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

AUTHENTICATION

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

Michelle Fountain

Project leader, Deputy Head of Pest and Pathogen Ecology

NIAB EMR

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Industry Representative

Signature .... Date ....

Report authorised by:

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AHDB

Signature .... Date ....

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GROWER SUMMARY

Objective 2. Neonetria canker of apple

Headline

- A combined approach of site-specific rootstock selection and the addition of specific soil amendments at planting time can help reduce Neonectria canker in newly planted orchards as part of an integrated disease management programme.

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 objective of Project TF 223a is to extend the experiments done in Project TF 223 that examined the effect of rootstock selection and the addition of soil amendments on canker number.

In the rootstock experiments, two sites in the UK were selected (Kent, Gloucestershire), while in the soil amendments experiments three sites were selected (two in Kent, one in Gloucestershire). The rootstock experiments evaluated a panel of six industry standard rootstocks alongside six advanced selections from the NIAB EMR rootstock breeding programme and two Geneva breeding programme selections (14 selections in total).

The amendment experiments evaluated the effect of arbuscular mycorrhizal fungi (AMF), plant growth promoting rhizobacteria (PGPR), Trichoderma and Biochar (at one of the sites) on reducing canker in newly planted orchards.

Summary of the project and main conclusions

Rootstock experiments

Canker numbers were assessed in 2020 in Spring (13-19 May in Kent, 21 May in Gloucestershire). For each tree, cankers were recorded according to their position on the tree where A = rootstock, B = mainstem or trunk of the tree and C, D, E = peripheral branches. Mainstem cankers (A+B), peripheral cankers (C+D+E) and total cankers (A+B+C+D+E) were recorded. The number of dead trees per rootstock was also recorded.

By the 2020 assessment, across all rootstocks, site 1 (Kent) had a 20.7 times higher total A+B+C+D+E canker number than site 2 (Gloucestershire) (Kent: 4803, Gloucestershire: 232).

Mean A+B+C+D+E canker number for Kent and Gloucestershire increased for most of the rootstocks between 2017-2020. G41 at Kent for example, had mean A+B+C+D+E canker of 0.03, but by 2020 this had increased to 27.29. G41 at Gloucestershire had a mean A+B+C+D+E canker in 2017 of 0.13 and in 2020 of 0.82.

At the Kent site, the highest mean A+B+C+D+E canker number and peripheral C+D+E canker number was lowest for M116 (7.10, 6.34) and MM106 (7.65, 6.46) while it was highest for the Geneva rootstocks G41 (27.29, 25.13) and G11 (21.54, 19.71).

At Gloucestershire, the lowest mean A+B+C+D+E canker number and peripheral C+D+E canker number was for M9 (337) with Golden Delicious interstock (0.11, 0) and EMR-004 (0.19, 0.19), while it was highest for G41 (0.82, 0.64) and M9 (EMLA) (0.81, 0.55).

By the 2020 assessment, at site 1 (Kent), 104 of the 448 trees (23.21%) had died, while at site 2 (Gloucestershire) 100 out of 560 trees (17.85%) had died.

Many of the NIAB EMR elite selections look promising for reduced canker, such as EMR-004 and EMR-003, while EMR-005 and EMR-006 are promising for reduced number of dead trees.

EMR-001 generally did not perform well, with the highest mainstem canker number and third highest peripheral canker number at Kent, and the fourth highest dead tree number for Gloucestershire and the sixth highest dead tree number at Kent.

Analysing data from both sites showed there is little relationship between tree vigour and canker number (R^2=0.0015).

Canker number is likely affected by site factors such as weather, orchard management and soil properties.

Soil amendment experiments

In 2020 (unlike 2019) there was no statistical difference between the unamended control and amended trees at any of the three sites.

There were slight decreases in mean tree vigour observed at all sites compared to the unamended control, except for AMF at site 1 which had an increase in vigour. It isn’t clear if this affected yield or other tree performance measures in 2020.

Main conclusions

Rootstock selection:

- Many of the NIAB EMR elite selections look promising for reduced canker, such as EMR-004 and EMR-003, while EMR-005 and EMR-006 are promising for reduced number of dead trees.
- At both sites, G41 had the highest mean number of A+B+C+D+E cankers.
- At both sites, tree death was higher with the M9 rootstocks [M9 (337) Golden Delicious interstock, M9 (337), M9 (EMLA)] and EMR-001.
- Analysing data from both sites showed there is little relationship between tree vigour and canker number (R^2=0.0015).
- Factors such as climate (temperature, rainfall, relative humidity), soil factors (organic matter content, waterlogging during autumn/winter, replant sites) and management factors (groundcover/mowing, tree spacing and scion cultivar selection) are likely to be having greater effects on canker number than the rootstock selection.

Soil amendments:

- There was no statistical difference between canker of the unamended control and the amended trees.
- There was a significant effect on vigour (trunk circumference) at sites 2 and 3, with decreases in mean tree vigour observed at all sites compared to the unamended control. It is not clear if this affected yield or other tree performance measures.

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 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 each year. This results in the financial burden for growers of replacing diseased/dead 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 as much of an effect on reducing canker.

Action points for growers

- It is important for growers to remain vigilant for cankers, identifying trees which are showing symptoms, pruning out cankers or removing heavily infected trees to prevent transmission to other trees and limiting abiotic stress of trees e.g. water stress, when planting out and establishing new orchards.
- Employing a range of canker reducing methods is recommended, as using a single method in isolation is unlikely to have as much benefit as a combined approach.

Objective 7.1 Improving the reliability of natural predation of pests

Headlines

- The use of wildflower mixes, earwig refuges and hoverfly attractants hastened the influx of natural enemies and reduced pest damage in newly established orchards.
- Effects on pests and natural enemies fluctuate between years and 2020 was the first year a rise in woolly apple aphid might have been detected in two of the six treated plots.

Background and expected deliverables

Establishing new crops requires substantial investment (~£35k/ha for apple). 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 several 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 alleyway establishment was successful in most orchards and the percentage coverage from the seed mix generally increased from 2018 to 2020.
- Not all species in the seed mix established. Red clover, yarrow and knapweed were the most abundant flowering species.
- In the early years, fewer aphids were observed in the treated plots in spring but not in summer. However, in 2020 there were more aphids overall in the treated plots and in at least two of the six treated plots, woolly apple aphids were higher in number. This should be a focus of future observations.
- More predatory spiders were found than earwigs in Wignests that had been deployed in treated plots, but anthocorids ladybirds and earwigs have also been observed. Some orchards still have relatively few earwigs even though they are in their third year.
- 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. Apple leaf curling midge was not assessed in 2019 or 2020.
- 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. Mites were not assessed in 2020.
- 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. Codling moth damage was too low to

analyse in 2020 but there were significantly fewer tortrix damaged apples in treated plots.

- 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 has 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, although it is important to observe effects on woolly apple aphid over the long term.
- Positive effects recorded included reduced numbers of pests including damage by codling moth, and higher numbers of natural enemies including hoverflies, spiders, and lacewings.
- Pest and natural enemy numbers need to be monitored in the long term.
- 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 listed in the table below (calculated in 2019).

	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: Wignest, 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 and 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.
- The 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 6-10 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 contact the soil. Following this, a period of rain or irrigation is desirable to encourage germination.
- The best time to sow in in the autumn.
- 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.
- Our orchards were also amended with earwig refuges (Wignests, Russell IPM) in each tree and hoverfly pheromone attractant. A similar hoverfly attractant product, MagiPal, is now available from Russell IPM.

SCIENCE SECTION

General Introduction

This is a short report on the continuation of the European apple canker (*Neonectria ditissima*) and Orchard Ecosystem Services (utilizing wildflowers, predator attractants and refuges) research that was set up to gather ongoing data from long-term trials and bridge a gap between years of AHDB funding in orchards.

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.

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.

Objective 2. Effect of roostock and soil amendments on Neonectria canker of apple

2.1 Effect of rootstock

Aim

Evaluation of the effect of rootstock on Neonectria canker of the scion (NIAB EMR/ADAS)

Introduction

Rootstocks are known to confer resistance/tolerance traits to various pests and diseases, for example woolly apple aphid, *Phytophthora* and *Neonectria*. Rootstocks are increasingly being considered as part of an integrated approach to canker control of particularly canker susceptible scion cultivars. The current objective evaluated the effect of a number of commonly used rootstocks as well as several advanced selections from the NIAB EMR and Geneva rootstock breeding programmes.

Materials and Methods

Orchard sites: Two sites in the UK were selected (Table 2.1.1). The first was the East Egham orchard, NIAB EMR, East Malling, Kent. The second was located at Herridges Farm, Gloucestershire.

Plant material: Fourteen rootstock selections were chosen, eight commercially relevant (to varying degrees due to vigour) rootstocks M9, M9 (EMLA), M9 (337), Geneva rootstocks (G11, G41), M26, MM106, M116, and six NIAB EMR elite selections (EMR001-006) (Table 2.1.2). In February 2016, common Gala cultivar scions were bench grafted on to all fourteen rootstock selections with one of the M9 (337) rootstocks having a Golden Delicious interstock grafted between it and the Gala scion. The six NIAB EMR advanced selection rootstock were sourced from Bruno Essner, Pepinieres Du Valois, France. The remaining rootstocks and Gala scion material were sourced from FP Matthews nursery, Tenbury Wells, Worcestershire. Grafted trees were grown on in pots in an outdoor nursery site 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 2017) they were prepared as bareroot trees and stored in commercial conditions (2°C in the dark, and the roots kept moist by being wrapped in damp hessian and watered regularly) until planting.

Table 2.1.1. Details of the two sites in the UK selected for the rootstock canker trial.

Site 1	Kent
Planted	29 March 2017
Description of planting site:	The site is situated amongst mature orchards in which *Neonectria ditissima* inoculum is prevalent, providing opportunities for natural infection.
Tree spacing:	3.5 x 1.75 m

Aerial view:

Trial layout:

Four replicates of 8 tree plots, arranged over four blocks (as determined by colour). A total of 448 trees in 56 plots.

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:

Four replicates of 10 tree plots per treatment. Each plot separated by mature Cox trees. A total of 560 trees in 56 plots.

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

Table 2.1.2. The rootstock selections, including one selection grafted with a M9 (337) rootstock and Golden Delicious cultivar interstock. Advanced selections from the NIAB EMR breeding programme are coded (EMR-001-006).

Rootstock selections
M9 (EMLA)
M9 (337)
MM106
M116
M26
M9 (337) with Golden Delicious interstock
G.11
G.41
EMR-001
EMR-002
EMR-003
EMR-004
EMR-005
EMR-006

Canker number assessments and number of dead trees: The 2020 canker number assessments were completed in spring (13-19 May in Kent, 21 May in Gloucestershire). For each tree, cankers were recorded according to their position on the tree as described by McCracken et al. (2003). A = rootstock, B = mainstem and C, D, E = peripheral (Figure 2.1.1). Mainstem cankers (A+B), peripheral cankers (C+D+E) and total cankers (A+B+C+D+E) were recorded. The number of dead trees per rootstock was also recorded.

Tree vigour: Trunk vigour was measured by trunk circumference (mm) around the trunk, 10 cm vertically above the graft union (Figure 2.2.1).

Statistical analyses: For each site, canker data was analysed with ANOVA. This included mainstem (A+B), peripheral (C+D+E), and total (A+B+C+D+E) cankers. An unbalanced design analysis was used due to dead trees altering tree numbers between rootstock selections.

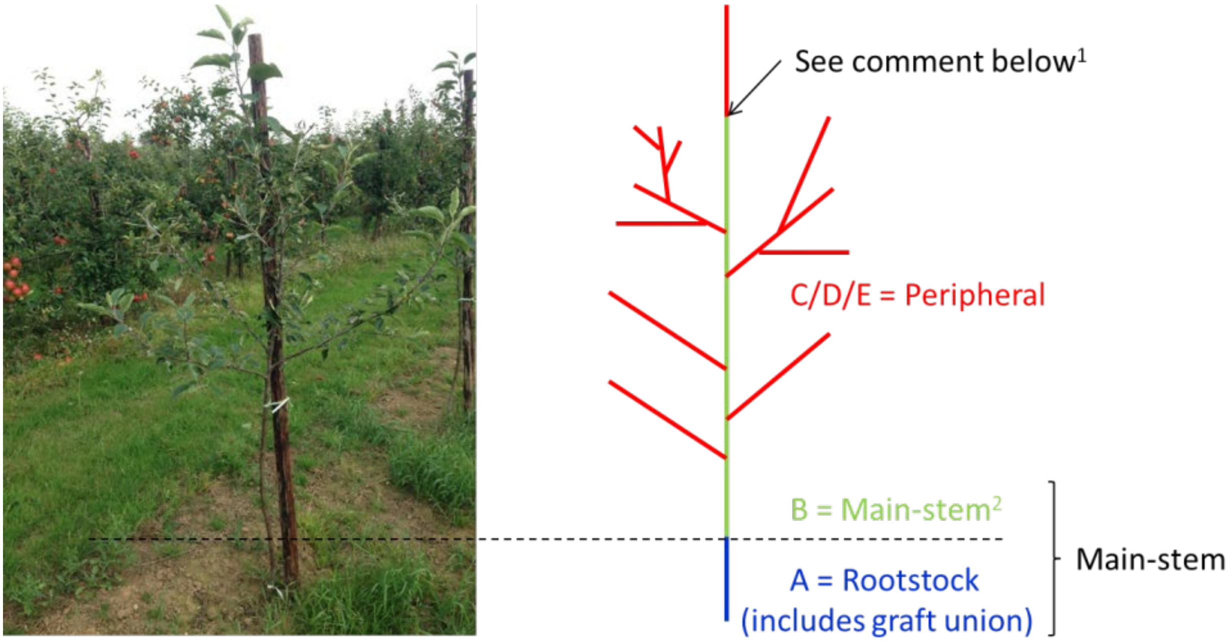


Figure 2.1.1. Diagram of the classifications of cankers based on their position on the tree. [1]note that there is a continuum between the mainstem and peripheral branch on the main leader; on wood which has grown since planting (i.e. < 3 years) were scored as peripheral and those on wood > 3 years were scored as mainstem. [2] cankers occurring on the interstock M9 with Golden Delicious interstock were scored as 'B' type canker.

Results (Tables and Figures are provided at the end of the results section)

Canker assessments: In 2020, site 1 (Kent) overall had higher total canker number (A+B+C+D+E) than site 2 (Gloucestershire) when cankers from all rootstocks were counted (Kent: 4803, Gloucestershire: 232). Mean A+B+C+D+E canker number for Kent and Gloucestershire generally increased between 2017-2020 (Table 2.1.3). At the Kent site, the highest mean A+B+C+D+E canker number and peripheral C+D+E canker number was lowest for M116: 7.10, 6.34, and MM106: 7.65, 6.46, while it was highest for the Geneva rootstocks (G41: 27.29, 25.13, and G11: 21.54, 19.71) (Table 2.1.4). At Gloucestershire, the lowest mean A+B+C+D+E canker number and peripheral C+D+E canker number was for M9 (337) with Golden Delicious interstock (0.11, 0) and EMR-004 (0.19, 0.19), while it was highest for G41 (0.82, 0.64) and M9 (EMLA) (0.81, 0.55) (Table 2.3.5). Comparing both sites M116, EMR-003 and EMR-004 had lower mean canker, while G41 had the highest. The statistical analyses showed that at both sites, canker number of mainstem (A+B), peripheral (C+D+E) and total (A+B+C+D+E) canker number was significantly different between rootstocks (Table 2.1.6).

The boxplot of Site 1 (Kent) 2020 assessment showed the lowest mainstem canker number was M116, EMR-002, EMR-003 and M9 (EMLA), and the lowest peripheral canker number was MM106, EMR-004, M9 (EMLA) and M116 (Figure 2.1.2). At Site 1, EMR-001 and G41 had the highest number of mainstem cankers, while both the Geneva rootstocks (G41, G11) had the highest peripheral canker number. The boxplot of Site 2 (Gloucestershire) showed that the lowest mainstem canker number was EMR-004 and EMR-003, while the lowest peripheral canker was for M9 (337) with Golden Delicious interstock and EMR-002 (Figure 2.1.3). The highest mainstem canker at Gloucestershire in 2020 was EMR-002 and MM106, and for peripheral cankers G41 and M9 (EMLA).

Number of dead trees: By the 2020 assessment, at Site 1 (Kent), 104 of the 448 trees (23.21%) had died, at Site 2 (Gloucestershire) 100 out of 560 trees (17.85%). At both sites, the NIAB EMR elite selections EMR-005, EMR-006 had the lowest number of dead trees (n= 3 for both at Kent, n=2 at Gloucestershire, Figure 2.1.4). In addition to EMR-005 and EMR-006 at Kent, M116 also had the lowest number of dead trees (n=3). Rootstocks with the highest number of dead trees at Kent was the NIAB EMR elite selection EMR-003 (n=13) followed by M9 (337) with Golden Delicious interstock (n=10). At Gloucestershire, M9 (337) with Golden Delicious interstock also had the highest number of dead trees (n=13), while M26 (n=12) had the second highest number of dead trees. Interestingly at Gloucestershire, M9 (337) with Golden Delicious interstock had the highest number of dead trees, however it had the lowest canker number. This is likely due to dead trees being excluded from canker number assessments after the tree had died.

Relationship between tree vigour (trunk circumference) and canker: The combined data from Sites 1 and 2 found there was only a very weak relationship between trunk circumference and canker number (R^2=0.0015, Figure 2.1.5). This indicates that there are likely other factors at play affecting canker number than tree vigour.

Table 2.1.4. Site 1 (Kent) mean canker of the fourteen rootstocks displayed as mainstem (A+B), peripheral (C+D+E), and total (A+B+C+D+E) cankers in spring 2020. Rootstocks are ordered lowest to highest total cankers from left to right.

	Rootstock													
Position of canker on tree	*M116*	*MM106*	*EMR-003*	*EMR-004*	*M9 (337) with GD interstock*	*EMR-006*	*EMR-002*	*M9 (EMLA)*	*M26*	*EMR-005*	*M9 (337)*	*EMR-001*	*G11*	*G41*
Mainstem	0.76	1.19	0.89	1.29	1.23	1.45	0.89	1.44	1.41	1.41	2.04	3.16	1.82	2.17
Peripheral	6.34	6.46	8.23	8.52	9.09	9.10	10.07	10.17	11.48	12.24	13.56	17.80	19.71	25.13
Total	7.10	7.65	9.12	9.81	10.32	10.55	10.96	11.61	12.89	13.66	15.60	20.96	21.54	27.29

Table 2.1.5. Site 2 (Gloucestershire) mean canker of the fourteen rootstocks displayed as mainstem (A+B), peripheral (C+D+E), and total (A+B+C+D+E) cankers in spring 2020. Rootstocks are ordered lowest to highest total cankers from left to right.

	Rootstock													
Position of canker on tree	*M9 (337) with GD interstock*	*EMR-004*	*EMR-003*	*M116*	*EMR-006*	*M26*	*EMR-001*	*EMR-005*	*G11*	*EMR-002*	*MM106*	*M9 (337)*	*M9 (EMLA)*	*G41*
Mainstem	0.11	0	0.06	0.19	0.24	0.32	0.23	0.24	0.11	0.52	0.47	0.47	0.26	0.18
Peripheral	0	0.19	0.18	0.14	0.18	0.14	0.23	0.24	0.41	0.13	0.29	0.33	0.55	0.64
Total	0.11	0.19	0.24	0.33	0.42	0.46	0.46	0.48	0.52	0.65	0.76	0.80	0.81	0.82

Table 2.1.6. ANOVA results from two sites (Kent and Gloucestershire) of canker number (mainstem, peripheral, total cankers) of 14 different rootstocks.

Location of canker	**Degrees of freedom**	***p-value***
Site 1: Kent		
Mainstem (A+B)	13	<0.001
Peripheral (C+D+E)	13	<0.001
Total (A+B+C+D+E)	13	<0.001
Site 2: Gloucestershire		
Mainstem (A+B)	13	0.007
Peripheral (C+D+E)	13	0.048
Total (A+B+C+D+E)	13	0.029

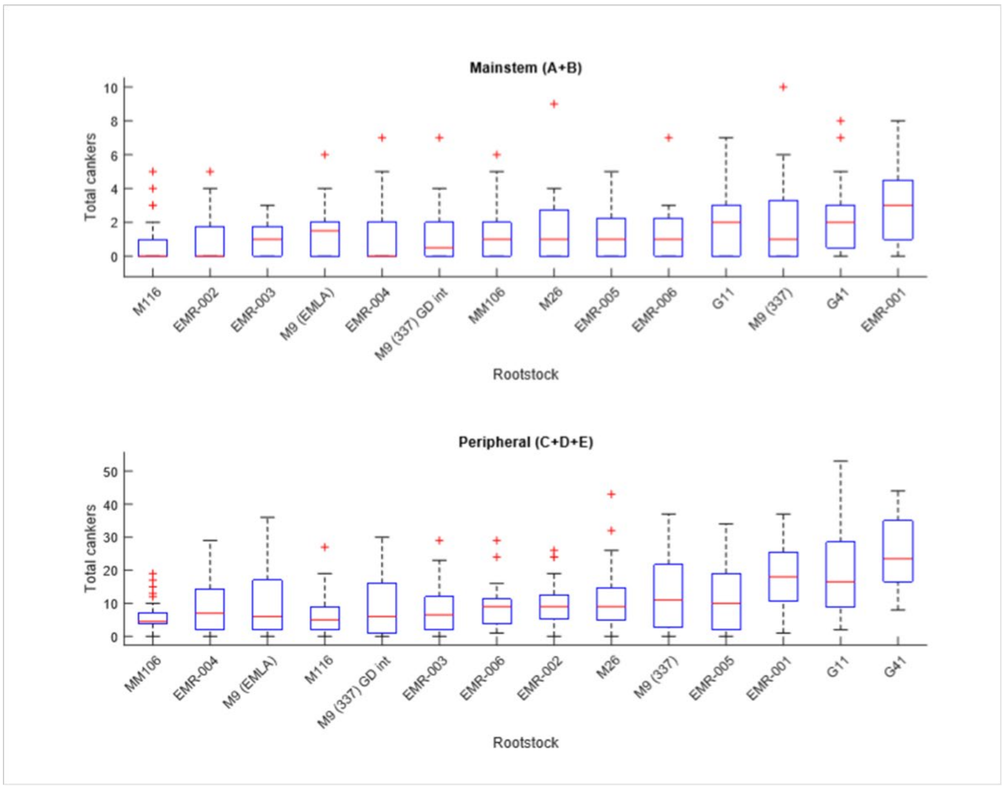


Figure 2.1.2 Boxplot of site 1 (Kent) sum of total mainstem (A+B) and total peripheral (C+D+E) cankers of the fourteen rootstocks displayed as in spring 2020. Rootstocks are ordered lowest to highest cankers from left to right.

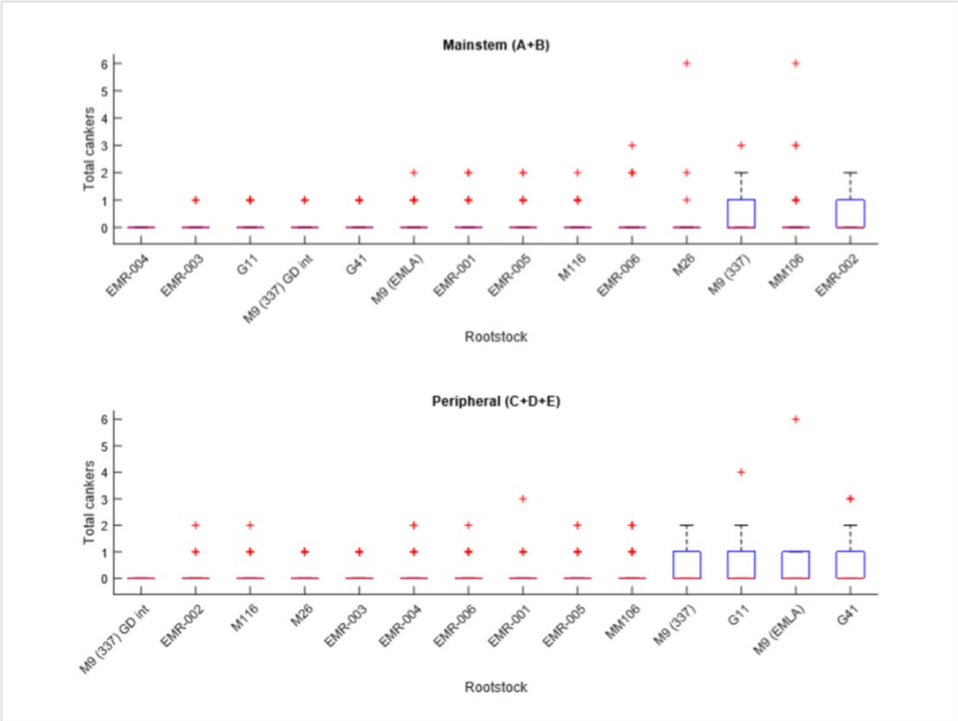


Figure 2.1.3 Boxplot of site 2 (Gloucestershire) sum of mainstem (A+B) and peripheral (C+D+E) cankers of the fourteen rootstocks in spring 2020. Rootstocks are ordered lowest to highest cankers from left to right.

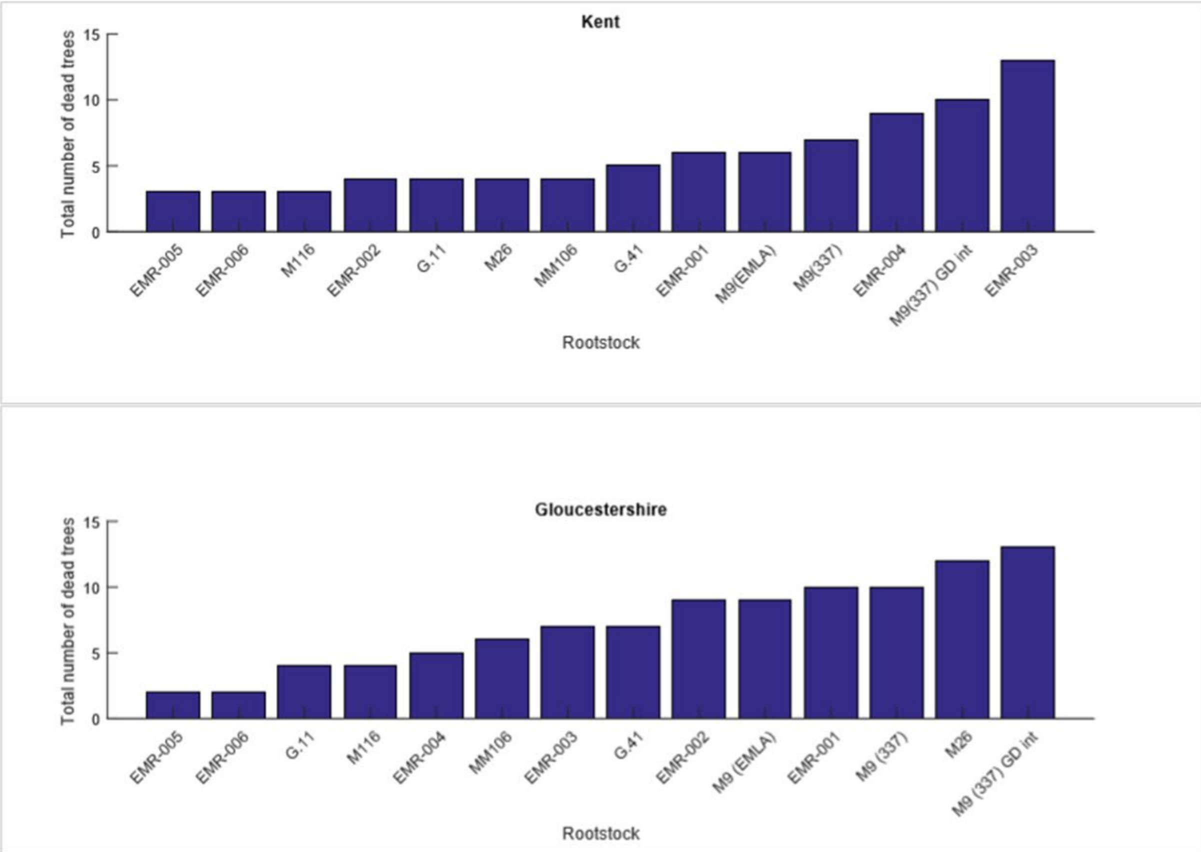


Figure 2.1.4. The number of dead trees per rootstock at Site 1 (Kent) and Site 2 (Gloucestershire) in 2020.

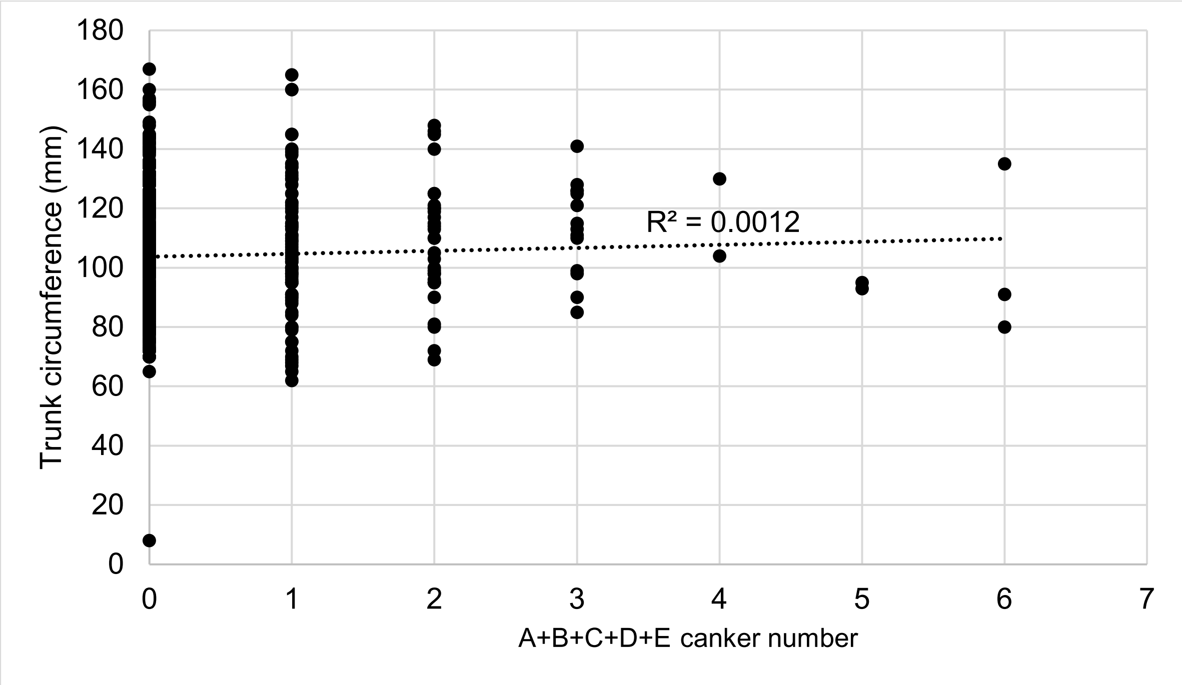


Figure 2.1.5. Number of cankers per tree plotted against trunk circumference (tree vigour) for the combined data from Kent and Gloucestershire. There was little relationship between the two variables (R^2 = 0.0015).

Discussion

In the 2020 assessments, canker number had increased at both sites in Kent and Gloucestershire. The total cankers on the mainstem and periphery in Kent were 20.7 times higher than Gloucestershire; the trees in Gloucestershire had fewer cankers. This indicates that rootstock does not have a strong effect on canker number, or that there is an interaction with the local environment. For example, site factors such as climate (temperature, rainfall, relative humidity), soil factors (organic matter content, waterlogging during autumn/winter, replant sites) and management, including groundcover/mowing, tree spacing, and scion cultivar selection may have a greater effect on canker number than the rootstock selection. Current work being completed at NIAB EMR in the BBSRC CankerLink project, is investigating fungal and bacterial endophyte communities associated with apple tree leaf scars (a key entry point for *Neonectria ditissima*, particularly in the autumn leaf fall period) and showed that 'site' was the strongest factor affecting endophyte assemblages.

In 2020, M9 rootstocks often had some of the highest canker numbers and numbers of dead trees e.g., M9 (337), M9 (337) with Golden Delicious interstock, M9 (EMLA) confirming observations by UK apple growers that the M9 series of rootstocks are particularly prone to canker. Regarding tree death, M9 (337) with Golden Delicious interstock was highest at Gloucestershire and second highest at Kent. This evidence would suggest this rootstock interstock selection combination is not a desirable choice.

Mainstem cankers are biologically significant as the infection may girdle and kill the tree. The rootstock EMR-001 had the highest number of mainstem cankers at the Kent site, while EMR-002 had the highest at Gloucestershire indicating they were 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 neighbouring trees.

Many of the NIAB EMR elite selections look promising for reduced canker such as EMR-004 and EMR-003, while EMR-005 and EMR-006 are promising for reduced number of dead trees. However, EMR-001 generally did not perform well, with the highest mainstem canker number and third highest peripheral canker number at Kent, and the fourth highest dead tree number for Gloucestershire, and the sixth highest dead tree number at Kent.

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 circumference) and canker, there was only a very weak correlation, indicating that vigour does not strongly affect canker. The BBSRC Link project (BB/P007899/1) has indicated that site and scion cultivar have a stronger effect on fungal communities associated with leaf scars on/in the scion wood than the effect of rootstock. These effects could also be the case for *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).

Conclusions

- By 2020, canker at both sites had increased for all rootstocks compared the 2017 assessment.
- In 2020, rootstocks were identified with lower canker number including M116, EMR-003, EMR-004. However, EMR-003 had the highest number of dead trees at the Kent site.
- At both sites, G41 had the highest mean number of meristem and peripheral cankers.
- At both sites, tree death was higher with the M9 rootstocks [M9 (337) Golden Delicious interstock, M9 (337), M9 (EMLA)], and EMR-001.
- There was a very weak relationship between tree vigour and canker number.
- Factors such as climate (temperature, rainfall, relative humidity), soil factors (organic matter content, waterlogging during autumn/winter, replant sites) and management factors (groundcover/mowing, tree spacing, and scion cultivar selection) are likely to be having greater effects on canker number than the rootstock selection.

2.2 Effect of soil amendments on Neonectria canker

Aim

Evaluation of treatments to improve tree health and establishment using soil amendments (NIAB EMR/ADAS).

Introduction

Previous research on Neonectria canker of apple, in particular the millennium trial (McCracken *et al*. 2003) showed that *N. ditissima* can infect trees in the nursery and remain asymptomatic within the tree. Once planted in the orchard, the tree experiences stress e.g., drought/water logging/replant disease, and canker symptoms are expressed. This objective aims to evaluate soil amendments to reduce canker and improve tree health over time.

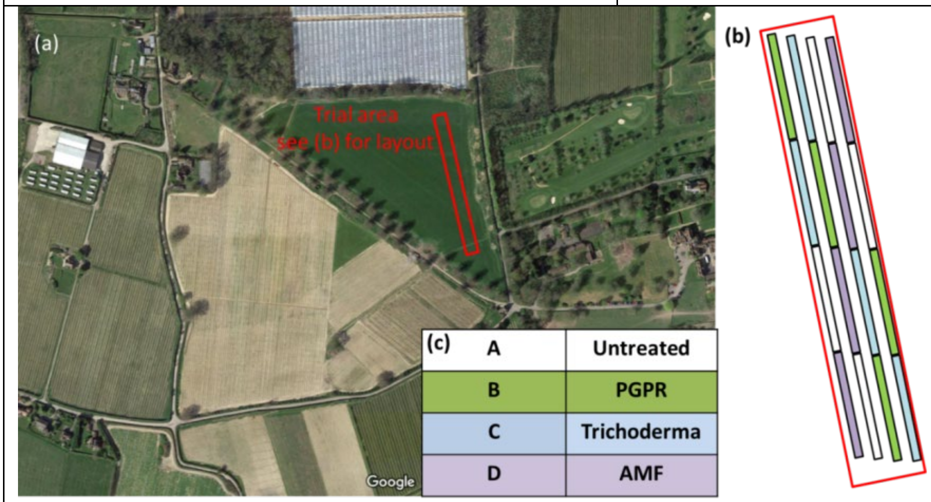
Materials and Methods

Sites

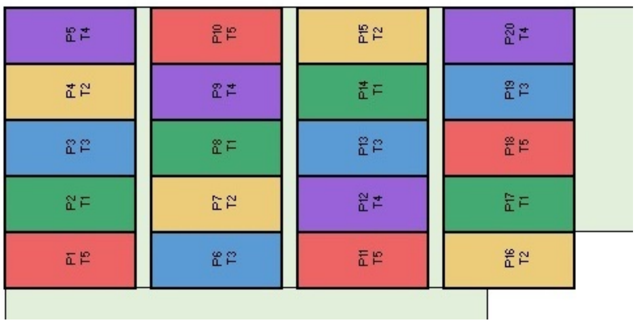
Two orchard sites in Kent were selected (Broadwater Farm, Kent, Friday Street Farm, Kent) and planted in 2016, with an additional site (Herridges orchard, Gloucestershire) planted in 2018 (Table 2.2.1).

Table 2.2.1 Orchard sites selected for the soil amendments in newly planted orchards.

Site 1	Friday Street Farm, Kent
Variety	Cv. Rubens
Number of trees	162/163 trees per amendment type, 650 trees total.
Planted	15/03/16



Site 2	Broadwater Farm, Kent
Variety	Cv. Gala (was intended to be Cv. Jazz but trees were not available when the trial was setup)
Number of trees	190 trees per amendment type, 760 trees total.
Planted	12/05/16

Site 3	Herridges orchard, Gloucestershire
Planted	12 April 2018
Variety	Leg Gala
Number of trees	45 trees per amendment type, 225 trees total.
	

Soil amendments

Details of the products used in the amendments trial and the application methods are listed in previous AHDB TF223 reports.

Assessments

Canker number assessments using the McCracken et al. 2003 protocol (described earlier in objective 2.1) were made in December 2020 due to UK COVID lockdown restrictions in spring/summer 2020. The number of dead trees on each site was also recorded.

Effect of tree vigour on canker number: Vigour was measured using tree circumference 10 cm above the graft union. Trunk circumference measurements were made on all trees at all sites. A regression analysis was performed with GenStat 19th edition, to determine any relationship.

Results (Tables and figures at end of results section)

Canker assessments

In the 2020 assessment, canker numbers on trees at site 1 (Friday Street Farm, Kent) amended with Trichoderma had the lowest mean A+B+C+D+E cankers (2.89) compared to the unamended control (3.29), AMF amended (3.35) and the PGPR amended (2.98) (Figure 2.3.2). However, this difference was not significantly significant to the unamended control (Table 2.2.2).

In the 2020 assessment, canker numbers at site 2 (Broadwater Farm, Kent) were low, and none of the soil amendment treatments were significantly different to the untreated control for A+B+C+D+E cankers (Table 2.2.2). AMF had the lowest mean A+B+C+D+E canker (0.64), with CarbonGold Biochar (0.89) and Trichoderma (0.95) also lower than the unamended control (0.96) (Figure 2.2.3).

In the 2020 assessment at site 3 (Herridges orchard, Gloucestershire) canker number was low particularly when compared to site 1. There was no significant difference between any of the amendments on A+B+C+D+E cankers compared to the unamended control (Table 2.2.2) 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 number of A+B+C+D+E cankers, however none of the amendments were significantly different to the unamended control (Table 2.2.2).

Vigour assessments

At Site 1 (Friday Street Farm, Kent) there was no significant difference in vigour between the amended trees (Table 2.2.4). AMF had the largest mean trunk circumference (mean 111.3

mm) compared to the unamended control (110 mm). At site 2 (Broadwater Farm, Kent), all the amendments had smaller mean trunk circumference compared to the unamended control (107.70 mm). However, these were not significantly smaller. At Site 3 (Herridges orchard, Gloucestershire), the untreated control also had the largest trunk girth, with PGPR closely following (80.06 mm). There was a significant difference here with the AMF+PGPR having mean trunk circumference of 75.69 mm.

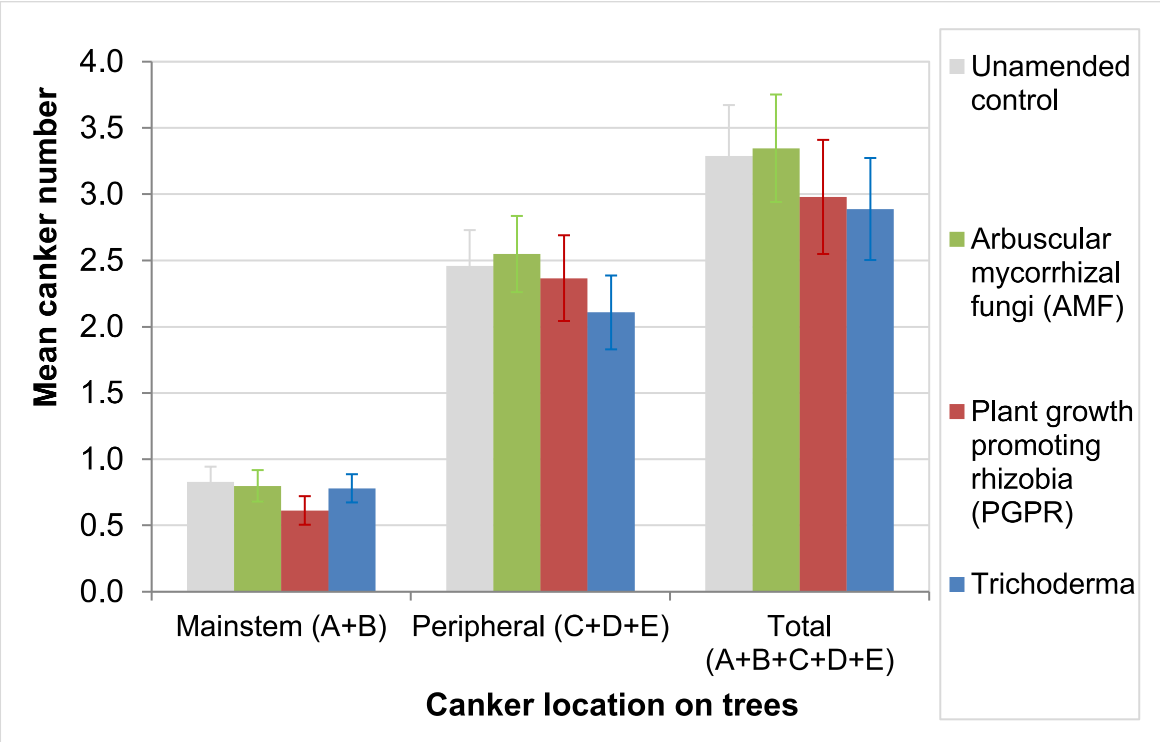


Figure 2.3.2 Mean number of cankers after treatment with soil amendments at Friday Street Farm, Kent (Site 1). Error bars indicate standard error of the mean. There was no significant difference in canker between the amended trees and the unamended control for any of the canker categories (mainstem A+B, peripheral C+D+E or total A+B+C+D+E, also see Table 2.2.2).

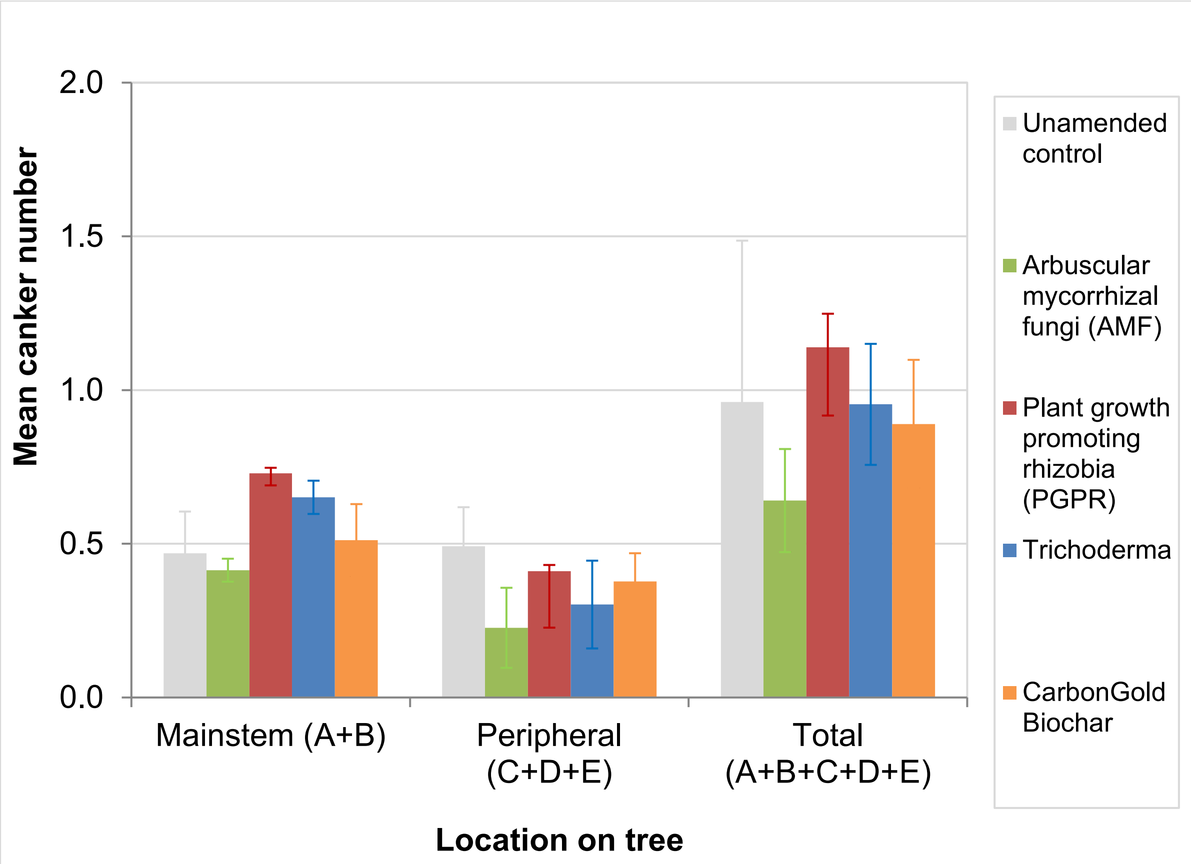


Figure 2.3.3 Mean number of cankers after treatment with soil amendments at Broadwater Farm, Kent (Site 2). Error bars indicate standard error of the mean. There was no significant difference in canker between the amended trees and the unamended control for any of the canker categories (mainstem A+B, peripheral C+D+E or total A+B+C+D+E, also see Table 2.2.2).

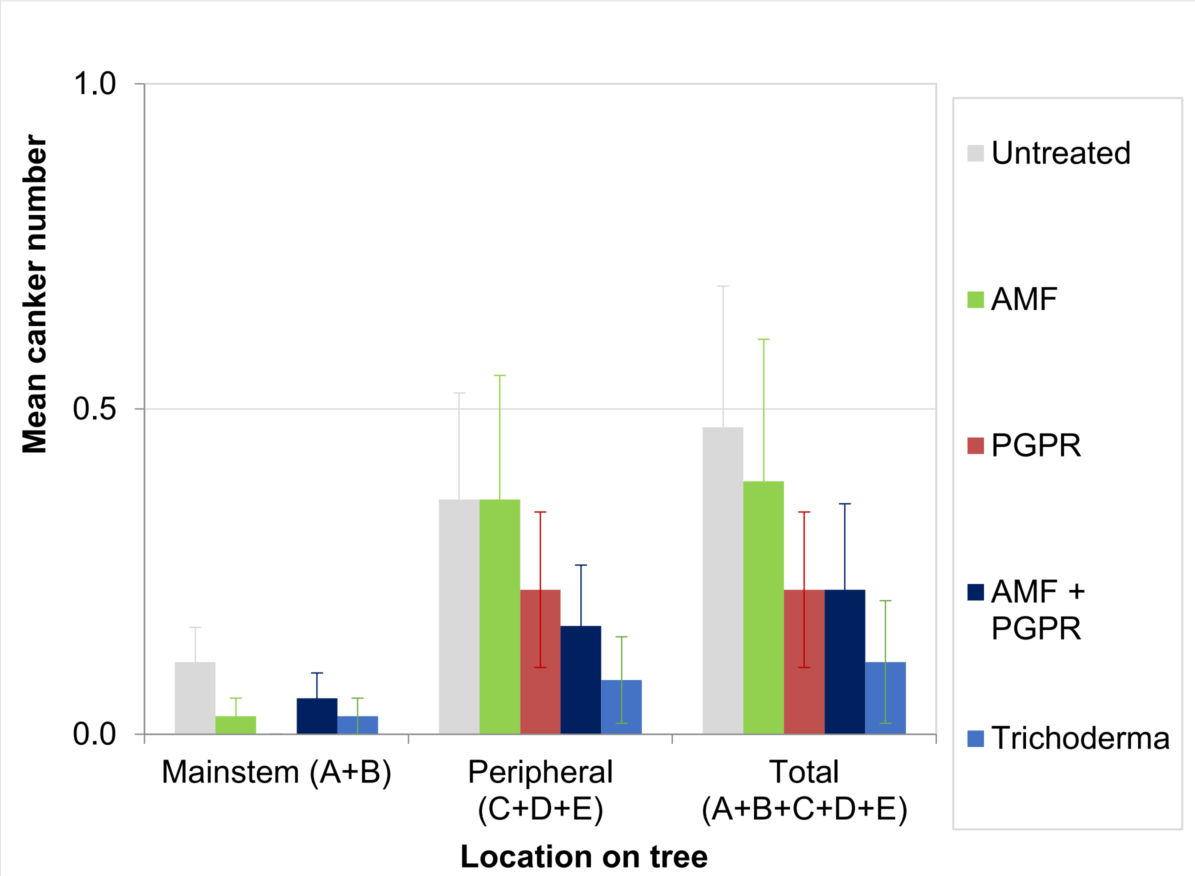


Figure 2.3.4 Mean number of cankers after treatment with soil amendments at Herridges orchard, Gloucestershire (Site 3). Error bars indicate standard error of the mean. There was no significant difference in canker between the amended trees and the unamended control for any of the canker categories (mainstem A+B, peripheral C+D+E or total A+B+C+D+E, 06/also see Table 2.2.2).

Table 2.2.2. ANOVA results of A+B+C+D+E canker number and soil amendments from the three orchard sites.

Site	Location of canker on tree	Degrees of freedom	p-value
1. Friday Street Farm	Total (A+B+C+D+E)	3	0.622
2. Broadwater farm	Total (A+B+C+D+E)	4	0.530
3. Herridges orchard, Gloucestershire	Total (A+B+C+D+E)	4	0.104

Table 2.2.3 Summary statistics for vigour (trunk circumference) for the three sites in the amendments trial.

Site	Amendment	Mean (mm)	Std dev	df; p-value
1. Friday Street Farm (Kent)	Unamended	110.00	15.38	3; 0.075
	AMF	111.30	17.14	
	PGPR	107.30	15.40	
	Trichoderma	108.10	16.66	
2. Broadwater Farm (Kent)	Unamended	107.70	12.02	4; 0.019
	AMF	106.80	9.36	
	PGPR	107.40	10.78	
	Trichoderma	104.40	10.04	
	Biochar	106.00	8.74	
3. Herridges orchard (Gloucestershire)	Unamended	80.97	4.88	4; <0.001
	AMF	79.69	4.39	
	PGPR	80.06	4.00	
	AMF+PGPR	75.69	3.91	
	Trichoderma	78.50	4.41	

Discussion

Site 1 had the highest canker of all sites in 2020, and Site 3 continued to have very low canker over time. This indicates there are site factors affecting canker number, even when amendments are applied. Site differences were also apparent in the rootstock experiments in the previous section.

Concerning the amendment type applied at planting time and canker in young trees, Trichoderma (Trianum G) had the most consistent effect in reducing canker number across sites and over time, reducing it the most effectively at Site 1 and Site 3. AMF was the most effective at reducing canker at Site 2, with the CarbonGold Biochar having some effect of reducing canker.

In the 2020 assessments, there were slight reductions in trunk circumference (vigour) observed at all sites compared to the unamended control, except for AMF at Site 1 which had an increase. It is not clear if this would affect yield or other tree performance measures.

At Site 3 in the 2020 assessment, the canker number was low despite favourable conditions during autumn 2019/winter 2020. The treatment with the lowest canker number was *Trichoderma*, which had performed well in 2019.

The comparison of canker across newly planted orchard sites and years revealed that canker across Site 1 and 2 increased over time. At Site 3, canker number was similar between the 2019 and 2020 assessments. This may well 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

- In 2020, there was no significant effect on canker between the amended trees compared to the unamended control.
- There was a significant effect on vigour (trunk circumference) at Sites 2 and 3 with decreased mean vigour compared to the unamended control. It is not clear if this affected yield or other tree performance measures.

- The effect of using amendments on canker of newly planted orchards since 2017 has been variable and will likely be most effective when used in combination with other canker control methods.
- It is important for growers to still be vigilant with visual inspection for cankers, identifying trees which are showing symptoms, pruning out cankers or removing heavily infected trees to prevent transmission to other trees, and limiting abiotic stress of trees e.g., water stress, when planting out and establishing new orchards.

Objective 7 - Improve Reliability of Natural Enemies

7.1 Enhance and accelerate the natural ecology in newly planted orchards

Introduction

Establishing new crops requires substantial investment (~£30k/ha for apple) and growers need confidence that their orchards will crop reliably, and 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 that can then be difficult to control.

In 2017 we applied interventions to newly planted orchards to establish this beneficial ecology more rapidly. In 2018 a perennial wildflower 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 regularly mown grass alleyways. Subsequently, flora affected arthropod abundance in treated plots compared to untreated. Fewer aphids were observed in treated plots during spring. 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. 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 predator attractant sachets in additional to floral alleyways may have also contributed to increased hoverfly adults in the treated plots. Arthropod numbers in 2018 were low and so results should be interpreted with caution.

In 2019 the floral cover of the sown species increased compared to the previous year. Red clover was the most common species in 2019 followed by common knapweed. More predatory spiders than earwigs were found in Wignests (Russell IPM, predator refuges) deployed in the treated plots. Most predatory spiders found in the refuges in 2019 belonged to family Araneidae. In 2019 anthocorids were also recorded in refuges. In the tree canopy, predatory spiders were the most common arthropod recorded in all seasons and most individuals collected belonged to Araneidae and Philodromidae family. Some species of 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. A subfamily of Linyphiidae, Erigoninae (also known as Micryphantids), have been reported preying on soft-bodied pests like aphids on various occasions (Nyffeler & Benz, 1988; Mansour & Heimbach, 1993). Also in 2019, predatory mites were found on apple leaves, with higher numbers recorded in treated plots. There were significantly fewer codling moth stings in treated plots in the autumn. Hoverfly adults were not significantly higher in the treated plots in autumn 2019.

Aim

Speed up the ecology of newly planted orchards to establish beneficial arthropods more quickly to mitigate losses due to pests and assess progress of floral establishment in orchard alleyways.

Methods

Sites:

The trial took place on six replicate apple orchards (blocks), sourced by Caroline Ashdown at Worldwide Fruit Ltd (WFL) (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.

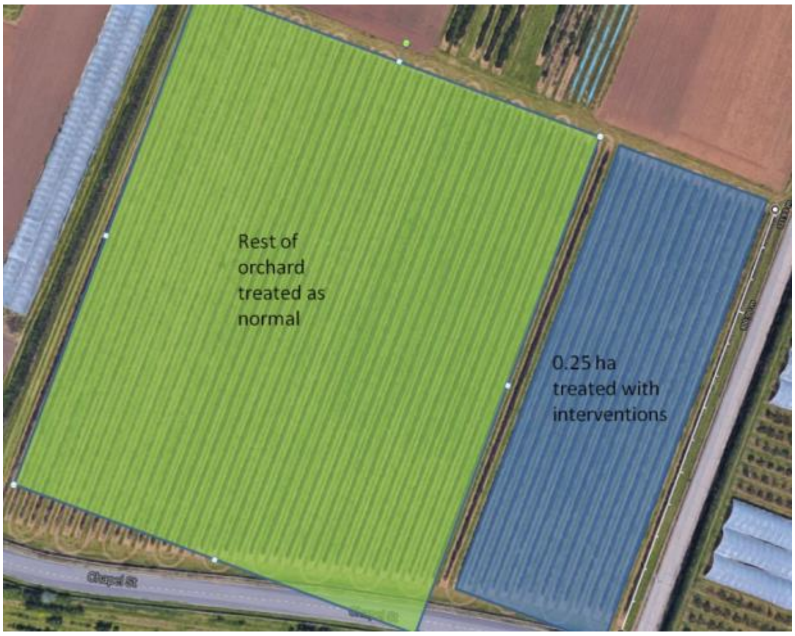


Figure 7.1.1. Example of an experimental block during the enhancing orchard ecology trial 2017 to 2020. Blocks were divided into 2 plots; an untreated plot (green), lacking ecological enhancement interventions and a treated plot (blue) with ecological enhancement interventions.

Table 7.1.1. Orchards (blocks), site managers and alleyway sowing dates for the enhancing orchard ecology trial 2017 to 2020.

Orchard (block)	Name	Address	Orchard, Variety	ha	spacing (m)	Trees planted	Notes/ planting	Row length (m)	Sown 2017
1	John Bray	A J Bray & Sons Ltd, Holmestall, Doddington Sittingbourne ME9 0HF	A12, Jazz	2.6	3.35x 1 or 1.2	Feb 2017	Every other row for 5 rows (10 rows)	144	Apr
2	Clive Chandler	Chandler & Dunn Ltd. Lower Goldstone Farm CT3 2DY	Broome, Gala	2.23	3.5x 1.25	Dec 2016	Every row (7 rows) for 0.25 ha of orchard	95	May
3	Clive Chandler	SEE ABOVE	Richards, Jazz	1.54	3.5x 1.25	Dec 2016	Every row (11 rows) for 0.25 ha of orchard	60	May
4	Peter Checkley	Howard Chapman Ltd. Broadwater Farm, West Malling, Kent ME19 6HT	New Barns, Gala	1.3	3.5x 1.5	April 2017	Every third, 0.25 ha, 5 rows	109	May
5	Jeremy Linsell	Chromesword Ltd, Braiseworth Orchards, Eye, Suffolk, IP23 7DS	Rectory, Jazz	1.13	3.25x 1.2	Jan 2017	4 rows every other row	144	April 2018
6	Charles Highwood	Sheerland Farm, Pluckley, Ashford TN27 0PN	Willow Wood Variety	2.28	4x1.5	May 2017	0.4 ha sown in every row	250	May

Treatments (Table 7.1.2.): *Perennial wildflower seed mix* was sown in 2017 at most sites but was resown where establishment was poor (e.g., Site 5) (Table 7.1.3), with some modifications.

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 implemented
Alleyway sowings [*1]	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 [*2]	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)

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

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

Table 7.1.3. Suggested and tested seed mix for orchard alleyway planting in the 0.25 ha 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" (predator 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. Refuges were renewed where necessary throughout the trial period.

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 each year in treated plots at the end of May, then replaced once mid-July. 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, mid-July 2019, and mid-July 2020. Crop husbandry was the growers' standard practice with less frequent mowing of the floral alleyways.

Assessments (2020)

In 2020, the assessments were slightly reduced from previous years due to Covid restrictions and slightly lower funding. Three assessments were made in the central rows of untreated and treated plots at each block. Assessments involved the following:

May

- Photographs of sward and tree stage were taken.
- 30 shoots were examined for the presence of aphids and total number of aphids in each shoot counted.
- 30 trees were checked for the presence of woolly apple aphid colonies.
- 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 trees were checked for the presence of Woolly apple aphid colonies.
- All fruit on 30 trees were examined and the number of fruits 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.
- 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 fruits 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.
- Wignests were open and arthropods inside them recorded and counted.

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 4-year trial perennial wildflower seed mixes successfully established to varying degrees in treated plots at most blocks with increasing forb diversity, evenness and structure observed; including block 5 which 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 so much though. Red campion developed in spring but was only recorded in the summer survey on one block and at a very low percent 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.

In 2019, a single more detailed seed mix assessment was made in summer; all forbs and grasses were identified to species level. In most blocks, coverage of the seed mix had increased since 2018 (Table 7.1.4). Red clover was still dominant along with common knapweed. In untreated plots, natural clover and unsown grasses were most common. Sward was higher in treated plots compared to untreated (p<.001, Fig. 7.1.4).

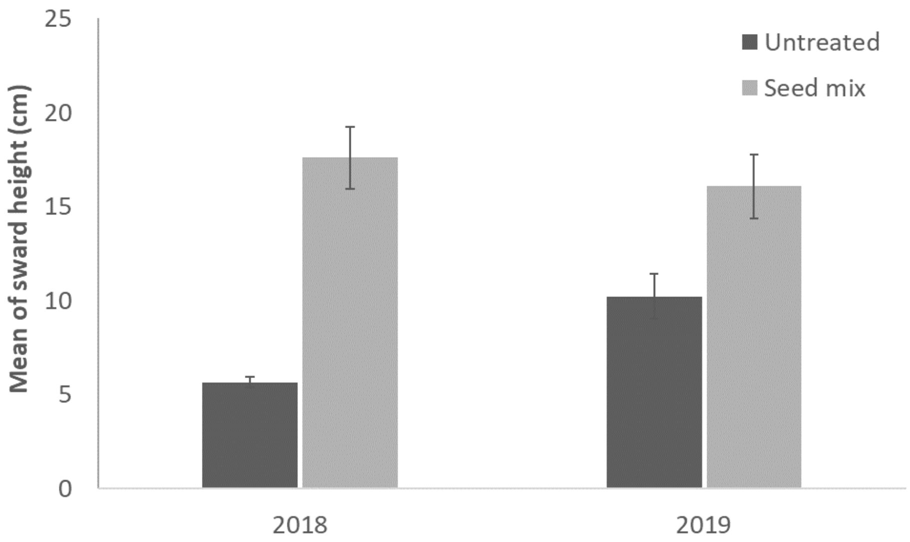


Figure 7.1.4. Sward height (cm) measured using the drop disk method in summer 2018 and 2019. Mean of 10 measurements per plot.

In 2019 the vegetation data was analyzed to test vegetation diversity compared to the untreated (Fig 7.1.5). Three tests were done (Table 7.1.5); *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 considers the number of species present, and 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.

In 2020, the sown seed mix coverage continued to increase in most blocks (Table 7.1.4). As in 2019 common knapweed remained one of the most common species in treated plots, however we also recorded an increase in yarrow coverage. In untreated plots unsown grasses were well established and the most common wildflower species were white clover and creeping buttercup. Sward height was not recorded in 2020.

Table 7.1.4. Percent vegetation cover of seed mixes, per treated site, in spring and summer 2018 to summer 2020.

Site	Season	Coverage of seed mix 2018 (%)	Coverage of seed mix 2019 (%)	Coverage of seed mix 2020 (%)
1	**spring**	61.5	-	-
	summer	81	64.5	69.3
2	**spring**	50	-	-
	summer	60	81.6	84.3
3	**spring**	29.5	-	-
	summer	48	83.6	83
4	**spring**	47	-	-
	summer	42	53.9	98
5	**spring**	-	-	-
	summer	-	-	72
6	**spring**	15.5	-	-
	summer	22	43.1	59

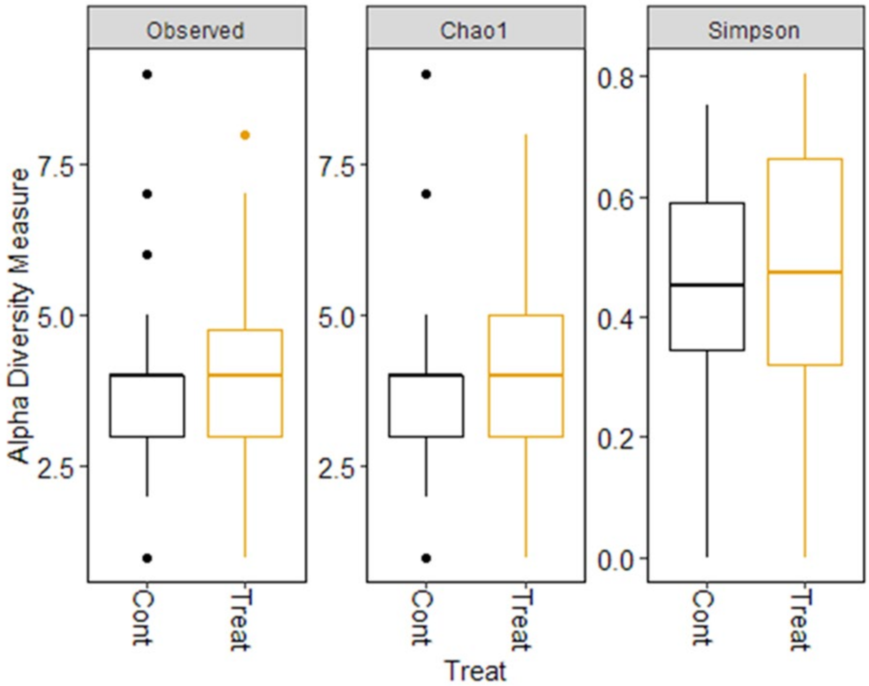


Figure 7.1.5. Vegetation diversity of treated (Treat) plots compared to the untreated (Cont) plots using 3 diversity index tests in 2019.

Aphid Monitoring

In spring 2018 more aphids were observed in untreated compared to treated plots ($p<.001$) (Fig. 7.1.6). Aphids were found on 4 untreated plots in 2018 and 2019 in spring, but only on 2 treated plots in 2018 and 1 in 2019 (too low for statistical analyses). In summer, the number of aphids increased in treated plots both years but were not significantly different compared to untreated (Fig. 7.1.6). Numbers of aphids in the summer months did not differ between treatments.

In 2020 number of aphids recorded on shoots did not show the same trend as previous years and significantly higher numbers were found in treated plots in spring ($p = .0087$) and summer ($p = .0043$) (Fig. 7.1.6). In spring and summer 5 of the 6 treated plots recorded high numbers of aphids when compared with their respective untreated plot.

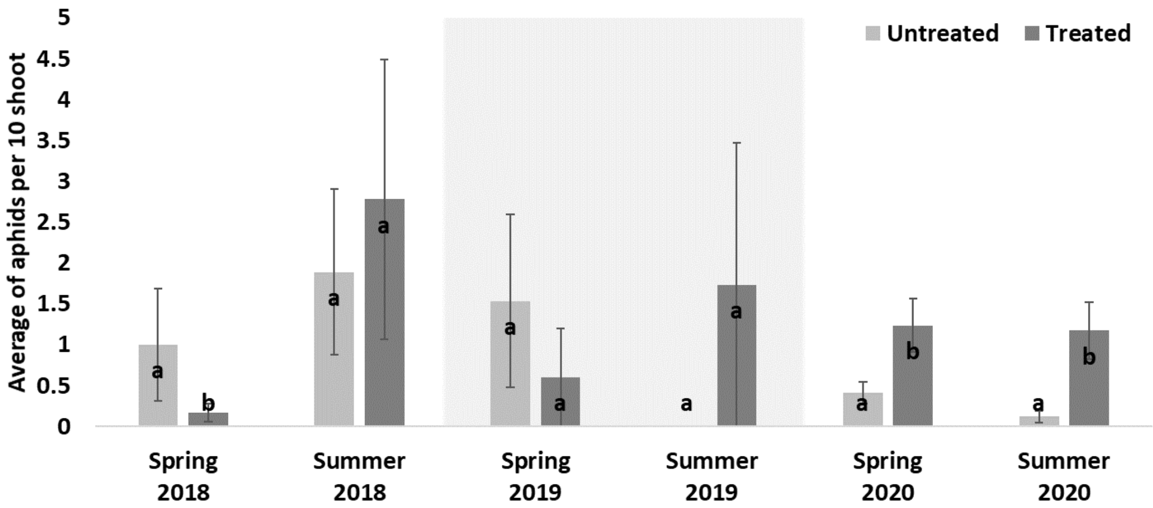


Figure 7.1.6. Mean number of aphids counted per 10 shoots from 10 sampled trees in untreated and treated plots in spring and summer of 2018 and 2019, and 2020. Significant labels only comparable between untreated and treated within the same season and year.

In 2020 a special focus was made in assessing woolly apple aphid colonies (*Eriosoma lanigerum*). In both spring and summer higher numbers of woolly apple aphid colonies were observed on treated plots when compared with untreated plots (Fig. 7.1.7). Numbers of woolly apple aphid recorded were only significant in summer (p = .0216). Aphids were only present on 2 of the 6 blocks so this significance should be taken with caution.

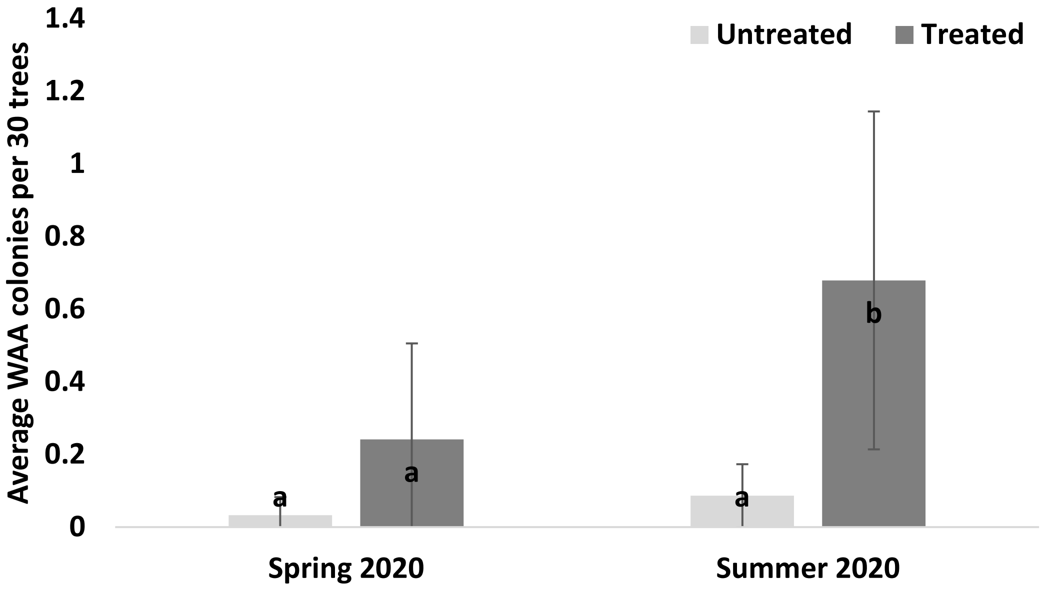


Figure 7.1.7. Mean number of woolly apple aphid colonies counted per 30 trees between untreated and treated plots in spring and summer of the enhancing orchard ecology trial 2020. Aphids were only present on 2 of the 6 blocks so this significance should be taken with caution. Significant labels only comparable between untreated and treated within the same season.

"Wignests" - Earwig refuges

Predatory spiders were the most abundant arthropod in refuges in 2018 and 2019. (Fig. 7.1.8). In April 2018 predatory spiders and earwigs were the only arthropods found in the refuges, with more predatory spiders than earwigs (mean = 0.561 and 0.061 respectively) 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 May 2019, predatory spiders were again the most common arthropod counted in refuges compared to earwigs (mean = 0.367 and 0.0333 respectively). However, on Site 2 there was a small increase in numbers of earwigs from 2018 to 2019. Site 5 was not assessed in 2019 since sowed seed mix was not establish the year before. Anthocorids were also recorded in 2019 (Fig. 7.1.8).

Most predatory spiders formally identified in the refuges belonged to the Araneidae family. This family is known to weave a web to catch prey 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).

In 2020, arthropods using the refuges were assessed later in August. Ladybirds were the most common arthropod found in refuges (0.743 per refuge) (Fig. 7.1.8). Although we recorded fewer predatory spiders using refuges than in previous years (0.194 per refuge), we also observed a high occurrence of spider egg sacs. On average, 1.967 spider sacs were found per refuge. No spider identification was done in 2020. Earwigs were present in smaller numbers compared to other arthropods. However, the overall number of earwigs per refuge was greatly driven by Site 3 were refuges had an average of 1.233 earwigs. As in 2018 and 2019, no earwigs were found on Sites 4, 5, and 6 in 2020. No correlation was found between numbers of arthropods present in the refuges.

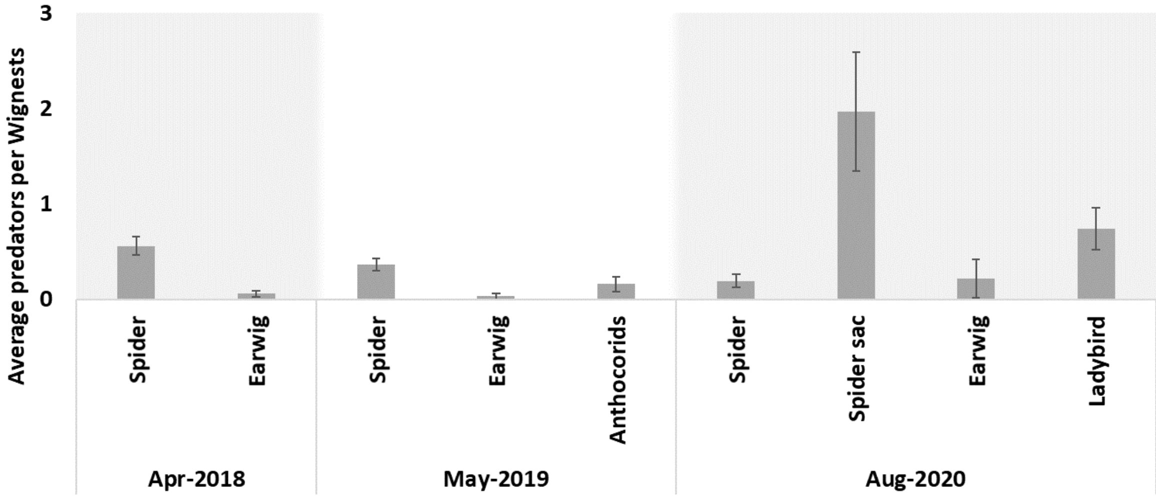


Figure 7.1.8. Average numbers of arthropod predators recorded in predatory refuges/Wignests in treated plots in spring 2018, 2019 and summer 2020.

Predator Monitoring

From 2018 to 2020 predatory spiders were the most common arthropod predator found every season (Fig. 7.1.9).

There was no significant increase in spiders or ladybirds in the treated plots, but lacewings (p=.047) numbers were significantly higher in the apple trees of the treated plots in the summer 2018 (Fig. 7.1.10). 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 (Fig. 7.1.9) and parasitoids were common but not statistically different between treatments.

In 2019 during all assessments a higher number of predatory spiders were recorded in treated plots compared to untreated; this difference was only significant in spring (p <.001). Most predatory spiders identified in 2019 belonged to the Araneidae and Philodromidae (Fig.7.1.13). 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=0.477) compared to untreated plots (D=0.558), however Linyphiidae was the only family with significantly higher numbers of individuals in the treated plots compared to untreated (*p* <.001, Fig 7.1.13).

Numbers of all other potential predators recorded in spring 2019 were too low for statistical analysis. In autumn 2019 slightly higher numbers of arthropods were recorded compared to spring and summer of the same year (Fig. 7.1.11) 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.

In 2020, numbers of predatory spiders recorded on each sampling occasion were like previous years (Fig. 7.1.9). Although numbers of predatory spiders were always higher in treated plots this difference was only significant in autumn (*p*=.0327). All other arthropods were recorded in low numbers in spring and no statistical difference was observed. In summer significantly more lacewings (*p*=.0278) and anthocorids (*p*=.0343) were found in treated plots (Fig. 7.1.12). There was no statistical difference in parasitoids and ladybirds between treatments. Harvestman were significantly higher in untreated plots when compared with treated plots (*p*=.0282) in summer. In autumn only the number of common leaf weevil (Phyllobius) was significantly higher in the treated plots (*p*=.0168). Numbers of ladybirds increased in both treated and untreated plots compared to summer. But at this time numbers were significantly higher in untreated plots when compared with treated plots (*p*=.0120).

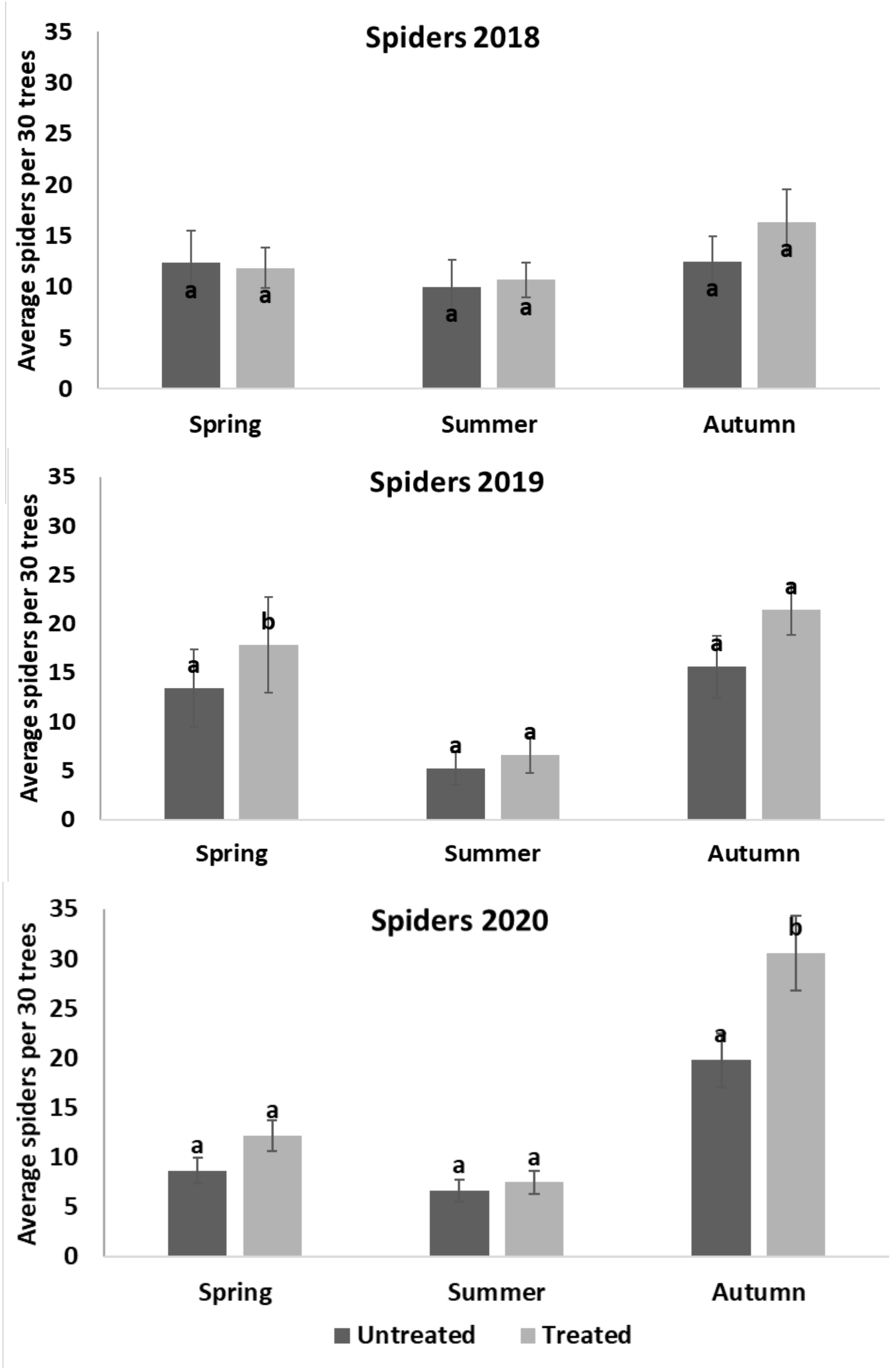


Figure 7.1.9. Mean and standard error of predatory spiders recorded by tap sampling 30 trees at untreated (Control) and treated (interventions) plots in spring, summer, and autumn in 2018 to 2020. Significant labels only comparable between untreated and treated within the same season and year.

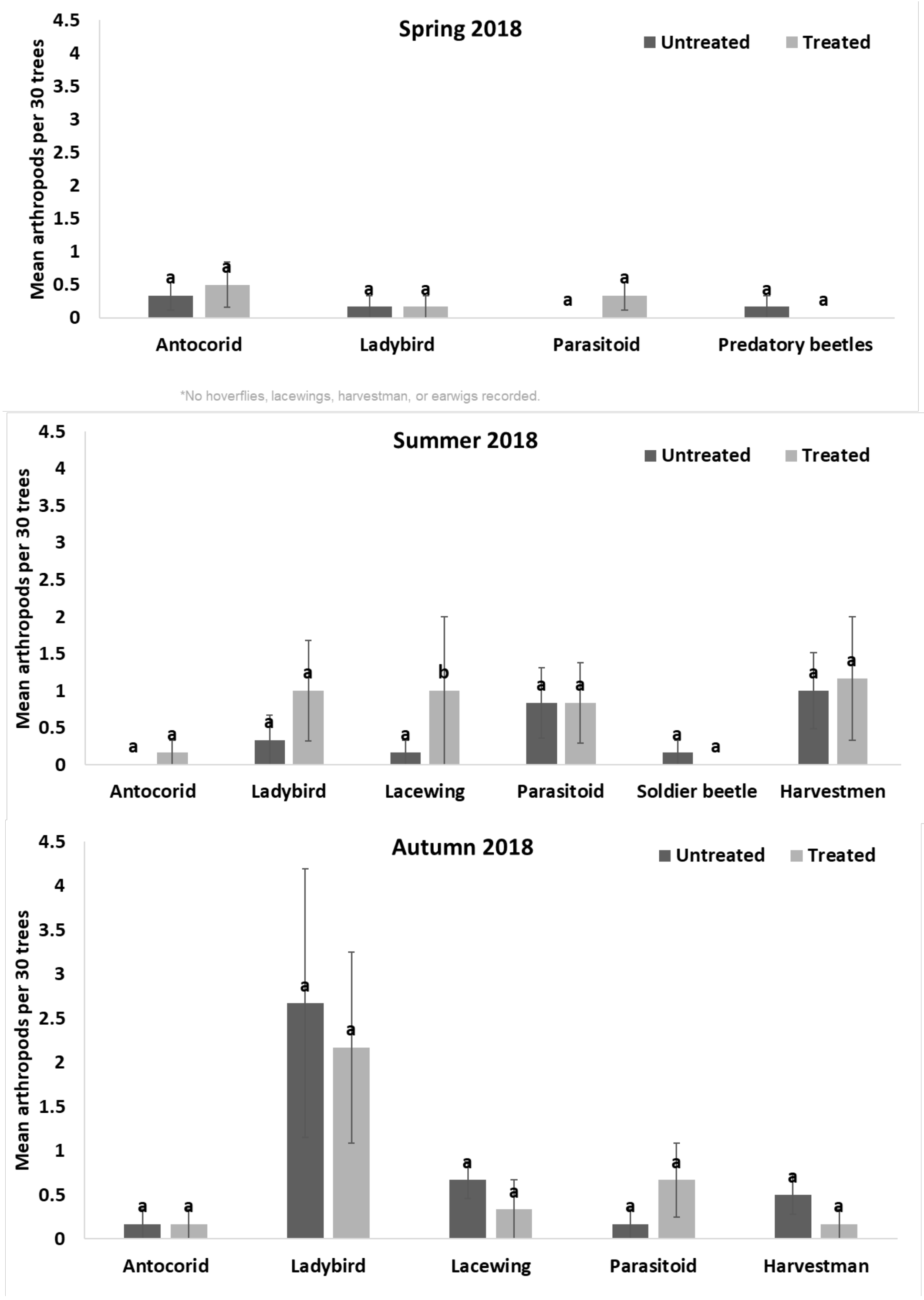


Figure 7.1.10. Mean and standard error of arthropods recorded by tap sampling 30 trees at untreated and treated plots in spring, summer, and autumn 2018. Significant labels only comparable between untreated and treated within the same species, season, and year.

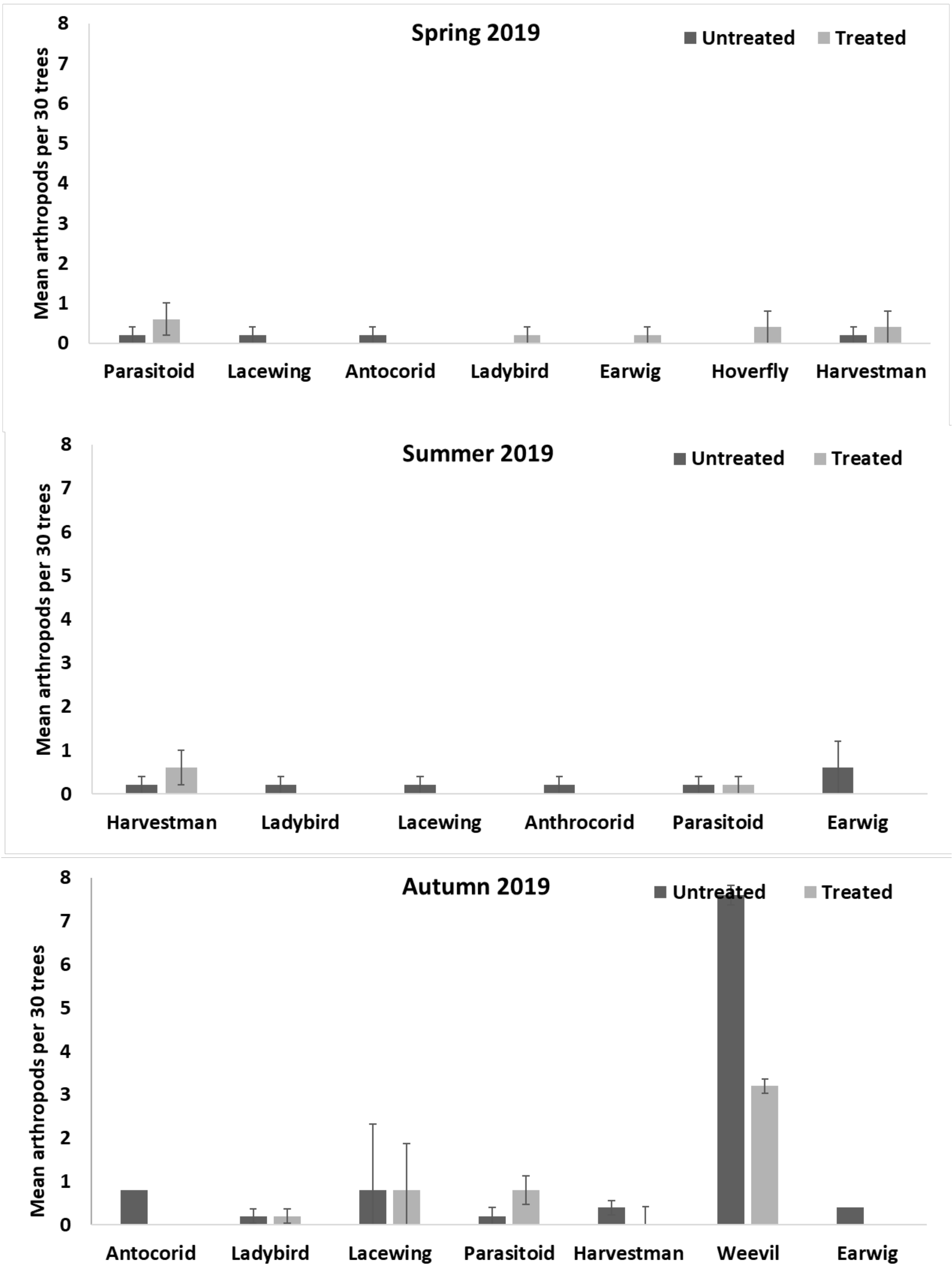


Figure 7.1.11. Mean and standard error of arthropods recorded by tap sampling 30 trees at untreated and treated plots in spring, summer, and autumn 2019. No significant differences found between arthropods of untreated and treated plots.

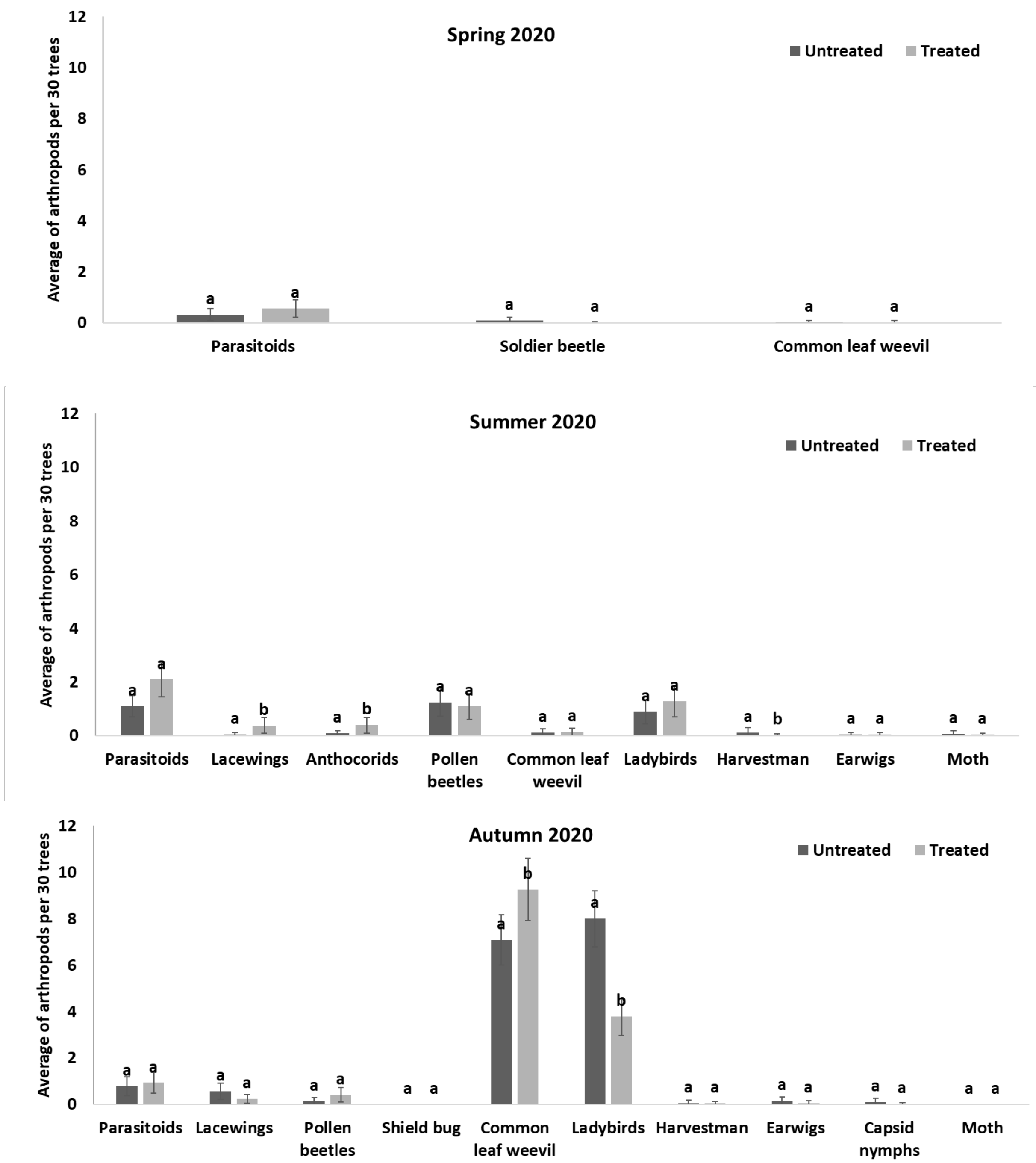


Figure 7.1.12. Mean and standard error of arthropods recorded by tap sampling 30 trees at untreated and treated plots in spring, summer, and autumn 2020. Significant labels only comparable between untreated and treated within the same species, season, and year.

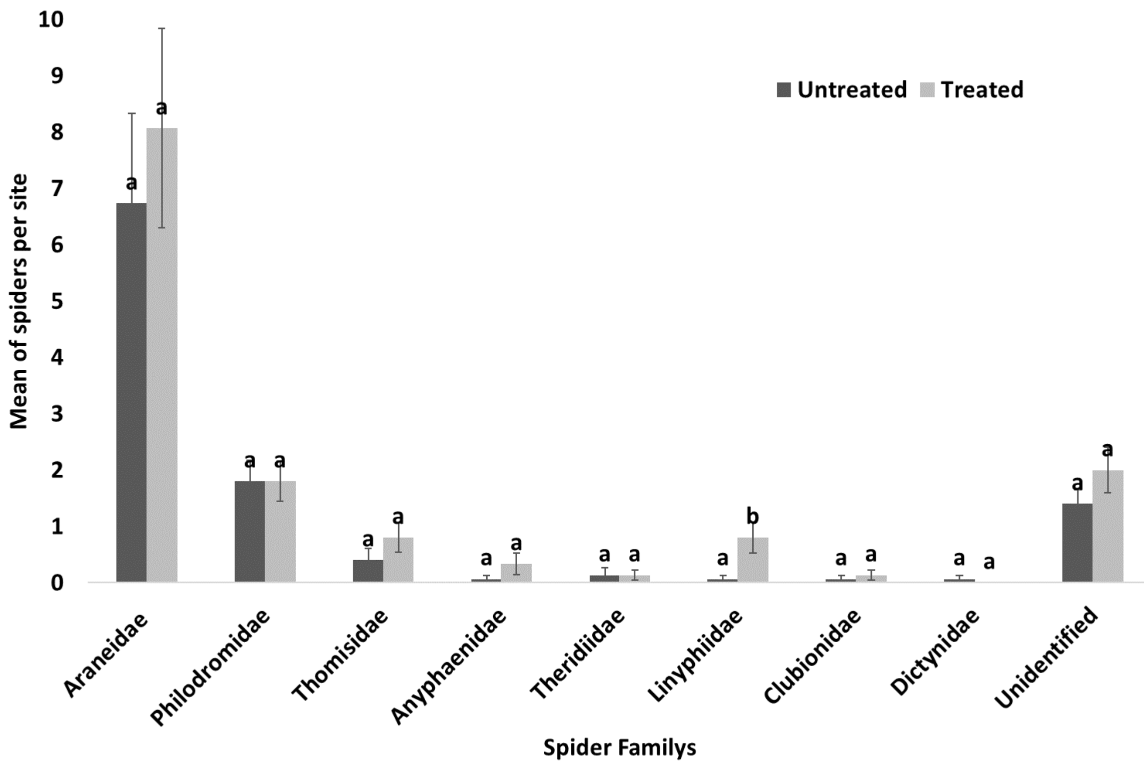
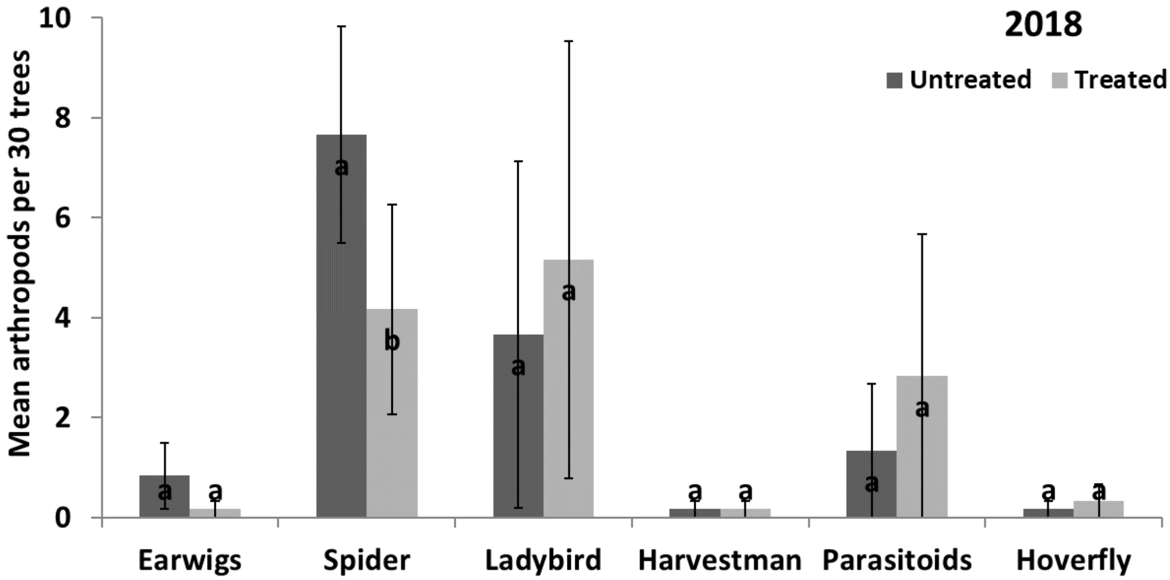


Figure 7.1.13. Mean numbers of predatory spiders within spider families identified from tap sampling apples trees in untreated and treated plots in 2019. Significant labels only comparable between untreated and treated within the same family.

Night assessment

There was no significant difference in earwig numbers in treated plots compared to untreated in both years (Fig. 7.1.14), but there was for predatory spiders. 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=.012) but not significant in 2019. Other beneficials recorded included ladybirds, harvestman, parasitoids, hoverflies and solitary bees (Fig. 7.1.14), but no significant difference was found between treated and untreated plots for any of these. No night assessments were done in 2020.



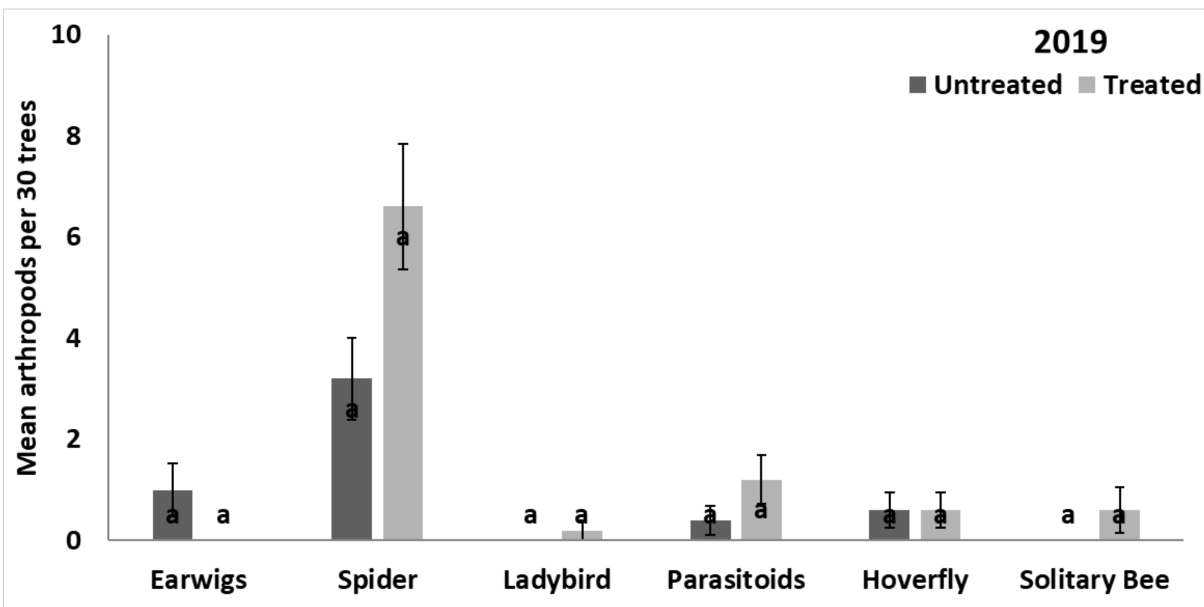


Figure 7.1.14. Mean numbers of predatory arthropods recorded from tap sampling apple trees in untreated and treated plots during the night assessments in 2018 and 2019. Significant labels only comparable between untreated and treated within the same taxa and year.

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 per 30 leaves compared to treated (0.20 and 1.40 respectively), but this difference was not significant. No assessment was carried out in 2020.

Codling Moth Damage and other pests

Codling moth (CM) stings (superficial sting central to a red region) and deep entry (Fig. 7.1.15) 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. 7.1.16). 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* <.001).

In summer 2019 no significant differences were found between CM damage in untreated and treated plots. In autumn CM stings decreased in treated plots compared to summer. CM stings were significantly less on treated plots compared to untreated at this time (*p* = .0346) (Fig. 7.1.17). 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. 7.1.17).

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. 7.1.16, Fig. 7.1.17). However, number of codling moth stings in untreated plots did not vary much 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. 7.1.16, Fig.7.1.17). In autumn 2018 there were fewer

CM sting damaged dropped apples (p=0.018) in the treated compared to untreated plots (Fig. 7.1.16). No CM deep entry damage was found in untreated plots and a very small number of fruits (0.333 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. 7.1.17).

Figure 7.1.15. Transversal cut of an apple with codling moth deep entry damage and larva

In 2020 CM damage was the lowest recorded since 2018 both in summer and autumn (Fig. 7.1.16, Fig. 7.1.18). In summer, an average of 3.35 damaged fruits with CM sting per 30 trees were recorded in untreated plots. In treated plots we observed an average of 2.98 damaged fruits with CM sting per 30 trees. CM deep entry damage was very low in both untreated and treated plots (0.199 and 0.161 damaged fruits per 30 trees, respectively). In autumn, on average fewer than 1 damaged fruit with CM sting per 30 trees were recorded on plots and CM deep entry damage was too low to analyse (Fig. 7.1.18). In 2020 trees had few dropped apples (overall mean of 3.5 drooped apples per tree) and damage was too low to analyse.

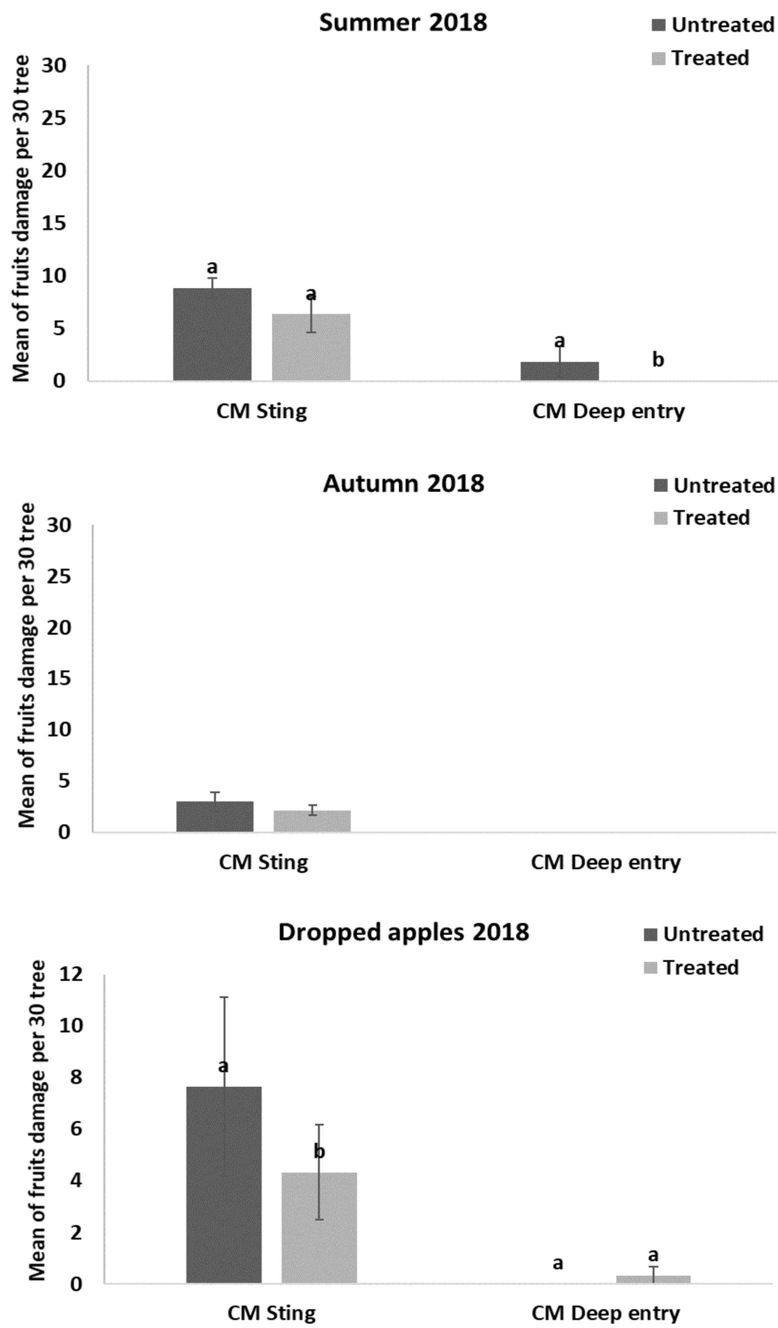


Figure 7.1.16. Mean numbers of apples per 30 trees with codling moth sting and deep entry damage in Untreated and treated plots 2018 Significant labels only comparable between untreated and treated within the same season and year.

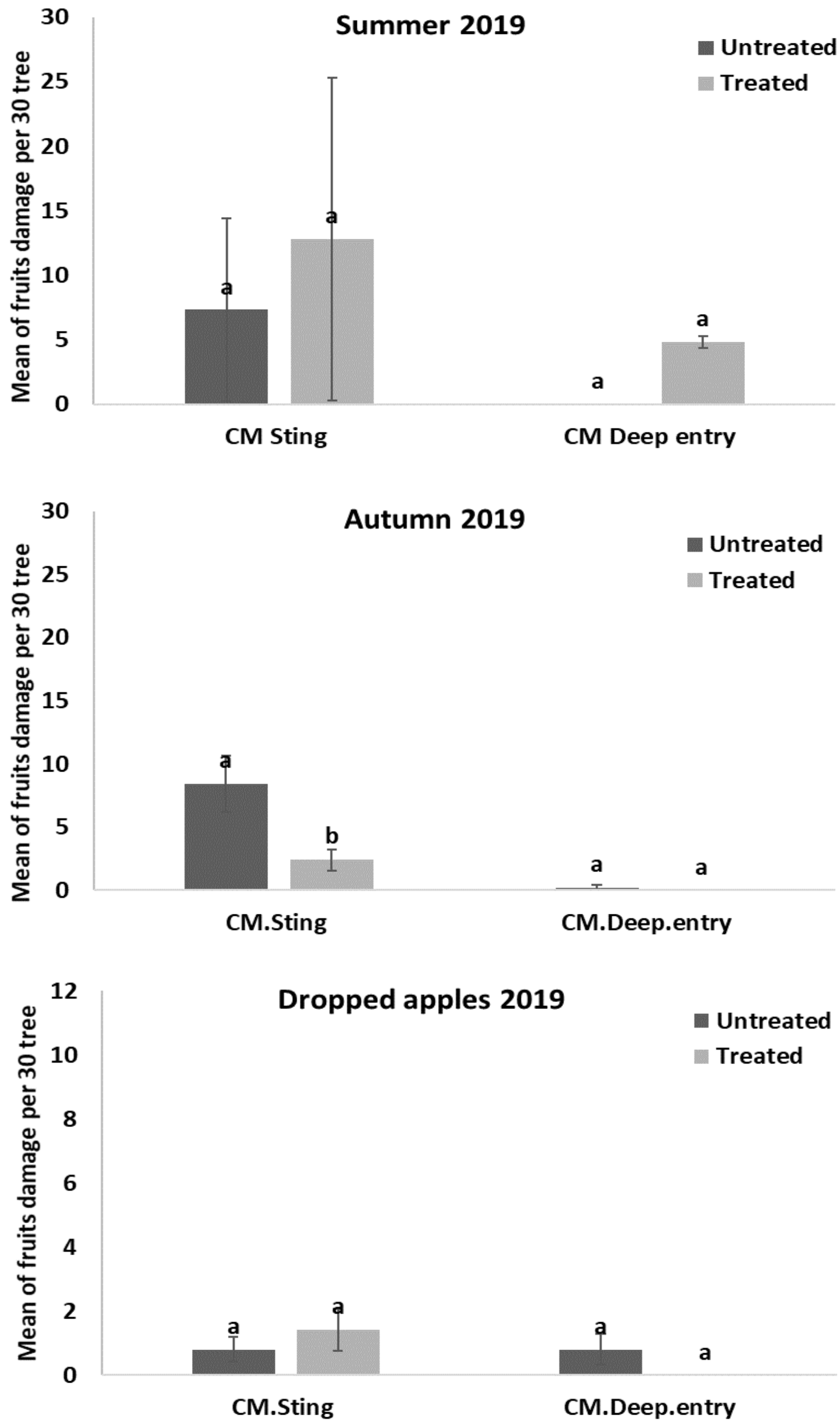
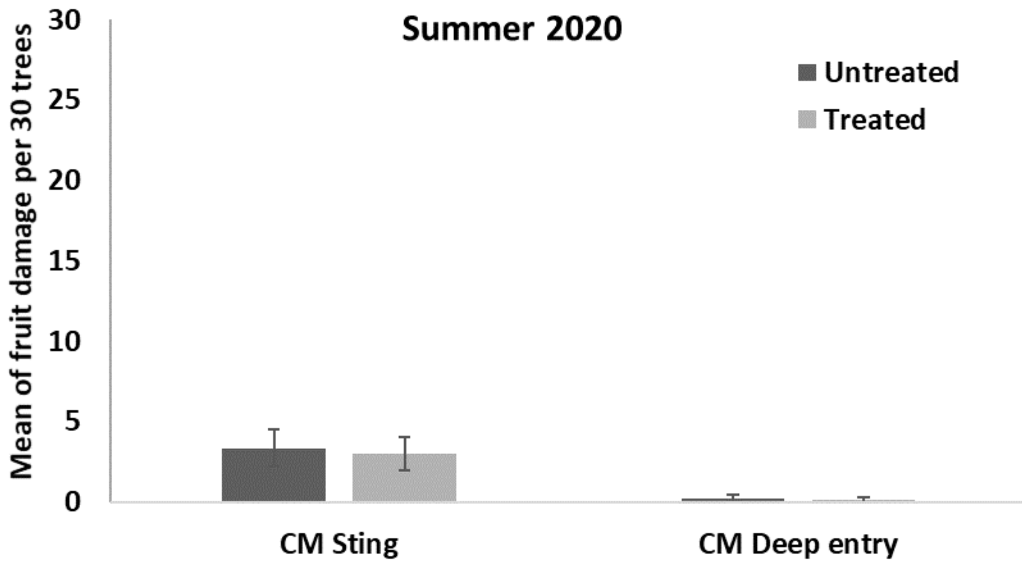


Figure 7.1.17. Mean numbers of apples per 30 trees with codling moth sting and deep entry damage in Untreated and treated plots in autumn from 2019. Significant labels only comparable between untreated and treated within the same season and year.



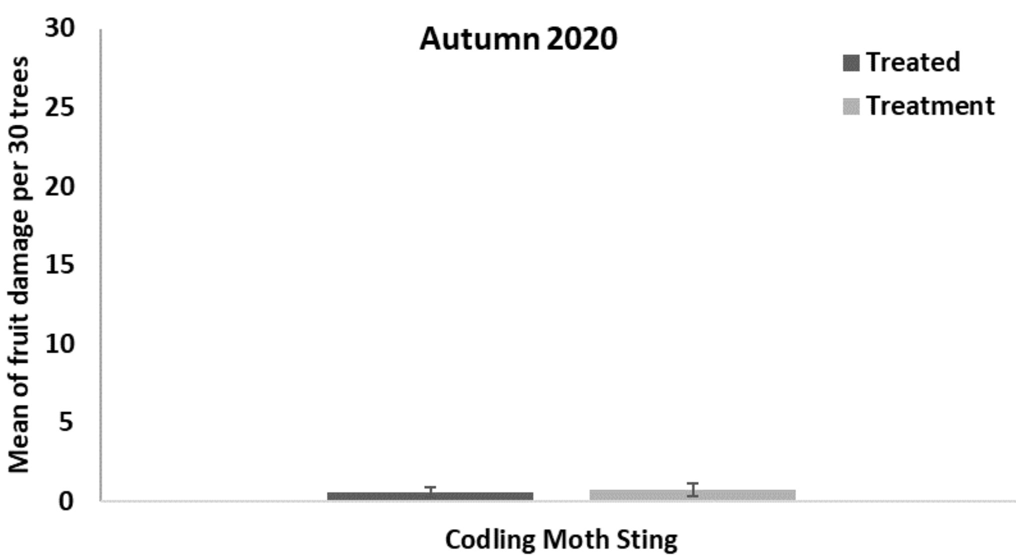


Figure 7.1.18. Mean numbers of apples per 30 trees with codling moth sting and deep entry damage in Untreated and treated plots in autumn from 2020. No significant differences were observed.

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

Rosy apple aphid and Rhynchites damage was only recorded in the summer 2018 (Fig. 7.1.19). However, numbers of fruits with rosy apple aphid damage were very low (0.333 fruits per 30 trees) and only recorded on one treated plot. There was no difference between tortrix damage found in untreated plots compared to 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. 7.1.20). However, fewer capsid damage and higher tortrix damage were recorded in summer 2019 when compared to 2018.

In 2020, only capsid and tortrix damage in summer and capsid damage in autumn was observed. In summer, fruits with capsid and tortrix damage was found in higher number in untreated plots when compared with treated plots (Fig. 7.1.21). Number of fruits with tortrix damage were significantly lower (p=.0242) in treated plots, but no statistical difference was observed for capsid damage. In autumn capsid damage was too low to analyse and no tortrix damage was found at any site. No damage was recorded in dropped apples.

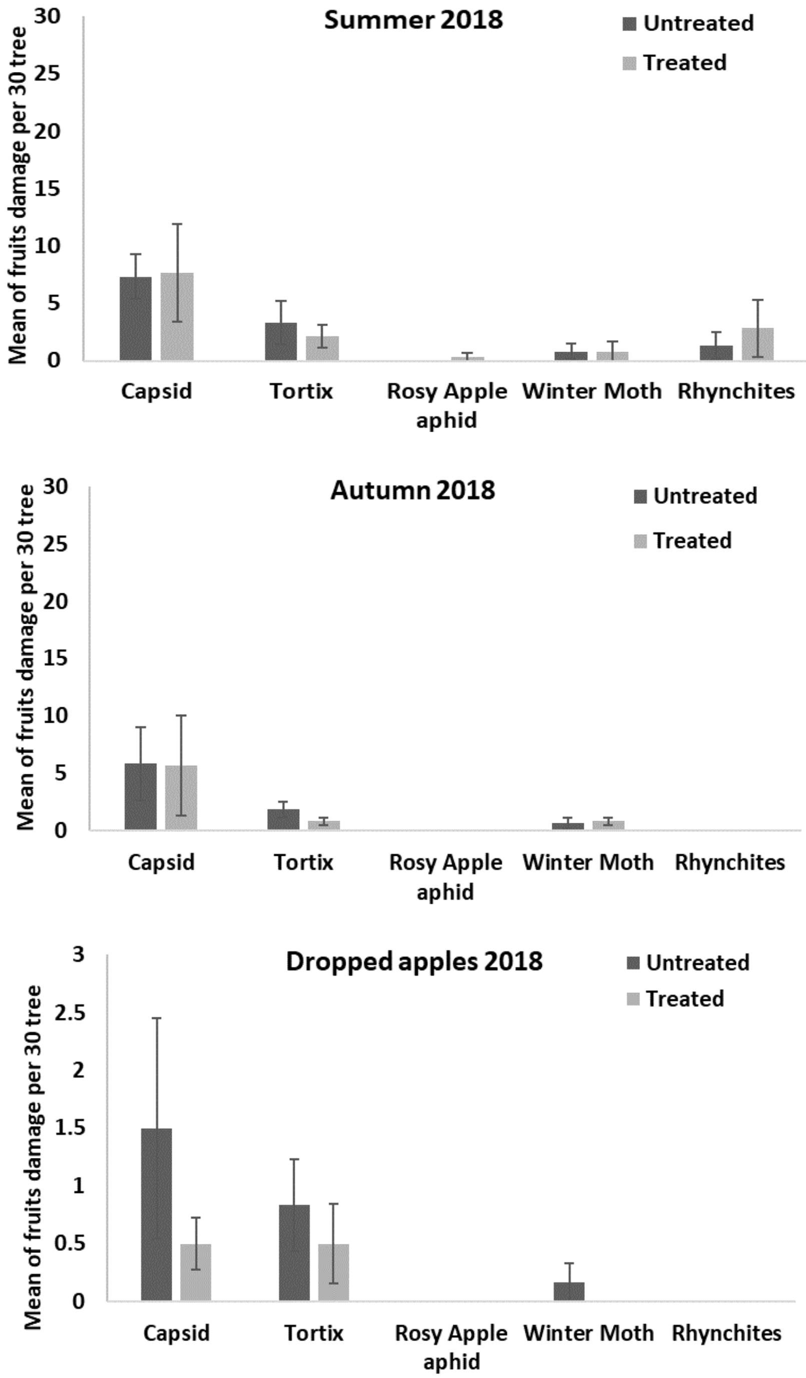


Figure 7.1.19. Mean numbers of apples per tree with damage from capsid, tortrix, rosy apple aphid, winter moth and Rhynchites in untreated and treated plots in summer and autumn of the enhancing orchard ecology trial 2018. No significant differences were observed. *Note that dropped apples are displayed on a smaller axis than previous damage.

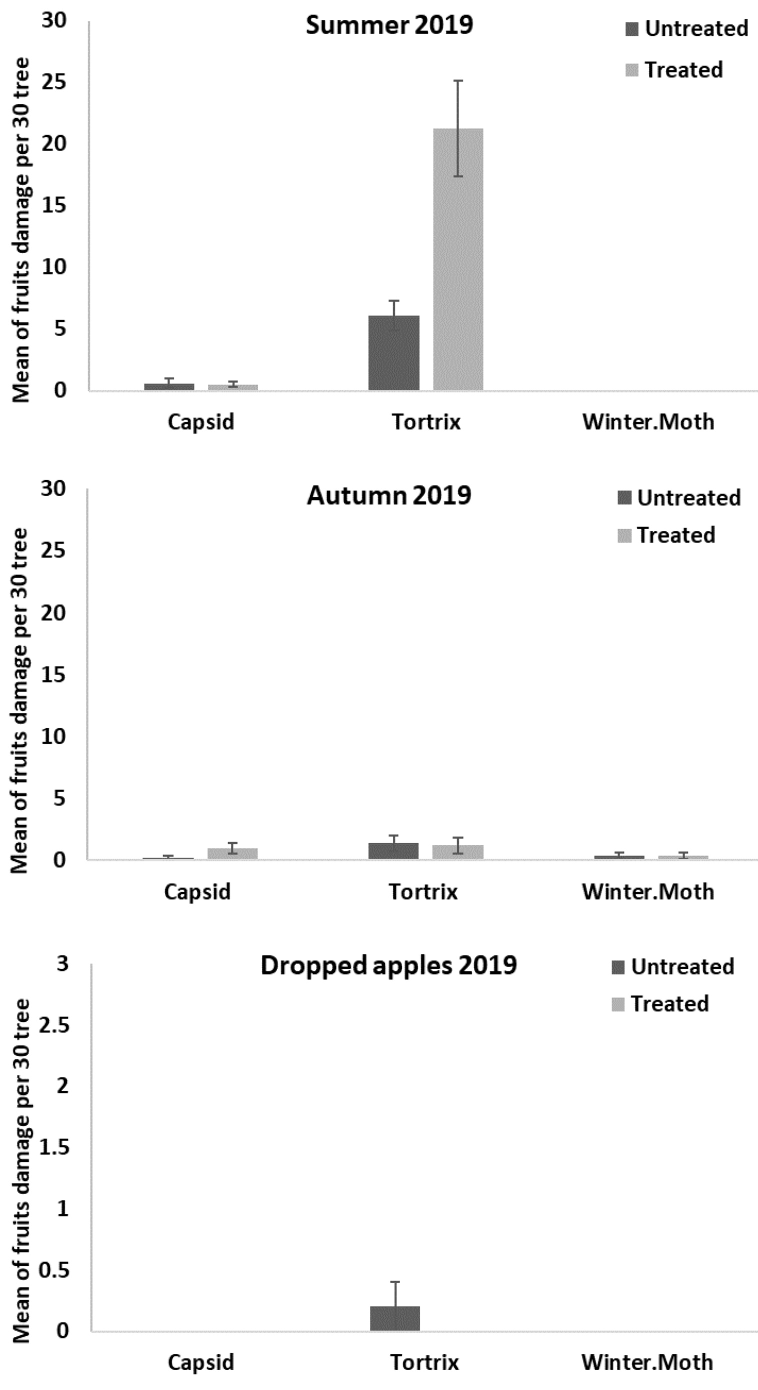


Figure 7.1.20. Mean numbers of apples per tree with damage from capsid, tortrix, rosy apple aphid, winter moth and Rhynchites in untreated and treated plots in summer and autumn 2019. No significant differences were observed. *Note that dropped apples are displayed on a smaller y-axis than previous damage.

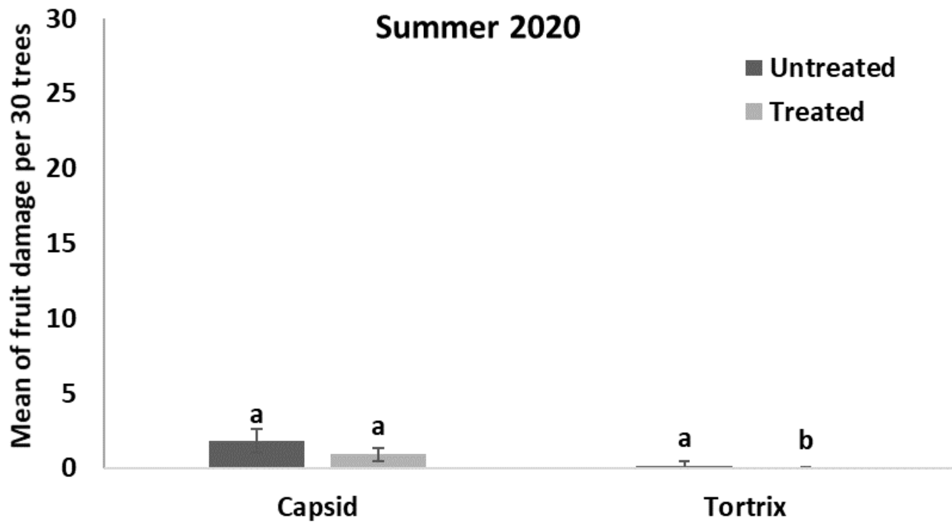


Figure 7.1.21. Mean numbers of apples per tree with damage from capsid, tortrix, rosy apple aphid, winter moth and Rhynchites in untreated (Control) and treated (Treatment) plots in summer 2020. *Note that dropped apples are displayed on a smaller axis than previous damage Significant labels only comparable between untreated and treated within the same taxa.

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.7.1.22), however this was not repeated in 2019 or 2020.

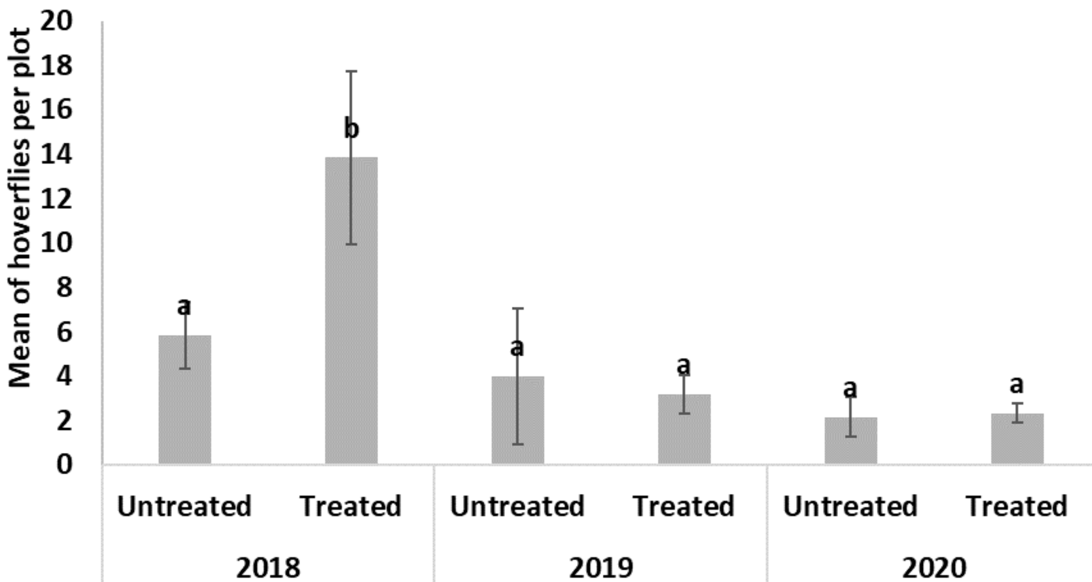


Figure 7.1.22. Mean numbers of hoverfly adults recorded on white sticky traps in treated and untreated plots (5 traps) from 2018 to 2020. Significant labels only comparable between untreated and treated within the same year.

Table 7.1.10. Summary of the effects that interventions to enhance apple orchard ecology had on beneficial arthropods during 3 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	2020
'Wignests'	Summer	91.75% predatory spiders 8.25% earwigs	68.1% predatory spiders, 4.5% earwigs 27.4% anthocorids	5.3% predatory spiders, 57.5% spider egg sacs 4.9% earwigs 32.3% ladybirds
Hoverflies	Summer	*p* <.001 (August)	*p* =.792 (July)	*p* =.611 (July)
Fruit damaged	Summer	Codling moth Deep entry, *p*<.001	×	×
	Autumn	×	Codling moth Sting *p*=.035	Tortrix *p*=.0242
dropped	Autumn	Codling moth Sting, *p*=.018	Few dropped fruit	Few dropped fruit
Aphids on shoots	Spring	*p*<.001	NSD	Overall aphids *p*=.0087
	Summer	NSD	Few aphids	Woolly apple aphid *p*=0.0216 (2 sites), Overall aphids *p*=.0043
Tree tapping	Spring	×	Predatory spiders *p*<.001	×
	Summer	lacewings *p*=.047	×	Lacewings *p*=.0278 Anthocorids *p*=.0343 Harvestman *p*=.0282
	Autumn	×	×	Predatory spiders *p*=.0327 Ladybirds *p*=.012
	Night assessment	NSD	×	×
Mites on leaves	Predatory mites	rust mites *p*<.001, Pred. mites *p*=.004, fruit tree red spider mite *p*<.001, (1 site)	Few mites	×

Conclusions

- Perennial wildflower cover increased in most plots from year to year.
- Not all species in the seed mix established. Red clover and yarrow were the most common in 2018. Red clover and common knapweed were most common in 2019 and common knapweed and yarrow in 2020.
- Sward height in treated plots was higher than in treated alleyways in 2018.
- In 2018 and 2019 fewer aphids were observed in treated plots in spring but not in summer. However, in 2020 significantly more aphids were observed in treated plots in spring and summer.
- In 2020 woolly apple aphid colonies were found in two of the treated plots in summer.
- More predatory spiders were found than earwigs in earwig refuges (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. In 2020, refuges were checked in Autumn and fewer predatory spiders were recorded. Higher number of spider egg sacs and ladybirds were found in the refuges when compared with previous years. Differences may be due to the season of assessment.
- Predatory spiders were the most common arthropod recorded in all seasons in all years. In 2019, most individuals collected belonged to Araneidae and Philodromidae family. 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 ($p<.001$).
- In 2018, no apple leaf curling midge damage occurred in treated plots compared to untreated. Apple leaf curling midge not assessed in subsequent years.
- 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. Mites numbers were not assessed in 2020.
- 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 in treated plots in autumn. In 2020,

CM damage was lower than previous years (in average 3 damaged fruits per 30 trees) and no significant difference was observed.

- In 2020, number of fruits presenting tortrix damage was significantly lower in treated plots in summer.
- Hoverfly adults were more abundant in the treated plots in autumn 2018 but there was no difference in 2019 and 2020.

Future work

For the first time a significant negative impact of the floral margins has been observed – woolly apple aphid. However, this was only in two orchards. It would be useful to continue to assess the impact of the treatments on this best and others over the long term.

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