Project title:	Improving integrated pest and disease management in tree fruit
Project number:	TF223
Project leader:	Dr Robert Saville East Malling Research
Report:	Annual report, March 2016 (Year 1)
Previous report:	None
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Date project commenced:	01/04/2015
Date project completed:	31/03/2020

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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 has principally focused on European apple canker, powdery mildew, codling and tortrix moths and apple fruit rhynchites weevil.

For ease of reading, this Grower Summary report is split into sections for each of the diseases and pests worked on in the first year.

Apple canker

Headline

- Early progress has been made in the development of a systematic approach to canker control from the nursery through to fruiting orchards.

Background and expected deliverables

The apple canker research within the project is embodied within Objective 2 which is to '*Develop an IPM strategy for apple canker control from nursery propagation to established orchards'*.

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.

The aim of this work is to identify IPM compatible practices which will reduce canker development in apple orchards. The focus is on nursery and early tree establishment phases. The work has been divided into several tasks;

(1) Develop a detection tool for Neonectria ditissima

(2) Evaluate susceptibility /resistance conferred by rootstock/interstock

(3) Evaluate whether biological soil amendments have an effect on canker development during the nursery phase and orchard establishment phase
(4) Evaluate novel application methods such as tree injection to target *Neonectria ditissima*

Summary of the project and main conclusions

Task 1: Develop a detection tool for Neonectria ditissima

Antibodies for the detection of *N. ditissima* are currently being developed by the Monoclonal Antibody Unit (MAU) at the University of Worcester. Several antibodies have been raised and assay optimisation will take place in year 2 to improve specificity.

Task 2: Evaluate susceptibility /resistance conferred by rootstock/interstock

Initial artificial inoculations have been conducted in the first year (whilst grafted trees are prepared for field evaluation). Artificial inoculations have shown a range of susceptibilities in rootstock accessions from those which limit lesion spread (MM106) to extensive disease progression (M9 clone 337). A larger panel of rootstocks (including Malling and Geneva series together with golden delicious interstock and advanced selections from the AHDB supported rootstock breeding club) are being grafted with a common Gala scion and will be evaluated in the field in 2017. This is a long term trial, which will be evaluated through the remaining life of the project. The results are expected to inform nursery and grower choice on rootstock/interstock.

Task 3: Evaluate whether biological soil amendments have an effect on canker development during the nursery phase and orchard establishment phase

Host stress has been hypothesised to be an important factor promoting canker disease expression. In this work, biological soil amendments (arbuscular mycorrhizal fungi, plant growth promoting rhizobacteria and Trichoderma) are being evaluated to determine whether they can be used commercially to reduce canker disease in orchards. The treatments are being evaluated both in a stool bed (representing the nursery phase) and in orchards (representing the orchard establishment phase). An additional treatment (biochar) has been included in one of the three trials as it has shown promising results in trials for ash dieback disease tolerance. This is a long term trial, which will be evaluated through the remaining life of the project. The results are expected to inform nursery and grower choice on the benefits of biological soil amendments at planting.

Task 4: Evaluate novel application methods such as tree injection to target *Neonectria ditissima*

A proof of concept trial for a tree injection system is being conducted in Spring 2016. The trial will evaluate various treatment categories (fungicides, biologicals, defence elicitors and plant health promoters) using a passive infusion device. Ultimately this technology may be used either in the nursery (to clean up mother trees used for scion wood) or by growers for spot treatment in newly establishing orchards.

Financial benefits

Traditional apple cultivars such as Cox and Bramley are on the decline in favour of new plantings of Braeburn, Gala, Cameo, Jazz, Kanzi, Zari and Rubens (increased in area by almost 50% since 2009). These cultivars offer the advantage of high consistent yields of first class apples that can compete with imported apples for retailer space. However, all these new cultivars are very susceptible to apple canker and annual tree losses due to tree death from trunk and systemic cankers (considered from nursery origin as latent infection in young trees) of around 10% or more are common. Establishment costs are £7 /tree or ~£30k/ha but the lack of effective methods to control canker has resulted in grubbing or extensive 'gapping up' of young orchards leading to financial losses. Canker also shortens the profitable life of an orchard and orchards will receive routine protectant sprays of fungicides pre- and post-harvest (average annual cost ~£700 /ha). Despite such stringent measures canker is not effectively controlled.

A systematic approach, from nursery propagation, through orchard establishment to established orchards could give effective canker control; reducing losses during tree establishment (targeting infection at the propagation phase) and improving efficacy of orchard control (novel and targeted applications).

Action points for growers

- At this stage in the project, it is too early to provide any action points for growers on canker control.

Apple scab and powdery mildew

Headline

- Test products are being compared with typical commercial fungicide programmes for the efficacy at controlling apple scab and powdery mildew.

Background and expected deliverables

The apple scab and powdery mildew research within the project is embodied within Objective 3 which is to *'Reduce reliance on fungicides for apple foliar disease control through promotion of plant health/resistance and off season 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 giving rise to fruit russet. 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.

The aim of the work carried out in the first year was to determine alternative products for the control of foliar diseases to complement a reduced conventional fungicide programme whilst maintaining or improving disease control.

Summary of the project and main conclusions

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. 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. Poor performing products will be removed from the treatment list whilst new products will be added. Promising treatments will be combined into programmes. By the end of the second year we expect to have a list of products with example programmes which growers can use to supplement the diminishing fungicide options available for season long foliar disease control.

Financial benefits

Routine sprays of fungicides for leaf disease control cost around £700/ha/annum with a large proportion spent on scab and mildew control. An integrated programme focused on reducing inoculum and promoting tree health/resistance could reduce fungicide applications whilst maintaining acceptable disease control. It is hoped that this could significantly reduce this typical spray programme cost.

Action points for growers

- At this stage in the project, it is too early to provide any action points for growers on scab or mildew control.

Codling and tortrix moths

Headline

- The RAK3&4 mating disruption system appeared to be very effective at disrupting male moth pheromone detection, but complete 'trap shut-down' (no moths captured) was not achieved for codling moth.

Background and expected deliverables

The codling and tortrix moth research within the project is embodied within Objective 6, which is to '*Develop improved apple IPM methods based on sex pheromone mating disruption and non-chemical controls (granulovirus) for codling and tortrix moths'*.

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

The aim of this work was to demonstrate the efficacy of sex pheromone mating disruption, alone versus in combination with granulosis viruses or nematodes, including effects on other pests and natural enemy populations. The effects will be examined over 2 growing seasons as the treatment with mating disruption pheromones is for long term control over a landscape scale.

Summary of the project and main conclusions

Two farms were selected, one in the South East and one in the West Midlands of England. Each farm was divided into RAK3&4 (supplied in kind by BASF) mating disruption (MD) system for control of codling moth (*Cydia pomonella* - CM) /tortrix moths (*Adoxophyes orana* - summer fruit tortrix - SFT and *Archips podana* - fruit tree tortrix - FTT) whilst the other half of each farm received the growers conventional spray programme. In addition, two plots on the MD side were treated with either codling moth granulosis virus (Cyd-X Xtra) and summer fruit tortrix moth granulosis virus (Capex) or Nemasys C (a.i. *Steinernema carpocapsae*). There was also an area (few rows) on the growers side of the farms which was left untreated with caterpillar sprays to determine what the damage would have been with no treatment at all.

Over six hectares on each farm was treated with RAK3&4. The trial data could not be analysed statistically as there were only two replicates (two farms). The trial will continue into

the 2016 growing season.

Assessments were made at each farm of the numbers of pests and natural enemies 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 moth and tortrix assessments, fruit that had dropped to the ground and tree fruits on whole trees were assessed. Other notable pest damage was also recorded.

At the South East site, the first generation flight of CM was above the threshold of 5 moths per trap for 5 weeks in the growers' conventional side of the farm, but only one week on the mating disruption side of the farm. Codling moth catches were very low at the site in the West Midlands. SFT was only present in low numbers at the farm in the South East and was not detected at the farm in the West Midlands. FTT moth catches were below threshold in the South East site but reached threshold in the West Midlands.

By the July assessment, conventional spray programmes, MD and virus applications had been made. There were some distinctions between the farms. The South East farm had higher numbers of earwigs. The West Midlands farm had higher numbers of woolly apple aphid (WAA) and harvestmen in the trees. There were also arthropod differences between the two halves of each farm, but at this time it is not known whether these are the result of the treatments or the location on the farm. For example there was a higher incidence of apple grass aphid (AGA) and lower incidence of earwigs on the conventional side of one farm and a higher incidence of AGA on the MD side of the other farm, where there were fewer earwigs.

By the harvest assessment, all of the treatments except nematodes had been applied. Encouragingly there were higher numbers of earwigs on the MD side of the farm in the South East compared to the conventionally sprayed plots and it remains to be seen if these earwig numbers continue to rise into year 2 of the trial. The site in the West had low numbers of earwigs overall.

On both farms the RAK3&4 system appeared to be very effective at disrupting male moth pheromone detection, but complete 'trap shut-down' (no moths captured) was not achieved for codling moth. There were some promising trends with the numbers of earwigs on both farms, being higher on the MD treated side of the farm. At the West Midlands site where there was a low CM pressure, there was negligible damage on the MD side of the farm and damage was comparable to the growers standard spray programme (see Table 6.9a below). At the South East site where the CM pressure was higher, there was higher damage in Early Windsor, Cox and Bramley varieties in the MD side of the farm (see Table 6.9a). These varieties were not present on the growers spray programme side.

Varieties which were on both sides – Gala and Braeburn – had similar CM damage to the

fruits in both grower spray programme and MD treatments. It may be advantageous at farms with medium to high pressure codling numbers to apply an additional Coragen to early ripening or vulnerable varieties where MD technologies are employed.

FTT pressure was high at one of the farms but there was no damage difference between the MD treatment and the grower's programme. At this time there was no evidence that additional sprays of viruses for CM and SFT had added benefit to the MD method.

Table 6.9a. *Summary of percentage codling moth (CM) and tortrix damage to dropped and tree fruit on both farms*

South East Farm					
Treatment	Variety	Dropped fruits		Tree fruits	
		CM	Tortrix	CM	Tortrix
Untreated	Braeburn	5.4	0.0	0.4	0.0
Growers programme	Gala	0.0	0.0	0.6	0.0
	Gala	0.0	0.0	0.3	0.0
	Gala	0.0	0.0	0.0	0.0
	Braeburn	4.5	0.0	0.3	0.1
MD only	E. Windsor	6.2	0.0	2.8	0.5
	Gala	0.0	0.0	0.7	3.1
MD + viruses	Bramley	2.1	0.3	1.5	0.2
	Cox	3.3	0.0	0.4	0.5
MD + nematodes	Gala	0.0	0.0	0.7	2.0
	Braeburn	5.8	0.3	1.2	0.4
West Midlands Farm					
Untreated	Royal Blush	10.3	0.0	0.7	0.1
Growers programme	Gala	0	0.0	0.1	0.1
	Red Windsor	0	0.0	0.1	0.2
	Cox	0	2.7	0	0.1
MD only	Gala	0	0.0	0.7	0.1
	Gala	0	0.0	0.1	0
MD + viruses	Gala	0	0.0	0	0.1
	Red Falstaff	0	0.0	0	0.1
MD + nematodes	Red Falstaff	0	0.0	0	0.3
	Gala	0	0.0	0	0

Financial benefits

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.

Action points for growers

- It may be advantageous at farms with medium to high pressure codling numbers to apply an additional Coragen to early ripening or vulnerable varieties where MD technologies are employed.

Apple fruit rhynchites

Headline

- There may be a window of opportunity to target weevils with control options both pre bloom and at petal fall – when females are likely to be laying eggs.

Background and expected deliverables

The apple fruit rhynchites research within the project is embodied within Objective 8, which is '*To improve the detection and monitoring of apple fruit rhynchites weevil and sawfly to enhance control by approved pesticides'*.

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.

The aim of this study was to investigate the presence of semiochemicals attractive to apple fruit rhynchites weevil.

Summary of the project and main conclusions

In the first year's work, volatile collections were made from field-collected male and female weevils and analysed. Significant quantities of any compounds associated with either sex of the weevils could not be reliably detected and no attraction was demonstrated using weevils as bait in orchards. However, it was shown that weevils entered the orchard in all varieites once bud scales were first visible and antennal responses were found in reaction to a flower bud compound. There was a window of opportunity to target weevils with control options both pre bloom and at petal fall – when females are likely to be laying eggs

Financial benefits

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.

Action points for growers

- At this stage in the project, it is too early to provide any action points for growers on apple fruit rhynchites control.

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 2015 season and has been submitted to the project coordinator. Analysed data will be made available in due course. The continued monitoring of scab virulence is important to understand fungus epidemiology and deployment of resistance genes in breeding programmes.

Objective 1	Surveillance	Task 2	Apple rot survey

Aim

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

Summary

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. Data from this season's survey was still being collected and collated at the time of writing.

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. Numbers 30% higher in woodlands in winter 2015-16 compared to the same period in the previous year.
	Summer fruit tortrix	Detected for the first time in the West Midlands during the 2015 growing season.
	Marmorated stink bug	Monitoring traps have been installed (none found as yet)
	Pear Bud Weevil	An incidental pest re-emerging since the introduction of reduced applications of insecticides for pear sucker. A factsheet has been produced and disseminated to growers and further work is being carried out to optimise a monitoring strategy. The AHDB factsheet can be found at: http://horticulture.ahdb.org.uk/publication/1715-pear-bud-weevil
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://secure.fera.defra.gov.uk/phiw/riskRegister/plant-health/documents/PLANT_DISEASE_FACTSHEET-Xanthomonas_arboricola_pv_pruni.pdf
	Xylella fastidiosa	A devastating bacterial disease which has a wide host range including *Prunus*. 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://secure.fera.defra.gov.uk/phiw/riskRegister/plant-health/documents/notifiable_diseases/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 based 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

The development of an antibody for the detection of *N. ditissima* has been subcontracted to the Monoclonal Antibody Unit (MAU) at the University of Worcester. Key tasks are summarised in Table 2.1. In brief; EMR's reference *N. ditissima* isolate, R09/05, was supplied to MAU. A solution of antigens (molecules which bind to the Ag-specific receptors of antibodies) was prepared. Six mice were immunized (antigen solution was introduced into the mice) twice over a 2 month period. After a further 14 day period, tail bleeds were carried out and an enzyme-linked immunosorbent assay (ELISA; a laboratory technique to measure the concentration of an antibody (or antigen) in solution) was used to determine whether any of the bleeds contained antibodies recognising *N. ditissima.* The ELISA identified the best candidates and the Monoclonal Antibody Unit are currently cloning the candidates. Validation will follow using antigen solutions prepared from 2 additional *N. ditissima* isolates; TL88 and R28/15 (positive control antigens), and antigen solutions prepared from 7 fungi commonly found in apple orchards (Fig. 2.1. negative control antigens); *Fusarium lateritium*, *Venturia inequalis* (scab), *Nectria cinnabarina* (coral spot), *Monilinia laxa* (brown rot), *Phomopsis/diaporthe*, *Colletotrichum acutatum* and *Botryosphaeria obtuse*.

Table 2.1. Diary sheet of key tasks carried out by the Monoclonal Antibody Unit (MAU) at the University of Worcester.

Antigen (for immunization) provided:	June 2015
Cross reactivity antigens supplied:	August/September 2015
Immunization commenced:	21st July 2015
Tail bleeds available:	9th September 2015
Tail bleed ELISAs carried out:	18th September 2015
Fusion 1:	14th October 2015
Identified cell lines cloned (x3):	November 2015 — December 2015
Cross reactivity studies:	January 2016

Figure 2.1. Negative control antigen collection. Isolates of species commonly found in apple orchards have been collected and sent to the monoclonal antibody unit at the University of Worcester to validate the antibodies generated.

Results

Two antibodies (UW 362 and UW 363) were initially tested for cross reactivity to positive antigens (prepared from *Neonectria ditissima*) and negative antigens (prepared from fungal species commonly found in apple orchards). Figure 2.2 shows the absorbance readings. Both antibodies recognise all three isolates of *N. ditissima* however the antibodies are also reacting to negative antigens. UW362 (blue) is the least specific reacting to all negative antigens and

particularly strongly with *Fusarium lateritium*, *Venturia inequalis*, *Nectria cinnabarina, Monilinia laxa* and *Botryosphaeria obtuse*. UW 363 (orange) is more specific, only recognising *Fusarium lateritium, Nectria cinnabarina* and *Botryosphaeria obtuse*. These species have the highest degree of relatedness to *Neonectria ditissima* of those represented in the negative antigen panel. Six more antibodies have been selected from a second fusion and will be evaluated to see whether specificity can be improved. Once the best antibodies have been selected based on results from the cross reactivity tests then further assay optimisation can be carried out to improve specificity.

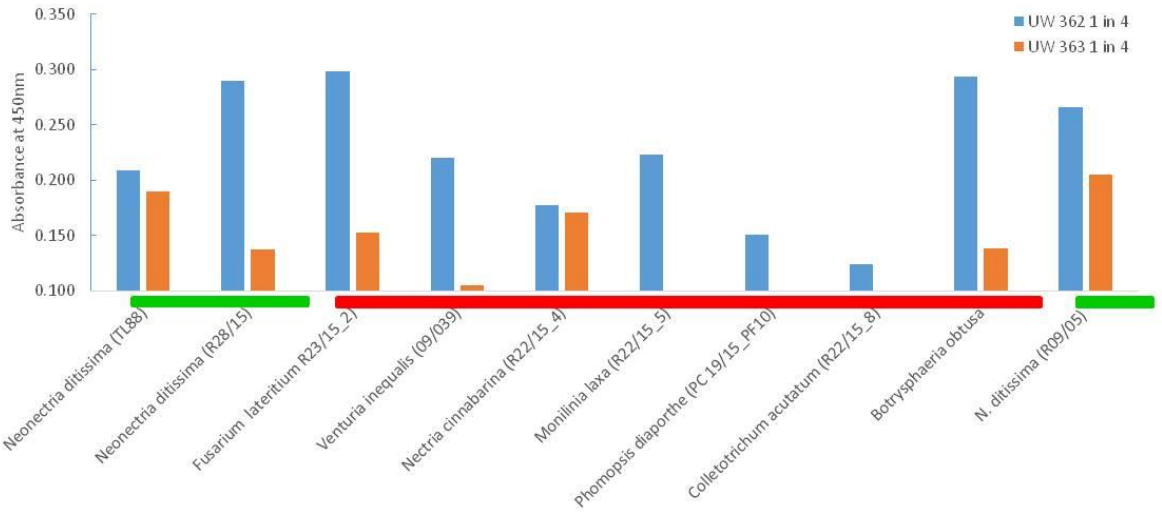


Figure 2.2. Cross reactivity assay showing 2 cell lines and how they react to positive antigens (*Neonectria ditissima*, green) and negative antigens (species commonly found in apple orchards, red).

Conclusions

- Antibodies have been raised which recognise *Neonectria dittisima*
- Further work is required to improve specificity

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 *Nectria*. 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 rootstock breeding club. 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 has been grafted during the winter of 2015/16 and will to be planted out during the winter of 2016/17.

Materials and Methods

Cold stored bare rooted rootstocks of various cultivars (Table 2.2) were potted up into 2 litre pots in June and established in a polytunnel. Established rootstocks were moved to a chilled glasshouse in August (Fig. 2.3), set at a maximum day temperature of 20°C with no additional lighting. Misting lines were hung under benches (with 360° misting units at approximately 60 cm intervals along the underside of the bench). These were placed on a timer, spraying for ten minutes at 6 hourly intervals to achieve a minimum humidity level of 80% RH.

Three leaves from each plant were removed; the fifth, tenth and fifteenth. The corresponding axillary bud was also removed. Inoculation points were prepared by cutting just below the bud wound, a little below the second abscission layer; the width of the incision was approximately 2-3mm. Within five minutes of cutting, 3µl of a conidial suspension (prepared from isolate R28/15 at a concentration of 1.1 $x10^5$) was placed onto the wound with micropipette. Inoculated wounds were covered with white petroleum jelly within five minutes of the droplet being absorbed and removed seven days later with a tissue. Lesion size was recorded after the first signs of infection became visible (Fig. 2.3). In total 6 assessments were carried out.

Table 2.2. Rootstock material to be evaluated.

Rootstocks evaluated in 2015[1]	**Rootstocks to be evaluated in 2016[2]**
EMLA M9	EMLA M9
M9 Clone 337	M9 Clone 337
M116	M116
MM106	MM106
EMR-001 (advanced selection)	EMR-001 (advanced selection) M26
	Geneva 11
	Geneva 41
	M9 Clone 337 with GD interstem
	EMR-002 to EMR-006 (advanced selections)

[1]Evaluated using artificial pathogenicity test. [2]To be grafted with common gala scion during winter 2015/16 and planted out in trial orchards during winter 2016/17

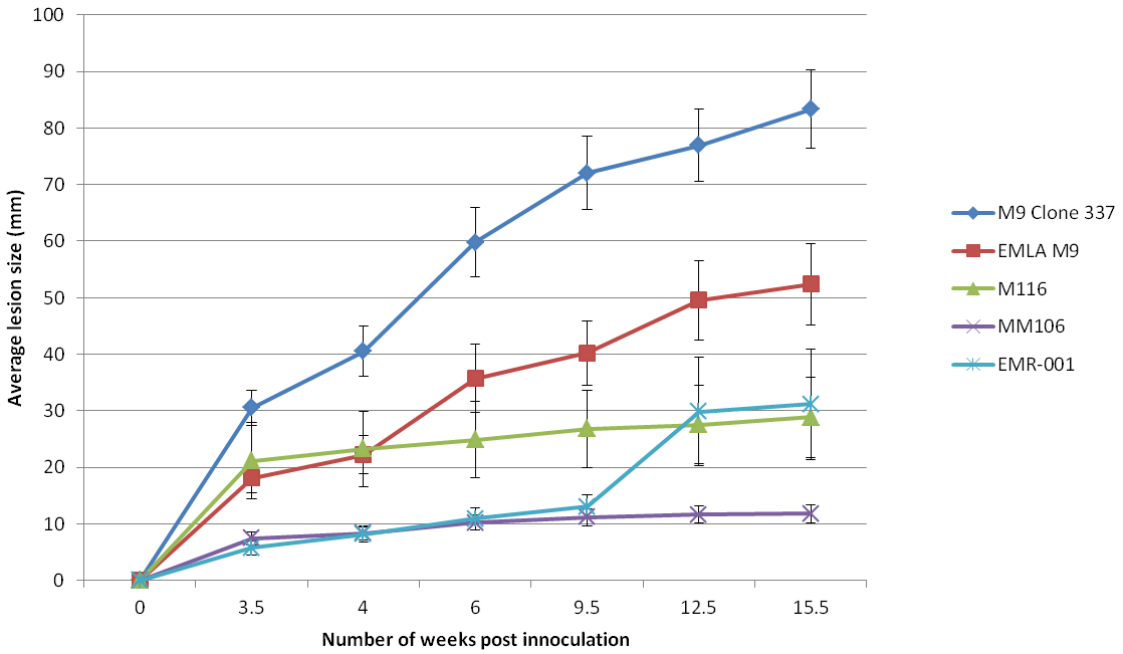
Figure 2.3. *N. ditissima* pathogenicity test. From left to right; chilled glasshouse compartment in which the pathogenicity tests were carried out, lesion developing from inoculation point and advanced symptoms causing dieback.

Results

Infection was successful using the artificial pathogenicity test adapted from Van De Weg (1987). Lesions developed from all but one inoculation point. No lesions developed from mock (water) inoculated controls (data not shown). Lesions became visible 24 days following inoculation and continued to expand for the remainder of the experiment (4 months following inoculation). The results (Fig. 2.4a) show that using this pathogenicity test M9 clone 337 was significantly more susceptible than the other lines and MM106 is significantly less susceptible.

The EMLA M9 clone is less susceptible than M9 clone 337. Lesions on M116 initially are as developed as EMLA M9 but expansion slows whilst EMLA M9 continues to expand at a greater rate. The advanced breeding line, EMR-001, initially contains infection spread, however 9.5 weeks following infection average lesion length increases markedly. Overall, using the area under disease progress curve (AUDPC) dataset (Fig. 2.4b) M9 clone 337 is significantly more susceptible to M9 EMLA clone which is significantly more susceptible than M116 followed by EMR-001 and MM106.

(a)



(b)

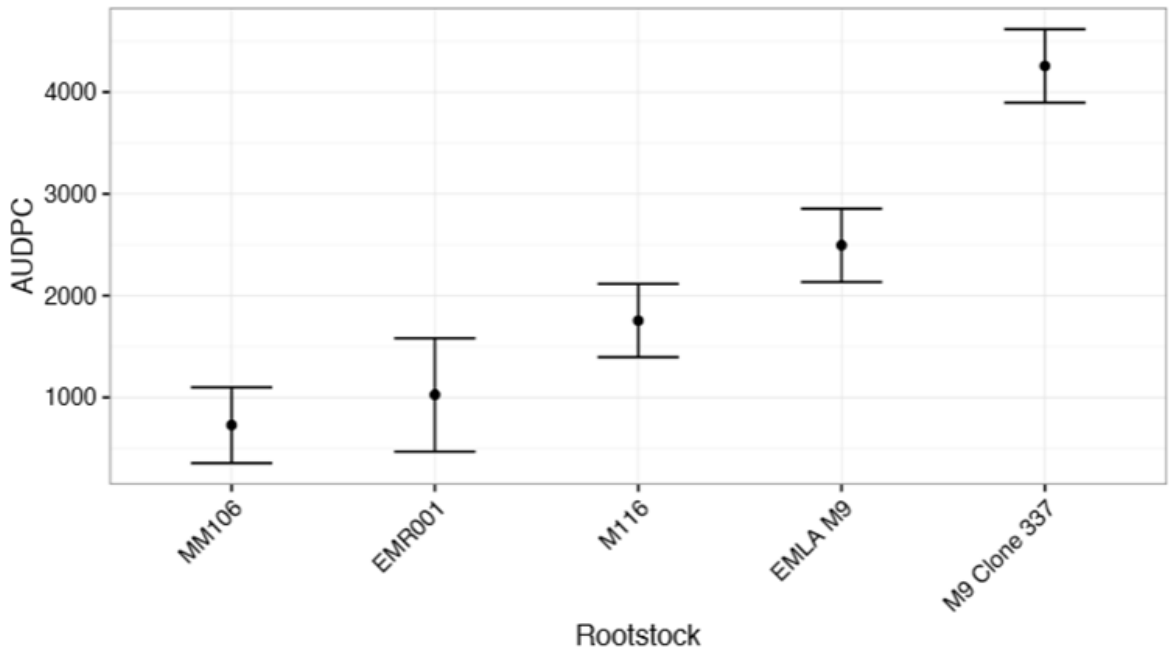


Figure 2.4. The development of *Neonectria ditissima* lesions on a panel of rootstock cultivars. (a) represented as lesion progression over the course of the experiment and (b) represented as Area under disease progress curve (AUDPC).

Discussion

The cultivars M116 and MM106 were selected for the panel as relatively resistant controls. This test has demonstrated that these cultivars are relatively resistant and will provide useful resistant controls in the field trial material to be evaluated in 2017 onwards. An advanced selection from the EMR breeding programme, EMR-001, groups with the resistant controls which is consistent with the pedigree of this selection which includes Robusta 5, a resistant rootstock cultivar. The two M9 clones were included to test the anecdotal evidence which suggested that the M9 clone 337, which is used widely on the continent, is relatively more resistant to the EMLA clone. This test suggests the opposite. It should be noted that this pathogenicity test measures only one component of susceptibility/resistance to this pathogen. The resistance to *N. ditissima* is thought to be multi-faceted, therefore other tests (i.e. natural inoculation in the field) may lead to a different result. Further work is being carried out to determine the mechanisms of resistance to *N. ditissima* in an AHDB funded PhD studentship.

Conclusions

- Rootstocks have differing susceptibility to *Neonectria ditissima*
- Differences are evident between clonal material (EMLA M9 and M9 clone 337)
- The field trial to be planted next 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

Based on previous research European apple canker (in particular the millennium trial, McCracken *et al*. 2003) it has been shown that *N. ditissima* can infect trees in the nursery and remain asymptomatic in the apple host. Once planted in the production site, where upon the tree can experience stress (drought/water logging/replant disease etc.), the disease is expressed. This objective aims to evaluate biological soil amendments to improve tree health

and establishment in the context of canker expression. The objective is to be conducted in two parts; (1) a stool bed trial will simulate the nursery phase of tree fruit production and (2) a replicated trial on newly planted orchards to simulate the establishment of new orchards on the production site. These are long term trials, requiring establishment and monitoring over time. The stool bed was planted in May 2015 and the layout and preliminary assessments are reported herein. The newly planted orchard trials (n=2) have been planted in February and March 2016.

Materials and Methods

Site

The rootstock bed was planted on 12th May 2015 on a 0.055ha site in EE211, East Malling Research. 1600 2 year old EMLA M9 7-9mm rootstocks were planted at 10 cm spacing arranged in 32 X 50 tree plots (Fig. 2.5). Stool bed 1, consisting of 16 plots, was used for the amendment trial. Stool be 2 was planted without soil amendments and shall be used as an experimental resource once established.

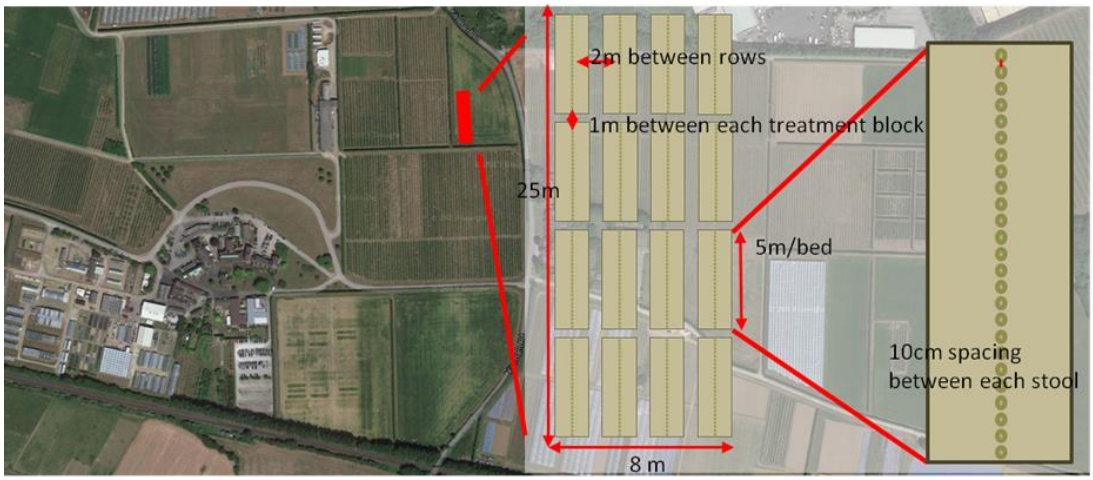


Figure 2.5. Planting position and plot layout of stool bed for soil amendment trial

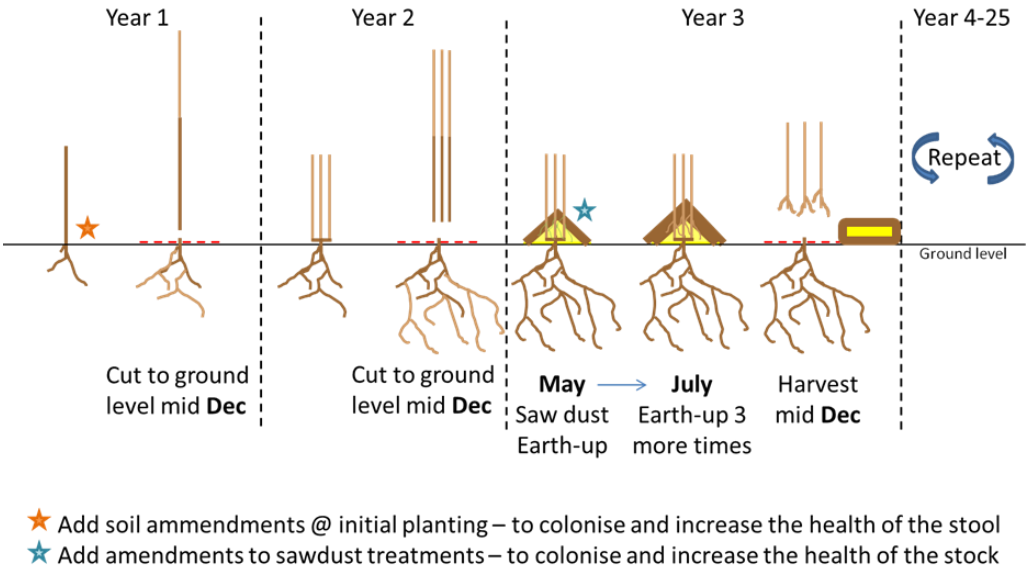


Figure 2.6. A schematic of the process of establishing a stoolbed.

Treatments

The trial was designed as a randomised block with each treatment replicated four times. The treatments, listed in Table 2.3, were added to the planting hole ensuring that the roots of each tree were covered. 25 ml of treatment was applied to each tree.

Table 2.3. Treatments used for biological amendments trial.

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

Assessments

The long term nature of this trial means that canker assessments will not commence until 3 years from planting, once the first rootstocks are harvested. During the establishment phase assessments have been conducted to quantify AMF colonisation and chlorophyll content, to infer plant health.

(i) AMF colonization was measured in the roots of a single tree selected from the centre of each untreated and AMF treated plot. Relative colonisation was determined using a method known as percentage root length colonisation (%RLC).

(ii) Chlorophyll content was measured using a SPAD devise which provides a non-invasive, non-destructive, rapid method to quantify chlorophyll content. SDAD readings were collected from the 5th leaf from the tip of extension growth of ten trees randomly selected from each plot.

Results

The %RLC results (Fig. 2.7) show the colonisation rates of 4 individual trees, selected from each of the 4 blocks, and their average for untreated and AMF treated plots. On average AMF treated plots have a significantly greater level of colonisation, however one of the untreated plots (plot 3) has equivalent colonisation to the treated plots suggesting natural colonisation from native populations of mycorrhizae. %RLC will continue to be measured through the establishment phase of the stool bed.

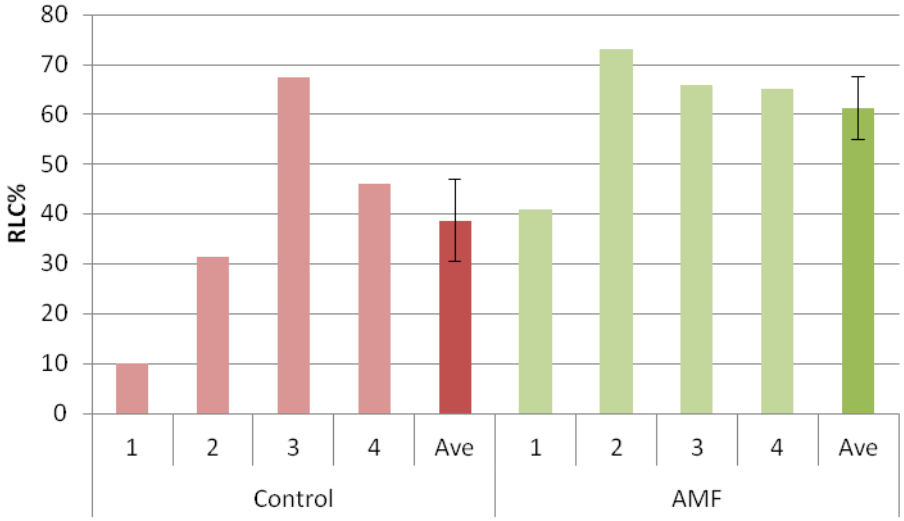


Figure 2.7. Mycorrhizae colonisation in control and Arbuscular Mycorrhizal Fungi (AMF) treated plots in stool bed trial. Percentage root length colonisation (RLC%) has been recorded for each replicate plot (1-4) and averaged (Ave). Error bars (on the average) represent Standard Error of the Mean (SEM) of the four replicates. Data courtesy of Plantworks Ltd.

The SPAD readings (Fig. 2.8) show that there are no significant differences between any of the treatments. The soil amendments tested are known to positively effect water and nutrient uptake and therefore improve plant health on numerous plant species including malus species. The absence of a significant difference in SPAD readings, which can be used to infer plant health, may be due to the natural colonisation of AMF (and other beneficial microorganisms) in the untreated plots as demonstrated in Figure 2.7, or may be a result of SPAD measurements being an inappropriate measure of plant health. Future measures to infer plant health could include extension growth rate and tree girth and more accurate devices now available to infer plant health by none destructive methods.

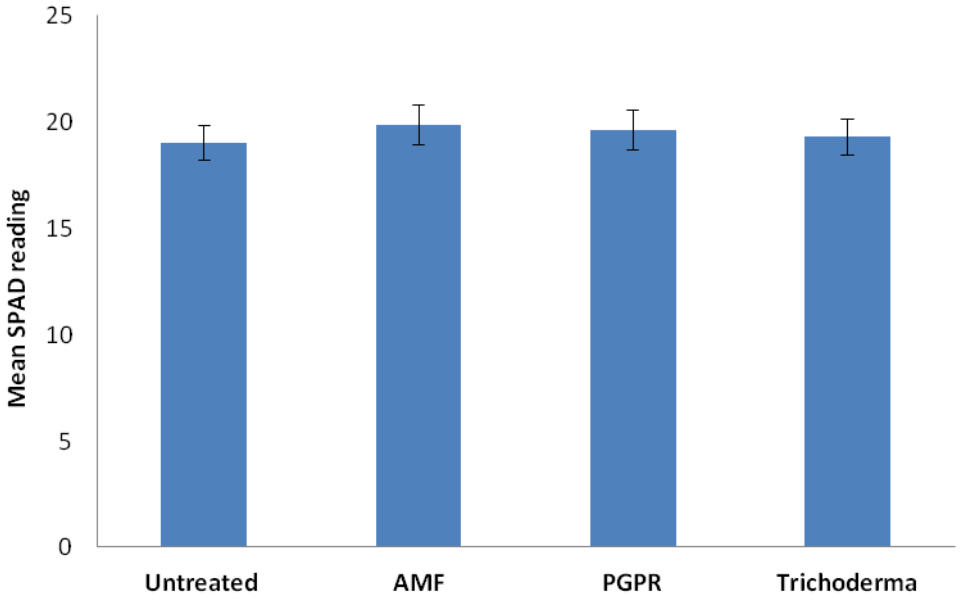


Figure 2.8. SPAD, a device which measures relative chlorophyll content, readings were taken from leaves of plants in each plot.

Conclusions

- These are long term trials which are currently in the establishment phase
- Initial findings suggest that native AMF present in the planting site is capable of interacting with the apple tree and that adding AMF inoculum to the planting hole increases colonisation.
- No effects on plant health have been observed in the growing season based on chlorophyll measurements.
- The effect on canker expression will not be evaluated until year 3 (stoolbed) and year 2 (newly established orchards).

Objective 2	Neonectria ditissima	Task 4	Novel application methods

Aim

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

Summary

Based on literature searches tree injection has been selected as the most promising and practical method of treatment delivery. To this end a collaboration has been established between Fertinyect, Bayer and 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 with the potential to formulate them to optimise efficacy in subsequent years trials. A protocol and treatment list has been prepared (Appendix 1). The trial is to commence in Spring 2016.

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)

Summary

No trials have been setup over the winter period as an Innovate UK (IUK) proposal has been developed which will cover this theme. Depending on the outcome of the proposal (due April 2016) work will either be funded through IUK or could be incorporated into this project potentially with proposed industry partners supporting the work in kind. A small pilot study has been setup through winter 15/16 to determine the major microbial groups which contribute to leaf degradation, an important component of controlling the overwintering form of apple scab. The study could form the basis of a new area of study and commercial exploitation which will be pursued with additional (IUK) external funding.

Objective 3	Apple foliar diseases	Task 2	Alternative treatments

Aim

Evaluate efficacy and persistence of alternative chemical treatments to fungicides (EMR, Yr 1-3)

Materials and methods

Site

Orchard EE190, located at East Malling Research. 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. Each plot consisted of three trees, separated from adjacent plots by single trees within the row and between rows.

Trial design

The trial was designed as a randomised block with each treatment replicated four times.

Treatments

All plots received a standard programme for pest and disease control (Appendix 2) and nutrients up to the start of the trial at green cluster (BBCH55/56). Thereafter the treatments in Table 3.1 and programmes in Table 3.2 were applied to the plots. Treatments for pests and nutrients were applied to all plots as necessary after the start of the trial. The fungicide Captan was applied at 2 kg/ha to all plots after the start of the trial for control of apple scab. Each programme was applied as a 9 spray programme at a spray interval dependent on the product, up to the end of shoot growth in August. A standard fungicide programme (P2) (based on Systhane, Cosine, Kindred, Topas) and an untreated control (P1) were included as a comparison. Test products were evaluated alone and in combination with the standard fungicide programme. Details of products used are given in Table 3.1.

Sprays were applied to plots using a Stihl motorised air-assisted knapsack sprayer at 500 L/ha following EMR SOP GEP 725. Treatments to all plots were applied using a tractor-trailed air-assisted orchard sprayer at the standard farm spray volume of 200 L/ha. Phenological stage at each application were recorded using BBCH crop growth scale. Records of daily maximum and minimum temperature and rainfall were taken from a weather station located at East Malling Research.

Table 3.1. Details of products evaluated in programmes in 2015

Product	Active ingredient	Product type	Rate of product / ha	Use
Systhane	myclobutanil	Fungicide	0.33 L	
Topas	penconazole	Fungicide	0.5 L	
Cosine	cyflufenamid	Fungicide	0.5 L	
Stroby	Kresoxim-methyl	Fungicide	0.2 kg	
HDC F191	Plant extract	Elicitor	2.5 L	7-14 day intervals
Cropbiolife	Flavonoids	Plant strengthener	300 ml	Early flowering, 50-100 blossom then monthly and 14 and 7 days pre-harvest
HDC F192	Antimicrobial polypeptides	Elicitor and plant strengthener	2L	Monthly from pre-flowering
SB invigorator	Various nutrients and natural products	Plant stimulant	2ml/L	Weekly sprays
Wetcit	Alcohol ethoxylate	Energiser adjuvant	0.2%	Improves fungicide efficacy
Ashton Tree Wash	Garlic extracts	Plant stimulant with antimicrobial properties	1:100 dilution	7-14 days
Proact	0.1% harpin protein	Elicitor and plant health regulator	70 g	2-3 times per crop
HDC F193	Natural compound	Elicitor	2.5 L/ha	

Table 3.2. Programmes, based on fungicides, growth promoters and elicitors evaluated in 2015 and dates treatments applied

Programme	Treatment	Product / Timing								
		1 **29 April**	**2** **8 May**	**3** **18 May**	**4** **28 May**	**5** **5 June**	**6** **12 June**	**7** **19 June**	**8** **29 June**	**9** **10 July**
		Early bloom	Full Bloom	Petalfall Start shoot growth	Fruitlet	Fruitlet	Fruitlet	Fruitlet	Fruitlet	Fruitlet End of shoot growth
1	Untreated	-	-	-	-	-	-	-	-	-
2	Fungicide	Systhane	Systhane	Cosine	Topas	Topas	Cosine	Systhane	Topas	Systhane
3	HDC F191/Fung	HDC F191	Systhane	HDC F191	Topas	HDC F191	Cosine	HDC F191	Topas	HDC F191
4	CBL	CBL		CBL		CBL		CBL		CBL
5	CBL/Fung	CBL	Systhane	CBL	Topas	CBL	Cosine	CBL	Topas	CBL
6	HDC F192	HDC F191		HDC F191		HDC F191		HDC F191		HDC F191
7	SBI	SBI	SBI	SBI	SBI	SBI	SBI	SBI	SBI	SBI
8	Wetcit/Fung	Wetcit	Systhane	Wetcit	Topas	Wetcit	Cosine	Wetcit	Topas	Wetcit
9	ATW	ATW	ATW	ATW	ATW	ATW	ATW	ATW	ATW	ATW
10	Proact	Proact		Proact		Proact		Proact		Proact
11	Proact/Fung	Proact	Systhane	Proact	Topas	Proact	Cosine	Proact	Topas	Proact
12	HDC F193	HDC F193	HDC F193	HDC F193	HDC F193	HDC F193	HDC F193	HDC F193	HDC F193	HDC F193

Assessments

Symptoms of phytotoxicity were checked for after each treatment and recorded. Records included any chlorosis / necrosis to foliage, growth regulatory effects to shoots and were assessed on a scale 0-5. (Table 3.3, EPPO Guideline PP 1/135(3)).

Table 3.3. 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

Initial and final fruit set and fruit drop were recorded. Two branches were marked on the central tree in each plot. Total number of flowers were recorded in blossom on 7 May, number of fruitlets recorded on 29 June and number of apples recorded in 1 September.

All assessments of powdery mildew were conducted on middle tree of each plot. Primary blossom was recorded on 28 April as total number of blossoms and number with mildew on 4 branches per tree. Secondary mildew was recorded weekly on 5 shoots per tree. The number of mildewed leaves was recorded in the top 5 leaves on each shoot, starting with the first fully expanded leaf.

The incidence of leaf and fruit scab was sporadic and at a low incidence and was not recorded.

At harvest yield per plot was recorded. A random sample of at 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 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 acceptable in Class 1 for Gala (EPPO Guideline PP 1/135 (3).

Chlorophyll content as a measure of tree health was assessed using a hand held SPAD meter. On 2 July 3 leaves down of 5 actively extending shoots of the central tree were measured with a SPAD meter. On 17 August, 3 leaves down of 5 fully extended shoots of the central tree were measured with a SPAD meter. Leaves either mildew-free, heavily infected with powdery mildew or with slight infection (see below) were also measured with a SPAD meter to look at the effect of mildew incidence on SPAD measurements.

Statistical analysis

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

Results and Discussion

Incidence of powdery mildew

The incidence of mildew in the orchard was very high due to high primary inoculum and unsprayed Cox guard rows (Table 3.4, Fig. 3.1), this disease pressure would not be expected in commercial orchards. Untreated plots rapidly increased to 100% secondary mildew. The best control was achieved by the standard fungicide programme (Programme 2) however the mildew levels in these plots was still above threshold of 10% for most of season. All test products delayed onset of epidemic with significantly less mildew in first 2 assessments but by later assessments only the products in combination with fungicide reduced the mildew epidemic, although not to commercially acceptable levels. This control can probably be attributed to the fungicide input rather than the test products.

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, the test treatments will be superimposed on these blocks. This will provide two disease pressures ensuring these are assessed in a commercially relevant disease pressure whilst ensuring sufficient disease pressure. Poor performing products will be removed from the treatment list whilst new products will be added. Promising treatments will be combined into programmes.

With regards to phytotoxicity, F191 and F193 showed leaf necrosis and leaf drop, especially F193 (Fig 3.2). The use of Captan as part of the general scab programme in combination with F193 may have exacerbated the phytotoxic effects of this test product.

There were no significant effects on fruit set but CBL and Wetcit/fungicide programmes indicate a trend towards better fruit set (Table 3.5). CBL is known to be most effective on yield traits when applied to crops which are under stress and so yield effect may become more or less pronounced dependent on the season. F191 resulted in significantly more russet (Table 3.6). Some treatments (Programmes 3, 5 and 12) gave increased fruit size but this was related to a reduction in another yield component, yield.

Plant health was assessed in July and August using a SPAD metre. In the first assessment none of the treatments gave significantly higher readings compared to the control, suggesting that none of the treatments improved tree health (Table 3.7 and Fig. 3.3a). The second assessment was conducted on older leaves and SPAD readings were much higher. The fungicide programme gave significantly higher SPAD reading than control. SBI and Proact were best of the test treatments (Table 3.7 and Fig. 3.3b). Heavily mildewed leaves had the lowest SPAD readings compared to partially mildewed and mildew-free leaves (Fig 3.3c). So SPAD measurements for treatment may have been complicated by effectiveness of mildew control. However, least mildewed leaves were selected for measurement where possible. As mentioned previously, measures to infer plant health in subsequent years of this project could include extension growth rate and tree girth and more accurate devices now available to infer plant health by none destructive methods.

Conclusions

- The powdery mildew pressure was very high in the experimental orchard providing a tough test for the treatments and not representing commercial reality
- The test products did delay the mildew epidemic relative to untreated control
- SB invigorator was the best performing test product
- F191 and F193 caused phytotoxic effects

Table 3.4. Mean % mildewed leaves (angular transformed) on apple cv. Gala following sprays of various treatments applied to apple trees from early flower at East Malling Research in 2015 (figures in brackets are back transformed data)

Programme	Treatment		Date assessed / % mildewed leaves							
		21 May	28 May	4 June	10 June	18 June	25 June	2 July	6 August	Overall mean
1	Untreated	63.8 (80.5)	84.2 (99.0)	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)	97.4
2	Fungicide	**13.3** (5.3)	**24.7** (17.4)	**15.8** (7.4)	**24.6** (17.4)	**31.4** (27.2)	**31.7** (27.5)	**39.0** (39.6)	**40.9** (42.9)	23.1
3	HDC F191/Fung	**20.1** (11.9)	**37.1** (36.4)	**42.1** (44.9)	**52.7** (63.2)	**50.9** (60.2)	**61.6** (77.4)	**61.2** (76.8)	**58.3** (72.4)	55.4
4	CBL	**51.0** (60.3)	**61.6** (77.3)	**77.9** (95.6)	**78.9** (96.3)	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)	87.1 (99.7)	91.2
5	CBL/Fung	**19.8** (11.4)	**42.1** (45.0)	**40.8** (42.7)	**46.2** (52.1)	**55.9** (68.6)	**61.0** (76.5)	**63.1** (79.5)	**70.3** (88.6)	58.1
6	HDC F192	**44.4** (49.0)	69.9 (88.1)	82.0 (98.1)	83.0 (98.5)	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)	91.7
7	SBI	**39.1** (39.7)	**53.8** (65.1)	**68.3** (86.3)	**68.3** (86.4)	84.9 (99.2)	84.1 (98.9)	**75.0** (93.3)	83.0 (98.5)	83.4
8	Wetcit/Fung	**20.0** (11.7)	**37.1** (36.4)	**38.8** (39.2)	**56.2** (69.0)	**57.8** (71.6)	**64.4** (81.3)	**64.5** (81.5)	**61.4** (77.1)	58.5
9	ATW	**49.2** (57.3)	**56.2** (69.1)	78.9 (96.3)	84.9 (99.2)	87.1 (99.7)	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)	90.2
10	Proact	**50.2** (59.1)	**67.3** (85.2)	**82.0** (98.1)	**78.9** (96.3)	90.0 (100.0)	90.0 (100.0)	87.1 (99.7)	83.4 (98.7)	92.1
11	Proact/Fung	**22.8** (15.0)	**49.9** (58.5)	**48.0** (55.2)	**53.9** (65.2)	**58.2** (72.2)	**62.8** (79.2)	**58.9** (73.3)	**59.6** (74.4)	61.6
12	HDC F193	**44.4** (48.9)	**63.5** (80.0)	**76.4** (94.4)	80.1 (97.1)	87.1 (99.7)	**79.8** (96.9)	**87.1** (99.7)	85.9 (99.5)	89.5
F Prob		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
SED (33)		5.412	7.729	5.687	6.259	5.065	4.548	4.299	5.205	
LSD (p=0.05)		11.011	15.726	11.571	12.733	10.305	9.253	8.746	10.590	

Figures in bold are significantly different from untreated

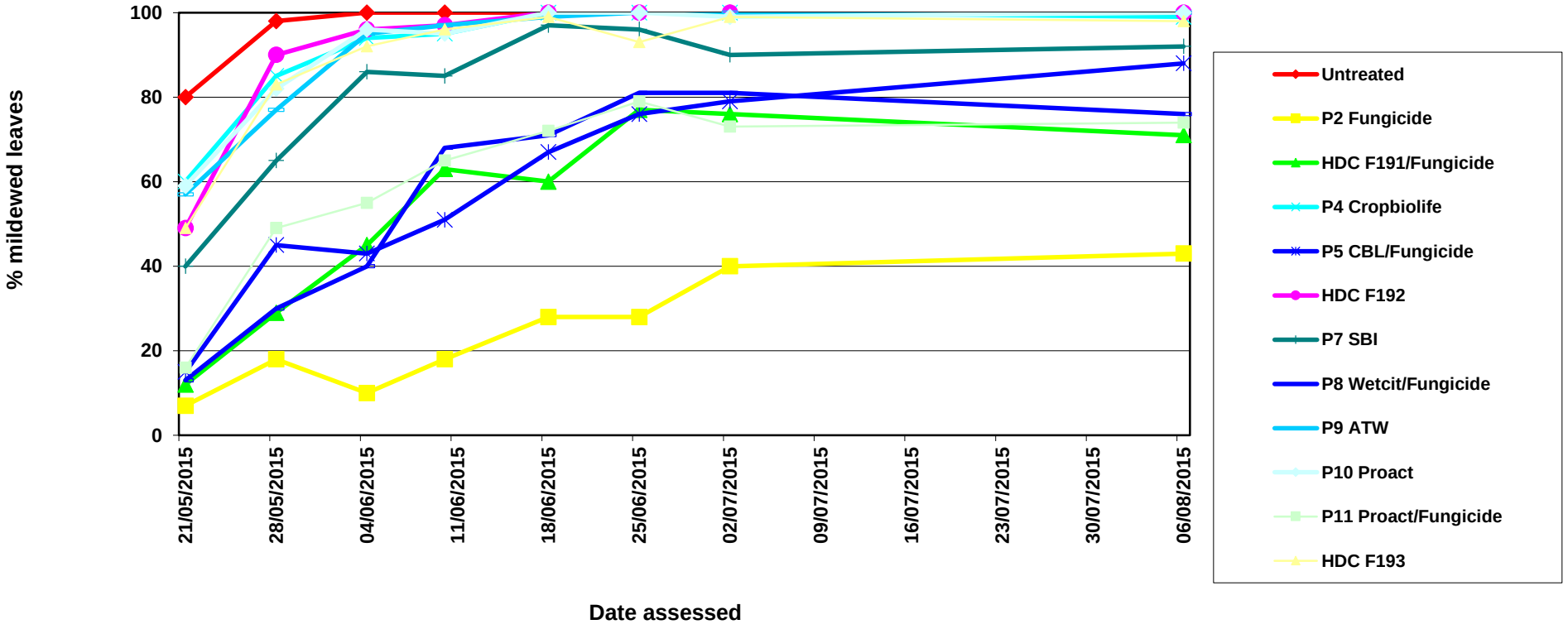


Figure 3.1. Percent mildewed leaves on apple shoots cv. Gala assessed at various times following treatment with various programmes in 2015

Table 3.5. 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 nine sprays of various programmes at East Malling Research in 2015. Figures in parenthesis are back-transformed means

Programme	Treatment	% Initial fruit set	% Final fruit set	% Fruit drop	Mean leaf necrosis score	Mean leaf fall score
1	Untreated	33.2 (29.9)	27.2 (20.9)	32.2 (28.4)	0	0.3
2	Fungicide	37.7 (37.4)	31.9 (27.9)	28.1 (22.2)	0	0
3	HDC F191/Fung	34.8 (32.6)	30.1 (25.2)	27.5 (21.3)	1	1
4	CBL	41.0 (43.0)	35.0 (32.8)	29.3 (23.9)	0	0
5	CBL/Fung	38.9 (39.5)	33.1 (29.8)	28.9 (23.3)	0	0
6	HDC F192	36.8 (36.0)	32.0 (28.1)	28.2 (22.4)	0	0
7	SBI	35.6 (33.9)	30.5 (25.7)	26.8 (20.3)	0	0
8	Wetcit/Fung	42.1 (44.9)	36.9 (36.0)	24.2 (16.8)	0	0
9	ATW	34.8 (32.6)	29.7 (24.6)	28.1 (22.2)	0	0
10	Proact	38.3 (38.3)	31.5 (27.2)	32.0 (28.0)	0	0.3
11	Proact/Fung	39.2 (40.0)	34.5 (32.0)	25.1 (18.0)	0	0
12	HDC F193	38.3 (38.4)	31.9 (27.9)	29.3 (24.0)	3	2
F Prob		0.918	0.800	0.891		
SED (33)		5.531	4.671	4.734		
LSD (p=0.05)		11.25	9.503	9.631		

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

Figure 3.2. Leaf necrosis on extension growth following treatment with HDC F193

Table 3.6. Effects of treatments on yield and fruit quality recorded as russet score, weight 100 fruit (kg) (In transformed) and % and weight (In transformed) of fruit > 65 mm diameter (square root transformed) on apple fruits cv. Gala recorded following nine sprays of various programmes at East Malling Research in 2015. Figures in parenthesis are back-transformed means. Figures in bold are significantly different from untreated.

Programme	Treatment	Fruit yield per plot kg	Mean russet score	Weight of 100 fruit kg	% fruit > 65 mm diameter	Weight of fruit >65 mm diameter
1	Untreated	43.8	16.0	2.0 (7.0)	0	-1.1 (0.3)
2	Fungicide	41.9	10.0	2.0 (7.2)	0.4 (0.2)	-1.3 (0.3)
3	HDC F191/Fung	31.9	**25.0**	2.1 (8.3)	**2.2** (4.8)	-0.2 (0.8)
4	CBL	36.5	11.8	2.1 (8.4)	1.7 (2.9)	-0.1 (0.9)
5	CBL/Fung	31.2	17.3	2.2 (8.8)	**2.9** (8.2)	**1.3** (3.6)
6	HDC F192	44.2	14.5	2.1 (7.9)	1.1 (1.2)	-0.4 (0.7)
7	SBI	42.0	13.8	2.0 (7.3)	0.3 (0.1)	**-3.0** (0)
8	Wetcit/Fung	44.6	9.5	2.0 (7.2)	0	-1.1 (0.3)
9	ATW	42.4	11.0	2.0 (7.4)	0.8 (0.6)	-1.7 (0.2)
10	Proact	36.0	13.5	2.1 (8.0)	1.2 (1.5)	-1.8 (0.2)
11	Proact/Fung	43.0	10.5	1.9 (7.0)	0.3 (0.1)	**-2.8** (0.1)
12	HDC F193	36.3	17.0	2.2 (8.6)	**2.7** (7.1)	-0.4 (0.7)
F Prob		0.225	0.039	0.053	0.023	<0.001
SED (33)		5.798	4.133	0.083	0.926	0.463
LSD (p=0.05)		11.797	8.408	0.168	1.884	1.032

Table 3.7. Effects of treatments on SPAD meter measurements conducted on 2nd July 3 leaves down of 5 actively extending shoots of the central tree and on 17 August, 3 leaves down of 5 fully extended shoots of apple trees cv. Gala following nine sprays of various programmes at East Malling Research in 2015.

Programme	Treatment	SPAD 2 July	SPAD 17 August
1	Untreated	14.8 a	42.4 a
2	Fungicide	13.4 ab	50.7 b
3	HDC F191/Fung	15.9 a	45.8 a
4	CBL	13.6 ab	45.6 a
5	CBL/Fung	13.5 ab	41.3 a
6	HDC F192	12.0 b	36.9 c
7	SBI	12.4 b	47.2 ab
8	Wetcit/Fung	12.7 b	43.7 a
9	ATW	12.1 b	39.5 ac
10	Proact	15.8 a	42.8 ac
11	Proact/Fung	13.9 ab	48.4 ab
12	HDC F193	12.4 b	34.9 c

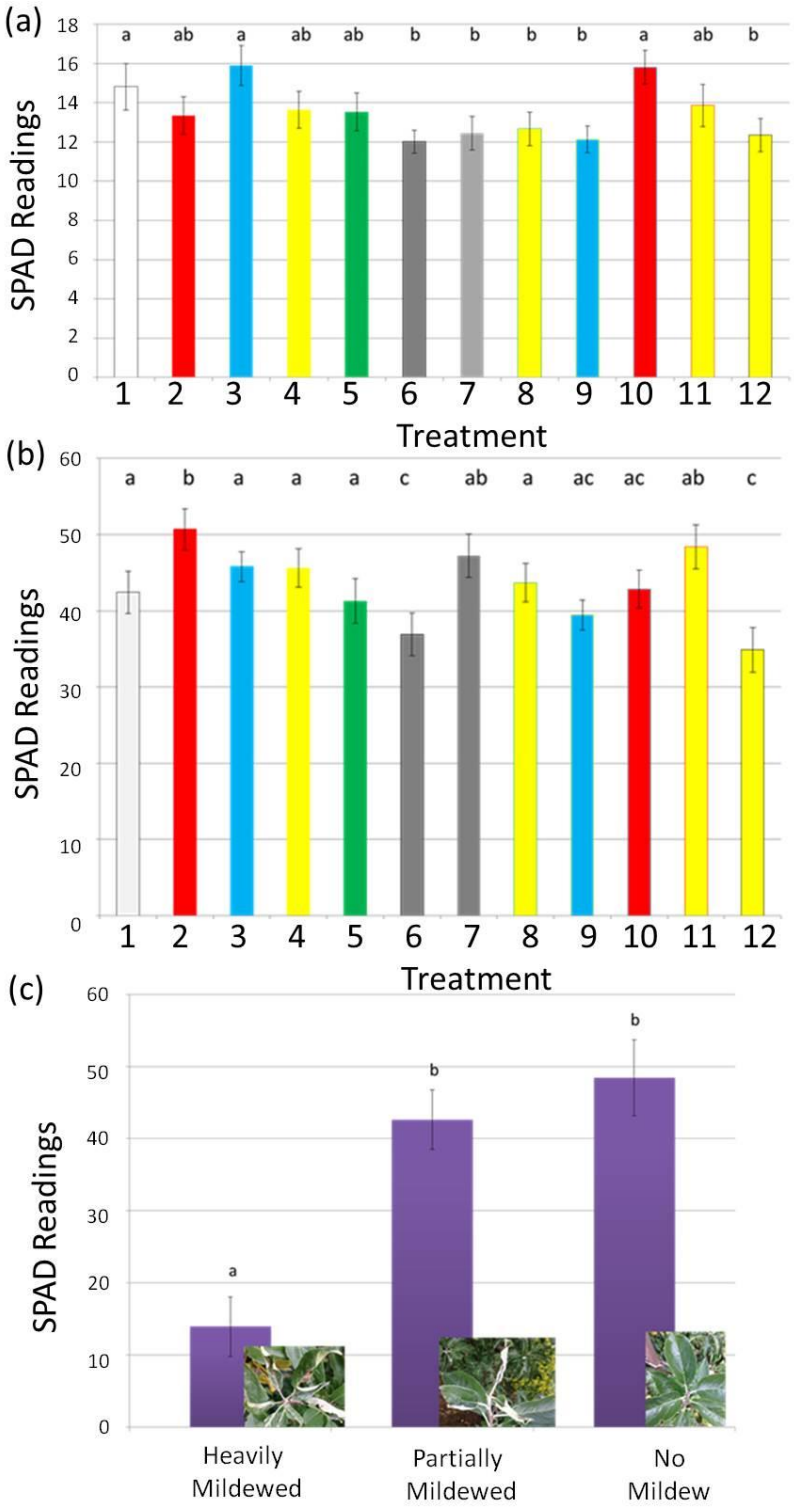


Figure 3.3. SPAD metre readings to determine chlorophyll content and infer plant health. SPAD readings were recorded on 02/07/15 (a) and 17/18/15 (b). Treatments = 1; Untreated, 2; Fungicide, 3; Reysa, Fungicide, 4; CropBiolife (CBL), 5; CBL, Fungicide, 6; TF-01, 7; SBI, 8; Wetcit, Fungicide, 9; ATW, 10; ProAct, 11; ProAct, Fungicide, 12; Requiem. SPAD readings were also recorded from leaves which were heavily mildewed, partially mildewed or clean (c).

Objective 6	Codling and tortrix moth	Task 1	Pheromone MD

Aim

Integrate pheromone mating disruption ±granulovirus into the control programmes for codling and tortricid moth in apple orchards whilst enhancing natural enemies and maintaining control of other pests (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 sprays 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 at least two companies are seeking UK registration. 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.

Aim: Demonstration of the efficacy of sex pheromone mating disruption, alone versus in combination with granulosis viruses or nematodes, including effects on pest and natural enemy populations. Economic benefits of this approach will be compared to standard spray programmes in the second year of the approach.

Materials and methods

Farms, Orchards and Site Managers

Below are the details of the 2 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 and 6.2, Figure 6.1 and 6.2). Each orchard was colour coded according to treatment. 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 was 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.

Table 6.1. 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 + gran)***	**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 + gran)***	**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)	**Broughton Meadow**	***Orchard 9 (MD+nem)***	**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+nem)***	**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

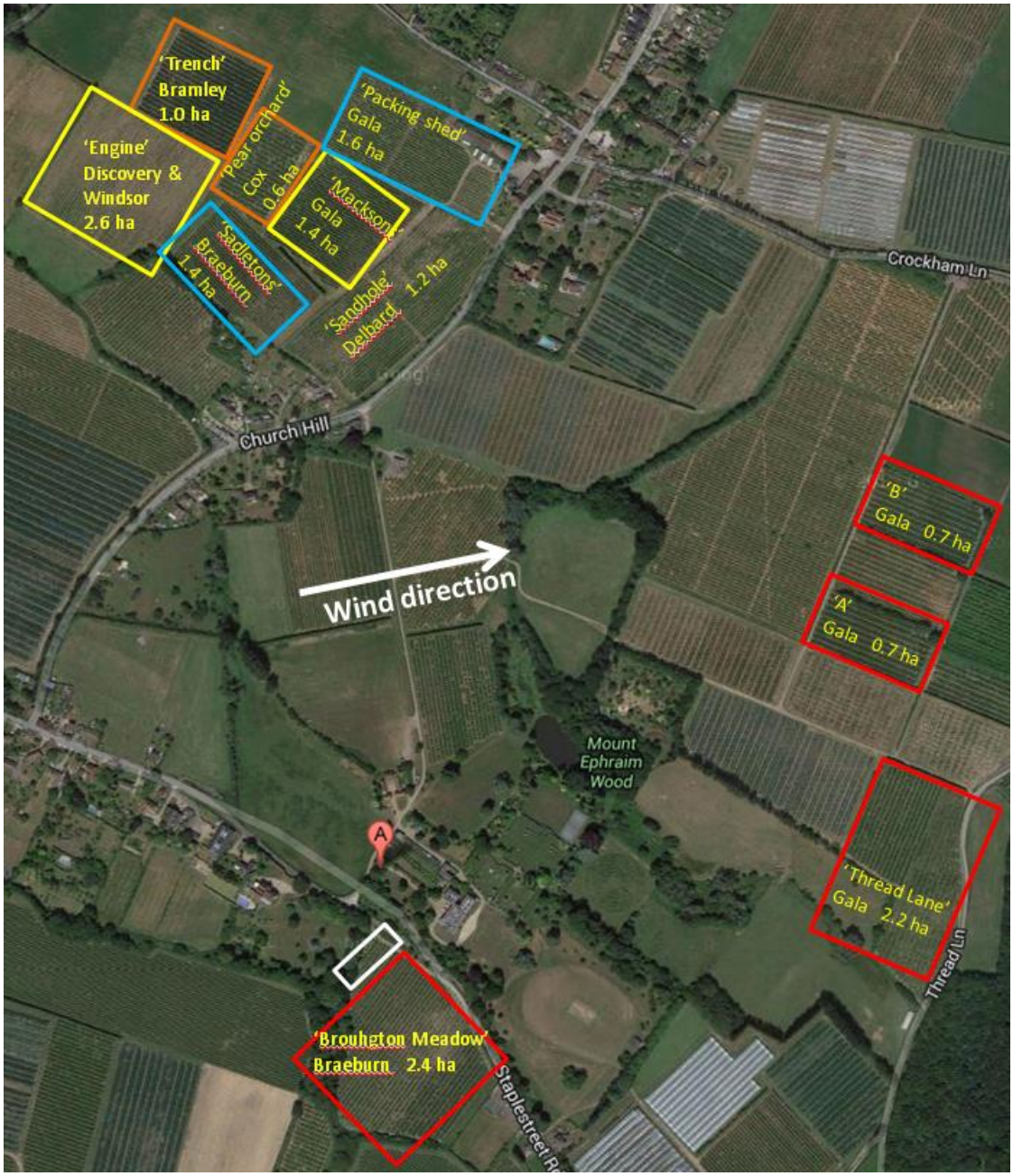


Figure 6.1. Map of Site 1 and the location of the treated plots (NB 'Sandhole' was grubbed in 2016).

Table 6.2. 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 + gran)***	**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 + gran)***	**Harwort M (Top)**
NGR	52.048928, -2.4214605	NGR	52.057715, -2.4169115
Variety	Red Windsor	Variety	Red Falstaff
Planting date	1999	Planting date	1999
Area (ha)	2.93	Area (ha)	0.9
Orchard 4 (Conv)	**C2**	***Orchard 9 (MD+nem)***	**Harwort M (Bottom)**
NGR	52.050062, -2.421203	NGR	52.057874, -2.4183277
Variety	Cox and Discovery	Variety	Red Falstaff
Planting date	1990	Planting date	1999
Area (ha)	0.9	Area (ha)	0.9
Orchard 5 (MD)	**M Linder (Bottom right)**	***Orchard 10 (MD+nem)***	**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 and the location of the treated plots**.**

Treatments

CONVENTIONAL: On each farm a block of orchards greater than ~6 ha was treated for codling moth and tortix moths using a standard grower spray programme.

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, Fig. 6.3). This treatment has longevity for CM of at least 9 months and was applied in April (Tables 6.3 and 6.4). Plots were adjacent to achieve a landscape effect (fewer orchard edges). Pheromone treated areas were downwind of the conventional plots of the farms to minimise dispersion of the pheromone into the grower conventional orchards. The devices (500 units per ha) were hung in the top third of the tree (as the pheromone drifts downwards) by EMR, BASF and ADAS staff under the supervision of BASF. This took approx. 2 man hours per ha. Products were supplied free of charge by the companies listed in Table 6.5.

GRANULOSIS VIRUS: Within each pheromone treated area, two large (~1 ha) sub-plots per farm were treated with a timed (by sex pheromone traps on the conventional side of the farm or RimPro) programme of sprays of codling moth granulosis virus (Cyd-X Xtra) and summer fruit tortrix moth granulosis virus (Capex).

- Cyd-X Xtra was applied at 100 ml/ha at a spray interval of 8 sunny days. The first application was made soon after egg lay and just before the first larvae hatched (7-10 days after the threshold of 5 moths per pheromone trap in a period of 2 weeks in the conventionally treated side of the farm (Tables 6.3 and 6.4)). Cyd-X Xtra targets the hatching larvae; the virus is ingested. The virus infects the digestive tract of the caterpillar causing disease that kills within 3-7 days. It should be applied as soon as the eggs of the first generation codling moth hatch. Site 2 predictions were made with RimPro (Alex Radu, Agrovista).
- Two applications (~10 days apart) of SFT granulosis virus (Capex) were applied to target the L3 overwintering larvae in April and then again for the summer generation in June/July (predicted with monitoring traps on the conventional side of the farms (Tables 6.3 and 6.4)). The goal was to expose all L3 larvae (L4 and L5 larvae are not as susceptible and usually do not die) to the virus; once the larvae start activity in spring. A spray 10 days later ensures a period of about 4 weeks with maximal 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. This could make Capex an excellent population management tool.

NEMATODES: There were two large (~1 ha) sub-plots per farm which received an application of nematodes in the autumn (Tables 6.3 and 6.4) when the average air temperatures were above 14°C. Nemasys C (a.i. *Steinernema carpocapsae*) supplied by BASF was applied at a rate of 1.5 billion nematodes/ha in a water volume of 1,000 l/ha. Nematodes were applied when the tree trunks and soil were thoroughly wet as a coarse spray on to the bark area, from ground to scaffolds (half way up the trunk). Applications were made late in the day to reduce exposure of the nematodes to UV.

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

Figure 6.3. RAK3&4 mating disruption device in top third of a tree and staff carrying out harvest assessments.

Table 6.3. Mt Ephraim Farm insecticide spray applications. Growers applied all products according to label recommendations

Date	Treatment	Active	Orchard treated
Conventional areas (red)			
17 Apr	Equity	chlorpyrifos	'A', 'B', Thread Lane, Broughton Meadow
11 May	Coragen	chlorantraniliprole	'A', 'B', Thread Lane, Broughton Meadow
15 Jul	Explicit	indoxicarb	'A', 'B', Thread Lane, Broughton Meadow
MD areas			
15 Apr	RAK3&4	*Cydia pomonella* *Adoxophyes orana* *Archips podana* sex pheromones	Mackson's, Trench, Pear Orchard, Packing Shed, Sadleton's, Engine (all except Red and White)
17 Apr	Capex	SFT granulovirus	Trench, Pear Orchard (Orange)
01 May	Capex	SFT granulovirus	Trench, Pear Orchard (Orange)
12 Jun	Cyd-X Xtra	CM granulovirus	Trench, Pear Orchard (Orange)
20 Jun	Cyd-X Xtra	CM granulovirus	Trench, Pear Orchard (Orange)
21 Oct	Nemasys C	*Steinernema carpocapsae*	Pack house and Saddletons (Blue)

Table 6.4. Site 2 complete insecticide spray records. Growers applied all products according to label recommendations

Date	Treatment	Active	Orchard treated
Conventional areas (red)			
09 Apr	Calypso	thiacloprid	C4, Oak, C2
24 Apr	Runner	methoxyfenozide	C2
15 May	Calypso	Thiacloprid	C2, Oak
05 Jun	Aphox	primicarb	C4
27/28 Jun	Coragen	chlorantraniliprole	C4, Oak, C2
08 Jul	Runner	methoxyfenozide	C4, Oak, C2
21/22 Jul	Coragen	chlorantraniliprole	C4, Oak, C2
21 Jul	Aphox	primicarb	C4
MD areas			
10 Mar	Cyren	chlorpyrifos	Harcourt Meadow, M Linder
20 Apr	RAK3&4	*Cydia pomonella Adoxophyes orana Archips podana* sex pheromones	M Linder (Top Right) (Bottom right), (Top left), (Bottom Left), Harcourt M (Top), (Bottom)
10 Apr	Gazelle	acetamiprid	M Linder & Harcourt Meadow all plots
20 Apr	Capex	SFT granulovirus	Orange plots (M Linder (BL), Harwort (T)
29 Apr	Capex	SFT granulovirus	Orange plots (M Linder (BL), Harwort (T)
15 May	Gazelle	acetamiprid	Harcourt Meadow all plots
03 Jul	Aphox	primicarb	M Linder all plots
09 Jul	Cyd-X Xtra	CM granulovirus	Orange plots (M Linder (BL), Harwort (T)
15 Jul	Cyd-X Xtra	CM granulovirus	Orange plots (M Linder (BL), Harwort (T)

24 Jul	Cyd-X Xtra	CM granulovirus	Orange plots (M Linder (BL), Harwort (T)
21 Oct	Nemasys C	*Steinernema carpocapsae*	Blue plots (Harwort (B), M Linder (TR)

Table 6.5. Products were supplied free of charge by the following;

Company	Product	Quantity	Contact
BASF plc	RAK3&4	16 ha in each year	Simon Townsend, Agronomy Manager, Specialist Products., BASF plc. Agricultural Products Division, PO Box 4, Earl Road Cheadle Hulme, Cheshire, SK8 6QG
Sentimol	CM Combo, SFT, FTT lures	132 lures for each moth sp.	David Loughlin, Director, Sentomol Ltd.
Andermatt Biocontrol AG	Capex	4 sprays for 8 ha in each year	Reto Flückiger, Technical Manager, Market Development, Stahlermatten 6 6146 Grossdietwil Switzerland
Certis	CydXtra	3 sprays for 8 ha / year	Alan Horgan, Technical Officer, Certis Europe 1 Riverside, Suite 5, Granta Park, Great Abington, Cambs, CB21 6AD

Experimental design and layout

Due to the size of the demonstration trial only 2 sites were treated, hence there were only 2 replicates. There were 2 subplots of each treatment at each farm (but these were pseudo-replicates and could not be analysed statistically). Detailed assessments of codling and tortrix moth and other pest damage and predator numbers were assessed in the centre of 10 plots at each site (4 pheromone, 4 pheromone + granulosis virus, 4 pheromone + nematode, 4 conventional, 2 untreated) on 3 occasions. The pheromone + nematode treatment will be assessed in 2016 only. All other treatments will be assessed in both 2015 and 2016.

Assessments

All project members attended the first sampling occasion (Site 1; 15 April and Site 2; 20 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 following varieties as they were ready to harvest.

There were 3 assessments;

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

Flight activity of codling and tortrix moths: 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 growers and 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 5 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 (adult/nymph), 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.

Tortrix caterpillar: 2 weeks after the application of Capex, trees in the centre of the MD only and MD + Granulosis virus plots, were inspected for 1 man hour. 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 apple 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 trees (every other tree) were raked out and counted (not June drop fruits). The number of apples 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 10-20 (>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 apple.

Second generation CM fruit damage (harvest assessment): Dropped fruit were assessed as above. Tree fruits in the centre of each plot were assessed by picking into picking buckets using the growers' standard for apple selection (size, shape, colour). All apples on each tree were counted. Any apples that had damage were dropped into a box beneath the tree for examination. Saleable apples were gently put into bins. Assessments for the damaged fruits were as 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:

The first generation flight of CM was above the threshold of 5 moths per trap for 5 weeks between 9 Jun and 8 July in the growers' conventional side of farm at Site 1 (Fig. 6.4). The grower applied Coragen (chlorantraniliprole) and Explicit (indoxicarb) against the first generation (Table 6.3). The numbers of codling moth on the MD side of the farm only exceeded 5 moths per trap in one week (8 Jul). However, in order to test the additive effect of granulosis virus applications of Cyd-X Xtra were made to the orange coded orchards at this farm on 12 and 20 June. The second generation of moths was below 3 per trap per week in all plots and hence spray applications were not considered necessary.

SFT numbers remained very low at this farm and although numbers did not reach threshold (30/trap/week) Capex was applied on 17 Apr and 01 May to the orange coded plots to time with the first generation. The second generation occurred in Aug/Sep but was again very small (Fig. 6.4).

FTT moth catches were also below threshold (<20 at peak) at this site and peaked in June and again in Aug/Sep, so no protectant products were applied in response to this pest (Fig. 6.4).

Codling moth catches were very low at Site 2 in 2015 even though the farm had reportedly had high CM catches in previous years (Fig. 6.4). No SFT moths were trapped at this farm in 2015. Despite this it was agreed to apply the Capex (20, 29 Apr) and Cyd-X Xtra (09, 15, 24 Jul). FTT numbers almost reached threshold levels in the untreated and growers conventional programme side of the farm (Fig. 6.4). There were virtually no FTT adults trapped in the MD side of the farm. Applications of Coragen (chlorantraniliprole) and Runner (methoxyfenozide) were applied to the conventional side of the farm from late June to late July, presumably to control FTT (Table 6.4).

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.

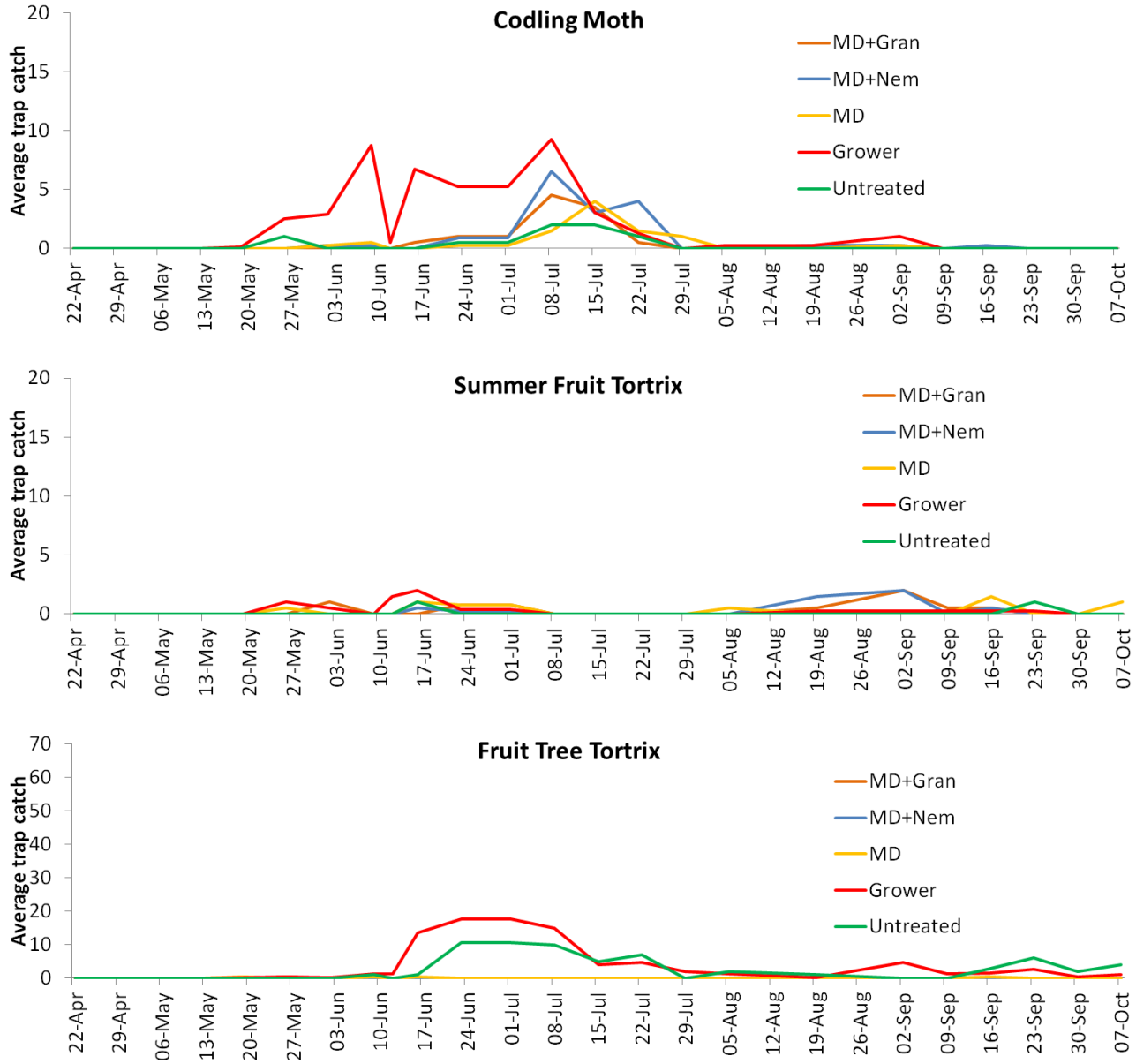


Figure 6.4. Mean numbers of codling moth, summer fruit tortrix and fruit tree tortrix moth in sex pheromone monitoring traps at Site 1 in 2015

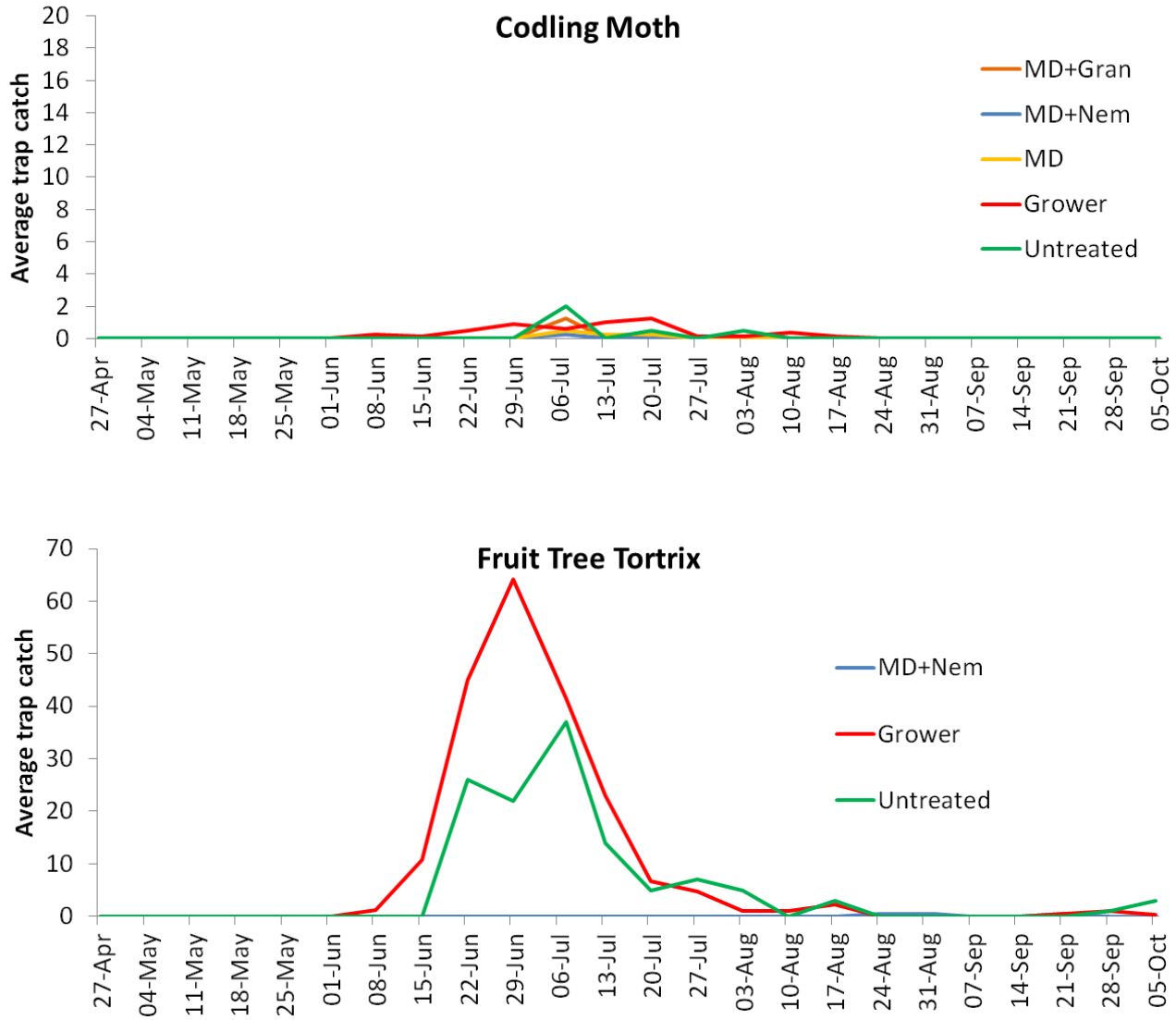


Figure 6.5. Mean numbers of codling moth and fruit tree tortrix moth in sex pheromone monitoring traps at site 2 in 2015. NB summer fruit tortrix moth was not found at this farm in 2015

Other pests and natural enemies – all assessments:

No treatments had been applied at the spring assessment, but this assessment acted as a baseline for the orchards in each farm. Fig. 6.6 shows the most common arthropod groups encountered at in each orchard. There was a clear difference between the abundance of arthropods between the two farms in April. Very few arthropods were found in the assessments at Site 2 although there was a slightly higher abundance of spiders compared to Site 1. Apple grass aphid (AGA) was the most abundant insect of note at Site 1 with reasonable numbers of Rosy apple aphid (RAA) also present. Although there are differences between the farms, within a farm the groups of invertebrates present were similar across the orchards.

At the July assessment (Fig. 6.7a) conventional, MD and virus applications had been made. There were some distinctions between the farms. Site 2 had higher numbers of WAA and harvestmen. Site 1 had higher numbers of earwigs, overall. It is well documented that earwigs are effective natural enemies of WAA. Figure 6.7b shows the numbers of woolly apple aphid plotted against the numbers of earwigs at each of the orchards at Site 2. In general where there are more earwigs (white bars) present there are fewer WAA (grey bars) colonies (more data will be needed for a thorough analyses). There were also arthropod differences between the two halves of each farm, but at this time it is not known whether these are the result of the treatments or the location on the farm. For example there is a higher incidence of AGA and lower incidence of earwigs on the conventional side of the farm at Site 1. In contrast there is a higher incidence of AGA on the MD side of Site 2, but there are fewer earwigs on this side of the farm.

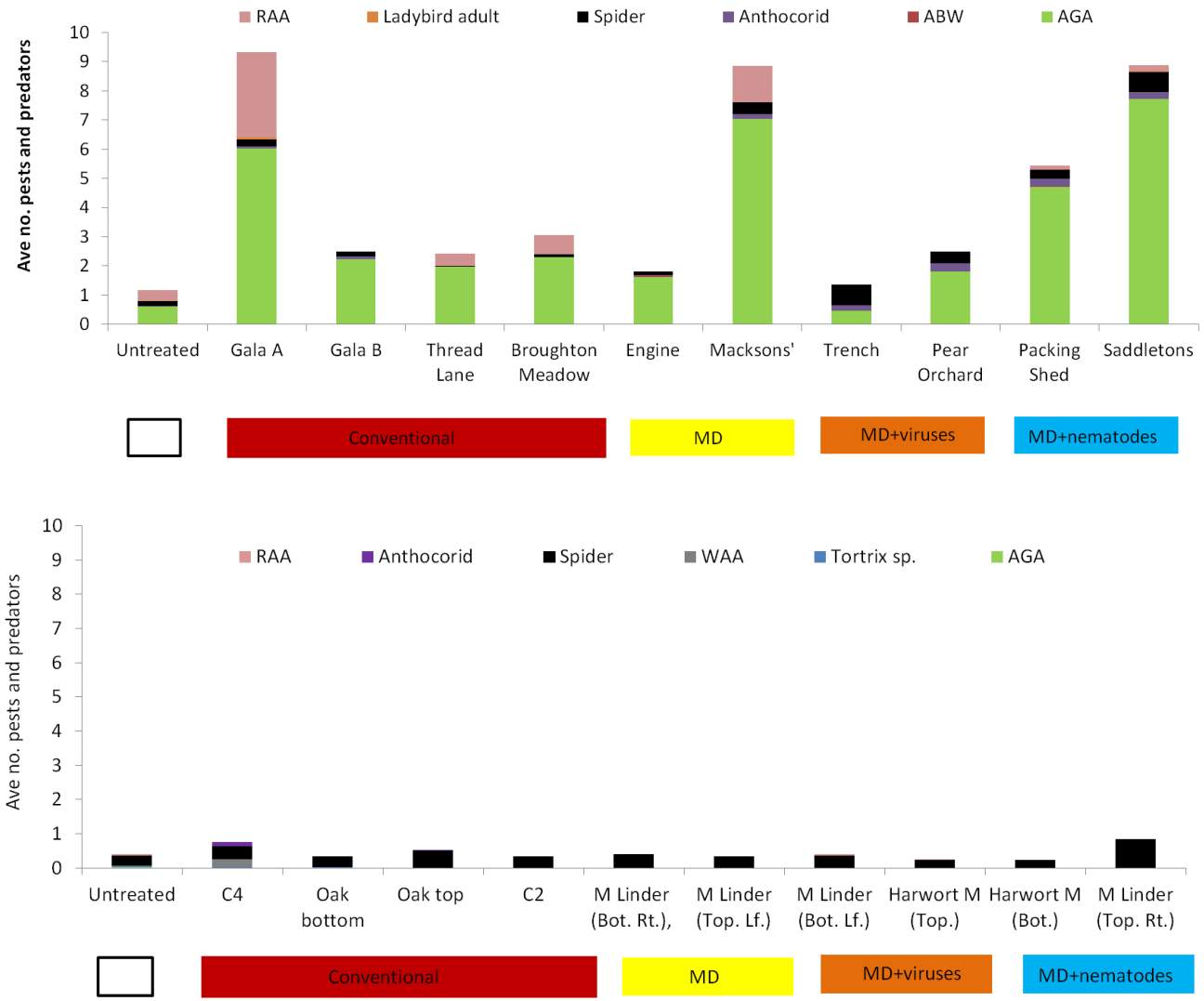


Figure 6.6. Spring assessments (pre-treatments) of dominant pests and natural enemies in each orchard at Mt Ephraim (top) and Site 2 (bottom) (April 2015); MD = mating disruption, RAA – rosy apple aphid, ABW = apple blossom weevil, AGA = apple grass aphid, WAA = woolly apple aphid, Tortrix sp. = tortricid caterpillar

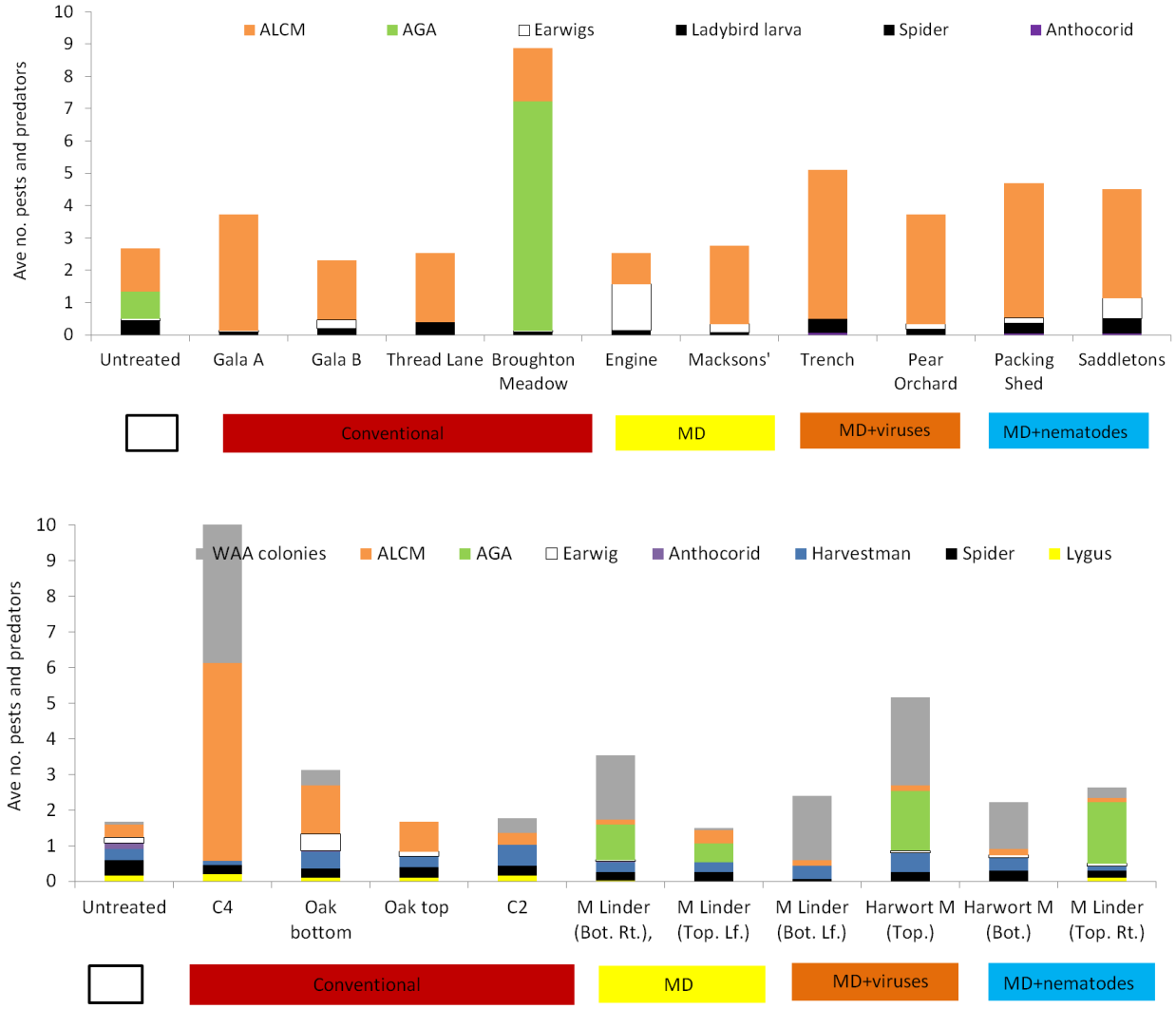


Figure 6.7a.

Summer assessments of dominant pests and natural enemies at Mt Ephraim (top) and b) Site 2 (bottom) (July 2015); MD = mating disruption, ALCM = apple leaf curling midge damaged shoots, WAA = woolly apple aphid

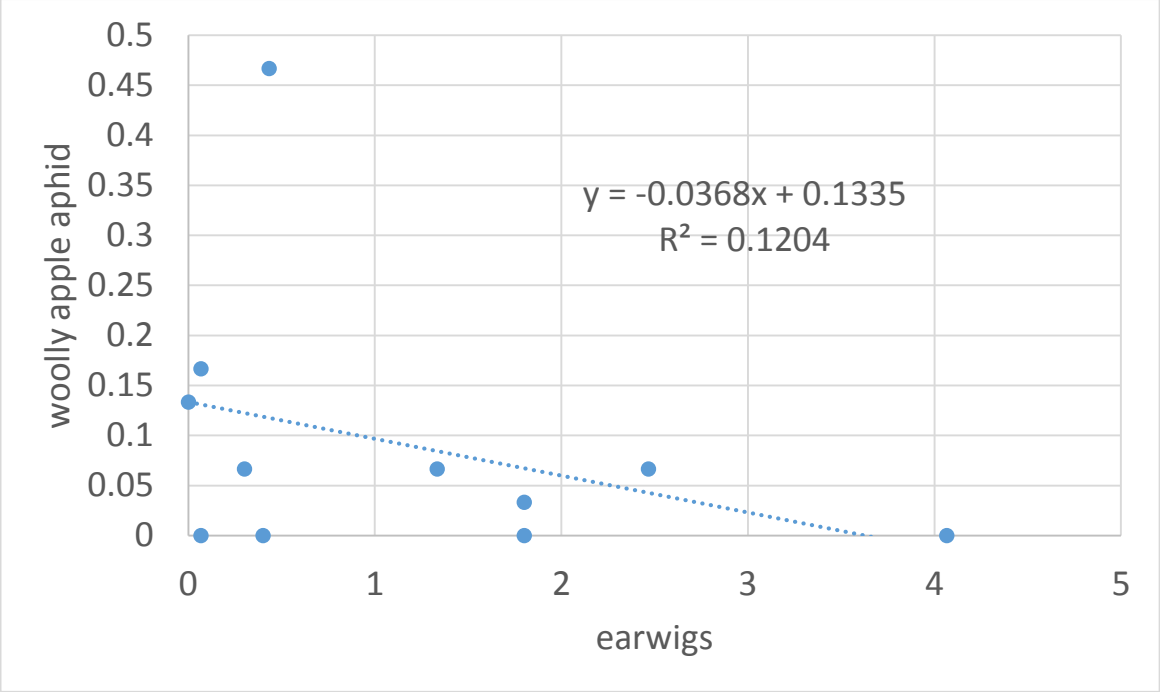


Figure 6.7b. Numbers of WAA plotted against numbers of earwigs at the Site 2 summer assessment

At the harvest assessment all of the treatments except nematodes had been applied (hence the nematode plots were MD only at this point). Encouragingly there were higher numbers of earwigs on the MD side of Site 1 compared to the conventionally sprayed plots and it remains to be seen if these earwig numbers continue to rise into the second year of the trial. No difference in earwig numbers was apparent at Site 2 (NB: WAA was not assessed at this time). Site 2 had a higher abundance of harvestmen (Opilione) and spiders than Site 1.

Tortrix caterpillar:

No tortrix caterpillars were found at Site 2 in the young leaf shoots (21 May). After searching the MD only and MD+virus plots at Site 1 (12 May) only 2 larvae were found in each plot. From the MD only plot, one adult emerged and one parasitoid. From the MD+ virus plot both larvae died before pupation.

First generation CM fruit damage:

At Site 1 at the first codling moth generation assessment (01 July) there were very few dropped fruits and as a consequence virtually no CM or tortrix damage was found. In addition only 2 fruits on the trees in Packing Shed orchard were found with tortrix damage. The highest CM damage (1.5%) was seen in the MD only treatment orchard with the variety Early Windsor. 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. In all other orchards at this farm the damage was 0.4% or lower (Table 6.6) overall. Virtually no tortix damage was found at Site 2 (29 July) at the first generation damage assessment (data not shown). The only notable CM damage was 9.5 % and 0.1 % of the dropped and tree fruit in the untreated plot (Oak – Royal Blush) and 8.3% of the dropped fruit in orchard C4 (Gala – Conventional treatment).

Table 6.6. Mean numbers of fruits damaged by first generation codling moth at Site 1. white = untreated, red = Conventional sprays, yellow = MD only, orange = MD + capex and Cyd-X Xtra, blue = MD = nematodes. CM = codling moth, DE = deep entry

		Dropped fruit		**Tree fruit**	
Orchard	**Variety**	**CM Sting**	**CM DE**	**CM Sting**	**CM DE**
Untreated	Braeburn	0	0.1	0.1	0
Gala A	Gala	0	0	0.3	0
Gala B	Gala	0	0	0.2	0
Thread Lane	Gala	0	0	0	0
B'ton Meadow	Braeburn	0	0	0	0
Engine	Early Windsor	0	0	0	1.1
Mackson's	Gala	0	0	0.2	0.1
Trench	Bramley	0	0.5	0	0.5
Pear Orchard	Cox	0	0.1	0	0
Packing Shed	Gala	0	0	0.3	0.1
Sadleton's	Braeburn	0	0	0.3	0.1

Second generation CM fruit damage (harvest assessment):

DROPPED FRUITS SITE 1: No CM deep entry (DE) damage was found on the convention side of site 1 at harvest (Table 6.7). Only two of the orchards on the MD disruption side of the farm had no DE damage (both Gala). Damage to 4 of the other orchards ranged from 1.6-6.2% damage, overall (the early variety having the highest damage level) and is considered significant. Other minor damage from tortrix, Rhynchites and capsid also occurred on the MD side of the farm (Table 6.7). There was some CM damage on in the untreated row on the conventional side of the farm (5.3% with CM stings), but similar damage was also seen on the treated side of this orchard (4.5%).

Table 6.7. Percentage of dropped fruits damaged at harvest by codling moth and other pests at Site 1. white = untreated, red = Conventional sprays, yellow = MD only, orange = MD + capex and Cyd-X Xtra, blue = MD = nematodes. CM = codling moth, DE = deep entry

Harvest Date	Orchard	Variety	CM DE	CM Sting	Tortrix	Early Caterpillar	Rhyn-chites	Capsid
12 Oct	Untreated	Braeburn	0.0	5.3	0.0	0.0	0.0	0.0
14 Sep	Orchard A	Gala	0.0	0.0	0.0	0.0	0.0	0.0
14 Sep	Orchard B	Gala	0.0	0.0	0.0	0.0	0.0	0.0
14 Sep	Thread Lane	Gala	0.0	0.0	0.0	0.0	0.0	0.0
12 Oct	B'ton Meadow	Braeburn	0.0	4.5	0.0	0.0	0.0	0.0
28 Aug	Engine	E. Windsor	5.2	1.0	0.0	2.1	0.0	0.0
14 Sep	Mackson's	Gala	0.0	0.0	0.0	0.0	0.0	0.0
28 Aug	Trench	Bramley	1.6	0.0	0.3	0.0	0.3	1.0
07 Sep	Pear Orchard	Cox	3.2	0.0	0.0	0.0	0.0	1.6
14 Sep	Packing Shed	Gala	0.0	0.0	0.0	0.0	0.0	0.0
12 Oct	Sadleton's	Braeburn	1.0	4.8	0.3	0.0	0.3	0.3

TREE FRUITS SITE 1: The untreated area on this farm had similar CM damage to the conventionally treated side of the orchard and is not considered a valid untreated area. DE damage on the conventional and MD sides of the farm ranged 0-0.1% and 0.1-1.0% respectively. Tortrix damage was also higher on the MD side of the farm (0.2-3.1%) compared to the conventional side (0-0.1%). The early ripening varieties, E. Windsor and Bramley, had the most CM damage; these varieties were not present on the conventional side of the farm. By comparing Gala and Braeburn (present on both sides of the farm) DE damage was similar (<0.2%) (Table 6.8).

There was more sawfly and early caterpillar damage on the conventional side and a higher frequency of Rhynchites and capsid damage on the MD side of the farm. This could be a result of spray programmes or the locality of the orchards on the farm. It is difficult to conclude at this time whether the addition of viruses improved pest moth control (orange plots) compared to MD only (yellow and blue plots, Table 6.8).

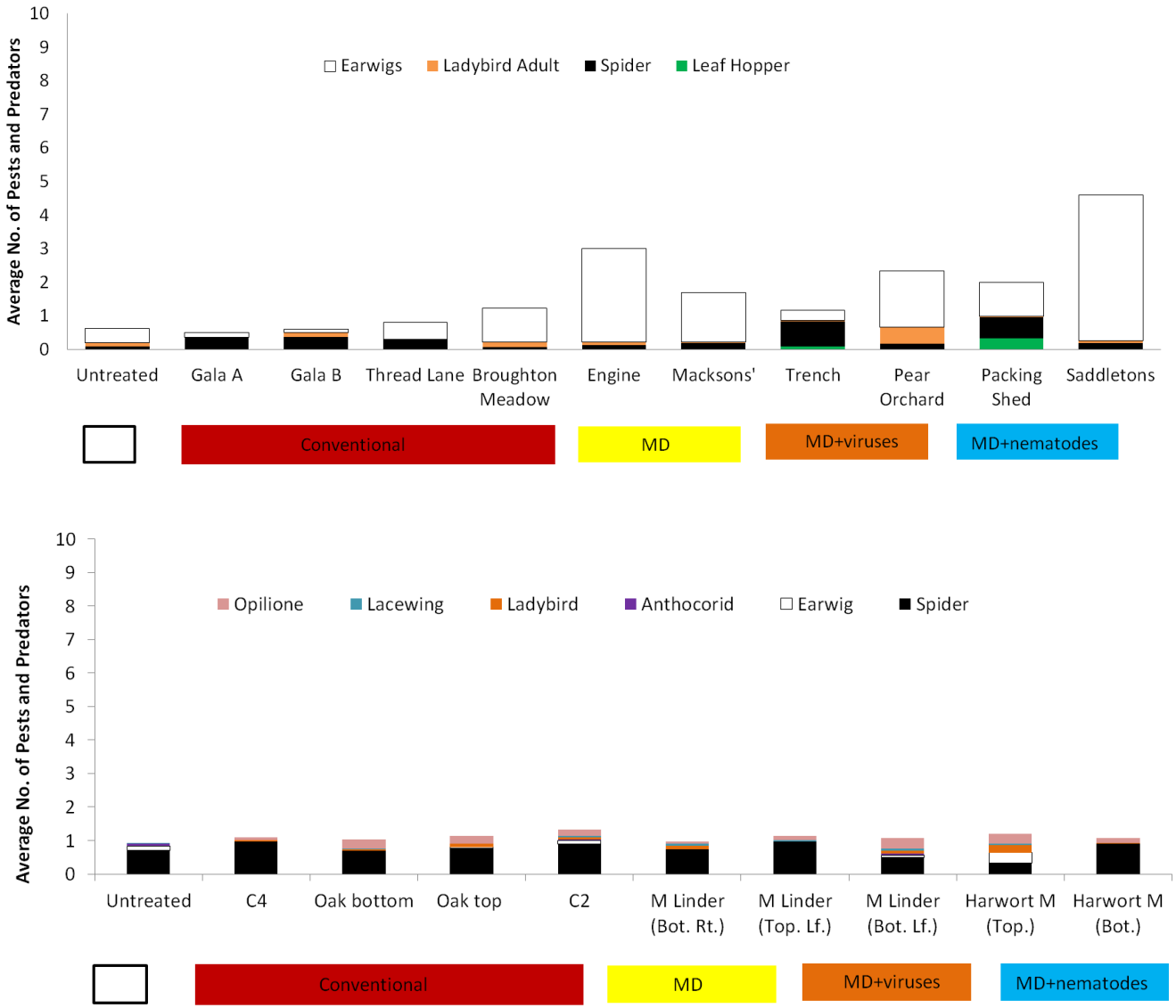


Figure 6.8. Autumn assessments of dominant pests and natural enemies at Mt Ephraim (top) and Site 2 (bottom) (7 and 9 Oct 2015); MD = mating disruption, Opilione = harvestmen

Table 6.8. Percentage of tree fruits damaged at harvest codling moth and other pests at Site 1. white = untreated, red = Conventional sprays,

Harvest Date	Orchard	Variety	CM DE	CM Sting	Tortrix	Early cater-pillar	Winter moth	Rosie apple aphid	Rhyn-chites	Capsid	Mussel scale	Sawfly
12 Oct	Untreated	Braeburn	0.1	0.2	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
14 Sep	Orchard A	Gala	0.1	0.2	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.3
14 Sep	Orchard B	Gala	0.0	0.1	0.0	0.3	0.1	0.0	0.0	0.0	0.0	0.3
14 Sep	Thread Lane	Gala	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.1	0.0	0.1
12 Oct	B'ton Meadow	Braeburn	0.1	0.2	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0
28 Aug	Engine	E. Windsor	1.0	0.7	0.5	1.0	0.4	0.1	0.0	0.0	0.0	0.0
14 Sep	Mackson's	Gala	0.2	0.2	3.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0
28 Aug	Trench	Bramley	0.7	0.3	0.2	0.0	0.1	1.0	0.0	0.0	0.2	0.0
07 Sep	Pear Orchard	Cox	0.2	0.2	0.5	0.1	0.2	0.0	0.2	0.4	0.4	0.0
14 Sep	Packing Shed	Gala	0.2	0.1	2.0	0.0	0.1	0.0	0.4	0.3	0.0	0.2
12 Oct	Sadleton's	Braeburn	0.1	0.7	0.4	0.0	0.1	0.0	0.2	0.1	0.0	0.0

yellow = MD only, orange = MD + capex and Cyd-X Xtra, blue = MD = nematodes. CM = codling moth, DE = deep entry

DROPPED FRUITS SITE 2:

CM trap catches were very low at this farm and correspondingly there was very little CM damage with the exception of the untreated area which had 0.8% damage in the dropped fruit (Table 6.9). The only significant damage was mussel scale in one orchard 13.6% in Harwort M (top part of the orchard).

Table 6.9. Percentage of dropped fruits damaged at harvest by codling moth and other pests at Site 2. white = untreated, red = Conventional sprays, yellow = MD only, orange = MD + capex and Cyd-X Xtra, blue = MD = nematodes. CM = codling moth, DE = deep entry

Harvest Date	Orchard	Variety	CM DE	CM Sting	Tortrix	Winter moth	Mussel scale
05 Oct	Untreated	Royal Blush	0.8	0.0	0.0	1.5	0.8
05 Oct	C4	Gala	0.0	0.0	0.0	0.0	0.0
09 Sep	Oak	Red Windsor	0.0	0.0	0.0	0.0	0.0
23 Sep	C2	Cox	0.0	0.0	2.7	0.0	0.0
23 Sep	M Linder (B R)	Gala	0.0	0.0	0.0	0.0	0.0
23 Sep	M Linder (T L)	Gala	0.0	0.0	0.0	0.0	0.0
23 Sep	M Linder (B L)	Gala	0.0	0.0	0.0	0.0	0.0
05 Oct	Harwort M (T)	Red Falstaff	0.0	0.0	0.0	0.0	13.6
05 Oct	Harwort M (B)	Red Falstaff	0.0	0.0	0.0	0.0	0.0
23 Sep	M Linder (T R)	Gala	0.0	0.0	0.0	0.0	0.0

TREE FRUITS SITE 2: There was very low CM damage in the conventional treated side of the farm. The untreated orchard had 0.5% DE damage and one Gala orchard on the MD side of the farm had 0.7% tree fruit damaged fruits. Damage by tortricids was fairly similar (0.1-0.3%, Table 6.10). The incidence of other pest damage in the orchards was similar to the dropped fruit above.

Table 6.10. Percentage of tree fruits damaged at harvest by codling moth and other pests at Site 2. white = untreated, red = Conventional sprays, yellow = MD only, orange = MD + Capex and Cyd-X Xtra, blue = MD = nematodes. CM = codling moth, DE = deep entry

Harvest Date	Orchard	Variety	CM DE	CM Sting	Tortrix	Winter moth	Capsid	Mussel scale
05 Oct	Untreated	Royal Blush	0.5	0.1	0.1	1.0	0.2	0.2
05 Oct	C4	Gala	0.1	0.0	0.1	0.0	2.5	0.1
09 Sep	Oak	R. Windsor	0.0	0.1	0.2	0.0	0.3	0.1
23 Sep	C2	Cox	0.0	0.0	0.1	0.0	0.0	0.2
23 Sep	M Linder (B R)	Gala	0.7	0.0	0.1	0.0	0.0	0.1
23 Sep	M Linder (T L)	Gala	0.1	0.0	0.0	0.3	0.1	0.0
23 Sep	M Linder (B L)	Gala	0.0	0.0	0.1	0.1	0.0	0.0
05 Oct	Harwort M (T)	R. Falstaff	0.0	0.0	0.1	0.0	0.2	3.9
05 Oct	Harwort M (B)	R. Falstaff	0.0	0.0	0.3	0.1	0.0	1.9
23 Sep	M Linder (T R)	Gala	0.0	0.0	0.0	0.1	0.2	0.1

Phytotoxicity: At the summer assessment in July 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 effected a small area of a couple of leaves per tree.

Figure 6.9. Photographs of phytotoxic damage to small areas of the leaves in direct contact with the RAK3&4 devices

Conclusions

It is difficult to draw conclusions from one year of data and because the trial is unreplicated but general trends in 2015 were;

- There are some promising trends with the numbers of earwigs (white bars) on both farms; being higher on the MD treated side of the farm
- Other predator trends will be further examined in 2016
- At the low CM pressure farm (Site 2) there was negligible damage on the MD side of the farm and damage was comparable to the growers standard spray programme (Table 6.11)
- At Site 1 where the CM pressure was higher there was higher damage in Early Windsor, Cox and Bramley varieties in the MD side of the farm (Table 6.11). These varieties were not present on the growers spray programme side of the farm. Varieties which were on both sides – Gala and Braeburn – had similar CM damage to the fruits in both growers spray programme and MD
- It may be advantageous at farms with medium to high pressure codling numbers to apply an additional Coragen to early ripening or vulnerable varieties where MD technologies are employed

- At Site 2 where the pressure from FTT was high damage by tortrix caterpillars was very low in both control methods
- There was no evidence that additional sprays of viruses for CM and SFT had added benefit to the MD method.

Table 6.11. Summary of percentage codling moth (CM) and tortrix damage to dropped and tree fruit on both farms.

Site 1					
Orchard	Variety	Dropped fruits		Tree fruits	
		CM	Tortrix	CM	Tortrix
Untreated	Braeburn	5.4	0.0	0.4	0.0
Gala A	Gala	0.0	0.0	0.6	0.0
Gala B	Gala	0.0	0.0	0.3	0.0
Thread Lane	Gala	0.0	0.0	0.0	0.0
B'ton Meadow	Braeburn	4.5	0.0	0.3	0.1
Engine	E. Windsor	6.2	0.0	2.8	0.5
Mackson's	Gala	0.0	0.0	0.7	3.1
Trench	Bramley	2.1	0.3	1.5	0.2
Pear Orchard	Cox	3.3	0.0	0.4	0.5
Packing Shed	Gala	0.0	0.0	0.7	2.0
Sadleton's	Braeburn	5.8	0.3	1.2	0.4
Site 2					
Orchard	Variety	Dropped fruits		Tree fruits	
		CM	Tortrix	CM	Tortrix
Untreated	Royal Blush	10.3	0.0	0.7	0.1
C4	Gala	0	0.0	0.1	0.1
Oak	Red Windsor	0	0.0	0.1	0.2
C2	Cox	0	2.7	0	0.1
M Linder (B R)	Gala	0	0.0	0.7	0.1
M Linder (T L)	Gala	0	0.0	0.1	0
M Linder (B L)	Gala	0	0.0	0	0.1
Harwort M (T)	Red Falstaff	0	0.0	0	0.1
Harwort M (B)	Red Falstaff	0	0.0	0	0.3
M Linder (T R)	Gala	0	0.0	0	0

Objective 8	Rhynchites weevil and sawfly	Task 1	Biology and Semiochemicals of Rhynchites weevil

Aim

Identification of biology and semiochemicals attractive to apple fruit rhynchites weevil (EMR/NRI, Yr 1)

Introduction

Damage by apple fruit rhynchites, *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% fruit are common and losses >5% are not unusual. Hawthorn and blackthorn are the pest's usual hosts. Damage to apple is caused by feeding punctures in young developing fruitlets during and after blossom. Females sever the stems of apple fruitlets after laying eggs and the development then probably occurs on the fruit on the ground (Massee 1954; Alford 1984). The pest causes damage at low population densities and the weevils are difficult to spot whilst they are feeding or egg laying. The extent of damage only becomes apparent when the characteristic corky scars develop and when it is too late to take action.

The weevil can be controlled by sprays of chlorpyrifos or thiacloprid (Calypso) but the former cannot be used during or after blossom and growers are reluctant to use thiacloprid during flowering because of risk to bees. In addition, chlorpyrifos is broad spectrum and can damage other beneficial insects in the orchard and both chlorpyrifos and thiacloprid are damaging to earwigs.

It would be beneficial to develop a sensitive, species-specific semiochemical based monitoring trap for this pest and be able to predict more accurately when and if to apply treatments. However, it is not known whether *R. aequatus* produces a sex or aggregation pheromone, when it is produced or which sex produces it. Many weevils are known to produce sex or aggregation pheromones, e.g. strawberry blossom weevil and pepper weevil (male produced aggregation pheromones), but in others pheromones do not seem to be so important, e.g. apple blossom weevil. Nothing is known about pheromones of Rhynchitidae. In a previously funded 2 year AHDB project (TF209) in the laboratory, males and females were able to identify each other and successfully mate resulting in eggs being laid. In a field experiment no statistical significances were identified to suggest attraction was occurring between males and females. However, in the second year of the project significantly more

female weevils were caught on trees not baited with weevils suggesting competition for egg laying sites/ dispersion.

Weevil volatile collections were made, but analyses of these collections by gas chromatography coupled to mass spectrometry (GC-MS) were unsuccessful and no sex specific compounds were identified. When the collections were tested by electroantennographic (EAG) recording from antennae of Rhynchites, good responses were obtained to collections made in the presence of food but not to those made without. Analyses of the collections by GC coupled to EAG recording showed a small EAG response to the large amount of benzyl alcohol in collections made with apple buds present. This compound may play a role in attraction of Rhynchites to host plants. In this project we

- Focused on the collection of newly emerging virgin males and females for semiochemical identification by including entrainment and EAG
- Identified growth stages of apple cultivars and wild hosts in correlation with appearance of rhynchites in hedgerows and orchards to further identify the window of spray opportunity before flower opening

Materials and methods

Sites and site manager

Wiseman orchard (cv's. Gala, Discovery, Ecolette, Fiesta, Queen Cox, Saturn) at East Malling Research, New Road, East Malling, Kent, ME19 6BJ by kind permission of Graham Caspell, farm manager.

Collection of newly emerged adults: Orchards and hedgerows previously infested with apple fruit Rhynchites were searched and trees tap sampled weekly over a white sheet on warm sunny days from 17 Feb 2015 (apple trees dormant). Six apple cultivars were sampled weekly until first catches were made on 07 Apr (individual found with soil on elytra suggesting it had just emerged from pupation). The sex of the weevils, the apple variety and growth stage was also recorded using the EPPO growth stage keys. A description of other host species (blackthorn and hawthorn) growth stage were also noted. Temperature, humidity and wind speed were recorded at the time of collection. Assessments were done on 23, 30 Apr, 07, 13, 21, 29 May, 05 and 10 Jun. Data was collated depending on growth stage of the tree (not date). No Rhynchites harmful sprays were applied to Wiseman orchard.

Entrainment of insects: Field collected weevils were sexed and taken back to the lab to be used for volatile collections (Table 8.1.1). Lighting was on between 0900 and 0130 h and off between 0130 and 0900 h. Insects were contained in silanised glass vessels (12 cm x 5 cm)

with a food source and air drawn in (200 ml/min) through an activated charcoal filter (20 cm x 2 cm; 8-10 mesh) and out through a collection filter consisting of Porapak Q (200 mg; 50/80 mesh) held between glass wool plugs in a Pasteur pipette (4 mm dia) (Controlled Temperature (CT) room 2 at EMR). The apparatus was cleaned by passing a continuous air flow through for 24 h before the collections began.

Table 8.1.1. Record of volatile collections made from Rhychites weevils

No.	Date collected	flow rate (ml/min)	Date begun	Insect in	Start time	Date ended	Species	male	female	food
1	07-Apr	250	07-Apr	16:30	17:00	08-Apr	*Rhynchites*	5	0	Apple leaf
2	07-Apr	250	07-Apr	16:30	17:00	08-Apr	*Rhynchites*	0	3	Apple leaf
3	07-Apr	250	08-Apr	15:00	15:00	09-Apr	*Rhynchites*	4	2	Apple leaf
4	07-Apr	250	08-Apr	15:00	15:00	09-Apr	*Malus*	0	0	Apple leaf
5	09-Apr	800	10-Apr	10:30	13:30	13-Apr	*Rhynchites*	5	0	Apple leaf
6	09-Apr	800	10-Apr	10:30	13:30	13-Apr	*Rhynchites*	0	4	Apple leaf
7	09-Apr	800	10-Apr	10:30	13:30	13-Apr	*Rhynchites*	2	2	Apple leaf
8	09-Apr	800	10-Apr	10:30	13:30	13-Apr	*Malus*	0	0	Apple leaf
9	09-Apr	800	10-Apr	10:30	13:30	13-Apr	**Blank**	0	0	Blank
10	17-Apr	800	17-Apr	16:30	16:45	17-Apr	*Rhynchites*	0	4	Apple bud
11	17-Apr	800	17-Apr	16:30	16:45	17-Apr	*Rhynchites*	4	0	Apple bud
12	17-Apr	800	17-Apr	16:30	16:45	17-Apr	*Rhynchites*	1	1	Apple bud
13	17-Apr	800	17-Apr	16:30	16:45	17-Apr	**Blank**	0	0	Blank
14	24-Apr	800	24-Apr	14:00	14:00	29-Apr	*Rhynchites*	5	0	Apple bud
15	24-Apr	800	24-Apr	14:00	14:00	29-Apr	*Rhynchites*	0	5	Apple bud
16	24-Apr	800	24-Apr	14:00	14:00	29-Apr	*Malus*	0	0	Apple bud
17	24-Apr	800	24-Apr	14:00	14:00	29-Apr	**Blank**	0	0	Blank

Analysis of volatile collections: Porapak filters were sent to NRI where they were extracted with dichloromethane (1 ml). The extracts were analysed by GC-MS using a CP3800 GC coupled to a Saturn 2200 MS (Varian). A polar GC column was used (DBWax 30 m x 0.25 mm i.d. x 0.125 µ film thickness) and oven temperature was programmed from 40°C for 2 min then at 10°C/min to 250°C. Selected samples were also analysed by GC-MS using a HP6890 GC and HP5973 MS with a non-polar column (DB5) to confirm identification of compounds. Compounds were identified by their GC retention times on the two phases, mass spectra and comparison with authentic synthetic compounds.

EAG: Newly emerged weevils were collected and sent to NRI for electroantennography.

Weevils were housed individually until they could be used. They were provided with apple buds or flowers as food.

Results

Appearance of Rhynchites in apple trees: No Rhynchites weevils were collected on apple trees which were in dormancy. The first weevils were found on 07 April on apple trees that had inner bud scales visible. Date was not a good predictor of weevil abundance in the trees (Fig. 8.1.1). In addition, there were variable numbers of weevils in the different varieties (not statistically tested), e.g. 4 times the numbers of weevils were found in Saturn compared to Ecolette. The reasons for this are not known but Saturn broke dormancy (13 Mar) 10 days before Ecolette (23 Mar) (Table 8.1.2).

Before petal fall there was a spray 'window' of 6-23 days depending on variety (Table 8.1.2). After petal fall, weevils were found in apple trees for at least 13 days. Weevils were present in the orchard until 30 mm diameter. Recording was stopped at this point as numbers had declined (Fig. 8.1.1). Very few Rhynchites were found in other hosts in 2015; only one male in blackthorn on the same date they were first found in the apple orchard.

When data was presented as mean numbers of weevils per tree for a given stage of tree development it gave a far better predictor of peak numbers in the trees (not analysed, Fig. 8.1.3). Numbers were highest in the trees at petal fall, but present from leaf development.

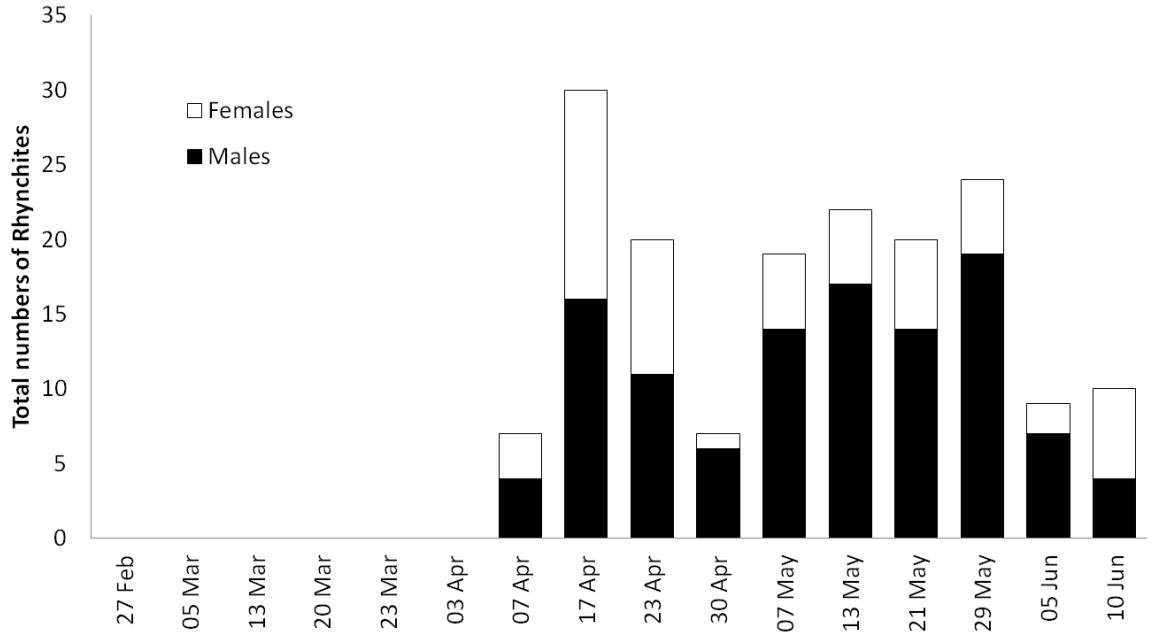


Figure 8.1.1. Total numbers of Rhynchites found in all apple varieties surveyed over the period of the trial.

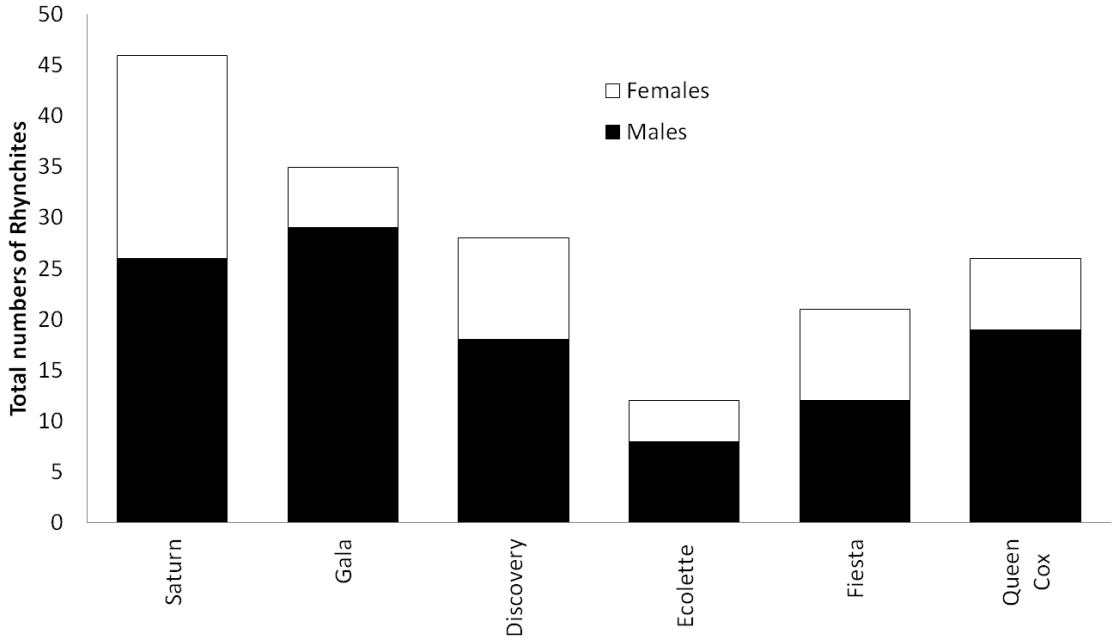


Figure 8.1.2. Numbers of weevils in apple varieties ordered (left to right) by variety to break dormancy first.

Table 8.1.2. Development of flowering in the different apple varieties and potential to target Rhynchites with controls

Variety	Date weevils first found	Date flowers open	No. days pre- flowering	Date end flowering	No. days post-flowering
Gala	07 Apr	30 Apr	23	21 May	20
Discovery	07 Apr	23 Apr	16	21 May	20
Ecolette	17 Apr	30 Apr	13	21 May	13
Fiesta	17 Apr	23 Apr	6	21 May	20
Queen Cox	07 Apr	30 Apr	23	21 May	20
Saturn	07 Apr	30 Apr	23	21 May	20

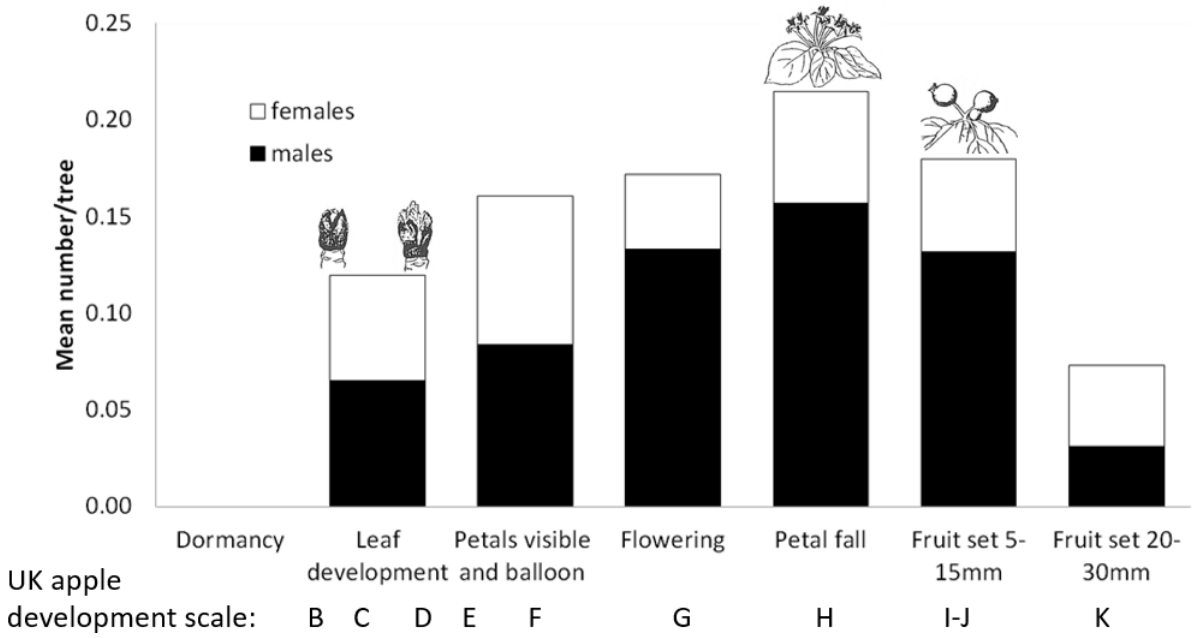


Figure 8.1.3. Mean numbers of male and female Rhynchites in Wiseman apple orchards according to tree development stage.

Analysis of volatile collections: Samples were analysed by GC-MS on a polar column (Fig. 8.1.4-8.1.6)

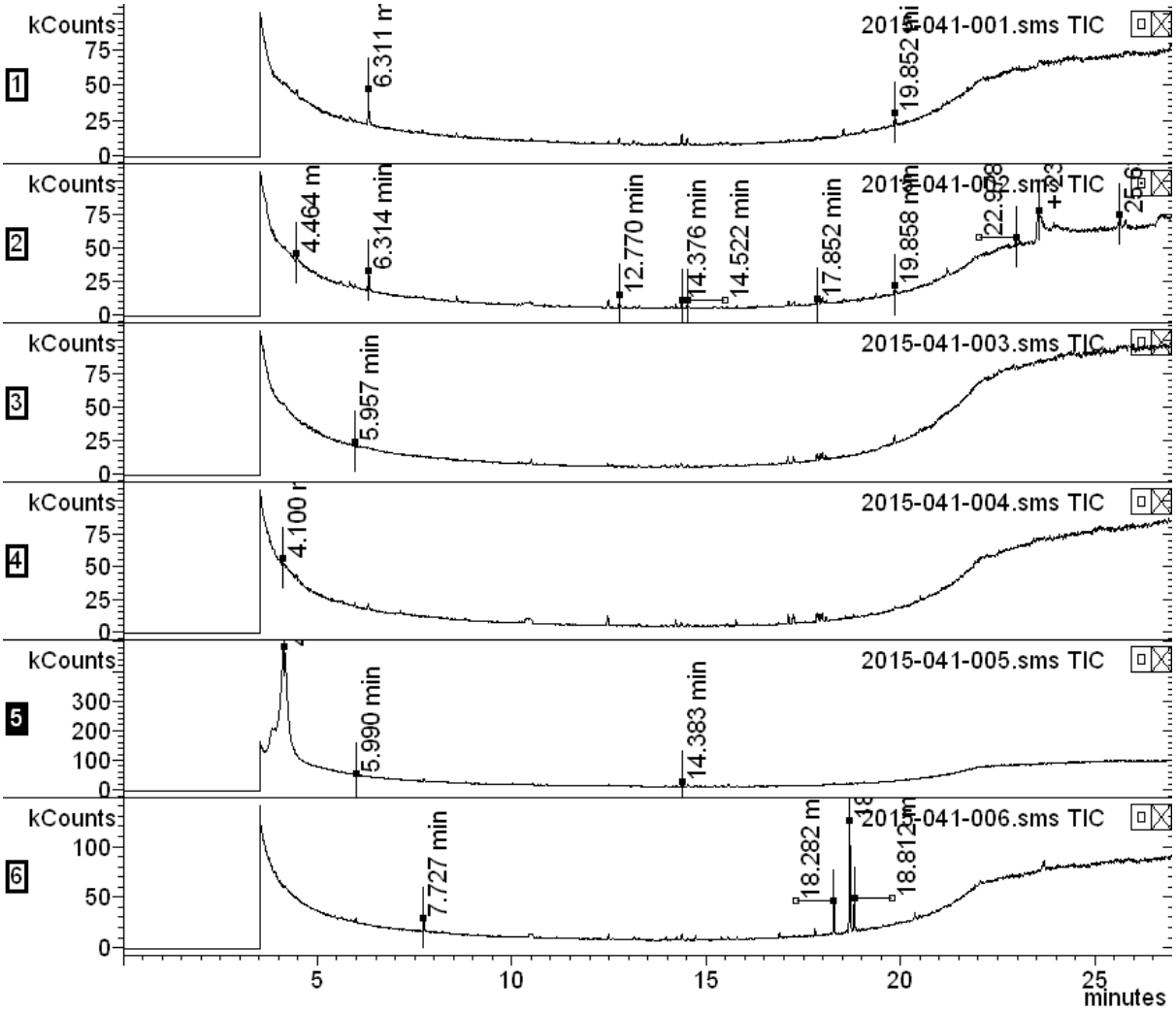


Figure 8.1.4. GC-MS Analyses on polar GC column of samples 1-6 (from top).

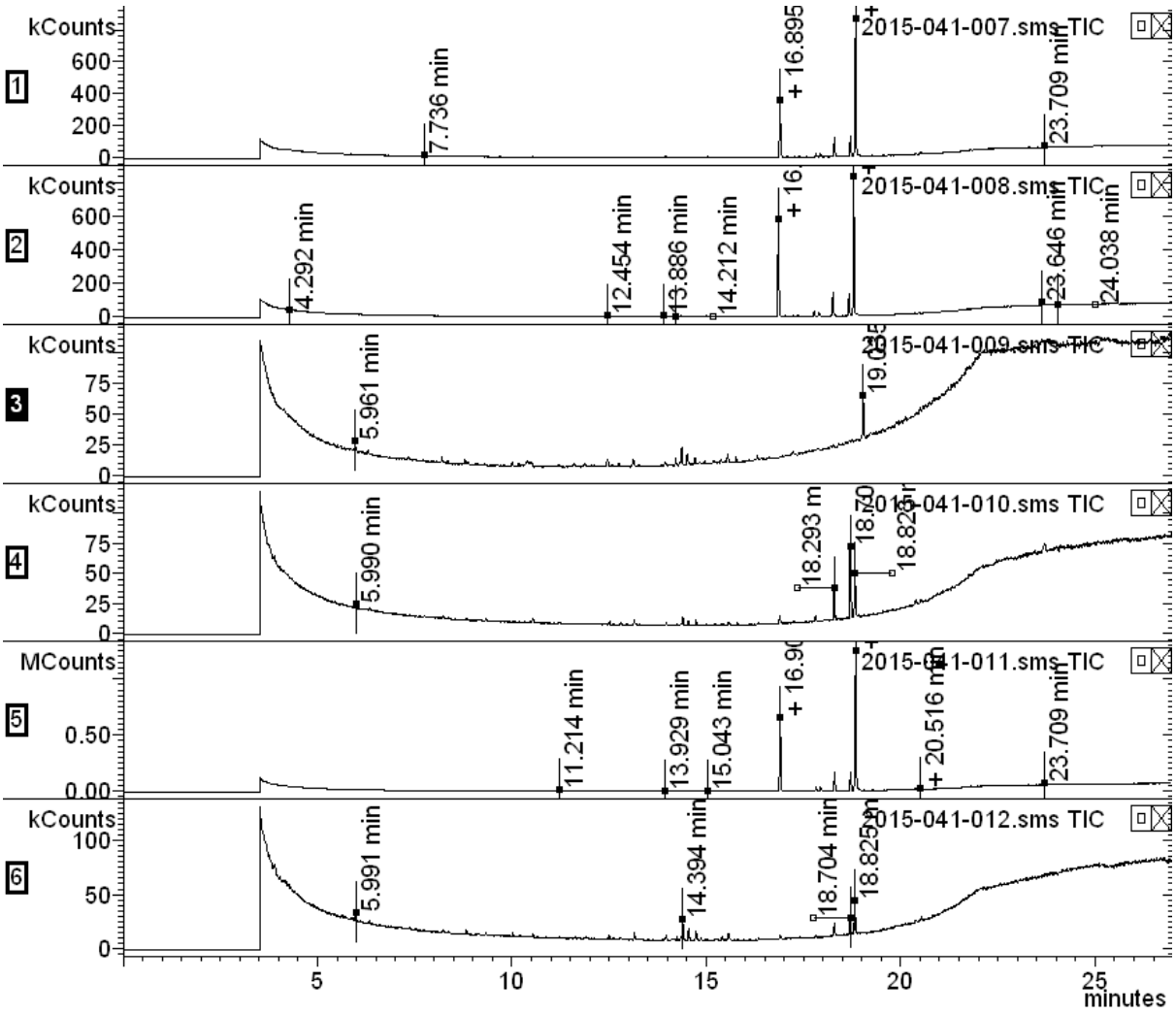


Figure 8.1.5. GC-MS Analyses on polar GC column of samples 7-12 (from top).

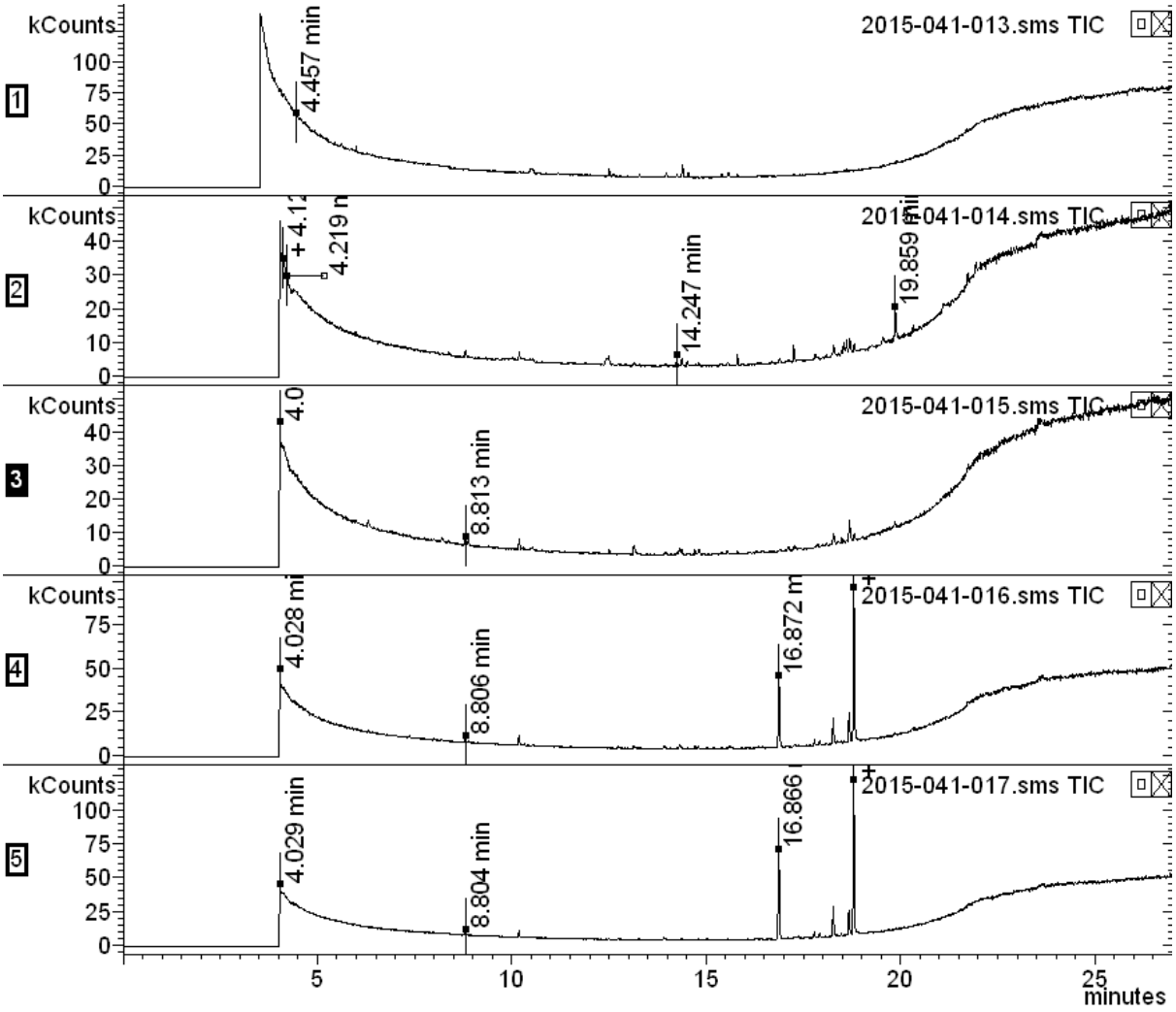


Figure 8.1.6. GC-MS Analyses on polar GC column of samples 13-17 (from top).

Some of the samples (7) showed a group of peaks later in the analysis, but otherwise no significant amounts of any other compounds were observed. Unfortunately, the same compounds were observed in collections from females (#6), males (#11), both sexes (#7) and the apple only (#8, 16) and empty (#17) blanks.

The compounds were identified as saturated and unsaturated hydrocarbons which were not seen in previous analyses of volatiles from *Rynchites*. It was realised that these were very similar to compounds obtained in large quantities from sawflies, and comparison with traces from GC-MS analyses of volatiles collected from gooseberry sawfly previously showed that they were essentially identical (Fig. 8.1.7).

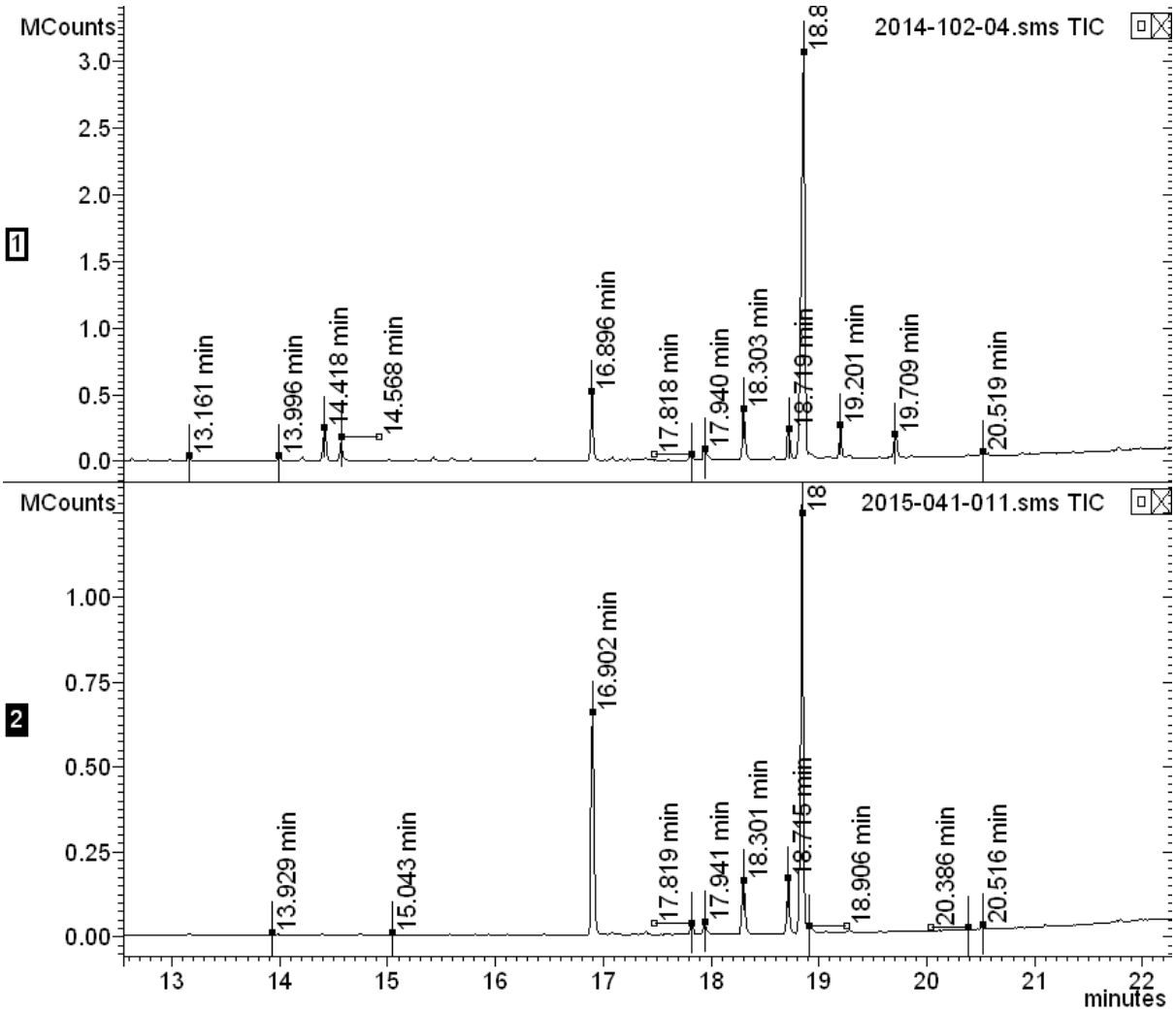


Figure 8.1.7. Comparison of GC-MS analyses of volatiles from gooseberry sawfly (upper) and *Rhynchites* males (#11, lower) on polar GC column (heneicosane 16.90 min; docosane 17.82 min; (*Z*)-9-docosene 17.94 min; 4-methyldocosane 18.30 min; tricosane 18.72 min; (*Z*)-9-tricosene 18.80 min).

It therefore seems most likely that the hydrocarbons are contaminants from previous volatile collections. The contamination may have been in the collection apparatus or in the Porapak filters. The former is unlikely because collections from the same collection chambers were free of the hydrocarbons.

EAG Analyses: No stable EAG preparations were obtained, possibly because of the age of the insects. Previous experience has shown that for stable EAG preparations it is important to use as fresh insects as possible.

Conclusions

- There was no suggestion with field testing or laboratory chemical analyses that Rhynchites weevils produced a long range sex or aggregation pheromone.
- Compounds associated with either sex of the weevils could not be reliably detected
- There was some suggestion that females are repelled by other females at egg laying/ fruit development
- EAG responses were found in response to benzyl alcohol and could be associated with feeding source detection
- Tap sampling 100 trees resulted in 7 weevils being found at first detection. This means that tap sampling 20 weevils would only result in one weevil being detected
- However Rhynchites weevils were present in the apple trees from the stage ‘bud scales first visible’, after dormancy
- There was also a tendency to find more weevils in apple varieties that broke dormancy earlier
- Weevils were less numerous in the trees once the fruitlets reached 15-30 mm in diameter.

General Discussion

European apple canker is a devastating disease which requires a multifaceted approach to achieve control. Work has commenced on 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 work is planned to evaluate whether tree injection is a practical strategy to control the disease. 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 in the first year and will be combined into programmes and evaluated in Year 2 together with strategies to reduce overwintering inoculum to make in season control more attainable.

Targeting codling moths through mating disruption, virus and nematode treatment is a long term strategy with effects accruing over several seasons. This project is evaluating these strategies over 2 years. Early indications are promising with higher levels of natural predators in MD treated plots relative to grower standard plots whilst achieving equivalent or better codling and tortrix control.

This project has increased our understanding of the biology of apple fruit rhynchites weevil identifying potential attractant and repellent compounds and the presence of the weevils in the orchard in relation to tree development. This information will inform monitoring strategies for this pest.

Forward planning

		2016				2017
		Apr-Jun	Jul-Sept	Oct-Dec	Jan-Mar	
1	**Surveillance**					
1.1	Scab virulence		Assess indicator cv.			
1.2	Apple rot survey				Year 2 survey	
1.3	Invasives	Ongoing surveillance				
2	**Neonectria**					
2.1	Detection/endophytes	Validate and optimise antibody				Utilise detection tools
2.2a	Rootstock/interstock	Graft and grow on rootstock panel with common scion			Plant out at trial sites (ADAS)	Assess canker expression
2.2b	Soil amendments (i) stoolbed (ii) newly established orchard	Continue establishment of (i), Assess (ii)				Assess (i) and (ii)
2.3	Novel application methods	Setup injection trial	? Secutur trial	Assess injection trial		
2.4	Season long control	Commence 2018				
3	**Apple foliar diseases**					
3.1	Over-wintering	Pending IUK outcome				
3.2	Alternative treatments	Year 2 trial + persistence study				
3.3	IPM trials					Year 3 programme trial
4	**Stone fruit diseases**					
4.1	Over-wintering	Pending IUK outcome				
4.2	IPM trials					Field trial
4.3	Bacterial canker	Commence 2018				Exploratory studies
5	**Optimising spray coverage**					
5.1	Linking spray coverage to biological efficacy	Commence 2018				
6	**Codling and tortrix moth**					
6.1	Pheromone MD	Year 2 trials on same farms				
7	**Enhancing natural predation**					
7.1	Newly planted orchards		Sourcing sites			Ecology enhancement trials
7.2	Dynamic charts	Training for monitoring in demonstration orchards				
8	**Rhynchites weevil and sawfly**					
8.1	Biology & Semiochemicals of Rhynchites weevil	Finished				
8.2	Semiochemicals of Sawfly	Commence entrainment ahead of schedule				
9	**Phytophagous mites**					
9.1	Elicitors and invigorators					Assessments (obj. 4.2)

Knowledge and Technology Transfer

Field visit

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

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

References

Christine Hapke, Julia Kirchert , Erich Dickler and Claus P.W. Zebitz 2001 Pheromones for Insect Control in Orchards and Vineyards. Combination of pheromone and an additive for the control of codling moth, Cydia pomonella. IOBC wprs Bulletin Vol. 24(2) 37-41.

Van De Weg, W. E. (1987). Note on an inoculation method to infect young apple seedlings with Nectria galligena Bres. *Euphytica* 36, 853–854

Appendices

Appendix 1: Tree injection protocol

HDC tree fruit crop protection Research Protocol (Draft)	
Researcher:	Robert Saville / Angela Berrie
Topic:	Objective 2 – Novel methods of treatment application to manage canker
Period:	JULY 2015 to JULY 2016

Title: Apple: Evaluation of novel methods of treatment application to manage canker with a focus on tree injection for the delivery of curative treatments.

Milestones

Mark out trial:	28th February 2016
Complete treatments	15th April 2016
Assessments	June 2016
Complete experiment report:	31st March 2017

Compliance, (Internal, ORETO or GLP)

N/A

Authorisation (Statistician, Project leader)

Dr R Saville

Dr P Brain, EMR Statistician

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Objectives

1. A proof of concept that tree injection could be used effectively for canker control.
2. To determine treatments to trial further in subsequent years of the project.

Study design and data analysis

Experiments will be done with a randomised block design with up to 10 replicates, each replicate consisting of one tree and subjected to Analysis of variance in Genstat. All products will be included in one experiment.

Site

Church fields east (CE231) located at East Malling Research. The orchard, planted in 2013, 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 trees will be used in this trial.

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

Treatments

Treatments will be applied to the plots at bud burst [green tip, BBCH 07-09) which is expected around mid-March to early April. Treatments will be applied early in the morning (pre 10:00) to maximise uptake. The treatments will be applied with the Fertinyct system.The devices are dosed using Cone Luer Lock following the instructions in the following videos (For liquids https://www.youtube.com/watch?v=3ylAd50pBzc and for solids: https://www.youtube.com/watch?v=mMxyfIJnjWw)

Standard treatments for pests, foliar disease and nutrients will be applied to all plots throughout the season. Based on fertinyect's experience the wound will be left open to heal in the first years trials.

Table 2. Treatment list to be tested

Product	Active ingredient	Formulation (e.g. EC, SL,WG etc)	Product type e.g. Fungicide, biological, defence elicitor etc)	Recommended foliar rate of product
HDC F198	Experimental	WG	Fungicide+defence elicitor	3kg/ha
HDC F199	Experimental	SC	Fungicide	281ml/ha
HDC F197	Experimental	SC	Biological	10L/ha
HDC F200	Experimental	WP	Biological	4kg/ha
CropBioLife	Preformed Phenolics	?	Plant health promoter	300ml/ha
HDC F201	Experimental	WG	Defense elicitor	3.75kg/ha
Fertinyct – Protect	Magensium Phosphite	?	Defense elicitor	Pre formulated
Folicur	Tebuconazole	EW	Fungicide	600ml/ha
Cercobin (Certis)	Thiophanate-methyl	WG	Fungicide	1.1kg/ha
UNTREATED	-	-	-	-

Table 3. Treatment rates

Product	Recommended foliar rate	Expressed as % assuming 1000L/ha	Injection rate (x 10) expressed as %	Rate/L	Rate/200ml device
HDC F198	3kg/ha	0.3	3	30 g	6 g
HDC F199	281ml/ha	0.0281	0.281	2.81 ml	562 µl
HDC F197	10L/ha	1	10	100 ml	20 ml
HDC F200	4kg/ha	0.4	4	40 g	8 g
CropBioLife	300ml/ha	0.03	0.3	3 ml	600 µl
HDC F201	3.75kg/ha	0.375	3.75	37.5 g	7.5 g
Fertinyct – Protect	Pre formulated	-	-	-	-
Folicur	600ml/ha	0.06	0.6	6 ml	1.2 ml
Cercobin (Certis)	1.1kg/ha	0.11	1.1	11 g	2.2 ml
UNTREATED	-	-	-	-	-

Table 4. Treatment properties

Product	Solubility (in Water)	pH
HDC F198	16/111.3	3,0 - 4,0 at 1 % (23 °C) (deionized water)
HDC F199	16	5,5 - 8,0 at 100 % (23 °C)
HDC F197	Dispersible	5,2 - 5,4
HDC F200	Dispersible	?
CropBioLife		
HDC F201	111.3	3,0 - 4,5 at (23 °C)
Phosphite (KH_2PO_3)		4
Fertinyct – Protect		4
Folicur	36	pH 5.0 - 8.0 at 1 % (23 °C) (deionized water)
Cercobin (Certis)		

Methods, assessments and records

Meteorological records

Records of daily maximum and minimum temperature and rain fall will be taken from a weather station located at East Malling Research.

Growth stages at application

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

Phytotoxicity

Symptoms of phytotoxicity will be checked and recorded. Records will include any chlorosis / necrosis to foliage, growth regulatory effects to shoots, assessed on a scale 0-5 (EPPO Guideline PP 1/135(3)).

Canker assessments

Three assessments will be conducted;

(1) Canker size

The size (width and length at longest point) of each of the cankers will be measured at the start of the trial prior to treatment application (Mid-March) and again at the end of the trial (June).

(2) Canker sporulation

Canker washings will be collected using established methods (see below) at the start of the trial prior to treatment application (Mid-March) and again at the end of the trial (June) following a rain event.

(3) New canker formation

New cankers forming on the previous years' extension growth will be counted and expressed as new cankers per tree in June.

Protocol for canker washings

To assess canker activity prior to treatment application each canker on the tree is washed with 50ml of distilled water from a hand held sprayer. The washings are collected in a plastic tube via a plastic funnel. Collected washings are spun down in a centrifuge, the supernatant discarded and the remainder resuspended in 2ml of distilled water. A drop of Thymol is added to each tube to prevent spore germination. The tubes are sealed and stored at 4oC until counted. The spore concentration is counted using a haemocytometer slide.

The effect of the treatment on canker sporulation is assessed by washing the cankers as described above. Cankers will be assessed for sporulation at the end of the trial in June, following a rain event (to ensure active sporulation).

Potted trees will be injected with a dye suspension to demonstrate the uptake of a solution within the apple tree vascular tissue. Destructive sampling of the dye treated samples will be undertaken 1 week after treatment application.

A note on formulations:

Formulation	Formulation in full	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 taking 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

Composition of carrier in Ynyect devices;

Name	Chemical Formula	CAS No.	Percentage
Potassium Nitrate*.	KNO3	7757-79-1	0.1%
Monoammonium Phosphate *	(NH4)2PO4	7722-76-1	0.04%
Ammonium Sulphate *	(NH4)2SO4	7783-20-2	0.3%
Water	H2O	7732-18-5	>99%

Appendix 2: Treatments applied to plots in EE190 prior to start of trial and to all plots during the trial in 2015

Date applied	Product	Type	Rate / ha
26 March	Dithianon WG	Fungicide	0.75 kg
2 April	Dithianon WG Indar	Fungicide	0.75 kg 1.0 L
13April	Systhane + Captan	Fungicide	0.33 L 1.0 kg
16 April	Pyrinex	Insecticide	1 L
28 April	Captan	Fungicide	2.0 kg
12 May	Captan Difference Calypso Insegar	Fungicide Fungicide Insecticide Insecticide	2.0 kg 0.2 L 0.375 L 600 g
26 May	Captan	Fungicide	2.0 kg
10 June	Captan	Fungicide	2.0 kg
19 June	Captan Mainman	Fungicide Insecticide	2.0 kg 0.14 kg
30 June	Steward	Insecticide	250 g
8 July	Captan	Fungicide	2.0 kg
23 July	Nimrod Coragen	Fungicide Insecticide	1.4 L 175 ml
6 August	Nimrod	Fungicide	1.4 L
27 August	Coragen	Insecticide	175 ml