Project title:	Improving integrated pest and disease management in tree fruit
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Project leader:	Dr Michelle Fountain NIAB EMR
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Date project commenced:	01/04/2015
Date project completed:	31/03/2020

DISCLAIMER

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

AUTHENTICATION

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

Michelle Fountain

Project leader, Deputy Head of Pest and Pathogen Ecology

NIAB EMR

Signature .... Date 16 June 2020.........................

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GROWER SUMMARY

Project TF 223 was a five-year project which commenced in April 2015 and was completed in March 2020. The project investigated solutions to the key tree fruit diseases and pests, namely: European apple canker, scab, powdery mildew, *Monilinia* species and bacterial canker affecting stone fruit, codling and tortrix moths including Blastobasis, pear sucker, apple fruit rhynchites weevil, apple sawfly, pear weevils and phytophagous mites. It also included surveillance or emerging insect pests and diseases including the brown marmorated stink bug. This Grower Summary includes information on the entirety of the five-year project. For ease of reading, it is split into sections for each of the diseases and pests worked on.

Objective 1. Surveillance

Headline

- During this project new and invasive pests have been reported and useful links and summaries can be found in the science section of the 2020 report.

Background and expected deliverables

This objective aimed to keep the industry briefed of ongoing pest and disease issues along with emerging pest and disease threats, which could ultimately lead to yield losses in tree fruit. The information gathered will also help to inform future research targets and priorities. Activities included the monitoring of scab virulence on indicator trees, undertaking apple rot surveys and horizon scanning for emerging and future pest and disease threats to the UK tree fruit industry.

Summary of the project and main conclusions

Scab virulence

- An indicator orchard was planted at NIAB EMR, containing 16 different *Malus* hosts which represent a range of apple scab resistance genes, as part of a large global project (VINQUEST) in which the same indicator cultivars were planted at over 30 sites in 24 different countries. These trees have been monitored for Scab lesions over the past 5 years.

- Resistant breaking strains of scab which have overcome *Rvi6* (formerly known as *Vf*, the most extensively used resistence gene for scab resistance in modern day varieties) have been observed at the UK site.
- Resistance breakdown in *M. floribunda* 821, the source of the *Rvi6* scab resistance gene, was again confirmed in 2019 as it had been in the previous three years.
- No breakdown was seen on the trees in a plot of the domesticated cultivar Priscilla which carries the *Rvi6* gene.
- Scab was also found on the indicator genotypes for the *Rvi3* and *Rvi8* genes; unlike *Rvi6* these genes cannot be found in any commercially available cultivars.

Apple rot survey

This task is a continuation of the apple rot survey which has been undertaken over the last century. The survey involves visiting packhouses during the months of January – March to determine the type and incidence of rot causing pathogens.

2018/2019

A total of 26 visits and 44 consignments of apples were examined. On average actual losses ranged from 0.01 to 5% with an overall mean of 1.2 %. The highest losses were recorded in Cox and Bramley which is as expected as these cultivars are stored at 3.5 – 4 ^{o}C, whereas the other cultivars are stored at around 1-2 ^{o}C. Neonectria rot was the most prevalent rot with an overall incidence of 24% and was the main rot in Braeburn (32.8%), Gala (29.7%) and Jazz (35.2%) reflecting the susceptibility of these cultivars to Neonectria. Brown rot (Monililnia) was the next most prevalent rot (19.3%) followed by *Neofabraea* (Gloeosporium, 16.2%), Botrytis (9.8%), Penicillium (8.8%) and Phytophthora (7.8%). Gloeosporium accounted for 58.8% of rots in Cox, most likely due to the extended storage of the samples assessed into April and the warm wet conditions pre-harvest. There was a higher incidence than previous years of Phytophthora rot in the late harvested cultivars Braeburn and Jazz, due to the high rainfall pre harvest. The data is summarised in Table GS1 together with the data from the previous four year's of apple rot surveys.

In summary

- The overall mean losses to fungal rot pathogens changed slightly between the sampled years, with 2.6% losses occurring in 2015/16, 1.5% in 2016/17, 1.6 % in 2017/18 and 1.2% in 2018/19.

- The pathogens causing the highest volume of rots in all sampled years were *Neonectria*, followed by *Monolinia* and *Neofabraea* (*Gloeosporium*).
- *Botrytis*, *Penicillium* and *Phytophthora* had similar incidence each year.
- A new apple rot pathogen, *Neofabraea kienholzii,* was reported for the first time in the UK.
- Different apple cultivars are more susceptible to certain pathogens. For instance, *Botrytis* tends to be more prevalent in Jazz, associated with missing stalks, whereas brown rot is more prevalent in Cox and Bramley.

Table GS1. Average loss (%) attributed to each rot pathogen during 2018/19 storage season. Data is compiled from 44 apple samples. Overall averages for 2017/18, 2016/17 and 2015/16 are included for comparison. [1]Core rots includes Fusarium and Phomopsis rots recorded separately.

Cultivar	Brown rot	Botrytis	Penicillium	Phytophthora	Neonectria	Gloeosporium	Fusarium	Mucor	Botryosphaeria	Phomopsis	Stalk	Eye	Cheek	[1]Core	No. samples	Loss (%)	Loss range %
Braeburn	11.9	18.6	13.9	13.6	32.8	7.6	0	0.4	0	0	0	0	1.1	0	10	0.23	<0.1-0.8
Bramley	34.5	2.0	8.2	0	8.1	2.2	5.2	0.4	0	32.5	0	0.3	2.2	14.3[1]	10	1.9	<0.1-3.6
Cox	12.3	5.3	4.8	2.7	14.4	58.8	0	0.3	1.0	0	0	0	0.4	0	5	2.3	0.5-3.0
Gala	36.4	5.1	6.1	0.8	29.7	10.6	0	0.6	0	0	0	0	0	0	9	0.5	0.01-0.7
Jazz	1.5	18.0	11.0	22.0	35.2	1.7	0	0.6	0	0	0	0	0	0	10	1.0	<0.1-5.0
Overall mean 2018/19	19.3	9.8	8.8	7.8	24.0	16.2	1.04	0.5	0.2	6.5	0	0.06	0.7	2.9	44	1.2	-
Overall mean 2017/2018	7.6	27.8	10.6	4.5	32.4	10.6	2.9	0	0	0.4	1.3	0.1	1.0	0.3	32	1.6	-
Overall mean 2016/17	19.3	9.7	11.2	1.6	31.3	12.4	0.4	4.2	0	0	2.0	0.6	1.5	5.6	52	1.5	-
Overall mean 2015/16	13.3	8.3	6.3	6.4	40.3	9.3	0.5	3.0	0	0	2.2	0	1.2	7.3	60	2.6	-

Invasive pests and diseases

A detailed summary for each pest and disease listed below is presented in the Science Section of this report with useful links.

- *Xylella fastidiosa* continues to be the biggest threat to UK horticulture including tree fruit crops but has not been detected in the UK to date.
- Another notifiable bacterial pathogen is *Xanthomonas arboricolae, pv. pruni.* which causes shot holing symptoms on leaves of plum and sweet cherry. Currently, it has only been reported on *Prunus laurocerasus* (cherry laurel) in the UK.
- *Drosophila suzukii* numbers (monitored only at NIAB EMR) rose slightly in 2019 compared to 2018.
- Summer fruit tortrix was detected for the first time in the West Midlands during the 2015 growing season and it is recommended that growers now monitor for this pest in the region using pheromone traps alongside codling moth and fruit tree tortrix monitoring traps.
- Brown marmarated stink bug (BMSB) was identified in the UK for the first time in Hampshire, in 2019.
- A new complex of shield/stink bugs have been found damaging apple and pear crops in recent years, including the forest bug (*Pentatoma rufipes*).
- A weevil found in pear orchards which has been damaging spring flower and leaf buds over the last two to three years, was identified as *Anthonomus spilotus* by the Natural History Museum and NIAB EMR in 2017. This pest is believed to be new to the UK. It has also recently been identified as an invasive pest in Belgium. Progress was made on the estimation of damage and the susceptibility to specific crop protection products. More details are included in the Science Section (Objective 9).
- The Royal Horticultural Society reported sightings of pear shoot sawfly (*Janus compressus*) in 2016. This has not been seen in commercial pear crops as far as we are aware.
- A new species of aphid, green citrus aphid (*Aphis spiraecola*) was reported in apple orchards the South East of England in 2018. This species is difficult to distinguish and is more resistant to aphicides than many other aphid species.

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

Overall conclusions from Objective 1 Surveillance in this project

- During this project new and invasive pest have been reported and useful links and summaries can be found in the science section of this report.
- These include, *Drosophila suzukii*, Summer fruit tortrix in the West Midlands, Brown Marmorated stink bug, Forest bug, Common Green Shieldbug, Mottled Shield Bug, *Anthonomus spilotus*, Pear Shoot sawfly, Apple maggot fly, Black and white citrus longhorn, False codling moth, Grapevine phylloxera, Ambrosia beetle on nursery stock, Gypsy moth, Magdalis beetle on pear, *Rhagoletis cingulate*, Green Citrus Aphid, American plum borer, European grapevine moth, Peach fruit moth, Oriental fruit fly, European Corn borer, Diaporthe causing apple leaf spots, *Neofabraea kienholzii* and *Xanthomonas arboricolae, pv. Pruni.*
- Resistance breaking strains of scab which have overcome *Rvi6* (formerly known as *Vf,* the major scab resistance gene used in modern varieties) have been observed at a UK site.
- *Neonectria* was the largest cause of storage rot in all years sampled, followed by *Monolinia* and *Neofabraea* (*Gloeosporium*).
- A new apple rot pathogen, *Neofabraea kienholzii,* has been reported for the first time in the UK.
- *Xylella fastidiosa* continues to be the biggest threat to UK horticulture but has not been detected the UK to date.

Financial benefits

Current, emerging and newly introduced pests and disease can have a devastating effect on yield and economic return to fruit businesses. Surveillance work ensures the ongoing monitoring of these threats, helping to inform future research priorities. For instance, before the spotted wing drosophila arrived in the UK, its spread to the USA and mainlaind Europe was identified and a UK SWD industry working group was set up (involving AHDB) to provide guidance to growers on how to identify and manage it when it arrived on our shores. This helped the industry to prepare for this existential threat and avoid serious financial losses.

Action points for growers

- Continue to use the rot risk assessment tool available in the AHDB/DEFRA apple best practice guide to limit loss of apples in store.
- Monitor for summer fruit tortrix moth in the west of England.
- Monitor for native and invasive stink bugs.
- Keep an eye on the trade press for important announcements from the animal and plant health agency (APHA) about invasive pests and disease which will affect your business such as *Xylella fastidiosa.* See https://www.jic.ac.uk/brigit/ for more information and partake in a survey of the insect vector at http://www.spittlebugsurvey.co.uk

Objective 2. Neonetria Canker of Apple

Headline

- A combined approach of careful rootstock selection, use of certain soil amendments at planting time and the application of wound protection treatments when pruning, can make a positive contribution to reducing incidence of Neonectria canker in newly planted orchards.

Background and expected deliverables

Neonectria canker caused by *Neonectria ditissima* is a devastating disease of apple which has been increasing in significance over the past 10-15 years as the industry has changed agronomic practices and cultivar choice. This project objective examined the effect of various factors such as development of an immunological based assay to detect *N. ditissima*, rootstock/interstock choice, the use of biological soil amendments, trunk injections to deliver active ingredients to trees and pruning wound protection treatments. Work from other projects including two BBSRC LINK projects which have AHDB and direct industry involvement and two AHDB funded PhD studentships, will contribute to the development of a systems approach for canker control from the nursery to the orchard.

Experiments were established on two sites to determine the effect of rootstock and biological soil amendments on canker incidence. The rootstock trials are evaluating a panel of industry standard rootstocks alongside several advanced selections from the NIAB EMR and Geneva rootstock breeding programmes. The amendment experiments evaluated the effect of arbuscular mycorrhizal fungi (AMF), plant growth promoting rhizobacteria (PGPR), Trichoderma and Biochar (at one of the sites) in newly planted orchards, as well as a stoolbed site to simulate a nursery scenario.

Summary of the project and main conclusions

A summary of results of all the work packages within this objective on Neonectria canker are provided below.

Development of an immunological based tool for Neonectria canker detection

- An Enzyme Linked Immunosorbant Assay (ELISA) protocol was optimized to detect *Neonectria ditissima* antigens in plant material.

- An antibody (1B10) was identified which gives good resolution in cross reactivity tests between *Neonectria ditissima* antigens and antigens from other fungi commonly found in UK apple orchards.
- With further refinement, this assay can be used to improve our understanding of the biology of *N. ditissima.*
- This diagnostic tool is being used in Project CP 161 with the intention of developing a sampling strategy to deploy its use in the nursery.

Rootstock/interstock

- Rootstock trials were established at two sites: at NIAB EMR, Kent (site 1) and at a commercial orchard in Gloucestershire (site 2). Conflicting results were recorded between sites.
- At site 1, the rootstocks MM106 and M116 had consistently lower levels of canker, while G.41 and G.11 had higher levels.
- At site 2, the rootstocks G.11 and G.41 had lower levels of canker, whilst MM106 and M116 had higher levels. The rootstocks were subsequently DNA fingerprinted and identities confirmed as correct.
- On further examination of the data for peripheral cankers from natural infection over three years at site 1, rootstock M9 (EMLA) showed significantly lower canker than the other rootstocks while G.41 had the highest canker number. There were no significant differences between the other 12 rootstocks at site 1.
- Combining three years of data (2017-2019) of peripheral cankers from natural infection at site 2, there was no significant difference in canker expression between the different rootstocks.
- Combining two years of data from artificial inoculation (2018-2019) from site 1 showed no significant difference in canker between rootstocks.
- Gala scions grafted to the NIAB EMR advanced selection EMR-001 had higher canker numbers at both sites.
- Factors such as site, scion cultivar selection and apple replant disease, are likely to be having some effect on canker incidence.
- Tree vigour does not appear to play a role in canker number.

Soil amendments

- *Trichoderma harzianum* (Trianum G) was the most promising amendment for reducing canker.

- A saving of >£1050.38 per 1,000 trees planted was calculated for Trichoderma amended trees at one of the trial sites.
- There was a significant effect of the amendment AMF+PGPR on fruit size at one of the trial sites, but no effect of any of the amendments on fruit number, fruit weight, or vigour (as measured by trunk girth).

Novel methods of treatment application to manage canker

- Trunk injections were found to effectively distribute some of the active compounds through trees.
- However, none of the chemistry tested to date has shown sufficient efficacy for the control of symptomatic cankers.

Future work could include a greater number of active ingredients representing a wider range of modes of action. Products could include those that affect plant hormones such as Bion, in addition to Trichoderma products which have shown promise in New Zealand trials, neither of which were tested in the current work due to current regulations not permitting their use.

Pruning wound protection treatments

- Application of wound protectant treatments using secateurs with a chemical dispenser to pruning cuts with Folicur (tebuconazole) with or without Blocade polymer, as well as the biological treatment T34 + Blocade polymer can significantly decrease the incidence of canker infection.
- T34 + Blocade polymer and both treatments that used Folicur ie. Folicur + Blocade and Folicur alone, had the lowest canker percentage compared to the other tested pruning wound treatments. T34 used on its own did not reduce canker.
- There was good callousing of host tissue at the cut site after application of Folicur.
- As of April 2020, Folicur can only be applied once in any given year, either before the first leaves are fully expanded or after the harvest of the final crop.
- The use of chemistry such as Folicur (tebuconazole) is increasingly being phased out in the UK with the current EAMU expiring on 28/02/2023.
- T34 was used under experimental approval and would need CRD approval for use on apple.

Key conclusions from Objective 2 Neonectria canker over the entire project

- Development of an immunological based tool for Neonectria canker detection was investigated.
- Rootstocks were identified with reduced canker, however there was conflict with the same rootstocks between the two tested sites.
- *Trichoderma harzianum* (Trianum G) was the most promising amendment for reducing the number of dead trees caused by canker in newly planted orchards with potential savings of >£1050.38 per 1,000 trees planted.
- Application of wound protectant treatments to pruning cuts with Folicur (tebuconazole) with or without a polymer, using secateurs with a chemical dispenser, can significantly decrease the incidence of canker infection.

Financial benefits

This work has established practical approaches growers can use to reduce losses to canker in their orchards including rootstock selection and the addition of biological soil amendments. Growers commonly remove trees with main stem cankers in the first five years of orchard establishment and canker is known to cause tree death of >10% of newly planted trees. This incurs the financial burden of replacing diseased trees and years of delayed fruit production. Employing a range of canker reducing methods is recommended, as using single methods in isolation may not have significant benefits.

Action points for growers

- It is still important to be vigilant with visual inspection, identifying trees which are showing canker symptoms and limiting abiotic stress as far as possible when planting out and establishing new orchards.
- Employing a range of canker reducing methods is recommended, as using a single method in isolation may not have significant benefits.

Objective 3. Apple Foliar Diseases

Headline

- Alternating conventional fungicides with biostimulant and physical acting products, can reduce reliance upon fungicides whilst maintaining acceptable mildew control and fruit quality.

Background and expected deliverables

Most UK apple cultivars are susceptible to powdery mildew, particularly Braeburn, Gala and Cox. The disease overwinters as mycelium in fruit or vegetative buds, which emerge as mildewed blossoms or shoot tips in spring (primary mildew). Spores from the primary mildew spread to developing shoots to initiate the secondary mildew epidemic. Mildew colonises fruit buds in June/July and vegetative buds at the end of shoot growth in late summer, where it remains dormant until the following spring. Under favourable humid conditions above 18°C, the fungus can infect leaves and produce sporing colonies in four to five days. Mildew inoculum level is the key factor in determining the seasonal epidemic. Therefore, control strategies depend on maintaining primary mildew at a low level. Season-long protection is essential, which can amount to 10 to 15 fungicide sprays.

With the continuing pressure to reduce reliance upon conventional fungicides, the industry needs to develop novel and alternative control measures for apple powdery mildew. A number of elicitors, biostimulants, biocontrol and physical control products are available to growers, but their success has shown great variation depending on seasonal weather conditions and disease pressure.

Work in this project aimed to find methods of reducing levels of over-wintering mildew and develop ways of improving the reliability and use of alternative control products.

Summary of the project and main conclusions

Reducing levels of overwintering mildew

Efforts were made to investigate the use of fungal and bacterial parasites, applied to apple trees in late summer as a means of antagonising the pathogen over the winter and reducing levels of overwintering inoculum. Trials to incorporate the mycoparasite *Ampelomyces quisqualis* (AQ10) in overwintering buds to reduce mildew inoculum were inconclusive. Plans to repeat the trials in 2018 applying both AQ10 and a novel bacterial parasite towards the end of shoot growth in late summer were hampered by early termination of growth due to the hot dry conditions. It is therefore planned to reassess this approach in a different project.

Improving reliability of alternative control products

Ways of improving tree health along with the tree's ability to withstand fungal infection were assessed using a range of substances within reduced fungicide control programmes and these were compared to traditional routine fungicide programmes. Various nutrients, substances reported to act as biostimulants that improve plant health and their ability to resist disease, and adjuvants that have a physical impact on mildew were included to assess their incidental effect on powdery mildew. Such substances can't be used for control of powdery mildew, but the knowledge of incidental effects on mildew may help inform a managed programme which could reduce fungicide use. Products based on potassium bicarbonate were not included in the trials as the efficacy of these products was already known.

Over the first three years of the project, the use of a range of substances to improve tree health were evaluated in small-plot replicated trials at NIAB EMR on Gala apples, with and without fungicides. From these trials promising incidental effects on mildew were seen for:

- Cultigrow (a potential biostimulant based on flavonoids)
- Trident (a silicon-based nutrient)
- Mantrac Pro (manganese nutrient)

Products which physically controlled mildew included SB Invigorator (a blend of surfactants) and the adjuvant Wetcit (a natural adjuvant based on alcohol ethoxylate) which can be used in combination with plant protection products.

In the final two years of the project the incidental effect of these substances were evaluated in season-long programmes with reduced fungicide use and compared for mildew control with a seven-day fungicide programme.

In 2019, three different programmes were evaluated in a large plot trial (six rows of 70 trees) in an orchard at NIAB EMR with alternating rows of Gala and Braeburn. The programmes were evaluated from early blossom and applied by a tractor-trailed orchard sprayer at 200 L/ha.

Two of the three were based on Cultigrow, applied monthly, with either Mantrac or Trident applied every two weeks. The other was based on Trident and Mantrac alternating every two weeks. SB Invigorator was applied as a separate spray (should not be mixed with other products) in all programmes and Cultigrow was used with the adjuvant Wetcit.

Fungicides were applied at 14-day intervals with the same product used as in the standard seven-day fungicide programme. Captan was included for scab control when necessary. Primary blossom mildew for both cultivars was low but there was a high incidence of primary vegetative mildew on Braeburn. Secondary mildew on extension shoots was assessed every week from petal fall.

The exact products used and the timing of applications in each of the four programmes (1-4) is laid out in the table below.

The incidence of primary mildew, particularly the primary vegetative mildew, in the trial orchard was higher than expected and appeared to be reduced by the early fungicide programme applied to the routine plots. Secondary mildew on Braeburn was higher than on Gala, indicating higher susceptibility to mildew. Initially the best control for both cultivars was achieved by the standard seven-day fungicide programme. However, by July all three trial programmes were performing as well as the standard programme with secondary mildew around 5-10% mildewed leaves. The standard seven-day fungicide programme was effective in controlling the early mildew resulting from the high primary mildew on the Braeburn. Starting all three trial programmes with a seven-day fungicide programme for the first few sprays would probably have resulted in comparable control throughout the season but with reduced fungicide input. There was no phytotoxicity noted in the trial from the programmes applied.

Programmes for powdery mildew control applied to apple cvs Braeburn and Gala in 2019.

Programme	Product / Timing													
	24 Apr	1 May	6 May	15 May	21 May	29 May	5 Jun	12 Jun	19 Jun	26 Jun	3 Jul	10 Jul	17 Jul	24 Jul
Growth stage	**Braeburn** **30% flower**	**Late flower**	**Petal fall**	**End flower**	**End flower**	**Petal fall +**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**End shoot growth**
	Gala **Early flower**	**Full flower**	**Full flower**	**Late flower**	**End flower**	**Petal fall +**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	
1 Fungicide 7 days	Flint	Sercadis + Captan	Flint	Sercadis	Topas + Captan	Talius	Cosine + Captan	Topas	Flint	Cosine	Sercadis	Topas	Flint	Talius
2 CBL/Mantrac	Mantrac + Flint	CBL+Captan	Mantrac + Flint	SBI	Topas + Captan + Mantrac	CBL+Wetcit	Cosine + Captan + Mantrac	SBI	Mantrac + Flint	CBL+ Wetcit	Sercadis + Mantrac	SBI	Mantrac + Flint	SBI
3 Mantrac/Trident	Mantrac + Flint	Trident + Captan	Mantrac + Flint	SBI	Topas + Captan + Mantrac	Trident	Cosine + Captan + Mantrac	SBI	Mantrac + Flint	Trident	Sercadis + Mantrac	SBI	Mantrac + Flint	SBI
4 CBL/Trident	Trident + Flint	CBL + Captan	Trident + Flint	SBI	Topas + Captan + Trident	CBL + Wetcit	Cosine + Captan + Trident	SBI	Trident + Flint	CBL + Wetcit	Sercadis + Trident	SBI	Trident + Flint	SBI

Main conclusions

- Plots treated with programmes 2, 3 and 4 which alternated between applications of conventional fungicides and biostimulants or physical control products received half the number of conventional fungicides compared to the routine treatment.
- The results show that by combining alternative products with fungicides it is possible to reduce fungicide inputs, while still maintaining mildew control and fruit quality.

The results from these trials show that there is potential for reducing fungicide inputs by improving the health of the tree and its ability to resist powdery mildew infection through the use of substances with biostimulant and physical properties. As the biostimulant products boost plant resistance to disease, they act slowly and require frequent applications from an early stage of growth to be most effective. The products that increase the tree's physical ability to resist infection, act more directly and could be used to intervene if mildew incidence was increasing. In a commercial situation, the key to effective mildew control is regular monitoring of mildew incidence on shoots during crop inspections so appropriate decisions on product use can be made. Full details of all of the records collected between the treatments and post harvest are laid out in the Science Section of this report.

Financial benefits

A high incidence of powdery mildew in apple orchards significantly reduces yield and fruit quality. Generally, 10-15 conventional fungicide sprays are required to control powdery mildew and to ensure buds are free from overwintering mildew. There are now decreasing numbers of effective conventional products available to control mildew. The use of effective alternative products will help growers to reduce their reliance upon conventional products and ensure that mildew can continue to be controlled, preventing economically damaging disease thresholds being reached.

Action points for growers

- Alternative products should be used in programmes combined with mildew monitoring.
- The key to effective mildew control is regular monitoring of mildew incidence on shoots during crop inspections. Decisions on product use can then be adjusted to the identified mildew risk.
- Growers and agronomists should consult the AHDB Apple Best Practice Guide online on how best to do this.

- Alternative products can reduce mildew and boost plant health, although they act slowly and require frequent applications from an early stage of growth to be most effective.

Objective 4 - Stone Fruit Diseases

Headline

- Progress has been made in identifying new control measures for brown rot and bacterial canker of cherry.

Background and expected deliverables

Brown rot of cherry

Brown rot (caused by Monolinia species) is one of the principal diseases causing yield loss in plum and cherry crops in the UK. Total losses are difficult to quantify as infection can occur throughout the season from blossom time through to harvest and during the storage period. Post-harvest development of brown rot limits the storage potential of plums and cherries. To gain control, growers currently rely heavily on the use of conventional fungicides such as Signum (boscalid + pyraclostrobin) and Switch (cyprodinil + fludioxonil) applied both during blossom and close to harvest.

This project aimed to evaluate newly available control products including plant health promotors, biological control agents and fungicides, which in combination, could provide a more effective programme for brown rot control.

Bacterial canker of cherry

Pseudomonas syringae pathovars; *syringae* (Pss), *morspronorum* race 1 (Psm1) and *morspronorum* race 2 (Psm2) cause a destructive disease called bacterial canker on prunus species. The disease reduces yields due to cankers girdling branches and trunks causing wilting and tree death. Until now growers have relied on copper treatments at leaf fall to reduce bacterial populations and control this disease. However copper is no longer permitted for use as a plant protection product. In this project, investigations have focussed on both bacteriophages and cultural control as alternatives to copper use.

Bacteriophages, often simply referred to as phages, are natural antimicrobial agents with very specific modes of action. They can control individual bacterial populations/strains and have therefore minimal unintended consequences in terms of inhibiting beneficial organisms. Moreover, phages are considered safe for human consumption. This objective focused on i) finding and characterising native UK phages against prunus canker pathogen and ii) testing their efficacy on plants to provide proof of concept for their use in disease management.

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

Summary of the project and main conclusions

Brown rot of cherry

A range of control products were tested including a biostimulant, an elicitor, the biofungicide Serenade, standard fungicides (Signum and Switch) and an untreated control. The coded product HDC F266 was effective in reducing brown rot and Botrytis rot on cherries. However, this is not currently approved for use on cherry and further work is required to secure an approval before growers can benefit from it.

Good orchard hygiene is also important for brown rot control. The work that most growers now undertake to remove all damaged, diseased and mummified fruit, both from trees and the orchard floor, to reduce damage from spotted wing drosophila has had a major impact on reducing brown rot infection and spread. Such fruit removal has the benefit of reducing sources of inoculum of *Monolinia* spp. and *Botrytis.*

Bacterial canker of cherry

Initial bacteriophage work identified more than 70 different phages which were effective against bacterial canker. In a subsequent cherry tree trial, a mix of four phage isolates were applied prior to leaf fall and their efficacy was observed. On the cultivars Van and Roundel, phage treatments decreased bacterial population by approximately 10-fold (90%) in comparison to a water control, which is comparable to current chemical and biocontrol products.

CRD/HSE approval was obtained for field trials of UK phages against *Pseudomonas syringae.* The permit is valid until June 2022. The 2019 experiments provided evidence for phage biocontrol and should be followed up in collaboration with a suitable biocontrol producer to deliver phage treatment against bacterial canker.

In the cultural control trial, tunnel skins were retained post-harvest in tunnels and were compared to those where tunnel skins were removed after harvest. Leaving tunnels covered until leaf fall was found to reduce canker progression in trees that already have canker. Covering cherry trees prior to blossom, as well as after harvest appeared to have the largest impact on the canker progression. Leaving the crop covered until leaf fall may also help to suppress weed seedling germination during late summer and early autumn.

Main conclusions

- The coded product HDC F266 was effective for reducing brown rot and Botrytis rot of cherry.
- A large collection of more than 70 bacteriophages isolated from UK orchards has been established and characterized.
- Using the data obtained in this project in 2018 and early 2019 we have successfully obtained CRD/HSE approval for field trials of UK phages against the cherry canker pathogen *Pseudomonas syringae*. The permit is valid until June 2022 and should enable further trials.
- 2019 experiments provide solid proof-of-principle for phage biocontrol and should be followed up in collaboration with a suitable biocontrol producer to deliver phage treatment against prunus canker to the growers as soon as possible.
- Extending the period cherry trees are covered for during the season can result in a reduction of new canker infections in orchards with existing infections.

Financial benefits

The use of effective products such as the coded HDC F266 is an effective method for reducing brown rot and Botrytis rot of cherry and will make significant reductions in crop loss caused by these diseases.

The phages tested in this project were found to be specific, efficient and robust enough to be considered as a future canker control which could help to reduce financial losses caused by this disease. The investment in more trials and collaboration with a plant protection producing company is required to expedite product development.

The tunnel covering trial assessed a simple cultural control that can reduce the spread of canker with no significant increase in cost to growers. This will lead to decreased losses to yield and increased profit.

Action points for growers

- The coded product HDC F266 was found to be an effective method for reducing brown rot and Botrytis rot of cherry. Approval will need to be granted before legal use of this product can be made.
- No commercial phage product is available yet for canker control but if growers have a serious outbreak of bacterial canker on prunus or other host, they should inform NIAB EMR who are seeking trial sites for future work.
- The period that cherry trees are covered during the season should be extended to reduce the spread of canker at critical times during rain events.

Objective 5 – Optimise spray coverage for key pest and disease targets

At the outset of this project, it was decided that the work on this objective would be dependent on progress in a TSB (former name of Innovate UK) funded project which was developing equipment to determine the optimum coverage of spray deposits for foliar pest and disease control. Due to the progress made in that project, it was ultimately decided in consultation with the TF223 programme management group, not to proceed with this work objective, however this did enable additional work to be conducted (Obj. 9 and 10) which focused on emerging issues.

Objective 6 – Codling, Tortrix, and Blastobasis 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

Codling moth is the most important pest of apples and is also an important pest of pears in the UK. Most spray control products employed on these crops are targeted towards these moths. Control is usually good, but populations are not reduced to such low levels that spraying is reduced in subsequent years, so growers find it difficult to reduce dependence on spray control. A number of novel approaches to the management and control of codling, tortrix and blastobasis moths in apple orchards were investigated in this objective. Sex pheromone mating disruption technology is one approach that offers a sustainable way of reducing damage and reducing local codling moth populations in the long term. Sex pheromones can also be deployed in monitoring traps to improve our knowledge of pest appearance and population development within an orchard. Predatory nematodes may also offer an alternative form of control.

Sex pheromone mating disruption

The original 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 were examined over two growing seasons as treatment with mating disruption pheromones is targeted at long-term control on a landscape scale.

Nematodes

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

Blastobasis pheromone

Larvae of the moth *Blastobasis lacticolella*, Rebel, 1940 (Synonym: *decolorella*) (Lepidoptera: Blastobasidae) (Figure 10.1) feed on the surface of apple and pear fruits in mid- and late-summer, often where clusters are touching, causing large open, scallop-shaped wounds in the flesh, and making affected fruit unmarketable. Very severe damage can result if the pest is allowed to increase over a number of years unchecked, especially on short stalked varieties such as Bramley and Egremont Russet which are very susceptible.

Growers currently have no means of identifying whether they have a problem other than the occurrence of damage the previous year, which is often confused with damage caused by other apple moth pests. It is also difficult to time sprays accurately against blastobasis. Sprays are likely to be most effective when they are applied against hatching eggs.

During the life of this five-year project, it was noticed that the less frequent use of conventional spray control products to reduce the occurrence or residues in harvested fruit, coupled with increased use of pheromone mating disruption and granulovirus for control of codling and tortrix moths, led to increased incidence of blastobasis moth and the subsequent occurrence of occasional but severe outbreaks of blastobasis damage. The resulting need to employ control sprays for blastobasis negates the benefits conferred by using RAK3+4 for mating disruption of codling moth and tortrix moths.

Pheromone traps are the easiest way of monitoring the flight activity and egg laying period of moth pests. There is a clear commercial need to develop a pheromone monitoring trap for blastobasis so that growers can determine whether they have a problem and determine the optimum time to apply control products.

Summary of the project and main conclusions

Sex pheromone mating disruption

Two farms, one in the South East and one in the West Midlands of England, offered to demonstrate the RAK3+4 mating disruption system. However, in the second year of work, the West Midlands farm was over sprayed with chlorantraniliprole (Coragen) so this was not used for monitoring, and an additional farm in the South East that had been treated with the RAK3+4 mating disruption (MD) system for three years was monitored instead. Each farm was divided into two halves. One half was treated wth 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). The other half received the growers conventional spray programme. 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.

In both years at each farm, assessments were made 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 (codling, summer fruit tortrix and fruit tree tortrix) were monitored weekly in each orchard using sex pheromone traps. For codling moth and tortrix assessments, both fruit that had dropped to the ground and those attached to trees were assessed. Other notable pest damage was also recorded.

Although few moths were captured in the pheromone monitoring traps on the MD side of the farms, the RAK3+4 did not cause complete trap shut-down (no moths in traps), indicating that some males may have been able to locate and mate with female moths. Some minor damage was observed in RAK3+4 treated orchards, but was comparable, with orchards receiving a conventional spray programme. Some orchards on the mating disruption sides of the farm received an additional chlorantraniliprole (Coragen) spray when trap moth catches of four or more were recorded per week, or where early ripening cultivars which are more vulnerable to codling moth were present.

There was some concern over tortrix caterpillars in the young shoots in the spring at Site 1. These were reared through and found to be summer fruit tortrix (SFT). However over 50% of the caterpillars were parasitized by wasps. Two sprays of granulovirus (Capex), 10 days apart, killed the majority of remaining caterpillars in the affected orchards.

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

In both years of the study there was more first-generation codling moth damage in the early ripening cultivars Early Windsor and Bramley.

There was notable damage from two pests in the second year on the MD side of the farms. Blastobasis caused damage to fruit at harvest and woolly aphid was abundant in some orchards on the MD side of farms in orchards that had lower numbers of earwigs. These pests would normally be controlled with conventional spray applications targeted at CM and tortrix and, in previous years, a spring spray of chlorpyrifos, respectively.

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

In conclusion, the mating disruption was effective at low population densities, but at higher densities, some additional sprays were required when monitoring trap catch thresholds were exceeded.

Nematodes

Using the orchards in the MD trials (above) we used sentinel cages of codling moth larvae attached to the trunks of apple trees with the grower's spray equipment. These were targeted with a mixture of *Steinernema carpocapsa* (Nemasys C) and *Steinernema feltiae* (750 million of each sp. per ha) at high water volumes to the cages. Because we did not get good infection of the larvae, believed to be because the cage mesh prevented droplets containing the nematodes reaching the larvae, we used a series of laboratory tests to give a 'best' chance for nematodes to locate and infect codling moth larvae and pupae.

Using a Birchmeier B245 motorised mist blower it was possible to infect codling moth larvae/pupae with nematodes, even when they were hidden within sentinel cages. Codling moth pupae were less susceptible to nematode infection than larvae. In the cages sprayed with 50 % and full dose nematodes, 62.5 % and 100 % died as a result of infection, respectively. These experiments show there may be some efficacy of the nematode sprays against codling moth larvae in the field and the tests should now be repeated in the field with larvae in cardboard rolls without the mesh cages.

Dissected codling moth larvae infested with nematodes

Blastobasis pheromone

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

Field trapping was repeated in 2018 and once again blends of (Z)-11-hexadecenal and (Z)-11-hexadecenyl acetate failed to attract *Blastobasis lacticolella* moths in field trapping tests, even though this species was clearly present as indicated by catches in light traps. Rearing *B. lacticolella* adult moths from larvae collected in the field proved a real challenge, but some were reared through to adult. Extracts of the pheromone glands of female moths were made from both moths collected in the field which were probably mated and from virgin female moths reared from larvae in the laboratory. In analyses of extracts by GC-MS, potential pheromone components including (Z)-11-hexadecenal, (Z)-11-hexadecenyl acetate, (Z)-5-decenyl acetate and (Z)-5-decenol could not be detected. However, (Z,Z,Z)-3,6,9-Nonadecatriene was identified as a potential component of the female sex pheromone but was subsequently shown to be present in extracts from both female and male moths and did not attract male *B. lacticolella* moths in the field.

Main conclusions from this objective

- 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.
- In laboratory studies codling moth larvae were vulnerable to commercially available pathogenic nematodes.
- A putative blastobasis moth pheromone was tested but did not attract blastobasis males.

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 use of conventional control products will be of financial benefit to growers, may enhance natural predators in the crop and benefit the wider environment. The estimated cost comparison of RAK3+4 mating disruption system compared to a conventional spray programme for codling moth and tortrix moth are presented in the table below.

Estimated cost comparison of RAK3+4 mating disruption system compared to conventional spray for codling moth and tortrix moth.

Cost/ha (£)	RAK 3+4	Conventional
Cost	£240-300	-
Person hours	2	1 (as part of fungicide round)
Cost of labour	Minimum, £8.20/hour (inc. NI&AL) **£16.40**	£20-25
Monitoring	same	same
Coragen	-	£71-85 (per spray) x2
Runner	-	£44-75 (per spray)
TOTAL	**£256.40-£316.40**	**£206-270 (chemicals only)**

Action points for growers

- Mating disruption technologies can offer a similar level of control of codling and tortrix moths to conventional spray pgorammes, but where pest pressure is medium to high, It may be advantageous to apply an additional Coragen to early ripening or vulnerable apple and pear cultivars.
- Growers should closely monitor for other pests which may occur because of the limited use of Lepidopteran control products. In particular sporadic tortrix species and blastobasis caterpillars.
- Even if growers have not had previous experience of blastobasis in orchards it would be wise to continue to monitor as populations may build up locally over years.
- Growers and agronomists should consult the AHDB Apple Best Practice Guide online on how best to do this.

Objective 7.1 Improving the reliability of natural predation of pests

Headline

- The use of wildflower mixes, earwig refuges and hoverfly attractants have hastened the influx of natural enemies and reduced pest damage in newly established orchards.

Background and expected deliverables

Establishing new crops requires substantial investment (~£35k/ha for apple) and growers need confidence that their orchards will crop reliably, and that fruit will find a profitable market. Ecological succession is the observed process of change in the species structure of an ecological community over time. The community begins with relatively few pioneering plants and animals and develops through increasing complexity until it becomes stable or self-perpetuating, as a climax community.

Newly planted orchards have an un-established ecosystem. The recently tilled ground in newly planted orchards often has minimal, simplified or absent vegetation cover with a low diversity of annual plant species resulting in low pollen and nectar provision and low refugia and structure. The tree bark and canopy are simple compared to older established trees affording little availability for predatory arthropods to gain refuge. Hence, local, populations of natural predators and pollinators have not built up and established in new orchards leading to random, sporadic, attacks from a number of pest species which can then be difficult to control.

The aim of this work was to apply interventions to newly planted orchards to hasten the establishment of beneficial ecology.

Summary of the project and main conclusions

Six replicate commercial apple orchards were chosen in 2017 and secured for experimental purposes through help from Caroline Ashdown at Worldwide Fruit Ltd. In each orchard, 0.25 ha was treated with ecological enhancement interventions.

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

- Seeded floral alleyways were successful in most orchards and percentage coverage from the seed mix seemed generally increased from 2018 to 2019.

- Not all species in the seed mix established. Red clover and yarrow were the most common in 2018. Red clover was also one of the most common in 2019 along with common knapweed.
- Sward height in treated plots was higher than in untreated alleyways in both years but only significantly in 2018.
- In both years fewer aphids were observed in the treated plots in spring but not in summer.
- More predatory spiders were found than earwigs in Wignests deployed in treated plots in spring 2018 and 2019. In 2019 anthocorids were also found in refuges. Most predatory spiders found in the refuges in 2019 belonged to the family Araneidae.
- Predatory spiders were the most common arthropod recorded in apple trees in all seasons in both years. In 2019 most belonged to the Araneidae and Philodromidae families. Some species of the Philodromidae, like *Tibellus macellus*, primarily feed on aphids, accounting for over half the total prey they ingest when available (Huseynov 2008).
- Linyphiidae was the only family with significantly higher numbers of individuals in the treated plots compared to untreated. A subfamily of Linyphiidae, Erigoninae (also known as Micryphantids), are reported preying on soft-bodied pests, like aphids (Nyffeler & Benz 1988; Mansour & Heimbach 1993).
- In 2018, no apple leaf curling midge damage occurred in treated plots compared to untreated (insert percentage damage?). Apple leaf curling midge was not assessed in 2019.
- In 2018, fewer predatory mites and fruit tree red spider mites were found in treated plots compared to untreated. However, the opposite was observed for rust mites and spider mites. In 2019 only predatory mites were found, with higher numbers recorded in treated plots.
- In 2018, significantly fewer codling moth deep entry damage was recorded on treated plots in summer and significantly fewer codling moth stings were recorded on treated plots in the dropped apple assessment. In 2019, codling moth stings were significantly less frequent in the treated plots in autumn.
- There were significantly more hoverfly adults in the treated plots in autumn 2018. It is not known if this is the consequence of the attractant sachet and/or the floral alleyways. This effect was not observed in summer 2019.Statistical analysis on all data have to be interpreted with caution since numbers of arthropods were low in the orchards.

Main conclusions

- Positive benefits have been shown over two seasons following sowing wildflowers in alleyways in newly planted orchards.
- Orchards were also amended with earwig refuges in each tree and hoverfly pheromone attractant.
- Positive effects recorded included reduced numbers of pests including damage by codling moth, and higher numbers of natural enemies including hoverflies, spiders, and lacewings.
- Perennial wildflower mixes in orchard alleyways also have the potential to outcompete undesirable weed species.

Financial benefits

The costs of implementing this system of management incorporating wildflower mixes, earwig refuges and hoverfly attractants are laid out in the table below.

The estimated costs associated with implementing floral resource alleyways and natural enemy attractant	Per unit	Per ha	Time (hours)
Seed Mix for 1 ha; every other row	-	~£152-310	-
Sowing/Drilling and Rolling over large area (Minimal ground prep because new orchard)	Large areas	New orchard £28	8 hours for 10 ha
Hoverfly attractant (7x7 m spacing)	£2.70/device 196/ha	£529.20 (£265 – half rate)	-
Cost of Labour (2019) Inc. NA + PEN	£8.77/hr	-	1
Deploying hoverfly attractant	-	£35.08	4
Reduced cost due to less mowing through labour and fuel		£ ?	Faster moving sprayer
OPTIONAL: Wignests, marketed by AgroVista		~50/pack @ £43.87/50 for 1-19 packs or £40.62/50 for 20+ packs	
Total		~£480-902	

Action points for growers

- New orchards, and even existing orchards, should be provisioned with pollen, nectar and structural resources to provide pollinators and natural enemies with habitat and food to increase their numbers.
- Selection of perennial wildflower seed mix should be largely driven by soil type.
- It is recommended to use a perennial mix which should be regularly cut to 10 cm in the first year to encourage establishment. The plants will flower from year 2.
- In preparation for sowing, soil should be weed free and have a fine tilth. Once the wildflower seeds are broadcast (not drilled) they should be rolled to help seeds make contact with the soil. Following this, a period of rain or irrigation is desirable to encourage germination.
- Sowing can be done in the autumn or spring.
- Seed mixes should contain a range of native open, legume, and complex flower types with non-competitive grass species making up a high percentage of the mix.
- From year 2, in general, one cut before fruit harvest is recommended or maybe an additional midsummer higher cut – depending on weather conditions.

Objective 7.2 Determining pear sucker/predator thresholds for spraying

Headline

- Sprays targeted against pear sucker can be avoided where the numbers of pear sucker eggs do not exceed 1,000 and the numbers of natural enemies were greater than 10 per 30 branch beat samples.

Background and expected deliverables

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

There are large differences in earwig populations between orchards, and Project TF 196 has demonstrated that use of conventional spray products and their timing may be, at least partly responsible for lower than optimum popuations. However, anecdotal evidence is showing that earwigs can be distributed unevenly within an individual orchard.

The aim of this study was for the entomology team at NIAB EMR to collaborate with commercial pear growers and their staff and train them to record pear sucker pest and predator numbers. This will help them to make more informed decisions on whether and when to apply control measures and contribute to data for a potential model for predator/prey thresholds.

Summary of the project and main conclusions

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

Records of pear sucker eggs, nymphs and adults, along with ladybirds, earwigs and anthocorids in the perceived low, medium, and high pear sucker pressure orchards were completed from March to September. It was found that in general, sprays could be avoided where there were <1,000 pear sucker eggs per 30 shoots per week and >10 natural enemies per 30 shoots per week. More work is needed to determine the threshold of nymphs. In addition, we ascertained that a mix of natural enemies (earwigs, anthocorids and ladybirds) provide resilience to pear sucker control.

Financial benefits

Close monitoring of pear sucker and natural enemies can prevent the application of unnecessary sprays and conserve natural enemies which control pear sucker. This will reduce the need for applications of products needed to control 'honey dew' on trees. The reduction of pear sucker in the crop reduces crop loss through the maintenance of fruit quality and prevents damage to overwintering bud and tree health.

Action points for growers

- Sprays targeted against pear sucker can be avoided where the numbers of pear sucker eggs do not exceed 1,000 and the numbers of natural enemies were greater than 10 per 30 branch beat samples.
- Monitor pear sucker life stages in the crop to improve the timing of spirodiclofen (Envidor) applications and prevent use of unnecessary sprays.
- A recently published study has also shown that spirotetramat (Batavia) can offer good control of early and late stage nymphs of *C. pyri*, both on the shoot tips and on cluster leaves in the central part of the tree. Spirotetramat is also reported to be safe to anthocorids.
- Use the monitoring of natural enemies such as earwigs, anthocorids and ladybirds alongside pear sucker monitoring to inform likely future control options that avoid damaging these predators.
- Consider releases of commercially produced anthocorids early on if numbers of natural enemies are low, but think about the surrounding habitat to encourage long term resilience in anthocorid populations (hazel, willow, hawthorn and nettle are good alternative hosts).
- Be considered with the choice, numbers and timing of spray applications. Think about spray frequency and impact on natural enemies.

Objective 8.1 - Apple fruit rhynchites

Headline

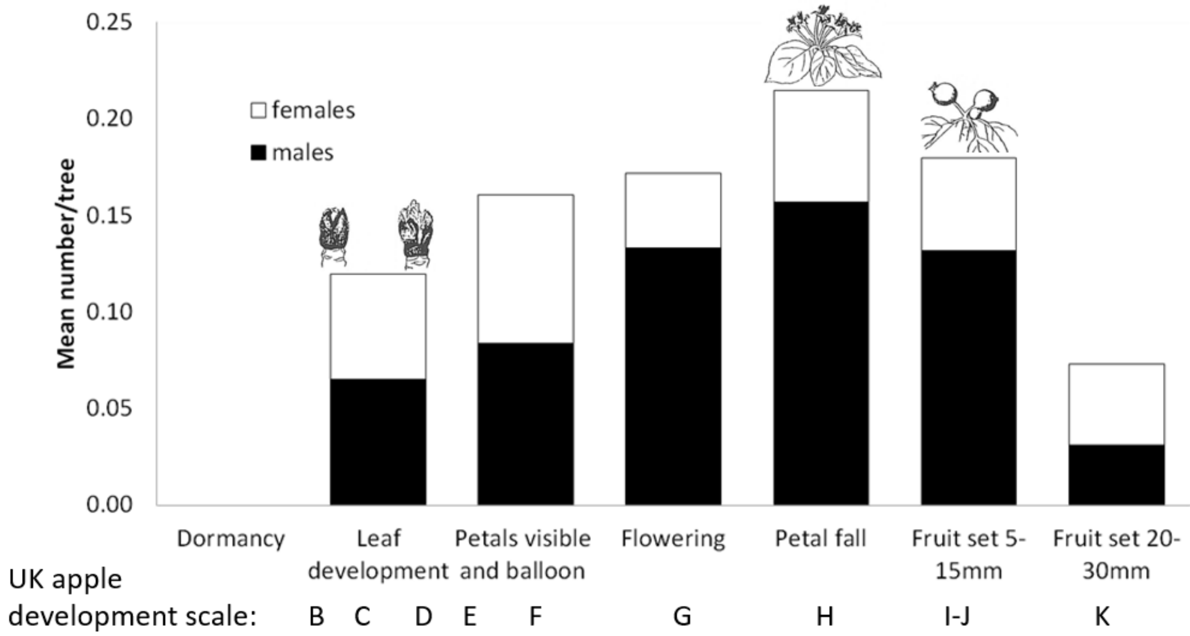
- There may be a window of opportunity to target weevils with control options both pre bloom and at petal fall – when females are likely to be laying eggs.

Background and expected deliverables

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 use of crop protection products. 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 would 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, but as a consequence we also undertook more detailed phenological studies of the pests' occurrence in orchards.

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 analyzed. 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 cultivars 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.



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

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 use of crop protecton products. Losses of 1% of fruit are common and losses >5% are not unusual. Although the research work done in this study has not successfully identified semiochemcals produced by this weevil, new information on the influx of the weevil to apple orchards has been gathered and this will allow growers to improve the timing of application of control products, thereby improving the effectiveness of control.

Action points for growers

- Target control options for apple fruit Rhynchites just pre or post blossom.

Objective 8.2 Apple sawfly

Headline

- Attempts to identify the sex pheromone of the apple sawfly for use in monitoring and mating disruption studes were unsuccessful.

Background and expected deliverables

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

Summary of the project and main conclusions

Apples containing apple sawfly larvae were collected in spring 2015, 2016, 2017, 2018 and 2019 from an unsprayed orchard at NIAB EMR. The apples were placed onto compost in mesh covered bins. Larvae were allowed to crawl out from the fruits and enter the compost. As apple sawfly has only one generation per year these were maintained outside until spring 2016 and spring 2017. However, no apple sawfly adults emerged and pupae were found to be infected with either bacteria or fungus, even when in 2017, bins were maintained with lids to prevent over wetting from rain. The previous winter had been very wet and it was speculated that the soil may have become too wet outside.

In spring 2017 and 2018, apple sawfly infected apples were collected again and kept in Bugdorm cages under cover. As the larvae emerged from the apples and began to 'wander' they were transferred into smaller plant pots of compost. Six were kept at ambient conditions in an outside area under cover and two were stored at 6°C for 2 months in 2017 and 5 months in 2018 to attempt to simulate a cold period. Again, no adults emerged.

Larvae collected in 2019 were kept in terracotta pots outdoor to mimic more realistic conditions. However, when the compost was removed in the spring, at the time of writing, it was once again very wet and to date no adults have emerged. Future work should deploy emergence cages, dig up orchard soil for flotation, and/or catch live adults in orchards in spring.

Financial benefits

- No financial benefits have arisen from this study.

Action points for growers

- No action points have arisen from this work.

Objective 9. *Anthonomus spilotus* in pear

Headline

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

Background and expected deliverables

A new pest of pear was identified in the first years of this project by NIAB EMR and the Natural History Museum. The weevil is from the *Anthonomus* family of weevils known to feed and develop in buds and fruits of plants. Unlike *Anthonomus piri*, *A. spilotus* feeds and lays eggs in spring blossom and, later, leaf buds. In order to control the weevil it is likely to be necessary to target sprays in the spring, before the flower clusters open. Work in this objective aimed to establish the activity period, lifecycle and toxicity of commonly used control products. More research is needed to establish damage thresholds and to improve precision of spray timing.

Summary of the project and main conclusions

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

Fewer than 10% of the flowers in a truss were damaged by adult feeding and fewer than 16% were damaged by larvae. Greater flower and leaf damage was observed when eggs/larvae were present. Hence the damage to flowers at 1 weevil per 40 taps is not the main consideration as only 1 of the 6 flowers in a cluster is normally destroyed and only 3-4 pear fruits can set to harvest on a single truss. The main consideration is the damage to leaves and photosynthetic ability for future years.

Even at very low levels of weevils (~1 per 40 tree taps) ~60% of new leaves were damaged later in the season. We have not been able to set a damage threshold for this because the resultant health to the tree cannot be estimated in this project. The majority of buds usually had 1 to 3 damage holes although buds with more punctures could be found.

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

In laboratory tests in 2016, acetamiprid (Gazelle) only gave 50% control, but thiacloprid (Calypso) at full and half field rate gave 80-90% mortality. Thiacloprid, lambda-cyhalothrin (Hallmark), and pyrethrins (Spruzit) were the most effective products against *A. spilotus* in the laboratory. High mortality and rapid negative behavioural effects were observed in these treatments. However it should be noted that, in this experiment, weevils received a direct application of the control product. In a pear crop this scenario is less likely and weevils may be more likely to come into contact with dried residues.

In 2018 we determined whether product efficacy can be improved through stimulating ingestion of the control agents, spinosad (Tracer) and indoxacarb (Steward). Calypso was the most effective product against *A. spilotus* in the laboratory trial where shoots had been sprayed with products and then weevils allowed to feed. 100% mortality in 9 days after ingestion was observed compared to the control group (40%).

In 2019 we examined the best timing of control measures in growers' orchards. A fully replicated randomised design was established in four Kent orchards known to have moderate populations of *A. spilotus*. The spray applications were supported by the growers and Avalon Produce staff. All planned applications were made, but numbers of weevils in spring 2019 were too low to establish meaningful results and there was no difference in damage to flowers or leaf shoots with either pre or post blossom applications of Calypso or Steward.

Main conclusions

- In this 2019 field trial a spray application of Calypso or Steward before or after blossom had no effect on feeding damage or numbers of *A. spilotus*.
- The population of weevils may have been too low to show benefits from the product application.
- Calypso has been effective against *A. spilotus* in laboratory tests in previous years.
- More extensive studies are needed to confirm the effectiveness and best timing of application of these products in the field to control *A. spilotus* and other spring damaging weevils.
- The loss of Calypso approval will mean that growers will only be left with broadspectrum prodcts to control weevils. This I not desirable as these are harmful to

natual enemies. However Gazelle gave 50% mortality in laboratory tests and could be used to keep populations in check.

Financial benefits

Larvae in flower buds feed on flowers, but then also feed on emerging leaf shoots. This could affect yield but also the health of trees over the long term. It is essential to calculate thresholds for spraying and spray timing. It is estimated that a female weevil in the *Anthonomus* family can lay around 25 eggs in her lifetime. Although the levels of damage to flowers, fruits and leaf tissue appear to vary, failure to monitor for the pest and employ control measures where necessary could lead to financial losses where significant damage occurs.

Action points for growers

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

Objective 10 – Brown Marmarated Stink Bug

Headline

- Adults of brown marmorated stink bug (BMSB) were reported and confirmed in Hampshire in 2018 and 2019, although no breeding populations have yet been detected.

Background and expected deliverables

The surveillance objective of this project (Objective 1) provides the opportunity for ongoing activities to continue and be reported. Such activities include the monitoring for the invasive pest BMSB. This objective aims to keep the industry up to date with the pest and disease threats which ultimately lead to yield losses and provides information for the industry to inform future research targets and priorities.

Summary of the project and main conclusions

Monitoring BMSB using sentinel pheromone traps in the South East, the East of England and South Wales did not detect any incursions of the pest. However, internet searches revealed reports of adults of this invasive pest free in the environment at two sites in Hampshire, and the species identification was confirmed. No establishment (detection of breeding populations inferred by the presence of nymphs or egg masses) has yet been reported.

Financial benefits

Current, emerging and newly introduced pests and disease can have a devastating effect on yield and economic return to your business. This objective enables the ongoing monitoring of these threats helping to inform future research priorities. BMSB alone represents a huge potential threat to multiple crops, and has caused extensive losses to tree fruit (including pear and apple) in North America and continental Europe.

Action points for growers

- Look out for news in the trade press or important announcements from the animal and plant health agency (APHA) about invasive pests and disease which will affect your business, such as BMSB and *Xylella fastidiosa.*
- Commercially produced traps to monitor BMSB are also available.

SCIENCE SECTION

General Introduction

This 5 year project set out to develop and implement strategies to manage key tree fruit diseases and pests, namely: European apple canker, apple scab, apple powdery mildew, blossom wilt and brown rot of stone fruit, and bacterial canker of stone fruit, codling, tortrix and blastobasis moths, pear sucker, weevils and apple sawfly. In light of future chemical control withdrawals, and ongoing consumer and environmental concerns about over reliance on chemicals, a focus on incorporating Integrated Pest & Disease Management (IPDM)-compatible approaches with conventional chemical control has been adopted for each of the disease and pest targets. This project has more relevance in the last couple of years as chemical controls have been withdrawn or have more restrictions. Many of the approaches in this project rely on non-chemical controls and future novel approaches to pest and disease control in fruit trees.

European apple canker, caused by the fungus *Neonectria ditissima*, has become one of the most important diseases for the industry in recent years due to increased planting of canker susceptible varieties. The disease is causing significant financial losses; from tree death during the establishment phase, loss of fruiting wood due to the pruning of canker wood, 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 may give more effective canker control and reducing losses during tree establishment.

Apple foliar diseases require season-long control. For scab and mildew, 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, control measures can break down during the growing season resulting in disease epidemics. Mildew epidemics, in extreme cases, can defoliate 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 may reduce fungicide applications whilst maintaining acceptable disease control.

Losses resulting from blossom wilt and brown rot of stone fruit caused by Monolinia spp. 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 fruits in one punnet can lead to food retailers rejecting whole consignments. Bacterial canker of stone fruit 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 IPDM 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.

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

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

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

There are large differences, between orchards, in earwig populations and Project TF 196 has demonstrated that pesticide use and timing may be, at least partly, responsible. However, anecdotal evidence is showing that earwigs can be patchily distributed within an individual orchard. The Innovate UK earwig project is making good progress with a marketable device which could be used in newly planted trees to help encourage natural predation of pests. This will be available from 2020 to growers, produced by Russell IPM and marketed by Agrovista the device is named 'Wignest'. We hypothesise that orchard niche availability has a significant influence on beneficial arthropod populations and subsequent pest control.

Project TF 218 determined the most important predatory hoverfly species in apple orchards and explored whether the adults can be enhanced by attraction with plant volatiles. The results have fed into this project to harness more hoverflies in newly planted apple orchards.

Monitoring by visual inspection for apple sawfly (*Hoplocampa testudinea*) adults is generally too difficult for growers and agronomists and damage is often done before the pest is noticed, control then being scheduled for the following year or missed. Growers currently rely on sprays of thiacloprid (Calypso) for control. This product is unlikely to be available for use in 2021. Semiochemical based pest specific monitoring traps for these pests would be a significant advancement, aiding decisions on the need for and timing of sprays. Alternatives to thiacloprid and chlorpyrifos for control of these pests are also needed.

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

Objective 1 - Surveillance

1.1 Scab virulence

Aim

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

Summary

This task involves the monitoring of an indicator orchard at NIAB EMR, planted as part of a large global project in which the same indicator cultivars are planted at over 30 sites in 24 different countries. It is hoped that the network will expand in the years to come to give a more global picture as most of the participating countries are currently in Europe. The data collected from each participating group is compiled by the project coordinator based in Switzerland. Scab incidence was recorded at the end of August 2019 and has been submitted to the project coordinator. In the NIAB EMR orchard severity of the disease in 2019 was much higher than 2018 and slightly higher than the other years of monitoring (since 2015). Resistance breakdown in *M. floribunda* 821, the source of the Rvi6 scab resistance gene (formerly known as Vf - the most extensively used R gene in breeding for scab resistance breeding), was again confirmed as in the previous three years. However, no breakdown has yet been seen on the trees in the plot of the domesticated cultivar Priscilla which carries the Rvi6 gene. Scab was also found on the indicator genotypes for the Rvi3 and Rvi8 genes; unlike Rvi6 these genes cannot be found in any commercially available cultivars. Isolates of scab from cultivars containing resistance genes have been collected for DNA extraction to determine the genetic changes in the population which has resulted in breaking the resistance this may in turn aid the identification of new sources of resistance. Patocchi et al (2020) report the findings from this global project thus far and how they can impact the use of these genes in future apple breeding.

1.2 Apple Rot Survey

Aim

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

Introduction

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

Materials and Methods

Two commercial pack houses in Kent (F W Mansfield and Newmafruit Farms) were visited weekly from 17 January until 11 April, a total of 26 visits and 44 consignments of apples examined. At each visit the fruit being graded at the time was assessed by estimating the number of rots on the grading line in relation to the volume of fruit being graded to give a % loss. The rots responsible for the loss were assessed by examining up to 100 rotten fruit from the rot bin and identifying the fungus present. Any rots not identified were sampled and identified by culturing the rot onto potato dextrose agar.

Results and Discussion

The incidence of rots per hundred rotted fruit for four dessert (Braeburn, Cox, Gala, and Jazz) and one culinary apple cultivar (Bramley) are summarised in Table 1.2.1. On average actual losses ranged from 0.01 to 5% with an overall mean of 1.2 %. The highest losses were recorded in Cox and Bramley which is as expected as these cultivars are stored at 3.5 - 4°C, whereas the other cultivars are stored at around 1-2°C.

Weather risk: The weather in April (79.8 mm rain) was wet with above average rainfall. Less rain was recorded in May (38.4 mm) but with significant wet days at the end of blossom, the main period for infection of fruit by Neonectria, giving a moderate risk for Neonectria fruit rot especially in orchards with a high incidence of canker. June and the first half of July were hot and dry, but the end of July (26.8 mm rain) and August (67.4 mm rain) were warm and wet giving favourable conditions for some storage rots such as Gloeosporium/Neofabraea and ensuring active Phytophthora in the soil. However, the September harvest period was relatively dry 35.6 mm rain at EMR but wetter elsewhere (53.4 mm rain at Sittingbourne) resulting in a variable Phytophthora rot risk for fruit harvested in September (Cox and Gala) depending on site. Wetter conditions were recorded in late October (33.6 mm) and especially in November (103.2 mm) for the later harvested cultivars Braeburn and Jazz indicated a high Phytophthora risk for these cultivars in orchards at risk (Table 1.2.2).

Rots and cultivars: Losses in Gala were on average 0.5 % and mainly caused by brown rot and Neonectria as expected from the moderate risk for Neonectria at the end of blossom and the high incidence of canker in many Gala orchards. Losses in Cox were relatively high and mainly attributable to Gloeosporium/Neofabraea. This is due to the favourable weather in July and August but also due to the Cox being stored for longer than usual due to the market influences. The five batches of Cox assessed were sampled between late February and mid - April. In most seasons Cox is generally sold by the end of February. Losses in Jazz were on average 1% but ranged up to 5%. Most losses were due to Neonectria, however in two samples picked in mid - November, most losses (> 2%) were due to Phytophthora (Table 1.2.2). Losses in Braeburn were on average 0.2% and due to a combination of rots including, brown rot, Botrytis, Penicillium, Phytophthora and Neonectria. Losses in Bramley were around 1.9% and mainly attributable to brown rot and Phomopsis, the later causing stalk, eye, and core rots. In previous years Fusarium was the main fungus identified causing rots at these sites with Phomopsis also present. The reasons for the change in significance are not clear but may be due to the high temperatures in the summer. Bramley with the open calyx is prone to core rots, which were recorded in 9/10 samples and ranged from 0-37% of rots. The cultivar Cameo is also prone to core rot, but no Cameo samples were assessed. The incidence of core rot in other cultivars is much lower and generally not important.

Fungal rots: As in other years brown rot and Neonectria rot were the main causes of rotting. The incidence of Gloeosporium/Neofabraea rot was higher than in previous years and recorded in all cultivars. Botrytis was recorded in all cultivars but at higher incidence in Braeburn and Jazz, and mostly associated with damage especially missing stalks. The average incidence of rots in the previous three years is also included in Table 1.2.1. Rot incidence was very similar with the greatest change in Botrytis incidence which decreased compared to 2017/18 and Phomopsis which increased compared to the previous three years.

Table 1.2.1 Average loss (%) attributed to each rot pathogen during 2018/19 storage season. Data is compiled from 44 apple samples. Overall averages for 2017/18, 2016/17 and 2015/16 are included for comparison. [1]Core rots includes Fusarium and Phomopsis rots recorded separately.

Cultivar	**Brown rot**	**Botrytis**	**Penicillium**	**Phytophthora**	**Neonectria**	**Gloeosporium/ Neofabraea**	**Fusarium**	**Mucor**	**Botryosphaeria**	**Phomopsis**	**Stalk**	**Eye**	**Cheek**	**[1]Core**	**No. samples**	**Loss (%)**	**Loss range %**
Braeburn	11.9	18.6	13.9	13.6	32.8	7.6	0	0.4	0	0	0	0	1.1	0	10	0.23	<0.1-0.8
Bramley	34.5	2.0	8.2	0	8.1	2.2	5.2	0.4	0	32.5	0	0.3	2.2	14.3[1]	10	1.9	<0.1-3.6
Cox	12.3	5.3	4.8	2.7	14.4	58.8	0	0.3	1.0	0	0	0	0.4	0	5	2.3	0.5-3.0
Gala	36.4	5.1	6.1	0.8	29.7	10.6	0	0.6	0	0	0	0	0	0	9	0.5	0.01-0.7
Jazz	1.5	18.0	11.0	22.0	35.2	1.7	0	0.6	0	0	0	0	0	0	10	1.0	<0.1-5.0
Overall mean 2018/19	19.3	9.8	8.8	7.8	24.0	16.2	1.04	0.5	0.2	6.5	0	0.06	0.7	2.9	44	1.2	-
Overall mean 2017/2018	7.6	27.8	10.6	4.5	32.4	10.6	2.9	0	0	0.4	1.3	0.1	1.0	0.3	32	1.6	-
Overall mean 2016/17	19.3	9.7	11.2	1.6	31.3	12.4	0.4	4.2	0	0	2.0	0.6	1.5	5.6	52	1.5	-
Overall mean 2015/16	13.3	8.3	6.3	6.4	40.3	9.3	0.5	3.0	0	0	2.2	0	1.2	7.3	60	2.6	-

Table 1.2.2 Phytophthora risk in relation to harvest date and actual losses due to rots and incidence of Phytophthora rot in some apple samples.

Apple harvest date	Rain (mm) in 15 days pre-harvest >20mm = Risk	Cultivar	% loss due to rots (% Phytophthora)
31 August	19.8	Bramley	3.6 (0)
10 September	15.2	Cox	2.5 (0)
14 September	31.6	Cox	0.5 (6.5)
20 September	2.8	Gala	0.2 (0)
25 September	26.8	Gala	0.5 (0)
12 October	6.0	Braeburn	0.4 (0)
31 October	30.6	Braeburn	<0.1 (12.1)
18 November	59.4	Braeburn	0.2 (55.6)
10 November	65.6	Jazz	2.0 (78.1)

1.3 Invasives

Aim

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

Summary

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

	Species	Action Taken
Pests	*Drosophila suzukii*	National monitoring programme and wide-ranging research programme ongoing. Attendance of Northern Europe SWD group in Belgium has resulted in a collaboration to develop a predictive model. *D. suzukii* numbers were high in April and late summer in 2017 compared to previous years, but lower in the spring of 2018 because of a cooler spring. In 2017, damage was seen in early June bearing strawberry and autumn ripening raspberry, blackberry and grape. However, probably due to the previous experience and revised management of cherry, fewer incidences of cherry damage were reported. Activity in the traps peaked to almost double winter 2016/17. Numbers were similar during raspberry harvest in 2018, winter activity and mean numbers over the whole year similar to 2017. Numbers (monitored only at NIAB EMR) rose slightly in 2019.
	Summer fruit tortrix	Summer fruit tortrix was detected for the first time in the West Midlands during the 2015 growing season and it is recommended that growers now monitor for this pest in the region using pheromone traps alongside codling moth and fruit tree tortrix monitoring traps. Damage was

	reported in the West Midlands in 2017 but the species was not confirmed.
NEW: Marmorated stink bug	Sentinel traps for *Halyomorpha halys* were placed in municipal gardens, commercial fruit farms and in gardens in 2018 but no BMSB have been detected to date. However there have been two reports of BMSB adults in Hampshire. These adults were found free in the environment, not obviously associated with imported goods or luggage and the two sites were in fairly close proximity.
NEW: Forest bug	https://www.cabi.org/ISC/abstract/20153399346 and https://www.britishbugs.org.uk/heteroptera/Pentatomidae/pentotoma_rufipes.html *Pentatoma rufipes* is a native but emerging pest of apple and pear in the UK. To date is has caused up to 40% damage to crops in certain orchards. It overwinters as an early stage nymph and feeds on early fruitlets severely distorting mature fruits. A review has been published by AHDB (2020), authored by Dr Glen Powell at NIAB EMR giving details of the limited research to date.
NEW: Common Green Shieldbug	https://www.britishbugs.org.uk/heteroptera/Pentatomidae/palomena_prasina.html *Palomena prasina* increasing reports of fruit damage in Belgium are being recorded from this, UK native, shield bug.
NEW: Mottled Shield Bug	https://www.britishbugs.org.uk/heteroptera/Pentatomidae/rhaphigaster_nebulosa.html *Rhaphigaster nebulosa* first recorded in Britain from the London area in 2010, also becoming a pest of tree fruit, in Belgium.
Anthonomus spilotus	This is new to the UK (Morris et al. 2017) and an AHDB factsheet was produced by M. Fountain and S. Raffle in 2018. The pest has also been recently identified as an invasive pest in Belgium (see Objective 9).
Pear Shoot sawfly	The RHS reported sightings of Pear Shoot sawfly, *Janus compressus* in 2016. This has not been seen in commercial pear as far as we are aware. This ‘occasional’ pest of pear in Europe effects the shoots causing symptoms similar to fire blight – shepherd crook shaped tips

	caused when the larvae feed inside the shoots. A paper was sent to Chris Nicolson for inclusion in the ADAS notes in 2017.
Apple maggot fly	http://entnemdept.ufl.edu/creatures/fruit/tropical/apple_maggot_fly.htm *Rhagoletis pomonella,* native to North America, originally fed on the fruit of wild hawthorn (*Crataegus* spp.), but then became a primary pest of cultivated apples in northeastern United States and southeastern Canada. Adults emerge from the ground during early summer. Pupae may remain inactive and do not emerge until the second year. The female punctures the skin of the fruit with her ovipositor and lays eggs singly in the pulp. Eggs hatch in five to 10 days. Larvae develop slowly in the green fruit and usually do not complete their growth until the infested fruits have dropped from the tree. Larval development is two weeks to three or more months in hard winter varieties. Hosts include: apple, *Prunus* spp., *Vaccinium macrocarpum,* and peach. Larvae have been found in *Pyrus* spp. Damage: irregular, winding tunnels in fruit which turn brown, causing premature dropping of fruit.
Black and white citrus longhorn	https://www.cabi.org/isc/datasheet/5556 *Anoplophora chinensis* is black and shiny, with white pubescence. Length 19-40 mm. Recognized by long antennae reaching to at least the end of the body. >26 families of living tree hosts including Citrus, *Malus domestica*, apricot, European pear. Egg is elongate, subcylindrical, white (6 mm long) and laid through bark (T-shaped slit) close to ground level. Larva is elongate, cylindrical, up to 56 mm long and bores into the stem destroying the pith and vascular system later entering heart wood, tunnelling up and down. Considerable amounts of frass (small cylindrical pellets of sawdust) and wood pulp are ejected through holes in the bark. Adults eat young leaves, branches and bark of the tree. At 20°C, 57% of the individuals completed their development 306 to 704 days after oviposition. Lower developmental threshold temperatures for eggs and young larvae 6.7 and 11.6°C, respectively. Tropical and subtropical regions one generation per year; further north one generation every 2 years.

	Although intercepted at ports or found in association with plants recently imported from Asia, it is not presently known to be established in the USA or Canada. First published record occurring on natural vegetation in Europe was in 2001. Eradication efforts are underway in Italy.
False codling moth	https://www.cabi.org/isc/datasheet/6904 *Thaumatotibia leucotreta* is a pest in tropical Africa but has failed to invade other areas as yet. Eggs: Flattened, oval, diameter 0.9 mm. Larva: When young yellowish-white with dark spots, up to 15 mm long, bright red or pink. Pupa: tough silken cocoon amongst debris or in the soil. Adult: Strongly dimorphic: Male wingspan 15-16 mm, female 19-20 mm. In both sexes the forewing pattern consists of a mixture of grey, brown, black and orange-brown markings, the most conspicuous being a triangular marking in the outer part of the wing, against the hind margin, and a crescent shaped marking above it. Seen in Europe where imported with produce from Africa. Detection of a single adult male in trap in California, in 2008. Pest of *Capsicum* (peppers), *Prunus persica* (peach). Probably low risk except glasshouse crops.
Grapevine phylloxera	https://www.cabi.org/isc/datasheet/56511 *Viteus vitifoliae* or *Daktulosphaira vitifoliae*. Globular aphid, 1.6-1.8 mm long and 1.0-1.2 mm wide. Native to North America and introduced into other continents (South and Central America, Africa, Oceania) in nineteenth century. Its introduction into European vineyards in the 1860s led to extremely severe losses and was considered as a major disaster. Destruction stopped by the grafting of European grapevine cultivars onto American rootstocks. Present in the UK from 1980's with few occurrences. Very limited capacity for natural spread if it remains more or less confined to the root system in the radicicolae form (as it does in Europe). Difficult and costly to eradicate. Symptoms: initially a few dead or declining contiguous vines in a vineyard. Gallicolae form: Small galls, about the size of half a pea, develop on the leaf surface, sometimes so numerous as to cover the entire leaf. Radicicolae form: Numerous knots or galls form on grapevine roots, with rotting of roots, yellowing of foliage and

	general decrease in vigour of the vines. Death of susceptible vines may result within 3-10 years. Complex alternation between an aerial, leaf-feeding form and the root-feeding form (gallicolae and radicicolae, respectively). However, *V. vitifoliae* can also persist parthenogenetically as the root-feeding form, without the leaf-feeding stage of the cycle. On cultivars of European grapevine (*V. vinifera*) grafted onto American rootstocks, normally infests only the underground parts of the plant and undergoes an incomplete cycle of seasonal development, with no change of feeding site. The winter is passed in the form of first- and second-instar nymphs on the nodules or galls on vine roots (European grapevines). In European cultivars of *V. vinifera* grafted onto American rootstocks, radicicolae become active, feeding on the roots, as soon as growth starts in the spring. Continue to multiply parthenogenetically through the summer. It is reported that sexuparous forms appear, but the gallicolous aphids do not normally develop on the leaves, and the aerial life-cycle is therefore not completed in Europe. However, pers. comm. with R. Saunders is that leaf symptoms, blistering, can occur every 3-4 years especially in Sauvingnon Blanche.
Ambrosia beetle on nursery stock	https://www.cabi.org/isc/datasheet/57038 *Xyleborinus saxesenii* (fruit-tree pinhole borer), native, not invasive, but should be considered a high-risk quarantine pest. This is because members of the tribe *Xyleborini* (*Xyleborinus* plus related genera) are inbreeding, with the males mating with their sisters within the parental gallery system before dispersal. Thus, the introduction of only a few mated females may lead to the establishment of an active population if suitable host plants can be found and environmental conditions are satisfactory. A very wide range of host plants. Any woody material of suitable moisture content and density may be all that is required. *X. saxesenii* has a high rate of increase due to its large brood sizes, almost all of which are females. The direct risk of establishment of populations of *X. saxesenii* outside its present range, followed by further spread of the species, should be considered very serious. A number of species of ambrosia beetle that normally attack only weakened host trees seem to be changing their

	habits and attacking healthy trees, either as exotics or in their native ranges (Kühnholz et al. 2003). Although such a change has not been noted for *X. saxesenii*, it would considerably increase its potential for causing economic damage to crop and forest trees.
Gypsy moth	https://www.cabi.org/isc/datasheet/31807 *Lymantria dispar.* Captured in light traps at higher frequency in 2018 (17 in one night in one light trap). It can damage fruit trees. Hatching larvae usually start feeding on flushing buds and later on newly-expanded leaves. High populations often result in total tree defoliation, often across a large spatial area. There is a pheromone identified.
Magdalis beetle on pear	Identified as minor pest of pear in 2018 (documented in Masse) causing superficial foliar damage. *M. armigera* is historically associated with elm and apple, in the spring months.
Rhagoletis cingulate	https://www.cabi.org/isc/datasheet/47051 Infestations in several cherry growing regions of Germany in sour cherries. Identified in UK in 2018. Due to the 3-4 weeks later emergence compared to *R. cerasi* sweet cherries mostly not affected by *R. cingulata*. Chemical control Exirel (cyantraniliprole), SpinTor (spinosad), Karate (lambda-Cyhalothrin) or netting.
Green Citrus Aphid	In 2018, Csaba Borbély identified 9% of the collected individual aphids from UK apple orchards (mostly south east) as *Aphis spiraecola*. Pear-shaped body with two black cylindrical siphunculi or cornicles on the posterior of the abdomen (1.2 - 1.7 mm in length). Uniform yellowish-green to green body, pale brown head, and pale brown legs and antennae. Winged forms have a dark brown thorax with a green abdomen. Hosts, Citrus, apple, hawthorn, pear, quince. Host damage: infested flower buds may fall off the plant, honeydew excreted by aphids, coats the outside of fruits and leaves, and promotes the growth of sooty mould fungus that inhibits photosynthesis, weakens the plant, and makes fruit unattractive, feeds on the underside of new growth, heavy infestation may result in severe curling and distortion. Spirea

	aphids are capable of transmitting *Citrus tristeza* virus (CTV). Common in Europe on sprayed orchards.
American plum borer	https://www.cabi.org/ISC/abstract/20113215871 *Euzophera semifuneralis* is a moth of the family Pyralidae. Found throughout the United States, southern Canada and parts of Mexico. Adults in the southern part of the range emerge from April through September. They live for 1–3 weeks. Larvae feed on a wide range of plants, including plum, peach, cherry, Chinese plum, pear, apple, apricot, and walnut. Plum and other drupe and pome fruit trees are favoured. Larvae bore into the bark of their host at scars, wounds, or crevices where bark scales offer concealment and protection. Larval mines are very shallow and irregularly shaped, cave-type burrows between wood and the outer bark. The galleries are usually loosely packed with frass. Larval feeding lasts 30–38 days. Pupation takes place in burrows under the bark in loosely spun silken cocoons partially surrounded by dark excrement pellets. The pupal stage lasts 24–33 days for the overwintering generation but may be completed in as few as 10 days for summer generations. Up to five generations occur annually in central Texas, but only two generations in Virginia, Delaware and Michigan.
European grapevine moth	https://www.cabi.org/isc/datasheet/42794 *Lobesia botrana* The original geographic distribution of follows a clear Palaearctic pattern. Now in central Africa (Ethiopia, Eritrea and Kenya). Records from northern Europe (Finland and Sweden) must be considered as incidental. More recently reported in vineyards of Chile (2008), California (2009) and Argentina (2010) (Ioriatti et al., 2012). It was declared eradicated from California in 2016 (NAPPO, 2016). Reported in South Africa in 2019. Host plants; wide host range recorded, grapevine is the major host crop. Wild hosts, *Daphne gnidium* is the major food plant. On inflorescences (first generation), neonate larvae firstly penetrate single flower buds. Symptoms are not evident initially, because larvae remain protected by the top bud. Later, when larval size increases, each larva agglomerates several flower buds with silk threads forming

	glomerules (nests) visible to the naked eye, and the larvae continue feeding while protected inside. Larvae usually make one to three glomerules during their development which provide protection against adverse conditions. Despite the hygienic behaviour of larvae, frass may remain adhering to the nests. On grapes (summer generations), larvae feed externally and penetrate them, boring into the pulp and remaining protected by the berry peel. Larvae secure the pierced berries to surrounding ones by silk threads to avoid falling. Frass may also be visible. Each larva is capable of damaging between 2 and 10 berries, and up to 20-30 larvae per cluster may occur in heavily attacked vineyards. If conditions are suitable for fungal or acid rot development, a large number of berries may be also affected by *Botrytis cinerea*, *Aspergillus carbonarius* and *Aspergillus niger*, which result in severe qualitative and quantitative damage. Damage is variety-dependent: generally it is more severe on grapevine varieties with dense grapes, because this increases both larval installation and rot development. Larval damage on growing points, shoots or leaves is unusual.
Peach fruit moth	https://www.cabi.org/isc/datasheet/11401 *Carposina sasakii* (Lepidoptera: Carposinidae) is not currently regulated in the EU although *C. niponensis*, a valid species of no economic significance that was previously mistakenly synonymised with *C. sasakii*, is regulated in Annex IIAI of 2000/29 EC. *C. sasakii* is a well-defined species that is recognised as a major pest of apples, peaches and pears in eastern China, Japan, Korea and Far East Russia. It is not known to occur in the EU. Adults emerge in the spring or early summer. Eggs are laid on host fruits. Larvae burrow into the fruit to develop. Infested fruits often drop early. Larvae exit fruit and overwinter in the soil. In the more southern areas of distribution, there can be two or more generations per year. Import of host fruit provides a potential pathway into the EU. *C. sasakii* occurs in a range of climates in Asia, some of which occur in the EU. Wild and commercially grown hosts are available within the EU. It has the potential to establish within the EU where there could be one or two generations per year. Impacts could

	be expected in apples, pears and other rosaceous fruit crops. The level of impacts would be uncertain.
Oriental fruit fly	https://www.cabi.org/isc/datasheet/17685 *Bactrocera dorsalis*. Highly invasive species. Native to Asia, now found in at least 65 countries, including parts of America and Oceania, and most of continental Africa (sub-Saharan countries). The potential risk of its introduction to a new area is facilitated by increasing international tourism and trade, and is influenced by changes in climate and land use. Can easily disperse as it has a high reproductive potential, high biotic potential (short life cycle, up to 10 generations of offspring per year depending on temperature), a rapid dispersal ability and a broad host range. The economic impact would result primarily from the loss of the export markets and the costly requirement of quarantine restrictions and eradication measures. Over 300 species of commercial/edible and wild hosts, *B. dorsalis* has the broadest host range of any species of *Bactrocera*. It is a serious pest of a wide range of fruit crops throughout its native range and wherever is has invaded. The major hosts are apple, guava, mango, peach and pear.
NEW: European Corn borer	https://www.cabi.org/isc/datasheet/46129 *Ostrinia nubilalis* (Hübner, 1796) A rare migrant before the 1930's, this species then began to colonise the area around London and the south-east and has spread to several other areas since. The males are darker than the females, and usually slightly smaller. The single generation flies in June and July, and the main food plant in Britain is mugwort (Artemisia vulgaris), although abroad it is often a pest on maize crops. Increasing pest on apple and hops.
Diaporthe causing apple leaf spots	A higher incidence of leaf spotting was observed on various apple varieties (particularly Braeburn and Cox) during the 2016 growing season. Resulting in defoliation in some cases. The causative agent was isolated and morphologically identified as the genus *Diaporthe* (formerly *Phomopsis*). Subsequently sequenced to determine species level identification as *Diaporthe rudis/viticola.*

Neofabraea kienholzii	Part of the group of pathogens which cause Gloeosporium/Neofabraea rot *Neofabraea kienholzii* had not been reported in the UK before but was picked up as part of the rot survey. A new disease report was published to inform the scientific community. Kingsnorth J, Perrine J, Berrie A, Saville R, 2017. First report of *Neofabraea kienholzii* causing bull's eye rot of apple in the UK. *New Disease Reports* 36, 15. [http://dx.doi.org/10.5197/j.2044-0588.2017.036.015]
Xanthomonas arboricolae, pv. pruni	A notifiable bacterial disease which causes shot holing symptoms on leaves. Plum and sweet cherry are both hosts. Currently only reported on *Prunus laurocerasus* (cherry laurel) in the UK. More information can be found on the DEFRA factsheet found at https://planthealthportal.defra.gov.uk/assets/factsheets/x-arboricola-pv-pruni-factsheet.pdf
Xylella fastidiosa	A devastating bacterial disease which has a wide host range including *Prunus*. The disease is vectored by plant hoppers of various species. Currently present in Mediterranean countries in Europe. Plant Health and Seeds Inspectorate (PHSI) are coordinating the national response to the threat of this disease to UK industry and environment. DEFRA have produced a Factsheet about this disease which can be found at: https://planthealthportal.defra.gov.uk/assets/factsheets/xylellaFastidiosa2015.pdf Current demarcated outbreaks are in southern Italy, the PACA region of France and Corsica, a site in Germany between Saxony and Thuringia, on mainland Spain in the Valencia region, and in all the Balearic Islands. In April, Spain detected X. fastidiosa for the first time in olive trees near Madrid, outside the current outbreak area in the region of Valencia. There has also been a finding on *Polygala myrtifolia* plants in a glasshouse in Almeria. See https://www.spittlebugsurvey.co.uk/ and https://www.jic.ac.uk/brigit/ for more information.

Objective 2. Neonectria Canker of Apple

2.2 Rootstock/interstock

Aim

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

Introduction

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

Materials and Methods

NIAB EMR

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

Table 2.2.1. The rootstocks and interstock to be evaluated. All rootstocks were grafted with Gala scion.

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

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

Sites

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

Site 1	Kent
Planted	29 March 2017
Description of planting site:	The site is situated amongst mature orchards in which *Neonectria dittisima* inoculum is prevalent providing opportunities for natural infection.
Tree spacing:	3.5 x1.75 m

Aerial view:

Trial layout:

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

WINDBREAK

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

ALLEY WAY

(grey box) = 4 spare tree stations

Site 2	Gloucestershire
Planted	14 March 2017
Description of planting site:	The trial was planted on the site of an old Cox orchard. 2 cox trees were left in the ground between each plot to serve as an inoculum source throughout the trial.
Tree spacing:	1.83 x 3.66 m

Aerial view:

Trial layout:

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

N ↑

WOODLAND

↑

6 MORE ROWS OF APPLE TREES

↓

Row 1	5G	5	2G	2	2G	13	2G	8	2G	11	2G	9	10G
Row 2	9G	4	2G		2G	12	2G	10	2G	14	2G	3	6G
Row 3	5G	1	2G	6	2G	7	2G	11	2G	1	2G	7	9G
Row 4	2G		2G	14	2G	12	2G	4	2G	8	2G	9	10G
Row 5	5G	6	2G	3	2G	13	2G	2	2G	5	2G	10	6G
Row 6	8G	14	2G	5	2G		2G	7	2G	9	2G	13	2G
Row 7	2G	3	2G	8	2G	4	2G	6	2G	1	2G	10	8G
Row 8	6G	2	2G	11	2G	12	2G	10	2G	5	2G	13	2G
Row 9	2G	3	2G	11	2G	6	2G	12	2G	9	2G	1	6G
Row 10	2G	7	2G	4	2G	8	2G		2G	14	2G	2	2G

↑

4 MORE ROWS OF APPLE TREES

↓

HEDGE

Soil/Meteorological records: Records of daily maximum and minimum temperature and rain fall were taken from a weather station located at the orchards. Soil condition records are also available.

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

Artificial inoculations: Artificial inoculations were completed during the leaf fall period in autumn 2018 at both the Kent orchard and Gloucestershire orchard. This was to produce identical infection conditions to determine any differences between the rootstocks. At NIAB EMR, artificial inoculation was completed on 29 October 2018, and at the Gloucestershire orchard, inoculation was completed 16-17 November 2018. At each orchard, eight trees per treatment (2 replicate trees per block from 4 blocks) were selected. Six infection sites were made on each tree: five leaf scars and one bud scar. The leaf scar is the infection route which best represents the natural infection route. Bud scar infection is an additional method used by NZ researchers to account for different scion/rootstock/interstock combinations losing their leaves at different times making it difficult to compare accessions using leaf scar inoculations alone. Prior to wounding, inoculation points were marked with coloured paint marker pens below the leaf or bud scar as follows; red for leaf scar, yellow for bud scar. Leaf scars were created by removing a leaf gently by hand whilst bud scars were made by dislodging the bud with the thumb. All wounds were made immediately prior to inoculation. The marked scars were inoculated with 5 µl of *N. ditissima* Hg199 spore suspension of $1x10^5$ conidia ml^{-1} suspended in sterile distilled water using a pipette. Mock inoculated controls on each inoculated tree, were prepared as above using one leaf and one bud scar per tree, sterile distilled water was used instead of a spore suspension. These were marked with coloured paint marker pens as follows; blue/yellow for mock bud scar and blue/red for mock leaf scar.

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

Assessments
Site 1 - Kent

The 2019 assessments were completed in spring. For each tree, cankers were recorded according to their position on the tree as described by McCracken et al. (2003). A = Rootstock, B = Main stem and C, D, E = Peripheral (Figure 2.2.1). Peripheral cankers in the combined years 2017-2019 were also analysed. Tree vigour as measured by trunk girth 10 cm above the graft union and canker were analysed to test any effect on canker. The number of dead trees was also recorded.

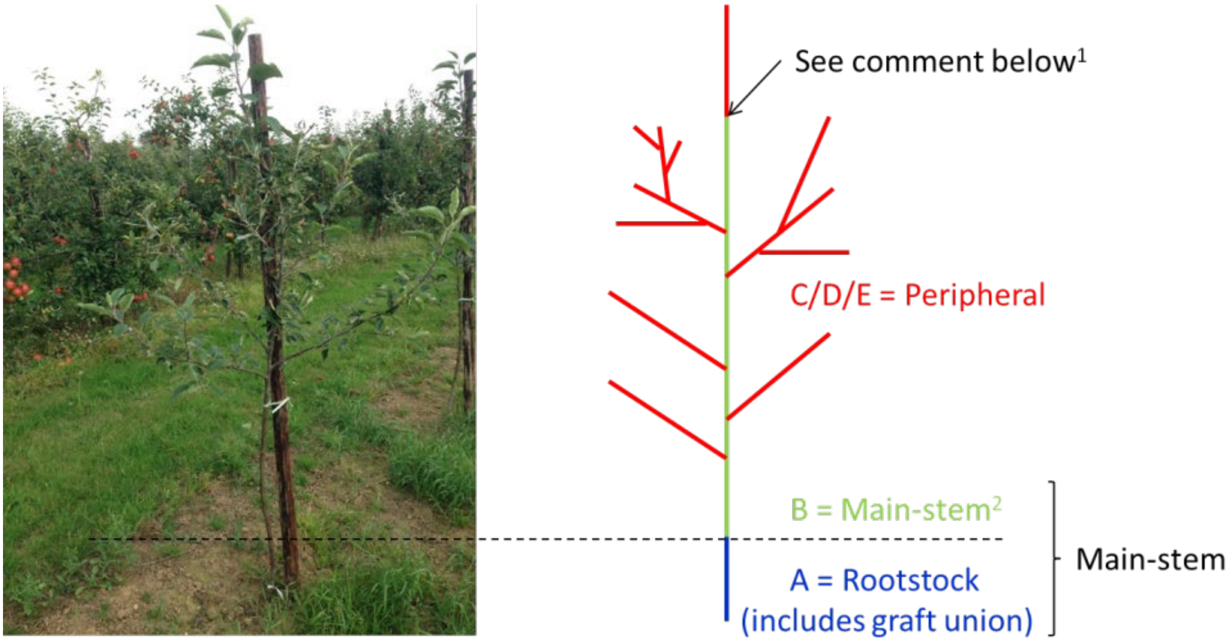


Figure 2.2.1. Diagram of the classifications of cankers based on their position within the tree. [1] note that there is a continuum between the main-stem and peripheral branch on the main leader; on wood which has grown since planting (i.e. < 3 years) were scored as peripheral and those on wood > 3 years were scored as main-stem. [2] cankers occurring on the interstem in treatment 8 (M9 with Golden Delicious interstem) were scored as 'B' – main stem

Statistical analyses: Each individual dataset was analysed by ANOVA. The natural infections were analysed using individual canker locations (A, B, C, D, E) and total cankers (A+B+C+D+E) per tree. Main stem cankers (A+B) and peripheral cankers (C+D+E) were also of interest.

Site 2 – Gloucestershire

The site was located in Gloucestershire in a block of 400 Cox trees, which was planted at a spacing of 1.83 m x 3.66 m in 1998. Old trees and roots were removed in sections of 10 trees (one plot) from the orchard early in 2017. These plots of 10 trees were interspersed with two canker infected mature guard trees and each row contained six plots (Figure 2.2.2). The Gala scions listed in Table 2.2.1 were planted into each plot on 14-15 March 2017, with 10 trees per plot. Each treatment was replicated four times, giving a total of 560 trees in 56 plots.

Figure 2.2.2. Experimental trial set up at Site 2 (Gloucestershire) in 2017

Artificial inoculations of canker were performed on 16-17 November 2018. In each treatment plot two trees were selected, leaves carefully removed to produce leaf scars and marked with permanent paint. The marked leaf scars were inoculated with 5 µl of *N. ditissima* Hg199 spore suspension of 1 x 10^5 conidia ml^{-1} suspended in sterile distilled water using a pipette. Another set of marked leaf scars were treated with water as a control. Germination tests showed 86% germination rate for the spore suspension after use.

Trees were assessed for canker on 22 November 2019. Main stem A+B and peripheral C+D+E cankers were counted for each tree as outlined above. Samples of canker were taken and isolated to check for canker infection. Tree death and cause was noted, as well as any additional damage not related to canker. The data were analysed using ANOVA.

Peripheral cankers in the combined years 2017-2019 were also analysed. Tree vigour as measured by trunk girth 10 cm above the graft union and canker were analysed to test any effect on canker. The number of dead trees was also recorded.

Tree vigour as measured by trunk girth 10 cm above the graft union and canker were analysed to test any effect on canker incidence. The number of dead trees was also recorded.

Results

Natural infection: In 2019 at Site 1 canker number from natural infection was higher than 2018. For example, the highest mean canker number per tree for peripheral cankers was G41 with 5.2, while the highest mean mainstem canker number per tree was EMR-001 with 1.8. Mainstem (A+B) canker was significantly higher in EMR-001, compared to MM106, M116,

EMR-005, EMR-002, M26, M9 (337), M9 (EMLA), and G41. Peripheral cankers were significantly higher in G41 and G11, than MM106, M116, M9 (337), EMR-006 and M9 (EMLA) (Figure 2.2.3). Regarding total canker number, G41 had significantly higher number than MM106, M116, and EMR-002 (Figure 2.2.4).

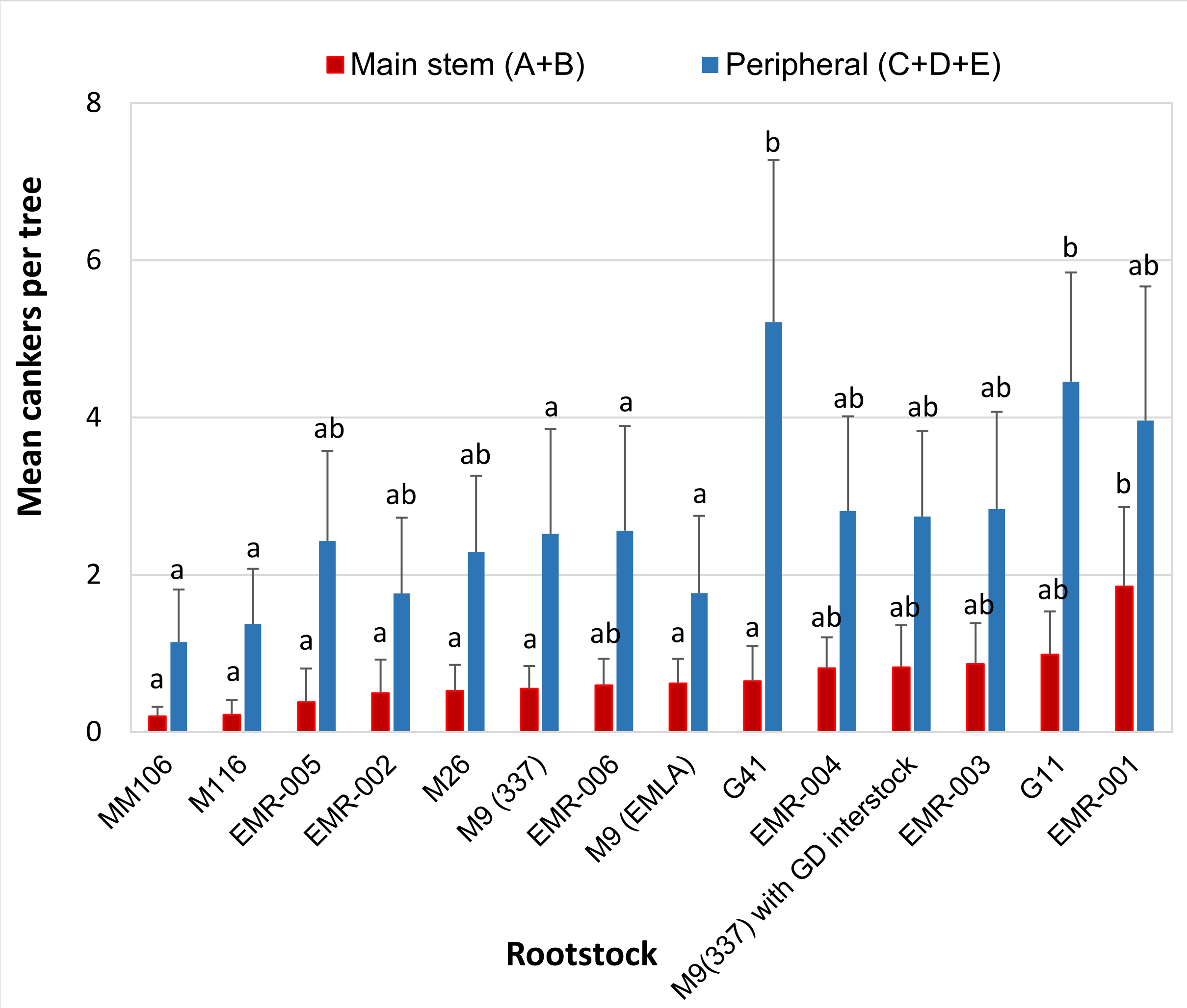


Figure 2.2.3. Mean number of mainstem and peripheral cankers from natural infection on apple trees grafted with 14 different rootstocks at Site1 (Kent). M9 (337) interstock GD has a Golden Delicious interstock grafted between the rootstock and the Gala scion

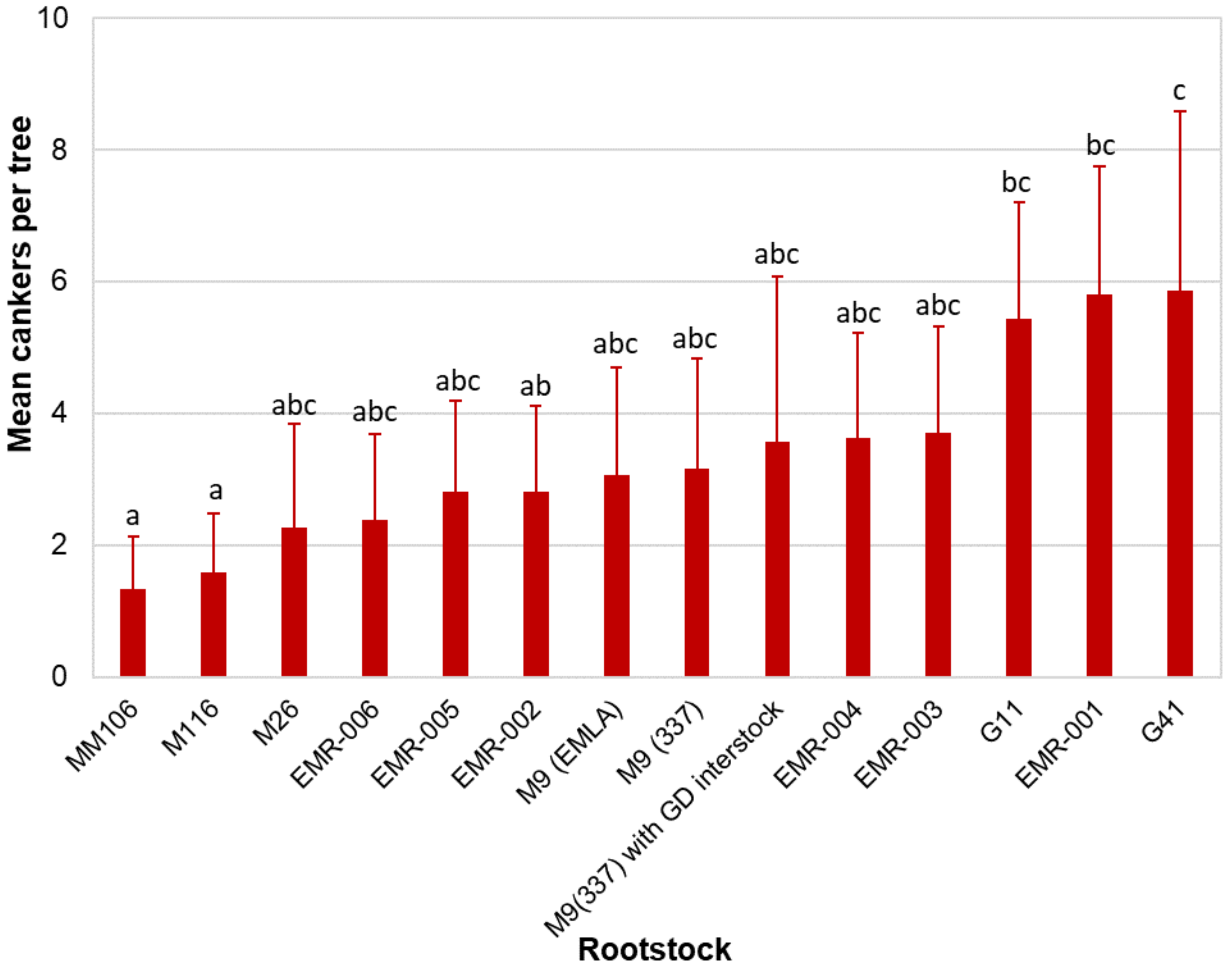


Figure 2.2.4. Mean number of total cankers from natural infection on apple trees grafted with 14 different rootstocks located at Site 1 (Kent)

Artificial inoculation: In 2019, the percentage of infected leaf scars >95% for all rootstocks (Figure 2.2.5). The highest were EMR-001 (98.75 %), M9 (337) (98.5 %) and M26 (95.48%), while the lowest were M9 (EMLA) (95.13 %), M116 (95.75 %) and MM106 (95.95 %).

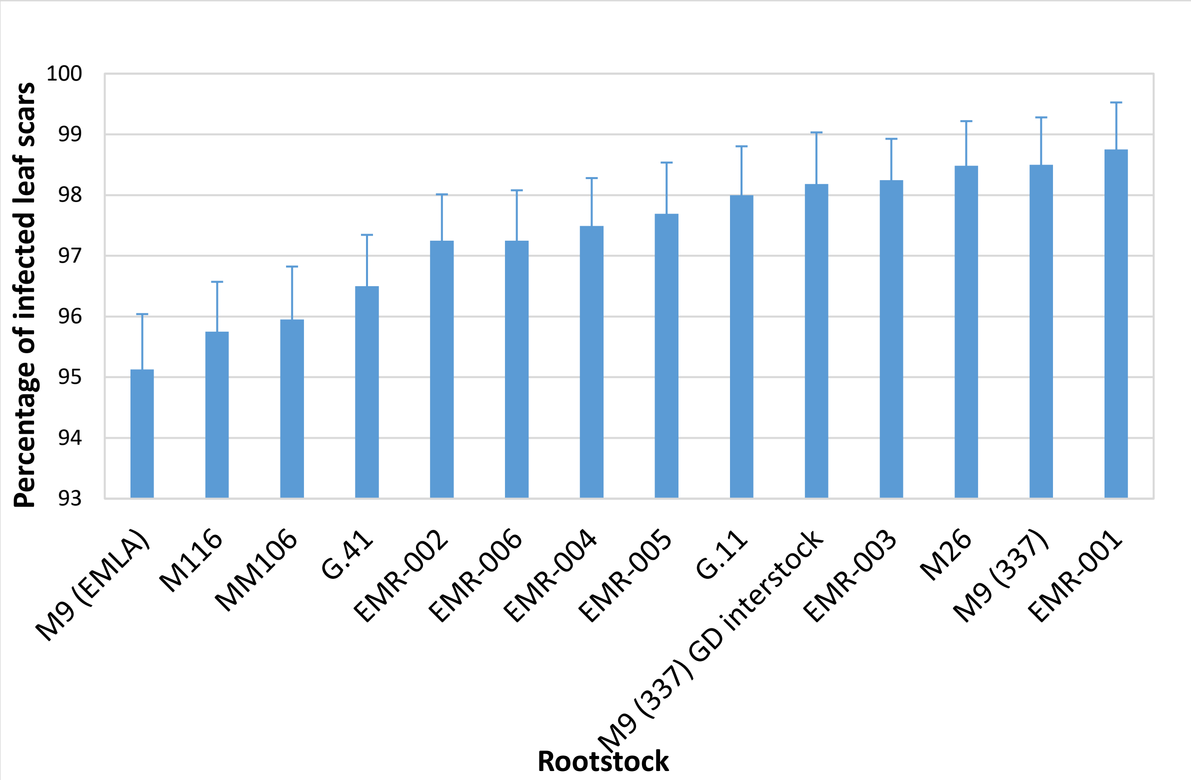


Figure 2.2.5. Percentage of leaf scars infected after inoculation with *N. ditissima* spores at Site 1 (Kent) in 2019. There was no significant difference between rootstocks.

Tree vigour

It was hypothesised that increased tree vigour, measured here using trunk girth, may be associated with reduced canker. Therefore, mean trunk girth 10 cm above the graft union was plotted against mean canker per tree. There was a very weak correlation between increased trunk girth and mean canker number per tree R^2=0.1054. The highest mean canker number was for trees with 81.25 mm and 94.76 mm girth, while the lowest was 93.8 mm and 105.79 mm girth (Figure 2.2.6).

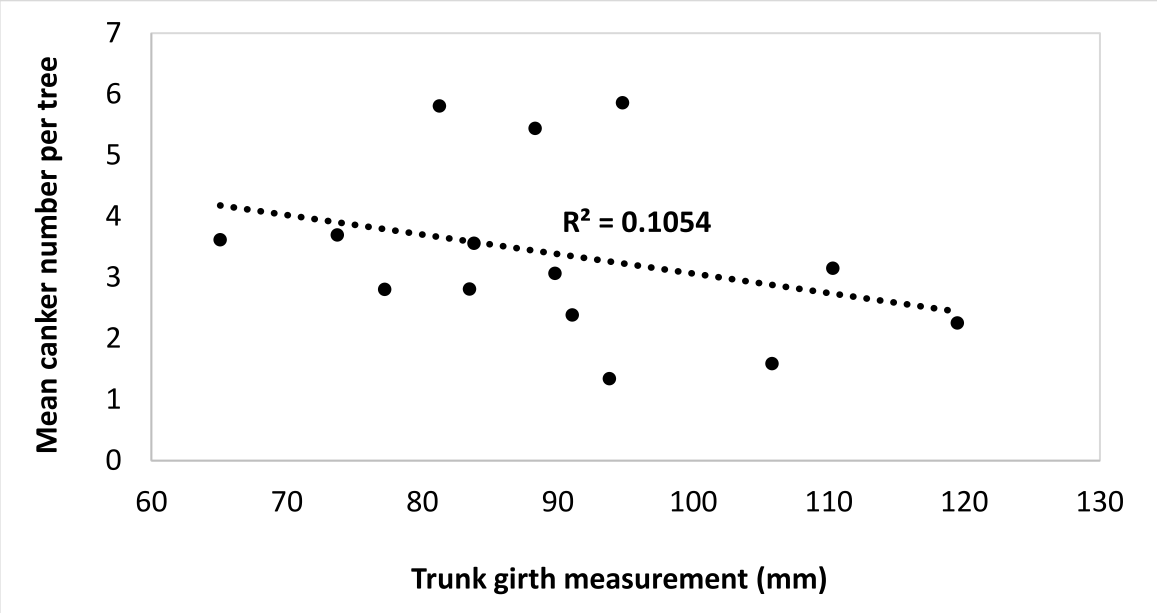


Figure 2.1.6. Mean trunk girth 10 cm above the graft union plotted against mean total canker number per tree at East Egham in the 2019 assessment. The linear regression and coefficient of variation (R^2) are also displayed

Number of dead trees: The rootstocks M116 and EMR-005 had the lowest number of dead trees (n=1 each), while G41 and M9 (EMLA) had the highest number of dead trees (n=7 each, Figure 2.2.7). Those rootstocks with the highest number of dead trees accounted for 21.8 % of the total trees for each of those rootstock cultivars. The M9 rootstocks were generally on the higher end of dead tree number ≥5 trees dead per rootstock selection.

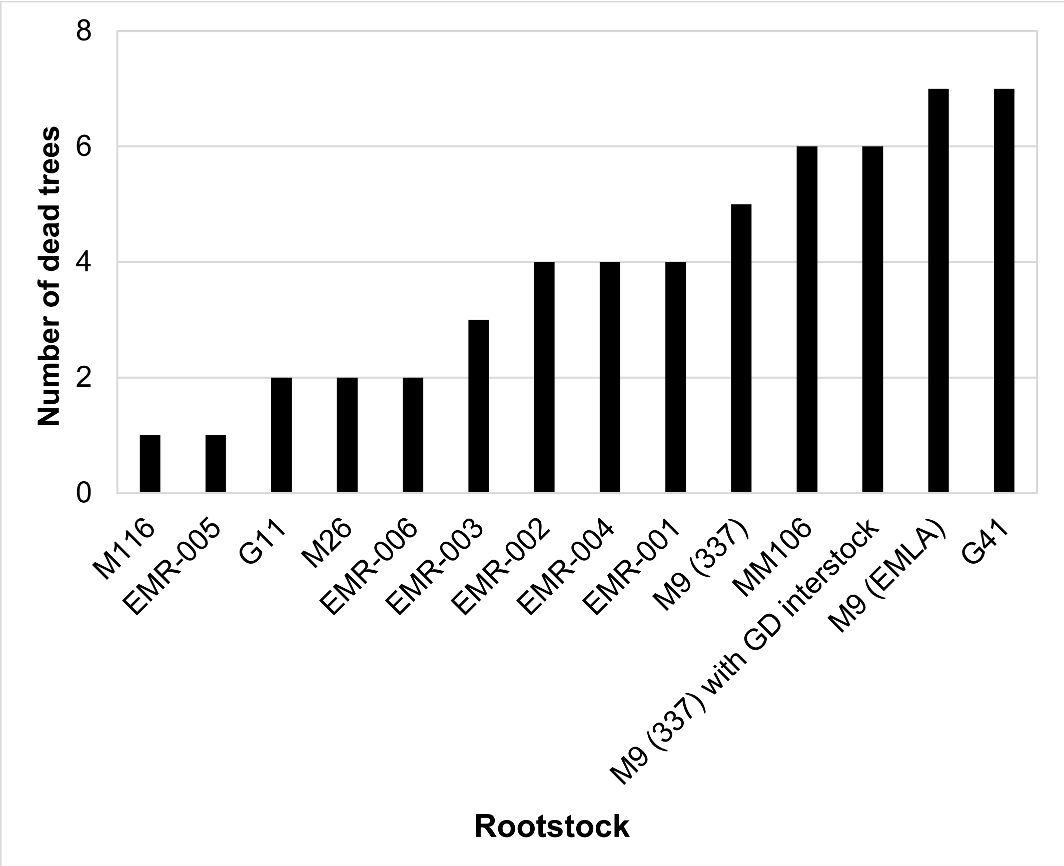


Figure 2.1.7. The number of dead trees observed in the Site 1 (Kent) rootstock trial in 2019

Site 1 (Kent) combined 2017-2019 peripheral cankers from natural infection: There was a significantly different (lower) canker number between M9 (EMLA) and G.41 rootstocks (p=0.005, df=13, Figure 2.2.8). M9 (EMLA), M116 and MM106 had the lowest number of cankers, while G.41, G.11 and EMR-001 had the highest.

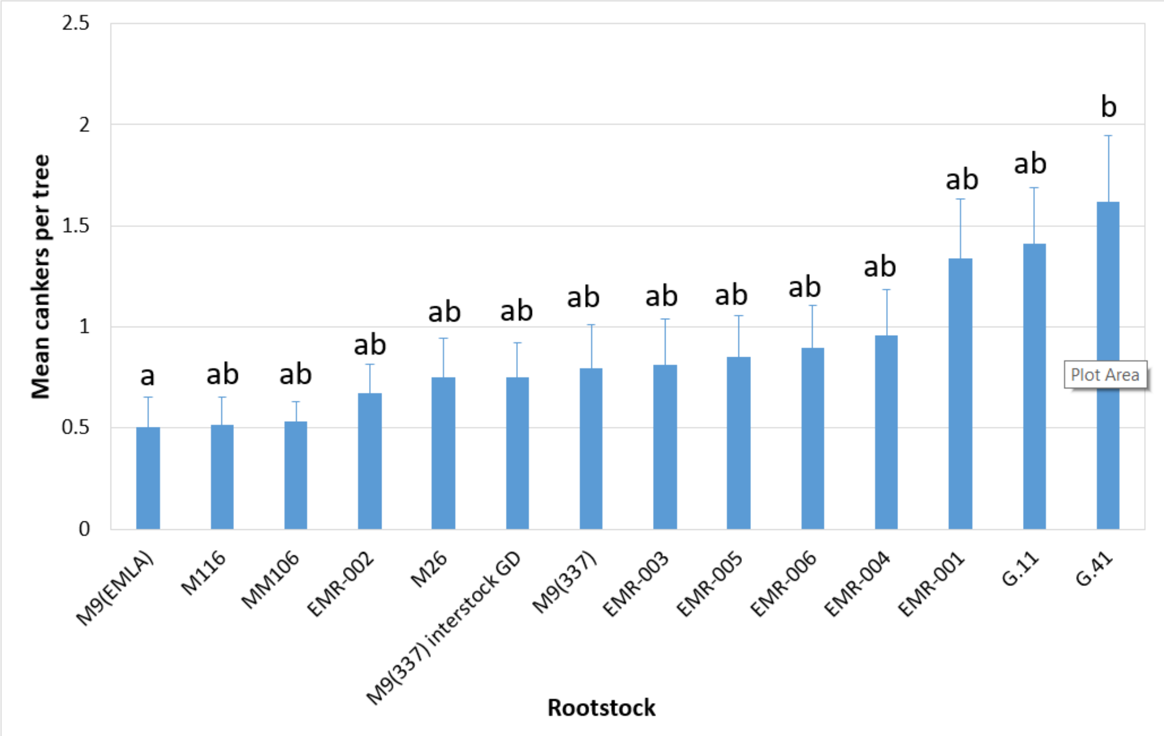


Figure 2.2.8. Site 1 (Kent) combined data from 2017-2019 sum of peripheral cankers from natural infection. There was a significant difference between canker of M9 (EMLA) and G.41. There was no significant difference between the other twelve rootstocks

Site 1 (Kent) combined 2017-2019 peripheral cankers from artificial inoculation

There was no significant difference between the combined 2017-2019 peripheral cankers from artificial inoculation at Site 1 (Kent) (P=0.996, df=13, Figure 2.2.9). However, the lowest number of infected leaf scars was EMR-006 and EMR-002, while the highest were EMR-004 and EMR-001.

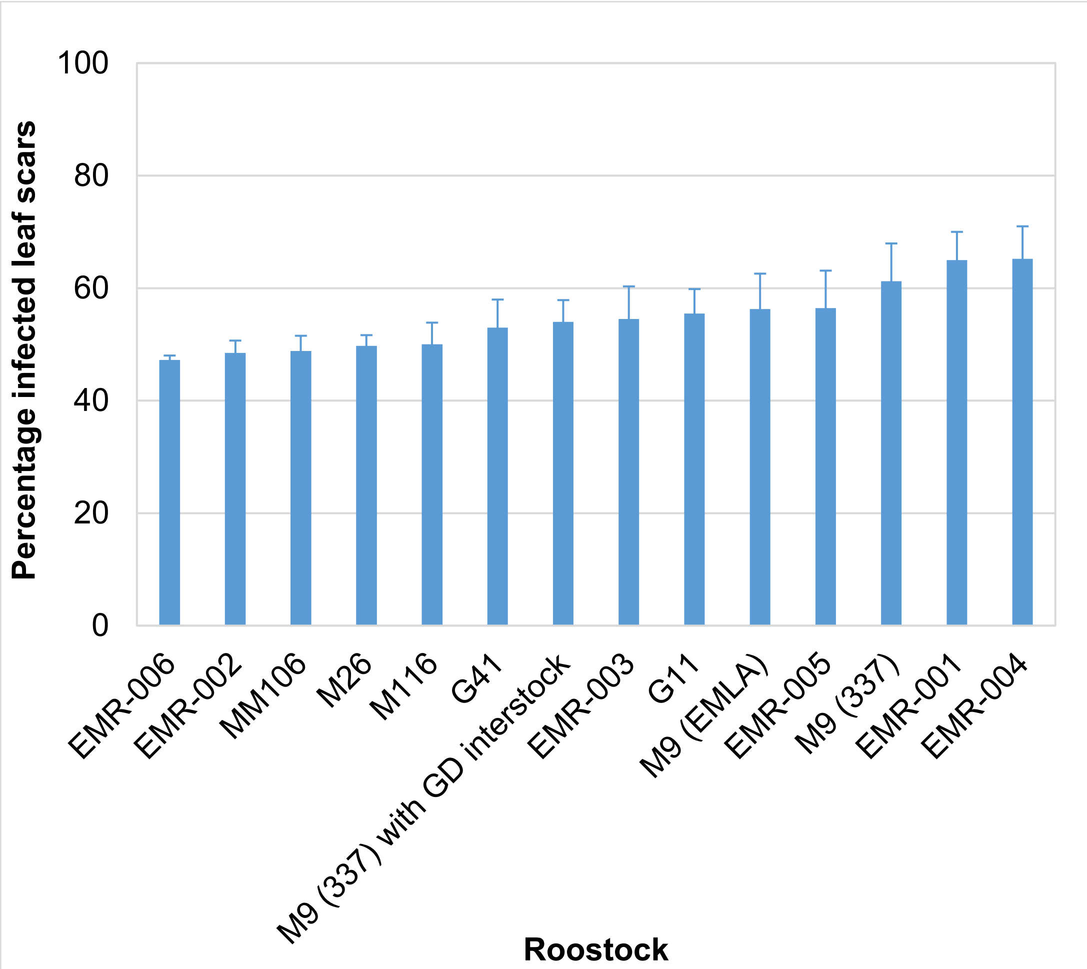


Figure 2.2.9. Mean percentage infected leaf scars from combined 2018 and 2019 data at Site 1 (Kent). Error bars represent standard error of the mean. There was no statistically significant difference between rootstocks)

Site 2 – Gloucestershire

Natural infection: The mean number of cankers from natural infection was low for all rootstocks (grand mean of 0.69, Figure 2.2.10), although the overall number of infections increased during the trial. There were statistically significant differences between the rootstocks, when looking at the mainstem cankers (A+B), A cankers alone, total canker numbers and number of dead trees due to canker (Table 2.2.2). The rootstocks with the highest mean number of mainstem cankers (A+B) were M9 (337), EMR-002 and M26 (0.88, 0.80 and 0.73 respectively). The rootstock with the lowest number of mainstem cankers was EMR-004 (0.15). The mean number of peripheral cankers showed a similar distribution to mainstem cankers, with a lower overall mean of 0.19 cankers. The rootstock with the highest mean number of peripheral cankers was M9 (337) with 0.33, followed by MM106 (0.30), whilst the lowest were EMR-006 and M116 (0.03 and 0.10 respectively).

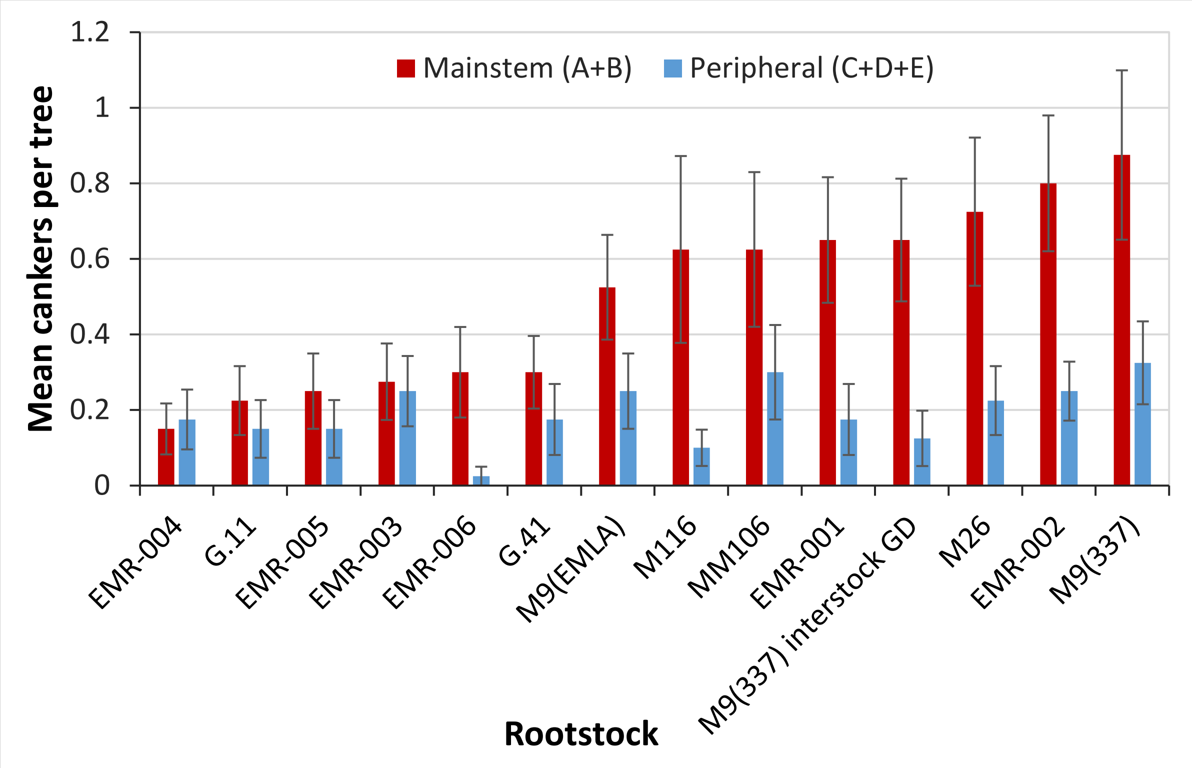


Figure 2.2.10. Mean number of cankers of apple trees with 14 different rootstocks infected from natural inoculum at Site 2 (Gloucestershire). M9 (337) interstock GD has a Golden Delicious interstock grafted between the rootstock and the scion. Error bars represent standard error of the mean

In 2019, a total of 83 trees were recorded as dead (14.8%, Figure 2.2.11). Of these, 78 were dead as a result of canker, with 22 of these dying between spring 2018 and autumn 2019. In the majority of these cases the mainstem canker noted in year one had girdled the tree resulting in tree death. The remaining five trees died because of other causes, such as rabbit damage. There was a statistically significant difference in the number of each rootstock dying with M26 having the most trees dying (12 trees, 30 % of planted trees) and EMR-006 having the fewest (one dead tree, 2.5 % of planted trees).

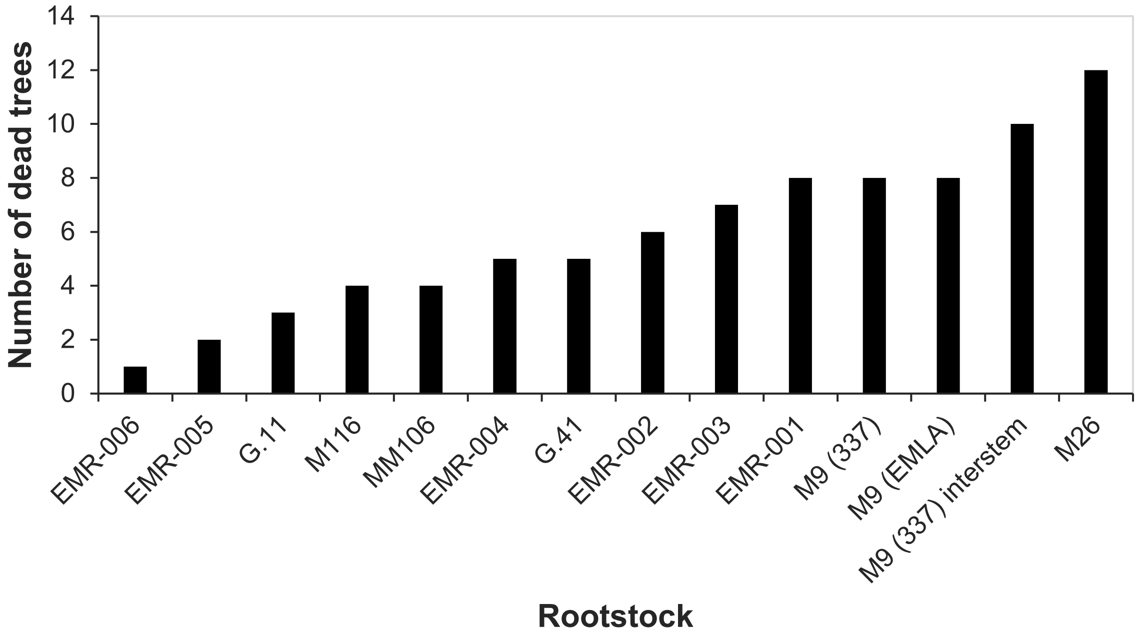


Figure 2.2.11. Number of dead trees at Site 2 (Gloucestershire) as a result of canker infections. M9 (337) interstem has a Golden Delicious interstock grafted between the rootstock and the scion.

Table 2.2.2. ANOVA results of number of cankers on apple trees with 14 different rootstocks infected from natural inoculum located at Site 2 (Gloucestershire). Bold p-values indicate significant differences.

Location of canker	**Degrees of freedom**	***p-value***
Rootstock (A)	13	**0.037**
Mainstem (B)	13	0.068
Peripheral (C)	13	0.766
Peripheral (D)	13	0.181
Peripheral (E)	13	0.086
Rootstock + main-stem (A+B)	13	**0.005**
Peripheral (C+D+E)	13	0.564
Rootstock + Mainstem + Peripheral (A+B+C+D+E)	13	**0.005**
Dead	13	**0.023**

Artificial inoculation: There was a statistically significant difference in the proportion of leaf scars infected with canker in the inoculation trial at Site 2 (Gloucestershire) (P <0.001, d.f. = 13). The rootstocks with the highest proportion of leaf scars infected through artificial inoculation were M9 (EMLA) (90 %), followed by EMR-002 (82.5 %), M9 (337) (75 %) and EMR-001 (75 %) (Figure 2.2.12). The rootstocks with the lowest proportion were G.11 (22.5 %), M116 (35 %).

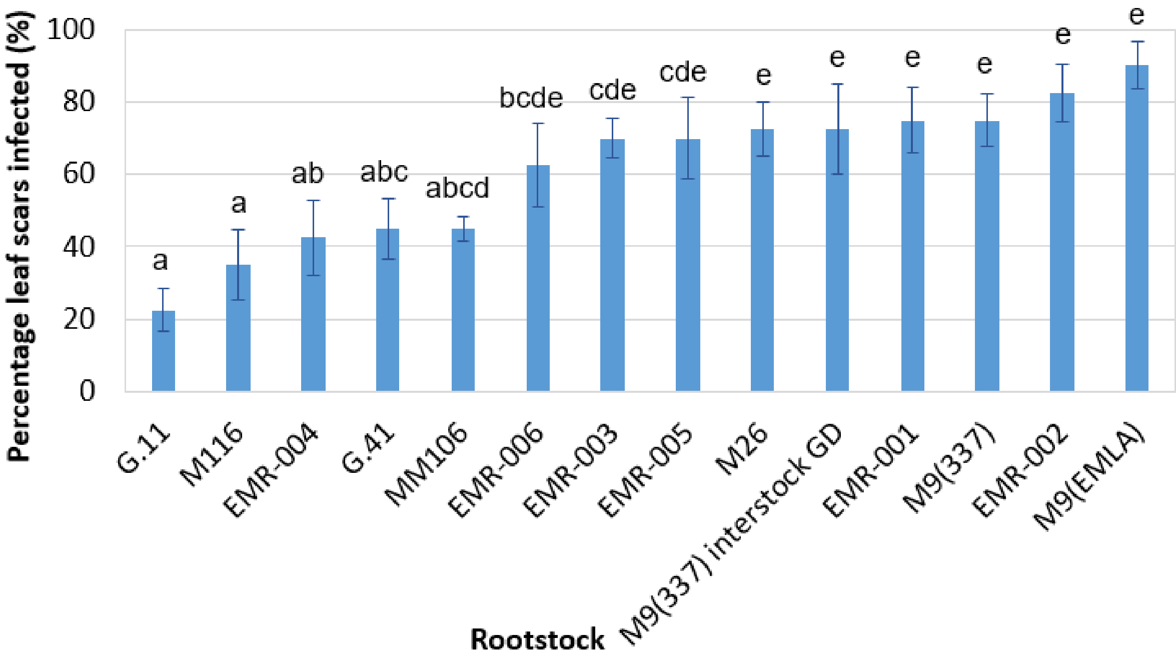


Figure 2.2.12. The proportion of scion leaf scars infected at Site 2 after artificial inoculation in the 2019 assessment year. Error bars represent standard error of the mean.

Tree Vigour

There was a very weak relationship between tree vigour (as measured by trunk girth) and canker at Site 2 (Gloucestershire) (R^2=0.0311). Some trees with smaller trunk girth e.g. 72.55 mm had low canker number, while some of those with larger girth e.g. 96.45 mm, had higher canker number (Figure 2.2.13).

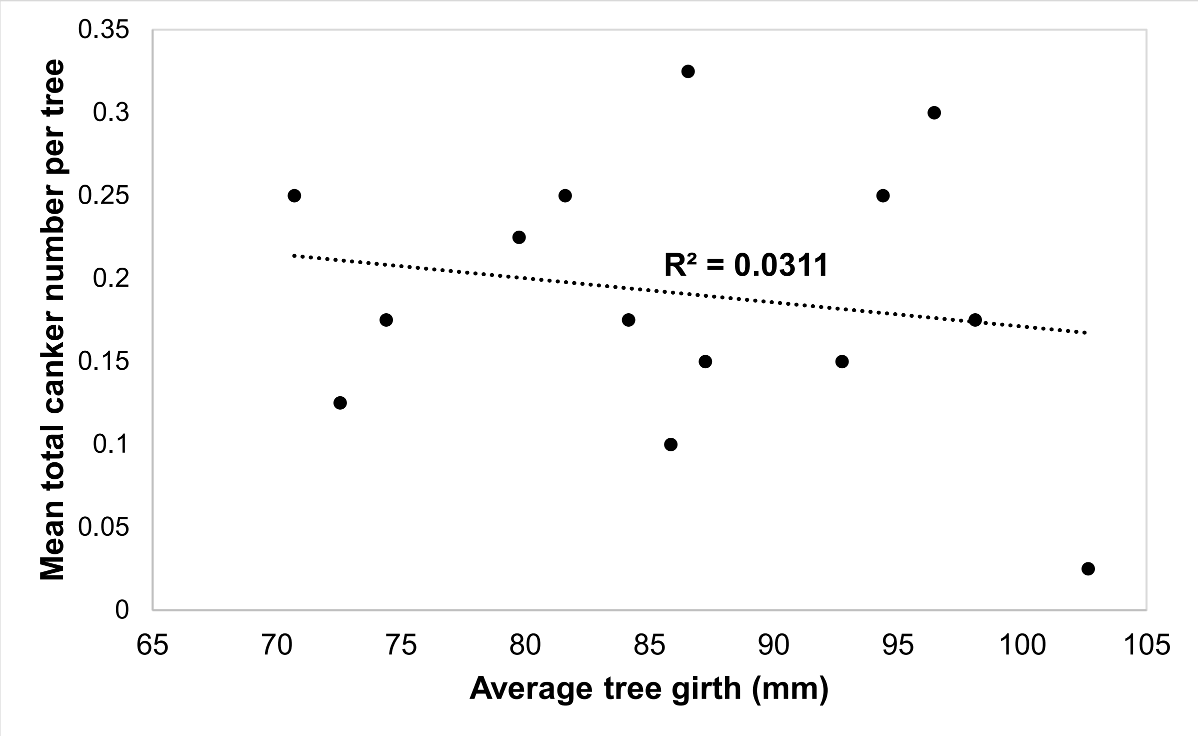


Figure 2.2.13. Mean trunk girth 10cm above the graft union plotted against mean total canker number per tree at Site 2 (Gloucestershire) in the 2019 assessment

Site 2 (Gloucestershire) combined 2017-2019 natural infection: The peripheral cankers at Site 2 were cut out after each assessment, which allowed the recorded canker numbers to be added together to give a total number of cankers in the period 2017 to 2019. When the peripheral cankers (C+D+E) for Site 2 were totalled across the three years of assessments (six assessments in total) there was no significant difference in the average number of cankers per tree between treatments (P = 0.572, Figure 2.2.14). A similar pattern was seen in the over peripheral canker infection to that of the leaf scar inoculations at Site 2 (Gloucestershire). EMR-006, EMR-004, M116, G.41 and G.11 had the lowest number of infections over the three assessment years.

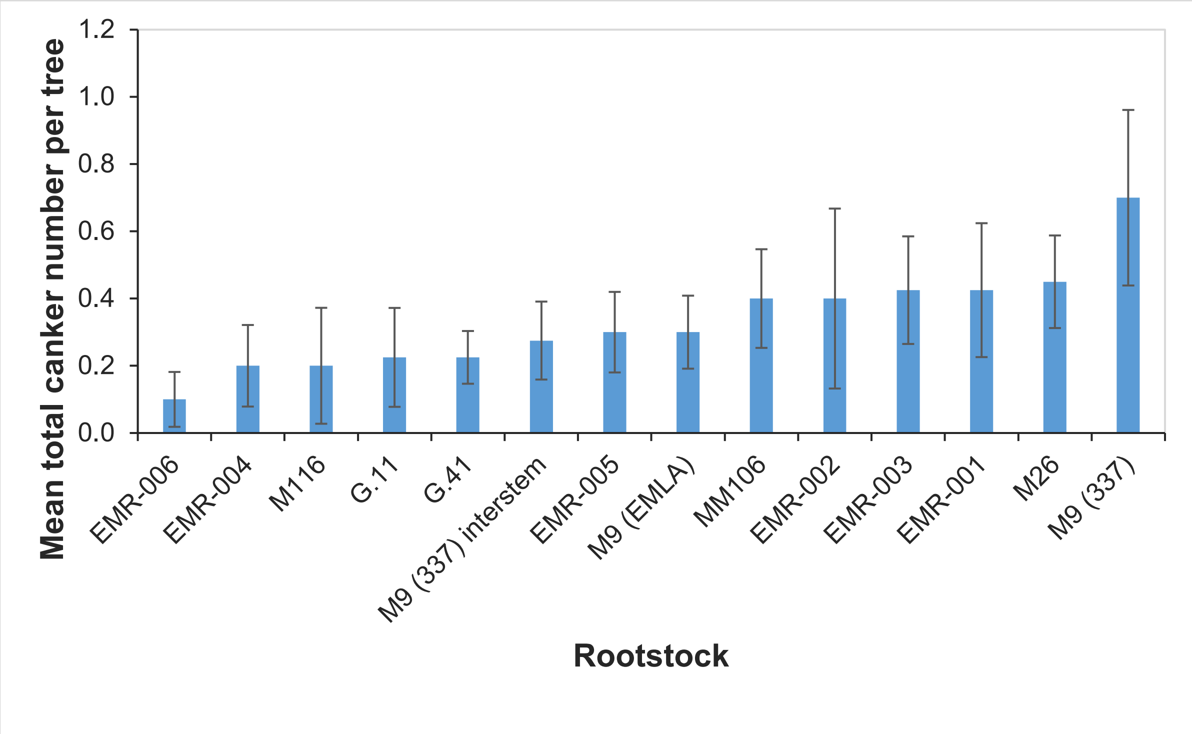


Figure 2.2.14. Site 2 (Gloucestershire) combined data from 2017-2019 mean total canker number per tree of peripheral cankers from natural infection. There were no statistically significant differences between rootstocks. Error bars represent standard error of the mean

Comparison of canker across sites and years: A comparison of the mean canker number from natural infection and mean leaf scar infection from artificial inoculation from the two sites is provided below (Table 2.2.3).

Table 2.2.3. Mean canker number from natural infection and mean leaf scar infection from artificial inoculation for both sites (1. Kent 2. Gloucestershire) using available data in the assessment years 2017-2019.

		Mean canker number (A+B+C+D+E type) from natural infection													
		Rootstock													
Site	**Assessment date**	M9 (EMLA)	M9 (337)	G.41	G.11	MM106	M116	M26	M9 (337) with GD interstock	EMR-001	EMR-002	EMR-003	EMR-004	EMR-005	EMR-006
Site 1	2017	0.25	0.13	0.03	0.06	0.75	0.16	0.16	0.22	0.10	0.16	0.13	0.10	0.03	0.31
	2018	0.08	0.03	0.14	0.06	0.07	0.04	0.07	0.07	0.11	0.09	0.05	0.09	0.03	0.06
	2019	2.40	3.10	5.90	5.40	1.30	1.60	2.80	3.6	5.80	2.30	3.70	3.60	2.80	3.20
Site 2	2017 (5/10/17)	0.23	0.65	0.18	0.18	0.58	0.55	0.50	0.38	0.18	0.43	0.15	0.10	0.15	0.20
	2018 (15/5/18)	0.25	0.63	0.18	0.15	0.55	0.60	0.60	0.38	0.23	0.45	0.18	0.10	0.23	0.18
	2019 (17/5/19)	0.53	0.83	0.30	0.23	0.60	0.63	0.60	0.65	0.60	0.70	0.20	0.13	0.28	0.33
		Mean leaf scar infection (%) from artificial inoculation													
		Rootstock													
Site	**Assessment date**	M9 (EMLA)	M9 (337)	G.41	G.11	MM106	M116	M26	M9 (337) with GD interstock	EMR-001	EMR-002	EMR-003	EMR-004	EMR-005	EMR-006
Site 1	2018	22.50	25.64	12.82	15.38	5.13	8.57	2.50	10.53	35.14	2.50	12.50	36.84	17.14	2.63
	2019	95.10	98.50	96.50	98.00	95.90	95.80	98.50	98.20	98.80	97.30	98.20	97.50	97.70	97.30
Site 2	2019 (22/2/19)	90.00	75.00	45.00	22.50	45.00	35.00	72.50	72.50	75.00	82.50	70.00	42.50	70.00	62.50

Discussion

Natural infection

In 2019, there was an overall lower incidence of natural infection at Site 2 (Gloucestershire) compared to Site 1 (Kent). However, the levels of infection at both sites have started to accumulate throughout the trial. In 2019, the weather was wetter than the extremely dry year of 2018, which was more conducive to natural infection. At Herridges, as in previous years, the majority of natural cankers were present on the mainstem compared to peripheral, whereas at East Egham there was a higher number of peripheral cankers compared to mainstem. Whilst peripheral (C, D, E) cankers are thought to result from orchard infection, mainstem (A, B) cankers are thought to originate in the nursery. The trees for both trial sites came from a common source. The discrepancy in peripheral and mainstem canker expression between the two sites suggests that site (soil type, replant etc) and environmental factors (stress following planting, weather events etc) play a significant role in canker incidence.

M9 rootstocks often had some of the highest canker numbers, confirming observations by UK apple growers. Mainstem cankers are biologically significant as the infection may girdle and kill the tree. The rootstock EMR-001 had high numbers of mainstem canker at both sites indicating it was likely infected in the nursery during propagation and may be more susceptible to infection during the propagation and establishment period. Peripheral cankers may not immediately kill the tree; however, they are a source of inoculum that may spread to the mainstem of the infected tree or to neighbouring trees. The number of mainstem cankers sometimes matched the trend of peripheral cankers such as MM106 and M116 at Site 1 (Kent) and EMR-004 and G.11 at Site 2 (Gloucestershire) having lower numbers for both types. However other rootstocks had high numbers for one type and lower for the other type. EMR-006 at Site 2 (Gloucestershire) had very low peripheral canker number, however it had a moderate number of mainstem cankers. EMR-005 at Site 1 (Kent) had a relatively low number of mainstem cankers, however it had a moderately high number of peripheral cankers. This indicates that it is not always clear that selecting a certain rootstock will have a predictable and consistent effect on lower canker incidence.

Comparing the 2019 natural infection data between the two sites, Site 1 (Kent) had a much higher number of cankers, both mainstem and peripheral. Within each site, Site 1 (Kent) had a higher number of peripheral cankers compared to mainstem, while Site 2 (Gloucestershire) had lower numbers of peripheral cankers compared to mainstem. This is likely due to site and environmental factors e.g. soil types, soil waterlogging, rainfall, temperature as well as biological factors e.g. soil microbial communities. At both sites, we would expect the same level of mainstem canker infections that would have originated in the nursery as all plants for both sites were sourced from the same nursery. However, this is not what we observed, hence the site, environmental and biological factors are likely affecting canker.

In the 2019 assessments, there was conflict between the results of the two sites. For example, natural infections of the Geneva rootstocks (G.11, G.41) at Site 1 (Kent) were higher, while those at Site 2 (Gloucestershire) were lower. MM106 and M116 at Site 1 had the lowest within site canker for both mainstem and peripheral, while these two rootstocks at Site 2 were among the highest particularly for mainstem cankers and peripheral canker of MM106. There was some agreement between the sites. Examples include EMR-001 which had higher canker number of both mainstem and peripheral cankers, EMR-005 which had lower canker mainstem canker at both sites, and generally the M9 rootstocks [M9 (337), M9 (337 with GD interstock, M9 (EMLA)] had moderate to high canker number at both sites.

Regarding dead trees, at Site 2 (Gloucestershire), 14.8 % died as a result of mainstem cankers, most of which were recorded in the first year of the trial. At Site 1 (Kent), depending on the rootstock, tree death was up to 21.8 %. If over one fifth of trees need to be replaced by growers, and depending on the size of the orchard, this results in thousands of pounds to replace dead and cankered trees, as well as delayed fruit production for a number of years. This reiterates that nursery infections are a key component to developing a canker management programme and that the nursery stage is a key target to reduce canker problems in the orchard.

Artificial inoculation

At Site 1 (Kent), artificial inoculations all had >95% infected leaf scars. The inoculation spore concentration was high, and the environmental conditions were more conducive (cooler, higher rainfall) to disease than in 2018. M9 (EMLA) (95.1 %), M116 (95.8 %), and MM106 (95.9 %) had a lower percentage of infected leaf scars while EMR-001 (98.8 %) had the highest. The higher incidence of EMR-001 reflects the natural infection data.

At Site 2 (Gloucestershire), the proportion of infected leaf scar data showed slightly different results compared with the natural infection at the site, although the trends were generally the same. In the artificial inoculation trial, M9 (EMLA) had the highest proportion of leaf scars infected with canker, the other M9 rootstocks also had high infection rates of over 70 %, reflecting what was seen in the natural infections. The lowest number of artificial infections was seen on the G.11 rootstock trees, which was low in mainstem cankers as well as peripheral cankers. M116 also had the second lowest infection rate in the artificially inoculated trial and the second lowest peripheral cankers in the natural infection trial. EMR-006 had the lowest peripheral cankers in the natural infection trial, however, when artificially inoculated over 60 % of the leaf scars were infected with canker when assessed. This was the sixth lowest infection rate during the trial (lowest infection was 22.5 %). This result contrasts with the artificial inoculation at Site 1 (Kent), where EMR-006 had one of the lowest infection rates.

It has been observed internationally that trees on very vigorous rootstocks may be better able to cope with canker infections. However, at both sites when analysing vigour (trunk girth) and canker from natural infection, there was only a very weak correlation indicating that vigour does not strongly affect canker. A BBSRC Link project (BB/P007899/1) has indicated that site and scion cultivar have a stronger effect on endophytic (microbes living within the plant) fungal communities (at scion leaf scars) than the effect of rootstock. These effects could also be extrapolated to *N. ditissima*. The factors governing canker infection are complex and clearly more than vigour related. They likely include other factors which are being investigated in other projects including cultivar (scion and rootstock) tolerance/susceptibility (BBSRC; BB/P000851/1 and AHDB studentship CP141), site selection and scion cultivar selection (BBSRC; BB/P007899/1), environmental factors [temperature, water stress, BBSRC; BB/P007899/1] and nutrition (CTP-FCR studentship), and potentially, the effect of endophyte communities on disease antagonism and expression (BBSRC; BB/P007899/1 and AHDB studentship CP161).

The comparison of canker over time and between sites with natural infection showed that by 2019 canker number had increased for all rootstocks at both sites. By the 2019 assessment, canker number at Site 1 (Kent), had up to 28 times higher mean total canker number than at Site 2 (Gloucestershire) eg. EMR-004 roostock had 3.6 cankers at Site 1 versus 0.13 cankers at Site 2.

Conclusions

- Rootstocks were identified with reduced canker within sites. However, there was conflict between sites.
- Canker from natural inoculum at both sites increased over time for all rootstocks, particularly the 2019 assessment.

- At Site 1 (Kent), MM106 and M116 had consistently lower canker, while G.41 and G.11 had higher. However, MM106 and M116 would not be useful to growers using existing growing systems as they are too vigorous.
- At Site 2 (Gloucestershire), G.11 and G.41 had lower canker, whilst MM106 and M116 had higher. DNA fingerprinting confirmed the genotypes were correct suggesting that the inconsistencies in virour between the two sites may be a result of the variable responses of the different rootstock genotypes to site specific effects (such as apple replant disease for example).
- EMR-001 had higher canker at both sites.
- The 2019 artificial inoculation canker incidence at Site 2 did show significant differences in the infections of leaf scars. G11 and M116 had significantly lower incidence than the M9 suite of rootstocks, EMR-002, EMR-001 and M26.
- There was only a very weak trend at both sites between increasing tree vigour and reduced canker.
- By the end of the trial in 2019, tree death at Site 1 was highest with G41, the M9 types and MM106 whilst the lowest were M116 and EMR-005. At Site 2 the highest number of tree deaths was recorded for M26, the M9 types and EMR-001.
- Combining the three years of data (2017-2019) of peripheral cankers from natural infection at Site 1 showed significantly lower canker with M9 EMLA and G.41 (which had the highest canker number) but no significant differences between the other twelve rootstocks.
- Combining the two years of data from artificial inoculation (2018-2019) of the Site 1 data showed no significant difference in canker between rootstocks.
- Combining the three years of data (2017-2019) of peripheral cankers of natural infection at Site 2, there was no significant difference in canker expression between the different rootstocks.
- Factors such as site selection including weather, scion cultivar selection, endophytes and apple replant disease are likely playing roles in canker incidence.

2.3 Soil amendments

Aim

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

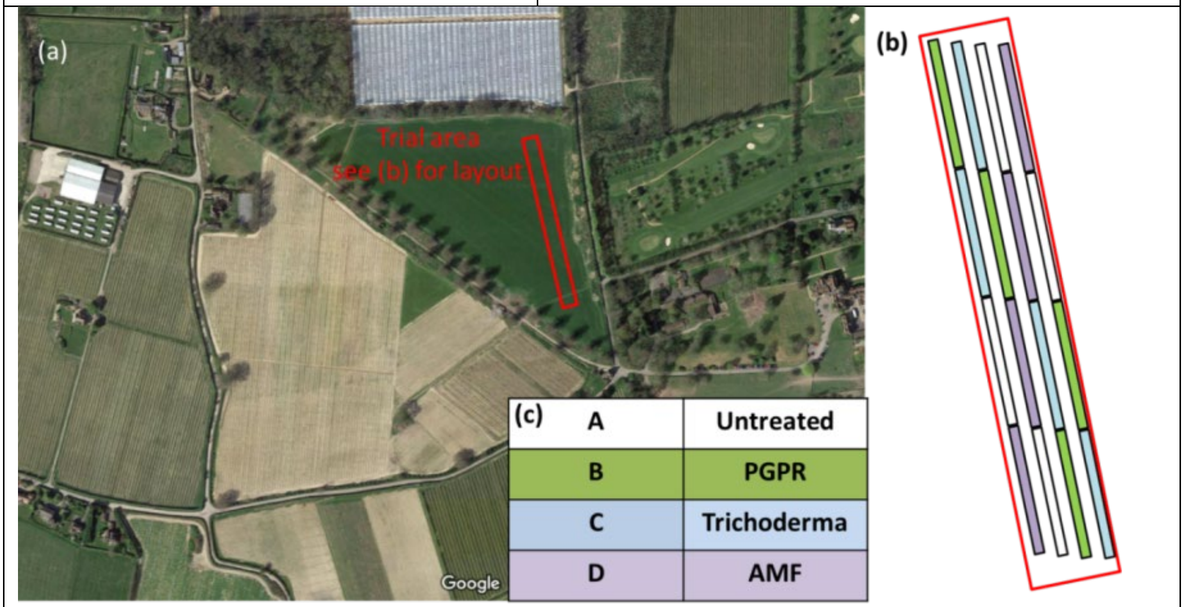
Introduction

Previous research on European apple canker, in particular the millennium trial (McCracken *et al*. 2003) has shown that *N. ditissima* can infect trees in the nursery and remain asymptomatic in the apple host. Once planted in the production site, where upon the tree can experience stress (drought/water logging/replant disease etc.) the disease is expressed. This objective aims to evaluate biological soil amendments to improve tree health and establishment in the context of canker expression. The objective was conducted in two parts; (1) a replicated trial on newly planted orchards to simulate the establishment of new orchards on the production site, and (2) a stool bed trial will simulate the nursery phase of tree fruit production. These are long-term trials, requiring establishment and monitoring over time. The stool bed was planted in May 2015. The newly planted orchard trials were planted in 2016 and assessments have been carried through to 2019 as part of the long-term monitoring of this trial. An additional trial was planted in the West of England (Gloucestershire) in 2018 to test soil amendments in different soil and weather conditions.

Materials and Methods

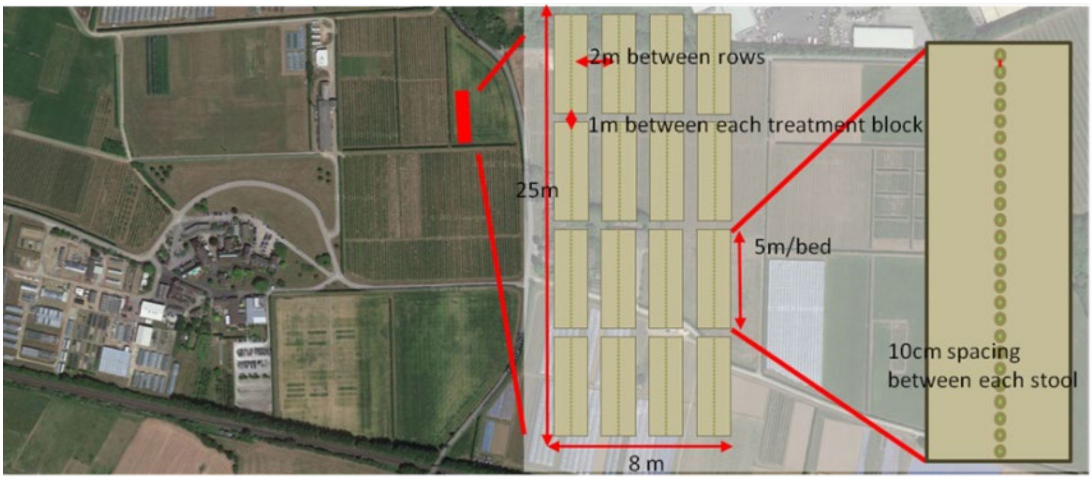
Sites

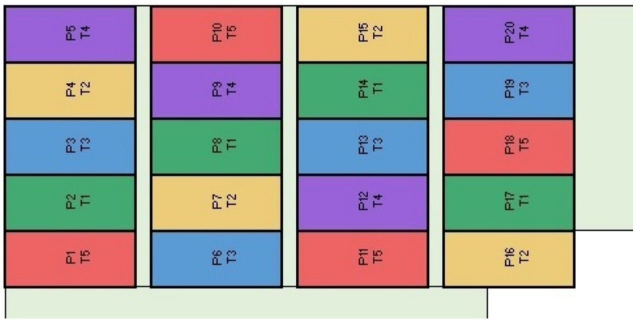
Site 1	Kent
Variety	Cv. Rubens
Planted	15/03/16



Site 2	Kent
Variety	Cv. Gala (was intended to be Cv. Jazz but trees were not available when the trial was setup)
Planted	12/05/16

Site 3 (Stoolbed)	Kent
Variety	EMLA M9
Planted	12/05/15



Site 4	Gloucestershire
Planted	12 April 2018
Variety	Leg Gala
	

Soil amendments

Soil amendments were applied as described in previous reports.

Assessments

Canker assessments caused by natural inoculum at Sites 1,2 and 4 were completed using the method as described by McCracken *et al*. (2003) and as used in the rootstock interstock trials (section 2.2.). At Site 1 canker was assessed on 17/5/19, Site 2 on 10/05/19, and Site 4 on 25/11/19. At Site 3 (stoolbed) shoots were harvested in winter 2019 (10/1/2019), cold-stored and size graded in winter 2019 (14/02/2019), planted out in spring 2019 (10/3/2019), and canker assessed in summer 2019 (07/2019).

At Site 4, yield data was also collected from a subset of trees (4 trees per plot) on 16/09/19. This included total number of apples per tree, total weight and apple size. Data from all sites was analysed using ANOVA.

Vigour assessments: vigour was measured using tree circumference 10 cm above the graft union. At the newly planted orchard sites (Sites 1, 2, 4) measurements were made at Sites 1 & 2 on 10/19 trees (32 trees per plot), and Site 4 on 25/11/19 (n= 3 trees per plot).

Colonisation of apple roots by AMF: To determine if amending the planting hole with a commercial inoculant of AMF increased colonisation above the background level of colonisation, Plantworks Ltd, the commercial inoculant supplier for AMF in this trial, tested a subsample of roots from the AMF treated (n=3 samples, subsampled 4 times) and the untreated control (n=3 samples, subsampled 4 times) from Site 2. NIAB EMR also tested roots from the stoolbeds at Site 3 (n=4 samples for AMF treated, and n=4 samples for the untreated control). Proportion of root length colonisation (%RLC) was calculated.

Cost-benefit analysis

A cost-benefit analysis has been conducted using the best performing biological amendment (Trianum G) against the potential loss of trees through main stem B-type scion cankers that lead to tree death. The dataset used for this analysis is the percentage of trees with B-type scion cankers from the unamended control at Site 2 (30%) and the percentage of trees with B-type cankers in the Trichoderma (Trianum G) amended (9%). Therefore, the figure of 21% reduction of tree death was used in the calculations (Table 2.3.1). It was assumed all trees with B-type cankers would have to be replaced.

Table 2.3.1. Figures used in the calculation of the cost-benefit analysis of newly planted trees in the unamended control versus trees amended with Trichoderma (Trianum G).

Amendment	Number of trees with B type lethal scion cankers	Total trees	% of trees with B type lethal cankers compared to total trees	Untreated control % minus Trichoderma treated %
Unamended	55	186	30	21
Trichoderma (Trianum G)	17	189	9	-

Results

Colonisation of apple roots after amednment with AMF

Root length colonisation (RLC%) was 4.23 times higher in the AMF treated than the untreated control in the samples collected from Site 2 (Table 2.3.2). The 5.73% colonisation in the unamended controls represents the colonisation from native AMF that are in the soil when the trees were planted. The RLC% was 1.43 times higher in the NIAB EMR stoolbed root samples treated with AMF compared to the unamended control. The higher level of colonisation in the unamended control (14%) in these samples may reflect the time since planting (4 years) and the proximity to the field margin (see site plans) leading to higher levels of natural AMF colonisation. No equivalent inexpensive tests are available to confirm colonisation of the other biological treatments used in this trial.

Table 2.3.2 AMF root length colonisation (RLC %) subsampling of newly planted orchard trees after addition of AMF amendment to newly planted orchard soil at Site 2 and stoolbed at Site 3

Site	Sample #	AMF treated (RLC %)				No AMF treated control (RLC %)			
Site 2	1	15	22	32	22	12	0	2	0
	2	29	8	39	34	7	9	17	0
	3	14	15	48	13	8	6	3	5
	Mean	**19.3**	**15**	**39.6**	**23**	**9**	**5**	**7.3**	**1.6**
	Grand mean	**24.2**				**5.7**			
	Sample #	**AMF treated (RLC %)**				**No AMF treated control (RLC %)**			
Site 3	1	15.3				11.3			
	2	5.3				0.7			
	3	28.0				31.3			
	4	31.3				12.7			
	Mean	**20.0**				**14.0**			

Assessments from natural infection

Site 1 – Kent

Trees amended with Trichoderma (TrianumG) had significantly (P-value 0.006) fewer total cankers per tree (mean 0.8), than the untreated control (mean 2.0) and AMF (mean 2.1, Figure 2.3.2). Trichoderrma also had significantly lower scion canker number (B type) than the untreated control, PGPR and AMF.

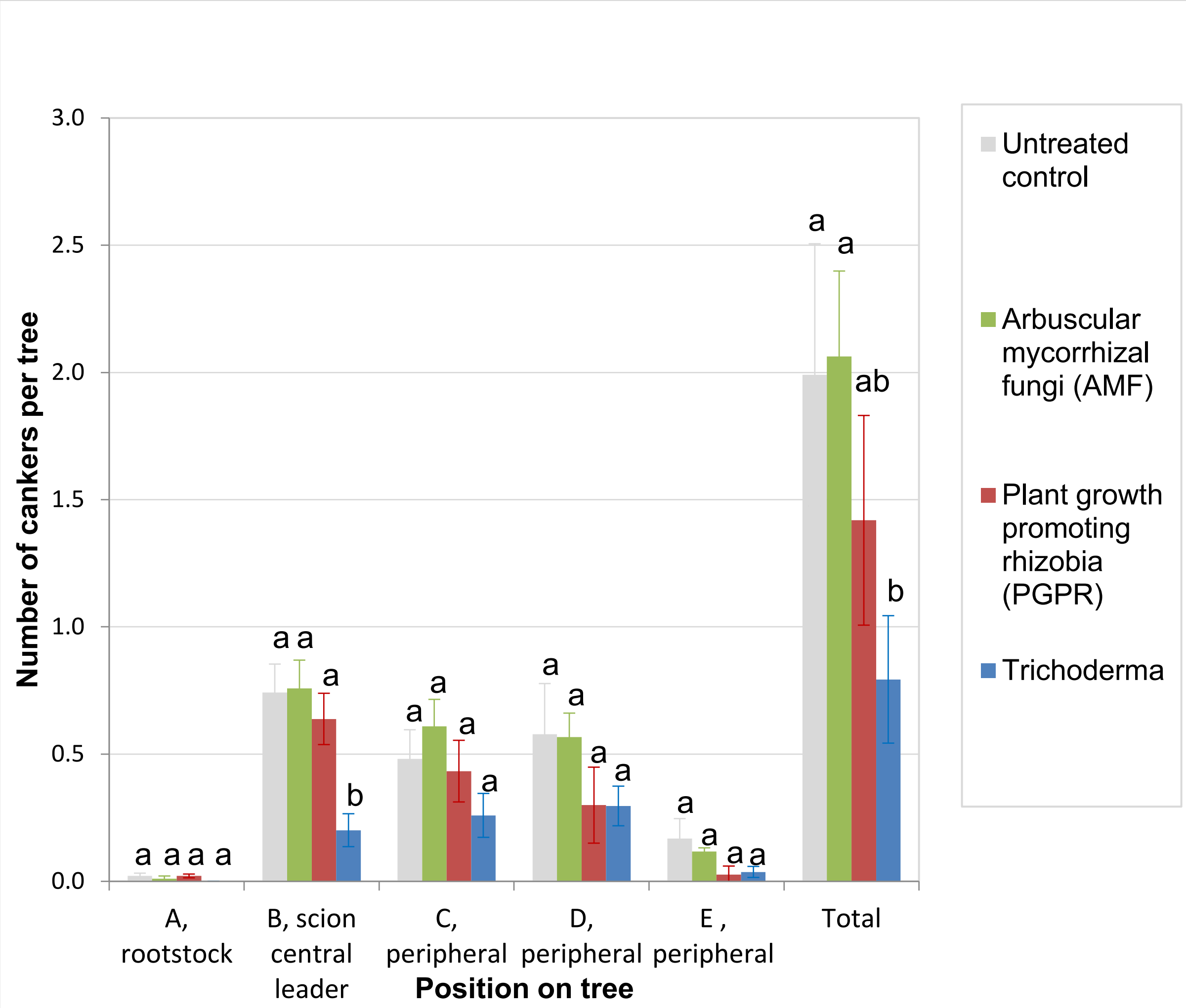


Figure 2.3.2 Mean number of cankers per tree after treatment with soil amendments at Site 1, Kent.

Site 2 – Kent

Overall canker numbers from natural infection were low, and none of the soil amendment treatments were significantly different to the untreated control at Site 2 (Figure 2.3.1). Trichoderma had the highest mean canker number per tree at 0.33, while AMF had the lowest at 0.09.

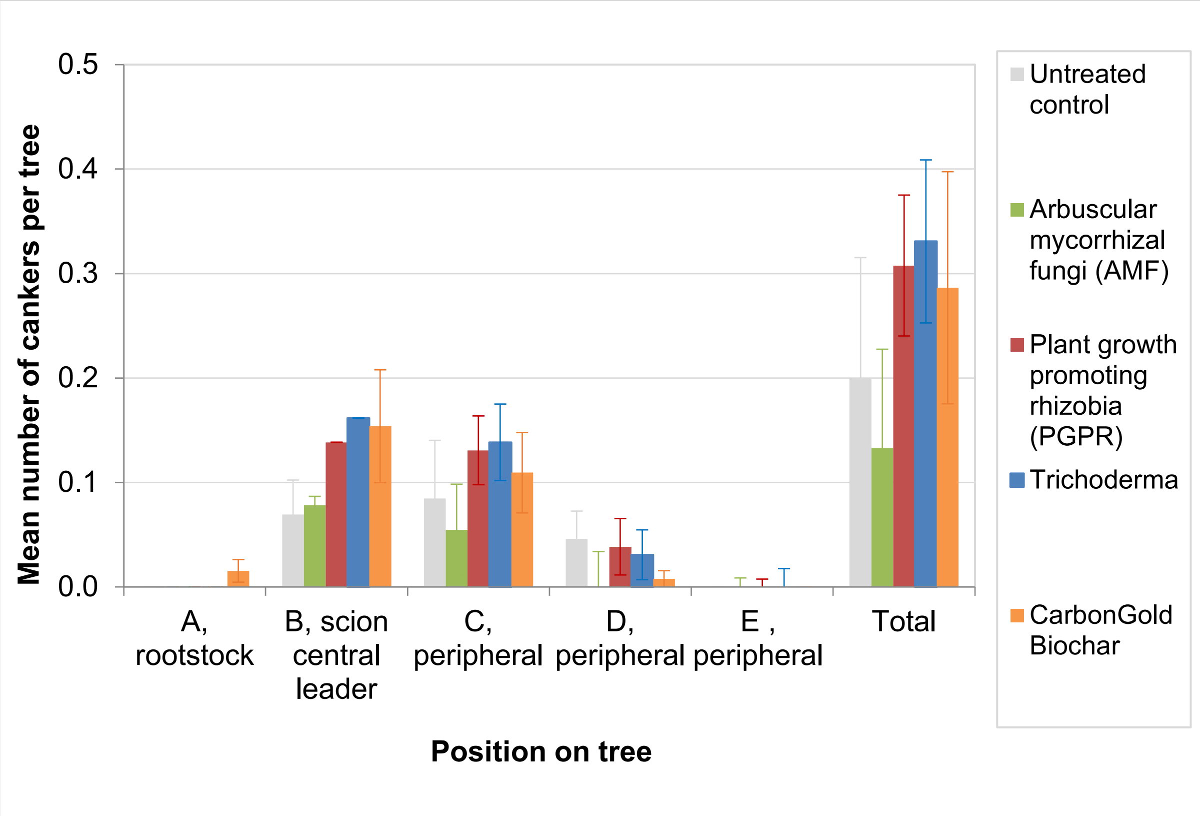


Figure 2.3.1 Mean number of cankers per tree after treatment with soil amendments at Site 2. No significant differences between any of the treatments on canker were found.

Site 3 – Kent stoolbed

There were very low levels of infection with means of less than 3.5 % across all the tested amendments (Figure 2.3.3). There was no significant difference between the percentage canker in the amended and unamended control.

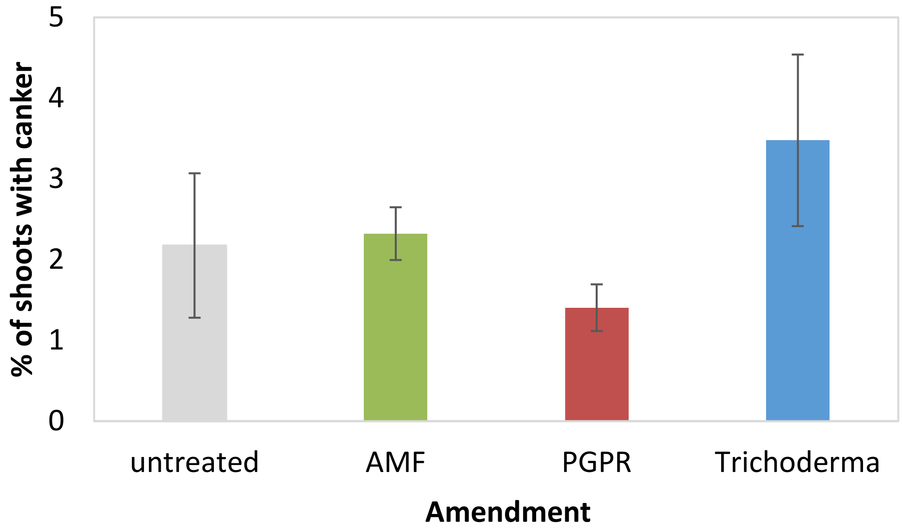


Figure 2.3.3 Percentage of shoots with canker after treatment of stoolbed with soil amendments at Site 3, Kent. There was no statistical difference of canker of shoots between amendments.

Site 4 – Gloucestershire

The natural levels of infection were quite low overall, canker was recorded mainly on C, D and E peripheral shoots, with some canker on the mainstem. Overall, there was no significant difference between any of the treatments in total number of cankers recorded as a result of the low canker incidence (Table 2.3.3). The untreated control and the AMF treated plots had the highest incidence of canker on peripheral shoots, whilst the incidence was lower in the other three treatments (Figure 2.3.4). The Trichoderma treatment had the lowest total number of cankers, and although this was not statistically significantly different (P=0.078) to any of the other treatments in this system with such low disease incidence and so few replicates this could be indicative of a treatment elicited effect.

Table 2.3.3. Statistics testing the effect of soil amendments on canker incidence at Site 4

Location of canker	Degrees of freedom	*p-value*
Rootstock (A)	13	N/A (0 cankers)
Mainstem (B)	13	0.404
Peripheral (C)	13	0.809
Peripheral (D)	13	0.190
Peripheral (E)	13	0.216
Rootstock + main-stem (A+B)	13	0.404
Peripheral (C+D+E)	13	0.144
Rootstock + Mainstem + Peripheral (A+B+C+D+E)	13	0.078

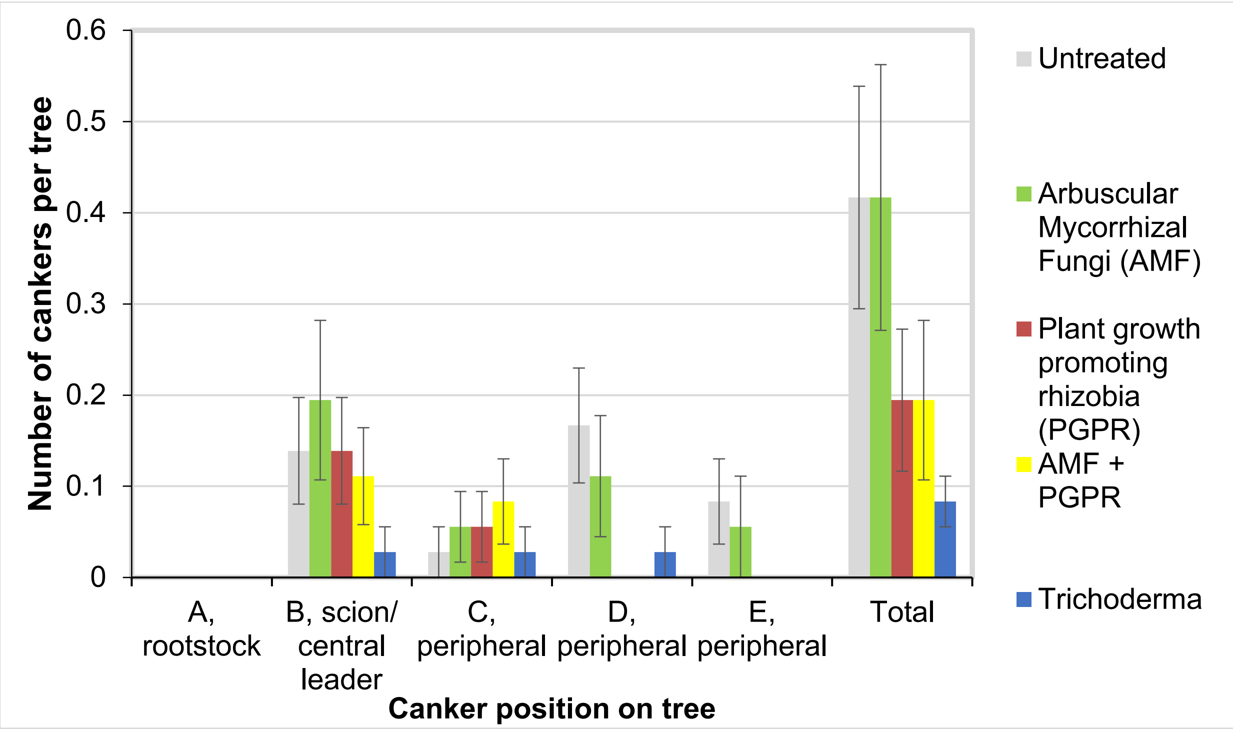


Figure 2.3.4. Mean number of cankers per tree after treatment with the addition of soil amendments at Site 4. Differences were not statistically significantly different.

Comparison of canker of newly planted orchards across all sites and the Site 3 stoolbed from 2017-2019

A comparison of canker of newly planted orchards across all sites and the Site 3 stoolbed from 2017-2019 is provided below (Table 2.3.4). By 2019, Site 1 had the highest canker number compared to other newly planted orchard sites. The Trichoderma/Trianum G treatment at that site had the lowest canker (0.8 mean cankers per tree), while the AMF had the highest (2.1 mean cankers per tree). At Site 2 by 2019, AMF had the lowest canker number (0.1 mean cankers per tree), slightly lower than the untreated control (0.2 mean cankers per tree). At Site 4, canker for all amendment types had reduced in the 2019 assessment compared to 2018.

Table 2.3.4 Comparison of canker of newly planted orchard trees with soil amendments across all sites, and the Site 3 stoolbed, with available data from 2017-2019.

Soil amendments			
Newly planted orchards	*Assessment year*	*Amendment*	*Mean canker number per tree (A+B+C+D+E cankers)*
Site 1 Kent	2017	AMF	0.1
		PGPR	0.0
		Trichoderma/TrianumG	0.0
		Control	0.1
	2018	AMF	0.3
		PGPR	0.1
		Trichoderma/TrianumG	0.1
		Control	0.3
	2019	AMF	2.1
		PGPR	1.4
		Trichoderma/TrianumG	0.8
		Control	2.0
Site 2 Kent	2017	AMF	0.0
		PGPR	0.0
		Trichoderma/TrianumG	0.1
		Carbon Gold Biochar	0.1
		Control	0.0
	2018	AMF	0.2

		PGPR	0.2
		Trichoderma/TrianumG	0.3
		Carbon Gold Biochar	0.4
		Control	0.2
	2019	AMF	0.1
		PGPR	0.3
		Trichoderma/TrianumG	0.3
		Carbon Gold Biochar	0.3
		Control	0.2
Site 4 Gloucestershire	2018	AMF	0.6
		PGPR	0.7
		AMF + PGPR	0.7
		Trichoderma/TrianumG	0.2
		Control	0.4
	2019	AMF	0.4
		PGPR	0.2
		AMF + PGPR	0.2
		Trichoderma/TrianumG	0.1
		Control	0.4
			Mean cankers per block
Stoolbed Site 3 Kent	2018	AMF	3.8
		PGPR	3.8
		Trichoderma/TrianumG	3
		Control	3.5
	2019	AMF	2.3
		PGPR	1.4
		Trichoderma/TrianumG	3.5
		Control	2.2

Vigour assessments

Site 1 – Kent

There was no significant difference in vigour between the amended trees at Site 1 (P=0.135, Figure 2.3.5). AMF and the unamended control had the largest girth (mean 102 mm)

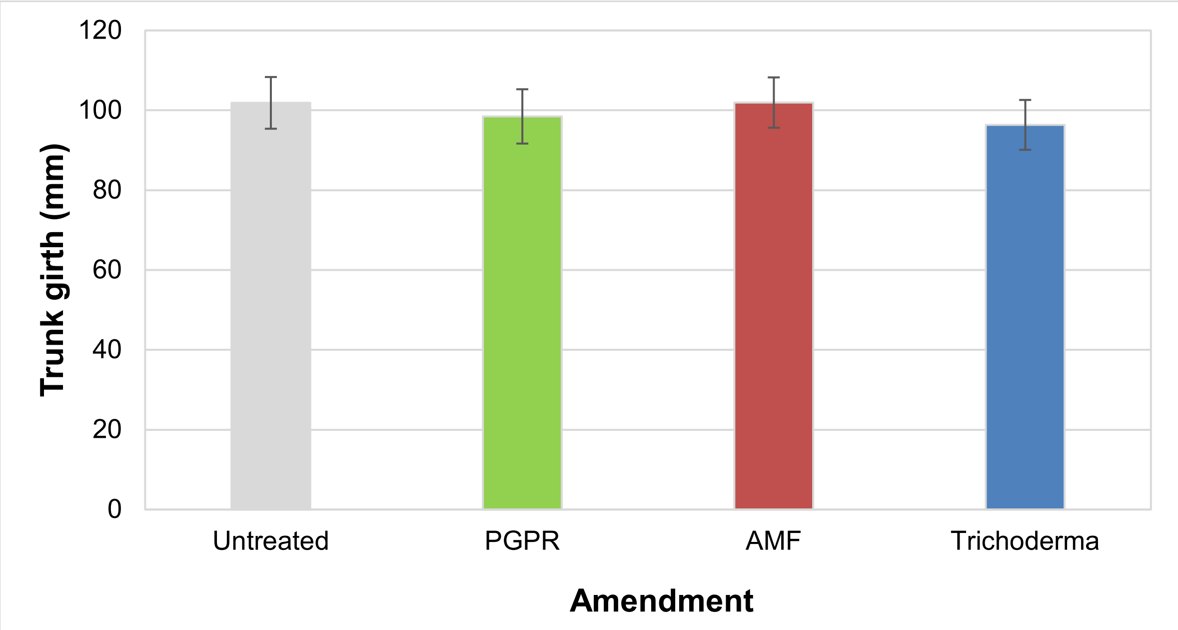


Figure 2.3.5. Mean trunk girth of trees with soil amendments applied at planting time at Site 1. Differences across treatments were not statistically significant (p=0.135). Error bars represent standard error of the mean.

Site 2 – Kent

The PGPR amended trees had significantly higher girth (99.43 mm) than the Trichoderma amended (96.24mm). The untreated, Biochar, and AMF amended were not significantly different to each other (Figure 2.3.6).

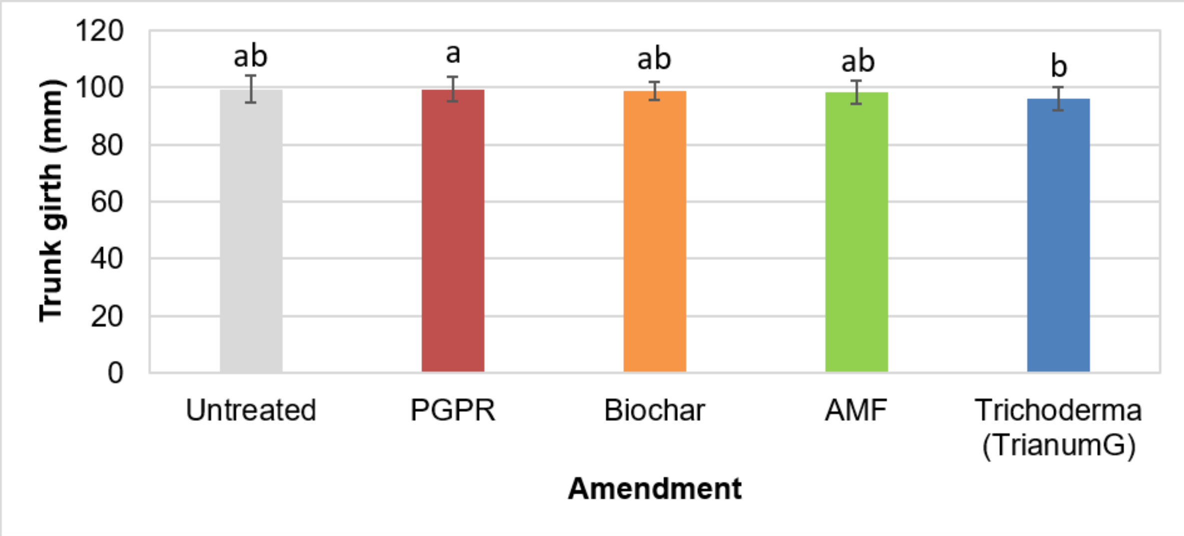


Figure 2.3.6. Mean trunk girth of trees with soil amendments applied at planting time at Broadwater Farm. Error bars represent standard error of the mean. Differences across treatments were not statistically significant.

Site 4 - Gloucestershire

The untreated control had the largest trunk girth and the AMF + PGPR had the smallest girth. However, the difference was small and there was no statistically significant difference in the girth of the trees in any of the treatments (P = 0.097; Figure 2.3.7).

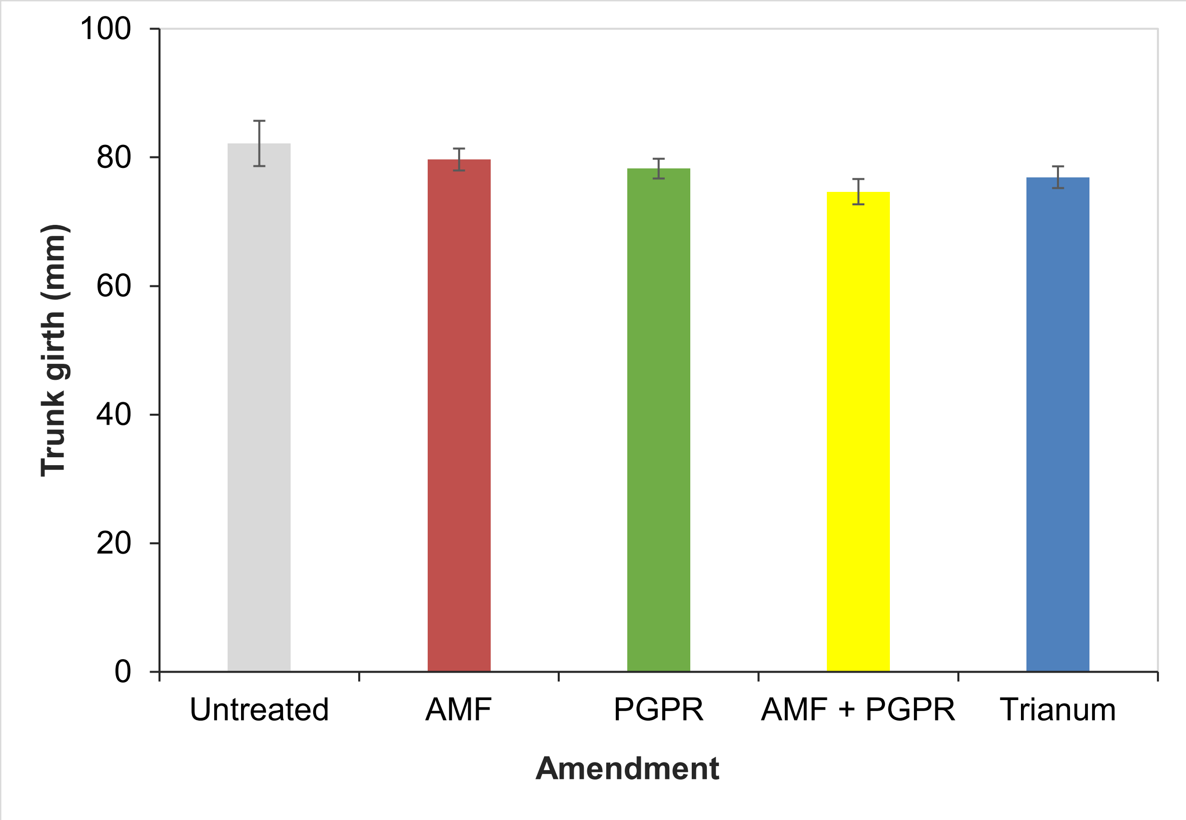


Figure 2.3.7. Mean trunk girth of trees with soil amendments applied at planting time at Site 4. Error bars represent standard error of the mean. Differences across treatments were not statistically significant.

Yield (Site 4 only)

The treatment of AMF + PGPR had the highest yield per tree and the most apples per tree when harvested in September 2019, followed by the Trianum (Trichoderma) treatment, however, these differences were not statistically significant compared to the other treatments (Figures 2.3.9- 2.3.10). The difference between the highest yield and the lowest (in the untreated control) was over 1 kg per tree.

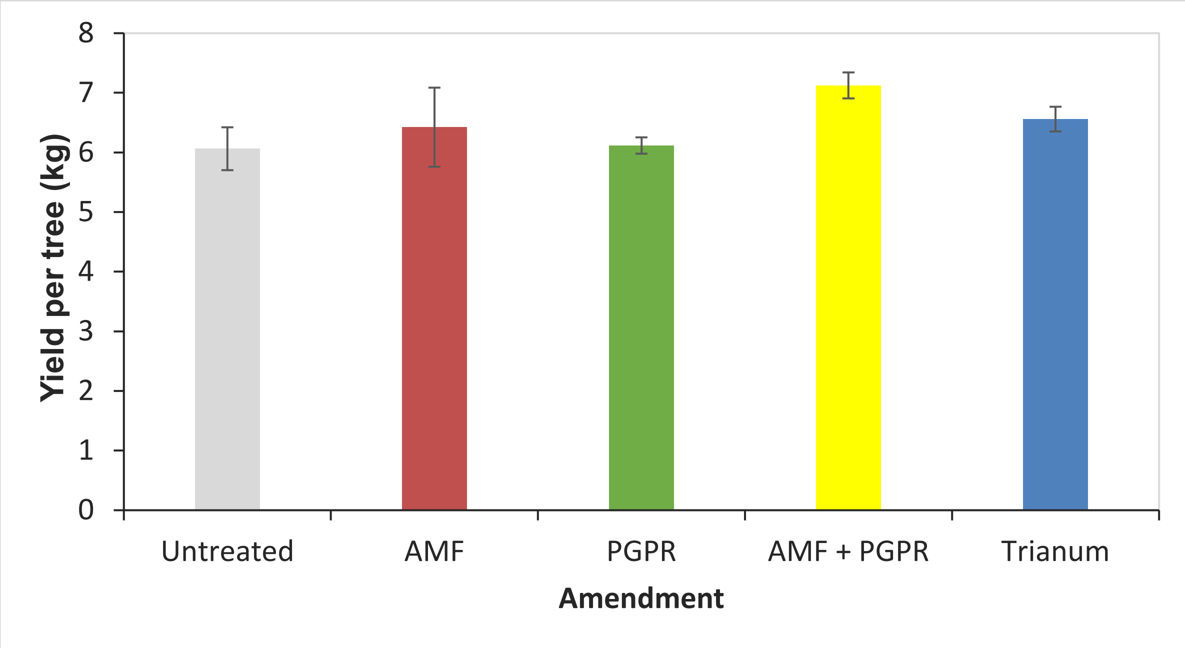


Figure 2.3.9. Mean yield per tree (kg) of amended trees. Error bars represent standard error of the mean. Differences across treatments were not statistically significant (P = 0.146).

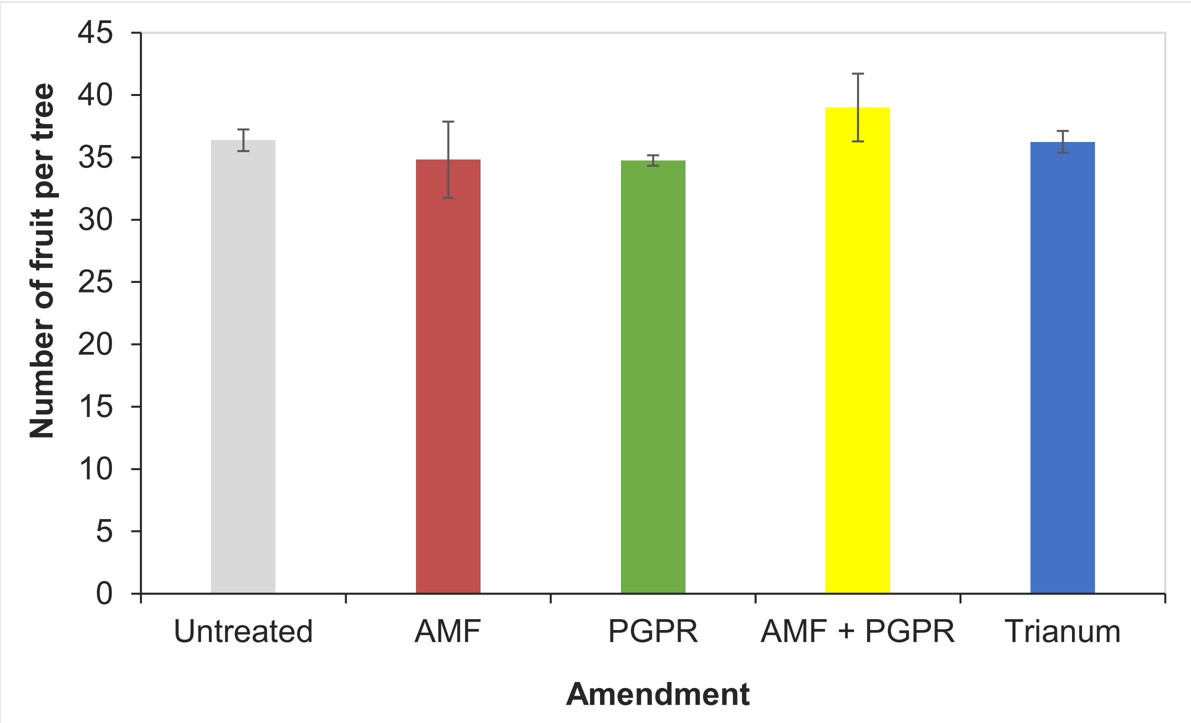


Figure 2.3.10. Mean number of fruits per tree in the soil amendment trial at Site 4. Error bars represent standard error of the mean. Differences across treatments were not statistically significant (P = 0.569).

There was a significant difference in the fruit size with the different soil amendments between AMF + PGPR and the other treatments including the untreated control. The AMF + PGPR treatment had the largest average fruit of 75.9 mm compared with the other treatments. The untreated plots had the smallest apples, which were an average of 69.2 mm (Figure 2.3.11).

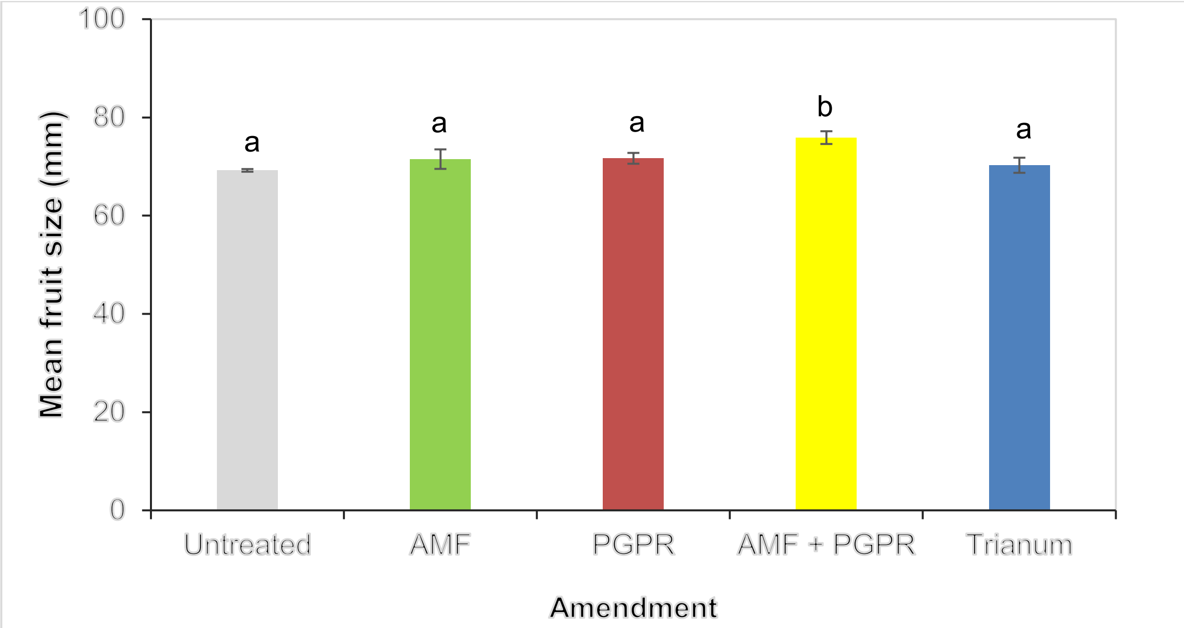


Figure 2.3.11. Average fruit size (mm) in the soil amendment trial. Error bars represent standard error of the mean. Differences across treatments were statistically significant ($P < 0.001$).

Cost-benefit analysis

Over £2051.36 in costs were associated with replacing dead trees over 5 years in an unamended 1000 tree orchard, assuming a 30% incidence of tree death. While the cost of using the Trichoderma (Trianum G) amendment was £536.10, with associated tree death calculated at 9%. This makes a potential saving of >£1050.38 per 1000 tree orchard (Table 2.3.5).

Table 2.3.5. Cost-benefit analysis of using Trianum G (*Trichoderma harzianum*) on a newly planted orchard of 1000 trees versus unamended. The percentage of total unamended trees with B type canker eg. 30% from Site 1 in the 2019 assessment was used. The 2019 assessment represents a cumulative amount of B-type canker since planting in 2016. B-type scion cankers are considered lethal and the assumption to remove trees and replace these trees was used. Labour costs and yield losses are not included in the analysis.

Costs with Trianum G amendment application for 1000 newly planted trees.
A 25 g scoop is added when planting trees, at a cost of £0.43 per tree based on £87.17 for a 5kg tub container of the product.

Category	Item	Quantity	Price (£)	Price per tree (£)	Price for 1000 trees (£)	Total with VAT
Amended	Trianum G	5 Kg	87.17	0.43	430	516.00
	Postage	1 x	11.75			14.10

Dead trees per year	Quantity of dead trees (based on 9% tree death)	Price of replacing trees @ £4 per tree	Total with VAT
Yr 1	90 out of 1000	360.00	432.00
Yr 2	8.1 out of 90	32.40	38.88
Yr 3	<1	<4	<0.2
Yr 4	<1	<1	<0.2
Yr 5	<1	<1	<0.2

Total costs including VAT £1000.98

Costs without Trianum G amendment of replacing additional dead trees over 5 years.

Category	Dead trees per year	Quantity of trees (based on 30 % tree death)	Price of replacing trees @ £4 per tree	Total with VAT (£)
Untreated	Yr 1	300 out of 1000	1200.00	1440.00
	Yr 2	90 out of 300	360.00	432.00
	Yr 3	27 out of 90	108.00	129.60
	Yr 4	8.1 out of 27	32.40	38.88
	Yr 5	2.43 out of 8.1	9.72	10.88

Total costs including VAT £2051.36

Grand total of replacing dead trees over 5 years incl VAT (£)	
Without Trianum G amendment	2051.36
With Trianum G amendment	1000.98
	1050.38

Discussion

At Site 2 in the 2019 assessment, AMF had the lowest mean number of cankers, while Trichoderma had the highest. This conflicts with the results of the Site 1 where Trichoderma amended trees had the lowest total canker number, significantly lower than the untreated control and AMF. At Site 1, the B type scion central leader canker number of Trichoderma treated trees was significantly lower than the other treatments and the untreated control. Mainstem (A, B) type cankers are hypothesised to mainly originate from the nursery, so this result shows that there is some effect of suppressing the canker from newly planted trees with previous nursery infection. There was no statistical difference of % canker of shoots at the Site 3 stoolbed, however Trichoderma had the highest % of shoots with canker.

At Site 4 (Gloucestershire) in the 2019 assessment, there was no significant effect of the soil amendments on the presence of canker on the trees. The treatment with the lowest canker incidence was the Trichoderma, which had performed well in the previous year and at the other trial sites.

The trees treated with AMF + PGPR had the highest number of C peripheral cankers and the smallest tree girth. AMF + PGPR produced the highest yield per tree, with many large fruits produced on these trees. At an average of over 75 mm per apple, it is likely that some of these will have fallen into the oversize category for fruit specification.

The Trichoderma treated trees produced the second highest yield in this trial, whilst the control had the lowest yield (with a difference of 0.5 kg per tree). Trichoderma was comparable to the untreated control in terms of fruit size and number of fruits per tree, suggesting that the individual fruit were heavier in the Trichoderma treatment. Yield data collection in subsequent years would be required to confirm this observation.

The comparison of canker across newly planted orchard sites and years revealed that canker at Sites 1 and 2 increased over time. At Site 4 and the Site 3 stoolbed canker generally decreased over time, however were still very low, Site 4 for example for all amendments were ≤0.7 mean A+B+C+D+E cankers per tree in the 2018 assessment year and ≤0.4 in the 2019 assessments. This may be due to site related factors such as soil type (sand/silt/clay ratio), soil condition (water logging, dry), aspect, and weather (temperature, rainfall, humidity, wind).

Conclusions

- Trichoderma shows potential as a soil amendment treatment to reduce canker, with variation between sites.
- A saving of >£1050.38 per 1000 tree planting was calculated for Trichoderma amended trees in a newly planted orchard.
- Trichoderma treated trees were also shown at Site 4 (Gloucestershire) to maintain yield, fruit number and fruit size.
- At Site 4 (Gloucestershire), AMF + PGPR produced the highest yield, eg. the highest number of apples per tree, the highest mean fruit size, and the largest fruit. However, with a mean of over 75 mm per apple it is likely that some of these will have fallen into the oversize category for fruit specification.
- No detrimental effects on vigour were found after adding amendments. Therefore, amendments can be applied to reduce canker without a loss of vigour.
- The effect of using amendments on canker of newly planted orchards and stoolbeds is variable and will likely be most effective when used in combination with other canker control methods.

Summary of methods and results for Objective 2 Neonectria canker over the course of the project

A summary of the results of canker experiments across sites and years is provided in Table 2.3.6. This information will help inform a IPDM strategy for UK apple growers and industry.

Table 2.3.6. Summary of methods and results to control Neonectria canker over the course of the project.

Method	*Key results*	*Recommendations and notes*
Tree injection using Fertinyect system	The injection devices tested have been shown to effectively distribute active ingredients through trees. None of the chemistry tested to date has shown sufficient efficacy for the control of symptomatic cankers including Cercobin (Certis), HDC F198, HDC F199 (x5), HDC F199 (x5) in 2 devices, HDC F199 (x10), HDC F200.	Trunk injection using the Fertinyect system can be used successfully as a method of systemic product application. Any new product would need to be tested individually to see if this method works for that product, and if there are any associated side effects such as phytotoxicity.
Pruning wound protection	T34 + BlocCade, Folicur (tebuconazole) + BlocCade, and Folicur (Tebuconazole) alone significantly reduced the development of cankers on pruning wounds.	Currently, Folicur can only be applied once in any given year, either before the first leaves are fully expanded or after the harvest of the final crop. The current EAMU for Folicur (tebuconazole) is expiring on 28/02/2023.
Development of an immunological based tool for *N. ditissima* detection	An Enzyme Linked Immunosorbant Assay (ELISA) protocol was optimized to detect Neonectria ditissima antigens in plant material. An antibody (1B10) was identified which gives good resolution in cross reactivity tests between Neonectria ditissima antigens and antigens from other fungi commonly found in UK apple orchards.	With further refinement, this assay can be used to improve our understanding of the biology of *N. ditissima.* This assay is being used an AHDB studentship (CP162) to understand the colonisation of N. ditissima following infection
Rootstock/interstock	Canker incidence increased over time at both sites.	Growers need to use rootstocks that are suited to their individual sites for reduced canker. Adapting orchard design with experience

	Rootstocks were identified with reduced canker, however there was conflict with the same rootstocks between the two tested sites e.g. MM106, M1116, Geneva rootstocks. EMR-001 (an advanced selection from the EM rootstock breeding programme) had higher canker at both sites. The M9 rootstocks (EMLA clone, 337 clone and 337 with Golden Delicious interstock) had a higher number of dead trees at both sites. There was no clear trend with vigour and canker.	Based on these experiments the NIAB EMR elite selection EMR-001 is not recommended for reduced canker and this has been fed back to the EM rootstock breeding club to inform variety descriptions Factors such as site selection including weather, scion cultivar selection, endophytes and apple replant disease are likely playing roles in canker.
Soil amendments Newly planted orchards	Trees treated with Trichoderma (Trianum G) at Site 1 had a significantly reduced canker number. There was also variation with amendments, for example, only one out of three sites had a significant canker reduction with Trichoderma treated trees. In the 2017 assessment, canker was generally very low (≤0.1 mean canker number per tree) for all amendments and the unamended control at Sites 1 and 2. In the 2018 assessment, another newly planted orchard site had been added, Site 4 (Gloucestershire), and the stoolbed at Site 3. Canker was still generally very low at all newly planted sites (≤0.7). Trees amended with Trichoderma had the lowest canker at two of the three newly planted sites. In the 2019 assessment at Site 2, AMF had slightly reduced canker compared to Trichoderma, PGPR, CarbonGold and the unamended control. At Site 1, Trichoderma and PGPR had reduced canker, with Trichoderma being the lowest (0.8 cankers per tree compared to 2 in the unamended control).	A potential saving of >£1050.38 per 1000 trees was calculated over 5 years at one trial site following treatment with Trichoderma (Trianum G) The effect of using amendments on canker of newly planted orchards is variable and will likely be most effective when used in combination with other canker control methods.

Stoolbed	In the 2018 assessment infection levels were very low with 3.5 cankers per block in the unamended control. Trichoderma was the only amendment that reduced canker (mean 3 cankers per block). AMF and PGPR actually had increased canker (3.8 cankers per block). In the 2019 assessment infection levels were even lower with 2.2 cankers per block in the unamended control. PGPR had the lowest canker 1.4 cankers per block while Trichoderma had higher canker with 3.5 cankers per block.	Results of amendments with the stoolbeds were variable. PGPR for example had higher canker than the unamended control in 2018 and lower in 2019. While Trichoderma had lower in 2018 and higher in 2019.
Tree vigour (as measured by trunk girth)	There was no significant difference of amendments on trunk girth at Site 1 or Site 4. There was a significant difference at Site 2 with PGPR having larger girth than Trichoderma. However, there were no significant differences between the unamended control and any of the amendment treated trees.	The amendments did not increase vigour/girth compared to the untreated control at any of the sites.
Yield, fruit number and fruit size – Site 4 in 2019 only	AMF + PGPR had the highest yield per tree, the most apples per tree, and the highest mean fruit size. The difference between the highest yield and the lowest (in the untreated control) was over 1 kg per tree. The only statistically significant difference was fruit size (AMF + PGPR treated were significantly larger than the other amendments and the unamended control).	Addition of AMF + PGPR to newly planted trees may increase yield and fruit size compared to no amendment.

Objective 3 - Apple Foliar Diseases

Task 3.2. Evaluate efficacy and persistence of alternative chemical treatments to fungicides (NIAB EMR Year 5)

Introduction

In 2018 a replicated small plot orchard trial on cv. Gala, the mildew control achieved by combined programmes of fungicides, elicitors / biostimulants (Cultigrow, Trident or Mantrac) and physical control products (Wetcit or SB Invigorator) were compared with that achieved by fungicides only applied at either 7- or 14-day intervals. There was no untreated control.

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

- The incidence of primary mildew in the trial orchard was higher than expected and the warm wet weather at the end of May 2018 was very favourable for mildew such that the overall incidence of secondary mildew in the trial was higher than commercially acceptable.
- Over the ten weekly assessments the lowest incidence of secondary mildew was in the plots receiving the 7-day fungicide programme.
- Highest mildew incidence was generally in the plots receiving the 14-day fungicide programme.
- The plots receiving the programmes combining fungicides with biostimulants and other alternative chemicals had generally significantly less mildew over the ten assessments than those receiving the 14-day fungicide programme indicating some benefit from the alternative treatments.
- There were no phytotoxic symptoms seen on the leaves but programmes 4 and 6 resulted in significantly lower fruit set than programme 1 (7-day fungicide programme),

possibly due to Wetcit applied in blossom, but this is not clear. The reduced fruit set was not seen in programme 8 which also included Wetcit at the same timings.

- There were no significant effects of treatments on yield, fruit russet, fruit colour or fruit size, even in treatments 4 and 6 which had reduced fruit set.

Objectives

The main objectives of the trial in 2019 was to largely repeat the 2018 trial to evaluate the control of mildew achieved with alternative products combined into programmes with fungicides. In view of the problems experienced in 2018 with the high incidence of primary mildew in the orchard and the unsprayed Cox guard rows, a new orchard was used for the 2019 trial. The orchard included cvs. Gala and Braeburn, giving the opportunity to evaluate treatments on another cultivar. Large plots were used, which limited the number of programmes evaluated and the replication but avoided the use of unsprayed guard rows and was more comparable to commercial practice.

Materials and Methods

Site: The trial was located in orchard MP196, located at NIAB EMR. The orchard was planted in 2012 and is 1.7 ha in size and consists of single alternate rows of Gala and Braeburn on M9 rootstock with 1.85 m between trees in the row and 3.5 m between rows.

Trial design: The trial was designed as large plots of six rows of 37 trees consisting of three rows of Braeburn and three rows of Gala. Each of the ten treatments was replicated three times in a randomised block design (Fig. 3.2.1).

Treatments: All plots received a standard programme for pest and disease control and nutrients (Appendix 3.1) up to the start of the trial at early flower (BBCH59-60). Thereafter the products in Table 1 were applied in programmes 1-4 given in Table 3.2.2. Programme 1 was the standard fungicide programme. Programmes 2 was based on Crop Biolife (CBL) applied monthly with Mantrac. Programme 3 was based on Mantrac and Trident without CBL. Programme 4 was based on CBL applied monthly with Trident. SB Invigorator (SBI) was included in all programmes 2-4. Wetcit was used with CBL, unless CBL was applied with a fungicide. All programmes were applied at 7-10 day intervals. A total of 14 spray rounds were applied starting at early flower on 24 April and continuing until the end of shoot growth on 24 July. Captan for scab control was applied to all plots as needed. Treatments for pests and nutrients were applied to all plots.

Spray application: Sprays were applied using a tractor-trailed air-assisted orchard sprayer at 200 L/ha.

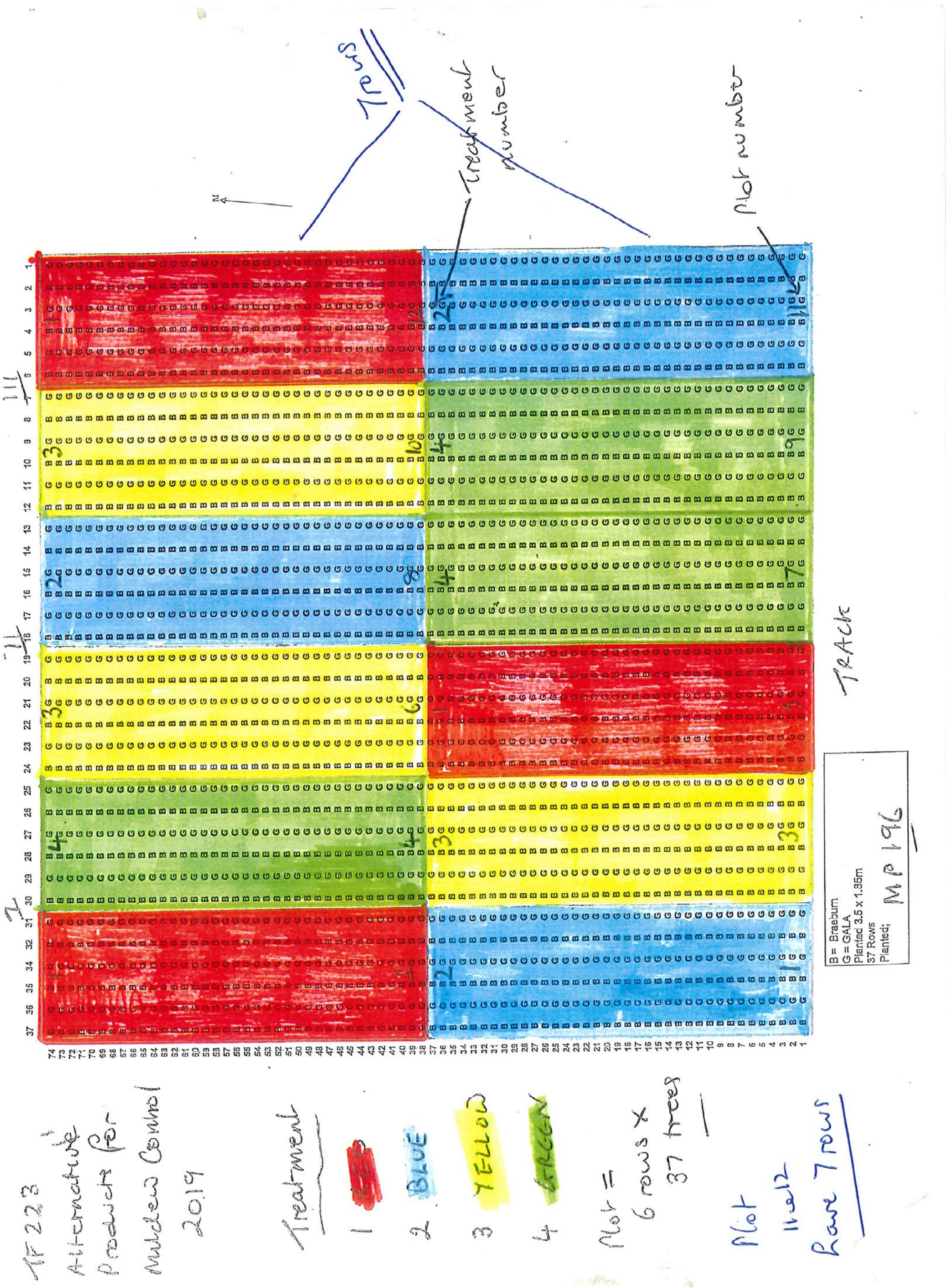


Figure 3.2.1. Trial plan of MP196

Table 3.2.1. Fungicides, elicitors, biostimulant products used in the programmes evaluated for control of powdery mildew in apple 2019.

Product	Active ingredient	Product type	Rate of product / ha	Harvest interval days	Latest use date assuming mid-September harvest	Number of sprays
Topas	penconazole	Fungicide	0.5 L	21	24/8	3
Cosine	cyflufenamid	Fungicide	0.5 L	14	31/8	2
Stroby	Kresoxim-methyl	Fungicide	0.2 kg	28	17/8	4
Fontelis	penthiopyrad	Fungicide	0.75 L	21	24/8	2
Sercadis	fluxapyroxad	Fungicide	0.3 L	35	10/8	3
Talius	proquinazid	Fungicide	0.25 L	49	28/7	2
Flint	trifloxystrobin	Fungicide	150g	14	31/8	4
Karma	Potassium hydrogen carbonate	mineral	5 kg	1	13/9	8
Kindred	meptyldinocap	Fungicide	0.6 L	Before end of bloom	-	2
Luna Privilege	fluopyram	Fungicide	0.15	14	31/8	3
Cultigrow CBL	flavonoids	Elicitor/biostimulant	500 ml	N/A	-	Blossom then monthly.
Wetcit	Alcohol ethoxylate	Energiser adjuvant	0.2 %	N/A	-	7-10 days

SB invigorator	Various nutrients and natural products	Physical action Controls various pests and mildew	1.0 ml/L	N/A	-	7-10 days Weekly sprays
Mantrac Pro	manganese	nutrient	0.5 L	N/A	-	5-6 applications from green cluster / pink bud
Trident (New)	Silicon 1%, Copper 2%, Zinc 4%	Nutrient / elicitor	1-3 L	N/A	-	7-10 days
Pek acid	Soluble P and K	fertiliser	0.75%	N/A	-	7-10 days

Table 3.2.2. Programmes for powdery mildew control applied to apple cvs Braeburn and Gala 2019 in MP196.

Programme	Product / Timing													
	24 Apr	1 May	6 May	15 May	21 May	29 May	5 Jun	12 Jun	19 Jun	26 Jun	3 Jul	10 Jul	17 Jul	24 Jul
Growth stage	**Braeburn** **30% flower**	**Late flower**	**Petal fall**	**End flower**	**End flower**	**Petal fall +**	-	-	-	-	-	-	-	**End shoot growth**
	Gala **Early flower**	**Full flower**	**Full flower**	**Late flower**	**End flower**	**Petal fall +**	-	-	-	-	-	-	-	
1 Fungicide 7 days	Flint	Sercadis + Captan	Flint	Sercadis	Topas + Captan	Talius	Cosine + Captan	Topas	Flint	Cosine	Sercadis	Topas	Flint	Talius
2 CBL/Mantrac	Mantrac + Flint	CBL+Captan	Mantrac + Flint	SBI	Topas + Captan + Mantrac	CBL+Wetcit	Cosine + Captan + Mantrac	SBI	Mantrac + Flint	CBL+ Wetcit	Sercadis + Mantrac	SBI	Mantrac + Flint	SBI
3 Mantrac/Trident	Mantrac + Flint	Trident + Captan	Mantrac + Flint	SBI	Topas + Captan + Mantrac	Trident	Cosine + Captan + Mantrac	SBI	Mantrac + Flint	Trident	Sercadis + Mantrac	SBI	Mantrac + Flint	SBI
4 CBL/Trident	Trident + Flint	CBL + Captan	Trident + Flint	SBI	Topas + Captan + Trident	CBL + Wetcit	Cosine + Captan + Trident	SBI	Trident + Flint	CBL + Wetcit	Sercadis + Trident	SBI	Trident + Flint	SBI

Table 3.2.3. Date and growth stage when treatments were applied in 2019.

Spray number	BBCH growth stage	Date treatment applied	Spray interval Days
1	Braeburn 63/65 Gala 61/62	23 April	-
2	Braeburn 67 Gala 65/66	1 May	8
3	Braeburn 67/69 Gala 65	6 May	5
4	Braeburn 69/70 Gala 67	15 May	9
5	Braeburn 70 Gala 67/69	21 May	6
6	Braeburn 71 Gala 70	29 May	8
7	Braeburn 72 Gala 71	5 June	7
8	Braeburn 72 Gala 72	12 June	7
9	Braeburn Fruitlet Gaka Fruitlet	19 June	7
10	Braeburn 73 Gala 72	26 June	7
11	Braeburn Fruitlet Gala Fruitlet	3 July	7
12	Braeburn 74 Gala 74	10 July	7
13	Braeburn 76 Gala 76	17 July	7
14	Braeburn 77 Gala 77	23 July	6

Assessments

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

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

Phytotoxicity: Symptoms of phytotoxicity were checked for after each treatment and recorded. Records taken were any chlorosis / necrosis to foliage, growth regulatory effects to shoots, assessed on a scale 0-5 (Table 3.2.4). In addition, initial and final fruit set and fruit drop were recorded. Two branches were marked on 10 trees of each cultivar in centre 2 rows in each plot. Total number of flowers were recorded in blossom on 23 April (Braeburn) and 9 May (Gala), number of fruitlets in June and number of apples recorded on 1 August.

Table 3.2.4. Foliage chlorosis/necrosis phytotoxicity scale, Source; EPPO Guideline PP 1/135(4).

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

Disease – Powdery mildew: All assessments were conducted on the middle two rows of each plot, on Gala and Braeburn, 10 trees per cultivar per row. Primary blossom was recorded on 1 May as the total number of blossoms and number with mildew on 4 branches on each of 10 trees per cultivar. Vegetative primary mildew was recorded on 22 May as the total number of mildewed shoots on each of 10 trees per cultivar per plot. Secondary mildew was recorded weekly from 29 May to 1 August on each of 10 shoots per cultivar per plot. The number of mildewed leaves in the top 5 leaves per shoot was recorded.

Harvest and fruit quality: At harvest, a random sample of 200 fruit were taken from 10 trees on each cultivar in each plot. Each 200-fruit sample was assessed as follows; weight of 200 fruit, weight, and number of fruit 65 mm or >, fruit colour and russet score. Russet was assessed on a scale of 0-4 where 0 = no russet, 1 = russet at stalk and calyx, 2 = russet on cheek but still acceptable as Class 1, 3 = rough russet and 4 = rough russet and cracking. Russet scores 0-1 are for Gala acceptable in Class 1 (EPPO Guideline PP 1/135 (4). Fruit colour was assessed as % red coloration on a scale of 0-4 scale where 0 = green, 1 = up to 25% red colour, 2 = 26-50% red colour, 3 = 51-75% red colour and 4 = 76-100% red colour. (EPPO Guideline PP 1/135 (4).

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

Table 3.2.5. Summary of treatment and assessment timings – NIAB EMR 2019.

Date	Record of work done (and operator responsible)
15 April	Trial marked out. 12 plots of 6 rows of 37 trees (JK/SC)
23 April	Number of flowers recorded on Braeburn (JK)
24 April	Spray 1 applied (Farm)
1 May	Spray 2 applied (Farm)
1 May	Primary blossom mildew assessed (AMB)
6 May	Spray 3 applied (Farm)
9 May	Number of flowers recorded on Gala (JK)
15 May	Spray 4 applied (Farm)
21 May	Spray 5 applied (Farm)
22 May	Primary vegetative mildew assessed (AMB)
29 May	Spray 6 applied Farm. Secondary mildew assessed (AMB)
5 June	Spray 7 applied (Farm)
6 June	Secondary mildew assessed (AMB)
12 June	Spray 8 applied (Farm)
13 June	Secondary mildew assessed (5 shoots only as raining) (AMB)
19 June	Spray 9 applied (Farm)
20 June	Secondary mildew assessed (AMB)
21 June	Initial fruit set recorded on Gala and Braeburn (JK, SC)
26 June	Spray 10 applied (Farm)
27 June	Secondary mildew assessed (AMB)
3 July	Spray 11 applied (Farm)
4 July	Secondary mildew assessed (AMB)
10 July	Spray 12 applied (Farm)
11 July	Secondary mildew assessed (AMB)
17 July	Spray 13 applied Farm. Secondary mildew assessed (AMB)
24 July	Spray 14 applied (Farm)
25 July	Secondary mildew assessed (AMB)
1 August	Secondary mildew assessed (AMB). Final fruit count on Gala and Braeburn (JK /SC)
September	Gala sampled 200 fruit per plot for fruit quality. Placed in cold store (TP)
October	Braeburn sampled 200 fruit per plot for fruit quality. Placed in cold store (TP)
November	Quality assessments – fruit size, russet and colour done on Gala and Braeburn (JK)

Results

Phytotoxicity and fruit set: No phytotoxicity was noted on leaves at any of the inspections or assessments. In Braeburn programmes 2-4 had significantly lower initial fruit set compared to the standard fungicide programme, but these differences were not continued in final fruit set and fruit drop where there were no significant differences between treatments.

For Gala there were no significant differences in initial fruit set but programme 2 resulted in a significantly lower final fruit set and a higher fruit drop compared to the other programmes (Table 3.2.6).

Table 3.2.6. Mean % fruit set (angular transformed) on apple cvs. Gala and Braeburn following 14 sprays of four programmes at NIAB EMR in 2019.

Programme	Gala			Braeburn		
	% Initial Fruit set	% Final fruit set	% Fruit drop	% Initial fruit set	% Final fruit set	% Fruit drop
1 Fungicide 7 days	71.3	58.3 ab	18.3 b	40.8 a	35.8	12.6
2 CBL/Mantrac	67.0	45.9 c	31.3 a	32.7 b	27.8	15.0
3 Mantrac/Trident	69.4	59.9 a	13.7 b	34.6 ab	29.0	16.3
4 CBL/Trident	66.2	54.9 b	16.8 b	32.6 b	29.5	9.7
F Prob	0.21524	<0.001	0.0040	0.08309	0.1517	0.1267
SED		1.45	0.125	2.86		
LSD (p=0.05)	NS	3.55	0.747	7.01	NS	NS

Disease – Powdery mildew

The incidence of primary blossom and vegetative mildew in the orchard was recorded at the start of the trial and is the mildew overwintered in the buds from the previous season (Table 3.2.9). The incidence of primary blossom mildew was low to moderate in Gala but moderate to high for Braeburn. Levels above 2% are considered high (Cross & Berrie, 1994). There were no significant differences between treatments. Primary vegetative mildew was also high, especially in Braeburn, indicating the higher susceptibility of this cultivar to mildew. Surprisingly, the plots allocated to the routine fungicide programme appeared to have less primary vegetative mildew than the other plots in both Gala and Braeburn with the difference significantly less in Braeburn. Several treatments had been applied by the time primary mildew was assessed on 22 May.

Secondary mildew on extension growth was assessed from the start of growth to the end, a total of 10 assessments at weekly intervals (Figures. 3.2.2 and 3.2.3 and Tables 3.2.7-3.2.9). Overall, the incidence of mildew was higher in Braeburn than Gala, again reflecting the higher susceptibility of this cultivar and significantly higher in May and June in programmes 2-4 compared to the routine programme. However, by July, mildew incidence had fallen to <20% mildewed leaves in both cultivars. From then onwards there was no significant difference in mildew incidence between the 4 programmes at most assessment dates and remained so until the final assessment where incidence had fallen to < 10% mildewed leaves for all programmes. Overall, there was significantly less mildew in the routinely treated plots compared to that in plots treated with programmes 2-4.

The number of products used in the programmes and their relative costs are summarised in Table 3.2.12. A total of 14 fungicides for mildew control were used in the routine programme compared to 7 in programmes 2-4. Total cost of programmes 2 and 3 were around £100-150 per ha cheaper than the standard fungicide programme whereas programme 4 was around the same cost.

Fruit quality: Fruit quality data - fruit russet, fruit colour and fruit size are presented in Tables 3.2.10 and 3.2.11. There were no significant differences between the 4 programmes for russet score and fruit weight and size. For fruit colour there were significant differences between treatments, but this was not consistent for the two cultivars.

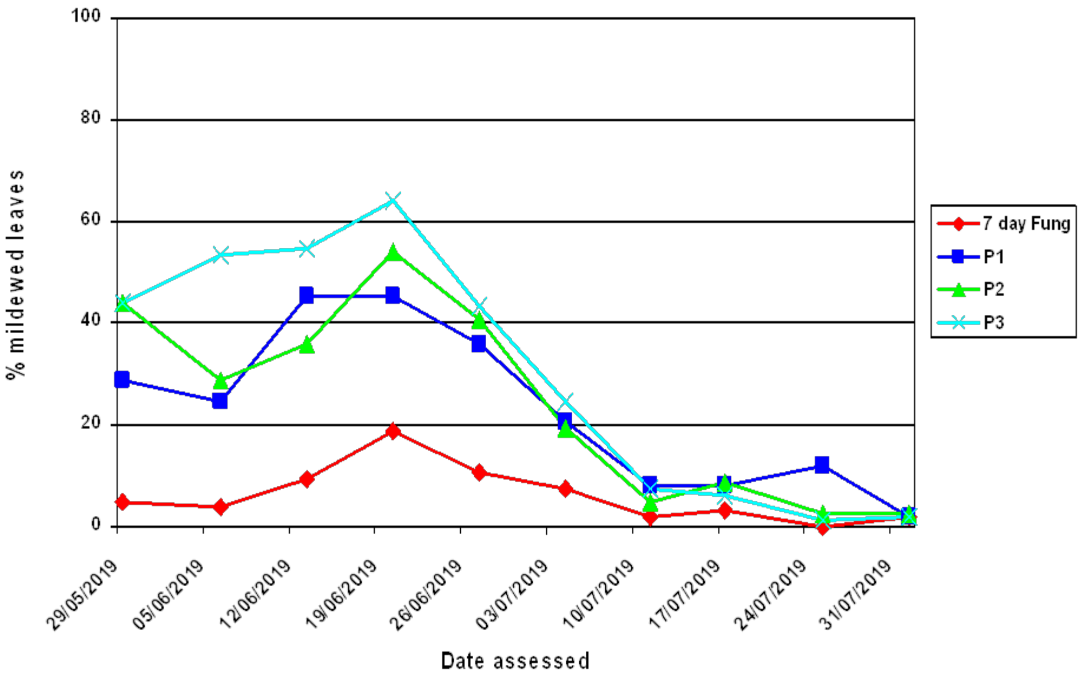


Fig. 3.2.2. Mean % mildewed leaves on apple shoots cv. Gala assessed at various times following treatment with 14 sprays of 4 different programmes of various products applied at NIAB EMR in 2019

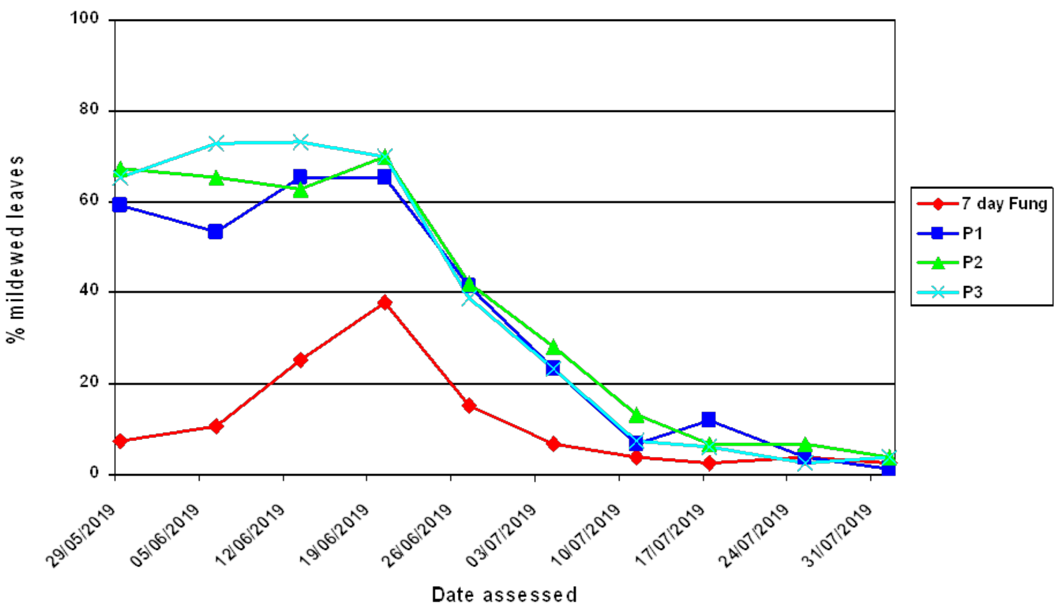


Fig.3.2.3. Mean % mildewed leaves on apple shoots cv. Braeburn assessed at various times following treatment with 14 sprays of 4 different programmes of various products applied at NIAB EMR in 2019.

Table 3.2.7. Mean % mildewed leaves (angular transformed) on apple cv. Gala following 14 sprays of various programmes, applied at NIAB EMR in 2019. (Figures in brackets are back transformed data). NS = Not significant. Figures with different letters are significantly different from untreated.

Programme	**% mildewed leaves**										
	29 May	**6 June**	**13 June**	**20 June**	**27 June**	**4 July**	**11 July**	**17 July**	**25 July**	**1 August**	**Overall mean mildew**
1 Fungicide 7 days	3.0 (4.7) b	2.6 (4.0) c	8.7 (9.3) b	18.6 (18.7) c	10.4 (10.7) b	7.2 (7.3) b	2.0 (2.0) c	3.3	0 b	2.0	4.5 (6.2) b
2 CBL/Mantrac	28.4 (28.7) a	24.3 (24.7) b	45.0 (45.3) a	45.3 (45.3) b	35.4 (36.0) a	20.3 (20.7) a	7.9 (8.0) a	8.0	12.0 (12.0) a	2.0	20.3 (23.1) a
3 Mantrac/Trident	44.0 (44.0) a	28.1 (28.7) b	35.9 (36.0) a	54.1 (54.0) ab	40.5 (40.7) a	19.2 (19.3) a	4.4 (4.7) b	8.7	0.9 (2.7) b	2.7	20.2 (24.1) a
4 CBL/Trident	43.8 (44.0) a	53.3 (53.3) a	54.7 (54.7) a	64.1 (64.0) a	43.3 (43.3) a	24.6 (24.7) a	7.1 (7.3) ab	6.0	0.5 (1.3) b	2.0	25.0 (30.1) a
F Prob	0.008	0.005	0.016	0.001	0.002	0.002	0.006	0.2246	0.03	0.8117	<0.001
SED (6)	1.23	1.09	1.33	0.37	0.32	0.12	0.068		0.79		0.11
LSD (p=0.05)	7.24	6.41	7.76	2.23	1.92	0.72	0.408	NS	4.65	NS	0.44

Table 3.2.8. Mean % mildewed leaves (angular transformed) on apple cv. Braeburn following 14 sprays of various programmes, applied at NIAB EMR in 2019. (Figures in brackets are back transformed data). NS = Not significant

Programme	**% mildewed leaves**										
	29 May	**6 June**	**13 June**	**20 June**	**27 June**	**4 July**	**11 July**	**17 July**	**25 July**	**1 August**	**Overall mean mildew**
1 Fungicide 7 days	7.1 (7.3) b	9.4 (10.7) b	24.4 (25.3) b	37.9 (38.0) b	15.2 (15.3) b	6.6 (6.7) b	4.0	2.7	4.0	2.7	8.6 (11.7) b
2 CBL/Mantrac	59.6 (59.3) a	53.4 (53.3) a	65.6 (65.3) a	65.8 (65.3) a	41.3 (41.3) a	23.3 (23.3) a	6.7	12.0	4.0	1.3	28.3 (33.2) a
3 Mantrac/Trident	67.6 (67.3) a	65.5 (65.3) a	62.9 (62.7) a	70.2 (70.0) a	42.0 (42.0) a	27.9 (28.0) a	13.3	6.7	6.7	4.0	32.4 (36.5) a
4 CBL/Trident	65.4 (65.3) a	74.2 (72.7) a	74.2 (73.3) a	70.5 (70.0) a	38.3 (38.7) a	22.7 (23.3) a	7.3	6.0	2.7	4.0	31.8 (36.3) a
F Prob	<0.001	0.004	0.018	0.002	0.018	0.012	0.189	0.133	0.970	0.8858	0.002
SED (6)	0.55	1.51	1.38	0.30	0.56	0.38					1.38
LSD (p=0.05)	3.25	8.83	8.09	1.76	3.31	2.26	NS	NS	NS	NS	5.44

Table 3.2.9. Mean % primary blossom mildew, primary vegetative mildew at the start of the trial and overall secondary mildew (overall mean of 10 assessments) % mildewed leaves (angular transformed) on apple cvs. Gala and Braeburn following 14 sprays of 4 different programmes, applied at NIAB EMR in 2019 (figures in brackets are original data). Figures with different letters are significantly different from untreated.

Programme	% primary blossom mildew		Mean number mildewed shoots per 10 trees		% secondary mildew Overall mean	
	Gala	Braeburn	Gala	Braeburn	Gala	Braeburn
1 Fungicide 7 days	1.0	1.3	4.0	11.7 c	4.5 (6.2) b	8.6 (11.7) b
2 CBL/Mantrac	0.7	2.3	8.3	41.3 a	20.3 (23.1) a	28.3 (33.2) a
3 Mantrac/Trident	0.3	2.7	12.0	28.7 b	20.2 (24.1) a	32.4 (36.5) a
4 CBL/Trident	0	1.0	12.7	27.0 b	25.0 (30.1) a	31.8 (36.3) a
F Prob	0.9298	0.9565	0.6304	<0.001	<0.001	0.002
SED (6)				12.16	0.11	1.38
LSD (p=0.05)	NS	NS	NS	16.12	0.44	5.44

Table 3.2.10. Effects of treatments on fruit quality recorded as russet score, colour score, weight 200 fruit (kg) and number and weight of fruit > 65 mm diameter on apple fruits cv. Gala following 14 sprays of 4 different programmes at NIAB EMR in 2019. Figures with different letters are significantly different from untreated. NS = Not significant. [1]Russet score = high score = high russet. [2]Colour score = higher score is better fruit colour.

Programme	Mean russet score[1]	Mean colour score[2]	Weight of 200 fruit kg	No. fruit > 65 mm diameter	Weight of fruit >65 mm diameter
1 Fungicide 7 days	157.7	763 ab	31.1	168.7	28.2
2 CBL/Mantrac	156.0	752 b	29.4	151.0	24.5
3 Mantrac/Trident	161.7	750 b	29.4	160.3	25.6
4 CBL/Trident	167.0	767a	28.4	150.7	23.9
F Prob	0.454	0.082	0.236	0.242	0.268
SED (6)		6.11			
LSD (p=0.05)	NS	14.94	NS	NS	NS

Table 3.2.11. Effects of treatments on fruit quality recorded as russet score, colour score, weight 200 fruit (kg) and number and weight of fruit > 65 mm diameter on apple fruits cv. Braeburn following 14 sprays of 4 different programmes at NIAB EMR in 2019. Figures with different letters are significantly different from untreated. NS = Not significant. [1]Russet score = high score = high russet. [2]Colour score = higher score is better fruit colour.

Programmes	Mean russet score[1]	Mean colour score[2]	Weight of 200 fruit kg	No. fruit >65 mm diameter	Weight of fruit >65 mm diameter
1 Fungicide 7 days	133.0	741.0 ab	36.2	178.0	33.7
2 CBL/Mantrac	112.3	733.3 b	33.8	171.7	30.7
3 Mantrac/Trident	139.7	764.3 a	33.9	178.0	31.5
4 CBL/Trident	129.3	746.0 ab	34.5	169.3	31.3
F Prob	0.2756	0.0943	0.8568	0.7973	0.8988
SED (6)		10.11			
LSD (p=0.05)	NS	24.74	NS	NS	NS

Table 3.2.12. Summary of products used in the different programmes and relative costs.

Item	Programmes			
	1. Fungicide 7 days	**2. CBL/Mantrac**	**3. Mantrac/Trident**	**4. CBL/Trident**
Mildew fungicides	14	7	7	7
Scab fungicides	3	3	3	3
CBL	0	3	0	3
Mantrac	0	7	7	0
Trident	0	0	3	7
SBI	0	4	4	4
Wetcit	0	2	0	2
Cost £ / ha				
Mildew fungicides	501.18	254.43	254.43	254.43
Biostimulants / wetters	0	152.40	102.40	248.46
Total Cost	**501.18**	**406.83**	**356.83**	**502.59**

Discussion

By switching to a commercial orchard at NIAB EMR which had not previously been used for trials it was hoped that the primary mildew incidence would be low. However, primary blossom and vegetative mildew were moderate to high, especially in Braeburn, indicating that mildew control at the end of the summer in 2018 had not been effective. A lower incidence of primary mildew was recorded in the plots assigned randomly to the routine programme which was unexpected and suggested that, since five rounds of treatments had been applied by the time of the primary vegetative mildew assessment on 22 May, the fungicides applied had eradicated the primary mildew. The difference in mildew incidence continued in the secondary mildew assessments, such that until early July mildew incidence was much higher in plots receiving programmes 2-4. Thereafter mildew incidence continued to fall in programmes 2-4 and was similar to that in the routine treated plots in both cultivars for the rest of the trial. This suggests that if the same fungicide programme at 7 day intervals had been applied in all 4 programmes for the first three rounds, before adopting the different programmes then there would likely have been similar control in all four programmes for the trial period. In this trial alternating fungicides with biostimulants and physical control products such as SBI, enabled fungicide inputs to be reduced by half while still giving effective control of powdery mildew, provided mildew incidence is monitored. This trial showed that Braeburn was more susceptible to mildew than Gala.

There were no obvious effects of treatments on fruit size and russet. There were small treatment effects on fruit colour but not consistent between cultivars. The cool nights in September ensured fruit colour was good in 2019. Most likely the effects of treatments on fruit colour would have been greater in a year when conditions did not favour fruit colour development. There were some significant effects of the treatments on fruit set. All programmes significantly reduced initial fruit set in Braeburn, but not in Gala. This may have been weather related as the Braeburn flowered earlier than the Gala. The fruit set effects were not continued through to final fruit set or fruit drop. In Gala however, programme 2 resulted in a significantly lower final fruit set with higher fruit drop than the other programmes. Reasons for this are not clear.

Conclusions

- The incidence of primary mildew, particularly the primary vegetative mildew, in the trial orchard was higher than expected and appeared to be reduced by the early fungicide programme applied to the routine plots. Primary mildew incidence was higher in Braeburn than Gala.
- The difference in primary mildew incidence was reflected in the subsequent secondary mildew epidemic with higher incidence recorded in plots treated with programmes 2-4 compared to the routine until early July. Thereafter mildew incidence decreased in all treatments with little difference between the routine and trial programmes in both cultivars with secondary mildew in both cultivars 0-10% mildewed leaves for the final assessments.
- Overall, significantly less mildew was recorded in the plots receiving the routine 7 day fungicide programme.
- Plots treated with programmes 2-4 which included biostimulants and physical control products received half the number of fungicides compared to the routine treatment.

- There were no phytotoxic symptoms seen on the leaves.
- There were no significant effects of treatments on fruit size and russet.
- There were small treatment effects on fruit colour but not consistent between cultivars.
- There were some significant effects of the treatments on fruit set. All programmes significantly reduced initial fruit set in Braeburn, but not in Gala. The fruit set effects were not continued through to final fruit set or fruit drop. In Gala programme 2 resulted in a significantly lower final fruit set with higher fruit drop than the other programmes.
- The results show that by combining biostimulants / elicitors and physical control products with fungicides in programmes for control of powdery mildew it is possible to reduce fungicide inputs, while still maintaining mildew control and fruit quality. Regular monitoring of mildew incidence is essential so that any increase in mildew incidence can be responded to.

Objective 4 - Stone Fruit Diseases

4.3 Bacteriophages against bacterial canker of cherry

Aim

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

Introduction

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

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

In the season 2018/2019 we made significant progress towards bringing phage treatment to the growers by obtaining a field trial permit to test phages in the cherry orchard on site and successfully run two different in planta assays showing phage efficacy on the host.

Methods

Characterisation of selected phage strains by Dr Mojgan Rabiey and Shyamali Roy (University of Reading)

The selected phages (MR13, MR14, MR16, MR18, Table 4.3.1) were tested against 15 pathogenic *P. syringae* strains from 3 main pathovars infecting cherry and 8 beneficial *P. syringae* strains using drop assay method, by spotting 5 µl of phage suspension (10^4-10^5 pfu ml^{-1}; pfu, phage forming units) on plates with a soft agar layer supplied with a specific bacterial isolate (OD_{600}=0.2). After incubation at 27°C for 24 hours, plates were checked for plaques; clear spots where phage infected bacterial cells lead to cell lysis. Transmission electron microscopy was used to image the morphology of the phage and estimate their size. Ten µl of 10^{10} pfu ml^{-1} phages was spotted on a copper coated carbon-formvar TEM grid and left to dry. Then 10 µl of sH_2O was spotted on the grid and left for 2 min. This was negatively stained with 10 µl of (1% w/v) Uranyl acetate for 2 minutes. Samples were analysed using a Phillips CM200 Transmission Electron Microscope at 80V and photos taken using AMT camera system software.

In planta assay on bean plants (University of Reading)

Briefly, phages were amplified, purified and bulked up for an in-planta assay. Bean plants were chosen as a simple model system and as a known host of *Pseudomonas syringae*. Planted beans were incubated at 22°C. Phage isolates were tested on 5 replicate plants arranged randomly. Bean leaves were sprayed with Pss 9097 bacterial strain at the concentration of 10^8 cfu ml^{-1} in phosphate buffer saline (PBS) followed by a single strain phage spray at 10^6 pfu ml^{-1} on PBS. Phage isolates MR13, MR14, MR16 and MR18 were used (Table 4.3.1). From each collected leaf, 4 disks were removed and placed into a 2ml tube containing 1ml PBS with two tungsten beads and homogenised at a speed of 6 m/sec for 10 seconds. Serial dilutions were prepared and 3 x 25 µl of neat, 10^{-1}, 10^{-2}, 10^{-3} serially diluted samples were spotted onto KB agar plates containing 100 µg ml^{-1} of Pseudomonas CFC supplement (cyclohexamide + cephalexin). Plates were incubated at 27°C and the number of colony forming units (cfu ml^{-1}) was recorded after 24 hours.

Table 4.3.1. Information on the UK phages used in 2019 experiments. Pss, *Pseudomonas syringae pv. syringae;* Psm1, *Pseudomonas syringae pv. morsprunorum* race 1; Psm2, *Pseudomonas syringae pv. morsprunorum* race 2.

Phage name:	Collection reference	Host range i.e. activity against cherry infecting *P. syringae* pathovars
MR11	Pss13	Pss
MR13	Psm1 10	Pss, Psm1 and Psm2
MR14	Psm1 11	Pss, Psm1 and Psm2
MR16	Pss1	Pss, Psm1 and Psm2
MR18	Pss19.2	Pss

Detached shoot assay

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

1) On 25 October 2018 more than 200 cherry cv. Sweetheart shoots were collected from rows 8 and 11 in RF181/182 orchard at NIAB EMR site

a. Shoots were appx. 30-40 cm in length

b. Collected from the end of the branches, i.e. the current season's growth

c. Leaves visibly yellowing but still attached

d. Upon collection the shoots were placed into saturated florists foam to prevent from drying.

2) The following day (26 October) the shoots (with leaves still attached) were inoculated with a mixture of Pss 9097 and Psm1 5244 strains

a. 10^8 cfu per ml of bacterial suspension consisting of PSM1 and PSS strains in 1:1 ratio to simulate high bacterial population size at leaf fall.

b. 0.6 l of inoculum was sprayed over 210 shoots (max 2.85x10^8 CFU per shoot)

c. Inoculated shoots were kept in polytunnel for 5 days

3) Five days post inoculation (dpi) leaves were manually stripped of the shoots and 180 most uniform shoots were randomly split into 3 experiments (Table 4.3.2) and treatments applied.

4) In experiment 1 and 2 the shoots were randomised in 6 blocks each block consisted of 4 shoots per treatment (24 shoots total). In experiment 3 shoots were randomised in 3 blocks with 4 shoots per treatment (12 shoots).

5) Each block consisted of a 20x30 cm tray with water saturated clean oasis foam. The shoots were pricked in the foam and enclosed in a large plastic bag to increase humidity.

6) The shoots are incubated in the poly tunnel at ambient temperatures to best simulate the natural infection process.

7) Frequency of successfully inoculated leaf scars per shoot was assessed on March 14 2019, which corresponded to and early bud break in the polytunnel environment. A dead bud next to a leaf scar was considered successful infection. The no of infected leaf scars and the total no of leaf scars on each shoot was counted and % infection calculated.

Table 4.3.2. Experimental set up in detached shoot assay. All shoots in all experiments were inoculated first with all leaves still attached (day 0); [a] bacterial spray was done with mixed bacterial culture (1:1) Pss 9097 and Psm1 5244 at total bacterial concentration of 5x10^7 CFU/ml; [b] phage cocktail comprised of phages: MR11, MR13, MR14, MR16, MR18; in 1:1:1:1:1 ratio, and total concentration of 10^6 PFU/mL; [c] Cuprokylt was applied at 7.5g/l. Leaves were manually stripped off the shoots to expose leaf scars.

	day 0	day 5							Shoots per group
EXP1	bacterial spray with leaves still attached	leaves removed to expose leaf scars			Water	30 min drying			24
					Phage [b]				24
					Cuprokylt [c]				24
EXP2			second bacterial spray [a]	30 min drying	Water	30 min drying			24
					Phage [b]				24
					Cuprokylt [c]				24
EXP3			second bacterial spray [a]	30 min drying	Water	30 min drying	third bacterial spray [a]	30 min drying	12
					Phage [b]				12
					Cuprokylt [c]				12

Small scale field trial: The aim was to prove that phage spray can reduce *Pseudomonas* population on trees in field condition. We have conducted a uniform inoculation of cherry branches/shoots followed by phage spray and quantification of bacterial population on the leaf surfaces in field conditions.

Plant material: We used trees of 4 different cherry cultivars, trees were of different ages and grown in different field conditions (Table 4.3.3).

Table 4.3.3. Plant material used in phage field efficacy trial.

Cultivar	No of trees	Shoots per tree	Tree age (years)	Grown in
Merton Heart	1	6	20+	field, uncovered
Roundel	1	6		
Van	1	6		
Sweetheart	9	2	3	polytunnel, covered

Experimental design

On cvs. M. Heart, Roundel and Van we marked 3 pairs of 1-year old shoots from 3 different positions around the tree. On smaller cv. Sweetheart trees only one pair of shoots was marked per tree. Shoots with no visual symptoms on their leaves were selected. Both members in each pair were from the same branch on the tree, ensuring similar background bacterial population and microclimate, a pair of shoots was therefore considered a block.

All shoots were uniformly and consistently inoculated with bacterial suspension (Pss 9097 overnight culture adjusted to 10e7 CFU/mL in water) using a calibrated hand sprayer. Six leaves per shoot were marked with a paint pen and sprayed on the upper and lower surface with inoculum. Inoculation was done in the morning between 10 and 11h.

Approximatlety 6 h after inoculation one shoot in each pair was sprayed with water (Control) or a mix of test phages (Table 4.3.4). Phages in stock concentrations were mixed in 1:1:1:1 volumetric ratio before diluting them 100-fold in water and spraying with calibrated hand sprayer on upper and lower surface of inoculated leaves. Water was sprayed on upper and lower surface of control leaves in the same way.

Table 4.3.4. Phages used in small field trial. Pss, *Pseudomonas syringae pv. syringae;* Psm1, *Pseudomonas syringae pv. morsprunorum* race 1; and Psm2, *Pseudomonas syringae pv. morsprunorum* race 2.

Phage name	Collection reference	Concentration (PFU/mL)	Host range
MR 10	Psm1 13	1.00E+10	Pss, Psm1 and Psm2
MR 13	Psm1 10	1.00E+08	Pss, Psm1 and Psm2
MR 16	Pss1	1.00E+08	Pss and Psm2
MR 18	Pss19.2	1.00E+12	Pss

Sampling

The leaves from experimental shoots were sampled: i) before the inoculation, one sample of leaves pooled from all shoots per tree, ii) immediately after inoculum has dried, two samples, one representing control and one phage designated shoots and iii) three days after inoculation / treatment. Three blemish free asymptomatic leaves were collected from each shoot. Two leaf discs were taken from each leaf, 6 leaf discs per shoot in total. Leaf discs were pooled in 2mL Eppendorf tube and homogenised in a Genogrinder shaker with steel ball bearings in water. Homogenate was serially diluted (10x, 100x and 1000x) and 10 uL of each dilution was plated in triplicates on *Pseudomonas* selective media (KingB + cephalexin + cyclohexamide). Two days after plating bacterial counts were recorded in each droplet on the plate and the size of bacterial population on leaves of each sample (6x leaf discs) were calculated.

Controls

To ensure high experimental quality several quality controls have been used. The effect of homogenisation on bacterial survival was tested using calibrated bacterial suspension before and after homogenisation was used in the test. The homogenisation method used had no detrimental effects on bacterial survival.

We sampled leaves from several shoots on each cultivar (several trees in case of cv. Sweetheart) before and after inoculation to ascertain background bacterial populations.

We have conducted in vitro plaque test to make sure that spraying itself did not inactivate phages. We have confirmed that the colonies counted were Pss by fluorescence under UV light.

Results

Phage characterisation by University of Reading

The four phage strains that had undergone a detailed host specificity assays in vitro have shown very different host specificity characteristics (Table 4.3.5). All strains have been able to infect at least one stain from the 3 main *Pseudomonas* pathovars infecting cherry (Pss, Psm1 and Psm2) and none of the beneficial *Pseudomonas* strains.

The best phage strain in terms of further studies and potential commercialisation was MR13. It could infect all tested strains pathogenic on cherry and also causal agent of bacterial speck of tomato (*P. s.* pv. tomato DC3000) and causal agent of angular leaf spot of cucurbits (*P. s.* pv*. lachrymans*) (Table 4.3.5). These results suggest that there are phages with various degrees of specificity present in nature and that careful selection is required to be able to formulate a mixture of phages that complement and reinforce each other's action.

Transmission Electron Microscopy was employed to image the morphology of the phage and estimate their size (Figure 4.3.1). All four phage exhibited typical head and tail morphologies associated with the order *Caudovirales*. The phages belonged to families *Myoviridae*, *Podoviridae* and *Siphoviridae*, with genetic material being dsDNA.

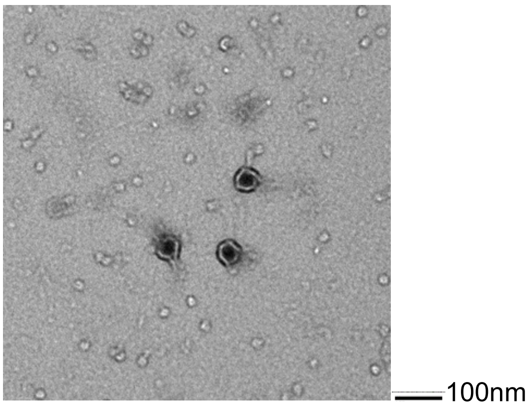


Figure 4.3.1. Morphology of phage MR14 determined by Transmission Electron Microscopy. This phage belongs to *Myoviridae*

Table 4.3.5. Summary of characterisation and bacterial host specificity results on a subset of phage isolates. Bacterial strains that were infected with a specific phage isolate are denoted with YES. Pss, *Pseudomonas syringae pv. syringae,* Psm1, *Pseudomonas syringae pv. morsprunorum* race 1, Psm2, *Pseudomonas syringae pv. morsprunorum* race 2.

Collection reference		Psm1 10	Psm1 11	Pss1	Pss 19.2
Isolated from		Leaf	Leaf	Soil	Soil
Phage size		Medium	Medium	Medium	Small
Phage family		Podoviridae	Myoviridae	Podoviridae	Podoviridae
Phage isolates		**MR13**	**MR14**	**MR16**	**MR18**
Pseudomonas isolates:					
Pathogenic strains	*PSS (9097)*	YES	YES	YES	YES
	Pss 9630	YES	YES	YES	YES
	Pss 9654	YES	NO	NO	NO
	PSM1 (5244)	YES	YES	YES	NO
	Psm1 5300	YES	NO	NO	NO
	Psm1 9326	YES	NO	NO	NO
	Psm1 9629	YES	NO	NO	NO
	Psm1 9646	YES	YES	YES	YES
	Psm1 9657	YES	NO	YES	NO
	PSM2 (5255)	YES	YES	YES	NO
	Psm2 5260	YES	YES	YES	YES
	Psm2 PSMR2 leaf	YES	NO	NO	NO
	Psm2 9095	YES	NO	NO	NO
	P. s. tomato DC3000	YES	YES	NO	NO
	P. s. lachrymans 789	YES	NO	NO	YES
Beneficial strains	*P. putida paw340*	NO	NO	NO	NO
	P. poae	NO	NO	NO	NO
	P. fluorescens ATCC 17400	NO	NO	NO	NO
	P. fluorescens F113	NO	NO	NO	NO
	P. fluorescens PFR37	NO	NO	NO	NO
	P. fluorescens PF.5	NO	NO	NO	NO
	P. fluorescens PF01	NO	NO	NO	NO
	P. fluorescens WCS 365	NO	NO	NO	NO

***In planta* assay on bean plants (University of Reading)**

At time zero, phage presence reduced the *Pss* population. There was a reduction between 10 and 100-fold in cfu per ml of bacteria for phage MR13, MR16 and MR18 compared to the control. Phage MR14 had a 10-fold reduction in cfu per ml of bacteria at 0h, but no reduction, thereafter, compared to the control. *Pseudomonas* population sizes (cfu/mL) were significantly reduced when phage isolates MR10, MR13 and MR18 were sprayed on bean leaves after inoculation (Figure 4.3.2). The reduction of *Pseudomonas* population due to these phages was significant already on day 0 and persisted until 96h after inoculation. Reduction was between 10 and 50-fold i.e., 90 to 95% reductions in comparison to the control. Phage MR14 reduced bacterial population by approximately 10-fold at 0h, but not in subsequent measurements stressing the importance of the right choice of phages and the importance of phage mixes to successfully control *Pseudomonas* populations *in planta*. In summary, this experiment yielded very positive results and has served as a proof of concept of the approach and another step towards field experiment on cherry trees.

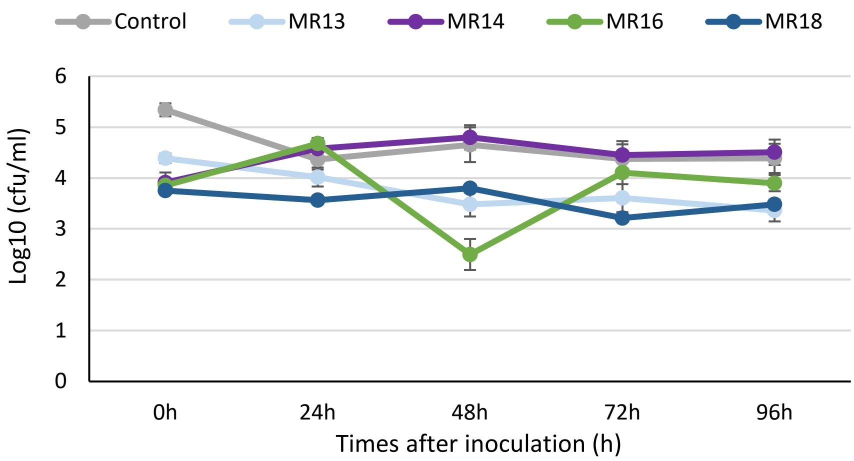


Figure 4.3.2. Population dynamics of *P. syringae* pv. *syringae* (*Pss*) on bean leaves with and without phage treatments with single phage MR13, Mr14, MR16 and MR18. Each value represents ±SEM

Detached shoot trials (set up 2018, assessed in 2019)

To mimic the leaf fall infections of the leaf scars we collected cv. Sweetheart one year old shoots from the orchards at leaf fall, inoculated them with a high dose of PSS and PSM1 bacterial mix and then sprayed either with water (neg. control), 5 phage mix, or Cuprokylt (pos. control). Shoots were incubated overwinter in fully randomised blocks in high humidity bags in polytunnel (Figure 4.3.3). We have tested that the spraying of the phages did not affect their activity by conducting in vitro plaque assay before and after the spraying (Figure 4.3.4).

Percentage of symptomatic leaf scars was assessed in March 2019. Altogether 3 different experiments were set up with different amount of disease pressure/inoculum used (Table 4.3.2). We did this to minimise the risk of having too high or too low disease pressure which could result in too much or too little disease for conclusive results.

Figure 4.3.3. An example of experimental blocks used in detached shoot test before and after bagging to ensure high humidity

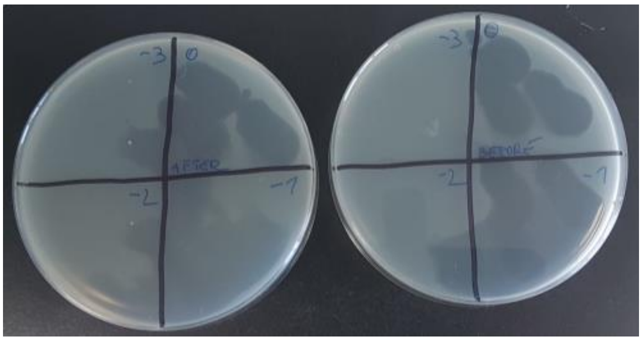


Figure 4.3.4. In vitro plaque tests to investigate if bacteriophages are affected by spraying method. Phage suspension was plated before spraying (right) and then passed though the sprayer as in standard experimental practice and plated after spraying (left). Infective phages causing plaques i.e. clear zones were present at the same concentration before and after spaying. Numbers on the figure represent dilution, 0=undiluted, -1 = 10x, -2=100x, -3=1000x (PHOTO: M. Papp-Rupar)

Despite our efforts we had relatively low disease incidence (between 5 and 20 % on average) and have failed to conclusively prove or disprove effectivity of phages in detached shoot assay. The main reasons were: i) very high variability of disease expression even in controlled conditions used and ii) partial or total contamination of a number of shoots with microorganisms infecting from the cut part of the shoot that was in oasis foam. We believe that large variability in disease expression within each experiment and treatment group (Figure

4.3.5) could be in part due to pre-existing infections of the buds from the field and variability in senescence of different shoots. The more senescent the leaves on the shoot at the time of stripping and inoculating, the less likely is that infection will take place due to natural defence layers produced by the plant. The contamination issue became apparent upon assessment where some shoots had no surviving buds and others only a few surviving buds on the tip of the shoots while the great majority of the shoots had only sporadic infection of few random leaf scars/buds. A closer inspection revealed that these highly infected shoots were most likely infected from the base of the shoot that was in contact with water infected with inoculum or other fungal/bacterial microorganisms rather than through leaf scars. These base shoot contaminations resulted in vascular tissue under the bark to change its colour from green to dark brown. However, because leaf scars could have been infected first followed by contamination from the base we have not excluded these shoots from the analysis.

If we look only at control group in the three experiments, we can see a trend in disease incidence with experiment 1 (single inoculationevent) having the lowest incidence below 5 % on average while experiment 2 and 3 (2 and 3 inoculation events respectively) with multiple inoculations had much higher incidence between 10 and 20% (Figure 4.3.5).

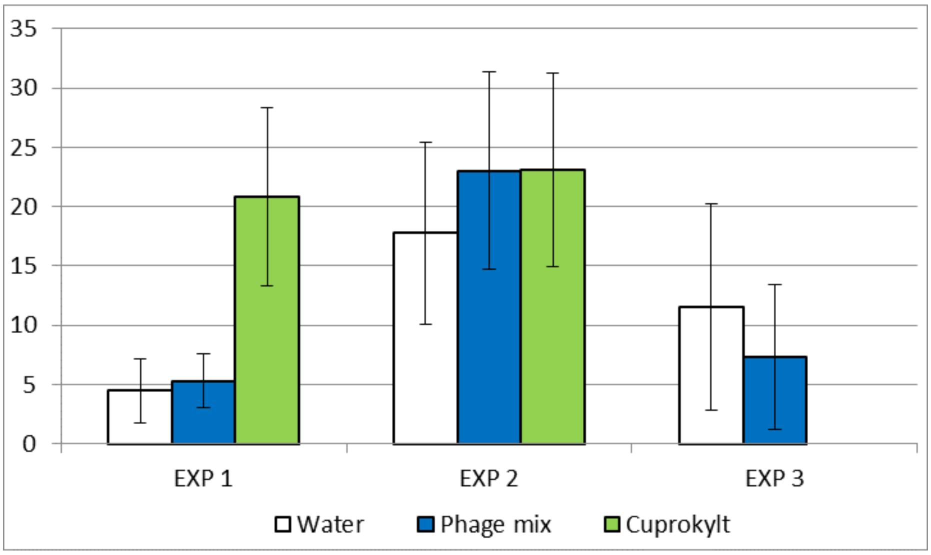


Figure 4.3.5. Disease incidence as an average percent of infected leaf scars per shoot in three different experiments. Error bars present +/- 1 standard error of the mean. In exp 1 and 2 N= 24, in exp 3 N=12

Overall, the difficulties with this assay were too great to allow for any conclusions on phage effectivity to be drawn from it. We expected problems with this assay and have conducted it mainly as a practise in case field permit for testing phages in 2019 would not be approved and we would need to continue with detached assays in completely confined environment. We have however managed to turn this assay into a valuable resource to support a chemical regulations directorate (CRD) application for a field trial permit for bacteriophage. We submitted a permit application with all the information on the phages we are using to HSE/CRD in Feb 2019. In March 2019 they responded with concerns that spraying phages in high amounts might result in their accumulation in the environment which could cause adverse effects despite their high specificity for pathogenic strains of *P. syringae*. We have managed to resolve this by conducting a blind experiment where shoots from the detached assay were coded and sent to University of Reading to quantify the amount of phages present on shoots after overwinter incubation. We showed that phages are below detection on healthy phage sprayed shoots even after incubation in stable atmosphere, protected from UV and rain. Phages were found in high quantity on shoots with obvious infected buds irrespective of treatment. This proved that phages are actually present in low amounts anyway and that spraying high quantities of phages does not resolve in accumulation even in ideal conditions. With this additional data CRD granted COP 2019/00527: PERMIT FOR TRIAL PURPOSES: ADMINISTRATIVE APPLICATION for field testing of UK phages in NIAB EMR orchards valid until 30 June 2022. This permit has enabled us to conduct orchard assays.

Small scale field trial

Using the controls above we were able to detect problems with the phage solutions used. Overall, we run the same experiment three times. In the first iteration (30/8/19) we used phages prepared by University of Reading in Aug 2018. In this case the concentration of infective phages as determined with in vitro plaque method was too low (below 10^6 in stock) to be efficacious. Indeed, in this experiment we recorded no differences between water and phage treated leaves in terms of bacterial population size. By 27/9/19 University of Reading prepared new phages and sent them to NIAB EMR. Another experiment was set up only to realise that phages must have been inactivated during the shipping. Matevz Papp-Rupar has therefore gone to the University of Reading to learn how to prepare highly concentrated and pure phages on site at NIAB EMR. Infectious phages ready for experimental were successfully prepared by 15/10/19. This coincided with the early leaf fall period in 2019 and limited the possibility of several successful experimental repeats in 2019. Here we only show the results of the third experiment where all experimental quality controls yielded expected results confirming validity of the test (Figure 4.3.6).

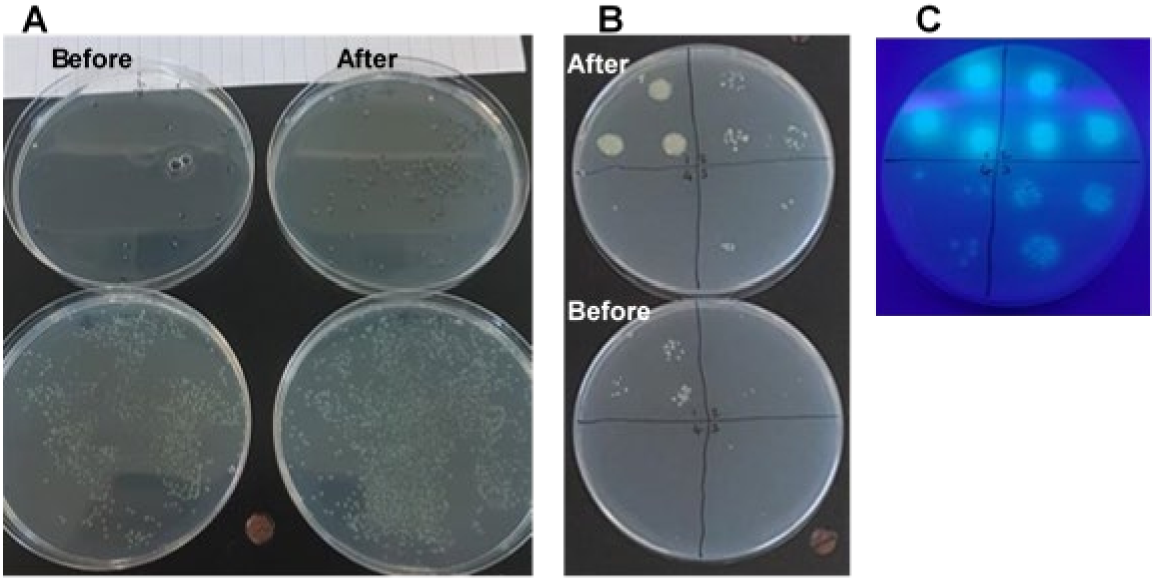


Figure 4.3.6. Experimental quality controls used. A) The effect of Genogrinder homogenisation on bacterial survival. The same calibrated bacterial suspension was plated before (left) and after the homogenisation procedure (right). No significant reduction of bacterial count was observed after homogenisation. B) The bacterial population on leaves was measured before and after inoculation with *Pss 9097* strain inoculum. The numbers in each quarter correspond to dilution factor 10x (1), 100x (2), 1000x (3) and 10,000x (4). The population after inoculation was about 100x higher than the background population before the inoculation. C) Confirmation of fluorescent pseudomonads under UV light. After counting, the plates were inspected under UV light to confirm that the colonies counted were fluorescent Pseudomonas as in inoculum.

Phage mix treatment has reduced the *Pseudomonas* population size on the leaves of cvs. Roundel and Van by appx 10-fold (90 % reduction) in comparison to water treated control (Figure 4.3.7). No reduction in population size vs water control was observed on cvs. Sweetheart and Merton Heart leaves (Figure 4.3.7). We believe that the apparent failure to reduce population size on the latter cvs. is mainly due to the physiological state of the leaves. Cvs. Sweetheart and Merton Heart had both fairly senescent yellowing leaves which exhibited leaf spots whilst cvs. Roundel and Van had very healthy and green leaves with no obvious signs of senescence at the time of the experiment (15/10/19).

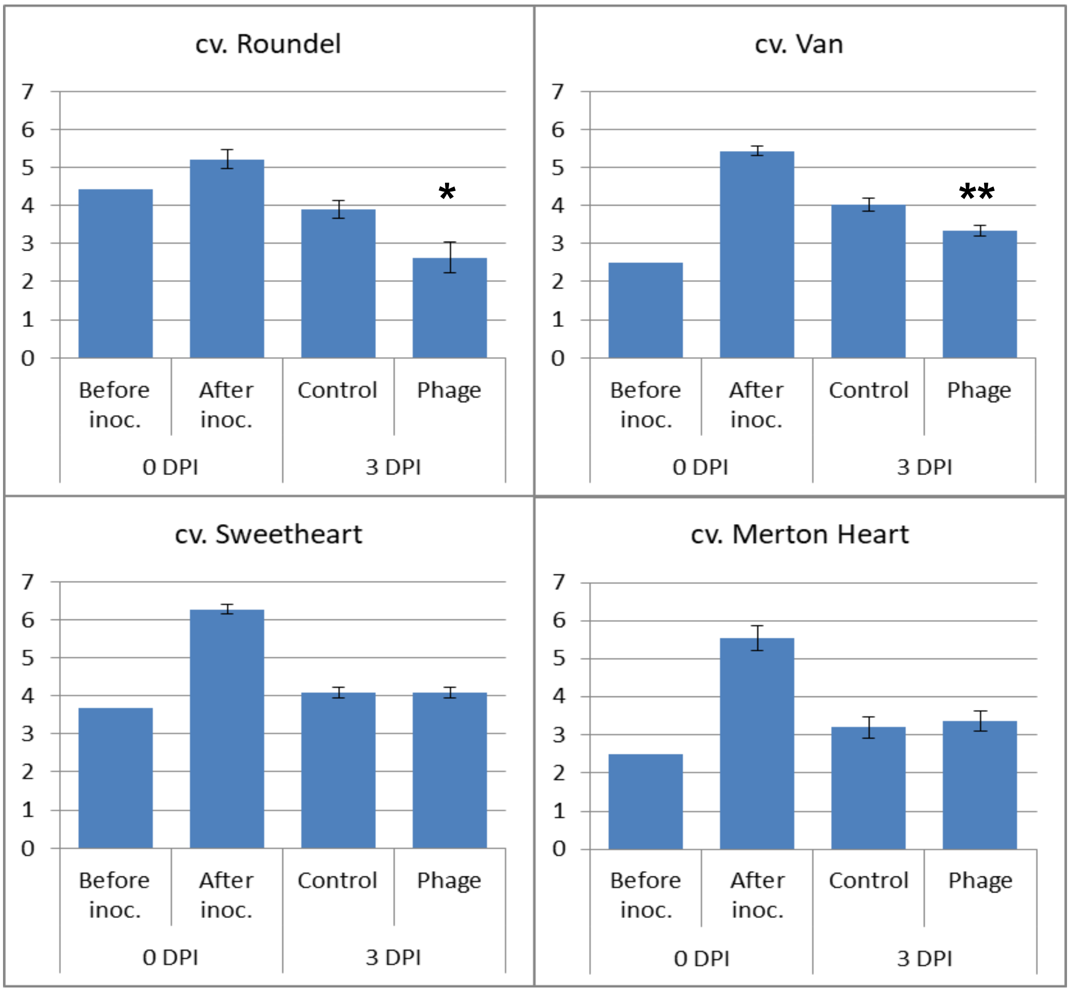


Figure 4.3.7. Results from the phage spray test in field conditions on four different cherry cultivars. Log10 *Pseudomonas* population sizes +/- SEM are shown. Populations were measured before and immediately after inoculum had dried on day 0 (0 DPI). Phage treatment was done on the same day, several hours after inoculation. *Pseudomonas* population were measured again at 3 days after inoculation (3 DPI) to determine if phage treatment reduced population sizes on leaves in comparison to water control. **, significantly different from control (t-test, p val=0.04); *, population reduced but less stat. significantly (t-test, P val=0.07)

On senescent leaves of Sweetheart and Merton Heart the *Pseudomonas* population levels applied by inoculation probably could not be sustained and, by day 3, reduced back to the background levels present before inoculation or lower (Figure 4.3.7). The bacteria remaining on the leaves after three days were probably already in the leaves prior to the inoculation and therefore less accessible to a single phage spray. Similarly, by day 3 *Pseudomonas* populations on water treated leaves of cultivar Roundel reduced just below the levels before inoculation indicating that the populations on leaves were diminishing during the experiment. Phage treated leaves however had far lower bacterial populations at day 3 than control (Figure

4.3.7) indicating that on healthy looking green Roundel leaves the majority of the *Pseudomonas* was on the surface, readily accessible to phage infection. The best example of phage efficacy was cultivar Van. Population at day 3 did decrease in comparison to population immediately after inoculation but remained higher than before inoculation (Figure 4.3.7). The population size before inoculation however was only measured once as a pooled sample representing many shoots on the tree and should therefore be taken as a guide rather than a set point. This does however suggest that bacteria were able to survive and multiply on the cv. Van leaves very late in the season, at fairly low temperatures of around 10 ̊C and high humidity (Figure 4.3.8). The phage treated cv. Van leaves had clearly reduced *Pseudomonas* population in comparison to the control (Figure 4.3.7) showing the efficacy of phages on leaves. These results are comparable to bean plant assays done in controlled environment conditions at The University of Reading where similar reduction of bacterial population (~10 fold or ~90%) was achieved. Despite these trials using unformulated phages diluted only in water and the difficulties faced in 2019 trials meaning that the phage were only applied once in field conditions, and late on in the season this work has provided some encouraging results. Commercial phages are formulated with UV and desiccation protection agents to extend and improve their action.

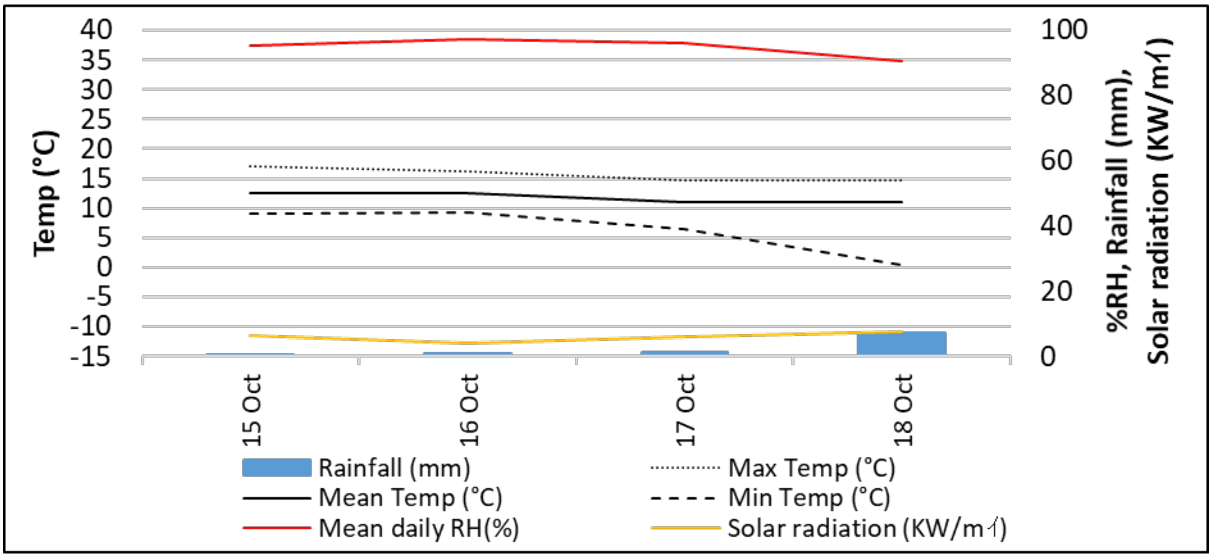


Figure 4.3.8. Weather data collected at East Malling weather station during the small scale field experiment

Discussion

The main effort of this year's work on bacteriophage control of bacterial canker was to establish the *in planta* efficacy of bacteriophages. We have attempted this in 3 different assays namely; a detached shoot assay, an *in planta* assay on beans in controlled environment and a small scale field trial on cherry trees. Although all 3 variants of detached shoot assays yielded inconclusive results on phage efficacy, we did manage to use them to support and successfully obtain the field trial permit from CRD/HSE which will enable us to field trial UK phages until 2022. This should enable us to put the phages through real efficacy testing in field conditions.

The first *in planta* assays were done by University of Reading where phage efficacy to reduce *Pseudomonas* populations were tested on bean plants. Four different phage isolates were sprayed on Pss inoculated leaves as single phage isolates and three of them reduced Pss populations significantly to up to 4 days after spraying (until the end of experiment). One phage isolate (MR14) only reduced the Pss population on the leaves on inoculation day. Afterwards the bacterial population sprayed with MR14 phage recovered to control levels within 24h (Figure 4.3.2). This signifies that initially bacteria were infected and inactivated but on subsequent days there was either a problem with phage replication which led to phage depletion or bacteria very quickly acquired resistance to the phage MR14. This brings to light the potential difficulties with single phage strain treatments. As a result, we used a mix of 4 phage isolates in the small-scale cherry field trial in order to avoid the emergence of bacterial resistance. Multiple phages will infect in a slightly different manner and it is very unlikely that a bacterial cell would develop resistance to all phages simultaneously. In the cherry trial we observed Pss population sizes at 3 days post phage treatment of cherry leaves to be able to detect emergence of bacterial resistance by 3 DPI. Mixed phage treatment significantly reduced Pss populations by approximatley 10-fold (90% reduction) on 2 cultivars; Roundel and Van. The reduction of bacterial populations on cherry leaves in the field was comparable to reduction on bean leaves in the constant environment of a growth chamber. Moreover, a single phage mix treatment achieved similar efficacy as 6 applications of AHDB 9829 (biocontrol agent) or 3 applications of AHDB 9936 (copper-based treatment) sprays which were shown to be the only efficacious products in the SCEPTREplus trials (SP19) in 2019. This is very encouraging in terms of commercial phage treatments and signifies that even unformulated phages diluted in water are robust enough to control bacterial populations in field conditions to the same levels as currently best available products. However, a controlled, replicated trial enabling direct side by side comparison in the same conditions is needed to confirm this. For good control in higher light levels and lower humidity in the summer, the use of UV and desiccation protection adjuvants will have to be used in the formulation of the final phage products to extend their efficacy.

Detailed characterisation of the four selected phage isolates with electron microscopy and i*n vitro* plaque assays on 23 different pathogenic and beneficial *Pseudomonas* strains have shown that the tested phages could not infect any beneficial *Pseudomonas* stains. The infection of non-*Pseudomonas* strains either in the orchard or in the post harvest chain is therefore very unlikely. This high specificity and the fact that phages are made of non-toxin protein and nucleic acids generally recognized as safe (GRAS) (Zacek et al. 2014) mean that they could be sprayed at any time in the season, and will not require a harvest interval meaning that crops can be treated right the way up to and including the harvest period. The adjuvants used in phage formulations will have to be similarly safe as well to enable year-round use. Alternatively, simple adjuvant free phage formulations could be used during the harvest if necessary.

Conclusions

In conclusion, this year's experiments provide encouraging results that the right combination of phage isolates in the right formulation could provide efficient and environmentally friendly control of bacterial canker for growers. Further study and involvement of plant protection product companies is required at this stage to push phages closer to market in the UK. Phage biocontrol products are already available for food borne human pathogens and plant pathogens (Zaczek et al. 2014). Listex P100 (Micreos, NED) is a well-known commercially available phage preparation developed for control of food-borne *Listeria monocytogenes*. Agriphage (OmniLytics, USA) is a registered product in the US for the control of bacterial diseases of tomatoes and peppers (*X. campestris* and *P. syringae* strains) (Buttimer et al. 2017). Erwiphage (Enviroinvest, HU), a product consisting of 2 different phage cocktails has been registered to control of fire blight (*Erwinia amylovora*). APS biocontrol in Scotland has developed a bacteriophage-based wash solution (Biolyse) for potatoes tubers, which is to be used for prevention of soft rot during storage (Buttimer et al., 2017). Availability of these commercial products is clear evidence that obstacles connected to market acceptability and legislation of phage-based biocontrol products can be overcome.

Future Work

We believe that the evidence presented here is sufficient to lend further inquiries into phage control of bacterial canker. The most important experiment still needed to aid commercialisation efforts would be season long experiment where phage sprays would be compared to untreated control and copper standard in a high disease pressure orchard. Such experiment could answer many research questions important for commercialisation and route to market such as: i) the effect of phages on *Pseudomonas* population sizes and bacterial strain composition across the seasons, ii) effect on different canker symptoms across the season, iii) frequency of phage resistance in bacteria in the field, vi) phage evolution in the field and v) the effect on non-target bacterial species and other disease in the field.

Since the bacterial canker on prunus was designated as of medium importance in the last AHDB tree fruit pest and disease research call with many higher priority pathogens listed, it is unlikely that the work on phages will be supported directly through AHDB in the short term. The urgency and potential impact of the disease if unchecked will require involvement of commercial partners from plant protection product producer to co-finance required research and tackle phage bulking up, shelf life, formulation and approval for commercial use. The fact that we obtained CRD approval for testing and that they are comparable products on the market in USA and EU are both very positive signs for future commercialisation of phage product in commercial horticulture in the UK.

Objective 4. Stone Fruit Diseases

Task 4.4. Tunnelling Cherries – observational study to assess effects of covering cherries on bacterial canker development

Introduction

Bacterial canker (*Pseudomonas syringae* pv. *morsprunorum* and *P. s*. pv. s*yringae*) in *Prunus* species infect trees in autumn through buds, wounds or leaf scars at leaf fall. These infections can develop into overwintering cankers, which lie dormant until spring when it re-emerges to infect the blossom. This pathogen is able to spread by water splashes during rain events. If water splashing can be reduced then there is the potential to limit the spread of this pathogen.

Anecdotal evidence have suggested that leaving the cover of tunnelled cherries on for longer after harvest may result in reduced bacterial canker development when compared to the standard current practice of removing the covers immediately after harvest. This current practice opens up the tunnel allowing light to reach leaves, which may positively affect potential yield in the following year. Observations on one grower site in Scotland where the covers were left on until after harvest seemed to suggest that there was less canker and a better yield the following year.

This observation trial on two grower sites assessed the effects of altering the timing of covering of cherry tunnels on disease incidence.

Materials and Methods

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

Trial Sites

Trial Site 1 – Herefordshire. Four tunnels were selected for the trial allowing comparison between both pre-blossom/post-blossom covering and post-harvest/leaf fall uncovering.	
Trial Site 2 - Kent. Two tunnels were selected for the trial and only the post-harvest covering was possible on this site.	

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

Standard treatments for pests, foliar disease and nutrients were applied to all plots throughout the season. In each tunnel ten trees were marked and recorded for baseline incidence of canker (November 2018 – Hereford). The trees were assessed again in July 2019, with a final assessment in October 2019.

Notes were also made of mummified fruit left on the tree. The cankers on the trees were monitored until October 2019 (25 – Hereford, 23 – Kent). Yield data was not able to be obtained for individual tunnels in the trials. The change in the number of cankers at each site was calculated. Statistical analysis was not performed on the data, as this was not a fully replicated and randomised trial.

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

Treatment	Description	Site 1 - Herefordshire	Site 2 - Kent
Treatment 1 (Control 1)	Pre-blossom covered, Post-harvest uncovered (BCHU)	Yes	No
Treatment 2	Pre-blossom covered, Post-harvest covered (BCHC)	Yes	No
Treatment 3 (Control 2)	Post-blossom covered, Post-harvest uncovered (PCHU)	Yes	Yes
Treatment 4	Post-blossom covered, Post-harvest covered (PCHC)	Yes	Yes

Figure 4.4.1. Cankers noted in observation trials 25 October 2019

Results

From 2018 to 2019 at the Herefordshire site there was the largest increase in the number of new cankers in the tunnel that had been covered after blossom and uncovered just after harvest (PCHU). There was no observed change in the number of cankers recorded in the tunnel that had been covered before blossom and uncovered at leaf fall (BCHC). The tunnel that had been covered after blossom but uncovered at leaf fall (PCHC) also resulted in a reduction of new cankers forming when uncovered later in the season.

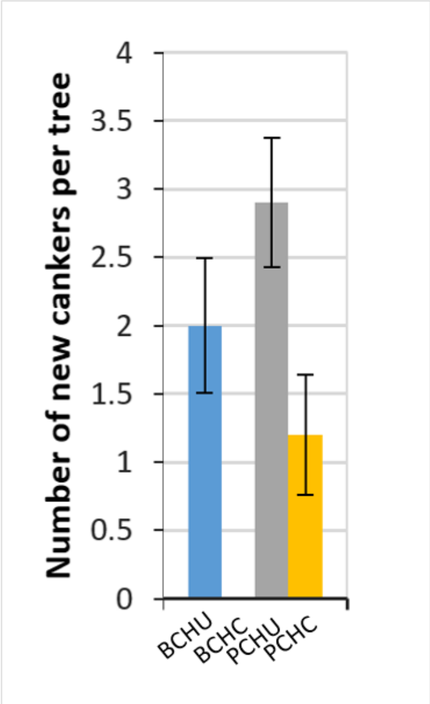


Figure 4.4.2. Mean new canker infections per tree of cherry at Site 1 from 2018 to 2019. BCHU = Pre-blossom covered, Post-harvest uncovered; BCHC = Pre-blossom covered, Post-harvest covered; PCHU = Post-blossom covered, Post-harvest uncovered; PCHC = Post-blossom covered, Post-harvest covered.

At the second site in Kent there was a lower total number of cankers present in the tunnels that had been left covered during the post-harvest period until leaf fall compared with those that were uncovered just after harvest (Figure 4.2.3). The difference between those that had been covered late into the season compared to those that had the tunnels removed after harvest was obvious at the late assessment dates at both sites, with oozing cankers visible in those that were not covered (Figure 4.4.4).

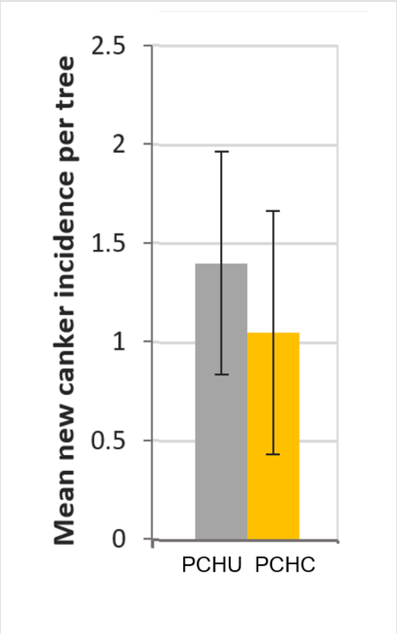


Figure 4.4.3. Mean new canker incidence per tree of cherry at Site 2 from 2018 to 2019. PCHU = Post-blossom covered, Post-harvest uncovered; PCHC = Post-blossom covered, Post-harvest covered.

Figure 4.4.4. Oozing canker infection in uncovered tunnel (left) compared to dry canker infection in trees left tunnelled until leaf fall in Kent.

Other observations

Mummified fruit were noted in all tunnels regardless of when the tunnels were put on or taken off during the trials. There was no difference in the numbers of mummified fruit found on the trees in any of the treatments. There was also no difference in the amount of shot holing in the leaves of the trees in the tunnels.

It was noted during the assessments for canker on the cherry trees that there were no germinating weed seedlings in the tunnels that had been left covered after harvest, compared with numerous seedlings in the tunnels that had been uncovered after harvest (Figure 4.4.5).

Figure 4.4.5. Weed seedling germination in tunnels at Site 1. Left – visible germination in uncovered cherries; Right – no germination in covered cherries

Discussion

At the Herefordshire site there were observational differences in the number of new cankers found in the cherry trees depending on when the crop was covered. Those that had been covered for the longest period had no overall change in the number of cankers on the trees. Those that had been covered for the shortest period, i.e. only from flowering to harvest, had the highest increase in the number of cankers. The trees covered from flowering until leaf fall had the second lowest increase in canker numbers. Covering the trees before blossom seemed to have some impact on the number of new canker infections, presumably occurring during flowering, although the overall impact was greatest in maintaining cover post-harvest.

The Kent site seemed to show a similar trend that leaving cherry tunnels covered until leaf fall can reduce the number of new cankers forming, particularly on peripheral branches. The differences were not as noticeable at this site. This could be due to the high disease pressure to start with and the tunnels being removed earlier in 2019.

The increased time spent being tunnelled will have reduced the amount of rain reaching the cherry trees, particularly in the key infection period of leaf fall when water splashes are most likely to transmit the pathogen to the wounds formed. Tunnelling during this time appeared to have more effect than just tunnelling prior to blossom, which is also another key infection period. The greatest observable effect combined tunnelling during both infection periods to protect the cherry trees.

Conclusions

- The results from this observational trial suggest that leaving tunnels covered until leaf fall can help to reduce canker progression in trees that already have canker.
- Having cherry trees covered prior to blossom as well as after harvest appeared to have the largest effect on the canker progression.
- Leaving the crop covered until leaf fall may also help to suppress weed seedling germination during late summer and early autumn.

Objective 7 - Improve Reliability of Natural Enemies

Task 7.1 Enhance and accelerate the natural ecology in newly planted orchards

Introduction

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

In 2017, we applied interventions to newly planted orchards to establish this beneficial ecology more rapidly.

In 2018, the seed mix applied to treated plots was successfully established in most orchards and caused evident changes in vegetation diversity, evenness, and structure on each replicate site. Not all species in the seed mix established, but of those that did, red clover and yarrow were the most common with a higher percentage of ground cover. As expected, sward height on treated plots was significantly higher than in the un-sown alleyways.

Subsequently, seed mix had varied effects on arthropod abundance in treated plots compared to untreated. Regarding pests, fewer aphids were observed in treated plots during spring but not summer. No apple leaf curling midge damage was recorded on treated plots compared to untreated and there were fewer fruits with codling moth damage in treatment plots compared to untreated, including significantly fewer codling moth stings on the dropped apples. With regards to natural enemies, there were lower numbers of predatory mites and fruit tree red spider mites in treated plots compared to untreated. However, the opposite was observed for rust mites and spider mites. There were significantly more predatory spiders than earwigs in earwig refuges deployed in the treated plots. Predatory spiders were the most common arthropod in all seasons. The use of attractant sachets significantly increased hoverfly adults in the treated plots, possibly by pulling hoverflies from untreated plots. However statistical findings from 2018's study should be interpreted with caution since numbers of arthropods were low.

In 2019, we aimed to continue the monitoring of arthropod diversity in all orchards and the evolution of the seed mix applied in 2017.

Aim

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

In 2019 we also identified predatory spiders to family and species where possible to discriminate between different functional groups (predation strategies).

Methods

Sites: The trial took place on six replicate apple orchards (blocks) (Table 7.1.1). Each block was divided into 2 plots: a treated plot (0.25 ha) and an untreated plot (Fig. 7.1.1). Plot position was randomised to avoid position effect bias. Minimum distance between blocks was 1 km.

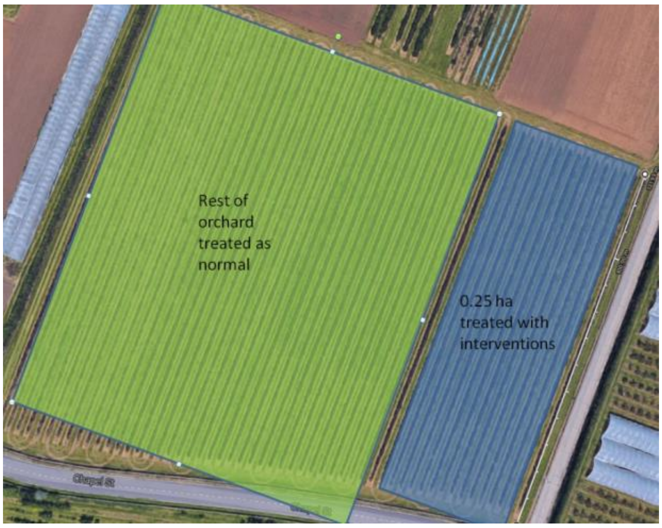


Figure 7.1.1. Example of an experimental block during the enhancing orchard ecology trial 2017 to 2019. Blocks were divided into 2 plots: an untreated plot (green), lacking ecological enhancement interventions and a treated plot (blue) with ecological enhancement interventions

Table 7.1.1. Orchards (blocks) and alleyway sowing dates for the enhancing orchard ecology trial 2017 to 2019.

Orchard (block)	Variety	ha	spacing (m)	Trees planted	Notes/ planting	Row length (m)	Sown 2017
1	Jazz	2.6	3.35x1 or 1.2	Feb 2017	Every other row for 5 rows (10 rows)	144	Apr
2	Gala	2.23	3.5x 1.25	Dec 2016	Every row (7 rows) for 0.25 ha of orchard	95	May
3	Jazz	1.54	3.5x 1.25	Dec 2016	Every row (11 rows) for 0.25 ha of orchard	60	May
4	Gala	1.3	3.5x 1.5	April 2017	Every third, 0.25 ha, 5 rows	109	May
5*	Jazz	1.13	3.25x 1.2	Jan 2017	4 rows every other row	144	Oct
6	Variety	2.28	4x1.5	May 2017	0.4 ha sown in every row	250	May

*Block 5 was not used for the spring, summer and autumn assessment 2019.

Treatments (Table 7.1.2.)

Seed mixes: In 2017, the seed mix (Table 7.1.3, with some modifications) was sown in treated plots at all blocks.

Table 7.1.2. Ecology enhancement interventions applied to treated plots during the enhancing orchard ecology trial 2017 to 2019.

Treatment	Detail	Target beneficial	Improve	Date implement
Alleyway sowings	Alleyway included Yarrow, Ox-eye daisy, Bird's foot trefoil, Self-heal, Red Campion, Red clover.	Pollinators, parasitoids, anthocorids, predatory spiders	Pest control inc. aphids, tortrix. Establish pollinator networks	At orchard establishment
"Wignest"	Innovate UK Bioactive predator refuge	Earwigs, predatory spiders, ladybirds	Aphids, caterpillar, codling moth	Autumn 2017
Hoverfly attractant	From AHDB TF 218	Hoverfly larvae	Aphid	From 2018 (2x applications, May/July)

Table 7.1.3. Suggested and tested seed mix for orchard alleyway planting in the 0.25 ha in the treated plot of the 6 blocks. NB to be mixed with high percentage (>70%) of non-competitive grasses (not specified in protocol).

Species	Common Name	Suggested mix %	Block 2 & 3 %	Block 4 & 6 %
Forbs species				
Achillea millefolium	Yarrow	2.0	3	2
Centaurea nigra	Knapweed	29.4	29	6
Leucanthemum vulgare	Oxeye daisy	5.9	6	4
Lotus corniculatus (wild type)	Birds foot trefoil	23.5	13	2
Prunella vulgaris	Selfheal	11.8	12	7
Silene dioica	Red Campion	11.7	12	6
Trifolium pratense (wild type)	Red Clover	15.7	10	1
Grasses species				
Agrostis capillaris	Highland common bentgrass	-	2.5	5
Cynosurus cristatus	Southland crested dogstail	-	2.5	10
Phleum bertolonii	Teno smaller catstail	-	2.5	5
Festuca rubra ssp. commutata	Chewings fescue	-	2.5	-
Poa pratensis	Evora smooth-stalked meadowgrass	-	5	16
Festuca ovina	Bornito sheeps fescue	-	-	20

Figure 7.1.2. Establishment of the seed mix sown in a treated plot at block 4 2017 (left) and 2019 (right)

Figure 7.1.3. Establishment of the seed mix sown in treated plots at blocks 2 (left side) and 3 (right side) 2017 (top) and 2019 (bottom)

"Wignests" (earwig refuges); Courtesy of the Innovate UK Bioactive predator refuge project (NIAB, NRI, WorldWide Fruit Ltd., Russell IPM, Fruition PO Ltd., Agrovista UK Ltd.), earwig refuges were deployed in the centre of treated plots at each block between 27 September and 13 October 2017 and left throughout the project's duration. One refuge was hung per tree between the tree and the support pole, attached to each tree by hanging onto the plastic tie using the hook provided on the refuge. Approximately 464 were deployed at each treated plot. The number of rows and length of row treated varied according to the layout of the orchards; 6 rows at Blocks 1 and 2, 9 rows at Blocks 3 and 4 and 6 - 4 rows at Block 5 (re-sown 2018). Of note, at Site 1 earwigs were already present in the yellow tree ties in 2017.

Hoverfly attractant: Hoverfly attractant sachets formulated by NRI consisted of a 5 x 5 cm polythene sachet containing 1.5 ml of methyl salicylate, phenylethanol and (E)-beta-farnesene and were deployed in treated plots end of May, then replaced once mid-July in 2018 and 2019. Sachets were evenly spaced at a rate of 180 sachets per hectare. To assess the presence of hoverflies White sticky traps were also deployed in early-August 2018 and mid-July 2019.

Crop husbandry involved the growers' standard practices.

Assessments (2019)

In 2019, three assessments were made in the central rows of untreated and treated plots at each block (except Block 5 since the mix failed to establish in 2017 and so was re-sown in April 2018). Assessments involved the following:

May

- Photographs of sward and tree stage were taken.
- Solitary bee nesting sites were assessed by examining the ends of 8 rows in the herbicide strip before the first tree (m^2)
- 30 shoots were examined for the presence of aphids and total number of aphids in each shoot counted.
- 30 earwig refuges (treated plots only) were held over a white tray and tap sampled 3 times then predators recorded. Predatory spiders were collected and brought back to the laboratory for identification to family, or species where possible.
- 30 branches in different trees were tap sampled for other predators. Predatory spiders were collected and brought back to the laboratory for identification to family, or species where possible.

- Deployed hoverfly attractant sachets (treated plots only).

July

- Photographs of sward and tree stage were taken.
- Percent coverage of grass species, forb species, moss and bare ground in alleyways were estimated using 10 measurements of 50 x 50 cm quadrats.
- 30 shoots were examined for the presence of aphids and total number of aphids in each shoot counted.
- 30 young leaves were collected and brought back to the laboratory to assess for the presence of rust mite and spider mite by light microscopy.
- Five, drop disk measurements of sward height were taken to estimate average sward height.
- All fruit on 30 trees were examined and the number of fruit with damage caused by first generation codling moth, capsid, tortrix and Rhynchites was counted. The total number of apples on sampled trees was also recorded.
- 30 branches in different trees were tap sampled for other predators. Predatory spiders were collected and brought back to the laboratory for identification to family, or species where possible.
- 30 trees were tap sampled for earwigs and other predators at night.
- Hoverfly attractant (treated plots only) was replaced. 5 white sticky traps were placed in the center of each plot for 1 week, after which the traps were collected and hoverflies counted.

August

- Photographs of sward and tree stage were taken.
- 30 trees were tap sampled for other predators. Predatory spiders were collected and brought back to the laboratory for identification to family, or species where possible.
- All fruit (including dropped fruit) from 30 trees were examined and the number of fruit with damage caused by second generation codling moth, capsid, tortrix and Rhynchites was recorded. The total number of apples on and under sampled trees was also recorded.

Regular communication was made between NIAB EMR staff and the growers/advisors.

Data loggers were deployed at each block to monitor temperature and humidity throughout the trial period.

Results

Seed mixes

Throughout the 3-year trial, seed mixes established to varying degrees in treated plots with most blocks increasing in forb diversity, evenness, and structure. The exception was Block 5 where the mix was re-sown in April 2018 (Table 7.1.4).

In 2018, from the sown seed mix, red clover and yarrow were the most successful species, with highest ground coverage. Red Campion also developed well but not on all blocks. Vegetation cover also changed from spring to summer, dominated by an increased coverage of red clover at most blocks, yarrow cover did not increase as much. Red Campion developed in spring but was only recorded in the summer survey on one block and at a very low percentage cover (1%). In 2018, some naturally established species remained in treated plots e.g. chickweed. In untreated plots, grass, natural clover, and plants from the Plantago genus were the most common species observed in both spring and summer (see Appendix 7.1).

In 2019, a single, more detailed seed mix assessment was made in the summer. During this assessment, all forbs and grasses were identified to species level. At most blocks, coverage of the seed mix had increased since 2018 (Table 7.1.4). Red clover was still one of the most well-established species along with common knapweed. In untreated plots, natural clover and unsown grasses were still most common. Sward was higher in treated plots compared to untreated, though not significantly; in 2018 the same trend was significant *(P* = <.001, Fig. 7.1.4).

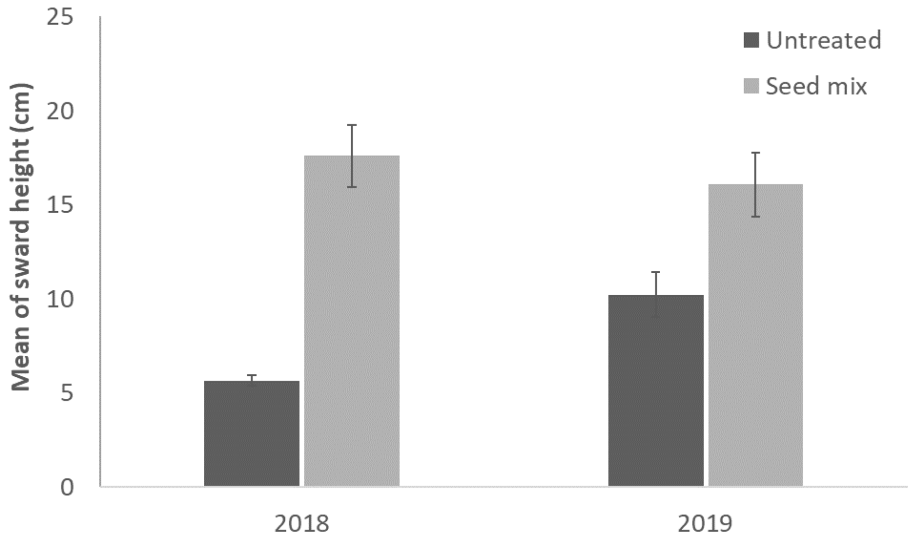


Figure 7.1.4. Sward height (cm) measured using the drop disk method in summer 2018 and 2019 (N=10)

In 2019, the vegetation data was analyzed to test vegetation diversity against the untreated (Fig. 7.1.5). Three tests were performed on the data; *Observed species* accounting for the number of species present in each sample/treatment, *Chao1 index* looking at the relative abundance of each species and *Simpson index* which takes into account the number of species present, as well as the relative abundance of each species. Although distinct species were found between treated and untreated plots, similar diversity indexes were obtained for both (Fig. 7.1.5), with no significant difference for all analyses.

Table 7.1.4. Percent vegetation cover of seed mixes, per treated site, in spring and summer 2018 and summer 2019.

Site	Season	Coverage of seed mix 2018 (%)	Coverage of seed mix 2019 (%)
1	spring	61.5	-
	summer	81	64.5
2	spring	50	-
	summer	60	81.6
3	spring	29.5	-
	summer	48	83.6
4	spring	47	-
	summer	42	53.9
5	spring	-	-
	summer	-	-
6	spring	15.5	-
	summer	22	43.1

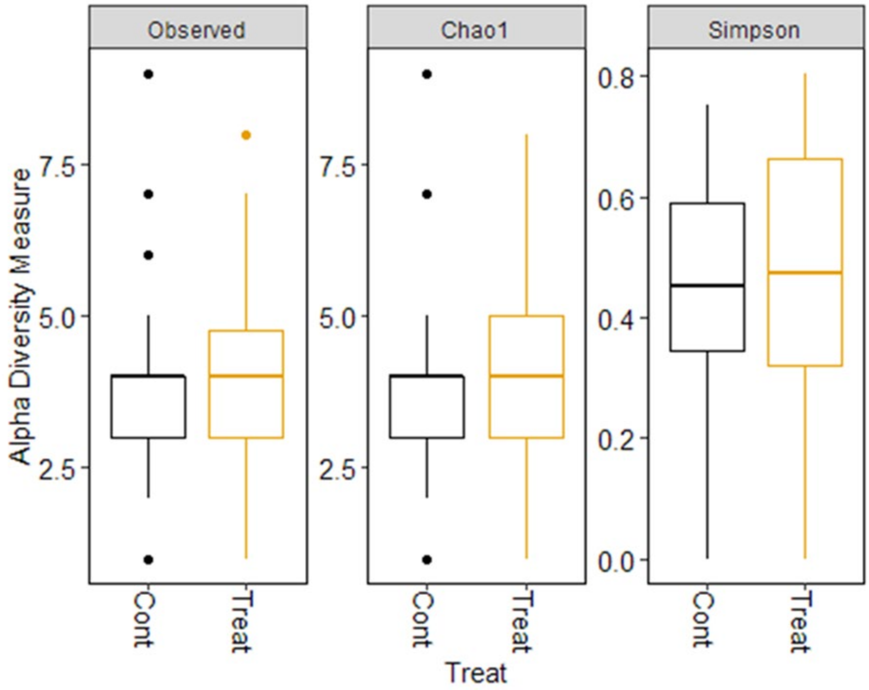


Figure 7.1.5. Vegetation diversity of treated (Treat) plots compared to the untreated (Cont) plots using 3 diversity index tests

Solitary Bee Nesting

Overall, the average number of solitary bee nests was not significantly different in both 2018 and 2019 between treated and untreated plots.

Aphid Monitoring

In spring of both 2018 and 2019, more aphids were observed in untreated plots compared to treated, significantly so in 2018 (P = <.001) (Fig. 7.1.6). Aphids were found on 4 untreated plots both years in spring, but only on 2 treated plots in 2018 and 1 in 2019. In summer, the number of aphids increased in treated plots both years (Table 7.1.5) but were not significantly different compared to untreated (Fig. 7.1.6). In summer 2019, numbers were too low for statistical analysis since aphids were only recorded on one treated plot at one block.

In summer 2018, aphids were found on 4 untreated plots compared to 3 treated plots. In 2018, no aphids were recorded in the treated plot at Block 5 in spring, but the highest number (10.7/10 shoots) was recorded in summer compared to all other plots. It is probable that the aphid numbers on Block 5 may be influencing the difference between untreated and treated in summer that year. In summer 2019, aphids were only found in the treated plot on Site 4.

Table 7.1.5. Mean number of aphids counted per 10 shoots from 10 sampled trees between untreated and treated plots during spring and summer of the enhancing orchard ecology trial 2018 and 2019.

	Spring 2018	Summer 2018	Spring 2019	Summer 2019
Untreated	1.000 ±0.683	1.889 ±1.010	1.533 ±1.057	0
Treated	0.167 ±0.114	2.778 ±1.708	0.600 ±0.600	1.733 ±1.733

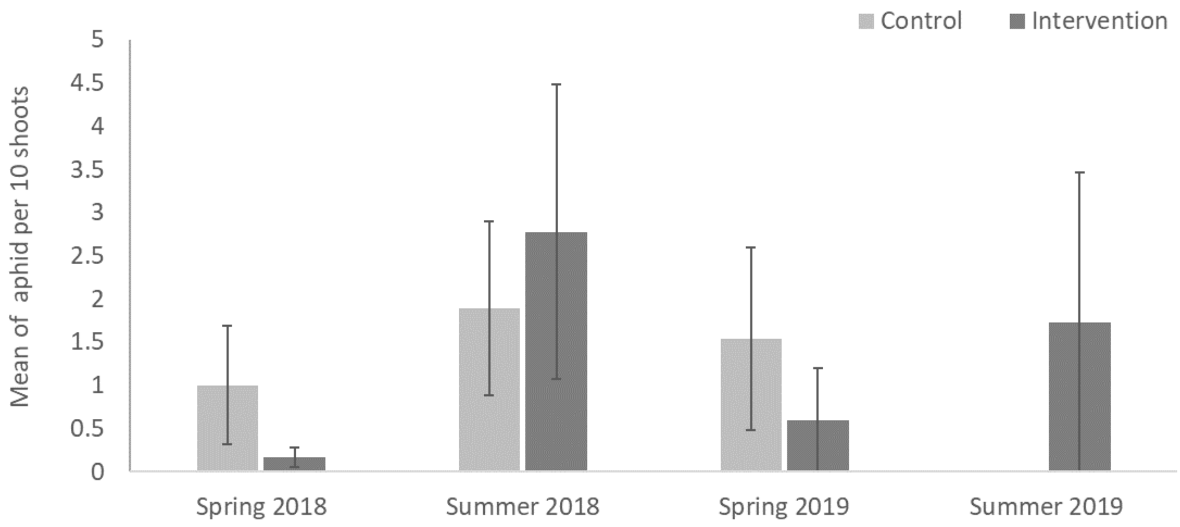


Figure 7.1.6. Mean number of aphids counted per 10 shoots from 10 sampled trees between untreated (Control) and treated (Intervention) plots in spring and summer of the enhancing orchard ecology trial 2018 and 2019.

"Wignests" - Earwig refuges

Predatory spiders were the most abundant arthropod counted in refuges in 2018 and 2019. (Fig. 7.1.7a). In 2018, predatory spiders and earwigs were the only arthropods found in the refuges, with significantly more predatory spiders than earwigs (mean = 0.561 and 0.061 respectively, P = <.001), even at Block 1 where earwigs were known to be present on trees with yellow ties. Overall, earwig numbers were low (0.2 per refuge). In 2019, predatory spiders were again the most common arthropod counted in refuges compared to earwigs (mean = 0.367 and 0.0333 respectively) (Table 7.1.6). However, on Site 2 there was a small increase in numbers of earwigs from 2018 to 2019 (Fig. 7.1.8). Site 5 was not assessed in 2019 since sowed seed mix was not established the year before. Anthocorids were also recorded in the Wignests in 2019.

Most predatory spiders formally identified in the refuges belonged to the Araneidae family. This family is known to weave a web to catch preys such as drifting, flying and hopping small and medium-sized insects (Hagen *et al*, 1999). Occasionally individuals from Philodromidae, Thomisidae, Anyphaenidae, Theridiidae and Clubionidae were also found but in very low numbers. Individuals from the Clubionidae family were exclusively found in refuges (0.4 per block, Fig. 7.1.7b).

Table 7.1.6. Percentage of Arthropods recorded in earwig refuges in treated plots of the enhancing orchard ecology trial spring 2018 and 2019.

	Predatory spider	**Earwig**	**Anthocorid**
2018	91.8 ±4.676	8.3 ±4.676	-
2019	68.1 ±9.959	4.6 ±4.545	27.4 ±10.958

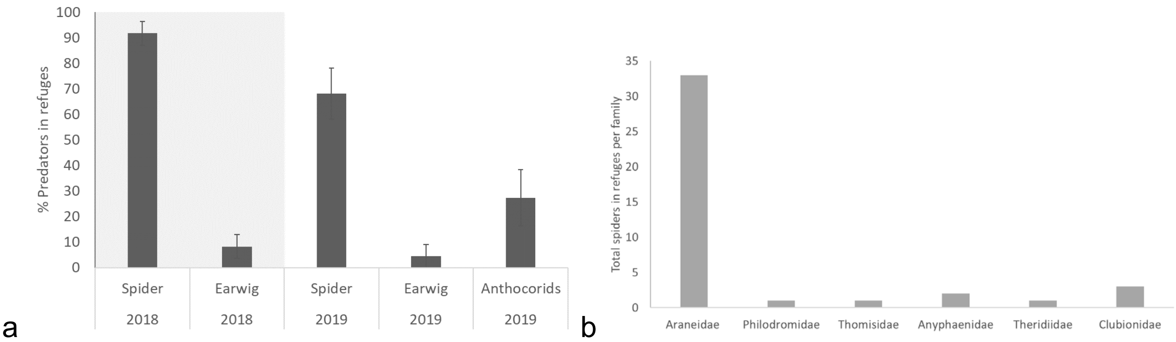


Figure 7.1.7. a) Percentage of arthropods recorded in earwig refuges and **b)** total numbers of spiders from different Families, in treated plots of the enhancing orchard ecology trial spring 2018 and 2019

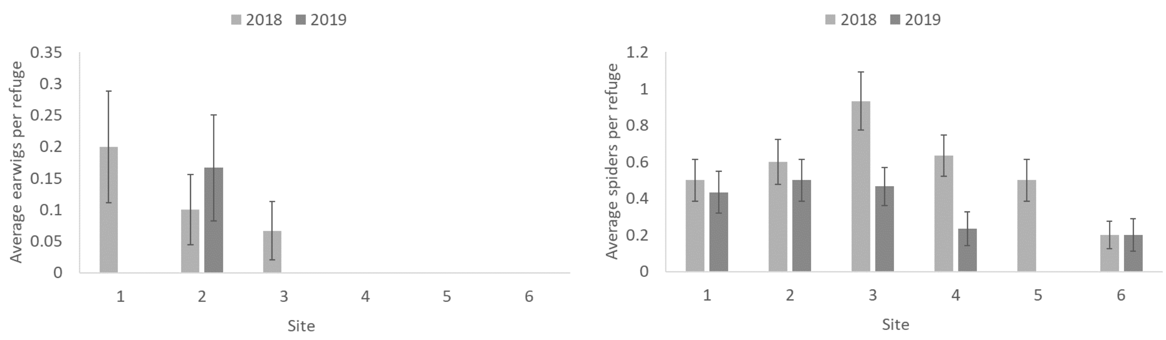


Figure 7.1.8. Mean number of earwigs (left) and predatory spiders (right) recorded per block (Site) in earwig refuges in treated plots of the enhancing orchard ecology trial spring 2018 and 2019

Predator monitoring in apple trees

In both 2018 and 2019 predatory spiders were the most common arthropod in apple trees.

There was no significant increase in spiders (p=0.719) or ladybirds (p=0.148) in the treated plots, but lacewings (p=0.047) numbers were higher in the apple trees of the treated plots in the summer 2018 (Fig. 7.1.9, Table 7.1.7). A similar response has previously been observed in a NIAB EMR PhD where coriander was sown among strawberry plants (Hodgkiss *et al.* 2019). In autumn 2018, spiders (p=0.080) and parasitoids (p=0.165) were common but not statistically different between treatments (Fig. 7.1.9). In 2019, during all assessments a higher number of predatory spiders were recorded in treated plots compared to untreated (Table 7.1.8); this difference was only significant in spring (P = <.001). Most predatory spiders identified in 2019 belonged to Araneidae and Philodromidae (Fig. 7.1.10). Overall, 8 predatory spider families were found: Araneidae, Philodromidae, Thomisidae, Anyphaenidae, Theridiidae, Linyphiidae, Clubionidae and Dictynidae. Using *Simpsons diversity* index, treated plots had higher predatory spider family diversity (D=0.523) compared to untreated plots (D=0.443), however Linyphiidae was the only family with significantly higher numbers of individuals in the treated plots compared to untreated (P = <.001, Fig 7.1.10).

Numbers of all other potential predators recorded in spring 2019 were too low for statistical analysis (Table 7.1.8). In autumn 2019 slightly higher numbers of arthropods were recorded compared to spring and summer of the same year (Fig. 7.1.9) but no significant difference was found for any species between treated and untreated plots. In 2019, earwigs were only recorded in one untreated plot on site 2 and therefore statistical analysis was not possible.

Night assessment of apple trees

There were low numbers of earwigs in the treated and untreated plots in both years (Fig. 7.1.11). Significantly more predatory spiders were present in untreated (0.83 per 30 trees) plots compared to treated (0.17 per 30 trees) in 2018 (P = 0.012) but this trend reversed in 2019. Other beneficials recorded included ladybirds, harvestman, parasitoids, hoverflies and solitary bees (Fig. 7.1.11.), but no significant difference was found between treated and untreated plots for any of these.

Table 7.1.7. Mean and standard error of arthropods recorded by tap sampling 30 trees at untreated and treated plots in spring, summer and autumn of the enhancing orchard ecology trial 2018.

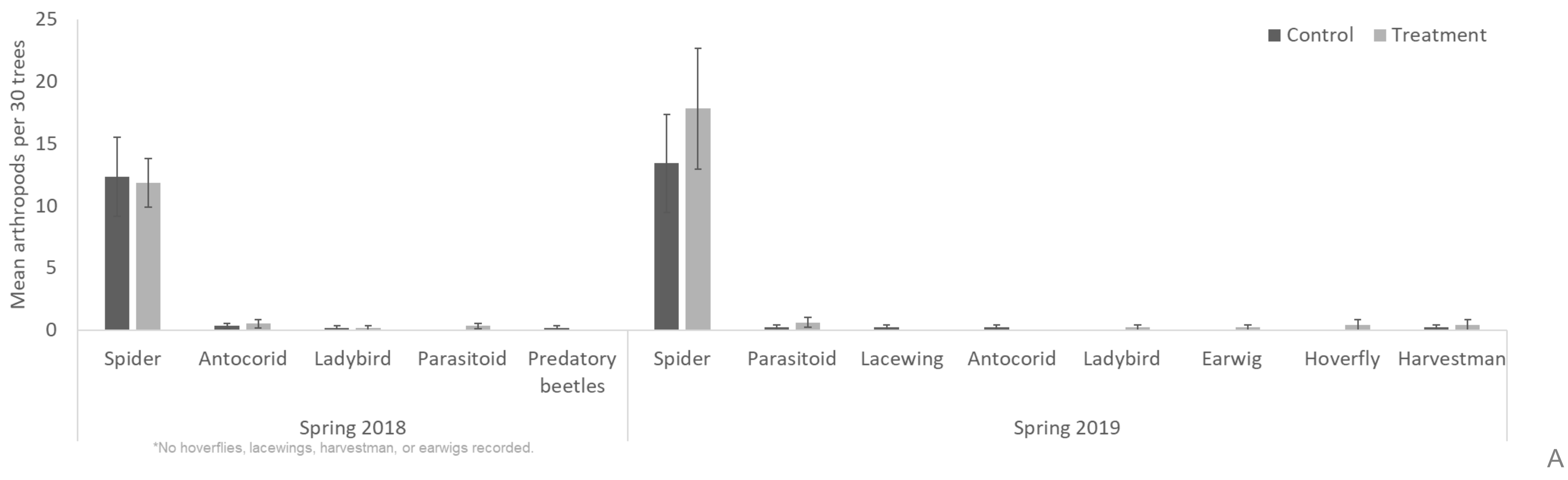
2018	Treatment	Predatory spider	Anthocorid	Ladybird	Lacewing
Spring	Untreated	12.33 ±3.19	0.33 ±0.21	0.17 ±0.17	-
Spring	Treated	11.83 ±1.97	0.50 ±0.34	0.17 ±0.17	-
Summer	Untreated	10.00 ±2.64	0	0.33 ±0.33	0.17 ±0.17
Summer	Treated	10.67 ±1.67	0.17 ±0.17	1.00 ±0.68	1.00 ±1.00
Autumn	Untreated	12.50 ±2.46	0.17 ±0.17	2.67 ±1.52	0.67 ±0.21
Autumn	Treated	16.33 ±3.20	0.17 ±0.17	2.17 ±1.08	0.33 ±0.33

	Treatment	Parasitoid	Soldier beetle	Harvestmen	Predatory beetles
Spring	Untreated	0	-	-	0.17 ±0.17
Spring	Treated	0.33 ±0.21	-	-	0
Summer	Untreated	0.83 ±0.48	0.17 ±0.17	1.00 ±0.52	-
Summer	Treated	0.83 ±0.54	0	1.17 ±0.83	-
Autumn	Untreated	0.17 ±0.17	-	0.50 ±0.22	-
Autumn	Treated	0.67 ±0.42	-	0.17 ±0.17	-

Table 7.1.8. Mean and standard error of arthropods recorded by tap sampling 30 trees at untreated and treated plots in spring, summer and autumn of the enhancing orchard ecology trial 2019.

2019	Treatment	Spider	Anthocorid	Ladybird	Lacewing	Parasitoid
Spring	Untreated	13.40 ±3.97	0.20 ±0.20	0	0.20 ±0.20	0.20 ±0.20
Spring	Treated	17.80 ±4.87	0	0.20 ±0.20	0	0.60 ±0.40
Summer	Untreated	5.20 ±1.66	0.20 ±0.20	0.20 ±0.20	0.20 ±0.20	0.20 ±0.20
Summer	Treated	6.60 ±1.86	0	0	0	0.20 ±0.20
Autumn	Untreated	15.60 ±3.17	0.80 ±0.37	0.20 ±0.20	0.80 ±0.37	0.20 ±0.20
Autumn	Treated	21.40 ±2.56	0	0.20 ±0.20	0.80 ±0.37	0.80 ±0.58

	Treatment	Earwig	Harvestman	Hoverfly	Weevil
Spring	Untreated	0	0.20 ±0.20	0	-
Spring	Treated	0.20 ±0.20	0.40 ±0.40	0.40 ±0.40	-
Summer	Untreated	0.60 ±0.60	0.20 ±0.20	-	-
Summer	Treated	0	0.60 ±0.40	-	-
Autumn	Untreated	0.40 ±0.40	0.40 ±0.24	-	7.60 ±5.15
Autumn	Treated	0	0	-	3.20 ±2.73



Mean arthropods per 30 trees
Control
Treatment
Spider
Antocorid
Ladybird
Parasitoid
Predatory beetles
Spider
Parasitoid
Lacewing
Antocorid
Ladybird
Earwig
Hoverfly
Harvestman
Spring 2018
Spring 2019
*No hoverflies, lacewings, harvestman, or earwigs recorded.
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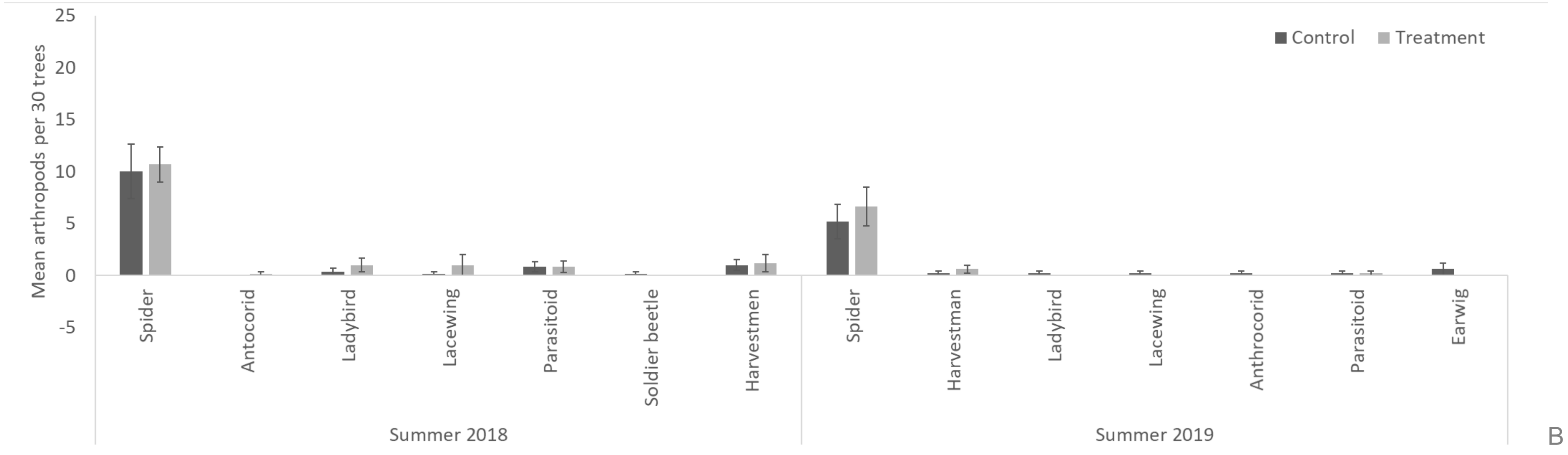

Mean arthropods per 30 trees
Control
Treatment
Spider
Antocorid
Ladybird
Lacewing
Parasitoid
Soldier beetle
Harvestmen
Spider
Harvestman
Ladybird
Lacewing
Anthrocorid
Parasitoid
Earwig
Summer 2018
Summer 2019
B

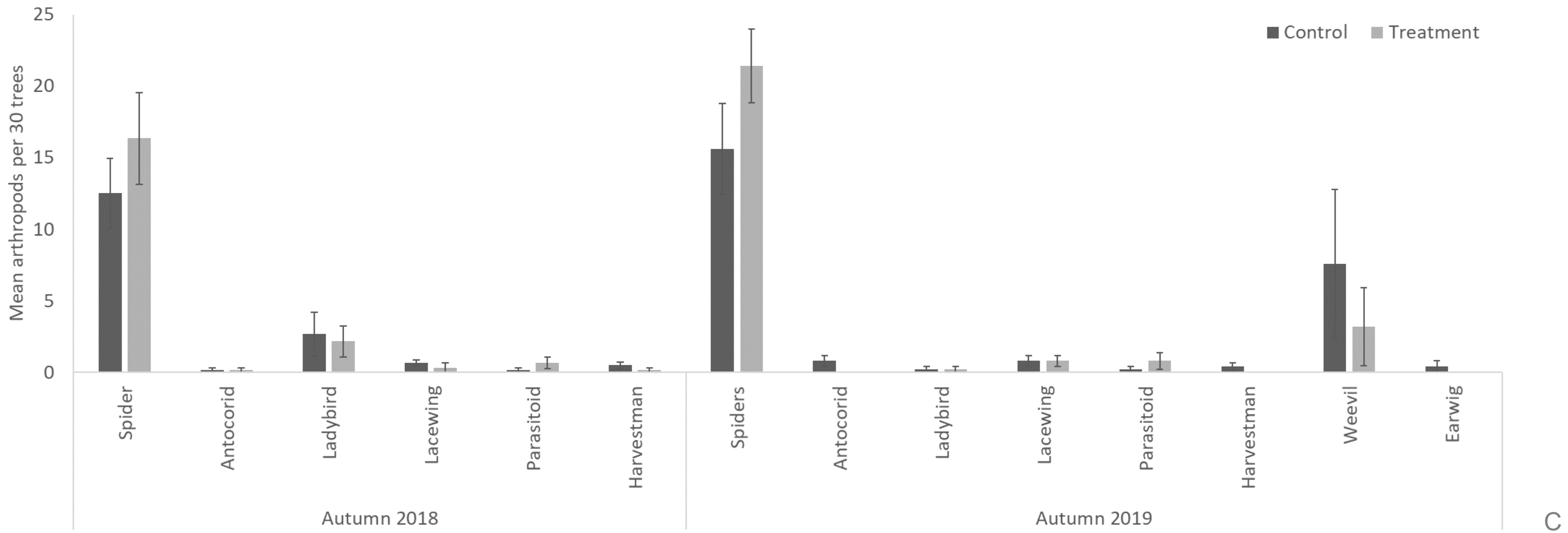

Figure 7.1.9. Mean and standard error of arthropods recorded by tap sampling 30 trees at untreated (Control) and treated (Treatment) plots in spring (A), summer (B) and autumn (C) of the enhancing orchard ecology trial 2018 and 2019

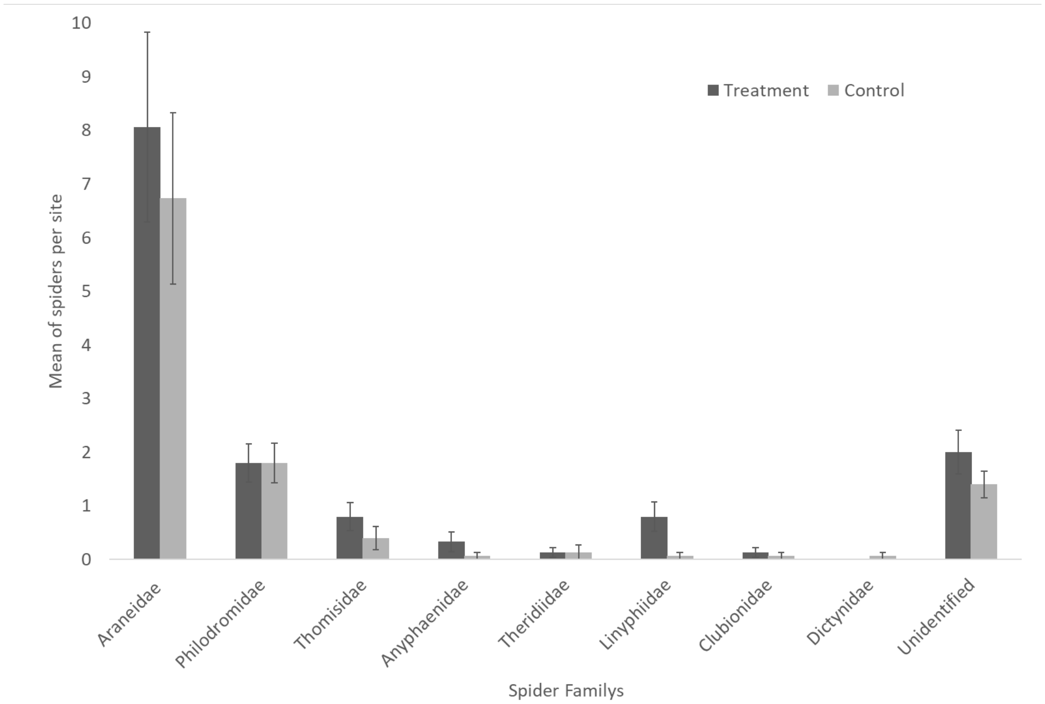

Figure 7.1.10. Mean numbers of predatory spiders within spider families identified when tap sampling apples trees in untreated (Control) and treated (Treatment) plots in spring, summer and autumn of the enhancing orchard ecology trial 2019

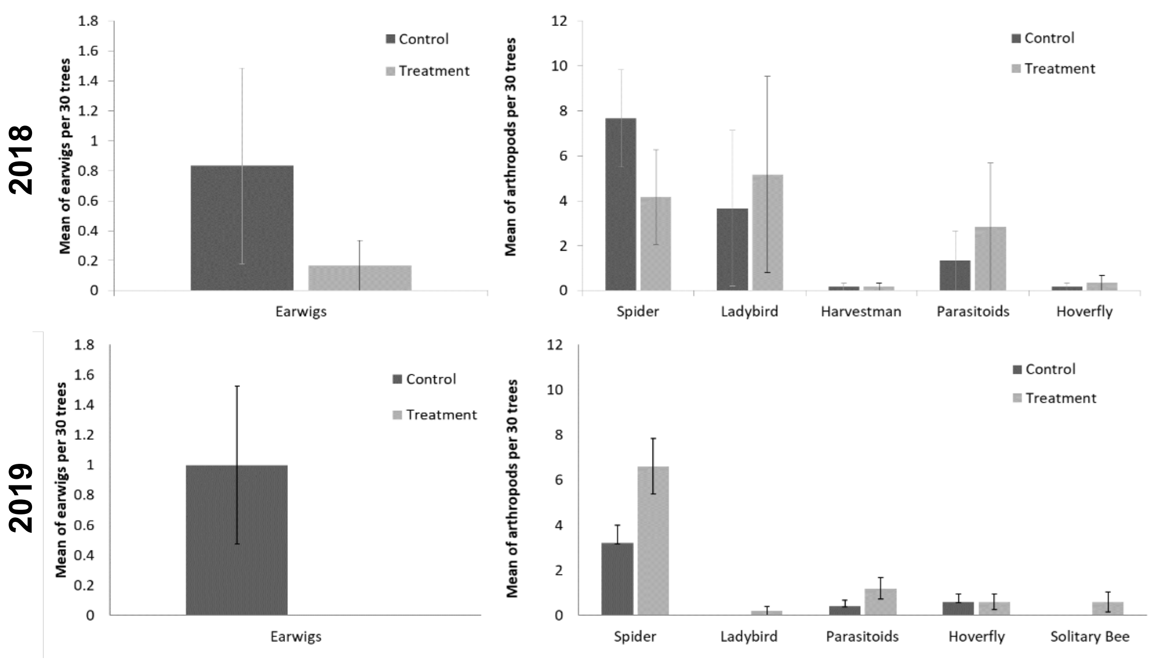

Figure 7.1.11. Mean numbers of predatory arthropods recorded from tap sampling apple trees in untreated (Control) and treated (Treatment) plots during the night assessment of the enhancing orchard ecology trial 2018 and 2019

Mites

In 2018, significantly more rust mites (*P* = <.001) were observed in treated plots (439.8 per 30 leaves) compared to untreated (195.3 per leaves). Three other taxa were recorded: predatory mites, fruit tree red spider mite (*Panonychus ulmi*), and other spider mites. There were significantly fewer predatory mites (*P* = .004) and fruit tree red spider mite (*P* = <.001) in treated plots compared to untreated. However, fruit tree red spider mite was only found in untreated and treated plots at Block 4. Other spider mites were more numerous on treated plots compared to untreated but only at Block 4.

In 2019, only predatory mites were recorded. Untreated plots had fewer predatory mites per 30 leaves compared to treated (0.20 and 1.40 respectively), but this difference was not significant.

Codling Moth Damage and other pests

Codling moth (CM) stings (superficial sting central to a red region) and deep entry (Fig. 7.1.12) were recorded in spring and summer of both years.

More fruits with codling moth stings were observed in untreated plots compared to treated in summer and autumn 2018 (Fig. 7.1.13). No CM deep entry damage was recorded on treated plots in summer and autumn. In 2018, treated and untreated plots were only significantly different for the deep entry damage on tree fruits in the summer *(P* = <0.001).

In summer 2019, no significant differences were found between CM damage in untreated and treated plots (Fig. 7.1.13.). In autumn CM stings decreased in treated plots compared to summer. CM stings were significantly fewer on treated plots compared to untreated at this time (*P* = .0346) (Fig. 7.1.13). CM deep entry damage to dropped apples was only recorded in the untreated plots during autumn 2019 but was too low for statistical analysis (Fig. 7.1.13).

Comparing both years, more damage was recorded in 2019. Treated plots recorded more stings and deep entry damage in summer 2019 than in the same period of 2018 (Fig. 7.1.13). However, number of codling moth stings in untreated plots did not vary for that same period between 2018 and 2019 (mean = 8.833 and 7.324 apples per 30 trees respectively). A greater decrease of codling moth stings and deep entry on treated plots from summer to autumn was recorded in 2019 compared to 2018 (Fig. 7.1.13).

Figure 7.1.12. Codling moth larvae in apple - deep entry damage

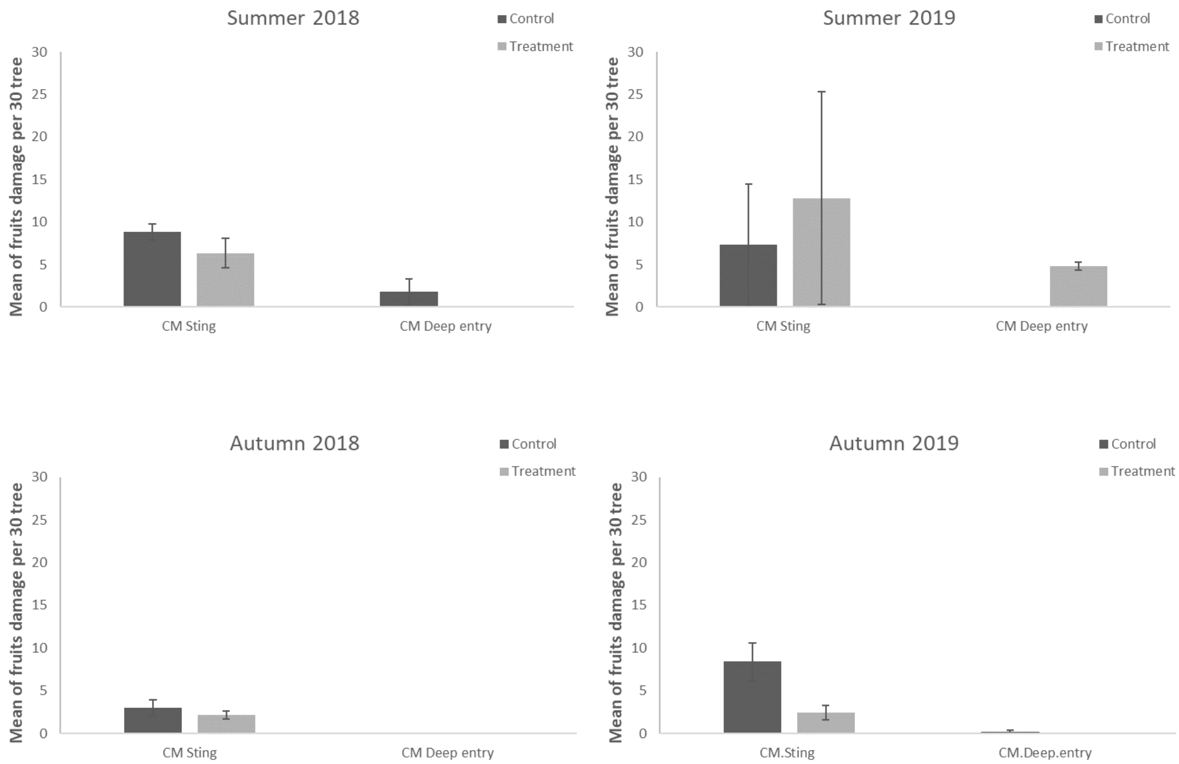

Figure 7.1.13. Mean numbers of apples per 30 trees with codling moth sting and deep entry damage in Untreated (Control) and treated (Treatment) plots in summer and autumn of the enhancing orchard ecology trial 2018 and 2019

In autumn 2018, there were fewer CM sting damaged dropped apples (p=0.018) in the treated compared to untreated plots (Fig. 7.1.14). No CM deep entry damage was found in untreated plots and a very small number of fruits (0.33 fruits per 30 trees) from one treated plot exhibited this damage. In 2019, no significant differences were recorded in damage to dropped apples. Numbers of CM sting and deep entry to dropped apples recorded were much lower in 2019 than in 2018. In fact, numbers were so low in 2019 that statistical analysis was not possible (Fig. 7.1.14).

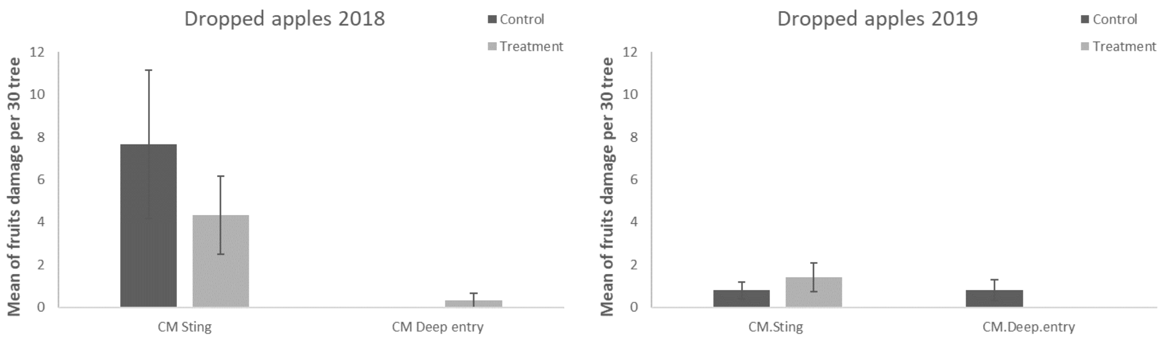

Figure 7.1.14. Mean numbers of dropped apples per 30 trees exhibiting codling moth sting and deep entry damage between untreated (Control) and treated (Treatment) plots in autumn of the enhancing orchard ecology trial 2018 and 2019

Damage from other pests including capsid, tortrix, rosy apple aphid, winter moth and Rhynchites was also observed during the fruit damage assessment (Fig. 7.1.15).

Rosy apple aphid and Rhynchites damage was only recorded in the summer 2018. However, the numbers of fruits with rosy apple aphid damage was very low (0.33 fruits per 30 trees) and only recorded on one treated plot. There was no difference between tortrix damage found in untreated plots compared to the treated. Winter moth damage was similar in untreated and treated plots in summer and autumn 2018, with very little damage found in the untreated (0.180 fruits per 30 tree). No difference was recorded for capsid damage between untreated and treated plots in summer and autumn 2018.

In 2019, only capsid and tortrix damage was recorded in summer with no significant numbers found in treated and untreated plots for both pests (Fig. 7.1.15). However less capsid damage and higher tortrix damage were recorded in summer 2019 when compared to 2018.

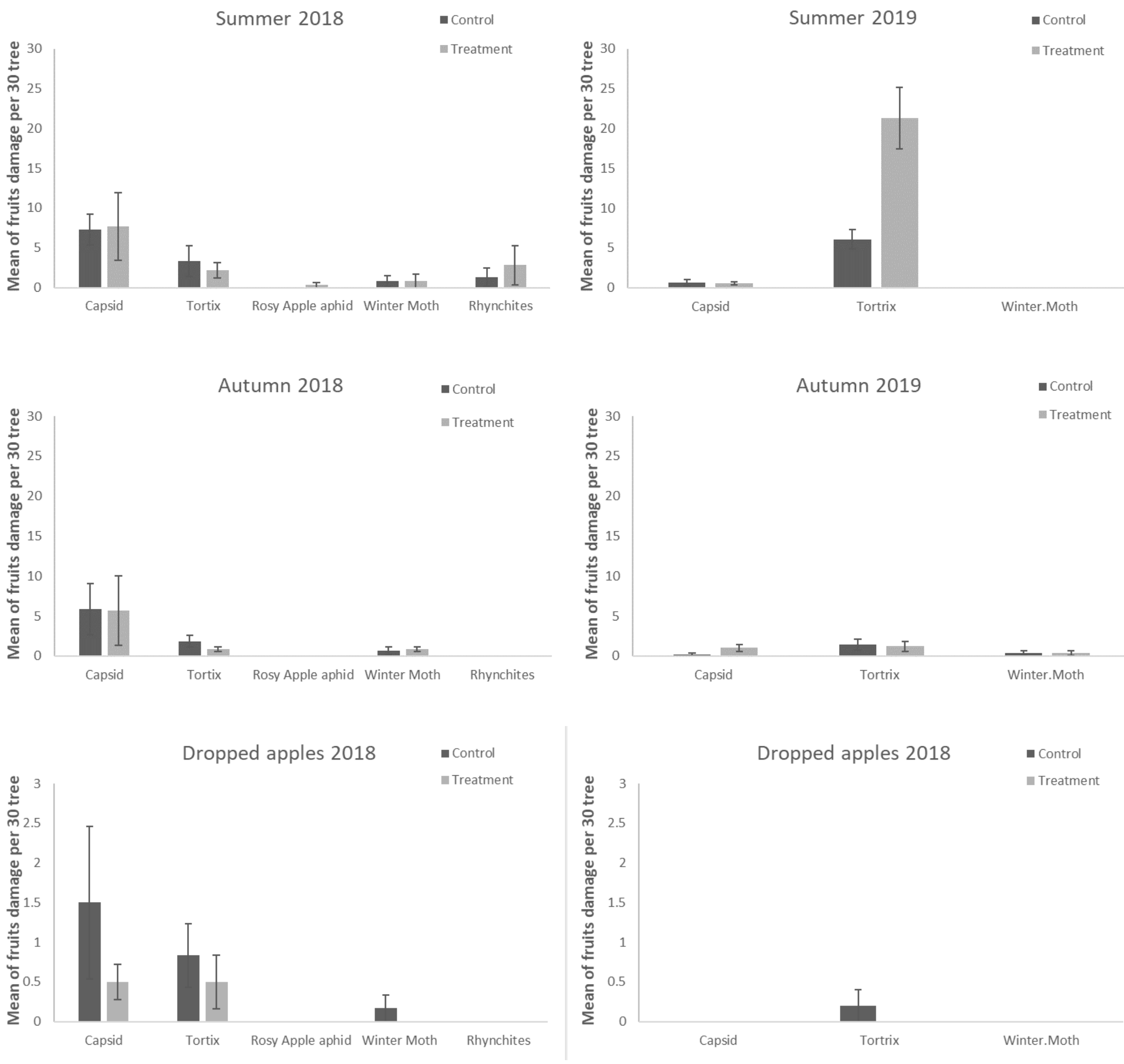

Figure 7.1.15. Mean numbers of apples per tree with damage from capsid, tortrix, rosy apple aphid, winter moth and Rhynchites in untreated (Control) and treated (Treatment) plots in summer and autumn of the enhancing orchard ecology trial 2018 and 2019. *Note that dropped apples are displayed on a smaller axis than previous damage

Hoverfly Assessment

Significantly more hoverfly adults were recorded on white sticky traps in the treated plots compared to untreated in autumn 2018 *(P* = <.001) (Fig.7.1.16). However, this was not repeated in 2019.

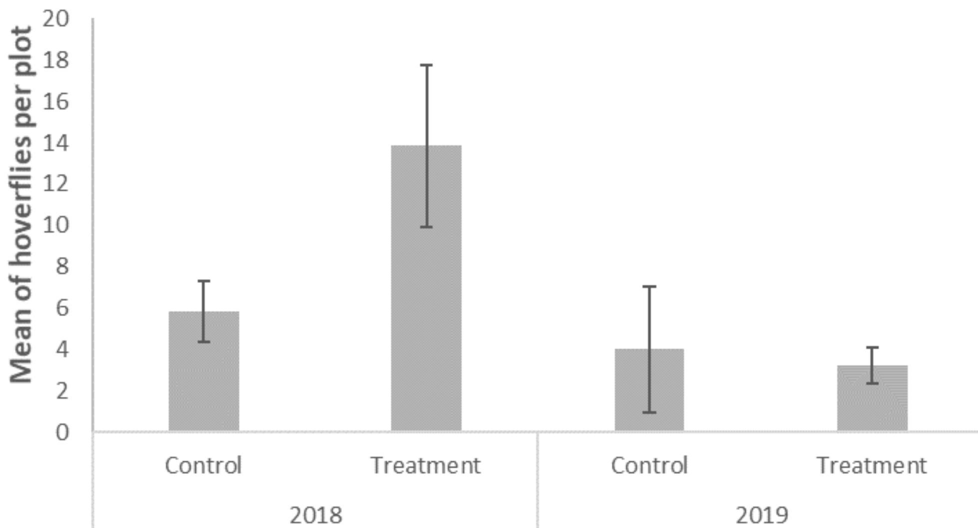

Figure 7.1.16. Mean numbers of hoverfly adults recorded on white sticky traps in treated (Treatment) and untreated (Control) plots (5 traps) in autumn of the enhancing orchard ecology trial 2018 and 2019

Table 7.1.9. Summary of the effects that interventions to enhance apple orchard ecology had on beneficial arthropods during 2 consecutive years of assessments after interventions were introduced. **Green = positive effect**, **red = negative effect**, and **black = no effect or insufficient data (x)**

Arthropod	Timing	2018	2019
'Wignests'	Summer	91.75% predatory spiders 8.25% earwigs	68.1% predatory spiders, 4.5% earwigs 27.4% anthocorids,
Hoverflies (sticky traps)	Summer	*P*<0.001 (August)	0.7918 (July)
Codling moth	Summer	Deep entry, *P*<0.001	×
	Autumn	×	Sting *P* = 0.035
	Autumn dropped apples	Sting, *P* = 0.018	Few dropped fruit
Aphids on shoots	Spring	*P*<0.001	NSD
	Summer	NSD	Few aphids
Tree tapping	Spring	×	Predatory spiders (*P*<0.001)
	Summer	lacewings (*P* = 0.047)	×
	Autumn	×	×
	Night assessment	NSD	×
Mites on leaves	Predatory mites	rust mites (*P*<0.001), predatory mites (*P* = 0.004), fruit tree red spider mite (*P*<0.001, at 1 site)	Few mites

Conclusions

- Seeded alleyways were successful in most orchards and percentage coverage from the seed mix seemed generally increased from 2018 to 2019.
- Not all species in the seed mix established. Red clover and yarrow were the most common in 2018. Red clover was also one of the most common in 2019 along with common knapweed.
- Sward height in treated plots was higher than in untreated alleyways in both years but only significantly in 2018.
- In both years fewer aphids were observed in the treated plots in spring but not in summer.
- More predatory spiders were found than earwigs in Wignests deployed in treated plots in spring 2018 and 2019. In 2019 anthocorids were also found in refuges. Most predatory spiders found in the refuges in 2019 belonged to family Araneidae.
- Predatory spiders were the most common arthropod recorded in apple trees in all seasons in both years. In 2019 most belonged to the Araneidae and Philodromidae families. Some species of the Philodromidae, like *Tibellus macellus*, primary feed on aphids, accounting for over half the total prey they ingest when available (Huseynov 2008).
- Linyphiidae was the only family with significantly higher numbers of individuals in the treated plots compared to untreated ($P < 0.001$). A subfamily of Linyphiidae, Erigoninae (also known as Micryphantids), are reported preying on soft-bodied pests, like aphids (Nyffeler & Benz 1988; Mansour & Heimbach 1993).
- In 2018, no apple leaf curling midge damage occurred in treated plots compared to untreated. Apple leaf curling midge was not assessed in 2019.
- In 2018, fewer predatory mites and fruit tree red spider mites were found in treated plots compared to untreated. However, the opposite was observed for rust mites and spider mites. In 2019 only predatory mites were found on apple leaves, with higher numbers recorded in treated plots.
- In 2018, significantly fewer CM deep entry damage was recorded on treated plots in summer and significantly fewer CM stings on treated plots in the dropped apple assessment. In 2019, CM stings were significantly less frequent in the treated plots in autumn.

- There were significantly more hoverfly adults in the treated plots in autumn 2018. It is not known if this is the consequence of the attractant sachet and/or the floral alleyways. This effect was not observed in summer 2019.

Objective 8 - Rhynchites Weevil and Sawfly

8.2 Sex pheromone of the apple sawfly

Aim

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

Introduction

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

Methods

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

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

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

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

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

All pots were moved into a 6 °C refrigeration unit in September to overwinter. The first set of pots were removed on 2 Jan 2019 into ambient temperature. Once again sawfly larvae failed to emerge – again the cocoons were empty.

In spring 2019, larvae were once again collected (6-11 June) and all larvae had emerged from apples by 24 June. After speaking to colleagues in the Netherlands a different approach was taken. All larvae were placed in two 40 cm diameter 40 cm deep terracotta pots (139 larvae in one, 108 in the other). Larvae tunnelled straight into the compost. Occasionally larvae were seen re-emerging and re-entering the soil. On 7 July 19 the terracotta pots were dug into ground in a sheltered spot at the back of the EMB building at NIAB EMR. A fine cloth mesh was taped around the top and a steel grid to prevent mammals digging laid over the top of this. Pots were labelled with contents (see photos). Finally, about 1 litre of water added to top as they were quite dry from being in office and no rain forecast for next 2 weeks.

Figure 8.2.1. a) collected fruitlets, b) larvae emerging from fruitlets and ‘wandering’, c) larvae burrowing down into d) potted substrates, e) in terrocota pots in soil, f) with mesh lid

On 11 March 2020, samples of soil were taken from the top, mid and bottom of the plant pots. There were 6 pupae in the in bottom, 2 in the middle and 1 from the top of the soil. These were dissected and all found to be dead. On 17 March, all soil was removed from pots and spread out on trays in bugdorm cages in a shed. At the time of reporting 18 April 2020, no sawfly have emerged. It is recommended that this is repeated, but that next time a layer of gravel is put on the bottom of the pots to encourage drainage.

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

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

Introduction

Incidence and damage caused by a weevil pest of pear was first reported to NIAB EMR in 2015. Subsequent reports were made to the entomology department at NIAB EMR as the weevil became more widespread across the South East UK. The weevil was initially thought to be the pear bud weevil (*Anthonomus piri*, Gyllenhal) (Fig. 9.1.1c), an uncommon species that is known to cause damage to pear. Investigation into the lifecycle in 2016 found that the weevil was laying its eggs in the closed flower and vegetative buds in spring (March- April). It was proposed that this could be *A. piri* adults that had overwintered and were laying their eggs in the spring. However, it was clear that further investigation was required to identify the weevil and determine its lifecycle and biology in UK pear orchards. In 2017, the weevil was confirmed as *Anthonomus spilotus*, Redtenbacher, 1847 by the Natural History Museum and was published in 2017 by NIAB EMR and NHM (Fig. 9.1.1). Presence of eggs, larvae, adults and feeding damage was observed from the beginning of sampling on 22 March and no adults were found after 12 June. This damage consisted of i) puncturing of the bud bracts by adult feeding, causing irregular growth and ii) larval feeding within leaf and flower buds leading to irregular growth, loss of buds and damaged flower buds. From the total buds collected a third had feeding damage and 7% of buds contained eggs, larvae or pupae which implied that feeding damage does not always mean that eggs, larvae, or pupae are present within the bud. In 2018, sampling for adults started earlier and adult activity, eggs in buds and adult feeding damage was recorded from 8 March until 6 June. Weevils fed on and laid eggs in flower and leaf buds depending on availability. The percentage of flower buds damaged by adult feeding was 22.6% and the percentage of flower buds damaged by larvae 0.7%. While 42.3% of leaf buds were damage by adult feeding, as in the flower buds only 0.7% of leaf buds were damaged by larvae. Therefore, most bud damage was a result of adult feeding. On average 1 weevil was counted per 40 tap samples in 2018. At this density only 1 flower per truss (6 flowers average) was destroyed. This is not significant considering that only 3-4 Conference fruits can set to harvest on a single truss. The main consideration is the damage to leaves and photosynthetic ability for future years. Even at very low levels of weevils (~1 per 40 tree taps) ~60% of new leaves were damaged later in the season. We have not been able to set a damage threshold for this because the resultant health to the tree cannot be estimated in this study.

Aim

Complementing previous work on the life cycle and effectiveness of products on *A. spilotus*, we tested the most effective products in the laboratory in the field for the control of *A. spilotus* adults pre and post petal fall. We also assessed pest damage and effects on natural enemies. We tested the;

- Effect of foliar applications of thiacloprid and indoxacarb at pre-blossom on weevil damage and adults.
- Effect of spray application of thiacloprid and indoxacarb at post-blossom on weevil damage and adult population.

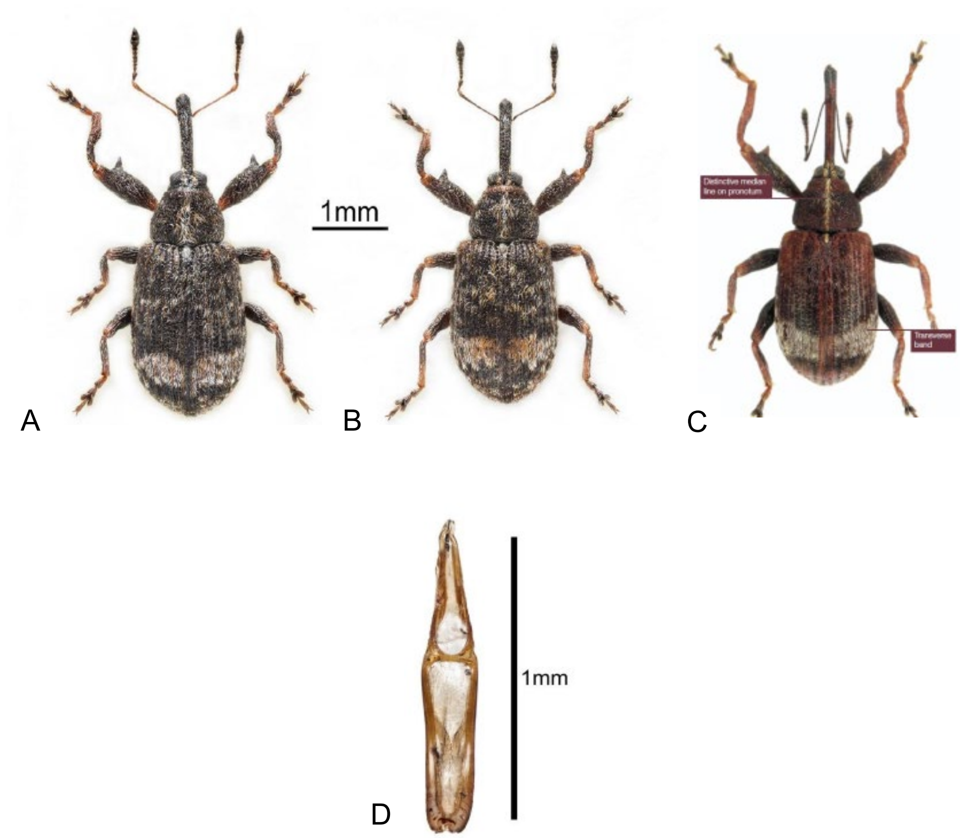

Figure 9.1.1. a) *A. spilotus* male, b) *A. spilotus* female, c) *A. piri*, d) *A. spilotus* median lobe of male genitalia (Harry Taylor. Figure A, B and D. 2017. Specimens in Natural History Museum; AHDB. Figure C. 2015).

Materials and Methods

Trial layout

Four pear orchards (blocks) cv. Conference, with historic populations of *A. spilotus* were selected for the spray trial. The same blocks had been monitored for *A. spilotus* the 2 previous years. All blocks were in Kent. Block 1 was located near Rochester while Blocks 2, 3 and 4 were situated in East Farleigh.

Each block was divided into 4 replicates of 5 treatments (see Table 9.1.1 for spray timings) except Block 4 where only 2 replicates of the 5 treatments were possible due to space constraints (Fig. 9.1.2). The position of plots in each block was randomised.

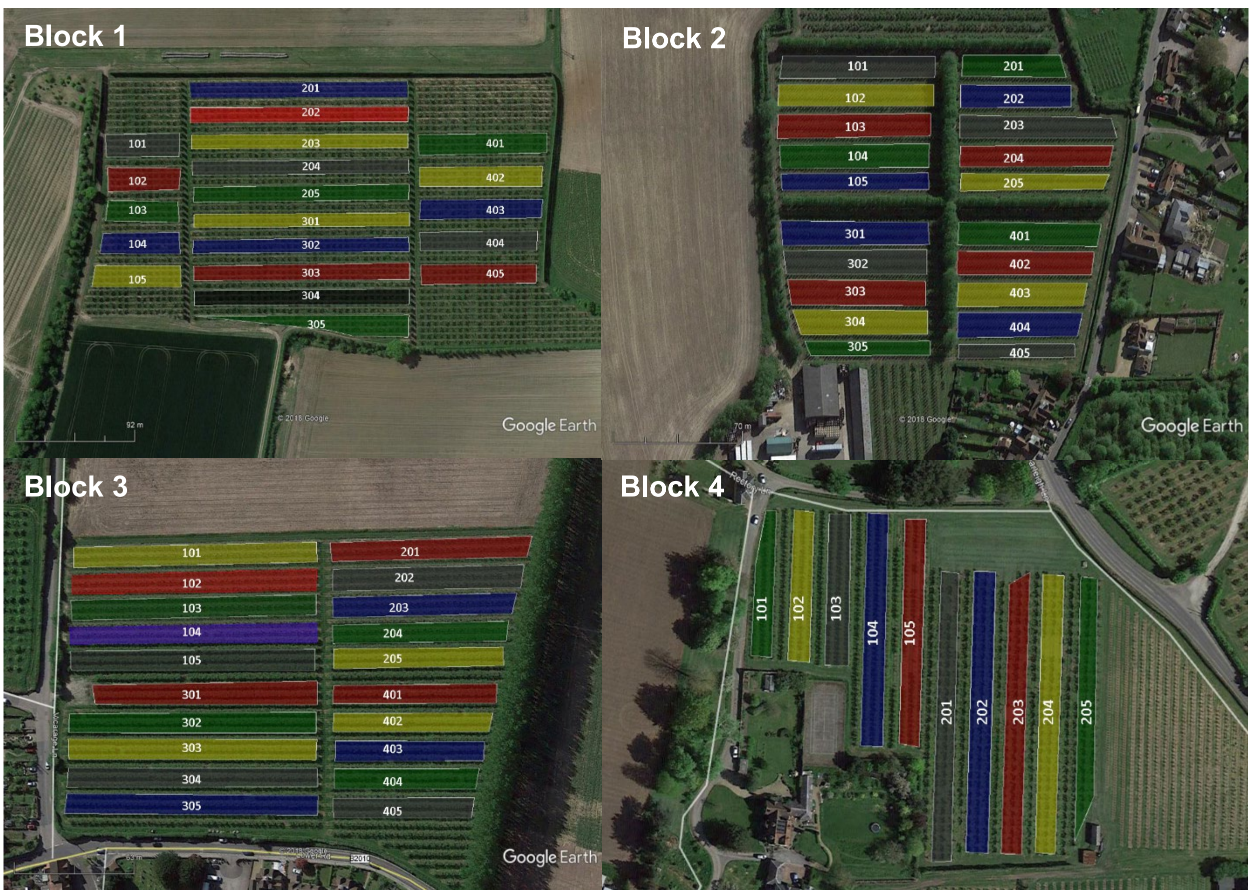

Figure 9.1.2. Position of randomised plots in Blocks 1, 2, 3 and 4

At Block 1, plots 201 to 305 were 3 row beds. The remaining plots were shorter in length so had 4 rows to ensure there were 30 trees to make assessments. All plots for this block had a guard row between them. Block 2 had 3 bed rows; each plot had 3 rows with no guard row (Fig. 9.1.3) as a 3-bed row offered enough protection from spray drift. At Block 3, plots were set up with 3 tree rows and no guard row as this block did not have enough rows to permit a guard row between each plot. Block 4 had the smallest area and could not accommodate 4 replicates of each treatment so only 2 replicates were set up, each with 3 tree rows and 1 guard row in between. The central row/rows of each plot were assessed.

Figure 9.1.3. Three row bed at block 2

Treatments

Products were applied by the growers and a member of Avalon staff using their own machinery at a rate of 500 l/ha. Application was supervised by a BASIS registered member of staff and assisted by PA1/6 qualified staff from NIAB EMR (Table 9.1.1). The growers applied a normal programme of plant protection products, avoiding products harmful to *A. spilotus*.

Red and black plots were sprayed when tree stage reached green cluster or white bud (Fig. 9.1.4). Blue and yellow plots were treated after petal fall (Fig. 9.1.5). Dates of spray applications and assessments are showed in table 9.1.2.

Table 9.1.1. Product details and field rate applied in this trial. * Label rate for pre-blossom application on pear**.**

Colour code	Product	a.i.	Field dosage/ha*	Max. No. of Applications	HI	Timing
Red	Calypso	thiacloprid	375 ml	2	14 d	Before blossom
Black	Steward	Indoxacarb	250 g	3	7 d	Before blossom
Blue	Calypso	thiacloprid	375 ml	2	14 d	After blossom
Yellow	Steward	Indoxacarb	250 g	3	7 d	After blossom
Green	Untreated	-	-	-	-	-

Figure 9.1.4. First spray timing, green cluster (left) at block 1 and white bud (right) at Block 2. Application on red (calypso) and black (steward) plots

Figure 9.1.5. Post-blossom (left) and spray application, petal fall at Block 4 (right)

Table 9.1.2. Dates of spray applications of Calypso and Steward. Dates of assessments performed in each block for feeding damage and adults of *A. spilotus*.

Block	Pre-blossom application	Post-blossom application	1^{st} assess. (white bud)	2^{nd} assess. (Petalfall)	3^{rd} assess. (fruitset)
1	25 March 2019	01 May 2019	01 April 2019	07 May 2019	31 May 2019
2	27 March 2019	03 May 2019	03 April 2019	13 May 2019	30 May 2019
3	27 March 2019	03 May 2019	03 April 2019	10 May 2019	29 May 2019
4	27 March 2019	01 May 2019	03 April 2019	07 May 2019	31 May 2019

Assessments: All assessments were made in the centre of each plot with all plots assessed each visit. Assessments were carried out depending on tree development stage.

Pre-assessment:

A pre-assessment was made before any treatment product was applied to assess the distribution of adult weevils and existing feeding damage on the different plots.

- A branch on 30 trees per plot was tap sampled. Number of weevil adults were recorded per tap sample then returned to the tree.
- Another branch was randomly selected from the same trees tap sampled and the number of leaf buds, flower buds and weevil feeding holes per type of bud were counted.

Pre-blossom spray:

- The assessment was made one week after spray application.
- A branch on 30 trees per plot was tap sampled. Number of weevil adults were recorded per tap sample then returned to the tree.
- Another branch was randomly selected from the same trees tap sampled and the number of leaf buds, flower buds and weevil feeding holes per type of bud were counted.
- Pre-blossom sprayed plots were assessed again after the post-blossom spray.

Post- blossom spray:

- Assessment was carried out one week after spray application.
- A branch on 30 trees per plot was tap sampled. Number of weevil adults were recorded per tap sample then returned to the tree.
- From the same trees tap sampled the recorder selected 10 random shoots and noted how many were damaged by weevil feeding.

Natural enemies: During tap samples numbers of natural enemies, including earwigs, parasitoids, spiders, ladybirds, lacewings and anthocorids were also recorded.

Results

Pre-blossom spray application

A pre-assessment of *A. spilotus* damage and adult numbers was made at all plots before treatments were applied to correct for the uneven distribution in damage and weevil numbers between plots that could affect the results of the study (Fig. 9.1.7) at the swollen bud stage.

The growth stage of trees was similar between blocks when treatments were applied (Fig. 9.1.6).

At swollen bud, before treatment application, a small difference was observed in feeding damage between sprayed and unsprayed plots. Weevil damage was similar across plots. At each assessment event (white bud, petal fall and fruit set) after spray application, a decrease in feeding damage was observed on all plots (treated or untreated, mean = 2.67). No significant differences were found between treated and untreated plots at any of the assessment timings.

Number of adult weevils recorded did not follow the same trend as feeding damage (Fig. 9.1.8). No significant differences were found between treated and untreated plots at any time point.

Figure 9.1.6. Tree growth stage at the 4 blocks the time of pre-blossom treatment application on red (Calypso) and black (Steward) plots. Blocks 1 to 4 (left to right)

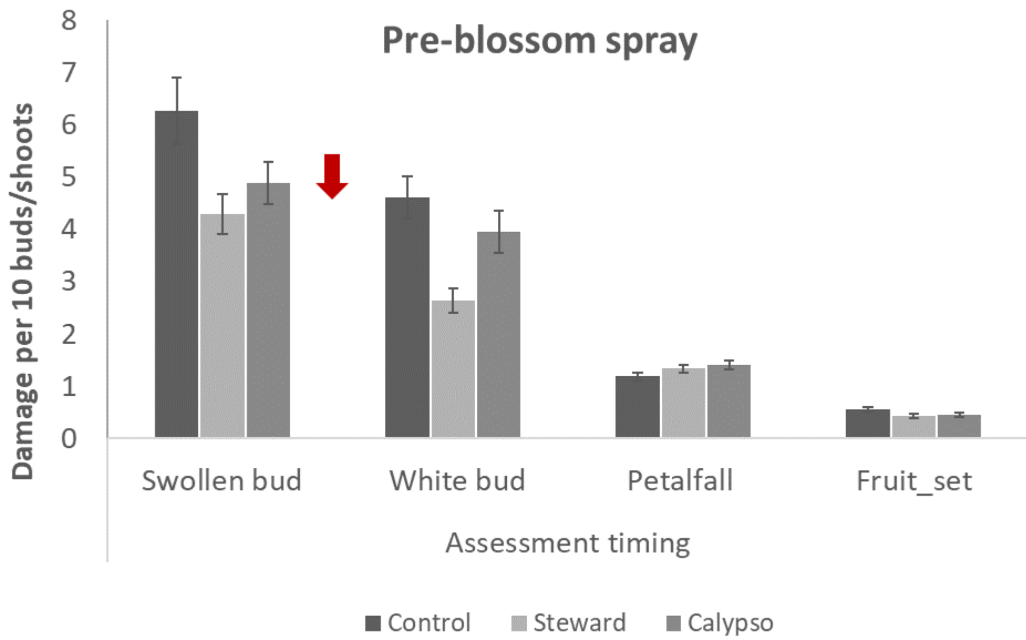

Figure 9.1.7. Mean of weevil feeding damage per 10 buds/shoots examined during pre-blossom assessments at treated (Steward, Calypso) and untreated (control) plots. Swollen bud was when the pre-spray assessment was made, the red arrow indicates the spray application of treatments on red (Calypso) and black (Steward) plots

Post-blossom spray application

Post-blossom plots were assessed at white bud stage when blocks were visited to make the first assessment on pre-blossom sprayed plots. At this time, post-blossom plots were not yet sprayed so this assessment was a pre-assessment to check feeding damage distribution (Fig. 9.1.10). All blocks exhibited a similar growth stage (Fig. 9.1.9).

At white bud, before the application of the sprays, all plots had a similar amount of weevil feeding damage which decreased over time. No significant difference was found between treated and untreated plots (Fig. 9.1.10).

The mean number of adult weevils did not relate to feeding damage recorded (Fig. 9.1.11). Number of adult weevils were low and not significantly different between treatments at white bud stage. No significant difference was found between treated and untreated plots for the rest of the trial.

Figure 9.1.9. Tree growth stage at the 4 blocks the time of post-blossom treatment application on blue (Calypso) and yellow (Steward) plots. Blocks 1 to 4 (left to right)

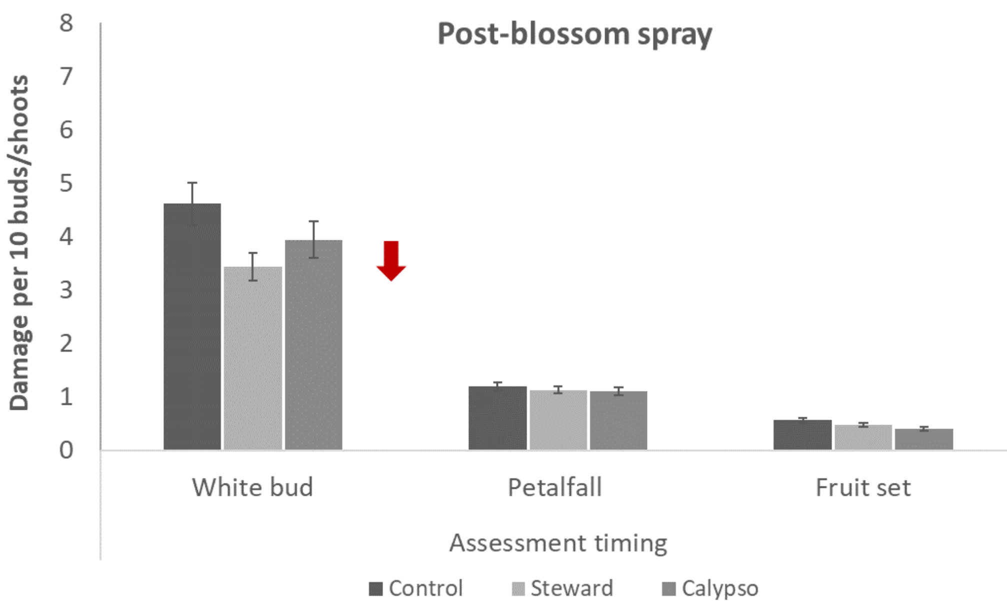

Figure 9.1.10. Mean of weevil feeding damage per 10 buds/shoots examined during examined during post-blossom assessments at treated (Steward, Calypso) and untreated (control) plots. The red arrow indicates the spray application of treatments on blue (Calypso) and yellow (Steward) plots

Natural predators

During 30 branch tap samples per plot natural predators per plot were also recorded. This included predatory spiders, ladybirds, lacewings, anthocorids, earwigs and parasitoids (Fig. 9.1.12). There was no indication that spray applications had a negative impact on natural predators. Most treated plots followed the same trend as the untreated control. At white bud Calypso (sprayed pre-blossom) had more anthocorids than the untreated control (*P*= 0.0316). However, by fruit set the numbers of anthocorids on the Calypso plots had declined significantly (*P*=.0265).

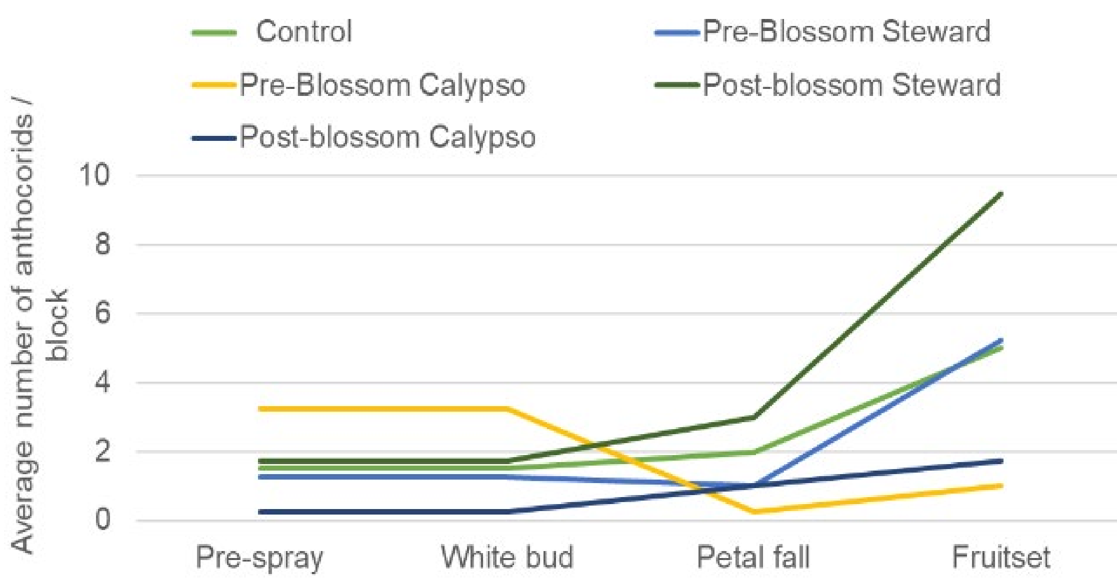

Figure 9.1.12. Mean number of anthocorids per block on sprayed and unsprayed plots

Discussion

No significant effect of the Calypso or Steward treatments on weevil numbers were recorded from both pre-blossom and post- blossom sprays. However, numbers of weevils were very low. Feeding damage in treated plots decreased during the trial.

Mean number of adult weevils recorded in 2019 were much lower than in 2018 for the same period (Fig. 9.1.13).

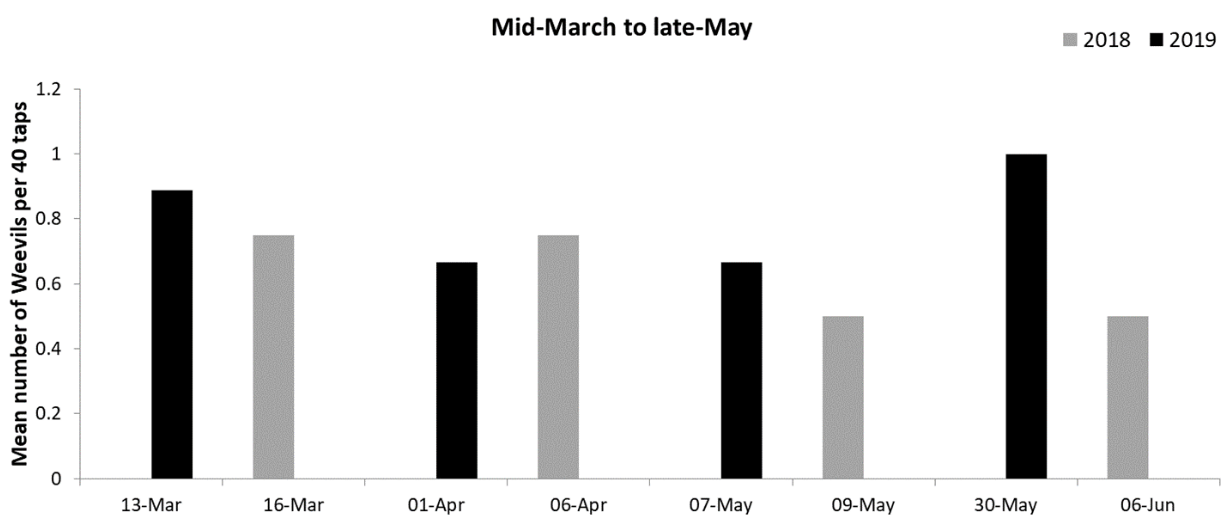

Figure 9.1.13. Mean number of adult weevils per 40 tree tap samples recorded during the same period in 2018 and 2019

There was high variability of feeding damage recorded between blocks (Fig. 9.1.14) making treatment effects difficult to conclude. No two blocks followed the same trend. Feeding damage decreased from mid-April 2019, then increased at the end of the monitoring period mid-May 2019.

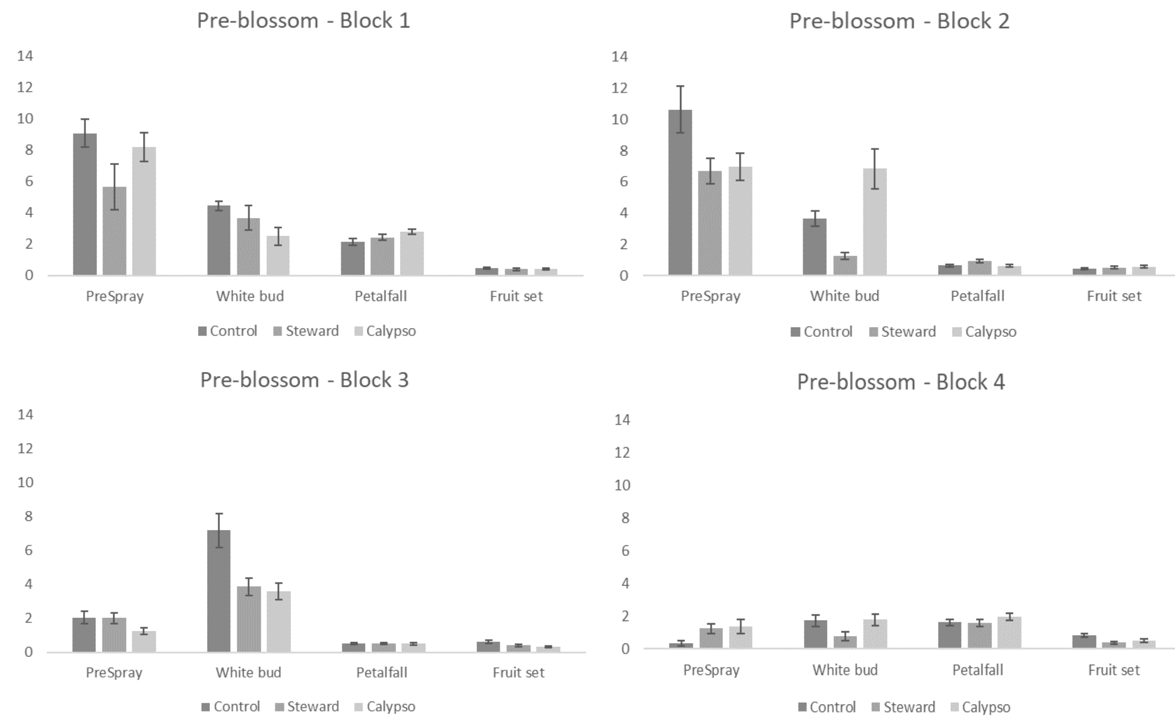

Figure 9.1.14. Average weevil feeding damage counted per 10 buds/shoots examined during pre-blossom assessments at treated (Steward, Calypso) and untreated (control) plots per block (1 to 4)

Conclusions

- In this field trial a spray application of Calypso or Steward before or after blossom had no effect on feeding damage or numbers of *A. spilotus*.
- The population of weevils may have been too low to show benefits from the product application.
- Calypso has been effective against *A. spilotus* in laboratory tests in previous years.
- More extensive studies are needed to confirm the effectiveness and best timing of application of these products in the field to control *A. spilotus* and other spring damaging weevils.
- The loss of Calypso from use means that really effective products are not currently available for weevil control. However, Gazelle (acetamiprid) gave 50% mortality in laboratory tests and could be used to keep populations in check.

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

Introduction

Brown marmorated stink bug (BMSB), an invasive pest native to East Asia, has become established in North America and several European countries (e.g. Switzerland, Italy, Germany, and France) in recent years. BMSB can travel long distances as a hitchhiker associated with imported goods and passenger luggage, and the insect has been intercepted entering the UK on several occasions (e.g. Malumphy, 2014). Bioclimatic modeling suggests that South East England is the most suitable region of the UK for establishment (Kriticos et al., 2017), but breeding populations have not yet been reported here. The insect poses a potential threat to UK horticulture as it can feed on and damage a wide range of plant species, including ornamentals, field crops and several tree fruit species (particularly apples).

When BMSB invades new countries it typically establishes initial populations feeding on ornamental plants and exotic tree species close to transport hubs and city centers, with spread outside urban areas and crop damage occurring later. As part of a small-scale surveillance programme during 2018, we raised awareness and appealed for reports of sightings of the pest, and placed pheromone traps at city center locations and sites of commercial fruit production in Southeast England. This activity has been continued during the 2019 season, using traps sited across a larger geographical area encompassing southern England and Wales.

Methods

The surveillance programme followed three strategies:

1. Pheromone trapping

"Pherocon BMSB STKY" rectangular (30 x 15 cm) double-sided clear sticky traps with high-dose 12-week pheromone lures (Trece Inc., USA) were used, containing two chemical components of the BMSB aggregation pheromone (Methyl E,E,Z-2,4,6-decatrienoate and Murgantiol). These pheromone traps provide effective, long-lasting detection of BMSB when populations of the pest are present (Weber et al., 2017). Traps were located at ten sites in England and Wales (Figure 10.1), each fixed to a horizontal tree branch (Figure 10.2) approximately 2.5 m from ground level (one trap per site). Trap sites for 2019 included apple and pear orchards in Kent and Suffolk, botanical and wildlife gardens in Cambridge and London, university campuses in Chatham and Reading, and an industrial site close to Cardiff Docks. Traps were initially installed between 29 May and 5 June 2019 and were checked weekly for signs of captured shield bugs. The sticky traps and lures were replaced after 12 weeks (in late August / early September) and the trapping continued for a second 12-week period (until November).

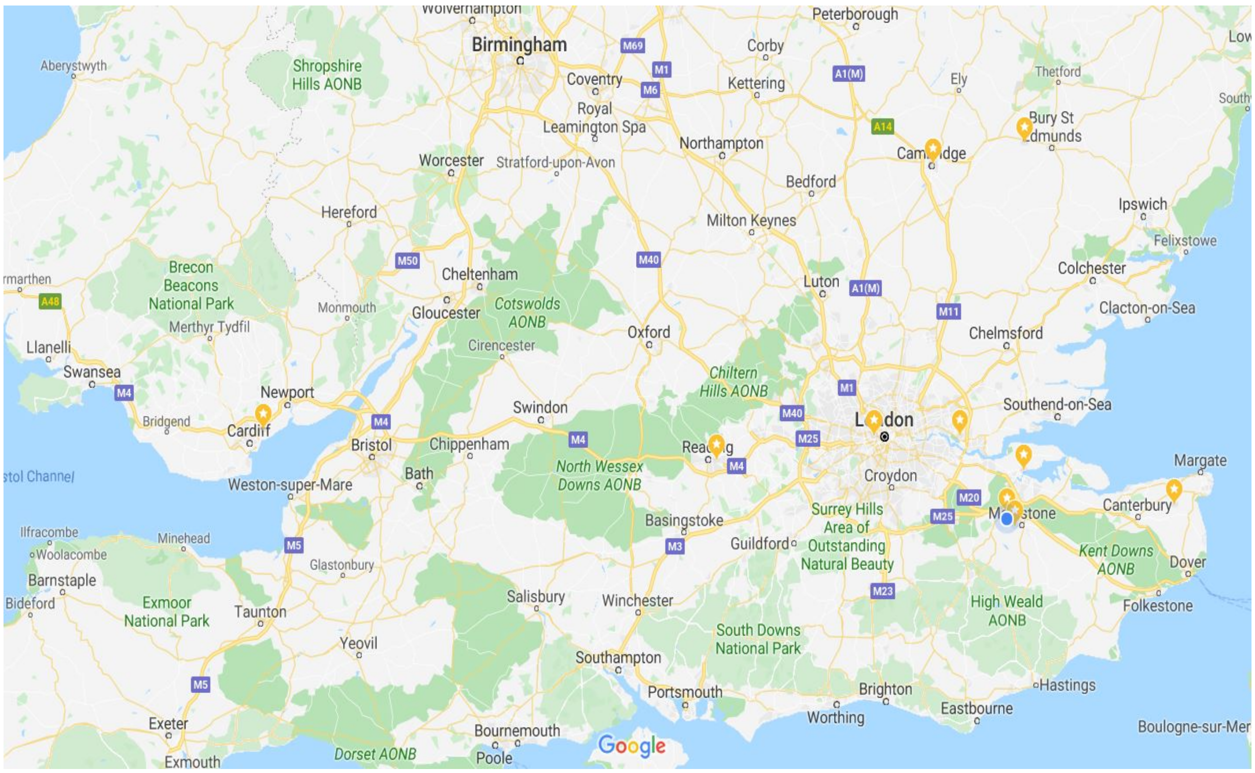

Figure 10.1. Sites of BMSB pheromone traps. Cambridge and London sites were urban locations, all other traps were positioned at sites of commercial fruit production

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

2. Alerts to growers and the general public

A NIAB EMR press release was issued on 1st June 2018 to publicize the pheromone monitoring programme and appeal for vigilance and reports of sightings. Growers and members of the public were requested to send specimens or images of any suspected BMSB to NIAB EMR for identification. These messages were also communicated in various articles that followed the press release, including the NFU's Horticulture Magazine, Fresh Produce Journal and the British Journal of Entomology and Natural History. The publicity efforts have continued through 2019 and 2020, particularly via presentations given by NIAB EMR staff to growers and agronomists (e.g. at multiple technical days including BIFGA, Berry Gardens, Agrovista and the AHDB Tree Fruit Day).

3. Searching for UK reports of BMSB using the internet and an amateur naturalist app.

Regular internet searches were carried out (using BMSB, UK, marmorated stink bug, and *Halyomorpha halys* as search terms) to become aware of and investigate reports of the invasive species associated with the UK. In addition, the iNaturalist app and website (**https://www.inaturalist.org/**) were used to check reports of BMSB in Europe posted by amateur wildlife enthusiasts.

Results

The pheromone monitoring traps caught no BMSB during 2019 and no shield bugs of any species. Emailed images and posted specimens continued to be sent to NIAB EMR for identification. Species mistaken for BMSB included two native UK species which superficially resemble BMSB (the forest bug, *Pentatoma rufipes* and the hairy shield bug, *Dolycoris baccarum*) in addition to two invasive species that have recently arrived and established in the UK (western conifer seed bug, *Leptoglossus occidentalis* and the mottled shield bug, *Rhaphigaster nebulosa*). No images or specimens of BMSB were received. Updated numbers of images / specimens are shown, combining numbers received in both 2018 and 2019 (Figure 10.3).

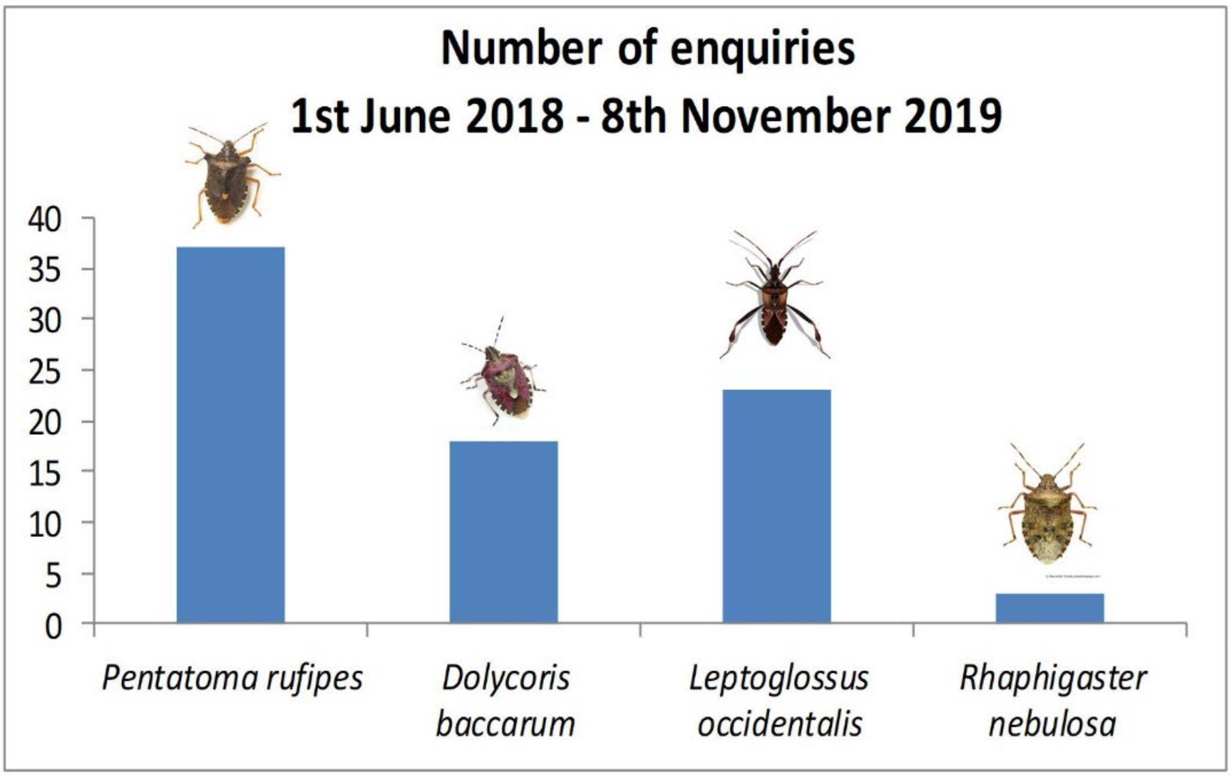

Figure 10.3. Number of plant bug species identified from images emailed and specimens sent to NIAB EMR between June 2018 and November 2019. Photo credits*: P. rufipes* © Chris Mattison, naturepl.com; *D. baccarum* © Life on White, picfair.com; *L. occidentalis* © seebugs.com; R. nebulosa © Alexander Slutsky, alsphotopage.com

Internet searches revealed a report (in November 2018) of an adult BMSB from Hampshire: https://www.pestcontrolnews.com/the-brown-marmorated-stink-bug-is-here-kicking-up-a-stink-in-the-uk-as-predicted-by-pcn/

This individual was intercepted by Mr Melvin Knapp, a pest control specialist. Mr Knapp provided images of this adult and the specimen itself for examination and identification by NIAB EMR – this was confirmed to be an adult male BMSB.

There is one iNaturalist record of the species, another adult found in April 2019 in the car park area of a Hampshire garden centre (see https://www.inaturalist.org/observations/23382471). The specimen was not collected but the photograph provides confirmation that this was another BMSB adult. This individual was reported within 20 miles of the previous UK record. Glen Powell (NIAB EMR) visited the garden centre site in June 2019. Several potential host tree species (*Sorbus, Ilex*) were found to be present. However, a search of these trees and the surrounding area did not reveal any signs of BMSB.

Discussion

Different species within the insect family Pentatomidae (stink / shield bugs) often share chemical components of their aggregation pheromones, resulting in significant cross-attraction of multiple species during pheromone monitoring programmes (Weber et al., 2017). The very low numbers of native species caught during the 24-week BMSB pheromone monitoring periods (only 3 native shield bugs caught during 2018, and none in 2019) is therefore encouraging and suggests that cross-attraction of non-targets is unlikely to be a problem during future UK BMSB monitoring using pheromones. However, some of our native pentatomids (particularly *P. rufipes* and *D. baccarum*) superficially resemble *H. halys* and are continuing to be mistaken for the invasive pest by growers, agronomists and members of the public (Figure 2). This highlights the value of continued future monitoring, combined with the provision of reliable identification methods. A current project, funded by the BBSRC through AHDB (CP 197), aims to develop a DNA-based identification method for this invasive species. This would help avoid confusion with the adults of other shield bug species but would also enable identification of the other life stages (egg masses and nymphs) that will be found once a breeding population establishes.

The two reports of BMSB adults in Hampshire are significant for two reasons. Firstly, these adults were found free in the environment, not obviously associated with imported goods or luggage (as has been the case for previous interception in the UK, e.g. Malumphy, 2014). Secondly, the two sites were in fairly close proximity, just a few miles from one another along the A31 main road between Farnham and Winchester. While this could be coincidental, with the adults arriving independently at the two sites having escaped from imported goods, it is also possible that there is a breeding population in Hampshire. It would therefore be worthwhile including Hampshire sites in a future pheromone trapping programme. In addition, targeted active surveys (using beating trays to sample likely host plants) should be carried out to supplement pheromone trapping, as proof of UK establishment of BMSB will require detection of nymphs and egg masses.

KNOWLEDGE AND TECHNOLOGY TRANSFER

2015

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

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

2016

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

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

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

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

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

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

21 July 2016: TF223 summer field visit, East Malling

2017

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

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

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

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

9 August 2017: National Association of cider Makers Orchard Walk at Weston's Caerswall Farm, Herefordshire, **Fountain and Saville**. Alternative pest control mechanisms, work on earwigs, and how the industry facing up to a post-chlorpyrifos and potential post-thiacloprid world. Overveiw of work on developing IPM programmes to control scab, mildew and canker.

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

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

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

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

2018

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

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

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

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

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

24 May 18: SOFT FRUIT WALK AT MOCKBEGGAR FARM ON TUESDAY 12 JUNE 2018 AT 5PM. Update on NIAB EMR research. **Fountain**

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

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

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

FACTSHEETS

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

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

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

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

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

2019

17 Apr 19 Talk to Lord Selbourne on entomology work at NIAB EMR, Pollinators and entomology **Fountain**

26-28 Jul 19 "IV Berries Festival" SERIDA Villaviciosa (Principality of Asturias, Spain) "Control strategies for Drosophila suzukii" **Fountain**

21 Jun 19 Innovation in Horticulture event, NIAB EMR, WET Centre: Fruit Quality attributes – research in to the role of beneficials and pollinators **Fountain**

Jul 19 Fruit Focus tour, Enhancing pest control by planting floral resources in and around strawberry crops **Fountain**

11 Sep 19 AHDB Fruit Agronomists' Day, NIAB EMR, 11 September, 2019

- Pear sucker monitoring (TF 223) **Celina Silva**
- New orchard biodiversity (TF 223) **Fountain**
- Apple powdery mildew (TF 223) **Angela Berrie**
- Apple canker research up-date (TF223) **Lucas Shuttleworth**

25-29 Nov 19 VIII Congress on Plant Protection Zlatibor, Serbia LIFECYCLE, DAMAGE AND CONTROL TO A NEW PEST OF PEAR IN SOME NORTHERN TEMPERATE PEAR ORCHARDS; ANTHONOMUS SPILOTUS **Fountain**

01 Oct 19 Canterbury Christ Church University 'Integrated Pest Management of Fruit Crops' **Fountain**

08 Oct 19 Agrii Fruit team, Throws Farm Essex. SWD, aphid control and forest bug **Fountain**

2020

13 Jan 20 Agrovista Grower Day, Black Horse Inn, Pilgrims Way, Thurnham, Maidstone, SWD, enhancing ecology and forest bug **Fountain**

23 Jan 20 BIFGA Technical Day, Ticehurst, East Sussex. Latest results on Neonectria canker of apple **Lucas Shuttleworth**

28 Jan 20 Agrovista Grower Day, White Lion, The Street, Selling, Faversham, SWD, enhancing ecology and forest bug **Fountain**

30 Jan 20 Herefordshire Hop Discussion Group, Plough Inn, Stoke Lacy, Herefordshire. Spider mite control in cherry **Fountain**

27 Feb 20 AHDB/NIAB EMR Tree Fruit Day, The Orchards Events Venue, East Malling, Kent

- An up-date on AHDB funded research into Neonectria canker of apple (Lucas **Shuttleworth**, NIAB EMR)
- Harnessing endophytes as an aid to apple canker control (Matevz **Papp-Rupar**, NIAB EMR)
- New research into the control of bacterial canker of cherry (Matevz **Papp-Rupar**, NIAB EMR)
- New approaches to apple powdery mildew control (Angela **Berrie**, NIAB EMR)
- Up-date on two shield bug pests – a native and an invader, (Glen **Powell**, NIAB EMR)
- Anthonomus spilotus – a climate change pest (Michelle **Fountain**, NIAB EMR)
- Enhancing the ecology of newly planted orchards (Celine **Silva**, NIAB EMR)

ACKNOWLEDGEMENTS

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A **massive** thanks to Dr Angela Berrie for all her help on this project.

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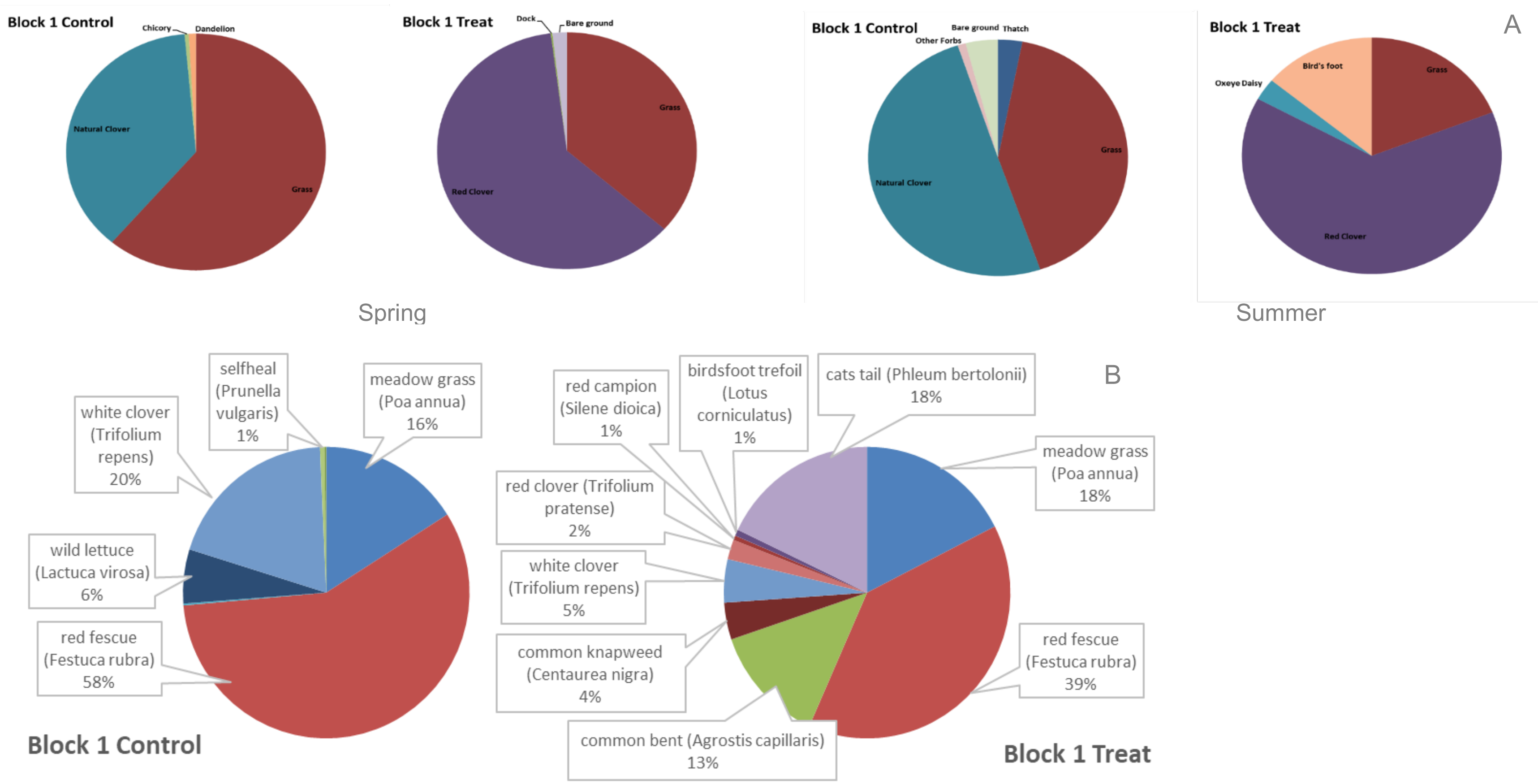
APPENDIX 3.1

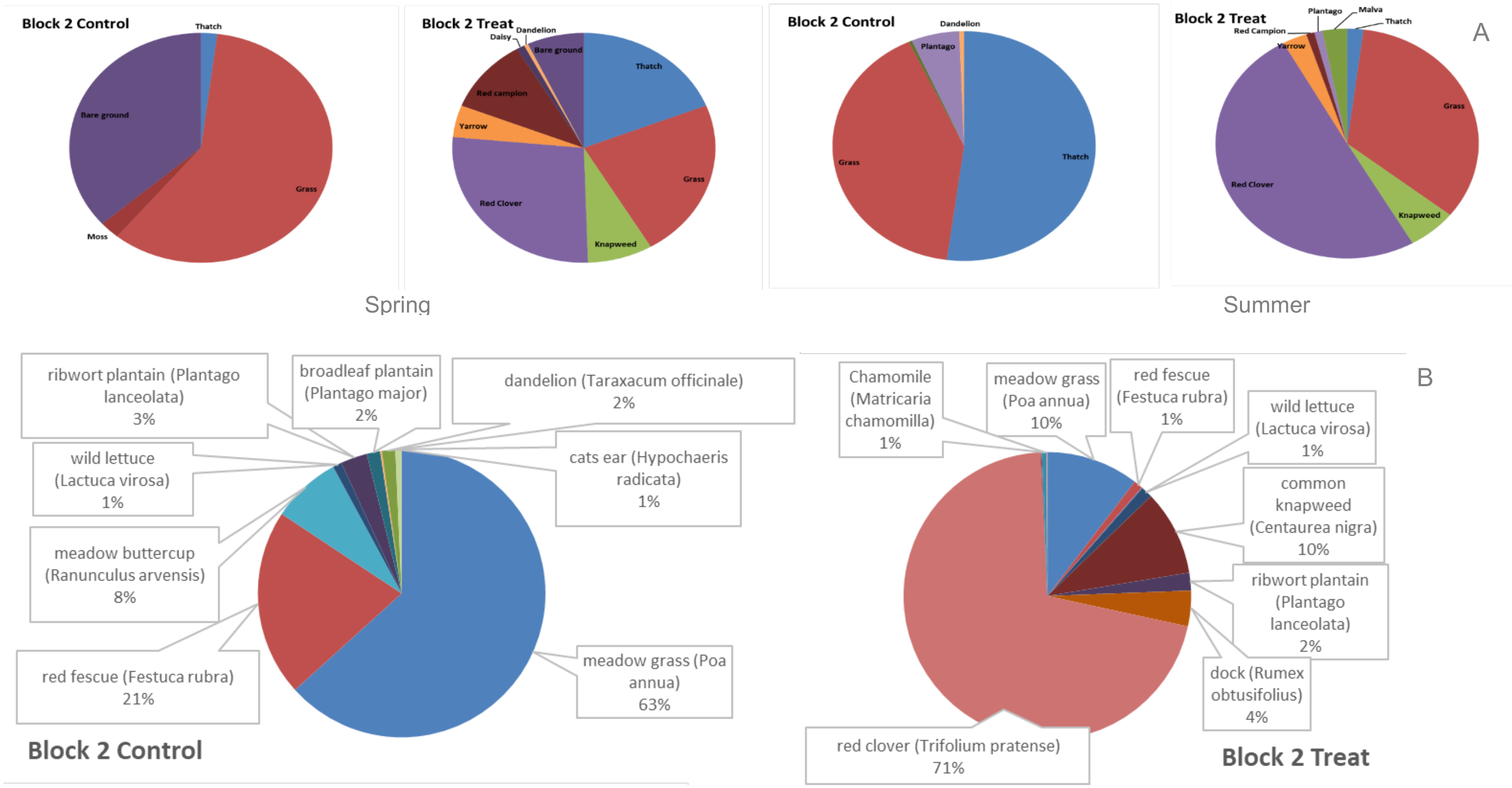
Table A. Treatments applied to orchard MP196 prior to start of trial and after end of trial and to all plots during the trial in 2019.

Date applied	Product	Type	Rate / ha
7 March	Cuprokylt FL	Fungicide	2 kg
20 March	Dithianon WG	Fungicide	0.5 kg
	Scala	Fungicide	1.1 L
29 March	Scala	Fungicide	1.1 L
	Alcoban	Fungicide	0.5 kg
	Calypso	Insecticide	0.375 L
11 April	Kindred	Fungicide	0.6 L
	Scala	Fungicide	1.1 L
	Calypso	Insecticide	0.375 L
14 May	Calypso	Insecticide	0.375 L
3 June	Batavia	Insecticide	1.5 L
13 June	Steward	Insecticide	0.25 kg
25 July	Coragen	Insecticide	0.175 L
31 July	Luna Privelege	Fungicide	0.225 L
	Mainman	Insecticide	0.14 kg
16 August	Topas	Fungicide	0.5 L
29 August	Coragen	Insecticide	0.175 L

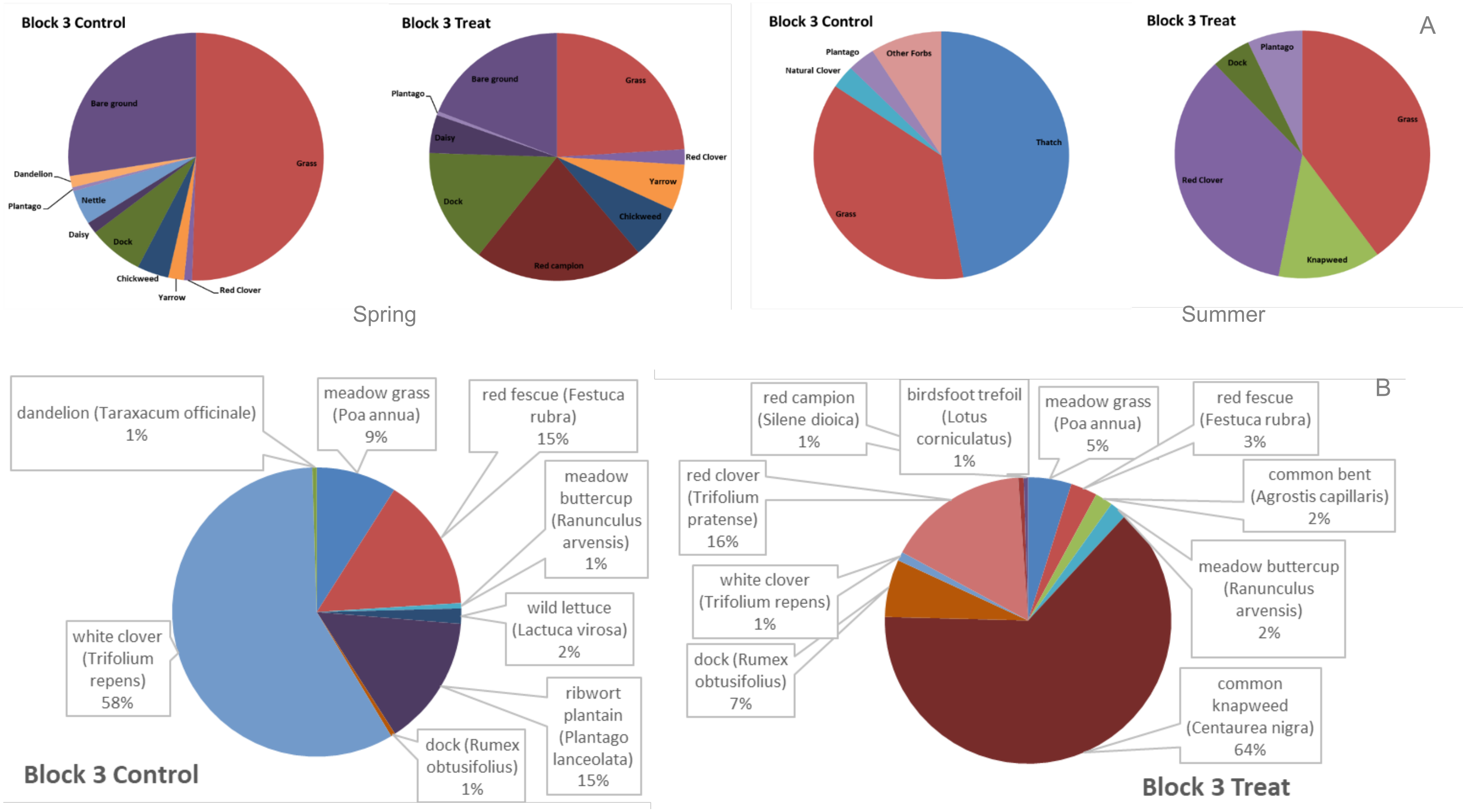
APPENDIX 7.1



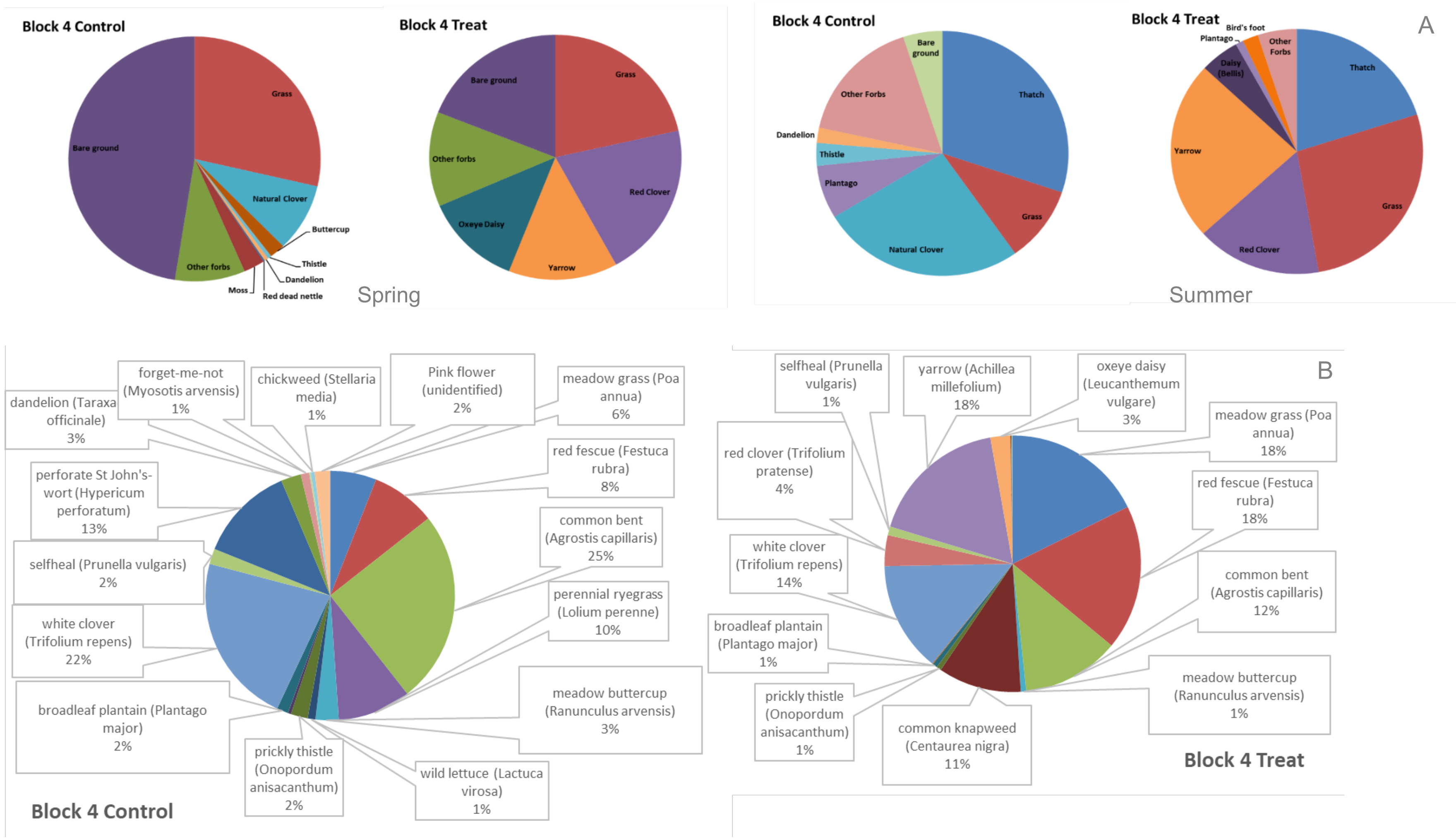
Vegetation cover in control and treatment intervention plots for block 1 in the A) spring and summer 2018 (upper charts) and B) summer 2019 (lower charts).



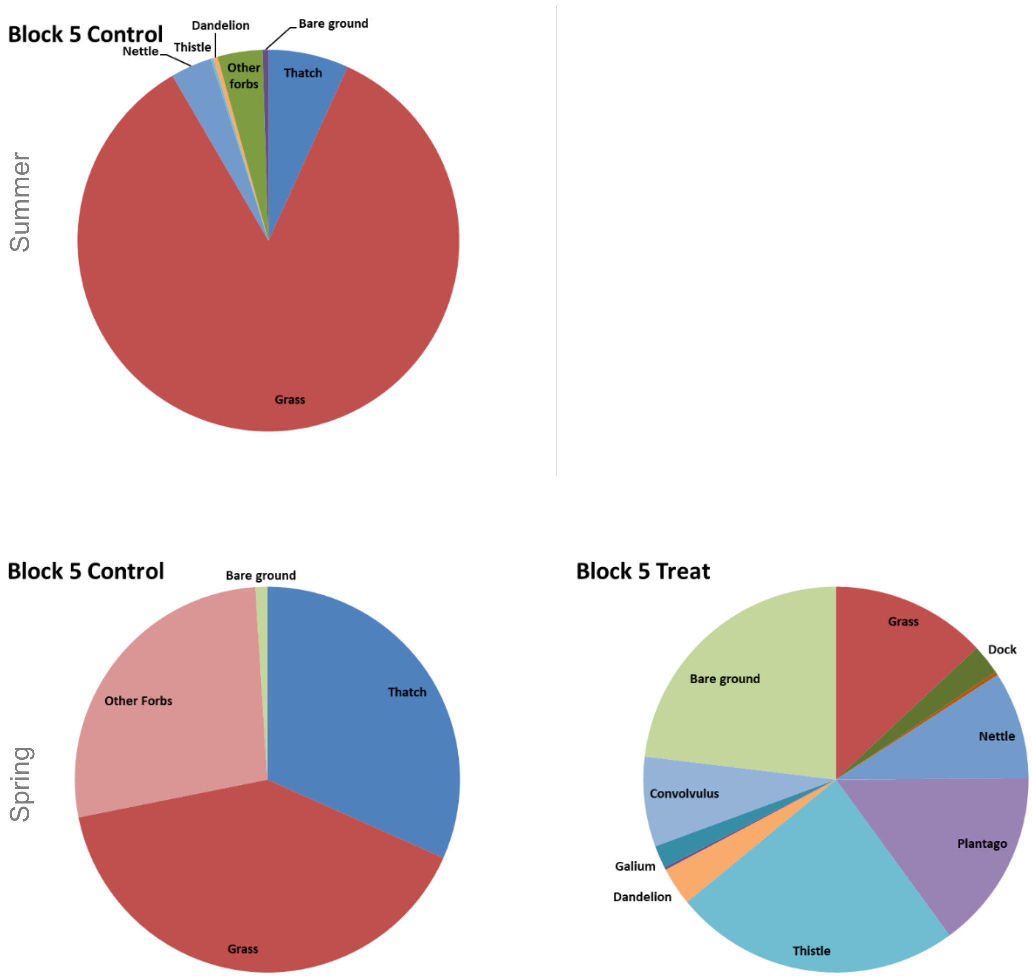
Vegetation cover in control and treatment intervention plots for block 2 in the A) spring and summer 2018 (upper charts) and B) summer 2019 (lower charts).



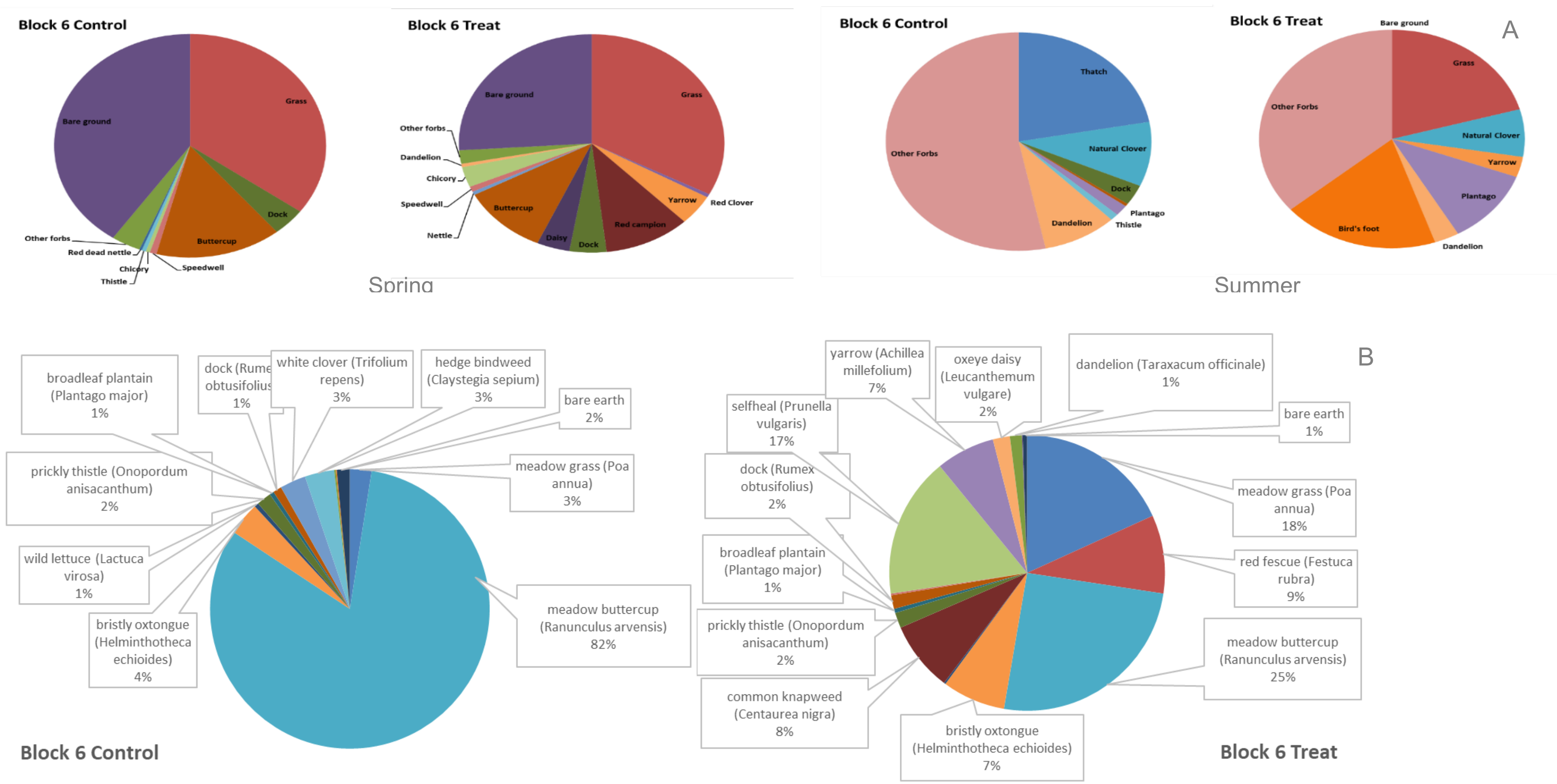
Vegetation cover in control and treatment intervention plots for block 3 in the A) spring and summer 2018 (upper charts) and B) summer 2019 (lower charts).



Vegetation cover in control and treatment intervention plots for block 4 in the A) spring and summer 2018 (upper charts) and B) summer 2019 (lower charts).



Vegetation cover in control and treatment intervention plots for block 5 in the spring and summer 2018. Block 5 was not assessed in 2019 as floral seed mix was re-sown in 2018.



Vegetation cover in control and treatment intervention plots for block 6 in the A) spring and summer 2018 (upper charts) and B) summer 2019 (lower charts).