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Project number:	TF223
Project leader:	Dr Robert Saville East Malling Research
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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 will investigate 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, pear sucker, apple fruit rhynchites weevil, apple sawfly 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. For ease of reading, this Grower Summary report is split into sections for each of the diseases and pests worked on in the second year.

Objective 1 - Surveillance

Headline

- Work continues to survey current and invasive pests and diseases of relevance in the UK.

Background and expected deliverables

Project TF 223 is a five year project which was commissioned to tackle a number of current pests and diseases affecting tree fruit crops. Objective 1 deals with the surveillance of existing and potential new invasive pests and diseases.

Summary of the project and main conclusions

Scab virulence

As part of a large pan-European project, orchards containing the same indicator cultivars have been planted in 25 European countries. The purpose of this study is to increase our understanding of scab populations, monitoring when and where the resistance is being broken and helping to inform the deployment of resistance genes in future cultivar releases.

Scab incidence is recorded each season and the data from each orchard is compiled by the project coordinator based in Switzerland. Analysed data will be made available as part of the wider project.

One result of note in the 2016 growing season at the indicator orchard planted at NIAB EMR was that the severity of the disease epidemic on the *Vf* (scab resistance gene) containing cultivars was much greater than assessments in previous years and comparable to the

disease incidence on Gala. This result suggests that the local scab population has broken the resistance conferred by *Vf*.

Apple rot survey

This task is a continuation of the apple rot survey which has been undertaken over the last century. The survey involves visiting pack houses during the months of January – March to determine the type and incidence of rot causing pathogens. A total of 60 samples were assessed over 25 visits this storage season. The overall average loss was 2.6% which is similar to recent, past surveys.

Nectria rot was the main rot identified in the 2015/16 survey with incidence being particularly high in canker susceptible cultivars where inoculum is prevalent; Gala (67% of total rots), Cameo (57%), Jazz (49%) and Braeburn (44%).

Brown rot (*Monililnia*) is the next most prevalent rot causing an overall average of 13% of total rots followed by **Gloeosporium** (9%), **Botrytis** (8%), **Phytophthora** (6%) and **Penicillium** (6%). Notably, Gleosporium was present in 58% of the samples which is a higher occurrence than in recent years (2012 - found in 47% of samples, 2013 - 18%, 2014 - 29%). A particularly high incidence was observed in Cox (28% of total rots) and Daliclass (35%). The higher incidence compared to recent years may have resulted from conditions conducive to the multiplication of the causative fungal species. These conditions include the autumn of the previous season (high rainfall), the winter which preceded the season (mild) and the long, drawn out spring all of which may have promoted canker development resulting in high levels of inoculum in the orchard approaching harvest.

Invasives

Xylella fastidiosa still remains a major threat for the UK horticultural industries. With such a large host range including horticultural crops such as Prunus, Vaccinium and Vitis along with a number of wild woodland species such as Quercus and Ulmus, the arrival of this pathogen in the UK would have a detrimental effect on the horticultural sector. At the time of writing there had been no reports of the disease in the UK but there is a heightened risk of it being accidentally introduced following discovery in Italy in 2013 and Corsica and mainland France in 2015. The Plant Health and Seeds Inspectorate (PHSI) are currently conducting surveys and inspections on host material coming into the UK. The main action for growers is to keep plant passports up to date and ensure plant material is not brought in from demarkated areas.

More information on this disease can be found at: (https://planthealthportal.defra.gov.uk/assets/factsheets/xylellaFastidiosa2015.pdf)

Xanthomonas arboricolae, pv. pruni is a notifiable bacterial disease which causes shot holing symptoms on leaves. Plum and sweet cherry are both hosts. To date, it has only been 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

Drosophila suzukii numbers continued to increase for the fourth year since its discovery in the UK in 2012. Numbers were 30% higher in woodlands in winter 2015-16 compared to the same period in the previous year. Fruit damage was reported in all monitored fruit growing regions, with the exception of Scotland.

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

Brown marmorated stink bug traps are in place at NIAB EMR and a terminal in Essex, but none were captured in 2015 or 2016.

A currently unidentified weevil has become an increasing problem in pear orchards. The weevil lays eggs in the flower bud in spring before it has opened. This project is investigating this further in Objective 10.

The RHS has reported sightings of Pear Shoot sawfly, *Janus compressus*. This ‘occasional’ pest of pear in Europe was identified in the UK in non-commercial pears and affects the shoots, causing symptoms similar to fire blight – hook shaped tips caused when the larvae feed inside the shoots.

Financial benefits

- No financial benefits are delivered from surveillance type work.

Action points for growers

- Growers and agronomists should be vigilant for signs or symptoms of new or invasive pests and diseases and report any to Defra's Plant Health Department.

Objective 2 – Neonectria ditissima

Project TF 223 is a five year project which was commissioned to tackle a number of current pests and diseases affecting tree fruit crops. Objective 2 deals with the surveillance of existing and potential new invasive pests and diseases. This Grower Summary reports separately on four different approaches to management and control of *Neonectria ditissima* being investigated in this project. They include work on:

1. Detection of *N. ditissima*
2. Rootstocks/interstocks
3. Soil amendments
4. Novel application methods

Detection

Headline

- An antibody has been selected that can detect *Neonectria ditissima* antigens in plant material.

Background and expected deliverables

Virus detection and elimination in industry base material has advanced hugely in the last 40 years whilst Nectria canker detection has got significantly worse. Propagation nurseries know that latent canker exists in trees but it rarely expresses itself either in the rootstock or the young tree on the nursery. Without better detection methods in rootstock stoolbeds, budwood and graftwood mother stock or indeed in the orchard, this situation will not improve. Understanding how the pathogen is transferred between the stages of tree and fruit production will be vital to develop management strategies to disrupt the disease cycle. The development of a detection tool will not only be invaluable for basic biological understanding of the pathogen but also has the potential to be developed for use by the industry.

Summary of the project and main conclusions

A short list of seven antibodies was selected from year 1. Cross reactivity tests were carried out with a panel of commonly occurring fungi in UK apple orchards to determine which antibodies were the most specific to the target species (*Neonectria ditissima*). *Botryosphaeria*

obtusa was consistently giving a stronger signal (colour change) relative to the other negative antigens for all of the antibodies. The cross reactivity of the antibodies with *Botryosphaeria* and *Neonectria* is of concern because both share a niche in apple as wood canker forming pathogens. Modifications to the ELISA protocol reduced the cross reactivity to *Botryosphaeria* improving the resolution between positive and negative antigens. A validation experiment demonstrated that the selected antibody could detect the presence of the canker pathogen in plant material.

Main conclusions

- An antibody (1B10) has been selected which gives good resolution in cross reactivity tests between Neonectria ditisima antigens and antigens from other fungi commonly found in UK apple orchards. An Enzyme Linked Immunosorbant Assay (ELISA) protocol has been optimised to provide maximum resoulution
- The antibody can detect *Neonectria ditissima* antigens in plant material.
- This work forms the basis of the development of a detection tool.

Financial benefits

With further refinement, outside of this project, this assay can be used to improve our understanding of the biology of *N. ditissima* and potentially developed as a detection tool for the industry. This tool will be used as part of various AHDB projects to increase our understanding of the spread of the disease in the host, from which it is hoped new control strategies can be developed. If developed for use by the industry, it could help propagation nurseries to remove any infected material within stock plants and significantly reduce incidence of the disease in fruiting trees.

Action points for growers

- At this stage of the project, no action points have been developed for growers.

Rootstocks/interstocks

Headline

- Two advanced selections from the NIAB EMR Rootstock Club show promise in conferring resistance to *N. ditissima*.

Background and expected deliverables

Rootstocks are known to confer resistance/tolerance traits to various pests and diseases such as woolly apple aphid, Phytophthora and Neonectria. Interstocks are being increasingly used to confer resistance to the particularly canker susceptible scion cultivars. This work on rootstocks and interstocks 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. The trials are being conducted in two phases; the first phase has evaluated relative resistance of the rootstocks alone using an artificial pathogenicity test and the second will evaluate relative resistance of a panel of rootstocks grafted with a common scion (cv. Gala) planted in the field. The material for the latter phase of this objective was grafted during the winter of 2015/16 and has been planted out during the winter of 2016/17. The purpose of this work is to provide evidence based information to nurserymen and growers to inform choice of rootstock and interstock in the context of European apple canker control.

Summary of the project and main conclusions

Rootstocks have been sourced from various nurseries and breeding programmes as described in the science section of Project TF 223. Rootstocks were bench grafted on to a common scion (cv. Gala) in February 2016. The trees were grown on in preparation for planting in field trials in winter 2016/17. In the meantime rootstock offcuts were retained and used to determine *N. ditissima* susceptibility of the rootstock cultivar in a detached stem pathogenicity test. Although disease progression is highly variable within experiments and across experiments, rootstocks have broadly differing susceptibility to *Neonectria ditissima.* The NIAB EMR advanced selections, EMR-004 and EMR-002, look promising canker resistant cultivars sharing the same significance grouping as MM106 as the least susceptible cultivars in the panel. All other cultivars are not significantly different from the most susceptible cultivar in this test, EMLA M9. The field trials which have now been planted will provide further information on rootstock and interstock influences on scion susceptibility.

Financial benefits

Planting a new orchard is a large, long-term investment. By conducting objective, controlled trials, the outputs from this task will help inform growers of decisions on rootstock choice in the context of canker susceptibility for future plantings.

Action points for growers

- Choice of rootstock/interstock is an important consideration when ordering trees for new plantings.

- Early results suggest that M9 rootstocks are conferring higher susceptibility to other rootstocks available on the market.
- New advanced selections from the NIAB EMR rootstock breeding programme are showing promise in terms of reduced canker susceptibility.

Soil amendments

Headline

- Long-term trials have been established to assess the benefit of biological soil amendments with respect to canker control at both the nursery phase and in newly established orchards.

Background and expected deliverables

This study aims to evaluate biological soil amendments:

- Arbuscular mycorrhizal fungi (AMF)
- Plant growth promoting rhizobacteria (PGPR)
- Trichoderma and Biochar (newly established orchard only)

The aim is to improve tree health and establishment in the context of canker expression. The work is to be conducted in two parts:

1. A stool bed trial will simulate the nursery phase of tree fruit production.
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 in 2017 will have reached the production phase so that rootstocks will be harvested from the stool bed for assessment in December 2017. Two newly planted orchard trials have been planted in 2016 and will be assessed through the remainder of the project. This task is expected to determine the value of biological soil amendments to reduce the impact of *N. ditissima* based on the hypothesis that the interaction with the beneficial microbes will provide the host with more water and nutrients and thus reduce the stress factors which can lead to the expression of a latent infection.

Financial benefits

The loss of trees to canker in the early stages of orchard establishment has financial consequences for both nurserymen and growers. The current cost of establishing an intensive

orchard is in the region of £28K per hectare (FAST Ltd, 2017). In commercial practice, with cultivars susceptible to *N. ditissima*, it is not uncommon for 10% of the trees to be lost each season in the first year or two after establishment. This incurs significant extra costs in replacing lost trees and results in years delay in repaying the outlay of establishing a new intensive orchard.

As part of an integrated approach this project is evaluating the benefit of biological amendments to increase the plant's resilience to transplanting and reduce the expression of the disease during the crucial early stages of orchard establishment. Reducing the incidence of the disease in the early years after establishment will reduce the time taken to repay the establishment costs.

Action points for growers

- The research into soil amendments has not yet reached a stage where action points can be recommended to growers.

Novel application methods

Headline

- Some control products show promise when applied for canker control through an inexpensive injection system.

Background and expected deliverables

Targeted treatment application has the potential to increase efficacy whilst reducing cost and environmental exposure. This study 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) and have great potential to be used for European apple canker control. A collaboration has been established between Fertinyect, Bayer and NIAB EMR to conduct proof of concept trials. This task will evaluate and demonstrate the benefits of targeting treatment application.

Summary of the project and main conclusions

Three trials were conducted. The first evaluated the uptake of a dye from the injection devices to assess the dynamics of product dispersal within the tree. The second and third trials evaluated the curative and protective effects of the treatments in field trials. The treatments

fell into one or more of the following categories; chemical, biological, defence eliciting and plant health promoting. The uptake and phytotoxicity of each of the treatments was recorded.

Despite high variability of the results, a synthetic fungicide product (HDC F199), a biological based product (HDC F200) and a defence eliciting product (Fertinyect protect), all performed well in the trials which assessed curative effects. Uptake issues were identified with certain products such as Cercobin, HDC F200 and HDC F197, which had over 75% of the product left in the devices after 3 days. The products which had poor uptake were available in forms known to be less amenable to injection systems. Some phytotoxic effects were observed, particularly in HDC F206 which caused necrosis of the leaves and the retardation of fruit development. Tests will continue in 2017 with the aim of reducing variability and taking forward the more promising products. Researchers in New Zealand are also working on tree injection for canker control. Results will be exchanged between NZ and UK enabling protocol and treatment list improvements.

Financial benefits

It is envisaged that once refined, an injection system will be available for use as part of an integrated programme to clean up mother trees in the nursery or to spot treat trees in young orchards to prevent trunk cankers girdling the stem.

Action points for growers

- At this stage in the project, no action points can be recommended to growers.

Objective 3 - Apple foliar diseases

Project TF 223 is a five year project which was commissioned to tackle a number of current pests and diseases affecting tree fruit crops. Objective 3 deals with the surveillance of existing and potential new invasive pests and 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

Over-wintering control strategies

The uptake and use of Biological Control Agents (BCA's) has been limited for disease control in orchard crops despite their great potential to reduce conventional products as part of an integrated pest management programme. Barriers for the uptake of BCAs in orchard systems include the higher cost/ha and their reduced and variable efficacy relative to conventional products. Successful control can be difficult to achieve during the season when environmental conditions are optimum for development of the pathogen. This study aims to improve our understanding of interactions between potential antagonists and the pathogen (or pathogen substrate) to inform control strategies which target the overwintering phase.

Apple powdery mildew (*Podosphaera leucotricha*) mainly overwinters as mycelium in floral and vegetative buds. *Ampelomyces quisqualis* (AQ) is a mycoparasite of powdery mildew. AQ10 (a commercial preparation of AQ) was one of the best performing BCAs in trials conducted as part of SCEPTRE when applied throughout the season and in combination with fungicides in a managed programme. However the control achieved was not commercially acceptable. One of the disadvantages of using AQ10 is the slow growth rate of this parasite. This has led to our proposed strategy to target the overwintering phase of the disease, offering a long interaction period between parasite and powdery mildew.

Apple scab (*Venturia inaequalis*) overwinters in leaf litter. Leaf litter management is an important tool for the management of this disease. By disrupting the lifecycle, inoculum is reduced the following spring. The most widely used strategy for leaf litter management in integrated fruit production is the use of autumn applications of urea. Previous studies have demonstrated urea has several modes of action; (1) Direct fungistatic effect of urea on perithecial development; (2) Increased abundance of microbial antagonists to *V. inaequalis*; (3) Accelerated leaf decomposition by (a) Increasing abundance and shift in microbial activity and (b) Increasing palatability of leaf litter to earthworms. New molecular tools are available to understand the microbial community shifts in environmental samples which offer the potential to develop more sustainable approaches to apple leaf litter management than urea.

Alternative treatments

In recent years there has been a reduction in available crop protection products for mildew control and an increase in the incidence of fungicide insensitivity. A number of alternative products are available on the market, which have plant health invigorating, plant defence eliciting or physical modes of action. This research will evaluate the efficacy of these products alone and as part of a programme for powdery mildew control, in order to reduce the reliance on a decreasing number of synthetic chemical based fungicide actives.

In Year 1, products which were evaluated included plant health invigorators, plant defence elicitors and products with a physical mode of action. The test products were evaluated in the field in programmes either with a reduced fungicide programme or alone. During the 2015 growing season powdery mildew disease pressure was high, particularly in the trial orchards which have very high levels of primary mildew due to carry over from previous seasons. This high disease pressure provided a demanding test for the programmes. The full fungicide programme performed best but even with a 7-10 spray interval, it was unable to keep the mildew epidemic below the 10% (commercial) threshold.

The test products alone did delay the epidemic relative to the untreated control but were unable to achieve commercially acceptable levels of control. Of the test products, SB Invigorator was the best performing product. Programmes in which test products were combined with reduced fungicides, performed better than test products alone but this improvement in performance was probably attributable to the fungicides.

The trial design for the 2016 trial season was amended to be more informative. The trial was conducted on a split plot design with half of the replicate blocks receiving a 7-day mildew programme based on fungicides and the other half receiving a 14-day mildew programme based on fungicides, with the test treatments being superimposed on these blocks. This provides two disease pressures ensuring test products are assessed under commercially relevant but sufficient disease pressure.

Summary of the project and main conclusions

Over-wintering control strategies

Trials were set up over the summer of 2016 to test whether the BCA is incorporated into the bud, whether the parasite can survive over winter and whether the stratergy is effective at reducing inoculum. The trial will compare AQ10 treatment with a winter treatment of conventional product + wetter and an untreated control. Spring assessments will be undertaken to determine the efficacy of these strategies and will be reported in next years' report.

We have used next generation sequencing technology to determine the early effects of urea on the microbial communities in leaf litter which could ultimately lead to the development of a biological product more sustainable than urea. Five Pseudomonad species have been

identified which are early colonisers in response to urea application and which are likely to be responsible for accelerated leaf litter breakdown and subsequent microbial succession in response to urea treatment.

Alternative treatments

In a replicated split plot orchard trial on Gala, 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 nine test alternative treatments (B204, Spore kill, SB Invigorator, Wetcit, Garshield, Mantrac Pro, HDC F230, HDC F231 and HDC F232) 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 B204 (three sprays at monthly intervals) and Mantrac Pro (nine sprays only). 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 (<40% - almost 100% mildewed leaves) and low (10-30% mildewed leaves) mildew plots in which to evaluate the test products.
- Treatment 4 (SB Invigorator) was the most consistent in reducing mildew.
- Treatment 5 (Wetcit) and Treatment 8 (HDC F230) were the next most consistent products.
- HDC F232, Mantrac Pro and B204 were the least effective.
- B204 appeared to have little effect on mildew incidence at the start of the trial but by the time the third application was made B204 treated plots had a significantly lower mildew incidence than the fungicide only plots.
- There was no significant effect of treatments on yield, but the lowest yield was recorded in plots treated with Treatment 4 (SB Invigorator) and Treatment 9 (HDC F231).
- Phytotoxicity was recorded on Treatments 5 (Wetcit), 8 (HDC F230), 9 (HDC F231) and 10 (HDC F232) as necrotic spotting on leaves. Wetcit also significantly reduced fruit set. HDC F230 and HDC F231 also caused some premature leaf drop. HDC F230 also increased fruit russet.
- There were no significant effect of treatments on fruit size or fruit colour.

Financial benefits

Foliar diseases require a great number of treatments through the season which not only incur a high cost (product and application) but can also reduce the quality of the produce (residues vs disease). In a regulatory climate of reducing availability of actives, alternatives are desperately sought.

Action points for growers

- Monitoring mildew epidemic is an important component of mildew management as it can inform the choice of product that is selected. The Apple Best Practice Guide, available online, offers guidance to do this.
- Some promising alternative products have been evaluated to be used in conjunction with a reduced fungicide programme, some of which are already available to UK growers.

Objective 4 – Stone fruit diseases

Headline

Early progress has been made to generate a collection of bacteriophage, bacteria killing viruses, which will be further characterised and evaluated in the remainder of the project.

Background and expected deliverables

Project TF 223 is a five year project which was commissioned to tackle a number of current pests and diseases affecting tree fruit crops. Objective 4 deals with the development of bacteria killing viruses to control bacterial canker in Prunus species.

Summary of the project and main conclusions

With the withdrawal of copper for biocidal use confirmed in 2016, treatment options for bacterial canker control in Prunus are no longer available causing significant concern for stone fruit growers. Phage therapy, using bacteria-killing viruses to prevent or cure an infection, may offer potential in the future as a targeted, non-toxic biocontrol agent.

Bacteriophage are one of the most abundant entities on the planet. Phage specific to the target host can be readily isolated wherever the host bacteria (in this case *Pseudomonas syringae syringae* and *Pseudomonas syringae morsprunorum)* can be found.

Soil and leaf samples were collected from stone fruit orchards around Kent, processed to collect any phage that may be present and plated on to Petri dishes containing a lawn of *P. syringae*, known as a ‘double-agar plaque assay’. The presence of phage in the sample

results in circular clearings in the agar called plaques. Phage morphology was determined using transmission electron microscopy (TEM) following isolation and purification. In total, 20 different phage morphologies were collected and these have been put into storage for future characterisation.

Financial benefits

The area of UK cherries is currently 600 ha, producing 4,500 tonnes and is worth £22.5 million (Source: British Summer Fruits). The area of UK plums is 825 ha, producing 7,200 tonnes and is worth £12.7 million (Source: Defra Horticultural Statistics 2014).

Bacterial canker has been a continuing problem for plum and cherry growers for many years. There are no definitive estimates for losses caused by bacterial canker and the impact of the disease on individual growers is likely to vary considerably depending on factors such as orchard age, intensity of production, etc. However, even a conservative estimate of average losses of ca. 5% p.a. would result in the cherry industry losing £1.12 million and the plum industry losing £635,000 p.a.

Developing a method of control would therefore save this level money and potentially more each year.

Action points for growers

- The research into phage specific to Pseudomonas species has not yet reached a stage where action points can be recommended to growers.

Objective 6 - Codling and tortrix moth

Project TF 223 is a five year project which was commissioned to tackle a number of current pests and diseases affecting tree fruit crops. Objective 6 deals with novel methods of controlling codling and tortrix moth species in apple orchards.

Headline

- The RAK3+4 mating disruption system can give comparable control of codling and tortrix moths to conventional spray programmes.

Background and expected deliverables

Codling moth is the most important pest of apples and is also an important pest of pears in the UK. Most insecticide sprays used on these crops are targeted specifically towards these moths. Control is usually good, but populations are not reduced to such low levels that

spraying is reduced in subsequent years. Sex pheromone mating disruption technology offers a sustainable way of reducing damage and reducing local codling moth populations in the long term.

The original aim of this work was to demonstrate the efficacy of sex pheromone mating disruption. It would be assessed alone and in combination with granulosis viruses or nematodes, whilst also measuring the effects on other pests and natural enemy populations. The effects were examined over two growing seasons as the treatment with mating disruption pheromones is for long term control over a wide scale. The sex pheromone mating disruption formulation (RAK3+4) was kindly supplied by BASF.

Summary of the project and main conclusions

Mating Disruption

Two commercial farms, one in the South East and one in the West Midlands of England were used. In the second year, the West Midlands farm was mistakenly over sprayed with Coragen by the host grower, so this site was not used for monitoring in that year. An additional farm in the South East was monitored instead. This had been treated with the RAK3+4 mating disruption (MD) system for three years. Each farm was divided into two halves. The first half was treated with the RAK3+4 mating disruption (MD) system for control of codling moth (CM), summer fruit tortrix (SFT) and fruit tree tortrix (FTT), whilst the other half received the grower's conventional spray programme. Over six hectares on each farm were subjected to mating disruption. The trial results could not be analysed statistically as there were only two replicates included.

In both years at each farm, the numbers of pests and natural enemies were assessed on three occasions; spring (pre-treatment); July (first generation codling damage) and harvest (second generation codling damage). All three pest moth species were monitored weekly in each orchard using sex pheromone traps. For codling and tortrix moth assessments, fruit that had dropped to the ground and fruits on whole trees were assessed. Other notable pest damage was also recorded.

Although few moths were captured in the pheromone monitoring traps on the MD side of the farms, the RAK3+4 system did not cause complete trap shut-down (no moths in traps) indicating that some males may have been able to locate and mate with female moths. Some minor moth damage was observed, but the results were comparable, like for like, with a conventional spray programme.

Some orchards on the mating disruption sides of the farm received an additional Coragen spray when trap moth catches were 4 or above per week or where early ripening varieties which are more vulnerable to codling moth were present. There was some concern over tortrix caterpillars in the young shoots in the spring at Site 1. These were reared through and found to be SFT. However over 50% of the caterpillars were parasitized by wasps. Two sprays of the granulovirus Capex, applied 10 days apart, killed the majority of remaining caterpillars in the affected orchards.

There were few observable differences in natural enemies between the RAK3+4 deployment and conventional spray programme over the trial period, including earwig numbers. However, as earwigs have a single generation each year, the study may not have been long enough to identify differences.

In the second year, there was more first generation CM damage in the early ripening varieties Early Windsor and Bramley. There was notable damage from two pests in the second year on the MD side of the farms. Blastobasis caused damage to fruit at harvest and woolly aphid was abundant in some orchards on the MD side of farms in orchards that had lower numbers of earwigs. These pests would normally be controlled with insecticide applications targeted at CM and tortirx moths and in the past, would have been controlled by the use of broad-spectrum products applied soon after petal fall to control spring pests.

The damage to fruit caused by codling moth at harvest was fairly similar between the MD and conventional sides of the farms. Tortrix caterpillar damage to the fruits was noticeably higher on the MD side of one farm compared to the conventional side.

Nematodes

A series of laboratory and field microcosm tests were instigated to test the efficacy of nematode sprays to target diapausing codling moth larvae in July and August in apple orchards. This work was kindly funded by BASF.

Using the orchards in the MD trials (above) the scientists attached sentinel cages of codling moth larvae to the trunks of apple trees. Using the grower's spray equipment, these were treated with a mixture of the predatory nematodes *Steinernema carpocapsa* (Nemasys C) and *Steinernema feltiae* (750 million of each sp. per ha) in high water volumes applied to the cages. Good infection of the larvae was not achieved, probably because the cage mesh

prevented droplets containing the nematodes reaching the larvae. As a result the scientists used a series of laboratory tests to give a 'best' chance for nematodes to locate and infect codling moth larvae and pupae. In the field, it was decided to employ a different approach. Using a Birchmeier B245 motorised mist blower, it was possible to infect codling moth larvae/pupae with nematodes, even when they were hidden within sentinel cages. Codling moth pupae were less susceptible to nematode infection than larvae. These experiments showed that there may be some efficacy of the nematode sprays when used against codling moth larvae in the field and the tests should now be repeated in the field with larvae in cardboard rolls without the mesh cages.

Main conclusions

The RAK3+4 mating disruption system gave comparable control of codling and tortrix moths to conventional spray programmes. However, certain apple varieties may be more vulnerable to damage and close monitoring of sporadic pests is essential. Growers may need to use supplementary spray applications to maintain commercially acceptable control.

In laboratory studies codling moth larvae were vulnerable to commercially available pathogenic nematodes.

Financial benefits

Codling moth control programmes typically cost growers more than £200/ha/annum. Even a low level of fruit damage (<0.3% fruits damaged) is economically unacceptable. Improving control and/or reducing spray use will be of financial benefit to growers. It may also enhance natural numbers of predators in the crop and benefit the wider environment.

Action points for growers

- The RAK3+4 mating disruption system can give comparable control of codling and tortrix moths to conventional spray programmes.
- It may be advantageous at farms with medium to high pressure of codling numbers to apply an additional Coragen to early ripening or vulnerable varieties where MD technologies are employed.
- Growers should closely monitor for other pests which may occur because of the limited availability of lepidopteran insecticides. In particular sporadic tortrix species and blastobasis caterpillars may be a risk.
- Growers and agronomists should consult the AHDB Apple Best Practice Guide online on how best to monitor for these pests.

Objective 7 - Pear sucker and natural enemies

Project TF 223 is a five year project which was commissioned to tackle a number of current pests and diseases affecting tree fruit crops. Objective 7 deals with pear sucker and the use of natural enemies to gain control in pear orchards.

Headline

- Six commercial pear orchards are being studied to improve our understanding of the levels of naturally occurring predators of pear sucker and their potential for commercial control.

Background and expected deliverables

Pear sucker, *Cacopsylla pyri*, is the major pest of pear. Sporadic population growth occurs in response to warm dry weather and also in orchards where significant populations of earwigs and anthocorids are not sustained. Evidence from other AHDB and Innovate UK projects has shown that earwigs are important control agents for aphids and pear sucker. Additional research in the USA also demonstrates predation of codling moth eggs. Aphid predators such as earwigs, hoverfly larvae, lacewing larvae, spiders and ladybirds are all 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 crop protection product use and timing may be, at least partly, responsible. However, anecdotal evidence is showing that earwigs can be unevenly distributed within an individual orchard.

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

Summary of the project and main conclusions

Six farms were involved in the study in 2016. All farm staff participating were trained in the monitoring technique at the start of the growing season. Each grower selected three orchards (high, medium and low pear sucker infestations) 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.

From March until September, in low, medium and high pear sucker infested orchards, numbers of pear sucker eggs, nymphs and adults, along with ladybirds, earwigs and anthocorids were recorded. The first peaks in pear sucker egg numbers were recorded either in mid to late-March or mid-April, depending on the location of the farm. The second generation of eggs were laid at the end of May and beginning of June with a subsequent smaller peak in pear sucker eggs in August. Anthocorids were released at one of the farms. In some orchards there was a late attack of pear sucker in September.

The majority of orchards never reached high numbers of pear sucker eggs. The exception was Farm 2, in a highly infested orchard, which reached 2,000 eggs per 30 shoots at the second egg laying peak at the beginning of June. Farms 1, 4 and 6 had significant numbers of earwigs and anthocorids and did not reach a peak of pear sucker eggs of more than 500/30 shoots. Farms 2 and 3 had very few natural enemies present in the trees.

Positive correlations existed between guilds of pear sucker averaged over the entire season. Hence where there were more adults there were more eggs and nymphs. There was a significant positive correlation between earwigs and anthocorids. 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 as a food source. Although this data is showing some trends, more seasonal data is required and future analyses could examine population trends over time.

Financial benefits

Close monitoring of pear sucker and 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 honeydew on trees. The reduction of pear sucker in the crop reduces crop loss through the maintenance of fruit quality and prevents damage to overwintering bud and tree health.

Action points for growers

- Monitor for pear sucker in the crop to accurately time Envidor applications and avoid sprays where unnecessary.

- Whilst monitoring for pear sucker, also monitor for natural enemies such as earwigs, anthocorids and ladybirds, to gauge the likely future control in the absence of sprays.

Objective 8 – Apple fruit rhynchites weevil and apple sawfly

Project TF 223 is a five year project which was commissioned to tackle a number of current pests and diseases affecting tree fruit crops. Objective 8 has been dealing with the search for sex pheromones for the apple fruit rhynchites weevil and apple sawfly, which could be used in a sex pheromone monitoring trap.

Headline

- Work has started to identify a sex pheromone for the apple sawfly.

Background and expected deliverables

In the first year of the project, the researchers sought to identify a sex pheromone for the apple fruit rhynchites weevil. As this was unsuccessful, attention has turned to the apple sawfly. 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

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

In spring 2016 apple sawfly infested apples were collected again and kept in drier conditions in compost filled bins (as above) in the laboratory until November, when the bins were transferred to outdoor conditions and covered to prevent too much rain entering. Initial analyses of 24 diapausing larvae have shown only three were alive. The bins will be brought

into room conditions in spring 2017 for emergence of adults and headspace volatile collection for pheromone identification.

Financial benefits

If it goes unnoticed in an apple orchard, the apple sawfly can cause very significant damage. Eggs are laid in flowers and young larvae feed just beneath the surface of the skin on developing fruitlets, leaving characteristic ribbon scars. The larvae can also consume the flesh of the developing fruits. Losses can be severe, particularly when the amount of blossom or crop set is light. In the past, broad spectrum insecticides used to control other spring pests soon after petal fall offered incidental control of apple sawfly, but the number of approved products available has diminished, so apple sawfly could become a bigger problem for growers in future.

The development of a sex pheromone for use in a monitoring trap will help to identify the need for a specific control spray and ensure that it is applied at the optimum time, thus avoiding unnecessary additional sprays at extra expense.

Action points for growers

- At this stage in the project, it is too early to offer any action points for growers based on the research done so far.

Objective 10 – Weevils in pear

Project TF 223 is a five year project which was commissioned to tackle a number of current pests and diseases affecting tree fruit crops. Objective 10 has been investigating a new weevil pest of pear and trying to understand the optimum time of the year to control it.

Headline

- A new damaging weevil pest of pear is being investigated.

Background and expected deliverables

A new pest of pear, still to be identified, is being investigated. The weevil is from the Anthonomus family of weevils known to feed and develop in buds and fruits of plants. Unlike *Anthonomus piri* (apple bud weevil), this weevil is feeding and laying eggs in unopened flower buds in the spring.

In order to control the weevil it is will be necessary to target sprays in the spring, before the flower clusters open. This research aimed to establish the activity period of the pest, its lifecycle and the toxicity of thiacloprid (Calypso) and acetamiprid (Gazelle) to the weevil (*Anthonomus* sp.).

Summary of the project and main conclusions

The weevil was found to be more active (tested by tap sampling a set number of trees) on warm still nights compared to in the daytime. It was active through March, peaking in numbers in mid-March. Mating occurred and females laid eggs in flower buds at bud swell.

In laboratory tests, when directly applying crop protection products to weevils with a Burkard benchtop sprayer, Gazelle did not provide effective control, but Calypso at full and half field rate resulted in 80-90% mortality of field collected weevils. Calypso caused detrimental effects to weevils within three days of application. More research is needed to confirm the identity and inform the complete lifecycle of the insect, including activity in autumn. More research is needed to identify the optimum spray timing during the season and during the day. Consideration should be given to natural enemies in each orchard. Weevils are very specific to orchards, so it is important not to spray every orchard, but to monitor orchards at night and spray where damage occurs.

Financial benefits

A single egg in a flower bud is likely to hatch and the larva destroy the flower. It is estimated that female weevils in the Anthonomus family can lay around 25 eggs, so it is clear that very significant numbers of flowers and fruits could be lost if the weevil is left uncontrolled.

It is therefore important in this work to identify the optimum time of day and time of the season to apply sprays of thiacloprid (Calypso) which is now known to offer control (based on the laboratory tests done in this project).

Action points for growers

- Monitor pear orchards after dark by tap sampling trees to look for weevil activity.
- Assess the amount of bud damage.
- Consider the effect of any applications of Calypso on natural enemies in the trees and only spray when and where it is really needed.
- Monitor each orchard, as not all orchards on a farm have the weevil.

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, apple fruit rhynchites weevil, apple sawfly and phytophagous mites. In light of future pesticide withdrawals, and ongoing consumer and environmental concerns about over reliance on pesticides, a focus on incorporating Integrated Pest Management (IPM)-compatible approaches with conventional pesticides is being adopted for each of the disease and pest targets.

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

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

Losses resulting from Monilinia sp. in stone fruit are hard to quantify because infection occurs throughout the season (blossom and fruit pre- and post-harvest). Post-harvest development

of brown rot limits the storage potential of UK stone fruit and a few rotten fruit in one punnet can lead to food retailers rejecting whole consignments. Bacterial canker is an orchard (and nursery) problem resulting in a loss of profitability from poor establishment, removal of affected trees and loss of fruiting wood. Novel IPM based strategies which complement a reduced fungicide programme will mitigate economic losses for growers, reduce residues for consumers and offer a much needed alternative to copper-based treatments which are no longer permitted for bacterial canker control.

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

Codling moth is the most important pest of apples and is also an important pest of pears in the UK. Most insecticide sprays on these crops are targeted towards it. Control is usually good, but populations are not being reduced to such low levels that spraying is reduced in subsequent years: growers are on an insecticide treadmill. Codling moth control programmes typically cost growers >£200/ha/annum. Even a low level of fruit damage (<0.3% fruits damaged) is economically unacceptable. Improving control and/or reducing insecticide use will be of financial benefit to growers, may enhance natural predators in the crop and benefit the wider environment. Sex pheromone mating disruption technology offers a sustainable way of reducing damage and reducing local codling moth populations in the long term.

Damage by apple fruit rhynchites weevil, *Rhynchites aequatus*, has been increasing in UK apple orchards and sometimes pear orchards in recent years, probably due to changing patterns of insecticide use. Losses of 1% of fruit are common and losses >5% are not unusual. The development of a sensitive, specific, semiochemical-based monitoring trap for apple fruit rhynchites will enable growers to minimise losses due to the pest, and target sprays against it only when they are needed.

Objective 1	Surveillance	Task 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 2016 season and has been submitted to the project coordinator. Analysed data will be made available as part of the wider project. One result of note in the 2016 growing season was the severity of the disease epidemic on the *Vf* (scab resistance gene) containing cultivars (Fig. 1.1) was much greater than assessments in previous years and comparable to the disease incidence on Gala. This result suggests that the local scab population has broken the resistance conferred by *Vf*. This finding illustrates the importance of this work, to monitor when and where the resistance is being broken and helping to inform the deployment of resistance genes in future releases.

Figure 1.1. Scab epidemic on the *Vf* containing indicator tree *Malus floribunda* in 2016.

Objective 1	Surveillance	Task 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 2015/16 storage season. In total 60 samples were assessed over 25 visits. The main cultivars sampled were Gala and Braeburn, reflecting the fact that these two cultivars are dominant in the UK industry. The overall average loss was 2.6% which is similar to recent past surveys. Losses of Cameo (10%) were very high but this is based on a single sample which was particularly affected by Nectria rot and core rot (likely Fusarium). Losses of Cox (4.8%) and Bramley (2.3%) were high but in line with the losses usually experienced in these varieties which is attributed to their higher storage temperature. The other apple varieties and pears sampled did not experience significant losses (all ≤1%). Nectria rot was the main rot identified in the 2015/16 survey with incidence being particularly high in canker susceptible varieties where inoculum is prevalent; Gala (67%), Cameo (57%), Jazz (49%) and Braeburn (44%). Brown rot (*Monililnia*) is the next most prevalent rot causing an overall average loss of 13% followed by *Gloeosporium* (9%), Botrytis (8%) *Phytophthora* (6%) and *Penicillium* (6%). Notably, *Gleosporium* was present in 58% of the samples which is higher than recent years (2012 (47%), 2013 (18%), 2014 (29%)). A particularly high incidence was observed in Cox (28%) and Daliclass (35%).

Table 1.1. The average loss (%) attributed to each rot pathogen during the 2015/16 storage season. The data is compiled from 60 samples.

Cultivar	Average % of loss attributed to each rot;															
	Brown rot	*Botyrtis*	*Phytopthora*	*Penicillium*	*Nectria*	*Gloeosporium*	*Fusarium*	*Mucor*	*Botryosphiria*	*Phomopsis*	Stalk	Eye	Cheek	Core	Number of samples	Loss (%)
Braeburn	5.8	2.4	16.2	5.2	44.2	11.3	0.0	14.6	0.0	0.0	0.0	0.2	0.0	0.0	13.0	0.4
Bramley	24.7	0.0	0.2	16.3	17.8	1.4	3.9	1.4	0.0	0.0	11.7	0.0	3.0	19.5	7.0	2.3
Cameo	2.1	0.0	0.0	2.8	56.6	7.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	31.5	1.0	10.0
Cox	20.7	4.3	6.5	6.0	32.2	23.7	0.0	1.7	0.0	0.0	5.0	0.0	0.0	0.0	8.0	4.8
Gala	7.6	7.5	9.1	1.7	67.6	5.3	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	16.0	0.9
Jazz	1.3	15.3	4.5	8.9	48.9	6.0	0.0	0.2	0.0	0.0	0.5	0.0	0.0	0.0	7.0	0.4
Other dessert	23.8	17.5	7.1	3.2	23.0	17.5	0.0	0.8	0.0	0.0	0.0	0.0	0.0	7.1	2.0	0.9
Pears	20.1	19.5	7.6	6.3	32.1	2.0	0.0	4.5	0.0	0.0	0.0	0.0	6.5	0.0	6.0	1.0
Overall average	**13.3**	**8.3**	**6.4**	**6.3**	**40.3**	**9.3**	**0.5**	**3.0**	**0.0**	**0.0**	**2.2**	**0.0**	**1.2**	**7.3**	**-**	**2.6**

Discussion

The 2015/16 rot survey results follow a similar trend to recent surveys with the following exceptions; (1) the incidence of Gloeosporium rot (caused by *Neofabreae* sp.) was higher than in recent years and may have resulted from the conducive conditions for the multiplication of for the causative fungal species; the autumn of the previous season (high rainfall), the winter which preceded the season (mild) and the long, drawn out spring all of which may have promoted canker development resulting in high levels of inoculum in the orchard approaching harvest. (2) Moderate (≥50mm/month) but consistent rainfall throughout the harvest period (August to November) resulted in a high incidence of *Phytophthora* in samples, although severity was relatively low suggesting growers are observing the rot risk assessment for this disease.

Objective 1	Surveillance	Task 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 International conferences IOBC, Greece and ICE, Florida Numbers 30% higher in woodlands in winter 2015-16 compared to the same period in the previous year. Damage seen in all soft fruit and cherry crops, but particularly blackberry. Fruit which take a long time to ripen are more vulnerable.
	Summer fruit tortrix	*Detected for the first time in the West Midlands during the 2015 growing season.*
	Marmorated stink bug	Monitoring traps were in place at NIAB EMR and a terminal in Essex, but no BMSB were captured in 2015 and 2016.
	Pear bud weevil	*Anthonomus piri was a particular problem in 2015 and 2016. This is likely because fewer insecticides are being applied for pear sucker in order to conserve natural enemies. See below for preliminary research volunteered on this pest by NIAB EMR in 2016.*
	Pear Shoot sawfly	*An emerging pest was reported by Jim Arbury of the RHS – Janus compressus. This 'occasional' pest of pear in Europe was identified in the UK in non-commercial pears and effects the shoots causing symptoms similar to fire blight – hook shaped tips caused when the larvae feed inside the shoots. A paper was sent to Chris Nicolson for inclusion in the ADAS notes.*
	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*
Diseases	*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*	Task 1	Detection

Aim

Develop a tool for *Neonectria* canker detection (EMR, Yr 1)

Introduction

Virus detection and elimination in industry base material has advanced hugely in the last 40 years but the matter of Nectria canker detection has got significantly worse. For nurseries the main difficulty that has always existed is that latent canker is known to exist in nursery trees but rarely expresses itself either in the rootstock or the young tree in the nursery. Without better detection methods both in rootstock stoolbeds, budwood and graftwood mother stock or indeed in the orchard, this situation will not improve. Understanding how the pathogen is transferred between the stages of tree and fruit production will be vital to develop management stratergies to disrupt the disease cycle. The development of a detection tool will not only be an invaluable tool for basic biological understanding of the pathogen but also has the potential to be developed for use by the industry.

Materials and methods

In the first year of this project six antibodies (1A1 G1E3, 1B10, 1D7 G3D3, 1F5, 3D8 61C8, and 6D12 C1E5) were generated by Gary Keane of the Monoclonal Antibody Unit (MAU) at the University of Worcester. A seventh antibody was acquired from Molly Dewrey (formally of Oxford University) who, together with Terry Swinburne, generated a *N. ditissima* specific antibody NG 1E4 (Dewey and Swinburne, 1995).

An antigen (molecules which bind to the Ag-specific receptors of antibodies) library was prepared consisting of positive antigens (extracted from *N. ditissima* isolates; R09/05, TL88 and R28/15) and negative antigens (extracted from six fungi commonly found in apple

orchards; *Fusarium lateritium*, *Nectria cinnabarina* (coral spot), *Monilinia laxa* (brown rot), *Phomopsis/Diaporthe*, *Colletotrichum acutatum* and *Botryosphaeria obtuse* (Fig. 2.1)).

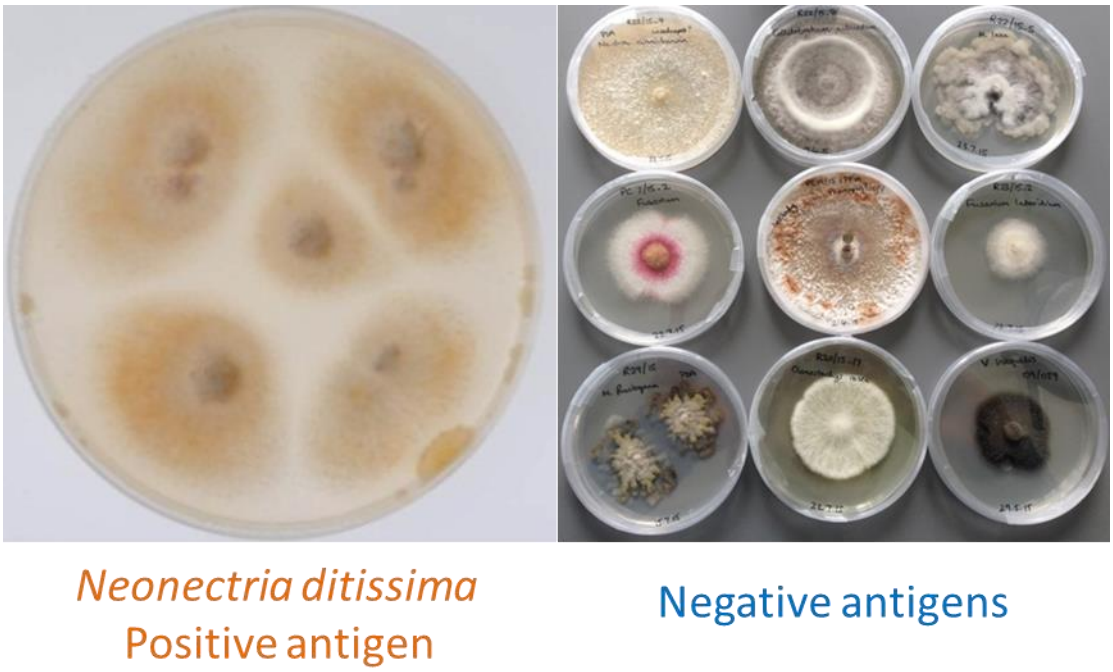


Figure 2.1. Positive and negative control antigen collection in culture.

Determining antibody specificity

An enzyme-linked immunosorbent assay (ELISA) is a laboratory technique to measure the presence/concentration of an antigen in solution). ELISA was used to determine the best antibody from the antibody panel and the optimum assay conditions. A plate trapped antigen (PTA) ELISA was developed, briefly; the antigen was incubated in the well overnight at 4°C, emptied and washed (x4) the following day with Phosphate Buffered Saline Tween-20 (PBST), hybridoma supernatant (containing the antibody) was added and incubated for 45 minutes at 30°C, followed by a further 4x washes with PBST, antimouse conjugated to biotin (which binds to specific antibody if present) was added and incubated for 45 minutes at 30°C, followed by 4x washes, streptavidine conjugated to peroxidase (4 x streptavidine molecules bind to each biotin if present) was added and incubated for 45 minutes at 30°C, followed by 4x PBST washes, TMB substrate (which changes colour if peroxidase is still present, i.e. bound) was added incubated in the dark for 20 minutes and then any colour change was measured by a spectral plate reader at 450nm. All reactions were at 100µl. All seven antibodies were tested for specificity to the antigen library.

Testing on plant material

Cankered branches were collected from a Gala orchard. Branch sections were surface sterilised with ethanol, bark tissue removed and wood shavings of the cambium layer were collected. Samples were taken from the leading edge, and 20 and 40 mm distal from the cankered leading edge. Wood shavings were incubated in the ELISA plate well overnight in PBST (general ELISA buffer) to allow antigens to bind to the well. An ELISA was conducted the following day.

Results and discussion

The antibody specificity tests are shown in Fig. 2.2. *Botryosphaeria obtusa* (antigen 7 in Fig. 2.2) was consistently giving a strong signal (colour change) in comparison to the other negative antigens. The cross reactivity of the antibodies with *Botryosphaeria* with *Neonectria* is of concern because both share a niche in apple as wood canker forming pathogens however modifications to the ELISA protocol, as discussed later, have reduced the cross reactivity to *Botryosphaeria* improving the resolution between positive and negative antigens.

NG 1E4 (Fig. 2.2a) was the least specific assay despite being a highly specific antibody when first published (see Fig. 1 in Dewry and Swinburne 1995). This may be due to the age of the hybridoma (antibody-containing) cell suspension, which despite being stored in optimum conditions for the past 20 years at Isis Innovation, University of Oxford, may have lost some of their activity.

1B10 has the greatest specificity of all of the antibodies tested here. The signal for the positive antigen (*N. ditissima*, orange bars in Fig. 2.2) preparations is strong; between 0.6 and 1 absorbance at 450 nm (Note that the y-axis changes for each antibody to facilitate interpretation between antigens within each assay). Whilst the signal for the negative antigens is very low <0.2 abs. 450 nm for all but *Botryosphaeria*. Subsequent assay refinement has improved assay specificity by measuring background signal (noise) in a negative sample (without antibody) for each antigen and normalising the results accordingly (Fig 2.3).

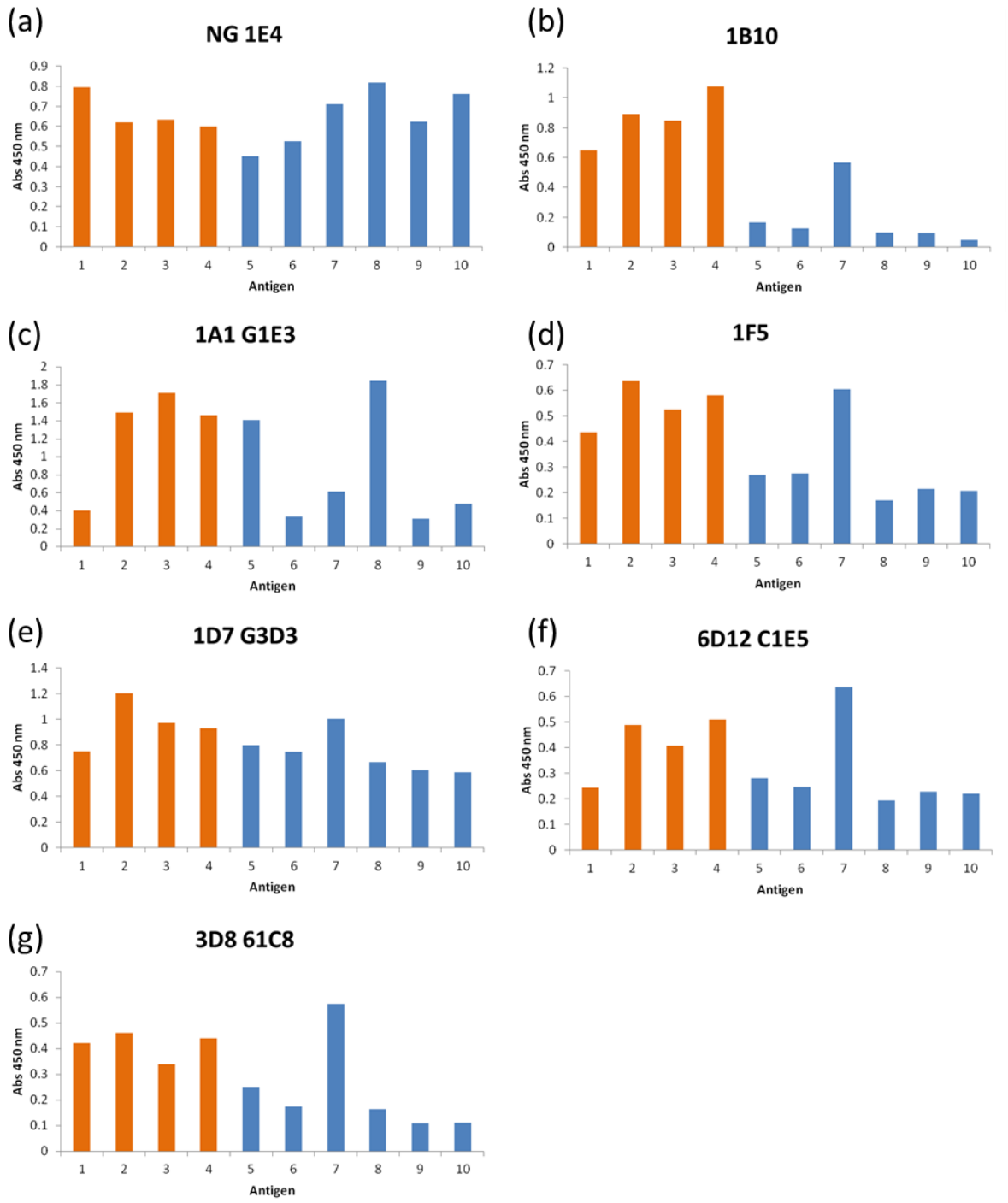


Figure 2.2. ELISAs to determine antibody specificity. Orange bars are positive antigens (various strains/preparations of *N. ditissima*), Blue bars are negative antigens. The negative antigens are as follows; 5 = *Fusarium lateritium;* 6 = *Monolinia laxa*; 7 = *Botryosphaeria obtusa*; 8 = *Nectria cinnabarina*; 9 = *Colletotrichum acutatum*; 10 = *Phomopsis sp*.

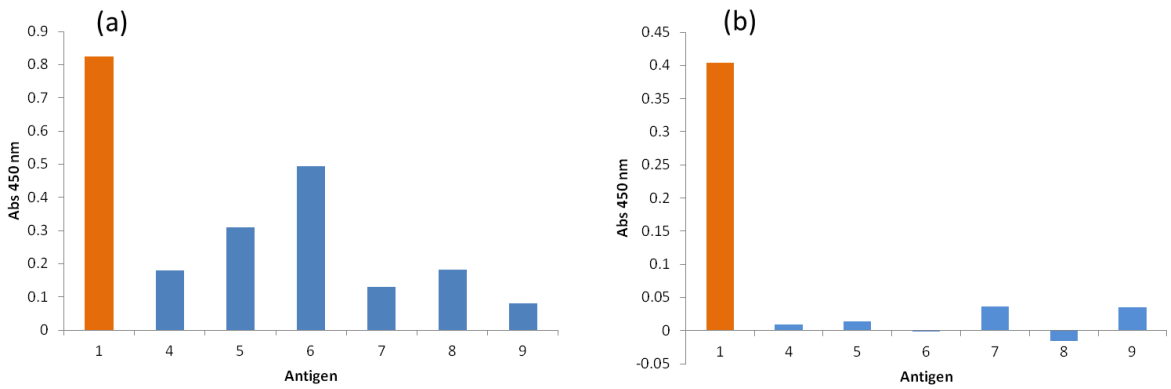


Figure 2.3. ELISA result using 1B10 before (a) and after (b) normalisation to background signal i.e. measuring background signal (noise) in a negative sample (without antibody) for each antigen and normalising the results accordingly. 1 = *Neonectria dittisima*; 5 = *Fusarium lateritium;* 6 = *Monolinia laxa*; 7 = *Botryosphaeria obtusa*; 8 = *Nectria cinnabarina*; 9 = *Colletotrichum acutatum*; 10 = *Phomopsis sp*.

The antibody showing the highest specificity in cross reactivity tests, 1B10, was used in an assay to detect *N. ditissima* in plant material (Fig. 2.4). The results show that *Neonectria* can be detected in symptomatic tissue, furthermore the assay may be useful for determining quantitative information with *Neonectria* being detected, albeit at lower levels, in tissue distal from the visible canker, consistent with the results from a study by Weber (2014) which used traditional culturing to determine the spread of *Neonectria* within the wood tissue. Further work is necessary to determine signal thresholds and to validate this preliminary study and that shall be carried out in AHDB TF PhD studentship CP161 – Understanding endophytes to improve tree health.

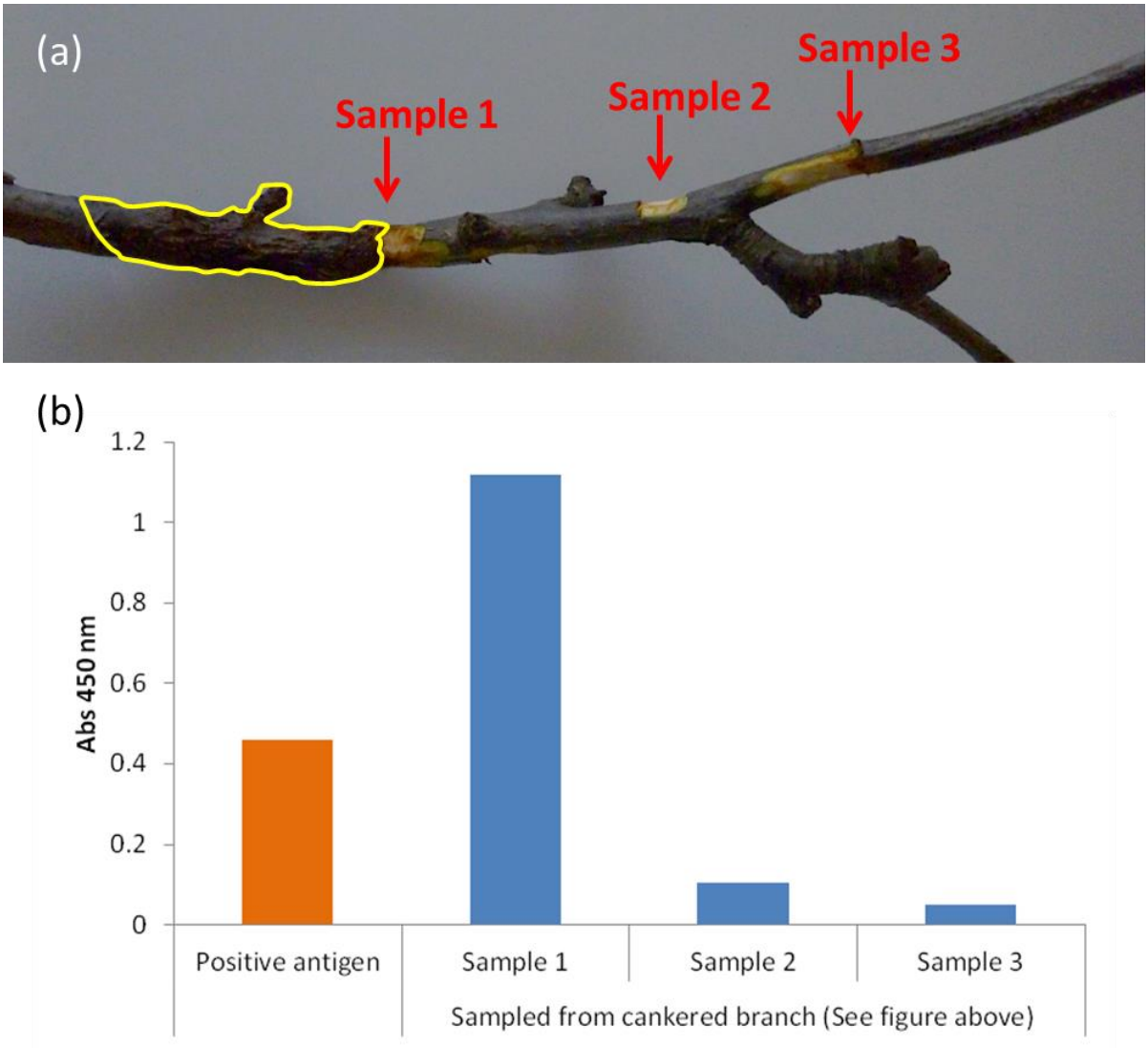


Figure 2.4. An ELISA with plant material. (a) a cankered branch of cv. Gala was sampled at the canker leading edge (sample 1), 20mm (sample 2) and 40mm (sample 3) distal from the canker. the visible cankered tissue is highlighted in yellow. (b) an ELISA using the best performing antibody, 1B10, with samples prepared from a cankered branch corresponding to figure (a) and a positive control antigen solution.

Conclusions

- ELISA assay conditions have been optimised and an antibody has been selected which shows good specificity to *N. ditissima*
- The assay is able to detect *N. ditissima* in plant material and early indications show that the pathogen spreads beyond the visible diseased tissue, and this is detectable using ELISA
- With further refinement, outside of this project, this assay can be used to improve our understanding of the biology of *N. ditissima* and used as a detection tool e.g. for

Objective 2, Task 3; to assess the rootstocks harvested from the soil amendments stool bed trial

Objective 2	Neonectria ditissima	Task 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*. Interstocks are being increasingly used to confer resistance to the 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. 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 herein) and the second will evaluate relative resistance of a panel of rootstocks grafted with a common (cv. Gala) scion planted in the field. The material for the latter phase of this objective was grafted during the winter of 2015/16 and will to be planted out during the winter of 2016/17.

Materials and Methods

The rootstocks sourced from various nurseries and breeding programmes (the advanced selections from the NIAB EMR breeding programme were supplied free of charge by Bruno Essner, Pepinieres Du Valois) are described in table 2.1. Rootstocks were bench grafted on to a common cv. Gala scion in February 2016. The trees were grown on in preparation for the field trial phase to be planted in winter 2016/17

The rootstock offcuts were retained and used to determine *N. ditissima* susceptibility of the rootstock cultivar in a detached stem pathogenicity test. Due to the poor quality of the Geneva series root stocks (G. 41 and G. 11) the offcuts were not retained for the detached stem pathogenicity test. Breifly; shoots were brought out of the cold store where they were stored, placed in oasis floral foam blocks and acclimatised to test conditions (16/8 h light/dark, 22/18 ºC day/night at constant humidity of 80% RH). Two buds on each shoot were inoculated. Inoculation points were prepared by cutting just below the bud to create a wound. An inoculum volume of 5µl of spore suspension ($1x10^5$ conidia/ml) was applied to the wound within five minutes of making the wound. Following inoculation, wounds were covered with white petroleum jelly, which was removed after seven days with a paper towel. During the first four days after inoculation relative humidity (RH) was increased to 90%. Mock (water) inoculated controls were included. Lesion length was recorded weekly. Eight replicate stems were

inoculated with two inoculation points per stem (pseudo replicates) providing a total of 16 replicates per rootstock. The experiment was repeated twice and data analysis was on both experiments combined.

Table 2.1. The rootstocks and interstock to be evaluated.

Root stock	Inter stock	Scion
M9 (EMLA)	-	Gala
M9 (337)	-	Gala
G.41	-	Gala
G.11	-	Gala
MM106	-	Gala
M116	-	Gala
M26	-	Gala
M9 (337)	Golden Delicious	Gala
EMR-001	-	Gala
EMR-002	-	Gala
EMR-003	-	Gala
EMR-004	-	Gala
EMR-005	-	Gala
EMR-006	-	Gala

Results

Infection developed at all inoculation points and mock (water) inoculations did not develop any symptoms. High variability in this dataset has resulted in a large LSD and few significant differences. The cultivars assessed can be classified into three statically different groups based on the final assessment (assessment 4, see Fig. 2.5), with those sharing a letter (a, b or c) not being significantly different from one another.

In the attached stem test presented in the previous TF223 report, which evaluated the susceptibility of a reduced panel of just five cultivars, the rootstocks cultivars were ranked from most susceptible as follows; M9 clone 337 > EMLA M9 > M116 > EMR-001 > MM106

with M9 clone 337 being significantly more susceptible than EMLA M9. Contrary to these results the detached stem test reported here places M9 EMLA as the most susceptible and both M116 and EMR-001 are ranked above M9 337, albeit none are significantly different, sharing the same letter, from each other owing to the variability inherent in this pathogenicity test. The NIAB EMR advanced selections, EMR-004 and EMR-002, look promising canker resistant cultivars sharing the same significance grouping as MM106 as the least susceptible cultivars in the panel. All other cultivars are not significantly different from the most susceptible cultivar in this test, EMLA M9. The early stages of disease progression (see assessments 1 and 2, Fig. 2.5.) clearly show that both the M9 clones (EMLA and 337) are more susceptible to disease spread initially, following assessment 2 M9 EMLA shows rapid lesion expansion whilst M9 337 slows. On the other hand EMR-001, EMR-003 and M116 experience rapid lesion expansion following assessment 2 having contained disease spread initially.

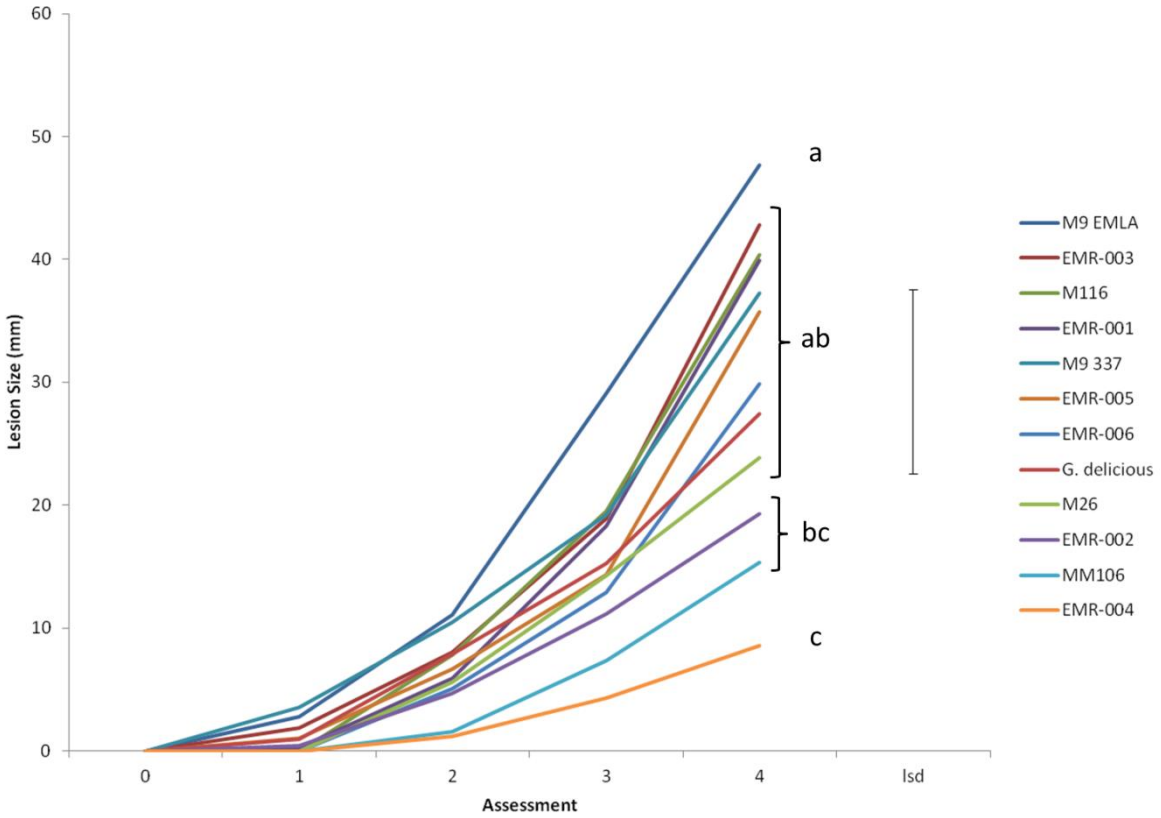


Figure 2.5. The development of *Neonectria ditissima* lesions on a panel of rootstock cultivars.

Discussion

Several controlled pathogenicity tests have been conducted in the first two years of this project to determine the innate varietal susceptibility of rootstock cultivars to complement the

results obtained from the field. The results relate directly to the susceptibility of the rootstock/interstock cultivar rather than the effects they confer on the scion (as will be determined in the field) and are independent of the vigour the rootstock confers on the scion. These tests have revealed broad groupings by susceptibility but due to the inherent variability of these tests, despite variables being controlled to the best of our abilities means that consistent rankings cannot be determined. Variability from year to year may in part be due to the quality of the wood i.e. some cultivars may have been grown to different growing, harvesting and storage processes prior to arrival at NIAB EMR making them more or less susceptible to canker which would contribute to variation over years. Sourcing all of the panel from a common source is not possible due to accessions being sourced internationally. Variation within experiment, giving rise to the large lsd, is difficult to control for, further work is being carried out in an AHDB funded studentship to better understand the host-pathogen interaction.

Conclusions

- Rootstocks have broadly differing susceptibility to *Neonectria ditissima* although disease progression is highly variable within experiments and across experiments
- The field trial to be planted this year will provide further information on rootstock and interstock influences on scion susceptibility

Objective 2	Neonectria ditissima	Task 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 maybe 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 reported in the previous report. The newly planted orchard trials (n=2) have been planted in this reporting year and are described herein. Root stocks will be harvested from the stool bed for assessment in December 2017 now that the stool beds have reached the production phase. The newly established orchards are to be assessed through the remainder of the project.

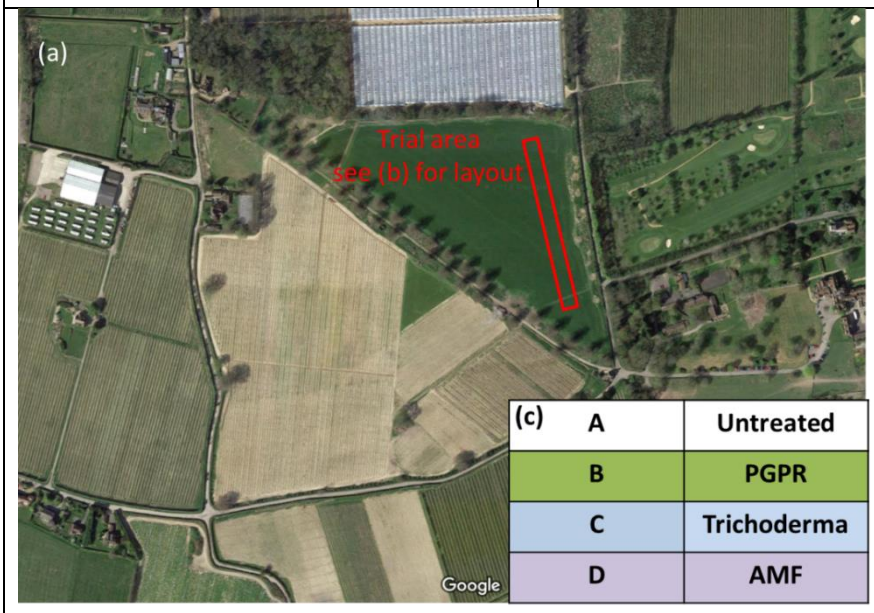
Materials and Methods

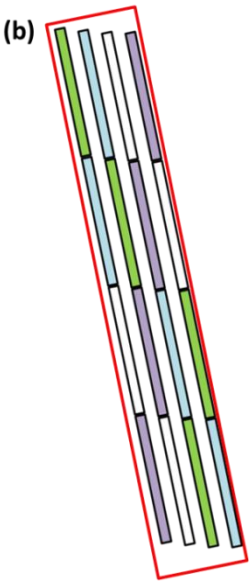
Sites

Two orchards were selected for this study. The sites and cultivars were selected based on what the grower was planting at the time the trial was setup. Susceptible cultivars were selected to increase the chances of disease expression and spread. The planting sites were kindly provided by Avalon Produce Limited and Worldwide Fruit Limited.

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





(c)	
A	Untreated
B	PGPR
C	Trichoderma
D	AMF

Site 2	Kent
Grid reference	51°16'55.9"N 0°24'35.1"E
Variety	Cv. Gala*
Planted	12/05/16
Producer organisation	Worldwide Fruit Limited

***** Cv. Jazz was initially chosen because of its susceptibility but due to inavailability of trees at host growers site we setup the trial in a cv. Gala orchard.

Treatments

Both trials were designed as a randomised block with each treatment replicated four times. The treatments, listed in Table 2.2, were added to the planting hole ensuring that the roots of each tree were covered.

Table 2.2. Treatments used for biological amendments trial.

Treatment	**Product (Supplier)**	**Species**	**Quantity per planting hole[1]**
Untreated	-	-	-
Arbuscular Mycorrhizae Fungi (AMF)	Rootgrow (Plantworks)	*Funneliformis mosseae* *Funneliformis geosporus* *Claroideoglomus claroideum* *Rhizophagus irregularis* *Glomus microaggregatum*	50 ml
Plant Growth Promoting Rhizobacteria (PGPR)	Experimental (Plantworks)	*Rhizobium* sp., strain IRBG74 *Bacillus amyloliquefacien* *Bacillus megaterium* *Derxia lacustris,* strain HL-12	50 ml
Trichoderma	TrianumG (Koppert)	*Trichoderma harzianum* strain T-22	25 ml
Biochar	Tree Soil Improver (CarbonGold)	Biochar + Mycorrhizae	25 ml

[1] As per manufacturer's instructions

Conclusions

- The nursery and newly established orchard phases of the trial have been setup
- As of this year assessments of canker development will commence and continue for the remainder of the project

Objective 2	*Neonectria ditissima*	Task 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) and have great potential to be used for European apple canker control. 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. Wound paints have traditionally been used to protect pruning wounds from *N. dittisima* however due to the extra labour required wound painting was seldom practiced and so products were removed from the market as there was a lack of demand. There are now new products on the market and a great economic incentive to protect pruning wounds particularly on high value canker-susceptible cultivars. A trial to evaluate the benefit of wound protection will commence in Spring 2017. A protocol and treatment list has been prepared (Appendix 1).

Materials and Methods

Site

Church fields east (CE231) located at NIAB EMR. The orchard, planted in 2013 by Avalon Produce Limited, consists of single alternate rows of Gala and Rubens on M9 rootstock. Row spacing is 3.5m and tree spacing is either 1m or 0.5m depending on the row. Rubens (canker-susceptible) trees were used in this trial.

Potted Cv. Bramley trees on MM106 maintained under polytunnel at NIAB EMR were used for the dye study.

Dye study

Ynyect devices were dosed with 3% Safrinine dye (a biological stain used in histology and cytology) giving a final concentration of 0.2%. Devices were inserted as per manufacturer's instructions. Trees were destructively sampled (trunk cut in transection every 10cm away from the injection point) at 2, 4, 8 and 24 hours following treatment and the distance of dye progression was determined.

Treatments

The treatments were applied with the Fertinyct ynyect delivery system. Treatments were applied as per manufacturer's instructions (see https://www.youtube.com/watch?v=2y0g-W44ipE).The devices were dosed using Cone Luer Lock syringes (see https://www.youtube.com/watch?v=q9E4sJSmON4). Standard treatments for pests, foliar disease and nutrients will be applied to all plots throughout the season. Based on fertinyect's experience the wound was left open to heal in the first year's trials.

Table 2.3. Treatment list for tree injection trials

Product	**Active ingredient**	**Formulation[1]**	**Product type**	**Rate/150ml device**
HDC F198	Experimental	WG	Fungicide+defence elicitor	4.5g
HDC F199	Experimental	SC	Fungicide	421.5µl
HDC F197	Experimental	SC	Biological	15ml
HDC F200	Experimental	WP	Biological	6g
B204	Preformed Phenolics	Pre-formulated	Plant health promoter	450µl
HDC F206	Experimental	WG	Defense elicitor	5.625g
Fertinyct – Protect	Magnesium Phosphite	Pre-formulated	Defense elicitor	-
Folicur	Tebuconazole	EW	Fungicide	0.9ml
Cercobin (Certis)	Thiophanate-methyl	WG	Fungicide	1.65ml
UNTREATED[2]	-	-	-	-

[1] abbreviations are explained in table 2.4. [2] Untreated were either injected with the carrier (Ynyect device) or holes were drilled but did not have an injection device applied (untreated).

Trial 1; Existing and new canker formation

Trees (cv. Rubens) were selected within the orchard which exhibit a distinct and active trunk canker. Selected trees were marked with spray paint at the base of the trunk, if multiple cankers were present on the trunk then the canker closest to the ground was the canker to be assessed.

Prior to treatment the area of the existing canker was measured The canker area was determined by multiplying the radius of the longest axis (major radius) with that of the shortest axis (minor radius) and multiplying by π. Due to low levels of sporulation it was decided canker washings would not be collected meaning a base line was not recorded. Treatments (Table 2.3) were applied on 16/05/16. Each treatment was replicated 7 times in a randomised block design. Two days after treatment artificial inoculations (wounding with file, inoculating with spore suspension (isolate Hg199 at 1.5 x 10^5 spores/ml) and coating with vaseline®) were made on the trunk 1.5m from the injection point. Treatment uptake and phytotoxicity assessments were carried out as described below. Canker area was measured for existing and inoculated cankers 7 months after treatment. Sporulation will be measured if deemed required in the spring (when spore numbers will be at their highest).

Trial 2; New canker formation

Healthy trees (cv. Rubens) were selected within the orchard. Selected trees were marked with spray paint at the base of the trunk. Treatments (Table 2.3) were applied on 14/06/16. Each treatment was replicated 5 times in a randomised block design. Two days after treatment artificial inoculations (wounding with file, inoculating with spore suspension (isolate Hg199 at 1.5 x 10^5 spores/ml) coating with vaseline®) were made on the trunk 1.5m from the injection point. Treatment uptake and phytotoxicity assessments were carried out as described below. Canker area was measured for inoculated cankers 5 months after treatment.

Uptake

The uptake of the treatments from the device was scored 3 days after being applied. The degree of uptake was scored on a 5 point scale; 1 = device empty; 2 = <25% full; 3 = 50% full; 4 = >75% full; 5 = full.

Phytotoxicity

Symptoms of phytotoxicity will be checked and recorded. Records included chlorosis / necrosis to foliage, growth regulatory effects to shoots and to fruit, assessed on a scale 0-5

(EPPO Guideline PP 1/135(3)) where 1 = 1-20% of tree affected, 2 = 21-40%, 3 = 41-60%, 4 = 61-80%, 5 = 81-100% affected.

Results

Dye study

Within 2 hours the dye reached the maximum distance that dye migration was observed, 30 cm above and 10 cm below the injection point (Fig. 2.6). The dye did not progress any further in the trees sampled 4, 8 and 24 hours after treatment application (data not shown). Dye migration towards the roots formed concentric circles potentially reflecting the active movement of the dye by xylem vessels down into the roots. Dye migration towards the apex seems less organised (Fig. 2.6).

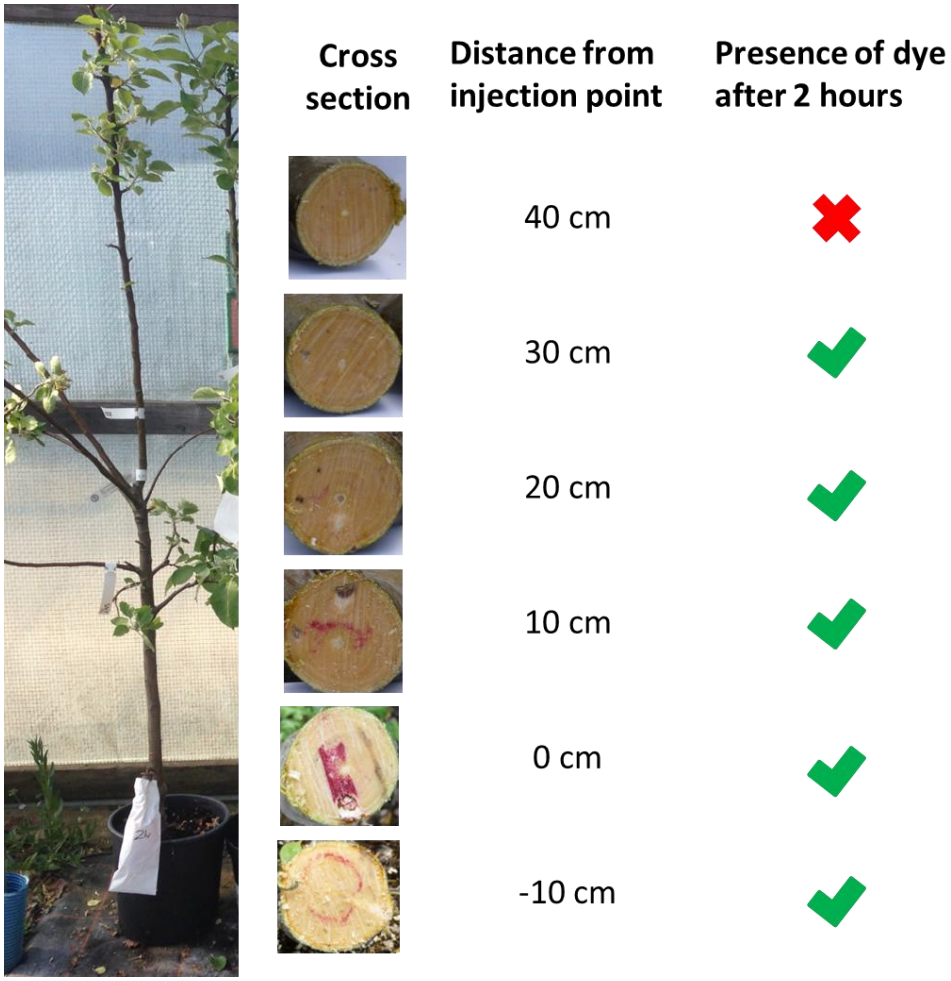


Figure 2.6. Experimental setup of dye study to demonstrate uptake from the ynyect delivery system. Cross sections were made every 10 cm and visually inspected for presence of dye.

Trial 1; Existing and inoculated (new) canker formation

An assessment of the treatments ability to be taken into the tree was conducted. Treatment uptake was generally 100 – 50% (0-2 on the scale, Fig. 2.7) however three treatments (Cercobin, HDC F200 and HDC F197) had over 75% (3 on the scale, Fig. 2.7) left in the devices after 3 days. Phytotoxic effects of some treatments were observed on the 06/06/16 (3 weeks after treatment application) and trees were assessed for chlorosis and necrosis. Untreated controls were showing signs of chlorosis which may have been due to the general poor condition of the trees due to the girdling of the stems. None of the treatments were significantly different from untreated control. Untreated and Ynject device (carrier solution) controls showed low levels of necrosis whilst necrosis was consistently recorded on trees treated with HDCF206 and Fertinyect protect.

The data for existing cankers (Fig. 2.8) shows that on average canker area increased from 16 to 171% depending on treatment. Cankers on untreated control increased in area by 147% on average. None of the treatments cause a significant reduction in canker expansion due to the high variability across replicates (particularly in untreated). However trends can be observed with all fungicides alone (Fig. 2.8, red bars) performing well, particularly HDC F198. HDC F200 was the best performing biological (Fig. 2.8, green bars) despite having poor uptake (Figure 2.7 a). B204 is the worst performing treatment.

The data for new canker development was highly variable and not informative, likely due to the varying degrees of canker lesion girdling at the base of the tree effecting treatment uptake and general plant health therefore the data for the inoculated cankers in Trial 1 is not shown. A second trial was commenced (Trial 2) to evaluate the effects of treatments on inoculated (new) cankers.

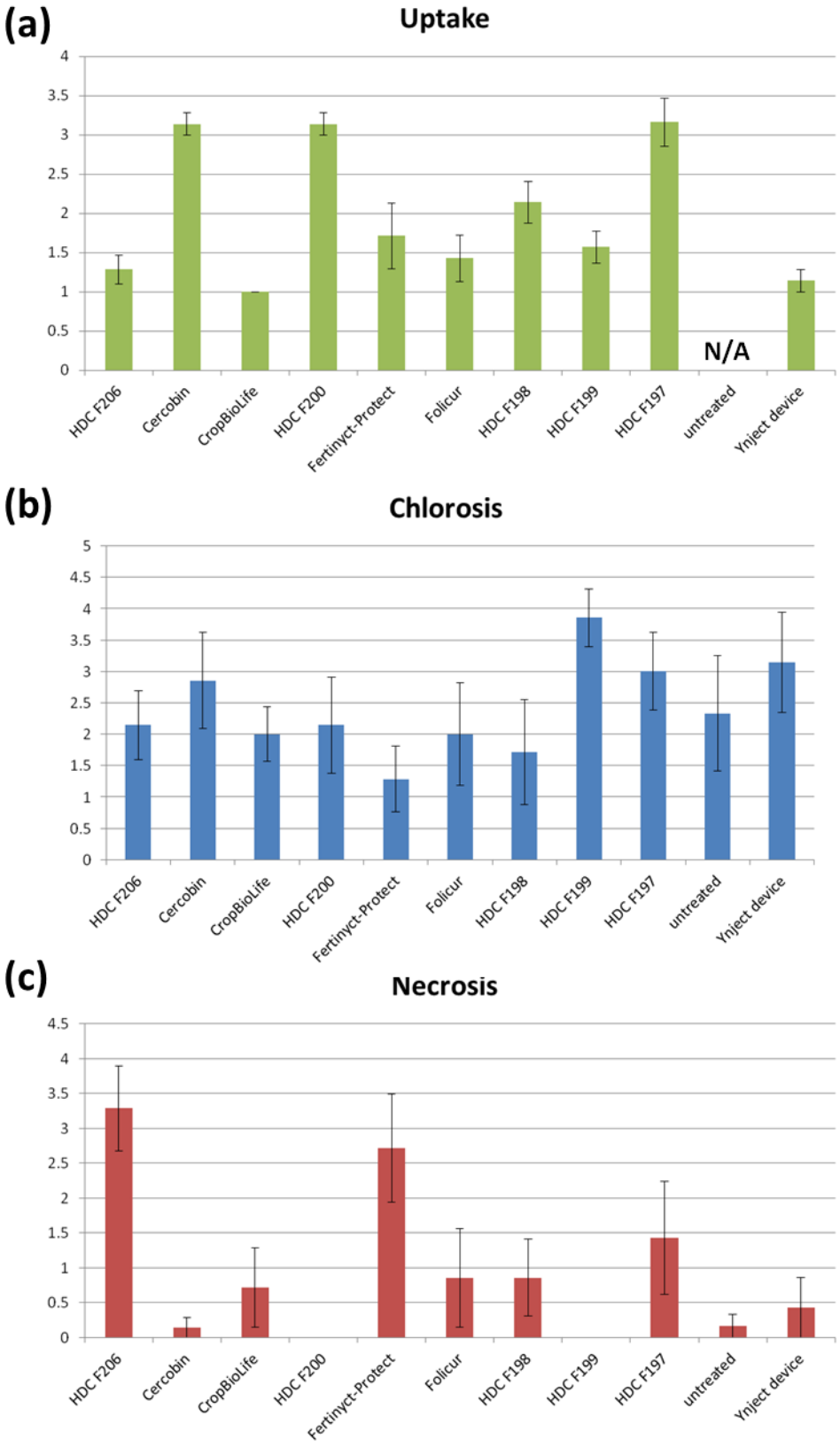


Figure 2.7. Treatment uptake and phytotoxicity assessments (chlorosis and necrosis) for tree injection Trial 1. All assessments are scored on a 1-5 scale as described in the materials and methods section. Error bars represent the standard error of the mean.

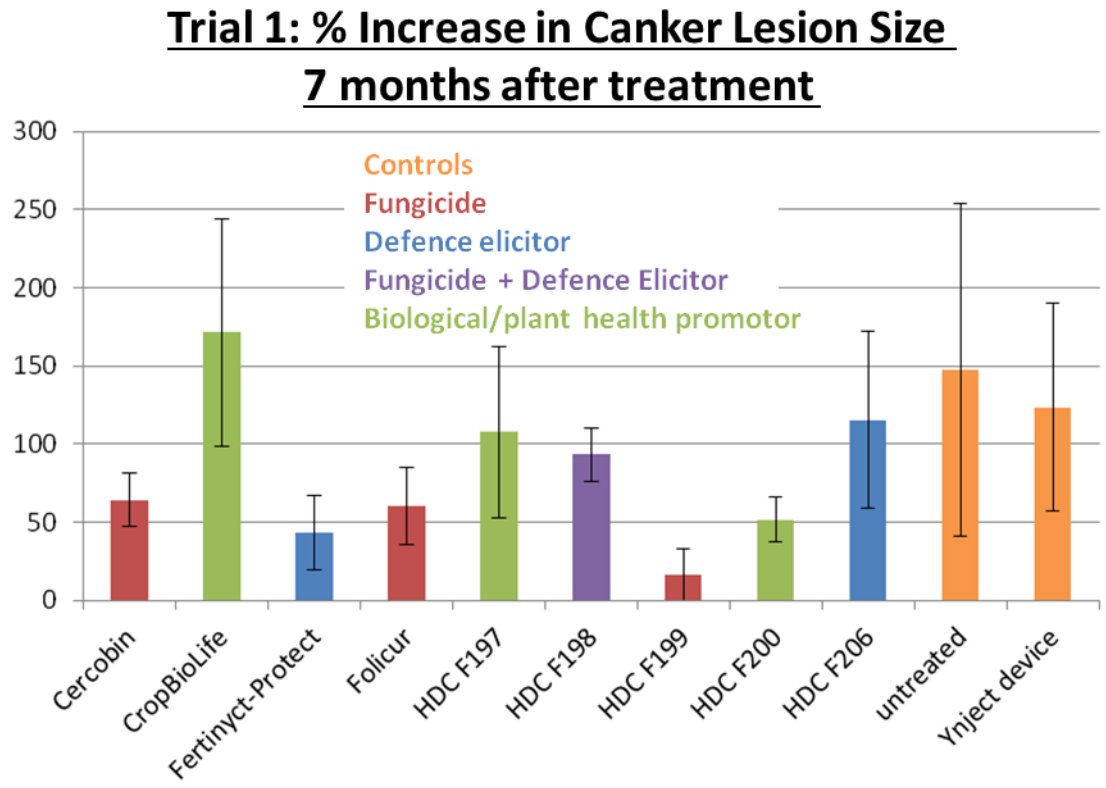


Figure 2.8. Percentage increase in the size of existing canker 7 months after treatment application in Trial 1. Treatments are colour coded according to their treatment category as described in Table 2.3.

Trial 2; Inoculated (new) canker formation

An assessment of the treatments ability to be taken into the tree was conducted. Treatment uptake (Fig. 2.9a) was very consistent with the previous trial with poor uptake (<25%) of Cercobin, HDCF200 and HDCF197, in addition uptake was poor for Fertinyct- Protect and HDC F198. Phytotoxic effects of the treatments were noted on the retardation of fruit development in this trial which was assessed on the 10/08/16. HDC F206 had a clear negative effect on fruit development, with on average over 60% of fruit affected relative to untreated control in which <5% of fruit were below average size (Fig. 2.9b and c).

The data for new cankers (Fig. 2.10) shows that cankers developed at all inoculation points, apart from the no Vaseline® control, demonstrating the importance of Vaseline® to create conditions conducive to disease development. Average new canker size varied from 9.3 to 18.4 cm^2 depending on treatment. No treatments significantly reduced new canker size relative to untreated control. Notably HDC F206 treated trees had significantly larger cankers develop at the inoculation point compared with the controls.

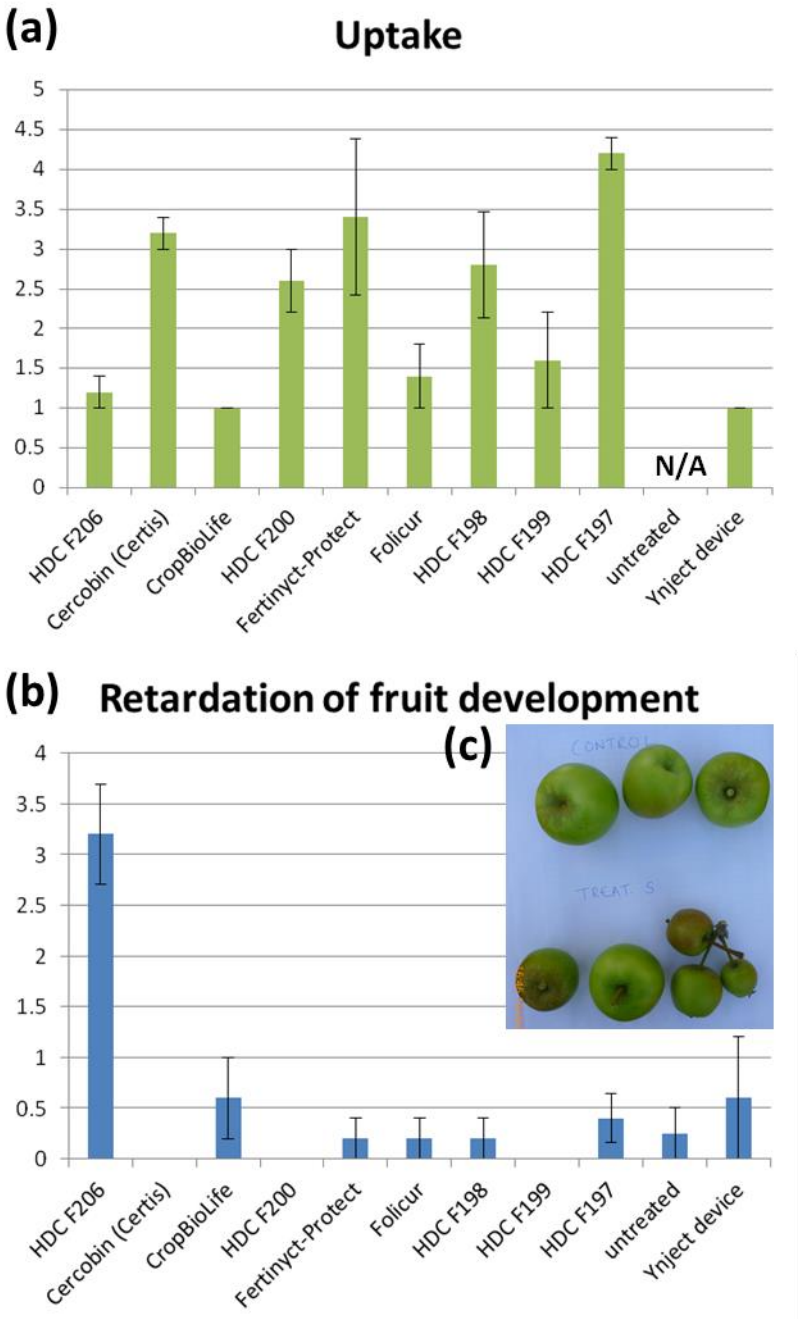


Figure 2.9. Treatment uptake and phytotoxicity assessments (retardation of fruit development) for tree injection Trial 2. All assessments are scored on a 1-5 scale as described in the materials and methods section. Error bars represent the standard error of the mean. Picture inset (c) shows representative fruit from untreated control (top) and HDC F206 (bottom).

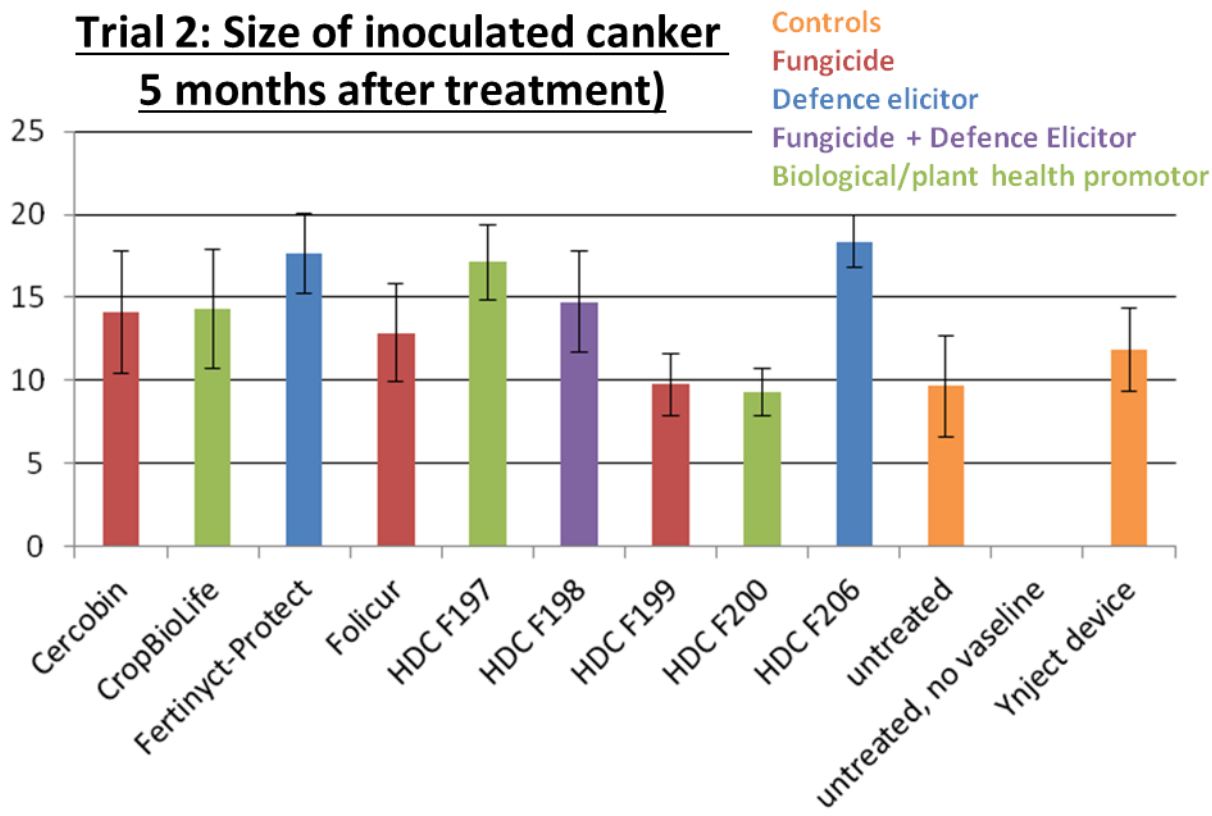


Figure 2.10. The size (cm^2) of inoculated cankers 5 months after treatment application in Trial 2. Treatments are colour coded according to their treatment category as described in Table 2.3.

Discussion

Measuring the growth of an existing canker following treatment (as in Trial 1) evaluated the curative properties of the treatments tested whilst measuring the formation of a new, artificially inoculated canker (as in Trial 2) evaluated the protective properties.

Although none of the results were statistically significant in Trial 1, due to the variability of this approach, trends were evident in the data with the potential to take treatments forward in future trials which use an improved method to evaluate efficacy. Apple cultivar Rubens was selected as it is known to be highly susceptible to canker, it may be better to select a variety which is slightly more tolerant of canker (e.g. cv. Gala) so that trees are not completely girdled during the course of the experiment. Artificial inoculations several months prior to treatment to produce uniform (age, position and size) cankers for treating will also improve the issue of variation.

None of the treatments in Trial 2 significantly reduced the size on the new, inoculated cankers. This may suggest that the treatments have not got protective effects or it may suggest that the effects of the treatment did not reach the inoculation point (which was 1.5 m above the

injection point and in the cambium (green tissue beneath the bark) layer) which would suggest that the treatment did not have systemic activity when delivered in this way. In practice, if shown to work this strategy is more likely to be used as a curative (treating existing or emerging cankers) rather than a protective (treating would-be-cankers) treatment however the former still requires treatments which have systemic activity to be effective.

There was a marked difference in the uptake of treatments (Fig 2.7a and 2.9a) which relates to their formulation (Table 2.3). In these trials the variation in uptake scores was consistent across both experiments. Generally the biological treatments (HDC F197 and 200) were not taken up effectively. The treatment which was taken up the least was HDC F197, a suspension concentrate, which is the least suitable formulation for injection devices (Table 2.4), whilst HDC F199 (also a suspension concentrate) was taken up as effectively as Folicur (oil in water emulsion) which are considered the most suitable. Formulation will be an important consideration for the future selection and development of promising treatments

Table 2.4. Table of formulations from the most suitable for injection devices (top) to the least suitable (bottom)

Decreasing suitability for injection devices

Formulation code	Formulation	Comments
EC	Emulsifiable concentrate	Easy dosing of device and homogeneous distribution. Higher the volume, higher the chance of separation.
SL	Soluble Concentrate	Must determine solubility of the active ingredient
WG	Water dispersible granule	Will have some solid drop out of solution but this can be taken up by the syringe when dosing. Must determine solubility of granule in water
SP	Soluble powder	Prepare mixture with water prior to dosing device. Must determine solubility of the powder in water
SC	Suspension concentrate	Possible but cannot guarantee the active is distributed throughout the plant

Pytotoxic effects were noted for certain treatments in both trails albeit manifesting in different forms. In Trial 1 necrosis was particularly evident on HDC F197 (a biological), Fertinyct protect and HDC F206 (both defence elicitors), whilst in Trial 2, HDC F206 retarded fruit development markedly. The rates of application were calculated based on multiplying the foliar rate recommended on the label by 10 as advised by the technical team at fertinyect. This is a general rule of thumb which may not be as effective in some treatment categories, such as defence elicitors, where the effects on the host are amplified.

Residues in the fruit and the effects on other diseases was not assessed this year due to a large treatment list and limited resource but it will be considered in future trials to inform how systemic the treatments are (and whether residue in the fruit should be a concern) and whether the treatments have additional benefits on disease control.

Conclusions

- An inexpensive and easy-to-use delivery system has been selected and successfully trialled
- Treatments have been identified which have curative effects
- Some formulation issues and phytotoxic effects of treatments observed
- Tests will continue in 2017 with the aim of reducing variability
- Researchers in New Zealand are also working on injection for canker control. Results will be exchanged between NZ and UK enabling protocol and treatment list improvements over 2 seasons per year

Objective 3	Foliar disease	Task 1	Overwinter innoculum

Aim

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

Introduction

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

Powdery mildew (*Podosphaera leucotricha*) mainly overwinters as mycelium in floral and vegetative buds. *Ampelomyces quisqualis* (AQ) is a mycoparasite of powdery mildew. Commercial preperations of AQ, such as AQ10 have been used in greenhouse and field-grown vegetable crops, usually with reduced fungicide inputs, to achieve disease control. AQ10 was one of the best performing BCAs in trials conducted as part of SCEPTRE when applied throughout the season and in combination with fungicides in a managed programme, however the control achieved was not commercially acceptable. One of the disadvantages of using AQ10 is the slow growth rate of this parasite. This has led to the strategy proposed here; to target the overwintering phase of the disease offering a long interaction period between parasite and powdery mildew. Trials were setup over the summer of 2016 to test whether the BCA is incorporated into the bud, whether the parasite can survive over winter and whether the strategy is effective at reducing inoculum. The trial will compare AQ10 treatment with a winter treatment of conventional pesticide + wetter and an untreated control. Assessments will take place in Spring and will be reported as an interim report when results are available.

Apple scab (*Venturia inaequalis*) overwinters in leaf litter. Leaf litter management is an important tool for the management of this disease. By disrupting the lifecycle, inoculum is reduced the following spring. The most widely practiced strategy for leaf litter management in integrated fruit production is autumn applications of urea. Previous studies have demonstrated urea has several modes of action; (1) Direct fungistatic effect of urea on perithecial development; (2) Increase abundance of microbial antagonists to *V. inaequalis*; (3) Accelerates leaf decomposition by (a) Increasing abundance and shift in microbial activity

and (b) Increasing palatability of leaf litter to earthworms. We have used next generation sequencing technology to determine the early effects of urea on the microbial communities in leaf litter.

Materials and Methods

Leaves were collected at leaf fall in Autumn 2015 from an orchard which receives low management inputs. Leaves were placed in net bags and half were treated by drenching in urea (5% w/v). Bags were pinned down to the orchard floor (Fig. 3.1) and sampled over the next 2 months (1, 2, 4 and 8 weeks following treatment). DNA was extracted from the microbial fraction of the leaf litter. Samples were processed through the metagenomic pipeline for ITS (eukaryote) and 16S (prokaryote) identification using the Illumina MiSeq next generation sequencing platform.

Figure 3.1. Net bags containing leaf litter pinned down on orchard floor (left). Leaf litter 16 weeks after experiment was setup from control (top right) and Urea treated (bottom right) samples.

Results and Discussion

Urea treatments had a clear visual effect (Fig. 3.1) on the breakdown of leaf litter during the first 16 weeks following treatment. We determined the main microbial groups responsible for the differences in the early stages of leaf decomposition in response to urea using a next generation sequencing approach. Figure 3.2 shows that the eukaryotic (which includes fungi) community is similar early on in leaf decomposition regardless of treatment but diverges over the sampling period. This may be a result of eukaryotic succession occurring over a longer time frame, compared to prokaryotes and this has not been captured in the sampling period of this study. The prokaryotic (which includes bacteria) community profile is initially quite distinct between treatments but converges over time. This result suggests that treatment

effects are short term with early colonisers of non-urea treated leaf litter potentially reducing the C-to-N ratio of plant material through the fixing/release of N thus mimicking the addition of urea. The initial treatment differences are caused by changes in the abundance of two groups (genus) of bacteria; *Pseudomonas* and *Hymenobacter* (Fig. 3.3, light blue and olive green respectively). *Pseudomonads* increase in abundance and species diversity in the early stages, returning to similar levels to untreated control samples within 8 weeks. *Hymenobacter* are more abundant in untreated controls in the initial stages of leaf decomposition. No major treatment effects were seen in fungal community profiles within the sampling period (data not shown). *Pseudomonads* are well recognised as general leaf decomposers and the application of next generation technologies provides a tool to measure their abundance and species diversity over time. *Hymenobacter*, more abundant in the untreated control in early samples have a lower nitrogen requirement compared to *Pseudomonads* and therefore fill the ecological niche in low nitrogen (non-urea amended leaf litter) environments.

These results are consistent with the culture dependent studies in the 1950's showing that the abundance and identity of prokaryotic microbes are influenced by the addition of urea in the early stages of leaf decomposition, in particular the pseudomonads (general leaf decomposers and antagonists). Succession of eukaryotic communities occurs over a longer time frame, interestingly very few recognised fungal antagonists of *V. inaequalis* were identified in this study but may be expected to increase in later stages of decomposition beyond the sampling period of this study. A repeat of this study is in progress over the winter of 2016/17 with the addition of a 16 week sample to capture longer term eukaryotic succession. A sister experiment is concurrently being carried out in Julius Kühn-Institut, Germany.

Conclusions

- Biological alternatives are being explored for overwinter use to reduce disease epidemics the following season and thus provide the best chance of success for an in season programme which includes alternatives to conventional pesticides (Obj. 3. 2)
- We are actively collaborating with European researchers at Julius Kühn-Institut, Germany (leaf degradation study) and as part of a H2020 project proposal (apple powdery mildew control using AQ10) in this area.

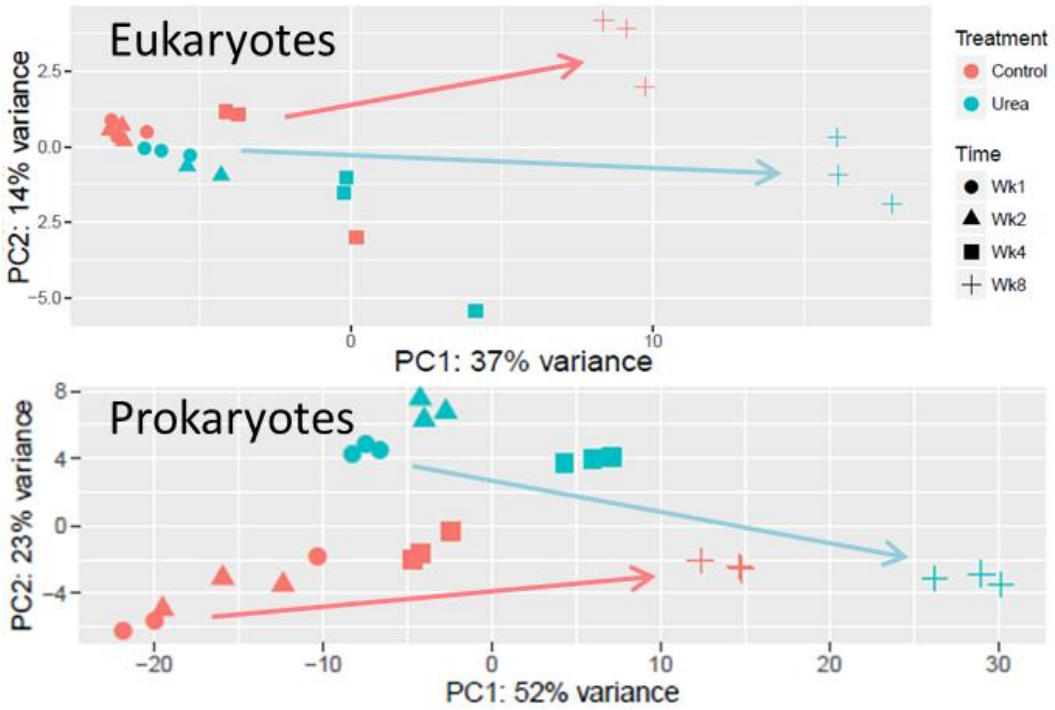


Figure 3.2. Principle component analysis plots of eukaryotic and prokaryotic community profiles over time in control (peach) and urea treated (turquoise) samples. The eukaryotic community is similar early on in leaf decomposition regardless of treatment but diverges over the sampling period. The prokaryotic community profile is initially quite distinct but converges over time.

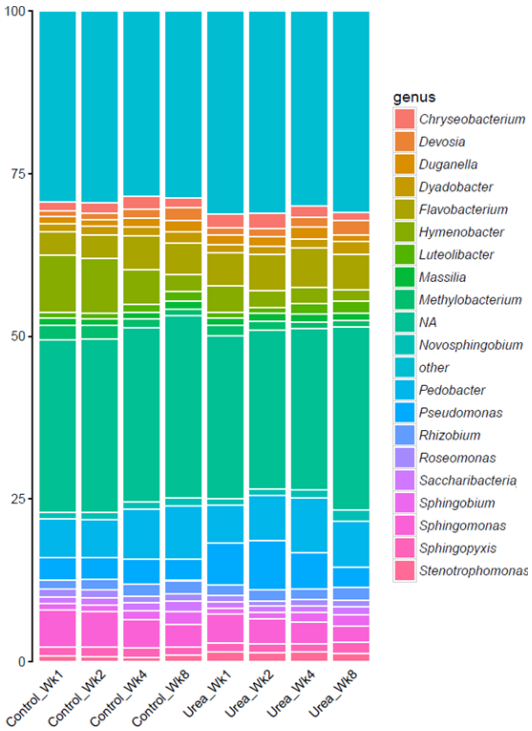


Figure 3.3. Changes in the prokaryotic communities (genus level) over time (x-axis) in control (first 4 columns) and Urea-treated (last 4 columns) samples.

Objective 3	Apple foliar diseases	Task 2	Alternative treatments

Aim

Evaluate efficacy and persistence of alternative chemical treatments to fungicides (NIAB EMR Year 2)

Introduction

Year 1 summary - Products which were evaluated included plant health invigorators, plant defence elicitors and products with a physical mode of action. The test products were evaluated in the field either as part of a reduced fungicide programme or alone.

During the 2015 growing season powdery mildew disease pressure was high, particularly in the trial orchards which have very high levels of primary mildew due to carry over from previous seasons. The high disease pressure provided a demanding test for the programmes. The full fungicide programme was the best performing but even with a 7-10 day programme, it was unable to keep the mildew epidemic below the 10% (commercial) threshold.

The test products alone did delay the epidemic relative to the untreated control but were unable to achieve commercially acceptable levels of control. Of the test products, SB invigorator was the best performing product. Programmes in which test products were combined with reduced fungicides, performed better than test products alone but this improvement in performance was probably attributable to the fungicides.

In order to ensure 2016 trials are more informative the trial design is going to be modified. The trial will be conducted on a split plot design with half of the replicate blocks receiving a 7 day mildew programme based on fungicides and the other half receiving a 14 day mildew programme based on fungicides, with the test treatments being superimposed on these blocks. This will provide two disease pressures ensuring test products are assessed under commercially relevant disease pressure whilst ensuring sufficient disease pressure.

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 a split plot with mildew incidence (high and low) as the main plots and the ten 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 ten test treatments designed as a randomised block with each treatment replicated six times overall (Fig. 3.4). The test treatments were on small 3 tree plots.

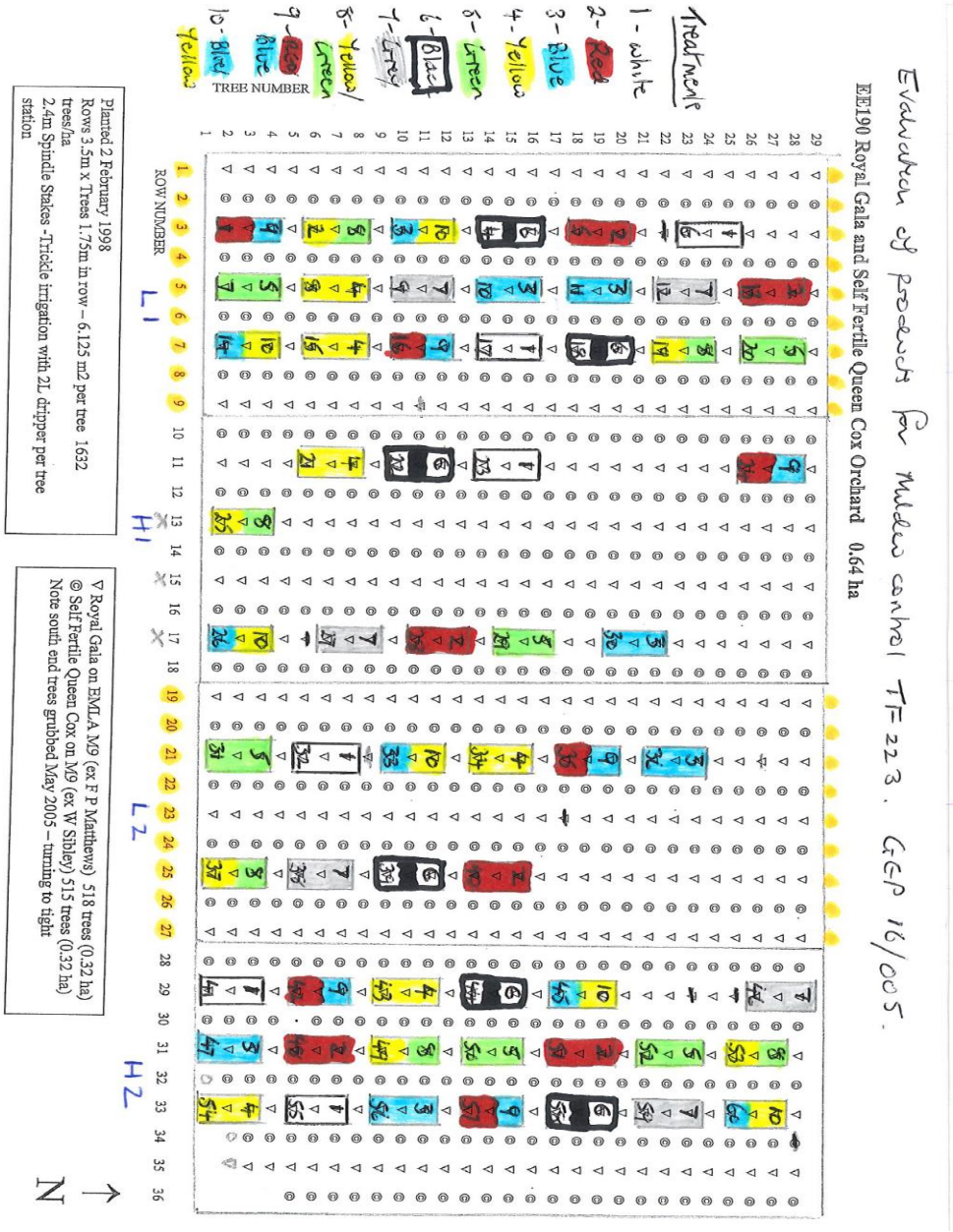


Figure 3.4. Trial plan showing main treatments L=low mildew incidence, 7 day fungicide programme and H=high mildew incidence, 14 day fungicide programme. Within each L or H block the sub treatments 1-10, are replicated one or two times, giving six replicates in total.

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 is labelled as untreated i.e. 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 11 applications (Table 3.3) apart from Treatment 2 – B204 – which was applied monthly (a total of 3 applications) and Treatment 7, which was applied 9 times, missing the last two applications. Treatments for pests and nutrients were applied to all plots as necessary after the start of the trial.

Table 3.1 Elicitor / plant strengthener products evaluated for effects on powdery mildew in apple 2016. All products were applied at 7 day intervals apart from B204 which was applied monthly

Treatment	Product	Active ingredient	Product type	Rate of product / ha	Use
1	Untreated	-	-	-	-
2	B204	flavonoids	Plant strengthener. Promising results in strawberry mildew trial. Also effects on fruit set and fruit quality	500 ml	Early flower then monthly
3	HDC F229	Didecyldimethylammonium chloride	Adjuvant	50 ml / 100 L water	7-10 day intervals
4	SB invigorator	Various nutrients and natural products	Plant stimulant. Controls various pests and mildew. Physical MoA	2ml/L**	7-10 days
5	Wetcit	Alcohol ethoxylate	Energiser adjuvant Physical MoA	0.4 %	7-10 days
6	Garshield	Garlic extracts	Plant stimulant with antimicrobial properties	1:100 dilution	7-14 days
7	Mantrac Pro	manganese	nutrient	0.5 L	5-6 applications from green cluster / pink bud
8	HDC F230	Silicon type product + minerals	Nutrient / elicitor	3 L	7-10 days
9	HDC F231	Natural plant oils + Silwett	elicitor	3 L +	7-10 day intervals
10	HDC F232	laminarin	elicitor	1 L	7-10 day intervals

** SB Invigorator was supplied in a double concentrate formulation for the 2016 trials but the rate used was the old one thus double the rate was unintentionally applied.

Table 3.2 Fungicides applied to Blocks L and H in EE190 during trial in 2016

Date applied	14 day interval blocks (H)		7 day interval blocks (L)	
	Product	**Rate / ha**	**Product**	**Rate / ha**
3 May	Kindred + Captan + Mainman	0.6 L + 2 kg + 0.14 kg	Kindred + Captan + Mainman	0.6 L + 2 kg + 0.14 kg
11 May			Systhane + Captan	330 ml + 2 kg
18 May	Systhane + Captan	330 ml + 2 kg	Systhane + Captan	330 ml + 2 kg
25 May	Calypso + Insegar	375 ml + 600 g	Nimrod + Calypso + Insegar	1.1 L + 375 ml + 600 g
1 June	Systhane + Captan	330 ml + 2 kg	Systhane + Captan	330 ml + 2 kg
9 June			Nimrod	1.1 L
15 June	Cosine + Captan	0.5 L + 2 kg	Cosine + Captan	0.5 L + 2 kg
21 June			Nimrod	1.1 L
30 June	Systhane + Captan	330 ml + 2 kg	Systhane + Captan	330 ml + 2 kg
6 July	Steward	250 g	Nimrod Steward	1.1 L 250 g
14 July	Cosine + Captan	0.5 L + 2 kg	Cosine + Captan	0.5 L + 2 kg
21 July	Nimrod + Coragen	1.1 L + 175 ml	Nimrod + Coragen	1.1 L + 175 ml

Treatment application

Sprays were applied to the 3 tree plots for treatments 1-10 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.

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 3.3 Date and growth stage when Treatments 2-10 were applied to sub plots in each block in 2016

Spray number	BBCH growth stage	Date treatment applied	Spray interval Days / B204 application date
1	59/60 Early flower	9 May	B204
2	65 Full bloom	20 May	11
3	69 + Petal fall	27 May	7
4	71 Fruitlet	3 June	7
5	32 Shoots about 20% final length	10 June	7 B204
6	33 Shoots up to 30 % final length	21 June	11
7	34 Shoots 40% final length	28 June	7
8	35/36 Shoots 50% final length	8 July	10
9	37 Shoots 70% final length	15 July	7 B204
10	38 Shoots 80% final length	25 July	10
11	39 Shoots 90% + of final length or stopped	1 August	7

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 3.4). In addition initial and final fruit set and fruit drop were recorded. Two branches were marked on the central tree in each sub plot. Total number of flowers were

recorded in blossom (24 May), number of fruitlets were recorded in June (22 June) and number of apples recorded in August (8 August).

Table 3.4 Foliage chlorosis/necrosis phytotoxicity scale, Source; EPPO Guideline PP 1/135(3)

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 middle tree of each plot. Secondary mildew was recorded weekly on 5 shoots per tree from 25 May-28 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.

Yield

All fruit were harvested on 14 September from the middle tree in each plot and the weight (kg) recorded.

Fruit quality

At harvest (14 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 (3).

Statistical analysis

Data was analysed by ANOVA as a split plot with high and low mildew as the main plots and the ten 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 in bold are significantly different from fungicide only control plots.

Table 3.5 Summary of treatment and assessment timings – NIAB EMR 2016

Activity	**Date**
Trial orchard EE190 at EMR. Alternate rows of Cox and Royal Gala. Trial on cv. Royal Gala marked out	27 April
Main block fungicide programme started. Sprays then applied at 7 or 14 day intervals	3 May
First treatments applied to sub plots at early flower	9 May
Phytotoxicity check. None seen	19 May
Second spray applied	20 May
Blossom count	24 May
First assessment	25 May
Third spray applied	27 May
Second assessment	2 June
Fourth spray applied	3 June
Third assessment	9 June
Fifth spray applied	10 June
Fourth assessment	15 June
Sixth spray applied	21 June
First fruit count	22 June
Fifth assessment	23 June
Seventh spray applied	28 June
Sixth assessment	30 June
Seventh assessment	7 July
Eighth spray applied	8 July
Eighth assessment	14 July
Ninth spray applied	15 July
Ninth assessment	21 July
Tenth spray applied	25 July
Tenth assessment	28 July
Eleventh spray applied	1 August
Final fruit count	8 August
Phytotoxicity assessment	11 August
Harvest	14 September
Fruit quality assessments	11-26 October

Results

Phytotoxicity

No phytotoxicity was noted at the first assessment in May. Most trees showed a low incidence of necrotic spotting on leaves on the extension growth, thought to be due to a fungal leaf spot, which was particularly prevalent in 2016. However, as the sprays were progressively applied excessive necrotic spotting was noted on the leaves of trees receiving Treatments 5, 8, 9 and 10. Some leaf drop was also recorded particularly on Treatments 8 and 9 (Table 3.6). There was no significant interaction between phytotoxicity parameters (necrosis, leaf drop, fruit set and fruit drop) and the main plot effects of low and high mildew incidence. Therefore the data presented in Table 3.6 is the overall mean of six replicates.

Table 3.6 Mean % initial fruit set, final fruit set and fruit drop (angular transformed) and phytotoxicity as leaf necrosis and leaf fall scores recorded on apple cv. Gala following eleven sprays of various products, applied in addition to a fungicide programme applied at 7 or 14 day intervals at NIAB EMR in 2016. Figures in brackets are back transformed means. Figures in bold are significantly different from untreated.

Treatment	Product	Mean leaf necrosis score	Mean leaf fall score	% Initial fruit set	% Final fruit set	% Fruit drop
1	Untreated	0.7	0.2	42.8 (46.2)	37.8 (37.6)	23.1 (15.4)
2	B204	0.2	0	44.8 (49.7)	39.2 (40.0)	24.3 (17.0)
3	HDC F229	0.5	0	43.0 (46.5)	36.8 (36.0)	28.3 (22.4)
4	SB invigorator	0.8	0	40.1 (41.5)	33.5 (30.4)	30.6 (25.9)
5	Wetcit	**2.4**	0	**35.6 (33.9)**	**31.3 (27.0)**	26.4 (19.8)
6	Garshield	0.5	0	48.2 (55.5)	42.8 (46.2)	23.0 (15.3)
7	Mantrac Pro	0.3	0	44.0 (48.3)	38.2 (38.3)	26.3 (19.6)
8	HDC F230	**2.9**	**0.7**	40.2 (41.7)	36.2 (34.9)	21.9 (13.9)
9	HDC F231	**3.2**	**1.2**	41.3 (43.6)	34.3 (31.8)	28.5 (22.8)
10	HDC F232	**1.7**	0.3	43.4 (47.2)	37.7 (37.5)	21.8 (13.8)
F Prob		<0.001	<0.001	0.021	0.026	0.803
SED (36)		0.262	0.217	2.936	2.903	5.663
LSD (p=0.05)		0.532	0.439	5.955	5.887	11.485

Leaf necrosis and leaf fall were recorded on a score of 0-5 See Materials and Methods.

Figure 3.5 Fungicide only Healthy leaves

Figure 3.6 Treatment 5 Leaf necrotic spotting

Figure 3.7 Treatment 8 Leaf necrotic spotting

Figure 3.8 Treatment 9 Leaf necrotic spotting

Figure 3.9 Treatment 10 Leaf necrotic spotting

Treatments 5, 8, 9 and 10 all had significantly more necrotic spotting on leaves than the fungicide controls (Treatment 1). In addition, Treatments 8 and 9 also resulted in significantly greater leaf drop. There was no leaf drop recorded for most treatments. The leaf drop recorded for Treatment 1 was probably associated with the higher incidence of mildew.

Most treatments had no significant effect on fruit set or fruit drop apart from trees treated with Treatment 5 which set significantly less fruit.

Disease – Powdery mildew

The incidence of primary blossom and vegetative mildew in the orchard was high, but 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 3.10 and 3.11). An incidence of more than 60% mildewed leaves was recorded in the high mildew blocks at the first assessment rising to nearly 100% secondary mildew in June before dropping to < 40% secondary mildew at the final assessment. By contrast in the low mildew blocks, mildew incidence in May was around 30% mildewed leaves falling to around 10 % 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 3.7 is the overall mean of six replicates. Treatment 4 SB Invigorator was the most consistently effective product significantly reducing mildew incidence compared to the fungicide only control at all assessment dates. Treatment 5 Wetcit and Treatment 8 HDC F230 were almost as effective. Treatment 10 HDC F232 appeared to be ineffective for mildew control. Treatment 2 B204, did not show any significant reductions in mildew incidence until the last 3 assessments after the third application had been made. The overall mean for the ten sub treatments for repeated measures analysis is given in Table 3.8.

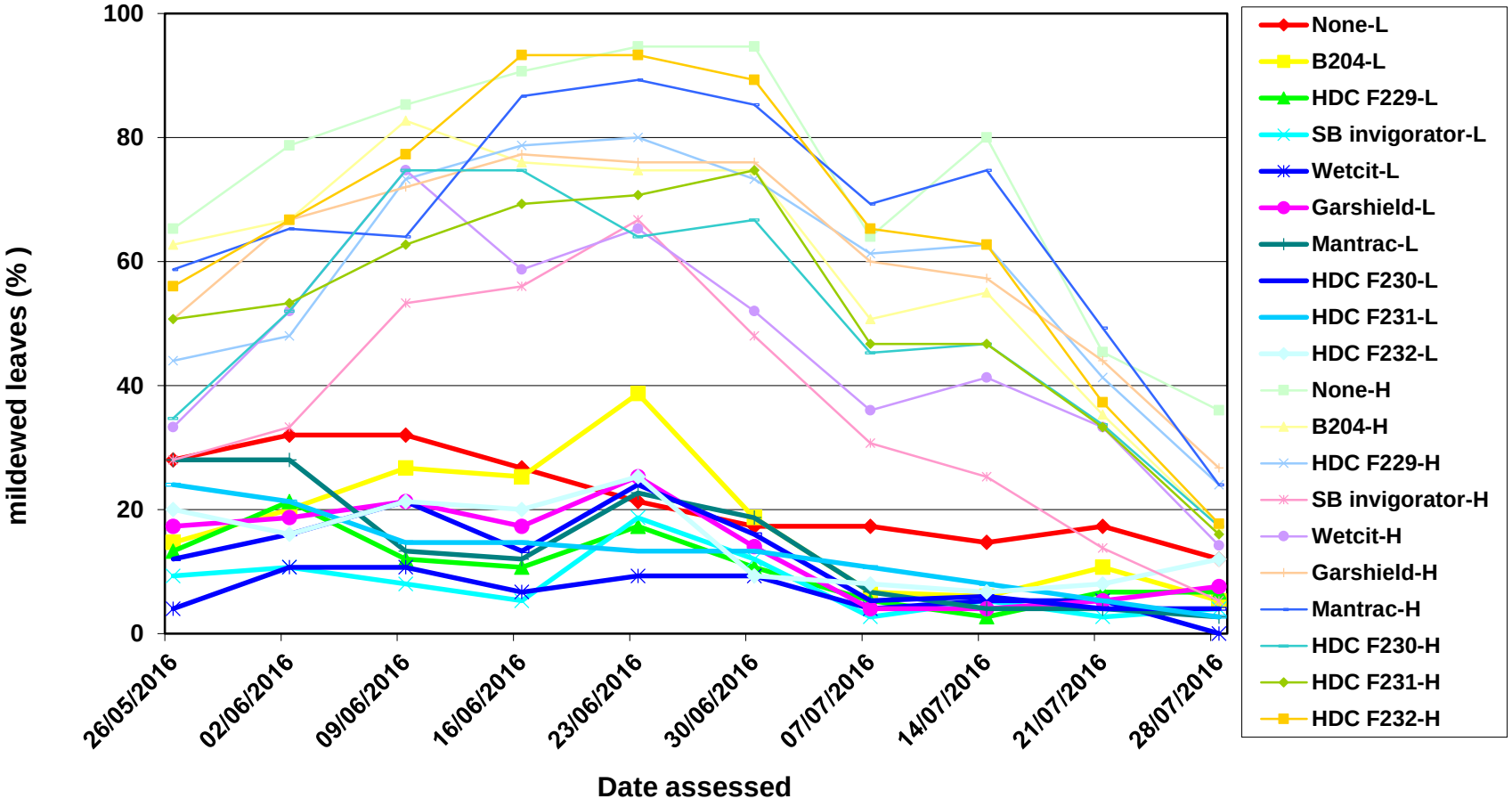


Figure 3.10. Mean % mildewed leaves on apple shoots cv. Gala assessed at various times following treatment with 11 sprays of various products applied in addition to a fungicide programme applied at 7 (L) or 14 (H) day intervals at NIAB EMR in 2016.

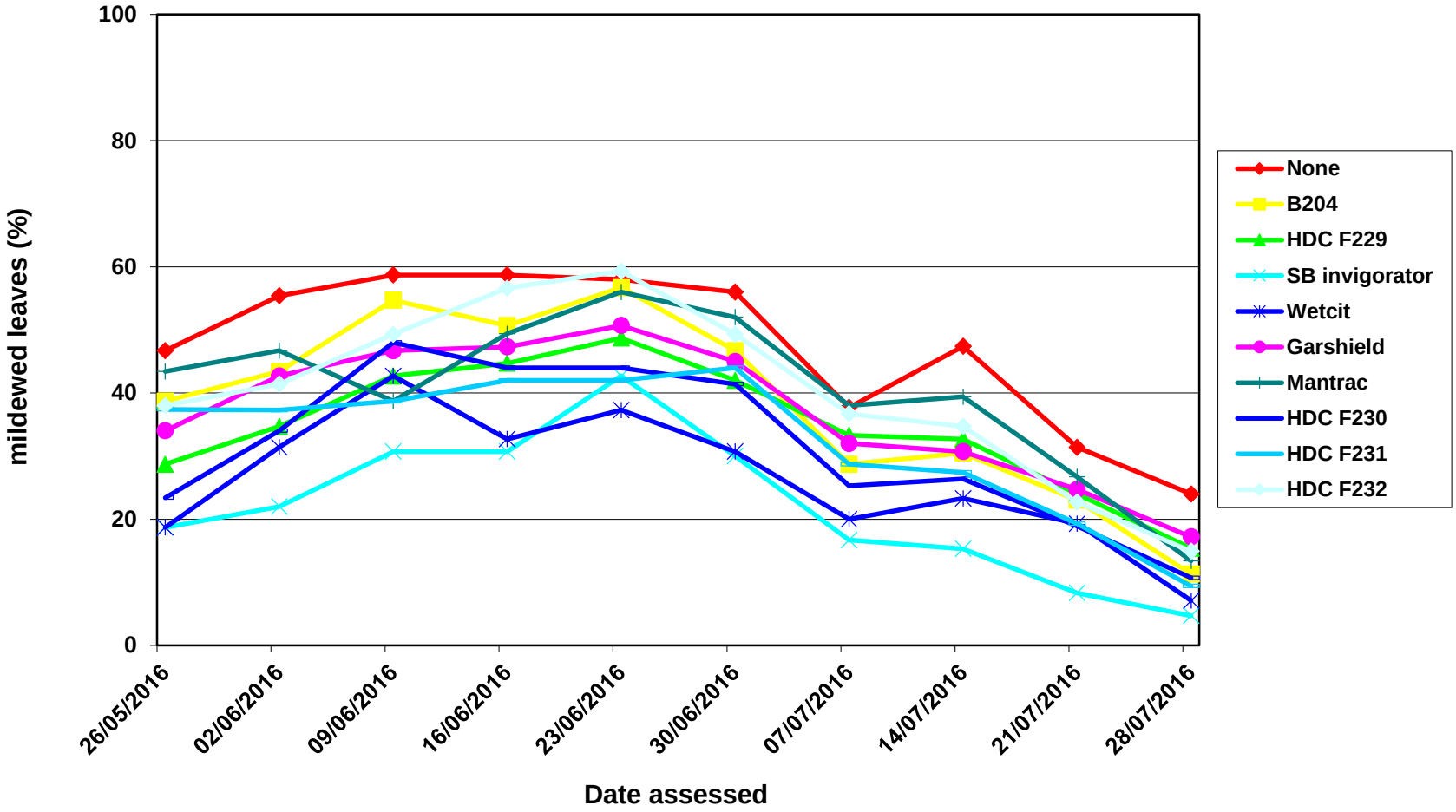


Figure 3.11. Data from Fig 6 presented as the mean of high (14 day fungicide programme) and low (7 day fungicide programme) mildew blocks.

Table 3.7. Mean % mildewed leaves (angular transformed) on apple cv. Gala following eleven sprays of various products, applied in addition to a fungicide programme applied at 7 or 14 day intervals at NIAB EMR in 2016. Figures in brackets are back transformed data.

Treatment	Product	Date assessed / % mildewed leaves									
		25 May	**2 June**	**9 June**	**15 June**	**23 June**	**30 June**	**7 July**	**14 July**	**21 July**	**28 July**
1	Untreated	42.7 (46.0)	48.6 (56.3)	50.4 (59.4)	53.3 (64.3)	52.1 (62.3)	50.4 (59.4)	38.4 (38.6)	43.5 (47.4)	32.8 (29.4)	28.5 (22.7)
2	B204	37.2 (36.5)	50.6 (42.3)	48.3 (55.8)	45.6 (51.0)	49.2 (57.3)	42.8 (46.2)	30.1 (25.1)	**30.6 (26.0)**	27.4 (21.1)	**17.7 (9.3)**
3	HDC F229	**31.5 (27.2)**	**35.7 (34.0)**	**39.6 (40.6)**	**40.5 (42.2)**	**44.4 (49.0)**	**39.0 (39.5)**	31.2 (26.8)	**30.2 (25.3)**	27.3 (21.0)	20.6 (12.4)
4	SB invigorator	**22.9 (15.2)**	**25.4 (18.4)**	**31.2 (26.9)**	**29.8 (24.7)**	**39.8 (40.9)**	**30.3 (25.4)**	**20.6 (12.4)**	**20.6 (12.4)**	**14.6 (6.3)**	**10.1 (3.1)**
5	Wetcit	**22.3 (14.4)**	**32.5 (28.9)**	**39.4 (40.2)**	**30.9 (26.4)**	**34.6 (32.2)**	**30.6 (25.8)**	**22.9 (15.2)**	**26.6 (20.0)**	22.8 (15.0)	**10.9 (3.6)**
6	Garshield	**32.6 (29.0)**	**39.4 (40.3)**	42.8 (46.1)	45.6 (51.1)	45.8 (51.4)	**40.5 (42.2)**	30.1 (25.2)	**29.6 (24.3)**	27.3 (21.0)	23.1 (15.4)
7	Mantrac Pro	40.9 (42.8)	43.0 (46.5)	**37.4 (36.9)**	44.6 (49.3)	49.9 (58.5)	46.2 (52.1)	36.0 (34.6)	37.0 (36.2)	27.0 (20.6)	**16.9 (8.5)**
8	HDC F230	**28.0 (22.1)**	**34.6 (32.3)**	43.6 (47.6)	**41.2 (43.4)**	**41.3 (43.5)**	**39.2 (40.0)**	**27.7 (21.7)**	**29.5 (24.2)**	**21.4 (13.4)**	**16.9 (8.4)**
9	HDC F231	37.3 (36.7)	**37.2 (36.6)**	**37.4 (36.9)**	**39.1 (39.9)**	**39.1 (39.7)**	**40.2 (41.7)**	30.9 (26.4)	**28.1 (22.2)**	24.2 (16.8)	**14.0 (5.8)**
10	HDC F232	37.2 (36.5)	**39.0 (39.5)**	44.6 (49.3)	52.2 (62.4)	54.0 (65.4)	44.5 (49.1)	33.8 (30.9)	**32.0 (28.1)**	26.3 (19.7)	**19.1 (10.7)**
F Prob		<0.001	<0.001	0.005	0.001	<0.001	0.002	0.006	0.013	0.091	0.004
SED (36)		3.809	3.596	4.412	5.331	3.657	4.669	4.272	5.124	5.013	4.229
LSD (p=0.05)		7.724	7.292	8.948	10.812	7.418	9.468	8.664	10.392	10.166	8.585

Figures in bold are significantly different from untreated

Table 3.8. Mean (overall mean of 10 assessments) % mildewed leaves on apple cv. Gala following eleven sprays of various products, applied in addition to a fungicide programme applied at 7 or 14 day intervals at NIAB EMR in 2016.

Treatment	Product	Overall mean
1	Untreated	44.1
2	B204	37.0
3	HDC F229	34.0
4	SB invigorator	24.5
5	Wetcit	27.3
6	Garshield	35.7
7	Mantrac Pro	37.9
8	HDC F230	32.3
9	HDC F231	32.8
10	HDC F232	38.3
F Prob		<0.001
SED (36)		2.260
LSD (p=0.05)		4.584

Yield

Yield data for high and low mildew blocks and the overall mean for the 10 sub treatments is presented in Table 9. There were no significant effects of treatments on plot yield, although the lowest yields were recorded in Treatments 4 and 9.

Fruit quality

Fruit quality data - fruit russet, fruit colour and fruit size are presented in Table 3.10. 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 3.6 is the overall mean of six replicates. There were no significant effects of treatments on fruit size or colour. Treatment of trees with Treatment 8 (HDC F230) resulted in russeted fruit compared to the fungicide only plots and all other treated plots.

Table 3.9. Effects of treatments on yield of apple fruits cv. Gala recorded following eleven sprays of various products, applied in addition to a fungicide programme applied at 7 or 14 day intervals at NIAB EMR in 2016.

Treatment	Product	Yield per plot kg Low mildew	Yield per plot kg High mildew	Yield per plot kg Mean
1	Untreated	34.5	24.0	29.3
2	B204	34.9	24.9	29.9
3	HDC F229	27.4	31.6	29.5
4	SB invigorator	20.1	22.5	21.3
5	Wetcit	26.5	23.3	24.9
6	Garshield	33.7	24.9	29.3
7	Mantrac Pro	32.0	26.7	29.3
8	HDC F230	30.0	27.7	28.9
9	HDC F231	21.5	20.8	21.2
10	HDC F232	29.2	26.7	27.9
F Prob				0.107
SED ()				3.640
LSD (p=0.05)				7.382

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 4, SB Invigorator, was the most consistent in reducing mildew, confirming results from 2015. Although not significant, this product also resulted in the lowest yield which may require further investigation. Treatment 5 Wetcit and Treatment 8 HDC F230 were next most consistent products. However, both these products caused necrotic spotting on leaves. HDC F230 also

resulted in russeted fruit and Wetcit reduced fruit set. Wetcit in this trial was used at a higher rate than in 2015 when no phytotoxicity was recorded. HDC F232, Mantrac Pro and CropBiolife were least effective. B204 appeared to have little effect on mildew incidence at the start of the trial but by the time the third application was made B204 treated plots had a significantly lower mildew incidence than the fungicide only plots. It is therefore possible that the effects are cumulative over the season. There is also some suggestion that the effects of B204 are cumulative over several seasons and that treatments in successive trials should have been applied to the same plots. This will be considered for trials in 2017.

Conclusions

- 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
- Treatment 4 SB Invigorator was the most consistent in reducing mildew
- Treatment 5 Wetcit and Treatment 8 HDC F230 were next most consistent products
- HDC F232, Mantrac Pro and B204 were least effective
- B204 appeared to have little effect on mildew incidence at the start of the trial but by the time the third application was made B204 treated plots had a significantly lower mildew incidence than the fungicide only plots
- No significant effect of treatments on yield, but lowest yield recorded in plots treated with Treatment 4 SB Invigorator and Treatment 9 HDC F231
- Phytotoxicity was recorded on Treatments 5 (Wetcit), 8 (HDC F230), 9 (HDC F231) and 10 (HDC F232) as necrotic spotting on leaves. Wetcit also significantly reduced fruit set. HDC F230 and HDC F231 also caused some premature leaf drop. HDC F230 also increased fruit russet
- No significant effect of treatments on fruit size or fruit colour

Table 3.10. 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 eleven sprays of various products, applied in addition to a fungicide programme applied at 7 or 14 day intervals at NIAB EMR in 2016. Figures in parenthesis are back-transformed means

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	Untreated	59.8	215.7	9.2	3.2 (10.1)	1.8
2	B204	76.3	251.2	8.8	2.6 (6.7)	1.1
3	HDC F229	71.7	239.5	7.9	1.3 (1.6)	0.4
4	SB invigorator	72.2	249.4	8.1	1.5 (2.2)	0.5
5	Wetcit	74.7	234.5	8.8	2.7 (7.4)	1.3
6	Garshield	68.0	241.9	8.3	1.8 (3.2)	0.5
7	Mantrac Pro	79.0	232.5	8.2	1.4 (2.0)	0.4
8	HDC F230	**109.8**	269.4	8.7	2.0 (3.9)	0.6
9	HDC F231	73.0	235.5	7.9	1.3 (1.8)	0.5
10	HDC F232	78.0	247.8	9.0	2.5 (6.3)	0.9
F Prob		0.009		0.436	0.242	0.136
SED (36)		10.660		0.650	0.821	0.488
LSD (p=0.05)		21.619		1.319	1.665	0.990

Figures in bold are significantly different from untreated

Objective 3	Apple foliar diseases	Task 3	Alternative treatments (protected)

Aim

To conduct controlled environment studies to determine efficacy, persistence and systemic nature of alternative products

Summary

A poly tunnel trial was conducted using MM106 (scab susceptible) rootstocks. This trial was inoculated with scab. The trees were sprayed to run-off with the treatments; Reysa (Elicitor A), Proact (Elicitor B), Systhane (positive control) and water (negative control). The rootstocks were treated 7 days or 3 days prior to inoculation. Field inoculum (collected from naturally infected leaves) of *V. inaequalis* was prepared ($1x10^5$ spores/ml) and 2ml was applied to the youngest leaves of actively extending shoots. The inoculated shoots were covered for 24 hours with bags to maintain humid conditions for the fungus to germinate and infect the leaves. An assessment was conducted once sporulating scab lesions became evident (27 days post inoculation). The number of sporulating lesions on the upper and lower surfaces of the 3 youngest leaves at the time of inoculation was scored for each tree.

Overall the fewest lesions were visible on Systhane-treated plants (Fig. 3.12). Systhane had the greatest systemic activity and persistence as the treatment had similar efficacy whether applied 7 or 3 days prior to inoculation and the number of lesions was reduced, relative to water control, on leaves that were yet to emerge at the time of treatment. Both elicitor treatments had similar efficacy as water after 7 days. Elicitor A reduced the number of lesions significantly compared to untreated rootstocks at 3 days, suggesting localised/short term effects are provided by this treatment. Elicitor B reduced lesions further after multiple applications (results not shown), suggesting the effects of this treatment accrue over time. These findings will be important to consider when advising growers on spray programmes.

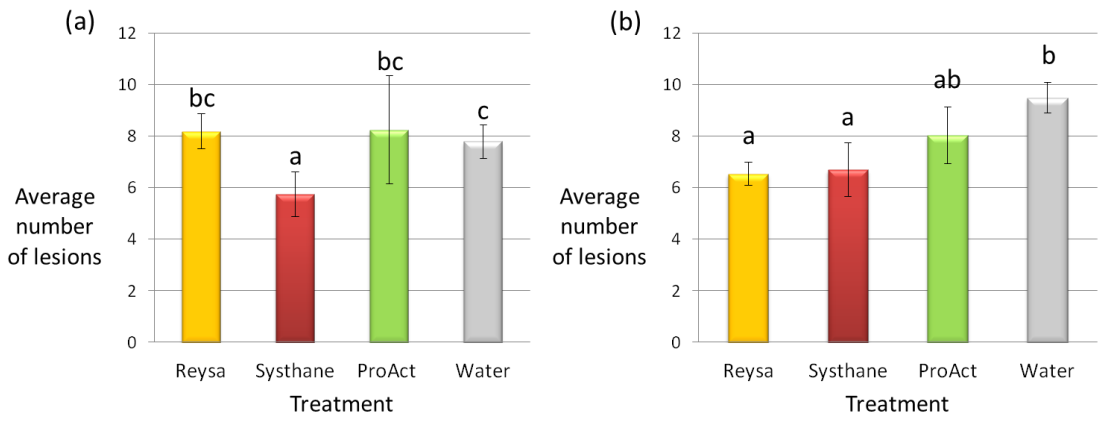


Figure 3.12. Average number of lesions on rootstocks treated either 7 (a) or 3 (b) days before inoculation.

The persistence (number of days the treatment had an effect following application) and systemic nature (ability to have an effect in the new leaves which were not exposed to the treatment can be determined. Systhane is both persistant (over 7 days) and systemic, Reysa has short term effects (3 days) but does have systemic activity. Proact, in this system at least, does not seem to show persistence or systemic effects.

Objective 4	Stone Fruit Diseases	Task 3	Bacterial Canker

Summary

With the withdrawal of copper for biocidal use confirmed in 2016 treatment options for bacterial canker control in prunus are no longer available causing significant concern for stone fruit growers. Phage therapy, using bacteria-killing viruses to prevent or cure an infection, may offer potential in the future as a targeted, non-toxic biocontrol agent. To explore this possibility a preliminary study was undertaken over the 2016 summer vacation by Billy Quilty (kindly supported by the British Society of Plant Pathologists (BSPP) undergraduate vacation bursary scheme). The project title was "Collection and characterization of native bacteriophage; a potential novel biocontrol agent to treat bacterial canker of *Prunus*".

Bacteriophage are one of the most abundant entities on the planet, and as such phage specific to the target host can be readily isolated wherever the host bacteria (in this case *Pseudomonas syringae syringae* and *Pseudomonas syringae morsprunorum)* can be found. Soil and leaf samples were collected from prunus orchards around Kent, processed to collect any phage that may be present and plated on to Petri dishes containing a lawn of *P. syringae*, known as a 'double-agar plaque assay'. The presence of phage in the sample results in circular clearings in the agar called plaques (Fig. 4.1a). Phage morphology (Fig. 4.1b) was determined using transmission electron microscopy (TEM) following isolation and purification. In total 20 diferent phage morphologies were collected and these have been put into storage for future characterisation.

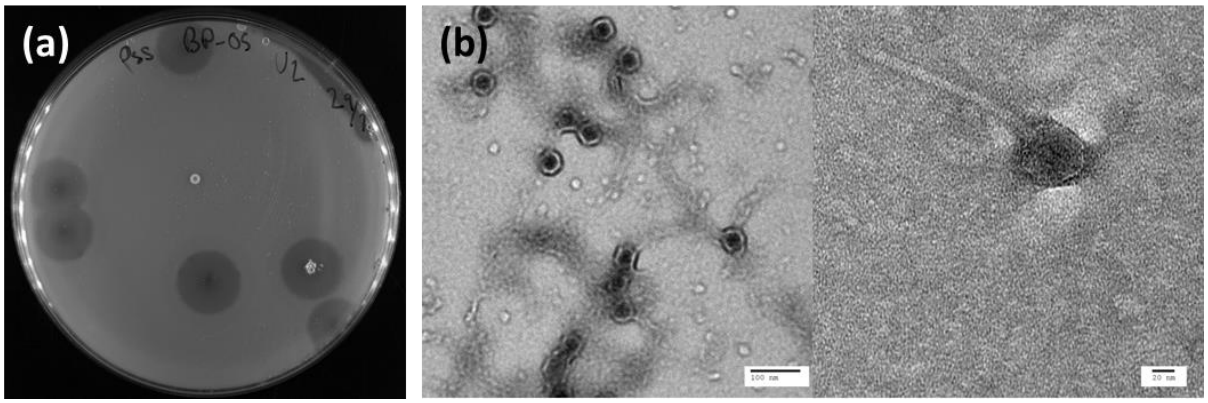


Figure 4.1. Preliminary study to collect and characterise bacteriophage. (a) a double-agar plaque assay where plaques (circular clearings) in the lawn of *Pseudomonas syringae* signify bacteriophage presence. (b) Transmission electron microscopy (TEM) of some of the isolated bacteriophage.

Objective 6	Codling and tortrix moth	Task 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)

Introduction

UK growers rely on programmes of sprays of pesticides to control codling and tortrix moths. This is effective but relies on programmes of multiple applications of insecticides at 2-3 week intervals from June to September, which can be costly and result in fruit residues. The numbers of sprays required appears to be increasing, possibly due to climate change, providing an increasingly favourable environment for the pest. The problems with this chemical approach are: (1) populations are not being reduced to such low levels that spraying is reduced in subsequent years; (2) intensive spraying of pesticides has adverse effects on natural enemies in the crop; (3) there is a risk of pest resistance developing (as has occurred in southern and central Europe already); (4) residues occur at harvest. Sex pheromone mating disruption is now used to successfully control codling and tortrix moths in most other countries in Europe. Currently this method is not adopted in UK horticulture as no suitable products are approved but this is likely to change in the near future as BASF have gained approval for use in the UK from 2017. Furthermore, entomopathogenic nematodes and granulosis virus products are available for both codling moth and summer fruit tortrix moth

and if used in conjunction with sex pheromone mating disruption could lead to long term population suppression. There is a need for the UK industry to move away from dependence on pesticides by adopting these practices in preparation for future pesticide withdrawals. We hypothesise that the combined use of these alternative methods of codling and tortrix moth control could not only decrease codling and tortrix moth populations, leading to long term population suppression, but boost natural enemy populations in orchards reducing the need to control of other pests.

Year 2 aims:

- Demonstration of the efficacy of sex pheromone mating disruption including effects on pest and natural enemy populations.
- Demonstration of the efficacy of nematodes on infesting codling moth larvae.
- Economic benefits of this approach will be compared to standard spray programmes.

Materials and methods

Farms, Orchards and Site Managers

The two trial farms from Year 1 were used in Year 2 and an additional third farm was also included due to complications through the year at Site 2. Below are the details of the three farms and multiple orchards involved in the trial and a map of the trial sites with the location of the orchards used (Tables 6.1, 6.2, 6.3, Figures 6.1, 6.2, 6.3).

At Site 1 there was 6.0 ha of conventionally treated orchard and 8.6 ha of mating disruption (MD) treated orchard. Three rows of Broughton Meadow orchard at this site were used as an untreated control.

At Site 2 there were 6.0 ha of conventionally treated orchard and 6.8 ha of MD treated orchard; 0.27 ha of ‘Oak’ orchard at this site were used as an untreated control. It should be noted that two of the orchards used in 2015 had been grubbed during the winter and were replaced by alternating rows of cultivars Rosette and Spartan (Harwort – Orchards 8 and 9).

At Site 3, 8.5 ha of orchard was treated conventionally and 10.3 ha MD treated. The orchards assessed at this site were a mix of apple and pear. In contrast to Year 1 the plots with additional granulosis virus and nematodes were removed because of the grubbed orchards

and some pest problems. In addition Certis could not supply codling moth granulosis virus at the beginning of 2016. Instead Capex for summer fruit tortrix was applied where needed as part of the MD integrated programme. Nematodes were tested separately, with additional funding from BASF, for proof of concept that they could infect codling moth larvae. Hence the main treatments at all sites were either Conventional Spray Programme or Mating Disruption (MD).

On 12 May a conference call was held with the steering group (Rachel Lockley, Scott Raffle, Nigel Kitney, Michelle Fountain, Chris Nicolson, Nigel Jenner and Tom Hulme) because Site 2 had been over-sprayed with chlorpyrifos on 8 March. Following this meeting and the summer steering meeting on 21 July it was decided that no more assessments were to be done at this site. In addition to the early chlorpyrifos treatment the whole farm, including the MD only, was sprayed with Coragen on 23 July. Instead an assessment was done on a farm in East Kent where MD had been used for 3 seasons (Site 3).

Table 6.1. Orchard details and treatment programmes at Site 1 (Kent)

Orchard 1 (Conv)	**'A'**	***Orchard 6 (MD)***	**Mackson's**
NGR	51.303789,0.96611	NGR	51.307867,0.957956
Variety	Gala	Variety	Gala
Planting date	1996	Planting date	2002
Area (ha)	0.7	Area (ha)	1.4
Orchard 2 (Conv)	**'B'**	***Orchard 7 (MD)***	**Trench**
NGR	51.305104,0.966883	NGR	51.309048,0.955811
Variety	Gala	Variety	Bramley
Planting date	1996	Planting date	1990
Area (ha)	0.7	Area (ha)	1.0
Orchard 3 (Conv)	**Thread Lane**	***Orchard 8 (MD)***	**Pear Orchard**
NGR	51.302018,0.966754	NGR	51.308216,0.956712
Variety	Gala	Variety	Cox
Planting date	1991	Planting date	1994
Area (ha)	2.2	Area (ha)	0.6
Orchard 4 (Conv and untreated)	**Broughton Meadow**	***Orchard 9 (MD)***	**Packing Shed**
NGR	51.300086,0.958943	NGR	51.308538,0.958858
Variety	Braeburn	Variety	Gala
Planting date	1985 Grafted 2005	Planting date	1996/ 2010-grafted
Area (ha)	2.4	Area (ha)	1.6

Orchard 5 (MD)	**Engine**	*Orchard 10 (MD)*	**Sadleton's**
NGR	51.30835,0.954738	NGR	51.307331,0.956497
Variety	Discovery/Windsor	Variety	Braeburn
Planting date	2010	Planting date	2004
Area (ha)	2.6	Area (ha)	1.4

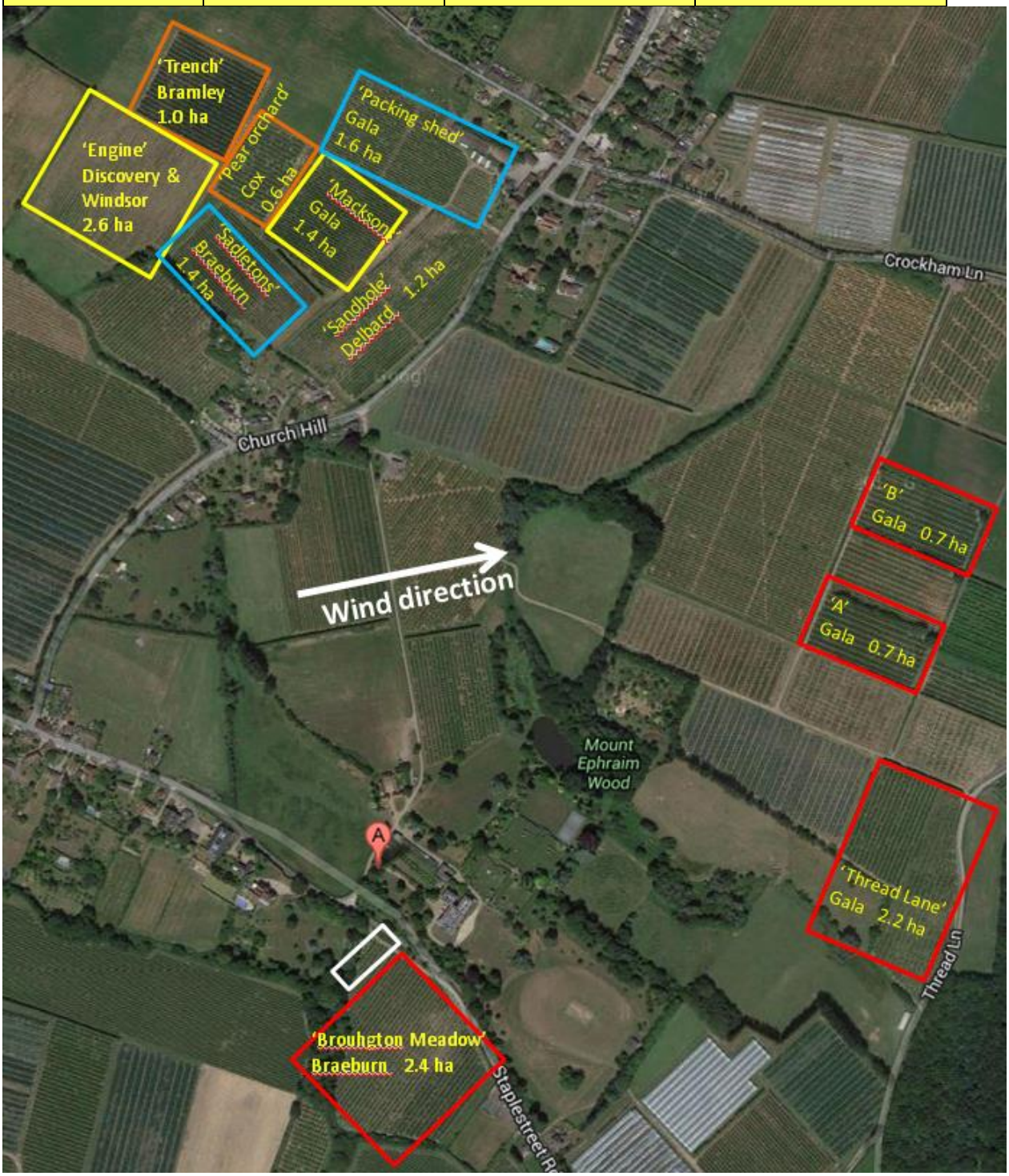


Figure 6.1. Map of Site 1 and the location of the treated plots (NB 'Sandhole' was grubbed in 2016)

Table 6.2. Orchard details and treatment programmes at Site 2 (West Midlands)

Orchard 1 (Untreated)	**Oak**	***Orchard 6 (MD)***	**M. Linder (Top left)**
NGR	52.047872, -2.421203	NGR	52.058903, -2.414122
Variety	Royal blush	Variety	Gala/Red Windsor
Planting date	1999	Planting date	2010
Area (ha)	0.27 ha	Area (ha)	1.25
Orchard 2 (Conv)	**C4**	***Orchard 7 (MD)***	**M. Linder (Bottom Left)**
NGR	52.049614, -2.4240784	NGR	52.059193, -2.4163536
Variety	Gala	Variety	Gala/ Red Windsor
Planting date	1993	Planting date	2008
Area (ha)	2.17	Area (ha)	1.25
Orchard 3a+b2 (Conv)	**Oak**	***Orchard 8 (MD)***	**Harwort M (Top)**
NGR	52.048928, -2.4214605	NGR	52.057715, -2.4169115
Variety	Red Windsor	Variety	Rosette and Spartan
Planting date	1999	Planting date	2015
Area (ha)	2.93	Area (ha)	0.9
Orchard 4 (Conv)	**C2**	***Orchard 9 (MD)***	**Harwort M (Bottom)**
NGR	52.050062, -2.421203	NGR	52.057874, -2.4183277
Variety	Cox and Discovery	Variety	Rosette and Spartan
Planting date	1990	Planting date	2015
Area (ha)	0.9	Area (ha)	0.9
Orchard 5 (MD)	**M Linder (Bottom right)**	***Orchard 10 (MD)***	**M. Linder (Top Right)**
NGR	52.058639, -2.4170403	NGR	52.058296, -2.4142078
Variety	Gala & Red Windsor	Variety	Gala/Red Windsor
Planting date	2008	Planting date	2010
Area (ha)	1.25	Area (ha)	1.25

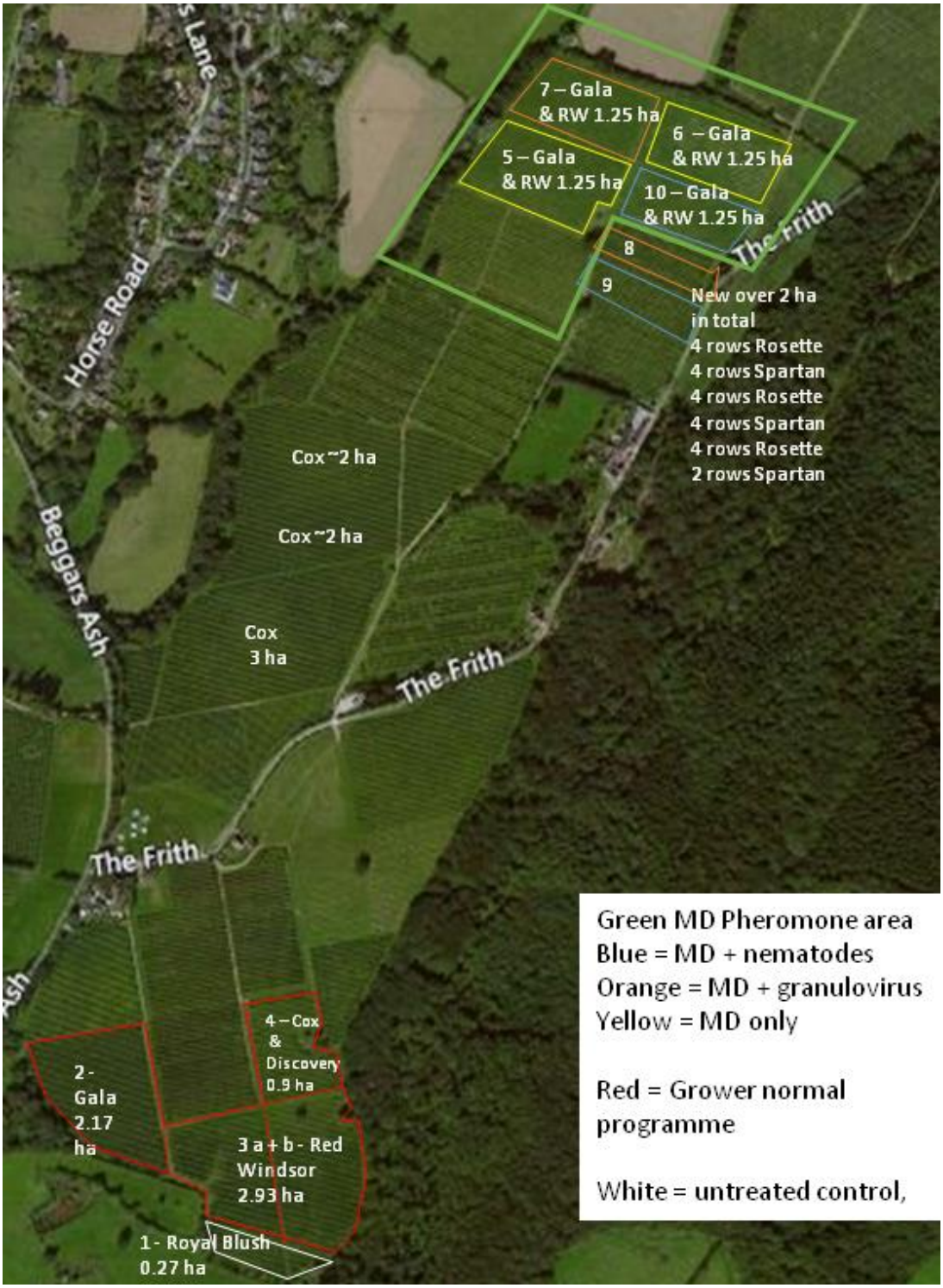


Figure 6.2. Map of Site 2 in Year 2 and the location of the treated plots. NB: orchards south of orchard 5 were grubbed at the end of the first year of the experiment.

Table 6.3. Orchard details and treatment programmes at Site 3 (Kent)

Orchard 1 (Conv)	**B1**	***Orchard 6 (MD)***	**9629**
NGR	51.317897, 1.246212	NGR	51.314577, 1.240773
Variety	Braeburn Apple	Variety	Gala Apple
Planting date	2005	Planting date	2010
Area (ha)	1.75	Area (ha)	2.80
Orchard 2 (Conv)	**B2**	***Orchard 7 (MD)***	**R1**
NGR	51.318562, 1.244828	NGR	51.314308, 1.242638
Variety	Braeburn Royal Apple	Variety	Rubens Apple
Planting date	2011	Planting date	2007
Area (ha)	2.07	Area (ha)	0.95
Orchard 3 (Conv)	**R3**	***Orchard 8 (MD)***	**R4**
NGR	51.309757, 1.243439	NGR	51.315845, 1.242679
Variety	Rubens Apple	Variety	Rubens Apple
Planting date	2008	Planting date	2010
Area (ha)	2.03	Area (ha)	2.64
Orchard 4 (Conv)	**3201**	***Orchard 9 (MD)***	**P1**
NGR	51.311441, 1.245451	NGR	51.315138, 1.244783
Variety	Cabaret	Variety	Conference Pear
Planting date	2016	Planting date	1981
Area (ha)	0.95	Area (ha)	2.11
Orchard 5 (Conv)	**P4**	***Orchard 10 (MD)***	**P3**
NGR	51.310628, 1.244530	NGR	51.313778, 1.239769
Variety	Conference Pear	Variety	Conference Pear
Planting date	1.70	Planting date	1982
Area (ha)	1.70	Area (ha)	0.85
		Orchard 11 (MD)	**P5**
		NGR	51.316491, 1.241195
		Variety	Conference Pear
		Planting date	2011
		Area (ha)	0.90

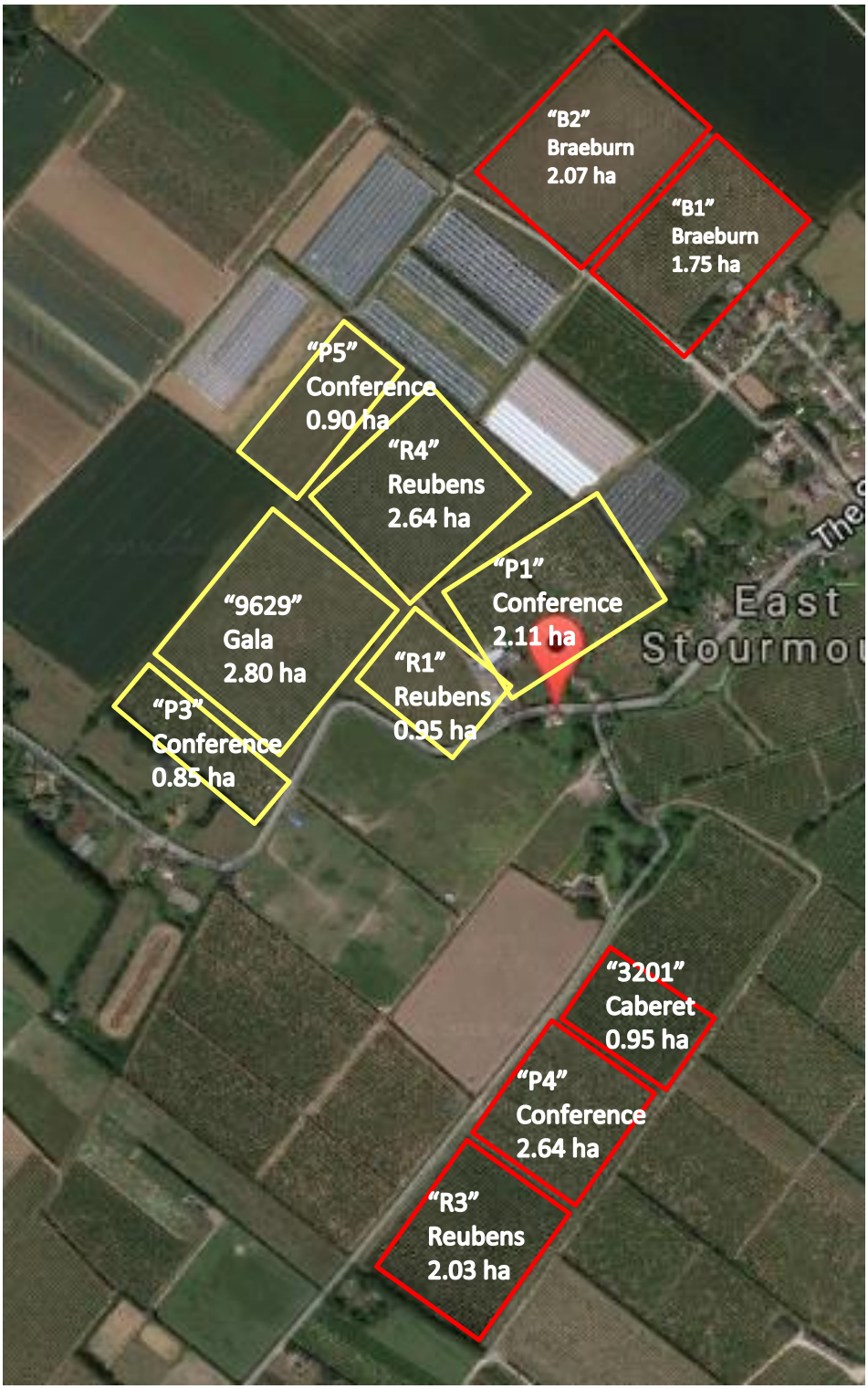


Figure 6.3. Map of Site 3 and the location of the treated plots

Treatments

CONVENTIONAL: On each farm a block of orchards greater than ~6 ha (see tables above) was treated for codling moth and tortrix moths using a standard grower spray programme (Tables 6.4 and 6.5).

MATING DISRUPTION: On each farm a block of orchards greater ~6 ha were treated with combined codling [*Cydia pomonella*] (CM)/tortrix moth [*Adoxophyes orana* (SFT) and *Archips podana* (FTT)] sex pheromone mating disruption formulation (RAK3+4, supplied in kind by BASF). This treatment has longevity for CM of at least 9 months and was applied in April to all sites. Plots were adjacent to achieve a landscape effect (fewer orchard edges). The devices (500 units per ha) were hung in the top third of the tree (as the pheromone drifts downwards) by NIAB EMR, BASF and ADAS staff under the supervision of BASF. This took approx. 2 man hours per ha. For Sites 1 and 2 this was the second year RAK3+4 had been applied while it was the third year of use at Site 3.

GRANULOSIS VIRUS: Two applications (13 and 23 May, ~10 days apart) of SFT granulosis virus (Capex) were applied to target the L3 overwintering larvae in two Gala orchards (Mackson's and Packing Shed) at Site 1. This was in reaction to close monitoring by the agronomist and NIAB EMR and the identification of significant numbers of overwintered summer fruit tortrix moth caterpillars (Table 6.4). This was to expose L3 larvae (L4 and L5 larvae are not as susceptible and usually do not die) to the virus during the active feeding period. A spray 10 days later ensures a period of about 4 weeks with maximum virus on the trees. Capex is not a fast acting insecticide; larvae usually survive L3 and L4 without being active and die in L5 usually in the periphery of branches. After their death billions of virus particles are released into the orchard. Caterpillars were collected pre and post application to assess efficacy and maintained in the laboratory to assess for survival.

UNTREATED: On the conventional treated side of the Site 1 farm there was one small area of untreated trees, for comparison, to evaluate what the codling moth damage would have been. See *Farms, Orchards and Site Managers* (above).

Table 6.4. Site 1 insecticide spray applications. Growers applied all products according to label recommendations

Date	Treatment	Active	Orchard treated[a]
15/04/2016	RAK3+4	*Cydia pomonella* *Adoxophyes orana* *Archips podana* sex pheromones	All MD orchards (Mackson's, Trench, Pear Orchard, Packing Shed, Sadleton's, Engine)
25/04/2016	Explicit	Indoxacarb	Mackson's(MD), Packing Shed(MD)
25/04/2016	Runner	Methoxyfenozide	'A', 'B', Thread Lane, Broughton Meadow
25/04/2016	Calypso	Thiacloprid	All orchards except unsprayed control rows of Broughton Meadow
13/05/2016	Capex	SFT granulovirus	Mackson's(MD) Packing Shed(MD)
20/05/2016	Calypso	Thiacloprid	Mackson's(MD) Packing Shed(MD), 'A', 'B', Thread Lane, Broughton Meadow
23/05/2016	Capex	SFT granulovirus	Mackson's(MD) Packing Shed(MD)
12/07/2016	Coragen	Chlorantraniliprole	Engine(MD)
30/07/2016	Coragen	Chlorantraniliprole	'A', 'B', Thread Lane, Broughton Meadow

[a]conventional orchard unless stated

Table 6.5. Site 3 insecticide spray applications. Growers applied all products according to label recommendations

Date	Treatment	Active	Orchard treated[a]
22/03/16	Calypso	Thiacloprid	P1(MD), P3(MD), P4, P5(MD)
28/04/16	Mainman	Flonicamid	B1, B2, R1(MD), R3, R4 (MD), 3201, 9629(MD)
27/05/16	Coragen	Chlorantraniliprole	R3, P4
06/06/16	Calypso	Thiacloprid	B1, B2, R1(MD), R3, R4(MD), 3201, 9629(MD)
17/06/16	Aphox	Pirimicarb	B1
24/06/16	Coragen	Chlorantraniliprole	R3, 3201, P4
12/07/16	Mainman	Flonicamid	R1(MD), R3, R4 (MD), 3201, 9629(MD)

[a]conventional orchard unless stated

Experimental design and layout

Due to the unavailability of CydX in 2016 the decision was taken to treat the sites as straight comparisons between MD treatment and conventional control. The addition of a third site was due to an overspray of chlorpyrifos to all orchards in early spring 2016 and Coragen in July 2016 at Site 2. 2016 was the third year that RAK 3+4 had been used on half of the farm at Site 3 while the other half of the farm was treated conventionally. Some of the orchards at Site 3 were pear (cv. Conference) but this was deemed a good replacement for Site 2. Detailed assessments of codling and tortrix moth and other pest damage and predator numbers were assessed in the centre of the plots.

It is not possible to do full statistical analysis of the data created through the assessments carried out at these sites. Due to the lack of replication (differing cultivars between sites and treatment types, differing treatments between 2015 and 2016) it is only possible to make general observations about the data and findings.

Assessments

All project members attended the first sampling occasion at Site 1 (15 April) and Site 2 (21 April) so that methods were standardised. The first harvest assessment on the earliest ripening variety was done by most project members and then the teams split into groups of 3-4 for the remaining varieties as they were ready to harvest. There were 3 assessments:

1) At deployment of MD devices, 2) 1st Codling damage, 3) Harvest

Flight activity of codling and tortrix moths (Sites 1 & 2 only): Sex pheromone/ pear ester kairomone "combo" traps were used for codling moth (CM) and sex pheromone traps for summer fruit tortrix moth (SFT) and fruit tree tortrix moth (FTT). One trap for each species was deployed in each orchard and monitored weekly by the science staff. The traps were located 10 m in from the edge of the plot in the central row ~10 m apart. Traps were hung in the upper third of the tree canopy, maintaining a foliage free area around the trap openings and visible to filtered sunlight. The lures for each species were replaced every 4 weeks.

- The CM trap catch threshold was a single catch of 4 or more moths per trap per week in May-July (1st gen., fruit less susceptible) and 3 per trap per week in August-September (2nd gen., fruit more susceptible).
- The trap threshold for SFT in June and Aug/Sep was 30 moths/trap/week.
- The trap threshold for FTT in Jun/early Jul and Aug/Sep was >30 moths/trap/week

Other pests and natural enemies – all assessments: In the centre of each orchard one branch of 30 trees in the centre of each plot was tap sampled over a white tray. Numbers of predators

including earwigs, spiders, ladybirds (adult/larvae), hoverfly larvae, lacewing larvae etc. were recorded.
Counts were also made of notable pests including weevils and capsids.
A separate assessment of aphids and apple leaf midge was made. For apple leaf curling midge 10 shoots on 30 trees were examined and the number of shoots effected per 10 shoots recorded. For aphids 30 shoots in the centre of each plot were assessed and the numbers of aphids per shoot recorded.
For woolly apple aphid (WAA) 30 trees were searched (including branches and trunk) and the numbers of colonies counted. A score scale was used as follows: none; 1 = slight - a couple of colonies at most; 2 = moderate - more than 4 colonies on tree but not high in canopy and not on fruit; 3 = severe colonies large and frequent in top of canopy and on fruit.

Tortrix caterpillar (Site 1 only): Trees in the centre of the MD plots were inspected for caterpillars during the April assessment. Further inspections in the Packing Shed and Mackson's orchards were made pre and post Capex application; on 6 May trees in the centre of orchards were inspected for ½ person hour and then inspected again a week after the first application of Capex (Engine was also inspected at this time). Any larvae discovered were collected and kept singly in petri dishes on a small piece of blue roll soaked in distilled water with apple leaves as food. Larvae were kept until death or adult development.

First generation CM fruit damage: For the first fruit damage assessment the total numbers of fruitlets on each of 5 randomly selected trees in the centre of each plot were counted (using a clicker counter) so that estimates of the percentage fruits damaged could be made.
The fallen fruits under each of the 10-20 apple trees (every other tree) were raked out and counted (not June drop fruits); for the Conference pear orchards at Site 3 this was done for 6 trees. The trees in orchard 3201 at Site 3 were so young they had little fruit and therefore 1000 fruit were assessed over 250 trees. The number of fruit with superficial CM (sting) and deep entry (DE fully penetrated by larvae) damage and tortrix damage were recorded; using a knife to cut open apples.
All the fruitlets on each of the 6-20 (>1000 fruits) randomly selected trees in the central area of each plot (every other tree) were inspected for codling moth damage. This was done by looking over the tree and inspecting each fruit.
At Site 2 a rapid assessment was carried out by ADAS and BASF in August (after application of Coragen to whole farm in July) to assess fruit damage.

Second generation CM fruit damage (harvest assessment at Site 1 only): Dropped fruit were assessed as above. All tree fruits in the centre of each plot were counted and assessed by picking into picking buckets. Any apples that had damage were dropped into a box beneath the tree for examination as first generation damage assessment above.

Experimental permits, crop destruction and grower compensation for crop losses: BASF obtained from the UK Chemical Regulations Directorate consumer assessed experimental permits for all the MD treatments required for this work so that destruction of fruit treated with the product was not required. Other products were approved for use on UK apple.

Phytotoxicity: Each time an assessment was made each plot was examined for any symptoms of phytotoxicity.

Results and Discussion

Flight activity of codling and tortrix moths (Sites 1 & 2 only)

The first generation flight of CM was above the average threshold of 4 moths per trap on 9 June in the growers' conventional side of the farm at Site 1 (Figure 6.4), although only one of the 4 orchards had more than the threshold. After this week the numbers dropped back down below threshold until 14 July when the levels were above threshold and remained so into the following week before falling back below threshold for the remainder of the season.
The average number of codling moth on the MD side of the farm didn't exceeded threshold although the threshold was met in the traps in a couple of individual orchards in mid-July when a spray of Coragen was recommended for those orchards.

SFT numbers remained very low at this farm and numbers did not reach threshold (30/trap/week) (Figure 6.4).

FTT moth catches were higher at this site in 2016 than 2015 when they were below threshold (<20 at peak). Levels were above threshold through late June and early July on the conventional side of the farm, with a second (smaller) peak in early September where only a couple of orchards had higher than threshold catches (Fig. 6.4). There were only 5 FTT moths caught across the year on the MD side of the farm.

Codling moth catches were very low at Site 2 (Fig. 6.5). This could have been because of the spray of Coragen, however, the Coragen was applied below the recommended threshold. CM numbers were also low in 2015 (even though the farm had reportedly had high CM catches in previous years).

No SFT moths were trapped at this farm this in 2015 or 2016.
FTT numbers reached threshold levels in the untreated and growers conventional programme side of the farm on 14 June and were then above threshold for a four week period between 28 June (the summer peak with a mean of 66 for the conventional side of the farm) and the 19 July (Fig. 6.5). There were no FTT adults trapped in the MD side of the farm.

On both farms the MD RAK3+4 system appeared to be very effective at disrupting male moth pheromone detection, but complete trap shut down was not achieved for codling moth.

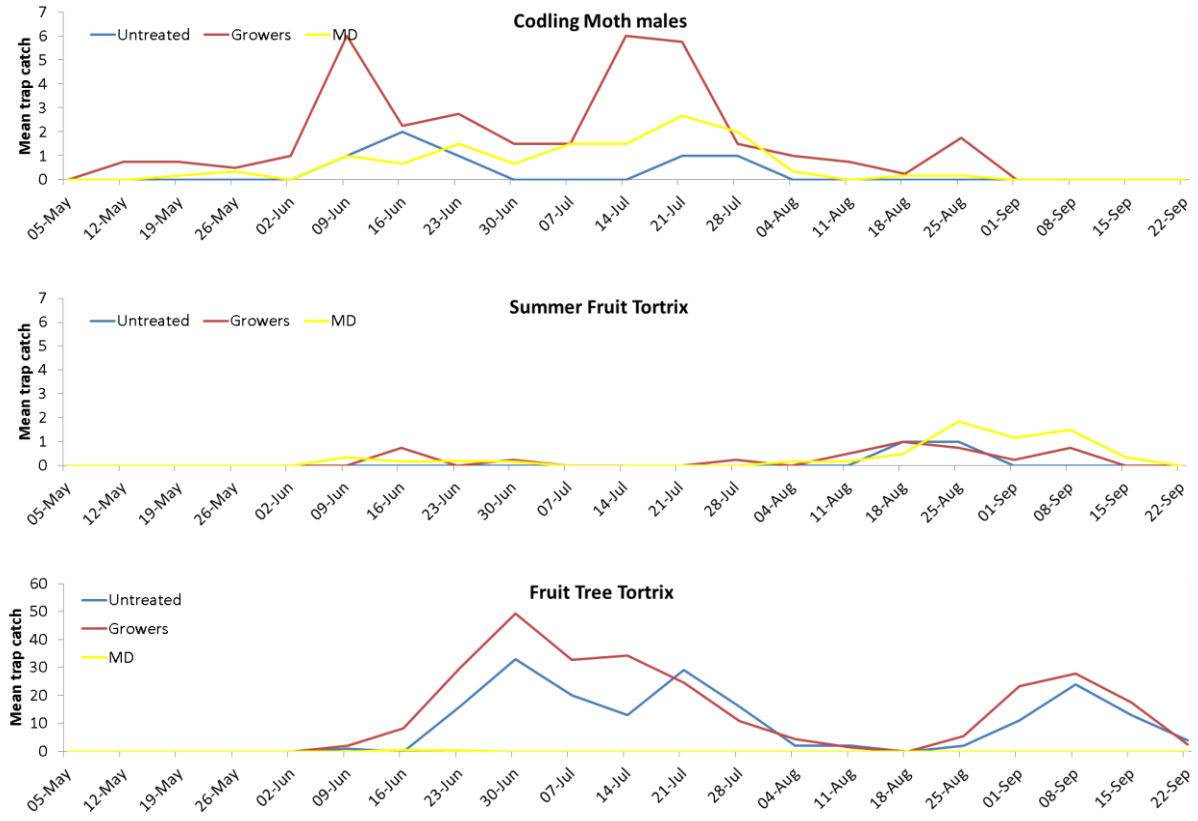


Figure 6.4. Mean numbers of codling moth, summer fruit tortrix and fruit tree tortrix moth in sex pheromone monitoring traps at Site 1 in 2016

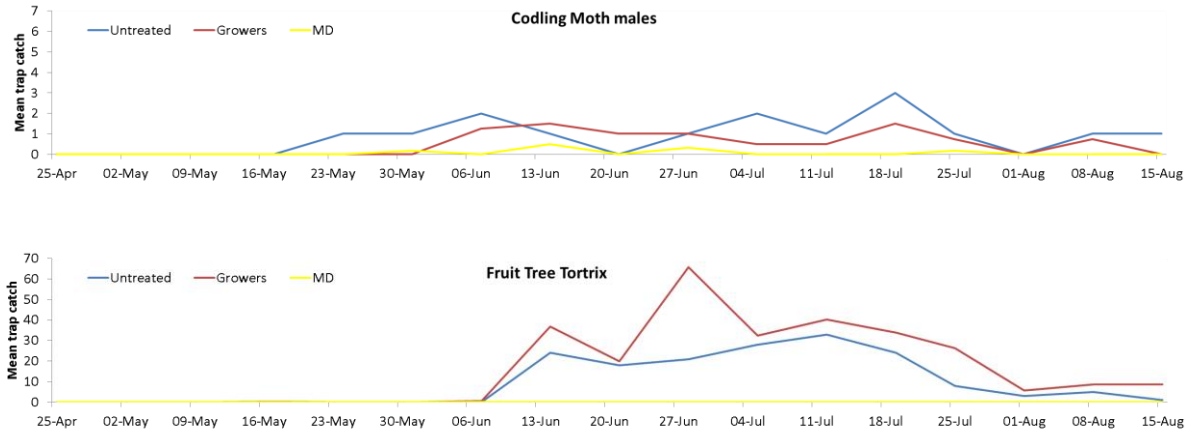


Figure 6.5. Mean numbers of codling moth and fruit tree tortrix moth in sex pheromone monitoring traps at site 2 in 2016. NB summer fruit tortrix moth was not found at this farm in 2016

Other pests and natural enemies – all assessments:

At the spring assessment at Site 1 Rosy Apple Aphid (RAA) and Apple Grass Aphid (AGA) were less common across both MD and conventional sides of the farm in 2016 compared to 2015 (Fig. 6.6). Tortrix caterpillars, identified as SFT, were more common in 2016 on the MD side of the farm (14), but also in Orchard B (2) on the growers' conventionally sprayed side.

All orchards at Site 2 had an un-recommended spray of Chlorpyrifos in early spring 2016 and in the April assessment spiders were the only arthropod group of note (Figure 6.7). One of the aims of the project was to build up earwig numbers; however, repeated applications of chlorpyrifos are unlikely to be compatible with earwigs.

At the July assessment at Site 1 there were few observable differences in in arthropod numbers between 2015 and 2016 (Figure 6.8). As noted in the year 1 report, there appeared to be more earwigs on the MD side of the farm, but this had not increased in any noticeable way in 2016.

At Site 3 no noticeable trends were observed in MD vs. conventional sprays after 3 years of MD deployment, but more earwigs were present in pear orchards compared to apple orchards (Figure 6.9). This has also been observed in an Innovate UK project at NIAB EMR. It was also observed that Orchards; Young, B2 and B1 never reached trap threshold and they were not sprayed, but did not have the RAK 3+4 system; they were upwind of RAK3+4 treated orchards.

At the harvest assessment at Site 1 numbers of earwigs on the conventional side of the farm were similar in both years (Figure 6.10). There was arguably a trend of fewer earwigs on the MD side of the farm compared to the previous year, 2015. It was notable that there was a higher incidence of WAA on the MD, in particular in Mackson's, Trench and Packing Shed orchards, compared to the previous year. It is well documented that earwigs are effective natural enemies of WAA. Figure 6.11 shows the numbers of woolly apple aphid plotted against the numbers of earwigs at each of the orchards at Site 1. It is notable that the highest WAA scores are where there are low numbers of earwigs (left side of chart).

Tortrix caterpillar

On 15 April caterpillars were found at low levels (<4) in Trench, Pear and Engine at Site 1. In Mackson's, however, 47 caterpillars were discovered in a 1 hour search. Some of these were reared through. Five adult SFT emerged and 9 parasitoid wasps (5 x small and 4 x large). This confirmed that the main tortrix larvae were SFT, but that many of them were parasitized. On 6 May small numbers of tortrix larvae were found in Mackson's and Packing Shed. Only 2 SFT emerged and 2 Dipteran parasitoids and one wasp parasitoid. On 20 May (after first Capex treatment) only one live larva in each of Mackson's, Packing Shed and Engine was found.

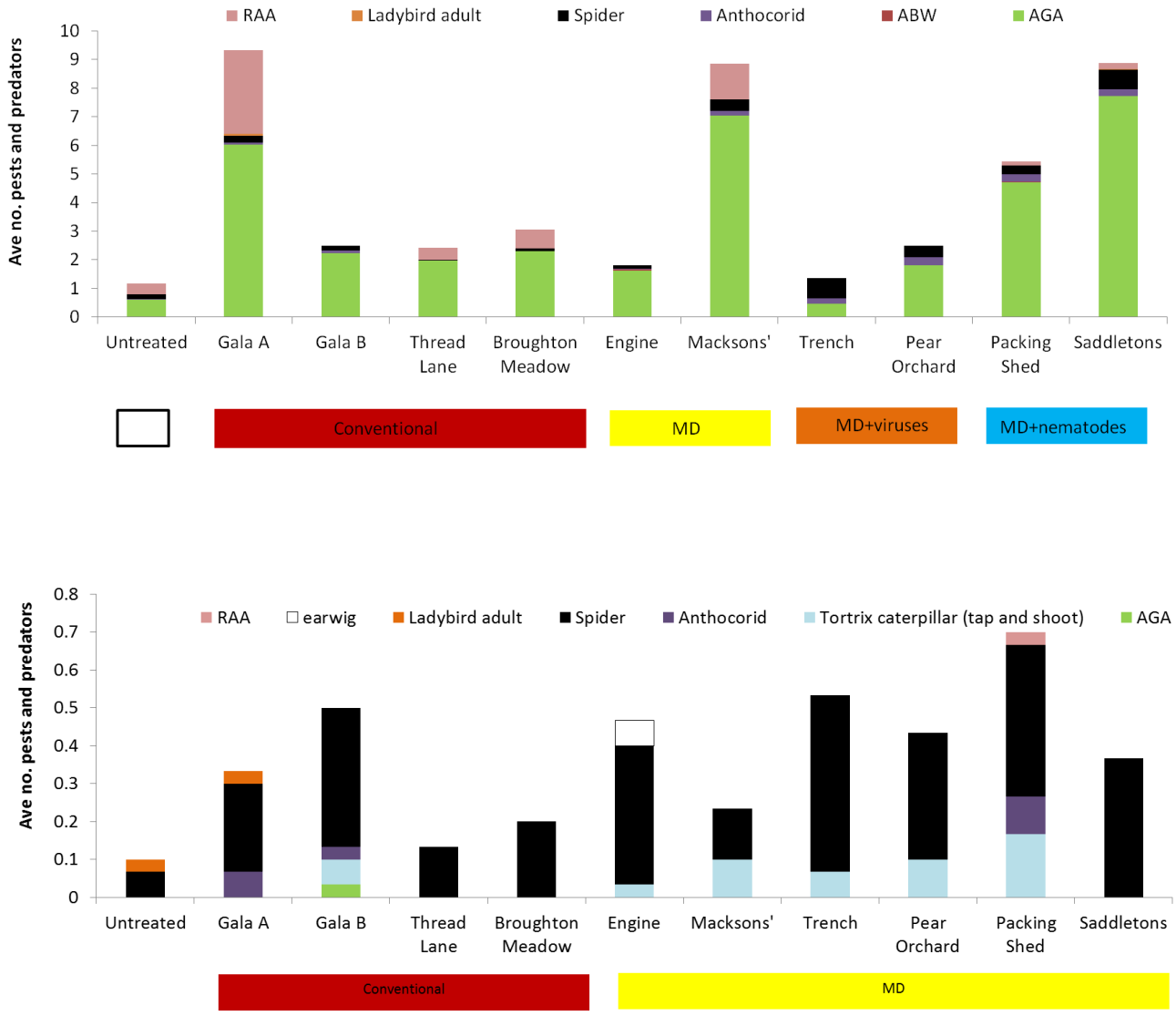


Figure 6.6. NB: different scales. Spring (April) assessments of dominant pests and natural enemies per tree in each orchard at Site 1 in 2015 (top) and 2016 (bottom); MD = mating disruption, RAA – rosy apple aphid, ABW = apple blossom weevil, AGA = apple grass aphid, WAA = woolly apple aphid, Tortrix sp. = tortricid caterpillar

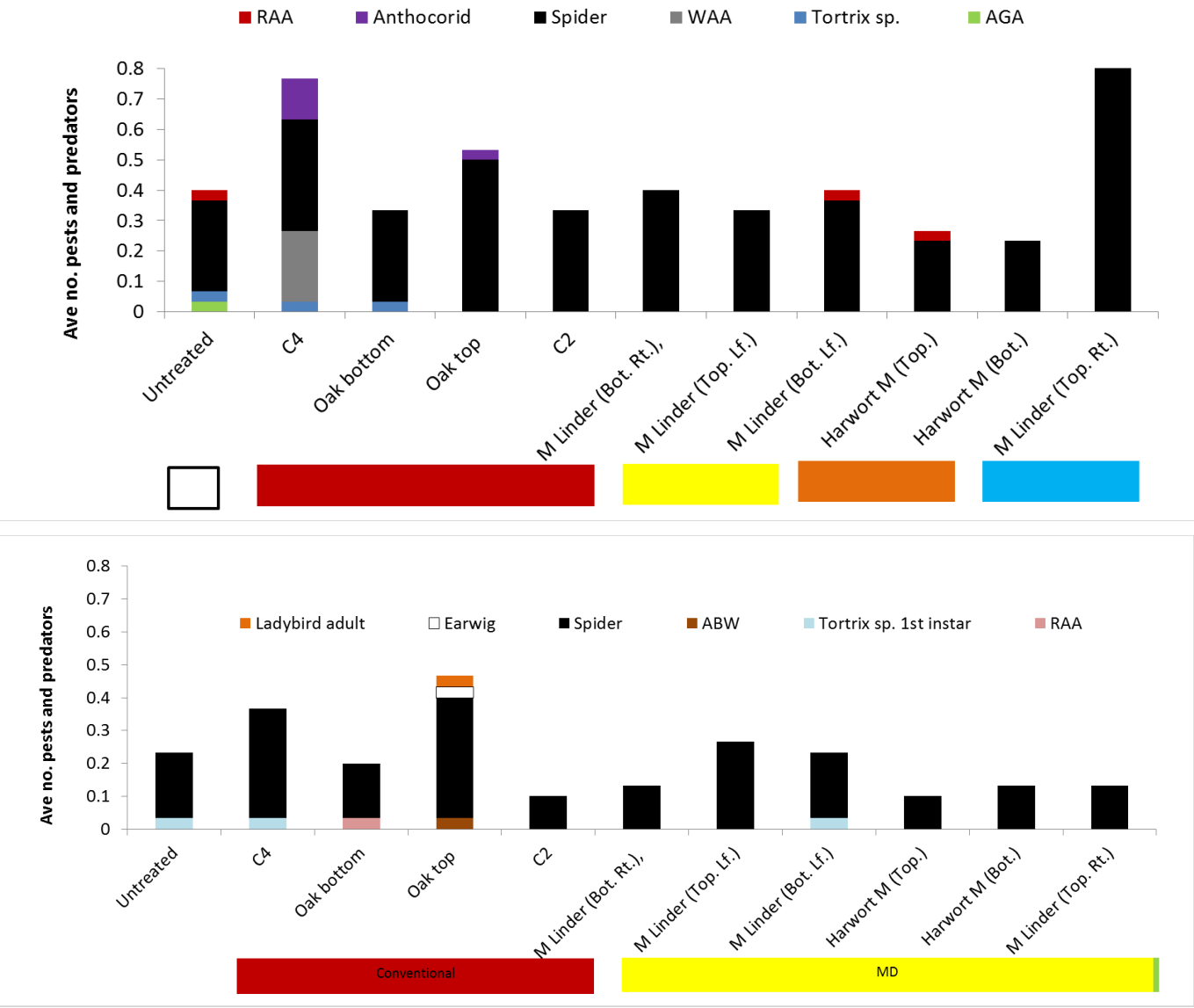
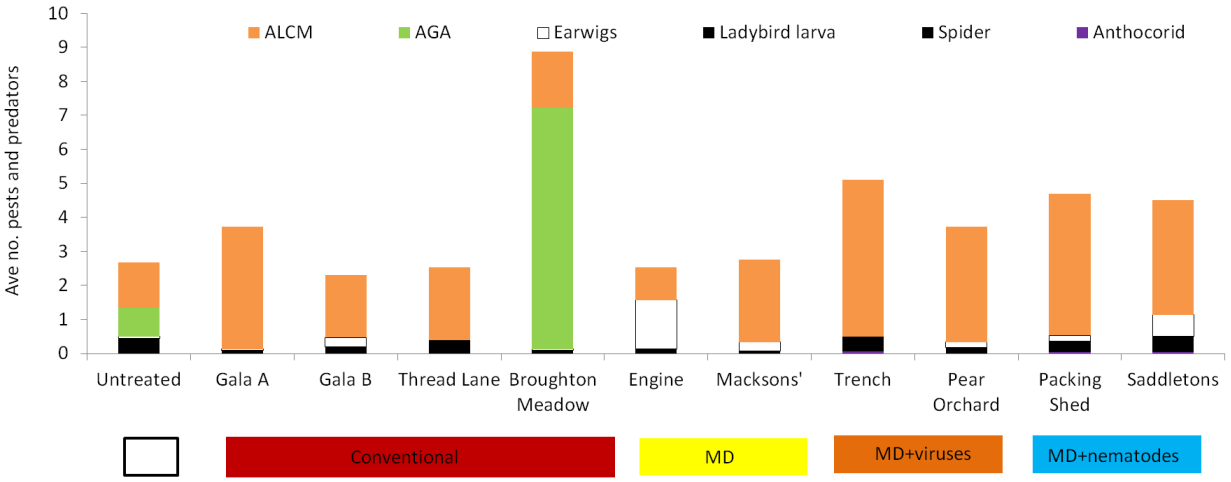


Figure 6.7. Spring (April) assessments (pre-treatments) of dominant pests and natural enemies per tree in each orchard at Site 2 in 2015 (top) and 2016 (bottom); MD = mating disruption, RAA – rosy apple aphid, ABW = apple blossom weevil, AGA = apple grass aphid, WAA = woolly apple aphid, Tortrix sp. = tortricid caterpillar. **NB** Site 2 was over sprayed and results are not comparable.



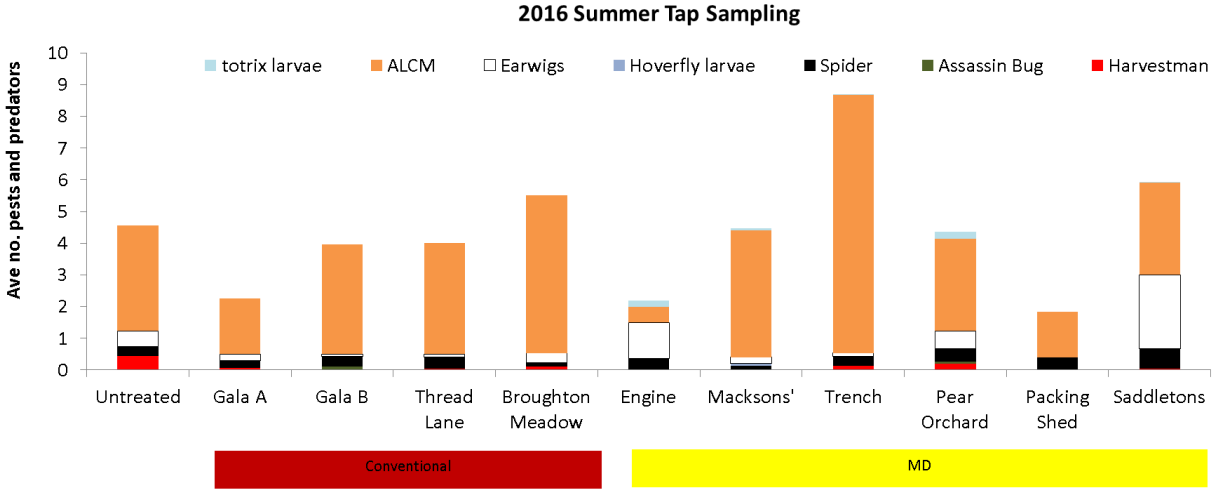


Figure 6.8. Summer assessments of dominant pests and natural enemies per tree at Site 1 in 2015 (top) and 2016 (bottom); MD = mating disruption, ALCM = apple leaf curling midge damaged shoots, AGA = apple grass aphid

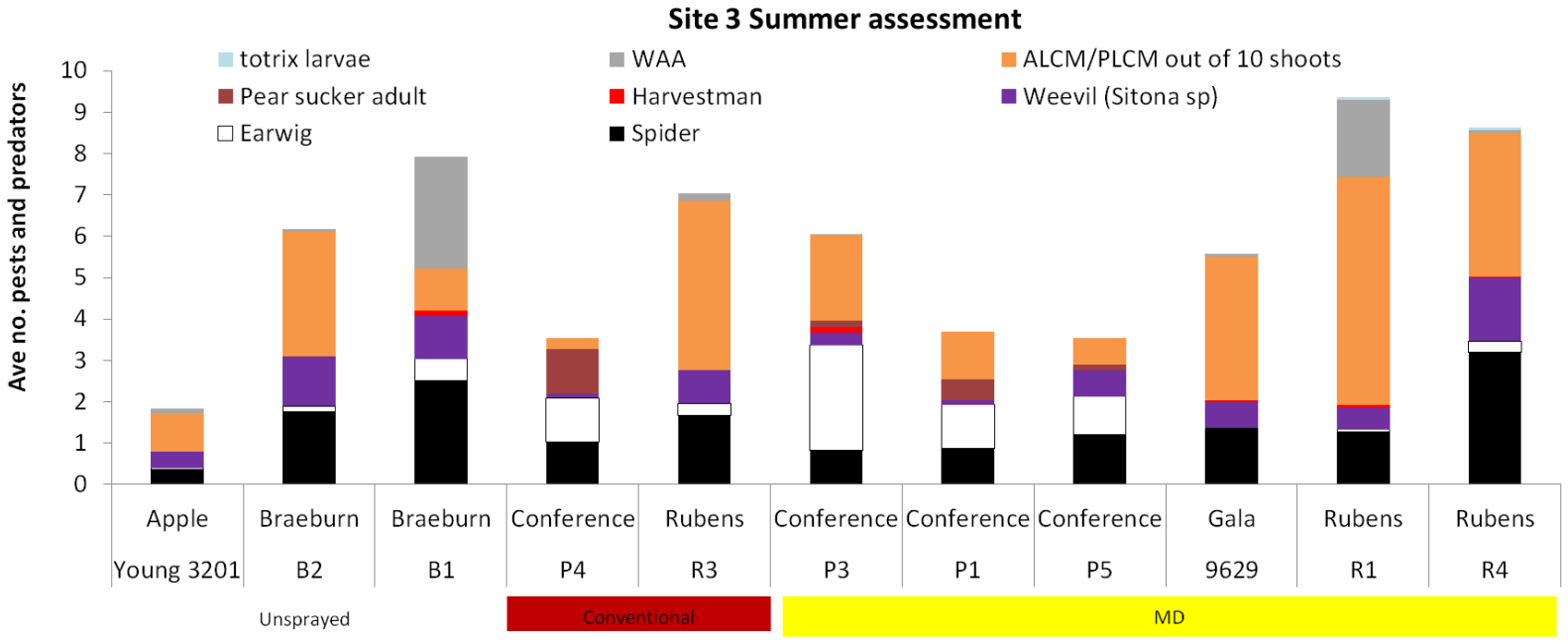


Figure 6.9. Summer assessments of dominant pests and natural enemies per tree at Site 3 in 2016; MD = mating disruption, ALCM/PCLM = apple/pear leaf curling midge damaged shoots, WAA = woolly apple aphid. NB because Young, B2 and B1 orchard never reached trap threshold they were not sprayed, but did not have the RAK 3+4 system; they were upwind of RAK3+4 treated orchards.

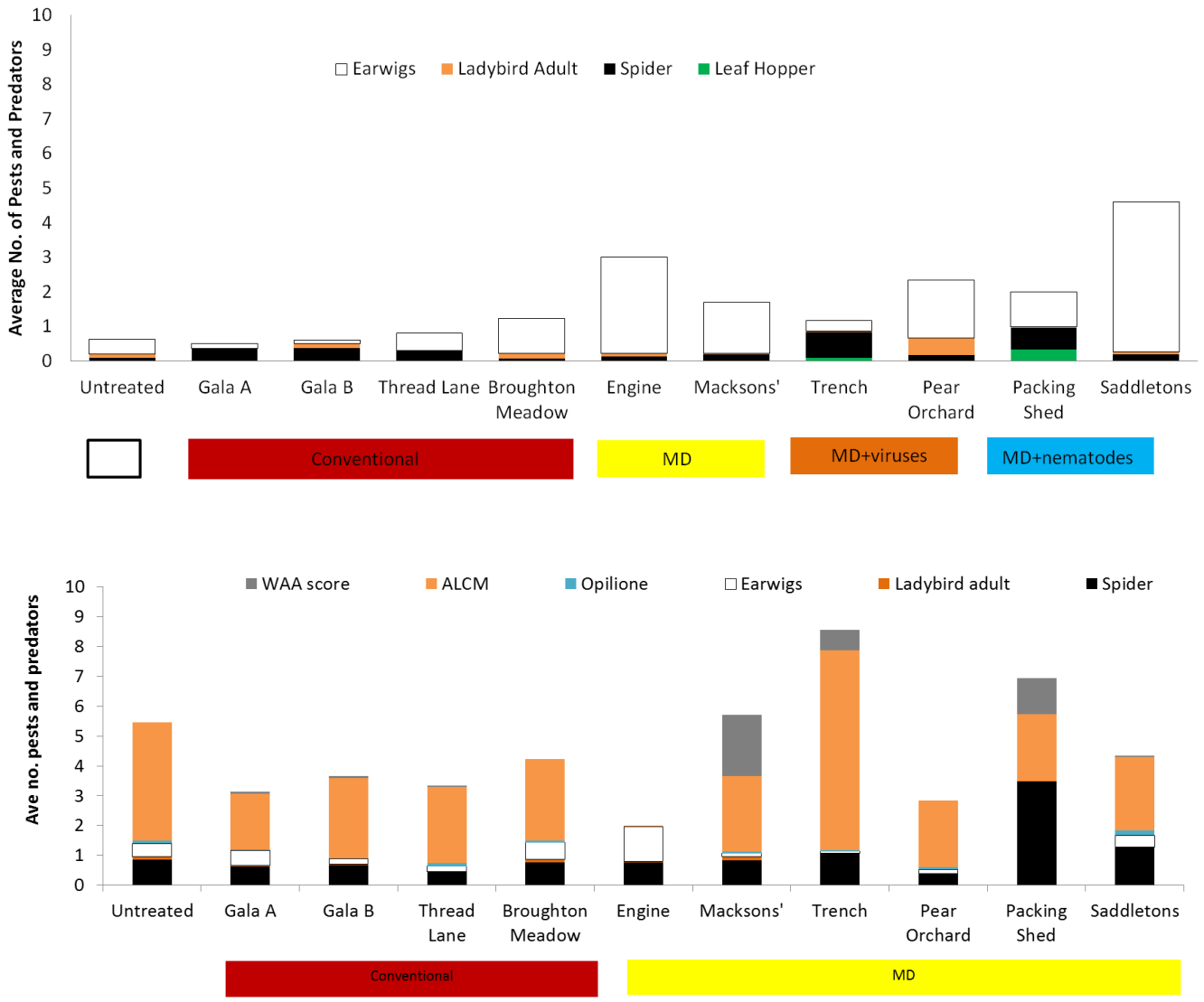


Figure 6.10. Autumn assessments of dominant pests and natural enemies per tree at Site 1 in 2015 (top) and 2016 (bottom); MD = mating disruption, Opilione = harvestmen, WAA score = Woolly apple aphid score (score scale: none; 1 = slight - a couple of colonies at most; 2 = moderate - more than 4 colonies on tree but not high in canopy and not on fruit; 3 = severe colonies large and frequent in top of canopy and on fruit.

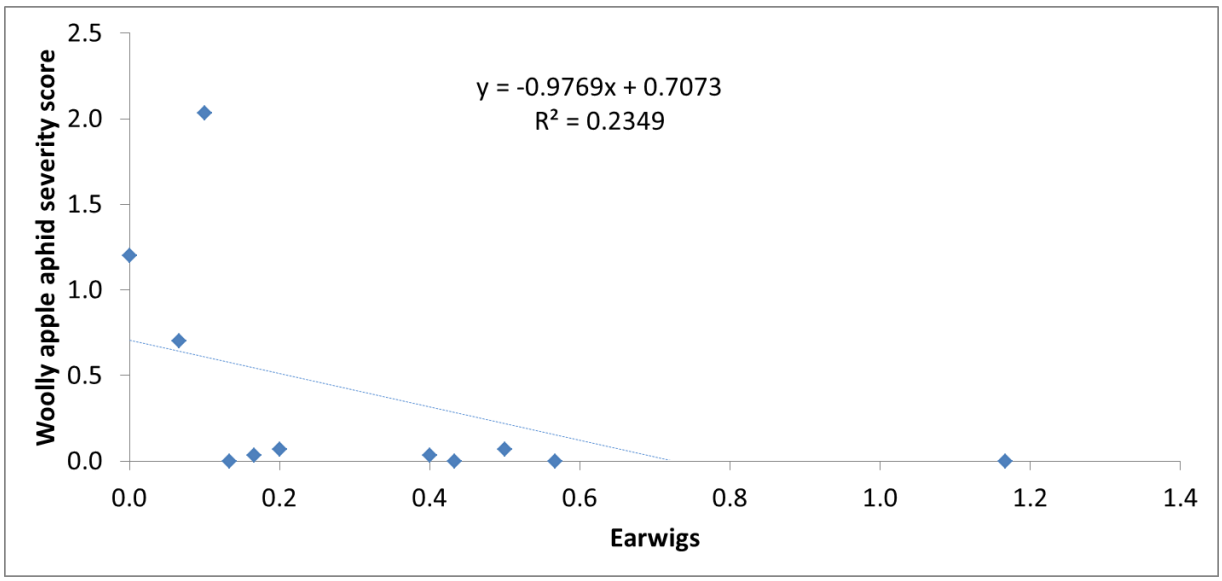


Figure 6.11. Correlation plot of woolly apple aphid score against earwig numbers in the orchards on Site 1 at the harvest assessment

First generation CM fruit damage

At Site 1, at the first codling moth generation assessment (13 July), there were very few dropped fruits with little or no CM or tortrix damage. The damage to tree fruit was minimal with damage no higher than 0.2% of fruit in a single orchard. There was more tree fruit damage on the conventional side of the farm compared to the MD where damage was only found in the early cultivar Early Windsor (Table 6.6). It is known that early ripening varieties are more vulnerable to CM larvae attack as the skins are softer earlier. It may be necessary to apply an additional insecticide application to early varieties.

As a result of the low arthropod numbers in spring and the subsequent Coragen application across the farm, Site 2 was assessed only at the end of August; only two of the conventional orchards were assessed and four of the MD orchards (Table 6.7). There was negligible damage found due to CM or tortrix and although there was higher damage from Rhynchites, mussel scale and sawfly on the conventional side of the farm. However, this could be a location effect.

Site 3 was assessed on 24 August and none of the dropped fruit was damaged by CM or tortrix caterpillars. Tree fruit damage due to CM was minimal with no orchard higher than 0.2% fruit (Figure 6.12). The apple trees on the MD treated orchards had between 0.3 % and 1% of tree fruit damaged due to tortrix, whereas there was little or no damage to pear and most of the conventionally treated apple orchards due to tortrix. The largest cause of damage to tree fruit at this site was by blastobasis which was predominantly in the MD orchards (Figure 6.12). The RAK 3+4 will have no effect on blastobasis and without use of pesticides targeted at CM and tortricids no protection is achieved for other incidental caterpillar species. This indicates that reliance on MD without careful and regular monitoring for other pests may cause an increase in less common pests that have not been a significant problem in the past.

Table 6.6. Mean numbers of tree fruits damaged by first generation codling moth at Site 1. white = untreated, red = Conventional sprays, yellow = MD

	Cultivar	CM Sting	CM Deep entry	Total damage	Average Fruit per tree	% CM tree fruit damaged
Untreated	Braeburn	0	0	0	405.8	0.0
Gala A	Gala	0.2	0	0.2	203.8	0.1
Gala B	Gala	0.1	0.2	0.3	127.4	0.2
Thread Lane	Gala	0.2	0	0.2	210.2	0.1
Broughton Meadow	Braeburn	0.3	0	0.3	350.0	0.1
Engine	E. Windsor	0.05	0	0.05	63.2	0.1
Mackson's	Gala	0	0	0	126.6	0.0
Trench	Bramley	0	0	0	133.4	0.0
Pear Orchard	Cox	0	0	0	216.6	0.0
Packing Shed	Gala	0	0	0	161.2	0.0
Sadleton's	Braeburn	0	0	0	236.4	0.0

Table 6.7. Overall mean numbers of tree fruits damaged by first generation codling moth at Site 2. white = untreated, red = Conventional sprays, yellow = MD

	Tortrix sp.	**Codling sting**	**Deep entry**	**Rhynchites**	**Capsid**	**Muscle scale**	**Sawfly**
Untreat	0.00	0.09	0.09	0.36	0.45	0.09	0.82
Conv	0.00	0.06	0.00	0.78	0.44	1.17	1.06
MD	0.00	0.00	0.00	0.00	0.40	0.00	0.42

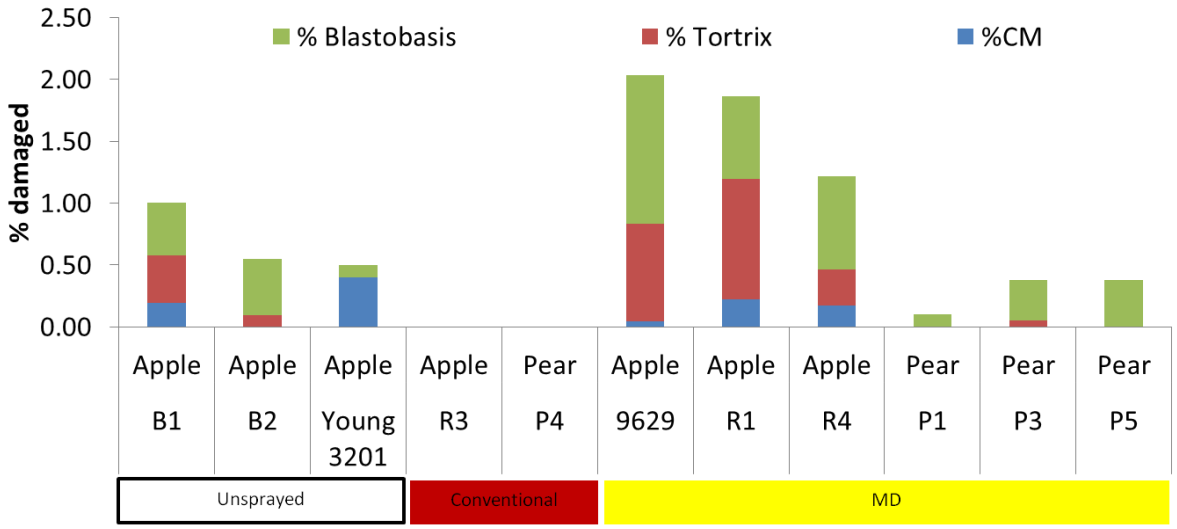


Figure 6.12. Percentage damage at the summer assessment (24 August) at Site 3 following 3 years of MD application

Second generation CM fruit damage (harvest assessment at site 1 only)

DROPPED FRUITS: Only two CM deep entry (DE) damaged dropped fruit were found on the conventionally treated side of Site 1 at harvest (Figure 6.13) in the Broughton Meadow orchard, however there was no dropped fruit damage due to CM in the untreated row. Three of the orchards on the MD disruption side of the farm had DE damage. 10% and over 20 % of the dropped fruit in Trench (Bramley) and Saddletons (Braeburn) respectively was CM damaged. In Trench 10.5% of the dropped fruit also had tortrix damage, but for the other orchards there was only minor damage from tortrix. There was also minor damage from Rhynchities weevil and Blastobasis caterpillars in the dropped fruit on the MD side of the farm.

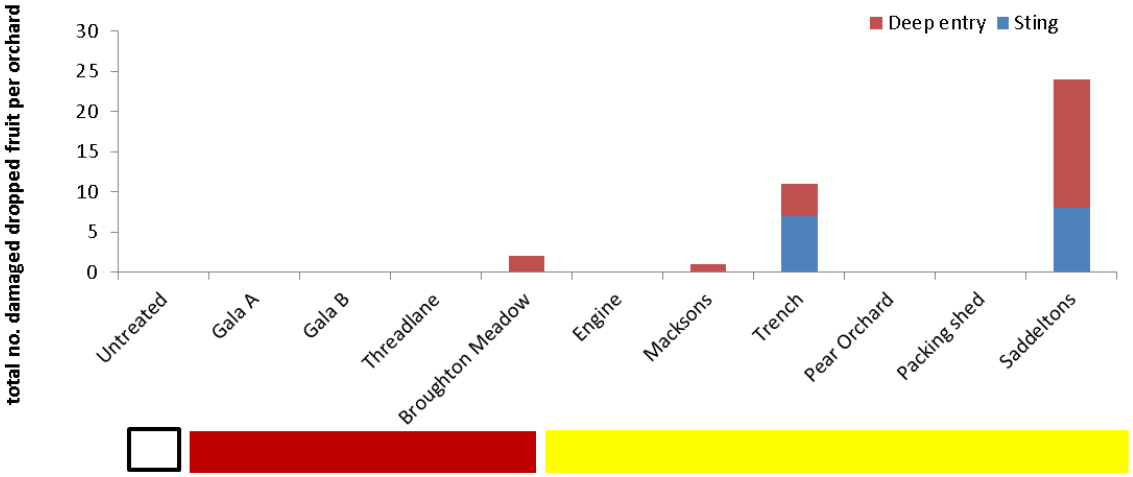


Figure 6.16. Total damage by codling moth to dropped fruit, per 5 trees, at harvest at Site 1

TOTAL FRUIT (Tree + dropped): The damage to all fruit caused by CM was fairly similar across the farm on the conventional (0.1-0.4%) and MD (0.06-0.6%) side of the farm, with the exception of the Bramley orchard (Trench 1.09%) on the MD side of the farm. A Bramley orchard on the conventional side of the farm was not available for comparison (Figure 6.17). Tortrix and Blastobasis caterpillar damage to the fruits was noticeably higher on the MD side of the farm compared to the conventional side. However, overall damage from caterpillars in each orchard, with the exception of the Bramley orchard, was generally below 2%.

As with 2015 the early ripening varieties, E. Windsor and Bramley, had the most damage; these varieties were not present on the conventional side of the farm (Figure 6.17).

By comparing Gala and Braeburn (present on both sides of the farm) damage was a little higher overall on the MD side for Tortrix in the Gala and for Blastobasis in the Braeburn (Figure 6.17). There was a small amount of Capsid and Rhynchites damage on the MD side of the farm with a little less on the conventional side, while there was a small amount of muscle scale in Orchard B.

There was a high level of WAA damage in the Gala orchard Mackson's (11.3% of fruit) and to a lesser extent Packing shed (1.4%) on the MD side and Orchard B (0.2%) on the conventional side of the farm.

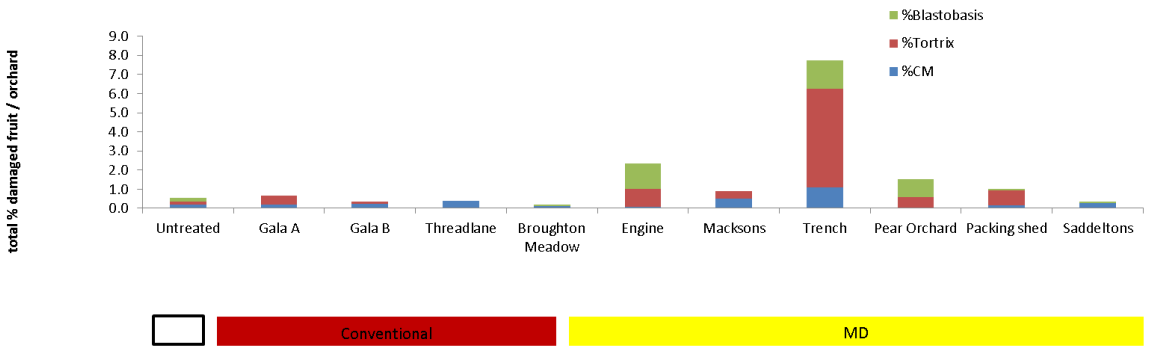


Figure 6.17. Total damage by caterpillars to dropped and tree fruit at harvest at Site 1

Phytotoxicity: In the first year some local damage to the leaves that had made contact with the RAK3+4 devices was seen. This was not considered significant as it only affected a small area of a couple of leaves per tree. There was no obvious increase in phytotoxicity this year.

Economics

Table 6.8 summarises the economic benefits of the conventional and RAK3+4 codling and tortrix programmes. Other benefits of the mating disruption approach could include;

- Protecting against a partial second generation without the need to consider harvest intervals
- Reduced probability of residue detection from insecticides in fruit
- Fruit coverage is not required by mating disruption as with conventional pesticides
- Less operator exposure to pesticide
- No re-entry interval into crop

Table 6.8. The comparative costs and risks of using conventional spray programmes vs RAK3+4 for codling and tortrix control. **NB:** these are based on low codling and tortrix pressure where no extra applications of insecticides are needed for either strategy. At high pest pressure or incidence of sporadic pests not covered by codling sprays in the RAK3+4 the cost of additional sprays will increase the cost of the control programme.

Cost/ha (£)	RAK ®3+4	Conventional
Cost	£240-300	-
Person hours	Minimum 2	1 (as part of fungicide round)
Cost of labour	Minimum, £8.20/hour (inc. NI&AL) = £16.40	£20-25
Monitoring	Should be as per Apple Best Practice Guide	Should be as per Apple Best Practice Guide
PPE	Nitrile gloves	Full
Specialist equip.	None – cost none	Tractor and sprayer – fuel, servicing and calibration
Coragen	?	£71-85 (per spray) x2
Runner	?	£44-75 (per spray)
TOTAL	**> £256.40**	**£206-270** **NB: fuel, servicing and calibration of equipment not included**

Conclusions

- Codling and tortrix MD was applied over two years to Site 1 and 3 years to Site 3. Site 2 was not comprehensively assessed in 2016.
- Data cannot be analyzed statistically as the trials were not replicated experiments and hence the following statements are based on observable trends in the data.
- Although few moths were captured in the pheromone monitoring traps on the MD side of the farms, the RAK3+4 did not cause complete trap shut-down (no moths in traps) indicating that some males may have been able to locate and mate with female moths. Some minor tortrix codling damage was observed, but was comparible, like for like, apple variety with a conventional spray programme.
- Some orchards on the mating disruption sides of the farm received an additional Coragen spray when trap catches in individual orchards was 4 or above per week or where early ripening varieties which are more vulnerable to codling moth were present.
- There was some concern over tortrix caterpillars in the young shoots in the spring at Site 1. These were reared through and found to be SFT, however over 50% of the caterpillars were parasitized by wasps and later in the season, Diptera parasitoids. Two sprays of Capex 10 days apart killed the majority of remaining caterpillars in the affected orchards.
- RAA and AGA were less common across both MD and conventional sides of the farm in 2016 compared to 2015.
- There were few observable differences in natural enemies between the first and second year or third year of RAK3+4 deployment at Site 1 or 3, respectively.
- Over the period of the study we did not identify a noticeable increase in earwigs from year 1 to year 2 at Site 1 where fewer insecticides had been used on the MD side of the farms. However, as earwigs have a single generation each year the study may not have been long enough to identify differences.
- It was notable that there was a higher incidence of WAA on the MD side of Site 1. High numbers of aphids were in orchards where there were lower numbers of earwigs.
- As with 2015 there was more first generation CM damage in the early ripening variety Early Windsor and the Bramley orchard. It is clear that the RAK3+4 is not giving complete control of moths, but is comparable with a conventional spray programme.
- There was notable damage from two pests at Site 1 and 3 in 2016 on the MD side of the farms. Blastobasis caused damage at harvest and WAA was abundant in some orchards at Site 1. These pests would normally be controlled with insecticide applications targeted at CM and tortirx and a spring spray of chlorpyrifos, respectively.
- The application of RAK3+4 needs to be coupled with monitoring for incidental pests and natural enemies.

- At harvest the damage to fruit caused by CM was fairly similar across Site 1 on the conventional (0.1-0.4%) and MD (0.06-0.6%) side of the farm, with the exception of the Bramley orchard (1.09%) on the MD side of the farm.
- Tortrix caterpillar damage to the fruits was noticeably higher on the MD side of the Site 1 compared to the conventional side.

Objective 6	Codling moth	Task 2	Nematodes

Aim

To test the efficacy of nematode sprays to target diapausing codling moth larvae in July and August in apple orchards.

NB: This work was supported and funded by BASF.

Materials and Methods

Sites 1 and 2 that were used for MD trials above were used for the nematode sprays and the untreated controls. Packing Shed, Sadletons, Harwort M (Bottom) and M. Linder (Top Right) were used for the nematode sprays and Mackson's, Engine, M. Linder (Top Left) and M. Linder (Bottom Right) were used as water only controls.

Sentinel codling moth cages were constructed of mesh (1.5 mm diameter) 19 cm tall and 10 cm diameter. Codling moth eggs were purchased from Andermatt 3-4 weeks before each trial commenced and were reared in the insect rearing facility at NIAB EMR. Larvae were allowed to develop on a growing media and then migrate into the cardboard rolls. Each cage contained 5-10 diapausing larvae on a roll of corrugated cardboard (Figure 6.18).

Figure 6.18. Sentinel codling moth cage showing construction and placement of corrugated cardboard refuge

Treatments

A mixture of *Steinernema carpocapsa* (Nemasys C) and *Steinernema feltiae*, one pack of 750 million of each sp. per ha were used on each spray occasion. Nematodes were stored at 4-5 °C.

Laboratory test. To test that the cages were an appropriate device for testing nematode efficacy a test was conducted to determine whether nematodes could get through the mesh into the cardboard rolls. A control using insect larvae known to be susceptible to the nematodes was used. Eight larvae of *Galleria mellonella* (Greater Wax Moth) were placed inside the cardboard refuge and then sprayed with a Birchmeier B245 motorised knapsack mistblower (routinely used to mimic conventional orchard spraying). The nematodes were applied at 1500 l/ha. The majority of conventional orchards have a planting density between 1500 and 2000 trees per ha. The 1500 trees per ha was chosen for convenience as this equates to 1 l per tree (applications of nematodes are targeted at the trunk).

The Knapsack sprayer was loaded with 1 l of water containing 1 Million nematodes (1.5 billion (US) / ha) of a 50:50 mix of both species. Two sentinel cages were then sprayed until the sprayer was empty. This was then repeated with the second two sentinel cages using 0.5 l of water only (equivalent to half the dose or a tree planting density of 3000 trees/ ha). After spraying, the cages were placed into individual polyethylene bags to maintain humidity for 24 hours. The larvae were individually reared in 7 cm plastic Petri dishes and monitored weekly for mortality. All of the treated larvae were dead due to nematode infestation within 7 days of the application, hence it was decided to proceed with the field trials.

Field trials. Laboratory reared codling moth were placed into 24 sentinel cages (3-4 larvae per cage). These were then tied into the trees of the four target orchards (6 per orchard) at Site 1 (21 July 2016) in comparison to a control (water only). As the sprays were to be targeted at the trunk of the tree the cages were suspended parallel to the trunk at approximately 30 cm from the ground. Sprays were applied using the growers own spray equipment, a Lochman RPS 15/90 UQH sprayer fitted with 14 blue Albuz hollow cones nozzles (7 per side) at 7 bar and 3.1 Kph, pulled by a New Holland TN75F tractor (Figure 6.19).

Figure 6.19. The spray equipment used at Site 1, 21 July 2016

Three applications of 1500 l/ha by the sprayer were required to ensure sufficient wetting, equivalent to 4500 l/ha. Therefore the plots were sprayed twice at 1500 l/ha with water only plus a single spray at 1500 l/ha of the nematode treatment.

Before the nematodes were applied they were pre mixed in a bucket of water and their viability checked. All filters, including end of line nozzle filters were removed from the sprayer. The nematode mix was then added to the spray tank, the tank was agitated to ensure thorough mixing of the nematodes, and a sample of the tank mix checked for viability.

A sample of nematodes was then sprayed onto a non-target area and the viability of the nematodes post nozzle was checked. The nematodes were then applied to the target areas of the orchards. A post spray sample of nematodes from the nozzles was again checked for viability.

At Site 2 with help from ADAS staff, the spray was applied with a Claas Nectis 267F tractor and Munckhof sprayer fitted with 14 green Albuz hollow cone nozzles (7 per side) at 18 bar and 6.8 Kph (Figure 6.20). To achieve sufficient wetness three passes of 900 l/ha were used to apply the water before the application of 900 l/ha of either nematodes or water depending on the treatment (as the tractor had no crawler gear it could not go slow enough with enough control on the slopes of the orchards to apply 1500 l/ha).

Figure 6.20. The spray equipment used at Site 2, on 17 August

Meteorological records

Sprays were applied in the evening to increase the humidity and drying time and to decrease the amount of incident UV (Table 6.8).

Table 6.8. Weather conditions at the time of spray application at two sites

Location	Date	Time	Dry temp °C	Wet temp °C	Humidity %RH	Wind
Site 1	21 July	17:00	24.5	22.0	88%	0
		20:00	21.0	16.0	75%	0
Site 2	17 Aug	17:40	25.0	20.0	77%	0
		19:00	21.0	17.0	80%	0

Laboratory tests. Due to the failure of the field trials to achieve infection of codling moth larvae with nematodes it was decided, in discussion with BASF, to test direct application of nematodes to codling moth larvae in comparison to wax moth larvae. Twelve larvae and twelve pupae of wax moth and seven larvae and twelve pupae of codling moth, were placed into individual 7 cm Petri dishes. Based on field rates of 1.5 billion (USA) nematodes/ha this is equal to 1.5 nematodes/cm^2. The average dimensions of a larvae/pupae was 5 mm diameter and 2 cm long equating to a surface area of 3.3 cm^2 (3.3 x 1.5 = 5 nematodes per larvae). A stock solution of 0.5 million nematodes/l was made up to give an easily achievable drop size of 0.01 ml. A 0.01 ml drop was dispensed on to each individual larva and left to dry. Five drops were examined under the microscope and the number of nematodes recorded (the mean number of nematodes per droplet was 5). The droplets were allowed to dry and each larvae/pupae was given a cardboard refuge and incubated for 7 days and examined 3 times each week for signs of nematode infection.

Sentinel Cages. Due to the failure of direct application to cause infection with nematodes we returned to the initial proof of concept method which involved spraying the sentinel cages using the motorised knapsack mistblower (07 October). There were 4 cages if wax moth larvae (12 per cage), 2 cages of wax moth pupae (12 per cage), 2 cages of codling moth larvae (9 per cage) and 2 cages of codling moth pupae (14 per cage).

Half of the number of cages were sprayed with water only as a control, the remaining half were sprayed as described previously. Each cage was then transferred to an individual polyethylene bag for 24 hours to maintain humidity. The viability of the nematodes as they exited the sprayer was checked before and after each application.

After 24 hours each cage was opened and each larvae/pupae removed and individually plated up on moist filter paper, with a cardboard refuge in a petri dish. Each replicate of each species was bagged individually and examined for signs of infestation 5 and 10 days post application. This was repeated on 11 November with the addition of apple sawfly pupae. Based on the number of individual's available 32 cages were prepared. Twelve cages with wax moth larvae (5 per cage), 12 cages with codling moth pupae (5 per cage) and 8 cages with apple sawfly pupae (3 per cage). Four cages each of codling moth, wax moth and apple sawfly pupae were sprayed with water as an untreated control; four cages of each species were sprayed with the full rate of nematodes; the remaining four cages of wax moth and codling moth were sprayed with a 50% rate of nematodes. Due to the limited availability of sawfly pupae, they were not checked for viability before spraying.

Assessments. For each experiment the larvae/pupae were dissected to examine for the presence of nematodes (Figure 6.21). Records were made of whether the larvae/ pupae were alive, dead, pupated, emerged or likely died from fungus/bacteria.

Figure 6.21. Codling moth larvae infested with nematodes

Results and Discussion

Pilot Laboratory test

The pilot test using spray equipment directly targeted into the sentinel cages resulted in 100% mortality of both treated codling moth and wax moth larvae due to nematode infestation (Figure 6.21).

Field trials

No mortality due to nematodes was observed in the field trails at Site 1 or 2. The nematodes were alive before, during and after application. This may have been due to the application by the sprayer. At 4500 l/ha, and run-off from the foliage, the sentinel cages were only mildly damp. The commercial sprayers used coarser droplet size than the motorised mist blower. It is possible these coarse droplets failed to penetrate the cage to the same extent as the mist.

Direct application in Laboratory

The direct application of nematodes to the larvae/ pupae failed to cause infection. This was probably due to the hydrophobic nature of the insect cuticle repelling the droplet and causing them to slide off before infection could occur.

Sentinel Cages in Laboratory

The wax moth larvae sprayed with water on 07 October did not develop nematode infection. Virtually all wax moth larvae sprayed with nematodes died from nematode infection. The wax moth pupae had 100% mortality due to nematode infection (Figure 6.22).

The codling moth larvae and pupae had high levels of natural mortality due to bacterial and/or fungal infections which were evident in the water treated cages. Codling moth larvae sprayed with nematodes showed a high mortality due to the nematodes (77.8 %) while the pupae seemed to be more resistant to nematode attack (28.6 % mortality, Figure 6.22).

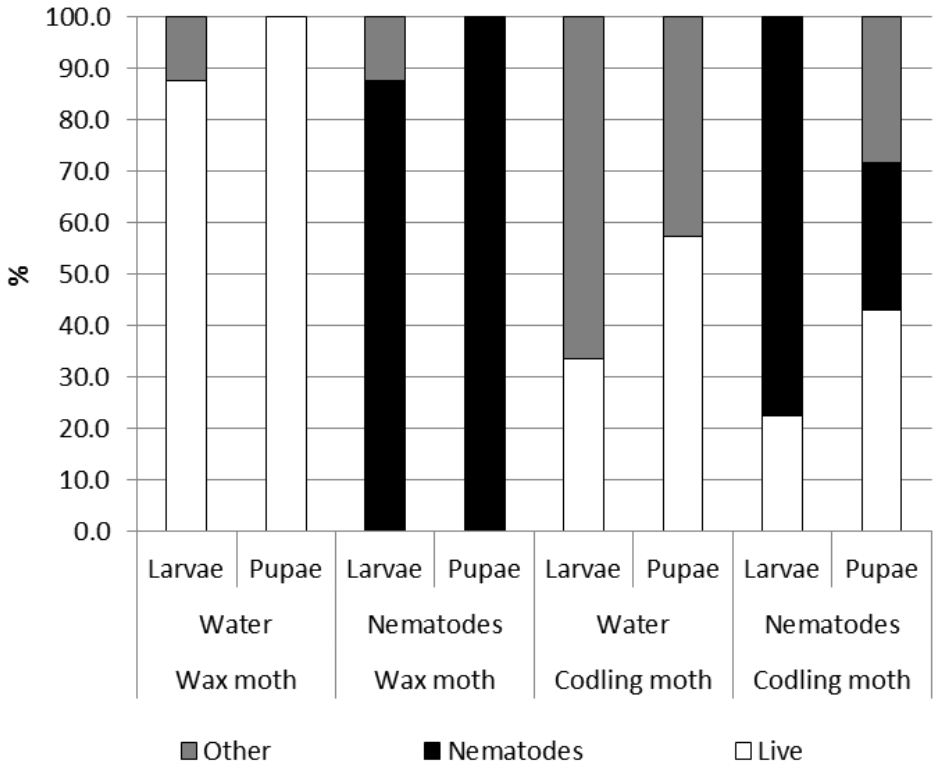


Figure 6.22. Percent mortality of the target species/life stages due to nematodes and other causes (fungal or bacterial) on 17 October

The wax moth larvae sprayed with water on 11 November did not die from nematode infection. Those sprayed with a full dose of nematodes all developed nematode infection. Only 2 wax moth larvae (12.5 %) treated with a 50 % concentration of nematodes survived (Figure 6.23).

The codling moth larvae had high levels of natural mortality due to bacterial and fungal infections which were evident in the water only treated cages. In the cages sprayed with 50 % and full dose nematodes, 62.5 % and 100 % died as a result of infection, respectively.

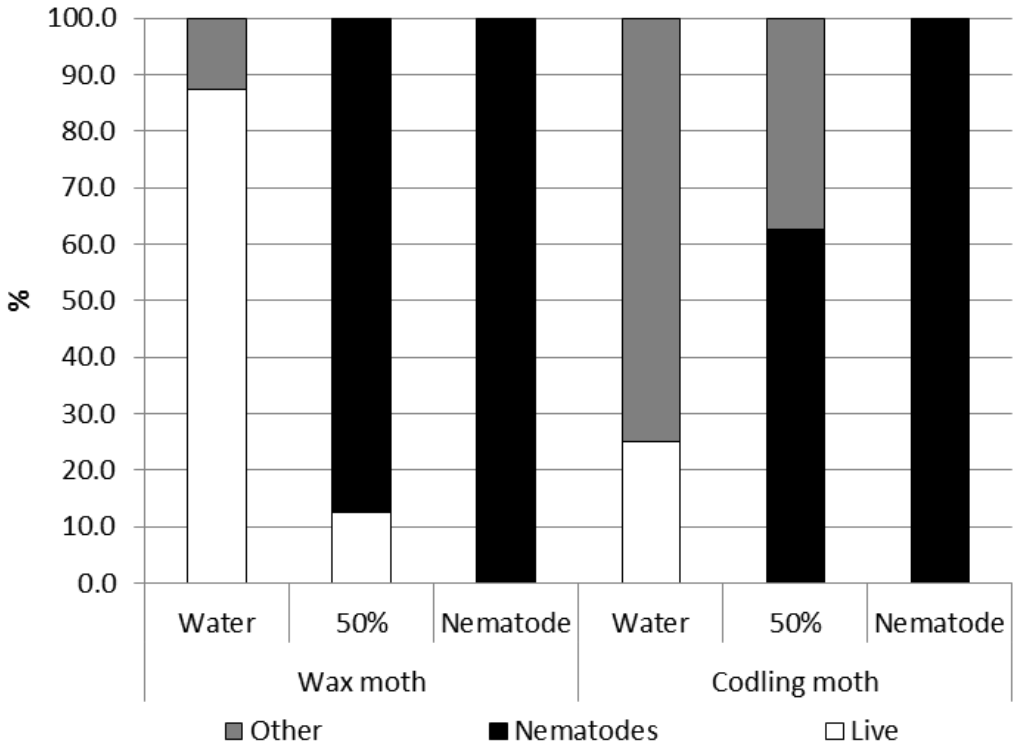


Figure 6.23. Percent mortality of the target species larvae due to nematodes and other causes (fungal or bacterial) 18 November

When the apple sawfly diapausing cocoons, sprayed on the 11 November, were dissected open to look for nematodes, it was discovered that of the 24 pupae used only 3 contained viable larvae. The rest had been dead for some time and only a dry husk was present inside the hard pupal case. Of the 3 live larvae 2 were in the water treated control and 1 in the full rate nematode spray. None of the larvae had developed a nematode infestation.

Conclusions

- Wax moth larvae were a good control to test for the viability of nematodes.
- Direct application of a droplet of 5 nematodes to the surface of larvae was not successful, probably because of the hydrophobic properties of the insect cuticle.
- Using a Birchmeier B245 motorized mist blower it was possible to infect codling moth larvae/pupae with nematodes, even when they were hidden within an artificial refuge.
- Codling moth pupae appear to be more resistant to nematode infection than larvae.
- These experiments do not rule out the efficacy of the nematode sprays against codling moth larvae in the field and the tests should now be repeated in the field with larvae in cardboard rolls without the mesh cages.

Objective 7	Improve reliability of natural enemies	Task 2	Dynamic pear sucker/ predator chart for growers

Task 7.2. Test and optimise a dynamic pear sucker/ predator chart for growers to use and gain better control of pear sucker (EMR, Yr 2-5)

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 we trained growers 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. David Long (Child's Farm) talked about his 5 year experience monitoring for the pest and its 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.

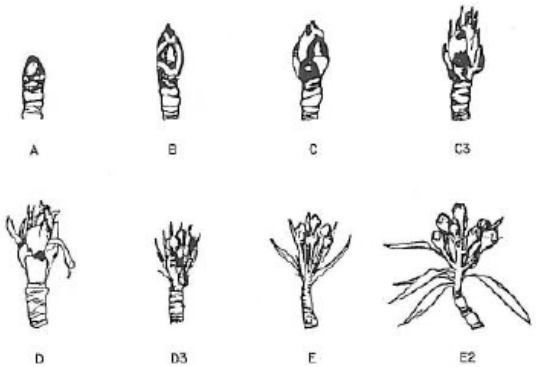
NIAB EMR devised a sampling method and record sheet which the persons responsible for reporting returned to NIAB EMR via email each week (Figure 7.1). The results were collated at least fortnightly and then shared with all participants.

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

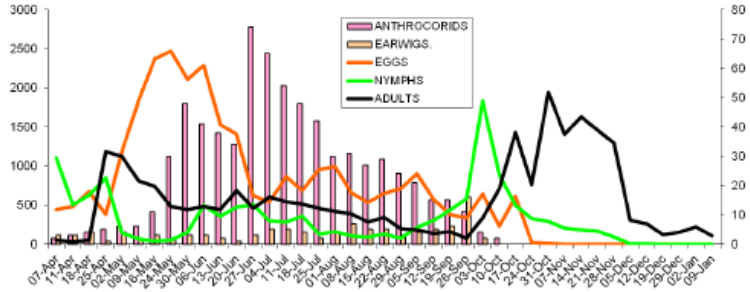
Name	Farm/Company	Person responsible for reporting
David Butler & William	GH DEAN	David Butler
Darren Wallis	AC GOATHAM	Darren Wallis
Nigel Jenner	AVALON	Ryan Williams
Russel Graydon	A SCRIPPS	Pam and Carol
Mark Chapman	AC HULME & SONS	Mark Chapman
Caroline & David Long & Tim Long	CHILD'S FARM	Elena/Katalina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

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)



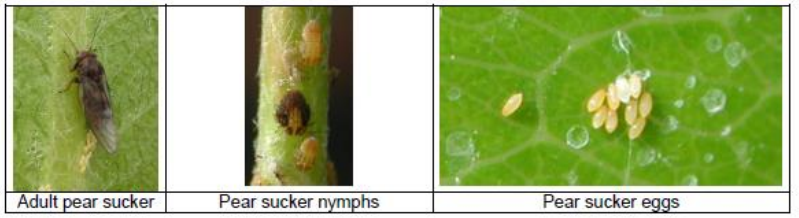
- Assess on the same day each week (e.g. Monday morning)
- Three orchards assessed (low, medium and high pear sucker pressure)
- 30 trees checked in each orchard in a W-shape
- Data should be entered onto an excel spreadsheet supplied by NIAB EMR and emailed weekly to michelle.fountain@emr.ac.uk



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



Monitoring predators

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

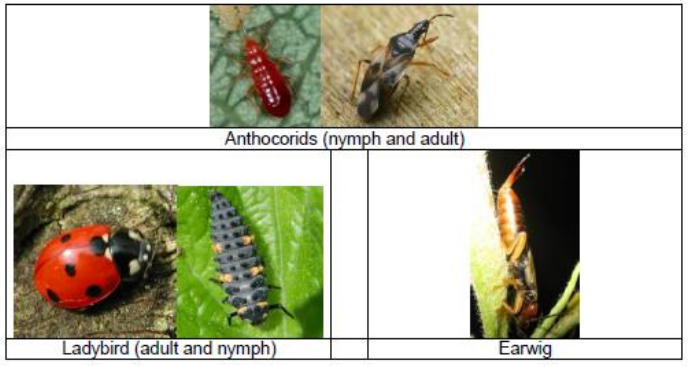


Figure 7.1. Sandard Operating Proceedure for monitirng pear sucker, key natural enemies and damage in pear orchards.

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. Unfortunately there were 2 breaks in data collection at Farm 3 at peak pear sucker times – April and June.

At Farms 1, 2 and 3 the first pear sucker eggs were laid in mid-April and mid to late March at Farms 4, 5 and 6. The second generation of eggs were laid at the end of May beginning of June with a subsequent smaller peak in pear sucker eggs in August. At Farm 5 anthocorids were released. There was a late attack of pear sucker in September; unfortunately the grower had stopped monitoring at this point and an Envidor was applied on 24 September. At this farm there were very few earwigs or ladybirds found and it was noted that multiple applications of sulphur were applied.

The majority of orchards did not reach large numbers of pear sucker eggs with the exception of Farm 2, high pressure orchard which reached 2000 eggs per 30 shoots at the second egg laying peak at the beginning of June. Anthocorids were ordered for this farm on 11 May and 11 July.

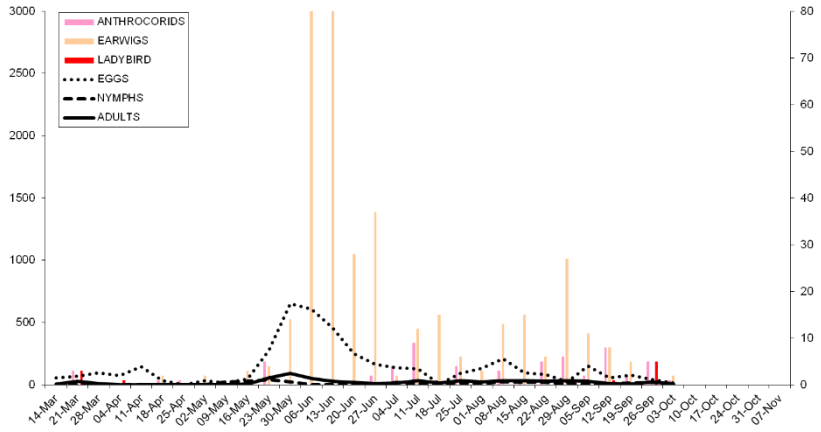
Farm 1, 4 and 6 had significant numbers of earwigs and anthocorids and did not reach a peak of pear sucker eggs of more than 500 / 30 shoots. Farms 2 and 3 had very few natural enemies present in the trees, but only Farm 2 had high populations of pear sucker in one orchard.

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. Although this data is showing some trends more seasonal data is required and future analyses could examine population trends over time.

It should be noted that these data analyses, to date, do not take into consideration the spray programmes or other 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 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



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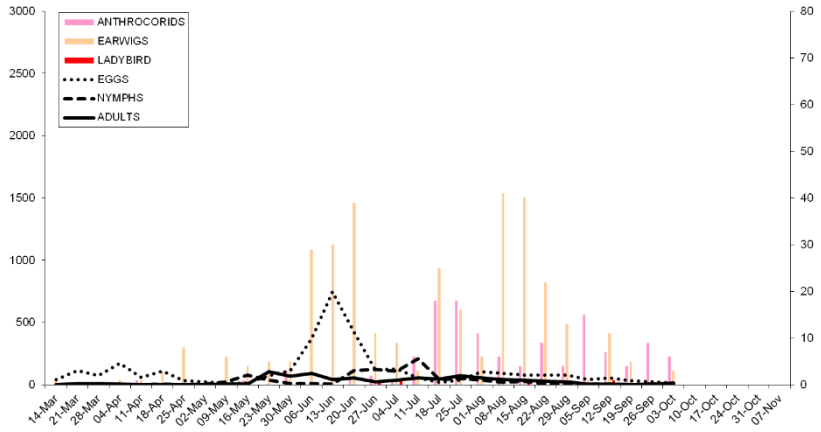

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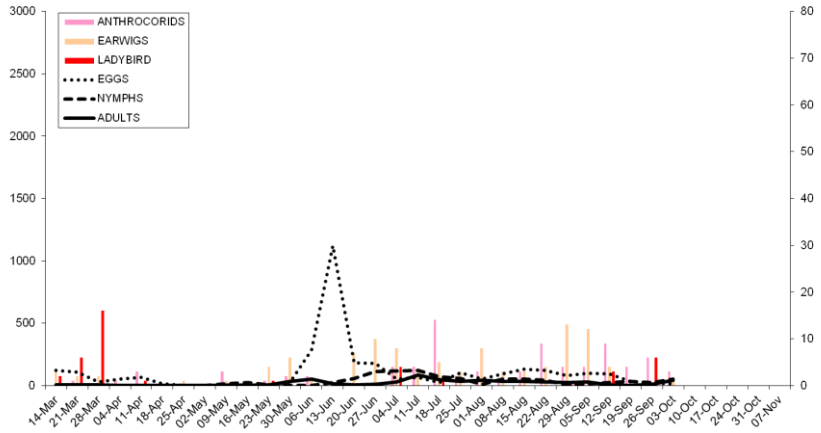

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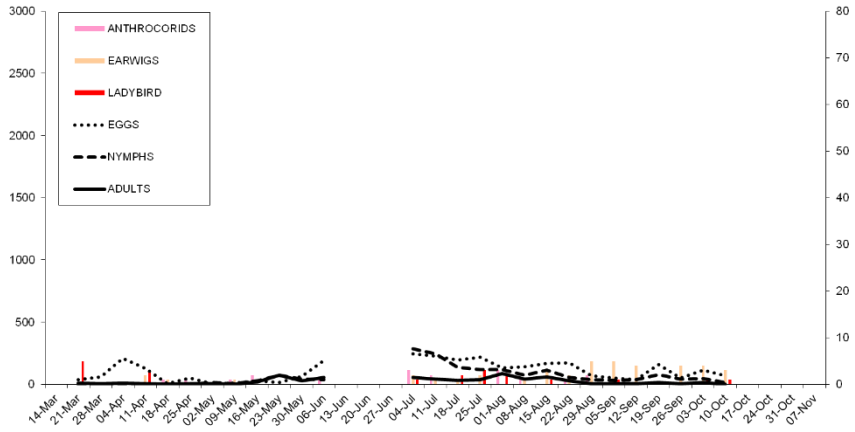
Figure 7.2. Farm 1 pear sucker and natural enemy monitoring in low, medium and high intensity pear sucker orchards during the growing season



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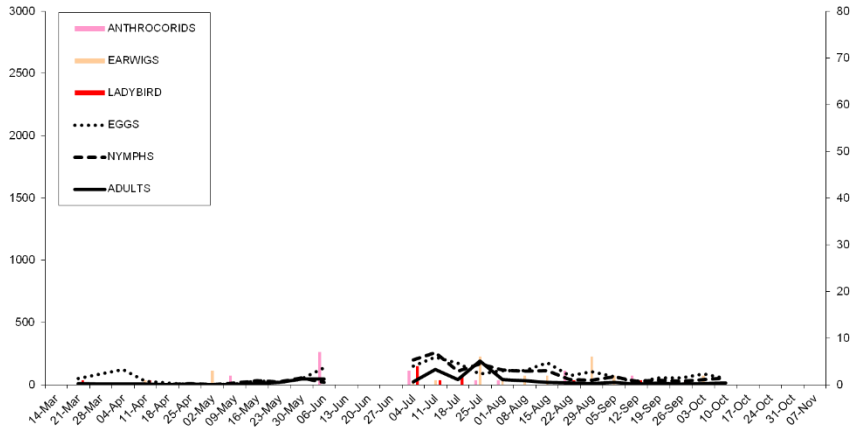

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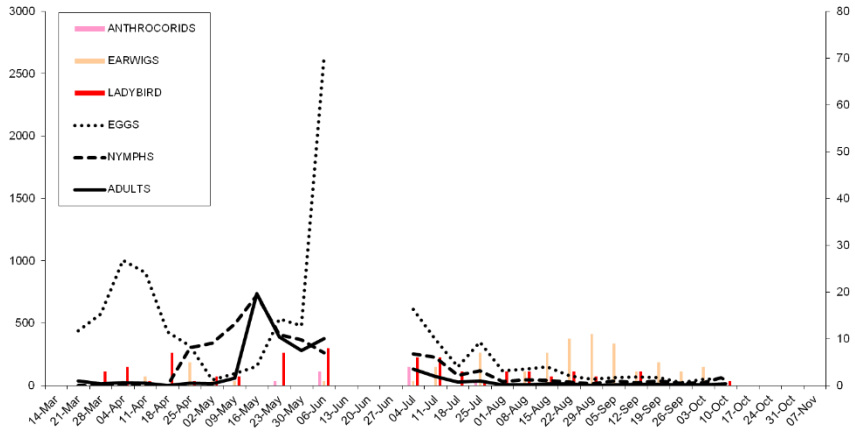

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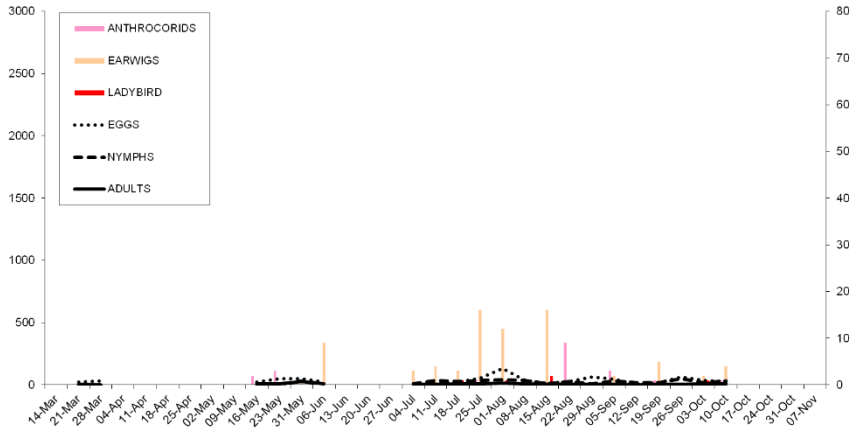
Figure 7.3 Farm 2 pear sucker and natural enemy monitoring in low, medium and high intensity pear sucker orchards during the growing season



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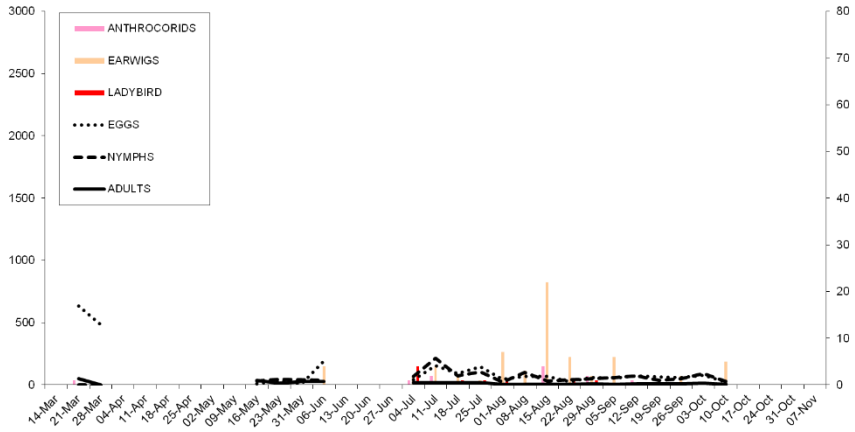

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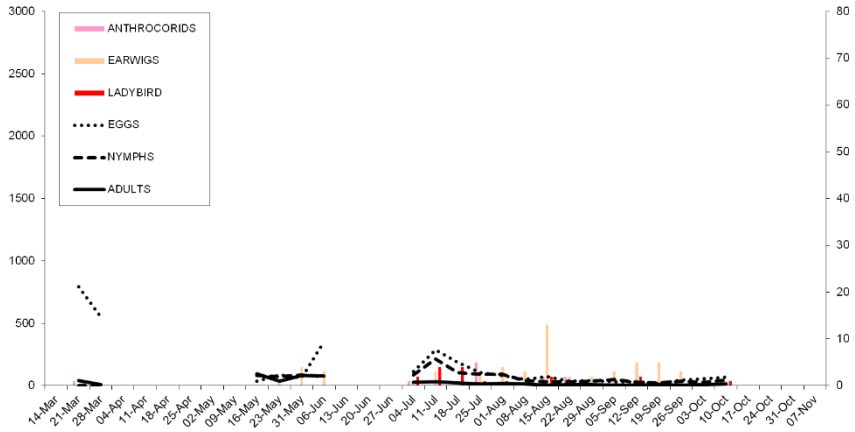

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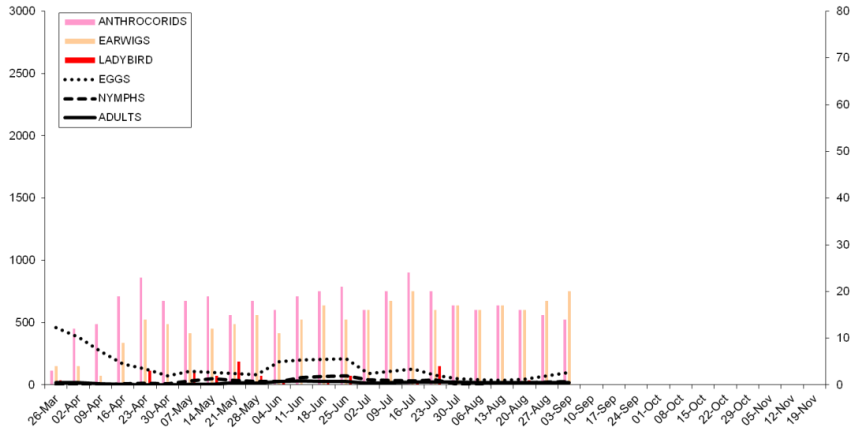
Figure 7.4. Farm 3 pear sucker and natural enemy monitoring in low, medium and high intensity pear sucker orchards during the growing season



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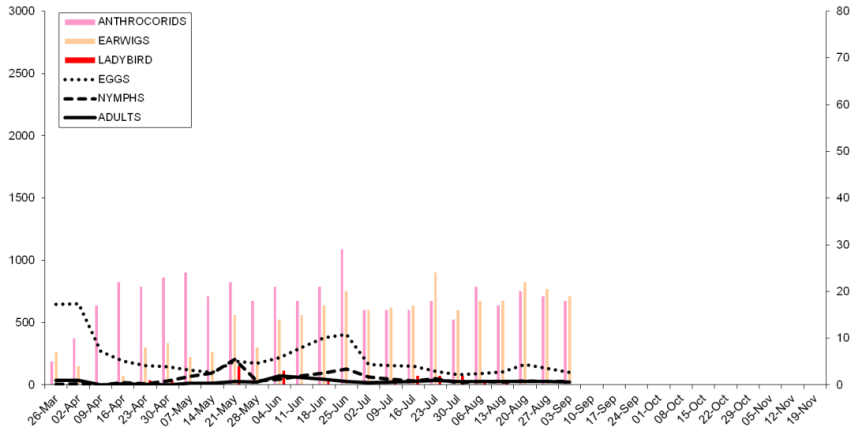

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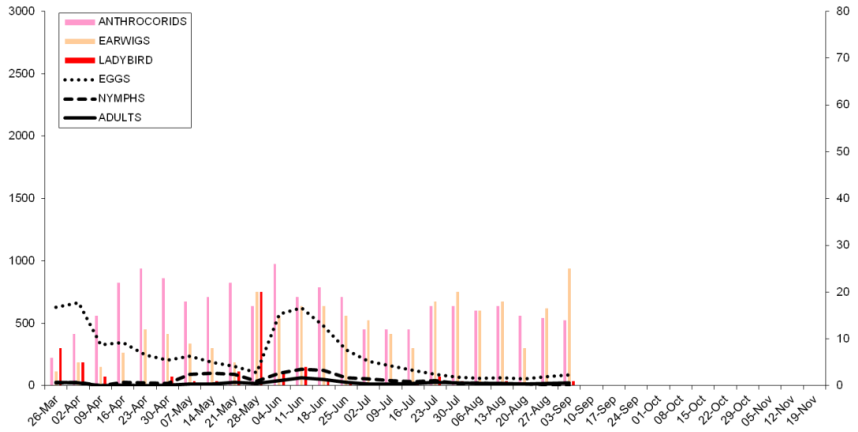

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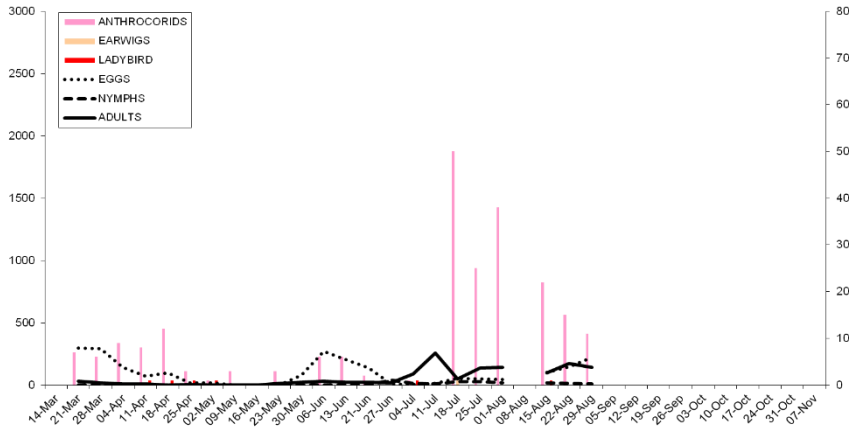
Figure 7.5. Farm 4 pear sucker and natural enemy monitoring in low, medium and high intensity pear sucker orchards during the growing season



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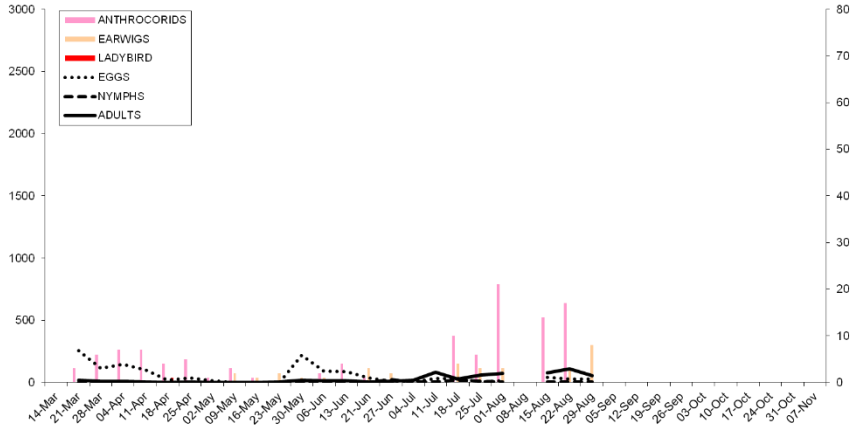

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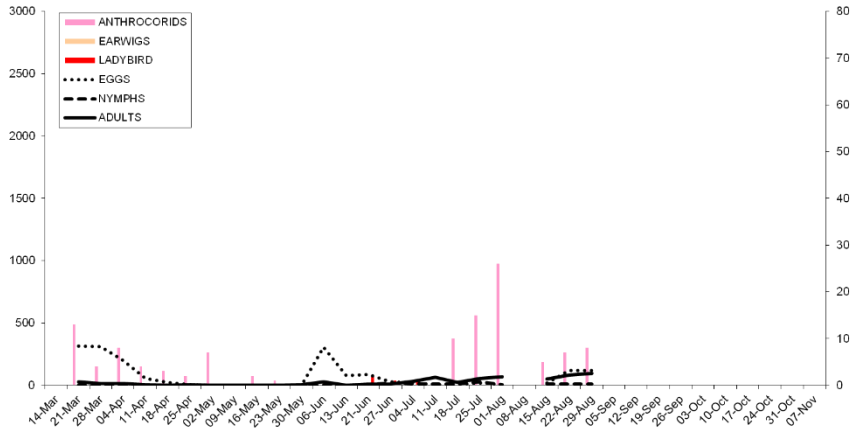

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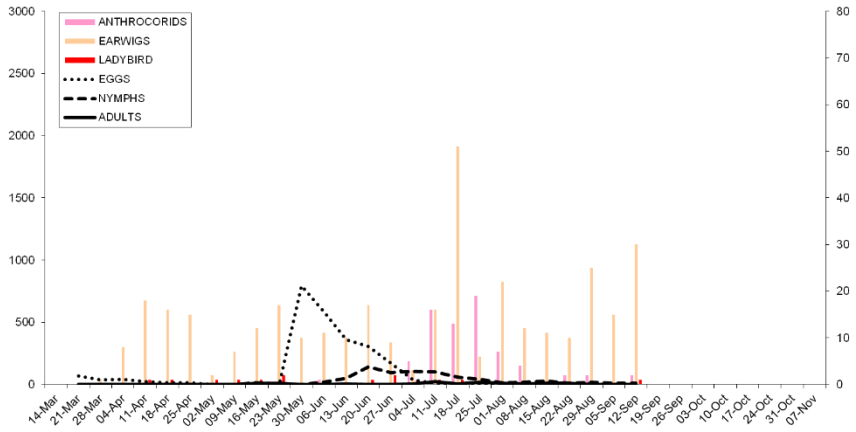
Figure 7.6. Farm 5 pear sucker and natural enemy monitoring in low, medium and high intensity pear sucker orchards during the growing season



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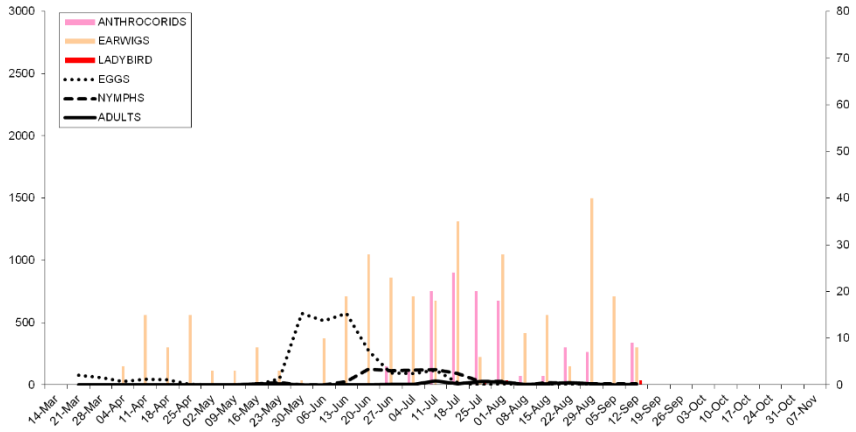

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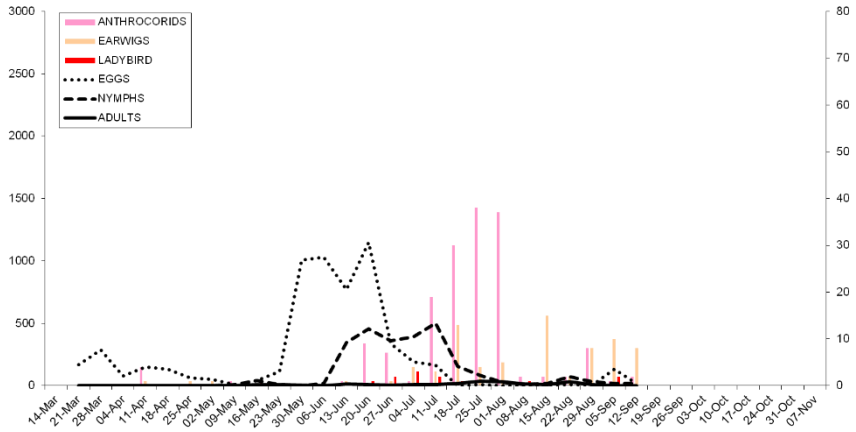

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Figure 7.7. Farm 6 pear sucker and natural enemy monitoring in low, medium and high intensity pear sucker orchards during the growing season

Table 7.3. Spray records for the farms including insecticides and sprays targeted against pear sucker and honeydew.

Farm	Perceived pear sucker pressure	Date	Product	Dose/ha	Volume rate	Area
1	Low	No data				
	Medium	21 Mar	Pyrinex 48	0.1	250 l	7.0
		19 May	Bittersaltz	6.0	330 l	
		30 May	Bittersaltz	6.0	330 l	
		9 Jun	Bittersaltz	6.0	330 l	
		21 Jun	Bittersaltz	6.0	330 l	
		9 Aug	**Coragen**	0.175	330 l	
	High	30 Mar	Calypso	0.375	400 l	7.27
		15 Jun	Runner	0.6	400 l	7.27
		21 Jun	Carpovirusine	1.0	800 l	7.27
		8 Jul	Carpovirusine	1.0	800 l	7.27
		1 Aug	Coragen	0.175	400 l	7.27
		28 Sep	Mag Sulph	4.0	400 l	7.27
2	Low	17 May	Bittersalz	5	450 l	1.16
		28 Apr	Mag Sulph	3.0	300 l	1.16
		24 Jun	Coragen	0.175	450 l	1.16
		13 Jul	Bittersalz	5	450 l	1.16
		21 Aug	Bittersalz	5	450 l	1.16
	Medium	1 Apr	Calypso	0.375	450 l	1.35
		17 May	Bittersalz	5	450 l	1.35
		28 Apr	Mag Sulph	3.0	300 l	1.35
		24 Jun	Coragen	0.175	450 l	1.35
		13 Jul	Bittersalz	5	450 l	1.35
		21 Aug	Bittersalz	5	450 l	1.35
	High	17 May	Bittersalz	5	450 l	3.84
		28 Apr	Mag Sulph	3.0	300 l	3.84
		7 Jun	Envidor	0.6	450 l	3.84
		15 Jun	Bittersalz	5	450 l	3.84
		24 Jun	Coragen	0.175	450 l	3.84
		13 Jul	Bittersalz	5	450 l	3.84
		20 Aug	Bittersalz	5	450 l	3.84
3	Low	21 Mar	Chlorpyrifos	1.0 l/ha	250 l	4.5 ha
	Medium (Radfield)	No data				
	High	22 Mar	Chlorpyrifos	1.0 l/ha	250 l	4.7 ha
4	Low	24 Mar	Headland Sulphur	3.0 l/ha	300 l	0.62 ha
		17 May	Headland Sulphur	3.0 l/ha	300 l	0.62 ha
		21 May	Mg Sulph Wetcit	7.5 kg/ha 1.5 l/ha	500 l	0.62 ha
		23 May	Headland Sulphur	3.0 l/ha	300 l	0.62 ha

		14 Jul	Mg Sulph	11.25 kg/ha	300 l	0.62 ha
		01 Aug	Mg Sulph	11.25 kg/ha	750 l	0.62 ha
		08 Aug	Mg Sulph	11.25 kg/ha	750 l	0.62 ha
	Medium	17 Mar	Headland Sulphur	3.0 l/ha	300 l	1.5 ha
		17 May	Headland Sulphur	3.0 l/ha	300 l	1.5 ha
		19 May	Mg Sulph Wetcit	7.5 kg/ha 1.5 l/ha	500 l	1.9 ha 1.9 ha
		23 May	Headland Sulphur	3.0 l/ha	300 l	1.5 ha
		29 May	Headland Sulphur	3.0 l/ha	300 l	1.5 ha
		10 Jun	Envidor Wetcit	0.6 l/ha 0.5 l/ha	750 l	1.9 ha 1.9 ha
		19 May	Mg Sulph	11.25 kg/ha	750 l	1.9 ha
	High	24 Mar	Headland Sulphur	2.0 l/ha	300 l	5.68 ha
		17 May	Headland Sulphur	2.0 l/ha	400 l	5.68 ha
		21 May	Mg Sulp Wetcit	7.5 kg/ha 1.5 l/ha	500 l	5.68 ha
		23 May	Headland Sulphur	3.0 l/ha	300 l	5.68 ha
		29 May	Headland Sulphur	3.0 l/ha	300 l	5.68 ha
		10 Jun	Envidor Wetcit	0.6 l/ha 0.5 l/ha	750 l	5.68 ha
		21 Jun	Headland Sulphur	2.0 l/ha	300 l	5.68 ha
		14 Jul	Mg Sulph	11.25 kg/ha	300 l	5.68 ha
		21 Jul	Mg Sulph Wetcit	11.25 kg/ha 1.5 l/ha	750 l	5.68 ha
		29 Jul	Mg Sulph	11.25 kg/ha	750 l	5.68 ha
		08 Aug	Mg Sulph	11.25 kg/ha	750 l	5.68 ha
		16 Aug	Mg Sulph	11.25 kg/ha	750 l	5.68 ha
5	Low and Medium	18 Mar	Surround	15.121	-	13.4
		19 May	Runner	0.599	-	13.4
		26 May	Sulphur	1.987	-	13.4
		3 Jun	Sulphur Bittersalz	2.016 2.016	-	13.4
		10 Jun	Sulphur	1.993	-	13.4
		14 Jun	Anthopak 500	1.193	-	13.4
		17 Jun	Sulphur Coragen	1.999 0.169	-	13.4
		24 Jun	Sulphur	1.999	-	13.4
		1 Jul	Sulphur	2.000	-	13.4
		8 Jul	Sulphur	2.000	-	13.4
		15 Jul	Sulphur	1.993	-	13.4
		18 Jul	Sulphur	1.999	-	13.4

		25 Jul	Bittersalz	6.250	-	13.4
		29 Jul	Sulphur Bittersalz	2.016 2.362	-	13.4
		08 Aug	Sulphur Bittersalz	2.016 2.362	-	13.4
		19 Aug	Sulphur Bittersalz	2.016 2.362	-	13.4
		30 Aug	Sulphur Bittersalz	2.016 2.362	-	13.4
		27 Sep	Sulphur Bittersalz Envidor	1.884 2.506 0.599	-	13.4
	High	18 Mar	Surround	15.121	-	3.95
		19 May	Runner	0.599	-	3.95
		26 May	Sulphur	1.987	-	3.95
		3 Jun	Sulphur Bittersalz	2.016 2.016	-	3.95
		10 Jun	Sulphur	1.993	-	3.95
		17 Jun	Sulphur	1.999	-	3.95
		24 Jun	Sulphur	1.999	-	3.95
		1 Jul	Sulphur	1.999	-	3.95
		8 Jul	Sulphur	1.999	-	3.95
		25 Jul	Sulphur	1.999	-	3.95
		18 Jul	Sulphur	1.999	-	3.95
		25 Jul	Bittersalz	6.25	-	3.95
		29 Jul	Sulphur Bittersalz Coragen	1.884 2.362 0.16	-	3.95
		8 Aug	Sulphur Bittersalz	1.884 2.506	-	3.95
		19 Aug	Sulphur Bittersalz	1.884 2.445	-	3.95
		30 Aug	Sulphur Bittersalz	1.884 2.506	-	3.95
		27 Sep	Sulphur Bittersalz Envidor	1.884 2.506 0.599	-	3.95
6	Low	09 Mar	Calypso	0.375	250 l	1.7
		17 Jul	Coragen	0.175	250 l	
		11 Jul	BitterSalz	5.0	250 l	
	Medium	09 Mar	Calypso	0.375	250 l	1.5
		05 Jun	Coragen	0.175	250 l	
		11 Jul	BitterSalz	5.0	250 l	
	High	26 Jun	Coragen	0.175	250 l	1.56
		26 Jun	BitterSalz	5.0	250 l	
		18 Jul	BitterSalz	5.0	250 l	

Objective 8	Rhynchites weevil and sawfly	Task 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)

Materials and Methods

Apple sawfly infected apples were collected in spring 2015 from Wiseman orchard at NIAB EMR and laid onto compost in mesh covered (top and bottom) bins. Larvae were allowed to crawl out and enter the compost. The three bins of apple sawfly larvae were kept outside until 22 January 2016 when they were brought into room temperature from outside. No apple sawfly adults emerged from these bins. Pupae were floated from the compost following NIAB EMR SOP 780 dissected and found to be infected with either bacteria or fungus. The previous winter had been very wet and it was speculated that the soil may have become too wet outside.

In spring 2016 apple sawfly infected apples were collected again and kept in dryer conditions in compost filled bins (as above) in the laboratory until November when the bins were transferred to outdoor conditions and covered to prevent too much rain into the bins. Initial analyses of 24 diapausing larvae have shown only 3 were alive. The bins will be bought into room conditions in spring 2017 for emergence of adults and headspace volatile collection for pheromone identification.

Objective 10	Pear Bud Weevil *Anthonomus piri*		Life cycle and control

Aim

Establish the activity period, lifecycle and toxicity of thiacloprid and acetamiprid on pear bud weevil (*Anthonomus sp.*).

Introduction

A new pest of pear, still to be identified is being investigated. The weevil is from the *Anthonomus* family of weevils known to feed and develop in buds and fruits of plants. Unlike *Anthonomus piri*, this weevil is feeding and laying eggs in unopened flower buds in the spring (Figure 8.1).

In order to control the weevil it is will 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 thiacloprid and acetamiprid on the weevil (*Anthonomus* sp.).

a)  b)  c) 

Figure 8.1. a) *Anthonomus sp.*damage to pear bud, b) egg inside bud, c) larvae in hollowed out bud

Materials and Methods

Activity period: Weevils were tap sampled from trees in daylight and then again at night to ascertain whether they were night active. Tap sampling was done at Beckets Conference pear orchard (Jim Gunyan's Farm, Maidstone). A transect was walked diagonally across the orchard on each visit stopping at 30 trees and beating one branch on each tree 3 times with a stick over a white trap. A head torch was used at night.

Grower field spray trial: A small unreplicated spray trial was planned. Four rows to the east of the orchard were left unsprayed and the rest of the orchard was sprayed with Calypso (thiacloprid) at the label rate by the grower on a warm evening on 9 March. The orchard was assessed on the evening of 14 March by tap sampling 30 trees on the treated and untreated rows. On 17 March 10 weevils were collected from another unsprayed orchard and dissected

to identify their sex. On the 30 March, 60 trees were tap sampled; 25 buds were collected for dissection for eggs and feeding damage. A tap sample of 30 trees was done at night on the 14 September at the original orchard where the spray trial was carried out but no weevils were found. Samples collected from growers/agronomist working were sent in and assessed.

Laboratory spray experiment: The two neonicotinoids (acetamiprid and thiacloprid) commonly used in top fruit orchards were tested at 100 and 50% field rate and compared to a distilled water control (Table 8.1).

Weevils were collected from a commercial pear orchard located in the South East on 29 March by tap sampling the canopy at night.

Treatments (Table 8.1) were made up to 1 litre with distilled water in graduated flasks. The spraying apparatus was a Burkard computer controlled sprayer (NIAB EMR Standard Operating procedure; APPENDIX, Table 1). The Burkard sprayer was calibrated to apply the maximum volume of liquid to each petri dish, 0.3 ml of the treatment at 10 PSI.

On 30 March weevils were transferred into labelled 5 cm petri dishes containing honey and paper towel segments saturated with water and left for 24 hrs in order to make sure the weevils were healthy. The following day weevils were transferred, using soft forceps, into inverted petri dishes (9 cm diameter, 63.63 cm^2 surface area) with a griddled lid ("base") and a paper filter disk at the bottom of the petri dish ("lid").

Table 8.1. Treatments applied with Burkard sprayer as 1000 l/ha

Active Ingredient	**Product**	**Mode of action**	**Field dosage**	**Recommended field spray volume (L/ha)**	**Field spray volume (L/ha)**	**Field concentration**
Water	Water	-	-	-	-	-
Acetamiprid	Gazelle	Nicotinic ACh receptor agonist	375 g/ha	500-1500	1000	0.375 g/l
Thiacloprid	Calypso	Nicotinic ACh receptor agonist	375 ml/ha	1000-1500	1000	0.375 ml/l
Acetamiprid	Gazelle	Nicotinic ACh receptor agonist	187.5 g/ha	500-1500	1000	0.1875 g/l
Thiacloprid	Calypso	Nicotinic ACh receptor agonist	187.5 ml/ha	1000-1500	1000	0.1875 ml/l

The weevils were sprayed with each treatment with the same volume of liquid. Some weevils moved under the filter paper or behind the rim of the mesh; this may have protected them from a direct application of the treatment. Using soft forceps the weevils were then immediately transferred back into the 5 cm petri dishes containing honey and water and maintained at 16^{o}C in a controlled temperature room (Figure 8.2). Trays holding petri dishes were placed inside polythene bags to prevent drying out. Weevils were maintained by topping up water and adding honey at every assessment.

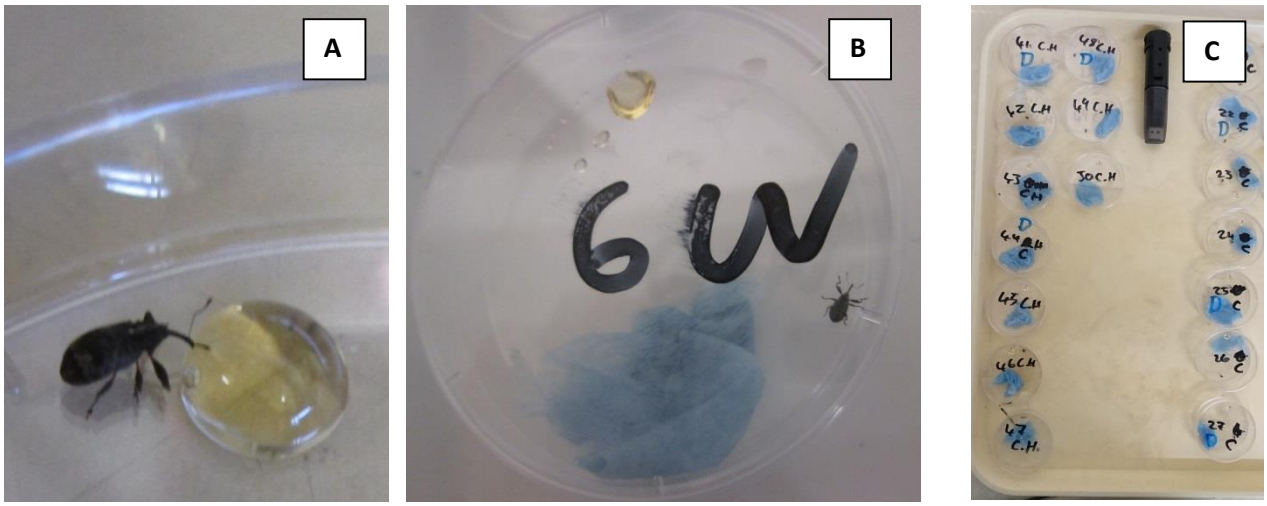


Figure 8.2. A) Weevil feeding on honey, B) pear bud weevil in 5cm petri dish with water and honey, C) Petri dishes and data logger

The weevils were assessed at the following time points after spray application until the weevils had made a full recovery or died: 0, 1.30, 16, 24, 96, 192 and 264h. 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

After the final assessment weevils were frozen, dissected and gender identified. When dissected, the females have a distinctive spermatheca which is in the shape of an apostrophe (Figure 8.3.C). Male weevils have an Aedeagus that is easily identified after dissection (Figure 8.3.B).

There were 10 replicates of the 5 treatments (50 petri dishes/pear bud weevils). The number of dead (or alive) out of 10 at the last date were analysed using a Generalised Linear Model

(GLM) with a Binomial distribution and a logit link. Pairwise comparisons between the five treatments were then carried out using t-tests on the means on the logit scale.

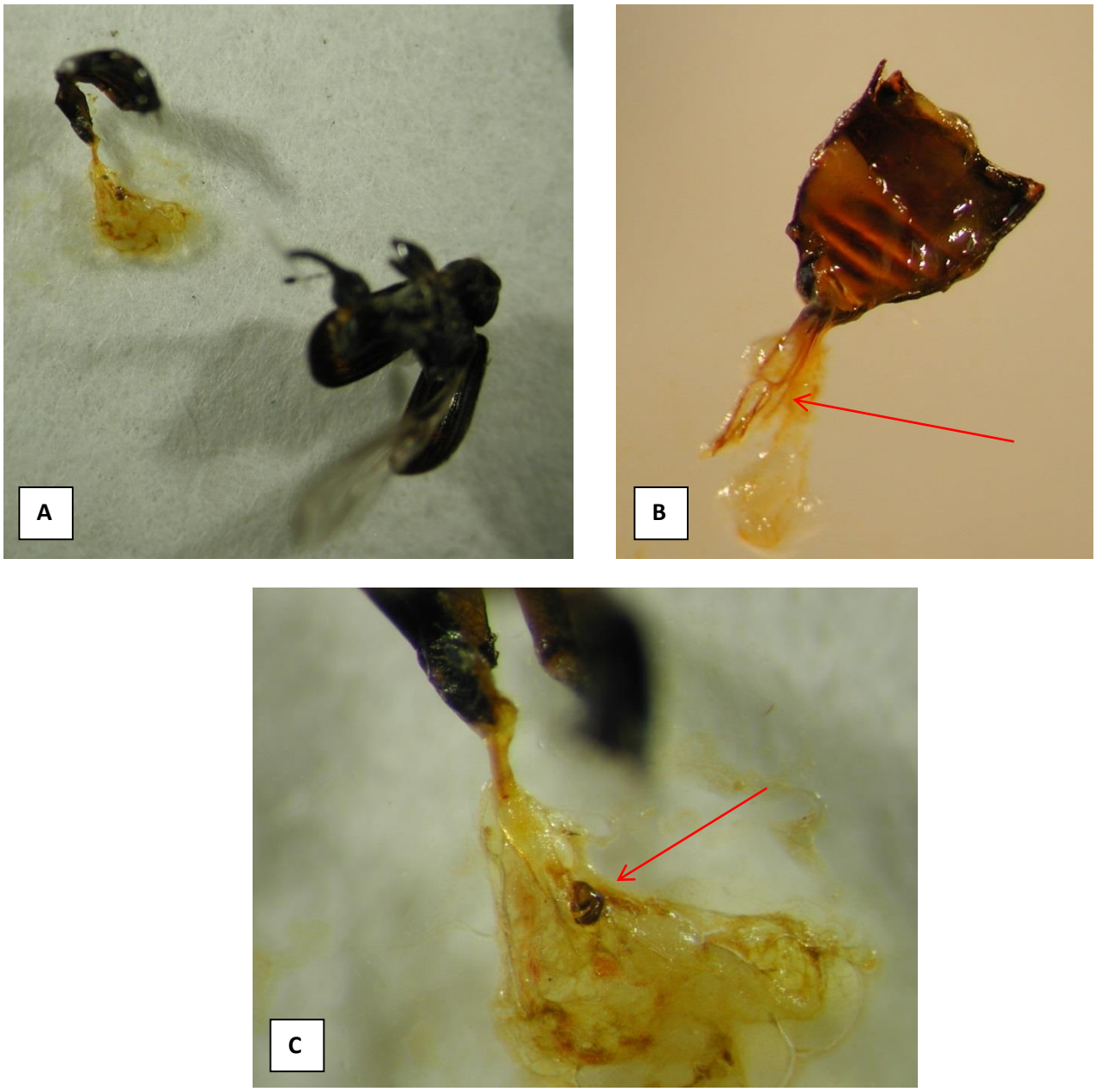


Figure 8.3. Dissected weevils A) Dissected female, B) Male penis, C) Dissected females spermatheca (red arrow)

Results

Activity period: Tap sampling in March at night and in the day indicated that the weevil was more active at night (Table 8.2). It was noted that the weevils found at this time were difficult to identify from *Anthonomus pomorum* by colour and pattern because of the high variation between individuals. The weevils were also dark in colour – similar to *A. pomorum*. There were many natural enemies in the trees at this time including centipedes, spiders and earwigs. There were also many alternative prey items including springtails and woodlice. When kept in culture the adult weevils were observed to feed on honey and were attracted to the buds of cut pear shoots. On 4 March, 20 pear buds were collected from the orchard and dissected open but no damage, weevil eggs or larvae were found.

Table 8.2. Results of tap sampling 30 pear trees and night and in the day for the weevil.

	2 Mar		**3 Mar**		**4 Mar**	
	Day	**Night**	**Day**	**Night**	**Day**	**Night**
Temp (Maidstone)	4-8°C	4-8°C	4-10°C	4-10°C	0-10°C	0-10°C
Weather	Very windy	Very windy	Slight Breeze	Slight Breeze	Slight Breeze	Slight Breeze
Notes weevils	Legs folded in	Legs folded in	Walking	Walking	Legs folded in	Legs folded in
Tree	16:30	19:00	15:30	19:00	12:20	19:10
TOTAL	**1**	**3**	**0**	**14**	**0**	**9**

Grower field spray trial: Nine weevils were found in 30 trees on the untreated side of the orchard and seven on the thiacloprid side of the orchard. However, six of the weevils on the treated side were moribund with the legs curled under the body. Weevils from the untreated side of the orchards were active and observed mating. Weevils were bought back to the laboratory, but by 16 March, two days after the assessment, those from the thiacloprid side of the orchard had died. From the 10 weevils that were collected on the 17 March, five were male and five were female. All females contained eggs. By 30 March only one weevils were found on 60 tap sampled trees. From the 25 buds collected five had weevil feeding damage but no eggs or larvae were found.

On 31 March, buds were bought in by an agronomist and half had feeding damage and an egg which had be laid just under the scale of one of the flower buds (Figure 8.4). Feeding damage appeared to go into the centre of the flower bud.

A tap sample of 30 trees was done at night on 14 September at the original orchard, but no weevils were found.

Figure 8.4. *Anthomonus sp.* egg laid in pear bud collected on the 31 March from pear orchard

Laboratory spray experiment: There was no statistical difference in percentage mortality between Gazelle treated weevils at half the maximum recommended concentration (0.1875 g/l) and at the maximum (0.375 g/l) and those treated with an untreated water control (Figure 8.5).

However weevil mortality (%) was significantly higher in those treated with Calypso (80-90%) at both concentrations (half-0.1875 ml/l and full-0.375 ml/l) than the untreated control (10%).

There were no significant differences in cumulative mortality at different time periods between treatments. However, Figure 8.6 shows the time taken after spray application for the weevils to die. The most effective treatment was Calypso which took eight days before 90% mortality was reached. After 10 days weevils were either dead or alive (Figure 8.6).

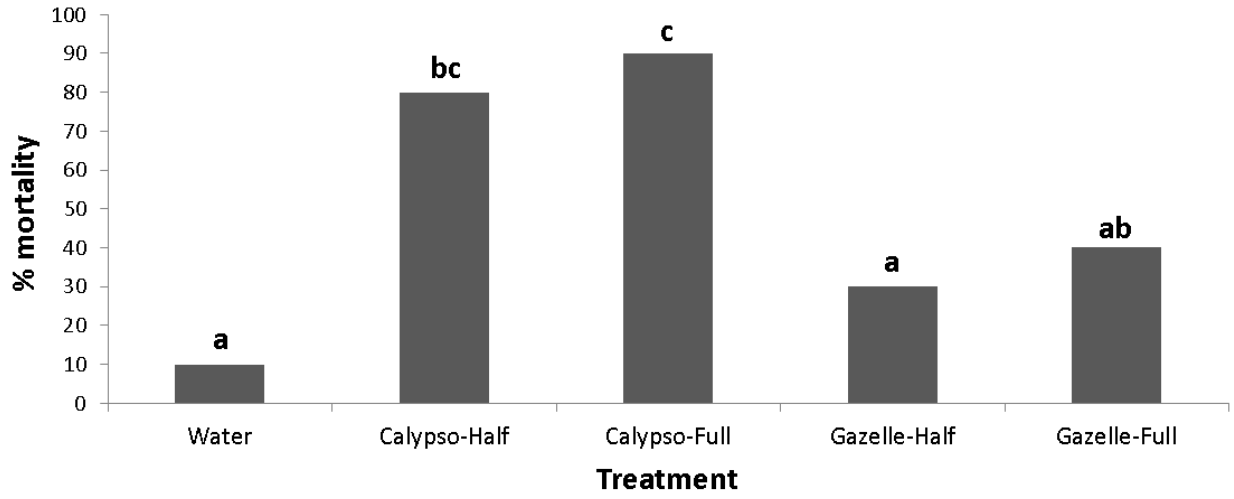


Figure 8.5. Cumulative mortality (%) 10 days after direct spray application.

Often weevils would “play dead” and it was difficult to determine whether the weevil was behaving abnormally.

There was no significant difference in the % mortality at the end of the trial between males and females and the treatments and dosages applied to the weevils. Seventeen of the 31 males died and 8 of 19 females.

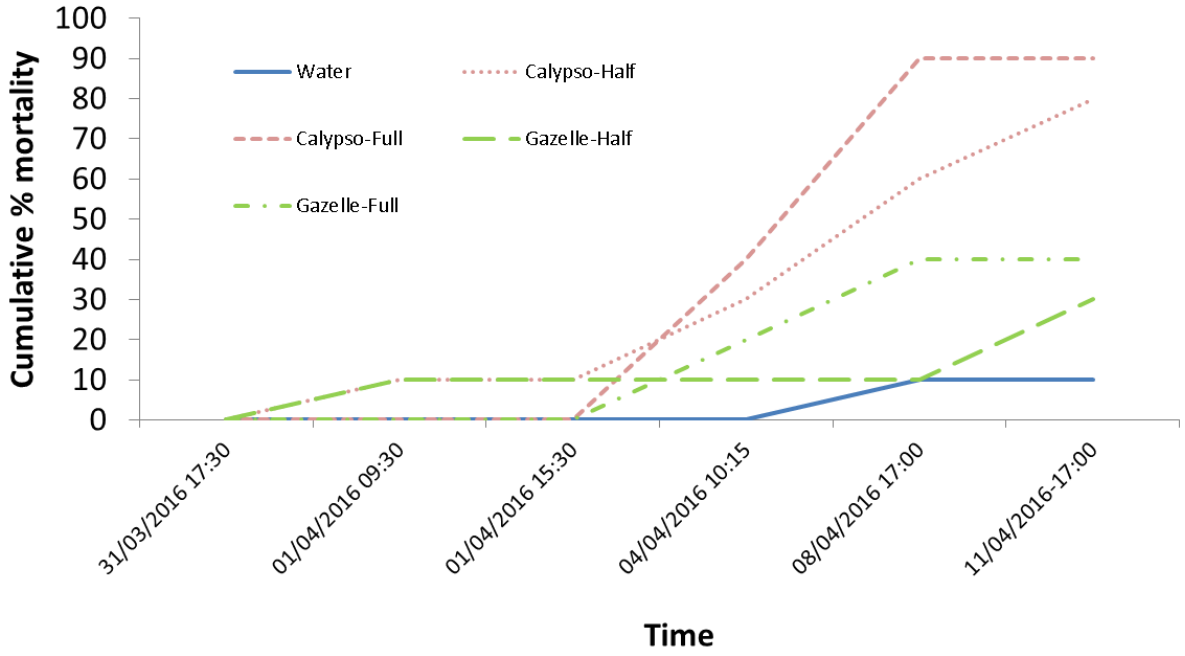


Figure 8.6. Cumulative % mortality of weevils at each assessment. For information only, no significant differences over time

Conclusions

- The weevil is active at night on warm still nights.
- The weevil was active through March, peaking in mid-March with egg laying.
- Mating occurred and females lay eggs in flower buds at bud swell.
- In laboratory tests Gazelle did not give effective control, but Calypso and full and half field rate gave 80-90% mortality.
- Calypso had effects on weevils within 3 days of application.
- Confirmation of the species is needed.
- More research is needed to inform the complete lifecycle of the insect, including activity in autumn (Figure 8.7).
- More research is needed on spray timing in the season and time of day orchards.
- Consideration should be given to natural enemies in each orchard.
- Weevils are very specific to orchards, hence it is important not to spray every orchard, but to monitor every orchard at night and spray where damage occurs.

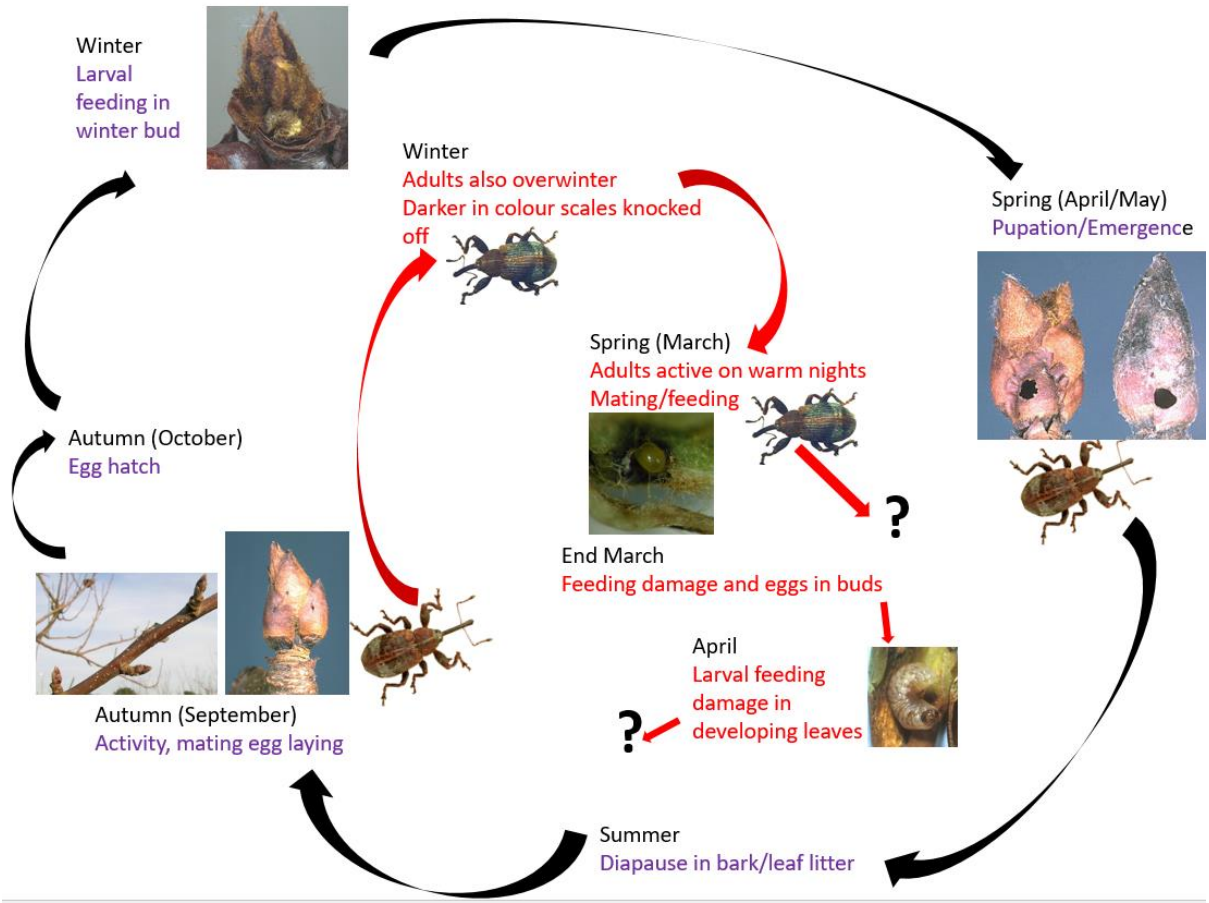


Figure 8.7. Life cycle of *Anthomonus piri* (black lines) and the *Anthonomus* sp. found in current orchards (red lines)

General Discussion

European apple canker is a devastating disease which requires a multifaceted approach to achieve control. Progress has been made on developing a detection tool to help increase our understanding of the disease. Long term trials have been established to look at the effects of rootstock/interstock and biological soil amendments on the susceptibility to this disease and the first year of trials to evaluate tree injection have been completed. In time these different approaches will be brought together to develop an IPM strategy for apple canker control from nursery propagation to established orchards.

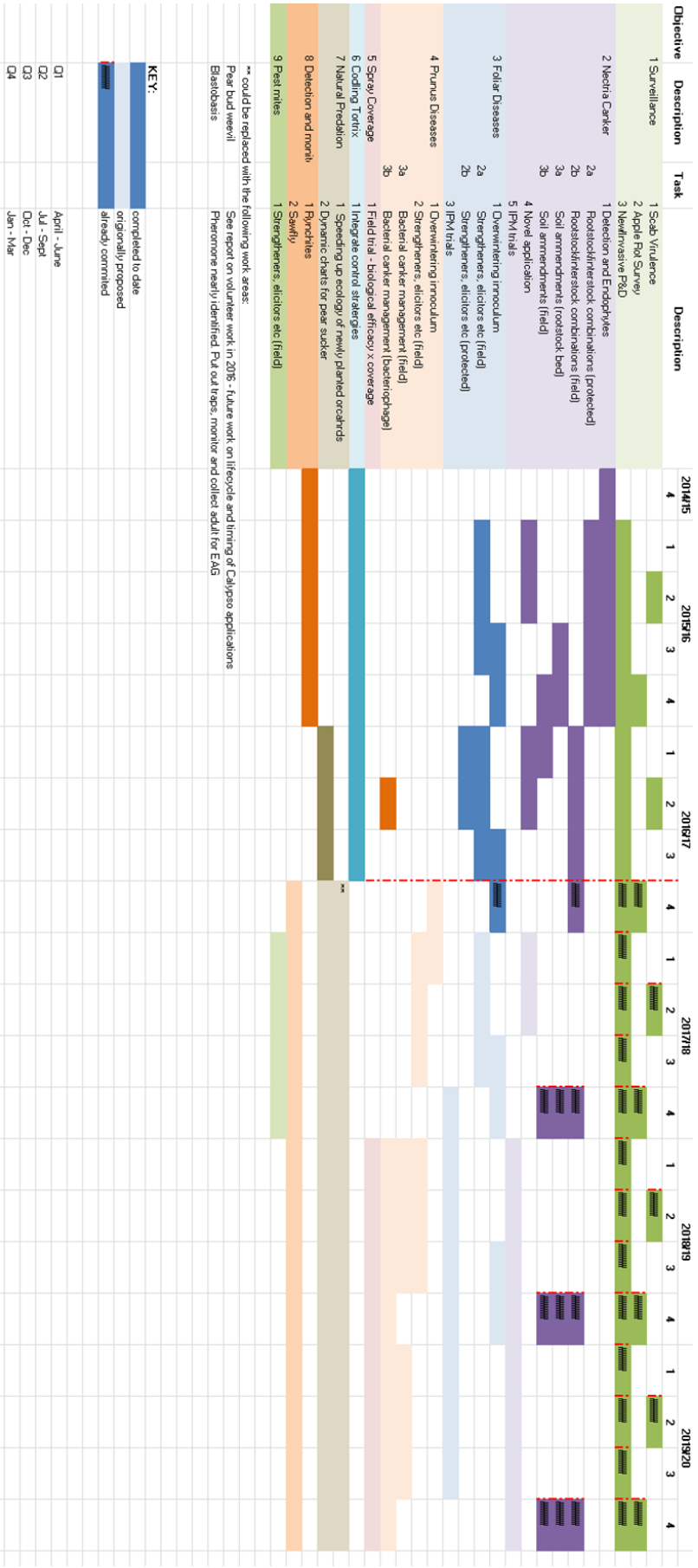
It is becoming increasingly difficult to control foliar diseases through the growing season with a reduced arsenal of conventional crop protection products. This project is evaluating new, alternative products and strategies to complement reduced fungicide programmes whilst maintaining commercially acceptable levels of disease control. Promising products have been identified building on findings from previous trials both within this project and without. Novel strategies are being developed for controlling overwintering inoculum, a key step to make in season control more attainable.

After two years' of trials on the same farms to determine the long term effects of the RAK3+4 mating disruption system this project has demonstrated that it gave comparable control of totrix and codling moths to conventional spray programs. The work has demonstrated that close monitoring of sporadic pests is needed as sporadic tortrix species and blastobasis caterpillars became incidental pests as a result of reduced applications of Lepidopteran insecticides.

Previous projects have shown the importance of natural predation for the control of pear sucker, this project is developing this concept by developing pest-preditor monitoring protocols for more effective pesticide use.

An additional piece of work, in light of the emergence of a new damaging weevil pest of pear has been investigated in this project. The identification and lifecycle of this pest is being determined in order to develop control strategies.

Forward planning



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 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.

Acknowledgements

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