocontrols for the aphid control.

Materials and Methods

Autumn control of aphids

Trials 2005 – 06 and 2006 – 07

Five spray timings of single applications of the aphicide thiacloprid (Calypso) were tested as single treatment (Table 5.1). An untreated control was included. Two adjacent cv. Glen Ample plantations at Clockhouse Farm, Linton, Kent, UK were used; Old Platt (No. 212) NGR TQ745 505 (54 rows 118.5 m long, spaced 2.74 m, total 1.75 ha) and Shaw Field (No. 211) NGR TQ 744 505 (48 rows 158.9 m long, spaced 2.74 m, total 2.09 ha). The areas sprayed consisted 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 the farm spray operator under the supervision of EMR staff. The sprayer had 4 air/spray jets per side. The forward speed was 6 km h^{-1}. Spray applications were made 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 3.43 l/nozzle/minute. There were four replicates of the six treatments. Blocks 1 and 2 were in Old Platt plantation and blocks 3 and 4 were in Shaw Field plantation. Plots were two rows wide and the full length of the plantation (~100 m) long, with two guard rows between plots.

Table 5.1. Treatments applied in autumn 2005 and 2006

Product	Active ingredient	Dose rate (/ha)	2005 applications	2006 applications
Calypso	480 g/l thiacloprid SC	250 ml	28 Aug	7 Sep
Calypso	480 g/l thiacloprid SC	250 ml	8 Sept	21 Sep
Calypso	480 g/l thiacloprid SC	250 ml	22 Sept	5 Oct
Calypso	480 g/l thiacloprid SC	250 ml	6 Oct	19 Oct
Calypso	480 g/l thiacloprid SC	250 ml	20 Oct	6 Nov
Untreated	-	-	-	-

The central 80 m in each plot was assessed for small raspberry aphid (*A. idaei*) and large raspberry aphid (*A. idaei*). The numbers of overwintering eggs were counted on a sample of 96 canes per plot (one cane per stool on each of 16 stools in each of six 8 m lengths of row per plot) on 19-24 January 2006 and 19 January 2007. Summer breeding stages were assessed on 20 April 2006 and 25 April 2007, before the first insecticide spray. Numbers of

growing shoots infested on a sample of 60 canes (10 in each of six 8m lengths of row per plot) were counted.

Trial 2007 – 08 and 2008 – 09

These trials tested three different timings of single sprays of pirimicarb (Aphox or Phantom), thiacloprid (Calypso) and pymetrozine (Plenum) for the control of aphids (Table 5.2) on the perimeter of a cv. Glen Ample plantation called Vanity (plot length 2 x 8 m, row width 2.75 m), owned by Robert Pascal, Clockhouse Farm, Coxheath, Kent UK. Sprays were applied at 500 l/ha with a Birchmieir motorised air assisted back pack sprayer by EMR staff. Spray applications were made to each side of the pair of rows in the bed. Assessments of winter eggs were done on 19 January 2007 and 14 January 2008 and summer breeding stages on 25 April 2008 and 27 April 2009.

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

Table 5.2. Treatments applied in autumn 2007

Product	Active ingredient	Dose rate (/ha)	2007 applications	2008 applications
Calypso	480 g/l thiacloprid SC	250 ml	21 Sep	9 Oct
Calypso	480 g/l thiacloprid SC	250 ml	5 Oct	23 Oct
Calypso	480 g/l thiacloprid SC	250 ml	19 Oct	12 Nov
Aphox or Phantom	50% w/w pirimicarb WG	280 g	21 Sep	9 Oct
Aphox or Phantom	50% w/w pirimicarb WG	280 g	5 Oct	23 Oct
Aphox or Phantom	50% w/w pirimicarb WG	280 g	19 Oct	12 Nov
Plenum WG	50% w/w pymetrozine WG	400 g	21 Sep	9 Oct
Plenum WG	50% w/w pymetrozine WG	400 g	5 Oct	23 Oct
Plenum WG	50% w/w pymetrozine WG	400 g	19 Oct	12 Nov
Untreated	-	-	-	-

Results

Trial 2005 – 06

Numbers of small raspberry aphid were too small to draw conclusions from the data. All of the Calypso spray timings greatly reduced populations of large raspberry aphid eggs that were found in the dormant period in January (Table 5.3). The different spray timings did not differ significantly when means were compared. Spray applications on 6 October reduced aphid populations by 97%. Heavy snowfall in mid-April 2006 caused the tunnels in Shaw

Field plantation (blocks 2 and 3) to collapse and this part of the experiment was lost. The assessment of adult large raspberry aphids was done on blocks 1 and 2 only. There were no significant treatment differences in the numbers of adults and nymphs in April due to variability in the data and the fact that only two replicates of data were available. However, all the spray treatments had smaller mean numbers of aphids than the control and the 20 October application date had the smallest numbers of aphids.

Trial 2006 – 07

Small raspberry aphid was not detected in the plantation. Sprays of Calypso in early to mid-October reduced the numbers of aphid eggs in the winter (Table 5.4). The numbers of large raspberry aphids in spring in the untreated control plots was low. However, the 19 October timing gave the best results, reducing aphid numbers by >95% (Table 5.4). The winter egg data showed a smooth time/response curve with a clear minimum at 19 October. The spring aphid data was more erratic.

Trial 2007 – 08

Calypso sprayed on the 19 October was the only treatment that was consistently significantly effective at reducing the numbers of aphid eggs and large raspberry aphids on the raspberry canes (Table 5.5).

Table 5.3. Mean numbers and square root transformed numbers of large raspberry aphid eggs found per 96 canes on 19-24 January 2006 and mean numbers of large raspberry aphid adults and nymphs per 60 canes on 20 April 2006. † Means followed by the same letter do not differ significantly (P = 0.05).

Date of Calypso application	Eggs/96 canes 19-24 Jan 06		Aphids/60 canes 20 April 06		
	n	√n †	Adults	Nymphs	Total
28 Aug	23.3	4.24 b	5.5	6.5	12.0
8 Sept	12.5	3.49 b	5.0	3.5	8.5
22 Sept	7.5	2.44 b	3.5	13.5	17.0
6 Oct	5.3	1.78 b	3.0	5.5	8.5
20 Oct	12.3	3.18 b	0.5	0.0	0.5
Untreated	145.8	10.73 a	5.1	30.9	36.0
Fprob		<0.001			NSD
SED (15 df)		1.658			
LSD (P = 0.05)		3.533			

Table 5.4. Mean numbers and square root transformed numbers of large raspberry aphid eggs found per 96 canes on 19 January 2007 and mean numbers of large raspberry aphid adults and nymphs per 60 canes on 25 April 2007. † Means followed by the same letter do not differ significantly (P = 0.05).

	Eggs/96 canes 19 Jan 07		Aphids/60 canes 25 April 07				
Date of Calypso application	Eggs		Adults	Nymphs	Mummies	Total	Total
	n	√n †	n				√n †
Calypso 7 Sep	17	2.67 a	5.5	25.9	1.0	32.4	9.39
Calypso 21 Sep	12	2.47 a	1.0	9.5	0.5	11.0	8.39
Calypso 5 Oct	6	1.9 b	6.3	27.1	0.4	33.8	10.56
Calypso 19 Oct	3	1.39 b	0.5	2.1	0.3	2.9	4.98
Calypso 6 Nov	49	5.82 a	16.6	59.7	0.8	77.0	12.54
Untreated control	86	7.02 a	6.8	13.8	0.4	21.1	6.95
Fprob		0.148					0.156
SED (15 df)		2.341					2.747
LSD (P = 0.05)		4.99					5.856

Table 5.5. Mean numbers of aphid eggs and large raspberry aphids on canes and plots, respectively. Actual means are shown; data was analysed using square root transformed means. † Means followed by the same letter do not differ significantly (P = 0.05).

	Aphid eggs/cane			Aphids/plot		
Date of treatment application	n	√n †		n	√n †	
Calypso 21 Sep	0.20	0.373	a	18	3.47	a
Calypso 5 Oct	0.40	0.438	a	10	2.67	a
Calypso 19 Oct	0.09	0.260	b	1	0.35	b
Aphox 21 Sep	0.53	0.665	a	84	6.12	a
Aphox 05 Oct	0.57	0.600	a	15	3.56	a
Aphox 19 Oct	0.11	0.327	b	22	3.82	a
Plenum 21 Sep	0.05	0.211	a	48	5.02	a
Plenum 05 Oct	1.38	0.747	a	27	3.46	a
Plenum 19 Oct	0.20	0.352	a	37	4.59	a
Untreated	1.43	0.970	a	125	8.32	a
F prob		0.255			0.422	
SED (d.f. 27)		0.2950			2.905	
LSD (P=0.05))		0.6052			5.961	

Trial 2008 – 09

In the final year of the autumn aphicides trial the effects of all products at all timings on eggs were still clearly visible. Calypso or Phantom sprayed in early to mid-October gave the best control of aphid eggs. However, the effects on subsequent adults in the spring were not

significant (Table 5.6). There was a large effect of block, i.e. the location around the edge of plantation had an influence on the numbers of large raspberry aphid. Total numbers counted on the West, South, East and North of the plantation were 38, 60, 130, 313, respectively. The reason for this breakdown in control is unclear, but high numbers of immigrating adults from the hedgerow could be a factor.

Integrated aphid management

In the final two years of the project counts of aphids (~50 leaves per tunnel), including records of the percentage parasitized, were made as part of an Integrated Pest and Disease Management Programme between April and July.

2009

Because this was the first year of the trials, no autumn aphicides were applied in 2008. At the Cambridge site releases of *Aphidius ervi* were made on 23 May and 12 June in the IPDM and 6 June in the Grower Standard tunnels in response to rising aphid numbers. Numbers of aphids began to rise in the IPDM plot at the Kent site on 15 July. An autumn aphicide was applied on 10 October 2009.

2010

At the Cambridge site releases of *Aphidius ervi* were made on 4 and 18 June in all of the plots. The plots at the Kent site were checked on 19, 26 April, 25 May and 26 July by EMR. Assessments were also made by the farm's agronomist. Only 2 *Macrosiphum euphorbiae*/ 50 shoots were found in the IPDM plot on 26 April. On 26 July, 19 and 14 *M. euphorbiae* were found on 50 shoots in the grower's and IPDM plots respectively. This was reported to the grower. Aphid populations were not treatment specific and infestations occurred sporadically despite regular releases of parasitoids.

Table 5.6. Mean number of aphid eggs and large raspberry aphids on canes and plots, respectively. Actual means are shown; data was analysed using square root transformed means. Treatments with different letters were significantly different. Means followed by the same letter do not differ significantly (P = 0.05).

	Aphid eggs/cane			Aphids/plot	
Date of treatment application	n	√n		n	√n
Calypso 9 Oct	0.0017	0.021	bc	6.8	2.52
Calypso 23 Oct	0.0052	0.036	b	7.5	2.34
Calypso 12 Nov	0.0090	0.080	b	15	3.16
Phantom 9 Oct	0.0063	0.040	b	18.8	3.60
Phantom 23 Oct	0.0023	0.024	bc	23.2	3.74
Phantom 12 Nov	0.0277	0.116	b	14.8	2.83
Plenum 9 Oct	0.0047	0.047	b	9.5	2.36
Plenum 23 Oct	0.0047	0.048	b	10.2	2.86
Plenum 12 Nov	0.0317	0.149	b	17	3.83
Untreated	0.1412	0.336	a	12.5	3.31
F prob		<.001			0.869
SED (d.f. 27)		0.057			1.130
LSD (P=0.05)		0.118			2.318

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References

Alford D.V. (2005) Pests of Fruit Crops. Manson Publishing; 2nd Edition pp 480 pages

Gordon, S.C., Woodford, J.A.T. and Birch, A.N.E. (1997). Arthropod pests of *Rubus* in Europe: pest status, current and future control strategies. *Journal of Horticultural Science* 72: 831-862.

Birch, A.N.E., Jones, A.T., Fenton, B., Malloch, G., Geoghegan, I., Gordon, S.C., Hillier, J. and Begg, G. (2002). Resistance-breaking raspberry aphid biotypes: constraints to sustainable control through plant breeding. *Acta Horticulturae* 587: 315-317.

Cross, J. V., Cubison, S., Harris, A., Harrington, R., 2007. Autumn control of rosy apple aphid, *Dysaphis plantaginea* (Passerini), with aphicides. Crop Protection 26: 1140–1149.

Helsen, H., 2001. Rosy apple aphid can also be controlled in autumn. Fruitteelt 91 (28): 12-13.

Helsen, H., Simonse, J., 2002. Influence of spray timing on the effectiveness of autumn control of the rosy apple aphid. Fruitteelt 92 (27): 12–14.

Jones, A.T. (2003). Important virus diseases of cane fruit crops and their control. *IOBC/WPRS Bulletin* 26(2): 169-175.

Wyss, E., 1997. Different strategies to control the rosy apple aphid *Dysaphis plantaginea* in organic fruit growing. Mitteilungen der Deutschen Gesellschaft fur allgemeine und angewandte Entomologie 11: 233–236.

Wyss, E., Daniel, C., 2004. Effects of autumn kaolin and pyrethrin treatments on the spring population of *Dysaphis plantaginea* in apple orchards. Journal of Applied Entomology 128: 147–149.

Objective 6. Integrated system

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

Task 6.1 - Devise an IPM strategy (years 4-5, all partners) and Task 6.2. - Test IPM in commercial crops (years 4-5; all partners)

Summary

Based on the research conducted in the first three years of the project, a Minimal Pesticide Residue Integrated Pest and Disease Management (IPDM) programme was devised and was tested and refined in years 4 (2009) and 5 (2010) of the project. The key features of this programme were:

1. Good crop hygiene and cane management, together with rapid fruit cooling and high quality cool chain marketing, to avoid the need for fungicide sprays for botrytis during flowering and fruiting.
2. Application of one-two sprays of a powdery mildew fungicide in the spring as soon as the tunnel is covered; then subsequent sprays of potassium bicarbonate for eradication of powdery mildew if the disease is observed.
3. Use of 50+ Agrisense raspberry beetle host volatile funnel traps with white cross vanes/ha. Sprays of Calypso are used only where trap catches exceed thresholds, indicating where local treatment is necessary (e.g. hot spots within tunnels, whole tunnels or field-grown crops in adjacent fields and whole farm level). Note that no Calypso sprays were applied in the trial in Kent (2009), even though the traps' catch threshold was greatly exceeded.
8. Application of a sex pheromone Attract and Kill treatment (SPLAT) for raspberry cane midge.
9. Removal of spent floricane soon after harvest in August.

10. Application of post-harvest fungicide sprays to control cane diseases on new spawn, starting in August.
11. Application of an autumn spray of thiacloprid (Calypso) for aphid control, supplemented with introductions of predators and parasites for biocontrol in summer.

The IPDM programme was implemented by growers in large (~ 1 ha) plots at Hugh Lowe Farm, Mereworth, Kent and Sunclose Farm, Cambridgeshire. Yields of waste and marketable fruit, the incidence of pest and disease damage, shelf life and the incidence of pesticide residues were assessed in the IPDM managed plots in comparison with a similar plot where the grower's standard pest and disease management programme was applied.

Introduction

The aims of the trial were:

1. To compare disease incidence and severity on canes and fruit of tunnel-grown raspberries under either Integrated Pest and Disease Management (IPDM) or a Grower Standard programme. IPDM to include cane manipulation and fungicide treatment, but no fungicides at flowering or between flowering and fruiting
2. To compare pest incidence and severity on canes and fruit of tunnel-grown raspberries and incidence in traps, under either IPDM or a Grower Standard programme. IPDM to include a grid of 50 monitoring traps per ha for raspberry beetle, used to direct local sprays of Calypso if a trap threshold is exceeded, the use of an Attract and Kill treatment for raspberry cane midge, late season aphicide sprays, and predatory mites for two spotted spider mite biocontrol
3. To compare fruit yield (Class 1 and waste) from IPDM and Grower Standard tunnels
4. To determine if Class 1 fruit storage at 2-3°C rather than at 4-5°C following initial rapid field heat removal cooling (at 1-2°C) produces a lower incidence of mould within the maximum shelf-life interval of fruit (five or seven days from harvest). Moulds will include botrytis, penicillium and mucor/rhizopus
5. To determine if pesticide residue levels are lower using the IPDM strategy compared with the Grower Standard programmes.

Materials and methods

Sites

Kent site (Hugh Lowe Farms Ltd): At Hugh Lowe Farms, Barons Place, Mereworth, Maidstone, Kent ME18 5NF in Jubilee 1 (IPDM treatment) and Highwood's Bank (grower's standard treatment) raspberry plantations at Bulls Farm, Mereworth. The plantations were located at NGR TQ 665 548. The raspberry cv. was Tulameen planted in spring 2005. The row spacing = 2.2 m. Row length = ~180 m. Eight fruiting canes/m (on average three canes per stool). The whole plantation consists of four contiguous fields, Jubilee 1, Jubilee 2, Neville's Orchard and Highwood's Bank of total area 6.5 ha, rectangular in shape with the rows running N-S. Jubilee 1 (1 ha) was at the western end of the plantation and Highwood's bank (~ 1ha) at the eastern end. The IPDM tunnel/plot was about 0.13 ha and the Grower's tunnel/plot about 0.06 ha.

Cambridgeshire site (Sunclose Farm*):* Treatments were compared in tunnel crops of three to four year old cv. Octavia with eight fruiting canes/m (on average 4 canes per stool), that was covered at the start of flowering. Tunnels 1-6 were 96 m 3-row tunnels. Tunnels 12-14 were 82 m 2-row tunnels (Figure 2). Management was carried out under either an IPDM or a Grower Standard regime, summarised in Table 1. The pathology Grower area (tunnels 4, 5 and 6) was not used as the entomology Grower area due to the need for spatial separation between the two entomology treatments. The entomology Grower Standard area (tunnels 12-14) was not used as the pathology Grower Standard due to the difference in tunnel dimensions and numbers of rows, which is likely to influence the tunnel microclimate. The Grower Standard treatments were decided by the grower as the crop and any problems developed. Pest and disease and fruit assessments were carried out as detailed later, with some assessments only in the southern Xmas Tree Field tunnels.

Treatments

There were two treatments: (1) a Minimal Pesticide Residues Integrated Pest and Disease Management (IPDM) programme (See Tables 1 and 3) (2) the grower's standard pest and disease management programme to be decided by the grower as the crop and any problems develop. Each treatment was applied to one plot at each site

Kent site (Hugh Lowe Farms Ltd). The IPDM programme (Table 1) was applied to the fifth tunnel in from the western end of Jubilee 1 plantation, the grower's standard programme was applied to the rest of the plantation but records were taken from the ninth tunnel in from the

eastern edge of Highwood's Bank plantation. Note that the raspberry beetle traps and the raspberry cane midge attract and kill treatment were applied to the whole of Jubilee 1 (1 ha area).

Table 6.2.1. The IPDM treatment at Hugh Lowe Farms, Kent in 2009 and 2010

Growth stage	Date due by	Tasks for IPDM*	Date done 2009	Date done 2010
Post-harvest	Dec	Floricanes - select and tie-in as grower's standard practice		
Onset of growth	Wk 11	Deploy one raspberry cane midge sex pheromone trap in the centre of each plot and monitor weekly till September. Keep bases covered with cling film for confirmation by EMR. Refresh lures monthly	10 Mar	3 Mar
Onset of growth + 2-3 weeks	Wk 13-14	Spray Calypso (as no aphicide was applied in autumn 2008). Note that Tulameen is very susceptible to aphids	Not done	
Pre-flowering	Wk 14	Primocanes – first flush removal by herbicide, second flush by hand to 10/m		~4 May ~7 Jun
First midge catch	April	Apply 2.5kg (= five 500 g mastic gun cartridges) of raspberry cane midge SPLAT formulation in 5000 0.5g SPLAT strings 7 cm long and 3mm diameter (See Figure 1) containing 10 g of the raspberry cane midge sex pheromone racemate per ha (1 string/m row) to polythene or irrigation pipe near base of canes using mastic gun over IPDM 1 hectare area. See Figure 1	9 April	20 Apr
3 days after SPLAT treatment	April	Spray Decis at 600 ml of product in 200 l of water per ha to polythene mulch on which SPLAT has been applied	Done in the one IPDM tunnel	~23 Apr
3 wks before flowering	Wk 15	Set out 50 raspberry beetle funnel lure traps/ha in a grid + extras in likely beetle emergence sites near hawthorn, wild blackberry etc. Count numbers of beetles in each trap at start (5%) flowering. (Note that EMR will take additional weekly records for scientific purposes – see assessments below)	14 April	4 May
2 weeks pre-flowering	Wk 16	When flower buds are well developed but not open, apply a spray of Scala spray for botrytis	Amistar applied	Flower buds developed 04 May
Pre-flowering	Wk 17	Systhane 20 EW for rust and mildew just before disease symptoms are likely to first appear, as indicated by experience in previous years	Not done	Traps: 10 May-26Jul
First flower	Wk 18	Record total number of raspberry beetles in each trap (add in counts from previous weeks records by EMR). Spray Calypso in those tunnels where one or more traps catches total of more than five raspberry beetles	6 May 09	
Mid flowering	Wk 20	At least two weeks after Calypso use, make two introductions of *Phytoseiulus persimils* predators in vermiculite at 14-21 day interval against two-spotted mite. Adjust the introduction rate according to the intensity of two-spotted spider mite infestation: 1/cane for low infestation, 2/cane for medium infestation and 10/cane for high infestation	One application made	
Fruiting	Wk 24 – 31	At every pick throughout harvest, record yield of marketable (Class 1) and waste fruit. Picking will be done every 1-2 days depending on temperature. Label and keep waste fruit in refrigerator for weekly collection		

Table 6.2.1. The IPDM treatment at Hugh Lowe Farms, Kent in 2009 and 2010

Growth stage	Date due by	Tasks for IPDM*	Date done 2009	Date done 2010
		by EMR		
Fruiting (1 wk after first pick)	Wk 25	First cold storage regime treatment and shelf life assessment. Wednesday harvest and yield, but put aside two punnets from each of six pickers for two trays (Pick 1). Logger / tray. Transfer fruit to field heat removal area, then into cold storage regimes (Table 3)	17 Jun	
Fruiting	Wk 26	Second cold storage regime treatment and shelf life assessment. Wednesday harvest and yield, but put aside two punnets from each of six pickers for two trays (Pick 1). Logger / tray. Transfer fruit to field heat removal area, then into cold storage regimes (Table 3)	24 Jun	
Fruiting	Wk 27 (29 June)	Third cold storage regime treatment and shelf life assessment. Wednesday harvest and yield, but put aside two punnets from each of six pickers for two trays (Pick 1). Logger / tray. Transfer fruit to field heat removal area, then into cold storage regimes (Table 3)	1 Jul	
Fruiting	Wk 28	Fourth cold storage regime treatment and shelf life assessment. Wednesday harvest and yield, but put aside two punnets from each of six pickers for two trays (Pick 1). Logger / tray. Transfer fruit to field heat removal area, then into cold storage regimes (Table 3)	8 Jul	
Within 2 wks of end of 2009 harvest	Early August	Cut through spent floricane at base and then into two or three pieces, remove from crop rows and the tunnel completely with minimum damage to surrounding primocane. Cut out new primocanes with botrytis. Thin new primocanes to no more than 10/m. Primocane to be held upright and supported by wires or strings placed either side of crop rows. In October, post leaf fall primocane thinned to final stand of six-eight canes/m, clipped, to top support wire of trellis and tipped lightly back where excessively tall to minimise wind rock. Finally all cane cut back to within 20-25cm of tip fixed wire of trellis in late Jan-Feb 2010.		
2 wks after harvest	Mid August	Apply Folicur for first post harvest spray of fungicide (Table 3) for cane diseases	Not done	
4 wks after harvest	10-14 d later	Apply Signum for second post harvest spray of fungicide (Table 3) for cane diseases	Not done	
6 wks after harvest	10-14 d later	Apply Folicur for third post harvest spray of fungicide (Table 3) for cane diseases	Not done	
Autumn	10 October	Spray Calypso against aphids	Not done	
	Spring 2010	Burn off primocane first flush. Thin second flush to 10/m, removing debris from the tunnel		

Cambridgeshire site (Sunclose Farm)*:* The IPDM and Grower programmes are detailed in Tables 6.2.2a and b. All pesticide applications were done by the grower at standard dose rates using a tractor-mounted sprayer at the volume used on the holding (range 300 – 1000 L/ha depending on crop density) if not specified on the label. A fungicide programme was applied in spring and autumn as part of IPDM for the management of cane diseases. Autumn application started at least two weeks post harvest. Fungicide products were used alternately, with no more than two sprays of any product. Fungicide application in the

Grower Standard tunnels was determined by the grower. All tunnels received drenches against *Phytophthora* root rot. There were no flower fungicide sprays in IPDM Tunnels 1, 2 and 3. The Pathology Grower Standard Tunnels (4, 5 and 6) and Entomology Grower Standard Tunnels (12, 13 and 14) received Teldor at (1.5 kg/ha) once there was flowering throughout the tunnels (first open flowers, 10% to 15%) and Scala (2 ml/L) applied 14 days later at around 50% flowering.

Insecticide application in Grower Standard tunnels 12, 13 and 14 was determined by the grower, and Brigade was applied against midge, but not against beetle. Aphicide was applied in autumn 2008, and would have been applied to the set of IPDM tunnels in spring if aphids had been seen. Calypso was to have been applied for raspberry beetle control in the IPDM area if a threshold of five or more beetles were counted in any trap, but this was amended by EMR to request no spray. Biological control agents (predators and parasites) are used by the grower and so were employed against pests in all tunnels. The grower applied Decis at 600 ml in 200 L water, targeted at the base of the canes, in Xmas Tree Field three days after applying SPLAT midge sex pheromone to the drip irrigation tapes.

Because the IPDM programme was not agreed until winter 2008, there was no difference in autumn 2008 fungicide use between the Grower Standard and the IPDM programme for the 2009 fruit harvest. Fungicide treatments were applied in autumn 2009 for the 2010 harvest, the IPDM programme receiving three sprays and the Grower Standard two (Table 6.2.2). Application of Systhane 20 EW for powdery mildew and rust was carried out in October 2008 and so this was not applied pre-flowering in 2009 (but x 2 at 10 day interval in spring 2010).

Treatments were compared in tunnel crops of 3 to 4 year old cv. Octavia with eight fruiting canes/m (on average four canes per stool), that was covered at the start of flowering. Tunnels 1-6 were 96 m three-row tunnels. Tunnels 12-14 were 82 m two-row tunnels. Management was carried out under either an IPDM or a Grower Standard regime summarised in Table 1. The pathology Grower area (tunnels 4, 5 and 6) was not used as the entomology Grower area due to the need for spatial separation between the two entomology treatments.

Pest and disease and fruit assessments were carried out as shown in Table 6.2.2, with some assessments (as detailed) only in the southern Xmas Tree Field tunnels.

Table 6.2.2a. The IPDM and Grower Programme at Sunclose Farm, Cambridge in 2009

Activity in crop and target*	**Crop Growth stage**	**IPDM (Tunnels 1, 2, 3)**	**Pathology Grower Standard and Entomology IPDM (Tunnels 4, 5, 6)**	**Entomology Grower Standard (Tunnels 12,13,14)**
Summary of pesticide treatments		No fungicides during flowering; insecticides permitted	No insecticides except Decis; fungicides permitted	Fungicides and insecticides permitted
Fungicides - *Phytophthora*	Before leaf emergence	Shirlan (13 March)	Shirlan (13 March)	Shirlan (13 March)
Sheet put up		1 May	1 May	1 May
Fungicides - cane diseases	From 6 wks pre-flowering	Cuprokylt (4 May) Folicur (15 May) Scala (29 May)	Cuprokylt (4 May) Folicur (15 May)	Cuprokylt (4 May) Folicur (15 May)
Beetle traps	From 2-4 wks pre-flowering	Yes (22 April)	Yes (22 April)	No
Insecticide - beetle	From 2-4 wks pre-flowering	No	No	Maybe (but not done)
SPLAT + midge males	Pre flowering, second primocane flush	SPLAT (28 April) Decis (4 May) to cane base	SPLAT (28 April) Decis (4 May) to cane base	Chlorpyrifos (27 April)
Lepidopteran caterpillars		No	No	Brigade (1 June)
Fungicide - botrytis	Flowering	No	Teldor (6 June) Scala (20 June)	Teldor (6 June) Scala (20 June)
Biocontrol agents – Aphids + mites	Flowering	*Phytoseiulus persimilis* (23 May) *Aphidius ervi* (12 June)	*Phytoseiulus persimilis* (23 May) *Aphidius ervi* (12 June)	*P. persimilis* and *A. ervi* (6 June)
Sheets dropped		25 Aug	25 Aug	19 Aug
Fungicides – cane pests and diseases	Post harvest	Folicur (13 Oct) Plenum (13 Oct)	Signum (12 Oct) Plenum (12 Oct)	Signum (12 Oct) Plenum (12 Oct)
Floricane removal	Post harvest	Cut canes and carry out (21 Aug – 1 Sep)	Cut and hang. Leave prunings (21 Aug – 1 Sep)	Cut and hang. Leave prunings (21 Aug – 1 Sep)
Primocane selection	After leaf drop	6 canes/m and remove prunings	9-10 canes/m and leave prunings	9-10 canes/m and leave prunings
Phytophthora control	Post harvest	Shirlan (22 Oct) SL561A (28 Oct)	Shirlan (22 Oct) SL567A (28 Oct)	Shirlan (22 Oct) SL568A (28 Oct)

Table 6.2.2b. The IPDM and Grower Programme at Sunclose Farm, Cambridge in 2010

Activity in crop and target*	**Crop Growth stage**	**Full IPDM (Tunnels 1, 2, 3)**	**Pathology Grower Standard and Entomology IPDM (Tunnels 4, 5, 6)**	**Entomology Grower Standard (Tunnels 12,13,14)**
Fungicides – cane pests and diseases	Post harvest 2009	Folicur (13 Oct) Plenum (13 Oct)	Signum (12 Oct) Plenum (12 Oct)	Signum (12 Oct) Plenum (12 Oct)
Floricane removal	Post harvest 2009	Cut canes and carry out (21 Aug – 1 Sep)	Cut and hang. Leave prunings (21 Aug – 1 Sep)	Cut and hang. Leave prunings (21 Aug – 1 Sep)
Primocane selection	After leaf drop 2009	6-8 canes/m and remove prunings	10 canes/m and leave prunings	10 canes/m and leave prunings
Phytophthora control	Post harvest 2009	Shirlan (22 Oct) SL567A (28 Oct)	Shirlan (22 Oct) SL567A (28 Oct)	Shirlan (22 Oct) SL567A (28 Oct)
Floricane selection	Pre-budbreak March 2010	6 canes/m	8 canes/m	8 canes/m
Fungicides - *Phytophthora*	Before leaf emergence 2010	Shirlan (15 Mar) SL567A (22 Mar)	Shirlan (15 Mar) SL567A (22 Mar)	Shirlan (15 Mar) SL567A (22 Mar)
Fungicides - cane diseases	From 6 wks pre-flowering	Cuprokylt (15 Apr) Signum (25 May)	Cuprokylt (15 Apr) Folicur (28 May)	Cuprokylt (15 Apr) Folicur (28 May)
Beetle traps	From 2-4 wks pre-flowering	Yes (12 May)	Yes (12 May)	No
Insecticides		Decis (25 May to cane base)	Decis (25 May to cane base)	Equity (21 May) Decis (25 May to cane base)
SPLAT + midge males	Pre flowering, second primocane flush	SPLAT (21 May)	SPLAT (21 May)	No SPLAT
Tunnels covered		1-3 Jun	1-3 Jun	1-3 Jun
Fungicide - botrytis	Flowering	Switch (18 Jun)	Amistar (19 Jun)	Amistar (19 Jun)
Insecticides	Flowering			Calypso (30 Jun to base of cane)
Lepidopteran caterpillars		No	No	
Biocontrol agents Aphids + mites	Flowering	*Aphidius ervi* (4 and 18 Jun) *Phytoseiulus persimilis* (11- 18 Jun)	*Aphidius ervi* (4 and 18 Jun) *Phytoseiulus persimilis* (11- 18 Jun)	*Aphidius ervi* (4 and 18 Jun) *Phytoseiulus persimilis* (11- 18 Jun)
Rasp Midge	Flowering	Decis (29 Jul) to cane base	Decis (29 Jul) to cane base	Decis (29 Jul) to cane base
Tunnels uncovered		20-23 Aug	20-23 Aug	20-23 Aug
Canes pruned out	Post harvest	17-28 Aug	17-28 Aug	17-28 Aug
Fungicides/Insecticides – cane pests and diseases	Post harvest	Equity (14 Aug) Folicur (28 Aug) Signum (17 Sep)	Equity (14 Aug to base of cane) Folicur (28 Aug)	Equity (14 Aug) Folicur (28 Aug) Signum (17 Sep)

Fruit rot control by cold storage

Fruit was picked on four occasions and stored prior to assessment of mould incidence. Fruit picked by the grower's staff into standard punnets was put into cold storage within two hours. In addition to comparing fruit from the IPDM and Grower Standard tunnel, two different cold storage temperature regimes were used (Table 6.2.4).

Table 6.2.4. Cooling treatments applied to fruit from IPDM tunnel and Pathology Grower Standard tunnels (on four occasions at weekly intervals) at Kent and Cambridgeshire sites.

	Day 1 Wed	Day 2 Thur	Day 3 Fri	Day 4 Sat	Day 5 Sun	Day 6 Mon	Day 7 Tue	Day 8 Wed
Storage Regime	Field – heat removal	Cold-storage on farm		Simulated transport and shop display		Simulated storage at home (not refrigerated)		
Regime X with 2-3 ºC period	Pick and to field heat removal 1-2ºC	Move from 1-2ºC to 2-3ºC*	Leave in main cold store 2-3ºC*	Move from 2-3ºC to 16ºC	Leave in pack-house 16ºC	Assess Move from 16ºC to 20ºC	Leave in 20 ºC	Assess
Regime Y with 4-5ºC period	Pick and to field heat removal 1-2ºC	Move from 1-2ºC to 4-5ºC	Leave in shop cold store 4-5ºC	Move from 4-5ºC to 16ºC	Leave in pack-house 16ºC	Assess Move from 16ºC to 20ºC	Leave in 20 ºC	Assess

***** At the Cambridge site, punnets in Regime X actually remained in the field-heat removal store as closer supervision of their storage position was possible than in the main cold store.

Punnet lids were fitted on Day 2, after field heat removal. Fruit was assessed on Day 6 (whole punnet assessment) and Day 8 (individual fruit). Picks 2 and 3 at the Cambridge site were also assessed on Day 10 to see whether more mould was visible than at Day 8.

Raspberry beetle treatment

The raspberry beetle treatment for the IPDM plots was deployment of 50 raspberry beetle Agrisense Byturus funnel traps with moulded white cross vanes in a grid per ha through the plot, with additional traps placed in hawthorn hedgerows or other host plants in the vicinity. Total numbers of raspberry beetles in each trap were recorded.

Raspberry cane midge treatments

For raspberry cane midge, sex pheromone mating disruption treatments using SPLAT formulations were used. The work on raspberry cane midge is reported in section 3.6 above.

Experimental design and statistical analysis

The main IPDM experimental treatments (comparison of IPDM and Grower Standard strategies) were unreplicated, with one plot of each of the two treatments at each of the two sites (Cambs and Kent). No statistical analysis was possible

For the study of the effect of cold storage on latent botrytis there were two field treatments x two cold store treatments x four picks x six punnets. In the analysis, the effect of cold regime was assessed on each individual farm. The IPDM programme was evaluated across the two sites (Kent and Cambridge) in which four picks were treated as repeated measurements.

Assessments

Disease and pests assessments are given in Tables 6.2.5 and 6.2.6, respectively

Table 6.2.5. Disease assessments and approximate timings (IPDM and grower standard)

Visible botrytis – incidence of lesions and/or sclerotia. Assess 102 canes at 1 m intervals around the whole crop	End February/early March
Other cane diseases - incidence of cane blight, spur blight, cane spot. Assess 102 floricanes at 1 m intervals around the whole crop	End February/early March
Shelf-life test -12 punnets of marketable fruit per tunnel filled to the normal level. Place in cold storage regime and assess % moulds on Days 6 and 8	Weekly for four weeks starting on a Wednesday (start 1 week after first pick in June)
Botrytis and powdery mildew - % fruit affected in the crop. Assess 21 laterals bearing ripe fruit chosen at random; count number of fruit affected per lateral.	Weekly for four weeks starting on a Tuesday (start one week after first pick in June)
Powdery mildew and rust - % leaves affected. Assess 30 floricanes and 30 primocanes at 1m intervals around the whole crop	Within two weeks of final harvest (Aug/Sept)

Table 6.2.6. Pest assessments and approximate timings (IPDM and grower standard)

Counts of raspberry beetle adults in each trap.	Weekly from three-four weeks before flower till end of flowering
Counts of raspberry beetle/infested damaged fruit weekly during picking. Samples taken from the centre of each tunnels with a minimum of 1,000 fruits sampled per sampling point per occasion.	Weekly at harvest
Throughout season, from two weeks after first midge catch in pheromone traps, 30 ~10 cm long artificial splits made fortnightly towards the base of the primocane, 10 in the centre, and 10 towards each end of each central plot tunnel. Fortnightly counts of eggs/larvae in 30 splits. Length of splits measured so the numbers of larvae/cm of split could be calculated.	Fortnightly from start of catches in pheromone trap to end of season (September)
Counts of aphids including records of the percentage parasitized. The sampling size was adjusted to suit the level of infestation but a sample of 50 leaves per tunnel was the norm.	Monthly

Yield (IPDM and grower standard tunnels)

All ripe fruit was picked by farm staff, and weighed as marketable or waste fruit. The yields per tunnel at each pick and the accumulated yield were compared. The total row length of each tunnel was determined to obtain a yield per metre of crop; the length of rows with no or very thin crop measure and subtracted from the theoretical maximum.

Fruit sampling for shelf-life under two different storage regimes

Replicate fruit samples were picked from different locations in the tunnel (using different pickers). Each punnet of the six punnets per storage regime was analysed as a replicate per storage regime treatment. Replicate punnets were kept together in a tray. There were four pick dates and on each occasion the allocation of punnets from crop areas to each replicate were at random.

Punnets were sampled from the IPDM and the grower standard tunnel on each of four pick dates. Six punnets for the low temperature regime X (1-2 ˚C) and six for the farm cold storage regime Y were provided from each tunnel. There were slightly different numbers of fruit in each punnet (as they are filled by volume), but scores were % fruit with various moulds and % healthy.

Picking started once fruit was available throughout the tunnel (usually up to a week after the start of fruiting). Fruit was picked by the farm pickers on four occasions at seven day intervals. When picking, fruit samples in Kent were taken from all three rows, picking all fruit within an area of row until each punnet was filled. Punnets were picked from different locations in the tunnel (using different pickers harvesting to the same standard). At the Cambridge site, fruit for shelf-life testing was picked from the centre row of each tunnel.

Two Class 1 punnets were collected from each of six pickers in each tunnel. One punnet of each pair was put in a tray destined for X cold storage regime, and the other for Y cold storage regime. A punnet with a Tiny-Tag logger was placed in each tray of six punnets.

Subsequent movement of the trays between the storage regimes was carried out according to Table 3. The principal difference in shelf-life treatments was the use of different temperature cold stores to hold the fruit. Lids were put on punnets coming out of field-heat removal on Day 2. Trays moving to the pack-house were covered by net to reduce the chance of fruit fly contamination. During incubation at research laboratories, the four trays were spread out singly, near natural daylight but out of direct sunlight.

Fruit was assessed on Day 6 (whole punnet assessment) and Day 8 (individual fruit). The Day 6 assessment was carried out in the pack-house before moving the fruit, provided that there was sufficient illumination to see clearly. Each punnet was numbered and assessed separately. The Day 6 score was made through the punnet walls, scoring the number of fruit with and without any obvious mould or rot. It was not possible to identify the fungus at this stage. The Day 8 assessment (after seven days storage) required the fruit to be lifted out one at a time with minimal handling and examination for fungal growth. The total number of fruit per punnet with obvious mould or rot was counted.

The number of fruit in each of the following categories was determined:

- not affected by fungi (include fruit collapsed through being over-ripe, and fruit where mycelium has spread from an adjacent fruit)
- affected by botrytis. This typically has a coarse, woolly mycelium, which may develop grey sporulation on dark conidiophores (oval shaped spores).
- affected by fusarium. This typically has brilliant-white dense cotton-wool-like mycelium which may become pink (oval and sickle-shaped spores).
- affected by penicillium. This typically has little mycelium with abundant blue / green spore dust (spherical spores)
- affected by cladosporium. This typically has little mycelium with khaki green spore dust (lemon-shaped spores and elongate, knobbed spores).
- affected by rhizopus or mucor. Typically a network of fast-spreading mycelium, with spore bearing pin-heads (spherical spores).
- affected by other unidentifiable fungi
- affected by bacteria or other breakdown (not just maturity).

Fungi other than botrytis were recorded as ‘other’ if it was not clear. Each fungus was scored as present or absent on each fruit. Some berries had more than one fungus present and these were scored under each category. Overgrowth of fungus which was clearly from a neighbouring fruit was excluded because the records were to determine the number of fungal species which had grown out of the fruit, not the spread severity.

In-crop disease and pest assessments

Records from canes, leaves and fruit were taken from positions around each tunnel to produce a mean for each tunnel. Where possible, assessments were taken equally from

within three replicate blocks along the tunnel length. Assessments were of % infection or infestation. Accumulated counts of pests over the assessment period were compared between tunnels.

Pesticide residue analysis

Fruit were picked from the IPDM and Grower Standard tunnels (English sites only) at 5% and 75% fruit pick according to established marketing group protocols. Sampling and analysis for all fungicides and insecticides approved on raspberry was arranged directly between the grower and the marketing group.

Results

Total yield

Kent site (Hugh Lowe Farms Ltd): Table 6.2.7 gives the total yield in the two tunnels (IPDM and Standard treatment). Total yields/ha for the IPDM treatment were similar to those for the Grower Standard. The percentage waste for the IPDM and Grower Standard plots were 11.0% and 8.8% in 2009 and 17.5% and 16% in 2010, respectively. Thus there was slightly higher wastage in the IPDM plot than the Grower Standard but only marginally so. We cannot determine whether such differences were statistically significant as there were no replicates.

Cambridgeshire site (Sunclose Farms): Table 6.2.8 gives the total yield in the two tunnels (IPDM and Standard treatment). Mean yields/ha were 60% greater at Sunclose Farm than at Hugh Lowe Farms in 2009, but 4% lower in 2010. Total yields/ha in for the IPDM treatment were similar to those for the Grower Standard. The percentage waste for the IPDM and Grower Standard plots were 3.7% and 3.3% in 2009 and 4.1% and 3.9% in 2010, respectively. Thus there was slightly higher wastage in the IPDM plot than the Grower Standard but only marginally so. We cannot determine whether such differences were statistically significant as there were no replicates.

Table 6.2.7. Yields of Class 1 and waste raspberries picked by the farm staff at Hugh Lowe Farms for the two treatment tunnels

Year		IPDM (0.1188 ha)		Grower Standard (0.0495 ha)	
		Class 1 (kg)	Waste (kg)	Class 1 (kg)	Waste (kg)
19 Jun- 23 Jul 2009	total	1489	185	713	69
	/ha	11452	1421	11875	1150
3 Jun – 31 Jul 2010	total	1060	225	417	79
	/ha	8924	1892	8419	1600

Table 6.2.8. Yields of first class and waste raspberries picked by the farm staff at Sunclose Farms for the two treatment tunnels

Year		IPDM (0.1242 ha)		Grower Standard (0.1242 ha)	
		Class 1 (kg)	Waste (kg)	Class 1 (kg)	Waste (kg)
3 Jul- 13 Aug 2009	total	2567	98	2470	84
	/ha	20665	788	19890	673
15 Jul -14 Aug 2010	total	1163	50	1218	49
	/ha	9364	403	9807	395

Shelf life and the occurrence of botrytis and other fruit rots

Results are summarised in Table 6.2.9. The in-store shelf-life of fruit marketed by the major supermarkets is generally given as five days from picking.

Kent site: In 2009, for the first three picks, only a few fruit were rotted on day 6; in contrast 21% fruit from the standard storage regime were rotted, compared to 7% of the cold storage regime (Table 6.2.9). Many more fruit were rotted on day 8, when nearly 68% fruit were rotted. Analysis of variance showed that the cold storage regime resulted in significantly less rot than the standard regime: 58% vs 78%. Botrytis rot was the main rot and cladosporium came second. The incidence of botrytis rot for the cold storage regime (46%) was significantly less than for the standard regime (61%). This was also true for cladosporium, which was recorded in all four picks. In many cases the rot was present on individual drupelets or in the hollow centre of the fruit. Such rotting would have been difficult to see by viewing fruit through the punnet suggesting that the rot present on day 6 may have been an underestimate. In 2010, a very low incidence of fungal rotting occurred on day 6. On day 8, the amount of grey mould was very low, about 3-4%. Instead, the greater loss was mainly due to *Cladosporium* spp.: nearly 48% had rots caused by *Cladosporium* spp.

Cambridgeshire site*:* In 2009, virtually no disease was visible on fruit in the samples when assessed at this time (on Day 6) with six out of the 16 assessments of six punnets having no mould (Table 6.2.9). Punnets with mould had only one or two fruit with specks of mould, and the majority affected were in the first pick, when fruit picked was riper than normal. Across subsequent picks, five and four punnets out of 36 had mould from Tunnels 2 and 5, respectively on Day 6. Consequently, fruit were assessed on the eighth day, and tenth day after picking (picks 2 and 3 only) in order to provide disease levels sufficient to detect any significant effects from the crop management or cool-storage treatments tested in this work.

Around 70% of fruit remained unaffected by fungal growth at eight days after picking. The proportion was significantly greater in the grower management than the IPDM tunnel (pick 3 only), and at the cooler of the two storage regimes (pick 2 only). At all other picks, there was no significant effect of crop management or storage temperature on the proportion of fruit with no fungal growth (i.e. visibly healthy). The proportion of marketable fruit closely reflected the proportion of fruit with no fungal growth. At pick 1 only, around 40% of fruit in all treatment combinations were classed as damaged. This appeared as sunken drupes with blackening and was considered due to the collapse of some fruit retained on the crop for four days in order to accumulate sufficient fruit for harvest.

The predominant fungi that developed on fruit within eight days of picking were *Botrytis cinerea* (11% of all fruit), *Penicillium* spp. (16%) and *Cladosporium* spp. (21%). Other fungi, recorded occasionally, were *Mucor* spp. and *Fusarium* spp. The proportion of fruit with visible botrytis at eight days after picking was unaffected by crop management or cold storage regime, with one exception: at pick 3, there was a greater incidence of botrytis on fruit from the IPDM tunnel (mean of both storage regimes 9%) than the grower tunnel (mean 4%). The proportion of fruit with visible *Penicillium* spp. at eight days after picking was greater in the IPDM tunnel than the grower tunnel (mean of both storage regimes per tunnel) at picks 2, 3 and 4 (24 vs. 15%, 16 vs. 5% and 16 vs. 11% respectively). The cooler storage regime reduced *Penicillium* spp. at pick 2 only (14 and 24% affected respectively). The proportion of fruit with visible *Cladosporium* spp. was unaffected by crop management or cold storage regime with one exception: at pick 1, there was a greater incidence of the fungus on fruit from the grower tunnel (mean 43%) than the IPDM tunnel (mean 26%).

In 2010, no botrytis and low levels (<5%) of other diseases were visible on fruit at the assessment on day 6, with 87 - 96% marketable fruit. There was no significant effect of crop management or storage temperature on the proportion of healthy fruit except at pick 3. At pick 3, the cold storage regime showed a slight reduction in marketable fruit down from over 90% to 88/87%, this coincides with an anomaly shown by the temperature loggers where the standard store was in fact at times running at a lower temperature than the cold store, averaging ~2 and 3 °C respectively for the first two days in store. Over 60% of fruit remained unaffected by fungal growth even at eight days after picking. The proportion of healthy fruit was significantly greater in the grower management (79%) than the IPDM tunnel (65% pick 1 only, standard cooling regime only).

At all other picks, there was no significant effect of crop management or storage temperature on the proportion of fruit with no fungal growth (i.e. visibly healthy). The fungi that developed on fruit within eight days of picking were, *Penicillium* spp. (14-33% of all fruit), *Botrytis cinerea* (2-7% of all fruit) and *Cladosporium* spp. (<1% of all fruit). Other fungi, recorded occasionally, were *Mucor* spp. and *Fusarium* spp. The proportion of fruit with visible botrytis at eight days after picking was unaffected by crop management or cold storage regime.

Overall data analysis. Differences between two storage regimes or between two management strategies were not statistically significant. On average, about 50% of those fruit stored in the cold regime remained healthy on day 8, compared to the 48% when stored under the standard regime. About 51% of those fruit from the conventional management programme remained healthy on day 8, compared to the 47% for the IPM strategy.

There was virtually no difference in the amount of grey mould between two management strategies: on average, about 18% of fruit with grey mould for either of the management strategy. On the other hand, cold storage led to a significant ($P < 0.01$) reduction in grey mould on day 8 over the standard regime: 16% versus 20% grey mould on day 8.

Apart from *B. cinerea, Cladosporium* spp. and *Penicillium* spp. were the other commonly observed on rotten fruit on day 8. The former occurred mainly at the Kent site, particularly in 2010 (39% compared to 10% at the Cambridge site), whereas the latter occurred mainly at the Cambridge site (18% compared to 2% at the Kent site). Neither cold regime nor management strategy significantly affected the incidence of cladosporium rot on day. However, the IPM strategy appeared to increase slightly penicillium rot by 3% ($P = 0.03$).

Table 6.2.9. Incidence of *B. cinerea* and other fungi on raspberry fruit from commercial crops of cv. Octavia in Cambridge and cv. Tulameen in Kent, UK in 2009 and 2010; crops were grown to one of two management strategies (IPDM and Conventional) and fruit were subjected to one of two storage regimes (Cold and Normal)

Site and management strategy	Storage	Incidence of affected fruit 8 days after harvest 2009			2010		
		Botrytis	*Cladosporium*	*Penicillium*	*Botrytis*	*Cladosporium*	*Penicilliium*
Cambridgeshire							
IPDM	Cold	10	16	19	3	0	22
	Normal	13	18	20	2	0	22
Conventional	Cold	10	23	11	4	0	20
	Normal	11	25	14	4	0	17
Kent							
IPDM	Cold	44	20	0	3	55	7
	Normal	63	41	0.1	6	51	2
Conventional	Cold	45	23	0.3	3	40	4
	Normal	67	33	0	6	44	2

Fruit pesticide residue analyses

Pesticide residue analysis results are summarised in Tables 6.2.10 – 6.2.12.

Cambridgeshire (Sunclose Farm): In 2009, samples were taken from the Tunnels 2 and 5 in Xmas Tree Field and Tunnel 13 in Peters Field at both 5% and 75% fruiting. The samples were free from detectable residues of all but two of the 119 active ingredients analysed for, and these were below the maximum residue levels (MRLs) of 10 mg/kg. The fungicide fenhexamid (as found in Teldor) was detected at 0.09 mg/kg and 0.12 mg/kg in T5 and T13, respectively on 2 July at 5% fruiting. This followed Teldor at flowering on 6 June to only T5 and T13. No residues were detected by the 22 July.

The fungicide pyrimethanil (as found in Scala) was found in traces in all six samples. At 5% fruiting it was at 0.13 mg/kg in T2, 0.58 mg/kg in T5 0.72 mg/kg in T13. Scala was applied to all these tunnels, but T2 received it only pre-flowering on 29 May, while T5 and T13 received Scala on 20 June as a second spray at flowering. At 75% fruiting pyrimethanil was at 0.01 mg/kg in T2, 0.29 mg/kg in T5 and 0.13 mg/kg in T13. These were the residues remaining from pre-flowering or flowering sprays. Samples for pesticide residue analysis were not taken at the Kent site.

In 2010, azoxystrobin (as found in Amistar) was detected at 0.02 mg/kg in T5 (grower standard tunnel) at 5% fruiting. This followed Amistar applied as requested at flowering on 19th June. No residues were detected by pick at 75% fruiting.

The fungicides cyprodonil and fludioxonil (as found in Switch) were found in tunnel 2 (IPDM tunnel). At 5% fruiting cyprodonil was found at 0.04 mg/kg and fludioxonil at 0.06 mg/kg. Switch was applied only to the IPDM tunnels on the 18th June, one week after the start of flowering (later than intended by the programme). Flower tagging showed that the fruit picked for residue analysis at 5% fruiting would have been at flowering stage when the Switch was applied. The first pick for the cold storage regime was taken 3 days after the pick for residues at 5% fruiting. At 75% fruiting the same active ingredients were found at 0.01 mg/kg and 0.03 mg/kg respectively. These were residues remaining from the early flower spray.

Table 6.2.10. Pesticide residues (mg/kg) detected in raspberry fruit at 5% and 75% fruiting at the Cambridgeshire site in 2009

Site and treatment	Fenhexamid		Pyrimethanil	
	5%	75%	5%	75%
Cambs site				
IPDM (tunnel 2)	ND	ND	0.13	0.01
Grower (tunnel 5)	0.09	ND	0.58	0.29
Grower (tunnel 13)	0.12	ND	0.72	0.13

Table 6.2.11. Pesticide residues (mg/kg) detected in raspberry fruit at 5% and 75% fruiting - 2010

Site and treatment	Azoxystrobin (mg/kg)		Cyprodonil (mg/kg)		Fludioxonil (mg/kg)	
	5%	75%	5%	75%	5%	75%
Cambs site						
IPDM (tunnel 2)	ND	ND	0.04	0.01	0.06	0.03
Grower (tunnel 5)	0.02	ND	ND	ND	ND	ND

ND – not detected

Kent (Hugh Lowe Farms). Residue analysis was not done in 2009. In 2010, fruit picking started on 30 June and ended on 31 July. Full residues analysis of fruit samples taken on 22 July showed no residues to be present above the reporting limit (RL) in the IPDM plot, but a low residue of 0.02 mg/kg of the fungicide pyrimethanil (Scala) in the fruit from the grower's standard plot.

Table 6.2.12. Pesticide residues (mg/kg) detected in raspberry fruit at mid-fruiting – on 22 July 2010

Site/treatment	Sampling date	Pesticide	RL (mg/kg)	MRL (mg/kg)	Residue (mg/kg)
Kent site					
IPDM	22 July 2010	none detected	0	0	0
Grower	22 July 2010	pyrimethanil	0.01	10.0	0.02

Pest damage to fruits

The main cause of pest damage to fruit was raspberry beetle. The percentage of fruit damaged by raspberry beetle at the two sites in 2009 and 2010 are shown in Table 6.2.13.

Kent site (Hugh Lowe Farms): 0.26% and 0.57% of fruits were damaged or infested by raspberry beetle on the IPDM plot compared to 0.007% and 0.32% in the Grower Standard plot (sprayed with Calypso) in 2009 and 2010, respectively (Table 6.2.12). Thus there was slightly higher damage in the IPDM plot than the Grower Standard but only marginally so. We cannot determine whether such differences were statistically significant as there were no replicates.

Cambridgeshire site (Sunclose Farm): No raspberry beetle fruit damage was recorded in either the IPDM or the growers standard tunnel (Table 6.2.13).

Table 6.2.13. Estimates of and fruits damaged by raspberry beetle calculated from the harvest yields of Class 1 and waste fruit and the number of waste fruits damaged by raspberry beetle in 2009 and 2010

Site/year	**Treatment/ variety**	**% waste fruit damaged by raspberry beetle**	**% fruit damaged by raspberry beetle**
Kent 2009	IPDM	17.2	0.264
Tulameen	Grower‘s	0.2	0.007
Cambs 2009	IPDM	0	0
Octavia	Grower‘s	0	0
Kent 2010	IPDM	2.71	0.57
Tulameen	Grower‘s	1.69	0.32
Cambs 2010	IPDM	0	0
Octavia	Grower‘s	0	0

Pesticide use

At the Kent (Hugh Lowe Farms) site, the grower used very little pesticide in general and the IPDM programme resulted in very large reductions in pesticide use, with a 75% reduction (from four sprays to one spray) in 2009 and a 33.3% reduction (from six sprays to four sprays) in 2010 (Table 6.2.14). At the Cambridgeshire (Sunclose Farm) site, pesticide use was greater and perhaps more typical, the host grower being much more risk adverse. The IPDM programme resulted in a 9% reduction in pesticide use (11 sprays to 10) in 2009 and no reduction in 2010. These two examples, whilst showing the range by which pesticide use would be reduced, do not indicate the amount by which numbers of pesticide sprays would be reduced typically. It has been estimated that on average, numbers of sprays would reduce from nine per season for a typical growers programme to five for the IPDM, a reduction of 44% (Table 6.2.14).

Table 6.2.14. Numbers of conventional pesticides used

	IPDM				Growers				IPDM Typical	Growers Typical
	Kent (Tulameen)		Cambs (Octavia)		Kent (Tulameen)		Cambs (Octavia)			
	2009	2010	2009	2010	2009	2010	2009	2010		
Fungicides										
Pre-flower	0	1	4	4	1	1	3	4	2	2
During flowering/fruiting	0	0	0	1	0	0	2	1		2
Post fruiting	0	2	3	3	0	2	3	2	2	2
Total	0	3	7	8	1	3	8	7	4	6
Insecticides										
Pre-flower	1	1	1	1	2	1	1	2	0	1
During flowering/fruiting	0	0	0	1	1	1	0	1	0	1
Post fruiting	0	0	1	1	0	0	1	1	0	1
Total	1	1	2	3	3	2	2	4	1	3
Grand total	1	4	9	11	4	6	10	11	5	9

Comparative economics

Changes in costs due to typical implementation of the IPDM programme are summarised in Table 6.2.15. The annual total varies from £298 to £538/ha/annum, depending on the extent to which existing growers cane management practices, which vary considerably, have to be adapted. The cost per tonne is £25-45, assuming a typical yield of 12 tonne/ha.

Table 6.2.15. Changes in variable costs per ha per annum that are likely to occur typically as a result of implementing the IPDM programme in comparison with a typical grower's programme

Target pest/disease	Item	Cost/ha/annum £ (incl VAT)
	Additional costs for IPDM programme	
Botrytis/cane diseases	Up to 30 man hours @£8 per hour to manage cane and remove debris	0 to +240
	Increased costs for more rapid transport of fruit from field	+120
	Rapid cooling 12 t fruit/ha to 1-2 °C rather than 4-5 °C	+30
Raspberry beetle	50 Agrisense traps (life five years)	+156
	50 Agrisense lures life one season	+180
	10 man hours @ £10/hr to deploy and service traps	+100
Cane midge	Two sex pheromone monitoring traps deployed in typical 3 ha field	+24
	Three man hours @ £10/hr to deploy and service traps per 3 ha field	+10
	Cost savings in IPDM programme	
Raspberry beetle	One spray Calypso at 0.25 l/ha†	-44
Botrytis	Two sprays Teldor at 1.5 kg/ha†	-238
	Four man hours to apply 3 sprays	-40
	Net cost increase	+298-538

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

An HDC factsheet is being prepared for growers

Patents and publications

Patents

The raspberry cane midge sex pheromone has been patented internationally by EMR and NRI

The controlled release technology used for the raspberry beetle lures is protected by a patent owned by Suterra Ltd, the manufacturers of the lures.

Publications that have arisen directly out of this project

Anon., 2009. Agrisense Ltd. Technical brochure. IPM tools in Soft Fruit. March 2009.

Berrie, A.M., O'Neill, T., Wedgwood, E., Ellerker, B. Evaluation of alternative chemicals for control of botrytis in raspberries. IOBC Bulletin (in press)

Berrie, A.M., Xu, X., Wedgwood, E., O'Neill, T. 2011. Integrated management of Botrytis cinerea in protected raspberries to minimise fungicide residues in the fruit. Proceedings IOBC meeting, Budapest, September 2010.

Birch, A,N,.E., Allen, J. 2009. Pests in the firing line. Fresh Produce Journal, July 2009, pp 36-37

Birch, A.N.E., Williams, R.N., Gordon, S.C. 2010. Raspberry aphid. In: Ellis, M.A., Converse, R.H., Williams, R.N. & Williamson, B. (eds.). *Compendium of Raspberry and Blackberry Diseases and Pests.* 2nd edition. American Phytopathological Society, USA, (in press).

Birch, A.N.E., Shepherd, T. 2010. Integrated pest and disease management for high quality raspberry production. *Defra Horticulture LINK Annual Report*, 85pp.

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. 2007. Article in HDC News No. 130, promoting Hortlink 0175.

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.

Cross, J.V., 2010. To spray, or not to spray: That is the question. Horticultural Entomology in the 21st century. University of Greenwich Inaugural Professorial Lecture series 11 February 2010, 96 pp.

Cross, J. V. 2011. Alternatives to Pesticides: Recent Advances in Fruit Crop Protection That Could Be Transferred to Ornamentals. IPPS Conference Proceedings, Ipswich, 2010, 3-10.

Cross, J.V., Berrie, A.M., Xu, X., Gutteridge, C., O'Neill, T., Wedgwood, E., Allen, J., Hall, D.R., Farman, D., Gordon, S.C., Birch, A.N.E., Casagrande, E. 2006. Addressing Pesticide Residues in Raspberries through a New HortLINK Project. Proceeding of the 2006 ADAS/EMR Soft Fruit Conference, Sutton Coldfield, 5pp

Cross, J.V., Berrie, A.M. 2008. Development of zero pesticide residue Integrated Pest and Disease Management programmes for UK fruit crops. ICE 2008 Abstract.

Cross, J.V., Hall, D.R., Amarawardana, L. 2006. Pheromones of midge pests of fruit crops. National Fruit Show Handbook 2006

Cross, J.V., Hall, D.R., Fountain, M.T., Harris, A.L., Farman, D. 2008. Utilising sex pheromones of raspberry cane midge, *Resseliella theobaldi*, and apple leaf midge, *Dasineura mali*, for pest monitoring and control. ICE 2008 Abstract.

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

Cross, J.V., Berrie, A.M. Xu, X., O'Neill, T., Wedgewood, E., Allen, J., Hall, D. R., Farman, D., Birch, A.N.E, Mitchell, C., Jorna, C., Shepherd, T., Boonham, N., and Spence, N. 2009. Bearing down on inputs. HDC News Sept 2009, pp 16-17.

Cross J.V., O'Neill T.M., Birch N, Berrie A.M., Xu X. 2010. Raspberries without residues. HDC News 161, March 2010, pp. 20-21

Cross, J.V., Baroffio, C., Grassi, A., Hall, D.R., Łabanowska, B., Milenković, S., Nilsson, T., Shternshis, M., Tornéus, C., Trandem, N., Vétek, G. 2007. Monitoring raspberry cane midge, *Resseliella theobaldi*, with sex pheromone traps: results from 2006. IOBC-wprs Bulletin 39, 11-17

Hall, D., Shepherd, T., Fountain, M., Vétek, G., Birch, A.N.E., Jorna, C.S., Farman, D. & Cross, J. 2010. Investigation of attraction of raspberry cane midge, *Resseliella theobaldii*, to volatiles from wounded raspberry primocanes. *IOBC Working Group: Integrated Plant Protection in Fruit Crops, Subgroup: Soft Fruits. 7th Workshop on Soft Fruit Production,* Budapest, Hungary, 20-23 September 2010 (Talk). In: Cross, J. & Linder, C. (ed.) *7th Workshop on Integrated Soft Fruit Production,* IOBC, Ghent University.

O'Neill, TM, Berrie, A, Wedgwood E, Allen J & Xiangming X. 2009. Effect of canopy manipulation on cane and fruit botrytis in protected raspberry. *Communications in Agricultural and Applied Biological Sciences*, 74, 633-642.

O'Neill, TM, Wedgwood E, Berrie AM, Allen J & Xu X-M. Management of raspberry grey mould under protection. *Agronomy for Sustainable Development.* (drafted)

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

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

Xu X, Wedgwood E, Berrie AM, Allen J & O'Neill TM (2011). Epidemiology of strawberry and raspberry grey mould in open field and under protection. *Agronomy for Sustainable Development* (in press).

Xu X, Wedgwood E, Berrie A.M., O'Neill T.M. 2009. Infection of raspberry leaves by Botrytis cinerea in relation to leaf and cane age. *Communications in Agricultural and Applied Biological Sciences*, 74, 761-770.

Related publications that have arisen indirectly

Baroffio, C., Mittaz, C. 2010. Raspberry beetle: flight monitoring with semiochemical traps in Switzerland. IOBC/wprs Bulletin 54, pp. 397-400.

Blagogie, G.K.J. 2010. Mass trapping of the raspberry beetle and the strawberry blossom weevil. MSc Thesis. Norwegian Universities of Life Sciences, pp 1-62.

Birch, A.N.E., Begg, G.S. & Squire, G.R. 2011. How agro-ecological research helps to address global food security issues under EU's new IPM and pesticide reduction policies (Invited Review). Journal of Experimental Botany (in press).

Birch, A.N.E. & Begg, G.S. 2010. The future central role of IPM in EU crop protection: how can ecological research be put into practice? Proceedings Crop Protection in Northern Britain 2010*,* Dundee, Scotland, 23-24 February 2010, 9-14

Birch, A.N.E. 2010. The future central role of IPM in EU crop protection: how can ecological research be put into practice? Society for Experimental Biology. Food Security and Safety Symposium*,* University of Lancaster, 13-15 September 2010

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.

Gordon, S.C., Birch, A.N.E. 2010. Raspberry cane midge. *Compendium of Raspberry and Blackberry Disease and Insects*, American Phytopathological Society, USA, 2nd edition, (in press).

Łabanowska, B.H., Cross. J.V. Raspberry Cane Midge - *Resseliella theobaldi* (Barnes) – Flight And Eggs Laying Dynamics On Two Years Old Fruiting Raspberry And Date Of Control**.** Journal of Fruit and Ornamental Plant Research, 16, 315-323.

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.

Presentations and technology transfer activities

2006. A 30-minute lecture was given by J Cross to the ADAS/EMR Soft fruit conference entitled 'Addressing Pesticide Residues in Raspberries through a New HortLINK Project.' A summary of the presentation was published in the conference proceedings.(See Cross et al., 2006 above)

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

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 September 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

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

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

7 Feb 2008. Planning meeting at Bioforsk As. N Birch met with Nina Trandem to coordinate testing oh raspberry beetle traps and lures in Norway during 2008 in cognate Bioforsk funded research projects on organic and conventional farms.

18 March 2008. Sainsbury's Biopesticide/IPM Conference, 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

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

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

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

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

Jan 2009. Nick Birch, Jerry Cross and others in the Hortlink project assisted Agrisense Ltd with the design and wording of growers technical brochure: 'Integrated Pest Management tools in Soft Fruit - raspberry beetle and raspberry cane midge monitoring kits'.

19 May 2009. Tim O'Neill delivered a 15 minute lecture 'Integrated management of raspberry fruit botrytis' and the 61st International Crop Protection Symposium, Ghent, Belgium.

12 June 2009. J Cross gave a 20 minute presentation to the Defra Food and Farming staff entitled 'Crop Protection Research at East Malling' where the sex pheromone work on raspberry pests was briefly described.

July 2009. Nick Birch featured on STV news discussing the development and use of raspberry beetle trap and lure as a monitoring kit for Soft Fruit IPM.

July 2009. Raspberry beetle trap and lure featured on Beechgrove Garden TV programme; this opens opportunities to market the trap to gardeners (Agrisense and SCRI in discussion currently to further this option).

16 July 2009. SCRI hosted 'Fruit Future' event. HortLINK and RERAD Soft Fruit IPM research and outputs featured.

22 September 2010. A Berrie presented a paper at the IOBC soft fruit meeting in Budapest on integrated control of Botrytis in raspberry.

30 October 2009. J Cross gave a 40 minute presentation and lead a discussion to a large group of store managers and technologists from a multiple retailer on the subject of 'Reducing pesticides and pe4sticide residues in fruit' where the work in the project was reported in detail

17 November 2009. J Cross gave a presentation to > 100 growers and technologists from a soft fruit PO entitled 'Reducing residues in raspberries'

5 February 2010. J Cross gave a 1 hr lecture to Ashford Beekeepers on the subject of 'Crop protection in fruit crops and its risk to bees' where the alternative technologies for pest control on raspberries developed in the project were presented.

11 Feb 2010. J Cross gave inaugural professorial lecture at the University of Greenwich to a mixed audience of > 100 where the sex pheromone work in the project was outlined (See Cross, 2010 above).

9 June 2010. J Cross gave a 20 minute presentation to senior management from a multiple retailer entitled 'Crop Protection Research at East Malling' where the sex pheromone work on raspberry pests was briefly described.

23 July 2010. J Cross gave a 30 minute presentation and led a discussion amongst a gathering of technologists from a multiple retailer on the subject of 'Reducing pesticide residues in Fruit' where the outcomes of the project were presented.

30 September 2010. J Cross gave a 20 minute presentation to Defra entitled 'Crop Protection Research at East Malling' where the sex pheromone work on raspberry pests was briefly described.

4 November 2010. J Cross gave a training course to the technologists and store managers of a multiple retailer on the subject of IPM and pesticide residue reduction where the results of the project were reported.

15 February 2011. J Cross gave a 20 minute presentation to BBSRC staff entitled 'Crop Protection Research at East Malling' where the sex pheromone work on raspberry pests was briefly described.

16 February 2011. J Cross gave a presentation to the British Protected Ornamentals Association Conference near Daventry entitled 'Pheromones: Their use for pest monitoring and control' where the relevant findings of this project were described in detail as leanings for other UK Horticulture sectors.

15 March 2011. J Cross gave a 1 hr lecture to the Mid-Kent Beekeepers on the subject of 'Crop protection in fruit crops and its risk to bees' where the alternative technologies for pest control on raspberries developed in the project were presented.

16 March 2011. R Harnden and J Cross gave a 20 minute presentation entitled 'Advanced IPM: New pest and disease management techniques for fruit growers' to the HortLINK PMC where the achievements of this project and 7 others were overviewed