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Project number:	TF 223
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Date project commenced:	01/04/2015

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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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.

Michelle Fountain

Project leader, Deputy Head of Pest and Pathogen Ecology
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Signature ..... Date 31 Mar 2019.....

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GROWER SUMMARY

Background

Project TF 223 is a five year project which commenced in April 2015. The project is investigating solutions to the key tree fruit diseases and pests, namely: European apple canker, scab, powdery mildew, *Monilinia* species and bacterial canker affecting stone fruit, codling and tortrix moths including Blastobasis, pear sucker, apple fruit rhynchites weevil, apple sawfly, pear weevils and phytophagous mites. In the first year, work focused on European apple canker, powdery mildew, codling and tortrix moths and apple fruit rhynchites weevil. In the second year research focused on European apple canker, apple foliar diseases, bacterial canker of stone fruit, codling moth, tortirx moths, weevil affecting pear buds, pear sucker and associated natural enemies. In the third year, work continued on European apple canker and apple powdery mildew and we began trials for control of Monilinia diseases on stone fruit. Entomology work focused on blastobasis, a weevil affecting pear buds, pear sucker and their associated natural enemies (NE) and establishing trial sites to enhance NEs in newly established orchards. For ease of reading, this grower summary report is split into sections for each of the diseases and pests worked on in the fourth year. Full details of each objective are presented in the Science Section of this report.

Objective 1. Surveillance

Headlines

- Vf (scab resistance gene) breaking strains of scab have been observed in the UK
- A new apple rot pathogen, *Neofabrae kienholzii,* has been reported for the first time in the UK
- A new pest of pear, *Anthonomus spilotus,* has been reported for the first time in the UK
- A new species of aphid, Green citrus aphid - *Aphis spiraecola*, has been reported in South East England apple orchards which is more resistant to insecticides

Background and expected deliverables

The surveillance objective provides the opportunity for ongoing activities to continue and be reported. Such activities include the monitoring of scab virulence on indicator trees, undertaking an apple rot survey and horizon scanning for emerging and future pest and

disease threats to the UK tree fruit industry. This objective aims to keep the industry up to date with the pest and disease threats which ultimately lead to yield losses and provides information for the industry to inform future research targets and priorities.

Summary of the project and main conclusions

Scab virulence: This task involves the monitoring of an indicator orchard, planted as part of a large pan-European project in which the same indicator cultivars are planted in 25 European countries. As in previous years the severity of the disease epidemic on the *Vf* (scab resistance gene) containing cultivars was comparable to the disease incidence on Gala. This confirms that the local scab population has broken the resistance conferred by *Vf*.

Apple rot survey: Of the 52 samples assessed from the 16/17 storage season, overall average loss was 1.5%, lower than recent past surveys. Nectria rot was the most prevalent rot with an overall incidence of 33%, Brown rot (*Monililnia*) was the next most prevalent rot (19.3%) followed by *Gloeosporium* (12.4%), *Penicillium* (11.2%) and Botrytis (9.2%). *Phytophthora* was only found in a single sample.

Neofabraea kienholzii, a pathogen closely related to those which cause Gloeosporium rot has been reported for the first time in the UK. Gloeosporium rots (caused by *Neofabraea*) have been increasing in prevalence in recent apple rot surveys. *N. Kienholzii* adds to the list of *Neofabraea* known to occur in the UK (*N. perrenans* and *N. vegabunda*).

In 2017/18 there were relatively low overall losses (1.6%) from the rot survey, similar to the previous year and partly due to a relatively dry harvest period in September. Neonectria continued to contribute to losses in susceptible varieties such as Gala, Jazz and Cameo, although the weather around blossom was only moderately favourable. Changes in rot incidence of brown rot and Botrytis rot may be more related to the change in varieties assessed. Botrytis tends to be more prevalent in Jazz, associated with missing stalks, whereas brown rot is more prevalent in Cox and Bramley. In 2017/18 only one Cox sample was assessed, compared to eight in each of the previous two years.

***Drosophila suzukii* (spotted wing drosophila – SWD)** numbers were delayed in 2018, compared to previous years, because of the cooler spring. However, by the end of the year, numbers were similar indicating that generations increased through the season and did not seem to be deterred by the hot, dry, weather.

Summer fruit tortrix was detected for the first time in the West Midlands during the 2015 growing season and it is recommended that growers now monitor for this pest in the region using pheromone traps alongside codling moth and fruit tree tortrix monitoring traps.

Brown Marmarated Stink Bug (BMSB): Monitoring traps in the South East and East of England did not detect any incursions of the pest. Monitoring will continue in 2019.

Anthonomus spilotus: A weevil found in pear orchards which has been damaging spring flower and leaf buds over the last two to three years, was identified as *Anthonomus spilotus* by the National History Museum and NIAB EMR in 2017, and is new to the UK. It has also been recently identified as an invasive pest in Belgium. Progress was made on the estimation of damage and the susceptibility to specific control products. More details are found in Objective 10.

Pear shoot sawfly: The RHS reported sightings of pear shoot sawfly (*Janus compressus*) in 2016. This has not been seen in commercial pear as far as we are aware.

Green citrus aphid: A new species of aphid, green citrus aphid (*Aphis spiraecola*), was reported in South East of England apple orchards. This species is difficult to distinguish and is more resistant to aphicides.

A table of additional pest and disease threats relevant to tree fruit growers is presented in the science section of this report with links to useful resources.

Financial benefits

Current, emerging and newly introduced pests and disease can have a devastating effect on yield and economic return to businesses. This objective enables the ongoing monitoring of these threats helping to inform future research priorities.

Action points for growers

- Continue to use the rot risk survey available in the Apple Best Practice Guide on the AHDB website, to limit loss of apples in store
- Monitor for summer fruit tortrix moth in the west of England
- Keep an eye on trade press for important announcements from the animal and plant health agency (APHA) about invasive pests and disease which will affect your business such as *Xylella fastidiosa*

Objective 2. Neonectria

Headlines

- Long term trials were established to determine the effects of rootstock/interstock choice and biological soil amendments on susceptibility/tolerance to European apple canker
- Experiments have identified rootstocks which have reduced susceptibility to canker

- A biological treatment which reduces canker infection, particularly in stoolbeds was identified
- Application of wound protectant treatments to pruning cuts using secateurs with a chemical dispenser can significantly decrease the incidence of apple canker (*Neonectria ditissima*) infection

Background and expected deliverables

European apple canker caused by *Neonectria ditissima*, is a devastating disease of apple which has been increasing in significance over the past 10-15 years as the industry has changed agronomic practices and cultivar choice. This objective looks at various factors such as rootstock/interstock choice and the use of biological amendments which, together with work from other projects, will contribute to the development of a systems approach for canker control from the nursery to the orchard.

Traditionally used wound paints to protect pruning wounds from *Neonectria ditissima* have been removed from the market in the past few years due to the high labour costs required in application, resulting in a lack of demand. Newly available application products, such as chemical dispensers attached to pruning secateurs, have the potential to reduce labour costs involved with protecting pruning wounds from canker infection by treating them at the same time as hand pruning. An initial trial looking at wound protectants in 2017 indicated that biological and chemical products may have a protective effect on the pruning wounds when used in conjunction with a polymer to seal the wound. The purpose of this trial is to evaluate efficacy of a selection of protectants that can be applied to wounds during tree pruning

Summary of the project and main conclusions

Long-term trials have been established on multiple sites to determine the effect of rootstock/interstock and biological soil amendments on canker incidence and severity. The rootstock trials are evaluating a panel of commonly used rootstocks alongside several advanced selections from the NIAB EMR and Geneva rootstock breeding programmes.

The amendment trials are evaluating the effect of arbuscular mycorrhizal fungi (AMF), plant growth promoting rhizobacteria (PGPR), Trichoderma and Biochar in both newly planted orchards and stoolbeds. Trichoderma significantly reduced the incidence of canker in the stoolbed at one trial site. This treatment also reduced total canker incidence at a second orchard, although this was not statistically significant.

The protectant treatments used in the wound trial included a biological (coded product BCP511B), chemical (tebuconazole) and physical (BlocCade) treatment. The wound

treatments were applied in April 2018 to trees in the trial during pruning, using Felco 19 secateurs with a chemical dispenser. The marked cuts were then inoculated with canker spores 24 hours after application of the wound treatments to simulate an infection event. The trial was monitored and recorded for the presence of canker in the marked cuts as well as regrowth and any phytotoxic effects of the treatments.

Some degree of preventive effect was seen in all of the treatments, with the tebuconazole and tebuconazole + BlocCade treatments showing significant reduction of canker development. Although the biological treatment showed a reduction in canker development compared to the control, it was not significantly better. It is probable that this biological treatment did not have sufficient time to establish on the cuts before the wounds were inoculated resulting in reduced performance.

Regrowth was generally low for all treatments, although there was slightly higher regrowth in shoots treated with BCP511B, although the effect was not significant. No phytotoxic effects were seen on the trees. The branches treated with tebuconazole (Folicur) formed good callouses over the pruning wound.

Financial benefits

European apple canker is a devastating disease that has an economic impact from plants grown at nurseries, orchards, to fruit sold at stores. This project focuses on key areas within the supply chain to develop an integrated approach to canker control and reduce financial losses caused by the disease.

The use of preventive treatments on pruning wounds to reduce infection is a useful tool in developing an integrated approach to canker control. Application of treatments at the same time as hand pruning using secateurs with a chemical dispenser can reduce the labour cost of application to growers as it can be done in a single pass.

Action points for growers

- With the results generated to date, and those we will generate in the final year of the project, we will have a clearer idea of the rootstock/interstock varieties that are the most promising for reducing canker
- Application of Trichoderma appears to be promising for reducing canker particularly in stoolbeds. This experiment is being repeated this year
- It is still important to be vigilant with visual inspection, identifying trees which are showing canker symptoms and limiting abiotic stress as far as possible when planting out and establishing new orchards

- Treatment of pruning wounds with tubuconazole with or without a polymer can significantly reduce canker incidence even when high levels of inoculum are present
- The use of directed sprays of preventive treatments during hand pruning can be a useful tool to reduce canker development in an orchard

Objective 3 Foliar diseases

Task 3.1 Determine optimum timing of treatments to target the over-wintering phase of mildew to disrupt the lifecycle (NIAB EMR)

Headline

- A new approach to reducing over-wintering powdery mildew has been proposed.

Background and expected deliverables

The uptake of biological control agents (BCAs) has been limited for disease control in orchard crops despite their great potential to replace conventional control products as part of an integrated pest management programme. Barriers for the uptake of BCAs in orchard systems include the higher cost/ha and their reduced/variable efficacy relative to conventional products. If applied during the season when a pahtogen is developing rapidly, there is a delay before the BCA has time to establish and gain control. Crop damage therefore often occurs before control is achieved. This task aims to develop understanding of interactions between potential antagonists and the pathogen (or pathogen substrate) to inform strategies which can target the overwintering phase.

Powdery mildew (*Podosphaera leucotricha*) mainly overwinters as mycelium in floral and vegetative buds. *Ampelomyces quisqualis* (AQ) is a mycoparasite of powdery mildew. Commercial preparations of AQ such as AQ10, have been successfully used in greenhouse and field-grown vegetable crops to gain control, usually with reduced fungicide inputs. AQ10 was one of the best performing BCAs in the SCEPTRE project trials when applied throughout the season and in combination with fungicides in a managed programme. However the control achieved was not commercially acceptable. One of the disadvantages of using AQ10 is the slow growth rate of this parasite. This has led to the strategy proposed here; to target the overwintering phase of the disease offering a long interaction period between parasite and powdery mildew. Trials were set up over the summer of 2016 to test whether the BCA is incorporated into the bud, whether the parasite can survive over winter and whether the stratergy is effective at reducing inoculum. These trials were inconclusive. The objective of trials in 2018 was to re-evaluate the strategy with AQ10 and to include an alternative BCA –

a bacterial-based product from Bayer – which has been very effective in controlling strawberry powdery mildew.

Summary of the project and main conclusions

The trial was located in a Gala orchard at NIAB EMR. The plan was to target overwintering mildew in vegetative buds by applying treatments starting towards the end of shoot growth at the end of summer. However, shoot growth stopped early in 2018 due to the hot dry conditions in July so treatments were not applied. There are plans to repeat the trial in a new project.

Financial benefits

The quantity of primary mildew overwintering in fruit and vegetative buds is key to the new season mildew epidemic. For effective mildew control, primary mildew must be minimised. The availability of methods to reduce overwintering mildew would enable improved mildew control in the growing season with reductions in fungicide use and consequent savings in costs.

Action points for growers

- There are no action points at present

Task 3.2 Efficacy of alternative chemical treatments to fungicides (NIAB EMR)

Headline

- Alternative products show promise to control powdery mildew when incorporated into fungicide spray programmes

Background and expected deliverables

Foliar diseases of apple require season-long control. For powdery mildew control, susceptible cultivars require season long programmes of fungicides (~10-15 sprays) to protect shoots and buds and prevent high levels of over-wintering inoculum. Routine sprays of fungicides cost around £700/ha/annum with a large proportion spent on scab and mildew control. Despite such stringent measures, scab and mildew control can break down during the growing season resulting in disease epidemics. Mildew epidemics, in extreme cases, can defoliate affected trees reducing yield and causing russeting of the fruit. With a reduction in the availability of effective products against powdery mildew, due to changing regulations and fungicide insensitivity, new approaches to disease control need to be developed which are less dependent on conventional fungicides. This project aims to assess alternative treatments

based on physical and biological properties with the aim of reducing fungicide applications whilst maintaining acceptable disease control.

Summary of the project and main conclusions

The physical control products SB Invigorator and Wetcit have shown consistently good results as have the biostimulants Cultigrow CBL, Trident and Mantrac. In 2018, these were reassessed in combination with fungicides in programmes applied at 7- or 14-day intervals and compared with fungicide only programmes in a Gala orchard at NIAB EMR. The incidence of primary and subsequent secondary mildew in 2018 was high as a result of favourable weather conditions at the end of May. Over the ten weekly assessments, the lowest incidence of secondary mildew was found in the 7-day fungicide only programme and the highest in the 14-day fungicide only programme. Plots receiving the combined programme had significantly less mildew than those receiving the 14-day fungicide only programme, indicating some benefit from the alternative treatments. There were no phytotoxic effects of these treatments, but two of the treatments resulted in lower fruit set, so further evaluation of these products in programmes with fungicides is needed before recommendations are made to growers.

Financial benefits

A high incidence of powdery mildew in apple orchards reduces yield and fruit quality. Generally 10-15 sprays are required to control powdery mildew and to ensure buds are free of overwintering mildew. This is costly and with a limited number of effective fungicide products available control is not always ideal. Identifying effective alternative products reduces the dependence on fungicides and possibly also reduces costs.

Action points for growers

Some alternative products have been identified but further evaluation of these products in programmes with fungicides, particularly on their effects on fruit quality is needed before recommendations can be made to growers.

Objective 4. Stone fruit diseases

Headline

- Coded fungicide HDC F266 was effective in reducing brown rot and Botrytis rot on cherries

Background and expected deliverables

Losses resulting from Monilinia sp. in stone fruit are hard to quantify because infection occurs throughout the season (blossom and fruit pre- and post-harvest). Post-harvest development of brown rot limits the storage potential of UK stone fruit and a few rotten fruit in one punnet can lead to food retailers rejecting whole consignments. Two *Monilinia* species are present in the UK; *Monilinia laxa* and *Monilinia fructigena*. Currently diseases associated with Monilinia are controlled by 1) inoculum removal and 2) fungicides. The former was seldom practiced due to the associated increase in cost. However in recent years with the advent of spotted wing drosophila (SWD), removal of rotted and ripe fruit at harvest has become a management necessity to control SWD but with obvious benefits in rot control for fungal diseases. Fungicides are applied at blossom and pre-harvest including Signum and Switch, but are not totally effective and pre-harvest applications present a residue risk. This project will evaluate newly available products including plant health promotors, biological control agents and fungicides, which in combination could provide a more effective programme for brown rot control.

Due to the late frosts during the 2017 growing season which coincided with blossom and early fruitlet development, the yield within the trial orchard was significantly affected. In addition to the frost, the trial was severely hit by SWD prior to harvest, despite the use of weekly control sprays. Together the frost and the SWD damage meant that very little fruit was available for picking by harvest and it was impossible to draw any meaningful conclusions on the products evaluated. Therefore the trial in 2018 was largely a repeat of the 2017 work.

Summary of the project and main conclusions

In a small plot trial on cv. Skeena the control of blossom wilt and brown rot achieved by a range of coded test products, including a biostimulant and an elicitor (HDC F266, HDC F267, HDC F268, HDC F 269, HDC F270, HDC F271) was compared with that achieved by the biofungicide Serenade, standard fungicide products (Signum and Switch) and an untreated control. Treatments were applied as two sprays at blossom and two pre-harvest, except for HDC F271 (biostimulant) which was applied at three week intervals from blossom. Plots were assessed for blossom wilt soon after petal fall and for rots at harvest and in post-harvest tests after storage for three days and incubation at ambient temperature for 7 days. Yield and fruit size were also recorded. The results obtained were as follows.

The incidence of blossom wilt (*M. laxa*) was negligible. The incidence of rots at harvest was low (5% in untreated plots). There were no significant effects of treatments on rot incidence, but the lowest incidence of rots was recorded in Treatments 3 (HDC F266) and 4 (HDC F267) and in the standard treatment 2 (Signum/Switch). The rot incidence increased in post-harvest

tests to over 30% in untreated plots after 7 days' incubation. The lowest incidence after 3 days' incubation was recorded in Treatment 2 (Signum/Switch), Treatment 3 (HDC F266), Treatment 4 (HDC F267) and Treatment 8 (HDC F270). However, the differences were not quite significant compared to the untreated control. At the final assessment after 7 days' incubation the lowest rot (accumulated rot) incidence was again recorded in Treatment 2 (Signum/Switch), Treatment 3 (HDC F266) and Treatment 8 (HDC F268). Only Treatment 3 had significantly less rot than the untreated control. The effects of the treatments on the incidence of *M. fructigena* was not significant, however, the lowest incidence of *M. fructigena* was recorded in fruit treated with HDC F266 or HDC F268. All treatments apart fromT6 and T7 significantly reduced the incidence of *M laxa* with the lowest incidence in fruit treated with Treatments 3 (HDC F266) or 4 (HDC F267). All treatments, apart from T6, significantly reduced the incidence of Botrytis compared to the untreated control. The lowest incidence was in fruit treated with T3 (HDC F266) which performed significantly better than most other treatments. Several of the fungicides evaluated in this trial were effective in reducing rotting, in particular HDC F266 (Treatment 3) which was the most consistently effective of the fungicides tested. Of the alternative products tested HDC F269 and Serenade were ineffective. The effect of HDC F271, a biostimulant was variable. It was not effective in boosting plant resistance to *M fructigena* which is a wound pathogen but more successful in improving resistance to *M laxa* and *Botrytis.* There were no significant effects of treatments on yield or fruit size. There were no phytotoxic effects of any of the treatments.

Financial benefits

Brown rot is an important disease of cherries causing significant losses both in the orchard and post-harvest and limiting the storage of cherries to extend the marketing period. The availability of a range of effective fungicides to control the disease is vital to the profitability of the industry.

Action points for growers

- Orchard sanitation is important for brown rot control, removing all mummies from the orchard is important
- Blossom and pre harvest application of fungicides are generally required in most seasons
- The effective products identified in 2018 in this trial are not currently approved for use on cherries.

Task 4.3 Bacteriophages against bacterial canker in cherry

Headlines

- Large collection of native bacteriophages isolated from UK orchards have been established and partly characterised
- The first year of efficacy trials on detached cherry leaves have identified some phages with biocontrol potential

Background and expected deliverables

Pseudomonas syringae pathovars; *syringae* (PSS), *morspronorum* race 1 (PSM1) and *morspronorum* race 2 (PSM2) cause a destructive disease called bacterial canker on prunus species. This disease reduces yields; cankers can girdle branches and trunks causing wilting and tree death. Until now growers have relied on copper treatments at leaf fall to reduce bacterial populations. However copper is no longer permitted to be used as a plant protection. Moreover, there have been reports of emerging bacterial resistance to chemical control. Bacteriophages (phages) are natural antimicrobial agents with enormous potential to treat bacterial diseases. Phages very effectively reduce very specific bacterial populations and have therefore minimal unintended consequences in terms of inhibiting non target and beneficial organisms. This objective is focused on i) finding and characterising native UK phages against prunus canker pathogen and ii) test their efficacy on plants to provide proof of concept for their use in disease management.

Summary of the project and main conclusions

Research team from University of Reading have isolated 70 potential biocontrol phages different cherry orchards in UK. All isolated phages were active against a PSS strain and 10 phages were active against PSM1 and PSM2 strains. Six phage isolates were found to have broader host range with activity against PSS, PSM1 and PSM2 and are therefore good candidates for further characterisation and efficacy testing. Importantly, none of the isolated phages showed any activity against *Pseudomonas fluorescens*, beneficial bacteria related to canker pathogen, which demonstrates specific action of phages against pathogen bacteria.

In parallel to phage collection we have established a phage efficacy testing method. We have optimised a detached leaf assay where cherry leaves from the orchard were inoculated with PSS, PSM1 and PSM2 in laboratory conditions. Leaves were then treated with phages and necrotic lesion symptoms observed to ascertain their ability to control disease. This year we have used five phage isolates collected from NIAB EMR and Brogdale Collection sites during preliminary study in 2015. The best success was observed when PSM1 inoculated leaves

were treated with the phages. Three out of five isolates significantly reduced PSM1 lesions incidence (from 100% to 60%) and lesion size compared to untreated control establishing the first line of evidence for phage efficacy against cherry canker pathogen. We have also sprayed the five phage blend on inoculated detached shoots which are currently incubating and canker incidence will be assessed in March 2019 to confirm their efficacy in woody part of the plants.

In the next year we plan to select the best phages from our collection based on their characteristics on in-vitro agar assays in the lab. The phages with best antimicrobial potential will be tested on detached leaf and to confirm their activity on the plants. Phages will be also tested in an orchard field trial if approved by CRD.

Financial benefits

This project endeavours to speed up the development of new integrated approach to canker control and reduce financial losses caused by this disease. These approaches are still being evaluated and will be reported in subsequent reports.

Action points for growers

No action points at this time.

Objective 6. Codling and Tortrix Moth

Headline

- Early attempts to identify a sex pheromone from Blastobasis for monitoring purposes have been unsuccessful

Background and expected deliverables

Larvae of the moth *Blastobasis lacticolella*, Rebel, 1940 (Synonym: *decolorella*) (Lepidoptera: Blastobasidae) feed on the surface of apple and pear fruits in mid- and late- summer, often where clusters are touching, causing large open scallop-shaped wounds in the flesh and making attacked apples un-saleable. Very severe damage can result if the pest is allowed to increase over a number of years unchecked, especially on short stalked varieties such as Bramley and Egremont Russet which are very susceptible. Growers currently have no means of identifying whether they have a problem other than the occurrence of damage the previous year, which is often confused with damage caused by other apple moth pests. It is also difficult to time sprays accurately against Blastobasis.

A recent increase in the use of mating disruption techniques such as RAK 3+4 for codling and tortrix moth control along with use of granulovirus, has resulted in a reduction in application of broad-spectrum control products. Occasional but severe outbreaks of Blastobasis have consequently occurred, requiring application of products which negated the benefit of using mating disruption or granulovirus. There is a clear commercial need to develop a pheromone monitoring trap for Blastobasis so that growers can determine whether they have a problem and time insecticide applications correctly.

Summary of the project and main conclusions

Field trapping experiments with three potential pheromone blends based on previous work were carried out in Northern Ireland, Hereford and Kent. A number of moths were caught, but analysis of sample moths by DNA barcoding of COI gene locus and comparison with NCBI Database indicated that probably none were *Blastobasis lacticollela*. The majority identified were *Rhigognostis incarnatella* and six out of eight were from traps baited with blend C, 1:10 Z11-16:Ac : Z11-16:Ald. This species is related to the diamondback moth, *Plutella xylostella*, the pheromone of which is a 1:1 blend of Z11-16:Ac and Z11-16:Ald. These results confirmed that the lures were working as intended and would have trapped *B. laticollela* if the pheromone blend was correct and this species was present.

Field trapping was repeated in 2018 and once again blends of (Z)-11-hexadecenal and (Z)-11-hexadecenyl acetate failed to attract *Blastobasis laticolella* moths in field trapping tests, even though this species was clearly present as indicated by catches in light traps. Rearing *B. laticolella* adult moths from larvae collected in the field proved a real challenge, but some were reared through to adult. Extracts of the pheromone glands of female moths were made both from moths collected in the field which were probably mated and from virgin female moths reared from larvae in the laboratory. In analyses of extracts by GC-MS, potential pheromone components including (Z)-11-hexadecenal, (Z)-11-hexadecenyl acetate, (Z)-5-decenyl acetate and (Z)-5-decenol could not be detected. (Z,Z,Z)-3,6,9-Nonadecatriene was identified as a potential component of the female sex pheromone. However, it was subsequently shown to be present in extracts from both female and male moths and did not attract male *B. laticolella* moths in the field. Further work is required and growers who belived they have populations of Blastobasis in their orchards are encouraged to make contact with Michelle Fountain and her team at NIAB EMR.

Financial benefits

- No financial benefits have been identified at this stage of the project

Action points for growers

- No action points have been identified at this stage of the project

Objective 7. Natural predation of pests

Objective 7.1. Improving the reliability of natural predation of pests

Headline

- Six trial orchards have been set up to monitor the benefits of hastening the influx of natural predators into newly planted orchards to reduce pest damage.

Background and expected deliverables

Establishing new orchard crops requires substantial investment (~£35k/ha for apple) and growers need confidence that their orchards will crop reliably and that their fruit will find a profitable market. Ecological succession is the observed process of change in the species structure of an ecological community over time. The community begins with relatively few pioneering plants and animals and develops through increasing complexity until it becomes stable or self-perpetuating, as a climax community. Newly planted orchards have an un-established ecosystem. The recently tilled ground in newly planted orchards often has minimal, simplified or absent vegetation cover with a low diversity of plant species resulting in low pollen and nectar provision and low refugia and structure. The tree bark and canopy are simple compared to older established trees affording little availability for predatory arthropods to gain refuge. Hence, local, natural predators and pollinators have not built up and established in new orchards leading to random, sporadic, attacks from a number of pest species which can then be difficult to control.

In this project, work has been instigated to hasten the influx of natural predators in new orchards. Six replicate commercial apple orchards were chosen in 2017 and secured for experimental purposes through help from Caroline Ashdown at Worldwide Fruit. In each orchard, 0.25 ha is being treated with ecological enhancement interventions.

In each treated area, interventions included the sowing of alleyway seed mixes (including yarrow, ox-eye daisy, bird's foot trefoil, self-heal, red campion and red clover), and the provision of earwig refuges and hoverfly attractants. Each treated area is being assessed and compared to an untreated area of the same orchard throughout 2018 and 2019.

Summary of the project and main conclusions

In 2018, four of the six alleyway seed mixes established very well with over 50% coverage of sown species. Fewer aphids were observed in the apple trees on treated plots in spring. Unlike in the control plots, no apple leaf curling midge damage was found in the treated plots. Fewer fruit tree red spider mites and predatory mites were found in the treated plots than the control plots. However, in contrast, there were higher populations of rust mites and predatory mites in the treated plots than in the control plots. In the treated plots, there were fewer fruits with codling moth damage and also higher numbers of hoverfly adults. Given this is the first year of recording, the results should still be treated with caution.

Financial benefits

- No financial benefits have been identified at this stage of the project.

Action points for growers

- No action points have been identified at this stage of the project.

Objective 7.2. Dynamic pear sucker/ predator chart

Headline

- Threshold numbers of pear sucker eggs and natural enemies will enable growers to decide on the need to implement pear sucker control measures

Background and expected deliverables

Pear sucker, *Cacopsylla pyri*, is still the major pest on pear with sporadic population growth in relation to warm dry weather and in orchards where the numbers of earwigs and anthocorids is not sustained. Emerging evidence from other AHDB and Innovate UK projects is showing that earwigs are important control agents for aphids and pear sucker. Additional research in the USA also demonstrates predation of codling moth eggs. Earwigs, hoverfly larvae, lacewing larvae, spiders and ladybirds are able to penetrate the leaf rolls (galls) caused by the various apple aphid species.

There are large differences, between orchards, in earwig populations and Project TF 196 has demonstrated that plant protection product use and timing may be, at least partly, responsible. However, anecdotal evidence is showing that earwigs can be patchily distributed within an individual orchard.

The aim of this study is to enable more effective monitoring, control product use and natural enemy build-up in pear orchards. It is expected that the application of control product interventions will be better timed.

Summary of the project and main conclusions

Six farms were involved in the study in 2016, 2017 and 2018. All participants were trained in the monitoring technique at the start of the growing season. Each grower selected three orchards (high, medium and low pear sucker populations) on each farm and allowed time for a worker to systematically assess the chosen orchards each week. The results were collated at least fortnightly by NIAB EMR and then shared with all participants.

Records of pear sucker eggs, nymphs and adults, and ladybirds, earwigs and anthocorids in the perceived low, medium and high pear sucker pressure orchards were made from March to September. The records were scrutinised and it was concluded that in general, sprays could be avoided where there were <1,000 pear sucker eggs per 30 shoots per week and >10 natural enemies per 30 shoots per week. More work is needed to determine the threshold of nymphs.

Financial benefits

Close monitoring of pear sucker and natural enemies can help to avoid use of unnecessary sprays and conserve natural enemies which control pear sucker. This will reduce the need for applications of products needed to control honey dew on trees. The reduction of pear sucker in the crop prevents direct damage to fruits as well as damage to overwintering bud and tree health.

Action points for growers

- Monitor pear sucker stages in the crop to accurately time Envidor applications and avoid sprays where unnecessary
- Use the monitoring of natural enemies such as earwigs, anthocorids and ladybirds alongside pear sucker monitoring to track the likely future control by these predators in the absence of sprays
- Consider releases of anthocorids early on if natural enemies are low, but think about the surrounding habitat to encourage long term resilience in populations
- Be considered with the choice, numbers and timing of spray applications. Think about spray frequency and impact on natural enemies

Objective 8. Apple sawfly

Headline

- Attempts are being made to discover the sex pheromone of apple sawfly for future monitoring

Background and expected deliverables

Apple sawfly is a locally common and problem pest, particularly in organic orchards where products for effective control are not available. However, timing of application relies on knowing when the first flight is occurring and when females are laying eggs. This project aims to identify the sex pheromone of the apple sawfly for use in future monitoring and mating disruption studies.

Summary of the project and main conclusions

Apple sawfly larval infected apples were collected in spring 2015, 2016, 2017 and 2018 from an unsprayed orchard at NIAB EMR. The apples were placed onto compost in mesh covered bins. Larvae were allowed to crawl out from the fruits and enter the compost. As apple sawfly has only one generation per year these were maintained outside until spring 2016 and spring 2017. However, no apple sawfly adults emerged and pupae were found to be infected with either bacteria or fungus, even when in 2017 bins were maintained with lids to prevent over wetting from rain. The previous winter had been very wet and it was speculated that the soil may have become too wet outside.

In spring 2017and 2018 apple sawfly infected apples were collected again and kept in Bugdorm cages under cover. As the larvae emerged from the apples and began to 'wander' they were transferred into smaller plant pots of compost. Six were kept at ambient conditions in an outside area under cover and 2 were stored at 6^{o}C for 2 months in 2017 and 5 months in 2018 to attempt to simulate a cold period. To date no adults have emerged, but pots will be bought into room conditions in spring 2018 for emergence of adults and headspace volatile collection for pheromone identification.

Financial benefits

- No financial benefits have been identified at this stage of the project

Action points for growers

- No action points have been identified at this stage of the project

Objective 9. *Anthonomus spilotus* in pear

Headline

- New damaging weevil pest of pear blossom identified as *Anthonomus spilotus* and is new to the UK

Background and expected deliverables

A new weevil pest of pear has been identified. The weevil is from the *Anthonomus* family of weevils known to feed and develop in buds and fruits of plants. Unlike *Anthonomus piri*, *A. spilotus* feeds and lays eggs in spring blossom and leaf buds. In order to control the weevil it is likely to be necessary to target sprays in the spring, before the flower clusters open. This objective aimed to establish the activity period, lifecycle and toxicity of commonly used control products. More research is needed to establish thresholds and to target spray timing more precisely.

Summary of the project and main conclusions

Extensive field surveys and damage assessments were done on four affected orchards in Kent. *Anthonomus spilotus* adult activity, eggs in buds and adult feeding damage was recorded from 8 March until 6 June in 2018. Weevils fed on and laid eggs in flower and leaf buds depending on availability. The percentage of flower buds damaged by adult feeding was 22.6% and the percentage of flower buds damaged by larvae 0.7%. The percentage of leaf buds damaged by adult feeding was 42.3% and the percentage of leaf buds damaged by larvae was 0.7%. Hence most bud damage was the result of adult feeding.

Fewer than 10% of the flowers in a truss were damaged by adult feeding and fewer than 16 % were damaged by larvae. Greater flower and leaf damage was observed when eggs/larvae were present. Hence the damage to flowers at one weevil per 40 taps is not the main consideration as only one of the six flowers is normally destroyed and only three to four Conference fruits can set to harvest on a single truss. The main consideration is the damage to leaves and photosynthetic ability for future years.

Even at very low levels of weevils (~one per 40 tree taps) ~60% of new leaves were damaged later in the season. We have not been able to set a damage threshold for this because the resultant health to the tree could not be estimated in this study. The majority of buds usually had one to three damage holes although buds with more punctures could be found.

There were indications that population activity may be sensitive to significant temperature changes, but more data is needed to reach a more accurate conclusion.

In laboratory tests in 2016, Gazelle did not give effective control, but Calypso at full and half field rate gave 80-90% mortality. Calypso, Hallmark, Gazelle and Spruzit were the most effective products against *A. spilotus* in the laboratory. High mortality and fast negative behavioural effects were observed in these treatments. However note that in this experiment, weevils received a direct application of the product. In a pear crop this scenario is less likely and weevils may be more likely to come into contact with dried residues.

In 2018 we determined whether control product efficacy can be improved through stimulating ingestion of the actives, spinosad and indoxacarb. Calypso was the most effective product against *A. spilotus* in the laboratory trial where shoots had been sprayed with products and then weevils allowed to feed. 100% mortality in nine days after ingestion was observed compared to the control group (40%). In 2019 we will examine the best timing of control measures in growers' orchards.

Financial benefits

Larvae in flower buds feed on flowers, but then also feed on emerging leaf shoots. This could affect yield but also the health of trees over the long term. It is essential to calculate thresholds for spraying and spray timing. It is estimated that a female weevil in the *Anthonomus* family can lay around 25 eggs in her lifetime.

Action points for growers

- Monitor pear orchards weekly from February by inspecting for feeding holes in unopened flower buds and then later on in leaf buds
- Continue to monitor until May
- Make a careful decision over the need to use control measures and the choice of product so that natural enemies are not affected
- Continue to monitor for the pest after control methods have been used

SCIENCE SECTION

Introduction

This five year project sets out to develop and implement strategies to manage key tree fruit diseases and pests, namely: European apple canker, scab, powdery mildew, *Monilinia* species and bacterial canker affecting stone fruit, codling and tortrix moths, pear sucker, weevils, apple sawfly and phytophagous mites. In light of future pesticide withdrawals, and ongoing consumer and environmental concerns about over reliance on pesticides, a focus on incorporating Integrated Pest Management (IPM)-compatible approaches with conventional pesticides is being adopted for each of the disease and pest targets.

Apple canker (caused by *Neonectria dittisima*) has become an increasingly important disease for the industry in recent years mainly due to increased planting of canker susceptible varieties. The disease is causing significant financial loses; from tree death during the establishment phase, loss of fruiting wood due to the pruning out of cankers and losses of fruit from pre and post-harvest rots. Previous studies have shown that the disease can remain asymptomatic in the host tree during the nursery phase and then express once planted in the production orchard. Disease can also spread from local sources surrounding the production site. A systematic approach, from nursery propagation, through orchard establishment to established orchards could give effective canker control; reducing losses during tree establishment and improving efficacy of orchard control.

Apple foliar diseases require season-long control. For scab and mildew control, susceptible cultivars require season long programmes of fungicides (~10-15 sprays) to protect shoots and buds and prevent high levels of over-wintering inoculum. Routine sprays of fungicides cost around £700/ha/annum with a large proportion spent on scab and mildew control. Despite such stringent measures, scab and mildew control can break down during the growing season resulting in disease epidemics. Mildew epidemics, in extreme cases, can defoliate affected trees reducing yield and causing russeting of the fruit. Scab infection of fruit renders it unmarketable and can lead to cracking which serves as entry points for rot fungi which subsequently develop in store. An integrated programme focused on reducing inoculum and promoting tree health/resistance could reduce fungicide applications whilst maintaining acceptable disease control.

Losses resulting from Monilinia sp. in stone fruit are hard to quantify because infection occurs throughout the season (blossom and fruit pre- and post-harvest). Post-harvest development of brown rot limits the storage potential of UK stone fruit and a few rotten fruit in one punnet can lead to food retailers rejecting whole consignments. Bacterial canker is an orchard (and nursery) problem resulting in a loss of profitability from poor establishment, removal of

affected trees and loss of fruiting wood. Novel IPM based strategies which complement a reduced fungicide programme will mitigate economic losses for growers, reduce residues for consumers and offer a much needed alternative to copper-based treatments which are no longer permitted for bacterial canker control.

Optimising spray coverage has obvious financial and environmental benefits whilst increasing the efficacy of control. Particularly in light of the potential withdrawal of certain active substances, it will be more important than ever to achieve maximum efficacy from the remaining products. This project will facilitate the uptake of equipment being developed in a TSB project by demonstrating the equipment for practical applications (i.e. determining optimum coverage of spray deposits for foliar pest and disease control).

Ecological succession is the observed process of change in the species structure of an ecological community over time. The community begins with relatively few pioneering plants and animals and develops through increasing complexity until it becomes stable or self-perpetuating as a climax community. Newly planted orchards have an un-established ecosystem. The recently tilled ground in newly planted orchards often has minimal or absent vegetation cover with a low diversity of plant species. The tree bark and canopy are simple compared to older established trees affording little availability for predatory arthropods to gain refuge. Hence, local, natural predators and pollinators have not built up and established in new orchards leading to random, sporadic, attacks from a number of pest species which can then be difficult to control.

We hypothesise that by providing ground cover and predator refuges and attractants in new orchards and ‘seeding’ orchards with natural enemies, early on, this will help to mitigate sporadic pest invasions and enhance ecosystem services much more rapidly. The aim of this objective is to accelerate, enhance and monitor the natural biological processes evident in more established orchards whilst providing information which could be used in established orchards to augment and improve habitat conditions for beneficial insects.

Pear sucker, *Cacopsylla pyri*, is still the major pest on pear with sporadic population growth in relation to warm dry weather and in orchards where the numbers of earwigs and anthocorids is not sustained. Emerging evidence from the HortLINK Cherry and Plum project (TF 194) and an EMR TSB project is showing that earwigs are important control agents for aphids and pear sucker. Additional research in the US also demonstrates predation of codling moth eggs. In addition, earwigs, hoverfly larvae, lacewing larvae, spiders and ladybirds are able to penetrate the leaf rolls (galls) caused by the various apple aphid species.

There are large differences, between orchards, in earwig populations and Project TF 196 has demonstrated that pesticide use and timing may be, at least partly, responsible. However,

anecdotal evidence is showing that earwigs can be patchily distributed within an individual orchard. The TSB earwig project is making good progress with a marketable device which could be used in newly planted trees to help encourage natural predation of pests. This will be available from 2016 for use in this project (confidential). We hypothesise that orchard niche availability has a significant influence on beneficial arthropod populations and subsequent pest control.

Project TF 218 is determining the most important predatory hoverfly species in apple orchards and exploring whether the adults can be enhanced by attraction with plant volatiles. If successful we could incorporate this technology in the latter stages of this project. In addition a PhD project on based enhancing useful hoverfly species in strawberry could be used to inform flowering species for incorporation into orchard alleyways.

Monitoring by visual inspection for apple sawfly (*Hoplocampa testudinea*) adults is generally too difficult for growers and agronomists and damage is often done before the pest is noticed, control then being scheduled for the following year or missed. Growers currently rely on sprays of thiacloprid (Calypso) and/or chlorpyrifos for control. These products are fairly effective, but they are harmful to earwigs. Semiochemical based pest specific monitoring traps for these pests would be a significant advancement, aiding decisions on the need for and timing of sprays. Note that alternatives to thiacloprid and chlorpyrifos for control of these pests are also needed and is anticipated that testing of alternatives will be done through the new AHDB programme – IMPRESS. Project TF 220 is to examine the effects on earwig populations of early season (pre-petal fall) versus mid-season (fruit development) applications of one versus two sprays of acetamiprid (Gazelle) or thiacloprid in apple (2015).

EMR and NRI in HortLINK project HL01105 have identified the sex pheromone of the blackcurrant sawfly, *Nematus olfasciens*. Research has also begun on common gooseberry sawfly, *Nematus ribesii,* (TF 147) and is due to finish in early 2017. As the apple sawfly, *Hoplocampa testudinea*, is closely related to these two species (Tenthredinidae family) there is an opportunity to use the methods and information gathered from the other projects to identify the pheromone of the latter pest for more accurate monitoring and even mating disruption in future years.

Objective 1 - Surveillance

1.1. Scab virulence

Aim

Monitor scab virulence on indicator trees (EMR, Yr 1-5).

Summary

This task involves the monitoring of an indicator orchard at NIAB EMR, planted as part of a large pan-European project in which the same indicator cultivars are planted in 25 European countries. The data collected from each participating group is compiled by the project coordinator based in Switzerland. Scab incidence was recorded at the end of August 2018 and has been submitted to the project coordinator. Analysed data will be made available as part of the wider project. Severity of the disease in 2018 was lower than previous years of monitoring (since 2015). Resistance breakdown in *M. x floribunda 821,* the source of the *Rvi6* scab resistance gene (formerly known as *Vf* - the most extensively used R gene in breeding for scab resistance), was again confirmed as in the previous two years. However no breakdown has yet been seen on the trees in the plot of the domesticated cultivar Priscilla which carries the *Rvi6* gene. Scab was also found at a less severe level on the indicator genotype for the *Rvi8* gene; unlike *Rvi6* this gene cannot be found in any commercially available cultivars. Isolates of scab from cultivars containing resistance genes have been collected for DNA extraction to determine the genetic changes in the population which has resulted in breaking the resistance this may in turn aid the identification of new sources of resistance.

1.2. Apple rot survey

Aim

Undertake apple rot survey to monitor disease incidence (EMR, Yr 1-5)

Introduction

This task is a continuation of the apple rot survey which has been undertaken over the last century, most recently as part of the fellowship project. The survey involves visiting pack houses during the months of January – March to determine the type and incidence of rot causing pathogens.

Results

Weather in March (26.2 mm rain) and the first half of April (1.0 mm rain) was relatively dry, and less favourable for production of conidia on Neonectria cankers. Weather conditions from full bloom to the end of May (the main period of risk for Neonectria infection of fruit) were wetter (54.4 mm rain and 18/42 days with rain) giving a moderate risk for Neonectria fruit rot especially in orchards with a high incidence of canker. The second half of July (45.8 mm rain) and August (49.4 mm rain) were relatively wet giving favourable conditions for some storage rots such as Gloeosporium and ensuring active Phytophthora in the soil. However, the

September harvest period was relatively dry (21.4 mm rain at EMR) resulting in a low Phytophthora rot risk. The number of samples assessed for some varieties, particularly Braeburn and Cox was reduced due to these varieties being frosted in parts of Kent resulting in poor yields and reduced quantities in store.

Table 1.1 summarises the losses attributed to each rot pathogen during the 2017/18 storage season. In total 32 samples were assessed over 32 visits. The main cultivars sampled were Gala (8 samples), Bramley (9), Cox (1), Braeburn (1) and Jazz (8). The overall average loss was 1.6% which was similar to 2016/17. Losses of Gala (2.3%) and Jazz (3.2%) were higher than previously and mainly attributable to a Neonectria and Botrytis rots. Losses in the other apple varieties sampled ranged from 0.5-2.0%. Neonectria rot, as expected from the weather conditions around blossom, was the main rot identified in the 2016/17 survey with an overall incidence of 32.4%. Neonectria rot was particularly high in canker susceptible varieties where inoculum is prevalent; Gala (64.2%), Cameo (57.9%) and Jazz (52.6%). Botrytis was the next most prevalent rot causing an overall average loss of 27.8% followed by *Gloeosporium* (10.6%), *Penicillium* (10.6%) and Brown rot (7.6%). *Phytophthora* was only found in a two samples at very low incidence, which is as expected from the relatively dry harvest period.
The average incidence of rots in the previous two years is also included in Table 1.1. Rot incidence is very similar apart from a lower incidence of brown rot and a higher incidence of Botrytis in 2017/18.

Discussion

Relatively low overall losses (1.6%) were recorded during the 2017/18 apple rot survey similar to the previous year and partly due to a relatively dry harvest period in September. Neonectria continued to contribute to losses in susceptible varieties such as Gala, Jazz and Cameo, although the weather around blossom was only moderately favourable. Changes in rot incidence of brown rot and Botrytis rot may be more related to the change in varieties assessed. Botrytis tends to be more prevalent in Jazz, associated with missing stalks, whereas brown rot is more prevalent in Cox and Bramley. In 2017/18 only one Cox sample was assessed, compared to eight in each of the previous two years.

Table 1.1 The average loss (%) attributed to each rot pathogen during the 2017/18 storage season. The data is compiled from 32 samples. Overall averages for 2016/17 and 2015/16 are included for comparison

Apple cultivar	Average % loss attributed to each rot																
	Brown rot	**Botrytis**	**Penicillium**	**Phytophthora**	**Neonectria**	**Gloeosporium**	**Fusarium**	**Mucor**	**Botryosphaeria**	**Phomopsis**	**Stalk**	**Eye**	**Cheek**	**Core**	**No. samples**	**Loss (%)**	**Loss range %**
Braeburn	0	62.5	12.5	0	12.5	12.5	0	0	0	0	0	0	0	0	1	2.0	-
Bramley	37.3	6.7	9.7	0	15.3	0	13.4	0	0	2.5	3.8	0.6	6.7	0.7	9	1.3	0.5-5.0
Cameo	4.1	17.0	11.2	0	57.9	1.4	7.1	0	0	0	0	0	0	1.4	2	0.5	-
Cox	0	30.0	0	10.0	10.0	50.0	0	0	0	0	0	0	0	0	1	1.5	-
Gala	6.3	16.8	2.9	0	64.2	9.8	0	0	0	0	0	0	0	0	8	2.3	0.1-7.0
Jazz	0.9	32.7	10.7	1.2	52.6	0.6	0	0	0	0	0.3	0	0.4	0	8	3.2	0.1-5.0
Other dessert	4.8	29.1	27.0	20.2	14.3	0	0	0	0	0	4.8	0	0	0	3	0.6	0.1-1.0
Overall average	7.6	27.8	10.6	4.5	32.4	10.6	2.9	0	0	0.4	1.3	0.1	1.0	0.3	32	1.6	-
Overall average 2016/17	19.3	9.7	11.2	1.6	31.3	12.4	0.4	4.2	0	0	2.0	0.6	1.5	5.6	52	1.5	-
Overall average 2015/16	13.3	8.3	6.3	6.4	40.3	9.3	0.5	3.0	0	0	2.2	0	1.2	7.3	60	2.6	-

1.3. Invasives

Aim

Keep abreast of new and invasive pests and diseases (ALL, Yr 1-5)

Summary

This task allows for new and current invasive pests and diseases to be monitored and action taken. Action may involve consultancy (e.g. if an invasive or emergent problem is suspected by a grower then a field visit can be arranged). The plant clinic at NIAB EMR is also available for laboratory diagnostics. Further action, together with AHDB knowledge exchange and research managers, can include the generation of factsheets, articles in grower publications (e.g. fruit notes) and organisation of training courses to raise awareness. Recent and new invasive species which are currently causing concern for the UK tree fruit industry are summarised in table 1.2.

Table 1.2 Invasive species of pests and diseases of concern for the UK tree fruit industry

	Species	Action Taken
Pests	*Drosophila suzukii*	National monitoring programme and wide ranging research programme ongoing. Attendance of Northern Europe SWD group in Belgium has resulted in a collaboration to develop a predictive model. *D. suzukii* numbers were high in April and late summer in 2017 compared to previous years, but lower in the spring of 2018 because of a cooler spring. In 2017 damage was seen in early June bearing strawberry and autumn ripening raspberry, blackberry and grape. However, probably due to the previous experience and revised management of cherry, fewer incidences of cherry damage were reported. Activity in the traps peaked to almost double winter 2016/17. Numbers were similar during raspberry harvest in 2018, winter activity and mean numbers over the whole year similar to 2017.
	Summer fruit tortrix	Summer fruit tortrix was detected for the first time in the West Midlands during the 2015 growing season and it is recommended that growers now monitor for this pest in the region using pheromone traps alongside codling moth and fruit tree tortrix monitoring traps. Damage was reported in the West Midlands in 2017 but the species was not confirmed.
	Marmorated stink bug	Sentenal traps for *Halyomorpha halys* were placed in municipal gardens and on commercial fruit farms and in gardens in 2018 but no BMSB have been detected to date. See Objective 10 for more information.
	Anthonomus spilotus	This is new to the UK and an AHDB factsheet was produced by M. Fountain and S. Raffle in 2018. The pest has also been recently identified as an invasive pest in Belgium (see Objective 9).
	Pear Shoot sawfly	The RHS reported sightings of Pear Shoot sawfly, *Janus compressus* in 2016. This has not been seen in commercial pear as far as we are aware. This 'occasional' pest of pear in Europe affects the shoots causing symptoms similar to fire blight –

	shepherd crook shaped tips caused when the larvae feed inside the shoots. A paper was sent to Chris Nicolson for inclusion in the ADAS notes in 2017.
Apple maggot fly	http://entnemdept.ufl.edu/creatures/fruit/tropical/apple_maggot_fly.htm *Rhagoletis pomonella,* native to North America, originally fed on the fruit of wild hawthorn (*Crataegus* spp.), but then became a primary pest of cultivated apples in northeastern United States and southeastern Canada. Adults emerge from the ground during early summer. Pupae may remain inactive and do not emerge until the second year. The female punctures the skin of the fruit with her ovipositor and lays eggs singly in the pulp. Eggs hatch in five to 10 days. Larvae develop slowly in the green fruit and usually do not complete their growth until the infested fruits have dropped from the tree. Larval development is two weeks to three or more months in hard winter varieties. Hosts include: apple, *Prunus* spp., *Vaccinium macrocarpum,* and peach. Larvae have been found in *Pyrus* spp. Damage: irregular, winding tunnels in fruit which turn brown, causing premature dropping of fruit.
Black and white citrus longhorn	https://www.cabi.org/isc/datasheet/5556 *Anoplophora chinensis* is black and shiny, with white pubescence. Length 19-40 mm. Recognized by long antennae reaching to at least the end of the body. >26 families of living tree hosts including Citrus, *Malus domestica*, apricot, European pear. Egg is elongate, subcylindrical, white (6 mm long) and laid through bark (T-shaped slit) close to ground level. Larva is elongate, cylindrical, up to 56 mm long and bores into the stem destroying the pith and vascular system later enterering heart wood, tunnelling up and down. Considerable amounts of frass (small cylindrical pellets of sawdust) and woodpulp are ejected through holes in the bark. Adults eat young leaves, branches and bark of the tree. At 20°C, 57% of the individuals completed their development 306 to 704 days after oviposition. Lower developmental threshold temperatures for eggs and young larvae 6.7 and 11.6°C, respectively. Tropical and subtropical regions one generation per year; further north one generation every 2 years. Although intercepted at ports or found in association with plants recently imported from Asia, it is not presently known to be established in the USA or Canada. First published record occurring on natural vegetation in Europe was in 2001. Eradication efforts are underway in Italy.
False codling moth	https://www.cabi.org/isc/datasheet/6904 *Thaumatotibia leucotreta* is a pest in tropical Africa but has failed to invade other areas as yet. Eggs: Flattened, oval, diameter 0.9 mm. Larva: When young yellowish-white with dark spots, up to 15 mm long, bright red or pink. Pupa: tough silken cocoon amongst debris or in the soil. Adult: Strongly dimorphic: Male wingspan 15-16 mm, female 19-20 mm. In both sexes the forewing pattern consists of a mixture of grey, brown, black and orange-brown markings, the most conspicuous being a triangular marking in the outer part of the wing, against the hind margin, and a crescent shaped marking above it. Seen in Europe where imported with produce from Africa. Detection of a single adult male in trap in California, in 2008. Pest of *Capsicum* (peppers), *Prunus persica* (peach). Probably low risk except glasshouse crops.
Grapevine phylloxera	https://www.cabi.org/isc/datasheet/56511 *Viteus vitifoliae* or *Daktulosphaira vitifoliae*. Globular aphid, 1.6-1.8 mm long and 1.0-1.2 mm wide. Native to North America and introduced into other continents (South and Central America, Africa, Oceania) in nineteenth century. Its introduction into European vineyards in the 1860s led to extremely severe losses and was considered as a major disaster. Destruction stopped by the grafting of European grapevine cultivars onto American rootstocks. Present in the UK from 1980's with few occurrences. Very limited capacity for natural spread if it remains more or less confined to the root system in the radicicolae form (as it does in Europe). Difficult and costly to eradicate. Symptoms: initially a few dead or declining contiguous vines in a vineyard. Gallicolae form: Small galls, about the size of half a pea, develop on the leaf surface, sometimes so numerous as to cover the entire leaf. Radicicolae form: Numerous knots or galls form on grapevine roots, with rotting of roots, yellowing of foliage and general decrease in vigour of the vines. Death of susceptible vines may result within 3-10 years.

	Complex alternation between an aerial, leaf-feeding form and the root-feeding form (gallicolae and radicicolae, respectively). However, *V. vitifoliae* can also persist parthenogenetically as the root-feeding form, without the leaf-feeding stage of the cycle. On cultivars of European grapevine (*V. vinifera*) grafted onto American rootstocks, normally infests only the underground parts of the plant and undergoes an incomplete cycle of seasonal development, with no change of feeding site. The winter is passed in the form of first- and second-instar nymphs on the nodules or galls on vine roots (European grapevines). In European cultivars of *V. vinifera* grafted onto American rootstocks, radicicolae become active, feeding on the roots, as soon as growth starts in the spring. Continue to multiply parthenogenetically through the summer. It is reported that sexuparous forms appear, but the gallicolous aphids do not normally develop on the leaves, and the aerial life-cycle is therefore not completed in Europe. However pers. comm. with R. Saunders is that leaf symptoms, blistering, can occur every 3-4 years especially in Sauvingnon Blanche.
Ambrosia beetle on nursery stock	https://www.cabi.org/isc/datasheet/57038 *Xyleborinus saxesenii* (fruit-tree pinhole borer), native, not invasive, but should be considered a high-risk quarantine pest. This is because members of the tribe Xyleborini (Xyleborinus plus related genera) are inbreeding, with the males mating with their sisters within the parental gallery system before dispersal. Thus the introduction of only a few mated females may lead to the establishment of an active population if suitable host plants can be found and environmental conditions are satisfactory. A very wide range of host plants. Any woody material of suitable moisture content and density may be all that is required. *X. saxesenii* has a high rate of increase due to its large brood sizes, almost all of which are females. The direct risk of establishment of populations of *X. saxesenii* outside its present range, followed by further spread of the species, should be considered very serious. A number of species of ambrosia beetle that normally attack only weakened host trees seem to be changing their habits and attacking healthy trees, either as exotics or in their native ranges (Kühnholz et al. 2003). Although such a change has not been noted for *X. saxesenii*, it would considerably increase its potential for causing economic damage to crop and forest trees.
Gypsy moth	https://www.cabi.org/isc/datasheet/31807 *Lymantria dispar.* Captured in light traps at higher frequency in 2018 (17 in one night in one light trap). It can damage fruit trees. Hatching larvae usually start feeding on flushing buds and later on newly-expanded leaves. High populations often result in total tree defoliation, often across a large spatial area. There is a pheromone identified.
Magdalis beetle on pear	Identified as minor pest of pear in 2018 (documented in Masse) causing superficial foliar damage. *M. armigera* is historically associated with elm and apple, in the spring months.
Rhagoletis cingulate	https://www.cabi.org/isc/datasheet/47051 Infestations in several cherry growing regions of Germany in sour cherries. Identified in UK in 2018. Due to the 3-4 weeks later emergence compared to *R. cerasi* sweet cherries mostly not affected by *R. cingulata*. Chemical control Exirel (cyantraniliprole), SpinTor (spinosad), Karate (lambda-Cyhalothrin) or netting.
Green Citrus Aphid	In 2018 Csaba Borbély identified 9% of the collected individual aphids from UK apple orchards (mostly south east) as *Aphis spiraecola*. Pear-shaped body with two black cylindrical siphunculi or cornicles on the posterior of the abdomen (1.2 - 1.7 mm in length). Uniform yellowish-green to green body, pale brown head, and pale brown legs and antennae. Winged forms have a dark brown thorax with a green abdomen. Hosts: citrus, apple, hawthorn, pear, quince. Host damage: infested flower buds may fall off the plant, honeydew excreted by aphids, coats the outside of fruits and leaves, and promotes the growth of sooty mold fungus that inhibits photosynthesis, weakens the plant, and makes fruit unattractive, feeds on the underside of new growth, heavy infestation may result in severe curling and distortion. Spirea aphids are capable of transmitting *Citrus tristeza* virus (CTV). Common in Europe on sprayed orchards.
American plum borer	*Euzophera semifuneralis* is a moth of the family Pyralidae. Found throughout the United States, southern Canada and parts of Mexico. Adults in the southern part of the range emerge from April through September. They live for 1–3 weeks. Larvae

	feed on a wide range of plants, including plum, peach, cherry, Chinese plum, pear, apple, apricot, and walnut. Plum and other drupe and pome fruit trees are favoured. Larvae bore into the bark of their host at scars, wounds, or crevices where bark scales offer concealment and protection. Larval mines are very shallow and irregularly shaped, cave-type burrows between wood and the outer bark. The galleries are usually loosely packed with frass. Larval feeding lasts 30–38 days. Pupation takes place in burrows under the bark in loosely spun silken cocoons partially surrounded by dark excrement pellets. The pupal stage lasts 24–33 days for the overwintering generation but may be completed in as few as 10 days for summer generations. Up to five generations occur annually in central Texas, but only two generations in Virginia, Delaware and Michigan.
European grapevine moth	*Lobesia botrana* The original geographic distribution of follows a clear Palaearctic pattern. Now in central Africa (Ethiopia, Eritrea and Kenya). Records from northern Europe (Finland and Sweden) must be considered as incidental. More recently reported in vineyards of Chile (2008), California (2009) and Argentina (2010) (Ioriatti et al., 2012). It was declared eradicated from California in 2016 (NAPPO, 2016). Reported in South Africa in 2019. Host plants; wide host range recorded, grapevine is the major host crop. Wild hosts, *Daphne gnidium* is the major food plant. On inflorescences (first generation), neonate larvae firstly penetrate single flower buds. Symptoms are not evident initially, because larvae remain protected by the top bud. Later, when larval size increases, each larva agglomerates several flower buds with silk threads forming glomerules (nests) visible to the naked eye, and the larvae continue feeding while protected inside. Larvae usually make one to three glomerules during their development which provide protection against adverse conditions. Despite the hygienic behaviour of larvae, frass may remain adhering to the nests. On grapes (summer generations), larvae feed externally and penetrate them, boring into the pulp and remaining protected by the berry peel. Larvae secure the pierced berries to surrounding ones by silk threads to avoid falling. Frass may also be visible. Each larva is capable of damaging between 2 and 10 berries, and up to 20-30 larvae per cluster may occur in heavily attacked vineyards. If conditions are suitable for fungal or acid rot development, a large number of berries may be also affected by *Botrytis cinerea*, *Aspergillus carbonarius* and *Aspergillus niger*, which result in severe qualitative and quantitative damage. Damage is variety-dependent: generally it is more severe on grapevine varieties with dense grapes, because this increases both larval installation and rot development. Larval damage on growing points, shoots or leaves is unusual. https://www.cabi.org/isc/datasheet/42794
Peach fruit moth	*Carposina sasakii* (Lepidoptera: Carposinidae) is not currently regulated in the EU although *C. niponensis*, a valid species of no economic significance that was previously mistakenly synonymised with *C. sasakii*, is regulated in Annex IIAI of 2000/29 EC. *C. sasakii* is a well-defined species that is recognised as a major pest of apples, peaches and pears in eastern China, Japan, Korea and Far East Russia. It is not known to occur in the EU. Adults emerge in the spring or early summer. Eggs are laid on host fruits. Larvae burrow into the fruit to develop. Infested fruits often drop early. Larvae exit fruit and overwinter in the soil. In the more southern areas of distribution, there can be two or more generations per year. Import of host fruit provides a potential pathway into the EU. *C. sasakii* occurs in a range of climates in Asia, some of which occur in the EU. Wild and commercially grown hosts are available within the EU. It has the potential to establish within the EU where there could be one or two generations per year. Impacts could be expected in apples, pears and other rosaceous fruit crops. The level of impacts would be uncertain. http://www.efsa.europa.eu/en/efsajournal/pub/5516?utm_source=EFSA+Newsletters&utm_campaign=79bc4880ef-EMAIL_ALERTS_ALL&utm_medium=email&utm_term=0_7ea646dd1d-79bc4880ef-63949401

Diseases	Oriental fruit fly	*Bactrocera dorsalis*. Highly invasive species. Native to Asia, now found in at least 65 countries, including parts of America and Oceania, and most of continental Africa (sub-Saharan countries). The potential risk of its introduction to a new area is facilitated by increasing international tourism and trade, and is influenced by changes in climate and land use. Can easily disperse as it has a high reproductive potential, high biotic potential (short life cycle, up to 10 generations of offspring per year depending on temperature), a rapid dispersal ability and a broad host range. The economic impact would result primarily from the loss of the export markets and the costly requirement of quarantine restrictions and eradication measures. Over 300 species of commercial/edible and wild hosts, *B. dorsalis* has the broadest host range of any species of *Bactrocera*. It is a serious pest of a wide range of fruit crops throughout its native range and wherever is has invaded. The major hosts are apple, guava, mango, peach and pear. https://www.cabi.org/isc/datasheet/17685
	Diaporthe causing apple leaf spots	A higher incidence of leaf spotting was observed on various apple varieties (particularly Braeburn and Cox) during the 2016 growing season. Resulting in defoliation in some cases. The causative agent was isolated and morphologically identified as the genus *Diaporthe (*formerly *Phomopsis)*. Subsequently sequenced to determine species level identification as *Diaporthe rudis/viticola.*
	Neofabraea kienholzii	Part of the group of pathogens which cause Gloeosporium rot *Neofabraea kienholzii* had not been reported in the UK before but was picked up as part of the rot survey. A new disease report was published to inform the scientific community. Kingsnorth J, Perrine J, Berrie A, Saville R, 2017. First report of *Neofabraea kienholzii* causing bull's eye rot of apple in the UK. *New Disease Reports* 36, 15. [http://dx.doi.org/10.5197/j.2044-0588.2017.036.015]
	Xanthomonas arboricola pv. *pruni*	A notifiable bacterial disease which causes shot holing symptoms on leaves. Plum and sweet cherry are both hosts. Currently only reported on *Prunus laurocerasus* (cherry laurel) in the UK. More information can be found on the DEFRA factsheet found at https://planthealthportal.defra.gov.uk/assets/factsheets/x-arboricola-pv-pruni-factsheet.pdf
	Xylella fastidiosa	A devastating bacterial disease which has a wide host range including *Prunus*. The disease is vectored by plant hoppers of various species. Currently present in Mediterranean countries in Europe. Plant Health and Seeds Inspectorate (PHSI) are coordinating the national response to the threat of this disease to UK industry and environment. DEFRA have produced a Factsheet about this disease which can be found at: https://planthealthportal.defra.gov.uk/assets/factsheets/xylellaFastidiosa2015.pdf Current demarcated outbreaks are in southern Italy, the PACA region of France and Corsica, a site in Germany between Saxony and Thuringia, on mainland Spain in the Valencia region, and in all the Balearic Islands. In April, Spain detected *X. fastidiosa* for the first time in olive trees near Madrid, outside the current outbreak area in the region of Valencia. There has also been a finding on *Polygala myrtifolia* plants in a glasshouse in Almeria.

Objective 2. Neonectria ditissima

2.2 Rootstock/interstock

Aim

Evaluation of susceptibility of rootstocks to canker (EMR/ADAS, Yr 1-5)

Introduction

Apple rootstocks are known to confer resistance/tolerance traits to various pest and disease for example woolly apple aphid, *Phytophthora* and *Neonectria*. Rootstock and interstock choice is being increasingly considered as part of an integrated approach to canker control of particularly canker susceptible scion cultivars. This objective will evaluate the relative resistance conferred by a panel of rootstocks commonly used today alongside several advanced selections from the NIAB EMR and Geneva rootstock breeding programmes to inform these decisions. The trials are being conducted in two phases; the first phase has evaluated relative resistance of the rootstocks alone using an artificial pathogenicity test (reported previously) and the second phase are long term trials evaluating relative resistance of a panel of rootstocks grafted with a common scion (cv. Gala) planted at two field locations. Assessments of natural infections in the field provides the most representative results for field resistance however this takes time, therefore artificial inoculations will be used in conjunction with natural inoculation to provide information on relative resistance conferred by the rootstocks.

Materials and Methods

Plant material

The rootstocks sourced from various nurseries and breeding programmes are described in Table 2.2.1. Rootstocks were bench grafted on to a common scion (cv. Gala) in February 2016. The trees were grown on in pots outside at NIAB EMR. In order to promote feathering of the maidens the apex shoot was pinched out and slightly bruised (to remove apical dominance) as the shoot reached the top of the cane (July onwards). This task was performed as and when each tree reached the top of the cane, which varied depending on the rootstock. Once the trees were dormant (January) they were prepared as bare rooted trees and stored in commercial conditions (kept at 2°C in the dark, and the roots kept moist by being wrapped in damp hessian and watered regularly) until planting.

Table 2.2.1 The apple rootstocks and interstock to be evaluated

Treatment Number	Rootstock	Interstock	Scion
1	M9 (EMLA)	-	Gala
2	M9 (337)	-	Gala
3	G.41	-	Gala
4	G.11	-	Gala
5	MM106	-	Gala
6	M116	-	Gala
7	M26	-	Gala
8	M9 (337)	Golden Delicious	Gala
9	EMR-001*	-	Gala
10	EMR-002*	-	Gala
11	EMR-003*	-	Gala
12	EMR-004*	-	Gala
13	EMR-005*	-	Gala
14	EMR-006*	-	Gala

*Advanced selections from the NIAB EMR breeding programme are coded – material was kindly provided by Bruno Essner, Pepinieres Du Valois.

Sites

Bare rooted trees were planted at two trial sites in the spring of 2017 as described below.

Site 1	East Egham Orchard , NIAB EMR, New Road, East Malling, Kent, ME19 6BJ
Grid reference	51.287861, 0.43831340
Planted	29 March 2017
Description of planting site	The site is situated amongst mature orchards in which *Neonectria dittisima* inoculum is prevalent providing opportunities for natural infection.
Tree spacing	3.5 x1.75 m

Aerial view:

Trial layout:

4 replicates of 8 tree plots, arranged over four blocks (as determined by colour)

WINDBREAK

G.41	MM106	EMR-004	M9 (337)	EMR-003	M9 (337) interstock GD	M116	EMR-002
EMR-005	M9 (EMLA)	M116	EMR-006	M9 (EMLA)	M116	EMR-001	G.11
M116	EMR-004	G.41	M9 (EMLA)	EMR-002	EMR-004	M9 (337)	M9 (EMLA)
EMR-003	M9 (337) interstock GD	EMR-002	EMR-005	EMR-006	EMR-005	MM106	M9 (337) interstock GD
EMR-002	M9 (337)	MM106	EMR-001	M9 (337)	MM106	EMR-005	EMR-003
M26	EMR-001	EMR-003	M26	G.41	G.11	EMR-006	M26
EMR-006	G.11	M9 (337) interstock GD	G.11	EMR-001	M26	G.41	EMR-004

ALLEY WAY

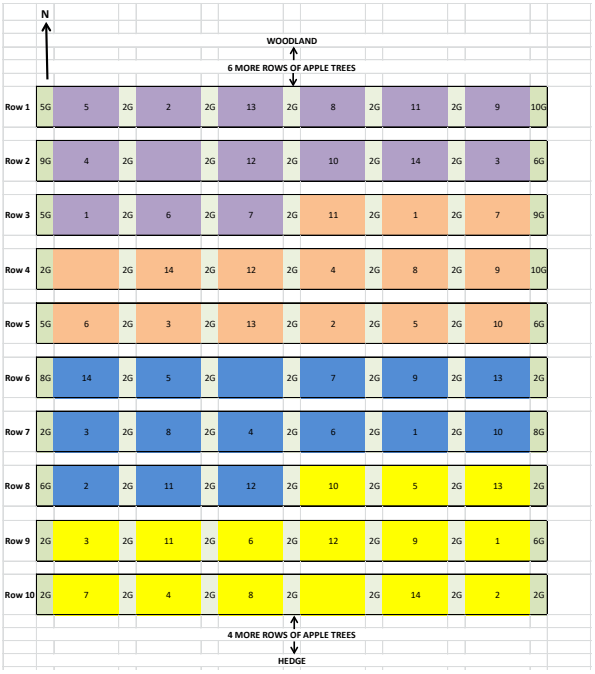
= 4 spare tree stations

Site 2	Herridges Orchard, Ketford Road, Poolhill nr. Newent, Gloucestershire. GL18 1LW
Grid reference	51.966956, -2.3953805
Planted	15 March 2017
Description of planting site	The trial was planted on the site of an old Cox orchard. Two Cox trees were left in the ground between each plot to serve as an inoculum source throughout the trial.
Tree spacing	1.83x3.66 m

Aerial view:

Trial layout:

4 replicates of 10 tree plots per treatment. Each plot separated by mature Cox trees



Natural infection

Where possible treatments effective against canker have been avoided and wounds left unprotected to promote the development of natural infections. On the commercial site canker specific treatments were omitted only where commercially acceptable.

Artificial inoculation (site 1 only)

Artificial inoculations were conducted in autumn 2017 in order to produce identical infection conditions across the treatments and to guarantee infection for determining differences between the treatments. In mid-November 2017 (16 - 17 November), eight trees per treatment (two replicate trees per block from four blocks) were selected. Six infection sites were made on each tree: five leaf scars and one bud scar. The leaf scar is the infection route which best represents the natural infection route. Bud scar infection is an additional method used by NZ researchers to account for different scion/rootstock/interstock combinations losing their leaves at different times making it difficult to compare accessions using leaf scar inoculations alone. Prior to wounding, inoculation points were marked with coloured paint marker pens below the leaf or bud scar as follows; red for leaf scar, yellow for bud scar. Leaf scars were created by removing a leaf gently by hand whilst bud scars were made by dislodging the bud with the thumb. All wounds were made immediately prior to inoculation. The marked scars were inoculated with 5 µl of *N. ditissima* Hg199 spore suspension of $1x10^5$ conidia ml^{-1} suspended in sterile distilled water using a pipette. Mock inoculated controls on each inoculated tree, were prepared as above using one leaf and one bud scar per tree, sterile distilled water was used instead of a spore suspension. These were marked with coloured paint marker pens as follows; blue/yellow for mock bud scar and blue/red for mock leaf scar.

The inoculations were done over two days; blocks 1 & 2 on 16 November, and blocks 3 & 4 on 17 November. The same inoculum suspension was used on both days and kept on ice in a fridge overnight. Germination tests following 24 hours showed a 98% germination rate for spore suspension plated at the beginning of both days reducing to 59% in the suspension brought back from the field after the second day of inoculation.

Assessments

<u>Site 1 - East Egham</u>

Assessments were completed in spring 2018. For each tree, cankers were recorded according to their position on the tree as described by McCracken *et al*. 2003. Briefly; A = Rootstock, B = Main stem and C, D, E = Peripheral (Figure 2.2.1). Dieback unrelated to canker was also recorded. Where possible i.e. when not integral to the tree, cankered

branches and dieback was removed. Cankers on the rootstock or main stem, which are integral to the tree were not removed.

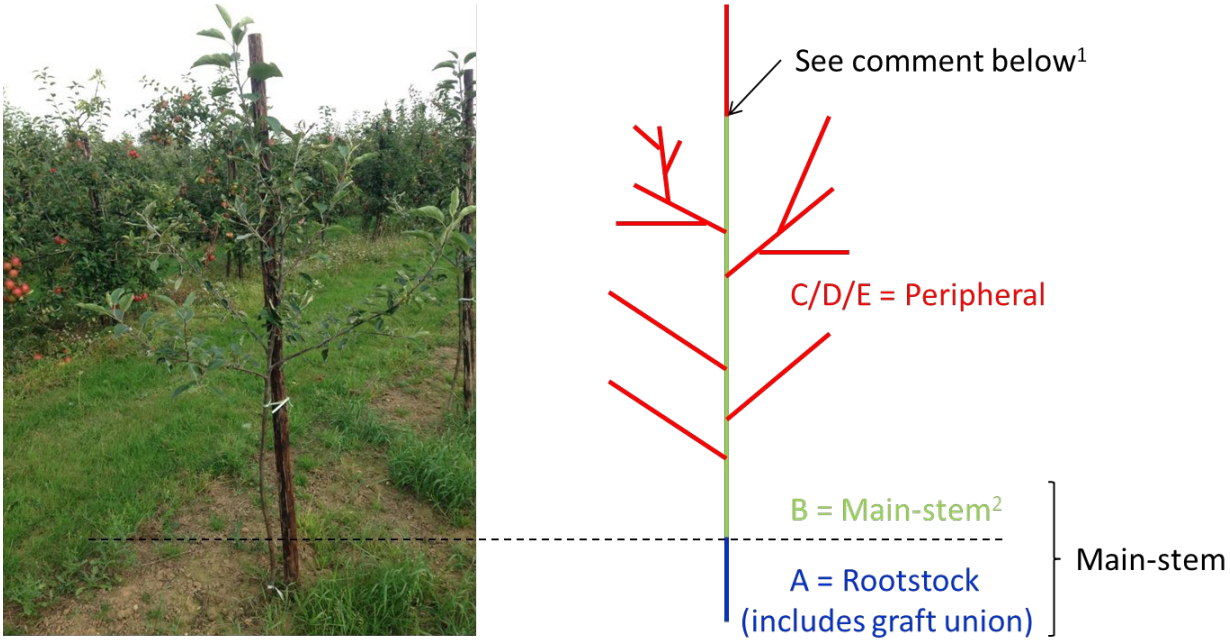


Figure 2.2.1 Diagram of the classifications of cankers based on their position within the tree. [1] note that there is a continuum between the main-stem and peripheral branch on the main leader; cankers on the 1 year wood were scored as peripheral and those on the ≥ 2yr wood were scored as main-stem. [2] cankers occurring on the interstem in treatment 8 (M9 with Golden Delicious interstem) were scored as 'B' – main stem

Statistical analyses

Each individual dataset was analysed by ANOVA. The natural infections were analysed using individual canker locations (A, B, C, D, E) and total cankers per tree.

Site 2 – Herridges Orchard

The site was located at Herridges Orchard, Gloucestershire in a block of 400 Cox trees, which was planted at a spacing of 1.83m x 3.66m in 1998. Old trees and roots were removed in sections of 10 trees (one plot) from the orchard early in 2017. These plots of 10 trees were interspersed with two canker infected mature guard trees and each row contained six plots (Fig. 2.2.2). The Gala scions listed in Table 2.2.1 were planted into each plot on 14/15 March 2017, with 10 trees per plot. Each treatment was replicated four times, giving a total of 560

trees in 56 plots. All trees were tied in and protected against rabbit damage by attaching mesh guards around the main stem.

Figure 2.2.2 Experimental trial set up at Herridges Orchard in 2017

Artificial inoculations of canker were performed on 19 October 2018. In each treatment plot two trees were selected, leaves carefully removed to produce leaf scars and marked with permanent paint. The marked leaf scars were inoculated with 5µl of *N. ditissima* Hg199 spore suspension of $1x10^5$ conidia ml^{-1} suspended in sterile distilled water using a pipette. Another set of marked leaf scars were treated with water as a control. Germination tests showed 86% germination rate for the spore suspension after use.

The trial was checked regularly throughout 2018 and the trees were assessed on 16 May and 11 October 2018 for canker development. Main stem A/B and peripheral C/D/E cankers were counted for each tree as outlined above. Samples of canker were taken and isolated to check for canker infection. Tree death and cause was noted, as well as any additional damage not related to canker. The data were analysed using ANOVA.

Results

Site 1 – East Egham

Natural infection

The mean number of cankers from natural infection was low for all rootstocks (grand mean of 0.24, Figure 2.2.3), and there was no statistically significant difference between the rootstocks (Table 2.2.2). However, the rootstocks with the highest mean number of mainstem

cankers (A+B) were M9 (EMLA) and M9 (337) GD interstock (0.47 and 0.41 respectively). The rootstock with the lowest number of mainstem cankers was EMR-005 (0.03). The mean number of peripheral cankers showed a different number and distribution compared to mainstem cankers, with a lower overall mean of 0.13 cankers. The rootstock with the highest mean number of peripheral cankers was G.41 (0.31), while the lowest were M9 (EMLA), M116, M9 (337) and M9 (337) interstock (0, 0.03, 0.06). There were only three rootstocks that had a higher number of peripheral cankers compared to mainstem cankers (G.41, G.11 and EMR-005).

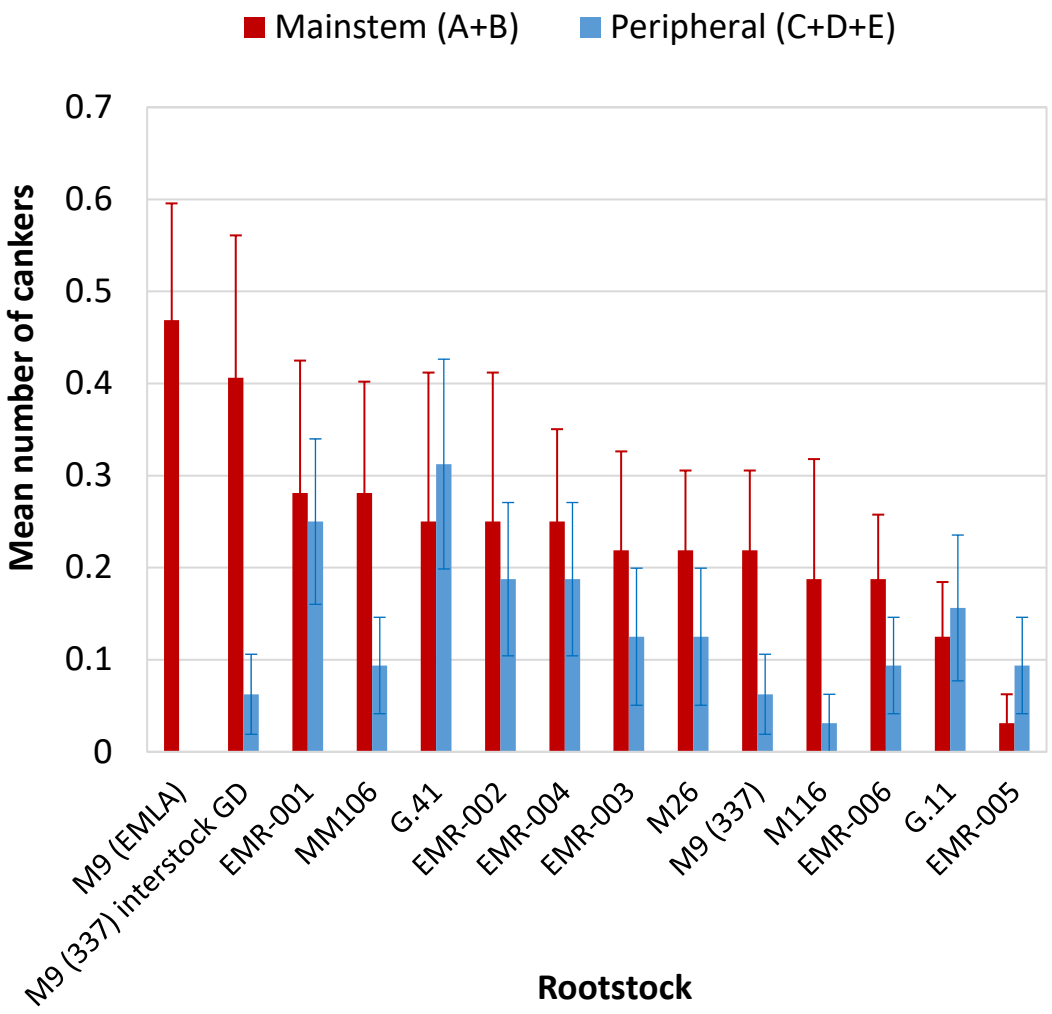


Figure 2.2.3 Mean number of cankers on apple trees with 14 different rootstocks infected from natural inoculum located at East Egham (NIAB EMR). M9 (337) interstock GD has a Golden Delicious interstock grafted between the rootstock and the scion

Table 2.2.2 ANOVA results of number of cankers on apple trees with 14 different rootstocks infected from natural inoculum located at East Egham (NIAB EMR)

Location of canker	Degrees of freedom	p-value
Rootstock (A)	13	0.122
Mainstem (B)	13	0.911
Peripheral C	13	0.122
Peripheral D	13	0.502
Peripheral E	13	N/A (all data values 0)
Rootstock + main-stem (A+B)	13	0.617
Peripheral (C+D+E)	13	0.074
Total (A+B+C+D+E)	13	0.595

Artificial inoculation

There were significant differences found between the proportions of leaf scars infected by artificial inoculation. M26, EMR-006, EMR-002, and MM106 were significantly lower than EMR-004 and EMR-001. The rootstocks with the highest proportion of leaf scars infected through artificial inoculation were EMR-004 (39.38%), followed by EMR-001 (36.88%), M9 (337) (27.5%) and M9 (EMLA) (22.5%) (Figure 2.2.4). The rootstocks with the lowest proportion were M26 (2.5%), EMR-002 (2.5%), EMR 006 (2.5%) and MM106 (5.63%).

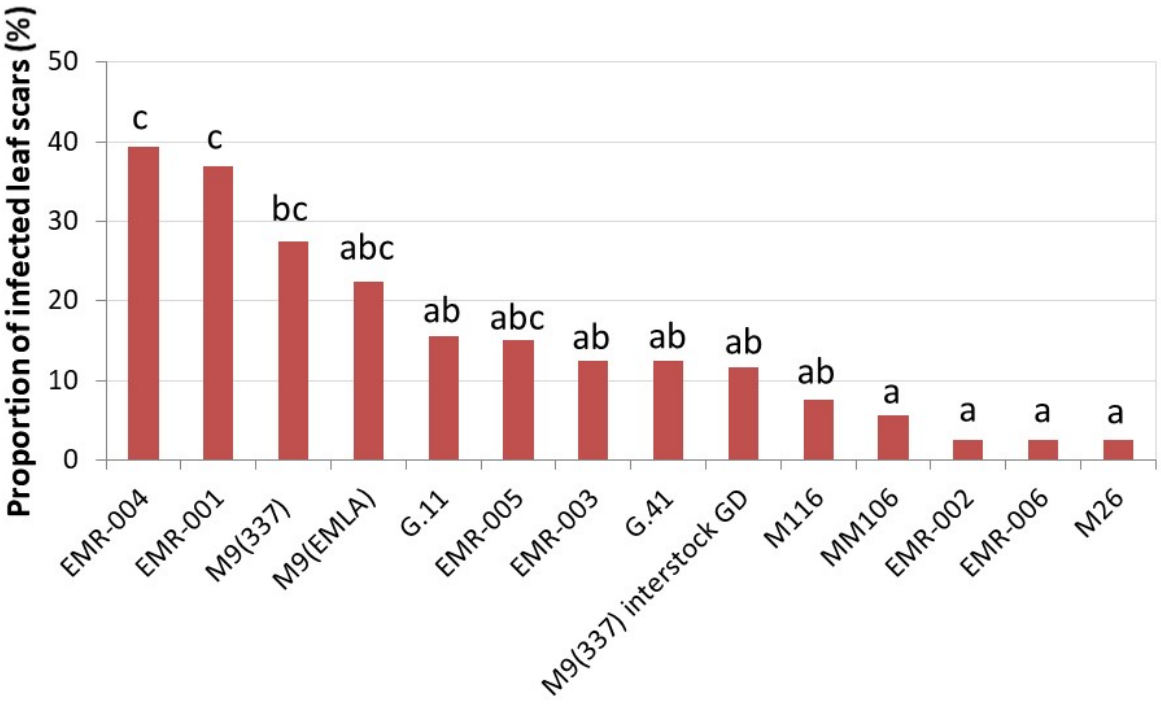


Figure 2.2.4 The proportion of scion (cv. Gala) leaf scars infected with Neonectria canker at the Egham site (NIAB EMR) after artificial inoculation with *N. ditissima* conidia. Letters above columns indicate the statistical results of the Fisher's exact test. Colums with the same letter are not significantly different to each other, those with different letters are significantly different

The rootstock with the highest mean canker length at inoculated leaf scars was EMR-004 (5.8 mm), followed by EMR-001 (5.3 mm) and M9 (337) (5.1 mm) (Figure 2.2.5). The lowest mean canker lengths were EMR-002 (0.2 mm), EMR-006 (0.4 mm), and M26 (0.4 mm). The ANOVA showed that canker length was not significantly different between the rootstock varieties (p=0.280).

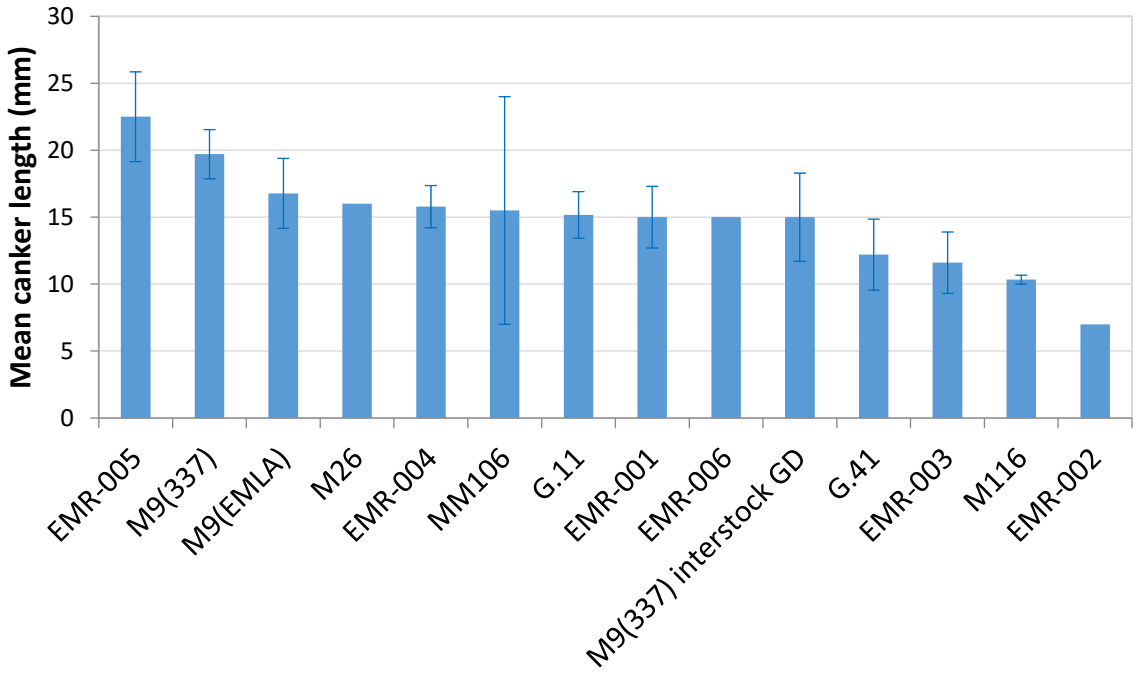


Figure 2.2.5 The mean canker length at leaf scars of scion (cv. Gala) artificially inoculated with *N. ditissima* conidia at the Egham site (NIAB EMR)

Site 2 – Herridges Orchard

Natural infection

At Herridges Orchard, a total of 61 trees were recorded as dead (10.8%). Of these, 56 had died as a result of canker, with 13 of these dying during 2017. In the majority of these cases the mainstem canker noted in year one had girdled the tree resulting in tree death. The remaining five trees died as a result of other causes, such as rabbit damage.

The infection rate of the newly planted trees was generally quite low across the orchard, at this point in the trial at Herridges Orchard. Main stem (A, B) and peripheral (C) cankers were noted on the trees at Herridges Orchard. The majority of canker recorded on the trial trees were on the main stem either below (A) or above (B) the graft union. Some peripheral (C) cankers were recorded.

A significant difference was seen in the average total canker across the rootstocks trialled (Table 2.2.4), with EMR-004 having the lowest total incidence of canker per tree and was significantly lower than the levels on M9(337) (Fig. 2.2.6). EMR-004 only had mainstem cankers below the union recorded in the trial. With the exception of EMR-001 and EMR-002, the advanced selections from the NIAB EMR breeding programme had lower total levels of

canker compared to the majority of the commercially used rootstocks (M116, M26, M9(337) and MM106). The Geneva series rootstocks (G.41 and G.11) were comparable in the levels of canker compared to the advanced NIAB EMR selections.

Table 2.2.4 Statistical analysis of the rootstock effect on canker developing from natural infections following at site 2 (Herridges Orchard). Figures significantly different from others are highlighted in bold

Rootstock	Total*	A	B	C
EMR-001	0.650	0.325	0.200	0.125
EMR-002	0.725	0.450	0.231	**0.050**
EMR-003	0.275	0.125	0.075	0.075
EMR-004	0.125	0.125	**0.000**	**0.000**
EMR-005	0.275	0.100	0.100	0.075
EMR-006	0.325	0.100	0.200	**0.025**
G.11	0.250	0.175	**0.050**	**0.025**
G.41	0.325	0.200	0.100	**0.025**
M116	0.650	0.350	0.250	**0.050**
M26	0.700	0.425	0.175	0.100
M9 (337)	0.925	0.325	0.475	0.125
M9 (337) interstem GD	0.650	0.300	0.300	**0.050**
M9 (EMLA)	0.525	0.300	0.200	**0.025**
MM106	0.600	0.325	0.275	**0.000**
Fprob	0.031	0.051	0.199	0.661
SED (39)	0.242	0.127	0.150	0.065
LSD (P = 0.05)	0.476	0.250	0.296	0.128

* Average total number of cankers. Main stem (A, B) and peripheral (C) cankers.

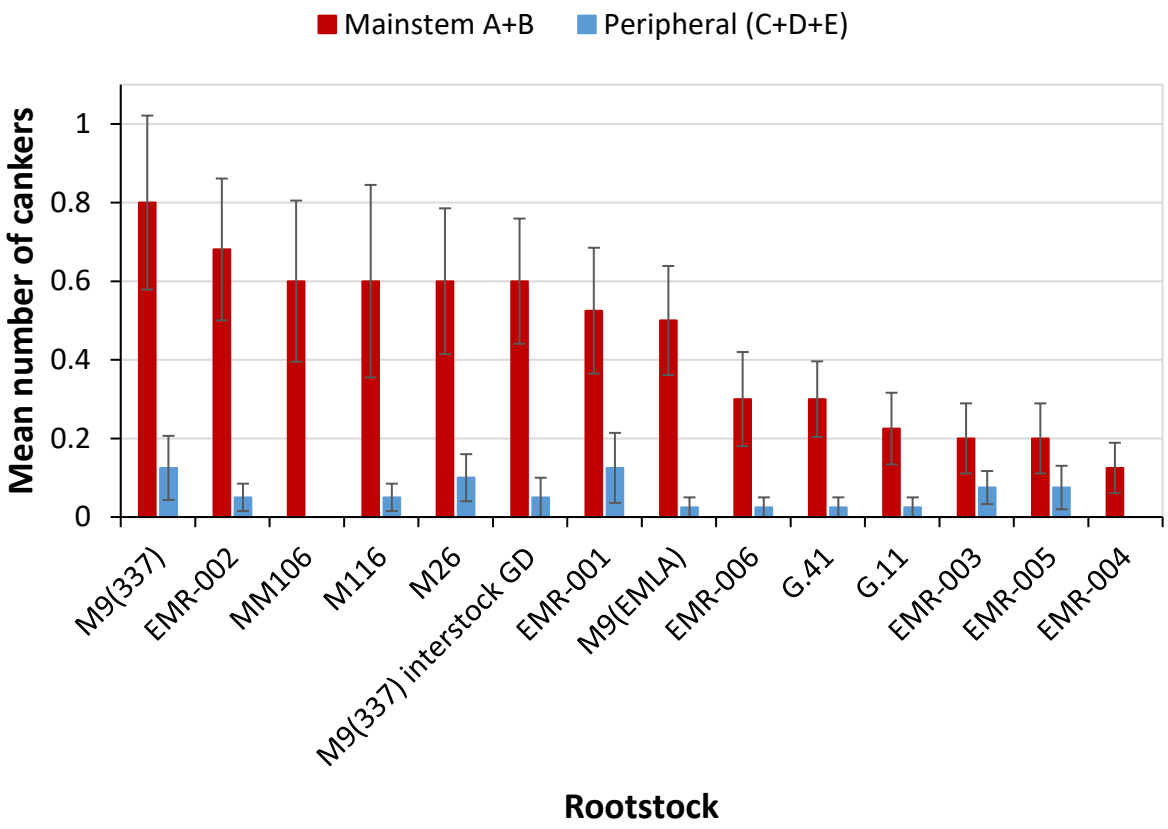


Figure 2.2.6 Naturally occurring canker at Herridges Orchard. The mean number of total cankers per tree is presented; Mainstem (red) = A (Rootstock) + B (Mainstem), and Peripheral (blue) = C + D + E peripherals

Discussion

Natural infection

There was an overall low incidence of natural infection at both trial sites. The majority of natural cankers were present on the mainstem (A and B types). McCracken *et al.* (2003) reported that cankers on the mainstem were more likely to originate from infections in the nursery and can express disease symptoms up to 3 years following planting. Therefore these A and B infections likely originated from the propagation phase in the nursery. Mainstem cankers are commercially significant as the infection may girdle and kill the tree. Peripheral cankers may not immediately kill the tree, however they are a source of inoculum that may spread to the mainstem of the infected tree or to neighbouring trees. The rootstock with the lowest number of mainstem natural cankers at the Egham site was EMR-005. This variety is worth investigating further due to its apparent reduced susceptibility to natural cankers. The M9 rootstocks are the most widely planted in the UK apple industry, and show the highest

number of mainstem cankers in both trials. This confirms the observations in industry regarding these rootstocks.

Artificial inoculation

The proportion of infected leaf scar data, and the mean canker length data were very similar for artificial inoculation at the Egham site. The rootstocks with the highest values for both datasets were EMR-004, followed by EMR-001, M9 (337) and M9 (EMLA), while the lowest were EMR-002, EMR 006 and M26. With the M9 (EMLA) rootstock the high value is echoed in the natural infection data.

The artificial inoculation performed in the autumn at the Herridges site will be assessed in 2019. This will give more comparable results of susceptibility of the trees to canker on this site compared to natural infection alone.

Conclusions

- Overall the presence of cankers from natural inoculum was very low on all sites.
- EMR-006 consistently had lower values for mainstem cankers, proportion of infected leaf scars and mean canker length at site 1 (East Egham)
- EMR-004 had the lowest incidence of natural canker infections at site 2 (Herridges), and also had the lowest infection rate in the initial test of rootstock material in 2016.
- In the artificial inoculation experiment at East Egham, EMR-006, EMR-002, M26 and MM106 had significantly lower incidence of canker. M9 (EMLA) consistently had higher values for mainstem cankers, proportion of infected leaf scars and mean canker length at site 1 (East Egham)
- The trial at site 2 (Herridges) has been artificially inoculated with *N. ditissima*, so an increase in infection should be evident in 2019
- M9 (337) had the highest natural infection rate of canker at site 2 (Herridges)
- Across all sites EMR-006 is looking the most promising rootstock, with consistently reduced canker incidence
- In the standardised artificial inoculation experiment, in addition to EMR-006, the rootstocks EMR-002, M26 and MM106 are looking most promising for reduced canker incidence
- Artificial inoculations were completed at Herridges orchard in 2018, so will be able to be compared with the East Egham data in 2019

Future work

- Continued assessment of natural and artificial canker development on the 14 rootstocks

2.3 Soil amendments

Aim

Evaluation of treatments to improve tree health and establishment using soil amendments (EMR/ADAS, Yr 1-5).

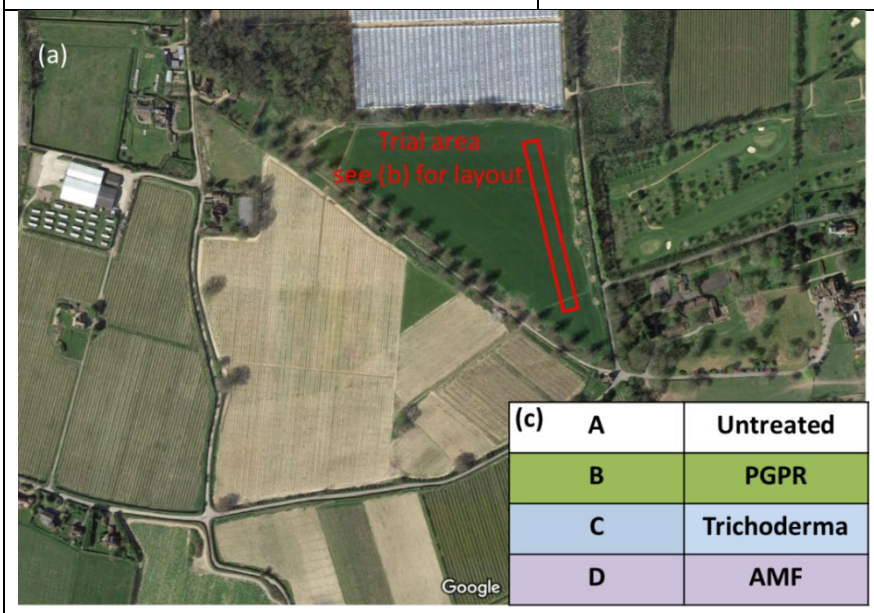
Introduction

Previous research on European apple canker, in particular the millennium trial (McCracken *et al*. 2003) has shown that *N. ditissima* can infect trees in the nursery and remain asymptomatic in the apple host. Once planted in the production site, where upon the tree can experience stress (drought/water logging/replant disease etc.), the disease is expressed. This objective aims to evaluate biological soil amendments to improve tree health and establishment in the context of canker expression. The objective is to be conducted in two parts: (1) a stool bed trial will simulate the nursery phase of tree fruit production and (2) a replicated trial on newly planted orchards to simulate the establishment of new orchards on the production site. These are long term trials, requiring establishment and monitoring over time. The stool bed was planted in May 2015 and this season was the first production cycle following a 2 year establishment phase. The newly planted orchard trials were planted in 2016 and assessments have been carried through to the most recent 2018 growing season as part of the long term monitoring of this trial.

Materials and Methods (NIAB/EMR)

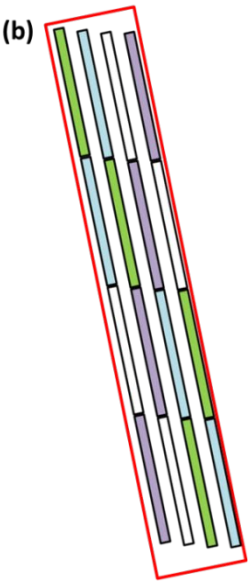
Site

Site 1	Friday Street - Kent
Grid reference	51°12'58.2"N 0°36'36.5"E
Variety	Cv. Rubens
Planted	15/03/16
Producer organisation	Avalon Produce Limited



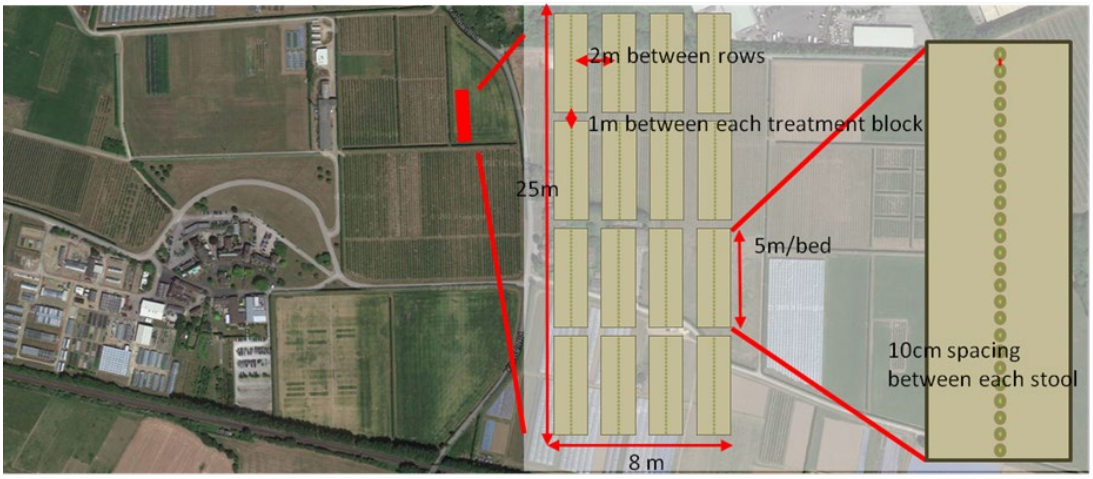
(c)

A	Untreated
B	PGPR
C	Trichoderma
D	AMF



Site 2	Broadwater - Kent
Grid reference	51°16'55.9"N 0°24'35.1"E
Variety	Cv. Gala (was intended to be Cv. Jazz but trees were not available when the trial was setup)
Planted	12/05/16
Producer organisation	Worldwide Fruit Limited

Site 3 (Stoolbed)	Egham - Kent
Grid reference	51.287328, 0.45690701
Variety	EMLA M9
Planted	12/05/15
Host	NIAB EMR



Treatments

Treatments were applied at planting as described in previous reports (Table 2.3.1).

Table 2.3.1 Treatments used for biological amendments trial to study the effects on tree health and establishment and on apple canker expression

Treatment No.	Treatment	Product (Supplier)	Species
1	Untreated	-	-
2	Arbuscular Mycorrhizae Fungi (AMF)	Rootgrow (Plantworks)	*Funneliformis mosseae* *Funneliformis geosporus* *Claroideoglomus claroideum* *Rhizophagus irregularis* *Glomus microaggregatum*
3	Plant Growth Promoting Rhizobacteria PGPR)	Experimental (Plantworks)	*Rhizobium* sp., strain IRBG74 *Bacillus amyloliquefaciens* *Bacillus megaterium* *Derxia lacustris,* strain HL-12
4	Trichoderma	TrianumG (Koppert)	*Trichoderma harzianum* strain T-22
5[1]	Biochar	Tree Soil Improver (CarbonGold)	Biochar + Mycorrhizae

[1]Treatment 5 (biochar) was used in site 2 only

Stoolbed trial

On 6 July 2017, once the shoots on the stoolbeds had reached sufficient height to replicate commercial practice (Figure 2.3.2) sawdust was applied to the base of the shoots of each stoolbed to prevent lignification at the base of the shoots and promote root development. For each plot the sawdust was amended with the respective biological product with which the plot was treated at planting to improve stool health, with the aim of also pre-colonizing the rootstocks. The quantity of inoculum applied (Table 2.3.2) was based on manufacturers recommendations.

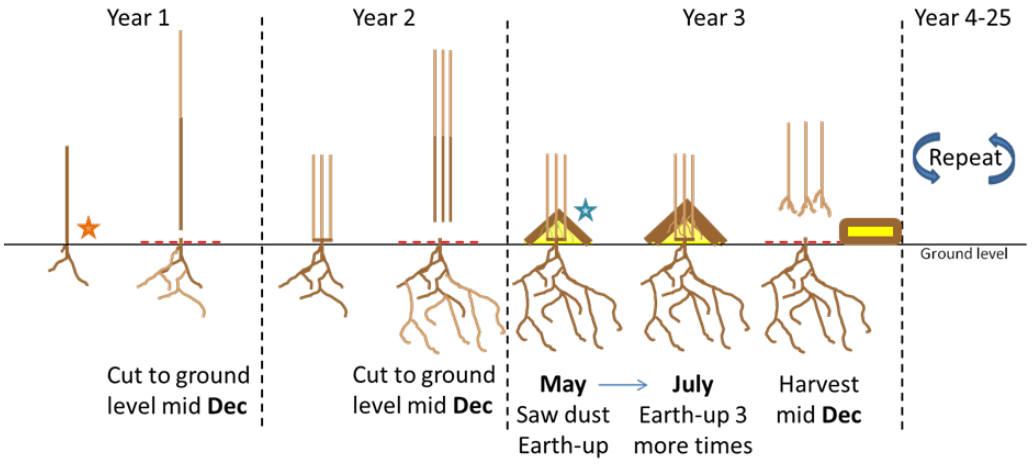


Figure 2.3.2 a) A schematic of the commercial practice of establishing and harvesting a stoolbed b) photographs of a bed in which amended sawdust has been applied and weighed down with soil

Table 2.3.2 Amendment rates incorporated into sawdust

Treatment	Product delivered/plant (ml)	Number of propagules/plant
Trichoderma	6.6	7.9×10^8
AMF	10	1.6×10^4
PGPR	10	1×10^9

Inoculations

In order to guarantee infection to determine differences between the treatments artificial inoculations were conducted in autumn 2017. Bud inoculations were carried out between the 28 and 29 November as follows; the 15th bud from the top of the stem was marked with a paint marker pen on 20 randomly selected shoots per plot. The marked buds of 15 shoots per plot were inoculated by wounding (dislodging the leaf bud with the thumb) and applying 5µl of inoculum (strain Hg199 at 4.6 x 10^5 spores/ml). The marked buds of the remaining 5 shoots per plot were mock inoculated, following the same protocol as above but applying sterile distilled water instead of a spore suspension. Inoculations took place over 2 days; Blocks 1, 2 and 3 were inoculated on 28 November 2017 and marked with yellow paint, Block 4 was inoculated on 29 November 2017 and marked with red paint. Mock inoculations were conducted over the two days at the same time as the inoculations and were all marked with blue paint. Germination tests were conducted after inoculation, 79% and 66% germination following 24 hours was recorded, after the first and second days of inoculation respectively.

Assessments

Stoolbed

The stoolbed was harvested on the 14/12/17 and the harvested rootstocks were subjected to the following assessments:

Stress test

In order to encourage the development of latent canker a protocol, described by Wenneker *et al.* (2017) has been developed to expedite the expression of disease symptoms. This protocol was implemented to encourage expression of disease symptoms of both inoculated and uninoculated rootstocks. Briefly, rootstocks grouped by plot were placed in buckets filled with moist sand which were placed in a climate controlled (99 %RH, 18°C) shipping container (Figure 2.3.3). Temperature and humidity were monitored continuously using a data logger. Rootstocks were monitored weekly for signs of symptom development. Each individually labelled inoculated stem is recorded for canker presence (incidence), canker size (severity) and latency period (time until disease expression). Results will be added in the Yr 5 report.

Figure 2.3.3 Experimental setup of the 'stress test' to expedite the symptom development at inoculation sites

Results

Stoolbed trial

The *Trichoderma* amendment resulted in a reduced number of cankers compared to all other treatments and the untreated control (Figure 2.3.4). There was a statistically significant difference in the number of cankers between the amendments (df=3, p=0.041). The multiple comparisons test showed a statistically significant difference between *Trichoderma* and PGPR. However, there was no significant difference between the untreated control and *Trichoderma*. There was also no significant difference between any of the other treatments using the multiple comparisons test. AMF had a lower mean number of cankers than the control between 03/1/18 and 07/03/18, however it then surpassed the control between 07/03/18 and 28/03/18. PGPR had a higher number of cankers than the control for the entire duration 03/01/18-28/03/18. The latency of symptom expression was 50-51 days, and was determined as the period between inoculation time (28-29/11/17) and canker expression for all biological amendment treatments, 18/01/18.

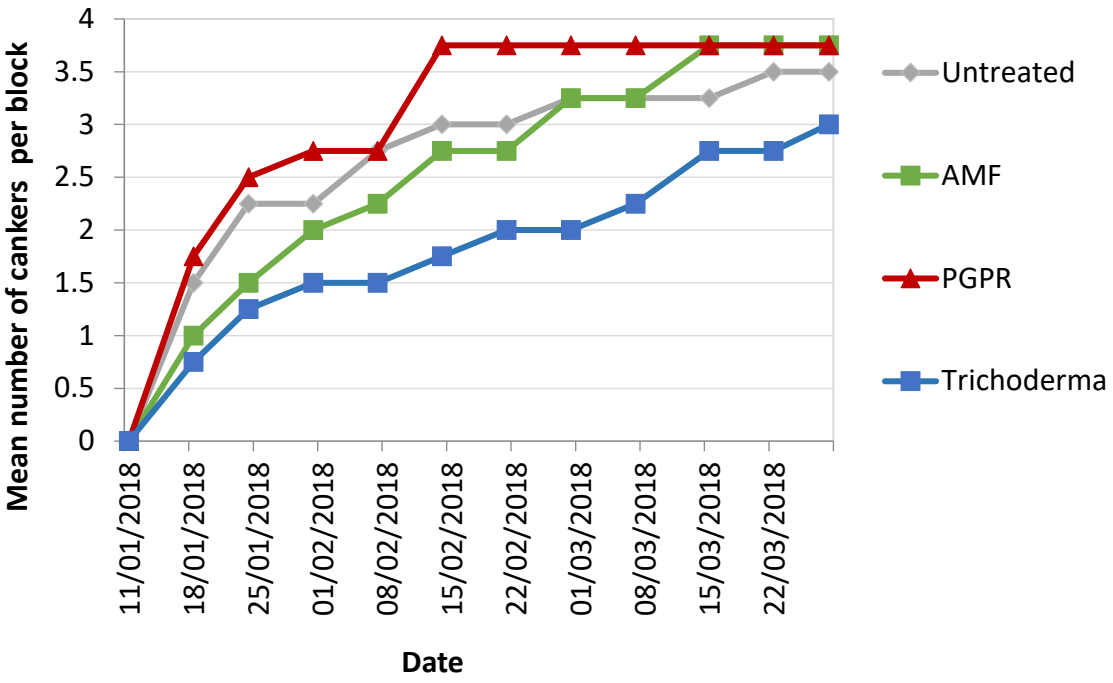


Figure 2.3.4 Disease progression curve displaying mean number of cankers developed under each biological treatment added to stoolbeds, representing the newly established orchard phase. Latency is determined by length of time until symptom expression

Newly established orchards

The overall number of cankers was very low at Broadwater with a grand mean canker number of 0.035 (Figure 2.3.5). No statistically significant differences between the amendment treatments were recorded (Table 2.3.3). There were no rootstock (A-type) cankers recorded for any of the trees treated with soil amendments. The number of mainstem cankers (B-type) was lowest for AMF. The untreated control had higher canker number than any of the trees with amendments added. PGPR, *Trichoderma* and CarbonGold were all very similar for mean mainstem B-type cankers (0.1, 0.11, 0.09, respectively). Regarding peripheral cankers, again, the untreated control had the highest number of C-type cankers (0.06). The amendment with the lowest C-type cankers was PGPR, while AMF, CarbonGold and *Trichoderma* were similar in canker number (0.03).

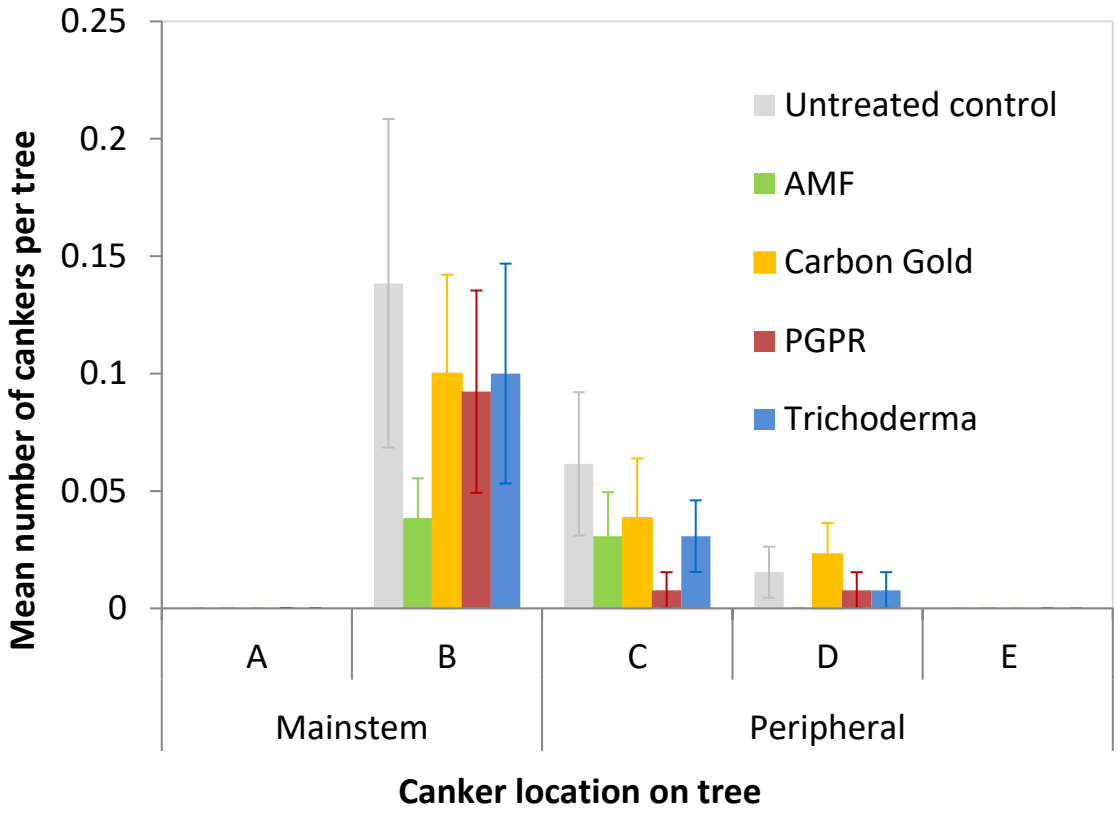


Figure 2.3.5 Mean number of cankers per tree at the Broadwater farm after treatment with various soil amendments. The assessment was completed on 11/5/2018

Table 2.3.3 ANOVA results table of cankers on apple trees at Broadwater farm treated with various soil amendments.

Location of canker	**Degrees of freedom**	***p-value***
Rootstock (A)	4	N/A
Mainstem (B)	4	0.446
Peripheral (C)	4	0.446
Peripheral (D)	4	0.355
Peripheral (E)	4	N/A

The overall number of cankers was also very low at Friday Street farm with a grand mean canker number of 0.04 (Figure 2.3.6). Peripheral cankers (C-type) were found to be significantly different between the amendments with the lowest number for *Trichoderma* and PGPR (Table 2.3.4). B-type mainstem cankers were the most prevalent at Friday Street. Interestingly, AMF had a higher number of B-type cankers than the untreated control. For C-type peripheral cankers AMF had a similar number of cankers to the control, while PGPR and *Trichoderma* were both lower than AMF and the control. For A-type cankers, these were only present with the *Trichoderma* amendment, however the mean number was very low (0.005). There were no E-type peripheral cankers for any of the amendments.

The heat map of cankered trees at the Broadwater farm and the Friday Street farm are presented in Figure 2.3.7. There was no clear trend with the location of cankers as they appear mostly random.

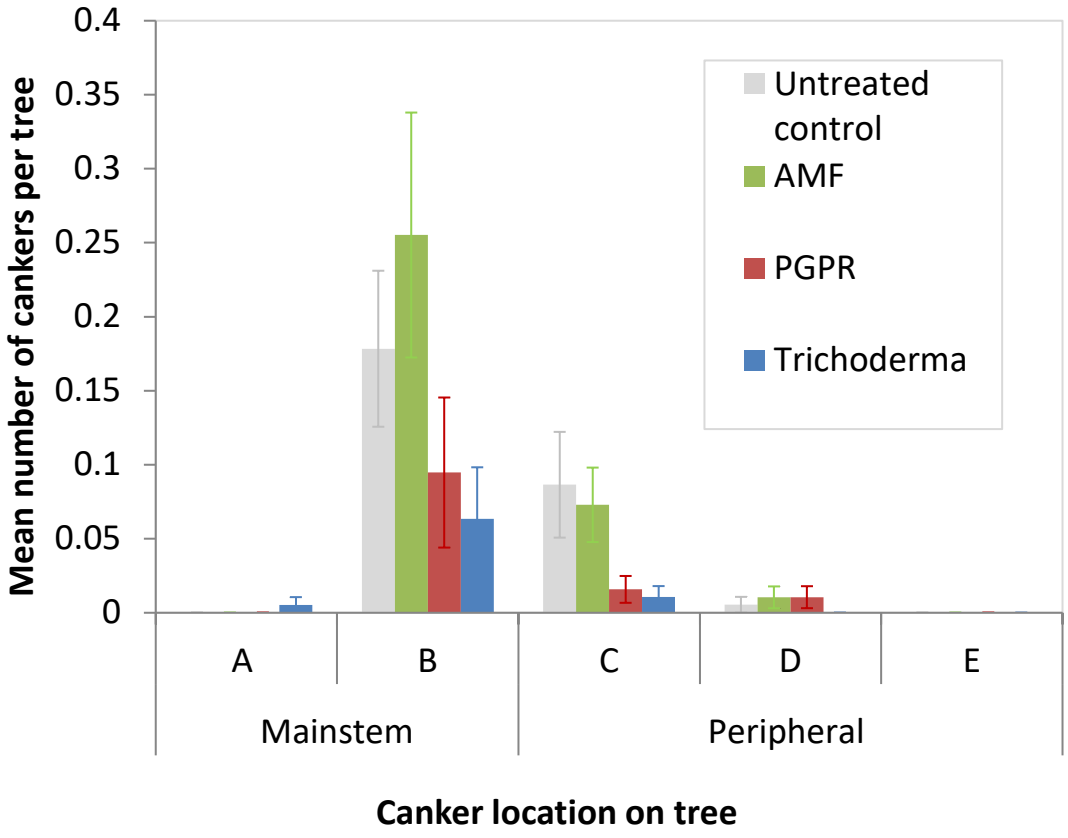


Figure 2.3.6 Mean number of cankers per tree at Friday Street farm after treatment with various soil amendments. The assessment was completed on 17/5/2018

Table 2.3.4 ANOVA results table of cankers on apple trees at Friday Street farm treated with various soil amendments. The statistically significant value is in bold

Location of canker	Degrees of freedom	*p-value*
Rootstock (A)	3	0.441
Mainstem (B)	3	0.091
Peripheral (C)	3	**0.011**
Peripheral (D)	3	0.551
Peripheral (E)	3	NA

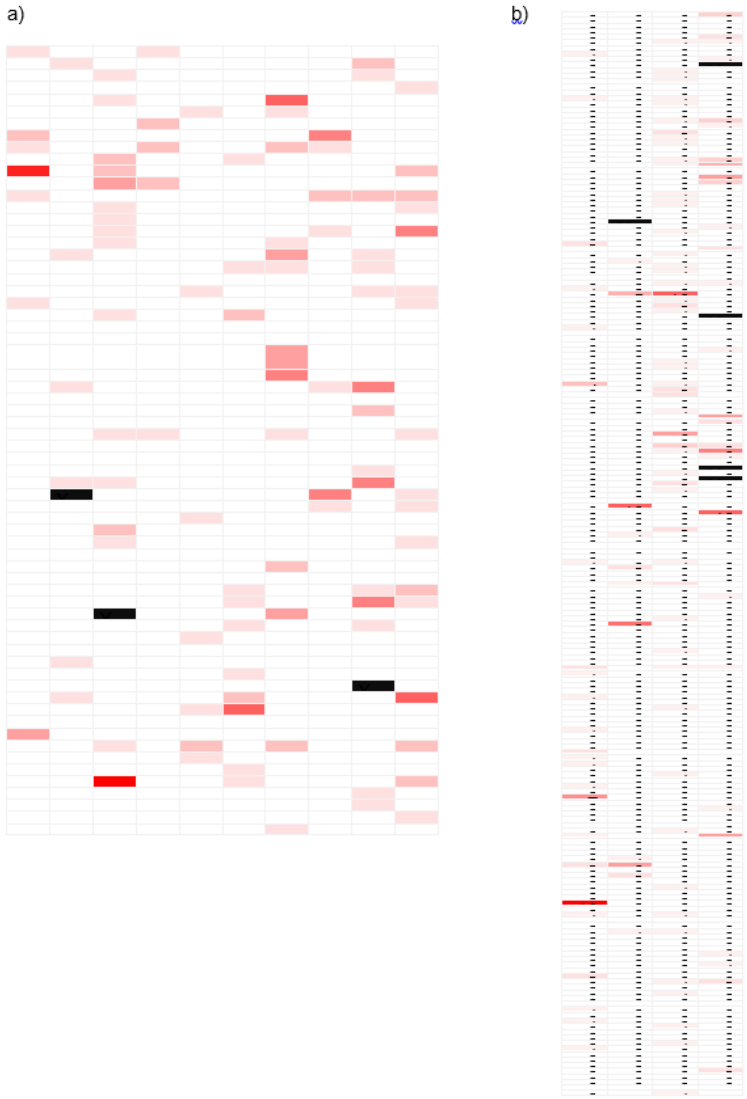
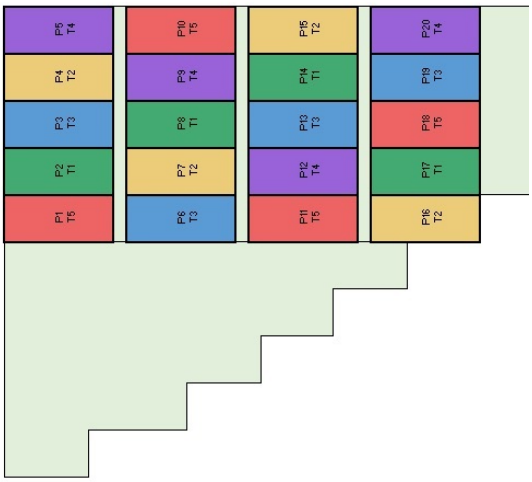


Figure 2.3.7 Heat map showing location of trees with cankers at Broadwater farm (a), and Friday Street farm (b). The intensity of the red colour indicates severity. The darker the colour the more severe the canker. Black indicates dead tree

Material and Methods (ADAS)

Site

The site was located at Herridges Orchard, Gloucestershire in a newly planted block of leg Gala trees (Figure 2.3.8). The trial was laid out in a randomised block design with four replicate blocks, each replicate block consisting of nine tree pseudo-replicates in late March. Each of the four replicate blocks had a guard tree row in between them. All products were included in one experiment comprising five treatments plus an untreated control (Table 2.3.5).



There were a total of 36 trees per treatment, and 180 trees for the whole trial.

Figure 2.3.8 Trial plan and set up at Herridges Orchard 12 April 2018

The leg Gala trees were planted on 12 April 2018, with the AMF and *Trichoderma harzianum* treatments applied to the planting holes immediately before planting. The PGPR treatments were applied as a drench around the tree two weeks after planting (Table 2.3.5), and covered with soil immediately afterwards.

Table 2.3.5 Treatments and timings of applications for each

Treatment No.	Treatment	Product (Supplier)	Species	Timing
1	Untreated	-	-	-
2	Arbuscular Mycorrhizae Fungi (AMF)	Rootgrow (Plantworks)	*Funneliformis mosseae* *Funneliformis geosporus* *Claroideoglomus claroideum* *Rhizophagus irregularis* *Glomus microaggregatum*	At planting
3	Plant Growth Promoting Rhizobacteria (PGPR)	Experimental (Plantworks)	*Bacillus megaterium* *Pseudomonas putida* *Pseudomonas fluorescens* *Azospirillum brassilense*	Two weeks after planting
4	AMF + PGPR	As above	As above	As above
5	Trichoderma	TrianumG (Koppert)	*Trichoderma harzianum* strain T-22	At planting

Standard treatments for pests, foliar disease and nutrients were applied to all plots throughout the season. Specific canker treatments were omitted by the trial host where commercially acceptable, however, sprays were applied for scab control that may have an incidental effect on canker.

An initial assessment of the trial was conducted on 6 June 2018, with any dieback of branches or shoots noted. The trial was monitored regularly during the growing season and the main assessment was performed on 14 November 2018. Main stem A/B and peripheral C/D/E cankers were counted for each tree as for the other sites. Data were analysed by ANOVA.

Results

During the initial assessment of the trial at Herridges Orchard dieback was noted on shoots of six trees. None of the trees in the trial were recorded as having mainstem cankers below the union (A) or cankers on E peripheral shoots. The natural levels of infection were quite low overall, canker was recorded mainly on C and D peripheral shoots, with some canker on the mainstem.

Overall there was no significant difference between any of the treatments in total number of cankers recorded (Table 2.3.6). Although not significant, the trees treated with *Trichoderma* had a lower canker incidence overall compared with the other treatments, with canker only recorded on the peripheral shoots (Figure 2.3.9). The incidence of canker on C peripherals in

the AMF + PGPR treatment was significantly greater than any of the other treatments and more canker was seen on D peripherals in the PGPR treatment. With the low natural canker incidence on the site these results should be treated with caution as they may be a result of natural variation.

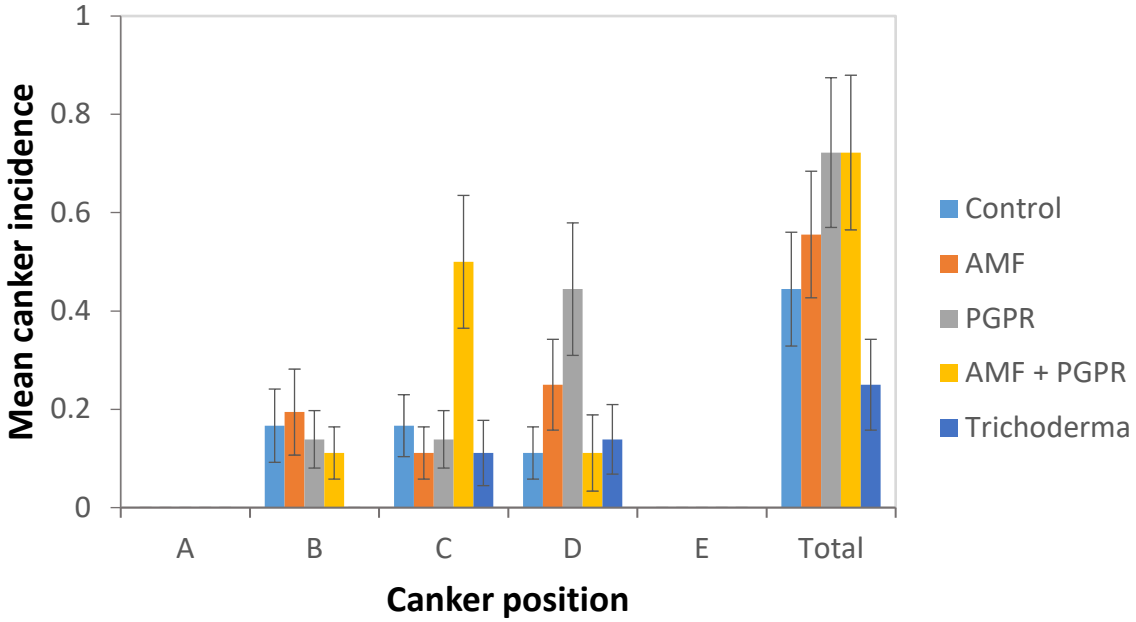


Figure 2.3.9 Effect of soil amendments on development of canker at Herridges Orchard

Table 2.3.6 Effect of soil amendments on development of canker at Herridges Orchard

Treatment	B	C	D	Total
Control	0.171	0.171	0.114	0.457
AMF	0.194	0.111	0.250	0.556
PGPR	0.139	0.139	0.444	0.676
AMF + PGPR	0.111	0.500	0.111	0.722
Trichoderma	**0.000**	0.118	0.147	0.265
Fprob	0.228	**0.003**	**0.043**	0.062
SED	0.088	0.116	0.127	0.187
LSD (P = 0.05)	0.176	0.228	0.250	0.369

Discussion

Overall, the number of cankers was very low at Broadwater, Friday Street and Herridges Orchard. At Friday Street, peripheral cankers (C-type) were found to be significantly different between the amendments with the lowest number for *Trichoderma* and PGPR. At Herridges Orchard the trees treated with *Trichoderma* had the lowest canker incidence. Friday Street overall had a higher number of mainstem B-type cankers (0.148), compared to Broadwater (0.095). Interestingly the AMF amendment at Friday Street had the highest number of B-type cankers, however at Broadwater AMF had the lowest number of B-type cankers. It is not clear why this is the case, but there was no significant difference between amendments for B-type cankers at either site, so it may simply represent biological variation.

In the stoolbed trial (East Egham) the *Trichoderma* amendment resulted in a significantly reduced number of cankers compared to PGPR, however was not significantly different to the untreated control. *Trichoderma* is worth investigating further as a stoolbed amendment. *Trichoderma* spp. have previously been shown to reduce other apple diseases such as scab (Doolotkeldieva & Bobusheva 2017) and ring spot (Kexiang et al. 2003). For all stoolbed amendments, the latency of symptom expression was 50-51 days in the stress trial. In nature, the symptom development may be longer due to a lower amount of environmental stress.

The heat maps (Fig. 2.3.7) showed there was no clear trend with regard to the location of cankers or severity, they appear to be randomly distributed. This indicates that there were effects of position (plot location) occurring in the experiments. Artifical inoculation at the Herridges site in 2019 may aid the understanding of the effects of the amendments on the incidence of canker.

Conclusions

- The overall number of cankers at the newly planted orchards at Broadwater, Friday Street and Herridges was very low
- A significant difference was found with the number of C-type peripheral cankers at the newly planted orchard at Friday Street with *Trichoderma* and PGPR having the lowest number
- At East Egham, the *Trichoderma* stoolbed amendment resulted in a reduced number of cankers compared to all other treatments and the untreated control throughout the sampling period. *Trichoderma* had significantly lower canker incidence than PGPR. However, it did not have significantly lower canker incidence than the untreated control

- There was no significant difference in the canker expression at Herridges Orchard in 2018, however *Trichoderma* had the lowest overall canker incidence
  The latency period for canker expression in the stoolbed trial was 50-51 days from inoculation time
- Heat maps showed no clear trend of the location of cankered trees. They appear randomly distributed at both sites

Future work

- Continue canker assessments of newly planted orchards at Broadwater, Friday Street and Herridges
- Continue assessments of canker in stoolbed trials
- Further investigation of *Trichoderma* spp. as a control measure for canker
  Herridges orchard trial will be artificially inoculated in 2019 to get higher infection in the treatments

2.4 Novel methods of treatment application to manage apple canker (EMR/ADAS, Yr 1-3)

Aim

To demonstrate the benefit, if any, of pruning cut protection

Introduction

Traditionally used wound paints to protect pruning wounds from *Neonectria ditissima* have been removed from the market in the past few years due to the high labour costs required in application resulting in a lack of demand. Newly available products, such as chemical dispensers attached to pruning secateurs, have the potential to reduce labour costs involved with protecting pruning wounds from canker infection by treating them at the same time as pruning. The initial pruning trial in 2017 indicated that *Trichoderma* may have a protective effect on the pruning wounds when used in conjunction with a polymer to seal the wound.

Material and Methods (ADAS)

Site: The site was located at Herridges Orchard, Gloucestershire in a block of 400 Cox trees. It was planted at a spacing of 1.83m x 3.66m in 1998. The orchard had been identified as having a high incidence of canker. The trial was conducted on one whole row in the orchard (Fig. 2.4.1).

Figure 2.4.1 Row of trees at Herridges Orchard pruned (left) and Felco 19 secateurs used to apply treatments

Treatments were applied on a dry day at the beginning of the season (12 April 2018). A total of six treatments were use in the trial (Table 2.4.1) including a control of water. Five shoots per tree were pruned and treatments were applied using Felco 19 secateurs with a chemical dispenser (Fig. 2.4.2). The treated shoots were spray painted and string tied around them so they could be returned to record canker incidence in the cut shoots.

Table 2.4.1 Novel application trial 2018 on a row of Cox apple trees at Herridges Orchard including five treatments for pruning cuts and a water control

Treatment	Active	Rates
Water	-	-
BlocCade	polymer	100 ml/L
Trichoderma	BCP511B	10.0 g/L
Folicur	tebuconazole	0.6 ml/L
BlocCade + Trichoderma	polymer + BCP511B	As above
BlocCade + Folicur	Polymer + tebuconazole	As above

Figure 2.4.2 Apple shoots pruned and treatments being applied

The trial was artificially inoculated with canker 24 hours after the initial application of the treatments to simulate an infection event after pruning. The marked cuts were inoculated with 5 µl of *N. ditissima* Hg199 spore suspension of $1x10^5$ conidia ml^{-1} suspended in sterile distilled water using a pipette. Germination tests 24 hours after inoculation showed a 78% germination rate for the spore suspension.

Standard treatments for pests, foliar disease and nutrients were applied to all plots throughout the season. Specific canker treatments were omitted by the trial host where commercially acceptable; however, sprays were applied for scab control that may have an incidental effect on canker.

The trial was checked regularly throughout 2018 and the trees were assessed on 14 November 2018. The number of cut shoots with canker, the regrowth on from the cut shoot and length of regrowth were measured. The trees were also assessed for canker on the branches and the tree main stem. Any phytotoxic effects were noted, with particular note of whether callouses were formed over the cut. The data were analysed using ANOVA.

Results

The background levels of canker in the orchard were high. There was a 98 % incidence of canker on the main stem of the trees used in the trial and 78 % of trees had canker on their branches. There was no significant difference in the number of trees with canker on the branches or main stem in the study blocks or in treatments. Necrosis or chlorosis to foliage was not noted during the interim assessments in 2018. Some differences were noted in the callousing of the pruning cuts at the final assessment in November, with those treated with Folicur showing good callousing and those with the *Trichoderma* showing poorer callousing (Fig. 2.4.3).

Figure 2.4.3 Cut shoots showing: canker (left - Untreated); no canker, but poor callousing (centre - *Trichoderma*); no canker with good callousing (right - Folicur) at assessment November 2018

Regrowth was generally low across the trial at this assessment date. BCP115B had the highest incidence of regrowth, with 30% of cuts showing some regrowth, however this was not significantly different to the other treatments (Fig. 2.4.4).

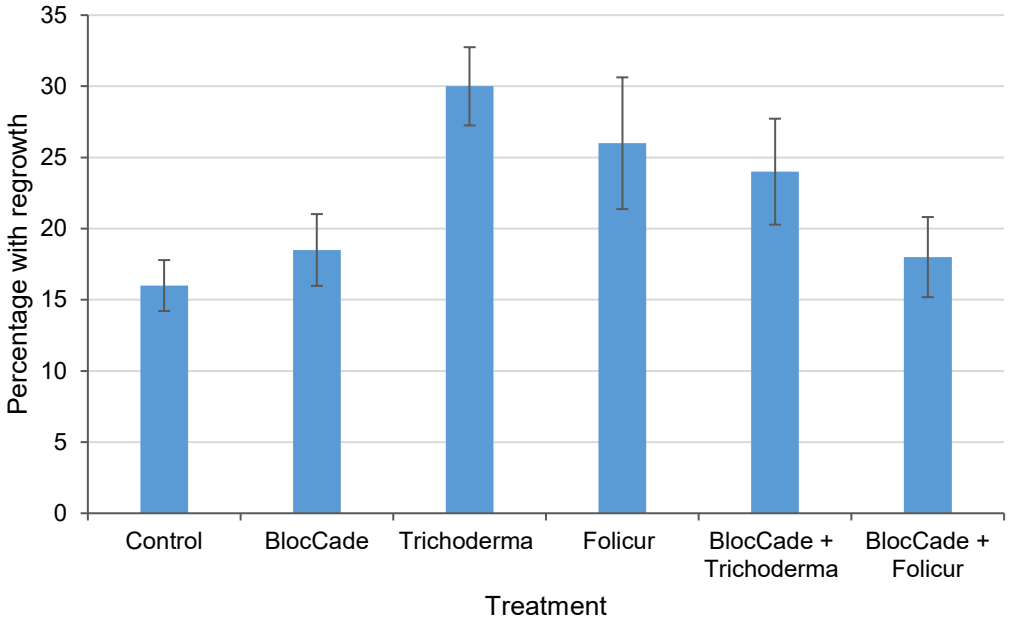


Figure 2.4.4 Percentage of cut shoots showing regrowth in November 2018. Differences are not statistically significant

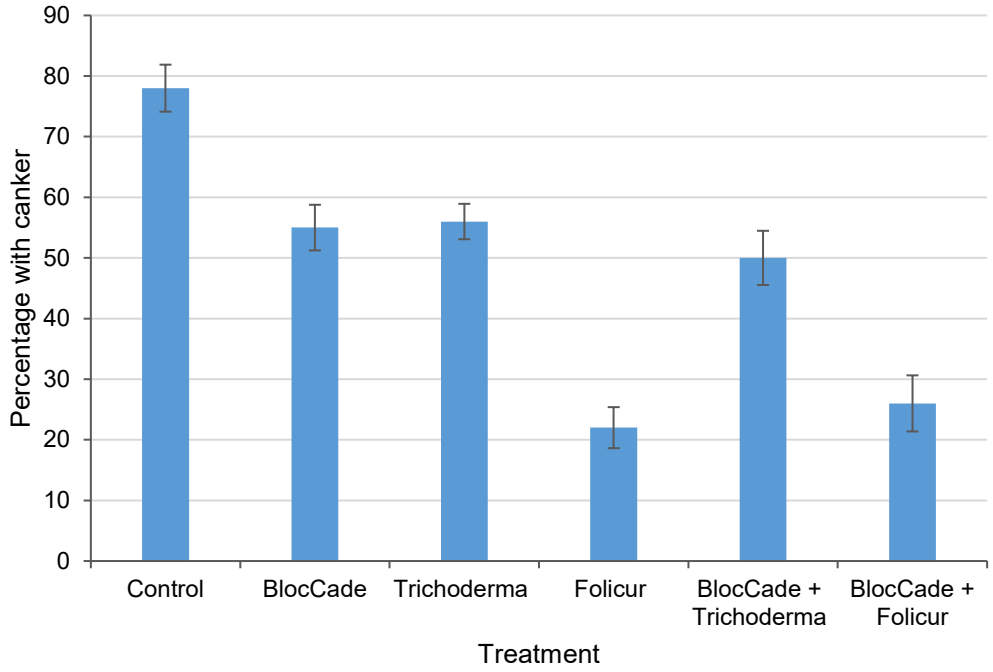


Figure 2.4.5 Proportion of cut shoots with canker present in November 2018. Differences are statistically significant ($p < 0.001$)

There were significant differences in the proportion of canker on the cut shoots (Table 2.4.2). The artificial inoculation of the pruning cuts was successful, with nearly 80% of the control cuts expressing canker symptoms (Fig.2.4.5). The treatments containing tebuconazole (Folicur) performed the best with the standalone treatment having the lowest incidence of canker in the trial. Both of the tebuconazole containing treatments had significantly lower incidence compared to the control.

Table 2.4.2 Effect of pruning cut treatments on development of canker at Herridges Orchard. Figures significantly different from others are highlighted in bold

Treatment	Percentage regrown	Percentage with canker
Control	16.0	78.0
BlocCade	18.5	55.0
BCP511B	30.0	56.0
Folicur	26.0	**22.0**
BlocCade + BCP511B	24.0	50.0
BlocCade + Folicur	18.0	**26.0**
Fprob	0.640	**< 0.001**
SED (45)	9.33	12.58
LSD (P = 0.05)	18.78	25.33

Discussion

Regrowth was generally low for all treatments, although there was slightly higher regrowth in shoots treated with *Trichoderma*, the effect was not significant. The branches treated with tebuconazole (Folicur) formed good callouses over the pruning wound, which was not always the case for the other treatments. Although canker was not necessarily recorded in the wounds that did not callous, these sites may act as an entry point for future infections.

The incidence of canker developing was significantly lower in the treatments containing tebuconazole compared to the water treated control. Although these treatments were not significantly better than the biological or physical products there was low canker development despite artificial inoculation with *N. ditissima* spores following pruning.

The same preventative effect was seen in the initial pruning trial in 2017, with both tebuconazole treatments showing significant reduction of canker development. In the previous trial the *Trichoderma* treatment showed some promise with the polymer sealant, so a species that was isolated from wood (as opposed to soil) was chosen for this trial. Although the treatment showed a reduction in canker development compared to the control, it was not significantly better. It is probable that this biological treatment did not have sufficient time to establish on the cuts before the wounds were inoculated resulting in reduced performance. This product may be most effectively applied during dry weather when there is a lower risk of immediate exposure of the wound to canker spores.

The trials in 2017 and 2018 have shown that the spray applicator significantly reduces the incidence of canker development compared to no treatment at the wounding stage. The most consistently promising treatment trialled was tebuconazle (Folicur) in both cases.

Conclusions

- Trials from 2017 and 2018 indicate that application of wound protection products at pruning can significantly reduce the incidence of canker on pruning cuts
- All treatments applied with this method reduced the level of canker over applying water alone
- Treatments with tebuconazole showed the lowest infection rate of all of the treatments
- The biological treatment (*Trichoderma*) may not have had sufficient time to establish before wounds were inoculated, application during dry weather may improve efficacy.

Objective 3 - Apple Foliar Diseases

Task 3.1 Overwintering inoculum

Aim

Determine optimum timing of treatments to target the over-wintering phase of scab and mildew to disrupt the lifecycle (EMR, Yr 1-4)

Introduction

The uptake of biological control agents (BCAs) has been limited for disease control in orchard crops despite their great potential to reduce conventional pesticides as part of an integrated pest management programme. Barriers for the uptake of BCAs in orchard systems include the higher cost/ha and their reduced/variable efficacy relative to conventional pesticides. Used in season, when the rate of pathogen development is usually at its greatest, results in a challenging environment for BCAs to suppress disease development. This task aims to develop understanding of interactions between potential antagonists and the pathogen (or pathogen substrate) to inform strategies targeting the overwintering phase.

Powdery mildew (*Podosphaera leucotricha*) mainly overwinters as mycelium in floral and vegetative buds. *Ampelomyces quisqualis* (AQ) is a mycoparasite of powdery mildew. Commercial preperations of AQ, such as AQ10 have been used in greenhouse and field-grown vegetable crops, usually with reduced fungicide inputs, to achieve disease control. AQ10 was one of the best performing BCAs in trials conducted as part of SCEPTRE when applied throughout the season and in combination with fungicides in a managed programme, however the control achieved was not commercially acceptable. One of the disadvantages of using AQ10 is the slow growth rate of this parasite. This has led to the strategy proposed here; to target the overwintering phase of the disease offering a long interaction period between parasite and powdery mildew. Trials were setup over the summer of 2016 to test whether the BCA is incorporated into the bud, whether the parasite can survive over winter and whether the stratergy is effective at reducing inoculum. These trials were inconclusive. The objective of trials in 2018 was to re-evaluate the strategy with AQ10 and to include an alternative BCA – Sonata (*Bacillus pumilis*) – which has been very effective in controlling strawberry powdery mildew.

Objective

To demonstrate the benefit of reducing primary mildew by targeting overwintering inoculum and to evaluate a new strategy to improve the efficacy of BCA's for mildew control

Materials and methods

Site: The trial was located in orchard EE190, located at NIAB EMR. The orchard was planted in 1998 and is 0.64 ha in size and consists of single alternate rows of Royal Gala and Self Fertile Queen Cox on M9 rootstock with 1.75 m between trees in the row and 3.5 m between rows. Cox trees were used in this trial which had previously been on a 14 day fungicide programme resulting in a high incidence of mildew in the plots

Trial design: The trial was designed as eight tree plots of cv. Cox. The trial plots were separated in the row by one tree, with a row of Gala trees between the trial rows. Each of the treatments was replicated six times in a randomised block design (Fig. 3.1.1).

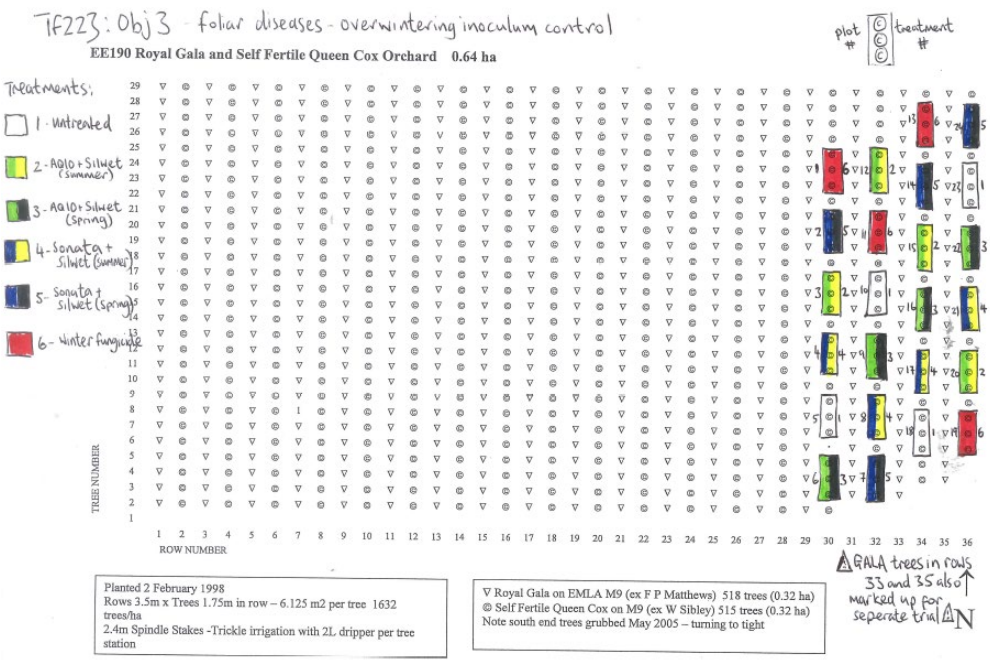


Fig. 3.1.1 Trial plan for reducing powdery mildew overwintering inoculum (2018)

Treatments: Treatments and treatment timings are given in Table 3.1.1. Standard treatments for pests, foliar disease and nutrients were applied to all plots throughout the season.

Treatment application: Sprays were applied to the eight tree plots for treatments using a Birchmeier motorised air-assisted knapsack sprayer at 500 L/ha following EMR SOP GEP 725. Treatments to the whole trial area were applied using a tractor-trailed air-assisted orchard sprayer at the standard farm spray volume of 200 L/ha.

Assessments

Meteorological records: Records of daily maximum and minimum temperature and rainfall were taken from a weather station located at NIAB EMR.

Growth stages at application: Phenological stage at each application will be recorded using the BBCH growth stage scale.

Phytotoxicity: Symptoms of phytotoxicity were checked and recorded. Records will include any chlorosis / necrosis to foliage, growth regulatory effects to shoots, assessed on a scale 0-5 (EPPO Guideline PP 1/135(4)).

Primary mildew assessments: An assessment of the number of mildewed vegetative buds (at petal fall) as a percentage of total buds will be conducted in the spring. Ensuring all the extending buds are quantified i.e. terminal and side shoots.

Ampelomyces quisqualis presence: Five mildewed shoots will be collected from each of five trees within each plot and observed beneath a microscope for parasitism with AQ. The results will be presented as proportion of buds with the interaction.

Table 3.1.1 Treatments evaluated in 2018 to target the over-wintering phase of mildew

Treatment number	Product	Active ingredient	Treatment timing	Recommended foliar rate of product
1	Untreated	-	-	-
2	AQ10 + Silwet	*Ampelomyces quisqualis* + silicon wetter	3 applications at the end of extension growth (July '18)	70g/ha + 0.05%
3	AQ10 + Silwet	*Ampelomyces quisqualis* + silicon wetter	3 applications at bud burst (March '19)	70g/ha + 0.05%
4	Sonata + Silwet	*Bacillus pumilus* + silicon wetter	3 applications at the end of extension growth (July '18)	5 L/ha + 0.05%
5	Sonata + Silwet	*Bacillus pumilus* + silicon wetter	3 applications at bud burst (March '19)	5 L/ha + 0.05%
6	Talius + Wetcit	proquinazid + wetter	Single treatment at high volume applied when trees are fully dormant (Dec/Jan) and timed so several dry days after application	0.25 L + 0.5%-1%

Results

Treatments with the BCAs were scheduled to start in mid-July to target the period when terminal buds on apple shoots seal up. Unfortunately the hot dry weather in July resulted in shoot growth stopping early. Hence the trial was not started.

The trial will be rescheduled for 2019.

Task 3.2 Evaluate efficacy and persistence of alternative chemical treatments to fungicides (NIAB EMR Year 4) ORETO 18/003

Background (3 year summary)

In a replicated split plot orchard trial on apple cv. Gala, main plots were sprayed with a standard fungicide programme at 7 or 14 day intervals to establish a high and low incidence of secondary mildew. Within these main plots 10 test alternative treatments (Cultigrow (CBL), SB Invigorator, Wetcit, B225, Trident) were applied by air-assisted knapsack sprayer at 500 L/ha to small three tree plots. Sub plot treatments were applied eleven times at 7-10 day intervals, apart from CBL (3 sprays at monthly intervals). Plots treated with CBL in 2016 were retained for a second year to evaluate the cumulative effects of this product. In addition CBL was evaluated with and without the addition of Wetcit. B225 was applied monthly or at 7-10 day intervals. Untreated plots were included which were the 7 or 14 day fungicide only programmes. Secondary mildew was assessed weekly on extension growth. Plots were also assessed for phytotoxicity, fruit set, yield and fruit quality. The results obtained thus far are summarised as follows:

- The 7 and 14 day programmes used as the main block treatments successfully established high (50%- almost 100% mildewed leaves) and low (40-60% mildewed leaves) mildew plots in which to evaluate the test products, although differences were not as great as in 2016
- Overall all treatments had significantly less mildew than the fungicide only plots
- Treatment 6 SB Invigorator was the most consistent in reducing mildew
- Treatments 2 (CBL 2 years), 4 (CBL + Wetcit) and 5 (Wetcit only) were next most consistent products
- CBL applied for a second year had almost significantly less mildew than in plots where CBL was applied for the first year, indicating a possible cumulative effect
- None of the treatments resulted in phytotoxicity or fruit russet
- No significant effect of treatments on yield
- No significant effect of treatments on fruit size or fruit colour

Aim

In 2018 we aimed to evaluate the control of mildew achieved with alternative products combined into programmes with fungicides.

Materials and methods

Site: The trial was located in orchard EE190, located at NIAB EMR. The orchard was planted in 1998 and is 0.64 ha in size and consists of single alternate rows of Royal Gala and Self Fertile Queen Cox on M9 rootstock with 1.75 m between trees in the row and 3.5 m between rows.

Trial design: The trial was designed as four tree plots of cv. Gala, which were located in the parts of the orchard which received the seven day fungicide programme in 2017 to ensure the least primary mildew. The trial plots were separated in the row by one tree, with a row of Cox trees between the trial rows. Each of the ten treatments was replicated four times in a randomised block design (Fig. 3.2.1).

Treatments: All plots received a standard programme for pest and disease control (Appendix 3.2) and nutrients up to the start of the trial at pink bud (BBCH57). The programmes detailed in Table 3.2.2, based on the products in Table 1 were then applied from pink bud. The programmes tested were based on combining fungicides, elicitors / biostimulants (Cultigrow, Trident or Mantrac) and physical control products (Wetcit or SB Invigorator). Programmes 3 and 4 were based on Cultigrow with either Wetcit or SB Invigorator; programmes 5 and 6 were based on Mantrac with either Wetcit or SB Invigorator; and programmes 7 and 8 were based on Trident with either Wetcit or SB Invigorator. All included fungicides applied at 14 day intervals. Programmes 1 and 2 were fungicide controls applied at either 7 or 14 day intervals as comparisons. Programmes 9 and 10 were based on either Cultigrow or Trident with decisions on other products based on the mildew incidence assessed weekly. There was no untreated control.

These programmes were followed for the first three sprays (Table 3.2.2). Then warm wet weather at the end of May resulted in rapid lush shoot growth and, as a consequence, a large increase in secondary mildew, especially on the unsprayed Cox guard rows. It was therefore decided to change the programme. A spray of potassium bicarbonate was immediately applied to the Cox guard rows to suppress mildew sporulation. The 14 fungicide programme was then applied to all plots including the Cox guard rows by orchard tractor sprayer at 200 L/ha. This meant that Programme 2 was not applied to the small plots and programme 1 was applied to the small plots every other spray round. A total of 10 treatment rounds were applied.

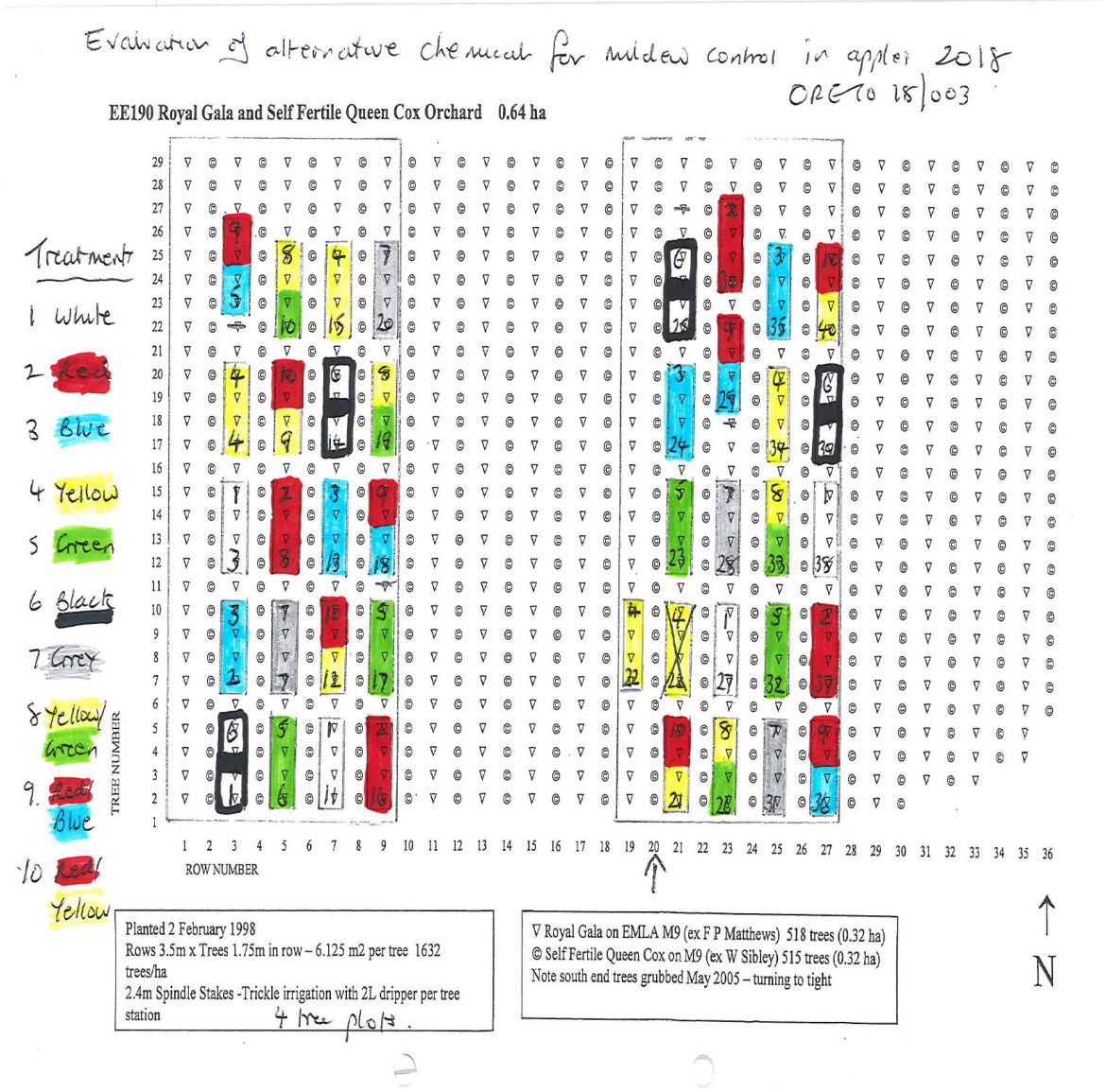


Figure. 3.2.1 Trial plan showing four tree plots located in two orchard blocks which had received 7 day mildew programme in 2017

Table 3.2.1 Fungicides, elicitors, biostimulant products used in the programmes evaluated for effects on powdery mildew in apple 2018

Product	Active ingredient	Product type	Rate of product / ha	Harvest interval days	Latest use date mid Sep harvest	Maximum number of sprays / Use
Topas	penconazole	Fungicide	0.5 L	21	24/8	3
Cosine	cyflufenamid	Fungicide	0.5 L	14	31/8	2
Stroby	Kresoxim-methyl	Fungicide	0.2 kg	28	17/8	4
Fontelis	penthiopyrad	Fungicide	0.75 L	21	24/8	2
Sercadis	fluxapyroxad	Fungicide	0.3 L	35	10/8	3
Talius	proquinazid	Fungicide	0.25 L	49	28/7	2
Flint	trifloxystrobin	Fungicide	150g	14	31/8	4
Karma	Potassium hydrogen carbonate	mineral	5 kg	1	13/9	8
Kindred	meptyldinocap	Fungicide	0.6 L	Before end of bloom	-	2
Cultigrow CBL	flavonoids	Elicitor/biostimulant	500 ml			Blossom then monthly
Wetcit	Alcohol ethoxylate	Energiser adjuvant	0.2%			7-10 days
SB invigorator	Various nutrients and natural products	Physical action. Controls various pests and mildew	1 ml/L			7-10 days, weekly sprays
Mantrac Pro	manganese	nutrient	0.5 L			5-6 applications from green cluster/pink bud
Trident (New)	Silicon 1%, Copper 2%, Zinc 4%	Nutrient / elicitor	1-3 L			7-10 days
Pek acid	Soluble P and K	fertiliser	0.75%			7-10 days

Table 3.2.2 Programmes evaluated for powdery mildew control in apple 2018 and treatment application dates

Programme	Treatment	Product / Timing											
	Growth stage	**Pink bud 4 May**	**15 May**	**24 May**	**4 Jun**	**12 Jun**	**18 Jun**	**21 Jun**	**26 Jun**	**27 Jun**	**3 Jul**	**10 Jul**	**17 Jul**
Overall spray							Flint			Cosine		Talius	
1	**Fungicide only 7 days**	Flint	Fontelis	Sercadis	Topas			Stroby			Topas		Sercadis
2	**Fungicide only 10-14 days**	Flint		Sercadis									
3	**CBL based A**	Flint + CBL	SBI	Sercadis	SBI	CBL		SBI			SBI	CBL	SBI
4	**CBL based B**	Flint + CBL	Wetcit	Sercadis	Wetcit	CBL		Wetcit			Wetcit	CBL	Wetcit
5	**Mantrac based A**	Flint + Mantrac	SBI	Sercadis + Mantrac	SBI	Mantrac		SBI	Mantrac		SBI	Mantrac	SBI
6	**Mantrac-based B**	Flint + Mantrac	Wetcit	Sercadis + Mantrac	Wetcit	Mantrac		Wetcit	Mantrac		Wetcit	Mantrac	Wetcit
7	**Trident based A**	Flint + Trident	SBI	Sercadis + Trident	SBI	Trident		SBI	Trident		SBI	Trident	SBI
8	**Trident based B**	Flint + Trident	Wetcit	Sercadis + Trident	Wetcit	Trident		Wetcit	Trident		Wetcit	Trident	Wetcit
9	**CBL based managed**	Flint + CBL	Kindred	Cosine	SBI	CBL		Stroby			Topas	CBL	Sercadis
10	**Trident based managed**	Flint + Trident	Kindred	Cosine + Trident	SBI	Trident		Stroby	Trident		Topas	Trident	Sercadis

Treatment application: Sprays were applied to the four tree plots for treatments 1-10 using a Birchmeier motorised air-assisted knapsack sprayer at 500 L/ha following EMR SOP GEP 725. Treatments to the whole trial area were applied using a tractor-trailed air-assisted orchard sprayer at the standard farm spray volume of 200 L/ha.

Table 3.2.3 Date and growth stage when treatments were applied in 2018

Spray number	BBCH growth stage	Date treatment applied	Spray interval Days
1	57/61	4 May	-
2	66/67	15 May	11
3	67/69	24 May	9
4	69/71	4 June	11
5	71/72	12 June	8
6	72	21 June	9
7	73	26 June	5
8	75	3 July	7
9	75	10 July	7
10	76	17 July	7

Table 3.2.4 Air temperature and humidity conditions at the time of spray applications

Date	At start of spray applications					At end of spray applications					Weather conditions
	Time	Temp °C		RH%	Wind speed (kmph) Direction	Time	Temp °C		RH%	Wind speed (kmph) Direction	
		Dry bulb	Wet bulb				Dry bulb	Wet bulb			
4 May	7.55	14	12	79.3	0	12.00	17	15	81	1.4 SE	Sunny
15 May	8.57	13.5	13.5	100	4.4 NM	12.15	19.5	18	86.5	3.7 N	Sunny / breezey
24 May	11.25	17.5	16.5	90.4	1.0 N	15.30	16	15.5	95	0.8 N	Overcast
4 June	11.15	16.5	16	95	1.7 NE	16.05	16.5	16	95	3.3 N	Overcast
12 June	12.45	15.5	15	94.9	2 NE	10.15	18.5	17.5	90.7	0	Overcast
21 June	7.50	13.5	13	94.6	3.8 NW	11.40	17.5	15	76.8	3.3 N	Sunny spells
26 June	8.23	17.5	17	95.2	0	10.10	22.5	19.5	75.6	3.0 N	Sunny spells
3 July	7.15	17	17	100	4.0 NE	10.55	21	18	74.7	4 E	Sunny spells
10 July	6.42	17	16.5	95.1	2.4 NW	9.12	15.5	15	94.9	2.6 NW	Cloudy
17 July	7.00	16	15.5	95	4 NW	10.15	21	18	74.7	3.1 SW	Sunny

Table 3.2.5 Percentage accuracy of spray applications (volume applied / volume required expressed as a percentage).

Treatment number	Spray date 4 May	15 May	24 May	4 June	12 June	21 June	26 June	3 July	10 July	17 July
1	116	112	107	112		104		106		104
2	115		89							
3	110	119	84	99	102	97		122	100	101
4	119	109	90	104	98	98		108	106	98
5	104	118	93	111	96	102	114	110	104	100
6	104	110	89	106	96	98	103	107	110	100
7	106	105	106	110	104	102	102	112	102	100
8	106	108	101	89	84	94	102	105	106	97
9	102	102	102	111	104	97		106	106	108
10	108	108	101	99	98	112	104	105	105	108

Assessments

Meteorological records: Records of daily maximum and minimum temperature and rainfall were taken from a weather station located approximately 500 m west of the trial orchard at NIAB EMR.

Growth stages at application: The phenological stage using the BBCH scale was recorded at application and assessment times (Table 3.2.3).

Phytotoxicity: Symptoms of phytotoxicity were checked for after each treatment and recorded. Records taken were any chlorosis / necrosis to foliage, growth regulatory effects to shoots, assessed on a scale 0-5 (Table 3.2.6). In addition fruit set was recorded. Two branches were marked on the central tree in each sub plot. Total number of flowers were recorded in blossom on16 May, number of fruitlets were recorded on 4 July.

Table 3.2.6 Foliage chlorosis/necrosis phytotoxicity scale, Source; EPPO Guideline PP 1/135(4)

0	No symptoms
1	1-5% leaves very slight
2	6-10% leaves slight
3	11-25% leaves moderate
4	26-50% leaves high
5	>50% leaves very high

Disease

Powdery mildew: All assessments of powdery mildew were conducted on the middle two trees of each plot. Secondary mildew was recorded weekly on five shoots per tree from 15 May-18 July, a total of ten assessments. The number of mildewed leaves was recorded in the top five leaves on each shoot, starting with the first fully expanded leaf and expressed as % leaves mildewed.

Yield: All fruit were harvested on 24 September from the middle two trees in each plot and the weight (kg) recorded.

Fruit quality: At harvest (24 September) a random sample of 100 fruit was taken from each plot. Each 100 fruit sample was assessed as follows: Weight of 100 fruit, number and weight of fruit >65 mm diameter, fruit colour and russet score. Russet was assessed on a scale of 0-4 where 0 = no russet, 1 = russet at stalk and calyx, 2 = russet on cheek 3 = rough russet and 4 = rough russet and cracking. Russet scores 0-1 are for Gala acceptable in Class 1. Fruit colour was assessed as % red coloration. on a 0-4 scale where 0 = green, 1 = up to 25% red colour, 2 = 26-50% red colour, 3 = 51-75% red colour and 4 = 76-100% red colour. (EPPO Guideline PP 1/135 (4).

Statistical analysis: Data was analysed by ANOVA. Mildew data were angular transformed prior to analysis. Repeated measures analyses were done for the mildew assessments with multiple dates. Percentage data was angular transformed prior to analysis except for % (or number) of fruit > 65 mm in diameter which was square root transformed. Figures with different letters are significantly different.

Table 3.2.7 Summary of treatment and assessment timings – NIAB EMR 2018

Activity	**Date**
2017 trial marked out. Treatment 2 plots retained same as in 2016. Other plots rerandomised JK	19 April
1st spray applied pink bud / early flower MP	4 May
Plots checked for Phytotox. None seen AMB	8 May
2nd spray applied Full bloom MP	15 May
Plots checked for Phytotox. None seen AMB	15 May

Secondary mildew assessed Top 5 leaves on 5 shoots per plot AMB	15 May
Initial flower count on two marked branches per plot JK	16 May
Secondary mildew assessed Top 5 leaves on 5 shoots per plot AMB	23 May
3rd spray applied End of flowering MP	24 May
Secondary mildew assessed Top 5 leaves on 5 shoots per plot AMB	30 May
Plots checked for Phytotox. None seen AMB	30 May
4th spray applied MP	4 June
Secondary mildew assessed Top 5 leaves on 5 shoots per plot AMB	6 June
5th spray applied Treatment 7 MP	12 June
Secondary mildew assessed Top 5 leaves on 5 shoots per plot AMB	13 June
Secondary mildew assessed Top 5 leaves on 5 shoots per plot AMB	20 June
6th spray applied MP	21 June
Fruit count on two marked branches per plot JK	23 June
7th spray applied MP	26 June
Secondary mildew assessed Top 5 leaves on 5 shoots per plot AMB	27 June
Plots checked for Phytotox. None seen AMB	27 June
8th spray applied MP	3 July
Secondary mildew assessed Top 5 leaves on 5 shoots per plot AMB	4 July
Fruit count on two marked branches per plot JK	4 July
9th spray MP	10 July
Secondary mildew assessed Top 5 leaves on 5 shoots per plot AMB	11 July
10th spray MP	17 July
Secondary mildew assessed Top 5 leaves on 5 shoots per plot AMB	18 July
Two middle trees in each plot harvested by Farm. Yield recorded and 100 fruit sample taken for quality assessments SC/AMB/RS	24 September
Fruit quality assessments – fruit size, russet and colour JK/TP/SC	November
Data input to computer and checked AMB	14 January

Results

Phytotoxicity: No phytotoxicity was noted on leaves at any of the inspections or assessments. However, fruit set (Table 3.2.8) was on average 10% less in plots receiving programmes 4 and 6. Reasons for this are not yet clear.

Disease

Powdery mildew: The incidence of primary blossom and vegetative mildew in the orchard was high, even though the blocks where the trial plots were located had received a seven day fungicide programme in 2017. Warm wet weather at the end of May resulted in rapid lush shoot growth and, as a consequence, a large increase in secondary mildew (Fig. 3.2.2). The overall mean for the 10 assessments is given in Tables 3.2.8 and 3.2.9. The highest mildew incidence was generally recorded in the plots receiving a 14 day fungicide programme only. Least mildew was recorded in plots receiving the 7 day fungicide programme, but this was still above the 10% mildewed leaves threshold for mildew for most of the season. The incidence of mildew in programmes combining fungicides with alternative fungicides was in general around 10% or more less than in the 14 day fungicide programme but higher than in the 7 day programme.

Yield: Yield data for the 10 programmes is presented in Table 3.2.8. There were no significant effects of treatments on plot yield.

Fruit quality: Fruit quality data - fruit russet, fruit colour and fruit size are presented in Table 3.2.10. There were no obvious differences between the 10 programmes.

Table 3.2.8 Mean % fruit set (angular transformed) and yield recorded on apple cv. Gala following ten sprays of various programmes at NIAB EMR in 2018. Figures in brackets are back transformed means.

Treatment	Product	% Fruit set	Mean total yield kg
1	Fungicide 7 days	33.4	87.9
2	Fungicide 14 days	29.6	82.2
3	CBL/SBI/Fung 14	32.8	80.1
4	CBL/Wetcit/Fung 14	21.0	84.6
5	Mantrac/SBI/ Fung 14	36.5	78.0
6	Mantrac/Wetcit/ Fung 14	24.1	75.6
7	Trident/SBI/Fung 14	35.5	74.7
8	Trident/Wetcit/Fung 14	36.0	82.7
9	CBL Managed	44.0	84.8
10	Trident Managed	34.0	78.3
F Prob		0.013	0.396
SED (27)		3.283	5.776
LSD (p=0.05)		6.737	11.85

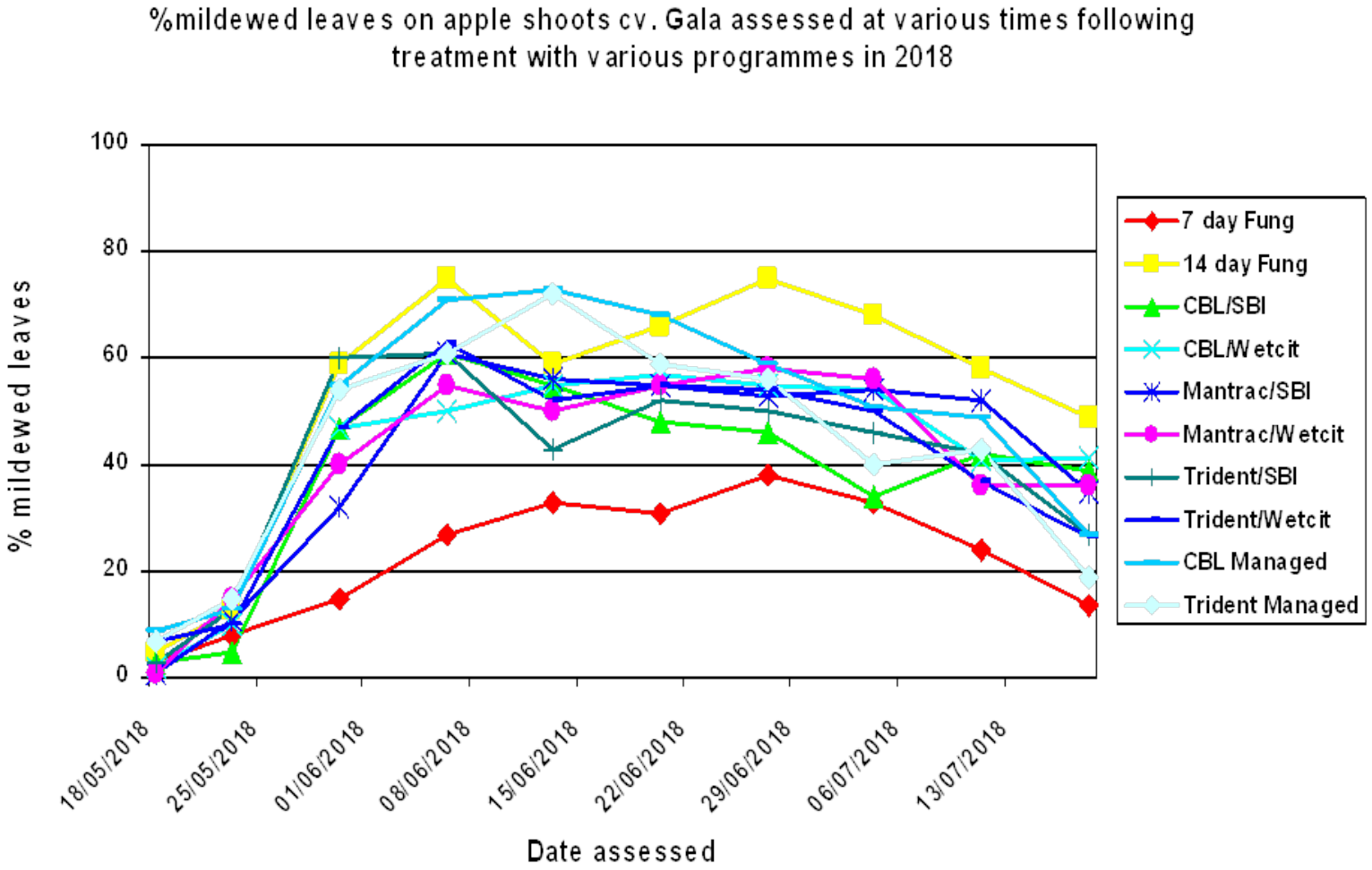


Figure 3.2.2 Mean % mildewed leaves on apple shoots cv. Gala assessed at various times following treatment with 12 sprays of 10 different programmes of various products applied at NIAB EMR in 2018

Table 3.2.9 Mean percentage mildewed leaves (angular transformed) on apple cv. Gala following 12 sprays of various programmes, applied at NIAB EMR in 2018 (Figures in brackets are back transformed data). Figures with different letters are significantly different from untreated.

Treatment	Product	% mildewed leaves										
		15 May	23 May	30 May	6 June	13 June	20 June	27 June	4 July	11 July	18 July	Overall mean mildew
1	Fungicide 7 days	7.0 (1.5)	16.4 (8.0)	22.1 (14.1) a	30.8 (26.3) a	34.9 (32.7) a	33.8 (30.9) a	37.6 (37.3) a	34.4 (31.9) a	28.9 (23.4) a	21.5 (13.4) a	28.3 (22.5) bcd
2	Fungicide 14 days	9.2 (2.5)	18.1 (9.7)	50.3 (59.2) d	60.1 (75.1) c	50.3 (59.2) cd	54.6 (66.5) cd	60.1 (75.1) c	55.8 (68.4) c	49.6 (58.0) d	44.6 (49.3) e	46.6 (52.7) abc
3	CBL/SBI/ Fung 14	5.1 (0.8)	11.1 (3.7)	43.3 (47.0) bcd	51.5 (61.3) bc	47.9 (55.0) bc	43.8 (47.9) b	42.7 (46.0) ab	35.5 (33.7) a	40.4 (42.0) bc	38.2 (38.3) cde	38.0 (38.0) bcd
4	CBL/Wetcit/Fung 14	5.8 (1.0)	15.9 (7.5)	43.3 (47.0) bcd	45.1 (50.2) b	47.9 (55.1) bc	49.1 (57.1) bcd	47.9 (55.1) b	47.3 (54.0) bc	39.6 (40.2) bc	39.7 (40.8) de	39.9 (41.2) a
5	Mantrac/SBI/ Fung 14	2.9 (0.3)	19.2 (10.8)	33.9 (31.1) b	51.5 (61.3) bc	48.5 (56.2) bc	47.9 (55.0) bcd	46.7 (53.0) ab	47.3 (54.0) bc	46.1 (52.0) cd	36.1 (34.7) cd	39.8 (41.0) cd
6	Mantrac/Wetcit / Fung 14	2.9 (0.3)	22.4 (14.5)	39.1 (39.9) bc	47.9 (55.0) bc	45.0 (49.9) bc	47.9 (55.0) bcd	49.8 (58.3) b	48.5 (56.1) bc	36.7 (35.7) ab	36.8 (35.9) cde	39.3 (40.2) ab
7	Trident/SBI/Fung 14	7.0 (1.5)	20.2 (11.9)	51.0 (60.3) d	52.1 (62.3) bc	40.8 (42.7) ab	46.1 (52.0) bc	45.0 (50.1) ab	42.7 (46.0) ab	40.4 (42.0) bc	30.8 (26.3) bc	39.0 (39.6) cd
8	Trident/Wetcit/ Fung 14	14.9 (6.6)	18.1 (9.6)	43.2 (46.9) bcd	52.8 (63.4) bc	46.1 (52.0) bc	48.0 (55.2) bcd	47.3 (54.1) ab	45.0 (50.0) b	37.3 (36.8) abc	30.6 (25.9) bc	39.3 (40.1) cd
9	CBL Managed	14.9 (6.6)	20.5 (12.3)	47.9 (55.1) cd	57.6 (71.3) c	58.7 (73.1) d	55.6 (68.1) d	50.6 (59.8) bc	45.6 (51.0) b	44.4 (48.9) bcd	31.1 (26.6) bc	43.6 (47.5) d
10	Trident Managed	12.9 (5.0)	22.1 (14.1)	47.3 (54.0) cd	51.6 (61.5) bc	58.1 (72.1) d	50.3 (59.2) bcd	48.5 (56.0) b	39.2 (39.9) ab	41.0 (43.0) bcd	25.8 (19.9) ab	40.7 (42.6) bcd
	F Prob	0.356	0.612	<0.001	0.006	<0.001	0.002	0.022	0.003	0.006	<0.001	<0.001
	SED (27)	5.987	5.276	4.67	6.048	4.507	4.244	4.982	4.625	4.299	3.984	1.879
	LSD (p=0.05)	12.284	10.826	9.582	12.409	9.247	8.707	10.222	9.49	8.822	8.174	3.855

Table 3.2.10 Mean (overall mean of 10 assessments) % mildewed leaves (angular transformed) on apple cv. Gala following 12 sprays of 10 different programmes, applied at NIAB EMR in 2018 (figures in brackets are back transformed data).

Treatment	Product	Overall mean
1	Fungicide 7 days	22.6
2	Fungicide 14 days	52.7
3	CBL/SBI/Fung 14	38.0
4	CBL/Wetcit/Fung 14	41.2
5	Mantrac/SBI/ Fung 14	41.0
6	Mantrac/Wetcit/ Fung 14	40.2
7	Trident/SBI/Fung 14	39.7
8	Trident/Wetcit/Fung 14	40.1
9	CBL Managed	47.6
10	Trident Managed	42.6
F Prob		<0.001
SED		1.879
LSD (p=0.05)		3.855

Discussion

The incidence of primary mildew in the trial orchard was relatively high and the use of small plots meant that the Cox guard rows were initially unsprayed at the start of the trial. Secondary mildew incidence in the trial was low at the start at the first two assessments in May and promising. However, following the warm weather at the end of May and the high rainfall, apple shoot growth was rapid and lush and very susceptible to mildew. Thereafter the epidemic rapidly increased with high incidence of mildew on the unsprayed guard rows. To counter this, it was decided to apply the 14 day fungicide programme by tractor spray to ensure the guard rows were also treated to reduce the mildew inoculum generated. Overall the incidence of secondary mildew in the trial was above the 10% mildewed leaves threshold of commercially acceptable mildew control, even in the seven day fungicide plots, which had the lowest mildew incidence of all the programmes. The highest mildew incidence was in the plots receiving the 14 day fungicide programme Most of the combined programmes gave

significantly better mildew control than the 14 day programme indicating some benefit from these treatments. As the mildew incidence overall was too high it is difficult to demonstrate the value of including the alternative treatments in programmes 3-10. In 2019 the combined programmes will be evaluated in larger plots in another orchard with a more commercially acceptable level of primary mildew.

Of some concern was the reduction in fruit set in programmes 4 and 6. Both included Wetcit which, when applied at a higher concentration in 2016, also reduced fruit set. The Wetcit was applied every 14 days which coincided with an application in blossom. Wetcit was also included in programme 8 at the same timings which did not result in reduced fruit set. Reasons for these effects are not clear. The effects on fruit set were not reflected in yield or fruit size where there were no significant effects of treatments.

There were no effects of treatments on fruit russet or fruit colour. Cool nights in August ensured that fruit colour development was good, so any effects of treatments would have been masked.

Table 3.1.11 Effects of treatments on fruit quality recorded as russet score, colour score, weight 100 fruit (kg) (In transformed) and number and weight (transformed) of fruit > 65 mm diameter (square root transformed) on apple fruits cv. Gala following 12 sprays of nine different programmes at NIAB EMR in 2018

Programme	Treatment	Mean russet score[1]	Mean colour score[2]	Weight of 100 fruit kg	No. fruit > 65 mm diameter	Weight of fruit >65 mm diameter
1	**Fungicide 7 days**	79.5	268.8	9.7	26.0	3.4
2	**Fungicide 14 days**	75.5	260.3	8.6	10.5	1.3
3	**CBL/SBI/Fung 14**	85.0	283.8	9.7	11.0	3.1
4	**CBL/Wetcit/Fung 14**	80.5	261.5	8.9	23.3	3.1
5	**Mantrac/SBI/ Fung 14**	82.8	271.3	9.4	18.8	2.5
6	**Mantrac/Wetcit/ Fung 14**	75.0	275.0	9.3	19.0	2.4
7	**Trident/SBI/Fung 14**	84.0	279.3	9.1	16.0	2.2
8	**Trident/Wetcit/Fung 14**	69.8	302.0	9.4	22.0	2.7
9	**CBL Managed**	82.5	286.8	10.1	27.0	3.5
10	**Trident Managed**	79.3	272.8	9.9	33.5	4.1
F Prob		0.625	0.380	0.418	0.285	0.520
SED (27)		7.527	16.675	0.629	8.949	1.144
LSD (p=0.05)		15.445	34.215	1.291	18.363	2.348

[1] Russet score = The higher the score the worse the russet
[2] Colour score = The higher the score the better the fruit colour

Conclusions

- The incidence of primary mildew in the trial orchard was higher than expected and the warm wet weather at the end of May 2018 was very favourable for mildew such that the overall incidence of secondary mildew in the trial was higher than commercially acceptable

- Over the ten weekly assessments the lowest incidence of secondary mildew was in the plots receiving the 7 day fungicide programme
- Highest mildew incidence was generally in the plots receiving the 14 day fungicide programme
- The plots receiving the programmes combining fungicides with biostimulants and other alternative chemicals had generally significantly less mildew over the ten assessments than those receiving the 14 day fungicide programme indicating some benefit from the alternative treatments
- There were no phytotoxic symptoms seen on the leaves but treatments 4 and 6 resulted in significantly lower fruit set than treatment 1 (7 day fungicide programme), possibly due to Wetcit applied in blossom, but this is not clear. The reduced fruit set was not seen in treatment 8 which also included Wetcit at the same timings
- There were no significant effects of treatments on yield, fruit russet, fruit colour or fruit size, even in treatments 4 and 6 which had reduced fruit set.

Objective 4 - Stone Fruit Diseases

4.2 In season control

Aim

IPM trials targeting diseases associated with *Monilinia* sp. integrating control of over-wintering inoculum and use of alternative treatments with a reduced fungicide programme (NIAB EMR/ADAS, Yr 3-4)

Summary

In a replicated small plot orchard experiment four experimental fungicides, a plant extract, a biostimulant and the biofungicide Serenade were compared with a standard fungicide programme based on Signum and Switch for control of blossom wilt, brown rot (*M. laxa* and *M. fructigena*) and Botrytis. An untreated control was included. Treatments were applied as two sprays at flowering and two pre-harvest except for the biostimulant which was applied at full bloom and then at 21 day intervals to harvest. The incidence of blossom wilt was assessed after petal fall. Rot incidence was recorded at harvest and in post-harvest tests at three and seven days incubation at ambient temperature following three days storage at 0°C. Yield and fruit size was also recorded. The results obtained are summarised as follows:

- The incidence of blossom wilt (*M. laxa*) was negligible
- The incidence of rots at harvest was low (5% in untreated plots). There were no significant effects of treatments on rot incidence, but the lowest incidence of rots was recorded in Treatments 3 and 4 and in the standard treatment (2)
- The rot incidence increased in post-harvest tests to over 30% in untreated plots after 7 days' incubation
- The lowest incidence after 3 days' incubation was recorded in Treatment 2 (Signum/Switch), Treatment 3 (HDC F266), Treatment 4 (HDC F267) and Treatment 8 (HDC F270). However, the differences were not quite significant compared to the untreated control
- At the final assessment after 7 days' incubation the lowest rot (accumulated rot) incidence was again recorded in Treatment 2 (Signum/Switch), Treatment 3 (HDC F266) and Treatment 8 (HDC F268). Only Treatment 3 had significantly less rot than the untreated control

- The effects of the treatments on the incidence of *M. fructigena* was not significant, however, the lowest incidence of *M. fructigena* was recorded in fruit treated with HDC F266 or HDC F268
- All treatments apart fromT6 and T7 significantly reduced the incidence of *M laxa* with the lowest incidence in fruit treated with Treatments 3 (HDC F266) or 4 (HDC F267)
- All treatments, apart from T6, significantly reduced the incidence of Botrytis compared to the untreated control. The lowest incidence was in fruit treated with T3 (HDC F266) which performed significantly better than most other treatments
- Several of the fungicides evaluated in this trial were effective in reducing rotting. In particular HDC F266 (Treatment 3) which was the most consistently effective of the fungicides tested
- Of the alternative products tested HDC F269 and Serenade were ineffective
- The effect of HDC F271, a biostimulant, was variable. It was not effective in boosting plant resistance to *M. fructigena* which is a wound pathogen, but more successful in improving resistance to *M. laxa* and *Botrytis*
- There were no significant effects of treatments on yield or fruit size
- There were no phytotoxic effects of any of the treatments

Introduction

Losses resulting from *Monilinia* sp. in stone fruit are hard to quantify because infection occurs throughout the season (blossom and fruit pre- and post-harvest). Post-harvest development of brown rot limits the storage potential of UK stone fruit and a few rotten fruit in one punnet can lead to food retailers rejecting whole consignments. Two Monilinia species are present in the UK; *Monilinia laxa* and *Monilinia fructigena*. Currently diseases associated with Monilinia are controlled by 1) inoculum removal and 2) fungicides. The former is seldom practiced due to the associated increase in cost, although the advent of SWD and the strategies in place to control this pest in cherries have improved this aspect. Fungicides are applied at blossom and pre-harvest including Bellis, Signum and Switch, but are not totally effective and pre-harvest applications present a residue risk. New products are available including plant health promotors, biological control agents and fungicides which in combination could provide a more effective programme for brown rot control. A trial was set up in 2017 to evaluate these new products. However, the late frosts in 2017 reduced fruit set and a severe infestation of SWD resulted in a very reduced fruit yield at harvest. Consequently there were too few fruit to enable meaningful results to be obtained. The trial was there repeated in 2018.

Objectives

1. To evaluate fungicides and elicitors for control of brown rot and Botrytis fruit rot in cherries
2. To assess whether test products cause any phytotoxicity, including fruit set

Methods

Site: The trial was located in a cherry plantation in Deadmans 187 at NIAB EMR. The orchard consisted of nine different varieties planted at various spacing (2m – 1.25m). Orchard planted in. Originally the trees were trained in three different systems (centre leader, Tasmanian bush and Univeg method). Over the past 2 years all trees have been converted to a centre leader.

Variety: The variety Skeena was used in the trial with a tree spacing of 1.25m – 2m. Skeena was chosen based on its susceptibility to cracking and Monilinia diseases.

Plot size and trial design: Each plot consisted of three trees, separated from adjacent plots by single trees within the row and between rows. Each treatment was replicated 4 times in a randomised block design.

Treatments: Details of the treatments applied are given in Table 4.2.1 and application dates in Tables 4.2.2-4.2.4. Treatments were applied during flowering and before harvest (to assess activity against *M. laxa* and *M. fructigena).* All fungicides were applied as a 4 spray programmes – 2 at blossom and 2 pre-harvest at the following timings. HDC F271 was applied at intervals of 21 days from early flower.

Treatment application: Sprays were applied using a Birchmeier motorised air-assisted knapsack sprayer at 500 L/ha following EMR SOP GEP 725.

Brown rot inoculum: To ensure that inoculum of *Monilinia* was present in the orchard, two introductions of inoculum were made. Firstly netted bags containing *M. laxa* infected fruit (plums) were placed in the central tree of each three tree plot during flowering (26 April). Secondly netted bags containing *M. fructigena* infected fruit (peaches) were placed in the central tree of each three tree plot during fruit ripening (26 June).

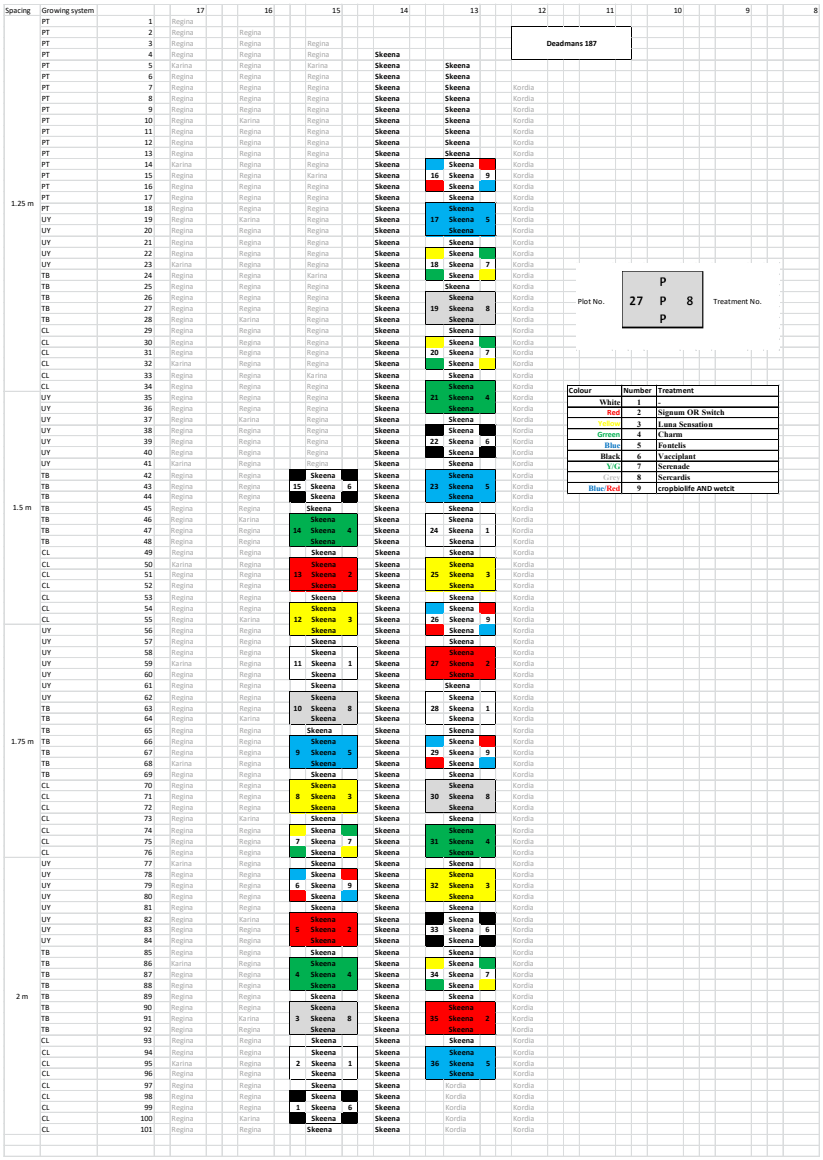


Figure 4.2.1 Plot plan 2018

Assessments

Meteorological records: Records of daily maximum and minimum temperature and rain fall were taken from a weather station located at NIAB EMR.

Growth stages at application: Phenological stage at each application and assessment were recorded

Phytotoxicity: Symptoms of phytotoxicity were checked after each treatment and recorded. Records included any chlorosis / necrosis to foliage, growth regulatory effects to shoots, assessed on a scale 0-5. (EPPO Guideline PP 1/135(4)).

Phytotoxicity scale

0 = No symptoms

1 = 1-5% leaves very slight

2 = 6-10% leaves slight

3 = 11-25% leaves moderate

4 = 26-50% leaves high

5 = >50% leaves very high

Disease assessments

Flowering: One – two weeks after petal fall the number of flower clusters with blossom wilt was assessed. Ten blossoms on each of five branches per tree were assessed, 50 clusters per plot.

Fruits: At harvest rot incidence was assessed on the tree. A random sample of 100 visually healthy fruit was picked from the middle tree of each plot. The fruit was then stored at 0°C for 3-5 days and then transferred to ambient (15-25°C) and assessed after 3 and 7 days. The rots were identified and incidence recorded as a proportion of total fruit.

Statistical analysis

Data was analysed by ANOVA. Percentage data was angular transformed prior to analysis. Figures with different letters are significantly different.

Table 4.2.1 Treatments evaluated for control of Monilinia diseases in 2018 at NIAB EMR

Treat	Category	Product	Active ingredient	Product rate per ha	Timing
1	Untreated	-	-	-	-
2	Standard fungicide	Signum + Switch	boscalid + pyraclostrobin cyprodinil + fludioxonil	0.75 kg 0.6 kg	Alternating sprays. 4 sprays 2 at blossom, 2 pre-harvest
3	Fungicide	HDC F266	Experimental	0.8 L	4 sprays 2 at blossom, 2 pre-harvest
4	Fungicide	HDC F267	Experimental	0.6 L	4 sprays 2 at blossom, 2 pre-harvest
5	Fungicide	HDC F270	Experimental	0.75 L	4 sprays 2 at blossom, 2 pre-harvest
6	Elicitor	HDC F269	Experimental	1L	4 sprays 2 at blossom, 2 pre-harvest
7	BCA	Serenade	Bacillus subtilis	10.0 L	4 sprays 2 at blossom, 2 pre-harvest4 sprays 2 at blossom, 2 pre-harvest
8	Fungicide	HDC F268	Experimental	0.3 L	4 sprays 2 at blossom, 2 pre-harvest
9	Biostimulant	HDC F271 + Wetcit	Flavonoids + wetter	500ml + 0.1%	First spray at full bloom then every 21 days there after

Table 4.2.2 Timing of treatments applied to cherry trees at NIAB EMR in 2018

Treatment	Start of flowering BBCH 57-60 23 April	Start of petal fall BBCH 67 3 May	15 May	5 June	25 June	10-15 days before harvest 29 June	3-5 days before harvest 6 July
1	-	-	-	-	-	-	-
2	Signum	Switch				Signum	Switch
3	HDC F266	HDC F266				HDC F266	HDC F266
4	HDC F267	HDC F267				HDC F267	HDC F267
5	HDC F270	HDC F270				HDC F270	HDC F270
6	HDC F269	HDC F269				HDC F269	HDC F269
7	Serenade	Serenade				Serenade	Serenade
8	HDC F268	HDC F268				HDC F268	HDC F268
9	HDC F271 + Wetcit		HDC F271 + Wetcit	HDC F271 + Wetcit	HDC F271 + Wetcit		

Table 4.2.3 Air temperature and humidity conditions at the time of spray applications

Date	At start of spray applications					At end of spray applications					
	Time	Temp ºC		RH%	Wind speed (kmph) Direction	Time	Temp ºC		RH%	Wind speed (kmph) Direction	Weather conditions
		Dry bulb	Wet bulb				Dry bulb	Wet bulb			
23 April	10.25	13.5	11.5	78.9	2.7 SW	13.30	15.5	14.0	85.0	1.5 SW	Sunny
3 May	9.30	11.5	9.5	77.5	3.0 WNW	12.30	13.0	11.5	83.8	4.0 WNW	40% cloud
15 May	13.40	22.0	21.5	95.7	1.2 SW	13.55	22.0	20.0	83.3	3.9 SW	Sunny
5 June	11.35	14.5	14.0	94.8	0	12.01	15.5	15.0	94.9	1.4 NE	Overcast
25 June	7.30	17.5	17.0	95.2	0	8.05	17.5	17.0	95.2	0	Sunny spells
29 June	8.10	16.5	16.5	100	1.0 NE	11.05	21.5	20.0	87.2	0	Sunny spells
6 July	7.12	18.5	18.5	100	0	10.06	22.0	20.5	87.3	0	Sunny spells

Table 4.2.4 Percentage accuracy of spray applications (volume applied / volume required expressed as a percentage)

Treatment	Spray date						
	23 April	**3 May**	**15 May**	**5 June**	**25 June**	**29 June**	**6 July**
2	110	116				99	105
3	114	116				101	105
4	118	117				101	100
5	93	118				101	86
6	109	121				105	105
7	95	123				100	101
8	108	116				100.6	109
9	106.5	-	110	101	99		

Results

Phytotoxicity: Phytotoxicity was assessed on four occasions. No symptoms were observed in any of the treatments.

Disease

Blossom wilt: Blossom wilt was assessed on 17 May. The incidence was negligible.

Rots at harvest: The incidence of rots assessed at harvest are shown in Table 4.2.5 and Fig. 4.2.2. The highest incidence of around 5% was recorded in untreated plots and those treated with Treatments 5-9. The lowest rot incidence was in plots treated with the standard programme of Signum / Switch or HDC F267 or HDC F266, however the differences were not quite significant.

Rots in post-harvest tests: The incidence of rots in post-harvest tests are shown in Table 4.2.5 and Fig. 4.2.3 - 4.2.6. Rot incidence increased post-harvest and in the final assessment at 7 days ranged from 5.8-48.4 %. The lowest incidence after 3 days' incubation was recorded in Treatment 2 (Signum/Switch), Treatment 3 (HDC F266), Treatment 4 (HDC F267) and

Treatment 8 (HDC F268). However, the differences were not quite significant compared to the untreated control. At the final assessment after 7 day' incubation the lowest rot (accumulated rot) incidence was again recorded in Treatment 2 (Signum/Switch), Treatment 3 (HDC F266) and Treatment 8 (HDC F268). Only Treatment 3 had significantly less rot than the untreated control. The effects of the treatments on the incidence of the individual rot species after 7 days' incubation is shown in Table 4.2.5 and in Figures 4.2.4-4.2.6. The effects of the treatments on the incidence of *M. fructigena* was not significant, however, the lowest incidence of *M. fructigena* was recorded in fruit treated with HDC F266 or HDC F268. All treatments apart fromT6 and T7 significantly reduced the incidence of *M. laxa* with the lowest incidence in fruit treated with Treatments 3 (HDC F266) or 4 (HDC F267). All treatments, apart from T6, significantly reduced the incidence of Botrytis compared to the untreated control. The lowest incidence was in fruit treated with T3 (HDC F266) which performed significantly better than most other treatments.

Yield: Yield data is presented in Table 4.2.6. Yield per plot was very variable. There were no significant effects of the treatments on yield.

Fruit quality: Fruit size (Weight of 100 fruit) is given in Table 4.2.6. Fruit size was also very variable. There were no significant effects of the treatments on fruit size.

Table 4.2.5 Mean percentage rots (angular transformed) at harvest and in post-harvest tests in cherry cv. Skeena following treatment with eight different products at NIAB EMR in 2018. Figures in brackets are back transformed data

Treatment	Product	Total % rot at harvest	Total % rot in post-harvest tests				
			Total % rot 3 days	Total % rot 7 days	*M. fructigena*	*M. laxa*	*Botrytis*
1	Untreated	12.8 (4.9)	14.6 (6.4)	34.8 (32.6) bcd	10.6 (3.4)	14.8 (6.6) bc	23.9 (16.4) de
2	Signum /Switch	7.0 (1.5)	8.5 (2.2)	21.6 (13.5) ab	15.2 (6.9)	4.5 (0.6) abc	10.4 (3.2) ab
3	HDC F266	2.5 (0.2)	6.0 (1.1)	13.6 (5.5) a	1.4 (0.1)	0 a	5.4 (0.9) a
4	HDC F267	7.4 (1.7)	12.2 (4.5)	27.7 (21.6) abc	12.7 (4.8)	0 a	15.7 (7.3) bc
5	HDC F270	10.9 (3.6)	14.1 (5.9)	28.5 (22.7) abc	16.2 (7.8)	4.9 (0.7) abc	9.9 (3.0) ab
6	HDC F269	12.7 (4.8)	20.5 (12.3)	44.1 (48.4) d	17.3 (8.9)	17.1 (8.7) c	26.8 (20.3) e
7	Serenade	12.5 (4.7)	20.0 (11.7)	42.5 (45.6) cd	24.0 (16.5)	16.0 (7.6) c	19.2 (10.8) cd
8	HDC F268	11.4 (3.9)	10.9 (3.6)	21.7 (13.7) ab	7.5 (1.7)	2.5 (0.2) ab	11.5 (4.0) b
9	HDC F271+ Wetcit	15.0 (6.7)	20.5 (12.3)	39.9 (41.2) cd	29.0 (23.5)	4.9 (0.7) abc	18.3 (9.8) cd
	F prob	0.086	0.096	0.004	0.061	0.048	<0.001
	SED (24)	3.847	5.393	7.524	7.824	6.311	2.849
	LSD p=0.05	7.939	11.131	15.528	16.147	13.026	5.880

Table 4.2.6 Mean yield and weight of 100 fruit of cherry cv. Skeena recorded at harvest at NIAB EMR in 2018

Treatment	Product	Total yield g	Weight of 100 fruit g
1	Untreated	12432.5	1035
2	Signum / Switch	14460	979
3	HDC F266	11575	1001
4	HDC F267	9247.5	1064
5	HDC F270	9730	1030
6	HDC F269	10345	1150
7	Serenade	9582.5	1011
8	HDC F268	5948	1104
9	HDC F271 + Wetcit	7685	1070
	F prob	0.051	0.157
	SED (24)	2329.6	58.7
	LSD p=0.05	4808.0	121.2

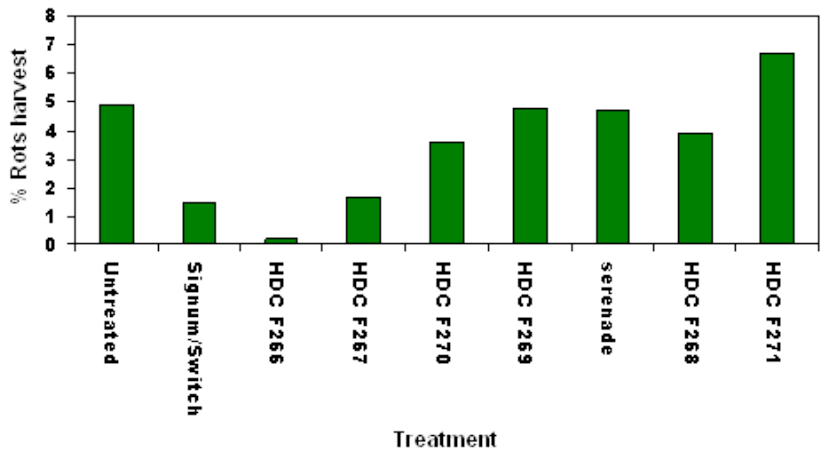


Figure 4.2.2 Percentage rots at harvest in cherry cv. Skeena at NIAB EMR in 2018 following sprays of various treatments

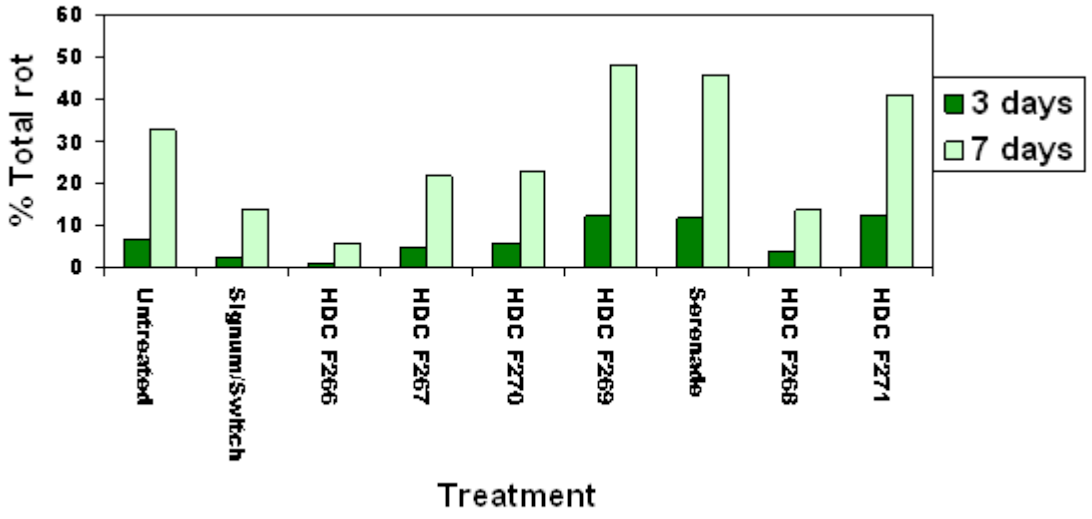


Figure 4.2.3 Percentage rots post-harvest in cherry cv. Skeena at NIAB EMR in 2018 following sprays of various treatments and 3 days and 7 days at ambient temperature after 3 days storage at 2ºC

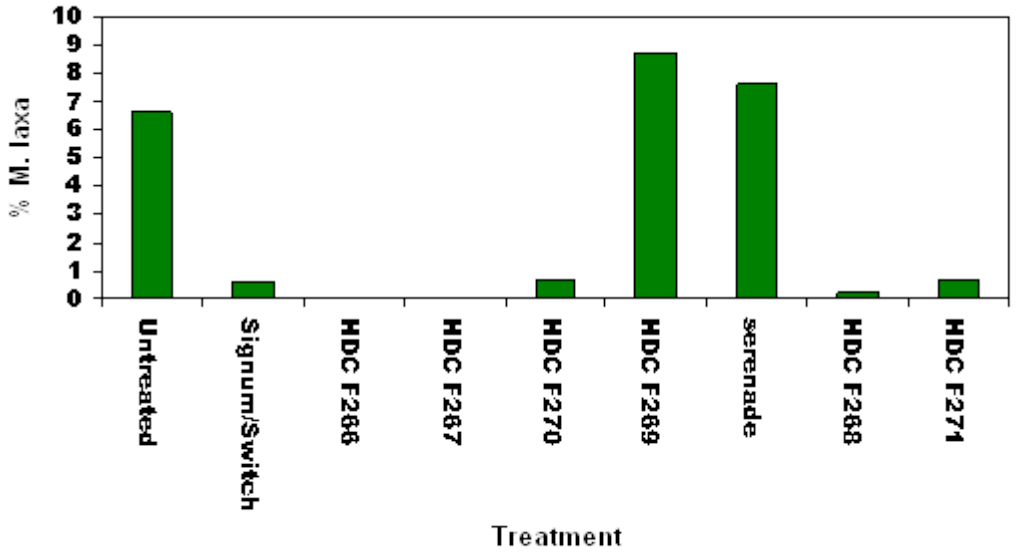


Figure 4.2.4 Percentage *M. laxa* rots post-harvest in cherry cv. Skeena at NIAB EMR in 2018 following sprays of various treatments and 3 days and 7 days at ambient temperature after 3 days storage at 2ºC

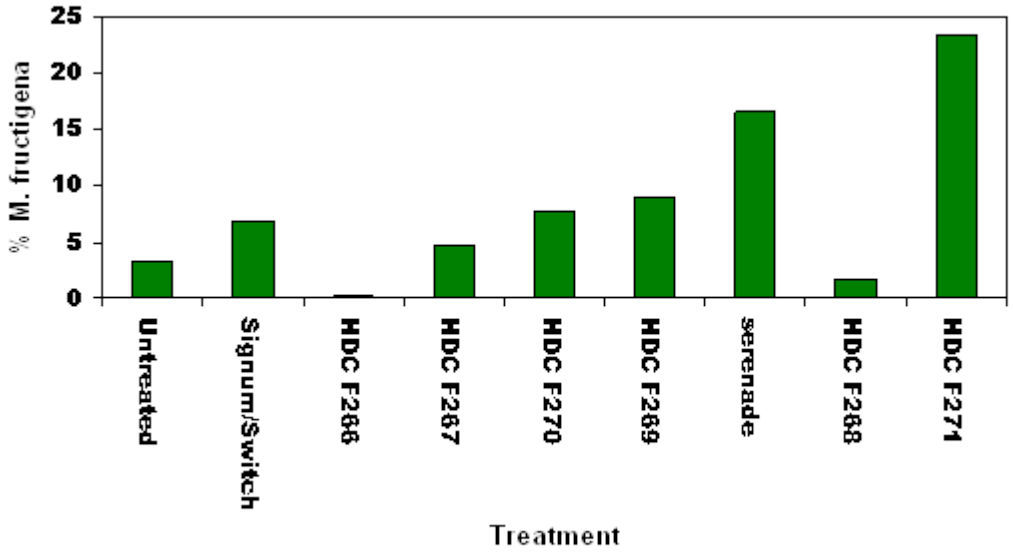


Figure 4.2.5 Percentage *M. fructigena* rots post-harvest in cherry cv. Skeena at NIAB EMR in 2018 following sprays of various treatments and 3 days and 7 days at ambient temperature after 3 days storage at 2ºC

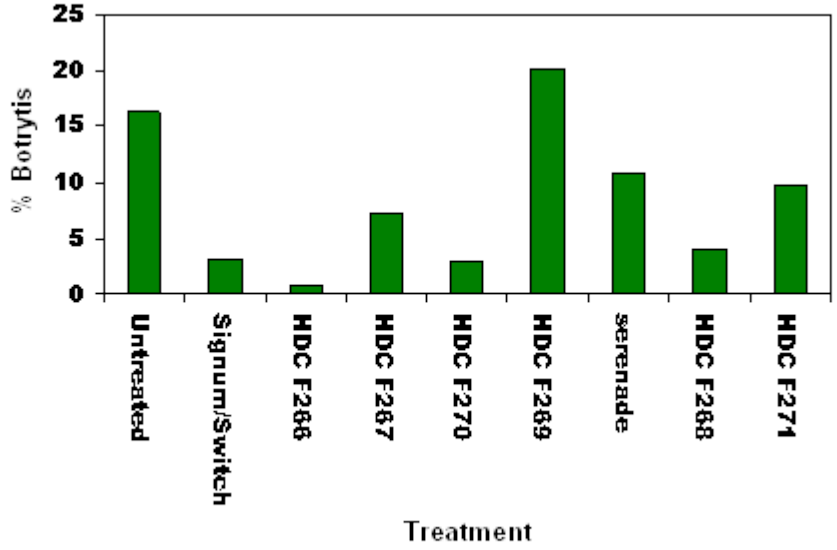


Figure 4.2.6 Percentage *Botrytis* rot post-harvest in cherry cv. Skeena at NIAB EMR in 2018 following sprays of various treatments and 3 days and 7 days at ambient temperature after 3 days storage at 2ºC

Discussion

Despite introducing *M. laxa* inoculum into the orchard at blossom time, the incidence of blossom wilt in the orchard was negligible. Rot incidence at harvest was still relatively low (around 5% in untreated plots), but developed in post-harvest tests to over 30% in untreated plots. The increase in rot incidence after harvest is a commercial problem and limits the short term storage of cherries which would allow more managed marketing of the fruit and more profitable returns for growers. Post-harvest rot problems in cherries are predominantly caused by *M. laxa*, *M. fructigena* and Botrytis. Fungicides currently available for cherries have limited efficacy, compared to products previously available such as iprodione and carbendazim. Several of the fungicides evaluated in this trial were effective in reducing rotting. In particular HDC F266 which was the most consistently effective of the fungicides tested. Of the alternative products tested HDC F269 and Serenade were ineffective. The effect of HDC F271 was variable. This is a biostimulant which acts by boosting the resistance of the host plant rather than any direct effect on the fungus. In this respect it was not effective in boosting plant resistance to *M. fructigena* which is a wound pathogen, but more successful in improving resistance to *M. laxa* and *Botrytis* which infect the fruit earlier and remain as symptomless infections at harvest, subsequently developing post-harvest. Products such as HDC F271 could be included with fungicides in programmes to boost the resistance of the plant and assist fungicides in reducing rotting post-harvest.

Conclusions

- The incidence of blossom wilt (*M. laxa*) was negligible
- The incidence of rots at harvest was low (5% in untreated plots). There were no significant effects of treatments on rot incidence, but the lowest incidence of rots was recorded in Treatments 3 and 4 and in the standard treatment (2)
- The rot incidence increased in post-harvest tests to over 30% in untreated plots after 7 days' incubation
- The lowest incidence after 3 days' incubation was recorded in Treatment 2 (Signum/Switch), Treatment 3 (HDC F266), Treatment 4 (HDC F267) and Treatment 8 (HDC F268). However, the differences were not quite significant compared to the untreated control
- At the final assessment after 7 days' incubation the lowest rot (accumulated rot) incidence was again recorded in Treatment 2 (Signum/Switch), Treatment 3 (HDC F266) and

Treatment 8 (HDC F268). Only Treatment 3 had significantly less rot than the untreated control

- The effects of the treatments on the incidence of *M. fructigena* was not significant, however, the lowest incidence of *M. fructigena* was recorded in fruit treated with HDC F266 or HDC F268
- All treatments apart fromT6 and T7 significantly reduced the incidence of *M laxa* with the lowest incidence in fruit treated with Treatments 3 (HDC F266) or 4 (HDC F267)
- All treatments, apart from T6, significantly reduced the incidence of Botrytis compared to the untreated control. The lowest incidence was in fruit treated with T3 (HDC F266) which performed significantly better than most other treatments
- Several of the fungicides evaluated in this trial were effective in reducing rotting. In particular HDC F266 (Treatment 3) which was the most consistently effective of the fungicides tested
- Of the alternative products tested HDC F269 and Serenade were ineffective
- The effect of HDC F271, a biostimulant was variable. It was not effective in boosting plant resistance to *M fructigena* which is a wound pathogen but more successful in improving resistance to *M laxa* and *Botrytis*
- There were no significant effects of treatments on yield or fruit size
- There were no phytotoxic effects of any of the treatments

4.3 Bacteriophages against bacterial canker in cherry

Aim

Proof of concept for using native bacteriophages against *Pseudomonas syringae*.

Introduction

Plum and cherry are major horticultural crops in the UK grown on over 1440 ha and worth over £27 M to the UK economy. Novel prunus crops (such as apricot and peach) and ornamental prunus also contribute to a growing industry sector. *Pseudomonas syringae* pathovars; *syringae* (Pss) and *morspronorum* (Psm), cause a destructive disease called bacterial canker on prunus species. Bacterial canker reduces yield, affecting profitability of the industry. The cankers caused by the disease girdle stems causing wilting and branch death, trunk cankers can result in tree death. Until now growers have relied on copper treatments at leaf fall, the period at which infection occurs, to control this disease. However copper is no longer permitted to be used as a plant protection product in the UK.

The lack of approved chemical control, emerging of resistance to chemical control and consumers' preference for organic produce have made significant push for alternative control of bacterial diseases. Bacteriophages (phages) as antimicrobial agents have enormous potential as an alternative for treating bacterial diseases. There are several advantages to using phage therapy. Phages are very effective reducing bacterial populations and also very host specific, affecting a narrow range of bacterial strains and have therefore minimal unintended consequences in term of inhibiting non target organisms. Constant and rapid phage evolution can potentially overcome bacterial resistance when it occurs. Phage therapies could be used as preventative treatment as well as therapeutic, to be applied to trees and act as a barrier to infection. Using phage therapies also has the added benefit of being organic and reducing the use of chemicals in environment. The Jackson lab at the University of Reading has successfully used phage therapy to target *Pseudomonas syringae* pv. *aesculi*, causative agent of horse chestnut blight. In this project we aim to *in vitro* characterise bacteriophages isolated from healthy and diseased trees in orchards across UK. We have developed assays to i) test their efficacy against disease causing strains of *Pss* and *Psm, ii)* cross-reactivity with other bacterial population on the plants and iii) conduct initial proof of concept work of using phages to control bacterial canker on plants or plant simulation assays.

Methods

Phage collection and characterisation by Dr Mojgan Rabiey and Shyamali Roy (University of Reading)

Soils and leaves from commercial and research cherry orchards in Kent were sampled for bacteriophages. Phosphate-buffered saline was added to the samples, vortexed, centrifuged and filtered (0.22 nm) to remove any bacterial cells. This filtrate was then plated with three virulent pathovars of Pseudomonas, i.e. *Pseudomonas syringae* pv. *syringae* (PSS isolate 9097), *P. syringae* pv. *morsprunorum* race 1 (PSM1 isolate 5244) and 2 (PSM2 isolate 5255) using a double-agar plaque assay. The presence of phage in the sample results in circular clearings in the agar called plaques (Figure 4.3.1). All phages are being further assessed for their host range against 19 pathogenic *Pseudomonas syringae* from PSS, PSM1 and PSM2 groups collected within UK and also against non-pathogenic and beneficial *Pseudomonas spp.* (*P. fluorescens*). Based on host specificity and plaque morphology, 18 phages were chosen for further study for characterisation by transmission electron microscopy and sequencing.

Four phages with large plaque size against PSS and efficient replication cycle (high PFU/ml in overnight culture) were selected to investigate efficacy of phage mixtures against PSS. All possible cocktails of four phages (Pss19.2, Psm1-10, Psm1-11, Psm1-13) were prepared at two concentrations (10^6 and 10^7 PFU/ml) and tested against PSS in vitro by double-agar plaque assay.

Detached leaf assays (Matevz Papp-Rupar, NIAB EMR)

Bacteriophages used in detached leaf assay (Table 4.3.1) were isolated from the orchards at NIAB EMR, East Malling and National Fruit Collection, Brogdale, during the undergraduate research project of Billy Quilty (mentors Robert J. Saville NIAB EMR and Rob Jackson, University of Reading) in 2016. The phages (Figure 4.3.1) were stored, revived and bulked up at University of Reading in May 2018. Before use, the phages were diluted to 10^5 PFU/ml in distilled water. Phages Psm1-10 and Pss19.2 were used undiluted.

Table 4.3.1. Information on the phages used in detached leaf assay. Concentration is shown in plaque forming unit (PFU) per ml

Phage name	Phages suspended in	Concentration	Host range i.e. activity against
Pss1	LB	10^10 PFU/ml	PSS and PSM2
Pss13	LB	10^10 PFU/ml	PSS
Pss19.2	LB	10^10 PFU/ml	PSS
Psm1-10	LB	10^5 PFU/ml	PSS, PSM1 and PSM2
Psm1-11	LB	10^5 PFU/ml	PSS, PSM1 and PSM2

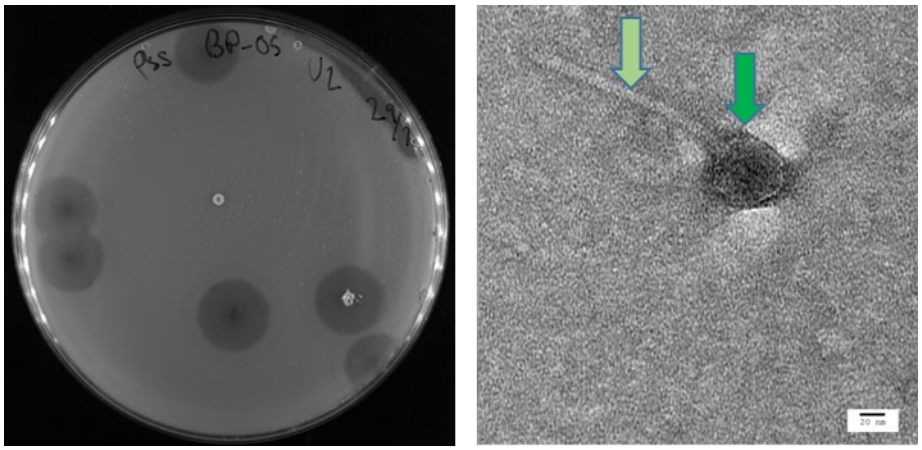


Figure 4.3.1 LEFT: Bacteriophages causing plaques i.e. clear zones where PSS bacteria died due to phage infection on agar plate. RIGHT: electron micrograph of bacteriophage from *Siphoviridae* family with hexagonal head (dark green and long tail light green (PHOTO: Billy Quilty)

Bacterial isolates used in detached leaf assays were isolated from UK orchards and characterised by Dr. Michelle Hulin, NIAB EMR. We used *Pseudomonas syringae* pv. *morsprunorum* race 1 (PSM1), race 2 (PSM2) and *Pseudomonas syringae* pv. *syringae* (PSS). Before each assay, single colony of each isolate was inoculated into sterile LB media and grown overnight at 27°C on a shaker (180 RPM). Concentration of bacteria used for inoculation was prepared by centrifugation to remove LB media followed by dilution in distilled water to reach optical density at 600 nm of 0.2 +/- 0.01 (appx 10E+8 CFU/ml).

SPRAY-SPRAY assay

In spray-spray assay we closely simulated the population distribution of bacteria on the leaf by spraying uniformly across the leaves and mimicked the commercial spray application of plant protection products by spraying bacteriophages.

1) Young (6 cm long) cherry leaves (cv. Sweetheart) were washed in 2% bleach and let to dry in laminar flow hood
2) 10 leaves were either inoculated with each strain of bacteria (PSM1, PSM2) and two leaves were mock inoculated with water (Table 4.3.2)
    a. PSS was omitted since previous experiments showed that it is too aggressive in this assay and thus too difficult to score
3) Small atomiser was used to spray bacterial inoculum or water mock inoculation in the leaves
    a. 200-300 µl per leaf was sprayed
4) Leaves were dried for 1 h before solution of phages or water control was sprayed
    a. 200-300 µl per leaf was sprayed
5) Leaves were dried for another 1h before we moved then in high humidity boxes
    a. Incubated with 16h light at 22C
6) At 5 and 7 days post inoculation number of lesions per leaf was counted and percentage of necrotic area were lesions merged visually estimated
    a. Number of lesions and necrotic area were summarised
    b. Area under disease progression curve (AUDPC) calculated and used for statistical analysis and visualization of data.

Table 4.3.2. Details on phages and bacterial strains used in SPRAY-SPRAY assay.

Treat-ment	Phage name	Phage conc. sprayed	Diluted in	Number of leaves inoculated PSM1	Number of leaves inoculated PSM1	MOCK (H_2O) inoculated
1	Pss1	10^5	ddH2O	10	10	2
2	Psm1-10	10^5	Non-diluted in LB	10	10	2
3	Psm1-11	10^5	Non-diluted in LB	10	10	2
4	Control (ddH2O)	-	-	10	10	2

DROP-SPRAY assay

Disease assessments on sprayed leaves was problematic. Large numbers of small lesions combined with larger areas where lesions had merged made it difficult to accurately estimate the disease severity. Hence, we developed a more controlled DROP-SPRAY assay. In DROP-SPRAY assay the bacterial inoculation drop size area was limited and sprayed bacteriophages over droplet inoculated leaves.

1) Young cherry leaves (cv. Sweetheart) were surface washed with 2% bleach
2) Each leaf was inoculated with 2x 5µl droplets of each bacterial strain and one water control droplet (total 7 droplet per leaf, Figure 4.3.2)
    a. All bacterial strains were used (PSM1, PSM2 and PSS)
    b. Droplets were left to dry in laminar flow hood
3) When bacterial droplets have dried we sprayed treatments (table 4.3.3) on the leaves until run off (200-300 ul per leaf) and randomised in three trays (20 leaves per tray)
4) Leaves were scored at 5 and 7 days after inoculation: 0= no lesion, 1= small lesion spots, 2= large lesion spots, 3= spots have merged into one larger lesion
5) Area under disease progression curve (AUDPC) based on the scores at 6 an 8 DPI was calculated and two replicated inoculated points on the same leaf were averaged before statistical analysis.

Table 4.3.3 Details on treatments used in DROP-SPRAY detached leaf assay

Treatment	Phage name	Concentration	Diluted in	Number of leaves
A	Pss1	10^5 PFU	H_2O	10
B	Pss13	10^5 PFU	H_2O	10
C	Pss19.2	10^5 PFU	H_2O	10
D	Psm1-10	10^5 PFU in LB	Not diluted. (in LB)	10
E	Psm1-11	10^5 PFU in LB	Not diluted. (in LB)	10
F	H_2O	/	H_2O	10

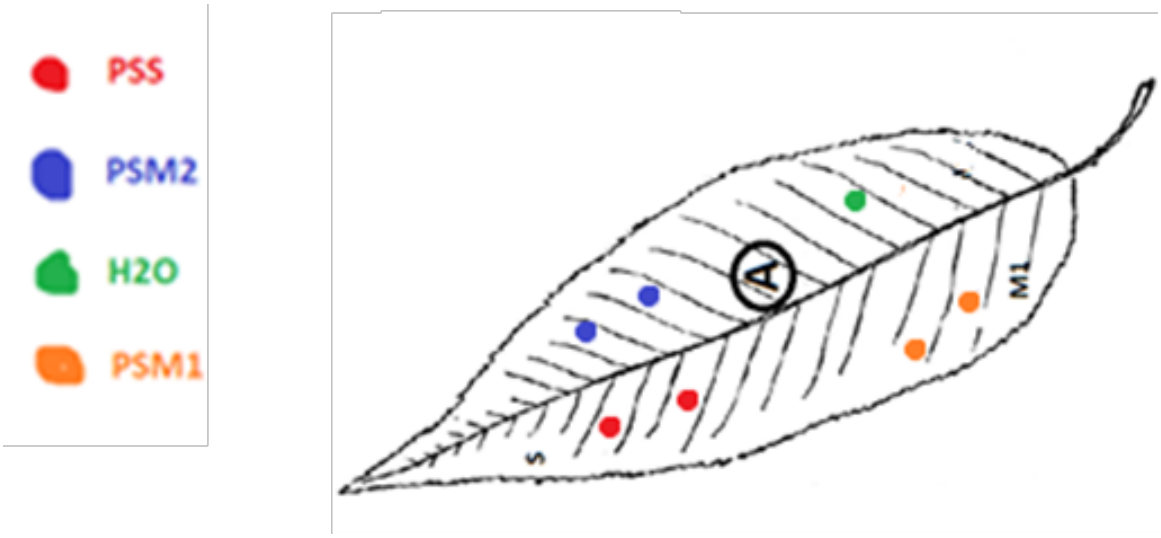


Figure 4.3.2 Drop-spray inoculation scheme with all 3 bacterial strains and water inoculation on each leaf

DROP-DROP assay

After conducting DROP-SPRAY assay we noticed that, even though bacterial droplets were dry, when we sprayed bacteriophages they spread out. This assay was developed to further limit the area of interactions between bacteria, flat leaf and bacteriophages to enable for more accurate disease severity assessment.

The leaves were drop inoculated with three different bacterial strains and water the same way as in DROP-SPRAY assay (Figure 4.3.2) with a few modifications:

1) We have coloured all inoculation droplets (5 µL) with brilliant blue food colouring to be able to track them through the experiment (Figure 4.3.3).
2) We have diluted all phages in LB instead of water for better comparison and used LB for control instead of H_2O.
3) Number of treatments was expanded to include industry standard 5 µl of Cuprokylt (7.5 g/L), phage cocktails (1:1 ratio) and control without blue colouring (Table 4.3.4)
4) Leaves were randomised in six blocks and incubated in high humidity trays in a plastic bags until assessment.
5) At 5 and 7 days post inoculation high resolution photos were taken and lesion that formed within blue coloured area were measured with ImageJ software
    a. Total of 1400 measurements over 2 assessment days.
6) Results were analysed as lesion incidence per block per bacterial suspension per treatment. Only points that develop lesions were used to calculate lesion progression as conditional AUDPC.

Figure 4.3.3 Blue stains on the leaves indication inoculation points after inoculation droplets have dried

Table 4.3.4 Details on treatments used in DROP-DROP detached leaf assay.

Treatment	Phage or treatment name	concentration	No. of inoc. leaves
A	Cuprokylt (positive control)	187 mg/25 ml	10
B	Pss1	10^5 PFU in LB	10
C	Pss13	10^5 PFU in LB	10
D	Pss19.2	10^5 PFU in LB	10
E	Psm1-10	10^5 PFU in LB	10
F	Psm1-11	10^5 PFU in LB	10
G	LB media	/	10
H	cocktail B+E+F	1:1:1 (vol. ratio)	10
I	cocktail E+F	1:1 (vol. ratio)	10
J	cocktail B+C+D+E+F	1:1:1:1:1 (vol. ratio)	10
K	LB media (bacteria without brilliant blue)		10

Detached shoot assay

In this assay we simulated the natural shot infection process through leaf scars at leaf fall to test if bacteriophages could directly replace the copper based products by reducing the canker causing *Pseudomonas spp.* populations at leaf fall.

1) On 25 October 2018 more than 200 cherry cv. Sweetheart shoots were collected from rows 8 and 11 in RF181/182 orchard at NIAB EMR site
    a. Shoots were appx 30-40 cm in lengths
    b. Collected from the end of the branches, i.e. this year's wood (2018)
    c. leaves visibly yellowing but still attached
    d. upon collection the shoots were pricked in saturated Oasis foam to keep to prevent drying
2) on 26 October the shoots were inoculated with mixture pf PSS and PSM1 strains
    a. leaves still attached
    b. 10^8 CFU per ml of bacterial suspension consisting of PSM1 and PSS strains in 1:1 ratio to simulate high bacterial population size at leaf fall.
    c. 0.6 l of inoculum was sprayed over 210 shoots (max 2.85x10^8 CFU per shoot)
    d. Inoculated shoots were kept in polytunel for 5 days

3) On 31 October leaves were manually stripped of the shoots and 180 most uniform shoots were randomly split into 3 experiments (Table 4.3.5).
4) In exp1 and 2 the shoots were randomised win 6 blocks each block consisted of 4 shoots per treatment. In exp. 3 shoots were randomised in three blocks with four shoots per treatment.
5) Each block consisted of a 20x30 cm tray with water saturated clean oasis foam. The shoots were pricked in the foam and enclosed in a large plastic bag to increase humidity.
6) The shoots are incubated in the poly tunnel at ambient temperatures to best simulate the natural infection process.
7) Frequency successfully inoculated leaf scars per shoot will be assessed in late January 2019 by scraping the bark of the shoots and observing browning of the cambium.

Table 4.3.5 Experimental set up in detached shoot assay; Leaves were manually stripped off the shoots to expose leaf scars.

							Shoots per group
EXP 1			Water control	30 min drying			24
			Phage [b]				24
			Cuprokylt [c]				24
EXP 2	bacterial spray [a]	30 min drying	Water control	30 min drying			24
			Phage [b]				24
			Cuprokylt [c]				24
EXP 3	bacterial spray [a]	30 min drying	Water control	30 min drying	bacterial spray [a]	30 min dryin g	12
			Phage [b]				12
			Cuprokylt [c]				12

[a] bacterial spray was done with mixed bacterial culture (1:1) PSS and PSM1 at total bacterial concentration of 5x10^7 CFU/ml; [b] phage cocktail comprised of phages: Pss1, Pss13, Pss19.2, Psm1 10, Psm1 11; in 1:1:1:1:1 ratio, and total concentration of 10^6 PFU/mL; [c] (7.5g/l).

Results

Phage collection

In total, 70 bacteriophages were isolated with activity against one or more virulent pathovars PSS, PSM1 or PSM2. Most of the active phages were isolated from the soil samples. All 70 isolated phages were active against PSS and a small proportion against PSM1 (7), PSM2 (6). Interestingly, several phage isolates were found with a broader host range capable of infection; two or more *Pseudomonas* strains from different groups. None of the isolated phages showed any activity against non-target *P. fluorescens.*

Table 4.3.6 Overview of the phage collection host range

Host range, i.e. effective against	No. of Phages
PSS	70
PSM1	7
PSM2	6
PSS+PSM1	7
PSS+PSM2	6
PSS+PSM1+PSM2	6

The cocktail assay indicated that when acting against a single bacterial strain on agar plates phage mixtures seem as effective as a single phage strains. The amount of plaques formed by a cocktail was very similar to the amount of plaques formed by the most effective single phage in the mix. In most, but not all cases, application of higher concentration of phages resulted in more plaques formed. The fact that higher phage concentration did not result in a higher amount of plaques could indicate that phage efficacy was already at its maximum at lower concentrations. Due to the single biological and technical repeat per phage cocktail, statistical analysis could not be conducted and, therefore, conclusions should not be drawn from this data until more repeats are performed.

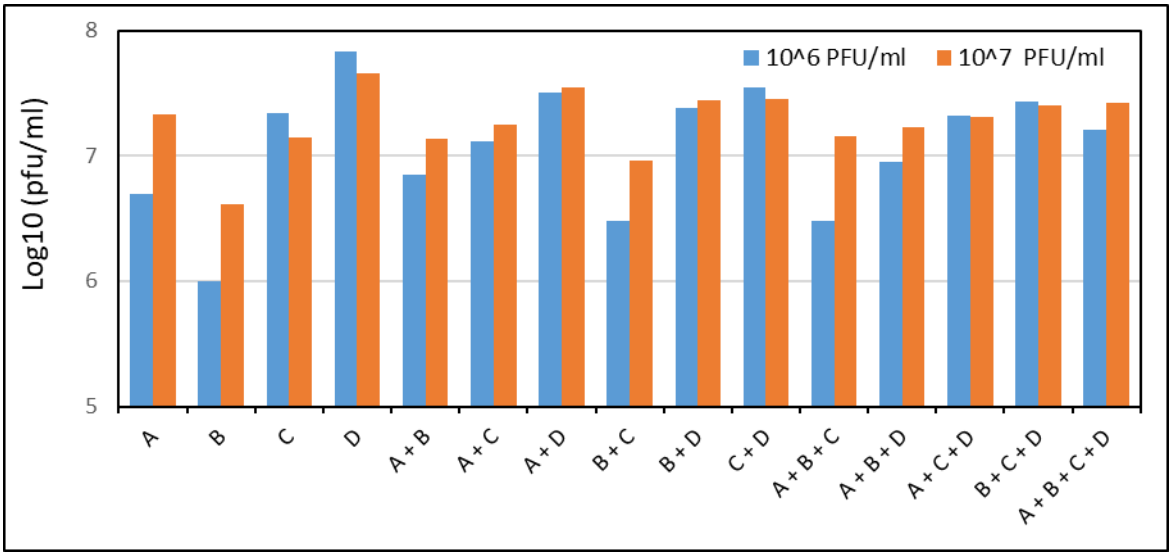


Figure 4.3.4 Blend assay of phage PSS19.2 (A), PSM1-10 (B), PSM1-11 (C), PSM1-13 (D) used individually and in combination in double-agar plaque assay. Phage dilution of 10^6 and 10^7 PFU/ml were used

Detached leaf assays results

SPRAY-SPRAY assay results

When bacteria (PSM1,PSM2) were spray inoculated on the young cherry leaves followed by a phage spray we observed formation of necrotic lesions of various sizes ranging small black spots (less than 1 mm across) to large necrotic lesions (few cm across) (Figure 4.3.5).

As expected, mock (H_2O) inoculated leaves showed no symptoms. We observed two spots on one leaf in the mock inoculated water control (data not shown) which could be attributed to natural bacterial populations present in the leaf that were not removed by the surface cleaning method. In all treatments PSM1 caused more pronounced symptoms with larger lesion area than PSM2.

Figure 4.3.5 Small black lesions (spots) and large necrotic area on PSM1 inoculated leaves (spray test)

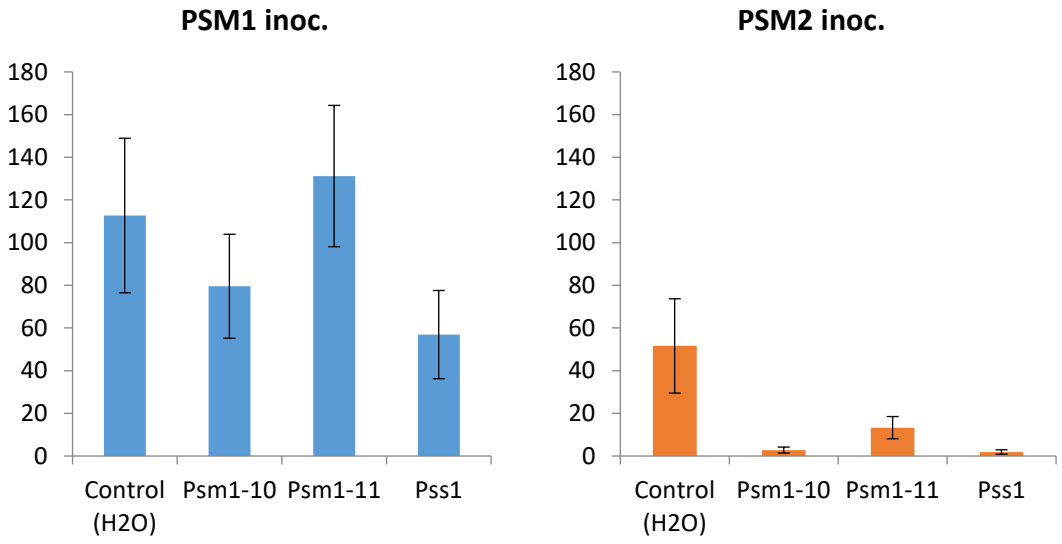


Figure 4.3.6 Area under disease progression curve (x axis) of control and three phage strains (y axis) measured on PSM1 and PSM2 inoculated leaves. Error bars represent +/- SEM

Bacteriophages decreased the amount of necrotic lesions observed in all cases; apart from when PSM1 inoculated leaves were treated with Psm1-11 phages solution where lesion area was slightly larger than in water control. This observation most likely stems from high amounts of nutrients in the LB media based phage solutions. Nutrients in phage treatments could mask the effects of phages by enabling faster bacterial growth compared to water control.

Due to unequal variances between treatment groups we performed robust one way ANOVAs with Tamhane and Games-Howell multiple comparisons post hoc tests. We observed approximately 40% reduction of AUDPC in phage treated leaves (Table 4.3.7). No statistically significant differences were observed between the treatments (Table 4.3.8 and 4.3.9). When comparing the efficacy of the phages across the two bacterial strains the same trend with phage Pss1 reducing necrotic lesion formation was observed followed by Psm1-10 and Psm1-11.

Table 4.3.7 Mean reduction of AUDPC on PSM1 and PSM2 inoculated leaves

Inoculation	Phage treatment	AUDPC reduction vs control
PSM1	Psm1-10	29%
	Psm1-11	-16%
	Pss1	50%
PSM2	Psm1-10	43%
	Psm1-11	34%
	Pss1	44%

Table 4.3.8 Statistical analysis of AUDPC from PSM1 inoculated leaves

Test of Homogeneity of Variances					
Variable	Levene Statistic	df1	df2	Sig.	
AUDPC	3.302	3	35	.031	
Robust Tests of Equality of Means					
Variable	test	Statistic[a]	df1	df2	Sig.
AUDPC	Welch	1.373	3	19.057	.281
	Brown-Forsythe	1.306	3	29.958	.291

DROP-SPRAY assay

Due to the high variably and uncertainty associated with visual assessment of necrotic area we first attempted to use image analysis to accurately determine necrotic lesion size. We were not able to capture images that could be reliably used for image analyses due to high curvature of young cherry leaves and changes to leaf shape upon lesion formation (Figure 4.3.5). To mitigate the assessment issues we decided to point inoculate bacterial strains which limited the location of possible lesion formations and enabled more reliable and refined lesion scoring system.

Lesion incidence (above 60%) and progression on PSS inoculated points was the highest among the three bacterial strains. There were no significant differences in lesion incidence between phage and water control treated and leaves (Figure 4.3.7, left). The lesion progression (AUDPC), however, was greater in all phage treatments and significantly increased in Psm1.11 and Pss1 phage treated leaves (Figure 4.3.7, right). This may be the result of the residual LB media in the phage solution which provided ample nutrient source to the bacteria in contrast to water control.

Table 4.3.9 Statistical analysis of AUDPC from PSM2 inoculated leaves

Test of Homogeneity of Variances					
	Levene Statistic	df1	df2	Sig.	
AUDPC	6.618	3	36	.001	
Robust Tests of Equality of Means					
		Statistic[a]	df1	df2	Sig.
AUDPC	Welch	2.985	3	17.867	.059
	Brown-Forsythe	4.215	3	10.112	.036
Pairwise comparison					
Test			Mean Difference (I-J)	Std. Error	Sig.
Tamhane	control	pss1	49.70003	22.12773	.271
		psm1-10	48.80001	22.14849	.287
		psm1-11	38.30002	22.70842	.544
Games-Howell	control	pss1	49.70003	22.12773	.182
		psm1-10	48.80001	22.14849	.193
		psm1-11	38.30002	22.70842	.379

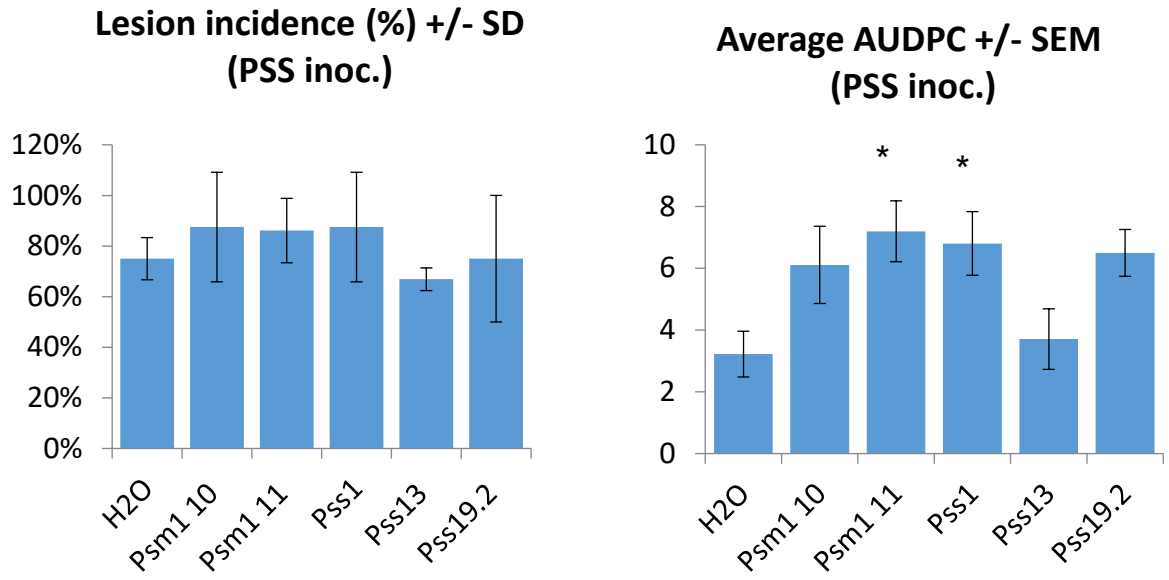


Figure 4.3.7 LEFT: Incidence of lesion formation when leaves were drop inoculated with PSS and sprayed with water or Phage solution. Error bars indicate +/- 1 SD (n= 3 blocks). Asterisk indicates means that are statistically significantly different from control (H_2O) (p val.(t test)<0.05). RIGHT: Conditional area under disease progression curve i.e. only inoculation points with successful inoculation are included. Error bars indicate +/- SEM over all leaves in all trays. Asterisk indicates means that are statistically significantly different from control (H_2O) (mean difference larger that pairwise Fisher LSD (p<0.05))

On PSM1 inoculated points we found no significant differences between groups in terms of incidence (Figure 4.3.8, left). When lesions formed they progressed with approximately the same rate in all treatments accept for Pss1 phage treated leaves where lesions seem to progress significantly faster (Figure 4.3.8, right). This result is could again be due LB media in the phage solution which diminished the control effect of phages.

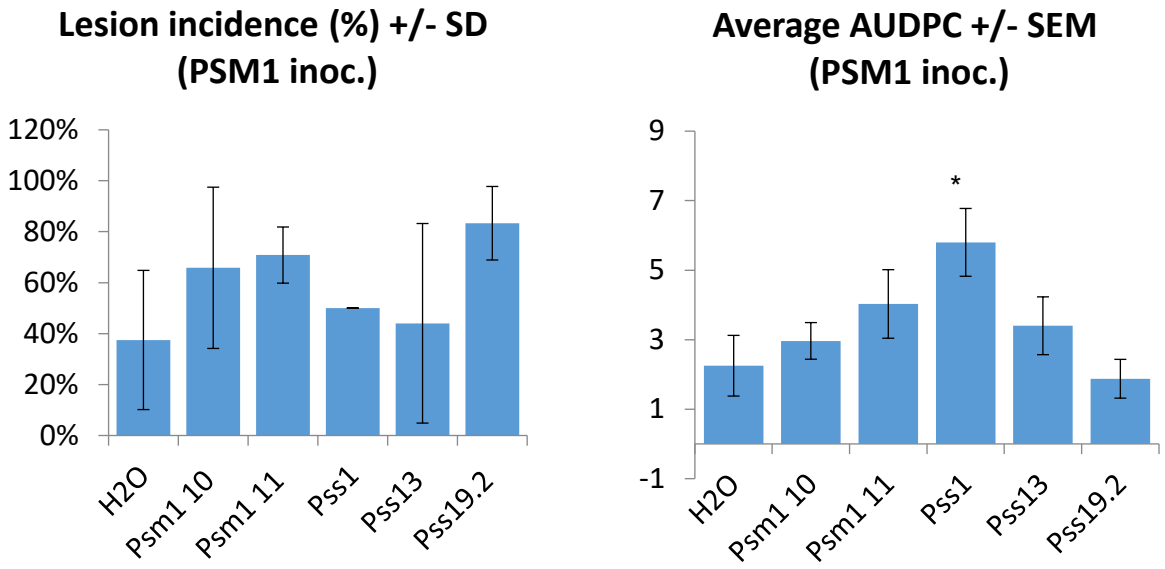


Figure 4.3.8 LEFT: Incidence of lesion formation when leaves were drop inoculated with PSM1 and sprayed with water or Phage solution. Error bars indicate +/- 1 SD over three blocks. Asterisk indicates means that are statistically significantly different from control (H_2O) (p val.(t test)<0.05). RIGHT: Conditional area under disease progression curve i.e. only inoculation points with successful inoculation are included. Error bars indicate +/- SEM over all leaves in all trays. Asterisk indicates means that are statistically significantly different from control (H_2O) (mean difference larger that pairwise Fisher LSD (p<0.05))

Lesion incidence in PSM2 inoculated points was between 20 and 60%, markedly lower than in PSS inoculations. On PSM2 inoculated leaves we found that Pss13 phage treated leaves showed significantly increased lesion incidence compared to control (Figure 4.3.9, left). With the low number of repeats used in analysis (incidence per block with three blocks was analysed) and a borderline t-test p-value of 0.046 this could be an artefact of the small experimental design. No significant differences were observed between treatments and control in terms of lesion progression (AUDPC) (Figure 4.3.9, right). Interestingly, phage Pss19.2 treated PSM2 inoculated leaves had less and smaller sessions than water control, differences however were not significant. We suspect that this result is also to some extent a consequence of the residual LB media in the phage solution which countered the control effect of phages.

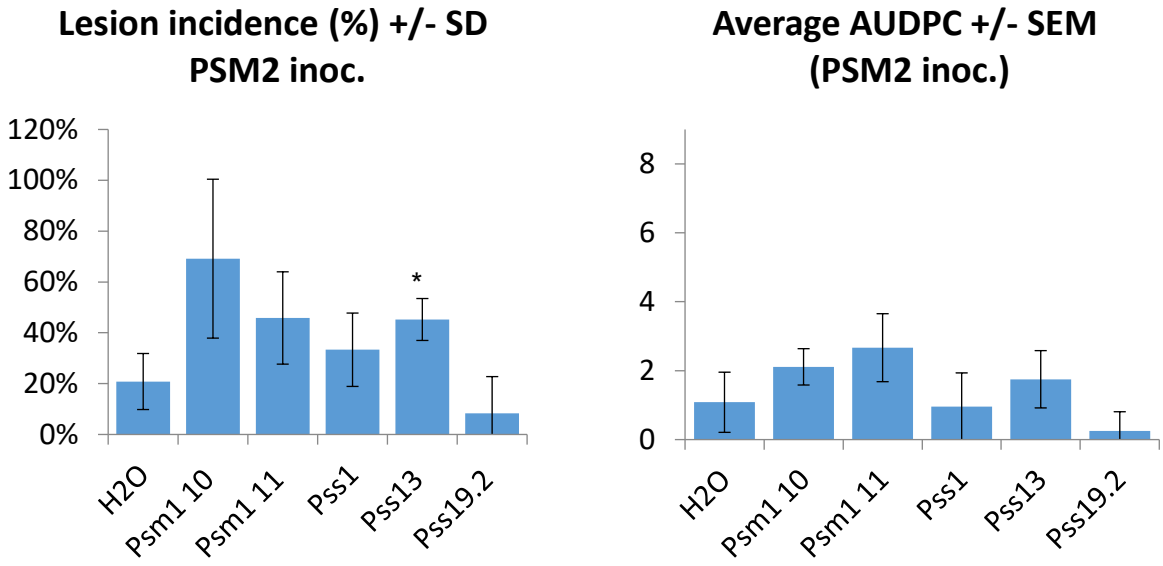


Figure 4.3.9 LEFT: Incidence of lesion formation when leaves were drop inoculated with PSS and sprayed with water or Phage solution. Error bars indicate +/- 1 SD over three blocks. Asterisk indicates means that are statistically significantly different from control (H_2O) (p val.(t test)<0.05). RIGHT: Conditional area under disease progression curve i.e. only inoculation points with successful inoculation are included. Error bars indicate +/- SEM over all leaves in all trays. Asterisk indicates means that are statistically significantly different from control (H_2O) (mean difference larger that pairwise Fisher LSD ($p<0.05$))

DROP-DROP assay

In this assay we tried to further optimise and standardise phage testing in detached leaves. Even though we drop inoculated the leaves in the DROP-SPRAY assay and let the bacterial suspension to dry before spraying we observed some indication that the drops were spreading from their original position upon spray application. This could have skewed our assessment which was only done on the area we marked for inoculation, and also introduced noise due to different spread on different leaves depending on curvature. In DROP-DDROP spray we added a drop of phage solution on the bacterial droplet after it dried to avoid issues described above. In order to apply phages precisely on the spot where bacterial drop have been dried we mixed food colourant brilliant blue (BB) with bacterial suspension. We confirmed that BB did not affect bacterial growth by plating PSS, PSM1 and PSM2 bacterial suspension after one hour of incubation with or without BB and counting colony forming units (CFU) 24 H afterwards. None of the bacterial strains were negatively affected by BB (Figure

4.3.10), on the contrary slightly higher counts were obtained when bacteria were incubated with BB. All bacterial drops in this assay therefore included BB to mark their position (Figure 4.3.3). To confirm that BB did not affect virulence on the leaf we also included a group of leaves inoculated with non-coloured bacterial strains in all subsequent assays to compare side by side with the virulence of coloured bacterial suspensions. No significant differences in lesion incidence or progression was observed between coloured (labelled as LB blue) and non-coloured bacteria (labelled as LB) (Figure 4.3.12-14).

The fact that the assay was done later in the season meant that even the youngest leaves on the trees were expanded further than in previous two assays. This enabled us to take reliable photographs (Figure 4.3.11) of the leaves that were amendable to image analysis with ImageJ and markedly improve reliability and accuracy of lesion scoring.

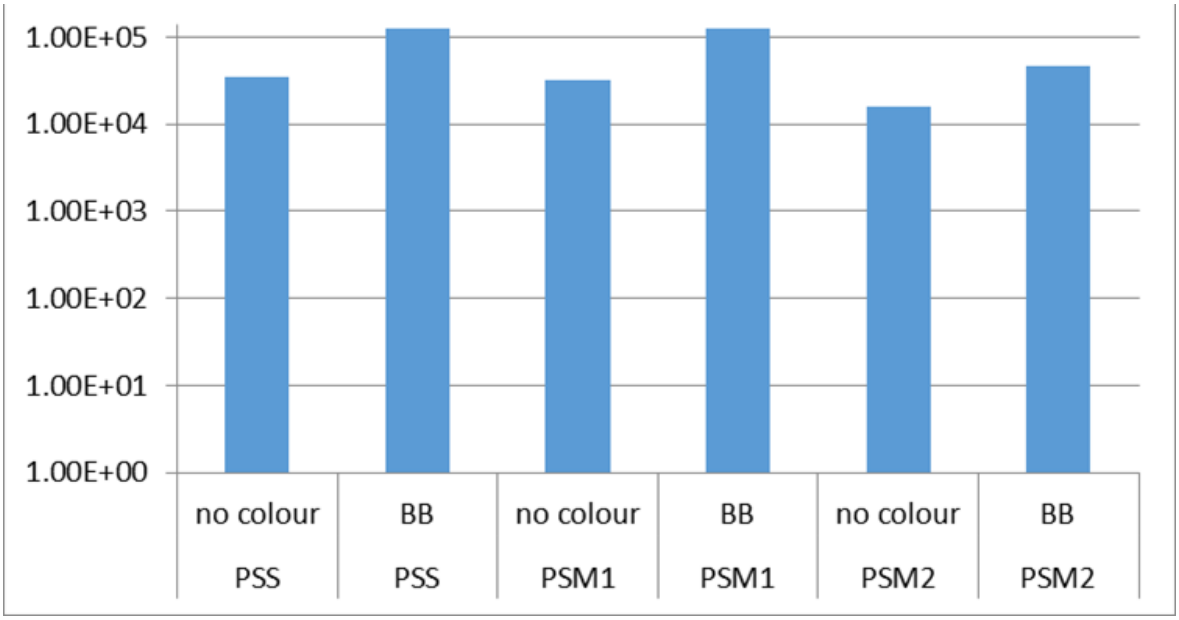


Figure 4.3.10 Colony forming units (CFU) count in 5 µL of bacterial suspension incubated with or without brilliant blue (BB). Results of the tests with all three bacterial strains (PSS, PSM1 and PSM2) are shown

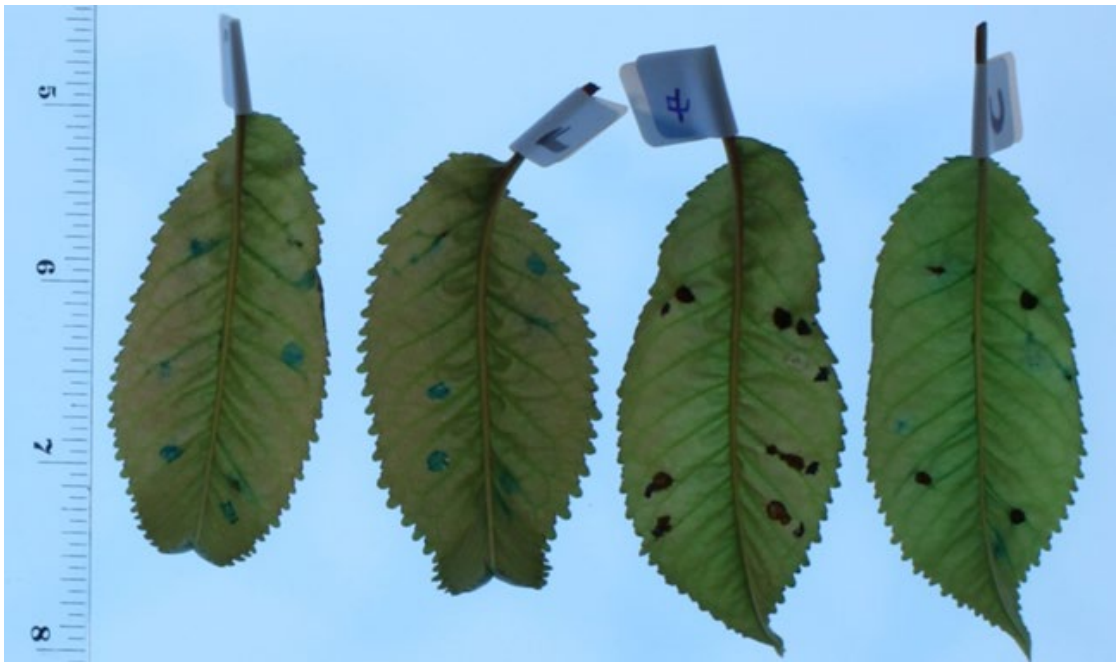


Figure 4.3.11 An example of high resolution image of leaves with lesions 5 DPI

No significant differences between LB (blue) and phage treatments were observed on PSS inoculated points (Figure 4.3.12). The only group that significantly reduced incidence and progression was as expected the positive control Cuprokylt. Lesion progression data in case of Cuprokylt had too few data points for reliable statistical testing and thus considered not significant. Treatment with Pss13 phage seems to significantly increase lesion incidence but not progression. This observation might be due to the fact that LB and LB (blue) controls did showed lower lesion incidence (40-60%) than expected. Most of the other groups had incidence between 60% and 80%.

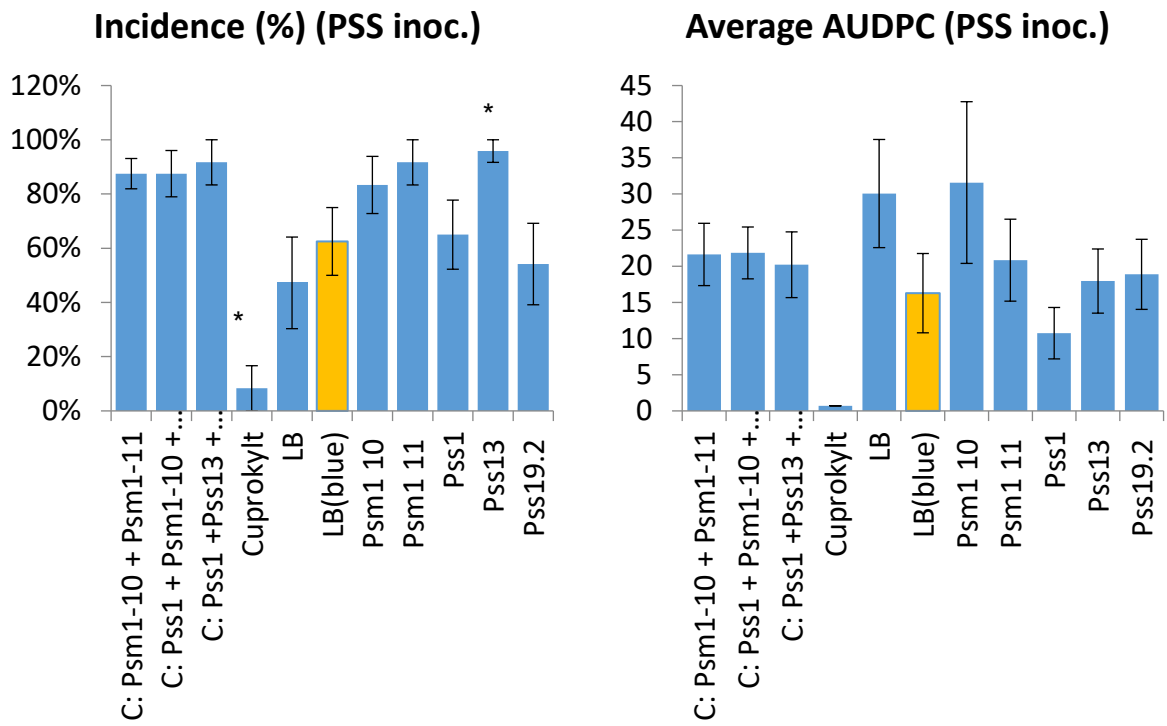


Figure 4.3.12 Incidence of lesion formation (%) (left) and conditional lesion progression (AUDPC) (right) on PSS inoculated points on the leaves. Treatments from left to right are phage cocktails (C) with 2, 3, and all five different phages followed by Cuprokylt (positive control) colourless "LB" and coloured "LB (blue)" negative controls followed by single phage solutions. All groups were compared to "LB (blue)" control group shown in orange

In contrast, in PSM1 inoculated points LB and LB (blue) groups had almost 100% lesion incidence and the highest lesion progression as expected (Figure 4.3.13, left). Cuprokylt, again, significantly reduced lesion incidence and progression. Single phage treatment with Pss13 phage also significantly reduced incidence and progression, but to lesser extent than Cuprokylt. Phage treatments with Pss1 and Pss19.2 also significantly reduced lesion formation but not progression, while Psm1.11 significantly reduced progression only. This is a very positive result especially since PSM1 bacterial strain is considered to be one of the main causes of bacterial canker in UK. Interestingly, when 2, 3 or 5 phages were mixed together in a cocktail they seem to have no effect on lesion incidence or progression. It could be due to competition of phages for the same infection route / binding site on the bacterial that results in mutual inhibition. This is in line with some of the *in-vitro* results from University of Reading where phage mixes resulted in less plaques than single strains (data not shown).

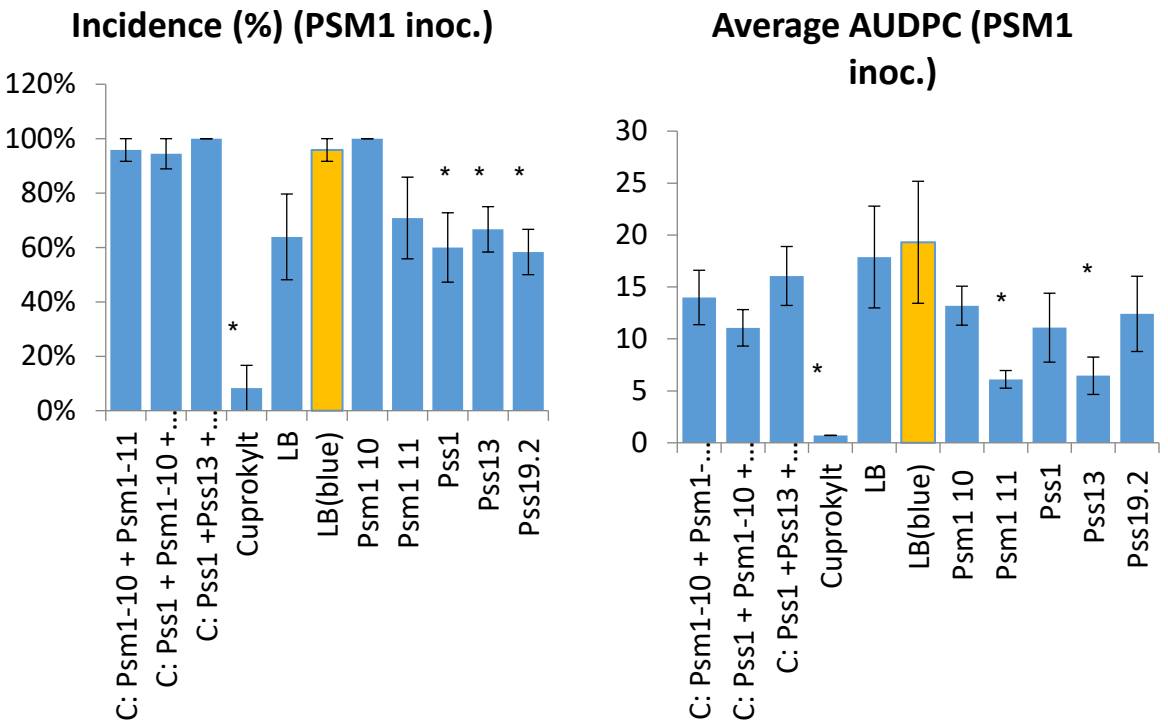


Figure 4.3.13 Incidence of lesion formation (%) (left) and conditional lesion progression (AUDPC) (right) on PSM1 inoculated points on the leaves. Treatments from left to right are phage cocktails with 2, 3, and all 5 different phages followed by Cuprokylt (positive control) colourless “LB” and coloured “LB(blue)” negative controls followed by single phage solutions. All groups were compared to “LB(blue)” control group shown in orange

The same as in DROP-SPRAY assay the PSM2 inoculated points had the lowest percent of successful lesion formation (between 5 and 40%) (Figure 4.3.14, left). The progression of lesions was also the lowest on the three strains with AUDPC under 10 in most cases (Figure 4.3.14, right). No statistically significant differences between LB (blue) control and the rest of the treatments were observed in lesion incidence or progression. Interestingly, most of the single phage (to the right of the control) and also phage cocktails treatments resulted in slightly reduced incidence and progression (AUDPC).

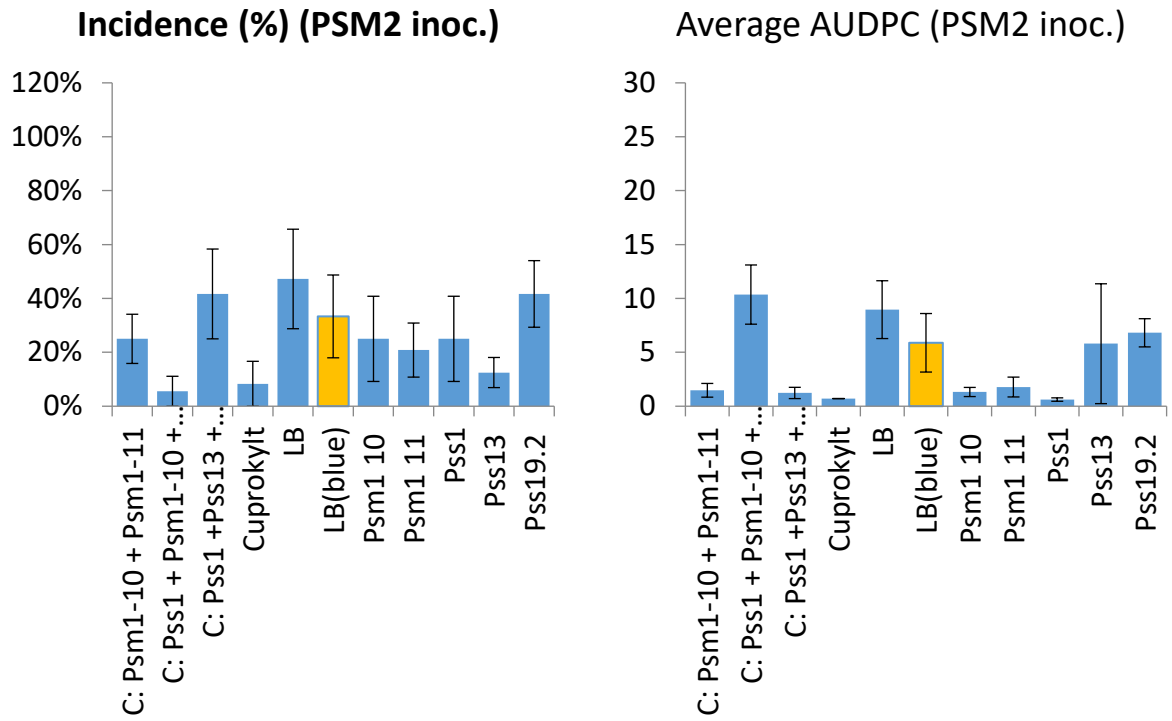


Figure 4.3.14 Incidence of lesion formation (%) (left) and conditional lesion progression (AUDPC) (right) on PSM2 inoculated points on the leaves. Treatments from left to right are phage cocktails with 2, 3, and all 5 different phages followed by Cuprokylt (positive control) colourless “LB” and coloured “LB(blue)” negative controls followed by single phage solutions. All groups were compared to “LB(blue)” group shown in orange

Detached shoot trials

Detached leaf test is very useful in terms of quick assessment of phage activity, but it does not reflect well the conditions in which most bacterial canker predominately infects trees in UK. To try to mimic the leaf fall infections of the leaf scars we collected more than 200 one year old shoots from the orchards at leaf fall, inoculated them with high dose of PSS and PSM1 bacterial mix and then sprayed either with 5 phage cocktail, water (neg. control) or Cuprokylt (pos. control). We obtained highly concentrated LB media free phages from Jackson lab to avoid masking phage activity by supplementing abundant nutrient source. We have tested that the spraying of the phages did not affect their activity by conducting in vitro plaque assay before and after the spraying. Inoculated and treated detached shoots are currently incubating at outside ambient temperature (polytunnel) in bags for high humidity. Percentage of symptomatic leaf scars will be assessed in the late January or early February 2019.

Figure 4.3.15 An example of experimental blocks used in detached shoot test before and after bagging to ensure high humidity

Discussion

The two main efforts of this year's work on bacteriophage control of bacterial canker were to collect a large library of native phages and to establish a good laboratory testing system for evaluation of their efficacy. The phages collected in a previous undergraduate project in Jackson lab at the University of Reading were used in the latter. The collection yielded more than 70 phage isolates of which several had exhibited activity against multiple pathogenic *P. syringae* strains (Figure 4.3.6) without effecting the non-pathogenic strains tested. These strains in mix or as single strains are the most promising and environmentally friendly control of bacterial canker to invest for the future.

In terms of efficacy testing the five phages previously collected in the Jackson lab, we conducted several interactions of detached leaf assay where we optimised inoculation and scoring procedures. These optimisations will ensure that we conduct future tests with the new set of phages as accurately and reproducibly as possible. One of the main issues in this year's detached leaf tests was the presence of LB media in the phage solution which increase the nutrient content of the treated areas and enabled bacteria to regrow very fast after the phages have reduced the populations. Due to this issue the results of the SPRAY-SPRAY and DROP-SPRAY assay should be interpreted with extreme caution or ignored completely since all phage treatment were compared to water instead of LB control.

Another potential issue with detached leaves is that in order to observe the lesion formation quite high bacterial concentration had to be used. This is even more pronounced in in drop inoculation where 10^4 CFU were used in a single drop, much higher than reported in nature (up to 10^6 per whole leaf). In high densities *P. syringae* can form a biofilm which can protect the colony against phages. Lower bacterial concentration will be used in drop inoculation in the future to reflect the natural densities.

Overall results were mixed. As expected, in both assays where phages were compared to water instead of LB treatment they don't significantly decrease incidence of lesion progression. Phage treatments were slightly decreasing lesion area in SPRAY-SPRAY assay with bacterial and phage densities most comparable to those that would be found in nature. On the contrary in DROP-SPRAY assay most of the phage treatments caused more lesions and faster expanding lesions as well.

Positive results were observed in the most accurate DROP-DROP assay where phages were compared to LB media as control. In the case of PSM1 inoculated points single phages significantly reduced lesion formation and progression even in presence of high nutrient LB media indicating that in absence of LB the effect could be even stronger (Figure 4.3.13). The

results from PSS and PSM2 inoculated points in the same assay however, showed no positive effects of phage treatment. This could be due to too high virulence of PSS and too low virulence of PSM2 and/ or phage specificity.

In terms of detached leaf assays we believe that symptom observation is not ideal marker for efficacy of phage mixes. This is due to the potential discrepancies between population size and virulence, i.e. symptom formation. Prolonged incubation times required for the symptom expression could be responsible for stressing the leaf and expediting symptom expression despite reduced bacterial population due to phage attack. Instead of symptom scoring we propose measuring bacterial population sizes by plating on agar or by molecular tools such as quantitative PCR (qPCR) to be used in the future assays to accurately assess whether phage treatments stably reduce bacterial population on leaves and shoots.

Future work

Future research will attempt to fully sequence the phages, and investigate the efficacy of phages individually and in cocktails in repeated detached leaf experiments, detached stem experiments and in orchard conditions (subject to CRD approval) to demonstrate the potential and practical use of phages as biocontrol agent.

4.4 Cultural Control

Aim

Tunnelling Cherries – observational study to assess effects of covering cherries on bacterial canker development

Introduction

Anecdotal evidence has suggested that leaving the cover of tunnelled cherries on for longer after harvest may result in reduced canker development when compared to the standard current practice of removing the covers immediately after harvest. This current practice opens up the tunnel allowing light to reach leaves, which may positively affect potential yield in the following year. Observations on one grower site in Scotland where the covers were left on until after seemed to suggest that there was less canker and a better yield the following year. This observation trial on two grower sites will assess the effects of altering the timing of covering of cherry tunnels of disease incidence and yield.

Materials and Methods

Two sites have been selected across UK, one in Herefordshire and one in Kent. The same variety (Summersun) was chosen on both sites as it is a susceptible variety and is a consistent variety across both observational sites. Trees in all of the tunnels selected were assessed to determine the levels of canker before the commencement of the trials.

Sites

Trial Site 1 – Lower Hope Estate, Sidnall Farm, Bromyard, Herefordshire. Four tunnels have been selected for the trial. Altering both the pre-blossom and post-harvesting was possible at this site.

Trial Site 2 – Little Sharsted Farm, Sittingbourne, Kent. Two cherry tunnels were selected for the trial and only the post-harvest covering was possible on this site.

The treatments can be found in Table 4.4.1. At site 1, there were two controls, one where the trees were covered pre-blossom and one where the tunnels were covered post-blossom. In both of these controls the tunnels are uncovered post-harvest. At site two there was a single control of pre-blossom covering with post-harvest uncovering.

Table 4.4.1 Timings of covering and uncovering of tunnels for the trial.

Treatment	Description	Site 1 - Herefordshire	Site 2 - Kent
Control 1	Pre-blossom covered, Post-harvest uncovered	Yes	Yes
Control 2	Post-blossom covered, Post-harvest uncovered	Yes	No
Treatment 1	Pre-blossom covered, Leaf fall uncovered	Yes	Yes
Treatment 2	Post-blossom covered, Leaf fall uncovered	Yes	No

Standard treatments for pests, foliar disease and nutrients were applied to all plots throughout the season. Weather station data will be collected from each site. In each tunnel ten trees were marked and recorded for baseline incidence of canker (14 November 2018 – Hereford, 28 November 2018 – Kent). Main stem A/B and peripheral C/D/E cankers were counted and recorded for each tree and photographs taken. Notes were also made of mummified fruit left on the tree. Timing of blossom and yield will be recorded in spring 2019 for each treatment.

Figure 4.4.1 Cankers noted in observation trials 14 November 2018

Future Work

The trials will be recorded throughout 2019, with blossom dates, leaf shot holing and yield being recorded as well as canker incidence.

Objective 6 - Codling and Tortrix Moth

6.1 Pheromone MD

Aim

Integrate pheromone mating disruption into the control programmes for codling and tortrix moth in apple orchards whilst enhancing natural enemies and maintaining control of other pests and reduce spray residues, and have long term detrimental impacts on populations of codling and tortrix moths (EMR/ADAS, Yr 1-2)

Summary

This objective was completed in 2016 and 2017

6.2 Blastobasis

Introduction

Larvae of the moth *Blastobasis lacticolella*, Rebel, 1940 (Synonym: decolorella) (Lepidoptera: Blastobasidae) (Figure 6.2.1) feed on the surface of pear and apple fruits in mid- and late-summer, often where clusters are touching, causing large open, scallop-shaped, wounds in the fruit flesh and rendering fruits un-saleable. Very severe damage can result if the pest is allowed to increase over a number of years unchecked, especially on short stalked varieties such as Bramley and Egremont Russet. Growers currently have no means of identifying whether they have a problem other than the occurrence of damage the previous year, which is often confused with damage caused by other apple moth pests. It is also difficult to time sprays accurately against Blastobasis. Sprays are likely to be most effective when they are applied against hatching eggs. Pheromone traps are the easiest way of monitoring the flight activity and egg laying period of moth pests.

The increased use of pheromone mating disruption and granulovirus and the move towards reducing the occurrence of pesticide residues on fruits and the removal of pesticides have meant that the chemicals that control Blastobasis are not always used. This has led to the occurrence of occasional but severe outbreaks of damage. In particular, in recent trials growers using RAK3+4 for mating disruption of codling moth and tortrix moths experienced outbreaks of Blastobasis requiring application of insecticide which negated the advantages of using mating disruption.

In addition, in 2018, Blastobasis caused significant damage on some strawberry farms as caterpillars burrowed under the calyx of the fruits.

In previous work by NIAB EMR and NRI, adult *Blastobasis* moths were collected by beat sampling. Pheromone glands of female moths were excised and pheromone extracted in solvent. In analyses by gas chromatography (GC) coupled to electroanntennographic (EAG) recording from the antennae of male moths two active components were detected. As these moths were most probably already mated, very little pheromone was present in the extracts and was not possible to identify the compounds fully. GC retention indices of the active compounds indicated these were a 16-carbon mono-unsaturated acetate and the corresponding aldehyde. It was not possible to determine the position or configuration of the double bond, although the GC retention data fitted those for the (Z)-11- isomers. Furthermore it was not possible to determine the relative amounts of the two compounds in the extracts.

In 2017, field trapping experiments with three potential pheromone blends based on previous work were carried out in Northern Ireland, Hereford and Kent. A number of moths were caught, but analysis of sample moths by DNA barcoding of COI gene locus and comparison with NCBI Database indicated that probably none were *B. lacticollela*. The majority identified were *Rhigognostis incarnatella* and six out of eight were from traps baited with blend C, 1:10 Z11-16:Ac : Z11-16:Ald. This species is related to the diamondback moth, *Plutella xylostella*, the pheromone of which is a 1:1 blend of Z11-16:Ac and Z11-16:Ald.

Figure 6.2.1 Adult *Blastobasis lacticolella*, Rebel, 1940

There is a clear commercial need to develop a pheromone monitoring trap for Blastobasis so that growers can determine whether they have a problem and time insecticide applications. The pest needs to be monitored routinely in orchards of high risk varieties (Bramley, Egremont

Russet), alongside normal sex pheromone trap monitoring for codling and tortrix moths. The aim of this work was to develop a sex pheromone trap for Blastobasis for use by UK apple growers. This will help growers to reliably know whether they have a developing problem with this pest and when to spray for it and will facilitate the use of RAK3+4 mating disruption for tortrix moth control in orchards. In 2018 this work was funded by BASF, the objectives were:

- Collect adult female Blastobasis in the field for pheromone gland extraction
- Collect Blastobasis larvae in the field and rear through to adults in the laboratory for pheromone gland extraction
- Prepare pheromone gland extracts and analyse by GC-MS and GC-EAG to detect potential pheromone components
- Identify and synthesise the chemical structures of potential pheromone components
- To demonstrate attractiveness of blends of synthetic pheromone components to Blastobasis in the field and develop a suitable lure for pest monitoring
- To test a lure and trap in commercial orchards for monitoring Blastobasis adult moths

Materials and Methods

Initial field trapping tests in Northern Ireland

Several contacts with experience of Blastobasis on orchards helped with the deployment of traps and monitoring and allowed us to survey their orchards. Bramley apple orchards were selected where *B. lacticolella* damage was confirmed in 2017 and, when possible, unmanaged or unsprayed orchard where chosen (Table 6.2.1).

The pheromone was deployed in green delta traps with sticky glue inserts in ten different apple orchards on 18 June (Table 6.2.1). Either three treated or untreated delta traps were hung at mid-canopy height, perpendicular to the row and >10 m apart or around the perimeter of the apple orchards. Lures were changed after 2 weeks (on 2 July).

The potential sex pheromone components, (Z)-11-hexadecenyl acetate (Z11-16:Ac) and (Z)-11-hexadecenal (Z11-16:Ald) were synthesised and formulated into polythene vials as dispensers at NRI. The lures were combinations of the two compounds in 10:1, 1:1 and 1:10 ratios (Table 6.2.2).

Table 6.2.1 Details of locations for testing pheromone lures for Blastobasis during 2018

Site No	Person responsible	Co-ordinates	Variety	Sprayed (Y/N)	Address
1	Patricia&Patrick Murray	54.671030, -6.269551	Bramley	N	Crumlin
2	Patricia&Patrick Murray	54.671416, -6.270576	Bramley	N	Crumlin
3	(S. MacAntsaoir), AFBI MI06	54.412624, -6.586802	Bramley	N	Armagh
4	(S. MacAntsaoir) AFBI Millennium M27+M9	54.409997, -6.587231	Bramley	N	Armagh
5	(S. MacAntsaoir) AFBI Fruit Wall	54.406790, -6.595700	Bramley	N	Armagh
6	Andrew Glass	54.433840, -6.553789	Bramley	Y	Kilmore
7	Andrew Price	54.392309, -6.487513	Bramley	Y	Portadown
8	Graham Hewitt	54.403625, -6.516376	Bramley	Y	Portadown
9	Sam Mc Niece	54.443726, -6.596863	Bramley	N	Portadown
10	Tommy Mc Glennon	54.394984, -6.695697	Bramley	N	Benburb

Table 6.2.2 Composition of lures used in trapping experiments for Blastobasis.

	Amount (µg)	
Lure code	**Z11-16:Ac**	**Z11-16:Ald**
2018/098A	1000	100
2018/098B	500	500
2018/098C	100	1000
2018/098D	-	-

Six delta traps (three with the pheromone, three untreated) were located in 10 apple orchards in Northern Ireland (coordinated by Francesco Maria Rogai, Sean Mac AntSaoir and Kieran Lavelle) (Figure 6.2.2).

Figure 6.2.2 Example of green delta trap deployed in an unsprayed apple orchard and hedgerow

Apple orchards were also tap sampled (on 19, 20, 21, 22 June and on 3, 4, 5, 6 July) with the intention of collecting adult Blastobasis moths for pheromone extraction and identification. Apple trees were also inspected for Blastobasis larvae and pupae.

In addition, from 18 to 21 June and from 2 to 5 July, a night sampling of *B. lacticolella* adults was done using light traps. The sampling took place at Murlough National Nature Reserve in Northern Ireland, where abundant presence of *B. lacticolella* was recorded over 2017. One Robinson moth trap with 125 W mercury vapour bulb (Figure 6.2.3) and four portable heat moth traps with 6 W actinic bulbs (Figure 6.2.4) were used thanks to the kind assistance of the moth expert Andrew Crory. The traps were deployed at 20:00 within a grassland and woodland at the nature reserve and assessed at 06:00 the following day. The lights attract moths to the traps where they fall down through a funnel and into a box containing egg trays, between which the moths could hide until identification (Figures 6.2.3 and 6.2.4). Adult *B. lacticolella* were placed into 35 ml individual tubes and kept refrigerated to ensure their survival until the gland extraction was carried out at NRI laboratories.

Figure 6.2.3 Robinson moth trap and inside the bottom of the trap with egg trays for moths to hide

Figure 6.2.4 Examples of portable heat moth traps in grassland and woodland

Field trapping test at NIAB EMR

A potential new pheromone component identified at NRI was tested in a pilot study in an unsprayed apple orchard (Wiseman), a beech hedgerow and woodland at Park Farm site at NIAB EMR.

Green delta traps were used baited with rubber septa impregnated with 1 mg (*Z,Z,Z*)-3,6,9-nonadecatriene (ZZZ3,6,9-19:H). There were 5 pheromone-baited traps and 5 unbaited traps.

Rearing Blastobasis larvae

We also received Blastobasis larvae collected in strawberry from a grower in the West Midlands on two occasions; 18 July and 21 August for rearing through to adults. Larvae were placed into Bugdorm cages with damp tissue paper at the bottom and fed with strawberry leaves, fruits and codling moth diet (Figure 6.2.5).

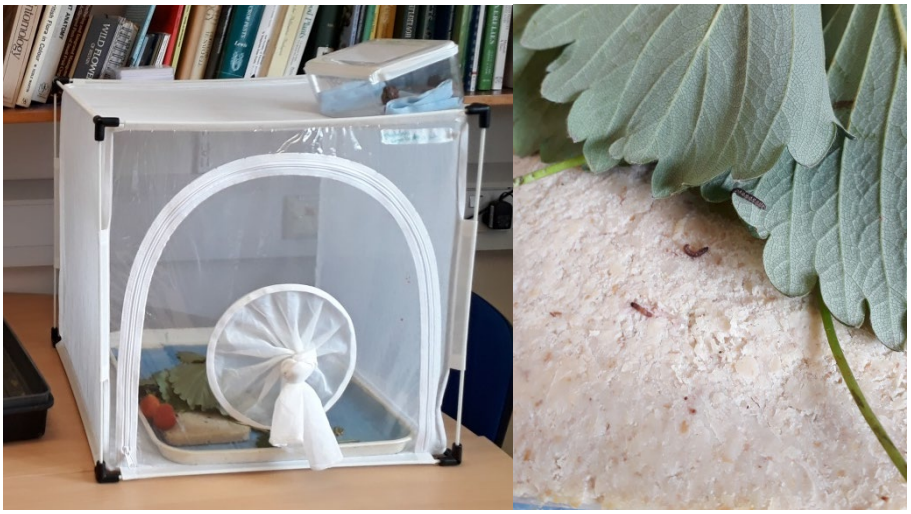


Figure 6.2.5 On the left Bugdorm cage with strawberry leaves, fruit and codling moth diet; on the right detail of Blastobasis larvae feeding on codling moth diet and leaves

After one week 60 cocooned larvae were moved into 35 ml individual tubes with a small piece of corrugated card (Figure 6.2.6). Some of these larvae were subjected to cooling at 4 or 10 °C for a week to stimulate pupation (Figure. 6.2.7).

As unmated adults emerged, they were sent to NRI for gland extraction. Emergence was very sporadic with emergence occurring in small numbers and most larvae not pupating. The majority of the larvae were held back in refrigeration and removed in January 2019.

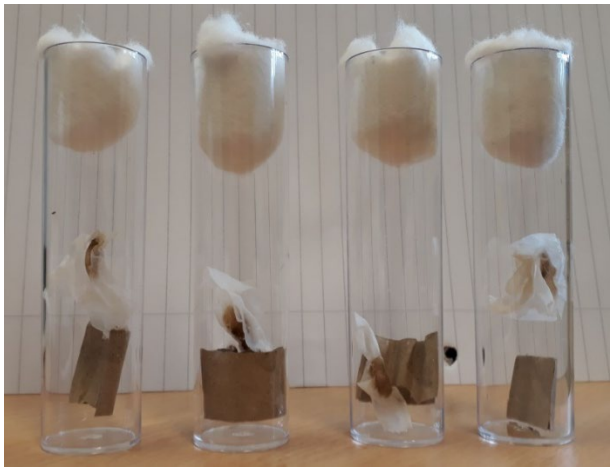


Figure 6.2.6 Cocooned Blastobasis larvae in individual tubes

Figure 6.2.7 *Blastobasis lacticolella* pupa and emerged adults

Preparation of pheromone gland extracts

The sex of adult Blastobasis moths was determined by anaesthetising the moth with carbon dioxide and gently squeezing the abdomen to expose the genitalia. Males were characterised by brushes and females by an ovipositor.

Pheromone gland extracts were prepared from females by excising the ovipositor directly into hexane (10 µl/ovipositor; Pesticide Residue Grade), allowing to extract for 10 min and then withdrawing the extract with a microsyringe and storing in a conical vial (1.5 ml) at -20°C. Extracts were prepared 1-1.5 h into the scotophase (period of darkness, especially one that is artificially imposed) as Blastobasis moths are thought to mate at dusk.

Analysis of pheromone gland extracts

Gland extracts were analysed by gas chromatography coupled to mass spectrometry (GC-MS) on a Varian CP-3800 GC coupled directly to a Saturn 2200 MS (Varian, now Agilent, Stockport, UK) using fused silica capillary columns (30 m x 0.25 mm i.d. x 0.25 µ film thickness) coated with non-polar VF5 (Varian) or polar DBWax (Agilent). Carrier gas was helium (1 ml min^{-1}), injection was splitless (250 °C and 220 °C respectively) and oven temperature was held at 40°C for 2 min then programmed at 10°C min^{1} to 250°C and held for 5 min. Retention indices of compounds were calculated from their retention times relative to those of *n*-alkanes and compounds were identified by comparison of their mass spectra and retention indices relative to those of authentic standards.

Gland extracts were also analysed by GC coupled to electroantennographic (EAG) recording from the antenna of a male moth. For the EAG preparation, an excised antenna was

suspended between two glass electrodes filled with saline solution (0.1 M potassium chloride with 1% polyvinylpyrrolidone to reduce evaporation) and connected by silver wires to a portable device consisting of integrated electrode holders, micromanipulators, and amplifier (INR-02; Syntech, Hilversum, The Netherlands, now Kirchzarten, Germany). GC Analyses were carried out on a fused silica capillary column (30 m x 0.32 mm i.d. x 0.25 µ film) coated with polar DBWax (Supelco) with the column outlet split 1:1 through equal lengths of deactivated fused silica tubing between the flame ionization detector and a heated outlet (250°C). The latter went into a silanised glass tube (4 mm i.d.) with air (300 ml/min) carrying the effluent to the EAG preparation. The signal was amplified x 10 and the amplifier was connected to the GC as a detector device. Data were processed with EZChrom Elite v3.0 (Agilent).

Results

Initial field trapping tests in Northern Ireland

No Blastobasis moths were caught either in the treated or untreated traps from all 10 sites. One specimen of *Xestia triangulum* was found in one treated trap at Site 1 on 22 June.

In total, 33 *B. lacticolella* and two *B. adustella* adults were captured in light traps, with 13 moths captured from 18 to 21 June and 22 from 2 to 5 July. These samples were either posted or delivered to NRI in order to proceed with sexing and gland extraction from female moths.

Pheromone identification

In all, six pheromone gland extracts were prepared and analysed (Table 6.2.3).

Table 6.2.3 Blastobasis pheromone gland extracts (prepared 1-1.5 h into the scotophase).

Date	Details	Extract	Ref
26/6/2018	5 adults brought back from N.I. 4 dead	1 female	2018/092/01
5/7/2018	7 adults in post from N.I. 4 dead, 3 alive, I female, 2 male	1 female	2018/092/02
9/7/2018	13 adults brought back from N.I.	4 females	2018/092/03
		1 male	2018/092/04
10/7/2018	2 adults from Jerry Cross; 1 dead	1 female	2018/092/05
18/9/2018	3 adults from culture	3 female	2018/092/06

Pheromone gland extracts were analysed by GC-MS on polar and non-polar GC columns (Figure 6.2.8). All clear peaks were examined for mass spectra typical of lepidopteran pheromone components. Single-ion scanning at *m/z* 61 was carried out to detect acetate esters. The presence of (*Z*)-11-hexadecenyl acetate and (*Z*)-11-hexadecenal, potential pheromone components identified previously was investigated by comparison with retention times and mass spectra of synthetic standards. In some classifications, Blastobasis is included in the Coleophoridae family. (Z)-5-Decenyl acetate and alcohol are common pheromone components in this family, and the presence of these was investigated in the extracts by comparison with synthetic standards from the NRI library.

No potential “lepidopteran-like” pheromone components could be detected except for a peak at Retention Indices 2022 on the polar GC column and 1966 on the non-polar column. The mass spectrum showed a base peak at *m/z* 79 and a probable molecular ion at *m/z* 262 (Figure 6.2.9) and the compound was identified as (*Z,Z,Z*)-3,6,9-nonadecatriene. However, this compound was subsequently found to be present in extracts from males, and thus is probably a cuticular hydrocarbon common to both sexes.

In GC-EAG analyses, it proved very difficult to get a circuit, probably because the antennae were dried out. Three GC-EAG runs were carried out on extracts 03 and 05 (e.g. Figure 6.2.10) and five on mixtures of the above synthetic standards (Figure 6.2.11), but no consistent responses were observed.

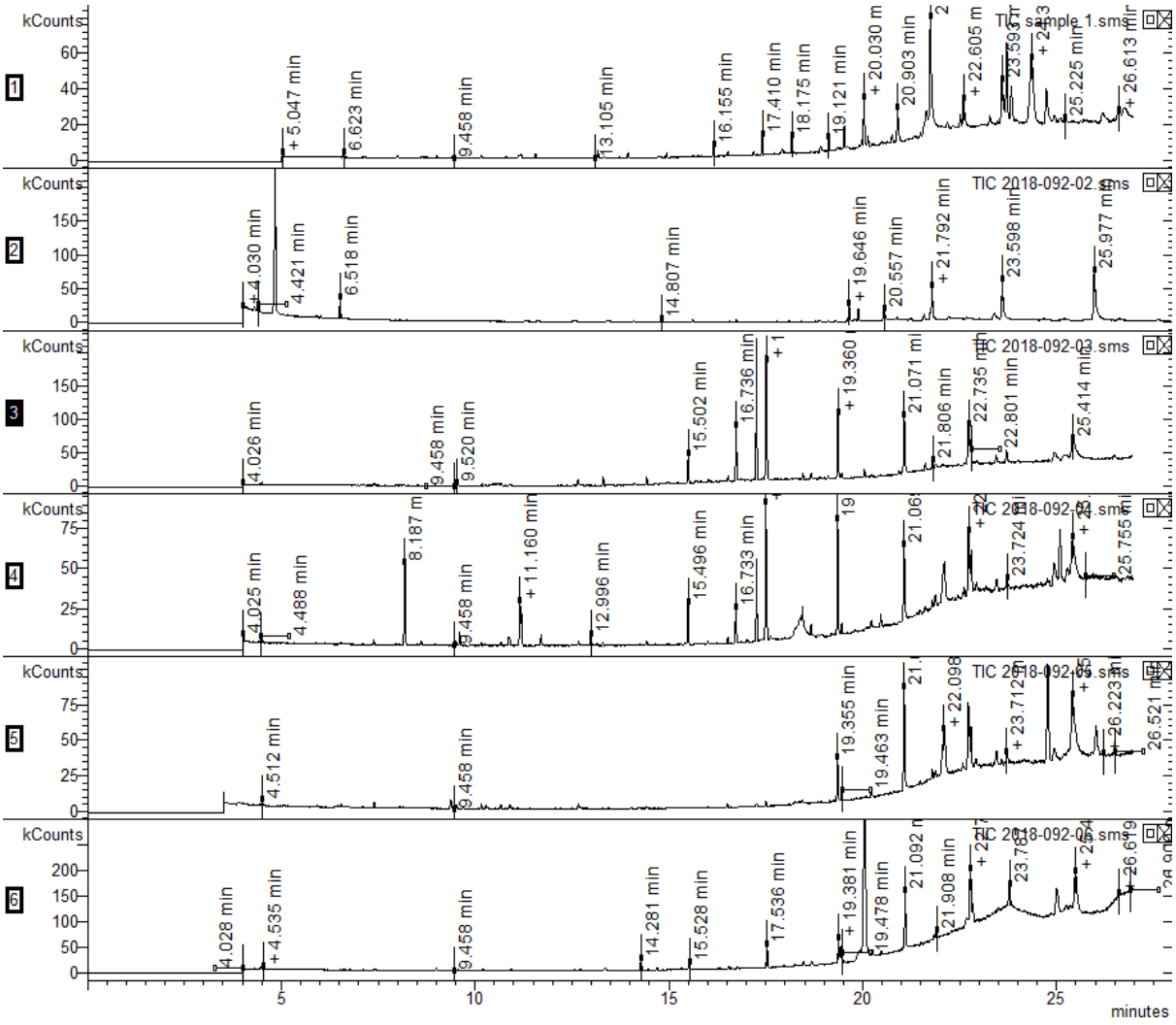


Figure 6.2.8 GC-MS Analyses of pheromone gland extracts from female Blastobasis *laticolella* on polar GC column (from top 2018/092/1-8)

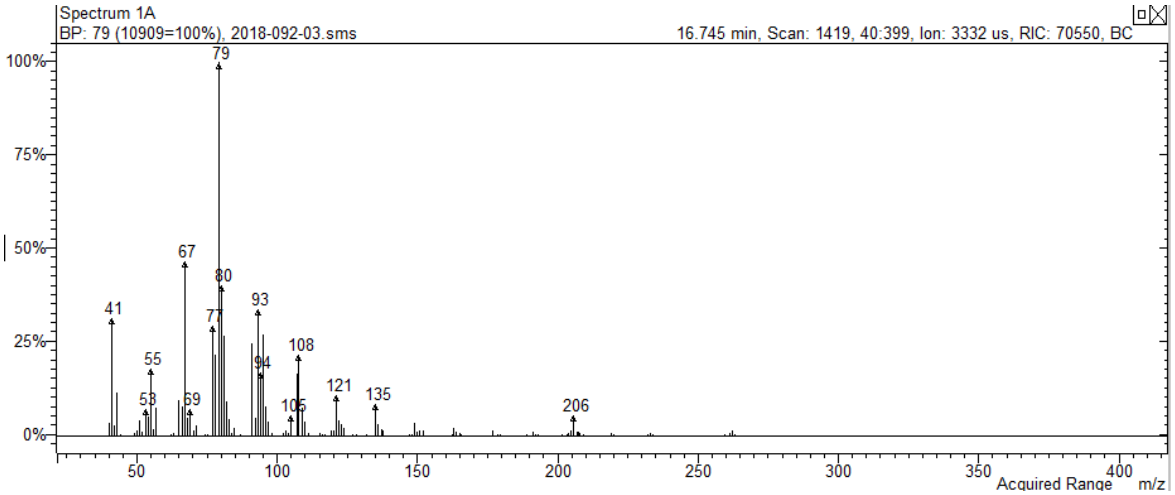


Figure 6.2.9 Mass spectrum of potential pheromone component identified as (*Z,Z,Z*)-3,6,9-nonadecatriene

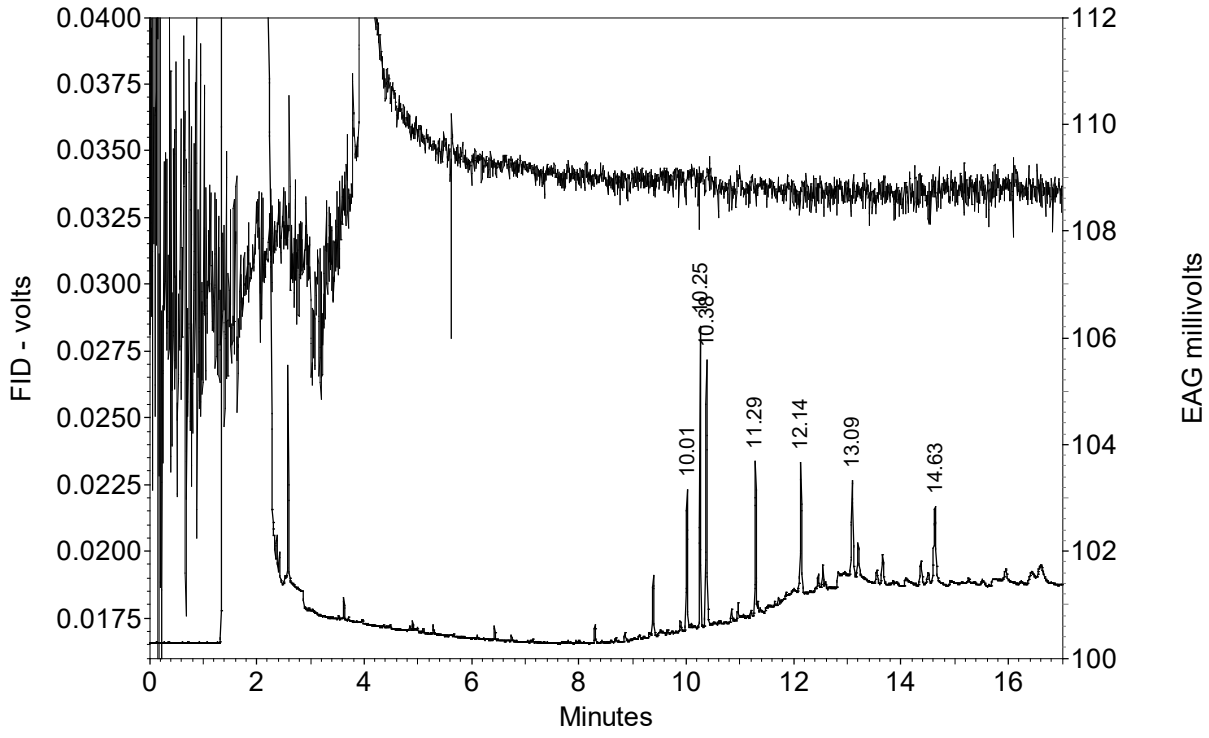


Figure 6.2.10 GC-EAG Analysis of pheromone gland extract from female Blastobasis *laticolella* (2018/092/03) on polar GC column (upper trace EAG, lower GC-FID; (*Z,Z,Z*)-3,6,9-nonadecatriene at 10.01 min)

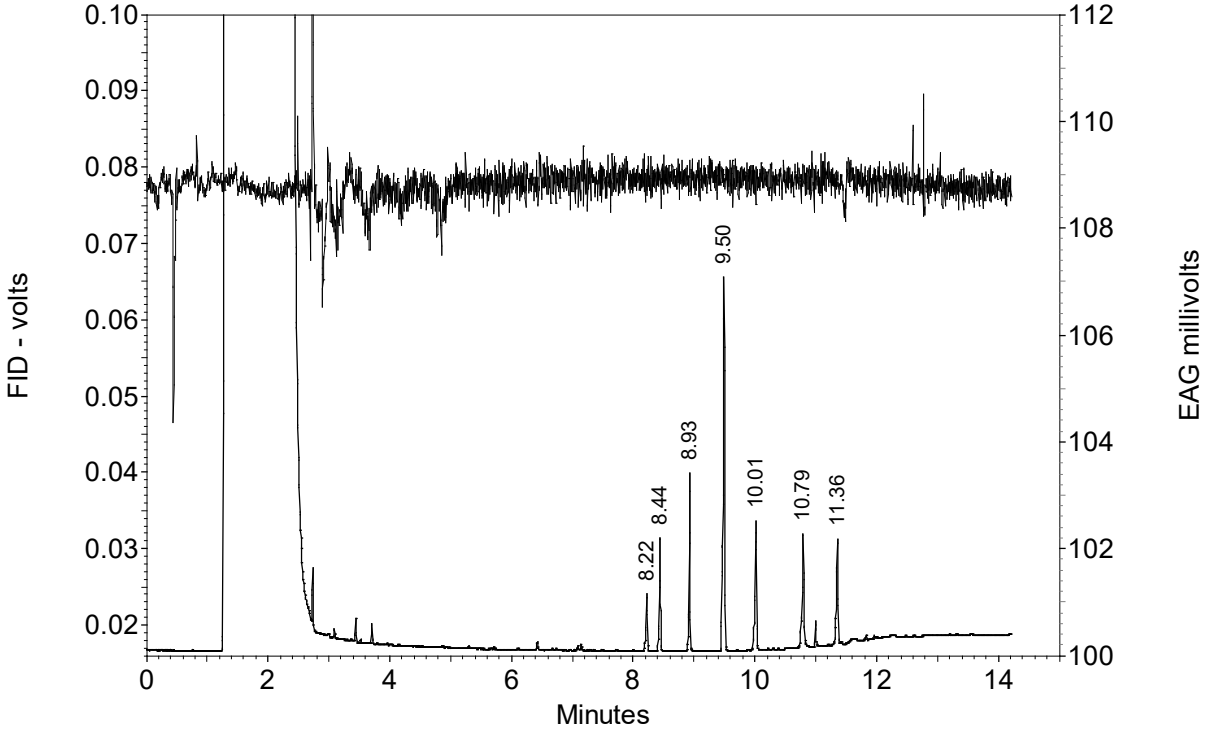


Figure 6.2.11 GC-EAG Analysis of synthetic standards with male *Blastobasis laticolella* EAG preparation on polar GC column (upper trace EAG, lower GC-FID; decyl acetate 8.22 min; (*Z*)-5-decenyl acetate 8.44 min; (*Z*)-5-decenol 8.93 min; BHT 9.50 min; (*Z,Z,Z*)-3,6,9-nonadecatriene 10.01 min; (*Z*)-11-hexadecenal 10.79 min; (*Z*)-11-hexadecenyl acetate 11.38 min)

Field trapping test at NIAB EMR

Some moths were caught in the traps at NIAB EMR for the pilot study but none were Blastobasis sp. In particular 20 *Ourapteryx sambucaria* (Lepidoptera; Geometridae; Ennominae) adult moths were caught in the 5 pheromone treated traps after one week from their deployment (Figure 6.2.12). *O. sambucaria* was previously reported to be attracted to (*Z,Z,Z*)-3,6,9-nonadecatriene by Subchev et al. (1986).

Figure 6.2.12 *Xestia triangulum* moth (left) and *Ourapteryx sambucaria* moths (right) caught on the sticky glue inserts in traps baited with (*Z,Z,Z*)-3,6,9-nonadecatriene at NIAB EMR

Conclusions

- As found previously, blends of (*Z*)-11-hexadecenal and (*Z*)-11-hexadecenyl acetate failed to attract *Blastobasis laticolella* moths in field trapping tests, even though this species was clearly present as indicated by catches in light traps
- Rearing adult *B. laticolella* adult moths from larvae collected in the field proved a real challenge, but some were reared through to adult
- Extracts of the pheromone glands of female *B. laticolella* moths were made from both moths collected in the field which were probably mated and from virgin female moths reared from larvae in the laboratory
- In analyses of extracts by GC-MS, potential pheromone components including (*Z*)-11-hexadecenal, (*Z*)-11-hexadecenyl acetate, (*Z*)-5-decenyl acetate and (*Z*)-5-decenol could not be detected
- (*Z,Z,Z*)-3,6,9-Nonadecatriene was identified as a potential component of the female sex pheromone of *B. laticolella* but was subsequently shown to be present in extracts from both female and male moths and did not attract male *B. laticolella* moths in the field

Future Work

Future work should focus on obtaining more virgin female moths from larvae in the laboratory in order to make and analyse gland extracts at different times into the scotophase. Collection of volatiles by entrainment could be investigated, but past experience has shown this approach to not be effective with the very small amounts of relatively involatile pheromone typically produced by moths.

Objective 7 - Improve Reliability of Natural Enemies

7.1 Enhance and accelerate the natural ecology in newly planted orchards

Aim

The overall aim was to speed up the ecology of newly planted orchards to establish beneficial arthropods more quickly to mitigate losses due to pests.

Introduction

Establishing new crops requires substantial investment (~£30k/ha for apple) and growers need confidence that their orchards will crop reliably and that their fruit will find a profitable market. Ecological succession is the observed process of change in the species structure of an ecological community over time. The community begins with relatively few pioneering plants and animals and develops through increasing complexity until it becomes stable or self-perpetuating, as a climax community. Newly planted orchards have an un-established ecosystem. The recently tilled ground in newly planted orchards often has minimal, simplified or absent vegetation cover with a low diversity of plant species resulting in low pollen and nectar provision and low refugia and structure. The tree bark and canopy are simple compared to older established trees affording little availability for predatory arthropods to gain refuge. Hence, local, natural predators and pollinators have not built up and established in new orchards leading to random, sporadic, attacks from a number of pest species which can then be difficult to control.

In 2017 we applied interventions to newly planted orchards in order to establish more rapidly the beneficial ecology.

Methods

Six replicate orchards were sourced by Caroline Ashdown at Worldwide Fruit (WFL) (Table 7.1.1). In each orchard, 0.25 ha was treated with ecological enhancement interventions. There were a maximum of two orchards per farm and orchards were separated by >1 km. The intervention treated area was randomised and the treated areas were assessed and compared to an untreated area of the same orchard.

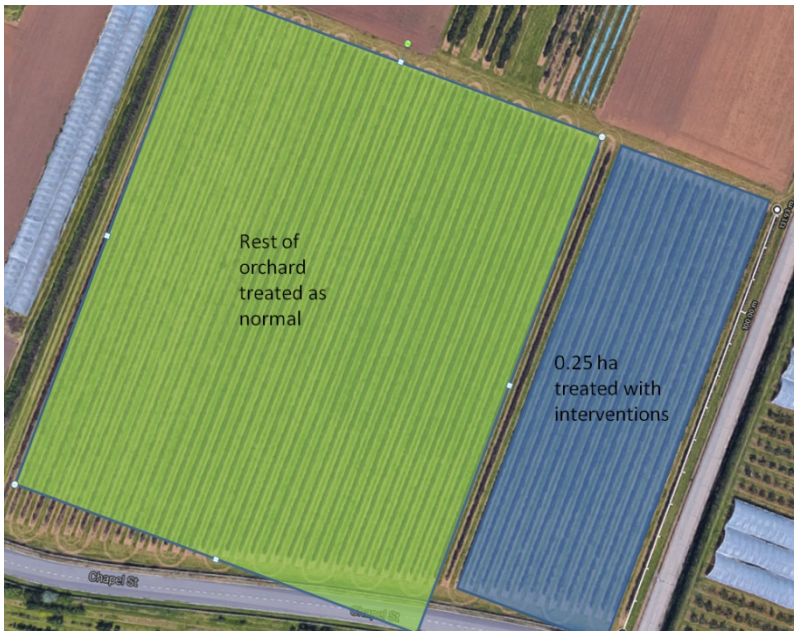


Figure 7.1.1 Example of orchard layout

Crop husbandry was the growers' normal programme of sprays. Regular communication has been established between NIAB EMR staff and the growers/advisors. A Stevenson's screen with two data loggers to record temperature and humidity every 30 minutes were deployed in each orchard. Photographs were taken of the forb establishment at each site in the autumn of 2017 and at each assessment.

The suggested seed mix (Table 7.1.3) was used, with some modifications on some sites. For example, Peter Checkley (Site 4) and Charles Highwood (Site 6) used a mix with 5% Highland common bentgrass, 10% Southland crested dogstail, 5% Teno smaller catstail, 20% Bornito sheeps fescue, 16% Evora smooth stalked meadow grass, 2% Yarrow, 6% Lesser Knapweed, 7% Self Heal, 2% Birdsfoot trefoil, 1% Essex red clover, 4% Ox-eye daisy and 6% Red Campion. Establishment at site 4 is shown in Fig. 7.1.8.

Table 7.1.1 Sites, sites managers and alleyway sowing dates

Orchard	Name	Address	Orchard, Variety	ha	spacing (m)	Trees planted	Notes/ planting	Row length (m)	Sown 2017
1	John Bray	A J Bray & Sons Ltd, Holmestall, Doddington Sittingbourne ME9 0HF	A12, Jazz	2.6	3.35x1 or 1.2	Feb 2017	Every other row for 5 rows (10 rows)	144	Apr
2	Clive Chandler	Chandler & Dunn Ltd. Lower Goldstone Farm CT3 2DY	Broome, Gala	2.23	3.5x1.25	Dec 2016	Every row (7 rows) for 0.25 ha of orchard	95	May
3	Clive Chandler	SEE ABOVE	Richards, Jazz	1.54	3.5x1.25	Dec 2016	Every row (11 rows) for 0.25 ha of orchard	60	May
4	Peter Checkley	Howard Chapman Ltd. Broadwater Farm, West Malling, Kent ME19 6HT	New Barns, Gala	1.3	3.5x1.5	April 2017	Every third, 0.25 ha, 5 rows	109	May
5	Jeremy Linsell	Chromesword Ltd, Braiseworth Orchards, Eye, Suffolk, IP23 7DS	Rectory, Jazz	1.13	3.25x1.2	Jan 2017	4 rows every other row	144	Oct
6	Charles Highwood	Sheerland Farm, Pluckley, Ashford TN27 0PN	Willow Wood Variety	2.28	4x1.5	May 2017	0.4 ha sown in every row	250	May

Table 7.1.2 Treatment interventions within the programme

Treatment	Detail	Target beneficial	Improve	Date implement
Alleyway sowings [*1]	Alleyway included Yarrow, Ox-eye daisy, Bird's foot trefoil, Self-heal, Red campion, Red clover.	Pollinators, parasitoids, anthocorids, spiders	Pest control inc. aphids, tortrix. Establish pollinator networks	At orchard establishment
Earwig refugia	Innovate UK Bioactive predator refuge [*2]	Earwigs, spiders, ladybirds	Aphids, caterpillar, codling moth	Autumn 2017
Hoverfly attractant	From AHDB TF 218	Hoverfly larvae	Aphid	From 2018

[*1] Further contacts - Colin Bird, Agrii and Megan Mckerchar PhD

[*2] NIAB, NRI, WorldWide Fruit Ltd., Russell IPM, Fruition PO Ltd., Agrovista UK Ltd.

Table 7.1.3 Suggested and tested seed mix for orchard alleyway planting in the 0.25 ha on the intervention side of the six orchards. NB to be mixed with high percentage (>70%) of non-competitive grasses

Species	Common Name	% By Weight
Achillea millefolium	Yarrow	2.0
Centaurea nigra	Knapweed	29.4
Leucanthemum vulgare	Oxeye daisy	5.9
Lotus corniculatus (wild type)	Birds foot trefoil	23.5
Prunella vulgaris	Selfheal	11.8
Silene dioica	Red Campion	11.8
Trifolium pratense (wild type)	Red Clover	15.7

Figure 7.1.8 Establishment at Site 4 in 2017

At Clive Chandler's orchards (Sites 2 & 3) a similar wildflower and grass mixture was sown, but in different proportions: 2.5% Highland common bentgrass, 2.5% Southland crested dogstail, 2.5% Teno smaller catstail, 2.5% Calliope red/chewings fescue, 5% Evora smooth stalked meadow grass, 29% Lesser Knapweed, 3% Yarrow, 6% Ox-eye daisy, 13% Birdsfoot trefoil, 12% Self Heal, 12% Red Campion and 10% Essex red clover. Establishment is shown in Fig. 7.1.9.

Figure 7.1.9 Establishment of the mix used at sites 2 & 3 in 2017

Earwig refuges, courtesy of the Innovate UK Bioactive predator refuge project (NIAB, NRI, WorldWide Fruit Ltd., Russell IPM, Fruition PO Ltd., Agrovista UK Ltd.), were deployed at each site between 27 September and 13 October 2017, with approximately 464 at each site, at a rate of one per tree in a block on the 0.25 ha side of the orchard. These were attached to each tree by hanging onto the plastic tie using the hook provided on the refuge. Where possible, and where rubbing would not occur, the refuge was placed between the tree and the support pole. These were always placed in a block at the centre of each enhanced ecology area, and the number of rows and length of row treated varied according to the layout of the orchards. At Sites 1 & 2 - 6 rows, at Sites 3 & 4 - 9 rows and at Sites 5 & 6 - 4 rows were treated. At Site 1 earwigs were already present in the yellow tree ties. The refuges are constructed from two grooved wooden sections which can easily be opened, and the blue plastic cap provides the attachment hook, and an initial food source.

A hoverfly attractant from NRI was deployed in the orchards in spring 2018 and consisted of 5 x 5 cm polythene sachets containing 1.5 ml of a mixture of methyl salicylate, phenylethanol and (E)-beta-farnesene. Sachets were placed in the six orchards at 7 x 7 m spacing making a total of 180 sachets per hectare. Attractant sachets were deployed in the end of May and replaced once in mid-July. White sticky traps were deployed in the beginning of August to assess the presence of hoverflies.

Assessments (2018)

In 2018 there were three assessments (spring, summer and autumn) in the untreated and treated half of each orchard. The assessments were undertaken in the centre of the treated section of the orchard and the centre of the untreated section of each orchard.

APRIL

- Photographs of sward and tree stage were taken
- Average percentage grass, forb, moss, bare ground were estimated using 10 measurements of 50 x 50 cm quadrats per orchard half. Forbs were identified where possible
- Solitary bee nesting sites were estimated by examining the ends of 8 rows in the herbicide strip before the first tree (m^2)
- 30 shoots were examined for the presence of aphids
- 30 earwig refugees were held over a white trap and tapped three times with the tree tapping stick and predators recorded
- 30 trees were tap sampled for other predators

JULY

- Photographs of sward and tree stage were taken
- Average percentage grass, forb, moss, bare ground were estimated using 10 measurements of 50 x 50 cm quadrats per orchard half in the alleyways
- 30 shoots were examined for the presence of aphids
- For the apple leaf curling midge assessment, 10 shoots on 30 trees were examined and the number of shoots affected per tree recorded
- 30 young leaves were collected to assess the presence of rust mite and spider mite (assessed in the lab by means of light microscope)
- A drop disk was used to make 10 measurements of sward height in each orchard and estimate the average height
- First generation of CM damage assessment on tree fruit from 30 trees in each plot (total numbers of apples on 5 trees was recorded)
- 30 trees were tap sampled for other predators
- 30 trees were tap sampled for earwigs and other predators at night

AUGUST

- Photographs of sward and tree stage were taken
- 30 trees were tap sampled for other predators
- Second generation of CM damage assessment on tree fruit from 30 trees in each plot (total numbers of apples on and under all trees was recorded)
- Five white sticky traps placed in the centre of each half of each plot for one week were collected in clinging film for counting and identification of the hoverflies

Results

Vegetation Coverage

The sown seed mix applied to the treated plots was successful to some degree in all blocks (Table 7.1.4), except for Site 5 were the sowing was repeated in April 2018 (Fig. 7.1.10, Fig. 7.1.11, and Fig. 7.1.12). In all blocks the sowings increased the vegetation diversity, evenness and structure (data to be analysed after final year of project). Some already established species were retained in these plots e.g. chickweed. From the sown seed mix red clover and yarrow were the most successful species, with higher ground coverage. Red Campion also developed well but not on all blocks. Vegetation cover also changed from spring to summer dominated by an increased coverage of red clover in most blocks. Yarrow cover did not increase so dramatically from spring to summer. Red Campion developed in spring but was only recorded in the summer's surveys on one block and at very low percentage cover (1%).

Grass, natural clover and plants from the Plantago genus were the most common species found in control plots in both spring and summer.

There was a significantly higher sward height in the treatment intervention plots compared to the control plots in the summer ($p<0.001$, Fig. 7.1.13).

Solitary Bee Nesting

Solitary bee nesting was assessed in the spring on the herbicide end of rows located in the control area and in the area where the intervention was implemented. The average numbers of solitary bee nests (0.11 nest/m^2) in the intervention treated area was not significantly increased compared to the control plots (0.066 nest/m^2, $p=0.295$).

Aphid Monitoring

In the spring more aphids were observed ($p<0.001$, Fig. 7.1.14) in the control plots (0.1 per 10 shoots) compared to the treated plots (0.017 per 10 shoots). Aphids were found on four control plots versus two treated plots. In the summer the number of aphids recorded increased, but was not significantly different between the control and the intervention plots ($p=0.080$). At this time aphids were observed on four control plots versus three treated plots. The treated plot on Site 5 did not record any aphids in spring but had the highest number of aphids (1.07 aphids per 10 shoots) compared to all other plots in summer. It is probable that the aphid numbers on Site 5 may be prompting the difference between control and treatment in summer.

Table 7.1.4 Percentage cover of each species in the seed mix successfully sowed, per treated site, in spring and summer 2018.

Site	Season	Yarrow	Knapweed	Oxeye daisy	Bird's foot trefoil	Selfheal	Red campion	Red clover	Total coverage of seed
1	**spring**	-	-	-	-	-	-	61.5	**61.5**
	summer	-	-	3	14	-	-	64	**81**
2	**spring**	4.5	8	-	-	-	10.5	27	**50**
	summer	3	6	-	-	-	1	50	**60**
3	**spring**	6	-	-	-	-	21.5	2	**29.5**
	summer	-	13	-	-	-	-	35	**48**
4	**spring**	14	-	12.5	-	-	-	20.5	**47**
	summer	24	-	-	2	-	-	16	**42**
5	**spring**	-	-	-	-	-	-	-	**-**
	summer	-	-	-	-	-	-	-	**-**
6	**spring**	4.5					10.5	0.5	**15.5**
	summer	3	-	-	19	-	-	-	**22**

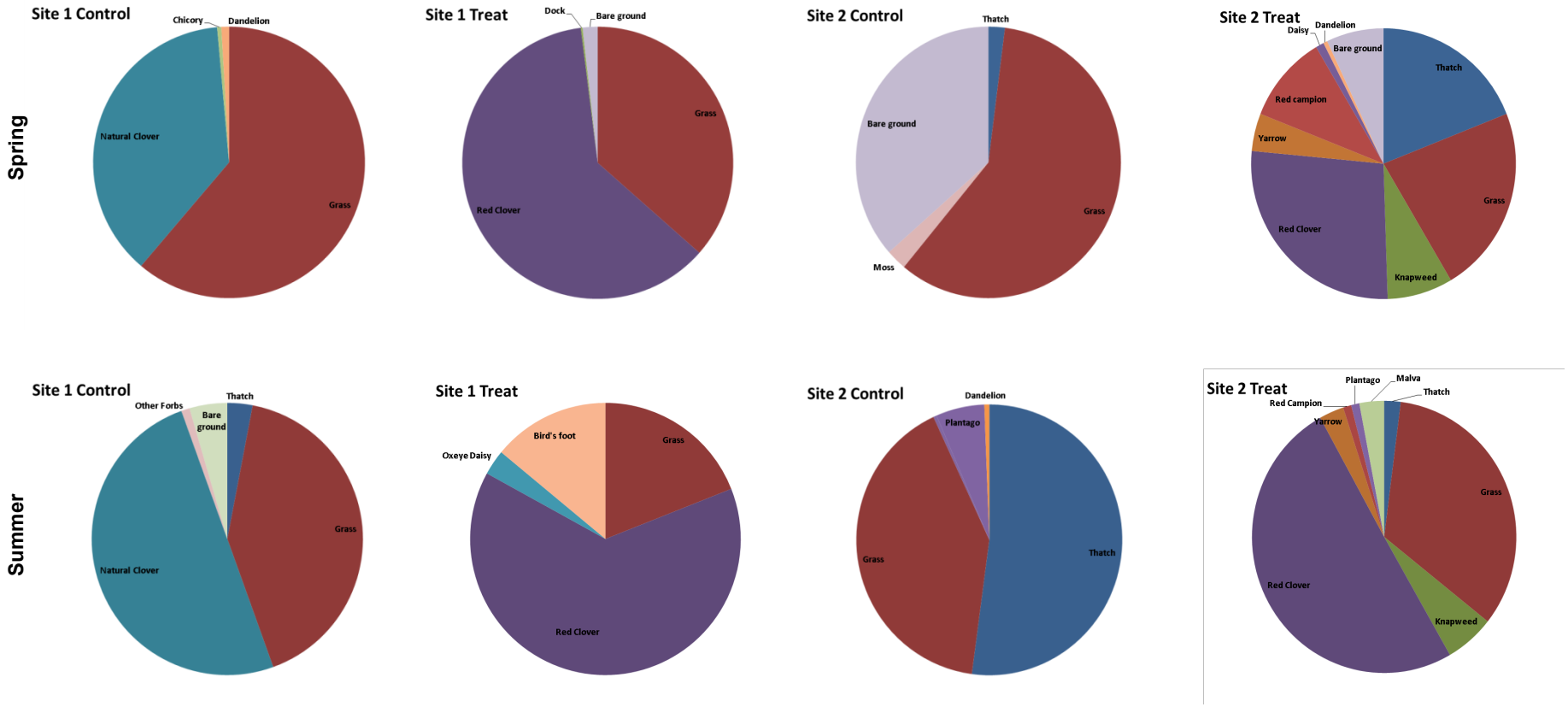


Figure 7.1.10 Vegetation cover in control and treatment intervention plots for Site 1 and Site 2 in the spring (upper charts) and summer (lower charts)

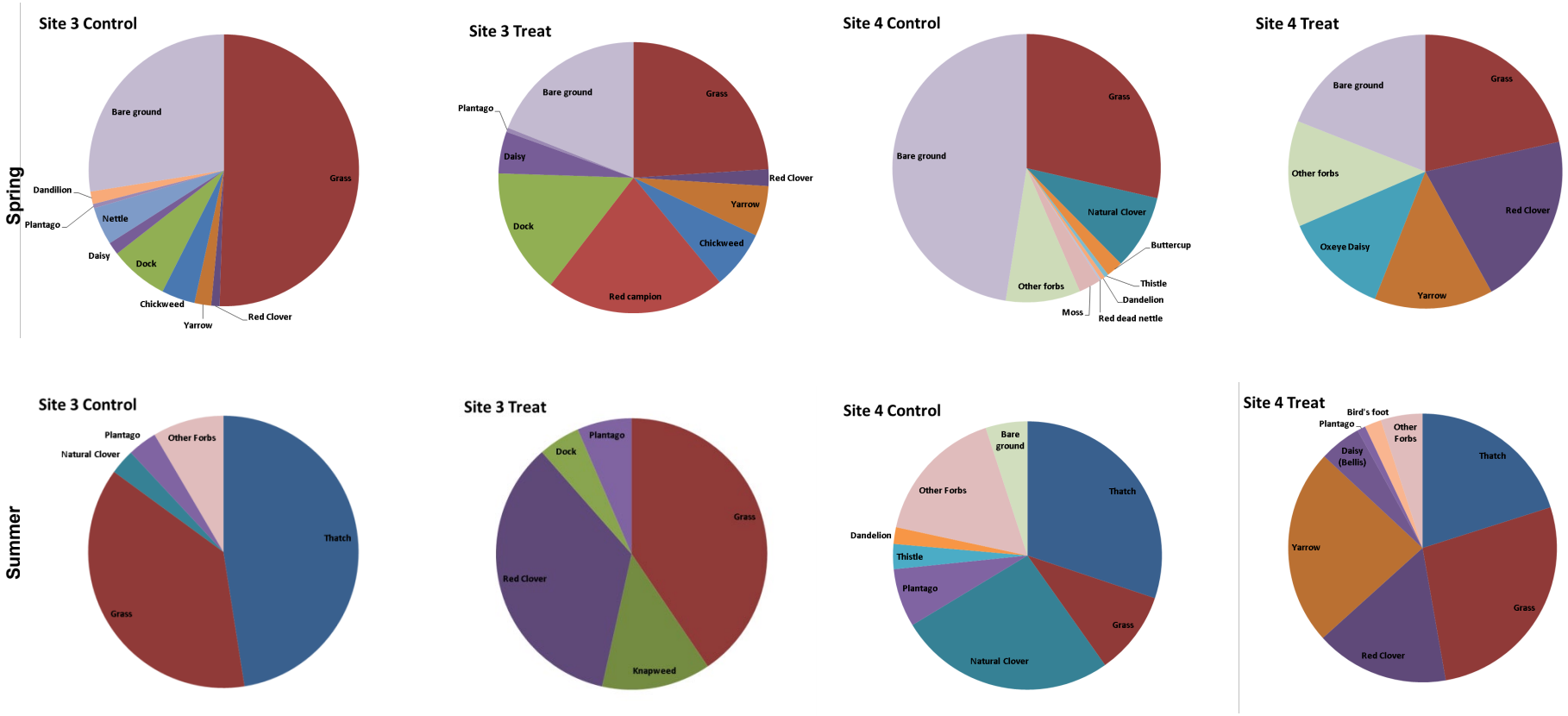


Figure 7.1.11 Vegetation cover in control and treatment intervention plots for Site 3 and Site 4 in the spring (upper charts) and summer (lower charts)

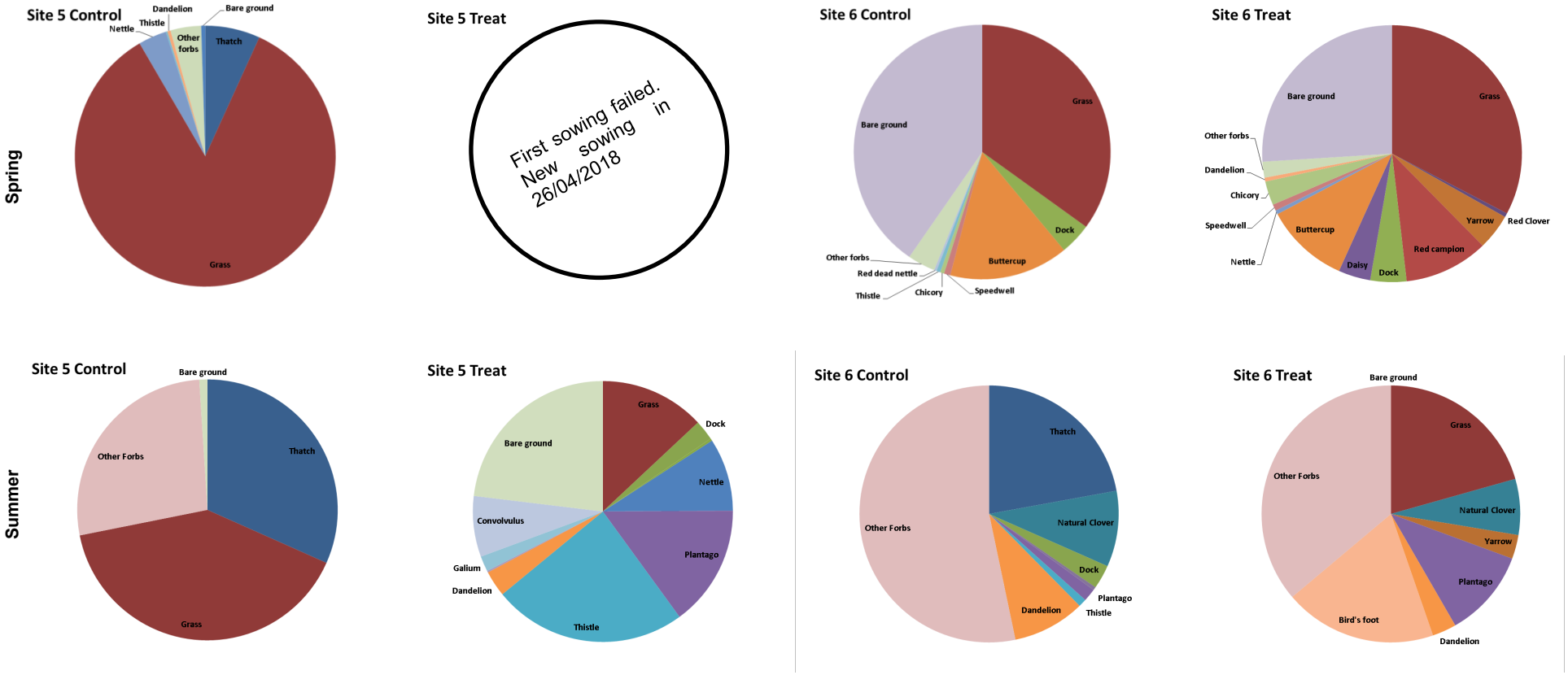


Figure 7.1.12 Vegetation cover in control and treatment intervention plots for Site 5 and Site 6 in the spring (upper charts) and summer (lower charts)

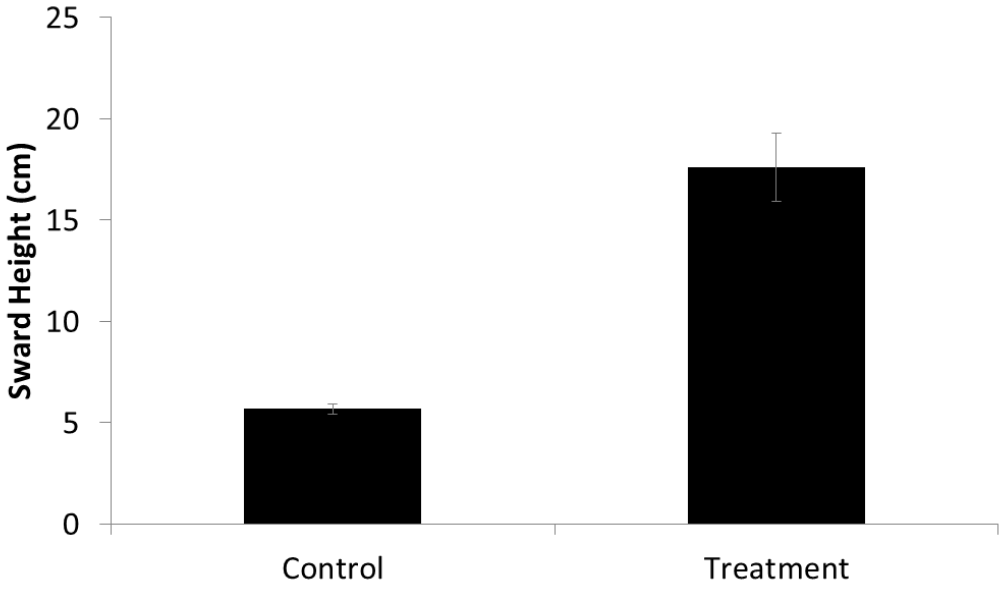


Figure 7.1.13 Sward height (cm) measured using the drop disk method in summer 2018. Mean of 10 measurements per plot

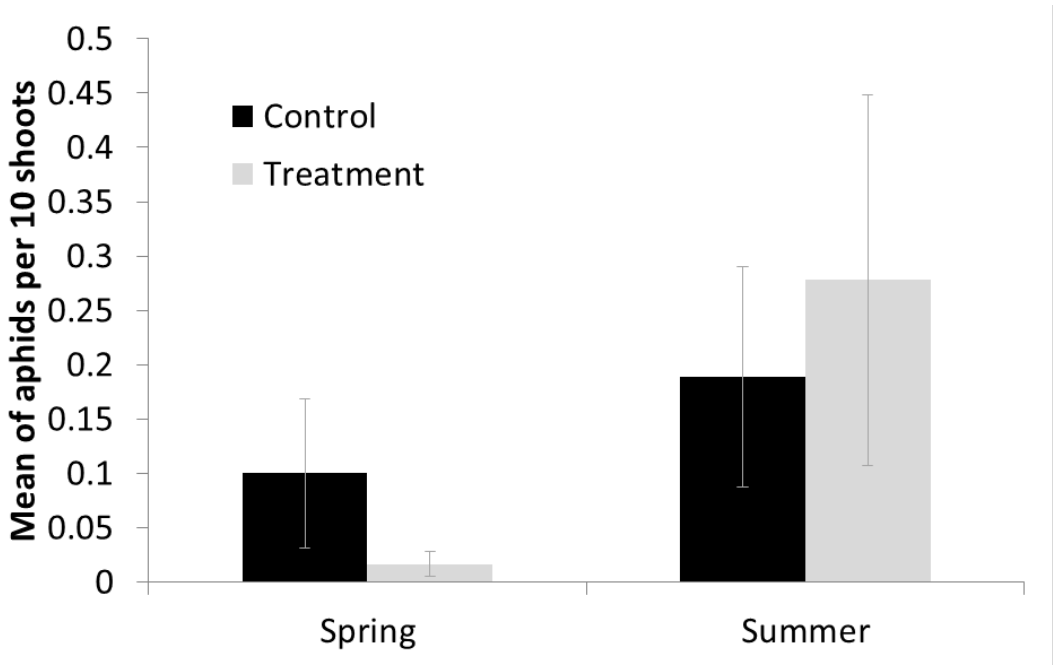


Figure 7.1.14 Mean number of aphids per 10 shoots, per plot, in control and treated areas in the spring and summer

Refuges Assessment

The biorefuges deployed in the treamtent intervention trees in 2017, where the seed mix was sown could not be compared to the controls. The content of refuges were assessed in the spring. Spiders (91.75%±4.68) and earwigs (8.25%±4.68) were the main arthropods found in the refuges. There were significantly more spiders than earwigs ($p<0.001$). A dry spring and summer in 2018 may have impacted the number of earwigs recorded. Even in Site 1 where earwigs were known to be present on trees with yellow ties the numbers observed were low (0.2 earwigs per refuge).

Predator Monitoring

Spiders were the most common arthropod found in all seasons and a more diverse set of arthropod predators were recorded in the summer (Fig. 7.1.15).

There was no significant increase in spiders ($p=0.719$) or ladybirds ($p=0.148$) in the treated plots, but lacewings ($p=0.047$) numbers were higher in the apple trees in the treated plots in the summer. A similar response has be observed in a NIAB EMR PhD where coriander was sown among strawberry plants (Hodgekiss et al. in press). In the autumn, spiders ($p=0.080$) and parasitoids ($p=0.165$) were common but not statistically different between treatments (Fig. 7.1.15).

A nocturnal assessment of earwigs and other predators was done in summer. There was no significant increase in earwig numbers in the treated plots ($p=0.088$).

Apple Leaf Curling Midge

Damage from apple leaf curling midge was evaluated in summer and was observed on two control sites from the six sites surveyed. We recorded a mean of 0.094 of damaged shoots per 10 shoots. In comparison no damage was found on shoots surveyed in the treatment plots. The difference between control and treatment intervention was significant ($p<0.001$).

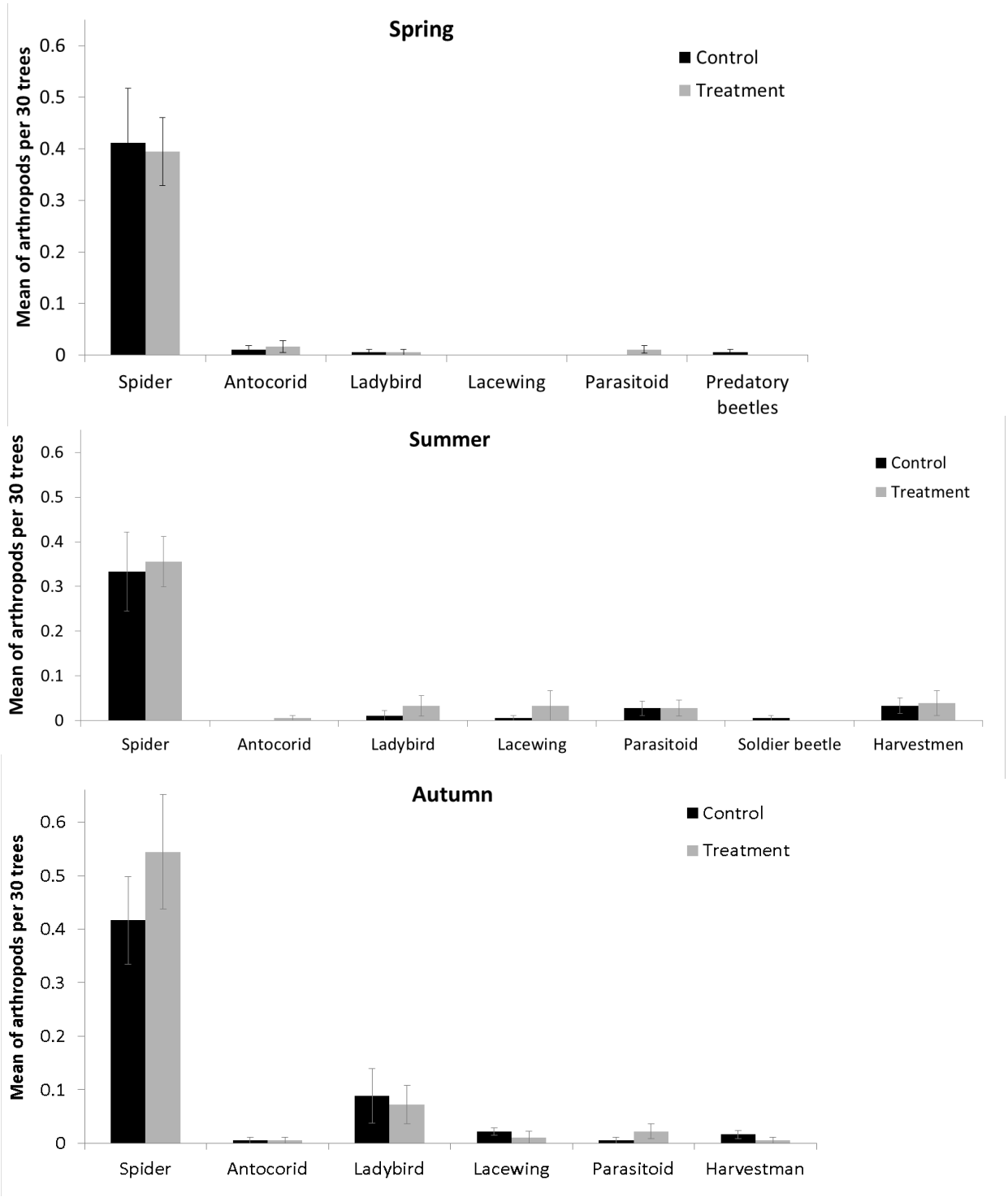


Figure 7.1.15 Mean numbers of arthropods recorded by tap sampling 30 trees on each control and treatment intervention plot in the spring, summer and autumn

Mites

Significantly more rust mites (p<0.001) were observed on treatment intervention plots than on control plots (Fig. 7.1.16a).

Three other taxa were recorded: predatory mites, fruit tree red spider mite (*Panonychus ulmi*) and other spider mites (Fig. 7.1.16b).

Predatory mites (p=0.004) and fruit tree red spider mite were fewer (p<0.001) in the treated plots compared to the control. However, fruit tree red spider mite was only found on the control and treated plots at Site 4.

Other spider mites were more numerous on the treatment plot compared to the control plot but only on Site 4.

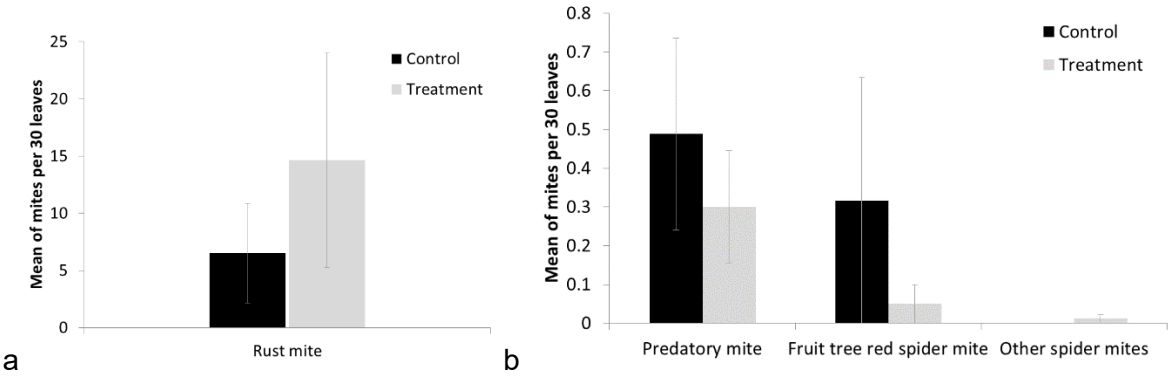


Figure 7.1.16 a) Mean numbers of rust mites per 30 leaves in the treatment intervention and control plots and **b)** Mean numbers of predatory mites, fruit tree red spider and other spider mites recorded per 30 leaves in each treatment and control plots

Codling Moth Damage

Codling moth (CM) stings (superficial sting central to a red region) and deep entry (a deeper damage hole) were recorded (Fig. 7.1.17).

More fruits exhibiting codling moth stings were observed in the control plots compared to the treated plots in the summer and autumn (Fig.7.1.18). No CM deep entry damage was recorded on treated plots in both summer and autumn. Treatment and control were only significantly different for the deep entry damage on tree fruits in the summer ($p<0.001$).

In the autumn there were fewer CM sting damaged dropped apples ($p=0.018$) in the treated compared to the control plots. No CM deep entry damage was found in control plots and a very small number of fruits (0.01 fruits per tree) from one treated plot exhibited this damage (Fig. 7.1.19).

Figure 7.1.17 Transversal cut of an apple with codling moth deep entry damage and larva

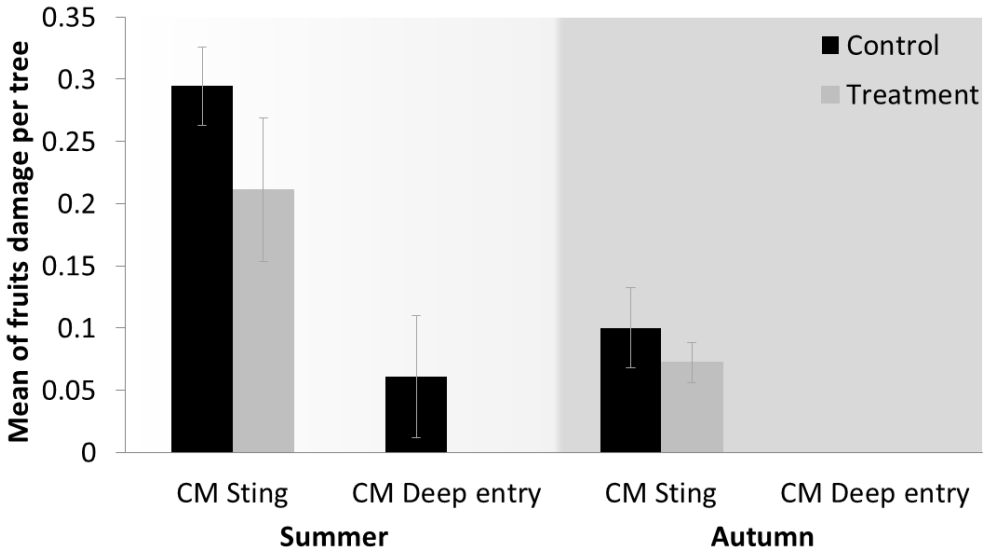


Figure 7.1.18 Mean numbers of fruits per tree with codling moth sting and deep entry damage in control and treated plots in the summer (left) and autumn (right)

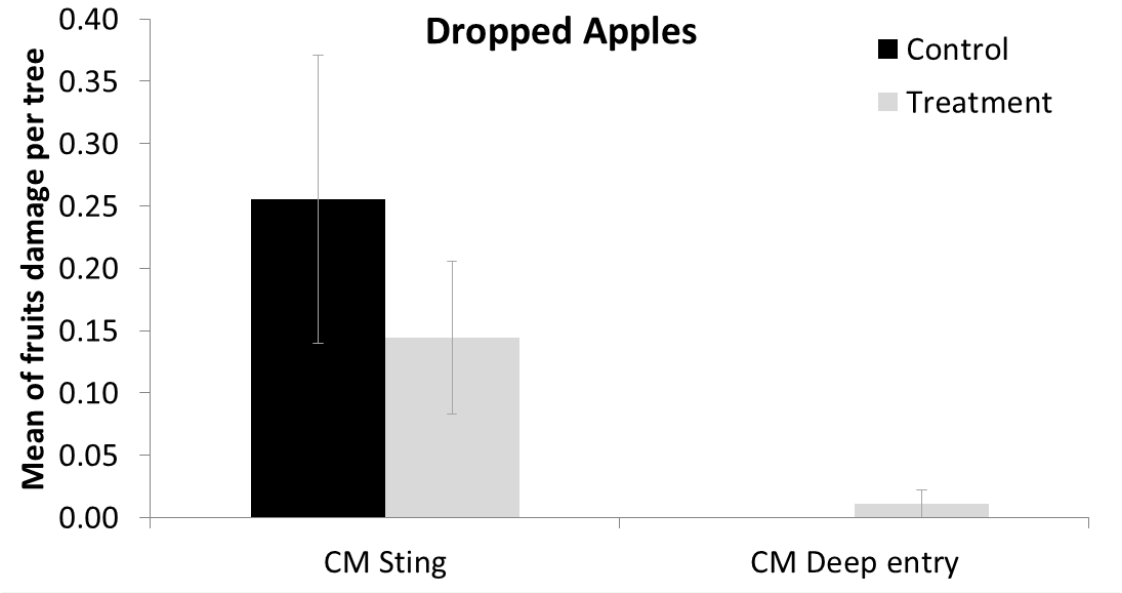


Figure 7.1.19 Mean numbers of dropped fruits per tree exhibiting codling moth sting and deep entry on control and treatment plots in the autumn

Damage from capsid, tortix, rosy apple aphid, winter moth and Rynchites was also observed during the fruit damage assessment (Fig. 7.1.20). Rosy apple aphid and Rynchites damage was only recorded in the summer. However the numbers of fruits with rosy apple aphid

damage was very low (0.01 fruits per tree) and only recorded on one treated plot. There was no difference between tortix damage found in control plots compared to treated plots. Winter moth damage was similar in control and treated areas in summer and autumn. Very little was found in the control (0.006 fruits per tree). No difference was recorded for capsid damage between the control and treated plots in the summer and autumn.

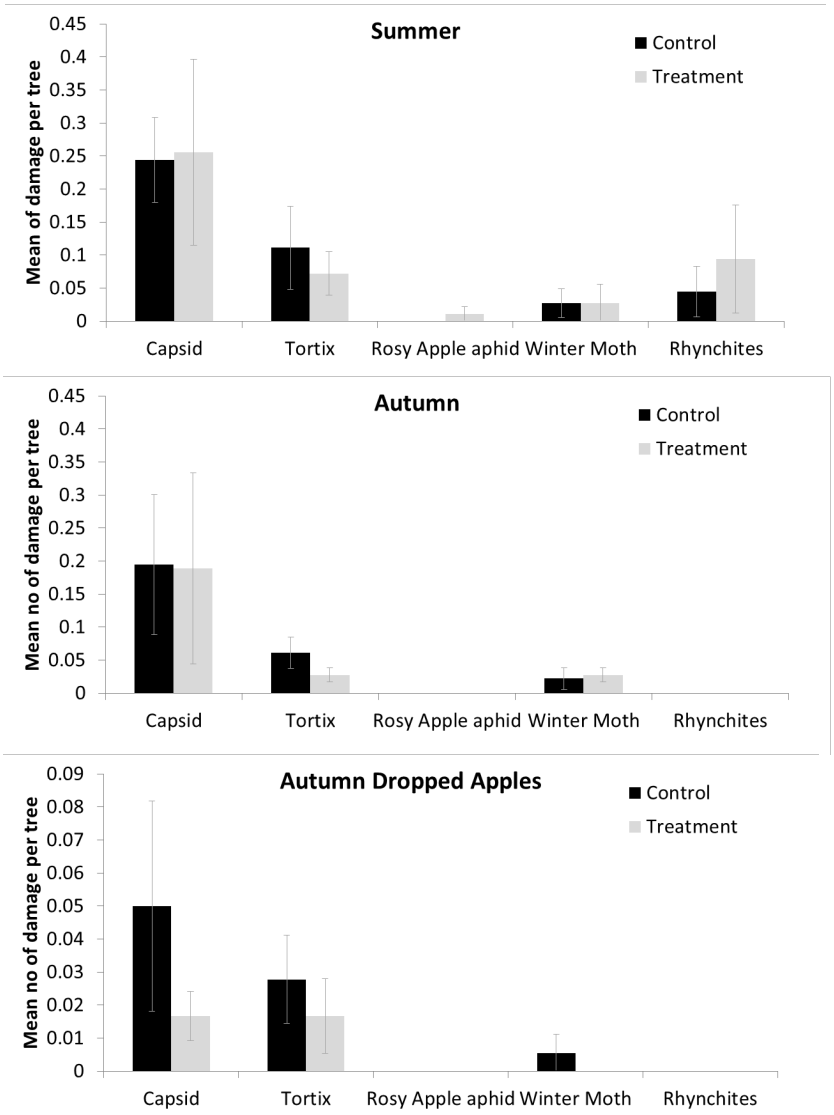


Figure 7.1.20 Mean numbers of fruits per tree with damage from capsid, tortix, rosy apple aphid, winter moth and Rhynchites in control and treated plots in summer and autumn. *Note that the Autumn Dropped Apples is on a smaller axis

Other predators, including spiders, ladybirds, harvestman, parasitoids and hoverflies were also recorded (Fig. 7.1.21). More spiders (p=0.012) were present in apple trees in control

plots compared to treated plots. Numbers of harvestman and hoverfly larvae were similar on control and treatment plots.

Hoverfly Assessment

Significantly more hoverfly adults were recorded on white sticky traps in the treatment plots compared to control plots ($p<0.001$, Fig. 7.1.22).

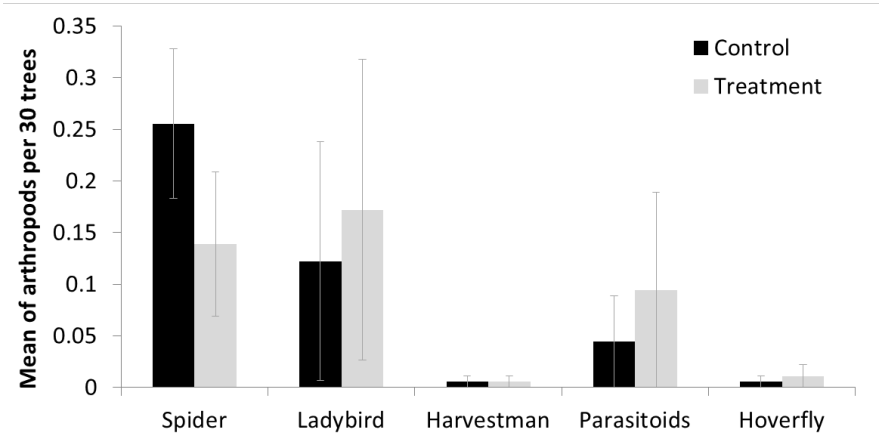


Figure 7.1.21 Mean numbers of predators recorded from tap sampling in control and treatment plots during the nocturnal survey in summer

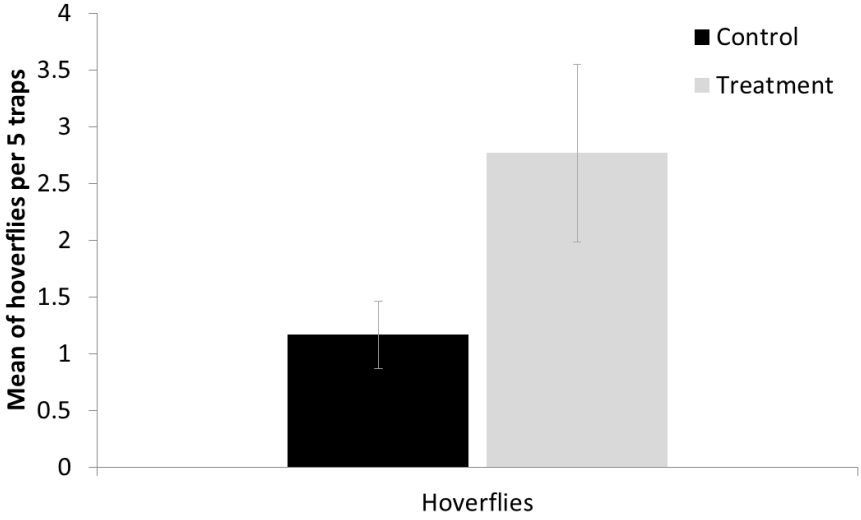


Fig. 7.1.22 Mean numbers of hoverfly adults recorded in treated and control plots in autumn on white sticky traps

Table 7.1.5 Summary table of vegetation, pest and natural enemy measures made at three timepoints in 2018 (not all measures made on all occasions). Arrows show a beneficial (green arrow) or detrimental (red arrow) effect of the ecological enhancement intervention one year after establishment in six newly planted orchards. Only data with statistical differences are reported (except vegetation diversity which will be analysed in 2019). b at one site only.

	Spring	Summer	Autumn
Vegetation diversity	↑	↑	-
Sward height	-	↑	-
PESTS			
Aphids	↓	-	-
Capsid damage	-	-	-
Rosy apple aphid damage	-	-	-
Apple leaf curling midge damage	-	↓	-
Rhynchites damage	-	-	-
Rust mite	-	↑b	-
Fruit tree red spider mite	-	↓	-
Tortrix damage	-	-	-
Codling moth deep entry fruit damage	-	↓	-
Codling damage in dropped apples	-	-	↓
BENEFICIALS			
Adult hoverflies	-	-	↑
Lacewings	-	↑	-
Spiders	-	-	↓
Earwigs	-	-	-
Ladybirds	-	-	-
Predatory mites	-	↓	-

Conclusions

- Seed mix application to treatment plots was successful in most orchards and caused evident changes in vegetation diversity, evenness and structure on each site
- Not all species in the seed mix established. Red clover and yarrow were the most common and found at a higher percentage of ground cover
- Sward height on treatment plots was significantly higher than in the un-sown alleyways
- Fewer aphids were observed on treated plots in spring but not in summer
- Significantly more spiders than earwigs were found in the refuges deployed on the treatment plots
- Spiders were the most common arthropod in all seasons
- No apple leaf curling midge damage was occurred in the treatment plots compared to the control plots, where a mean of 0.094 shoots damaged per 10 shoots was recorded
- Fewer predatory mites and fruit tree red spider mites were found on treated plots than in control plots. However the opposite was observed for rust mites and spider mites
- Fewer fruits with CM damage occurred in the treatment plots than control plots, including significantly fewer CM stings and in the dropped apple assessment
- The use of attractant sachets significantly increased hoverfly adults in the treated plots or pulled hoverflies from the control plots
- Statistic values on this study have to be interpreted with caution since numbers of arthropods were low

Future work

Continue with pest, beneficial and vegetation surveys in all three seasons depending on phenology of organism.

Identify spiders to family and species where possible to discriminate between different functional groups (predation strategies).

7.2 Dynamic pear sucker/predator chart for growers

Pear sucker, *Cacopsylla pyri*, is still the major pest on pear with sporadic population growth in relation to warm dry weather and in orchards where the numbers of earwigs and anthocorids is not sustained. Emerging evidence from other AHDB and Innovate UK projects is showing that earwigs are important control agents for aphids and pear sucker. Additional research in the US also demonstrates predation of codling moth eggs. Earwigs, hoverfly larvae, lacewing larvae, spiders and ladybirds are able to penetrate the leaf rolls (galls) caused by the various apple aphid species.

There are large differences, between orchards, in earwig populations and Project TF 196 has demonstrated that pesticide use and timing may be, at least partly, responsible. However, anecdotal evidence is showing that earwigs can be patchily distributed within an individual orchard.

The aim of this study was to enable more effective monitoring, pesticide use and natural enemy build-up in pear orchards. It is expected that the insecticide interventions will be better timed and applied.

Six farms were involved in the study in 2016, 2017 and 2018. All participants were trained in the monitoring technique at the start of the growing season. Each grower selected 3 orchards (high, medium and low pear sucker infested) on each farm and allowed time for a worker to systematically assess the chosen orchards each week. The results were collated at least fortnightly by NIAB EMR and then shared with all participants.

Records of pear sucker eggs, nymphs and adults, and ladybirds, earwigs and anthocorids in the perceived low, medium and high pear sucker pressure orchards were made from March to September.

Aim

Enable more effective monitoring, pesticide use and natural enemy build-up in pear orchards. It is expected that the insecticide interventions will be better timed and applied.

Materials and Methods

Each grower (Table 7.2.1) selected three orchards (high, medium and low pear sucker infested) on each farm and allowed time for a worker to systematically assess the chosen orchards each week. Farms and orchards in the results section have been anonymised.

NIAB EMR devised a sampling method and record sheet which the persons responsible for reporting returned to NIAB EMR via email each week (Figure 7.2.1). The results were collated at least fortnightly and then shared with all participants.

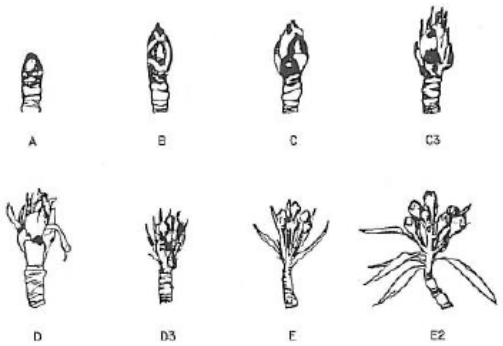
Table 7.2.1 Growers involved in pear sucker and predator monitoring and data collection.

Name	Farm/Company	Person responsible for reporting
David Butler & William	GH DEAN	David Butler
Darren Wallis	AC GOATHAM	Darren Wallis
Nigel Jenner	AVALON	Ryan Williams
Russel Graydon	A SCRIPPS	Pam and Carol
Mark Chapman	AC HULME & SONS	Mark Chapman
Caroline & David Long & Tim Long	CHILD'S FARM	Elena/Katalina
John Clark & Richard	FAST	-

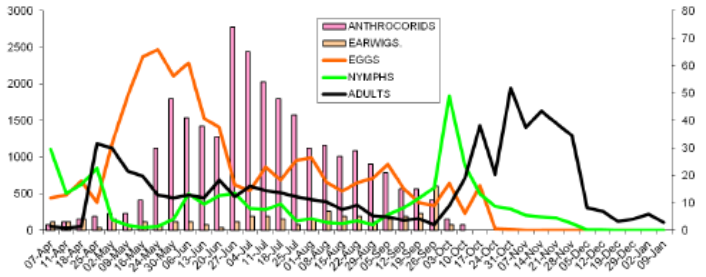
The orchards were coded 'farm_orchard'. Only one changed from 2016 to 2017 (H-H to H_G).

NIAB EMR (adapted from Standard Operating Procedure – GEP 729)	
Title : Assessment methods for pear sucker (*Cacopsylla pyricola* Förster)	
Author(s) : Michelle Fountain	Authorised by:
Date of Issue : 15 Feb 2016	Version No. : 1

- Assessments should begin at 'mouse ear' stage (growth stage D EPPO crop Growth stage keys No:2) (Cross & Berrie 2003)



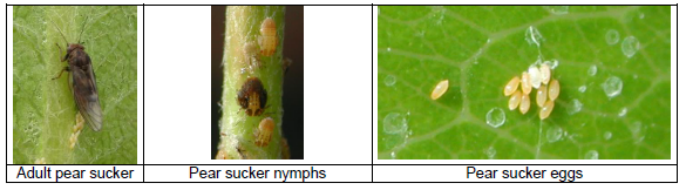
- Assess on the same day each week (e.g. Monday morning)
- Three orchards assessed (low, medium and high pear sucker pressure)
- 30 trees checked in each orchard in a W-shape
- Data should be entered onto an excel spreadsheet supplied by NIAB EMR and emailed weekly to michelle.fountain@emr.ac.uk



Example of data output

Monitoring pear sucker

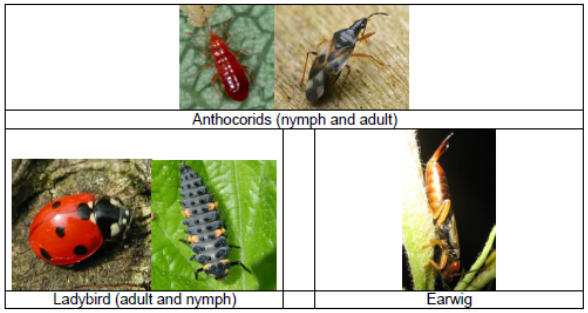
- Choose a tree and randomly walk to one branch
- Without disturbing the branch count the number of pear sucker adults on the whole branch (~30 cm)
- Then the number of eggs and nymphs (a hand lens is useful) – you may have to estimate if numbers are high
- In addition the degree of honeydew contamination on each sample should be scored on a 0-3 scale; 0 = none, 1 = slight, 2 = moderate, 3 = severe



Adult pear sucker	Pear sucker nymphs	Pear sucker eggs

Monitoring predators

- On the same trees tap the branch 3 times over a white tray and record numbers of earwigs, anthocorids and ladybirds.



Anthocorids (nymph and adult)		
Ladybird (adult and nymph)		Earwig

Figure 7.2.1 Standard Operating Proceedure for monitirng pear sucker, key natural enemies and damage in pear orchards

Results

Records of pear sucker eggs, nymphs and adults, and ladybirds, earwigs and anthocorids in the perceived low, medium and high pear sucker pressure orchards were made by most growers from March to September. Small numbers of pear sucker eggs were present from the beginning of monitoring in March 2016, 2017 and 2018. The first generation peaks were at the end of May to beginning of June in 2016, mid-May in 2017 and mid-May to beginning of June in 2018.

Most orchards did not have significant numbers of pear sucker eggs or nymphs. In 2018, pear sucker populations (eggs, nymphs and adults) had similar numbers compared to 2017 in most orchards. However, some orchards exhibited changes overtime.

Farm 1 never peaked above 1000 eggs and early season predators and later season natural enemy numbers appeared to control pear sucker in all years. In 2016, in some weeks, over 80 earwigs per 30 branches were present in orchard C_C. In 2017, the only insecticides applied were one spray of Runner early season and 3 sprays of Carpovirusine. Also in 2017 and 2018, over 100 earwigs per 30 branches were recorded in orchard C_G (Fig. 7.2.2). In 2018, Farm 1 management added 2 additional applications of Calypso early season and one Envidor spray in July to their spray programme.

It was noted, in 2017, that a lower peak of pear sucker eggs (<2000 /30 shoots) was an improvement on 2016 (>2000 eggs) at orchard G_M, at Farm 2 (Fig. 7.2.3). Earwig numbers and ladybird numbers were also lower in 2017 compared to 2016 and the spray programme differed very little, so the reason for this is unknown. It appears that an Envidor was not applied in 2017. Again in 2018, G_M orchard recorded an even lower peak of pear sucker eggs than previous years. The spray programme for G_M was reduced from three insecticides sprays in 2017 to one in 2018, although more sulphur applications were applied.

At Farm 4 (Fig. 7.2.4) earwig numbers were lower in 2017 compared to 2016. However in 2018 earwig numbers increased, especially in orchard H_G, where over 200 earwigs per 30 branches were recorded. Pear sucker eggs and nymphs were much lower in 2018 than previous years probably due to the presence of predators. 2018 spray records were similar to 2017.

Figure 7.2.6 shows the importance of monitoring beyond harvest as this is a time when there can be resurgence in egg laying and nymph hatch subsequently damaging overwintering bud. Farm 5 reported a late season pear sucker problem in 2016 and it was observed that the numbers of earwigs were generally low (< 3 each week). Also in 2017 and 2018, the

September monitoring recorded an increased in eggs and nymphs. Earwig numbers remained low in 2018 (< 6 each week). At this farm multiple applications of sulphur were applied in all years. The effect of these sprays on earwigs is not known.

In 2018 higher numbers of eggs and nymphs were found at Farm 6 compared to 2017 (Fig. 7.2.7). Also higher numbers of anthocorids were recorded on all three orchards at Farm 6. As anthocorid numbers peaked, pear sucker eggs and nymphs numbers declined probably because of predation.

In general, where there were at least 10 anthocorids in 30 tap samples each week, there is good control of pear sucker. A good example of this is Fig. 7.2.5 where in orchard H_S in 2017 there is a gap in predators and rise in the pear sucker egg numbers – although these levels are not considered detrimental.

In 2016 positive correlations existed between guilds of pear sucker averaged over the entire season (Table 7.2.2), hence where there were more adults, there were more eggs and nymphs. There was a significant positive correlation between earwigs and anthocorids (Table 7.2.2). Hence more earwigs were found where there were more anthocorids. This could be a consequence of crop management being more sympathetic to natural enemies on some sites. There was no correlation between mean seasonal numbers of earwigs or anthocorids and pear sucker guilds. Ladybirds were positively correlated with all pear sucker eggs and nymphs and may have been attracted to these guilds as a food source.

Some correlations obtained in 2016 remained similar in 2018. Positive correlations between the mean seasonal numbers of pear sucker adults and pear sucker eggs and nymphs were again obtained (Table 7.2.3). A significant positive correlation was observed between anthocorids and PS eggs and adults. This means that more anthocorids were recorded were more PS eggs or adults were also found. Also, ladybirds were positively correlated with anthocorids and guilds of pear sucker nymphs. The availability of a food source may be the cause for the correlations of pear sucker eggs and nymphs with anthocorids and ladybirds. Unlike 2016, there was no correlation between the mean seasonal numbers of anthocorids and earwigs in 2018.

It should be noted that these data analyses, to date, do not take into consideration the spray programmes or other unrecorded crop management practices for pests and natural enemies.

Table 7.2.2 Correlations and two sided T-test of pear sucker guilds and natural enemies from mean numbers throughout the 2016 season across all farms. PS = pear sucker.

Correlations

Anthocorids	-0.071						
Earwigs	-0.391	0.606					
PS Eggs	0.550	0.283	0.206				
Ladybirds	0.498	0.273	0.268	0.843			
PS Nymphs	0.466	-0.248	-0.112	0.801	0.628		
Total_PS	0.655	0.105	0.042	0.975	0.814	0.887	
	PS Adults	Anthocorids	Earwigs	PS Eggs	Ladybirds	PS Nymphs	Total_PS

Two-sided test of correlations different from zero; P value

Anthocorids	0.803						
Earwigs	0.149	0.017					
PS Eggs	0.034	0.307	0.462				
Ladybirds	0.059	0.326	0.335	<0.001			
PS Nymphs	0.080	0.374	0.692	<0.001	0.012		
Total_PS	0.008	0.709	0.883	<0.001	<0.001	<0.001	
	PS Adults	Anthocorids	Earwigs	PS Eggs	Ladybirds	PS Nymphs	Total_PS

Table 7.2.3 Correlations and two sided T-test of pear sucker guilds and natural enemies from mean numbers throughout the 2018 season across all farms. PS = pear sucker.

Correlations

PS Nymphs	0.4624						
PS Adults	0.5951	0.8119					
Anthocorids	0.2795	0.9571	0.7711				
Earwigs	0.1161	-0.1202	-0.1515	-0.0851			
Ladybirds	-0.243	0.4145	0.1187	0.4858	-0.1626		
Total_PS	0.9146	0.775	0.8251	0.6321	0.0135	-0.0002	
	PS Eggs	PS Nymphs	PS Adults	Anthocorids	Earwigs	Ladybirds	Total_PS

Two-sided test of correlations different from zero; P value

PS Nymphs	0.0826						
PS Adults	0.0193	<0.001					
Anthocorids	0.313	<0.001	<0.001				
Earwigs	0.6804	0.6695	0.5899	0.763			
Ladybirds	0.3829	0.1245	0.6734	0.0664	0.5625		
Total_PS	<0.001	<0.001	<0.001	0.0115	0.9618	0.9994	
	PS Eggs	PS Nymphs	PS Adults	Anthocorids	Earwigs	Ladybirds	Total_PS

The following section gives the phenology of the pest and natural enemies in each orchard. Below each set of charts is a summary table of the sprays targeted against honeydew and insecticides applied.

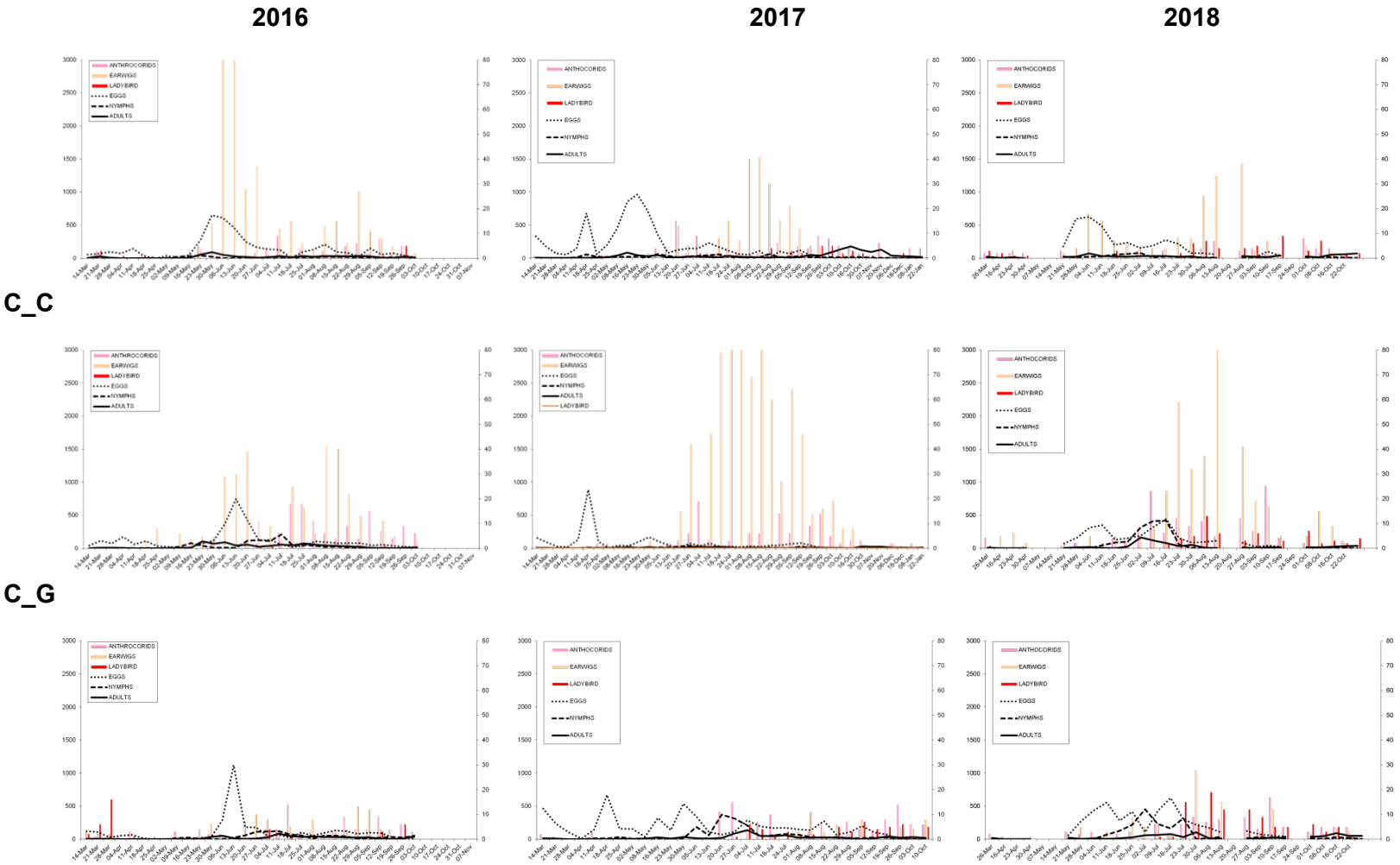


Figure 7.2.2 Farm 1 pear sucker and natural enemy monitoring in three orchards over three consecutive years during the growing season. Lines are on primary axis (eggs, nymphs and adults) and bars are on secondary axis (Anthocorids, earwigs and ladybirds)

Table 7.2.3 Farm 1 spray programme targeted against honeydew and insecticide applications that directly affect Anthocorids or Earwigs

Year	**Purpose of spray / Effect on pear sucker predators**	**Number of spray applications**
2016	Honeydew	4
	Harmful	1-2
	Harmless	1
2017	Honeydew	5-8
	Slightly harmful	0-1
	Harmless	3
2018	Honeydew	2
	Harmful	6
	Harmless	1

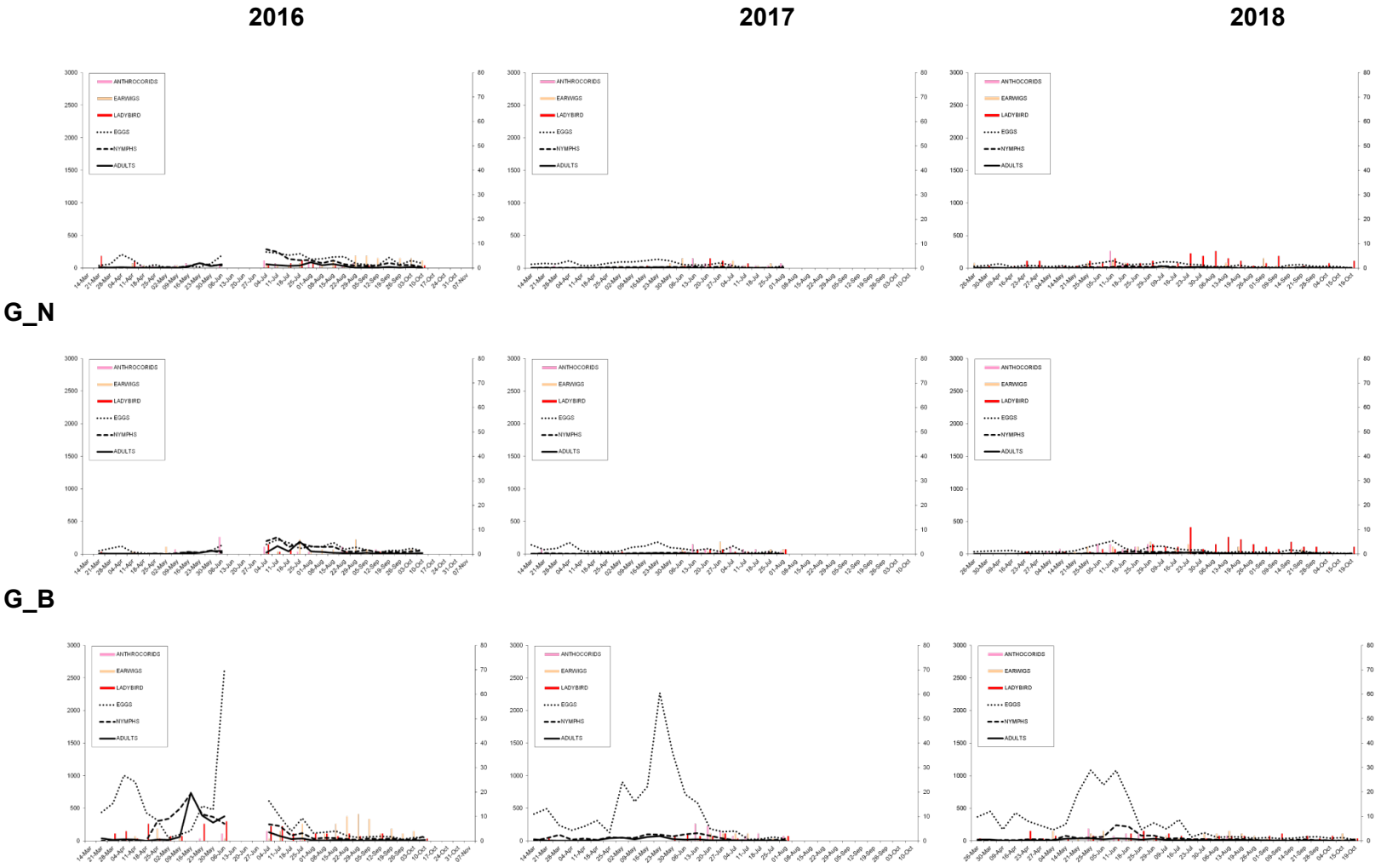


Figure 7.2.3 Farm 2 pear sucker and natural enemy monitoring in 3 orchards over 3 consecutive years during the growing season. Lines are on primary axis (eggs, nymphs and adults) and bars are on secondary axis (Anthocorids, earwigs and ladybirds)

Table 7.2.4 Farm 2 spray programme targeted against honeydew and insecticide applications that directly affect Anthocorids or Earwigs

Year	**Purpose of spray / Effect on pear sucker predators**	**Number of spray applications**
2016	Honeydew	5
	Slightly Harmful	1
	Harmless	2
2017	Honeydew	4-5
	Slightly Harmful	1
	Harmless	1
2018	Honeydew	7-8
	Slightly harmful	1
	Harmless	2

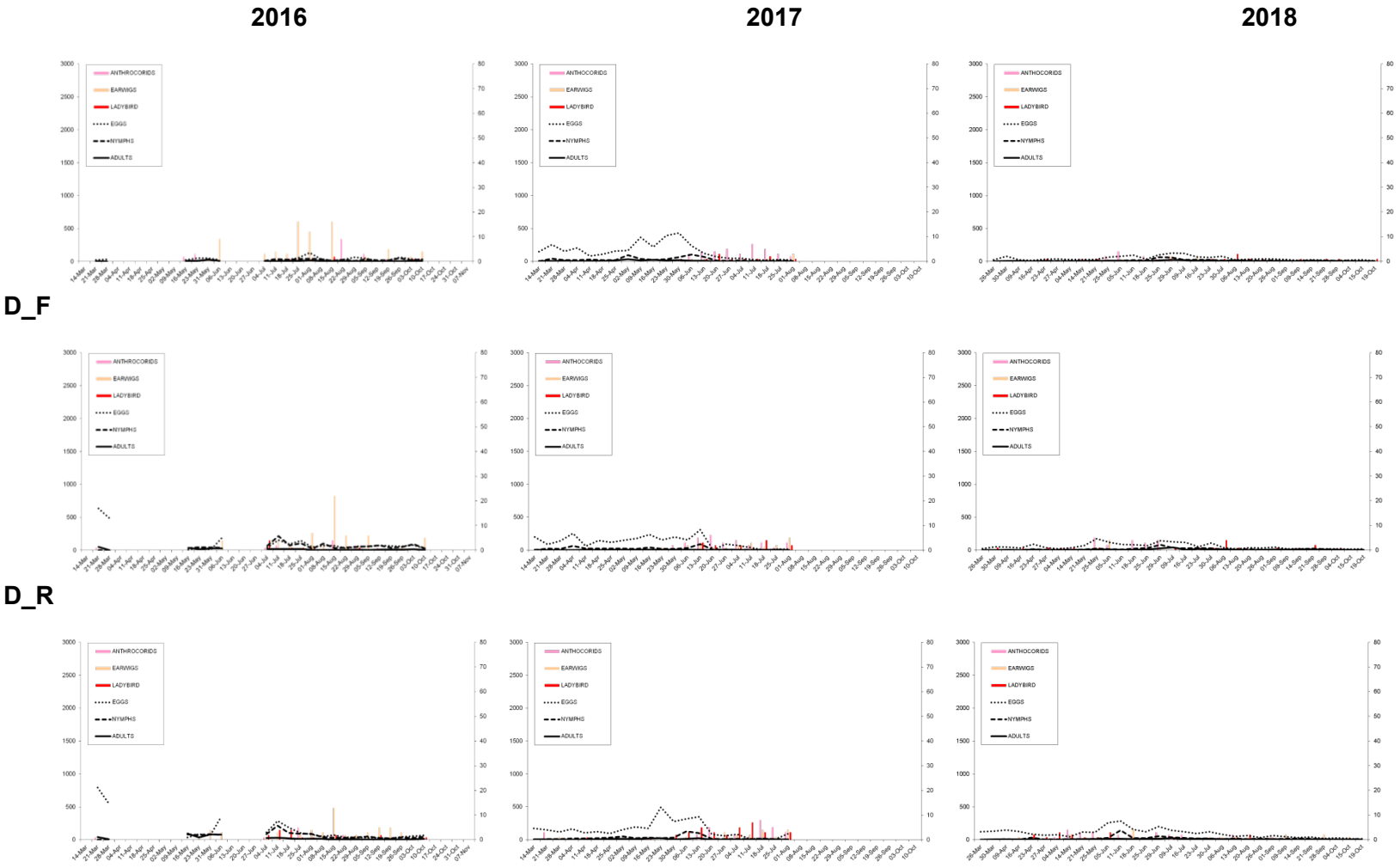


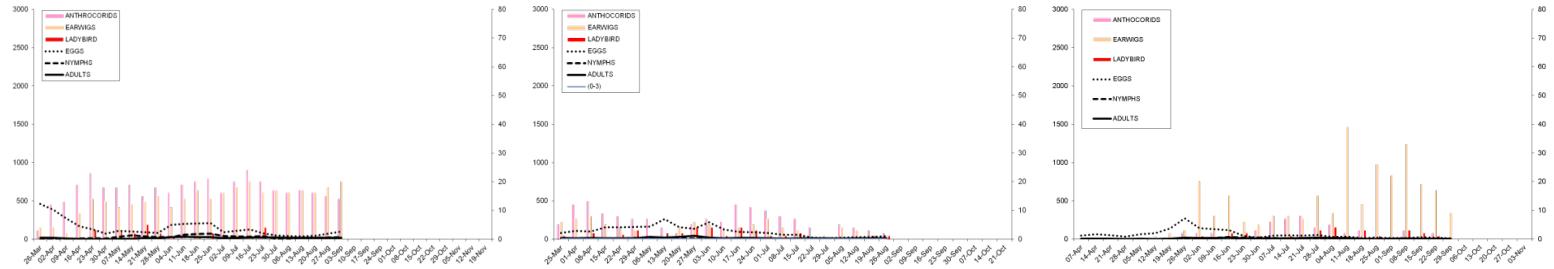
Figure 7.2.4 Farm 3 pear sucker and natural enemy monitoring in three orchards over three consecutive years during the growing season. Lines are on primary axis (eggs, nymphs and adults) and bars are on secondary axis (Anthocorids, earwigs and ladybirds)

Table 7.2.4 Farm 3 spray programme targeted against honeydew and insecticide applications that directly affect Anthocorids or Earwigs

Year	**Purpose of spray / Effect on pear sucker predators**	**Number of spray applications**
2016	Harmful	1
	Slightly Harmful	1
	Harmless	2
2017	Harmful	-
	Slightly Harmful	2
	Harmless	2
2018	Harmful	3
	Slightly harmful	0
	Harmless	3

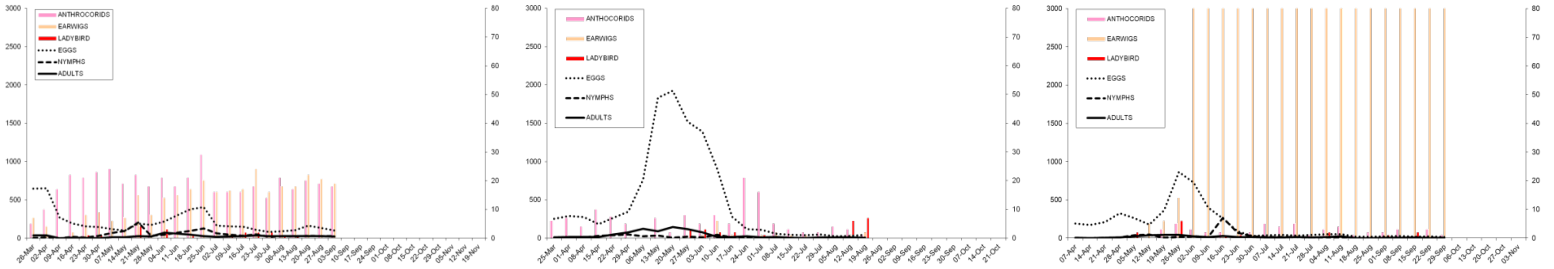
2016 **2017** **2018**

H_W



H_H

Altered to H_G



H_S

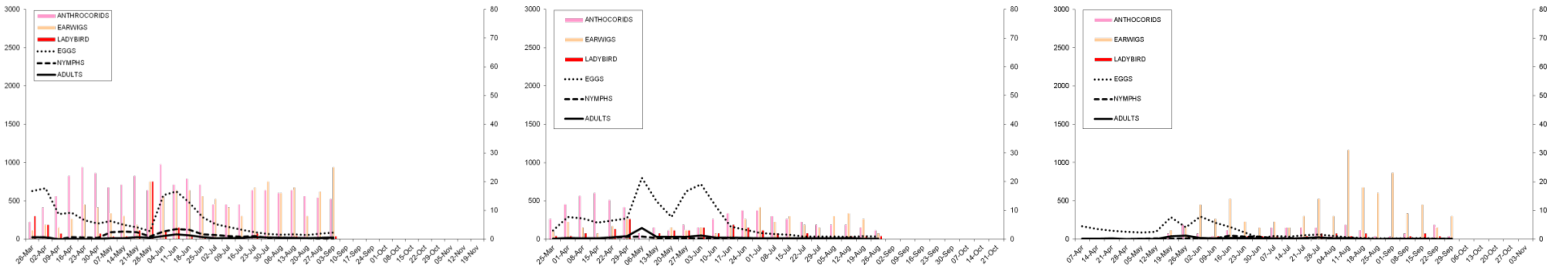


Figure 7.2.5 Farm 4 pear sucker and natural enemy monitoring in three orchards over three consecutive years during the growing season. Lines are on primary axis (eggs, nymphs and adults) and bars are on secondary axis (Anthocorids, earwigs and ladybirds).*change of orchard in year2

Table 7.2.5 Farm 4 spray programme targeted against honeydew and insecticide applications that directly affect Anthocorids or Earwigs

Year	**Purpose of spray / Effect on pear sucker predators**	**Number of spray applications**
2016	Honeydew	7-13
	Slightly Harmful	0-1
	Harmless	0-1
2017	Honeydew	9-14
	Harmful	1
	Slightly Harmful	3-2
	Harmless	1
2018	Honeydew	5-8
	Harmful	1-2
	Harmless	3-5

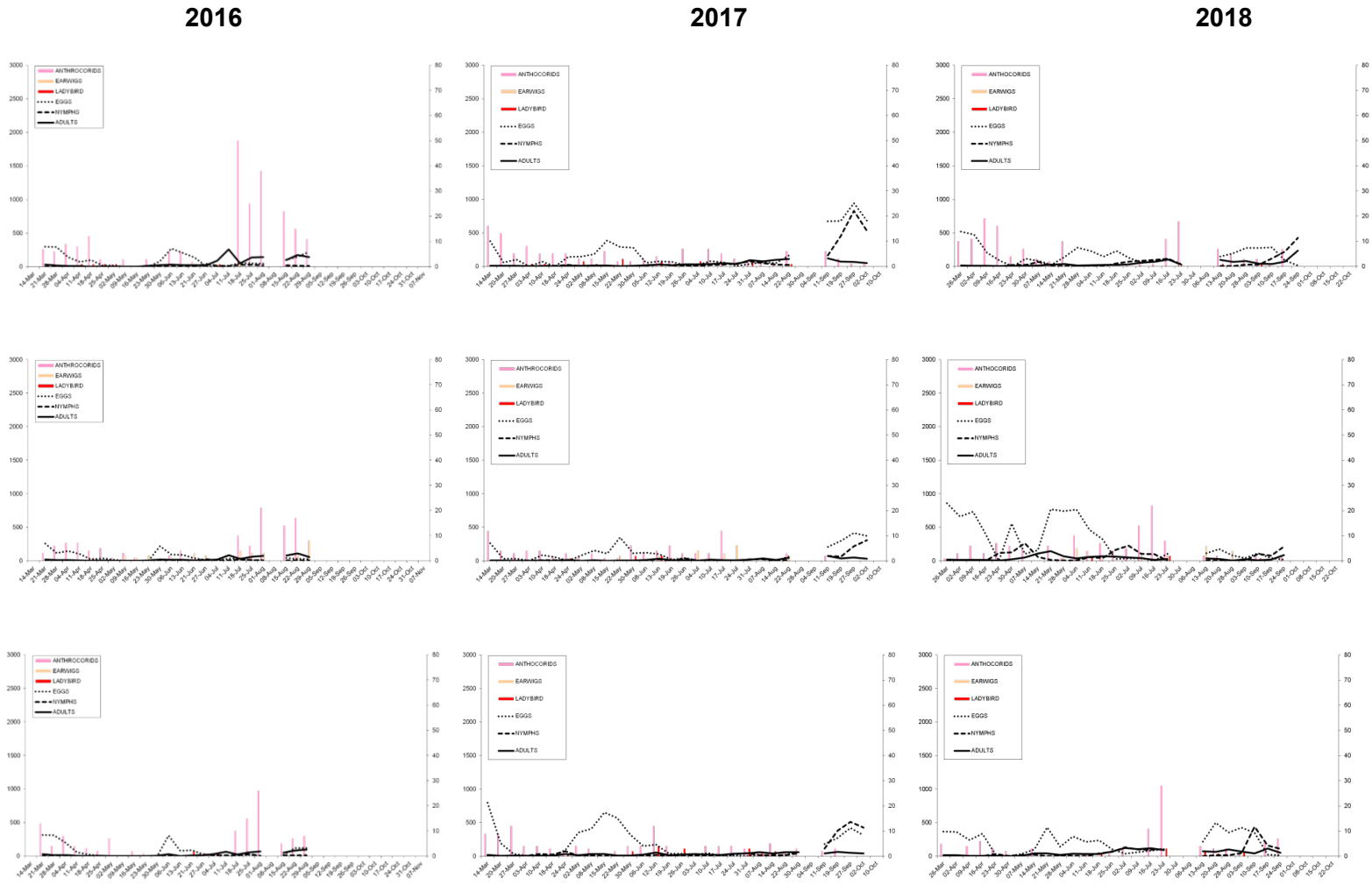


Figure 7.2.6 Farm 5 pear sucker and natural enemy monitoring in three orchards over three consecutive years during the growing season. Lines are on primary axis (eggs, nymphs and adults) and bars are on secondary axis (Anthocorids, earwigs and ladybirds)

Table 7.2.6 Farm 5 spray programme targeted against honeydew and insecticide applications that directly affect Anthocorids or Earwigs

Year	**Purpose of spray / Effect on pear sucker predators**	**Number of spray applications**
2016	Honeydew	24
	Slightly Harmful	2
	Harmless	3
2017	Honeydew	22
	Slightly Harmful	1
	Harmless	1
2018	Honeydew	22
	Slightly Harmful	3
	Harmless	6

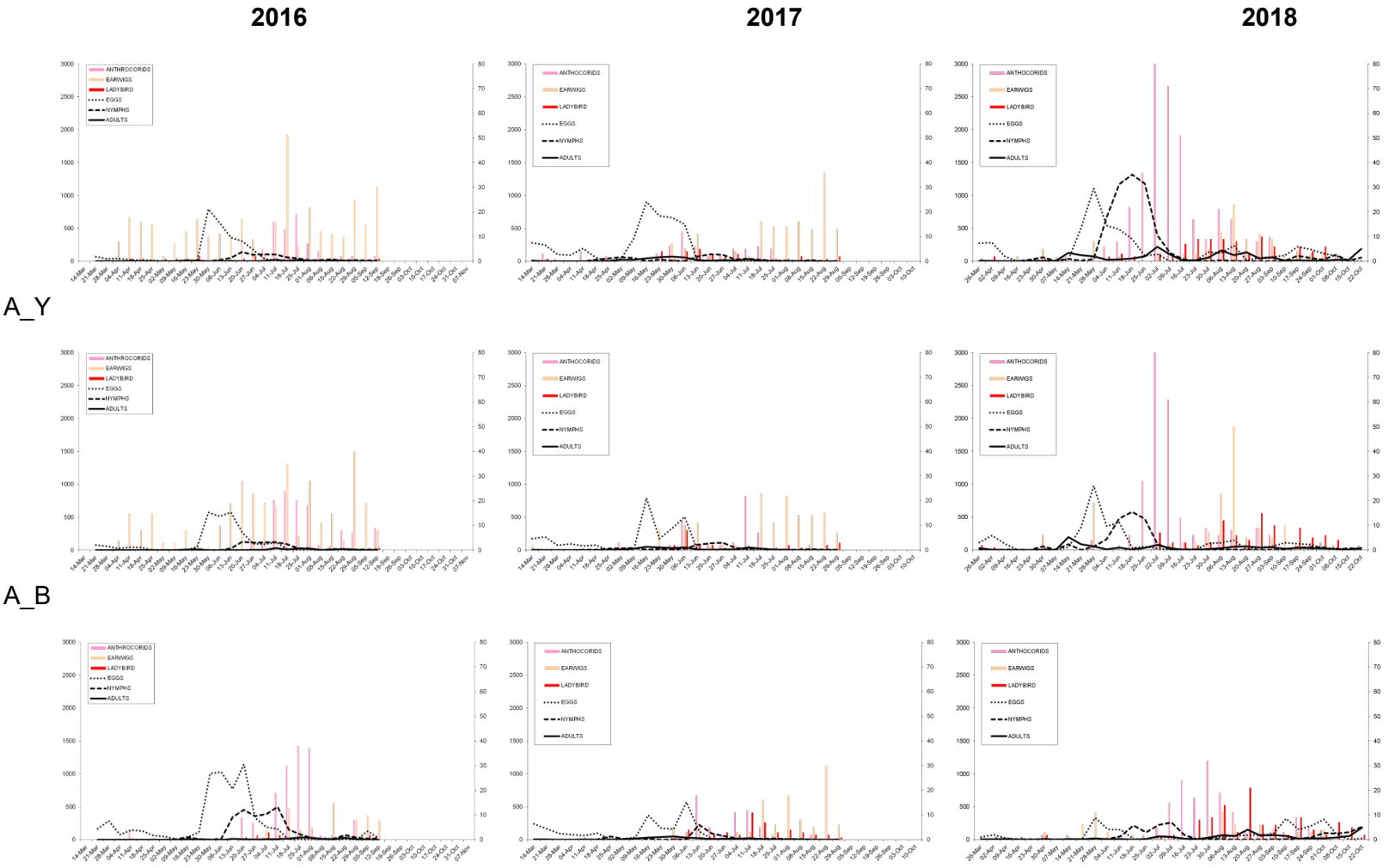


Figure 7.2.7 Farm 6 pear sucker and natural enemy monitoring in three orchards over three consecutive years during the growing season. Lines are on primary axis (eggs, nymphs and adults) and bars are on secondary axis (Anthocorids, earwigs and ladybirds)

Table 7.2.7 Farm 6 sprays targeted against honeydew and insecticide applications that directly affect Anthocorids or Earwigs

Year	Purpose of spray / Effect on pear sucker predators	Number of spray applications
2016	Honeydew	2-17
	Slightly Harmful	1-3
	Harmless	1-2
2017	Honeydew	6
	Slightly Harmful	0-1
2018	Honeydew	6-10
	Harmful	0-1
	Harmless	3-4

Conclusions

- It is important to monitor natural enemies (NE) alongside pear sucker life stages
- Enter into a spreadsheet to achieve an overall picture of when NE are detected and how this relates to the life stages of pear sucker
- Remember earwigs are nocturnal so you may underestimate them early in the spring
- Consider releases of anthocorids early on if NE are low, but think about the surrounding habitat to encourage long term resilience in populations
- Be careful with choice, numbers and timing of spray applications. Think about spray frequency and impact on NE
- Harmful, slightly harmful – think timing. Little is known about tank mixes and how they affect NE. Aim to achieve:

  <1000 pear sucker eggs per 30 shoots per week

  >10 natural enemies per 30 shoots per week

  A mix of natural enemies give resilience

Objective 8 - Rhynchites Weevil and Sawfly

8.2 Sex pheromone of the apple sawfly

Aim

Identify the sex pheromone of the apple sawfly for use in future monitoring and mating disruption studies (EMR/NRI, Yr 3-5).

Introduction

Apple sawfly is a locally common and problem pest, particularly in organic orchards where products for effective control are not available. However, timing of application relies on knowing when the first flight is occurring and when females are laying eggs. The aim of this project is to identify the sex pheromone of the apple sawfly for use in future monitoring and mating disruption studies.

Methods

Apple sawfly larval infected apples were collected in spring 2015 and 2016 from an unsprayed orchard at NIAB EMR. The apples were placed onto compost in mesh covered bins. Larvae were allowed to crawl out from the fruits and enter the compost. As apple sawfly has only one generation per year these were maintained outside until spring 2016 and spring 2017. However, no apple sawfly adults emerged and pupae were found to be infected with either bacteria or fungus, even when, in 2017, bins were maintained with lids to prevent over wetting from rain. The previous winter had been very wet and it was speculated that the soil may have become too wet outside.

In spring 2017 apple sawfly infected apples were collected, again, and kept in Bugdorm cages under cover. As the larvae emerged from the apples and began to ‘wander’ they were transferred into smaller plant pots of compost. Six were kept at ambient conditions in an outside area under cover and 2 were stored at 6°C for 2 months to attempt to simulate a cold period. Again no adults emerged and when the, few recovered, coccons were dissected it was observed that very few had survived (Table 8.2.1), even though two parasitoids had emerged on 12-26 March 2018.

Table 8.2.1 Numbers of pupae found in plant pots of compost initially inoculated with larvae

Date bought in	No. larvae in	Pupae	
10 Feb 18	20	2	One empty one dead adult
10 Feb 18	20	1	Empty cocoon
12 Mar 18	20	1	1 dead adult
12 Mar 18	7	0	
26 Mar 18	20	4	1 dead, others empty
Lab	20	0	
Lab	20	2	1 empty 1 dead adult
26 Mar 18	20	0	

The reason for this lack of successful emergence is still not clear, but could be related to entompathogens due to soil conditions. Hence in 2018 further collections were made (05-14 Jun), but this time larvae were allowed to burrow into different types of substrate in 30 cm tall pots (Fig. 8.2.1). Substrates included different blends of compost, coir, perlite, loam.

All pots were moved into a 6ºC refrigeration unit in September to overwinter. The first set of pots were removed on 2 Jan 2019 into ambient temperature.

Figure 8.2.1 a) collected fruitlets, b) larvae emerging from fruitlets and ‘wandering’, c) larvae burrowing down into d) potted substrates

Objective 9 - Pear Blossom Weevil (*Anthonomus spilotus*)

9.1 Further investigation into the lifecycle and the impact of *Anthonomus spilotus* in UK pear orchards

Introduction

Incidence and damage caused by a weevil pest of pear was first reported to NIAB EMR in 2015. Subsequent reports were made to the entomology department at NIAB EMR as the weevil became more widespread across the South East. The weevil was initially thought to be the pear bud weevil (*Anthonomus piri*, Gyllenhal) (Figure 9.1.1c), an uncommon species that is known to cause damage to pear. Investigation into the lifecycle in 2016 found that the weevil was laying its eggs in the closed flower and vegetative buds in spring (March- April). It was proposed that this could be *A. piri* adults that had overwintered and were laying their eggs in the spring. However it was clear that further investigation was required to identify the weevil and determine its lifecycle and biology in UK pear orchards. In 2017 the weevil was confirmed as *Anthonomus spilotus*, Redtenbacher, 1847 by the Natural History Museum and was published in 2017 by NIAB EMR and NHM. Presence of eggs, larvae, adults and feeding damage was observed from the beginning of sampling on 22 March and no adults were found after 12 June. This damage consisted of i) puncturing of the bud bracts by adult feeding, causing irregular growth and ii) larval feeding within leaf and flower buds leading to irregular growth, loss of buds and damaged flower buds. From the total buds collected a third had feeding damage and 7% of buds contained eggs, larvae or pupae which implied that feeding damage does not always mean that eggs, larvae or pupae are present within the bud.

Aim

Complementing previous work on the life cycle of *A. spilotus*, by better understanding the damage caused by the pest, will enable us to determine the optimum time and place to apply plant protection products, avoiding unnecessary applications. To achieve this we aimed to study:

- When adults emerge and move into the pear orchard canopy after diapause
- When adults start feeding on the buds and whether feeding damage correlates with the number of eggs laid in the buds
- Whether it is possible to predict the presence of the weevil using day length and temperature
- How many flowers in a truss are eaten by larvae until pupation and adult emergence
- If weevils release an aggregation pheromone

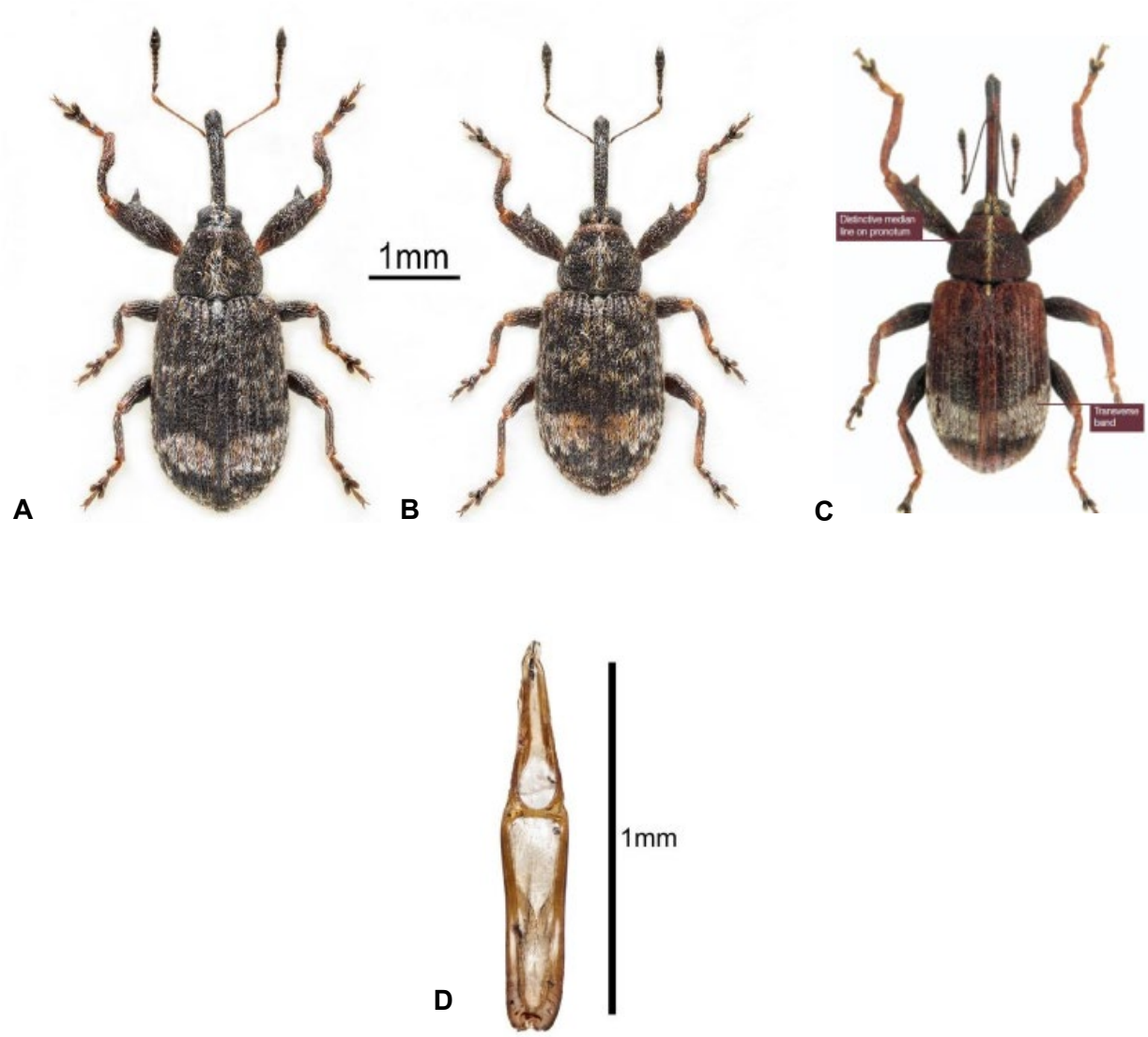


Figure 9.1.1 a) *A. spilotus* male**, b)** *A. spilotus* female**, c)** *A. piri*, **d)** *A. spilotus* median lobe of male genitalia (Harry Taylor. Figure A, B and D. 2017. Specimens in Natural History Museum; AHDB. Figure C. 2015)

Materials and Methods

Monitoring

Location: Four commercial pear orchards (sites) in the South East were monitored for *A. spilotus* once a week. To monitor for the presence of adults, 40 trees were tap sampled in each of the four orchards, following a W shape path, between 11 January and 6 June. One branch was tapped per tree by beating it three times over a white tray. Adult weevils were recorded and collected and later dissected in the laboratory to determine sex and fecundity.

To monitor for feeding damage (by counting the number of feeding holes), along with eggs and larvae, 40 random flowers and leaf bud samples were collected on each visit, then examined under a microscope at NIAB EMR (Figure 9.1.2).

Adult and earlier life stage feeding damage on leaf/vegetative and flower/mixed buds

Location: Within the same orchards, buds from pear trees were tagged to assess damage from weevil eggs/oviposition, larvae, cocoon and adult feeding.

From 21 March to 6 April, a total of 80 flower buds (damaged by feeding) were tagged in each orchard and, 20 undamaged flower buds were meshed as a control to exclude weevils (undamaged). Bud tagging was performed on two separate occasions per orchard (2x40 for the damaged buds and 2x10 for the control). The same procedure was done for leaf buds from 10 April to 18 May.

Buds were inspected upon blossom opening and leaf emergence, and then photographed to track damage. An assessment was made of whether the bud had adult feeding damage only or larval damage too.

From damaged buds, the numbers of blossoms were counted upon opening and the tip of the new growth (apical meristem) was examined for damage.

Entrainments - Weevils aggregation pheromone

Twenty individual adult weevils were collected from the field and entrained at a flow of 400 ml/h separately with a new pear bud/leaf. In addition 20 developing larvae/cocoons were collected from orchards and reared through to adult separately (virgins). These were entrained with a pear leaf after feeding on sugar solution and then dissected and sexed. Volatiles were collected from five samples of insects and one blank (Table 9.1.1). All six samples were provided with a pear leaf. Collections were sent to NRI for analyses. Collections were analysed by GC-MS on a polar GC column.

Table 9.1.1 Collections of volatiles from pear blossom weevil

	No. Insects		
NRI Code	Male	Female	Time (h)
2018/080/01	0	0	33
2018/080/02	1	2	33
2018/080/03	1	0	33
2018/080/04	0	1	33
2018/080/05	0	1	33
2018/080/06	0	1	33

Results

Adults and damage monitoring

In 2018, the first adult identified was a male, recorded on 16 February, when the flower buds were still in the dormant growth stage. Adult weevils and feeding damage were documented until fruit set. The 40 leaf and flower buds collected from each site, at each visit, were dissected using forceps and needles under a light microscope to look for the presence of feeding damage, eggs, larvae (Fig. 9.1.2d) and cocoons. No feeding damage, eggs, larvae or cocoons were found on/in the flower and leaf buds.

On 8 March, at swollen bud stage, the first flower buds exhibiting feeding damage and presence of eggs were found on Site 2 (Fig. 9.1.3). This coincided with the detection of the first adult female on the same site. A week later, all sites had flower buds with feeding damage and eggs, except for Site 3.

A. spilotus eggs, on flower buds, were documented from 8 March, at the beginning of swollen bud stage, until 17 April, at white bud stage, reaching a peak on 28 March (Fig. 9.1.3). Larvae were found from 6 April until 9 May, peaking on 10 April while the cocoon stage was only observed on one monitoring occasion (26 April). The monitoring of flower bud damage ceased on 23 May, since buds/flowers were no longer available for collection, as fruit was developing.

The first feeding damage on leaf buds occurred later than flower buds on 28 March at all sites (Fig. 9.1.4). On this date, eggs were found on Site 2 only. However, similar to flower buds, a week later, all sites showed the presence of eggs in leaf buds. Leaf buds holding eggs were found over four weeks from 28 March to 17 April peaking on the 10 April. *A. spilotus* larvae in leaf buds were recorded from 17 April until 23 May but were only documented on half of the sites.

Figure 9.1.2 a) feeding damage on a flower bud, **b**) Flower buds before collection at swollen bud stage, **c**) microscope observation of a bud, and **d**) Early stage larvae of *A. spilotus*

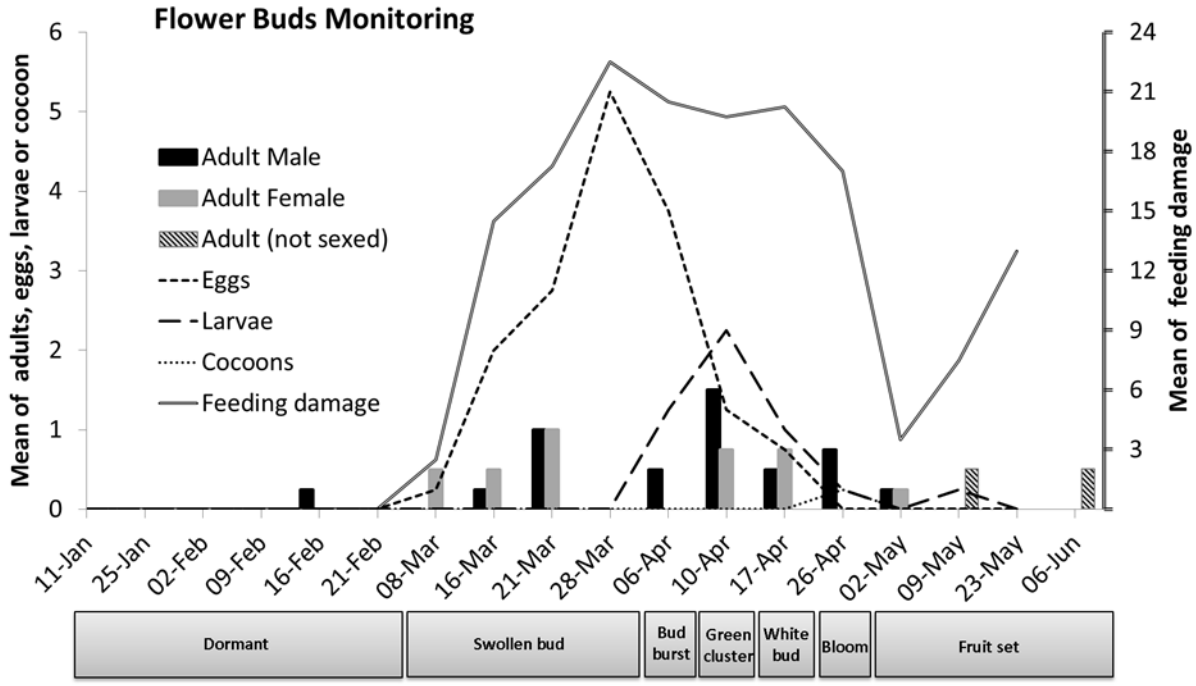


Figure 9.1.3 Mean number of *A. spilotus* eggs, larvae, cocoons, adults (primary axis) and feeding damage (secondary axis) on/in forty flower buds and the total mean of adult males and females tap sampled from forty branches on different trees, from four sites in the South East monitored between January and early June. Chronological bar indicates the growth stage of the flower buds

Cocoon stages of weevil development were found from 26 April to 23 May on the same sites where larvae were observed (Figure 9.1.4).

The first adult weevil was found before any feeding or development damage was recorded on 16 February (Figure 9.1.4). A similar number of male and female adults were found through all the growth stages until the last monitoring date at fruit set.

For each flower and leaf buds a total of 2800 buds were collected during the monitoring interval. A total of 633 flower buds showed damage from adult feeding, 64 contained pear blossom weevil eggs and 20 buds had larvae. For leaf buds, 351 exhibited adult feeding damage, 25 had eggs and 19 buds had larvae. One cocoon was found in flower buds whilst 10 cocoons were identified in leaf buds.

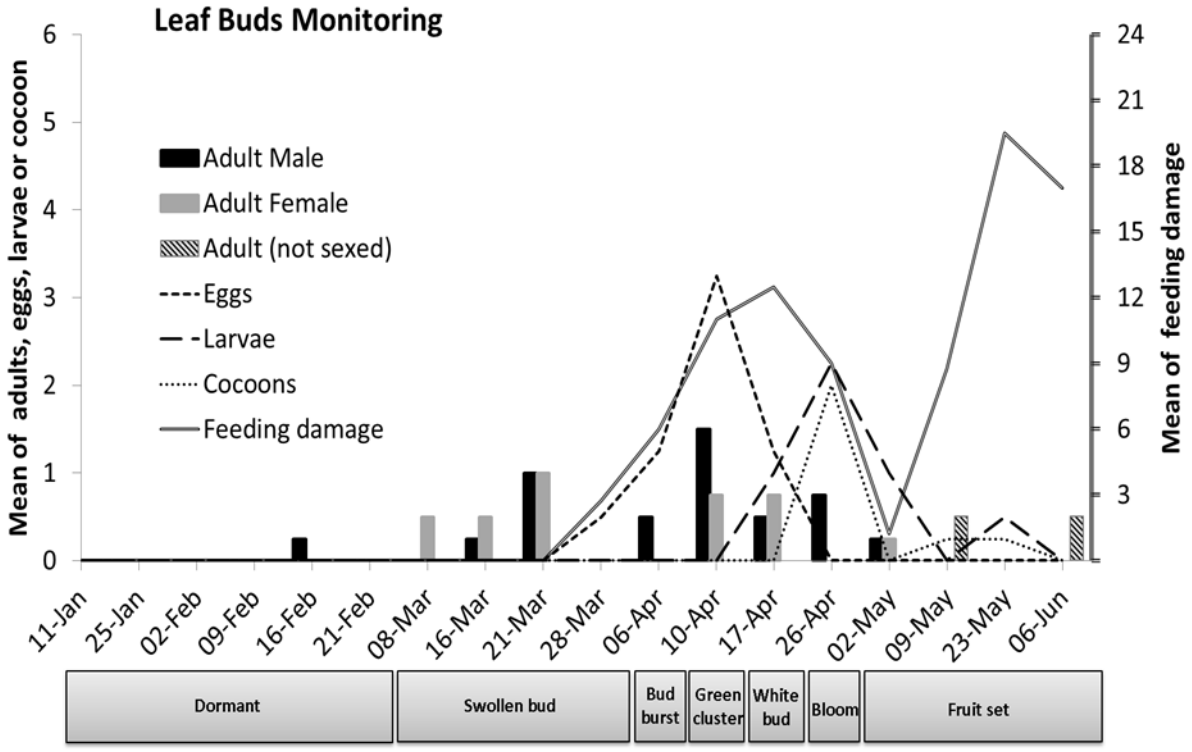


Figure 9.1.4 Mean numbers of *A. spilotus* eggs, larvae, cocoons, adults (primary axis) and feeding damage (secondary axis) on/in forty leaf buds and the total mean of adult males and females tap sampled from forty branches on different trees, from four sites in the South East monitored between January and early June. Chronological bar indicates the growth stage of the flower buds

On all sites, feeding damage to flower and leaf buds occurred during a similar period (8 March to 6 June) (Fig. 9.1.5) but a slight variation was observed between the numbers of damaged buds. Site 1 had the smallest numbers of flower and leaf buds with damage while Site 2 had the highest numbers with Site 3 in-between. However on Site 3 fewer damaged leaf buds were recorded. There was also a considerable decrease in damage on the 2 May monitoring visit in all orchards (Fig. 9.1.6).

A. spilotus adults were found at Sites 2, 3 and 4 (Fig. 9.1.5). We did not recover any adult weevils from tap sampling at Site 1, where damage was also lower. Adult weevils were more frequent at Site 2 throughout the monitoring period, where consistently higher damage occurred.

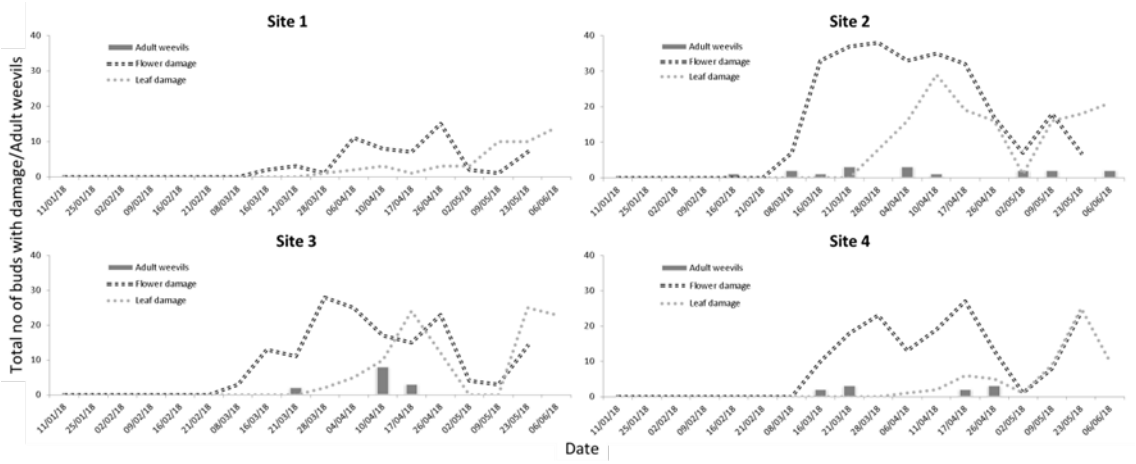


Figure 9.1.5 Total number of buds with feeding damage on/in forty flower and leaf buds and the total number of adults tap sampled from forty branches on different trees, for each site monitored between January and early June 2018

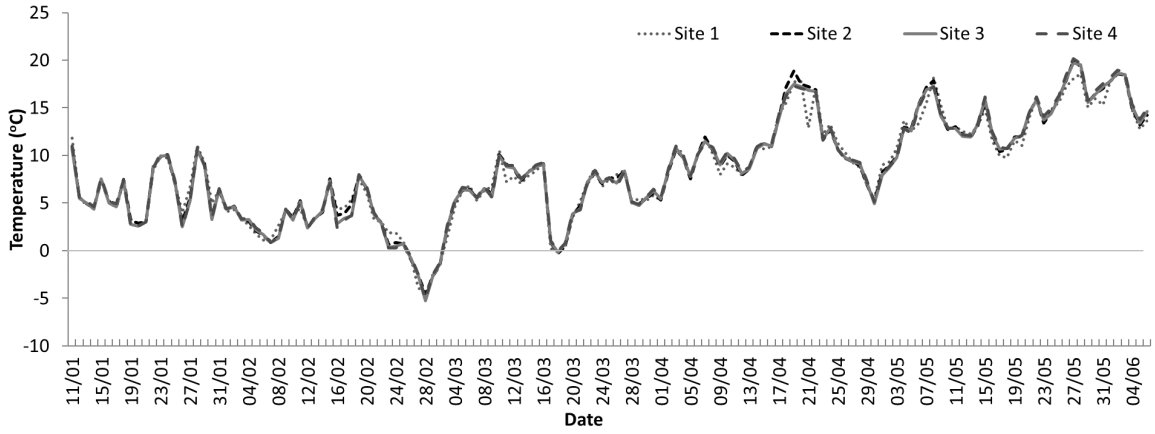


Figure 9.1.6 Average temperature (°C) for each site monitored between January and early June 2018

Adult development and feeding damage on leaf and flower buds

Forty flower and leaf buds with damage, per site, were tagged on two separate occasions (total of 320 buds for each damaged flower and leaf bud). Flower buds were tagged in late March and early April while leaf buds were tagged later between 10 April and 18 April when suitably developed. All buds tagged were chosen because they exhibited damage in line with the damage made by *A. spilotus*. Similarly on both occasions 10 flower and leaf buds

presenting no damage were tagged and protected with mesh to be used as control. The trusses/shoots that developed from the tagged buds were assessed for damage to flowers and leaves at bloom stage (25/26 April) (Fig. 9.1.7).

When assessing damage to the trusses we recorded damage occurring to flowers and also to the leaves that develop around the flowers of the mixed bud (Fig. 9.1.7a/b). Identification of damage focused on the presence of black necrotic spots and curled, shrunken leaves. On flowers one or more petals were brownish and shrivelled, or the reproductive organs damaged.

Approximately 10% of flowers in a truss were damaged by adult feeding and the number increased to 16% when an egg or larvae was present (Table 9.1.1). The exclusion meshed control recorded about 1.98% of damage of flowers in a truss. Leaves around the flowers in the truss were more damaged than the flowers in the truss by adult feeding. However, there was approximately the same damage to flowers and leaves when an egg or larvae developed (Table 9.1.1).

There was approximately 42% of adult feeding damage to leaves from tagged bud leaves compared to 5% of damage leaves on the control shoots. Leaf buds that were identified as containing eggs or larvae led to 60% of leaves damaged once the shoot was developed.

The mean numbers of flowers per truss observed was 6.1, the mean number of leaves per truss was 5.6 and the mean number of leaves per growing shoot was 6.5. Using this information we were able to determine how many flower and leaves on average will be affected when damage from either adult or development feeding is identified (Fig. 9.1.8). Damage from adult feeding on leaf buds led in average to 2.7 leaves being affected while damage from larval development affected about 3.9 leaves from the average 6.5 leaves present in a shoot. Leaf buds used as control had 0.3 leaves with damage.

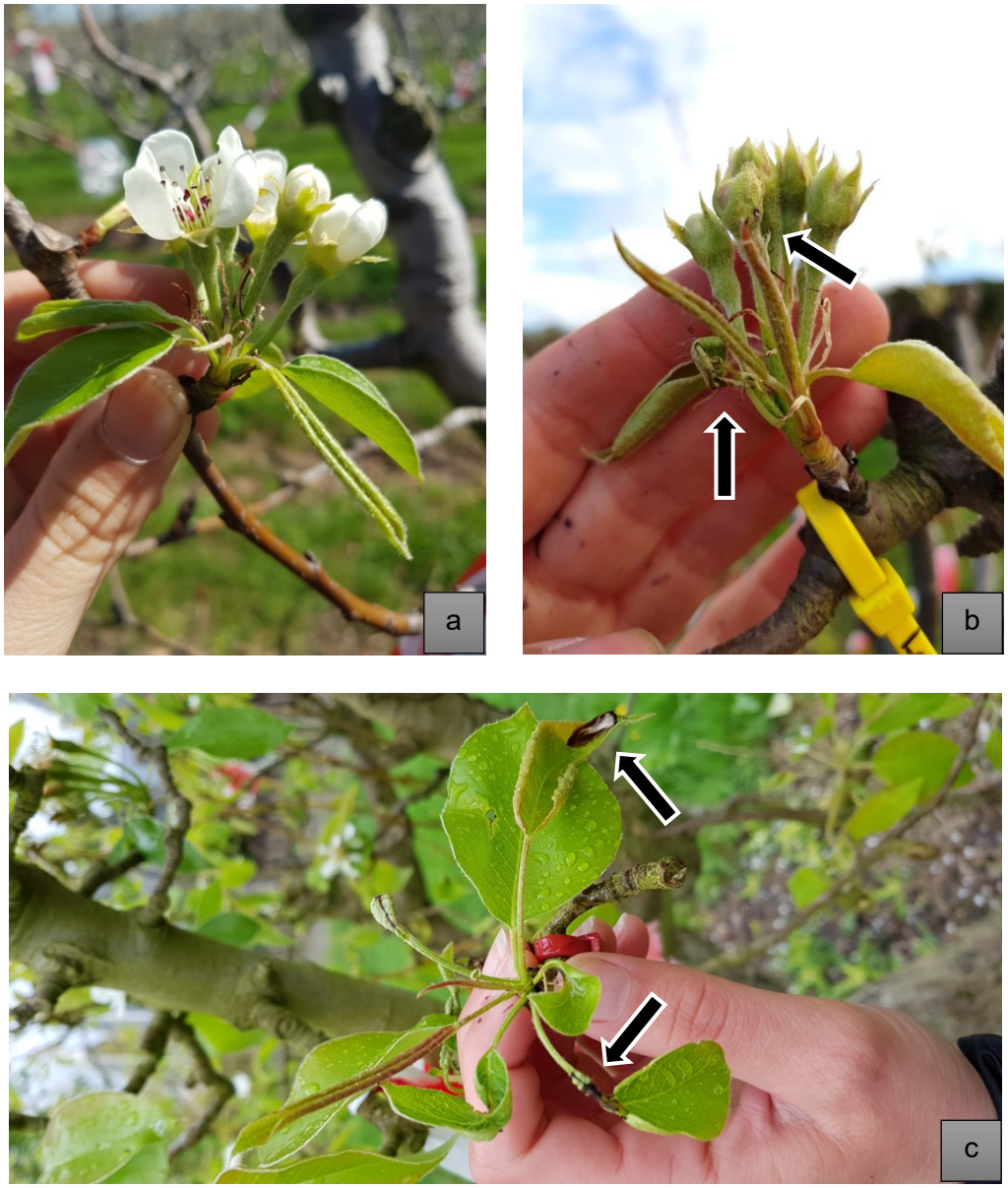


Figure 9.1.7 a) Pear flower truss including flower and leaves – mixed bud, **b**) pear flower truss with damaged bud and leaf, **c**) pear leaf shoot with curled, black necrotic leaves and shrinkage damaged

Table 9.1.1 Mean percentage of damage caused by adult and larval stage feeding on truss and leaf shoots

Damage	% Adult Feeding	% Larval	% Control
Flowers in the truss	10.76 ±1.38	16.02 ±5.19	1.98 ±2.72
Leaves in the truss	20.75 ±1.38	15.01 ±5.32	26.24 ±2.72
Leaves on the shoot	42.28 ±1.51	60.18 ±2.88	5.11 ±2.72

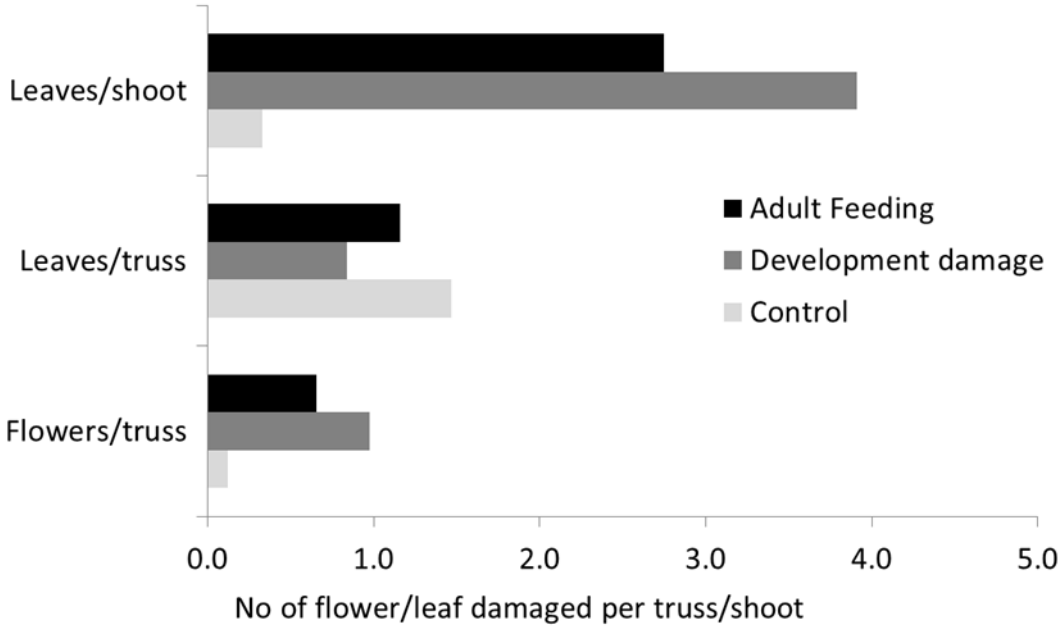


Figure 9.1.8 Number of flowers or leaves damaged per truss or shoot from adult and egg or larvae feeding and from meshed buds (control)

In trusses it was observed that adult feeding causes the loss of 0.7 flowers per truss and development feeding 1.0 flower per truss (Fig. 9.1.8). Only 0.1 flowers of the controls displayed damage similar to weevil/larvae feeding. In each truss an average of 1.2 leaves was damaged by adult feeding and 0.8 by larval development feeding. Leaf damage in control trusses showed an unexpected high value of 1.5 leaves per truss.

In the tagged buds the numbers of holes on one single bud differed (Fig. 9.1.9). The majority of flower and leaf buds collected, 118 and 107 respectively, had only one hole. But buds with two and three holes were also recorded in large numbers. Eight or above feeding holes were observed on a few flower buds, but no leaf buds had more than seven holes.

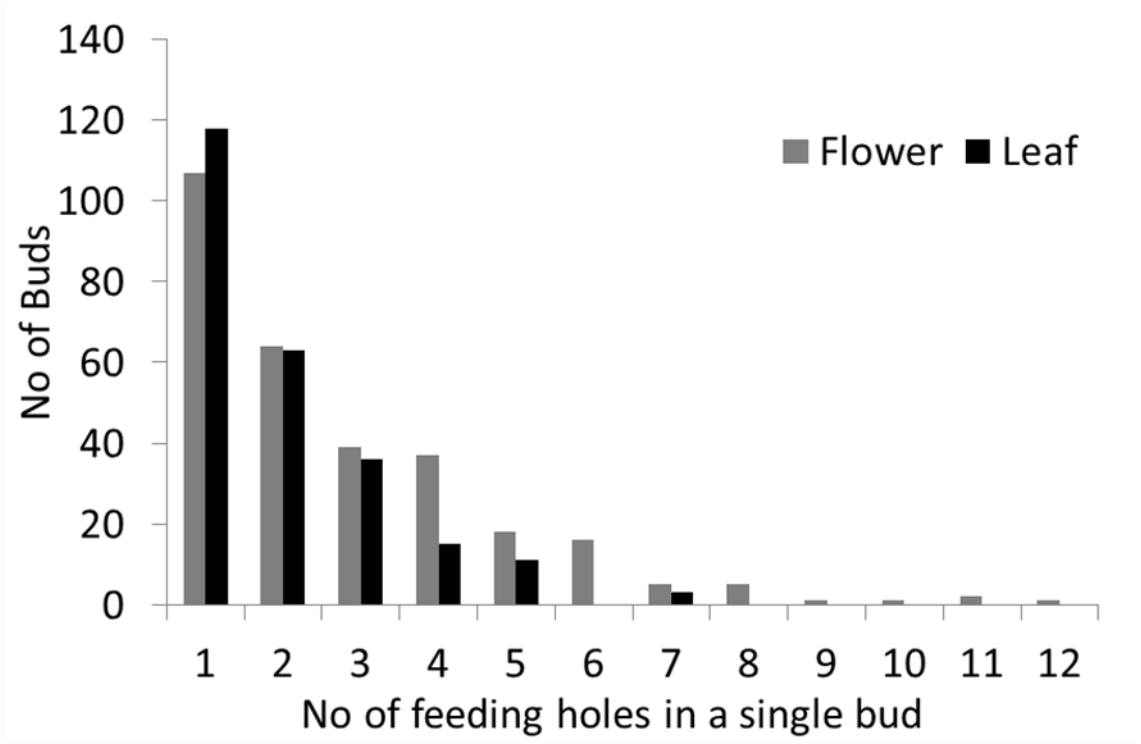


Figure 9.1.9 Frequency of number of holes counted on each flower and leaf bud tagged

Aggregation pheromone – entrainment

All collections seemed very similar with the main peaks corresponding to plant volatiles (*E*)-2-hexenal, (*Z*)-3-hexenol and (*Z*)-3-hexenyl acetate, as well as small amounts of other plant volatiles such as linalool, β-caryophyllene and methyl salicylate (Figure 9.1.10).

There were no apparent differences in collections from males and females. The Grandlures could not be detected by single ion scanning at the appropriate retention times using m/z 109 for Grandlure I, m/z 136 for Grandlure II and m/z 152 for Grandlures III and IV.

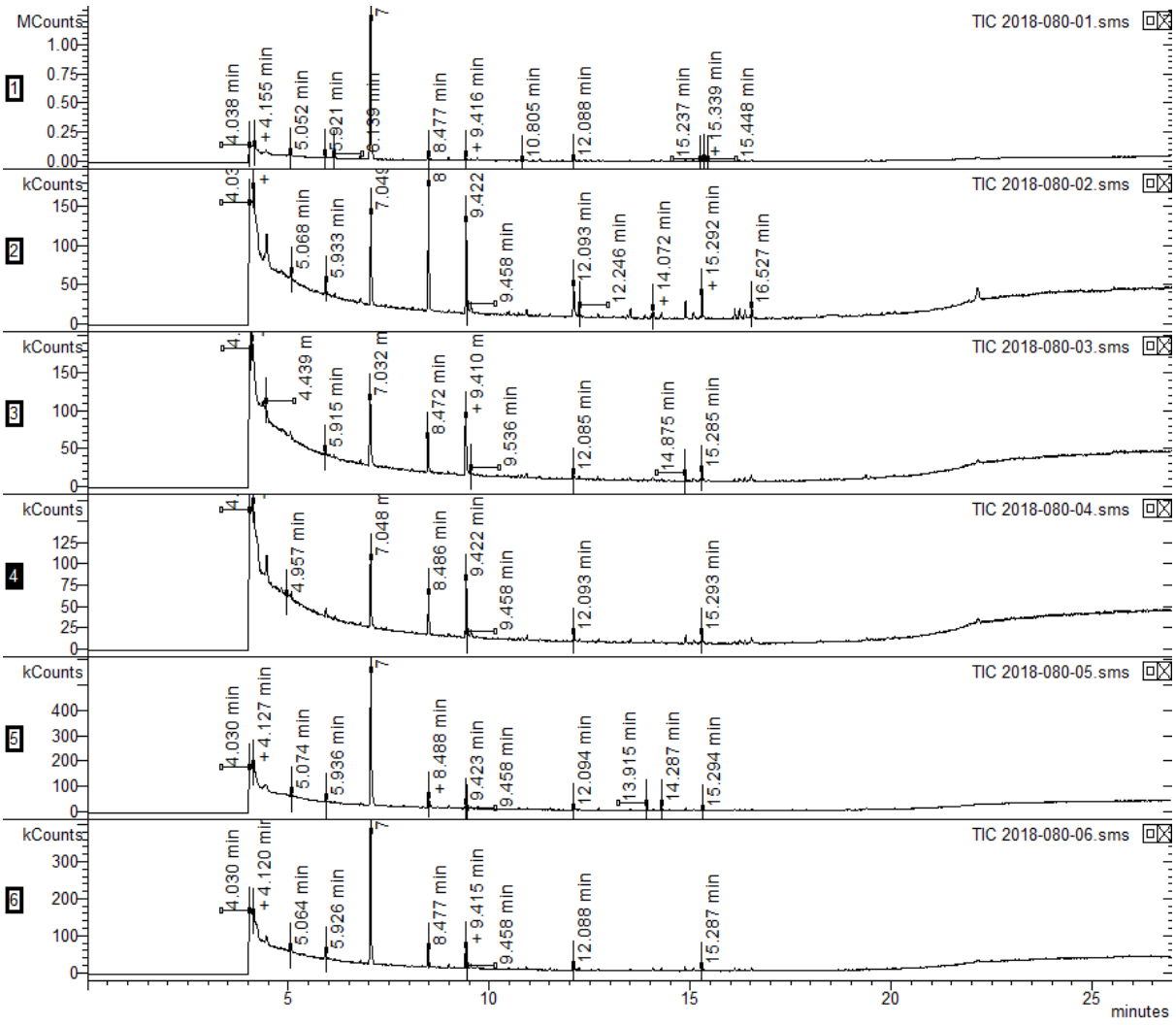


Figure 9.1.10 GC-MS Analyses of collections from pear blossom weevils from top 2018/080/01 – 02; polar GC column; (*E*)-2-hexenal 7.04 min, (*Z*)-3-hexenyl acetate 8.48 min, (*Z*)-3-hexenol 9.42 min)

Discussion

Monitoring was carried out from 11 January until 06 June. The first *A. spilotus* was found on 16 February and identified as a male, but no feeding damage was recorded at this time. This may be because buds were still at dormant stage and thus less attractive to feeding. Many Anthonominae weevils, emerging from overwintering, feed on digestible plant parts and even phloem tissue from cut-off shoots, until a more preferred food source, like buds, becomes available (Burke, 1976). Some female *Anthonomus* do not begin to lay eggs straight after emergence from diapause, e.g. *A. pomorum,* the apple blossom weevil, which also damages buds (Burke, 1976).

Female *A. spilotus* were found four weeks later and this coincided with feeding damage and the presence of eggs in flower buds. As with 2017's observations, the majority of buds with holes had no eggs nor larvae in them, suggesting that the damage was mainly from feeding adults. Weevils seemed to feed and lay eggs first on flower buds and then on leaf buds, although some literature suggests that *A. spilotus* adults may be more likely to attack leaf buds (Burke, 1976). This may be due to leaf buds developing later and flower buds becoming less available later on. In both flower and leaf buds, eggs were found up to 17 April which is in agreement with the literature that reports that females do not lay eggs after early May (Pussard, 1930). A decrease in adult feeding damage and number of larvae and cocoons was observed in early May on both flower and leaf buds (Figs. 9.1.3 and 9.1.4). A drop in temperature was recorded in late April and could have reduced weevil feeding activity (Fig. 9.1.6). A natural decline of the overwintered adult population may have also contributed to this reduced feeding activity. But more observational data would be needed to support this.

After this date adult feeding damage increased again, especially on leaf buds since the amount of flower buds available was becoming scarce. This could be attributed to the emergence of the new individuals that are reported to emerge closer to June (Pussard, 1930). A low number of weevils were collected during the monitoring period maybe due to individuals being more active at night, while the tap sampling was made during the day (Morris et al. 2017).

The presence of feeding damage was recorded on all four sites (Fig. 9.1.5). However Site 1 had less feeding damage and no adult weevils were found.

When feeding damage was observed on the flower bud, the developing truss had, on average, 0.7 and 1.2 of flowers and leaves damaged, respectively. Damage caused by larval development resulted in a loss of 1.0 and 0.8 flower and leaves per truss, respectively. The numbers recorded for the leaf buds are much higher. On bud leaves we recorded 2.7 leaves damaged per shoot when adult feeding was identified and 3.9 leaves when the damage

referred to larval development. This data suggests that adult feeding and egg laying on leaf buds can be more damaging to the tree than the damage caused to the flower buds and subsequent fruit yield. Therefore, potentially, future tree growth could be affected with high populations of weevil, beyond that seen in this study. An unexpected high number of leaf damage similar to weevil damage was recorded on control trusses. These flower buds were inspected for the presence of holes and since no damage was identified they were meshed and protected from any contact with weevils. It is unlikely that injuries to the leaf may have been caused by *A. spilotus*.

Literature states that *A. spilotus* females can puncture a single bud up to eight times for feeding and egg laying (Pussard, 1930). We recorded buds with between 1 to 12 punctures. However the majority of both bud types exhibited one to three punctures. A small number of flower buds had more than seven holes. Literature suggests the higher the number of punctures made to a bud, the lower the chance that the larvae will be able to develop (Pussard, 1930).

Conclusions

- *Anthonomus spilotus* adult activity, eggs in buds and adult feeding damage was recorded from 8 March until 6 June
- Weevils fed on and laid eggs in flower and leaf buds depending on availability
- The percentage of flower buds damaged by adult feeding was 22.6% and the percentage of flower buds damaged by larvae 0.7%
- The percentage of leaf buds damaged by adult feeding was 42.3% and the percentage of leaf buds damaged by larvae was 0.7%
- Hence most bud damage was result of adult feeding
- Fewer than 10% of the flowers in a truss were damaged by adult feeding and fewer than 16 % were damaged by larvae
- Greater flower and leaf damage was observed when eggs/larvae were present
- Hence the damage to flowers at 1 weevil per 40 taps is not the main consideration as only 1 of the 6 flowers is normally destroyed and only 3-4 Conference fruits can set to harvest on a single truss. The main consideration is the damage to leaves and photosynthetic ability for future years
- Even at very low levels of weevils (~1 per 40 tree taps) ~60% of new leaves were damaged later in the season. We have not been able to set a damage threshold for this because the resultant health to the tree cannot be estimated in this study

- The majority of buds usually had one to three damage holes although buds with more punctures could be found
- There were indications that population activity may be sensitive to significant temperature changes, but more data is needed to reach a more accurate conclusion
- The current recommendation would be to apply thiacloprid (Calypso), pre-bloom, on a warm day once adults and feeding damage to the unopened flower buds are detected
- However, it may not be necessary to do this every year and growers should monitor damage to the leaves after blossom to inform a decision on spraying in the following spring
- It is recorded that damaged buds containing pupae drop to the ground, however the maintenance of habitat for overwintering natural enemies important in resistant pear sucker control needs to be maintained. Hence cleaning up debris could be detrimental
- To date a potential pheromone has not been identified for this weevil

Future work

- Because 2018 was a cold spring it would be valuable to collect another year of data on the phenology of this pest
- More data is needed to determine the effect of temperature on weevil feeding activity
- More collections of weevils should be made for pheromone identification

9.2 Determine whether insecticide efficacy can be improved through stimulating ingestion of the insecticides, spinosad and indoxacarb on *Anthonomus spilotus*

Introduction

Cultural, biological and chemical controls could be incorporated into an integrated pest management system for the control of *A. spilotus*. Many cultural techniques deployed for the control of apple blossom weevil (*A. pomorum*, Linnaeus) in apple orchards could be applied for the control of *A. spilotus* in pear.

The weevil may have been introduced on imported pear trees, therefore it is important for growers to consider the introduction of new material on to their farms and apply good hygiene practices (Morris et al., 2017). An example of good hygiene would be to check for the presence of the weevil on imported pear trees (feeding damage) on arrival and when transporting or storing clean pear trees ensuring they are kept separate from any infected orchard or material.

Monitoring should be done to determine whether *A. spilotus* is present. It is recommended to tap sample 50 branches over a white tray when monitoring for the related weevil pest *A. pomorum* (Apple Best Practice Guide, AHDB, 2017). Tap sampling in areas where infestation has been observed in previous years will also improve the likelihood of identifying the presence of the pest. Checking closed buds for adult feeding/oviposition damage is also carried out to determine if the weevil has become established within the crop (AHDB, 2017). Monitoring should begin no later than March, before the adult weevils begin laying eggs.

A. spilotus is parasitized by two parasitic wasps; *Pteromalus varians*, Forst (Pteromolid) and *Microbracon discoideus*, Wesm (Braconid) (Thompson, 1943; CABI, 1931). Both parasitoids have been recorded in the British Isles (Dale-Skey et al., 2016; Broad et al., 2016). However the level of control in pear orchards in the UK is unknown and cannot be relied upon to keep weevil numbers below an economic threshold. Therefore the use of crop protection products for control of *A. spilotus* may be required.

In laboratory tests in 2016, Gazelle did not give effective control, but Calypso at full and half field rate gave 80-90% mortality. Calypso, Hallmark, Gazelle and Spruzit were the most effective insecticides against *A. spilotus* in the laboratory. High mortality and fast negative behavioural effects were observed in these treatments. However note that, in this experiment, weevils received a direct application of the insecticide. In a pear crop this scenario is less likely and weevils may be more likely to come into contact with dried residues.

Aim

Determine whether insecticide efficacy can be improved through stimulating ingestion of the insecticides, spinosad and indoxacarb.

Materials and methods

Adult weevil collection: More than forty live *A. spilotus* were collected per orchard visit by tap sampling on 15 May, for test A, and 22 May, for test B, from a commercial pear orchard in the South East of England. Weevils were starved for 24 hours (Test A) and 5 hours (Test B) to stimulate them to feed in the test.

Treatments: Three tree fruit plant protection products (PPPs) were tested (Table 9.2.1). Treatments were made up in glass graduated flasks with 5% sugar solution for Test A or distilled water for Test B. Further information on the crop protection products (Manufacturer, IRAC number, formulation etc.) is available in Appendix 9.2.

Test A: Weevils were transferred into a Perspex box (10x10x20 cm) each containing one sugar feeder (a mixture of sugar solution and insecticide), along with leaves from an unsprayed orchard at NIAB EMR (Fig. 9.2.1). One weevil was placed in each box and allowed to feed on the sugar feeder for 4 days. Each weevil was then removed using soft forceps into a clean petri dish containing water and a clean sugar feeder. Each treatment was replicated ten times.

Test B: Leaf shoots from trees located in an unsprayed orchard at NIAB EMR were washed in water, dried, and dipped for 5 seconds in a solution of field rate insecticide (Table 9.2.1). Shoots were left to dry in a fume hood cabinet for 4 hours. After drying, each shoot was added to a Perspex box (10x5 cm) containing one weevil. *A. spilotus* adults were allowed to feed on the shoots for 24 hours. An unsprayed small apical fragment of a young leaf was placed in a 5 cm diameter petri dish with a drop of honey for nutrition and a square of wet paper towel to maintain humidity (> 40% RH) (Fig. 9.2.2). Each weevil was then transferred from the Perspex box to a labelled petri dish where they were later assessed at each time point (details below).

Table 9.2.1 Product details of treatments tested in the laboratory on *A. spilotus*. *Based on manufacturers label for product ** Based on extension of authorisation for minor use on pear (n.o. 20142130) (apple use at 0.25 – 0.375 l/ha)

Treatment code	Product	A.I.	Field rate	Per litre of
1	Water	Water	NA	Sugar solution
2	Tracer	Spinosad	0.15 ml/l*	Sugar solution
3	Steward	Indoxacarb	0.25 g/l*	Sugar solution
4	Calypso	Thiacloprid	0.375 ml/l**	Sugar solution

Figure 9.2.1 Perspex box containing sugar feeder with insecticide treatment sugar solution, and *A. spilotus* feeding on pear leaves

Figure 9.2.2 a) Pear blossom weevil in 5 cm Petri dish with water and honey after exposure to treatments in Test B, **b)** *A. spilotus* feeding on shoot

Assessments

The weevils were assessed at time points (0, 24, 48, 72, 120, 168, 216 hours) after exposure to the insecticides and until the weevils had made a full recovery or died. Weevils at each assessment were scored as;

a. Healthy living weevil
b. Affected (abnormal behaviour, convulsive movements, lethargy etc.)
c. Moribund (very little movement, unable to stand after turning over)
d. Dead

Experimental design and statistical analysis

There were 10 replicates of 4 treatments in each test; A and B (2x 40 weevils). All analyses were carried out using a GLM with the Poisson distribution and a log-link. The pairwise comparisons were carried out using the difference in the deviances from fitting the GLM with the two treatments paired, versus fitting all treatments.

Results

Test A: Weevils were placed in Perspex boxes with a feeder containing sugar solution with a field rate concentration of one insecticide. All weevils were alive at the time of the first assessment (0 h). Within the first 24h, all treatments showed a higher mortality when compared to the control (Fig. 9.2.3). Weevils exposed to Calypso were all dead (100%) after 24 h of exposure (p=0.001). Steward and Tracer caused a mortality of 70% and 50%, respectively, although none of the treatments were significantly different from the control. We also recorded a mortality of 40% for the control weevils.

When data was combined all three treatments caused 100% weevil mortality by 48 h and were significantly different from the control (p=0.010). However the control also observed a high mortality of 60%. Assessments were stopped at 48 h time point since only the control had remaining live weevils.

Calypso, Steward, and Tracer had affected/moribund weevils immediately after exposure, but only Calypso was significant (p=0.001) when compared with the control (Fig. 9.2.4). 100% of the weevils displayed affected behaviour or moribund features from the beginning of exposure to Calypso. Treatments with Steward and Tracer led to 80% and 90% of affected/moribund weevils, respectively, but with no significant difference from the control. The control showed 40% affected/moribund weevils at the 0 h assessment point.

After treatment, within 24 hours, all weevils exposed to Calypso had died. 40% and 30% of weevils were affected/moribund after exposure to Tracer and Steward respectively. The affected/moribund result for Calypso (p=0.012) and Steward (p=0.023) 24 hours post treatment was significantly different from the control (10%).

All *A. spilotus* were dead 48 h after all treatments. There were no affected/moribund weevils observed in the control on this last assessment.

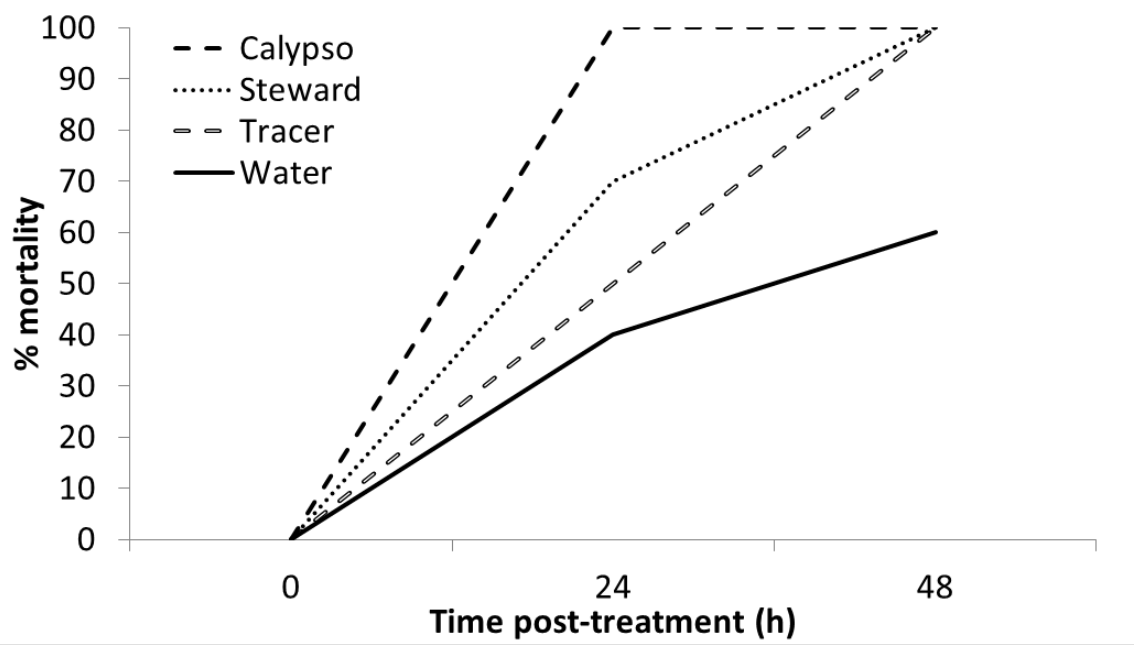


Figure 9.2.3 Percentage mortality of *A. spilotus* in Test A after contact with a 5% sugar solution of field rate insecticide for each treatment and pure 5% sugar solution for the control. Assessments were carried out at 0, 24 and 48 hours post-treatment

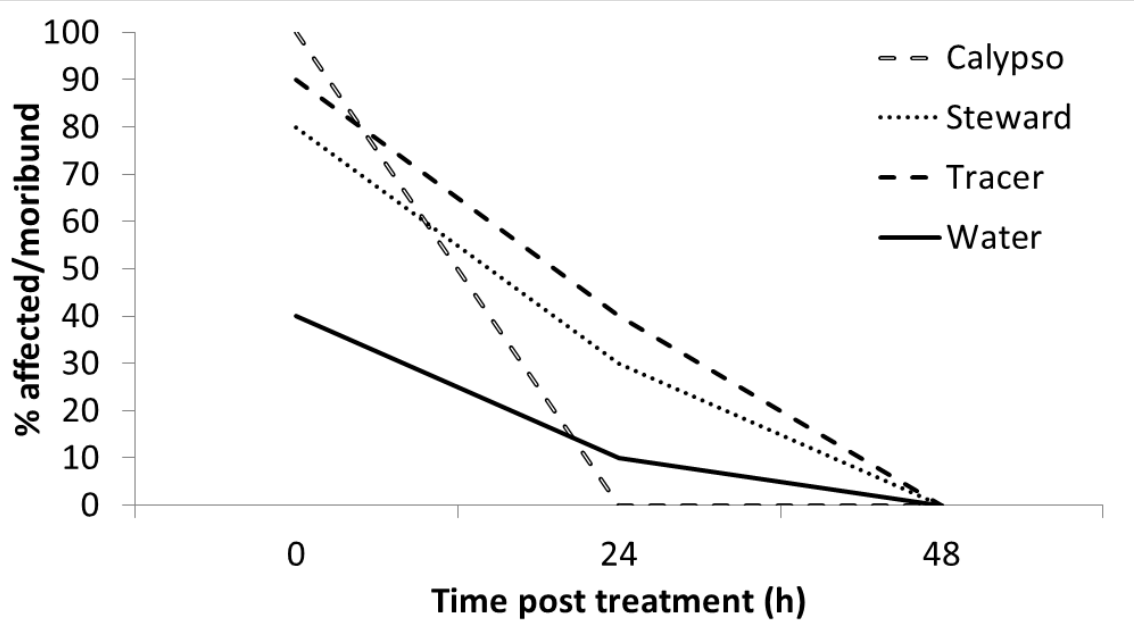


Figure 9.2.4 Percentage of *A. spilotus* adults affected or moribund after contact with a 5% sugar solution of field rate insecticide for each treatment and pure 5% sugar solution for the control. Assessments done at 0, 24 and 48 hours post-treatment

Test B: All weevils were alive immediately after exposure (0 h) to Calypso, Steward and Tracer and also in the control group (Fig. 9.2.5). Individuals that fed on shoots dipped in Calypso had the highest mortality at all time points. At 24 h after exposure, Calypso had killed 50% of weevils in the experiment. The same mortality was observed at the 48 h assessment, but both results were non-significant when compared to control mortality. At day 5 (120 h), Calypso increased weevil mortality significantly ($p=0.005$) to 80%. Again, this value was continued until day 7 (168 h) and remained significantly different from the control ($p=0.005$). A significant ($p=0.001$) increase in mortality to 100% was recorded at the day 9 (216 h) after treatment.

Shoots treated with Steward killed 40% of weevils within the first 24 h. This was constant until 168 h after treatment and then increased to 70% at 216 h. None of the time points were significantly different when compared to the control group.

Weevils that fed on shoots dipped in Tracer showed a lower percent mortality of the three treatments at all time points (Fig. 9.2.5). At 48 h after treatment fewer weevil were dead with Tracer (10%) compared to the control group (20%). Weevil mortality with Tracer treatment increased to 30% at 120 h after treatment and further increased to 40% at 216 h, but was not significantly different from the control.

At 0 h post contact with shoots dipped in a field rate insecticide solution, a higher percent of weevils exposed to Calypso, Steward and Tracer (100%, 40%, and 70% respectively), were affected/moribund compared to the distilled water control (0%) (Fig. 9.2.6). However, only the effects of Calypso (p=0.000) and Tracer (p=0.003) were significantly different from the control. Weevils affected by Calypso then decreased at 24 h, 120 h and 216 h (Fig. 9.2.6) due to increased mortality. At 24 h, 50% (p=0.003) of the weevils were affected/moribund, at 120 h 20% (p=0.001) and 216 h (p=0.012) all weevils were dead.

A significant percentage (70%, p=0.003) of weevils was affected by Tracer from 0 h assessment. This percentage decreased to 20% after 24 h and then to 10% at 120 h but these results were not significant compared to the control. At the last assessment time (216 h) no weevils were recorded as affected or moribund for weevils exposed to Tracer since they were either healthy or dead.

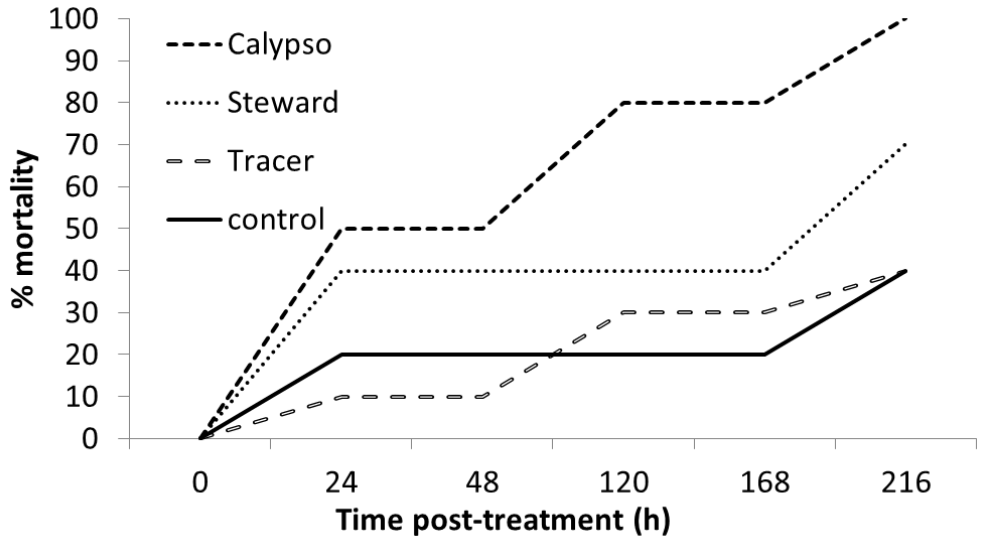


Figure 9.2.5 Percentage mortality of *A. spilotus* adults after contact with a shoots dipped in a field rate insecticide solution for each treatment or distilled water (control). Assessments were done at 0, 24, 48, 120 (5 days), 168 (7 days) and 216 (9 days) hours post-treatment

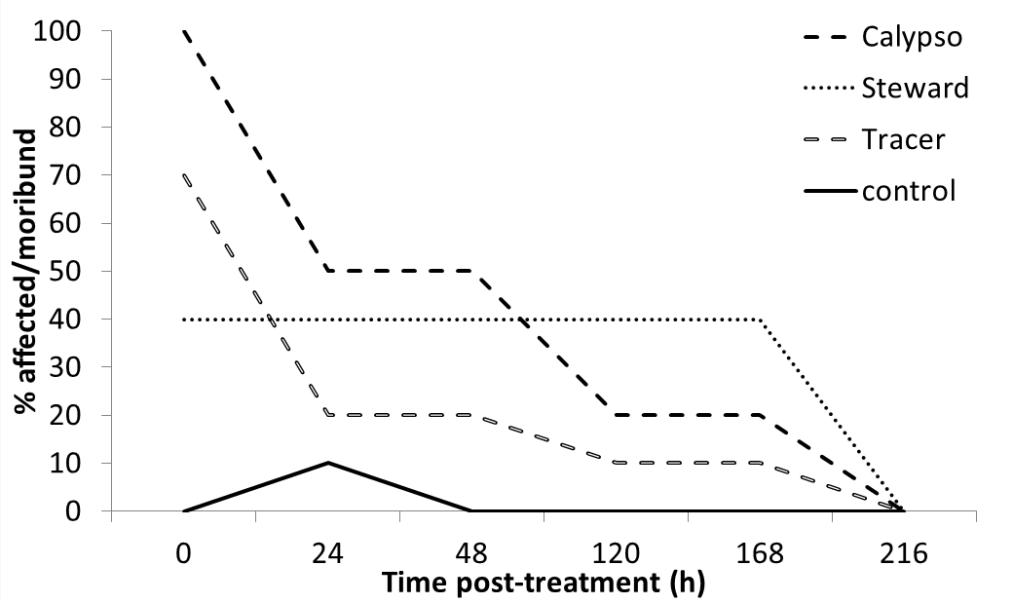


Figure 9.2.6 Percentage affected or moribund *A. spilotus* adults after contact with shoots dipped in a field rate insecticide solution for each treatment or distilled water (control). Assessments were done at 0, 24, 48, 120 (5 days), 168 (7 days) and 216 (9 days) hours post-treatment

Weevils exposed to Steward showed an affected/moribund percentage of 40% at the first time point (0 h). This value was constant until 168 h after treatment. When compared to the control group results were significant at 120 h and 168 h time points (p=0.018). As observed with Calypso and Tracer experiments, no affected/moribund weevils were recorded with the Steward treatment at the 216 h time point.

After 216 h, Calypso demonstrated to be the only treatment that had a significant difference in percentage mortality of *A. spilotus* (100%) compared to the control group (40%) (Fig. 9.2.7).

Although weevil mortality with Steward was higher than the control, this difference was not significant (p=0.174). By the end of the experiment (216 h) Tracer treatment had the same mortality (40%) as the control.

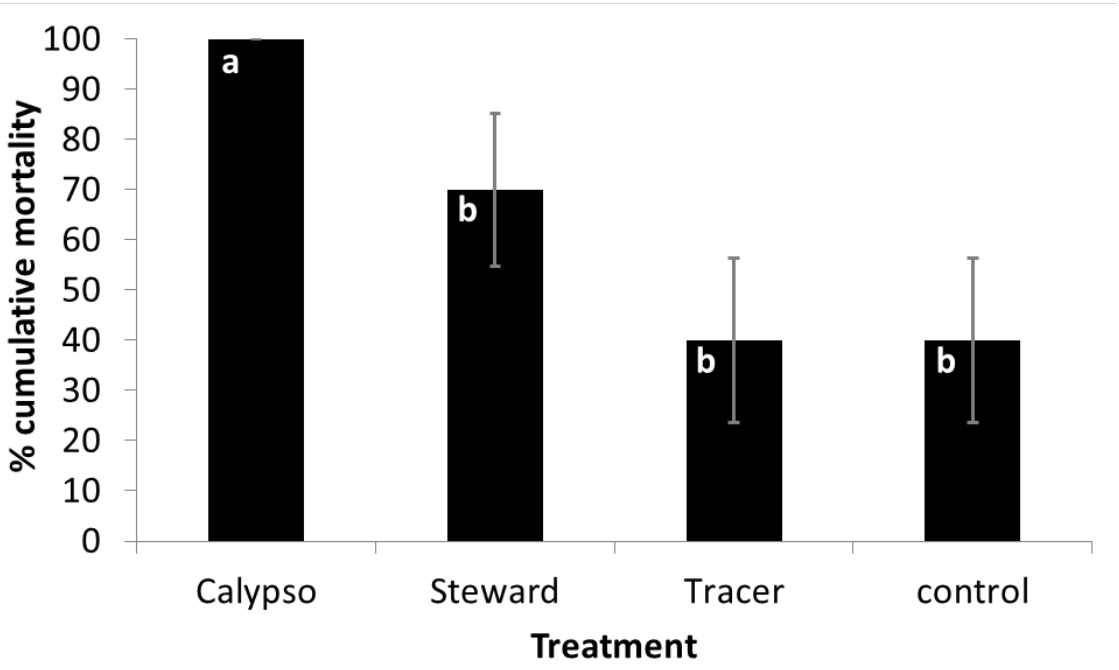


Figure 9.2.7 Cumulative percentage mortality of *A. spilotus* adults after contact with shoots dipped in a field rate insecticide solution for each treatment or distilled water (control). Significant differences indicated by different letters

Discussion

In these experiments we aimed to assess if a specific insecticide was more effective by topical application or if ingested by the weevils. For Test A we introduced the insecticide solution in a feeder mixed with a 5% sugar solution (Fig. 9.2.1). Within the first 48 h 100% *A. spilotus* mortality was observed (p=0.010). However, in the control group *A. spilotus* also had a considerable mortality (60%). In addition, in all treatments, weevils were not observed feeding on the solutions. Therefore, it was not possible to determine if the high mortality was a result of weevils feeding on the insecticide solutions.

A different approach was then taken: unsprayed pear leaf shoots were treated with the insecticides and offered to the weevils (Test B) to ensure weevils would ingest the insecticide.

The fastest and more effective insecticide was Calypso which killed and affected significantly more weevils than other treatments (Fig. 9.2.6, Fig. 9.2.7). In 2017, weevils treated with a topical application of Calypso did not die as quickly. However more weevils were affected/moribund for significantly longer than all other treatments and it was also concluded that Calypso gave the highest level of control compared to the other treatments. Weevils that

fed on plant material treated with Calypso recorded a higher cumulative mortality (100%) than those tested by topical application from 2017 (81.3%). However because these results were in different years they cannot be compared directly.

The topical application of Steward was not effective in 2017, recording less than 10% cumulative mortality. Steward ingestion, in Test B, seemed to be more effective killing 40% of the weevils in 24 h. It also had a significant negative effect on the same percentage of weevils from 48 h up to 168 h after treatment. 10% of the affected/moribund weevils recovered while the other 30% were dead by 216 h leading to a cumulative mortality of 70%.

Tracer was the least effective insecticide in this trial. About 10% of the weevils that fed on shoots treated with Tracer died in the first 24 h. 70% of individuals were affected or moribund right after feeding on the treated plant material. However most of them (40%) recovered before the 24 h post-treatment assessment. Tracer was the slowest of all insecticides to kill weevils and only had more mortality (30%) than the control (20%) after 120 h post-treatment. No spinosad insecticide was tested in 2017 for topical application.

These results suggest that Calypso and Steward are more effective when weevils feed on plant material treated with insecticide rather than when it is applied topically. Tracer acted slower and had less effect on the individual's behaviour.

Pear growers are highly reliant on beneficial predators for the control of significant pests such as pear sucker, aphid, midges, codling moth and caterpillars (AHDB, 2015; HDC, 2014). Earwigs, *Orius* and anthocorids are all voracious predators of pear sucker (AHDB, 2015). It is paramount that the use of insecticides is carefully assessed to determine if timing and conditions are appropriate to ensure the application is the most effective on the pest.

Conclusions

- Calypso was the most effective insecticide against *A. spilotus* in this laboratory trial causing 100% mortality nine days after ingestion (compared to 40% for the control group)
- Tracer had the lower mortality percentage from the three insecticides tested for all time points
- The results also suggest that insecticides tested in this trial are more effective when ingested by *A. spilotus* than when topically applied

Future work

- Spray timing and efficacy for control of *Anthonomus spilotus* in pear
- Calypso (Table 2, Appendix 1) applied by growers and growers own machinery under supervision of NIAB EMR at the recommended field concentration

Objective 10. Brown marmorated stink bug (*Halyomorpha halys*) surveillance

Introduction

Brown marmorated stink bug (BMSB), an invasive pest native to East Asia, has become established in North America and several European countries (e.g. Switzerland, Italy, Germany and France) in recent years. BMSB is able to travel long distances as a hitchhiker associated with imported goods and passenger luggage, and the insect has been intercepted entering the UK on several occasions (e.g. Malumphy, 2014). Bioclimatic modeling suggests that South East England is the most suitable region of the UK for establishment (Kriticos et al., 2017), but breeding populations have not yet been reported here. The insect poses a potential threat to UK horticulture as it is able to feed on and damage a wide range of plant species, including ornamentals, field crops and several tree fruit species (particularly apples). When BMSB invades new countries it typically establishes initial populations feeding on ornamental plants and exotic tree species close to transport hubs and city centres, with spread outside urban areas and crop damage occurring later. As part of a small-scale surveillance programme during 2018, we raised awareness and appealed for reports of sightings of the pest, and placed pheromone traps at city centre locations and sites of commercial fruit production.

Methods

The surveillance programme followed two strategies:

1. **Pheromone trapping**

"Pherocon BMSB STKY" rectangular (30 x 15 cm) double-sided clear sticky traps with high-dose 12-week pheromone lures (Trece Inc., USA) were used, containing two chemical components of the BMSB aggregation pheromone (Methyl E,E,Z-2,4,6-decatrienoate and Murgantiol). These pheromone traps provide effective, long-lasting detection of BMSB when populations of the pest are present (Weber et al., 2017). Traps were located at ten sites in South East England (Figure 10.1), each fixed to a horizontal tree branch (Figure 10.2) approximately 2.5 m from ground level (one trap per site). Traps were initially installed in April / May 2018 and checked weekly for signs of captured shield bugs. The sticky traps and lures were replaced after 12 weeks (in July) and the trapping continued for a second 12-week period (until October).

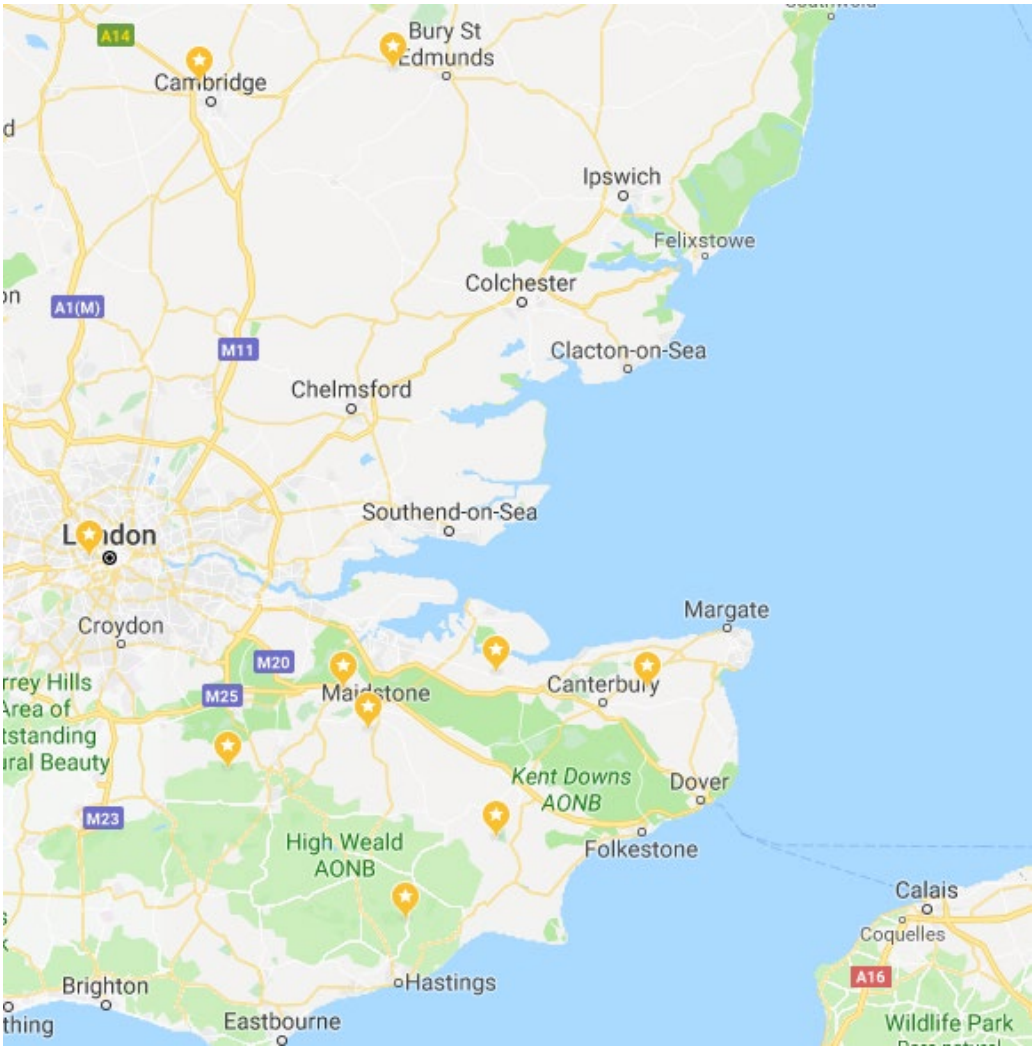


Figure 10.1 Sites of BMSB pheromone traps. Cambridge and London sites were urban locations, all other traps were positioned at sites of commercial fruit production

Figure 10.2 BMSB pheromone trap *in situ* with black pheromone lures visible, fixed next to the double-sided sticky trap.

2. Alerts to growers and the general public

A NIAB EMR press release was issued on 1st June 2018 to publicise the pheromone monitoring programme and appeal for vigilance and reports of sightings. Growers and members of the public were requested to send specimens or images of any suspected BMSB to NIAB EMR for identification. These messages were also communicated in various articles that followed the press release, including the NFU's Horticulture Magazine, Fresh Produce Journal and the British Journal of Entomology and Natural History.

Results

The pheromone monitoring traps caught no BMSB and very few shield bugs of any species. Only three individual shield bugs were trapped, belonging to three different species already known to be resident in the UK. Images sent by e-mail for identification included two native UK species which superficially resemble BMSB (the forest bug, *Pentatoma rufipes* and the hairy shield bug, *Dolycoris baccarum*) in addition to two invasive species that have recently arrived and established in the UK (western conifer seed bug, *Leptoglossus occidentalis* and the mottled shield bug, *Rhaphigaster nebulosa*). No images or specimens of BMSB were received (Figure 10.3).

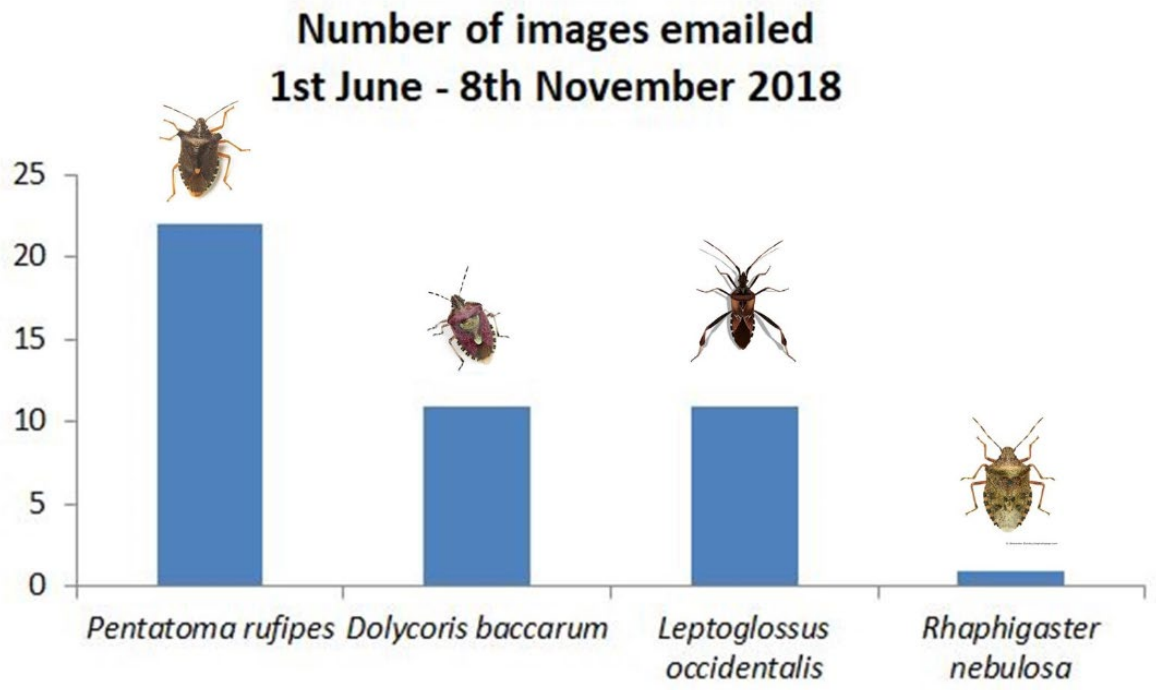


Figure 10.3 Number of plant bug species identified from images emailed to NIAB EMR by 45 separate sources. Photo credits*: P. rufipes* © Chris Mattison, naturepl.com; *D. baccarum* © Life on White, picfair.com; *L. occidentalis* © seebugs.com; R. nebulosa © Alexander Slutsky, alsphotopage.com

Discussion

Different species within the insect family Pentatomidae (stink / shield bugs) often share chemical components of their aggregation pheromones, resulting in significant cross-attraction of multiple species during pheromone monitoring programmes (Weber et al., 2017).

The very low numbers of native species caught during the 24-week BMSB pheromone monitoring period is therefore encouraging and suggests that cross-attraction of non-targets is unlikely to be a problem during future UK BMSB monitoring using pheromones. However, some of our native pentatomids (particularly *P. rufipes* and *D. baccarum*) superficially resemble *H. halys* and are likely to be mistaken for the invasive pest by growers, agronomists and members of the public (Figure 2). This highlights the value of continued future monitoring, combined with the provision of expert identification of suspected BMSB based on specimens and photographs.

KNOWLEDGE AND TECHNOLOGY TRANSFER

2015

12 August 2015 TF223 summer field visit, open meeting, Mount Ephraim

19 November 2015 **Saville**: Association of Applied Biologists IPM: THE 10 YEAR PLAN – using biocontrols more effectively in tree fruit crops

2016

12 January 2016 **Fountain**: Agrovista Conference (Brands Hatch) – talk on Rhynchites

27 January 2016 **Saville & Fountain**: BIFGA day – talk about Apple rots/Neonectria and Rhynchites respectively.

17 March 2016 **Fountain**: Pear Grower – pear sucker and predator monitoring training at David Long, Childs Farm

23 February 2016 **Saville**: AHDB Tree fruit day – Neonectria ditissima

12 July 2016: a farm walk entitled 'Pollinators, Predators and Productivity' at Lower Goldstone Farm. **Fountain** talked on Codling control.

20 July 2016: Fruit Focus (East Malling), **Saville** hosted a tour stop on Euroupean apple canker

21 July 2016: TF223 summer field visit, East Malling

2017

17 January 2017: Agrovista Conference (Brands Hatch), **Fountain and Saville** talked about Pear bud weevil and Canker respectively.

25 January 2017: BIFGA Technical Day (Ticehurst), **Saville** talked on European apple canker; The general practitioner's approach.

28 February 2017: EMR/AHDB tree fruit day (East Malling), **Berrie, Fountain and Saville** talked on Mildew, Codling, pear bud weevil and Canker respectively.

26 – 30th June 2017: 11th International IOBC - WPRS Workshop on Pome Fruit Diseases, Jūrmala, Latvia. **Berrie and Saville** presented on Apple Powdery Mildew and European Apple Canker.

9 August 2017: National Association of cider Makers Orchard Walk at Weston's Caerswall Farm, Herefordshire, **Fountain and Saville**. Alternative pest control mechanisms, work on

earwigs, and how the industry facing up to a post-chlorpyrifos and potential post-thiacloprid world. Overveiw of work on developing IPM programmes to control scab, mildew and canker.

13 September 2017: AHDB Agronomist day at NIAB EMR. **Saville, Berrie and Fountain** spoke and demonstrated work on European apple canker, Apple powdery mildew and Weevils in pears

19 September 2017: ADAS/AHDB Growing Media workshop at Frank P Matthews, Tenbury Wells, Worcs. **Nicholson** spoke on soil amendments for canker control.

Kingsnorth J, Perrine J, **Berrie A, Saville R**, 2017. First report of Neofabraea kienholzii causing bull's eye rot of apple in the UK. New Disease Reports **36**, 15. [http://dx.doi.org/10.5197/j.2044-0588.2017.036.015]

Morris M.G., Howard Mendel, Barclay M.V.L., Booth R.G., **Cannon M F.L., Conroy C.E., Csokay L.K., Faulder C, Fountain MT and Jay C.N**. (2017) Anthonomus spilotus Redtenbacher, 1847 (Curculionidae) new to Britain, a pest in pear orchards in Southern England. The Coleopterist, 26(2): 117-122.

2018

23 and 25 January 2018: **Cannon and R. Saville**: *Anthonomus spilotus* (Pear blossom weevil) – A new pest in UK pear orchards? And The latest work on European Apple Canker at NIAB EMR. Agrovista Cider growers day, Ledbury Rugby Club, Ross Rd, Ledbury HR8 2LP and Agrovista Desert apple growers day, Mercure Hotel, Brands Hatch for dessert growers

31 January 2018: Rothamsted Research BCPC Pests and Beneficials Review Michelle **Fountain** - Successful application of biocontrols in outdoor horticultural crops

31 January 2018: British Independent Fruit Growers' Association (BIFGA) technical day, Wadhurst, East Sussex. **Jay and R. Saville** presented on Pear weevil and Tree fruit diseases respectively.

22 February 2018: AHDB/EMR Association Tree Fruit Day – M. **Fountain, M. Cannon, A. Berrie and R. Saville** spoke on SWD Research, Pear bud weevil, Pear sucker and natural enemy monitoring, Blastobasis, speeding up the ecology in new orchards, Apple powdery mildew and European apple canker.

7 March 2018: Presentation to fruit researchers at University of Aarhus, Denmark by Angela **Berrie** entitled "Minimising Residues on Apple"

24 May 18: Soft fruit walk at Mockbeggar Farm on Tuesday 12 June 2018. Update on NIAB EMR research. Michelle **Fountain**

10 Jun 18: LEAF Open Farm Sunday, Tuesley Farm, Surrey. Bumblebees in horticultural crops – on behalf of BBSRC. Attended by Michael Gove. Michelle **Fountain**

25 Sep 18: Visitors from FAS/USDA (US Embassy, London). Entomology research at NIAB EMR. Michelle **Fountain**

Oct 2018: Michelle **Fountain and Scott Raffle** – story board on enhancing ecology for AHDB website.

FACTSHEETS

Factsheet 11/18. Managing spider mites on cherry. Michelle **Fountain**

Anthonomus spilotus - Pear blossom weevil. Michelle **Fountain**

Factsheet 12/18. Earwig friendly spray programmes in apple and pear crops. Michelle **Fountain**

28 February 2019 EMR Association/AHDB Horticulture, Tree Fruit Day, Technical Up-Date on Tree Fruit Research

- Surveillance for new pests and diseases in tree fruit (Glen **Powell** and Matevz **Papp-Rupar**, NIAB EMR)
- Enhancing the ecology of newly planted orchards (Celina **Silva**, NIAB EMR)
- New research into Anthonomus spilotus in pears (Michelle **Fountain**, NIAB EMR)
- The latest results on apple canker research (Lucas **Shuttleworth**, NIAB EMR)
- Understanding the impact of endophytes on tree health (Leone **Olivieri**, NIAB EMR)
- Bacteriophages for the control of cherry bacterial canker (Matevz **Papp-Rupar**, NIAB EMR)

ACKNOWLEDGEMENTS

Thanks to the ADAS and NIAB EMR glasshouse, farm and trials staff for assisting in trials. We are grateful to the growers and packhouse operatives who have assisted through the hosting of trials and sampling and finally to the programme management group for their engagement and advice in this project.

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APPENDICES

Appendix 3.2 Treatments applied to orchard EE190 prior to start of trial and after end of trial and to all plots during the trial in 2018

Date applied	Product	Type	Rate / ha
3 April Bud burst	Difference + Scala	fungicide	0.2 L + 1.0 L
13 April	Delan Pro + Scala	fungicide	2.5 kg + 1.0 L
25 April	Captan + Kindred	fungicide	0.6 L + 2 kg
1 May	Fontelis + Scala + PeK Acid	fungicide	0.75 L + 1.0 L + 5 kg
11 May	Captan	fungicide	2 kg
17 May	Captan	fungicide	2 kg
17 May	Calypso + Runner	insecticide	0.375 L + 0.6 L
1 June	Pot bicarb	fungicide	5 kg
6 June	Captan	fungicide	2 kg
13 June	Coragen + Mainman	insecticide	0.175 L + 0.14 kg
27 June	Cosine	fungicide	0.5 L
27 June	Batavia	insecticide	1.5 L
4 July	Steward	insecticde	0.25 kg
10 July	Talius	fungicide	0.25 L
6 August	Coragen	insecticide	0.175 L
30 August	Steward	insecticde	0.25 kg

Appendix 7.2 Spray records for Task 7.2

Spray records for the farms including insecticides and sprays targeted against pear sucker and honeydew in **2016**.

Farm	Date	Product	Dose/ha	Volume rate	Area
C_C	No data				
C_G	21 Mar	Chlorpyrifos	0.100 l	250 l	3.83
C_G	19 May	Bittersaltz	6.000 kg	330 l	3.83
C_G	30 May	Bittersaltz	6.000 kg	330 l	3.83
C_G	9 Jun	Bittersaltz	6.000 kg	330 l	3.83
C_G	21 Jun	Bittersaltz	6.000 kg	330 l	3.83
C_G	9 Aug	Coragen	0.175 l	330 l	3.83
C_S	30 Mar	Calypso	0.375 l	400 l	7.27
C_S	15 Jun	Runner	0.600 l	400 l	7.27
C_S	21 Jun	Carpovirusine	1.000	800 l	7.27
C_S	8 Jul	Carpovirusine	1.000	800 l	7.27
C_S	1 Aug	Coragen	0.175 l	400 l	7.27
C_S	28 Sep	Mag Sulph	4.000 l	400 l	7.27
G_N	17 May	Bittersalz	5.000 kg	450 l	1.61
G_N	28 Apr	Mag Sulph	3.000 l	300 l	1.61
G_N	24 Jun	Coragen	0.175 l	450 l	1.61
G_N	13 Jul	Bittersalz	5.000 kg	450 l	1.61
G_N	21 Aug	Bittersalz	5.000 kg	450 l	1.61
G_B	1 Apr	Calypso	0.375 l	450 l	1.35
G_B	17 May	Bittersalz	5.000 kg	450 l	1.35
G_B	28 Apr	Mag Sulph	3.000 l	300 l	1.35
G_B	24 Jun	Coragen	0.175 l	450 l	1.35

G_B	13 Jul	Bittersalz	5.000 kg	450 l	1.35
G_B	21 Aug	Bittersalz	5.000 kg	450 l	1.35
G_M	12 Mar	Calypso	0.375 l	450 l	3.84
G_M	17 May	Bittersalz	5.000 kg	450 l	3.84
G_M	28 May	Mag Sulph	3.000 l	300 l	3.84
G_M	7 Jun	Envidor	0.600 l	450 l	3.84
G_M	15 Jun	Bittersalz	5.000 kg	450 l	3.84
G_M	24 Jun	Coragen	0.175 l	450 l	3.84
G_M	13 Jul	Bittersalz	5.000 kg	450 l	3.84
G_M	20 Aug	Bittersalz	5.000 kg	450 l	3.84
D_F	21 Mar	Chlorpyrifos	1.000 l	250 l	4.50
D_F	01 Jul	Runner	0.400 l	330 l	4.50
D_F	11 Jul	Coragen	175 ml	330 l	4.50
D_F	10 Aug	Coragen	175 ml	330 l	4.50
D_R	No data				
D_P	22 Mar	Chlorpyrifos	1.000 l	250 l	4.70
D_P	02 Jul	Runner	0.400 l	330 l	4.70
D_P	12 Jul	Coragen	175 ml	330 l	4.70
D_P	10 Aug	Coragen	175 ml	330 l	4.70
H_W	24 Mar	Headland Sulphur	3.000 l	300 l	0.62
H_W	24 May	Headland Sulphur	3.000 l	300 l	0.62
H_W	24 Mar	Sulphate of ammonia (SOA)	150.0 kg	-	0.62
H_W	21 May	Mg Sulph	7.500 kg	500 l	0.62
H_W	23 May	Headland Sulphur	3.000 l	300 l	0.62
H_W	29 May	Headland Sulphur	3.000 l	300 l	0.62
H_W	25 Jun	Sulphate of ammonia	75.00 kg	-	0.62

H_W	14 Jul	Mg Sulph	11.25 kg	300 l	0.62
H_W	01 Aug	Mg Sulph	11.25 kg	750 l	0.62
H_W	08 Aug	Mg Sulph	11.25 kg	750 l	0.62
H_W	13 Aug	Mg Sulph	11.25 kg	750 l	0.62
H_H	24 Mar	Headland Sulphur	3.000 l	300 l	1.50
H_H	24 Mar	Sulphate of ammonia	150.0 kg	-	1.50
H_H	17 May	Headland Sulphur	3.000 l	300 l	1.50
H_H	19 May	Mg Sulph	7.500 kg	500 l	1.90
H_H	23 May	Headland Sulphur	3.000 l	300 l	1.50
H_H	29 May	Headland Sulphur	3.000 l	300 l	1.50
H_H	10 Jun	Envidor	0.600 l	750 l	1.90
H_H	25 Jun	Sulphate of ammonia	75.00 kg	-	1.90
H_H	17 Jul	Mg Sulph	11.25 kg	300 l	1.90
H_H	01 Aug	Mg Sulph	11.25 kg	750 l	1.90
H_H	08 Aug	Mg Sulph	11.25 kg	750 l	1.90
H_H	13 Aug	Mg Sulph	11.25 kg	750 l	1.90
H_S	24 Mar	Headland Sulphur	3.000 l	300 l	5.68
H_S	24 Mar	Sulphate of ammonia	150.0 kg	-	5.68
H_S	17 May	Headland Sulphur	3.000 l	400 l	5.68
H_S	21 May	Mg Sulp	7.500 kg	500 l	5.68
H_S	23 May	Headland Sulphur	3.000 l	300 l	5.68
H_S	29 May	Headland Sulphur	3.000 l	300 l	5.68
H_S	06 Jun	Envidor	0.600 l	750 l	5.68
H_S	21 Jun	Headland Sulphur	3.000 l	300 l	5.68
H_S	25 Jun	Sulphate of ammonia	75.00 kg	-	5.68
H_S	14 Jul	Mg Sulph	11.25 kg	300 l	5.68
H_S	15 Jul	Mg Sulph	11.25 kg	750 l	5.68

H_S	29 Jul	Mg Sulph	11.25 kg	750 l	5.68
H_S	29 Jul	Coragen	0.175 l	750 l	5.68
H_S	08 Aug	Mg Sulph	11.25 kg	750 l	5.68
H_S	16 Aug	Mg Sulph	11.25 kg	750 l	5.68
S_L4 / S_M4	18 Mar	Surround	15.121 kg	-	13.4
S_L4 / S_M4	05 May	Karamate	2.000 kg	-	13.4
S_L4 / S_M4	19 May	Karamate	1.999 kg	-	13.4
S_L4 / S_M4	19 May	Karamate	1.999 kg	-	13.4
S_L4 / S_M4	19 May	Runner	0.599 l	-	13.4
S_L4 / S_M4	26 May	Headland Sulphur	1.987 l	-	13.4
S_L4 / S_M4	03 Jun	Karamate	2.000 kg	-	13.4
S_L4 / S_M4	03 Jun	Headland Sulphur	2.016 l	-	13.4
S_L4 / S_M4	03 Jun	Bittersalz	2.016 kg	-	13.4
S_L4 / S_M4	10 Jun	Headland Sulphur	1.993 l	-	13.4
S_L4 / S_M4	14 Jun	Anthopak 500	1.193 Flask	-	13.4
S_L4 / S_M4	17 Jun	Headland Sulphur	1.999 l	-	13.4
S_L4 / S_M4	17 Jun	Coragen	0.169 l	-	13.4
S_L4 / S_M4	24 Jun	Headland Sulphur	1.999 l	-	13.4
S_L4 / S_M4	1 Jul	Headland Sulphur	2.000 l	-	13.4
S_L4 / S_M4	8 Jul	Headland Sulphur	2.000 l	-	13.4
S_L4 / S_M4	8 Jul	Explicit	0.250 kg	-	13.4
S_L4 / S_M4	15 Jul	Headland Sulphur	1.993 l	-	13.4
S_L4 / S_M4	18 Jul	Headland Sulphur	1.999 l	-	13.4
S_L4 / S_M4	25 Jul	Bittersalz	6.250 kg	-	13.4
S_L4 - S_M4	29 Jul	Headland Sulphur	1.884 l	-	13.4
S_L4 / S_M4	29 Jul	Bittersalz	2.362 kg	-	13.4
S_L4 / S_M4	29 Jul	Coragen	0.160 l	-	13.4

S_L4 / S_M4	08 Aug	Headland Sulphur	1.993 l	-	12.4
S_L4 / S_M4	08 Aug	Bittersalz	2.506 kg	-	12.4
S_L4 / S_M4	19 Aug	Headland Sulphur	1.956 l	-	12.4
S_L4 / S_M4	19 Aug	Bittersalz	2.445 kg	-	12.4
S_L4 / S_M4	30 Aug	Headland Sulphur	2.500 l	-	12.4
S_L4 / S_M4	30 Aug	Bittersalz	2.500 l	-	12.4
S_L4 / S_M4	27 Sep	Sulphur	2.995 l	-	13.4
S_L4 / S_M4	27 Sep	Bittersalz	2.995 kg	-	12.4
S_L4 / S_M4	27 Sep	Envidor	0.599 l	-	12.4
S_H3	18 Mar	Surround	15.121	-	3.95
S_H3	05 May	Karamate	2.000 kg	-	3.95
S_H3	19 May	Karamate	2.000 kg	-	3.95
S_H3	19 May	Runner	0.599 l	-	3.95
S_H3	26 May	Headland Sulphur	1.987 l	-	3.95
S_H3	3 Jun	Karamate	2.000 kg	-	3.95
S_H3	3 Jun	Headland Sulphur	2.016 l	-	3.95
S_H3	3 Jun	Bittersalz	2.016 kg	-	3.95
S_H3	10 Jun	Headland Sulphur	1.993 l	-	3.95
S_H3	17 Jun	Karamate	1.999 kg	-	3.95
S_H3	17 Jun	Headland Sulphur	1.999 l	-	3.95
S_H3	17 Jun	Coragen	0.169 l	-	3.95
S_H3	24 Jun	Headland Sulphur	1.999 l	-	3.95
S_H3	1 Jul	Headland Sulphur	2.000 l	-	3.95
S_H3	8 Jul	Headland Sulphur	2.005 l	-	3.95
S_H3	8 Jul	Explicit	0.250 kg	-	3.95
S_H3	15 Jul	Headland Sulphur	1.993 l	-	3.95
S_H3	18 Jul	Headland Sulphur	1.999 l	-	3.95

S_H3	25 Jul	Bittersalz	6.250 kg	-	3.95
S_H3	29 Jul	Headland Sulphur	1.884 l	-	3.95
S_H3	29 Jul	Bittersalz	2.362 kg	-	3.95
S_H3	29 Jul	Coragen	0.160 l	-	3.95
S_H3	8 Aug	Headland Sulphur	1.993 l	-	3.95
S_H3	8 Aug	Bittersalz	2.506 kg	-	3.95
S_H3	19 Aug	Headland Sulphur	1.956 l	-	3.95
S_H3	19 Aug	Bittersalz	2.445 kg	-	3.95
S_H3	30 Aug	Sulphur	2.500 l	-	3.95
S_H3	30 Aug	Bittersalz	2.500 kg	-	3.95
S_H3	27 Sep	Sulphur	2.995 l	-	3.95
S_H3	27 Sep	Bittersalz	2.995 kg	-	3.95
S_H3	27 Sep	Envidor	0.599 l	-	3.95
A_Y	09 Mar	Calypso	0.375 l	250 l	1.70
A_Y	27 May	Headland Mg	3.000 l	250 l	1.70
A_Y	16 Jun	Coragen	0.175	250 l	1.70
A_Y	25 Jun	Wetcit	0.500 l	250 l	1.70
A_Y	11 Jul	BitterSalz	5.000 kg	250 l	1.70
A_B	09 Mar	Calypso	0.375 l	250 l	1.50
A_B	27 May	Headland Mg	3.000 l	250 l	1.50
A_B	05 Jun	Coragen	0.175 l	250 l	1.50
A_B	11 Jul	BitterSalz	5.000 kg	250 l	1.50
A_R	03 Jun	Headland Mg	3.000 l	250 l	1.56
A_R	26 Jun	Coragen	0.175 l	250 l	1.56
A_R	26 Jun	BitterSalz	5.000 kg	250 l	1.56
A_R	18 Jul	Wetcit	0.500 l	250 l	1.56
A_R	18 Jul	BitterSalz	5.000 kg	250 l	1.56

Spray records for the farms including insecticides and sprays targeted against pear sucker and honeydew in **2017**

Farm code	Date	Product	Dose/ha	Volume rate	Area (ha)
C_C	27 Apr	Runner	0.600 l	400l	5.35
C_C	20 May	Carpovirusine	1.000 l	1000l	5.35
C_C	12 Jun	Carpovirusine	1.000 l	1000l	5.35
C_C	26 Jun	Carpovirusine	1.000 l	1000l	5.35
C_G	27 Apr	Runner	0.600 l	400l	3.83
C_G	20 May	Carpovirusine	1.000 l	1000l	3.83
C_G	12 Jun	Carpovirusine	1.000 l	1000l	3.83
C_G	26 Jun	Carpovirusine	1.000 l	1000l	3.83
C_S	27 Apr	Runner	0.600 l	400l	7.27
C_S	20 May	Carpovirusine	1.000 l	1000l	7.27
C_S	12 Jun	Carpovirusine	1.000 l	1000l	7.27
C_S	26 Jun	Carpovirusine	1.000 l	1000l	7.27
G_N	17 Mar	Calypso	0.357 l	-	1.61
G_N	26 Apr	Epso Microtop	5.000 kg	-	1.61
G_N	06 May	Epso Microtop	5.000 kg	-	1.61
G_N	05 Jun	Coragen	0.175 l	-	1.61
G_N	05 Jun	Bittersalz	5.000 kg	-	1.61
G_N	17 Oct	Bittersalz	5.000 kg	-	1.61
G_B	17 Mar	Calypso	0.380 l	-	1.35

G_B	26 Apr	Epso Microtop	5.000 kg	-	1.35
G_B	06 May	Epso Microtop	5.000 kg	-	1.35
G_B	05 Jun	Coragen	0.175 l	-	1.35
G_B	05 Jun	Bittersalz	5.000 kg	-	1.35
G_B	17 Oct	Bittersalz	5.000 kg	-	1.35
G_M	17 Mar	Calypso	0.380 l	-	3.84
G_M	16 Apr	Bittersalz	3.000 kg	-	3.84
G_M	26 Apr	EPSO Microtop	5.000 kg	-	3.84
G_M	06 May	EPSO Microtop	5.000 kg	-	3.84
G_M	05 Jun	Coragen	0.175 l	-	3.84
G_M	05 Jun	Bittersalz	5.000 kg	-	3.84
G_M	05 Jul	Runner	0.600 l	-	3.84
G_M	17 Oct	Bittersalz	5.000 kg	-	3.84
D_F	02 Jun	Runner	0.600 l	400l	4.50
D_F	02 Jun	Envidor	0.600 l	400l	4.50
D_F	19 Jun	Coragen	175.0 ml	400l	4.50
D_F	02 Aug	Coragen	175.0 ml	500l	4.50
D_R	02 Jun	Runner	0.600 l	400l	7.00
D_R	02 Jun	Envidor	0.600 l	400l	7.00
D_R	19 Jun	Coragen	175.0 ml	400l	7.00
D_P	05 Jun	Runner	0.600 l	400l	4.70
D_P	05 Jun	Envidor	0.600 l	400l	4.70
D_P	20 Jun	Coragen	175.0 ml	400l	4.70
D_P	13 Jul	Coragen	175.0 ml	400l	4.70
H_W	08 Mar	Sulphate of ammonia	75.00 kg	-	0.62
H_W	9 Mar	Calypso	0.375 ml	500l	0.62
H_W	30 Mar	Calypso	0.375 ml	-	0.62

H_W	20 Apr	Runner	0.600 l	-	0.62
H_W	20 Apr	Mg Sulphate	5.000 kg	-	0.62
H_W	26 Apr	Lime	250.0 kg	-	0.62
H_W	12 May	Mg Sulphate	5.000 kg	-	0.62
H_W	16 May	Mg Sulphate	5.000 kg	-	0.62
H_W	27 May	Mg Sulphate	5.000 kg	-	0.62
H_W	05 Jun	Mg Sulphate	5.000 kg	-	0.62
H_W	13 Jun	Mg Sulphate	5.000 kg	-	0.62
H_W	14 Jun	Mg Sulphate	5.000 kg	-	0.62
H_W	21 Jun	Sulphate of ammonia	75.00 kg	-	0.62
H_W	15 Jul	Coragen	0.175 ml	-	0.62
H_G	08 Mar	Sulphate of ammonia	75.00 kg	-	5.50
H_G	20 Apr	Runner	0.600 l	-	5.50
H_G	20 Apr	Mg Sulphate	5.000 kg	-	5.50
H_G	10 May	Mainman	0.140 kg	-	5.50
H_G	10 May	Mg Sulphate	5.000 kg	-	5.50
H_G	13 May	Mg Sulphate	6.000 kg	-	5.50
H_G	16 May	Mg Sulphate	5.000 kg	-	5.50
H_G	23 May	Kieserite	200.0 kg	-	5.50
H_G	24 May	Envidor	0.600 l	500l	5.50
H_G	27 May	Mg Sulphate	5.000 kg	-	5.50
H_G	01 Jun	Mg Sulphate	6.000 kg	380l	5.50
H_G	01 Jun	Agricolle	1.140 l	380l	5.50
H_G	05 Jun	Mg Sulphate	5.000 kg	-	5.50
H_G	13 Jun	Mg Sulphate	5.000 kg	-	5.50
H_G	14 Jun	Mg Sulphate	5.000 kg	-	5.50
H_G	23 Jun	Mg Sulphate	5.000 kg	-	5.50

H_G	15 Jul	Coragen	0.175 ml	-	5.50
H_S	08 Mar	Sulphate of ammonia	125.0 kg	-	5.68
H_S	09 Mar	Calypso	0.375 l	500l	5.68
H_S	20 Apr	Runner	0.600 l	-	5.68
H_S	20 Apr	Mg Sulphate	5.000 kg	-	5.68
H_S	26 Apr	Lime	250.0 Kg	-	5.68
H_S	12 May	Mg Sulphate	5.000 kg	-	5.68
H_S	16 May	Mg Sulphate	5.000 kg	-	5.68
H_S	27 May	Mg Sulphate	5.000 kg	-	5.68
H_S	01 Jun	Envidor	0.600 l	-	5.68
H_S	05 Jun	Mg Sulphate	5.000 kg	-	5.68
H_S	13 Jun	Mg Sulphate	5.000 kg	-	5.68
H_S	14 Jun	Mg Sulphate	5.000 kg	-	5.68
H_S	21 Jun	Sulphate of ammonia	75.00 kg	-	5.68
H_S	21 Jun	Mg Sulphate	5.000 kg	-	5.68
H_S	15 Jul	Coragen	0.175 ml	-	5.68
S_L4 / S_M4	17 Mar	Surround	13.77 kg	-	13.4
S_L4 / S_M4	20 Mar	Kieserite	151.7 kg	-	13.4
S_L4 / S_M4	12 Apr	Karamate	1.479 kg	-	13.4
S_L4 / S_M4	12 Apr	Runner	0.592 l	-	13.4
S_L4 / S_M4	27 Apr	Karamate	1.997 kg	-	13.4
S_L4 / S_M4	11 May	Karamate	1.997 kg	-	13.4
S_L4 / S_M4	25 May	Karamate	1.479 kg	-	13.4
S_L4 / S_M4	04 Jun	Calcifert (Lime)	380.2 kg	-	13.4
S_L4 / S_M4	09 Jun	Bittersalz	2.500 kg	-	13.4
S_L4 / S_M4	15 Jun	Headland Sulphur	2.466 l	-	13.4
S_L4 / S_M4	15 Jun	Bittersalz	2.466 kg	-	13.4

S_L4 / S_M4	15 Jun	Coragen	0.165 l	-	13.4
S_L4 / S_M4	27 Jun	Headland Sulphr	2.466 l	-	13.4
S_L4 / S_M4	27 Jun	Bittersalz	2.466 kg	-	13.4
S_L4 / S_M4	07 Jul	Headland Sulphur	2.003 l	-	13.4
S_L4 / S_M4	07 Jul	Bittersalz	2.497 kg	-	13.4
S_L4 / S_M4	07 Jul	Explicit	0.250 kg	-	13.4
S_L4 / S_M4	14 Jul	Anthopak 500	0.986 Flask	-	13.4
S_L4 / S_M4	18 Jul	Headland Sulphur	1.973 l	-	13.4
S_L4 / S_M4	18 Jul	Bittersalz	2.466 kg	-	13.4
S_L4 / S_M4	28 Jul	Headland Sulphur	2.003 l	-	13.4
S_L4 / S_M4	28 Jul	Bittersalz	2.497 kg	-	13.4
S_L4 / S_M4	07 Aug	Headland Sulphur	1.973 l	-	13.4
S_L4 / S_M4	07 Aug	Bittersalz	2.466 kg	-	13.4
S_L4 / S_M4	19 Aug	Bittersalz	2.497 kg	-	13.4
S_L4 / S_M4	19 Aug	Explicit	0.250 kg	-	13.4
S_L4 / S_M4	25 Aug	Bittersalz	0.247 Kg	-	13.4
S_H3	17 Mar	Surround	13.77 kg	-	3.01
S_H3	20 Mar	Kieserite	151.7 kg	-	3.95
S_H3	12 Apr	Karamate	1.479 kg	-	3.58
S_H3	12 Apr	Runner	0.592 l	-	3.58
S_H3	27 Apr	Karamate	1.997 kg	-	3.58
S_H3	11 May	Karamate	1.997 kg	-	3.58
S_H3	25 May	Karamate	1.479 kg	-	3.58
S_H3	04 Jun	Calcifert (Lime)	380.2 kg	-	3.95
S_H3	09 Jun	Bittersalz	2.500 kg	-	3.58
S_H3	15 Jun	Headland Sulphur	2.466 l	-	3.58
S_H3	15 Jun	Bittersalz	2.466 kg	-	3.58

S_H3	15 Jun	Coragen	0.165 l	-	3.58
S_H3	27 Jun	Headland Sulphr	2.466 l	-	3.58
S_H3	27 Jun	Bittersalz	2.466 kg	-	3.58
S_H3	07 Jul	Headland Sulphur	2.003 l	-	3.58
S_H3	07 Jul	Bittersalz	2.497 kg	-	3.58
S_H3	07 Jul	Explicit	0.250 kg	-	3.58
S_H3	14 Jul	Anthopak 500	0.986 Flask	-	3.58
S_H3	18 Jul	Headland Sulphur	1.973 l	-	3.58
S_H3	18 Jul	Bittersalz	2.466 kg	-	3.58
S_H3	28 Jul	Headland Sulphur	2.003 l	-	3.58
S_H3	28 Jul	Bittersalz	2.497 kg	-	3.58
S_H3	07 Aug	Headland Sulphur	1.973 l	-	3.58
S_H3	07 Aug	Bittersalz	2.466 kg	-	3.58
S_H3	19 Aug	Bittersalz	2.497 kg	-	3.58
S_H3	19 Aug	Explicit	0.250 kg	-	3.58
S_H3	25 Aug	Bittersalz	0.247 Kg	-	3.58
A_Y	16 Apr	Bittersalz	3.000 kg	250l	1.70
A_Y	23 Apr	Epso Microtop	5.000 kg	250l	1.70
A_Y	04 May	Epso Microtop	5.000 kg	250l	1.70
A_Y	02 Jun	Bittersalz	5.000 kg	250l	1.70
A_Y	25 Jun	Bittersalz	5.000 kg	250l	1.70
A_Y	05 Jul	Mg Sulphate	5.000 kg	250l	1.70
A_Y	05 Jul	Runner	0.600 l	250l	1.70
A_B	16 Apr	Bittersalz	3.000 kg	250l	1.50
A_B	23 Apr	Epso Microtop	5.000 kg	250l	1.50
A_B	04 May	Epso Microtop	5.000 kg	250l	1.50
A_B	02 Jun	Bittersalz	5.000 kg	250l	1.50

A_B	25 Jun	Bittersalz	5.000 kg	250l	1.50
A_B	05 Jul	Mg Sulphate	5.000 kg	250l	1.50
A_B	05 Jul	Runner	0.600 l	250l	1.50
A_R	24 Apr	Epso Microtop	5.000 kg	250 l	1.56
A_R	05 May	Epso Microtop	5.000 kg	250 l	1.56
A_R	16 May	Epso Microtop	5.000 kg	250 l	1.56
A_R	27 May	Bittersalz	5.000 kg	250 l	1.56
A_R	29 Jun	Bittersalz	5.000 kg	250 l	1.56
A_R	12 Oct	Bittersalz	5.000 kg	250l	1.56

Spray records for the farms including insecticides and sprays targeted against pear sucker and honeydew in 2018

Farm code	**Date**	**Product**	**Dose/ha**	**Volume rate**	**Area (ha)**
C_C	05 Apr	Calypso	0.375 l	450 l	5.35
C_C	05 Apr	Solfa WG	3.000 kg	450 l	5.35
C_C	30 May	Runner	0.600 l	400 l	5.35
C_C	30 May	Calypso	0.375 l	450 l	5.35
C_C	04 June	Carpovirusine	1.000 l	1000 l	5.35
C_C	27 June	Carpovirusine	1.000 l	1000 l	5.35
C_C	11 July	Envidor	0.600 l	400 l	5.35
C_C	27 July	Magnesium Sulphate	4.000 kg	400 l	5.35
C_C	20 Sep	Magnesium Sulphate	4.000 kg	400 l	5.35
C_G	05 Apr	Calypso	0.375 l	450 l	3.83
C_G	05 Apr	Solfa WG	3.000 kg	450 l	3.83
C_G	30 May	Runner	0.600 l	400 l	3.83
C_G	30 May	Calypso	0.375 l	450 l	3.83
C_G	04 June	Carpovirusine	1.000 l	1000 l	3.83
C_G	27 June	Carpovirusine	1.000 l	1000 l	3.83

C_G	11 July	Envidor	0.600 l	400 l	3.83
C_G	27 July	Magnesium Sulphate	4.000 kg	400 l	3.83
C_G	20 Sep	Magnesium Sulphate	4.000 kg	400 l	3.83
C_S	05 Apr	Calypso	0.375 l	450 l	7.27
C_S	05 Apr	Solfa WG	3.000 kg	450 l	7.27
C_S	30 May	Runner	0.600 l	400 l	7.27
C_S	30 May	Calypso	0.375 l	450 l	7.27
C_S	04 June	Carpovirusine	1.000 l	1000 l	7.27
C_S	27 June	Carpovirusine	1.000 l	1000 l	7.27
C_S	11 July	Envidor	0.600 l	400 l	7.27
C_S	27 July	Magnesium Sulphate	4.000 kg	400 l	7.27
C_S	20 Sep	Magnesium Sulphate	4.000 kg	400 l	7.27
G_N	07 Mar	Headland Sulphur	2.000 l	450 l	1.61
G_N	29 Mar	Headland Sulphur	2.000 l	450 l	1.61
G_N	06 Apr	Calypso	0.375 l	450 l	1.61
G_N	11 Apr	Headland Sulphur	2.000 l	450 l	1.61
G_N	07 May	Epso Microtop	5.000 kg	450 l	1.61
G_N	17 May	Epso Microtop	5.000 kg	450 l	1.61
G_N	27 May	Epso Microtop	5.000 kg	450 l	1.61
G_N	16 June	Coragen	0.175 l	450 l	1.61
G_N	26 June	Bittersalz	5.000 kg	450 l	1.61
G_N	06 July	Karamate	2.000 kg	450 l	1.61
G_N	06 July	Bittersalz	5.000 kg	450 l	1.61
G_N	16 July	Karamate	2.000 kg	450 l	1.61
G_B	07 Mar	Headland Sulphur	2.000 l	450 l	1.35
G_B	29 Mar	Headland Sulphur	2.000 l	450 l	1.35
G_B	11 Apr	Headland Sulphur	2.000 l	450 l	1.35
G_B	07 May	Epso Microtop	5.000 kg	450 l	1.35
G_B	27 May	Epso Microtop	5.000 kg	450 l	1.35

G_B	16 June	Coragen	0.175 l	450 l	1.35
G_B	26 June	Bittersalz	5.000 kg	450 l	1.35
G_B	06 July	Karamate	2.000 kg	450 l	1.35
G_B	06 July	Bittersalz	5.000 kg	450 l	1.35
G_B	16 July	Karamate	2.000 kg	450 l	1.35
G_M	07 Mar	Headland Sulphur	2.000 l	450 l	3.84
G_M	29 Mar	Headland Sulphur	2.000 l	450 l	3.84
G_M	06 Apr	Calypso	0.375 l	450 l	3.84
G_M	11 Apr	Headland Sulphur	2.000 l	450 l	3.84
G_M	07 May	Epso Microtop	5.000 kg	450 l	3.84
G_M	17 May	Epso Microtop	5.000 kg	450 l	3.84
G_M	27 May	Epso Microtop	5.000 kg	450 l	3.84
G_M	26 June	Bittersalz	5.000 kg	450 l	3.84
G_M	06 July	Karamate	2.000 kg	450 l	3.84
G_M	06 July	Bittersalz	5.000 kg	450 l	3.84
G_M	16 July	Karamate	2.000 kg	450 l	3.84
D_F	01 Apr	Calypso	0.375 l	250 l	4.50
D_F	01 May	Steward	0.200 kg	330 l	4.50
D_F	07 Jun	Batavia	1.500 l	500 l	4.50
D_F	11 Jun	Clayton Courage	0.175 l	400 l	4.50
D_F	02 Jul	Runner	0.600 l	400 l	4.50
D_F	18 Jul	Coragen	175.0 l	400 l	4.50
D_R	01 Apr	Calypso	0.375 l	250 l	7.00
D_R	01 May	Steward	0.200 kg	330 l	7.00
D_R	07 Jun	Batavia	1.500 l	500 l	7.00
D_R	11 Jun	Clayton Courage	0.175 l	400 l	7.00
D_R	02 Jul	Runner	0.600 l	400 l	7.00
D_R	18 Jul	Coragen	175.0 ml	400 l	7.00
D_P	31 Mar	Calypso	0.375 l	250 l	4.70

D_P	06 Apr	Surround WP	12.50 kg	250 l	4.70
D_P	03 May	Steward	0.200 kg	330 l	4.70
D_P	08 Jun	Batavia	1.500 l	500 l	4.70
D_P	12 Jun	Clayton Courage	0.175 l	400 l	4.70
D_P	02 Jul	Runner	0.600 l	400 l	4.70
H_W	13 MAr	SOA	175.0 kg	*	0.62
H_W	19 Mar	Calypso	0.375 ml	300 l	0.62
H_W	19 Mar	Headland Sulphur	3.000 l	300 l	0.62
H_W	21 Mar	Headland Sulphur	3.000 l	300 l	0.62
H_W	16 Apr	Kieserite	200.0	300 l	0.62
H_W	09 May	Runner	0.600 l	300 l	0.62
H_W	09 May	Magnesium Sulphate	5.000 kg	300 l	0.62
H_W	25 Jun	Magnesium Sulphate	7.500 kg	500 l	0.62
H_W	25 Jun	Soap	1.500 kg	500 l	0.62
H_W	25 Jun	Coragen	0.175 ml	300 l	0.62
H_W	25 Jun	Magnesium Sulphate	5.000 kg	300 l	0.62
H_W	03 Jul	Magnesium Sulphate	5.000 kg	300 l	0.62
H_W	17 Jul	Coragen	0.175 ml	300 l	0.62
H_G	13 Mar	SOA	175.0 kg	*	6.00
H_G	21 Mar	Headland Sulphur	3.000 l	*	6.00
H_G	16 Apr	Kieserite	200.0 kg	*	6.00
H_G	09 May	Runner	0.600 l	300 l	6.00
H_G	09 May	Magnesium Sulphate	5.000 kg	300 l	6.00
H_G	26 May	Magnesium Sulphate	7.500 kg	500 l	6.00
H_G	26 May	Soap	1.500 kg	500 l	6.00
H_G	15 Jun	Batavia	1.500 kg	500 l	6.00
H_G	25 Jun	Coragen	0.175 ml	300 l	6.00
H_G	25 Jun	Magnesium Sulphate	5.000 kg	300 l	6.00
H_G	17 Jul	Coragen	0.175 ml	300 l	6.00

H_S	13 Mar	SOA	175.0 kg	*	5.68
H_S	19 Mar	Calypso	0.375 ml	300 l	5.68
H_S	19 Mar	Headland Sulphur	3.000 l	300 l	5.68
H_S	21 Mar	Headland Sulphur	3.000 l	*	5.68
H_S	28 Mar	Lime	250.0 kg	*	5.68
H_S	09 May	Runner	0.600 l	300 l	5.68
H_S	07 Jun	Magnesium Sulphate	7.500 kg	500 l	5.68
H_S	07 Jun	Soap	1.500 l	500 l	5.68
H_S	20 Jun	Magnesium Sulphate	7.500 kg	500 l	5.68
H_S	20 Jun	Soap	1.500 l	500 l	5.68
H_S	25 Jun	Coragen	0.175 ml	300 l	5.68
H_S	25 Jun	Magnesium Sulphate	5.000 kg	300 l	5.68
H_S	28 Jun	Magnesium Sulphate	7.500 kg	500 l	5.68
H_S	28 Jun	Soap	1.500 l	500 l	5.68
H_S	03 Jul	Magnesium Sulphate	5.000 kg	300 l	5.68
H_S	17 Jul	Coragen	0.175 ml	300 l	5.68
S_L4 S_M4 S_H3	11 Apr	Anthopak 500	1.901 Flask	*	13.40 13.40 3.58
S_L4 S_M4 S_H3	13 Apr	Headland Sulphur	2.028 l	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	19 Apr	Headland Sulphur	2.028 l	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	01 May	Kamarate	1.996 kg	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	16 May	Kamarate	1.521 kg	250 l	13.40 13.40 3.58
S_L4	24 May	Headland Sulphur	2.028 l	250 l	13.40

S_M4 S_H3					13.40 3.58
S_L4 S_M4 S_H3	24 May	Bittersaltz	2.535 kg	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	24 May	Insegar WG	0.596 kg	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	01 Jun	Kamarate	1.584 kg	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	01 Jun	Headland Sulphur	2.091	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	01 Jun	Bittersaltz	2.598 kg	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	06 Jun	Headland Sulphur	2.028 l	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	06 Jun	Bittersaltz	2.535 kg	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	15 Jun	Coragen	0.174 l	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	15 Jun	Headland Sulphur	2.028 l	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	15 Jun	Bittersaltz	2.535 kg	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	19 Jun	Sprayguard	0.221 l	666 l	13.40 13.40 3.58

S_L4 S_M4 S_H3	22 Jun	Headland Sulphur	2.028 l	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	22 Jun	Bittersaltz	2.535 l	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	28 Jun	Headland Sulphur	2.028 l	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	28 Jun	Bittersaltz	2.535 kg	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	05 jul	Kamarate	1.984 kg	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	05 jul	Headland Sulphur	1.996 l	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	05 jul	Bittersaltz	2.472 kg	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	05 jul	Explicit	0.250 kg	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	12 Jul	Headland Sulphur	1.996 l	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	12 Jul	Bittersaltz	2.503 kg	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	18 Jul	Headland Sulphur	1.996 l	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	18 Jul	Bittersaltz	2.503 kg	250 l	13.40 13.40 3.58

S_L4 S_M4 S_H3	26 Jul	Bittersaltz	2.503 kg	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	26 Jul	Clayton Courage	0.175 l	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	03 Aug	Bittersaltz	2.503 kg	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	08 Aug	Bittersaltz	2.503 kg	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	14 Aug	Anthopak 500	1.901 Flask	*	13.40 13.40 3.58
S_L4 S_M4 S_H3	15 Aug	Bittersaltz	2.35 kg	250 l	13.40 13.40 3.58
S_L4 S_M4 S_H3	15 Aug	Steward	0.250 kg	250 l	13.40 13.40 3.58
A_Y	16 Mar	Headland Sulphur	2.000 l	250 l	1.70
A_Y	22 Mar	Headland Sulphur	2.000 l	250 l	1.70
A_Y	30 Mar	Headland Sulphur	2.000 l	250 l	1.70
A_Y	02 Apr	Calypso	0.375 l	500 l	1.70
A_Y	07 Apr	Headland Sulphur	2.000 l	250 l	1.70
A_Y	14 May	Epso Microtop	5.000 kg	250 l	1.70
A_Y	23 May	Epso Microtop	5.000 kg	250 l	1.70
A_Y	After 31 May	Coragen	0.175 l	250 l	1.70
A_Y	20 Jun	Kamarate	2.000 kg	475 l	1.70
A_Y	20 Jun	Coragen	0.175 l	475 l	1.70
A_Y	29 Jun	Kamarate	2.000 kg	475 l	1.70

A_Y	29 Jun	Bittersaltz	5.000 kg	475 l	1.70
A_Y	04 Jul	Bittersaltz	5.000 kg	500 l	1.70
A_Y	07 Jul	Bittersaltz	5.000 kg	475 l	1.70
A_Y	03 Oct	Bittersaltz	5.000 kg	300 l	1.70
A_B	16 Mar	Headland Sulphur	2.000 l	250l	1.50
A_B	22 Mar	Headland Sulphur	2.000 l	250l	1.50
A_B	30 Mar	Headland Sulphur	2.000 l	250l	1.50
A_B	07 Apr	Headland Sulphur	2.000 l	250 l	1.50
A_B	14 May	Epso Microtop	5.000 kg	250 l	1.50
A_B	23 May	Epso Microtop	5.000 kg	250 l	1.50
A_B	After 31 May	Coragen	0.175 l	250 l	1.50
A_B	20 Jun	Kamarate	2.000 kg	475 l	1.50
A_B	20 Jun	Coragen	0.175 l	475 l	1.50
A_B	29 Jun	Kamarate	2.000 kg	475 l	1.50
A_B	29 Jun	Bittersaltz	5.000 kg	475 l	1.50
A_B	04 Jul	Bittersaltz	5.000 kg	500 l	1.50
A_B	03 Oct	Bittersaltz	5.000 kg	300 l	1.50
A_R	11 Apr	Calypso	0.375 l	500 l	1.56
A_R	13 May	Epso Microtop	5.000 kg	250 l	1.56
A_R	24 May	Epso Microtop	5.000 kg	250 l	1.56
A_R	04 Jun	Coragen	0.175 l	250 l	1.56
A_R	25 Jun	Headland Sulphur	2.000 l	250 l	1.56
A_R	25 Jun	Bittersaltz	5.000 kg	250 l	1.56
A_R	05 Jul	Headland Sulphur	2.000 l	250 l	1.56
A_R	13 Jul	kamarate	2.000 kg	250 l	1.56
A_R	13 Jul	Bittersaltz	5.000 kg	250 l	1.56
A_R	28 Jul	Coragen	0.175 l	250 l	1.56

