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Project leader:	Dr Robert Saville NIAB EMR
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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.

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GROWER SUMMARY

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, a weevil affecting pear buds, pear sucker and associated natural enemies. In the third year, reported herein, 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 third year. Full details of each objective are presented in the Science Section of the 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.

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 2016 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*).

Drosophila suzukii (SWD) numbers were particularly high in April and late summer in 2017 compared to previous years. Despite this, fewer incidences of cherry damage were reported, probably due to the previous experience and revised management of cherry. In autumn 2017 trap catches were almost double the previous year, at least partly due to a mild October and November in 2017. However, in general numbers and damage increased in most monitored regions in 2017. Fruit damage in wild blackberry was recorded for the first time in Scotland and the pest is now present in Ireland.

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.

No monitoring traps for **Brown Marmarated Stink Bug** (BMSB) were in place in 2017. However, a new trap has become available which is more specific and works on the 2 main phases of the lifecycle. These have been ordered and will be deployed in amenity gardens from April 2018.

A weevil found in pear orchards which has been, over the last 2-3 years, damaging spring flower and leaf buds was identified as *Anthonomus spilotus* by the Natural History Museum and NIAB EMR in 2017 and is new to the UK. It has also recently been identified as an invasive pest in Belgium. More details are provided under Objective 10.

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.

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 your business. 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 AHDB/DEFRA apple best practice guide to limit loss of apples in store.
- Keep an eye on trade press for important announcements from the animal and plant health agency (APHA) about invasive pests and diseases which will affect your business such as *Xylella fastidiosa.*

Objective 2. Neonectria

Headlines

- Long-term trials have been established to determine the effects of rootstock/interstock choice and biological soil amendments on susceptibility/tolerance to European apple canker.
- The first year of trials have identified treatments which reduce pruning wound infection by the canker pathogen.

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 including developing diagnostic tools, rootstock/interstock choice, biological soil amendments and novel delivery systems which, together with other projects will contribute to the development of a systems approach for canker control from nursery to orchard.

Summary of the project and main conclusions

A diagnostic tool has been developed and is currently being utilised in AHDB PhD studentship (CP161) to increase our understanding of latent non-symptomatic canker infections with the ultimate goal of developing a sampling strategy to deploy the diagnostic tool in the nursery.

Long-term trials have been established on multiple sites to determine the effect of rootstock/interstock and biological soil amendments on canker resistance/tolerance. The rootstock trials are evaluating a panel of rootstocks commonly used today alongside several

advanced selections from the NIAB EMR and Geneva rootstock breeding programmes. The amendment trials are evaluating the effect of arbuscular mycorrhizae fungi (AMF), plant growth promoting rhizobacteria (PGPR), Trichoderma and Biochar in both newly planted orchards and stool beds. These trials are now established but their long term nature means that we need to collect data over the remaining two years of the project before conclusions can be drawn.

Two treatment delivery systems have been evaluated in this project; a pruning wound protection device (the Felco19 system) and a tree injection system (fertiyect) with the aim of better targeting treatments to the vulnerable areas of the plant such as pruning wounds and eradicating systemic infection respectively. Using the Felco19 device, five treatments (Folicur (tebuconazole), blocade (a physical barrier), T34 (a Trichoderma strain) and combinations thereof) were evaluated against an untreated control. Folicur alone and Blocade in addition to Folicur or T34 all reduced infection significantly compared to the untreated control. Another year of trials will be carried out to confirm the benefits of these products and a Trichoderma species better adapted to aerial environments will be tested instead of T34. Fertinject devices provide an inexpensive and easy to use system for tree injection which effectively distributes treatments through the tree. Products evaluated to date (including conventional plant protection products, defence elicitors and biological based products) have not shown sufficient efficacy to recommend tree injection as part of an integrated programme. Next season's trials will develop a screening method to evaluate differerent actives with varying modes of action in controlled environment facilities.

Financial benefits

European apple canker is a devastating disease that has an economic impact through the chain from plants in the nursery to fruit in the store. This project endeavours to focus on key areas within the chain to develop an integrated approach to canker control and reduce financial losses caused by this disease. Currently these approaches are still being evaluated and will be summarised in subsequent reports.

Action points for growers

- It is hoped to develop a commercially available diagnostic tool to determine disease risk in the nursery.
- Results from the rootstock and soil amendment trials will inform best practice planting guidelines which mitigate against canker expression in the field. These will not be available until the end of the project.

- In the meantime it is important to be vigilant with visual inspection, roguing out any trees which are showing symptoms and limiting abiotic stress as far as possible when planting out and establishing new orchards.

Objective 3. Apple foliar diseases

Headline

- Alternatives to conventional fungicides are showing promise for in-season mildew control as part of a reduced fungicide programme.

Background and expected deliverables

Foliar diseases of apple 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. 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 develop an integrated programme focused on reducing inoculum, promoting tree health/resistance and evaluating 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 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. It was important to have these blocks differing in mildew incidence as the largest differences in mildew between the test products was in the high mildew plots. Most of the test products over the whole season significantly reduced mildew incidence compared to the fungicide only plots. SB Invigorator was the most consistent in

reducing mildew, confirming results from 2015 and 2016. AHDB9910 2 years, AHDB9910 + AHDB9904 and AHDB9904 only were almost as effective. AHDB9910 2 years had almost significantly less mildew than plots receiving AHDB9910 for the first year, indicating a possible cumulative effect of this product. Including AHDB9904 with AHDB9910 also improved performance, but this may have been due to the AHDB9904 only. AHDB9908 performed as well as in 2016 but did not cause leaf spotting or russeted fruit as in 2016. This is most likely due to a change in the wetter included in the formulation.

Financial benefits

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

Action points for growers

- Careful monitoring of the mildew epidemic is essential to rationalise fungicide use.
- Where primary mildew levels are high, prompt physical removal of mildewed blosoms and shoots is necessary. Alternative control strategies based on biological control products are being investigated.
- Alternative products for in-season control to supplement a reduced fungicide programme have been identified, but further evaluation needs to be conducted before programmes can be recommended.

Objective 4. Stone fruit diseases

Headline

- Evaluation of alternative treatments for Monilinia diseases commenced in the 2017 season but due to severe frosts and SWD damage the results were not possible to interpret – Trials will be repeated in 2018

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 both 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. Fungicides are applied at blossom and pre-harvest including Bellis, Signum, Switch, Systhane (and other myclobutanil containing products) 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.

Summary of the project and main conclusions

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 affected by SWD prior to harvest, despite weekly application of control products. 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.

Financial benefits

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

Action points for growers

- Orchard sanitation is important for brown rot control, removing all mummies and wood showing cankers from the orchard.
- Blossom and pre harvest application of fungicides are required dependent on risk. Trials in 2018 will evaluate new fungicide based and fungicide alternative treatments to add to the current registered products.

Objective 6. Codling and tortrix moth – Blastobasis monitoring

Headline

- This objective aimed to validate and optimise a pheromone trap for Blastobasis as part of an integrated programme for Codling and Tortrix control as more growers use the RAK 3+4 MD system.

Background and expected deliverables

Larvae of the moth Blastobasis lacticolella, Rebel, 1940 (Synonym:decolorella) (Lepidoptera: Blastobasidae) (Figure 10.1) feed on the surface of the pear and apple 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. 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. Increased use of pheromone mating disruption and granulovirus, the move towards reducing the occurrence of product residues on fruits and the loss of crop protection products have meant that the products 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 products which negated the advantages of using mating disruption. 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 can time product applications accordingly.

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 in all probability, none were *Blastobasis lacticollela* (Synonym decolorella). 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. In 2018 work will focus on obtaining virgin adults of Blastobasis laticollela for pheromone and molecular analysis, rather than further testing of candidate pheromone blends. Attempts will be made to rear larvae collected during 2017 and to collect pupae or adults from sites in Northern Ireland during 2018.

Financial benefits

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

Action points for growers

- Not action points have been identified at this stage of the project.

Objective 7.1 Improving the reliability of natural predation of pests

Headline

- Six trial orchards have been set up to monitor the benefits of speeding up the ecology of newly planted orchards in establishing beneficial arthropods more quickly to mitigate losses due to pests.

Background and expected deliverables

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.

Summary of the project and main conclusions

We applied interventions to newly planted orchards in order to establish more rapidly the beneficial ecology. The alleyway sowings are completed at all sites and most orchards have now established. Earwig refuges were deployed in autumn 2017. Monitoring in 2018 will provide detailed data on the establishment of orchards with and without interventions.

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

- A study of pear sucker and its natural enemies has been set up on six commercial pear farms.

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 AHDB Project TF 196 has demonstrated that the use and timing of crop protection products may be, at least partly responsible. However, anecdotal evidence is showing that earwigs can be distributed in patches within an individual orchard.

The aim of this study is to enable more effective monitoring, crop protection 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 and 2017. 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 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.

Financial benefits

Close monitoring of pear sucker and its natural enemies can prevent the application 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 reduces crop loss through the maintenance of high fruit quality and prevents 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.
- Monitor natural enemies such as earwigs, anthocorids and ladybirds alongside pear sucker populations to track the likely future control by these predators in the absence of sprays.
- Enter numbers and information into a spreadsheet to get an overall picture of when natural enemies 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 numbers of natural enemies are low, but think about the surrounding habitat to encourage long term resilience in populations.
- Be careful with spray application. Think about spray frequency and its impact on natural enemies
- Aim to achieve;

  <1,000 pear sucker eggs per 30 shoots per week

  >10 natural enemies per 30 shoots per week

Objective 8. Apple sawfly

Headline

Work has begun 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. The aim of this project is 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 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 two were stored at 6^{o}C for two months to attempt to simulate a cold period. To date, no adults have emerged, but pots will be brought 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

- A new damaging weevil pest of pear blossom has been identified as *Anthonomus spilotus* and is new to the UK.

Background and expected deliverables

A new pest of pear was identified. The weevil is from the *Anthonomus* family of weevils known to feed and develop in buds and fruits of plants. Unlike *Anthonomus piri* (pear bud weevil), *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 insecticides. More research is needed to establish thresholds and to target spray timing more precisely.

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 tap sampling tree branches to check for the presence of *Anthonomus spilotus*.
- Check for feeding holes in flower and leaf buds.
- Continue to monitor until May.
- Make a careful decision over the need to use control measures and the choice of product.
- Continue to monitor for the pest after control methods have been used.

SCIENCE SECTION

General Introduction

This 5 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 losses; 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 based on 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 maybe done through the new AHDB programme – SCEPTREplus. 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, 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 the 2017 season and has been submitted to the project coordinator. Analysed data will be made available as part of the wider project. As in 2016 the severity of the disease epidemic on the *Vf* (scab resistance gene) containing cultivars was comparable to the disease incidence on Gala. Confirming that the local scab population has broken the resistance conferred by *Vf*. Isolates of scab on the *Vf* containing cultivars have been collected for DNA extraction to determine the genetic changes in the population which has resulted in breaking the resistance which will in turn inform the identification of new sources of resistence.

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

Table 1.1 summarises the losses attributed to each rot pathogen during the 2016/17 storage season. In total 52 samples were assessed over 19 visits. The main cultivars sampled were Gala (15 samples), Bramley (9), Cox (8), Braeburn (7) and Jazz (7). The overall average loss was 1.5% which is lower than recent past surveys. Losses of Cox (4.6%) and Bramley (2.7%) were high but in line with the losses usually experienced in these varieties which is attributed to their higher storage temperature. The variety with the next highest losses, as with previous

surveys, was Gala (1.2%). The other apple and pear varieties sampled did not experience significant losses (all ≤1%). Nectria rot was the main rot identified in the 2016/17 survey with an overall incidence of 31.3%. Necria rot was particularly high in canker susceptible varieties where inoculum is prevalent; Gala (61%) and Jazz (68%), although loses due to nectria rot in Braeburn were equivalent to losses due to penicillium and gleosporium. Brown rot (*Monililnia*) is the next most prevalent rot causing an overall average loss of 19.3% followed by *Gloeosporium* (12.4%), *Penicillium* (11.2%) and Botrytis (9.2%). *Phytophthora* was only found in a single sample.

Table 1.1 The average loss (%) attributed to each rot pathogen during the 2016/17 storage season. The data is compiled from 52 samples.

	Average % of loss attributed to each rot;															
Cultivar	**Brown rot**	***Botyrtis***	***Penicillium***	***Phytopthora***	***Nectria***	***Gloeosporium***	***Fusarium***	***Mucor***	***Botryosphiria***	***Phomopsis***	**Stalk**	**Eye**	**Cheek**	**Core**	**Number of samples**	**Loss (%)**
Braeburn	11.2	10.3	23.9	0.0	23.5	22.5	0.0	7.2	0.0	0.0	0.0	0.0	1.2	0.2	7	0.2
Bramley	24.8	0.7	7.3	0.0	23.2	0.0	1.6	0.3	0.0	0.2	13.4	1.4	4.0	22.6	9	2.7
Conference	44.8	17.3	19.0	0.0	12.5	0.0	0.0	4.8	0.0	0.0	0.0	0.0	1.6	0.0	2	0.1
Cox	11.9	8.5	5.6	0.0	22.8	44.4	0.0	1.0	0.0	0.0	0.0	1.3	0.8	3.3	8	4.6
Gala	15.4	6.8	4.3	0.0	61.0	9.8	0.0	0.4	0.0	0.0	0.3	0.0	0.2	0.6	15	1.2
Jazz	0.8	14.2	2.9	0.0	67.8	0.0	0.0	14.3	0.0	0.0	0.0	0.0	0.0	0.0	7	0.5
Other dessert	26.0	9.9	15.2	10.9	8.6	10.2	0.9	1.6	0.0	0.0	0.0	1.6	2.7	12.5	4	1.5
Overall average	**19.3**	**9.7**	**11.2**	**1.6**	**31.3**	**12.4**	**0.4**	**4.2**	**0.0**	**0.0**	**2.0**	**0.6**	**1.5**	**5.6**	**-**	**1.5**

Discussion

Relatively low overall losses (1.5%) were recorded during the 2016/17 rot survey compared to recent years which is likely to be a result of a very dry harvesting window in the 2016 growing season with just 28mm of rain falling over September and October (East Malling weather station data). The very low levels of Phytophthora recorded (only found in a single sample) will have been a result of the exceptionally dry harvesting period which will have also reduced the incidence of the pathogens which can infect pre harvest such as Nectria and Gloeosporium rots.

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. The following table summarises recent and new invasive species which are currently causing 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. Drosophila suzukii numbers were particularly high in April and late summer in 2017 compared to previous years. Higher incidence of 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 is peaked to almost double winter 2016/17. Fruit damage was recorded for the first time in Scotland and the pest is now present in Ireland.
	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	No monitoring traps for Brown Marmarated stink bugs were in place in 2017. However, a new trap has become available which is more specific and works on the 2 main phases of the lifecycle. We will deploy 10 sentenal traps from March 2018.
Weevil of pear	The weevil which has been, over the last 2-3 years, damaging spring flower and leaf buds was identified as *Anthonomus spilotus* by NHM and NIAB EMR in 2017 and is new to the UK. It has also been recently identified as an invasive pest in Belgium. More details are in Objective 10. The finding was published; Morris M.G., Mendel M., Barclay, M.V.L. Booth R.G., Cannon M.F.L., Conroy C.E., Csokay L.K., Fisher C., Fountain M.T., 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.
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 effects 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 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. The maggots develop slowly in the green fruit and usually do not complete their growth until the infested fruits have dropped from the tree. Larval development: from 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. Egg is elongate, subcylindrical, white and about 6 mm long. Larvae is elongate, cylindrical, up to 56 mm long. 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 occuring on natural vegetation in Europe was in 2001. Eradication efforts are underway in Italy. Attacks many species of living tree (>26 families) including Citrus, *Malus domestica*, apricot, European pear. Eggs laid through bark (T-shaped slit) close to ground level. Larva bores into the stem and destroys the pith and vascular system later entering 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.
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. The full grown larva is about 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 1-1.2 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). Extremely 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 practically the entire leaf. Radicicolae form: Numerous knots or galls form on grapevine roots, with rotting of the roots, yellowing of the 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). 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.
Phoma/ 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 *Phomopsis*. Subsequently sequenced to determine species level identification as *Phomopsis 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 arboricolae, 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

Objective 2. Neonectria ditissima

2.2 Rootstock/interstock

Aim

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

Introduction

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 2.1. Rootstocks were bench grafted on to a common scion (cv. Gala) in February 2016. The trees were released in June and 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 2.1. The 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	29th 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 x1.75m

Aerial view:

Trial layout:

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

WINDBREAK

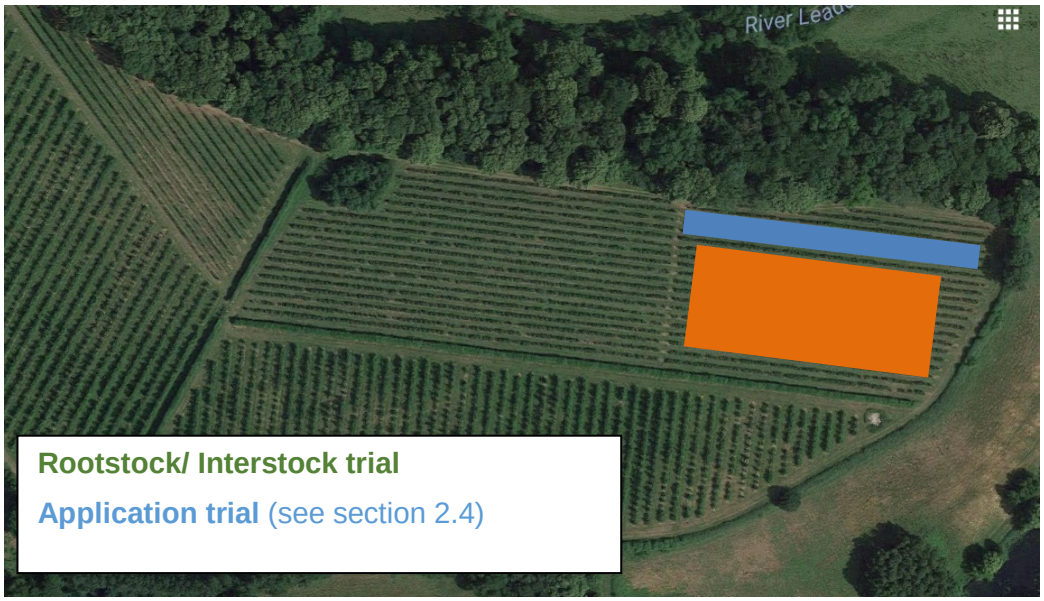
G.41		MM106		EMR-004		M9 (337)		EMR-003		M9 (337) interstock GD		M116		EMR-002	
EMR-005		M9 (EMLA)		M116		EMR-006		M9 (EMLA)		M116		EMR-001		G.11	
M116		EMR-004		G.41		M9 (EMLA)		EMR-002		EMR-004		M9 (337)		M9 (EMLA)	
EMR-003		M9 (337) interstock GD		EMR-002		EMR-005		EMR-006		EMR-005		MM106		M9 (337)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

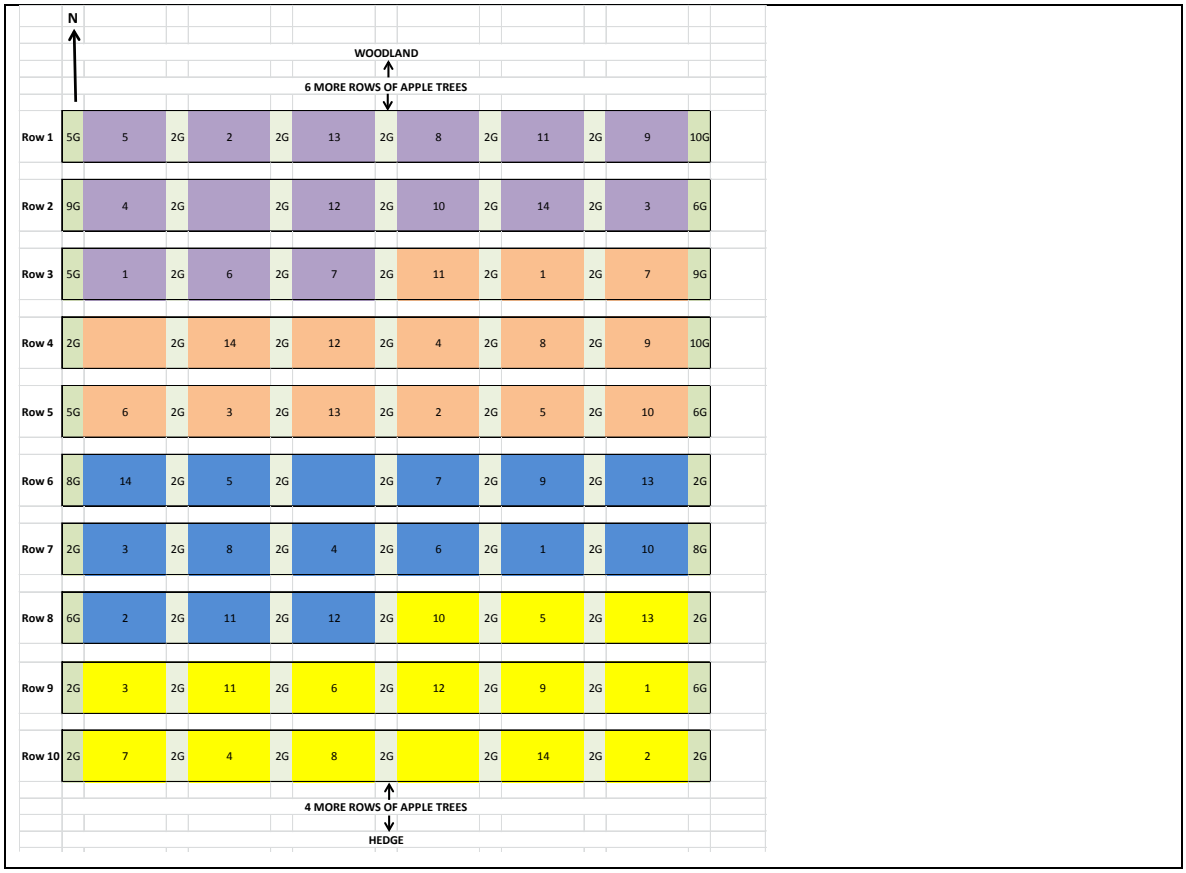
Site 2	Herridges Orchard, Ketford Road, Poolhill nr. Newent, Gloucestershire. GL18 1LW
Grid reference	51.966956, -2.3953805
Planted	15th March 2017
Description of planting site:	The trial was planted on the site of an old Cox orchard. 2 cox trees were left in the ground between each plot to serve as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

Aerial view:



Trial layout:

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



Natural infections

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.

Assessments

Site 1 - East Egham

Assessments were conducted on the 28th August 2017. 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 2.1). 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. A sample of the cankered branches was returned to the lab to confirm if *Neonectria dittisima* was the cause.

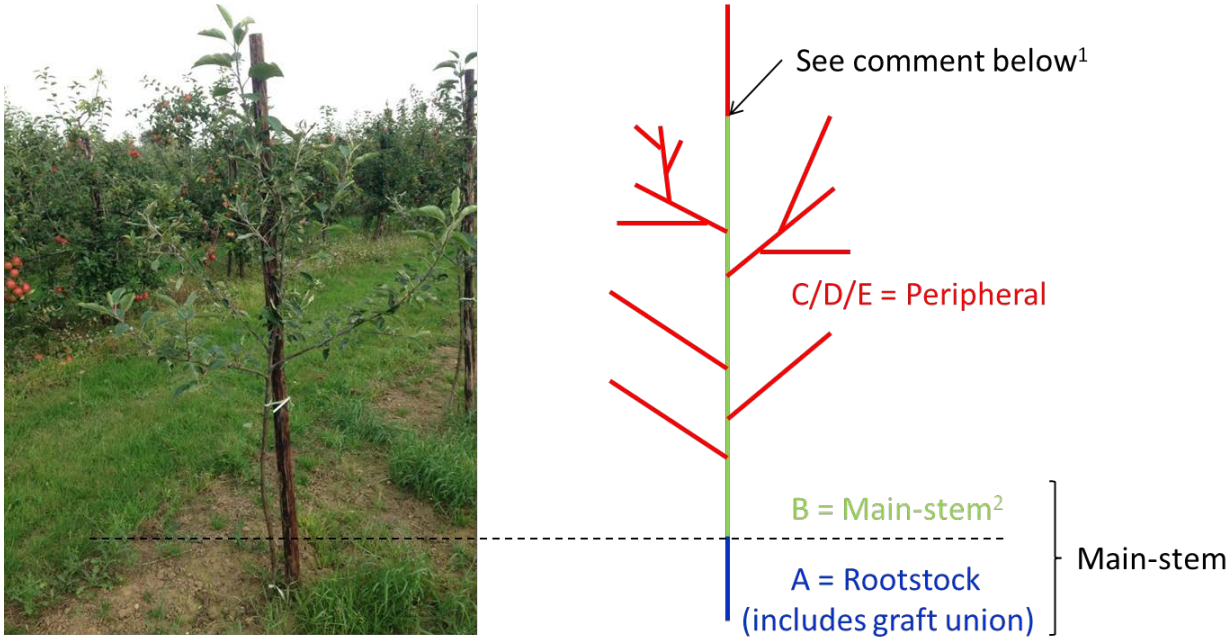


Figure 2.1. Diagram of the classifications of cankers based on their position within the tree. [1] note that there is a continuum between the main-stem and peripheral branch on the main leader; cankers on the 1 year wood were scored as peripheral and those on the ≥2yr wood were scored as main-stem. [2] cankers occurring on the interstem in treatment 8 (M9 with Golden Delicious interstem) were scored as 'B' – main stem.

Site 2 - Herridges Orchard

Assessments were conducted on 5th October 2017. Assessments were conducted as per site 1 above.

Artificial inoculations (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 (16th November, - 17th November), 8 trees per treatment (2 replicate trees per block from 4 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 5µl of *N. dittissima* 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 16th November, and blocks 3 & 4 on 17th 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. A frost was recorded on the night following the 16th (Minimum air temperature recorded at East Malling -1°C) and 17th (Minimum air temperature recorded at East Malling -0.3°C) November.

Statistical analyses

Each individual dataset was analysed by ANOVA. The two sites were analysed separately. Figures in bold highlight where rootstock effect was significantly different. An ANOVA was also performed on stacked data in which each type (position) of canker was analysed together. Fisher's protected least significant difference test was performed where there were significant differences.

Results

Site 1 – East Egham

Tree establishment was good and as of August 2017 (5 months after planting) ten trees (2%) had died mainly through the lack of root establishment (7) or the failure of the graft to take (3). Strimming was used as part of a weed control programme and 21 trees (5%) were severely damaged at the base due to poor strimmer control. A high level of dieback, not associated with Nectria canker, was noticeable early on in orchard establishment. The dieback was localised in the apex of the tree and was only present in certain treatments (Figure 2.2). During the canker assessment the dieback was recorded as number of branches affected and pruned out of the tree. Samples of the branches were returned to the laboratory to determine cause by isolation and found to be caused by a *Phomopsis* sp. The position of the dieback infection in the apex of the tree is consistent with infection through wounds created at heading back,

which may also explain the significant treatment (rootstock) effect as each rootstock was headed back at different times, based on when they reached the top of the cane, and inoculum presence and conditions for infection would have varied depending on when they were headed back.

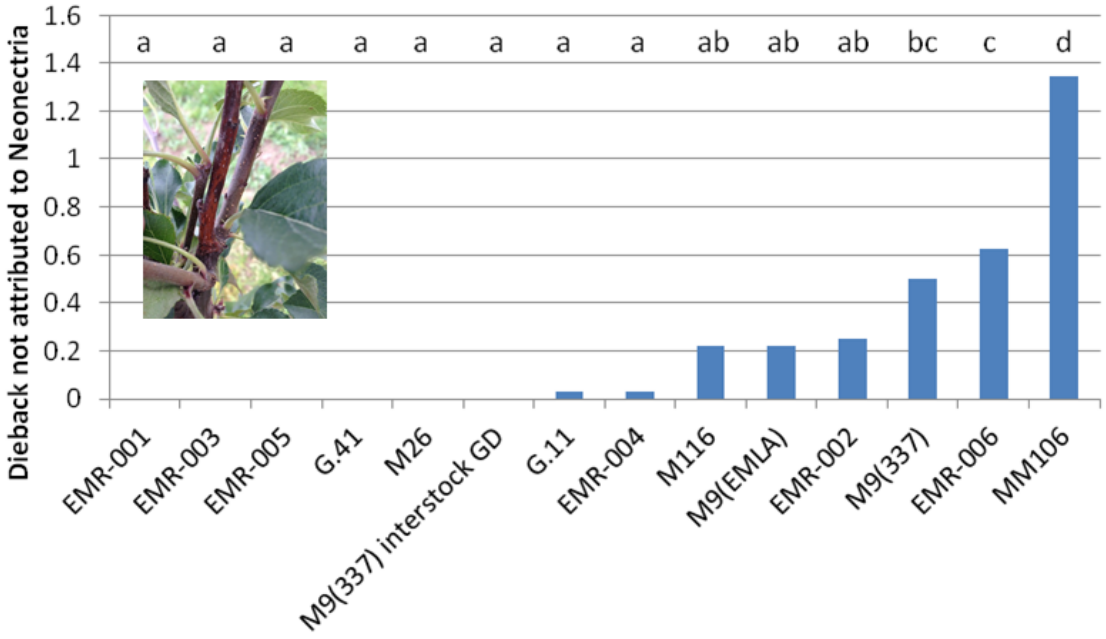


Figure 2.2. Dieback attributed to *Phomopsis* sp. (inset) rather the *Neonectria ditissima* at site 1 (NIAB EMR).

The Results reported in this trial are based on natural infections which occurred during the relatively low infection risk propagation and growing on phase and as a result natural infection was low (grand mean for total cankers per tree =0.19). The position of the cankers was recorded which show that cankers on the main stem (A and B types, grand mean = 0.07) were more frequent than peripheral cankers (C and D types, grand mean = 0.03) at this assessment.

A significant rootstock effect was shown for total cankers, and type A and C cankers. In each case MM106 was significantly different from the majority of other accessions tested (Figure 2.3a and Table 2.2). The incidence of canker is too low in this initial assessment to gain insight into the relationship between rootstock and canker susceptibility. Assessment of the artificial inoculations (due spring 2018) and further assessments of natural infection (2 assessments per year for the remainder of the project) will provide more information on the effect of rootstock on canker susceptibility.

<u>Site 2 – Herridges orchard</u>

Tree establishment at this site was good with a total of 15 (2.6%) trees recorded dead; 13 of which were a result of canker. As with site 1 dieback not associated with Nectria canker was recorded and removed during the assessment. MM106 had a higher incidence of non nectria associated dieback compared to other rootstocks (data not shown).

Nectria canker incidence was low across the orchard. The grand mean of total cankers at Herridges Orchard was 0.32 per tree. The canker incidence at site 2 was higher overall than the canker incidence at site 1 (0.19 per tree) which was due to a higher incidence of main stem canker. The majority of the cankers recorded were on the main stem (Figure 2.3b). No significant differences between rootstocks were observed at this initial assessment (Table 2.3).

Discussion

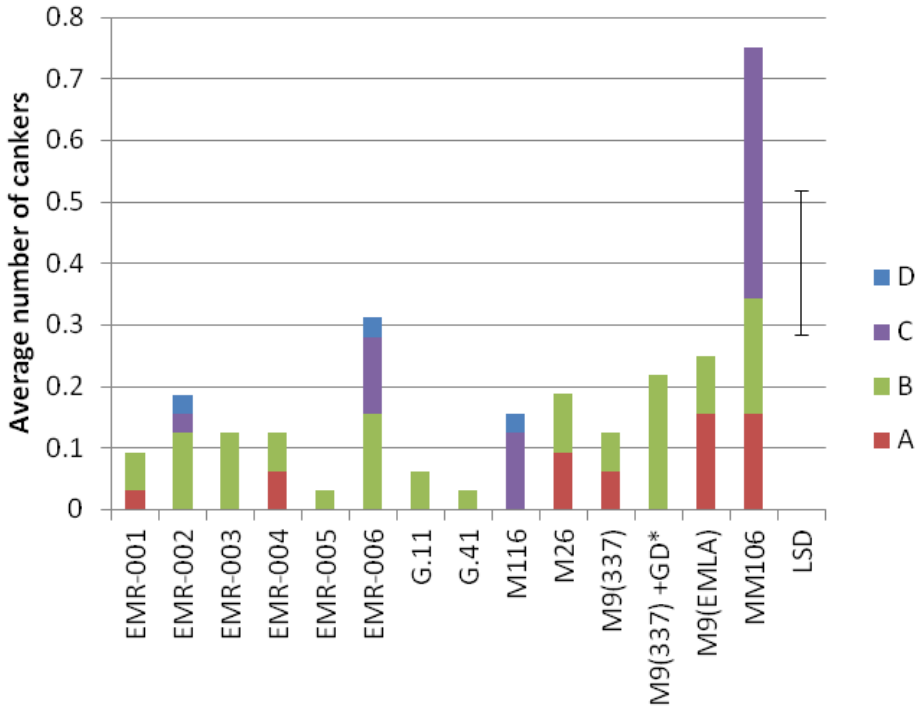
The trial orchards were planted in early 2017 and have established well. The initial assessment shows a low incidence of natural infection, which would be anticipated due to the trees not being exposed to the natural field inoculum during the main infection window (autumn leaf fall). These are long term trials requiring assessment over time. The trials have been planted in situations with high disease pressure so future assessments are expected to have a higher incidence of infection. Furthermore, we are able to artificially inoculate trees at site 1 providing the opportunity to provide the treatments with a consistent disease challenge which can be assessed for incidence and severity of canker.

The millennium trial (McCracken *et al.* 2003) provided empirical evidence that the cankers on the main stem ('A' and 'B' types) were more likely to originate from infections which took place in the nursery and can express disease symptoms up to 3 years following planting. The assessments at the two trial sites show that the majority of the cankers assessed were present on the main stem and so likely to represent infections which originated from the propagation phase. The higher incidence of canker recorded at site 2, a result driven by higher rates of main stem cankers, may be explained by the different planting site factors which can lead to varying degrees of stress which in turn can lead to the expression of latent canker. Since planting, the trees have been exposed to field inoculum through infection risk periods, particularly leaf fall 2017, and so it is expected that in future assessments peripheral cankers will dominate due to increased infections in the orchard.

The incidence of canker was higher in MM106 than expected, based on detached stem trials carried out in 2016 (see year 2 report) and there was a significantly higher level of dieback not associated with nectria in this treatment compared to other rootstocks. During the propagation phase, in order to encourage feathering the trees were headed back (apical

dominance removed by removing the apical shoot) as they reached the top of the bamboo cane, as a consequence each rootstock was headed back at different times depending on vigour they conferred to the scion. MM106 is a semi vigorous rootstock and as a result was headed back before the other treatments. It is likely that inoculum (*Phomopsis* and *Neonectria*) was present and conditions favourable for infection when the MM106 trees were headed back resulting in the wounds being infected which would be consistent with the majority of infection being localised in the apex of the tree.

a)



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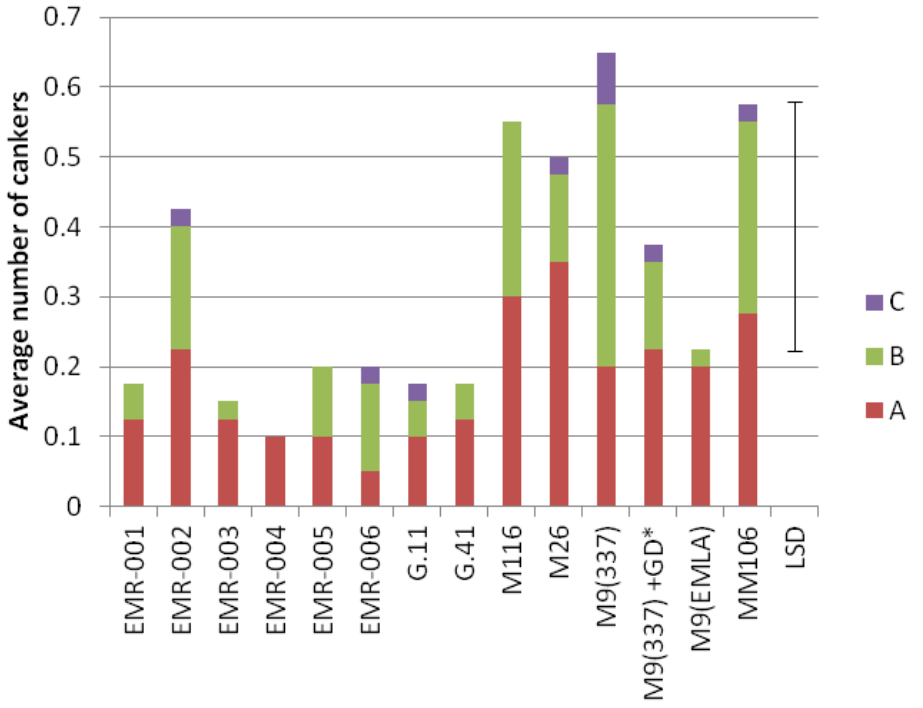


Figure 2.3. Assessments of naturally occurring canker at two trial sites; a) East Egham and b) Herridges Orchard. The average number of total cankers per tree is presented and each bar is further broken down into the position of each canker recorded; A = Rootstock (Red), B = Mainstem (Green), C = Peripheral from mainstem (purple) and D = peripheral from C-type (blue). * M9(337) + GD refers to M9 clone 337 with Golden Delicious (GD) interstem.

Table 2.2. Effect of rootstock on canker developing from natural infections following the first assessment at site 1 (East Egham). Figures significantly different from others are highlighted in **bold.**

Rootstock	Total	A	B	C	D
EMR-001	0.094	0.0313	0.062	0	0
EMR-002	0.188	0	0.125	0.0312	0.0312
EMR-003	0.125	0	0.125	0	0
EMR-004	0.125	**0.0625**	0.063	0	0
EMR-005	0.031	0	0.031	0	0
EMR-006	0.312	0	0.156	0.125	0.0312
G.11	0.063	0	0.063	0	0
G.41	0.031	0	0.031	0	0
M116	0.156	0	0	0.125	0.0312
M26	0.188	**0.0937**	0.094	0	0
M9(337)	0.125	**0.0625**	0.062	0	0
M9(337) interstock GD	0.219	0	0.219	0	0
M9(EMLA)	0.25	**0.1562**	0.094	0	0
MM106	**0.75**	**0.1562**	0.188	**0.4063**	0
Fprob	**0.011**	**0.011**	0.366	**0.004**	0.47
SED (39)	0.159	0.051	0.083	0.091	0.019
LSD (P=0.05)	0.322	0.103	0.168	0.185	0.038

Table 2.3. Effect of rootstock on canker developing from natural infections following the first assessment at site 2 (Herridges Orchard). No significant differences were observed.

Rootstock	Total	A	B	C
EMR-001	0.175	0.125	0.05	0
EMR-002	0.425	0.225	0.175	0.025
EMR-003	0.15	0.125	0.025	0
EMR-004	0.1	0.1	0	0
EMR-005	0.2	0.1	0.1	0
EMR-006	0.2	0.05	0.125	0.025
G.11	0.175	0.1	0.05	0.025
G.41	0.175	0.125	0.05	0
M116	0.55	0.3	0.25	0
M26	0.5	0.35	0.125	0.025
M9(337)	0.65	0.2	0.375	0.075
M9(337) interstock GD	0.375	0.225	0.125	0.025
M9(EMLA)	0.225	0.2	0.025	0
MM106	0.575	0.275	0.275	0.025
Fprob	0.16	0.305	0.331	0.754
SED (39)	0.215	0.114	0.143	0.036
LSD (P=0.05)	0.435	0.230	0.289	0.072

Conclusions

- The two trial sites have been planted and have established well
- An initial assessment has shown that mainstem cankers are more prevalent than peripheral cankers consistent with 1) infections originating in the nursery expressing in the field and 2) the fact that the trees had not been exposed to field inoculum through the main infection window at the time of assessment

It is not possible to draw Conclusions on the treatment (rootstock) effects yet. Artificial inoculations will be assessed in spring 2018 which will provide more information of treatment effect on canker susceptibility in addition to the data from the detached stem tests reported in year 2.

Future work

Continue assessments of natural and artificial disease development with a particular focus on artificial inoculation to guarantee infection and Results.

2.3 Soil amendments

Aim

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

Introduction

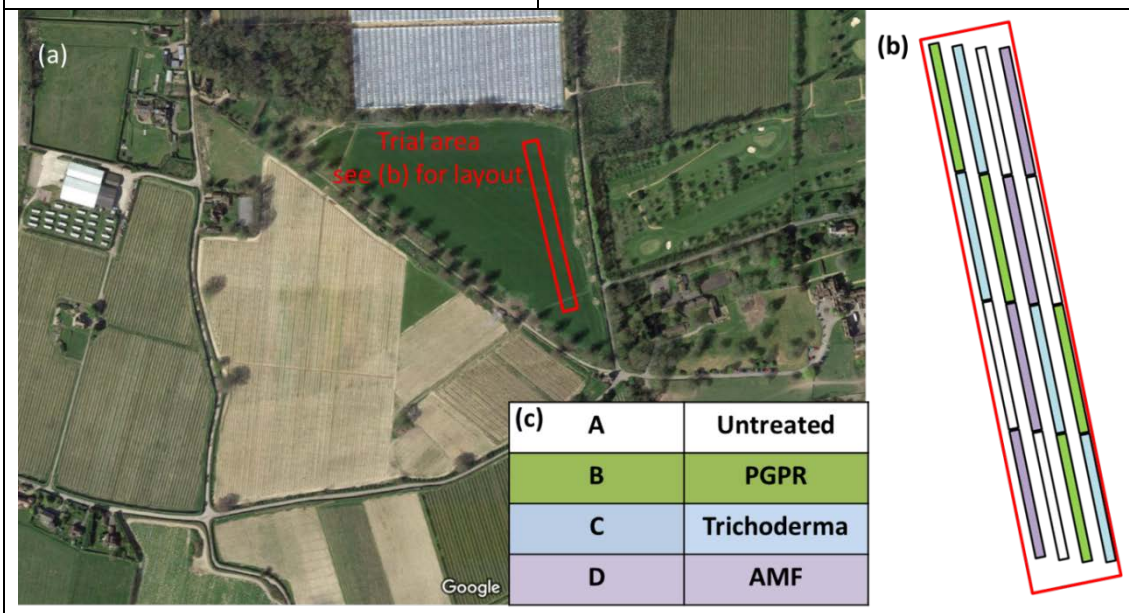
Previous research on European apple canker (in particular the millennium trial, McCracken *et al*. 2003) has shown that *N. ditissima* can infect trees in the nursery and remain asymptomatic in the apple host. Once planted in the production site, where upon the tree can experience stress (drought/water logging/replant disease etc.), the disease can be 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 out through 2017 growing season as part of the long term monitoring of this trial.

Materials and Methods

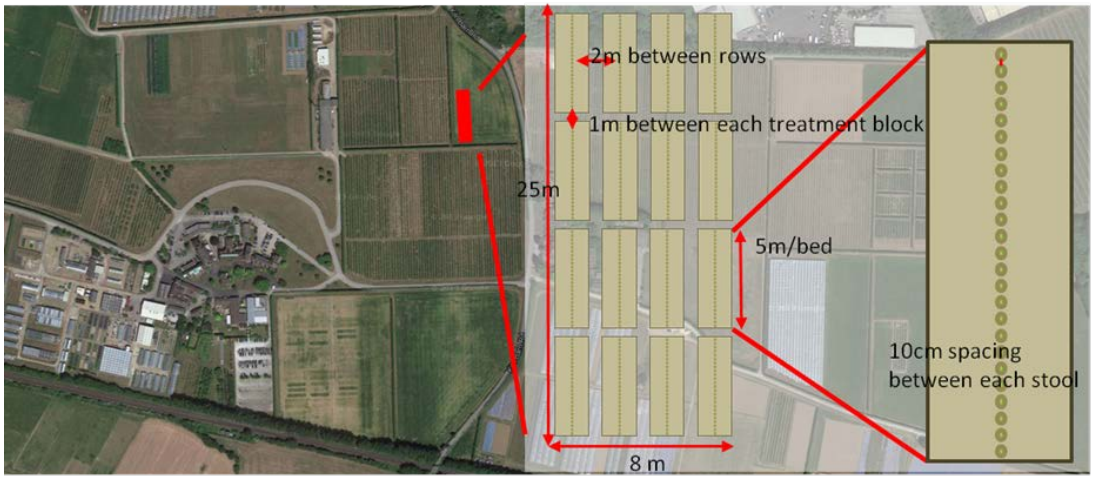
Site

Site 1	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



Site 2	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)	Kent
Grid reference	51.287328, 0.45690701
Variety	EMLA M9
Planted	12/05/15
Host	NIAB EMR



Treatments

Treatments (Table 2.4) were applied at planting as described in previous reports.

Table 2.4. Treatments used for biological amendments trial.

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 amyloliquefacien* *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

(1) Stoolbed trial

On 6th July 2017, once the shoots on the stoolbeds had reached sufficient height to replicate commercial practice (Figure 2.4) 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 2.5) was based on manufacturers recommendations.

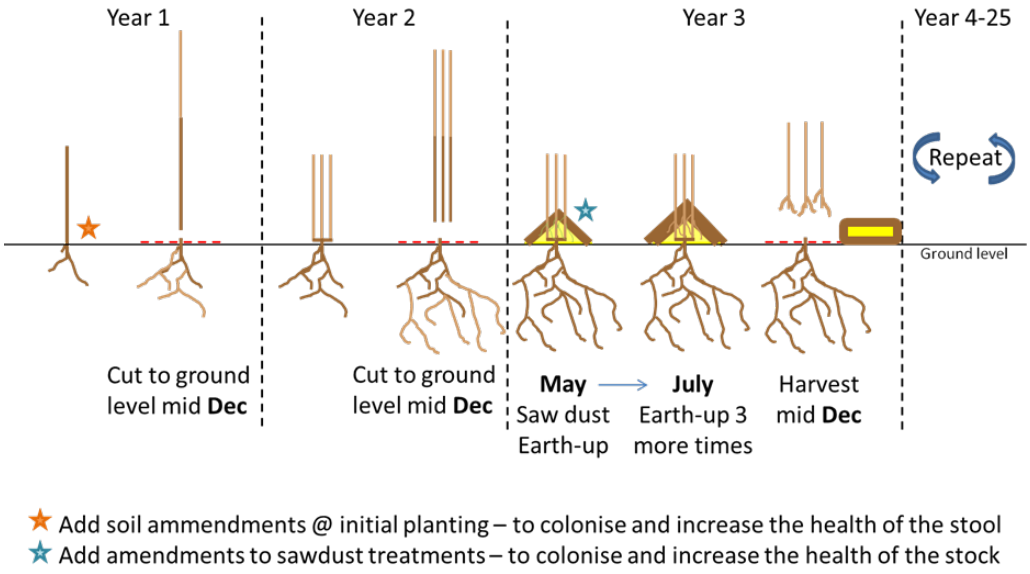


Figure 2.4 a) A schematic of the commercial practice of establishing and harvesting a stoolbed. b) photographs of a bed in which amended sawdust has been applied and weighed down with soil.

Table 2.5. 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 28th and 29th 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 28/11/17 and marked with yellow paint, Block 4 was inoculated on 29/11/17 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

(1) Stoolbed

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

Grading

Total rootstock number and rootstock size classification was recorded for each plot. Rootstocks were classified into the following size classifications: <9mm , 9-11mm and >11mm.

Root length colonisation (RLC)

Relative colonisation of AMF was determined in plots amended with AMF inoculum and compared with untreated plots using an established method known as percentage root length colonisation (%RLC). Briefly, fine roots were collected from the harvested rootstocks and stored in 10% KOH at 4°C until processing. Root samples were placed in cytology cassettes and incubated within beakers submerged in 20% (w/v) KOH at 90°C for one hour. Roots were rinsed in a sieve under running cold water and then submerged in 2% (v/v) HCl for at least an hour, HCl was discarded and roots were submerged in 0.05% (w/v) trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water) and incubated in a water bath for 1 hour at 90°C for staining. Casettes were destained by submerging cassettes in 50% (v/v) glycerol and then stored in a fridge until microscopic assessment. Colonisation was quantified by microscopic assessment.

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 2.5). 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).

Figure 2.5. experimental setup of the 'stress test' to expedite the symptom development at inoculation sites.

(2) Newly established orchards

Canker number and position was recorded on each tree in spring and autumn during 2017 according to McCracken *et al.* 2003. Cankers were categorised according to their position as follows; 'A' = Rootstock, 'B' = Main stem, 'C' – 'E' = Peripheral branches (Figure 2.1, Section 2.2). Representative samples of the cankered branches were returned to the laboratory for isolation to confirm *Neonectria ditissima*.

Overall cankers from the two assessment dates were analysed by ANOVA. Each site was analysed separately.

Results

Stoolbed trial

The quantity and quality (size) of the rootstocks harvested from each treatment block was recorded. The results (Figure 2.6) show that the majority of the rootstocks harvested were <9mm and proportions were similar across all treatments. There was an increase in rootstock number in the AMF treated stools.

To determine whether the biological treatments were colonising the rootstocks AMF colonisation was measured in the AMF amended plots and compared to the unamended control. Figure 2.7 shows that the untreated control had a background level of AMF colonisation and that the AMF treated plots had significantly more colonisation than the untreated controls. Techniques to measure the colonisation of the other amendments are not available in house and therefore were not conducted.

Artificial canker inoculations were conducted in Autumn 2017 and are being incubated in conditions to promote canker expression. At the time of writing assessments were ongoing therefore it is not possible to report the results of the canker inoculations at this time.

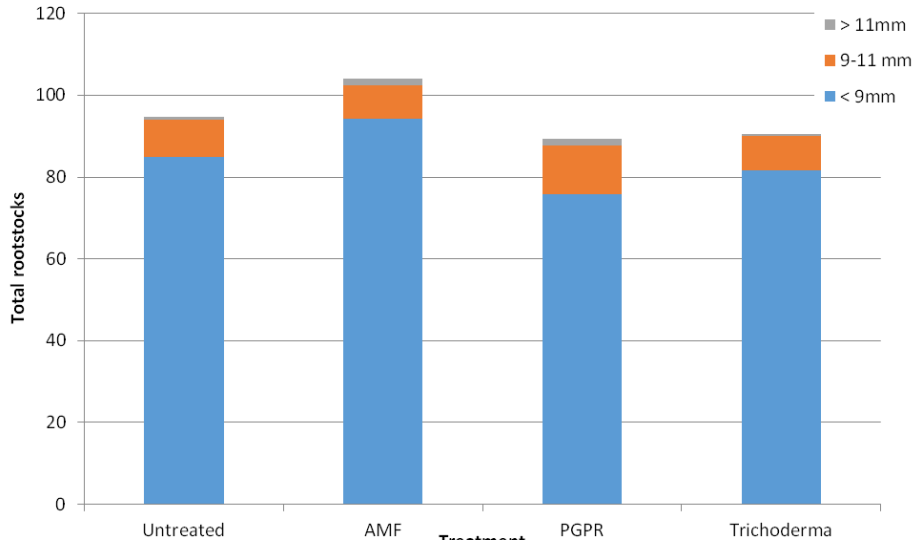


Figure 2.6. Average number of rootstocks harvested from each block categorised by size grade.

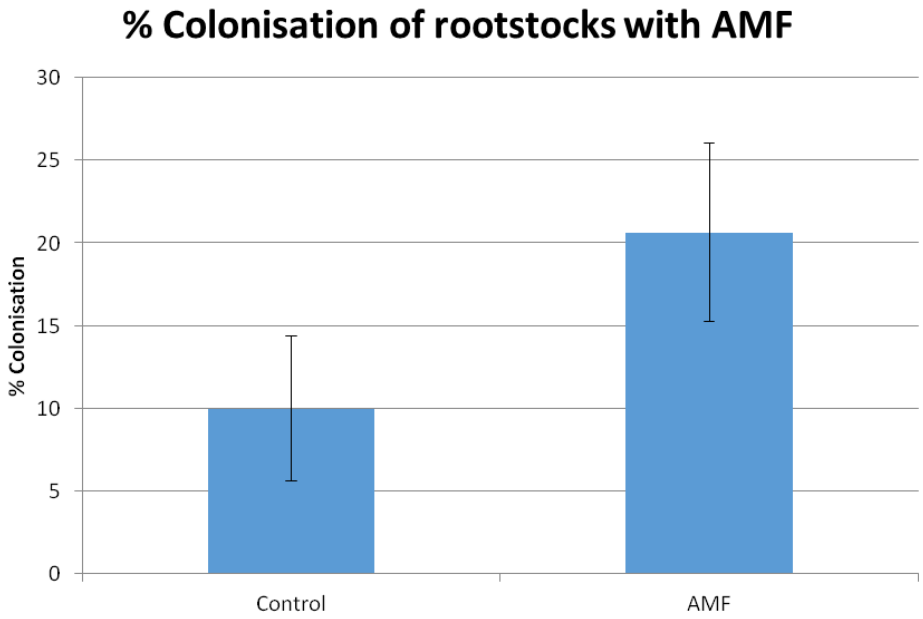


Figure 2.7. Colonisation of rootstock roots by AMF as determined by RLC.

Newly established orchards

Canker expression on the newly established orchard sites was assessed in spring and autumn of 2017. Total cankers recorded from both assessment are presented in Table 2.6 and Figure 2.8 for both sites. Canker incidence was low at both sites with a grand mean of 0.19 and 0.23 per tree at site 1 and 2 respectively.

No statistically significant differences between the treatments were recorded. At site 1 Untreated and AMF treated trees had higher incidence of canker than PGPR and Trichoderma treated trees. Cankers at this site mainly occurred on the main stem above the graft union (type ‘B’) and on the peripheral branches off the main stem (type ‘C’). At site 2 carbongold treated trees had the highest incidence of canker overall. The position of cankers recorded at this site was spread across ‘B’, ‘C’ and ‘D’ types.

Table 2.6. Effect of soil ammendments on canker developing from natural infections. Total infections over two assessments. No statistical significance was observed**.**

	Site 1 (Friday Street Farm)					Site 2 (Broadwater Farm)				
	A	**B**	**C**	**D**	**Total**	**A**	**B**	**C**	**D**	**Total**
AMF	0	0.115	0.209	0	0.325	0	0.062	0.0231	0.062	0.146
BIO	-	-	-	-	-	0	0.154	0.0462	0.146	0.346
PGPR	0	0.032	0.042	0.0053	0.079	0	0.108	0.0615	0.054	0.223
TRI	0	0.011	0.058	0.0053	0.074	0	0.078	0.0388	0.116	0.233
UNT	0	0.141	0.157	0	0.297	0	0.054	0.0233	0.132	0.209
F prob	-	0.354	0.072	0.573	0.091	-	0.251	0.651	0.115	0.1
sed	-	0.0415	0.0426	0.00531	0.0695	-	0.0496	0.02931	0.0433	0.0733
lsd (P=0.05)	-	0.0815	0.0836	0.01042	0.1365	-	0.0974	0.05755	0.085	0.1439

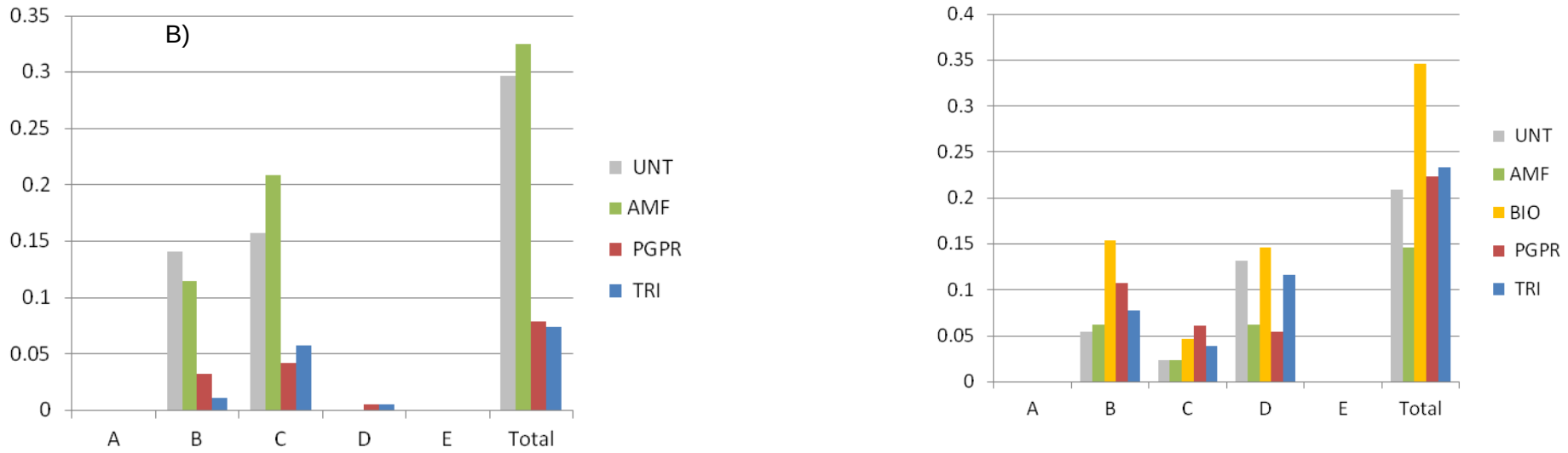


Figure 2.8 Assessments of naturally occurring canker at two trial sites; a) Site 1 and b) Site 2 to evaluate the effects of biological soil amendments on the expression of canker.

Discussion

Stool bed trial

Artificial inoculations of the rootstocks was carried out to ensure that the disease pressure was consistent across treatments and blocks. At the time of writing, assessments were still ongoing. Assessments include incidence, severity and length of time until expression of the symptoms to determine whether biological soil amendments are an effective strategy to increase resistance/ reduce susceptibility to canker.

In order to determine whether the rootstocks are being colonised by the soil amendments an RLC analysis was conducted on the AMF amended and unamended control rootstocks. It was found (as with previous assessments of the colonisation of the stool roots reported in year 2), that the unamended control is colonised by AMF from resident AMF found naturally in the soil. Although a background level of AMF is present the results show that amending the roots with AMF ensures a higher level of colonisation compared to the unamended control.

Although the principle aim of this trial was to determine the effect of soil amendments on canker susceptibility, the quantity and quality of the rootstocks harvested from the stool beds was also assessed. AMF treatment did have a positive effect on yield. The size of the majority of the root stocks harvested was below the commercial standard size of 9-11mm. This was the first production year of the stool bed and once the stool beds establish they will reach full production (and quality) potential.

Newly establishing orchards

The purpose of evaluating soil amendments in newly planted orchards was to test the following hypothesis 1) transplanting stress is thought to promote the expression of latent infection from the nursery, do biological soil amendments mitigate against the stress to reduce the expression of disease and 2) do the plant health promoting properties conferred by the soil amendments increase resistance to disease infection and development.

Latent infection from the nursery are more likely to be present on the rootstock and main stem and express within the first 3 years of orchard establishment (McCraken *et al,* 2003). Due to the low levels of canker development in ‘A’ and ‘B’ positions in both trials no significant differences between treatments were evident in the first two assessments of these trials. The use of two trial sites planted with susceptible varieties was intended to increase the chances of getting a meaningful level of latent canker to assess the effect of treatment but both trial sites have had low levels of expression in the first year of establishment. Ideally many more orchards would have been treated with the amendments to guarantee some of the trial sites had high levels of disease expression but this was not feasible in this study.

Assessments will continue through the remainder of this project as latent cankers from the nursery ('A' and 'B' types) continue to develop and local inoculum infect the peripheral branches ('C' – 'E' type cankers) in order to test the hypotheses set out above.

Conclusions

- The results from artificial inoculations conducted in the autumn are pending, once complete they will provide an insight into how increased plant health conferred by biological soil amendments affects canker incidence, severity and length of time until expression of symptoms.
- The first year of assessments on the newly established orchards show very low levels of disease development and therefore no significant differences in treatments could be observed. Assessments will continue through the remainder of the project.

Future work

- Assess canker incidence, severity and latency period for artificial inoculations of rootstock material treated with different amendments. This work will be repeated next year to observe treatment effects over multiple years.
- Continue monitoring canker development on the commercial trial sites

2.4 Novel application methods

Aim

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

Introduction

Targeted treatment application has the potential to increase efficacy whilst reducing cost and environmental exposure. This task explores novel application methods for treatments targeting *Neonectria ditissima*.

Tree injection systems are widely used in forestry and amenity sectors, have been trialled for apple foliar disease, fireblight and pest control in the USA (VanWoerkom *et al.* 2014). Tree injection has the potential to be used for European apple canker control where the disease develops in the wood and could be better targeted by delivering products through the vascular tissue. A collaboration has been established between Fertinyect, Bayer and NIAB EMR to conduct proof of concept trials. Fertinyect is a Spanish based company which manufacture

inexpensive tree injection systems. The Agchem company, Bayer, have agreed to provide treatments in kind for the first phase of trials. In 2016 trials an experimental product (HDC F199) was the best performing product, HDC F200 (biological) and Fertinyect Protect (defence elicitor) were the next best performing products.

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 wound protection products such as polymer coatings and dispensing systems, have the potential to reduce labour costs in protecting pruning wounds from canker infection by treating them at the same time as pruning. During the 2017 season trials have been conducted on both tree injection and wound protection products.

Materials and Methods

Tree Injection

Site

Church fields east (CE231) located at NIAB EMR. The orchard, planted in 2013, consists of single alternate rows of Gala and Rubens on M9 rootstock. Rubens trees with a row spacing of 3.5m and tree spacing of 0.5m were used in this trial.

Inoculation

Trees were inoculated on 05/04/17, two months prior to treatment application to ensure the disease was established and uniform across treatments at the point of treatment application. Trees were inoculated by creating a wound (2 x 1 cm groves made in the bark of the tree) at a defined height of 0.5m from ground level and applying a spore suspension prepared in the laboratory (isolate R6/17_3, 1.5×10^5 spores/ml). Inoculated wound sites were covered with petroleum jelly (Vaseline®) to create favourable conditions for infection.

Treatment application

The treatments were applied using the Fertinyect system following manufacturer's guidelines as described in earlier reports but with the following modifications; two weeks prior to treatment application the irrigation was turned off to dry the soil down in order to promote uptake of the treatments from the injection devices; Treatments were applied in the morning on a hot day. Treatments were applied on 26/05/17 before 10:00.

Standard treatments for pests, foliar disease and nutrients were applied to all plots throughout the season. The wound was left open to heal in these trials.

Assessments

Canker severity

The size (width and length at longest point) of each of the cankers were measured at 4 and 8 months from the date of treatment. A 12 month assessment of canker size and determining sporulation is still pending.

Table 2.6 Treatment list to be tested

Treatment	**Product**	**Active ingredient**	**Formulation**	**Product type**	**Recommended foliar rate of product**
1	**UNTREATED**	-	-	-	-
2	Cercobin (Certis)	Thiophanate-methyl	WG	Fungicide	1.1kg/ha
3	HDC F198	Experimental	WG	Fungicide+defence elicitor	3kg/ha
4	HDC F199 (x5)	Experimental	SC	Fungicide	281ml/ha
5†	HDC F199 (x5) in 2 devices	Experimental	SC	Fungicide	281ml/ha
6	HDC F199 (x10)	Experimental	SC	Fungicide	281ml/ha
7	HDC F200	Experimental	WP	Biological	4kg/ha
8	Fertinyct – Protect	Magnesium Phosphite	?	Defence elicitor	Pre formulated

† this treatment will see the first device inserted at the base of the tree (as with the others) and a second device equidistant above the inoculation point.

Phytotoxicity assessments

Symptoms of phytotoxicity were monitored following treatment application and recorded accordingly. Chlorosis / necrosis to foliage and growth regulatory effects on fruits were assessed on a scale 0-5 (EPPO Guideline PP 1/135(3)). Yield was recorded as total yield per tree and weight of 50 fruit.

Residue analysis

Fruit samples were collected 2 weeks following treatment application and 2 weeks prior to harvest. Samples were collected for treatments 2 (Cercobin), 3 (HDC F198) and 4 (HDC F199 x5). In each case 1 kg of fruit was collected from the middle of the canopy (1.5 M above the injection point). Samples were sent to a pesticide residue laboratory for analysis.

Table 2.7 Treatment rates

Treatment	**Product**	**Recommended foliar rate**	**Expressed as % assuming 1000L/ha**	**Injection rate (x 5‡) expressed as %**	**Rate/L**	**Rate/200ml device**
1	**UNTREATED**	-	-	-	-	-
2	Cercobin (Certis)	1.1kg/ha	0.11	0.55	5.5 g	1.1 g
3	HDC F198	3kg/ha	0.3	1.5	15 g	3 g
4	HDC F199 (x5)	281ml/ha	0.0281	0.1405	1.405 ml	281 µl
5	HDC F199 (x5) in 2 devices	281ml/ha	0.0281	0.1405	1.405 ml	281 µl
6	HDC F199 (x10)	281ml/ha	0.0281	0.281	2.81 ml	562 µl
7	HDC F200	4kg/ha	0.4	2	20 g	4 g
8	Fertinyct – Protect	Pre formulated	-	-	-	-

‡ unless otherwise stated

Pruning wound protection

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 (Figure 2.9).

Figure 2.9. Row of trees at Herridges Orchard pruned and Felco 19 secateurs used to apply treatments.

Treatments

Treatments were applied on a dry day at the beginning of the season (21 March 2017). A total of 6 treatments were used in the trial (Table 2.8) including a control of water. Five shoots per tree were pruned and treatments were applied using Felco 19 secateurs with a chemical dispenser (Figure 2.8). The treated shoots were marked with spray paint and string for future assessmentots.

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 2017 and the trees were assessed on 30 November 2017. The number of cut shoots with canker, the regrowth from the cut shoot and length of regrowth were measured. Canker incidence was recorded on the treated pruning wounds (Figure 2.10). The trees were also assessed for canker on the non-treated branches. Any phytotoxic effects were noted. The data was analysed using linear regression.

Table 2.8. Treatment list

Treatment	Product	Active	Rate of product / ha
1	Water	-	-
2	BlocCade	polymer	100 ml/L
3	T34	Trichoderma	10.0 g/L
4	Folicur	Tebuconazole	0.6 ml/L
5	BlocCade + T34	Polymer + Trichoderma	As above
6	BlocCade + Folicur	Polymer + Tebuconazole	As above

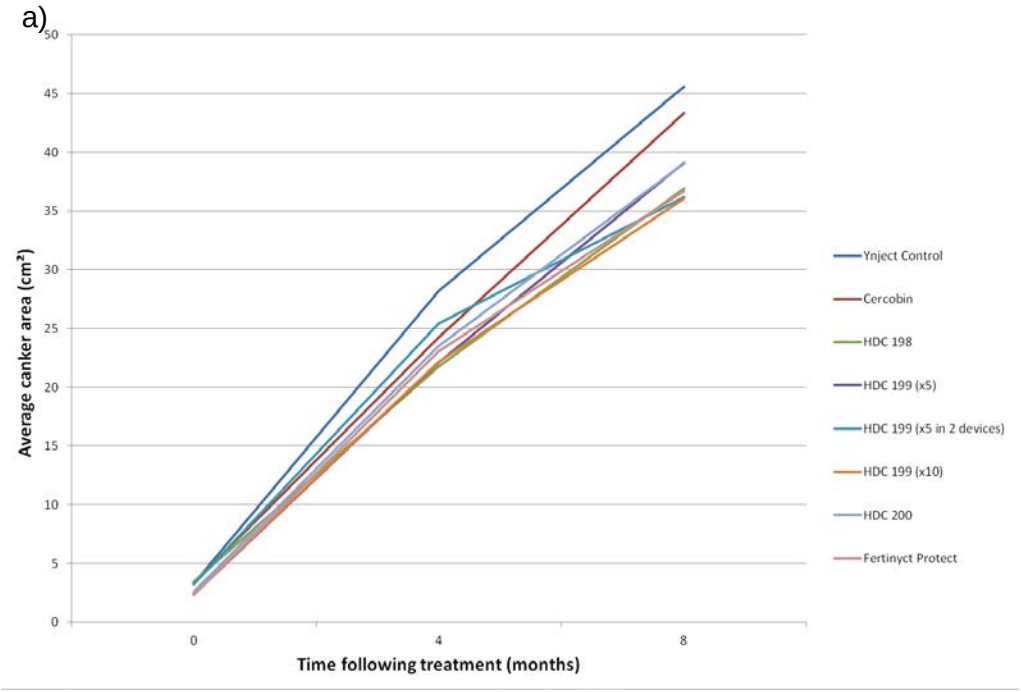
Figure 2.10. Cut shoots showing no canker (left) and canker (right) at assessment November 2017

Results

Tree injection

The growth of the inoculated cankers has been recorded over the first 8 months following treatment application. At the first assessment, recorded when the treatments were applied, the canker lesions were uniform in size (Figure 2.11a). The rate of canker lesion growth was greatest on control (treatment 1 – ynject) trees (Figure 2.11a) however the increased growth rate was not significantly different from the other treatments (Figure 2.11b). No significant differences in canker lesion growth or shape were observed between the treatment combinations (dosage and positioning of device in relation to the canker) of HDC F199. Despite phytotoxic effects (see below) conferred by treatment 8 (Fertinyect Protect) canker development was comparable to the other treatments.

a)



b)

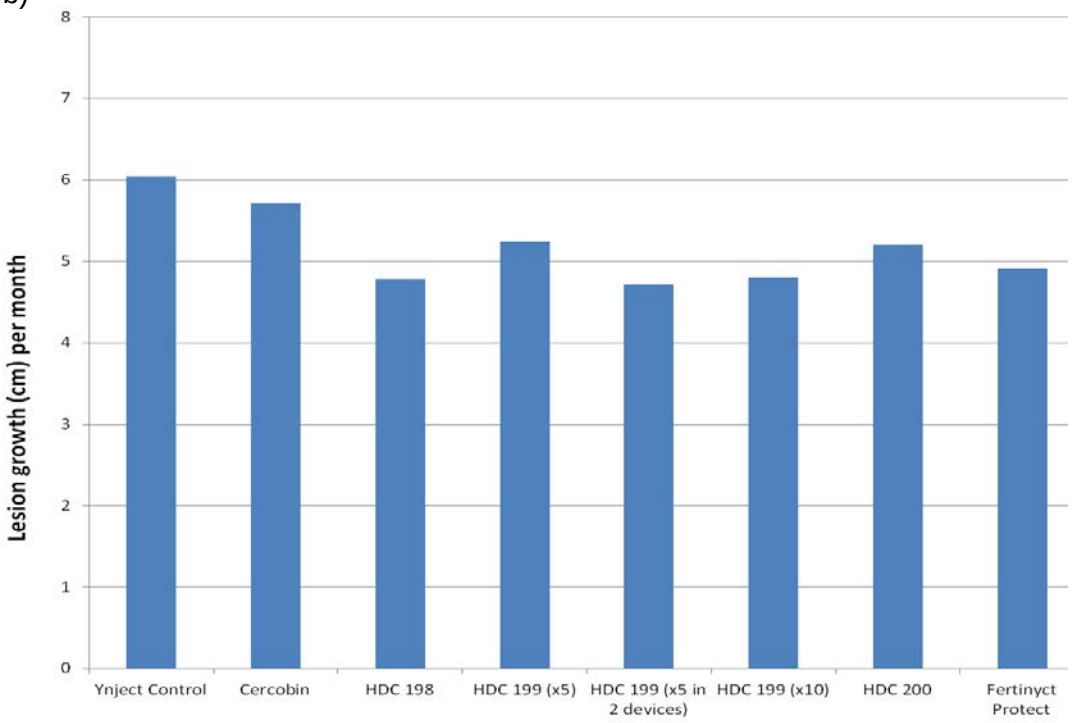


Figure 2.11. Canker severity for the 8 months following treatment application expressed as a) lesion development over time and b) lesion growth rate per month.

Uptake of treatments from the devices was assessed 5 days following treatment application to determine whether the formulations were suitable for tree injection systems. After 5 days devices for HDC F199 treatment combinations, Fertinyect protect and control devices were completely empty. Devices containing Cercobin and HDC F198 were half full while devices containing HDC F200 were 75% full, meaning only 25% of the treatment had been taken into the tree.

Phytotoxicity

Treatment 8 (Fertinyect Protect) caused severe leaf lamina necrosis including symptoms consistent with phosphite toxicity and growth regulatory effect on fruit development within 1 month of treatment application. No other treatments exhibited phytotoxic effects that were significantly different compared to the control (Figure 2.12).

Total yields and weight of 50 fruit were comparable across all the treatments apart from treatment 8 (Fertinyect Protect) where total yield was significantly (P= <0.001) reduced compared to the other treatments and related to the phytotoxicity recorded earlier in the season.

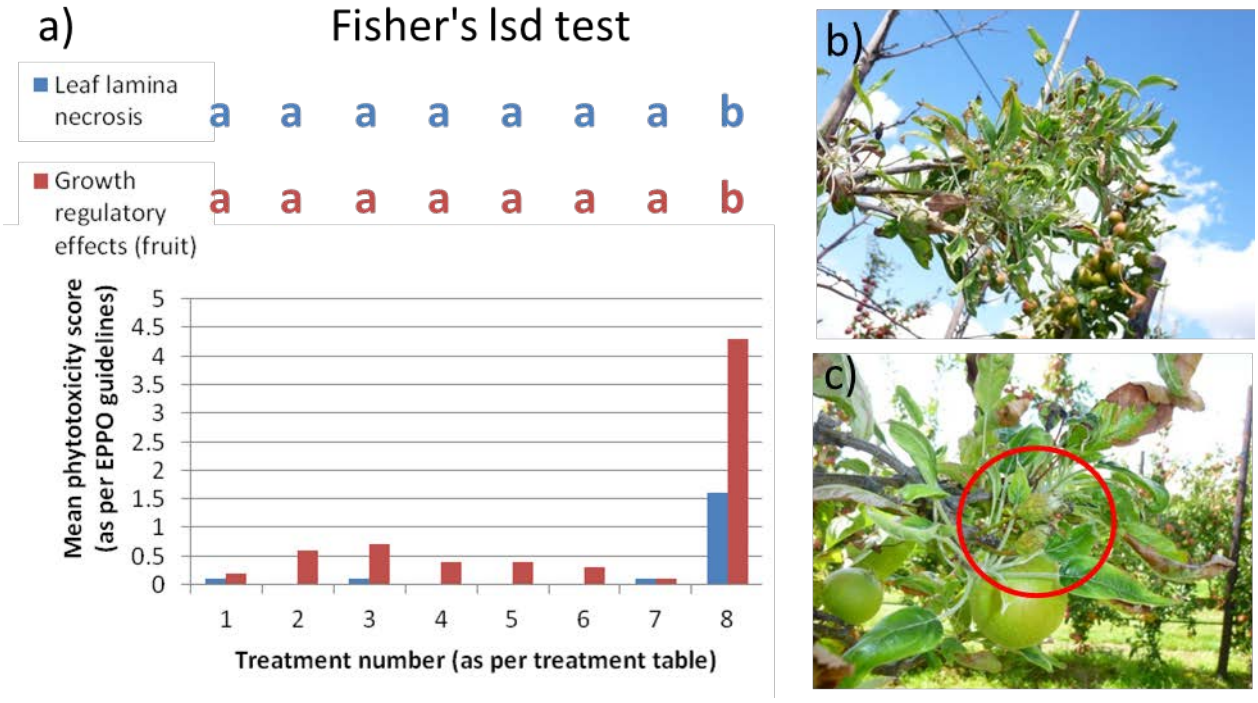


Figure 2.12. a) Phytotoxicity assessments for leaf lamina necrosis (blue) and Growth regulatory effects on fruit for each treatment as specified in Table 2.7. b) leaf necrosis and furling exhibited in trees treated with Fertinyect Protect and c) growth regulatory effects on the fruit in trees treated with Fertinyect Protect.

Residue analysis

Fosetyl aluminium was detected in the fruit tissue at 39.8 mg/kg two weeks after treatment application. By harvest (15 weeks after treatment) the residue had decreased to 6.6 mg/kg. The maximum residue limit for Fosetyl aluminium is 75 mg/kg and was not exceeded at either sampling date. Neither of the other active ingredients (Thiophanate-methyl or an experimental active) present in the treatments tested were not detectable in the fruit samples at either sampling point.

Pruning wound protection

The background levels of canker in the orchard were high. There was a 93 % incidence of canker on the main stem of the trees used in the trial and 76 % 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 assessment in November 2017.

On 13 of the trial trees a branch had been pruned accidently by the farm, however these were spread across the treatments. This did not affect the analysis because the proportion of canker on the remaining branches was calculated and the linear regression performed on this data.

Regrowth was generally low across the trial at this assessment date. Folicur had the highest incidence of regrowth, with 18 % of shoots showing some regrowth, however this was not significantly different to the other treatments (Figure 2.13). Treatments that contained T34 had the lowest proportion of regrowth on the cut shoots, with only two shoots having any regrowth (4 % of branches).

There were significant differences in the proportion of canker on the cut shoots ($p < 0.001$, d.f. = 5). Just over a quarter of the shoots on the control trees (treated with water) developed canker during the first recording period of the trial (Figure 2.14). When applied alone T34 had the highest incidence of canker on the cut shoots (40%), however, when applied with the addition of BlocCade a significantly lower incidence in the proportion of shoots infected with canker was observed (10%). Both treatments that had BlocCade in addition to the chemical or biological active had significantly lower proportion of shoots affected by canker when compared to the water control.

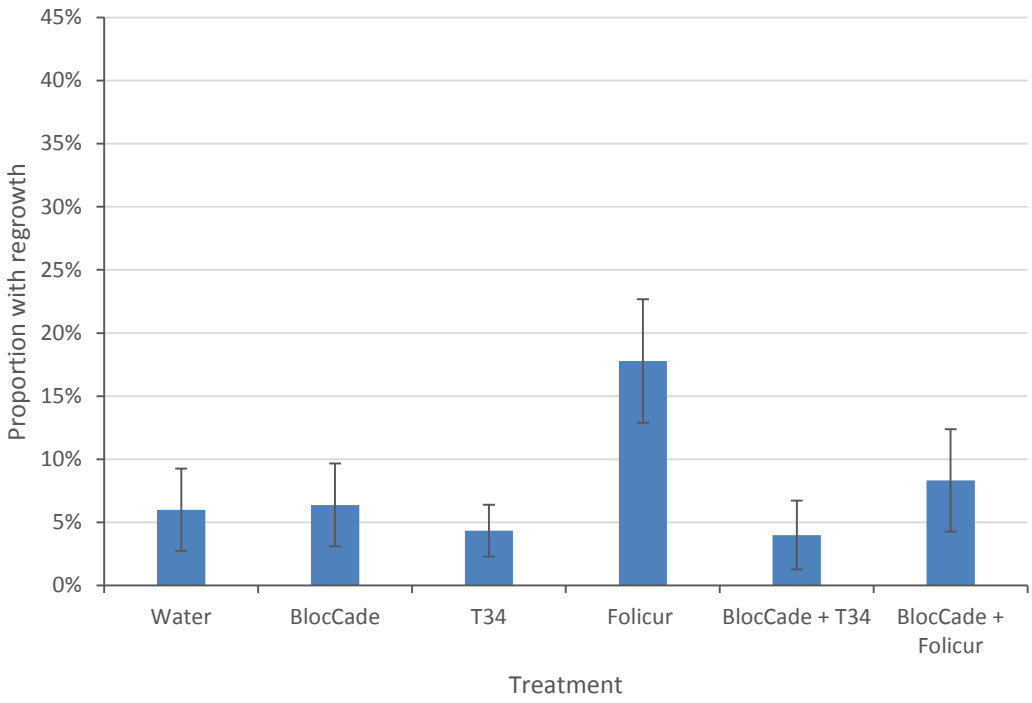


Figure 2.13. Proportion (%) of cut shoots showing regrowth in November 2017. Differences are not statistically significant.Error bars show standard deviation.

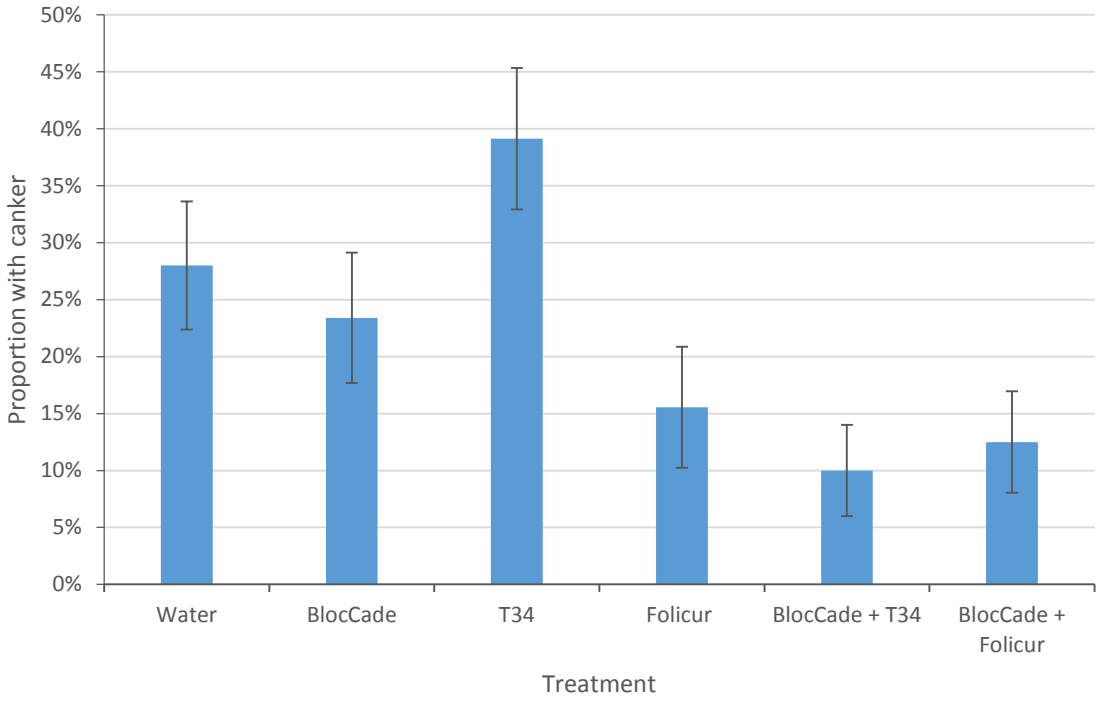


Figure 2.14. Proportion (%) of cut shoots with canker present in November 2017. Differences are statistically significant ($p < 0.001$, d.f. = 5). Error bars show standard deviation.

Discussion

Tree injection

Tree injection has the potential to be an effective delivery system at targeting the treatments to the tissue where the canker pathogen attacks. Potential applications in the industry would be to eradicate latent canker that is present in the tree but not showing symptoms (asymptomatic) and to eradicate symptomatic trunk cankers on establishing/established trees to prevent girdling and reduce inoculum presence in the orchard. Methods are not currently available to establish latent canker infections and therefore trials have focused on symptomatic cankers to test a variety of product categories (Table 2.7). The tree injection trials conducted in 2016 which used existing trunk cankers to measure efficacy of treatments showed highly variable results but three treatments (HDC F199 (experimental fungicide), HDC F200 (biological) and fertinyect protect (defence elicitor)) showed a trend towards reduced canker growth. The aim of trials in 2017 was to develop a methodology to reduce variability and to take forward the best performing treatments from 2016.

Trees were artificially inoculated with canker at the beginning of the season prior to treatment. At the point of treatment all inoculations had successfully established disease and canker area was measured. Although the growth rate of cankers on control trees was greatest there was no significant difference between control and other treatments. HDC F199 was the best performing treatment in 2016 trials and therefore was used at two different rates (5x and 10x foliar rate) and by adding a positional element (placing two devices; one above and one below the canker to determine whether canker lesion shape was affected due to the active ingredient being distributed from above and below the canker). None of the combinations of HDC F199 had any significant effect on canker size or shape.

The standard treatment used (Cercobin) is known to have efficacy against canker and the test treatments were selected based on their proven/presumed efficacy against the canker pathogen however the lack of efficacy observed in this trial may be due to 1) the treatments not being distributed to the tissue where the canker is present or not being present in sufficient concentrations, 2) the mode of action of the selected treatments is not suitable for this purpose. AHDB studentship CP161 has shown that the canker pathogen is present in both the cambium and the heartwood in symptomatic cankers both of which are served by conducting tissues (phloem and xylem respectively) so it would be expected that the pathogen would be exposed to the treatments but it is not known what concentration they are exposed at.

Following treatment fruit samples were collected at 2 and 15 week time points for residue analysis. Fosetyl Al, an active ingredient known to be highly motile through the plant was detected at both sampling points in the fruit demonstrating that the injection device can

distribute treatments through the tree. Despite distribution through the plant this treatment did not reduce lesion growth which may be due to the explanations set out above. The other two, less mobile, active ingredients tested were not detected suggesting these are concentrated in other tissues of the plant following uptake.

Uptake of the treatments from the devices is a passive process reliant on the trees transpiration stream. Treatments were applied early in the morning on a hot day and the soil was dried down for two weeks prior to treatment application by controlling the irrigation to ensure maximum uptake. Despite this trees treated with HDC F200 took up only 25% of the product from the device. Uptake of this product was poor in 2016 trials but the reduction of dose by 50% (making the product less viscous) in 2017 trials was hoped to improve uptake.

If an efficacious product is found then formulation will be a key consideration to promote product uptake and distribution.

Phytotoxicity consistent with phosphite toxicity (pers comm. Angela Berrie) was observed on treatment 8 (Fertinyect Protect) which led to leaf necrosis and growth regulatory effects on fruit development. On consultation with the manufacturers they suggest the issue was exacerbated by climatic conditions (hot and sunny) following treatment application.

Pruning wound protection

Regrowth was low for all treatments and although there was slightly higher regrowth in shoots treated with Folicur, the effect was not significant. Differences seen in 2017 may become more pronounced in future assessments in 2018.

This trial was conducted on a commercial site and therefore artificial inoculations to ensure infection were not possible. The site had high levels of inoculum present in the orchard however dry conditions prevailed through spring 2017 when infection would take place and as a result moderate levels of canker development were recorded in this trial. Initial indications show that the use of the treatments may aid in the reduction of canker on freshly cut branches when combined with the secateurs and spray bottle. Three treatments (BlocCade + T34, BlocCade + Folicur and Folicur) appeared to have some preventative effect, with fewer branches in these treatments infected with canker compared to the water control. The addition of BlocCade did not improve the efficacy of Folicur alone but did improve the efficacy of T34. When applied on its own, T34 did not have a preventative effect and the proportion of branches with canker was not significantly different to the control. The combination of BlocCade and T34 had an additive effect in reducing canker development. The differences seen between the BlocCade + T34 treatment and the T34 treatment alone may be because

T34 is adapted to a soil environment and therefore not well adapted to aerial environments, so the physical barrier has significantly enhanced its action by reducing factors such as desiccation and UV exposure associated with the aerial environment. T34 was used in this trial as it was recommended by Fargro Ltd. For use with the Felco 19 system. A product containing trichoderma isolates better adapted to aerial environments, such as VineVax, should be considered in any future work.

Conclusions

- The injection devices have been shown to effectively distribute active ingredients known to have systemic properties through the tree.
- None of the chemistry tested to date has shown sufficient efficacy for the control of symptomatic cankers.
- BlocCade + T34, BlocCade + Folicur and Folicur alone significantly reduced the development of cankers on pruning wounds.

Future work

Although some assessments will continue into 2018 the treatment delivery aspects of TF223 are due to be concluded in year 3 of this project however future work could include:

- The products that have been trialled to date have not shown the expected efficacy for the control of symptomatic canker. A greater number of active ingredients representing a wider range of MOA would be possible in a detached stem system in controlled environment facilities relying on product uptake by transpiration alone and so delivered independently of the injection system. Products could include active ingredients which alter/simulate plant hormones (e.g. Bion) and trichoderma products which have shown promise in trials in New Zealand.
- A repeat of the pruning wound protection trial taking forward promising products and replacing T34 with VineVax. To ensure higher levels of infection artificial inoculations of pruning wounds will follow treatment application.

Objective 3 - Apple Foliar Diseases

3.2 Alternative treatments

Aim

Evaluate efficacy and persistence of alternative chemical treatments to fungicides (NIAB EMR Year 3) ORETO 17/006

Introduction

Year 3 summary

In a replicated split plot orchard trial on 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 (AHDB9910, SB Invigorator, AHDB9904, AHDB9909, AHDB9907) 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 AHDB9910 (3 sprays at monthly intervals). Plots treated with AHDB9910 in 2016 were retained for a second year to evaluate the cumulative effects of this product. In addition AHDB9910 was evaluated with and without the addition of AHDB9904. AHDB9909 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 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 (AHDB9910 2 years), 4 (AHDB9910 + AHDB9904) and 5 (AHDB9904) were next most consistent products
- AHDB9910 applied for a second year had almost significantly less mildew than in plots where AHDB9910 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

Year 2 summary

In 2016, to overcome the difficulties of high incidence of mildew preventing evaluation of the biostimulants, the trial was designed as a replicated split plot orchard trial on cv. Gala, where the 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 9 test alternative treatments (AHDB9910, AHDB9905, SB Invigorator, AHDB9904, Garshield, AHDB9902, AHDB9908, AHDB9901 and AHDB9957) were evaluated. Sub plot treatments were applied eleven times at 7-10 day intervals, apart from AHDB9910 (3 sprays at monthly intervals) and AHDB9902 (Nine sprays only). Untreated plots were included which were the 7 or 14 day fungicide only programmes. The 7 and 14 day programmes used as the main block treatments successfully established high (<40% - almost 100% mildewed leaves) and low (10-30% mildewed leaves) mildew plots in which to evaluate the test products. Overall, all the test products significantly reduced mildew compared to the fungicide only plots. SB Invigorator was the most consistent in reducing mildew. AHDB9904 and AHDB9908 were next most consistent products.

AHDB9957, AHDB9902 and AHDB9910 were least effective**.** AHDB9910 appeared to have little effect on mildew incidence at the start of the trial but by the time the third application was made AHDB9910 treated plots had a significantly lower mildew incidence than the fungicide only plots. This suggested that there may be accumulative effects of the product

There were no significant effect of treatments on yield, but the lowest yield was recorded in plots treated with SB Invigorator and AHDB9901. There were no significant effect of treatments on fruit size or fruit colour. AHDB9904, AHDB9907, AHDB9901 and AHDB9957 caused necrotic spotting on leaves. AHDB9904 also significantly reduced fruit set. AHDB9907 and AHDB9901 also caused some premature leaf drop. AHDB9908 also increased fruit russet

The main objectives of the trial in 2017 were to evaluate new products and evaluate the possible cumulative effects of AHDB9910.

Materials and methods

Site

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

Trial design

The trial was designed as in 2016 as a split plot with mildew incidence (high and low) as the main plots and the eleven test treatments as sub plots. The trial orchard was divided into four main blocks which were randomly assigned to receive either a 7 day or 14 day interval spray programme for scab and powdery mildew control. Each programme was replicated twice. Within each block there were 2 or 1 replicates of each of eleven test treatments designed as a randomised block with each treatment replicated six times overall (Fig. 3.1). The test treatments were on small 3 tree plots. Plots treated with AHDB9910 in 2016 were retained. Plots treated with AHDB9902 in 2016 were omitted. The remaining plots were re-randomised for 2017 treatments.

Treatments

All plots received a standard programme for pest and disease control (Appendix 2) and nutrients up to the start of the trial at early flower (BBCH59/60). Thereafter the treatments in Table 3.1 and programmes in Table 3.2 were applied to the plots. Treatment 1 in Table 3.1 was untreated but this meant it only received the 7 or 14 day fungicide programme with no additional test treatment. All the test treatments in Table 3.1 were applied in addition to the 7 or 14 day fungicide programme. The test treatments were all applied at 7-10 day intervals, giving a total of 9 applications (Table 3.3) apart from AHDB9910 and Treatment 8 AHDB9909 – which were applied monthly (a total of 3 applications) and AHDB9906 which was applied every 14 days. Treatments for pests and nutrients were applied to all plots as necessary after the start of the trial.

Fig. 3.1 Trial plan showing main treatments L=low mildew incidence, 7 day fungicide programme and H=high mildew incidence, 14 day fungicide programme. Within each L or H block the sub treatments 1-10, are replicated one or two times, giving six replicates in total.

Table 3.1. Elicitor / plant strengthener products evaluated for effects on powdery mildew in apple 2017. All products were applied at 7-10 day intervals apart from Cropbiolife and B225 (Treatment 8) which were applied monthly

Treatment	Product	Active ingredient	Product type	Rate of product / ha	Use
1	Untreated Fungicide only	-	-	-	-
2	AHDB 9910	flavonoids	Plant strengthener.	500 ml	Blossom then monthly. Applied to same plots as in 2016
3	AHDB 9910	flavonoids	Plant strengthener.	500 ml	Blossom then monthly. Applied to different plots
4	AHDB 9910 + AHDB9904	Flavonoids + Alcohol ethoxylate	Plant strengthener. Addition of Wetcit said to improve performance	500 ml + 0.2%	CBL Blossom then monthly. Applied to different plots. Wetcit applied with CBL then 7-10 days
5	AHDB9904	Alcohol ethoxylate	Energiser adjuvant	0.2 %	7-10 days
6	SB invigorator	Various nutrients and natural products	Physical action Controls various pests and mildew	1ml/L	7-10 days Weekly sprays
7	AHDB9909	Botanical extract	Biostimulant and antifungal agent	1-2ml/ L	Apply as protectant at 1ml/L for low disease. Increase to 2m/L for severe disease pressure
8	AHDB9909	Botanical extract	Biostimulant and antifungal agent	500 ml	3 applications at 28 day intervals
9	AHDB9908	Silicon 1%, Copper 2%, Zinc 4%	Nutrient / elicitor	1-3 L	7-10 days
10	AHDB9907	Soluble P and K	fertiliser	0.75%	7-10 days
11	AHDB9906	Experimental	Surface disinfectant	5%	14 days 5 times over 70 day period

Table 3.2. Programmes, based on fungicides, growth promoters and elicitors evaluated in 2017

Programme	Treatment	Product / Timing								
		1 26 April	2 10 May	3 22 May	4 30 May	5 7 June	6 16 June	7 23 June	8 3 July	9 10 July
		Full bloom	End of flowering	End of flowering	Fruitlet	Fruitlet	Fruitlet	Fruitlet	Fruitlet	Fruitlet
1	Fungicide only	-	-	-	-	-	-	-	-	
2	AHDB9910 2 Years	AHDB9910			AHDB9910				AHDB9910	
3	AHDB9910	AHDB9910			AHDB9910				AHDB9910	
4	AHDB9910 + AHDB9904	AHDB9910 + AHDB9904	AHDB9904	AHDB9904	AHDB9910 + AHDB9904	AHDB9904	AHDB9904	AHDB9904	AHDB9910 + AHDB9904	AHDB9904
5	AHDB9904	AHDB9904	AHDB9904	AHDB9904	AHDB9904	AHDB9904	AHDB9904	AHDB9904	AHDB9904	AHDB9904
6	SB Invigorator	SB Invigorator	SB Invigorator	SB Invigorator	SB Invigorator	SB Invigorator	SB Invigorator	SB Invigorator	SB Invigorator	SB Invigorator
7	AHDB9909	AHDB9909	AHDB9909	AHDB9909	AHDB9909	AHDB9909	AHDB9909	AHDB9909	AHDB9909	AHDB9909
8	AHDB9909 Monthly	AHDB9909			AHDB9909				AHDB9909	
9	AHDB9908	AHDB9908	AHDB9908	AHDB9908	AHDB9908	AHDB9908	AHDB9908	AHDB9908	AHDB9908	AHDB9908
10	AHDB9907	AHDB9907	AHDB9907	AHDB9907	AHDB9907	AHDB9907	AHDB9907	AHDB9907	AHDB9907	AHDB9907
11	AHDB9906	AHDB9906	AHDB9906		AHDB9906		AHDB9906	AHDB9906		

Table 3.3. Fungicides applied to Blocks L and H in EE190 during trial in 2017

Date applied	14 day interval blocks		7 day interval blocks	
	Product	**Rate / ha**	**Product**	**Rate / ha**
3 May			Cosine	0.5 L
10 May	Systhane + Captan	450 ml + 2 kg	Systhane + Captan	450 ml + 2 kg
18 May			Topas	0.5 L
25 May	Systhane + Captan	450 ml + 2 kg	Systhane + Captan	450 ml + 2 kg
7 June			Topas	0.5 L
22 June			Topas	0.5 L
6 July	Talius	0.25 L	Talius	0.25 L
13 July	Nimrod	1.4 L	Nimrod	1.4 L

Treatment application

Sprays were applied to the 3 tree plots for treatments 1-11 using a Stihl motorised air-assisted knapsack sprayer at 500 L/ha following EMR SOP GEP 725. Treatments to the large blocks H and L were applied using a tractor-trailed air-assisted orchard sprayer at the standard farm spray volume of 200 L/ha.

Table 3.4. Date and growth stage when treatments 2-11 were applied to sub plots in each block in 2017

Spray number	BBCH growth stage	Date treatment applied	Spray interval Days
1	BBCH 65 Full bloom	26 April	-
2	BBCH 69 End Bloom	10 May	14
3	BBCH 69/71 End bloom	22 May	12
4	BBCH 72/32	30 May	8
5	BBCH 73/33	7 June	8
6	BBCH 73/34	16 June	9
7	BBCH 74/35	23 June	7
8	BBCH 76	3 July	10
9	BBCH 76/37	10 July	7

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 3.4).

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 3.5). 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 (8 May), number of fruitlets were recorded in June (26 June).

Table 3.5. 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 tree of each plot. Secondary mildew was recorded weekly on 5 shoots per tree from 11 May-18 July, a total of ten assessments. The number of mildewed leaves was recorded in the top 5 leaves on each shoot, starting with the first fully expanded leaf and expressed as % leaves mildewed. An additional assessment was done on 29 August of % leaf area mildewed on 5 leaves on 5 shoots per plot using a key of 0-5 where 1 = no powdery mildew, 2 = <10%, 3 = 10-25%, 4 = 25-50%, 5 = >50%.

Yield

All fruit were harvested on 25 September from the three trees in each plot and the weight (kg) recorded.

Fruit quality

At harvest (25 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 as a split plot with high and low mildew as the main plots and the eleven additional treatments as the sub plots. 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. Where there were no significant interactions between the main and sub plot treatments, the data were combined and presented as means of the six replicates. Figures with different letters are significantly different.

Table 3.6. Summary of treatment and assessment timings – NIAB EMR 2017

Activity	Date
Primary blossom mildew assessed on 50 blossoms per plot on 2016 plots AMB	
Primary vegetative mildew assessed on whole tree – Number of shoots and number mildewed on 2016 plots JK	19 April
2017 trial marked out. Treatment 2 plots retained same as in 2016. Other plots rerandomised JK	20 April
1st spray applied Full bloom GE	24 April
Initial flower count on 2 marked branches per plot JK	26 April
Plots checked for Phytotox. None seen AMB	8 May

2nd spray applied End blossom GE	9 May
Secondary mildew assessed Top 5 leaves on 5 shoots per plot AMB	10 May
Secondary mildew assessed Top 5 leaves on 5 shoots per plot AMB	11 May
3rd spray applied GE	17 May
Secondary mildew assessed Top 5 leaves on 5 shoots per plot AMB	22 May
4th spray applied GE	25 May
Secondary mildew assessed Top 5 leaves on 5 shoots per plot AMB	30 May
5th spray applied Treatment 7 B225 rate increased to 2ml per ha GE	1 June
Secondary mildew assessed Top 5 leaves on 5 shoots per plot AMB	7 June
Secondary mildew assessed Top 5 leaves on 5 shoots per plot AMB	8 June
6th spray applied GE	15 June
Secondary mildew assessed Top 5 leaves on 5 shoots per plot AMB	16 June
7th spray applied GE	22 June
Fruit count on 2 marked branches per plot JK	23 June
8th spray applied GE	26 June
Secondary mildew assessed Top 5 leaves on 5 shoots per plot AMB	3 July
9th spray applied GE	6 July
Secondary mildew assessed Top 5 leaves on 5 shoots per plot AMB	10 July
Secondary mildew assessed Top 5 leaves on 5 shoots per plot AMB	18 July
Final mildew assessment as leaf area mildewed on 5 leaves on 5 shoots per plot using score of 1-5 AMB	29 August
All 3 trees harvested by Farm. Yield recorded and 100 fruit sample taken for quality assessments SC/AMB/RS	25-26 September
Fruit quality assessments – fruit size, russet and colour JK/TP/SC	12-25 October
Data input to computer and checked AMB	25 January 2018

Results

Phytotoxicity

No phytotoxicity was noted at any of the assessments. There was no significant interaction between phytotoxicity parameter of fruit set and the main plot effects of low and high mildew incidence. Therefore the data presented in Table 3.7 is the overall mean of six replicates. Data on fruit set was variable due to frost damage to blossoms and fruitlets in April and May. The trial orchard is on a slight east / west slope with block 1 at the lowest point and with greater frost damage to flowers and fruitlets (mean fruit set 8.8%) than block 2 at the eastern higher end (mean fruit set 17.4%). There were almost significant effects of treatments on fruit set with the highest fruit set recorded in Treatment 6 (SB Invigorator) and the lowest in Treatment 9 (AHDB9908). However, caution must be placed on this data due to the variable effects of fruit set across the blocks due to frost damage.

Table 3.7. Mean % fruit set (angular transformed) recorded on apple cv. Gala following nine sprays of various products, applied in addition to a fungicide programme applied at 7 or 14 day intervals at NIAB EMR in 2017. Figures in brackets are back transformed means

Treatment	Product	% Fruit set
1	**Fungicide only**	14.9 (6.6) ab
2	**AHDB9910 2 Years**	20.6 (12.4) abc
3	**AHDB9910**	22.2 (14.2) bc
4	**AHDB9910 + AHDB9904**	22.1 (14.2) bc
5	**AHDB9904**	17.7 (9.2) ab
6	**SB Invigorator**	29.8 (24.7) c
7	**AHDB9909**	19.3 (11.0) ab
8	**AHDB9909 Monthly**	20.6 (12.4) abc
9	**AHDB9908**	12.4 (4.6) a
10	**AHDB9907**	15.0 (6.7) ab
11	**AHDB9906**	16.6 (8.1) ab
F Prob		0.069
SED (40)		4.823
LSD (p=0.05)		9.748

Disease – Powdery mildew

The incidence of primary blossom and vegetative mildew in the orchard was high. Warm dry weather at the end of March was very favourable for mildew sporulation on primaries and spread and infection of leaves on developing shoots. Hence the mildew risk was high and the disease developed rapidly. The 7 and 14 day fungicide programmes applied succeeded in achieving a high and low incidence of mildew in the main treatment blocks in which to evaluate the experimental products (Figures 3.2 and 3.3), but the differences were not as clear as in 2016. An incidence of more than 77% mildewed leaves was recorded in the high mildew

blocks at the first assessment rising to nearly 100% secondary mildew in June before dropping to 50% secondary mildew at the final assessment. In the low mildew blocks, mildew incidence in May was around 60% mildewed leaves falling to around 40 % mildewed leaves at the final assessment. Differences in the mildew epidemics between treatments were much greater in the high mildew blocks compared to the low mildew blocks.

At most assessment dates there was no significant interaction between the main treatment plots of high and low mildew. Therefore the data presented in Table 3.8 is the overall mean of six replicates. Treatment 6 SB Invigorator was the most consistently effective product significantly reducing mildew incidence compare to the fungicide only control at all assessment dates. Treatments 2 (AHDB9910 2 years), 4 (AHDB9910 + AHDB9904) and 5 (AHDB9904 only) were almost as effective. The overall mean for the eleven sub treatments for repeated measures analysis is given in Table 3.9. All treatments had significantly less mildew than the fungicide only plots. Mean leaf area mildewed scores are given in Table 3.10. The results are similar to the results expressed as % leaves mildewed given in Table 3.9.

Yield

Yield data for high and low mildew blocks and the overall mean for the 11 sub treatments is presented in Table 3.11. There were no significant effects of treatments on plot yield. Yields recorded in the low mildew blocks were in general lower than in the high mildew blocks, which is not as expected. This is because two of the three replicates in the low mildew were in the lowest part of the orchard and therefore more subject to the frost damage.

Fruit quality

Fruit quality data - fruit russet, fruit colour and fruit size are presented in Table 3.12. There was no significant interaction between fruit quality parameters and the main plot effects of low and high mildew incidence. Therefore the data presented in Table 3.12 is the overall mean of six replicates. There were no significant effects of treatments on fruit size, russet or colour.

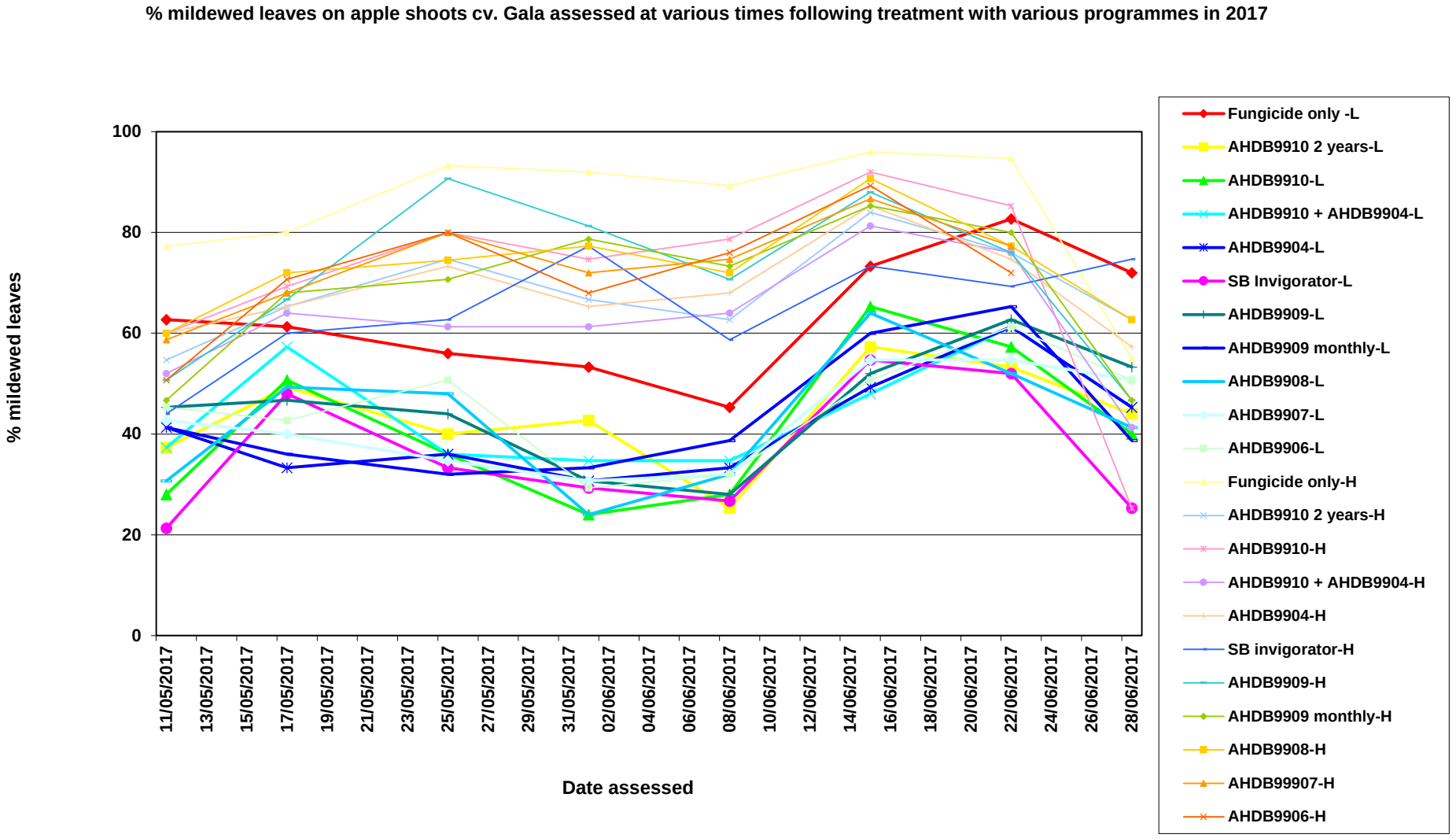


Fig.3.2. Mean % mildewed leaves on apple shoots cv. Gala assessed at various times following treatment with 9 sprays of various products applied in addition to a fungicide programme applied at 7 (L) or 14 (H) day intervals at NIAB EMR in 2017.

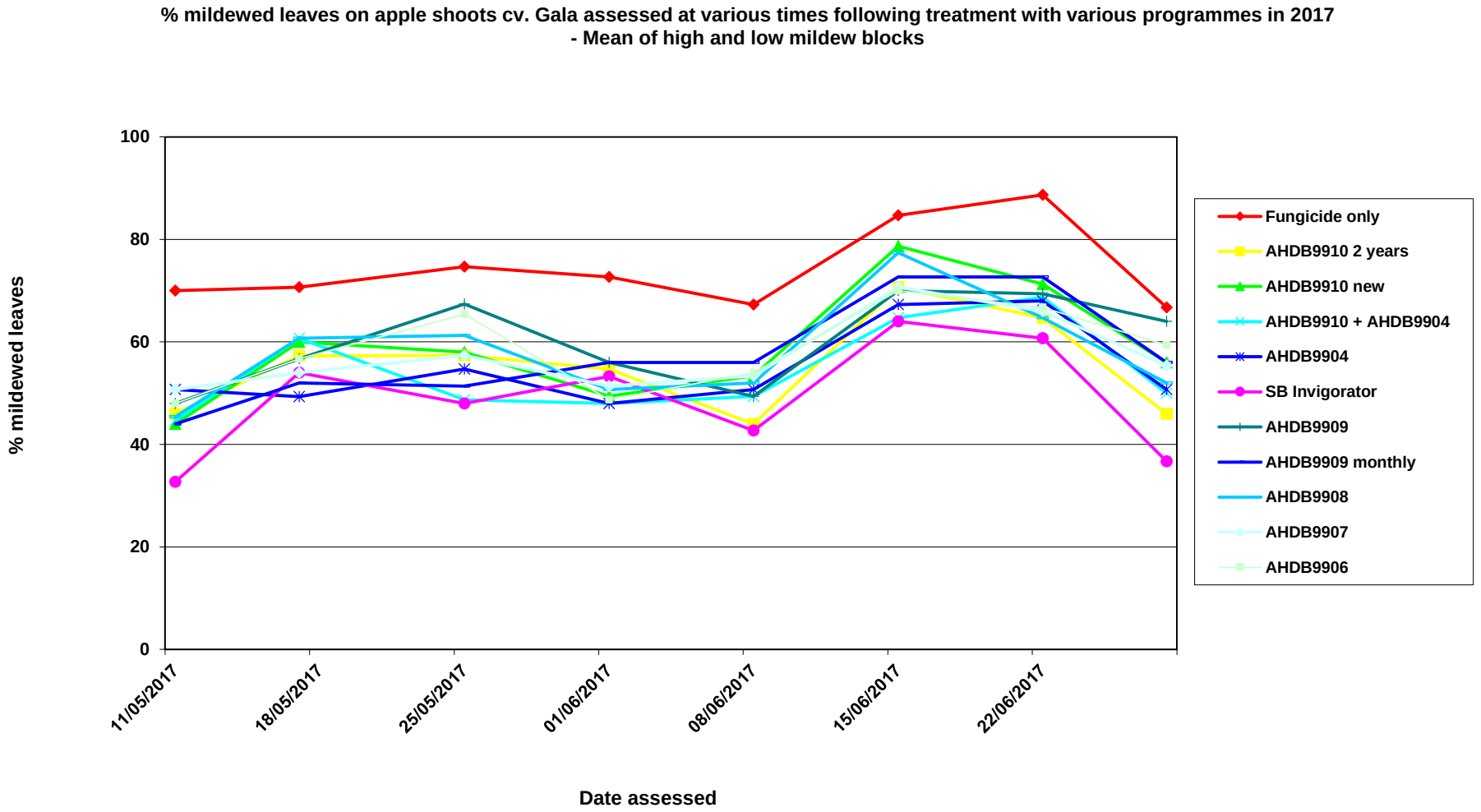


Fig. 3.3. Mean % mildewed leaves on apple shoots cv. Gala assessed at various times following treatment with 9 sprays of various products applied in addition to a fungicide programme applied at 7 (L) or 14 (H) day intervals at NIAB EMR in 2017. Mean of high and low mildew blocks.

Table 3.8. Mean % mildewed leaves (angular transformed) on apple cv. Gala following nine sprays of various products, applied in addition to a fungicide programme applied at 7 or 14 day intervals at NIAB EMR in 2017 . (figures in brackets are back transformed data)

Treatment	Product	% mildewed leaves								
		11 May	17 May	25 May	1 June	8 June	15 June	22 June	6 July	18 July
1	**Fungicide only**	57.4 (71.0) c	57.9 (71.8)	61.9 (77.8) e	60.5 (75.7) b	57.4 (71.0) c	71.4 (89.9) e	72.2 (90.7) b	54.8 (66.8) d	45.0 (50.0)
2	**AHDB9910 2 Years**	42.5 (45.7) ab	49.5 (57.8)	49.8 (58.3) abc	47.7 (54.7) a	41.5 (43.9) ab	58.0 (71.9) abcd	53.9 (65.3) a	42.8 (46.2) ab	36.1 (34.7)
3	**AHDB9910**	42.1 (44.9) ab	51.0 (60.4)	52.4 (62.8) bcd	44.6 (49.3) a	47.6 (54.5) ab	64.6 (81.6) de	58.6 (72.9) a	48.4 (55.9) bcd	44.3 (48.7)
4	**AHDB9910 + AHDB9904**	41.7 (44.2) ab	51.3 (60.9)	44.2 (48.6) a	43.7 (47.8) a	44.3 (48.8) ab	54.1 (65.6) ab	56.4 (69.4) a	45.0 (50.0) abc	39.0 (39.5)
5	**AHDB9904**	45.5 (50.9) b	44.7 (49.4)	48.3 (55.8) abc	43.9 (48.1) a	45.4 (50.6) ab	56.1 (68.9) abc	56.1 (68.9) a	45.4 (50.6) abc	40.5 (42.2)
6	**SB Invigorator**	33.8 (30.9) a	47.4 (54.1)	43.4 (47.2) a	47.3 (54.0) a	40.6 (42.4) a	53.4 (64.4) a	51.4 (61.0) a	36.8 (35.8) a	32.2 (28.4)
7	**AHDB9909**	43.7 (47.7) ab	49.1 (57.1)	58.4 (72.6) de	49.1 (57.2) a	44.7 (49.4) ab	58.1 (72.0) abcd	56.8 (69.9) a	53.7 (64.9) cd	38.3 (38.4)
8	**AHDB9909 Monthly**	41.3 (43.5) ab	46.8 (53.1)	46.2 (52.0) ab	48.9 (56.7) a	48.8 (56.6) b	61.5 (77.2) bcd	59.7 (74.5) a	51.1 (60.6) bcd	45.1 (50.1)
9	**AHDB9908**	42.6 (45.8) ab	52.3 (62.6)	51.9 (62.0) bcd	45.4 (50.7) a	46.2 (52.2) ab	63.4 (79.9) cd	53.9 (65.2) a	46.4 (52.5) bcd	36.0 (34.6)
10	**AHDB9907**	45.3 (50.6) b	47.5 (54.3)	49.9 (58.4) abc	45.9 (51.6) a	47.2 (53.9) ab	58.3 (72.3) abcd	54.7 (66.6) a	(48.1 (55.3) bcd	41.8 (44.5)
11	**AHDB9906**	43.8 (47.9) ab	49.3 (57.5)	54.4 (66.2) cde	44.1 (48.5) a	47.6 (54.6) ab	59.9 (74.9) abcd	54.9 (66.9) a	50.7 (59.9) bcd	35.3 (33.4)
F Prob		0.027	0.160	<0.001	0.003	0.022	<0.001	0.003	0.011	0.311
SED (40)		5.122	4.004	3.760	3.653	4.003	3.726	4.246	4.368	5.533
LSD (p=0.05)		10.351	8.093	7.600	7.354	8.090	7.530	8.582	8.829	11.182

Fi gures with different letters are significantly different from untreated

Table 3.9. Mean (overall mean of 9 assessments) % mildewed leaves (angular transformed) on apple cv. Gala following nine sprays of various products, applied in addition to a fungicide programme applied at 7 or 14 day intervals at NIAB EMR in 2017 . (figures in brackets are back transformed data)

Treatment	Product	Overall mean
1	**Fungicide only**	59.8 (74.8) c
2	**AHDB9910 2 Years**	46.9 (53.3) ab
3	**AHDB9910**	50.4 (59.3) b
4	**AHDB9910 + AHDB9904**	46.6 (52.9) ab
5	**AHDB9904**	47.3 (54.0) ab
6	**SB Invigorator**	42.9 (46.4) a
7	**AHDB9909**	50.2 (59.0) b
8	**AHDB9909 Monthly**	49.9 (58.5) b
9	**AHDB9908**	48.7 (56.4) b
10	**AHDB9907**	48.7 (56.5) b
11	**AHDB9906**	48.9 (56.8) b
F Prob		<0.001
SED (40)		2.252
LSD (p=0.05)		4.551

Table 3.10. Mean mildewed leaf area score assessed in August 2017 on apple cv. Gala following nine sprays of various products, applied in addition to a fungicide programme applied at 7 or 14 day intervals at NIAB EMR in 2017.

Treatment	Product	Mean mildew score
1	**Fungicide only**	2.64 e
2	**AHDB9910 2 Years**	2.19 ab
3	**AHDB9910**	2.36 bcde
4	**AHDB9910 + AHDB9904**	2.27 abc
5	**AHDB9904**	2.2 ab
6	**SB Invigorator**	1.99 a
7	**AHDB9909**	2.56 de
8	**AHDB9909 Monthly**	2.33 bcd
9	**AHDB9908**	2.2 ab
10	**AHDB9907**	2.53 cde
11	**AHDB9906**	2.41 bcde
F Prob		<0.001
SED (40)		0.140
LSD (p=0.05)		0.282

Table 3.11. Effects of treatments on yield of apple fruits cv. Gala recorded following nine sprays of various products, applied in addition to a fungicide programme applied at 7 or 14 day intervals at NIAB EMR in 2017.

Treatment	Product	Yield per plot kg Low mildew	Yield per plot kg High mildew	Yield per plot kg Mean
1	**Fungicide only**	79.6	94.7	87.2
2	**CBL 2 Years**	65.9	96.4	81.2
3	**CBL New**	75.4	113.2	94.3
4	**CBL New + Wetcit**	86.5	81.9	84.2
5	**Wetcit**	69.6	95.8	82.7
6	**SB Invigorator**	74.7	91.1	82.9
7	**B225**	83.6	103.5	93.6
8	**B225 Monthly**	73.0	104.9	89.0
9	**Trident**	47.8	110.1	79.0
10	**Pek Acid**	63.5	110.4	87.0
11	**QE23**	95.9	91.9	93.9
F Prob				0.974
SED (40)				13.946
LSD (p=0.05)				28.185

Discussion

The 7 and 14 day programmes used as the main block treatments successfully established high and low mildew plots in which to evaluate the test products. It was important to have these blocks differing in mildew incidence as the largest differences in mildew between the test products was in the high mildew plots. Most of the test products over the whole season significantly reduced mildew incidence compared to the fungicide only plots. Treatment 6 SB Invigorator was the most consistent in reducing mildew, confirming results from 2015 and 2016. Treatments 2 (AHDB9910 2 years), 4 (AHDB9910 + AHDB9904) and 5 (AHDB9904 only) were almost as effective. Treatment 2 (AHDB9910 2 years) had almost significantly less

mildew than plots receiving AHDB9910 for the first year, indicating a possible cumulative effect of this product. Including AHDB9904 with AHDB9910 also improved performance, but this may have been due to the AHDB9904 only. AHDB9908 performed as well as in 2016 but did not cause leaf spotting or russeted fruit as in 2016. This is most likely due to a change in the wetter included in the formulation.

Conclusions

- 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 (AHDB9910 2 years), 4 (AHDB9910 + AHDB9904) and 5 (AHDB9904 only) were next most consistent products
- AHDB9910 applied for a second year had almost significantly less mildew than in plots where AHDB9910 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

Table 3.12. 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 nine sprays of various products, applied in addition to a fungicide programme applied at 7 or 14 day intervals at NIAB EMR in 2017.

Programme	Treatment	Mean russet score	Mean colour score	Weight of 100 fruit kg	No. fruit > 65 mm diameter	Weight of fruit >65 mm diameter
1	**Fungicide only**	140.2	367.0	12.9	61.0	9.0
2	**CBL 2 Years**	152.2	366.9	12.7	58.7	8.8
3	**CBL New**	147.5	371.5	13.1	60.7	9.5
4	**CBL New + Wetcit**	152.4	357.7	12.2	52.4	7.6
5	**Wetcit**	147.2	367.8	12.9	61.9	9.1
6	**SB Invigorator**	138.2	362.2	12.9	51.8	8.2
7	**B225**	149.3	364.7	12.5	53.0	7.9
8	**B225 Monthly**	141.3	365.9	12.9	60.9	9.3
9	**Trident**	147.2	364.0	13.6	66.9	10.4
10	**Pek Acid**	152.0	359.8	12.1	50.4	7.6
11	**QE23**	134.2	354.0	12.8	60.9	9.0
F Prob		0.749	0.795	0.300	0.429	0.329
SED (40)		10.767	9.078	0.507	7.328	1.131
LSD (p=0.05)		21.761	18.347	1.024	14.811	2.285

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 (EMR/ADAS, Yr 3-4)

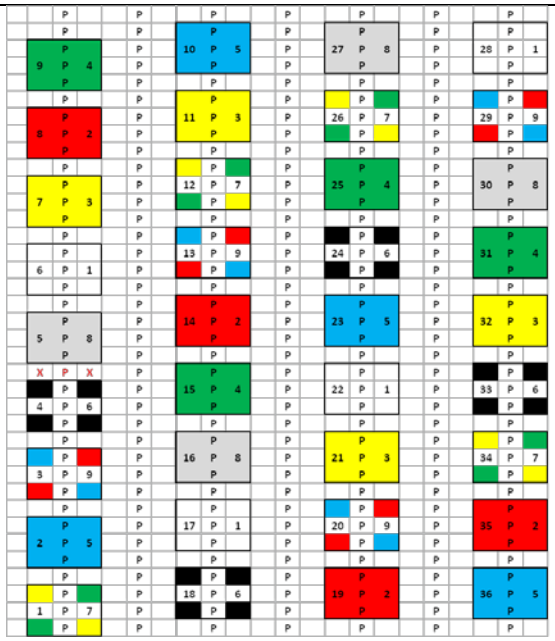
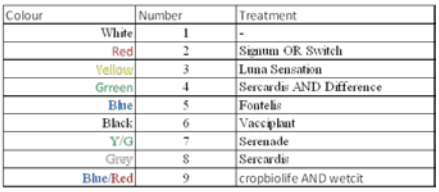
Introduction

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. Fungicides are applied at blossom and pre-harvest including Bellis, Signum, Switch, Systhane (and other myclobutanil containing products) 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.

Materials and Methods

Site

Farm	NIAB EMR, East Malling, New Road, Kent, ME19 6BJ
Orchard	Orchard RF181/182
Grid reference	N 51.2834, E 0.4421
Variety	Penny on Gisella 5 rootstock
Planted	2008/2009
Spacing	4.0 m between rows and 2.0 m in the row (1250 trees per hectare)

Crop information	Crop grown under polytunnels. Ends of tunnel protected by bird netting.
Trial aerial view	
Trial layout	  

Colour	Number	Treatment
White	1	-
Red	2	Signum OR Switch
Yellow	3	Luna Sensation
Grreen	4	Sercardis AND Difference
Blue	5	Fontelis
Black	6	Vaccplant
Y/G	7	Serenade
Grey	8	Sercardis
Blue/Red	9	cropbiolife AND wetcit

Plot number — Treatment number

Inoculum

To ensure Monilinia laxa inoculum was present in the trial mummified fruit were placed into net bags and hung amongst the canopy in each plot.

Treatments

Treatments (Table 4.1) were applied during flowering (to assess activity against M. laxa) and before harvest (to assess activity against M. laxa and M. fructigena). Treatment timing (Table 4.2) were start of flowering (BBCH 57-60), beginning of petal fall (BBCH 67), 10-15 days before harvest and 3-5 days before harvest. For the purposes of this trial even where label/EAMU restrictions permit <4 sprays/ season or have long (>14 day) harvest intervals all fungicides will be applied as 4 spray programmes to establish efficacy.

Table 4.1. Treatments

Treatment	**Category**	**Product**	**Active ingredient**	**Product rate per ha**
1	UNTREATED	-	-	-
2	STANDARD	Signum + Switch	boscalid + pyraclostrobin cyprodinil + fludioxonil	0.75 kg 0.6 kg
3	FUNGICIDE	Luna Sensation	fluopyram + trifloxystrobin	0.8 L*
4	FUNGICIDE	Sercardis + Difference	fluxapyroxad difenoconazole.	0.3 L** 0.2 L**
5	FUNGICIDE	Fontelis	penthiopyrad	0.75 L**
6	ELICITOR	AHDB9957	N/D	1L
7	BIOLOGICAL	Serenade	Bacillus subtilis	10.0 L *
8	FUNGICIDE	Sercardis	fluxapyroxad	0.3 L**
9	STIMULATOR	AHDB9910 + AHDB9904	Flavenoids + wetter	500ml + 0.1%

* maximum individual dose on (protected) strawberry; ** maximum individual dose on apple and pear

Treatment application

Sprays were applied using a Stihl motorised air-assisted knapsack sprayer at 500 L/ha. Pest crop protection programmes were as per standard farm practice for cherry.

Assessments

Flowering

At petal fall an assessment of the number of flower clusters with blossom wilt was conducted.

Table 4.2. Treatment timing

Treatment	Start of flowering (BBCH 57-60)	Beginning of petal fall (BBCH 67)	10-15 days before harvest	3-5 days before harvest
1	-	-	-	-
2	Signum	Switch	Signum	Switch
3	Luna Sensation	Luna Sensation	Luna Sensation	Luna Sensation
4	Sercardis + Difference	Sercardis + Difference	Sercardis + Difference	Sercardis + Difference
5	Fontelis	Fontelis	Fontelis	Fontelis
6	AHDB9957	AHDB9957	AHDB9957	AHDB9957
7	Serenade	Serenade	Serenade	Serenade
8	Sercardis	Sercardis	Sercardis	Sercardis
9	AHDB9910 + AHDB9904 were applied at full bloom then +21 days thereafter			

Fruits

The intention was to harvest 100 visually healthy fruit from the middle tree of each plot. However due to frost in the spring of 2017 and fruit damage caused by SWD the amount of healthy fruit was extremely low on many of the plots. Therefore up to 100 fruit were collected from all three trees from each plot. Fruit was stored at 0°C for 5 days and then transferred to ambient (15-25°C) and assessed following 5 days. Rot incidence and casual agent was recorded out of store and following shelflife.

Phytotoxicity

Symptoms of phytotoxicity were checked for after each treatment and recorded. If present chlorosis / necrosis to foliage and growth regulatory effects to shoots were assessed on a scale 0-5. (EPPO Guideline PP 1/135(3)).

Results

Frosts were recorded in April (20th and 27th) and May (10th) which coincided with blossom and early fruitlet (Figure 4.1) development. As a result of the frosts poor fruit set and subsequent yield were experienced across the trial orchard and in particular the two rows to the east of the trial (replicates 3 and 4). In addition to the frost the trial was severely hit by SWD prior to harvest, despite weekly insecticide sprays. SWD damage was particularly evident in the plots adjacent to the windbreak (the ends of the tunnels were not protected by SWD mesh). Together the frost and the SWD damage meant that very little fruit was available for picking by harvest. Rather than pick fruit from the middle tree only, as stipulated in the original protocol, all 3 trees in each plot were harvested in an attempt to get the 100 fruit required for post-harvest assessments. Overall the average fruit that was suitable for harvesting was 34 fruit per 3 tree plot, the frost and SWD pressure was not constant across the trial orchard (Figure 4.2), for instance in block 1 an average of 75 fruit were picked per plot whilst in the worst affected plots (block 3 and 4) an average of 8 and 9 fruit were suitable for harvesting

respectivley from the three tree plot.

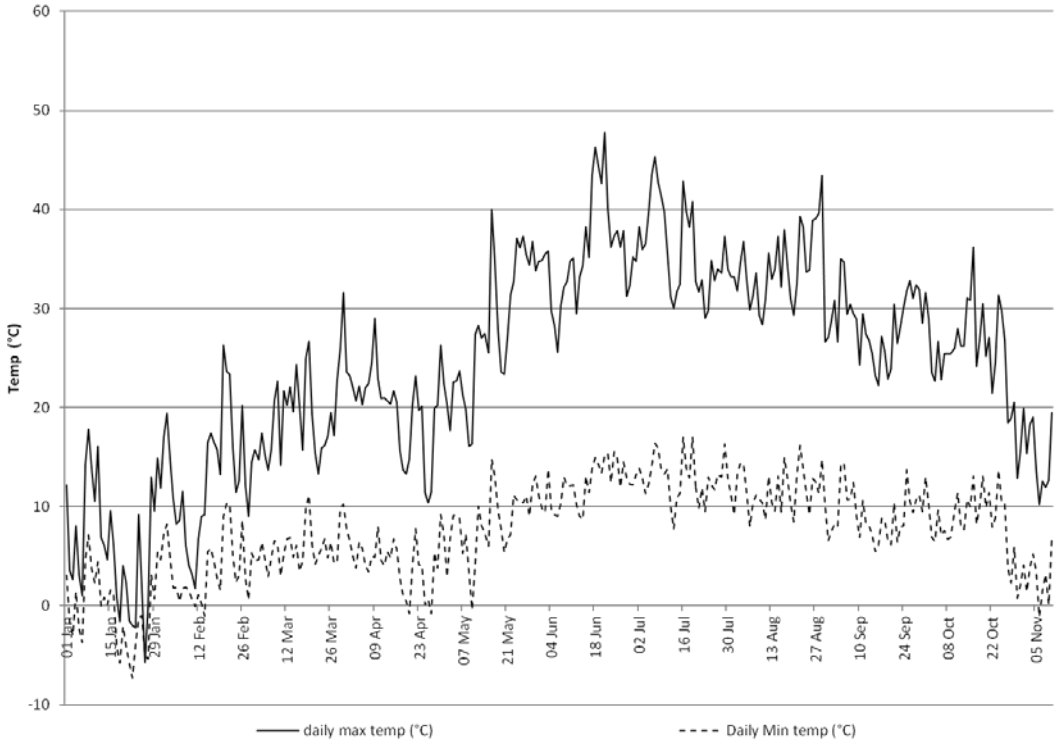


Figure 4.1. Daily min and max temperature recorded by the East Malling weather station during the 2017 season.

Disease assessments

A blossom wilt assessment on untreated controls showed high levels of dead blossoms (as a result of frost) but non showed Monilinia laxa sporulation despite conditions favourable for sporulation and therefore a full assessment was not deemed necessary.

Harvested fruit samples were stored in a cold store. Following 5 days fruit was assessed for rots and a low level of Cladosporium was recorded. Entry holes of SWD was also noted on some of the fruit. Following 5 days at ambient a ‘shelf life’ assessment was conducted. Overall Monilinia laxa and fructigena were present at very low levels (0.7 % and 1.4% respectively). Botrytis was the rot with the highest incidence (11.3% overall), Penicillium (2.3%), Cladosporium (5.4%) and Mucor (0.9%) were also present but at a low frequency. In consultation with a statistician due to the lack of full replication with a meaningful quantity of fruit and the confounding effect of SWD infestation it was not possible to analyse the data for treatment effects.

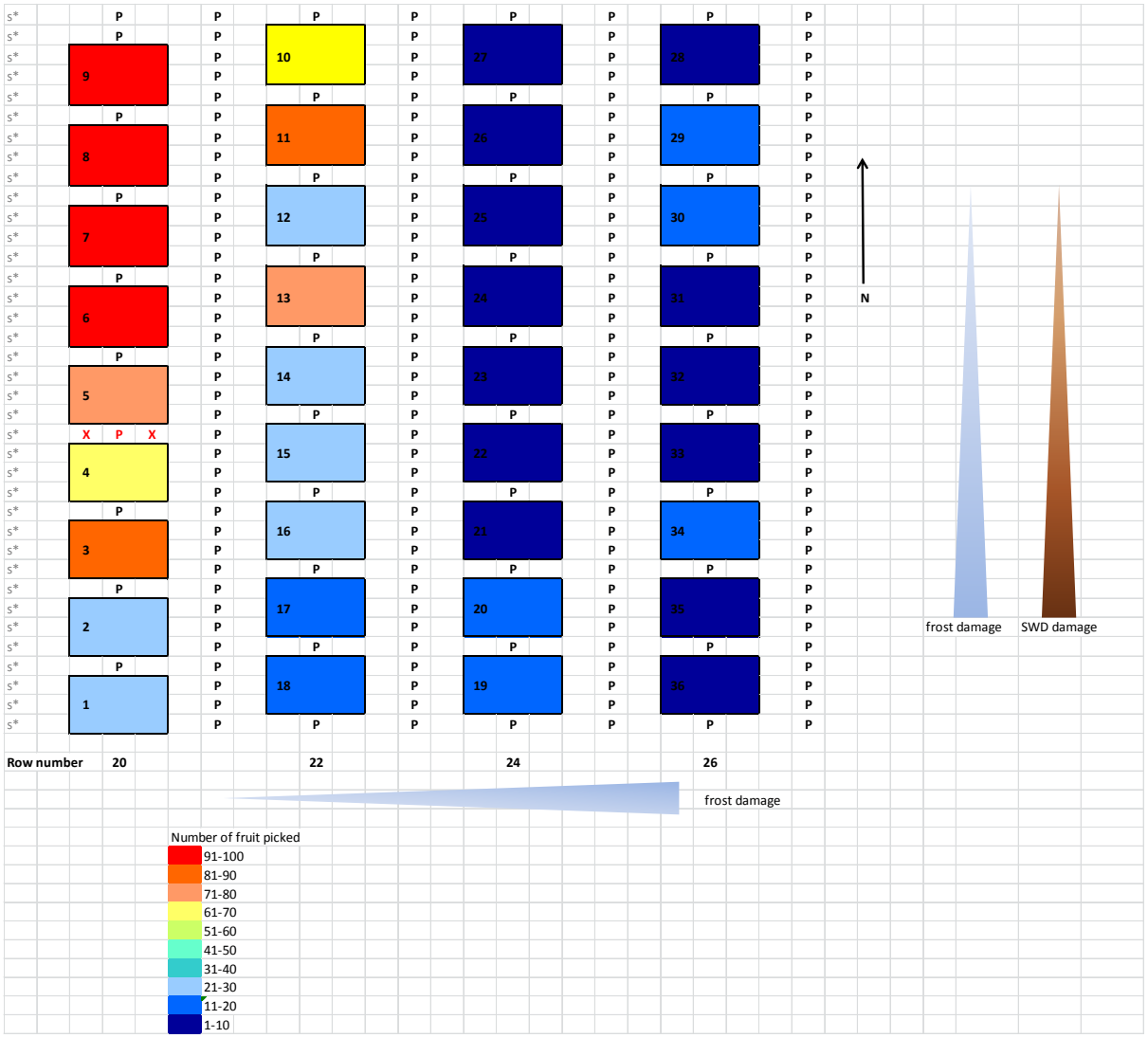


Figure 4.2. The effects of frost and SWD on the numbers of fruit suitable for picking at harvest. Fruit picked from each plot is represented by a colour key.

Discussion

The late frosts in spring 2017 greatly affected the trial because of a reduction in fruit available for post harvest assessments, a severe infestation of SWD at harvest reduced the amount of heathy fruit available for assessment further and confounded the interpretation of the post harvest data. The loss of fruit to frost is unavoidable (without significant investment) and is one of the many risks of conducting trials in the orchard. SWD is another risk which can be mitigated against and although a spray programme was applied through the growing season

the ends of the tunnel were not sealed with insect netting which could have reduced numbers. Future work will have to take these considerations into account.

Future work

- The field trial can be repeated on an alternative orchard made available at NIAB EMR for the 2018 season where the SWD and frost risk are reduced
- Elements of overwinter control developed in a PhD studentship will be incorporated into the programme

Objective 6 - Codling and Tortrix Moth

6.1 Pheromone MD

Aim

Integrate pheromone mating disruption into the control programmes for codling and tortricid 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 10.1) feed on the surface of the pear and apple 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. 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. 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 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.

Figure 10.1. 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. Objectives were:

- To produce lures with a range of pheromone loadings and test for attractiveness in the field;
- To collect or rear adult female Blastobasis and collect the pheromone by extraction or trapping volatiles;
- To identify the chemical structures of the pheromone components;
- To demonstrate attractiveness to a lure with a suitable blend and loading in the field and develop a suitable lure for pest monitoring;
- To test a lure and trap in commercial orchards for pest monitoring purposes.

Materials and Methods

Pheromone traps: Several contacts with experience of Blastobasis on orchards helped with the deployment of traps and monitoring and allowed us to survey their orchards (Table 10.1).

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 10.2).

Twenty replicates of each treatment were deployed in red delta traps with sticky glue inserts. The traps were hung at mid-canopy height, perpendicular to the row and > 10 m apart. Lures were changed every 4 weeks.

Table 10.1. Details of locations for testing pheromone lures for Blastobasis during 2017

Name	Organisation	Address
Graeme Cross	DAERA	
Sean Mac An tSaoir	Agri-Food and Biosciences Institute of Northern Ireland (AFBINI)	18a Newforge Lane, Belfast, Co Antrim, Northern Ireland, UK BT9 5PX
Will Dawes Steve Castle	Mount Ephraim	Hernhill Faversham, Kent ME13 9TX
Simon Mount	New Barn Farm	The Street, Stourmouth, Canterbury CT3 1HX
Cristian Marmandiu	Haygrove Ltd.	Redbank, Ledbury, Herefordshire HR8 2JL

Table 10.2. Composition of lures used in trapping experiments for Blastobasis.

	Amount (µg)	
Lure code	**Z11-16:Ac**	**Z11-16:Ald**
2017/055/A	1000	100
2017/055/B	500	500
2017/055/C	100	1000
2017/055D	-	-

Ten replicates were located in apple orchards in Northern Ireland (coordinated by Sean Mac An tSaoir), 10 in Kent (across two orchards) (coordinated by Chantelle Jay) and 8 in a Kiwiberry plantation in Ledbury by Cristian Marmandiu, Haygrove Ltd. Traps were deployed in randomised complete blocks of the four treatments and each orchard had at least two blocks.

Orchards with Blastobasis in previous years were also tap sampled (on 11, 26 May, 9, 23 June and 21 July at Mount Ephraim and on 11, 26 May, 9, 23 June and 5, 27 July at New Barn Farm) with the intention of collecting adult Blastobasis moths for pheromone extraction and identification. We also collected larvae from the Kiwi berry tunnel at Haygrove in September for rearing through to adults. At the time of writing these were currently being kept in winter conditions.

Sticky inserts from traps were posted to NIAB EMR from the Northern Ireland sites and checked at the Kent sites by NIAB EMR staff. Trap catches were assessed at least every two weeks from early May. Numbers of male and female Blastobasis per trap were recorded. Any potential Blastobasis moths from these traps were collected for molecular analysis.

Molecular analysis: The body of a whole moth, of size and shape similar to Blastobasis, was picked off each of 21 sticky traps (Table 10.3) and placed into individual 2 ml microtubes and placed into a freezer at -20°C. A possible Blastobasis larva was also placed into a 2 ml microtube and kept with the moths in the freezer until DNA extraction as a positive.

Table 10.3. Details of trap sites in Northern Ireland for collection of one Blastobasis-like larva and 21 single Blastobasis-like moths for extraction and identification of species by DNA barcoding.

Date	Tube No	Site	Block	Treatment	Blastobasis-like Moths	Small black stout moths
Sep/17	1	Hereford	-	-	Larva	
29/6/17	2	TM	6	C	47	0
29/6/17	3	TM	5	C	13	0
29/6/17	4	LG	9	C	3	0
29/6/17	5	AF	2	B	3	0
29/6/17	6	AF	1	C	15	0
29/6/17	7	JB	8	C	7	0
29/6/17	8	LG	10	C	7	0
9/6/17	9	LG	10	C	4	0
9/6/17	10	LG	9	C	7	0
9/6/17	11	TM	5	C	4	0
9/6/17	12	JB	8	C	12	0
9/6/17	13	AF	1	C	13	0
9/6/17	14	AF	2	B	9	0
9/5/17	15	GM	3	A	0	4
9/5/17	16	GM	3	B	0	4
9/5/17	17	GM	4	A	0	8
9/5/17	18	GM	4	D	0	3
9/5/17	19	JB	7	C	0	2
9/5/17	20	JB	8	B	0	8
9/5/17	21	LG	10	A	0	3
9/5/17	22	TM	6	C	23	0

DNA was extracted from the samples using a DNeasy Power soil kit (Qiagen) following the manufacturer's protocol but with the following amendments: Beads from the Powerbead tube were poured into the microtubes with the larva/moth samples on removal from the freezer. An additional two 4 mm ball bearings were added to the tube along with the 60 µl of buffer C1. The tubes were placed in a water bath at 60°C for 10 mins and inverted twice during this period. Tubes were then placed in a Genogrinder for 1500 rpm for 60 s instead of the 10 min vortex (as protocol). Protocol was then followed as published with all optional steps. DNA was quantified and quality-checked using a Nanodrop 1000 spectrophotometer (Thermo Scientific) and stored at -20°C.

DNA was diluted to between 1 ng/µl and 2 ng/µl in sterile water (Sigma) and PCR was performed to amplify part of the Cytochrome oxidase I (COI) gene (used as standard gene to identify insects), using 1X Moltaq basic buffer (Molzym), 0.2 mM dNTPs (ThermoFisher), 2 mM $MgCl_2$ (Molzym), 0.2 µM primer HCO (5'- -3'), 0.2 µM primer LCO (5'- -3'), 0.5 units Moltaq taq polymerase (Molzym), in a 25 µl reaction. The resulting PCR products were run on a 1.5% agarose gel at 100 V for 60 mins to check for successful amplification.

Those samples successfully amplified were sent to GATC Biotech as per their sample preparation request, namely, 5 µl DNA (XX-YY ng/µl) with 5 µl 5mM primer, each sample sent with both the forward and reverse primer, for DNA barcoding.

On receipt of results from GATC Biotech the forward and reverse sequences were aligned where possible using Genious software and the resulting sequence entered into the NCBI Blast tool for comparison to the sequences of species held within their database.

Results

The traps in NI were placed across five sites. These caught a number of potential Blastobasis moths, in addition to other moth species (Figure 10.2) with most moths caught by blend C (1:10 Z11-16:Ac : Z11-16:Ald). Darkening of the moths in the glue made visual identification difficult, hence the progression to molecular analysis for confirmation of species. Few moths were caught in the traps at the Kent sites and none were Blastobasis sp. No adult moths were caught from the tree traps in Kent.

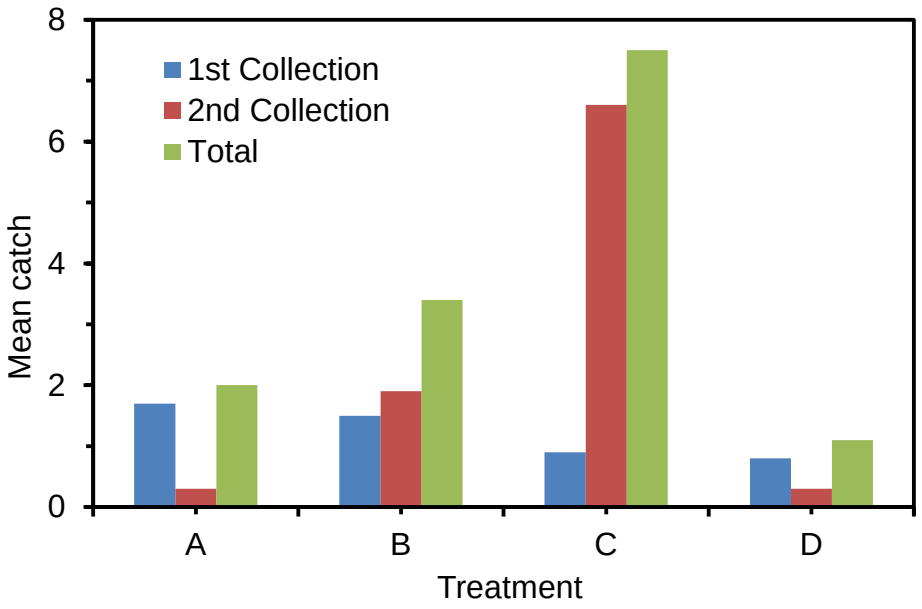


Figure 10.2. Mean catches of Blastobasis-like moths per trap in Northern Ireland (10 replicates)

Molecular analysis: Of the 22 samples, six showed no band on the agarose gel indicating they failed to amplify during PCR. The remaining 16 samples were sent to GATC Biotech for sequencing of which 5 failed, most likely due to the quality of DNA of those samples. The sequences fell into 3 distinct clades and input of the sequences into the NCBI Blast tool indicated 8 of the 10 moths identified were a 100% match to the Scotch smudge moth, Rhigognostis incarnatella (Steudel 1873) (Lepidotera: Plutellidae), one moth was a 100% match with the green pug moth, Pasiphila rectangulata L. (Lepidoptera; Geometridae) while the larva (sample 1) and one of the moths was a 94% match to Blastobasis adustella and a 93% match to Blastobasis lacticolella (Synonym: decolorella) (Table 10.4, Fig. 10.2.). Given that the sequence database does not contain the sequences for all of the Blastobasis species, it is possible that the NI species is another Blastobasis species. When these moths are matched back to the original descriptions, one was described as a 'Blastobasis-like moth', and the other as a 'small black stout moth', hence again highlighting the difficulties of identification from the glue-soaked samples. These Blastobasis sp. moths were collected from traps baited with blends B or C. Descriptions of Blastobasis species are shown in the Appendix.

Table 10.4. Moths collected on sticky traps identified by DNA barcoding of COI gene locus and comparison with NCBI Database.

Date	Sample No.	Pheromone blend	Species with closest match
Sep	1 (larva)		Blastobasis adustella (94% match)
29/6/17	2	C	Failed sequencing[2]
29/6/17	3	C	Rhigognostis incarnatella
29/6/17	4	C	Failed PCR[1]
29/6/17	5	B	Rhigognostis incarnatella
29/6/17	6	C	Rhigognostis incarnatella
29/6/17	7	C	Rhigognostis incarnatella
29/6/17	8	C	Rhigognostis incarnatella
9/6/17	9	C	Failed sequencing[2]
9/6/17	10	C	Failed sequencing[2]
9/6/17	11	C	Failed PCR[1]
9/6/17	12	C	Rhigognostis incarnatella
9/6/17	13	C	Rhigognostis incarnatella
9/6/17	14	B	Rhigognostis incarnatella
9/5/17	15	A	Failed PCR[1]
9/5/17	16	B	Failed sequencing[2]
9/5/17	17	A	Failed PCR[1]
9/5/17	18	D	Failed sequencing[2]
9/5/17	19	C	Blastobasis adustella (94% match)
9/5/17	20	B	Failed PCR[1]
9/5/17	21	A	Pasiphila rectangulata
9/5/17	22	C	Failed PCR[1]

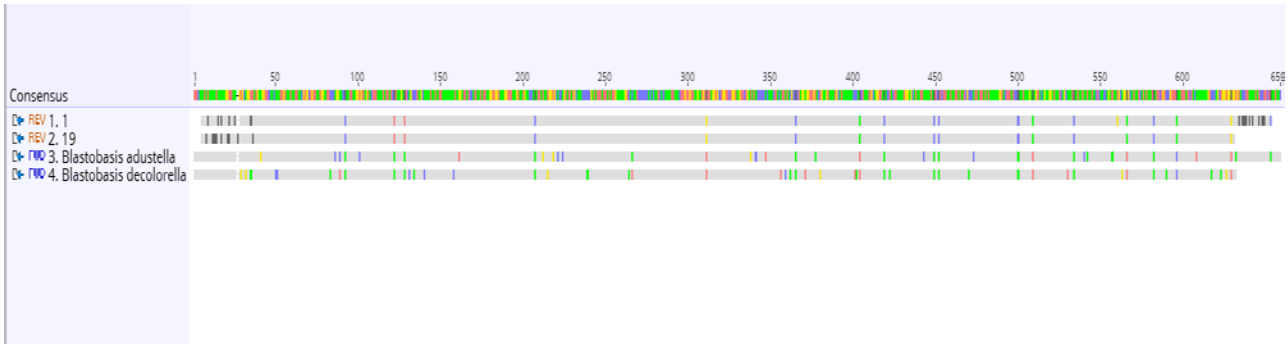


Figure 10.4. Data alignment from of the two samples that were Blastobasis sp. compared to the sequence for the two named species, B. adustella and B. lactocollela (Synonym: decolorella). '1' is the moth larva from kiwiberry and '2' is a moth from a sticky trap in NI.

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 (Synonym decolorella). 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.

Future work

In 2018 work will focus on obtaining virgin adults of Blastobasis laticollela for pheromone and molecular analysis, rather than further testing of candidate pheromone blends. Attempts will be made to rear larvae collected during 2017 and to collect pupae or adults from sites in Northern Ireland during 2018.

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.

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 2 orchards per farm and orchards were separated by >1 km. Figure 7.1.1 gives an ideal orchard layout, with Figures 7.1.2 onwards showing exact locations. The intervention treated area was randomised. The treated areas will be assessed and compared to an untreated area of the same orchard.

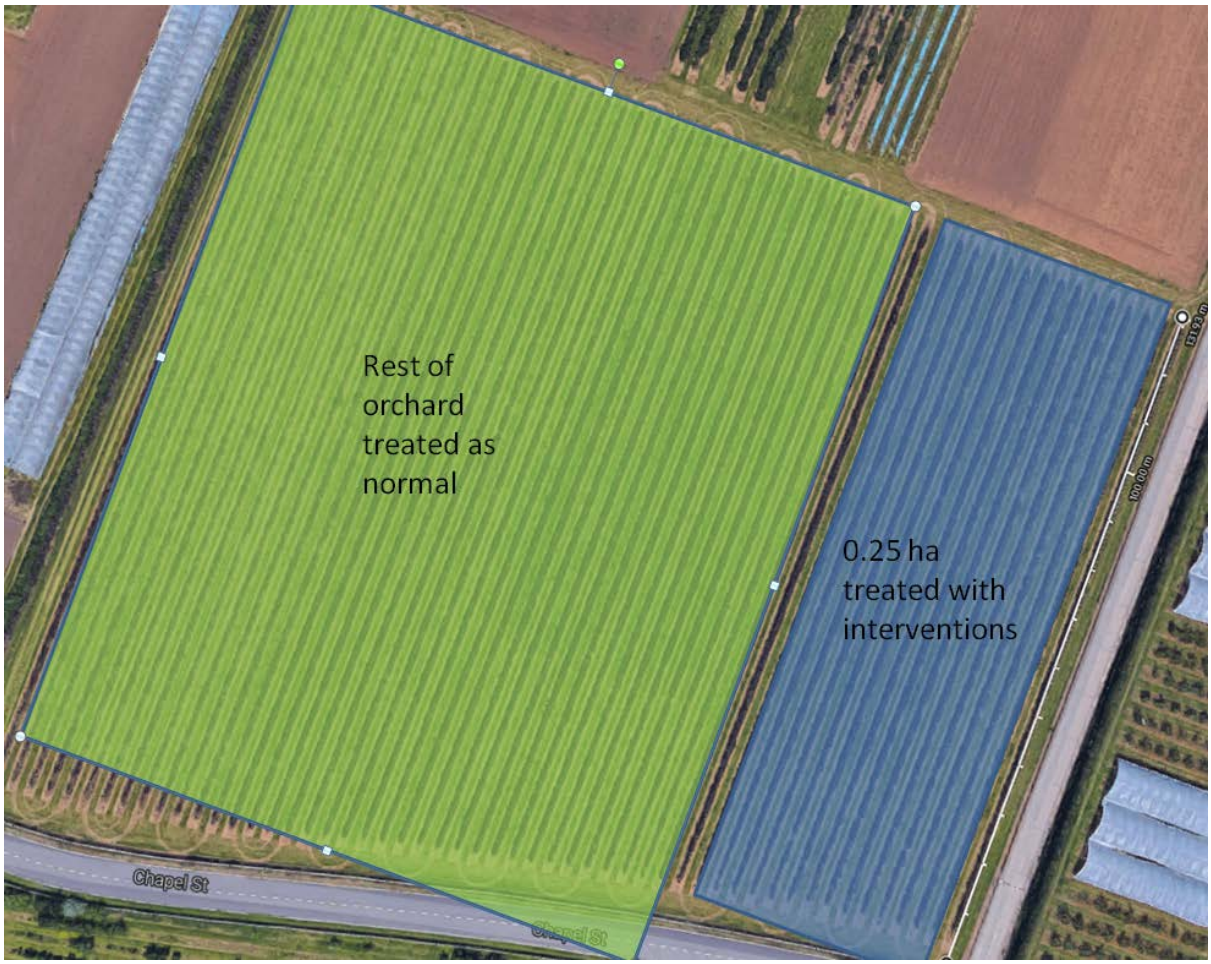


Figure 7.1.1. Example of orchard layout

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	3.35x1m or 1.2m	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

Figures 7.1.2-7.1.7 Orchard maps and location of treated area (Yellow is treated enhanced ecology area, Green is the untreated control area)

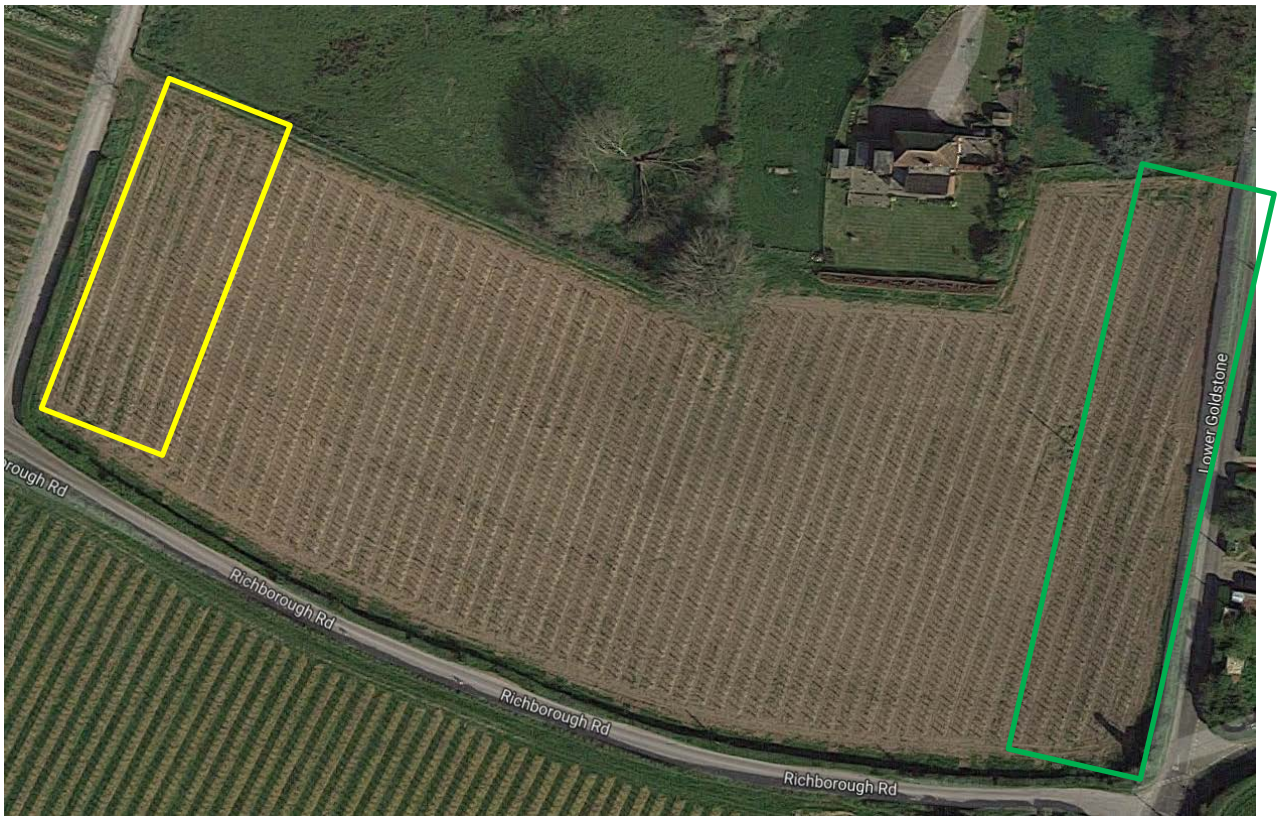
7.1.2. John Bray A12 Jazz

11 tree rows with every other alleyway sown with floral strip. Earwig refuges in centre 6 tree rows

7.1.3. Clive Chandler, Broome

8 tree rows with alleyway sown with floral strip. Earwig refuges in centre 6 tree rows

7.1.4. Clive Chandler, 'Richards'



12 tree rows with alleyway sown with floral strip. Earwig refuges in centre 9 tree rows

7.1.5. Peter Checkley, New Barns Gala

15 tree rows with every 3rd alleyway sown with floral strip. Earwig refuges in centre 9 tree rows

7.1.6. Jeremy Linsell, Rectory (farm area is upper right to the Braiseworth marker)

6 (5) tree rows with every other (adjacent) alleyway sown with floral strip. Earwig refuges in centre 4 tree rows

7.1.7. Charles Highwood 'Willow Wood'

Treatment applications (Table 7.1.2) were to include as many of the following treatments and be coupled with earwig sensitive spray programmes in the new orchards to encourage establishment of natural enemies.

Table 7.1.2. Potential interventions within the programme

Treatment	**Detail**	**Target beneficial**	**Improve**	**Date implement**
Alleyway sowings [*1]	Alleyway sowings should include 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
Solitary bee scrapes		Andrena	Fruit quality	Spring post planting
T. pyri prunings		Typhodromus pyri	Rust mite, spider mite	Spring post planting
Hoverfly attractant	From AHDB TF 218	Hoverfly larvae	Aphid	From 2018
Anthocorid releases		Anthocorids	Aphid	Bottles contain 500 adults. Release in spring at 5-6 release points /ha

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

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

Crop husbandry will be the growers' normal programme of sprays, but adjusted to be sensitive to the key life stage of earwigs. Regular communication has been established between NIAB EMR staff and the growers/advisors. A Stevenson's screen/white delta trap with two data loggers to record temperature and humidity every 30 minutes were deployed in each orchard. Photos were taken of the weed establishment at each site in the autumn of 2017. Each time the orchards are visited in 2018 a record will be taken of the crop growth stage.

Progress update

The alleyway sowings are completed at all sites (Table 7.1.1) and most orchards have now established. The suggested seed mix (Table 7.1.3) has been used, with some modifications on some sites. For example, Peter Checkley (Site 4) and Charles Highwood (Site 6) have 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 7.1.3. Suggested and tested seed mix for orchard alleyway planting in the 0.25 ha on the intervention side of the 6 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.

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.

Earwig refuges, courtesy of the Innovate UK Bioactive predator refuge (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, with 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 were treated, at Sites 3 & 4 - 9 rows were applied treatment 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 (Russek IPM) will be added in spring 2018.

Assessments (2018)

In 2018 there will be 3 assessments each year in the untreated and treated half of each orchard. The assessments will take place in the centre of the treated section of the orchard and the centre of the untreated section of each orchard. Pheromone monitoring traps for SFF, FTT and CM will be placed in each orchard to estimate tortrix pressure and checked and sticky inserts and lures changed on each assessment date.

APRIL

- Download temperature and RH data
- Solitary bee nesting sites will be estimated by walking a transect through the middle of each orchard and then calculated as number of next holes per metre.
- % bare ground will be estimated using 10 x 50x50 cm quadrats per orchard half in the alleyways.
- Numbers of insect visits to flowers will be assessed once at peak blossom.
- 30 shoots will be examined for the presence of aphids.
- 30 refuges will be opened and predators recorded.
- 30 trees will be tap sampled for other predators.

JUNE

- % bare ground will be estimated using 10 x 50x50 cm quadrats per orchard half in the alleyways.
- 30 shoots will be examined for the presence of aphids.
- Apple leaf curling midge 10 shoots on 30 trees will be examined and the number of shoots affected per 10 shoots recorded.

- 30 young leaves will be assessed for the presence of rust mite and spider mite 30 refuges will be opened and predators recorded.
- 30 trees will be tap sampled for other predators.
- Flowering strips will be estimated by taking 20 sward height records and 20 x 1 m^2 quadrate records of number and species of flower heads.
- First generation CM damage assessment on dropped and tree fruit from 20 trees in each plot.
- Tree trunks inspected for woolly aphid.

AUGUST

- % bare ground will be estimated using 10 x 50x50 cm quadrats per orchard half in the alleyways.
- 30 young leaves will be assessed for the presence of rust mite, spider miteand *T. pyri.*
- 30 refuges will be opened and predators recorded.
- 30 trees will be tap sampled for other predators.
- Second generation CM damage assessment on dropped and tree fruit from 20 trees in each plot

Summary

- The orchards have established well, as have the sowings in the majority of the orchards.
- Earwig refuges were deployed in autumn 2017.
- Monitoring in 2018 will provide detailed data on the establishment of orchards with and without interventions.

7.2 Dynamic pear sucker/predator chart for growers

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

On 17 March 2016 and 09 March 2017 we trained growers and their staff to use a current, established, template for pear orchard pest/predator assessment, developed for amateur farm staff to implement on farm (Table 7.1).

The course gave the background to the study including the original HortLINK project, lifecycle of pear sucker, Cacopsylla pyri, the description of the damage and how to identify the life stages, insecticide resistance, and the importance of natural enemies including habitat enhancement for natural enemies. The training aimed to;

1. Train farm staff in identification of sucker and its natural enemies so that growers can make informed decisions on if and when to apply control measures
2. Communicate regularly with entomologists at NIAB EMR on thresholds for control
3. Contribute to data for a potential model for predator prey thresholds

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. 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 7.1). The results were collated at least fortnightly and then shared with all participants.

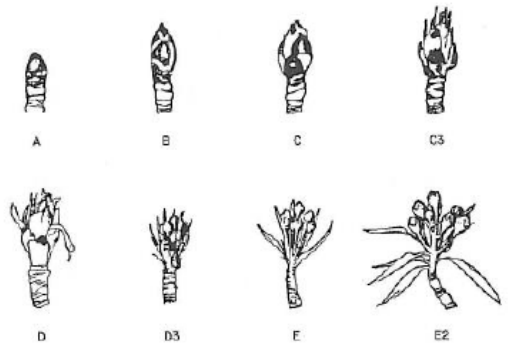
Table 7.1. Attendees of pear sucker and predator monitoring training day run by Michelle Fountain and David Long on 17 March 2016 and Michelle Fountain and Maddie Cannon on 09 March 2017

Name	Farm/Company	Person responsible for reporting
2016		
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 Killian	FAST	-
2017		
Nigel Jenner	AVALON	Mark Graves
Mark Chapman	AC HULME & SONS	Ivaylo Bashev

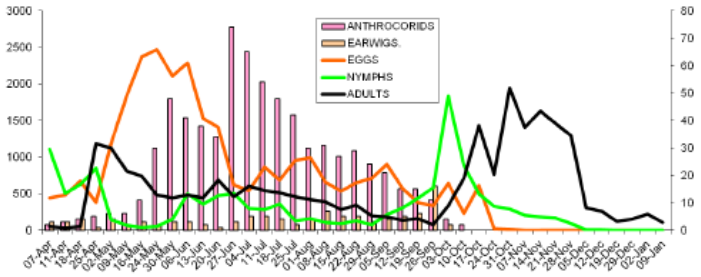
The orchards were coded 'farm_orchard'. Only one changed from 2016 to 2017 (H-H to H_G).

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

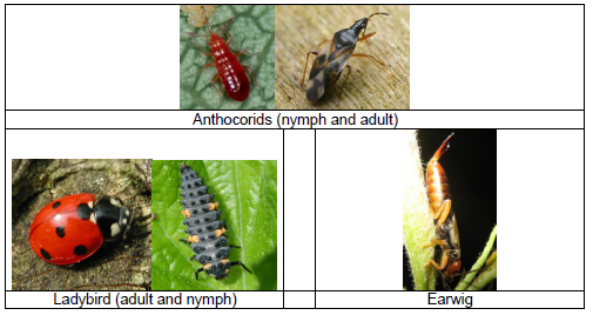
Monitoring pear sucker

- 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 7.1. Sandard 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 both 2016 and 2017 – March. The first generation peaks were at the end of May beginning of June in 2016 and mid-May in 2017.

In all orchards pear sucker egg numbers were similar in 2017 and 2016. Most orchards did not have significant numbers of pear sucker eggs or nymphs.

However it was noted that a lower peak of sucker eggs in 2017 (<2000 /30 shoots) was an improvement on 2016 (>2000 eggs) at orchard G_M, at Farm 2. 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.

Farm 1 never peaked above 1000 eggs and early season predators and later season natural enemy numbers appeared to control pear sucker. In some weeks over 80 earwigs per 30 branches were present in orchard C_C (Fig. 7.2). The only insecticides applied here were one spray of Runner early season and 3 sprays of Carpovirusine.

Figure 7.6 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 is observed that the numbers of earwigs are generally low (< 3 each week). At this farm multiple applications of sulphur were applied in 2016 and 2017. The effect of these sprays on earwigs is not known.

At Farm 4 (Fig 7.5) earwig numbers were lower in 2017 compared to 2016.

In general, where there are at least 10 anthocorids in 30 taps samples each week, there is good control of pear sucker. A good example of this is Fig. 7.5 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), 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). 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.

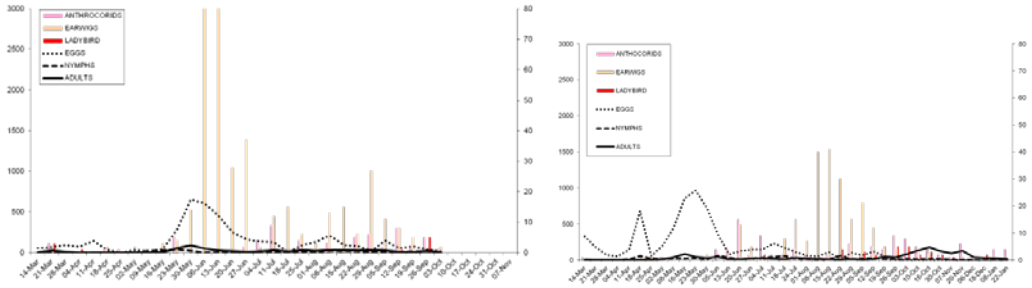
It should be noted that these data analyses, to date, do not take into consideration the spray programmes or other unrecorded crop management practices for pests and natural enemies.

Table 7.2. 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

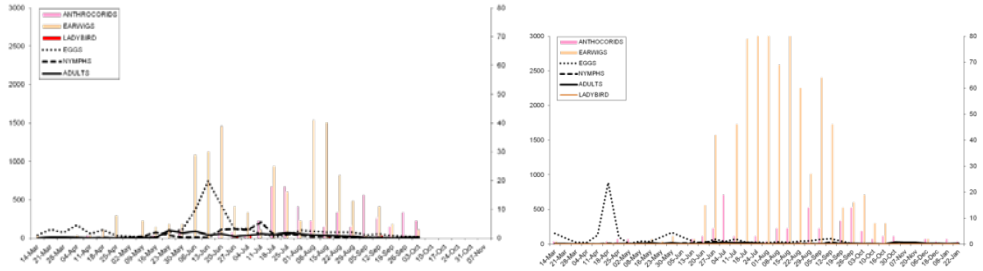
Correlations							
Anthocorids	-0.071						
Earwigs	-0.391	0.606					
PS Eggs	0.550	0.283	0.206				
Ladybirds	0.498	0.273	0.268	0.843			
PS Nymphs	0.466	-0.248	-0.112	0.801	0.628		
Total_PS	0.655	0.105	0.042	0.975	0.814	0.887	
Total_ear_anth	-0.244	0.912	0.879	0.276	0.301	-0.206	0.085
Two-sided test 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	
Total_ear_anth	0.381	<0.001	<0.001	0.320	0.275	0.461	0.764
	PS Adults	Anthocorids	Earwigs	PS Eggs	Ladybirds	PS Nymphs	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.

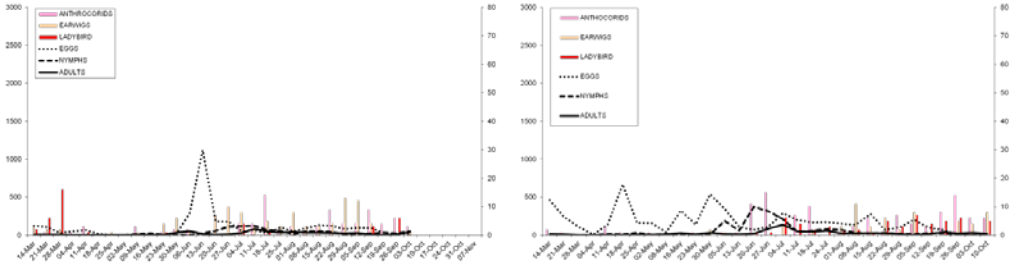
Orchard **2016** **2017**



C_C



C_G

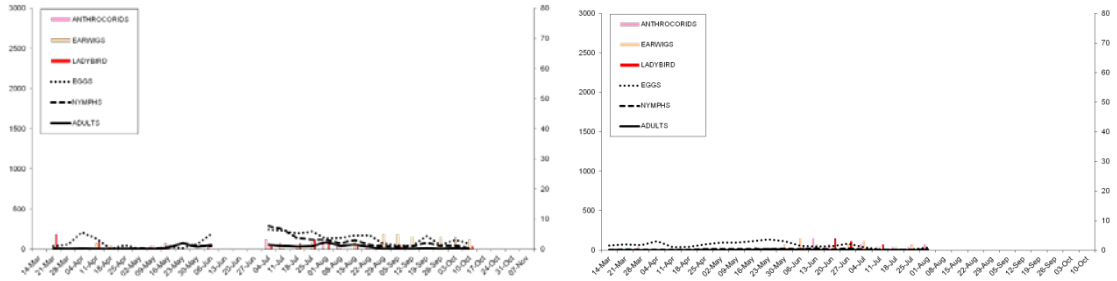


C_S

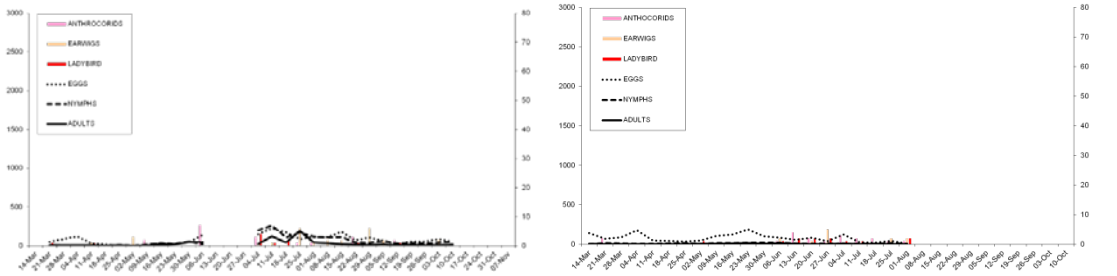
Figure 7.2. Farm 1 pear sucker and natural enemy monitoring in 3 orchards over 2 consecutive years during the growing season

2016	Honeydew	4
	Harmful	1-2
	Harmless	1
2017	Honeydew	5-8
	Slightly harmful	0-1
	Harmless	3

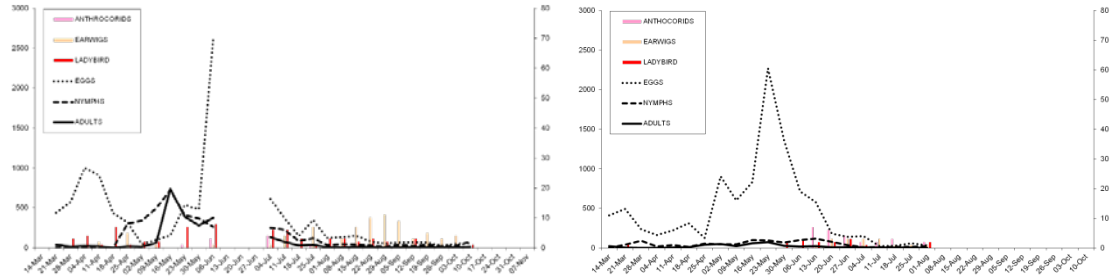
Orchard **2016** **2017**



G_N



G_B

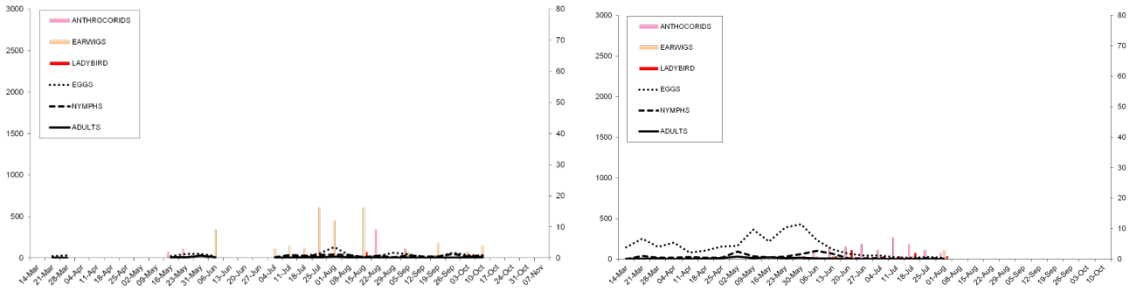


G_M

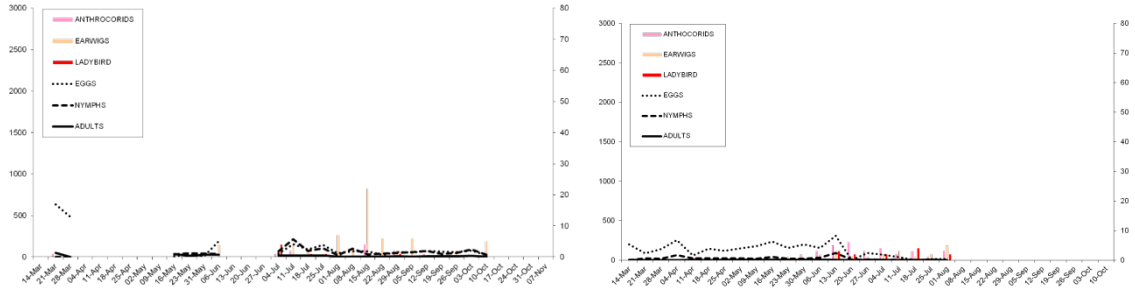
Figure 7.3 Farm 2 pear sucker and natural enemy monitoring in 3 orchards over 2 consecutive years during the growing season

2016	Honeydew	5
	Slightly Harmful	1
	Harmless	2
2017	Honeydew	4-5
	Slightly Harmful	1
	Harmless	1

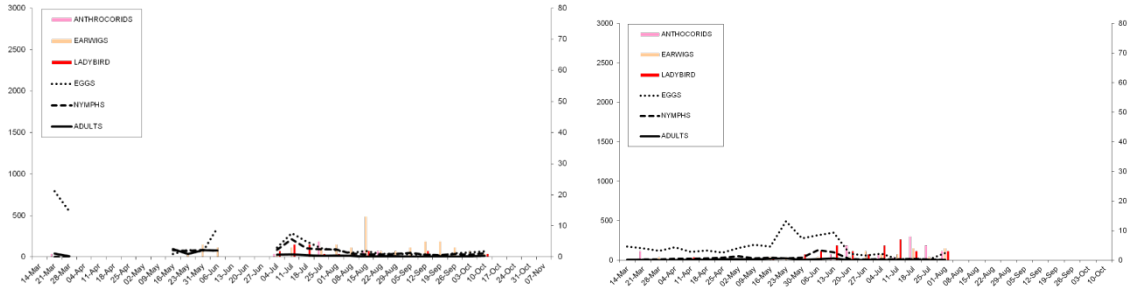
Orchard **2016** **2017**



D_F



D_R

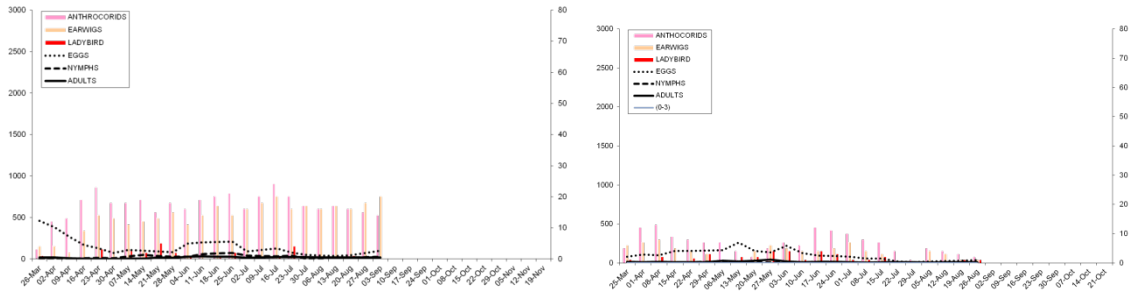


D_P

Figure 7.4. Farm 3 pear sucker and natural enemy monitoring in 3 orchards over 2 consecutive years during the growing season

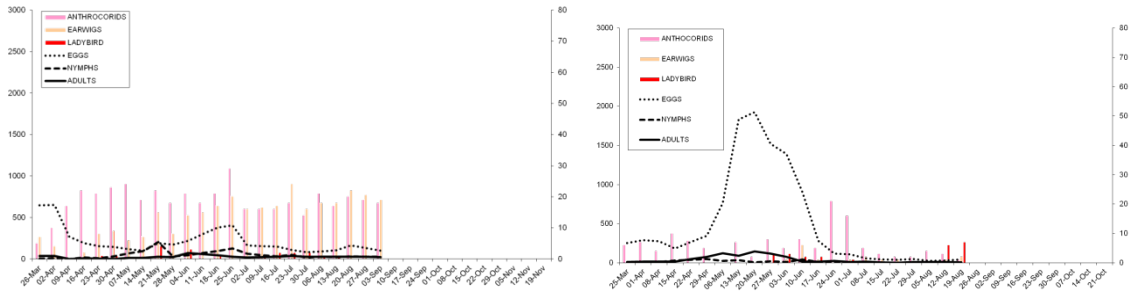
2016	Harmful	1
	Slightly Harmful	1
	Harmless	2
2017	Slightly Harmful	2
	Harmless	2

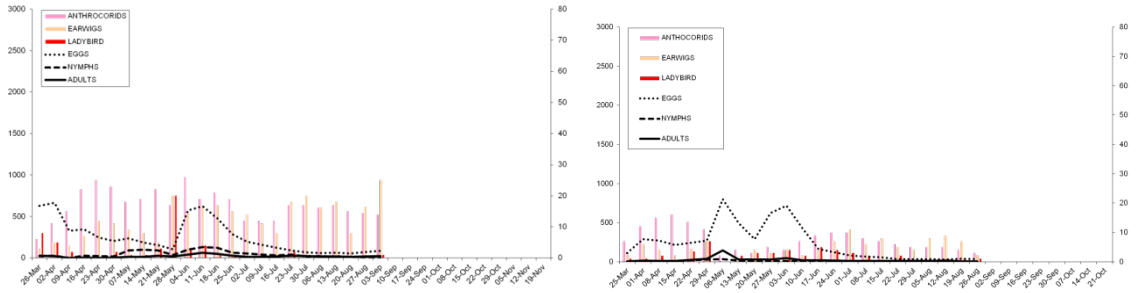
Orchard **2016** **2017**



H_W

H_H then *H_G



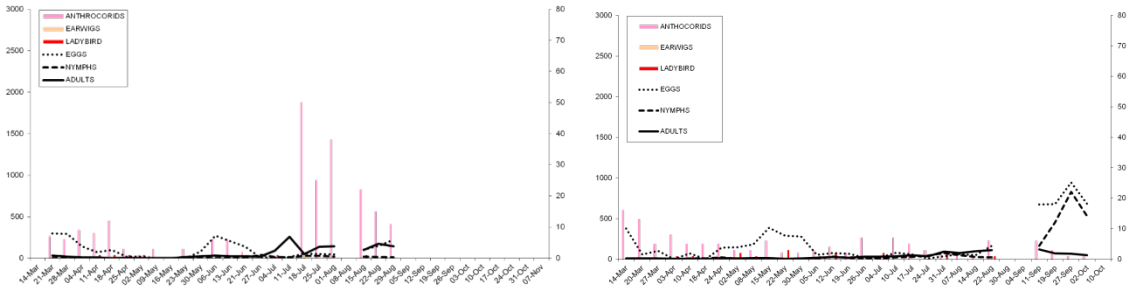


H_S

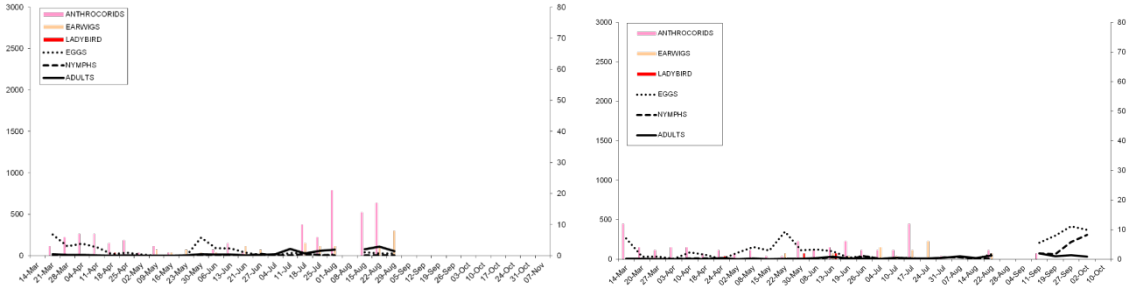
Figure 7.5. Farm 4 pear sucker and natural enemy monitoring in 3 orchards over 2 consecutive years during the growing season. *Note change of orchard in year 2

2016	Honeydew	7-13
	Slightly Harmful	0-1
	Harmless	0-1
2017	Honeydew	9-14
	Harmful	1
	Slightly Harmful	3-2
	Harmless	1

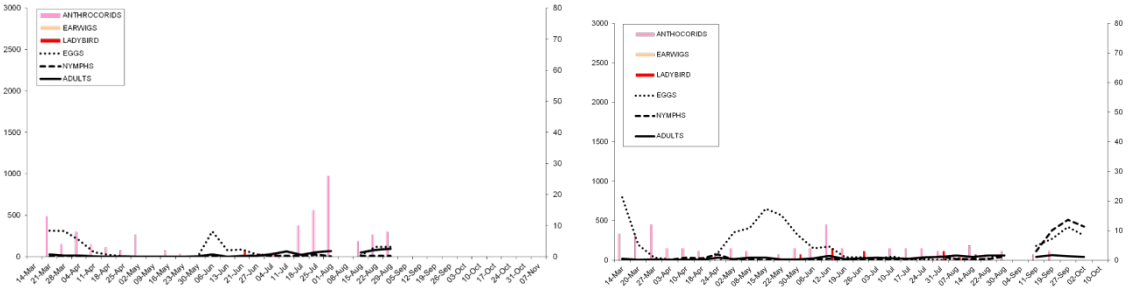
Orchard **2016** **2017**



S_L4



S_M4

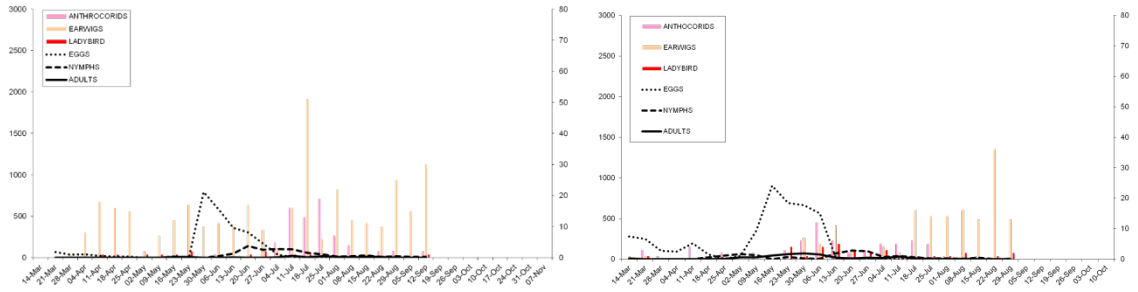


S_H3

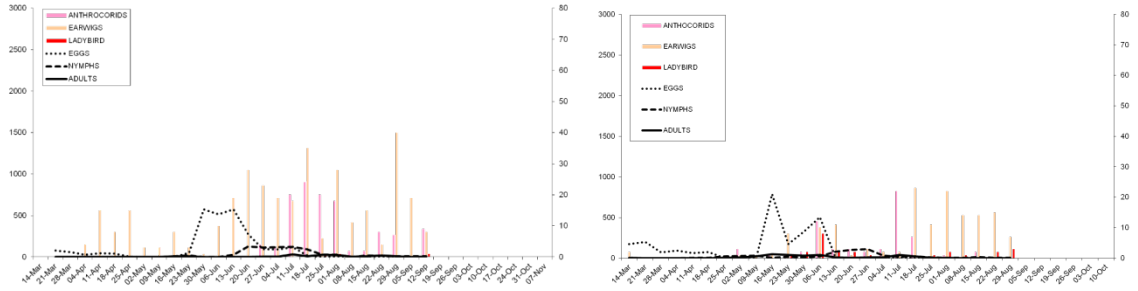
Figure 7.6. Farm 5 pear sucker and natural enemy monitoring in 3 orchards over 2 consecutive years during the growing season

2016	Honeydew	24
	Slightly Harmful	2
	Harmless	3
2017	Honeydew	22
	Slightly Harmful	1
	Harmless	1

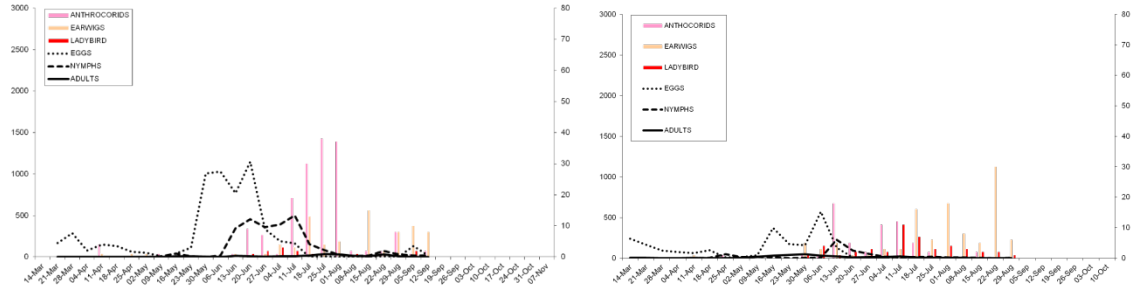
Orchard **2016** **2017**



A_Y



A_B



A_R

Figure 7.7. Farm 6 pear sucker and natural enemy monitoring in 3 orchards over 2 consecutive years during the growing season

2016	Honeydew	2-17
	Slightly Harmful	1-3
	Harmless	1-2
2017	Honeydew	6
	Slightly Harmful	0-1

Conclusions

- Important to monitor natural enemies (NE) alongside pear sucker life stages
- Enter into a spreadsheet and get 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 spray application. 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)

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.

Materials and Methods

Apple sawfly larval infested 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. As the larvae emerged from the apples and began to ‘wander’ they were transferred into 22x14x14 cm plant pots of compost. 6 were kept at ambient conditions in an outside area under cover and 2 were stored at 6 ^{o}C for 2 months to attempt to simulate a cold period. To date no adults have emerged, but pots are being bought into room conditions at intervals in spring 2018 for emergence of adults and headspace volatile collection for pheromone identification.

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

9.1 Identification and determination of the lifecycle of Anthonomus spilotus

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 wide spread across the South East. The weevil was initially thought to be the pear bud weevil (Anthonomus piri, Gyllenhal) (Figure 10.1.2C), 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.

Aim

The overall aim of this objective was to determine the lifecycle and confirm the identification of weevil pest of pear in UK pear production. To achieve this we aimed to;

- Closely record the timing of the life stages in at least one pear crop
- Culture the pest from egg to adult to allow for confirmation of the identity of the pest

Materials and Methods

Culturing: Flower and leaf buds containing eggs and larvae and hardened cases (Fig. 10.1.1 B-D) containing larvae or pupae were collected from the field. They were then placed in a 90 mm diameter petri dish containing wet paper towel and a food source of honey on the lid of the petri dish. They were then kept at ~20^{o}C until adult emergence was observed.

Identification: Identification of the weevil species was carried out by four entomologists at NIAB EMR using the key to species (and subgenera) within; “Handbooks for identification of British insects, Vol. 5, Part 17d True weevils (part III) (Coleoptera: Curculioninae, Baridinae, Orobitidinae)” (Morris, 2012) and through translating Balachowsky and Mesnils 1935 publication; Les Insectes Nuisibles aux Plantes Cultivees: Leurs moeurs Leur destruction (with the help of Julian Lecourt, NIAB EMR). Larval, pupal and adult weevil specimens were all sent to the Natural History Museum for the weevil to be identified. All specimens were collected by NIAB EMR by tap sampling or were cultured from leaf or flower buds containing eggs.

Lifecycle and biology

Location: Two commercial pear orchards near Maidstone in the South East were selected. Bud damage and adult specimens had been observed at both orchards in February 2017. Orchard A and B are located 1.6 miles from each other.

Eggs, larvae, pupae and feeding damage: A random sample of forty flower and leaf buds were collected from each orchard twice a week from 22 March (green cluster) to 2 May (fruit set) for flower buds and until 19 May for leaf buds (Chapman and Catlin, 1976). The buds were then examined under a light microscope at NIAB EMR. The number of buds with feeding damage (Fig. 10.1.6.A-C), the number of eggs (Fig. 10.1.3.A), larvae (Fig. 10.1.3.B) and pupae (Fig. 10.1.1E) were then recorded.

Bud damage: Sixty flower and leaf buds with weevil damage were tagged on the 27 March at green cluster they were then assessed for damage (0 = zero damage, 1 = 1-25% damage, 2 = 26 – 50% damage, 3 = 51 – 75% damage and 4 = 75 – 100% damage) up until 23 May at fruit set.

Adults: Forty branches were tap sampled over a white tray throughout the orchard and the number of adult weevils recorded from 22 March biweekly until the 19 May at which point sampling continued on a monthly basis until 3 November 2017.

Crop growth stage: The crop growth stage was recorded at each assessment using the classifications given in Chapman and Catlin (1976).

Results

Culturing weevils: Adult weevils were successfully cultured from buds containing eggs and larvae and hardened cases containing larvae or pupa (Fig. 10.1.1.A). Buds or hardened cases containing larvae (Fig. 10.1.1.B), pupae (Fig. 10.1.1.C) or emerging adults (Fig. 10.1.1.D) would move sporadically as the insect within moved. On average adults would emerge 18 days after buds/hardened cases were collected from the field. Cultured adults and adults collected from the field were used for identification.

Identification: Working through the identification key in Morris (2012), the weevils were quickly identified as being in the subgenus, Anthonomus, elytra had conspicuous transverse bands and the weevils were not a single colour. The weevil size (3-3.5 mm), the conspicuous pattern (transverse band) and the strongly bowed inner edge of the fore – tibia are traits of the rosaceae weevil pests; A. piri Kollar and A. pomorum Linnaeus. However the width of band in the weevil specimens collected is more horizontal than that of A. pomorum which is more V-shaped and widens to the outer edges of the wing case. Furthermore the line of pubescence in pronotum was conspicuous on the weevil specimens but not on A. pomorum. This pubescence is observed in A. piri, however A. piri had a differing transverse elytral pattern and the lifecycle of A. piri does not correlate with the presence of the weevil and the damage that was being recorded. Other species in the key (A. ulmi, De Geer) which had similar phenotypic traits to the specimens but its lifecycle did not correlate. Dr Michelle Fountain and Julien Lecourt, translated Balachowsky and Mesnils 1935 publication; Les Insectes Nuisibles aux Plantes Cultivees: Leurs moeurs Leur destruction, and found that the weevils characteristics and lifecycle fitted more with A. spilotus, Redtenbacher, 1847. A. spilotus is dissimilar to A. piri as being smaller and having differing elytral pattern and lacking the characteristic widened and raised third elytral interstices of that species (Morris et al., 2017) (Fig. 10.1.2.C).

The weevil specimens were sent to the Natural History Museum (NHM) and identification was confirmed fully (5 May 2017, Appendix 5) from a male specimen sent in which had the characteristic asymmetrical median lobe of the genitalia. This resulted in description jointly published by NIAB EMR and NHM (Morris et al., 2017) (Fig. 10.1.2.D). The male (Fig. 10.1.2.A)

and female (Fig. 10.1.2.B) A. spilotus rostrums differ. In the male it is dull, with deep punctures and a slightly shorter rostrum than the female. The female rostrum is shinier with fewer and less deep punctures.

Figure 10.1.1. A) Cultured Anthonomus spilotus adults **B)** Larvae in a hardened case **C)** Pupae within a hardened case **D)** Adult emerging from hardened case

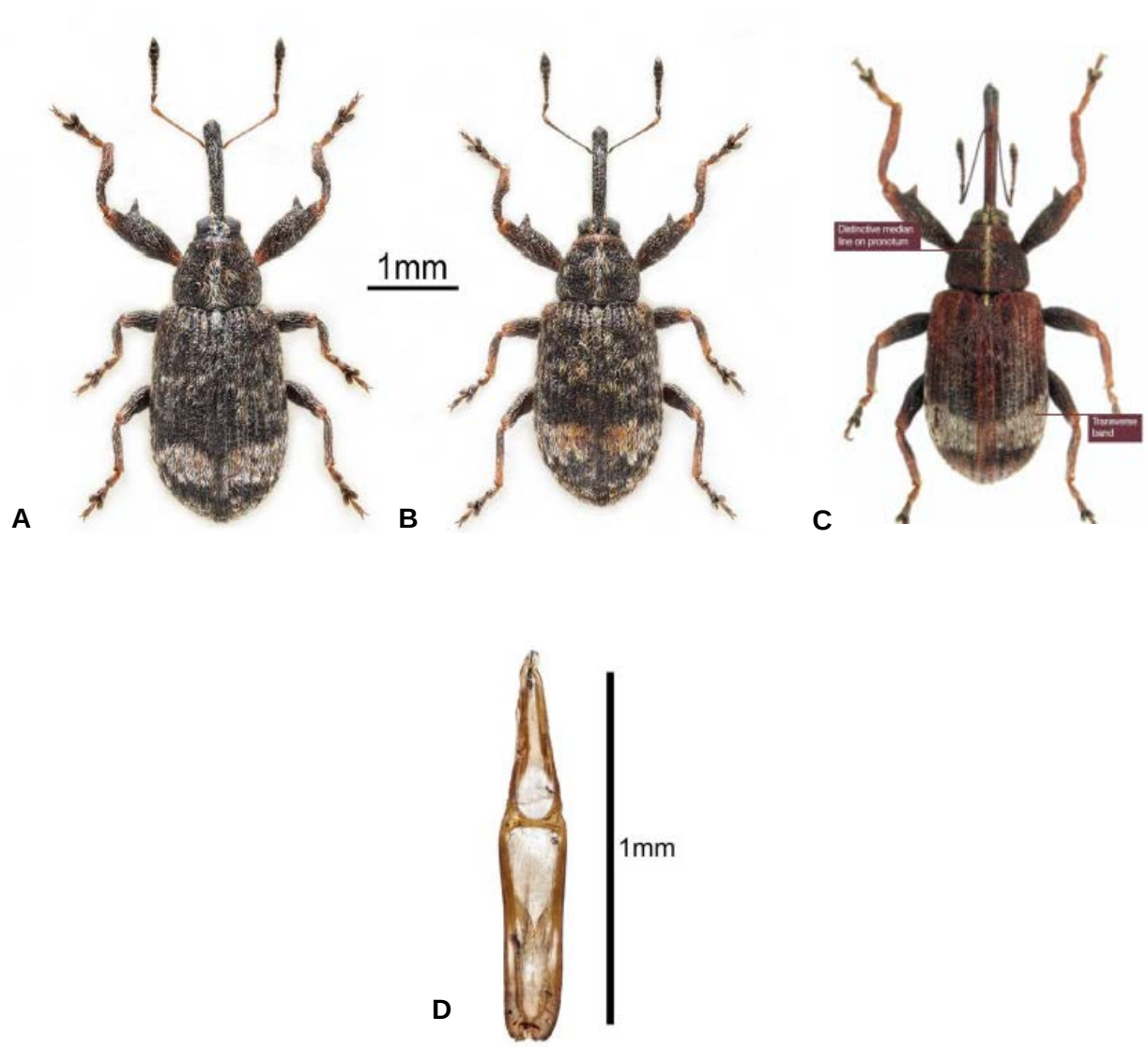


Figure 10.1.2. A) Anthonomus spilotus male, **B)** Anthonomus spilotus female, **C)** Anthonomus piri, **D)** Anthonomus spilotus median lobe of male genitalia. (Harry Taylor. Figure A, B and D. 2017. Specimens in Natural History Museum; AHDB. Figure C. 2015.)

Eggs, larvae, pupae, feeding damage and adults: Often if feeding damage was observed on the bracts of the buds, eggs, larvae and pupae would be located inside. In order to find eggs, larvae and pupae within the folds of the buds forceps and needles had to be used under a light microscope. Often in flower buds eggs would be laid close to the anthers and normally only one egg was laid in the bud, but sometimes two could be found (Fig. 10.1.3.A). Larvae were found feeding on the internal tissue of the flower (Fig. 10.1.3.B) or leaf (Fig. 10.1.3.C) bud. Larvae were also observed forming hardened cases from necrotic tissue/frass (Fig. 10.1.3.D). One pupa was observed in/on a leaf bud (Fig. 10.1.3.E).

Feeding damage was recorded on 56 leaf and 50 flower buds out of 160 buds collected in total at the first assessment, on 23 March 2017. Feeding damage was observed from green cluster onwards and could still be observed after fruit set. Eggs were found in both flower and leaf buds, however less than half the leaf and flower buds which had feeding damage had eggs. More eggs and larvae in total were recorded in leaf buds (55 eggs and 122 larvae) than in flower buds (7 eggs and 10 larvae) from 22 March to 12 May. No eggs were recorded in the buds collected after 26 April. No larvae were found after 12 May. Adults were recorded in the canopy from 22 March to 26 June (Figs. 10.1.4&5). One pupa was found in the buds collected.

There was little difference in the percentage of buds with feeding damage and the percentage of damaged buds with eggs, larvae and pupae present within the buds between orchard A and B (Table 10.1.1). From the 2720 pear buds collected, 33.1% had feeding damage. From buds with damage 6.9% had eggs, 14.7% had larvae, 0.1% had pupae. From 2720 buds collected 7.2% contained a life stage of A. spilotus (Table 10.1.1).

Figure 10.1.3. A) Two A.spilotus eggs in flower bud **B)** Larvae feeding on flower bud **C)** Larvae feeding on leaf bud **D)** Hardened larval/pupal case **E)** A. spilotus pupa

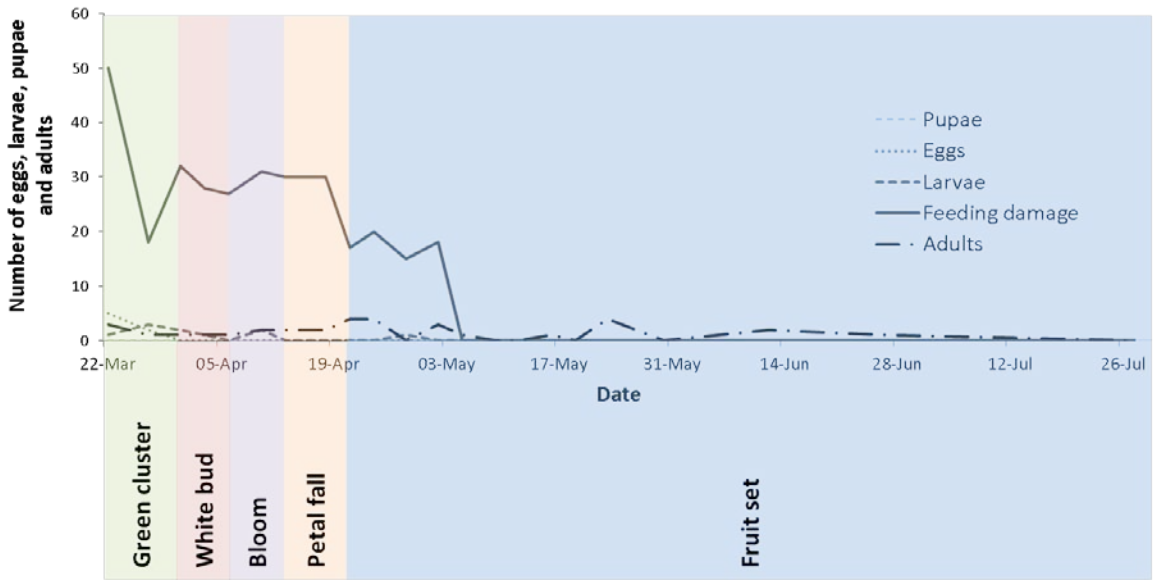


Figure 10.1.4. Total number of A.spilotus eggs, larvae, pupae and feeding damage on/in eighty flower buds and the total number of adults tap sampled from 80 trees, from two orchards in the South East monitored between mid- March and late July. Sampling continued until November, but no weevils were found after July

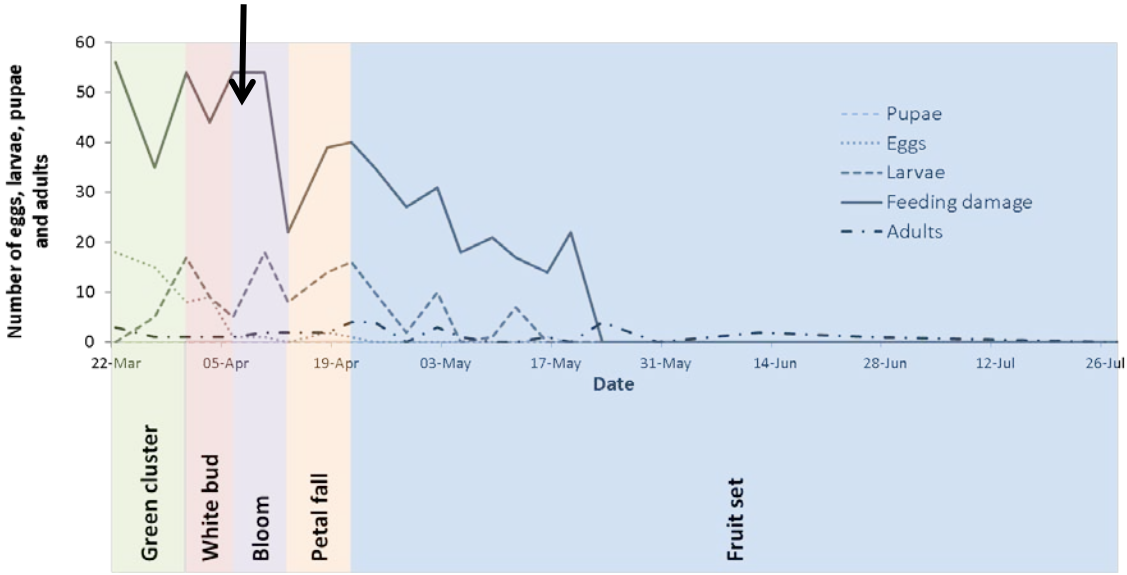


Figure 10.1.5. Total number of A.spilotus eggs, larvae, pupae and feeding damage on/in eighty leaf buds and the total number of adults tap sampled from 80 trees, from two orchards in the South East monitored between mid- March and late July. Arrow indicates when all leaves had fully emerged from their buds (6 April). Sampling continued until November, but no weevils were found after July

Table 10.1.1. Percentage of buds with feeding damage and percentage of damaged buds with eggs, larvae, and pupae present in the bud at orchards A and B. Numbers in brackets indicate the total number of damaged buds, eggs, pre-hatch eggs, larvae and pupae in all the buds collected from 22 March to 19 May.

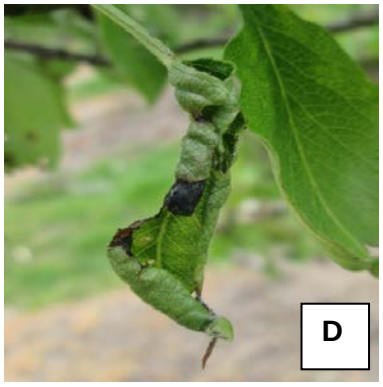
	Orchard A	**Orchard B**	**Orchard A + B**
Buds collected	1360	1360	2720
% Damaged buds	32.9 (447)	33.2 (452)	33.1 (899)
% buds with eggs, larvae and pupae	7.1 (96)	7.3 (99)	7.2 (195)
% Damaged buds with eggs	7.4 (33)	6.4 (29)	6.9 (62)
% Damaged buds with larvae	13.9 (62)	15.5 (70)	14.7 (132)
% Damaged buds with pupae	0.2 (1)	0 (0)	0.1 (1)

Bud damage: Adult feeding damage was observed on the bracts of closed leaf buds (Fig. 10.1.6.A&B), but not on flower buds which were at green cluster when buds were labelled thus damage caused by adult feeding could not be reliably recorded. However, adult feeding damage was observed on flower buds collected on 7 March from another site in the South East (Fig. 10.1.6.C). Necrosis was often observed around the circumference of the hole and liquid sap sometimes observed oozing out of the feeding site (Fig. 10.1.6.B). On some leaf buds seven feeding sites were observed on one bud. This puncturing caused the development of misshapen leaves (Fig. 10.1.6.D). Damage from larval feeding on the parenchyma of the leaf was characterised by the presence of irregular growth with twisting and bending of young leaflets which did not unfurl or grow properly and often contained a larvae within (Fig. 10.1.6.E). Damage from larval feeding on the flower buds led to poorly developed flowers (Fig. 10.1.6.F) or complete loss of the flower (Fig. 10.1.6.G). The impact adult feeding and larval feeding had on the quantity and quality of flower and leaf buds was difficult to quantify by giving a damage score.


A


B


C


D


E


F


G

Figure 10.1.6. A-B) Feeding damage on leaf buds, **C)** Feeding damage on flower buds, **D)** Damage on leaf from adult feeding **E)** Damage on leaf from larval feeding) Damage on flower from larval feeding **G)** Damaged of flower from larval feeding

Discussion

The weevil has been confirmed as Anthonomus spilotus, Redtenbacher, 1847 and a greater understanding of the biology and lifecycle of this pest in UK pear orchards has been established. A. spilotus is widely distributed throughout continental Europe and has been recorded in fourteen European countries from central and western Russia to Western Europe (France), south to Spain, Greece and Portugal reaching North Africa and northwards to Sweden and now Britain (Balachowsky and Mesnil, 1935; Caldara, 2013).In continental Europe the biology and lifecycle has been identified by a range of literature where the pest has been established for longer. The literature suggests that A. spilotus overwinters as an adult, feeding on spurs of smaller branches, young twigs and buds of host species (pear, medlar, hawthorn) until mid-March (Balachowsky and Mesnil, 1935; Scherf, 1964; Dieckmann 1968; Chevalier et al, 1963; Rheinheimer and Hassler, 2010). From mid- March until May the weevils mate and lay eggs in closed vegetative or flower buds on pear trees (Balachowsky and Mesnil, 1935). The larvae then develop within the leaf or flower bud as it grows (Balachowsky and Mesnil, 1935). The larvae forms a hardened case made of frass and develops into a pupa. The pupae then develop into adults which emerge in mid-June (Balachowsky and Mesnil, 1935). The adults do not lay eggs in the autumn and enter diapause to over winter. The preliminary data gathered in 2017 indicates a similar lifecycle and biology in UK pear orchards. Eggs and larvae were recorded in closed leaf and flower buds from the 22 March until the 12 May and adults were present in the canopy during this period. Larvae were recorded developing in leaf and flower buds and forming hardened cases and no adult weevils were found in the canopy after the 26 June through to November. Larvae would form hardened cases and then appeared to pupate on the orchard floor as only one pupa was found in the buds collected from the canopy.

The presence of feeding damage on leaf and flower buds did not always mean that eggs, larvae or pupae were present within the bud, with 78.4% of damaged buds having no eggs, larvae or pupae within. This was particularly the case early on where high feeding damage was observed; more than half buds collected had feeding damage, whilst low eggs and larval counts were recorded. From the buds collected 33% of buds had feeding damage and 7.2% contained eggs, larvae or pupae. This may have led to reduced bud quality and even loss of flower and leaf buds.

However, the impact the weevil had on the quantity and quality of the flower buds thus fruit produced wasn't quantified. The damage observed coincided with the characteristic damage identified in the literature (larval feeding on the parenchyma of leaf and flower buds and feeding/ oviposition punctures produced by adults on the surface of the bud). More eggs and larvae were observed in leaf buds than flower buds, however this was likely due to the fact that monitoring began in mid-March and egg laying in flower buds may have occurred earlier in the season, when the buds were closed.

Conclusions

- The weevil was confirmed as A. spilotus, Redtenbacher, 1847 by the Natural History Museum and was published in 2017 by NIAB EMR and NHM
- Eggs, larvae, adults and feeding damage was observed from the beginning of sampling 22 March in the two orchards
- Adults were not found in the orchards after the 12 June
- Damage caused by the weevil consisted of i) puncturing of the bud bracts by adult feeding, causing irregular growth, ii) larval feeding within leaf and flower buds leading to irregular growth, loss of buds and damaged flower buds
- The lifecycle and biology in UK orchards agrees with the literature
- A third of the total buds collected had feeding damage and 7% of buds contained eggs, larvae or pupae
- Feeding damage does not always mean that eggs, larvae or pupae are present within the bud
- An AHDB factsheet on A. spilotus is being published.

Future work

A threshold for spray treatment is urgently needed to avoid uneccesary applications of plant protection products. Future work should determine:

- 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 with day length and temperature

- How many flowers in a truss larvae eat until pupation and adult emergence
- What impact could the pest have on yield and fruit quality

9.2 Investigate the short-term toxicity of a number of insecticides 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 there is 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 (Figure 10.1.6.A-C) (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 parasioids 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.

Aim

To determine the efficacy of commonly used crop protection products in UK pear orchards for the control of A. spilotus (laboratory experiment). This should ideally be able to fit within an IPM system, i.e. within an earwig safe spray programme.

Materials and methods

Adult weevil collection

112 live A. spilotus were collected by tap sampling on 20 March 2017 from a commercial pear orchard in the South East of England.

Treatments

Seven tree fruit crop protection products were tested against an untreated distilled water control (Table 10.1.2). Exirel 10 SE is not currently approved on pear. Treatments (Table 10.2.1) were made up to 1 litre with distilled water in glass graduated flasks. Further information on the crop protection products (Manufacturer, IRAC number, formulation etc.) are in Appendix 2&3.

Treatment application

Weevils were transferred into a 9 cm petri dish each containing food (honey on lid) and water (paper towel segments saturated with water) and left for 24 hours to ensure weevils were healthy. After 24 hours weevils were held at 5^{o}C for 1 hour to sedate them. Each weevil was then placed using soft forceps into a new 9 cm diameter petri dish containing a filter paper disk in the base and a gridded mesh lid.

Treatments were applied directly to the weevils through the gridded mesh lid on the petri dish using a Burkard computer controlled bench top sprayer (Fig. 10.2.1, Burkhard Manufacturing Co. Ltd, Rickmansworth, England) following the NIAB EMR standard operating procedure (Appendix 1).

Once sprayed the weevils were immediately moved back to their corresponding petri dish (Fig. 10.2.2). They were then kept at 20^{o}C within a controlled temperature room. The humidity was maintained above 40 % relative humidity by placing the petri dishes on a tray and the tray within a polythene bag.

Table 10.2.1. Products 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), ***Based on EAMU20171134 emergency authorisation for use on cherry.

Treatment	a.i.	Product	Field rate	Amount per l (for spraying at 1000 l / ha)
1	Indoxacarb	Steward® Explicit™	250 g/ha*	0.250 g
2	Thiacloprid	Calypso®	375 ml/ha**	0.375 ml
3	Lambda-cyhalothrin	Hallmark	90 ml/ha*	0.09 ml
4	Cyantraniliprole	Exirel™	0.9 l/ha***	0.9 ml
5	Pyrethrum	Spruzit	12 l/ha*	12 ml
6	Acetamiprid	Gazelle	375 g/ha*	0.375 g
7	Chlorantraniliprole	Coragen®	175 ml/ha*	0.175 ml
8	Water	-	-	-

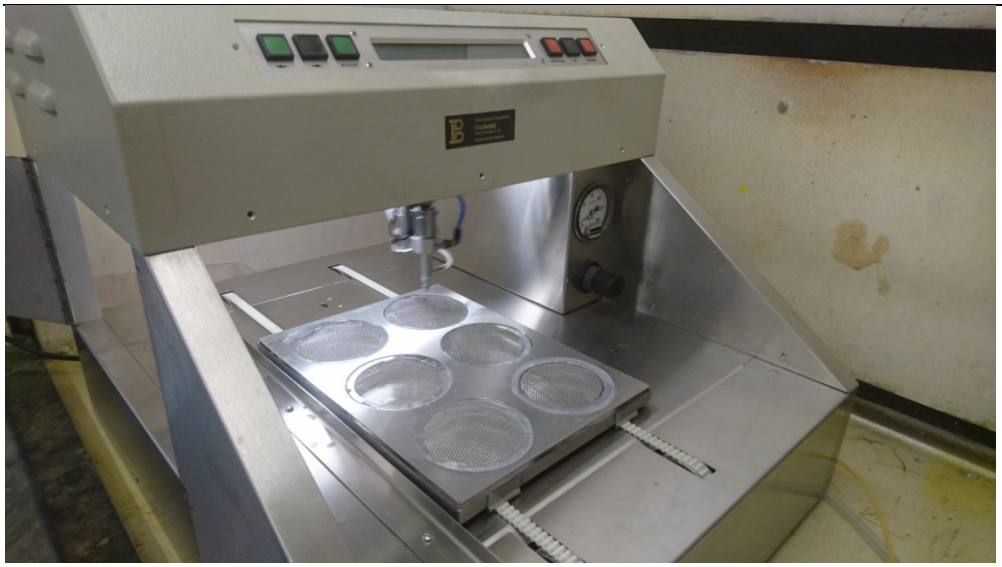


Figure 10.2.1. Burkard computer controlled bench top sprayer, griddled lids used to contain weevils in petri dish whilst applying spray treatment

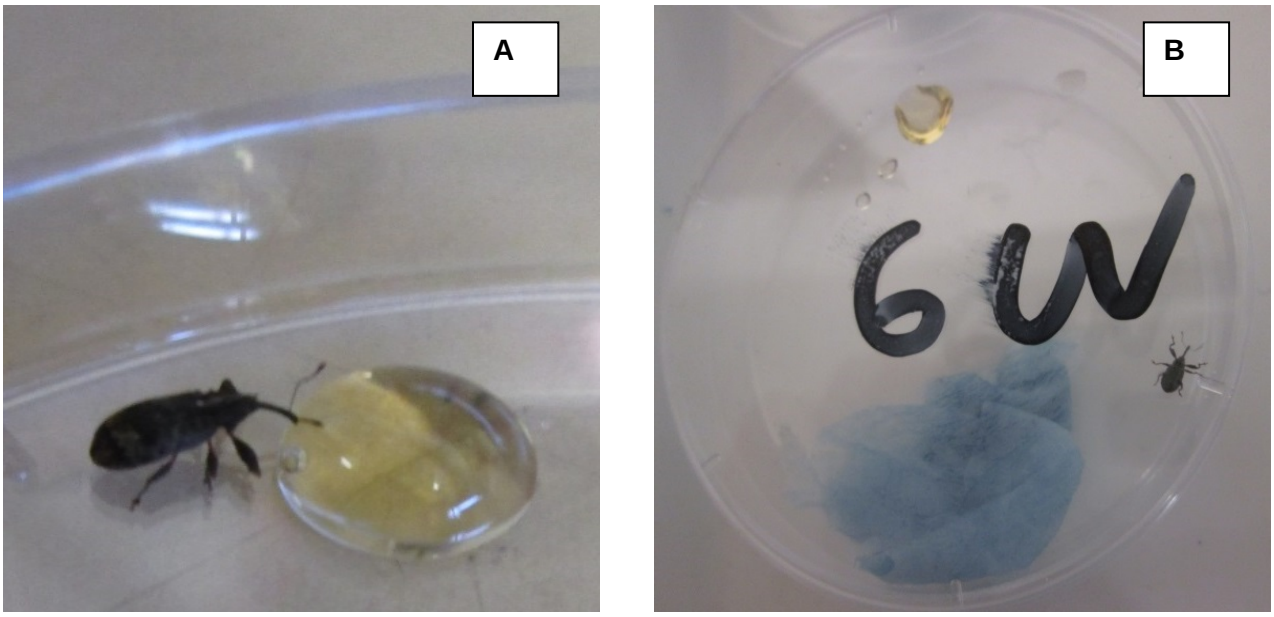


Figure 10.2.2. A) A. spilotus feeding on honey, **B)** pear bud weevil in 5 cm petri dish with water and honey

Assessments

The weevils were assessed at time points (0, 18.5, 24, 42.5, 48, 72, 144, 192, 240 hours) after spray application 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 16 replicates of 8 treatments (112 weevils). Mortality (number dead out of 16) was analysed using a Generalised Linear Model (GLM) with a binomial distribution and a logit link at each date. The same analysis was carried out comparing the status (a-d) out of 16 using a multinomial distribution.

Results

Immediately after spray application (0 hours) 87.5 %, 31.3 %, 81.3 %, and 43.8 % of weevils treated with Calypso, Hallmark, Spruzit and Gazelle were affected/moribund. The profiles (number of weevils healthy, affected, moribund, dead) of these treatments were signifciantly different from the untreated control ($P < 0.05$). No weevils died at this time point so the difference can be attributed to the number of weevils affected/moribund. After 24 hours the % of weevils affected/moribund decreased within all the treatments, except Coragen which decreased after 42.5 hours. The % of weevils affected/moribund by Calypso was higher than all other treatments throughout the experiment and began to decrease rapidly after 72 hours (Fig. 10.2.3). The profile of Calypso remained significantly different from all other treatments between 48 and 144 hours ($P < 0.05$).

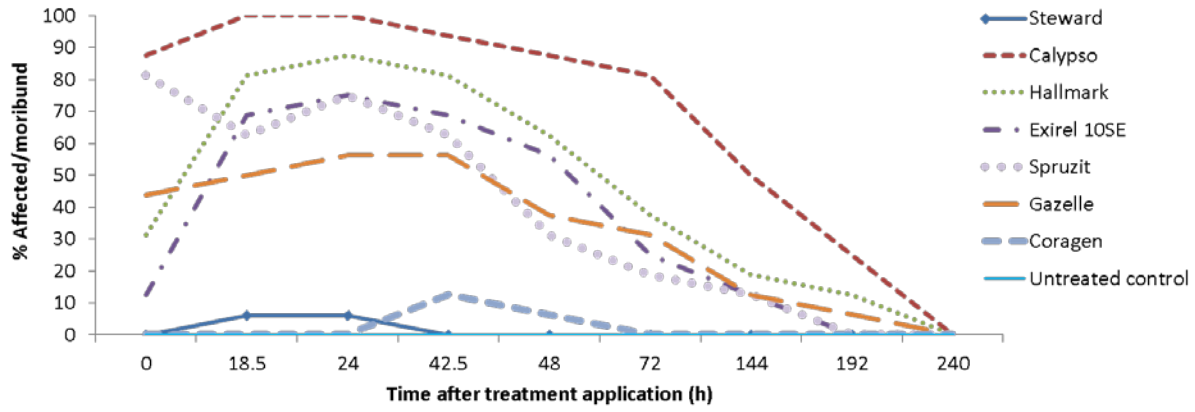


Figure 10.2.3. % affected/moribund of A. spilotus exposed to insecticides over time (240 hours/10 days)

After 18.5 hours significantly more weevils had died after being treated with Spruzit (18.75%) than all other treatments ($P = 0.034$) except Exirel 10SE (6.3%) ($P > 0.05$, Fig. 10.2.4). At 48 hours the % mortality of weevils treated with Spruzit (43.8%) was not significantly different from Hallmark which had increased to 25% ($P = 0.262$) after 48 hours, however was significantly higher than all other treatments ($P < 0.044$).

After 72 hours the % mortality of Calypso treated weevils increased to 18.8% and was no longer significantly less effective than Spruzit at 50% ($P = 0.059$) treated weevils and had become

significantly higher than the untreated control (P < 0.05). The % mortality in Calypso then continued to increase until higher than Spruzit. At 240 hours after treatment application all weevils had either been scored as healthy, and therefore were considered fully recovered, or had died.

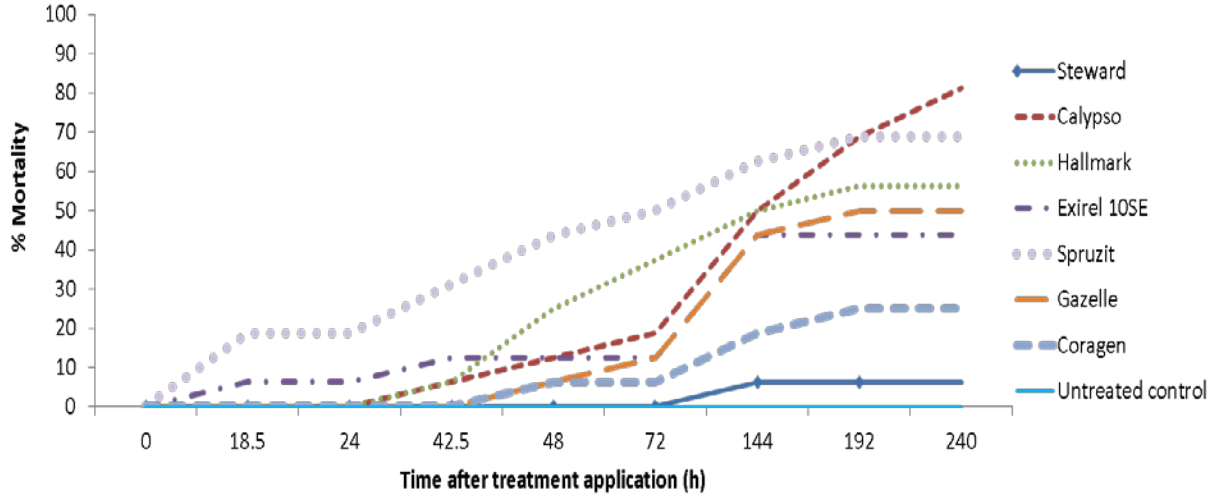


Figure 10.2.4. % Mortality of A. spilotus exposed to insecticides over time (240 hours / 10 days)

By 240 hours Calypso (81.3%), Spruzit (68.8%), Hallmark (56.3%), Gazelle (50.0%), Exirel 10SE (43.8%) and Coragen (25.0%) had significantly higher % mortality compared to the untreated control (0%) (P <0.05, Fig. 10.2.5). There was no significant difference in % mortality between Steward (6.3%) and the untreated control (P = 0.234). The diamides (Exirel 10SE and Coragen) were less affective than Calypso (P = 0.026 and 0.001) and were not significantly different from each other (P = 0.262). There was no significant difference between the sodium channel modulators, Spruzit and Hallmark (P = 0.464) or between weevils treated with the neonicotinoids, Calypso and Gazelle (P = 0.059), however 31% more weevils died in the Calypso treatment.

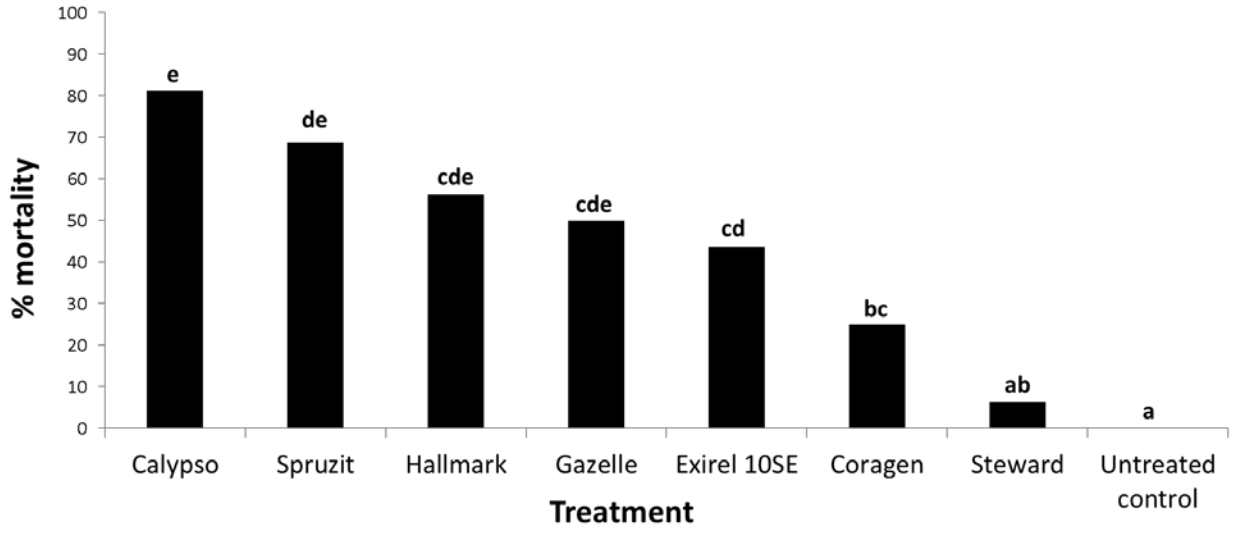


Figure 10.2.5. Cumulative mortality 240 hours of A. spilotus (10 days) after topical treatment application. Significant differences indicated by different letters

Discussion

Topical applications of Calypso, Spruzit, Hallmark, Gazelle, Exirel 10SE and Coragen all had a toxic effect on A. spilotus. Steward was not effective. The diamides, Exirel 10SE and Coragen, gave intermediate control. Initially the fastest acting insecticide was Spruzit which killed significantly more weevils than most other treatments. Weevils treated with Calypso did not die as rapidly. However more weevils were affected/moribund for significantly longer than all other treatments. Calypso gave the highest level of control (81.3%), 30% higher than Gazelle. Calypso is a recommended product for the control of other weevil species in fruit (A. pomorum) (AHDB, 2017). Calypso, Gazelle, Hallmark and Spruzit were all fast acting, stimulating negative behavioural characteristics in the weevil immediately after spray application, suggesting that these four plant protection products are the most effective against A. spilotus.

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). Pear sucker (Cacopsylla pyri) is one of the most important pests of pear in the UK. Earwigs, Orius and anthocorids are all veracious predators of pear sucker (AHDB, 2015). Therefore, the impact of insecticides on these predators can be particularly important. Gazelle was the only insecticide tested in this study found to be “harmless” to earwigs (Fountain and Harris, 2015). All four

insecticides were considered harmful to Orius and Anthocoris nemoralis. Only Coragen is considered harmless to Orius and Steward to Anthocoris nemoralis. Both were ineffective against A. spilotus in this laboratory study.

Conclusions

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 and weevils may be more likely to come into contact with dried residues. Steward, Coragen and Exirel 10SE are unlikely to be effective the field.

Future work

- Test the most effective laboratory products in field conditions at pre flowering
- Test the feeding toxicity of spinosyns and indoxicarb to A. spilotus

KNOWLEDGE AND TECHNOLOGY TRANSFER

2015

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

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

2016

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

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

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

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

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

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

21st July 2016: TF223 summer field visit, East Malling

2017

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

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

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

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

9th 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.

13th 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

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

2018

23rd and 25th January 2018: Cannon and 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

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

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

February 2018: Anthonomus spilotus (Pear blossom weevil) – Fountain: A new pest in UK pear orchards? Hutchinsons Agronomist Group, Northampton

22nd February 2018: AHDB/EMR Association Tree Fruit Day – Fountain, Cannon, Berrie and 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.

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

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 1. Paper published based on rot survey findings.

First report of Neofabraea kienholzii causing bull's eye rot of apple in the UK

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Apple rot surveys have been conducted since the 1930's in the UK. Together they help build a picture of how the rot profile has changed over time. In surveys conducted in the 1930's and the 1960's (Wilkinson, 1954; Preece, 1967), Neofabraea was the dominant causative agent of rot, but losses due to this pathogen became negligible in the 1980's (Berrie, 1989). More recent surveys found a resurgence of this disease in susceptible varieties (including cv. Cox's Orange Pippin) prompting identification of the species of Neofabraea responsible. Total actual losses due to rots have reduced significantly over the surveying period (for example total losses in cv. Cox's Orange Pippin have reduced 10 fold from 26.3% in the 1930's to 2.6% in the 2010's) thanks to advances in storage technologies. Rots attributed to Neofabraea spp. have reduced from nearly 50% to 15% in the same period but this still constitutes an economic loss to growers and packers (Saville et al., 2015).

Apple samples that exhibited discreet, circular "cheek" lesions which were light to dark brown, flat or slightly sunken, and firm, were collected from the packhouse during 2013 and 2014 surveys. Samples were returned to the lab and rinsed with sterile water and surface disinfected with 70% ethanol. Tissue from the leading edge beneath the skin was isolated and placed on potato dextrose agar. Plates were incubated at 20°C with a 16 hr photoperiod. Isolates were slow growing producing colonies of varying colour but with colony morphology characteristics typical of Neofabraea species (Fig. 1). DNA was extracted from axenic, single spore derived cultures, β-tubulin and ITS regions were amplified and sequenced. BLAST analysis revealed that in addition to N. perrenans and N. vegabunda isolates, which have been previously reported in the UK (Edney, 1983), a single isolate (G48) out of 49 tested

matched Neofabraea kienholzii with 100% identity. Repeating the above with a collection curated during the 2015 rot survey (growing season 2014), using the more phylogenetically informative β-tubulin region only, a single isolate (R4/15_2) out of 20 isolates tested had 100% identity to Neofabraea kienholzii. The sequences have been deposited in GenBank: Accession Nos. MF977989 (G48, ITS), MF983810 (G48, β-tubulin) and MF983809 (R4/15_2, β-tubulin).

To prove pathogenicity, Koch's postulates were conducted with isolate G48 (Fig. 2). Apples cv. Cox's Orange Pippin were surface sterilised, wounded with a sterile hypodermic needle and inoculated with 20 µl of conidial suspension (3 x 10^4 conidia ml^{-1}). Inoculation points were coated with petroleum jelly to maintain humidity. After 60 days incubation at 2°C, simulating commercial storage conditions for cv. Cox's Orange Pippin, lesions had developed on all inoculated apples characteristic of those caused by Neofabraea (Fig. 3). Non-inoculated control fruit showed no symptoms. Isolations from the leading edge of the lesion, as described above, confirmed the presence of Neofabraea.

This is the first report of Neofabraea kienholzii in the UK, where before only N. perrenans and N. vegabunda isolates were believed to be present (Edney, 1983). Previously named 'Neofabraea sp. nov.', Neofabraea kienholzii was first described by Spotts et al. (2009) and has since been reported in many apple (and pear) growing territories around the world. This data is important to contribute to a global picture of the distribution of this genus and so that control strategies can be tailored for the UK industry to mitigate losses due to apple rot diseases.

Figure 1: The variation of colony morphology of Neofabraea spp. isolated as part of the survey. All grown on potato dextrose agar and incubated at 20°C for four weeks. **Figure 2:** Neofabraea kienholzii (isolate G48) culture grown on potato dextrose agar after 28 days at 20°C. **Figure 3:** Infection of apple cv. Cox 's Orange Pippin with Neofabraea kienholzii (isolate G48), 60 days post inoculation and incubation at 2°C.

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Appendix 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

Appendix 3. Standard Operating Procedure for Burkard computer controlled bench top sprayer

1. Description

A bench top sprayer controlled by computer for applying known doses of pesticide to small areas.

2. Health and Safety Considerations

If pesticides are to be used then operation of this equipment should be conducted within a laboratory fume hood, suitable gloves and other personal protective equipment is also advised.

3. Preparation for use

The sprayer should be calibrated: (a) before each spray season; (b) when the sprayer has been dismantled; or (c) when any modifications or servicing has been conducted.

i) Read the user manual to familiarise yourself with the machine and its parts

ii) Check body of sprayer and lid for signs of contamination. Clean if necessary

iii) Turn the sprayer on at the rear of the machine

iv) Load the petri dish tray, against the guard rail

v) Check the compressed air cylinder for signs of damage

vi) Open the compressed air fully. Do not adjust the regulator

vii) Check the function of the "Air" supply by reading the value on the manometer.

viii) Remove the click stop cap from the air brush spray head

ix) Using the Menu and the arrow keys go to "maintenance menu" and accept "clean spray head" click on "air" to start the purge

x) Replace the click stop cap on the air brush at the desired number of clicks

4. Calibration

The sprayer should be calibrated before each spray session, when the sprayer has been dismantled, or when any modifications or servicing have been conducted. A record of each calibration will be kept in the equipment folder for this machine.

xi) Set up the sprayer as above and check operation

xii) Select a pressure for the air 5 psi gives a usable spray volume without disturbing insects on the target

xiii) Select a number of click stops. For calibration multiples of 2 are suitable

xiv) Fill the reservoir with a 1% solution of wetting agent

xv) Place a collecting vessel under the air brush

xvi) Press purge until spray emerges

xvii) On a laboratory balance take the weight of a Petri dish base and tear the value

xviii) Load the Petri dish tray with a Petri dish

xix) Load the tray in the sprayer and entre the “run menu” select the relevant programme and press “accept”

xx) Press “air” to start spraying.

xxi) The sprayer should spray the Petri dish

xxii) Remove the Petri dish and record the weight of sprayed liquid on a calibrated balance (GEP SOP 766)

xxiii) Repeat this a minimum3 times

xxiv) Open up the click stop cap 2 more clicks and repeat

xxv) Enter the data in to excel and plot the graph of □l cm2

xxvi) Select an appropriate dose

5. Application

a) Check the operating pressure 5 psi is usual

b) Check the clickstop setting

c) Fill and load the reservoir with the appropriate treatment compound

d) Place a collecting vessel under the air brush

e) Using the Menu and the arrow keys go to “maintenance menu” and accept “clean spray head” click on “air” to start the purge

f) When an adequate volume of spray has been dispensed press " air" to stop the purge and remove the collecting vessel

g) Select the appropriate stored template from the menu.

Available items are:

1 90 mm petri dish

2 90 mm petri dish

4 90 mm petri dish

6 90 mm petri dish

1 stn plate

2 stn plate

4 stn plate

1 90 mm petri dish small tray

4 90 mm petri dish small tray

(for none standard spray patterns a computer can be connected and patterns printed).

h) Load the Petri dish tray with Petri dishes

i) Load the Petri dish tray in to the sprayer

j) Press "accept to align the tray

k) Press "air" to start spraying

l) Repeat for as many Petri dishes as need treating

6. Cleaning

a) Remove the reservoir

b) Place a collecting vessel under the air brush

c) Select "maintenance menu" and accept "clean spray head" click on "air" to start the purge

d) When all spray remaining in the air brush is dispensed press "Air"

e) Load a reservoir of distilled water press “Air”

f) After spraying approximately 1 cm of the reservoirs volume remove the reservoir

g) Press “Air” when all spray remaining in the air brush is dispensed press “Air”

h) Load a reservoir of 100% ethanol

i) After spraying approximately 1 cm of the reservoirs volume remove the reservoir

j) Press “Air” when all spray remaining in the air brush is dispensed press “Air”

7. After spraying

a) Repeat the cleaning steps the ethanol prevents the air brush from rusting

b) Once the final ethanol clean has been conducted leave the air brush blowing for 5 minutes to thoroughly dry the air brush

c) Turn off the sprayer

d) Turn off the compressed air supply

e) Wipe all exposed surfaces of the sprayer to remove any pesticide contamination

f) Repeat this 3 times with alternately distilled water and then ethanol

Appendix 4. Further details on products used

Table 3. Products, manufacturer, Insecticide resistance action committee (IRAC) number, insecticide group, mode of action, formulation type, concentration of active ingredient in product and recommended spray volume.

Product	Manufacturer	IRAC #	Mode of action	Concentration of active ingredient in product.	Recommended spray volume L/ha
Steward	DuPont	22A	Voltage dependent sodium channel blocker	30% w/w	Adjust according to tree height/canopy density
Calypso	Bayer CropScience	4A	Nicotinic Ach receptor agonist	40.4 % w/w	500 – 1500
Hallmark	Syngenta	3A	Sodium channel modulator	100 g/l	200-2000
Exirel 10SE	DuPont	2B	Ryanodine receptor modulators	100 g/l	1000
Spruzit	Certis	3A	Sodium channel modulators	4.59 g/l	Adjust according to tree height/canopy density
Gazelle	Certis	4A	Nicotinic Ach receptor agonist	20 % w/w	1000-1500
Coragen	DuPont	2B	Ryanodine receptor modulators	200 g/l	200 - 1500

Appendix 5. Further details on products used

Product, Active ingredient, Formulation, Earwig toxicity score (Based on data from Fountain and Harris (2015) and Colvin and Cranshaw (2010)), Anthocoris nemoralis toxicity score (Biobest), Orius spp toxicity score (Biobest). The toxicity score is based on the percentage mortality of the insect after exposure to the insecticide. 1 = Harmless (<25%), 2 = Slightly harmful (25-50%), 3 = Moderately harmful (51-75%), 4 = Harmful (>75%). % indicates

Product	Active ingredient	Formulation type	Earwig toxicity score	Anthocoris nemoralis toxicity score	Orius spp toxicity score
Steward	indoxacarb	Suspension concentrate	4	1 nymph 1 adult	3 nymph 3 adult
Calypso	thiacloprid	Suspension concentrate	4	4 nymph 4 adult	4 nymph 4 adult
Hallmark	lambda-cyhalothrin	Capsule suspension	NT	4 nymph 4 adult	4 nymph 4 adult
Exirel 10SE	cyantraniliprole	Suspo - emulsion	NT	NT	NT
Spruzit	pyrethrum	Emulsifiable concentrate	4	4 nymph 4 adult	4 nymph 4 adult

Gazelle	acetamiprid	Soluble granule	1	3 nymph NT adult	4 nymph 4 adult
Coragen	chlorantraniliprole	Suspension concentrate	1	NT	1 nymph NT adult

NT = Not tested

Appendix 6. Toxicity scoring for Anthocoris nemoralis and Orius spp from the Biobest side effects manual

		acetamiprid		acetamiprid		indoxacarb	lambda-cyhalothrin	pyrethrins + piperonylbutoxide	cynaxypyr (chlorantraniliprole)
		s	i	s	i	s	s	s	s
Anthocoris nemoralis	Nymph	3	3	3	3	1	4	4	?
	Adult	?	2	?	2	1	4	4	?
	Persistance	1 week	?	1 week	?	-	?	?	?
Orius spp.	Nymph	4	2	4	2	3	4	4	1
	Adult	4	2	4	2	3	4	4	?
	Persistance	6 weeks	1 week	6 weeks	1 week	3 weeks	>8 weeks	1 week	?

Appendix 7. Moth species (information from UK moths www.ukmoths.org)

Distribution maps from 'NBN Atlas website at http://www.nbnatlas.org Accessed 29 Jan 18'

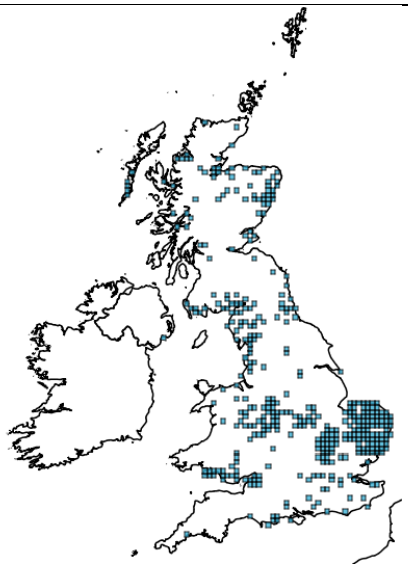
Species	**Rhigognostis incarnatella**
	Steudel, 1873
Size	Wingspan 17-21 mm
Information	Closely related to Diamond Back Moth and resembles a large and more brightly-coloured Plutella xylostella.
Abundance	Scarce
Location	Found locally in scattered locations from northern England to the Highlands of Scotland and parts of Ireland.
Life-history	The adults emerge in September and remain in this stage until around April, hiding in thick cover over the winter. The life history is not well known, but in Britain the larva is believed to feed on dame's violet (Hesperis matronalis). The pupa, like other members of the genus, is formed in a network cocoon on a leaf.
Image	
Photo credit	© Roy Leverton

Distribution	

Species	**Green Pug Pasiphila rectangulata**
	Linnaeus, 1758
Size	Wingspan 15-20 mm.
Information	Variable species in much of Britain, there are a number of very dark forms, some of which do not show any obvious green coloration.
Abundance	Fairly common.
Location	Its preferred habitats are woodland, parks and gardens, and bushy open places.
Life-history	It flies in June and July. The caterpillars feed on the blossoms of apple (Malus) and pear (Pyrus), as well as other fruit trees.
Image	

Photo credit	© Ian Kimber
Distribution	

Species	**Blastobasis lacticolella** [Synonyms: decolorella]
	Rebel, 1940
Size	Wingspan 18-21 mm.
Information	It is quite a variable species, some being very plain, others quite well-marked.
Abundance	Larvae will thrive on a wide variety of foodstuffs, including leaf-litter, vegetation, and stored products.
Location	Originally not a British species, this moth was accidentally introduced and appears to be established and expanding its range.
Life-history	The adult is nocturnal and comes readily to light in May and June and again in the autumn.
Image	
Photo credit	© Ian Kimber

Distribution	

Species	**Blastobasis adustella**
	Walsingham, 1894
Size	Wingspan 15-20 mm.
Information	Formerly known as B. lignea, this in fact was a misidentification of B. vittata, for which lignea is now a junior synonym. The larvae feed on a variety of foodstuffs, including decaying vegetable matter. Foodplant: In and on soft damp Dipsacus fullonum seedheads. January. (Emmet states 873 feeds on live and decaying conifer foliage, bird droppings and other dead or live organic matter, Sept.-June). Length: 5 mm to 9 mm in January. Head: Shiny dark chestnut brown. Frons slightly paler, with dark adfrontal border. Clypeus and labrum pale. Mandibles reddish brown with darker teeth. Prothorax (T1): Large oval pitchy brown subspiracular and subventral pinacula. Prothoracic shield: Large, covering all T1 dorsally and laterally down to the spiracle. Pitchy brown, darker than head. Divided by fine pale brown medial line. Thoracic legs: Coxa concolorous with venter. Femur and tibia dark brown. Tarsus and claw brown. Body: 9 mm larva described, 5 mm larva has paler abdomen. Dorsally brown, with a purplish tint in some light. Ventrally paler with prominent black spots on segments A1,2,7 and 8. Spiracles: Unobtrusive, small, concolorous. Pinacula: Large, shiny, pitchy brown. Setae: Translucent. Basally pale brown, fading distally. Anal plate: Shiny yellowish brown with a broad dark brown transverse band anteriorly. Prolegs: Concolorous with venter. Crochets brown.
Abundance	Common in many areas.
Location	This moth was introduced into Britain, and is now well established throughout.
Life-history	Nocturnal, occurring mainly in August and into September.

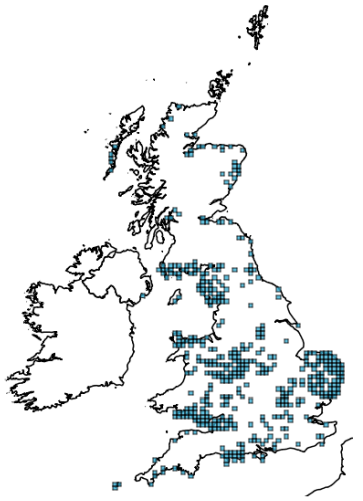
Image	 
Photo credit	© LH Image – Ian Smith, RH Image – Ian Kimber
Distribution	
Species	**Blastobasis rebeli** [Synonyms: wolffi]
	Karsholt & Sinev, 2004
Size	Wingspan c.13 mm.
Information	Only recently named.
Abundance	There are several records are from the same area over a few years, so it is assumed to be breeding, although the early stages have yet to be described.
Location	Recently discovered in Hampshire in 1998, there have been a number of records of this species, otherwise only known from Madeira.
Life-history	The adults have been encountered mostly in July, and generally attracted to mercury vapour light.

Image	
Photo credit	© Richard Coomber
Distribution	

Species	**Blastobasis phycidella**
	Zeller, 1839
Size	Wingspan c.18mm.
Information	The larval stages are not well described but it is thought to feed on plant debris.
Abundance	Scarce.
Location	This species occurs in central and southern Europe and prior to 1990 was only known in The British Isles from four specimens found at Southampton in 1930. In 1990 it was found to be resident on Guernsey when a number of individuals were discovered in June of that year.
Life-history	Adult moths have been found on the wing in June when they are attracted to light.
Image	
Photo credit	© Graham Wenman
Distribution	

Species	**Blastobasis vittata** [Synonyms: lignea]
	Wollaston, 1858
Size	
Information	The species is native to Maderia, along with other Blastobasis species such as B. adustella and B. lacticolella.
Abundance	Scarce
Location	The individual illustrated depicts the first British record of this species, attracted to light in a Lindfield, West Sussex garden on 15 June 2008. Since then there have been a couple more records, from West Sussex and Hampshire, and is suspected, rather like other Blastobasis species, to be a possible adventive colonist.
Life-history	
Image	
Photo credit	© Bob Foreman
Distribution	