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Date project commenced:	1 March 2015
Date project completed **(or expected completion date):**	31 March 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.

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CONTENTS

GROWER SUMMARY

The overall aim of the project is to advance and optimise on-farm integrated management of key pests and diseases of cane fruit. Within this project, it is planned to work on five differing objectives over the five year duration:

1. Investigate the infection process of *Phytophthora rubi* to inform the use of alternative or supplementary means to the use of chemical plant protection products for reducing the level of root rot in raspberries.

2. Develop and maintain IPM approaches to successfully control two-spotted spider mite whilst controlling spotted winged drosophila (SWD) and capsids with insecticides.

3. Develop and combine novel and current IPM approaches to successfully control blackberry leaf midge;

4. Establish cane management approaches on a model crop to optimise IPM strategies and spray penetration into canopies;

5. Disseminate research results to growers and translate research outputs into practical ‘ready to use’ techniques for immediate uptake on farms.

For ease of reading, this Grower Summary report is split into sections for each of the objectives (pests & diseases) being worked upon. The third year’s work (2017) concentrated on Objectives 1 and 2, so only these are reported on in this third annual report.

Raspberry root rot

Objective 1 – Investigate the infection process of *Phytophthora rubi* to inform the use of alternative or supplementary means to the use of chemical plant protection products for reducing the level of root rot in raspberries

Headlines

- In a trial to assess the efficacy of a range of control agents for *Phytophthora rubi*, no significant differences were recorded between treatments.
- A novel system of observing the behaviour of *P. rubi* zoospores has been developed.
- An experiment has been set up to investigate any beneficial effect from the application of biofungicides, either before winter or after potting in spring, on the susceptibility of long cane raspberries to *P. rubi* infection following either cold-storage or outdoor chilling.

Background and expected deliverables

Phytophthora root rot is now the most destructive disease of raspberries worldwide. Where raspberries have been grown in the soil *Phytophthora rubi* (previously known as *P. fragariae* var. *rubi*) is almost ubiquitous. Outbreaks of this disease across Europe at the same time in traditional raspberry-growing areas suggests that the disease has spread through the propagation network and has been distributed to farms in new planting material (Graham et al. 2011). Current approaches for Phytophthora control rely on fungicide applications twice per year either as a soil-applied drench or through the drip irrigation. SL567A (44.7% w/w metalaxyl-M) and Paraat (500 g/kg dimethomorph) can be used, although resistance developing in pathogens where products have only a single mode of action is a major concern.

The work in this project will focus on understanding the activity of non-conventional products that may improve root health and the production of propagation material that is more resistant to the disease. The work in 2017 was divided into three separate packages:

Work package 1 – To investigate the effects of a range of novel plant treatments on raspberry growth and their resilience to pests and diseases from propagation through to primocane production.
Work package 2 - To explore *P. rubi* zoospore behaviour with raspberry root exudates.
Work package 3 - To explore the effect of cold-storage of long cane raspberries on incidence & severity of *P. rubi* infection and the potential for protection using biofungicides.

Summary of the project and main conclusions

Work package 1 – To investigate the effects of a range of novel plant treatments on raspberry growth and their resilience to pests and diseases from propagation through to primocane production

Work in 2017 was a continuation of what began in 2015 and 2016. In 2015, modular Tulameen plants were raised at a propagation nursery in Oxfordshire and treated with a range of novel and traditional control products. These were compared to untreated control plants.

At the end of 2015, half of the plants remained at the propagation nursery where they were cold-stored as 'long-canes'. The other half were sent to ADAS Boxworth where they were cut back, before potting on in spring 2016 and artificially inoculated by *P. rubi* mycelium and zoospores.

The plants were grown-on at both an Oxfordshire soft fruit farm and ADAS Boxworth in 2016, where they were treated again with the same products to assess their effect on plant growth and pest and disease development. No phytotoxicity occurred at either site and no difference in vigour was recorded. Fruit was only harvested at the Oxfordshire site and no yield differences were seen between treatments in 2016. At Boxworth, by termination of the experiment in November 2016, a small increase in primocane number had occurred with the use of the biofungicides Prestop, Root Grow HYDRO, Serenade ASO and a coded product compared to the other treatments and untreated control, but no wilting had developed in any canes.

In 2017, the treatments were repeated in the Oxfordshire trial. A summary of all treatments used in 2015, 2016 and 2017 is set out in Table A below. Note that two of the treatments (Treatment 3 – HDC F201 and Treatment 4 – Root Grow HYDRO – HDC F204) were not applied in 2017. The former is only permitted for use on crops under permanent protection, while the Root Grow HYDRO is a mycorrhizal product which can only be applied when plants are potted up.

Table A. Summary of products and application timings made in 2015, at the propagators in multicell trays and then module pots, and application timings in 2016 and 2017 after the same plants were potted and grown on the Oxfordshire site.

Trt. no.	Treatment	Dilution rate and (product per 10L pot)*	Year: (Month product applied)
1	Untreated	-	-
2	Prestop	5 g/1 L water (5g)	2015: (April, May, July, October) 2016: (April, May, June)

	(*Gliocladium catenulatum*) [MAPP 15103]		2017: (April, May, June)
3	HDC F201	Not disclosed	2015: (April, May, July, October) 2016: (April, June)
4	Root Grow HYDRO (mycorrhiza species) (HDC F204)	7 g/1 L growing- media (70g)	2015: (April, May) 2016: (April)
5	Serenade ASO (*Bacillus subtilis*) (HDC F228)	10 L/ha using1000 L water (1 ml / m² *pro rata* to area of pot surface) (0.12ml per 10L)	2015: (April, May, July, October) 2016: (April) 2017: (April)
6	HDC F205	Not disclosed	2015: (April, May, July, October) 2016: (April, May, June) 2017: (April, May, June)
7	Paraat (dimethomorph) [MAPP 15445]	1.0 g/plant (1g)	2015: (April, May) 2016: (April) 2017: (April)

*Applying 1 L of diluted product per pot to give a drench of 10% of pot volume per 10 L pot. Treatments were applied up to three times following the treatment manufacturers' instructions.

Full details of the treatments and the methods of application are summarised in the Science Section of the report.

The Tulameen canes in the trial treatments were grown within a commercial raspberry plantation and received the same fertigation and crop protection spray programme as the surrounding crop. Paraat drenches by the grower were withheld from the experiment. The crop assessments made in 2017 are summarised in Table B below.

Table B. Dates and types of assessments and product application dates, Oxfordshire 2017.

Date	Procedure
7 April 2017	Pre-treatment assessment. Product applications.
9 May 2017	Phytotoxicity and floricane vigour assessment. Products applied.
23 May 2017	Phytotoxicity and floricane vigour assessment. Products applied.
7 June 2017	Phytotoxicity and floricane vigour assessment. Products applied.
19 June to 5 July 2017	Harvest assessments. Fruit yield. Mean berry weight.
29 June 2017	Phytotoxicity and primocane vigour assessment.

10 July 2017	Leaf samples for nutrient analysis.
6 September 2017	Final primocane vigour assessment.
4 December 2017	Suitability assessment of new cane produced in 2017.
15 January 2018	Final disease assessments of new floricane and roots.

In addition to these assessments, root samples were collected from the treatments in September 2017 for testing in bulk using a lateral flow device (LFD) for *Phytophthora* spp. and again in January 2018 when roots from each treatment were placed in float dishes to stimulate oomycete sporangia production. Fruit yields were recorded during harvest (June – July 2017) and on two harvest dates, mean berry weight and fruit quality were recorded.

No foliar disease, cane wilting or root rot were recorded in the trial, even in the untreated plots. Therefore no conclusions could be drawn regarding product effects on disease incidence or severity. Natural *Phytophthora* infestation may have started, as a faint positive was recorded on the LFD, but no sporangia developed in the root floats indicating it was not widespread.

In addition, there were no significant differences recorded, either between any of the treatments or between the treatments and the untreated control in plant vigour during crop growth, fruit harvest measurements or the number and strength of cane produced by January 2018. The treatments caused no adverse effects in the plants and had no apparent effect on growth promotion. There were different levels of nutrients in the leaves in July, with product HDC F205 recording higher than average levels of phosphorus and potassium. It is not clear why this occurred and what impact it might have on plant growth and physiology.

At the start of the 2017 growing season, plants treated with Serenade ASO produced significantly more new primocanes (spawn) than all other treatments. However, five canes per plant may be in excess of numbers actually required for retention as floricane.

When monitoring for Botrytis on floricanes pre-treatment in April 2017, those treated with HDC F205 had more Botrytis infection than all but the untreated and Root Grow Hydro treatments. This suggests there might be a reduction in resistance to this pathogen. However, after the final cane Botrytis assessment in January 2018, there were no significant differences between treatments. Pre-season Botrytis coverage ranged from 5% - 13% whereas post-season winter coverage was much higher at 53% - 68%. This indicates the time of year when cane Botrytis becomes particularly prevalent.

Work package 2 - To explore *P. rubi* zoospore behaviour with raspberry root exudates

Like other *Phytophthora* species, *P. rubi* zoospores are released by mature sporangia and swim towards raspberry root tips, before encysting and entering the root. It is thought that zoospores use chemotaxis to target the root. However, compared to other Phytophthora spp. relatively little is known about *P. rubi* and its exact mode of infection. Work therefore needs to be done to understand the behaviour of *P. rubi* zoospores (the infecting part of its lifecycle) to help in the search for robust control solutions.

Initial work was done with an isolate of *P. rubi* on the technique for producing sporangia and releasing zoospores as this differed from those standard for other Phytophthora species. Work has also been set up to develop techniques to investigate the movement of zoospores towards root exudates in laboratory conditions. Useful progress has been made but further work is required to refine the process.

Another experiment was undertaken to confirm that the isolate of *P. rubi* being used is pathogenic. A technique developed by the James Hutton Institute was employed. Three healthy Glen Moy plants were inoculated with the *P. rubi* isolate and grown inside a growth cabinet. Both root and stem sections from each plant were tested for *P. rubi* infection following the removal of the plants from the growth cabinet.

After removal of the plants from the cabinet, all three were wilting, indicating Phytophthora infection. The stem base was brown up to 20mm on all three plants and in the roots, browning was seen in every plant to varying extents. Sections of roots from each plant were removed and placed in soil water and observed. After 2 days, around 200 spores were seen around many of the roots from all plants. These zoospores indicate the presence of an oomycete. Tests on the stems of all three plants confirmed *P. rubi* on two plants.

In conclusion, the work confirmed that the isolate of *P. rubi* being used in the project is pathogenic to raspberry. The imaging and video capacity of ADAS laboratory equipment, alongside a novel observation set-up, enables clear monitoring of zoospore behaviour, for further work on raspberry exudates, chemical fungicides and other solutions.

Work package 3 - To explore the effect of cold-storage of long cane raspberries on incidence & severity of *Phytophthora rubi* infection and the potential for protection using biofungicides.

Long cane raspberries require a chilling period at the end of their propagation year in order to produce fruit in the following Summer. Some propagators place their containerised plants into cold-storage rather than leave them outside and risk inadequate chilling in a warmer Winter. In cold-stored strawberry there is experimental evidence that healthy plants become more susceptible to Phytophthora when planted in infested soil than those not cold-stored. Cold-stored plants can also succumb more readily to Phytophthora already in the crowns before storage.

It was therefore hypothesised that losses of cold-stored long cane raspberries in their fruiting year might follow as a result of increased plant susceptibility, potentially also linked to greater *P. rubi* inoculum pressure. This is because should long cane or module raised raspberries be infected by *P. rubi* before they are cold-stored, it is known that the pathogen can survive the period of storage on the roots. On returning the plants to ambient conditions and commencing watering, it is thought that this may trigger a mass zoospore release rather than a steady release. Cold-storage of healthy plants may also reduce their resistance to root infection.

In 2017, investigations were started to determine whether healthy cold-stored plants were more susceptible to *P. rubi* infestation in Spring than those left outside and also whether product application before or after Winter might reduce plant susceptibility. In 2018, work will be started with the same product applications and timings, but with *P. rubi* inoculation in November so that only the Autumn treatments will be protectant.

A trial was set up in summer 2017 at a propagation site in Oxfordshire where long cane Tulameen grown in 1.5 litre pots were chosen for experimentation. Two experiments were begun, one (Experiment 1) where canes were treated with drenches of control products in autumn 2017 and the other (Experiment 2) treated in spring 2018. In each experiment, half of the canes were cold-stored from December 2017 to March 2018 and half were left to stand in the field in ambient conditions.

In April 2018, all of the canes (both cold-stored and field-grown) were moved to ADAS Boxworth where they were potted into 5 litre pots (1 cane per pot) and those canes in Experiment 2 were drenched in the same way as Experiment 1 had been treated in Autumn 2017. One month later, all pots from both experiments (except untreated controls) were inoculated with *P. rubi*. Full assessments of cane height, vigour, disease incidence and root health were recorded throughout the trial. Full details of the treatments in each experiment are listed in Table C.

Table C. Products and number of applications in either Winter 2017 (Experiment 1) or Spring 2018 (Experiment 2). Inoculation with *P. rubi* in Spring 2018 (except T1) at ADAS Boxworth. Treatments 1-5 with cold storage are shaded in blue.

Experiment 1 (drenching in 2017 inoculation in 2018)		Experiment 2 (drenching and inoculation in 2018)	
T1 UT no *P. rubi* T2 UT T3 Prestop x2 T4 Serenade x1 T5 Paraat x1	Cold Store December 2017 to March 2018	T1 UT no *P. rubi* T2 UT T3 Prestop x2 T4 Serenade x1 T5 Paraat x1	Cold Store December 2017 to March 2018
T6 UT no *P. rubi* T7 UT T8 Prestop x2 T9 Serenade x1 T10 Paraat x1	Ambient outdoors December 2017 to March 2018	T6 UT no *P. rubi* T7 UT T8 Prestop x2 T9 Serenade x1 T10 Paraat x1	Ambient outdoors December 2017 to March 2018

Full results will be recorded in the spring and summer of 2018, then included in the next annual report in 2019.

Financial benefits

Raspberry root rot (caused by *Phytophthora rubi*) is the most devastating disease currently faced by cane fruit growers and in particular by raspberry producers. The disease spreads rapidly through the root system of the crop, leading to complete death of large areas of a plantation. Where severe, in soil grown crops, it commonly kills 75% of a raspberry plantation within two to three years of establishment. Although perhaps slower to spread in container grown crops, it has a similar effect in killing significantly large areas of a plantation within a few years of planting and establishment. Not only do growers make significant financial losses, they also incur additional labour costs in setting up new replacement plantations more frequently, along with the associated costs of establishing a new plantation along with the support system that goes with it.

Assuming a typical return for raspberries of £6.49/kg to growers (Defra Basic Horticultural Statistics 2014) and a yield of 14 tonnes/ha, then 75% crop loss would lead to a financial loss of £68,166/ha. Increasing the health of propagation material and providing material that is more resistant to the disease would not only significantly reduce such losses but lengthen the life expectancy of a raspberry plantation, thereby reducing the additional costs of re-establishing new plantations on a frequent basis.

Action points for growers

- Consider biological alternatives to plant protection products for the control of *Phytophthora. rubi.*
- A drench application of Serenade ASO to outdoor container raspberries may increase cane production.

Two-spotted spider mite

Objective 2 – Maintaining Integrated Pest Management of two-spotted spider mites whilst controlling spotted wing drosophila

Headlines

- Establishing populations of introduced and naturally occurring predatory mites early in the season can achieve control of two-spotted spider mite before any control sprays for SWD are required.
- Applying SWD control sprays over the top of a raspberry crop can provide refuges for predatory mites on the undersides of leaves, to limit the adverse effect of SWD control programmes on biological control systems.

Background and expected deliverables

A key current question for growers of soft fruit is how to maintain the successful Integrated Pest Management (IPM) approaches that have been developed over the past 10 years whilst applying crop protection products to control SWD. Two-spotted spider mite (TSSM) can be a devastating pest of raspberries, especially on crops grown under glasshouse or polytunnel protection and during hot weather. Control of TSSM with acaricides requires good spray cover, as most acaricides are contact acting. Effective leaf cover is difficult to achieve in raspberry crops which often have dense canopies. Recent changes in legislation have also meant that there is a limited range of acaricides for use in protected and outdoor raspberries and other cane fruit crops and it is likely that this trend will continue (e.g. abamectin is under threat due to potentially being an endocrine disrupter). The difficulties of applying sprays to a raspberry crop and restrictions on crop protection products mean that predators of TSSM are an important method for the control of this pest.

Phytoseiid predatory mites are the main natural enemies of TSSM. There are two main naturally occurring, overwintering, species in raspberry (predominantly *Amblyseius andersoni* but *Neoseiulus californicus* is also common). These mites naturally regulate TSSM populations to a greater or lesser extent, but not reliably. In recent years, growers have been successfully introducing *Phytoseiulus persimilis* predatory mites and the predatory midge *Feltiella acarisuga* for the control of TSSM mite in outdoor/protected raspberry and blackberry crops. However, information on side effects of crop protection products on biological control agents and experience in other countries, demonstrates that applications of products to control SWD such as spinosad (Tracer), lambda-cyhalothrin (Hallmark) and deltamethrin (e.g. Decis), can adversely affect these biological control agents leading to serious outbreaks of TSSM.

Outbreaks of TSSM and other mites, as a result of disruption to biocontrol by naturally occurring and introduced predatory mites, by sprays of products for SWD and/or capsid bugs, is an immediate serious threat which the UK cane fruit industry faces.

This study aims to address this problem in Year 3 through two specific objectives:

Objective 2.1**:** To develop and maintain IPM approaches to successfully control two-spotted spider mite whilst controlling SWD and other pests with insecticides.

Objective 2.2: To develop compatibility strategies for biocontrol of two-spotted spider mites (TSSM) by predatory mites with insecticide sprays for spotted wing drosophila (SWD) and capsids

Summary of the project and main conclusions in year 3

Objective 2.1**:** To develop and maintain IPM approaches to successfully control two-spotted spider mite whilst controlling SWD and other pests with insecticides.

This work was undertaken by ADAS. A commercial tunnel-grown raspberry crop of Maravilla was monitored between 29 June and 13 October 2017. Visits were made before and after a chemical control product was applied to control SWD. On each visit, records were made of numbers of TSSM, leaf area damaged by spider mites, numbers of *P. persimilis* and numbers and species of any naturally-occurring TSSM predators. An assessment of SWD adult emergence from treated fruit was made eight days after the SWD spray.

High numbers of TSSM and eggs were recorded on the preliminary assessment on 29 June when numbers of *Phytoseiulus persimilis* were still low following release by the grower on 29 May.

On the second assessment on 21 July, mean numbers of TSSM and eggs were significantly reduced. This reduction is likely to have been due to predation, not only by *P. persimilis* which had established well by this date, but also by four naturally-occurring predators; the predatory mite *Amblyseius andersoni*, the midge *Feltiella acarisuga,* the ladybird *Stethorus punctillum* and the predatory bug *Orius* sp.

On the third assessment on 2 August, immediately before the SWD spray was applied, mean numbers of TSSM and eggs were significantly reduced still further and this is likely to have been due to both predation by the combination of predators and to some of the floricane being cut back six days earlier on 27 July. Mean numbers of *P. persimilis* and *A. andersoni* were also significantly lower on this date than on the previous assessment and this is likely to have been due to both cutting back the floricane and to the reduced availability of spider mite prey.

On the fourth assessment on 9 August, seven days after the grower applied a tank mix of deltamethrin (Decis) for SWD control and thiacloprid (Calypso) for blackberry leaf midge control, mean numbers of *P. persimilis* mites and eggs were significantly lower (83% and 98% respectively) than on the previous, pre-spray date. This reduction is likely to have been due to both the harmful effects of Decis and Calypso and to the scarcity of TSSM prey. Both TSSM mites and eggs had reached very low numbers by this date which is likely to have been due to predation by the remaining predators. Mean numbers of *A. andersoni* and eggs were also lower than on the pre-spray date (55% and 67% lower respectively) but this reduction was not statistically significant. This predator seems to be more tolerant of crop protection products than *P. persimilis* and it is less dependent on TSSM for food as it will also feed on other prey and food sources such as pollen. Mean numbers of *F. acarisuga* and *S. punctillum* were both significantly lower than on the pre-spray assessment and this is likely to have been due to both the effects of the SWD spray and to scarcity of TSSM prey.

No SWD adults emerged from the fruit samples collected eight days after the SWD spray. Initially the grower intended to apply further SWD sprays but due to a combination of the spray on 2 August, good site hygiene and cut back of the floricane, further applications were not needed.

Both spider mite and predator numbers were very low on the final assessment on 13 October. TSSM damage to leaves did not increase during the monitoring period, but decreased by the final assessment. This was likely to have been due to any new leaves developing since the previous assessment showing less damage symptoms due to the decrease in numbers of spider mites.

Although the SWD spray is likely to have killed many of the spider mite predators, due to early good establishment, the predators had controlled the TSSM before the spray was applied and no acaricides were needed.

The results are neatly summarised in Figure A below.

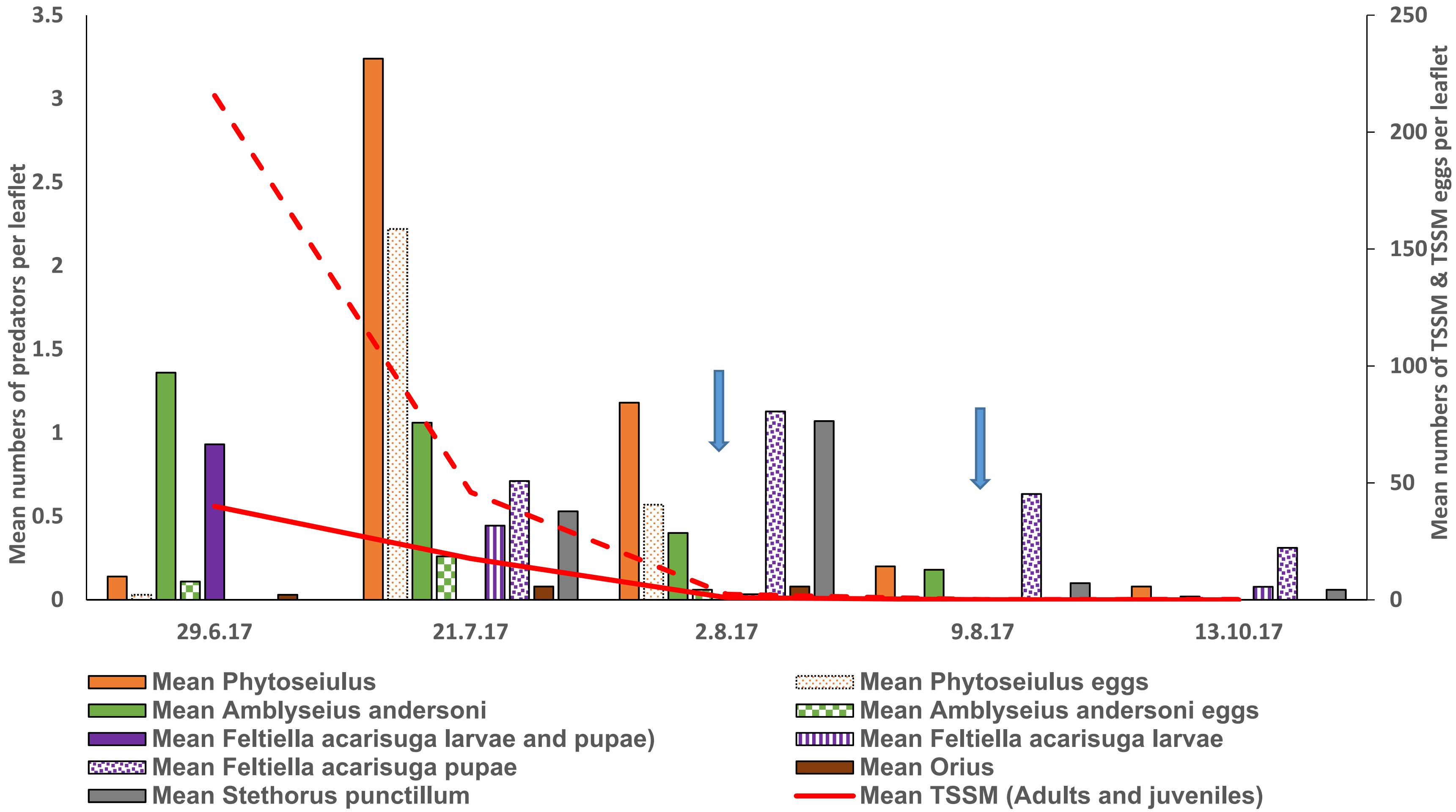

Figure A. Mean numbers of TSSM predators per leaflet (left hand axis) and mean numbers of TSSM and eggs per leaflet (right hand axis) on each assessment date

Objective 2.2: To develop compatibility strategies for biocontrol of two-spotted spider mites (TSSM) by predatory mites with insecticide sprays for spotted wing drosophila (SWD) and capsids

This work was undertaken by NIAB EMR. To maintain control of spider mite within a spray programme for SWD, it is assumed that the sprays may negatively affect the biocontrol programme. Leaving unsprayed refuges in the crop for commercially introduced and naturally occurring predatory mites may help to protect and maintain predatory mites. Therefore spray application methods which would provide good coverage on the upper leaf surface, but leave the lower leaf surface unsprayed were explored. To determine whether the method of spraying could be important, experiments were done in small purpose-built poly-tunnels to compare the same spray programme applied by two different spraying methods: pervasive canopy spraying using an air-assisted knapsack sprayer and a system of overhead spraying to give spray deposits mainly on the upper leaf surface (Figure B). The work began in 2015 (Year 1 of the project).

Figure B. A system of overhead spraying using nozzles directing sprays from above the crop canopy was set up in purpose built polythene tunnels.

In 2015, the effects of overall canopy spraying versus overhead misting application of a programme of sprays of deltamethrin (Decis/Bandu), spinosad (Tracer) and chlorpyrifos (Equity) on TSSM and naturally occurring predatory mites were compared using a suite of nine mini tunnels. The effects of date and treatment were significant. In early August, the numbers of natural phytoseiid mites were lower in both of the sprayed treatments. The numbers of TSSM then rose significantly in the sprayed plots from 17 August 2015. The numbers of SWD were lower in both of the treated plots.

In 2016, the same system of overhead spraying was used, with different nozzles to give a slightly larger droplet size. This resulted in less spray deposit on the underside of the leaves in the overhead spray treatment and although the natural phytoseiids were affected by the spray treatments, the effect could be mitigated by spraying from above. TSSM numbers were higher in the sprayed treatments (for all life stages with the knapsack spray). Introduced *P. persimilis* was less affected by the spray programme than anticipated; the spray programme did not negatively affect *P. persimilis* which significantly increased in the sprayed treatments compared to the control. The population development of *P. persimilis* followed that of the TSSM, albeit on a different numerical scale. Both methods of application, boom spraying and knapsack spraying, reduced the number of SWD compared to the control.

The work in 2017 repeated the 2016 experiment, again to determine the effects of overall canopy spraying verses overhead application of a programme of sprays of deltamethrin and spinosad on TSSM and predatory mites, both commercially introduced and naturally occurring.

In 2017, although it was not possible to determine any treatment effects for the TSSM and *P. persimilis* due to the low numbers per leaf, there were treatment effects for the naturally occurring phytoseiids. As in 2016, the sprays reduced the numbers of natural phytoseiids, however this effect could be mitigated by spraying from above. The assessment of spray deposition showed that there was less spray on the underside of the leaves in the overhead spray treatment, which could provide a refuge for predatory mites. The data also showed that the amount of spray deposited on the underside of leaves in the overhead spray treatment was highly variable.

As there were few *P. persimilis* motiles, it was not possible to determine the effect of the deltamethrin sprays in the field. However, bioassay work showed that with direct application of deltamethrin in the laboratory, almost all adults were killed within 24 hours. Therefore it is not believed that the commercially available strain of *P. persimilis* is resistant. The numbers of SWD were low in 2017 therefore no significant treatment effects could be determined.

Financial benefits

Before the spotted wing drosophila first arrived on UK shores, raspberry growers had refined their IPM programmes reasonably well and were gaining satisfactory control of two-spotted spider mite using biological and naturally occurring control programmes, primarily through the introduction of the predatory mite *Phytoseiulus persimilis* and sometimes complemented with other predatory midges such as *Feltiella acarisuga*.

The vital importance of controlling spotted wing drosophila at all costs, has resulted in a conflict with IPM programmes, given the nature of the crop protection products used for SWD control and the fact that they upset the predator/prey balance that is developed. However, failing to gain control of two-spotted spider mite can lead to serious reductions in the efficient photosynthetic area of the plant and this can lead to the production of small and shrivelled fruits and a subsequent reduction in the marketable yield of raspberry or other cane fruit crops.

Assuming a typical return for raspberries of £6.49/kg to growers (Defra Basic Horticultural Statistics 2014) and a yield of 14 tonnes/ha, then a 25% crop loss caused by two-spotted spider mite (a typical loss incurred) would lead to a financial loss of £22,722/ha. Developing a refined IPM programme on raspberries which can also cater for the control of other pests such as SWD and common green capsid, will significantly reduce such losses from two-spotted spider mite.

Action points for growers

- Aim to establish *P. persimilis* as early as possible and be aware of the contribution of naturally-occurring predators in the control of TSSM.
- Consider early release of *A. andersoni* for TSSM control before temperatures are suitable for *P. persimilis* as this predatory mite is more tolerant of low temperatures than *P. persimilis*. However, released predators of this species may be less tolerant of certain crop protection products such as pyrethroids, than naturally occurring populations.
- Wherever possible, use IPM-compatible plant protection products or those with the least harmful effects on biological control agents for control of all pests including SWD.
- As naturally occurring predatory mites, such as *A. andersoni*, may be harmed by plant protection products, consider leaving unsprayed refuges, for example by overhead spraying to reduce deposits on the lower leaf surface.
- Where unsprayed refuges are used, monitor regularly to ensure that other pests such as aphids are controlled, and treat within an IPM programme.
- Re-introduce *P. persimilis* for TSSM control where necessary.

SCIENCE SECTION

Objective 1: To determine the potential for alternatives to chemical fungicides for the reduction of Phytophthora root rot

Aim

WP 1.1: To investigate the effects of a range of novel plant treatments on raspberry growth and their resilience to pests and disease from propagation through to primocane production.

Introduction

Phytophthora root rot is now the most destructive disease of raspberries worldwide. Where raspberries have been grown in the soil *Phytophthora rubi* (previously known as *P. fragariae* var. *rubi*) is almost ubiquitous. Outbreaks of this disease across Europe at the same time in traditional raspberry-growing areas suggests that the disease has spread through the propagation network and has been distributed to farms in new planting material (Graham *et al*.,2011). Current approaches for Phytophthora control rely on fungicide applications twice per year either as a soil-applied drench or through the drip irrigation. SL567A (44.7% w/w metalaxyl-M) and Paraat (500 g/kg dimethomorph) can be used, however resistance development in pathogens because of limited modes of action is a major concern.

There is interest in the use of nutrients, elicitors and microbial products against pathogens such as *Phytophthora* spp. and also pests. Phosphites and silicon for example have been shown to prime plant defence responses and other products can stimulate systemic acquired resistance (SAR) whereby plants accumulate proteins which aid defence (Fauteux *et al.,* 2005). Microbes in biological products such as Prestop (*Gliocladium catenulatum*), Serenade ASO (*Bacillus subtilis*) and *Trichoderma* spp. in Trianum and T34 Biocontrol as well as competing with pathogens can also induce plant defences, and have been shown to improve efficacy when used in alternation with conventional fungicides.

Outline of preceding work

Raspberry plants of cv. Tulameen were raised as modules, in Year 1 (2015) of this project, at a propagation nursery in Oxfordshire from cuttings and treated on up to four occasions, depending on the treatment manufacturer's instructions. All liquid treatments were applied as a drench using a hand held spray gun that had been calibrated to ensure an accurate volume was applied to the plant modules. The granules of Root Grow HYDRO were sprinkled on to the compost surface and the cuttings were lifted out of the cells a little to allow the treatment to fall around the root plug. Plants were assessed for vigour and phytotoxicity throughout year one of the project. Details are given in the Annual Report on 2015.

Half of the plants at the end of 2015 remained at the propagation nursery and the other half relocated to a tunnel at ADAS Boxworth. The plants remaining at the propagation nursery were put into cold-storage over winter (2015/16) as they were required for long cane fruit production in 2016. The plants at Boxworth were cut back before potting-on in spring 2016 and artificial root inoculation by *Phytophthora rubi* mycelium and zoospores carried out.

Treatment with the microbial products from propagation allowed time for any plant defence stimulation claimed by the products, or protective colonisation of the plants by the beneficial microbes to take place before the stress of fruiting (which can aid infection). In 2016, investigations (reported in the Annual Report for 2016) focused on whether, once plants were potted for production and given further treatment with the same products, their growth was improved and the effects of any pest or disease reduced. Neither site in 2016 had any phytotoxicity, nor did vigour differ. Fruit yield was recorded in Oxfordshire and no treatment differences shown. At Boxworth a small increase in primocane production arose in 2016 with the use of all the biofungicides (Prestop, HDC F201, Root Grow HYDRO and Serenade ASO) compared with pots left untreated or given Paraat or the chemical HDC F205.

The first section of this report on 2017 deals with the second year of fruiting of the cv. Tulameen in Oxfordshire when products were re-applied at the same rates, with final assessment in January 2018. A further two experiments set up in 2017 at the same propagation site as 2015, but using long-canes and to be studied to the end of 2018, are then detailed. Finally, continuing work on zoospore behaviour is outlined.

Materials and Methods

2017 Oxfordshire

Figure 1.1. Raspberry plants cv. Tulameen May 2017 (top) prior to fruiting. (Bottom) following primocane selection for fruiting canes in the experiment at a commercial farm in Oxfordshire. December 2017.

In 2017, plants (one per 10 L pot) at the commercial farm in Oxfordshire were again treated and assessed (**Figure 1.1**). Pots had legs which raised them off the woven ground-cover material and each had a single dripper. Spring-water was used for irrigation. Re-treatment with the mycorrhizal product Root Grow HYDRO (HDC F204) was not possible as this is directed to be used at re-potting. One of the experimental pesticide products (HDC F201) was not re-applied, as its use did not allow application out of permanent protection. The crop was not tunneled. There were four pots per plot, with an unplanted pot between each plot and four replicate blocks. Treatments, summarised in **Table 1.1**, were applied at set rates and times during both 2016 and a further season in 2017. The products were made up in tap water in a

1 L bottle dedicated to each product and poured across the whole growing-media surface to give a 10% by volume drench. The rate for Paraat was based on the label recommendation for raspberries of 1 g per plant in a minimum of 200 ml water. The single drench of Serenade ASO was applied according to EAMU 0705 of 2013 for outdoor cane and bush fruit and calculating the amount of product based on the surface area of growing-media at the pot rim.

Table 1.1 Summary of products and application timings made in 2015, at the propagators in multicell trays and then module pots, and application timings after the same plants were moved to be potted into 10 L at the Oxfordshire site in 2016. 2017 applications are highlighted.

Trt. no.	Treatment	Dilution rate and (product per 10L pot)*	Year: Month applied
1	Untreated	-	-
2	Prestop [MAPP 15103]	5 g/1 L water (5g)	2015: (April, May, July, October) 2016: (April, May, June) 2017: (April, May, June)
3	HDC F201	Not disclosed	2015: (April, May, July, October) 2016: (April, June)
4	Root Grow HYDRO (HDC F204)	7 g/1 L growing- media (70g)	2015: (April, May) 2016: (April)
5	Serenade ASO (HDC F228)	10 L/ha using1000 L water (1 ml / m² *pro rata* to area of pot surface) (0.12ml per 10L)	2015: (April, May, July, October) 2016: (April) 2017: (April)
6	HDC F205	Not disclosed	2015: (April, May, July, October) 2016: (April, May, June) 2017: (April, May, June)
7	Paraat [MAPP 15445]	1.0 g/plant (1g)	2015: (April, May) 2016: (April) 2017: (April)

*Applying 1 L of diluted product per pot to give a drench of 10% of pot volume per 10 L pot. Treatments were applied up to three times following the treatment manufacturers' instructions.

The crop was managed as standard by the grower in the same way as other raspberry crops in the plantation, with all fertigation, insecticides and preventative fungicide applications (see Appendix 1), although withholding the normal farm application of Paraat to the plants in the experiment.

Table 1.2. Dates and types of assessments and product application dates, Oxfordshire 2017.

Date	Procedure
07 April 2017	Pre-treatment assessment. Product applications.
09 May 2017	Phytotoxicity and floricane vigour assessment. Product applications.
23 May 2017	Phytotoxicity and floricane vigour assessment. Product applications.
07 June 2017	Phytotoxicity and floricane vigour assessment. Product applications.
19 June to 05 July 2017	Harvest assessments.
29 June 2017	Phytotoxicity and primocane vigour assessment.
10 July 2017	Leaf samples for nutrient analysis.
06 September 2017	Final primocane vigour assessment.
04 December 2017	Suitability assessment of new cane produced in 2017.
15 January 2018	Final disease assessments of new floricane and roots.

The trial was assessed for phytotoxicity 2 weeks after each treatment application (**Table 1.2**). Any symptoms such as yellowing, distortion or necrosis that were likely to be as a result of product application were recorded. Plants were scored using a phytotoxicity scale of 0 to 9, with 0 being dead, 9 being healthy and comparable to the untreated control, and 7 being considered commercially acceptable.

Four weeks after each treatment application a more detailed assessment was carried out to assess the vigour of the plants and also to assess for the presence of key pests and diseases. These detailed assessments were carried out six times during the whole trial period. Vigour was recorded by measuring variables such as cane height, leaf size and the number of new primocane produced by each plant. A vigour score was awarded for each plant based on a visual assessment taking these variables into account. Plants were scored on a scale of 0 to 10, with 0 being dead and 10 being excellent vigour.

The final vigour assessment, on 4 December 2017, scored the height of new canes in each plot: short (<1.5m), medium (1.5m-1.8m), tall (>1.8m), thickness by visually categorising canes as either stout, medium or thin (**Figure 1.3**) and recording the number of canes in each category. Thin canes are less likely to produce good yields.

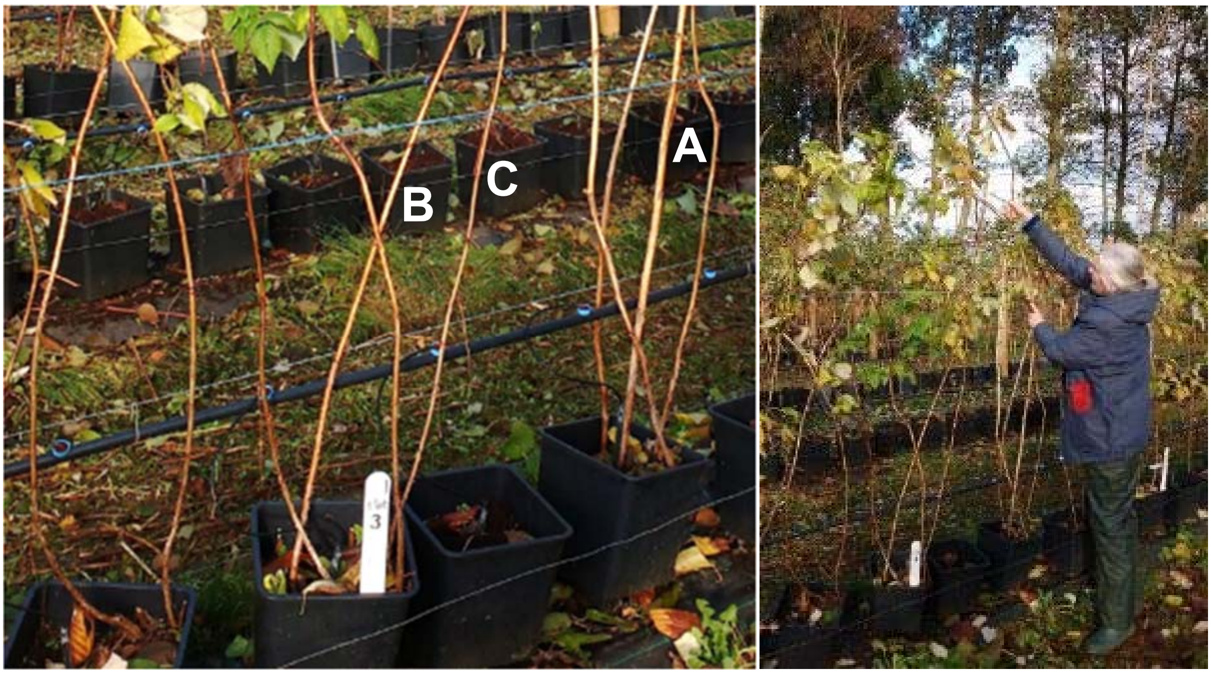

Figure 1.3. Examples (*left*) of cane thickness, stout (A), medium (B) and thin (C). Researcher (*right*) making a visual assessment of plant height, here holding a cane >1.8m in height. December 2017.

On 4 December 2017 and 15 January 2018, plants were assessed for any signs of disease, in particular cane damage. The second assessment was necessary in January 2018, to allow more time for the diseases to fully develop, to aid better identification, with advanced symptoms of spur and cane blight sought on susceptible varieties elsewhere on the site to aid assessment (**Figure 1.4**).

Figure 1.4. Cane diseases, botrytis (left) with black sclerotia, and spur blight (right) with pin-head sized spore bodies on mature primocanes. Both pathogens causing silvering of the epidermis, Oxfordshire, photographed not in the trial area. December 2017.

Roots samples were taken in September 2017, from three plants to allow laboratory checking for root pathogens. In January 2018, roots were sampled from the bottom of a pot from each treatment in Rep 1, as well as from two pots where canes had been too thin to be kept on.

A *Phytophthora* spp. Lateral Flow Device (LFD) was used with the September 2017 root samples. Root floats were made for each of the root samples, using fresh soil water to encourage the production of sporangia.

Fruit was picked, as commercially, three times a week, by farm staff over the harvest period, which commenced on 19 June 2017 and finished on 5 July 2017. Fruit was picked from the four pots in each plot, and split into two classes: class 1 (marketable fruit) and waste fruit. During two picks, 26 June & 3 July, researchers also recorded mean berry weight per plot and noted any recurring problems with the fruit in each plot such as over-softness, sun scorch, small size, or crumbly fruit.

Results

No significant differences were seen in phytotoxicity scores between the treatments at any of the assessments throughout the treatment application period (**Table 1.3**). All of the treatments scored a mean index of seven or above, meaning that all the plants were considered commercially acceptable after each treatment application was made.

Table 1.3. Mean phytotoxicity indices (0 = dead, 9 = no reduction in leaf quality) for raspberry plants throughout treatment application period in Oxfordshire in 2017.

Treat-ment	**Product**	**Mean Phytotoxicity Index**			
		09 May	**23 May**	**7 June**	**29 June**
1	Untreated	9.0	9.0	9.0	9.0
2	Prestop	9.0	9.0	9.0	9.0
3	HDC F201	9.0	9.0	9.0	9.0
4	Root Grow HYDRO	8.4	8.5	9.0	9.0
5	Serenade ASO	9.0	9.0	9.0	9.0
6	HDC F205	9.0	9.0	9.0	9.0
7	Paraat	9.0	9.0	9.0	9.0
	P-value	*Note 1*	*Note 1*	*Note 2*	*Note 2*

Note 1: ADAS statistician determined raw data variation was insufficient to produce any statistically significant differences. *Note 2*: No differences in raw data.

No significant differences were seen in plant vigour between treatments (**Table 1.4**) indicating good vigour throughout the 2017 trial period.

Table 1.4. Mean plant vigour indices (0 = dead, 10 = excellent vigour) for raspberry plants throughout the treatment application period, Oxfordshire 2017.

Treat-ment	**Product**	**Plant Vigour Index**				
		09 May	**23 May**	**07 June**	**29 June**	**6 September**
1	Untreated	7.00	7.82	8.31	6.25	6.25
2	Prestop	7.88	8.38	8.69	6.75	7.50
3	HDC F201	6.88	7.69	8.63	6.00	7.50
4	Root Grow HYDRO	7.00	7.69	7.94	6.50	6.75
5	Serenade ASO	7.06	7.88	8.50	6.00	7.25
6	HDC F205	7.69	8.56	8.50	8.00	6.75
7	Paraat	7.50	8.31	8.94	6.75	7.25
P value		0.236	0.248	0.336	0.345	0.769
L.S.D (d.f. 18)		0.960	0.891	0.842	1.862	1.879

Table 1.5 Average number of new cane (produced in 2017) per raspberry plant, after immature canes removed*. Oxfordshire, May 2017.

Treatment	Product	Average number of new cane per plant
1	Untreated	2.69
2	Prestop	3.69
3	HDC F201	2.63
4	Root Grow HYDRO	3.00
5	Serenade ASO	5.69
6	HDC F205	3.75
7	Paraat	3.69
P value		0.021
L.S.D (d.f. 18)		1.688

* Immature canes removed by grower, were not deemed mature enough to carry forward into next year's season.

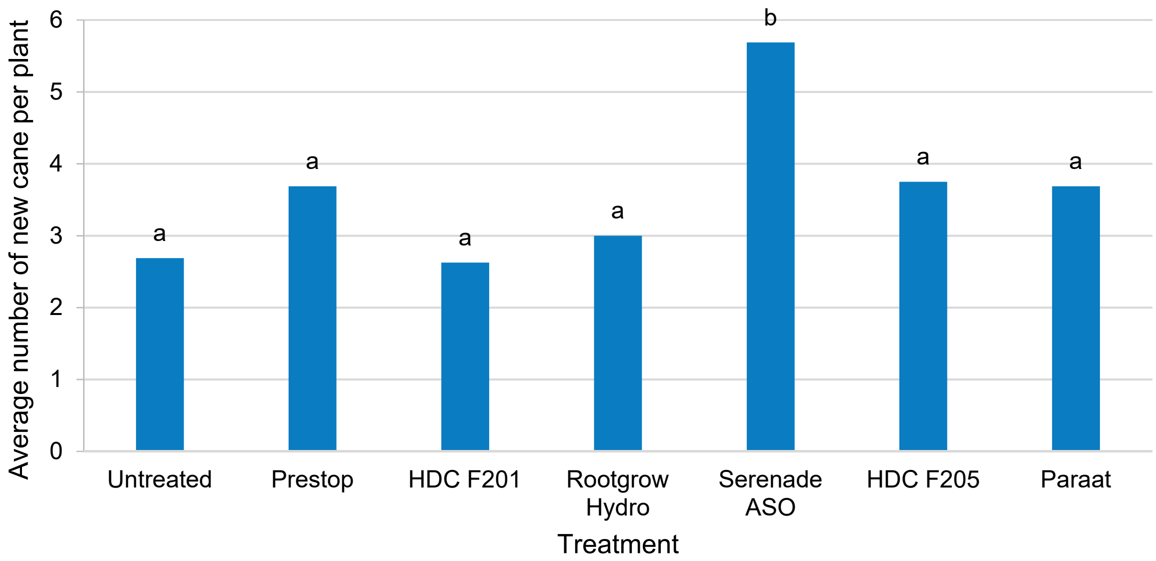

Figure 1.5. Average number of new cane per plant. Oxfordshire May 2017.

Plants treated with Serenade ASO, produced significantly (P = 0.021) more new canes than any other treatments, as shown in **Figure 1.5** where significant differences between treatments are denoted by a different letter.

In April 2017, the floricane of plants treated in 2016 with Prestop, HDC F201, Serenade ASO and Paraat had significantly lower (P = 0.014) coverage by cane botrytis than those which had received HDC F205 (Table 1.6 and Figure 1.6).

Table 1.6. Mean percentage of cane surface area covered by visible cane botrytis. Assessments conducted on floricanes (produced in 2016 and cut out in autumn 2017 after fruiting) before the April to June 2017 treatment applications and then in January 2018 on floricanes (produced in 2017 for fruiting in 2018). Oxfordshire, 2017/18.

Treatment	Product	% of cane surface with botrytis April 2017	% of cane surface with botrytis 15 Jan 2018
1	Untreated	9.48	56.9
2	Prestop	7.92	53.0
3	HDC F201	5.21	67.6
4	Root Grow HYDRO	10.83	63.5
5	Serenade ASO	6.87	56.3
6	HDC F205	13.85	68.2
7	Paraat	5.94	66.1
P value		0.014	0.315
L.S.D (d.f. 18)		4.674	16.24

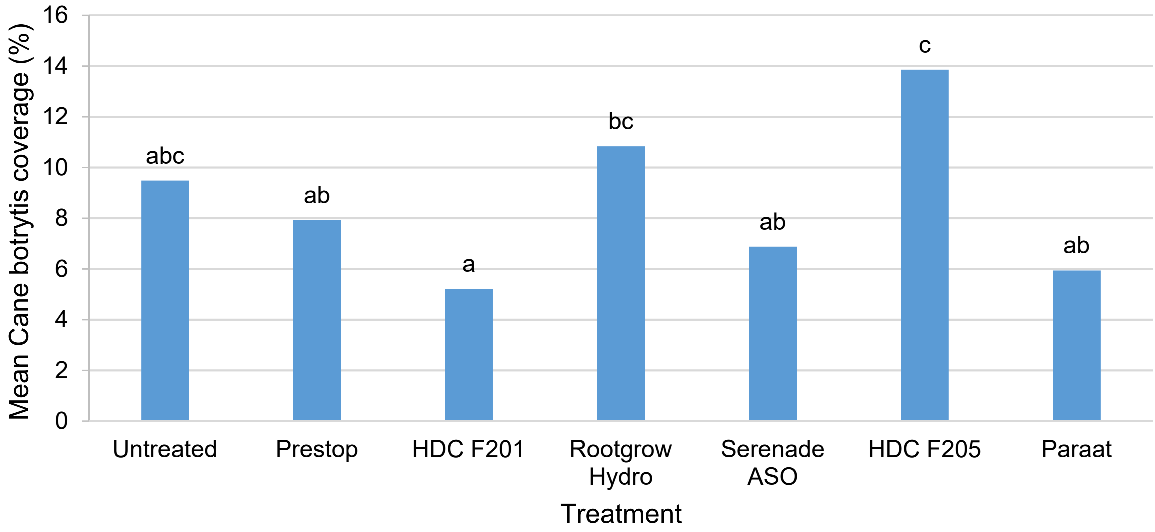

Figure 1.6. Cane botrytis % coverage on floricanes (cane produced in 2016) in April 2017 before any treatment applications in 2017 showing no significant differences between the untreated and treated plots, but a difference arose between the two coded products.

Treatment-wise differences are illustrated in **Figure 1.6,** where significant differences are denoted by a different letter. HDC F205 had more botrytis than all but the untreated and Root Grow Hydro. No significant differences in percentage coverage by cane botrytis were seen in the January 2018 assessment, but percentages were much higher, than in the April pre-season assessment. Botrytis was present up the length of the canes, with a mean surface area coverage of 57.3% below 1 m and similar (63.6%) above 1 m on the first cane recorded per pot. Pots had been cane-thinned in autumn to produce two or three floricanes per pot, but the obvious bleached sometimes “watermarked” botrytis symptoms with sclerotia (**Figure 1.7**) visible in January were not visible at the time of suitable cane (not thin or short) cane selection.

Figure 1.7. Botrytis bleaching and watermarking of the epidermis and sclerotial resting bodies on a floricane of cv. Tulameen within the experiment in Oxfordshire, January 2017

Harvesting information from the four pots per plot, showed that the total over eight harvests were not significantly different between treatments in either the mean total marketable yield (mean 2.05 kg), the mean total waste yield (mean 0.59 kg) or the mean berry weight (mean 6.05 g). A total of marketable plus unmarketable fruit was calculated, and all treatments were similar, with an average of 2.6 kg (**Table 1.7**).

Throughout the treatments, waste fruit was caused by sun scorch and some crumbling. During harvest, at times temperatures were in excess of 30^{o}C (**Figure 1.9**).

Table 1.7. Fruit assessments over all harvests, including total marketable yield, total waste yield, percentage marketable yield out of total, and average berry weight. June – July 2017.

		Over-all harvest yield records per plot (4 pots combined)			
Trt. No.	**Product**	**Mean total marketable (g)**	**Mean total waste (g)**	**Percentage marketable of total (%)**	**Mean berry weight (g)**
1	Untreated	1876	746	71.3	6.36
2	Prestop	2125	571	78.0	6.12
3	HDC F201	1996	673	74.8	5.52
4	Root Grow HYDRO	2144	447	81.6	6.01
5	Serenade ASO	1864	526	79.0	6.01
6	HDC F205	1896	602	75.0	6.16
7	Paraat	2137	593	78.7	6.23
P value		0.941	0.572	0.552	0.431
L.S.D (d.f. 18)		729.1	317.7	11.03	0.759

After the grower's autumn pruning-out of excess primocanes, the canes available for fruit production were recorded by height and width (**Table 1.8**) and analysis was done on the proportion of "suitable" canes (**Table 1.9**). Results might have differed if the immature canes were included, but in assessing after pruning, cane height and thickness were clearer to measure, and meant only canes that were deemed good enough to carry on to the next season, were included.

Table 1.8. Proportion of primocanes at certain heights and thicknesses. December 2017.

Trt. no.	Treatment	Plant height ranges (% of canes/range)			Plant thickness ranges (% of canes/width)		
		Tall	**Medium**	**Short**	**Stout**	**Medium**	**Thin**
1	Untreated	52.5	30.0	17.5	12.8	33.3	53.8
2	Prestop	62.8	23.3	14.0	18.6	16.3	65.1
3	HDC F201	51.4	28.6	20.0	14.3	37.1	48.6
4	Root Grow HYDRO	64.9	16.2	18.9	16.2	40.5	43.2
5	Serenade ASO	46.7	33.3	20.0	6.7	31.1	62.2
6	HDC F205	68.1	23.4	8.5	8.7	45.7	45.7
7	Paraat	68.3	19.5	12.2	12.2	31.7	56.1

Tall and medium height, and stout and medium thickness primocanes were deemed ‘suitable’, as opposed to thin and short canes. There were no differences between treatments, with 95.6% being suitably tall (a good number of lateral buds) and 46.5% being suitably stout (able to physically and nutritionally support the fruiting laterals).

Table 1.9 Proportion of suitable primocanes, by height and thickness in December 2017. Suitable canes were of tall or medium height and either stout or medium thickness.

Treatment	Product	% of canes of suitable height	Standard error of heights	% of canes of suitable thickness	Standard error of thickness
1	Untreated	82.6	5.9	43.6	7.6
2	Prestop	86.2	5.2	34.5	7.0
3	HDC F201	80.1	6.7	52.4	8.2
4	Root Grow HYDRO	80.9	6.4	56.9	8.0
5	Serenade ASO	80.0	5.9	37.8	7.0
6	HDC F205	91.4	4.0	54.4	7.0
7	Paraat	87.6	5.1	43.3	7.5
P value		0.684	-	0.259	-

Root assessments

A sample of roots were taken from across the trial area in September 2017, from a small proportion of roots that had browning. An LFD test confirmed the presence of *Phytophthora* spp. However no plants showed any symptoms of *P. rubi* infection.

Following the 15 January 2018 root assessment of one plant at random in replicate block 1, no *Phytophthora* spp. (or *Pythium* spp.) sporangia were produced in the root floats in water from any of these plants, and *Phytophthora* sp. LFD test results of roots were negative. None of the root balls examined in these pots had any rotted brown roots, as distinct from brown suberized / tannin stained healthy roots that were present in addition to white new roots (**Figure 1.8**).

Figure 1.8 Example of typical root development in the raspberry pots by 10 January 2018 showing good pot fill by healthy roots, with older healthy suberized roots and new white healthy roots visible. The roots shown are of an untreated plant (pot 2 in plot 7).

Environmental conditions

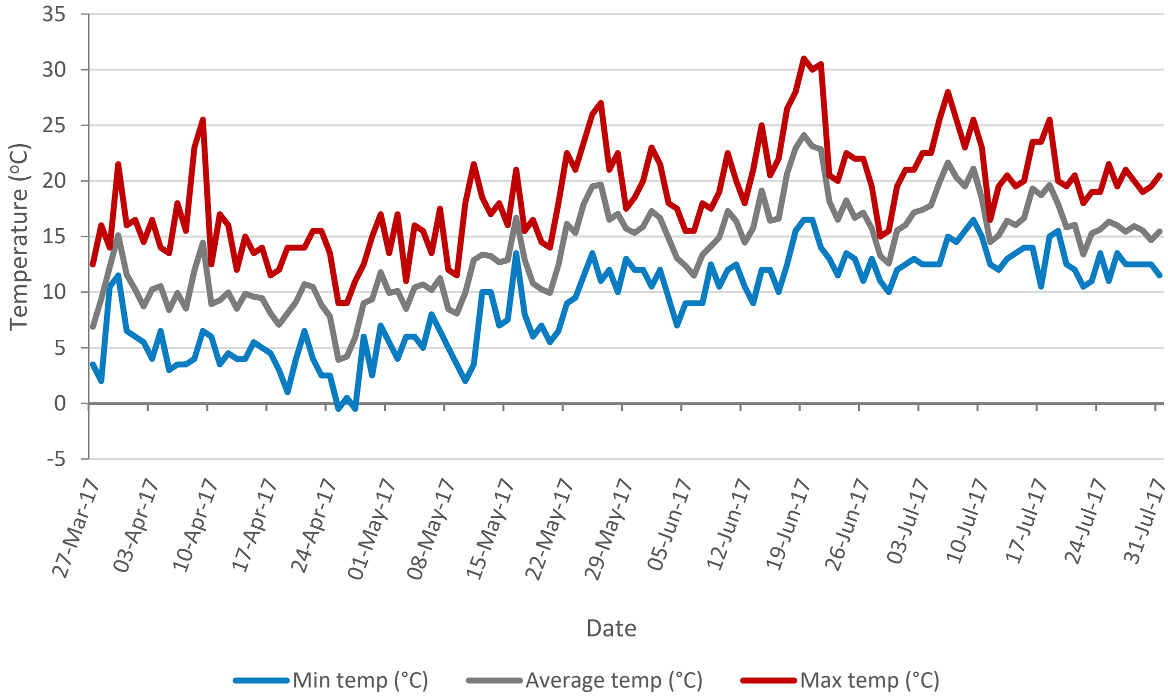

Figure 1.9. Minimum, mean and maximum daily air temperatures in the canopy between 27 March and 31 July 2017. Oxfordshire, UK. Harvest period 19 June to 5 July 2017.

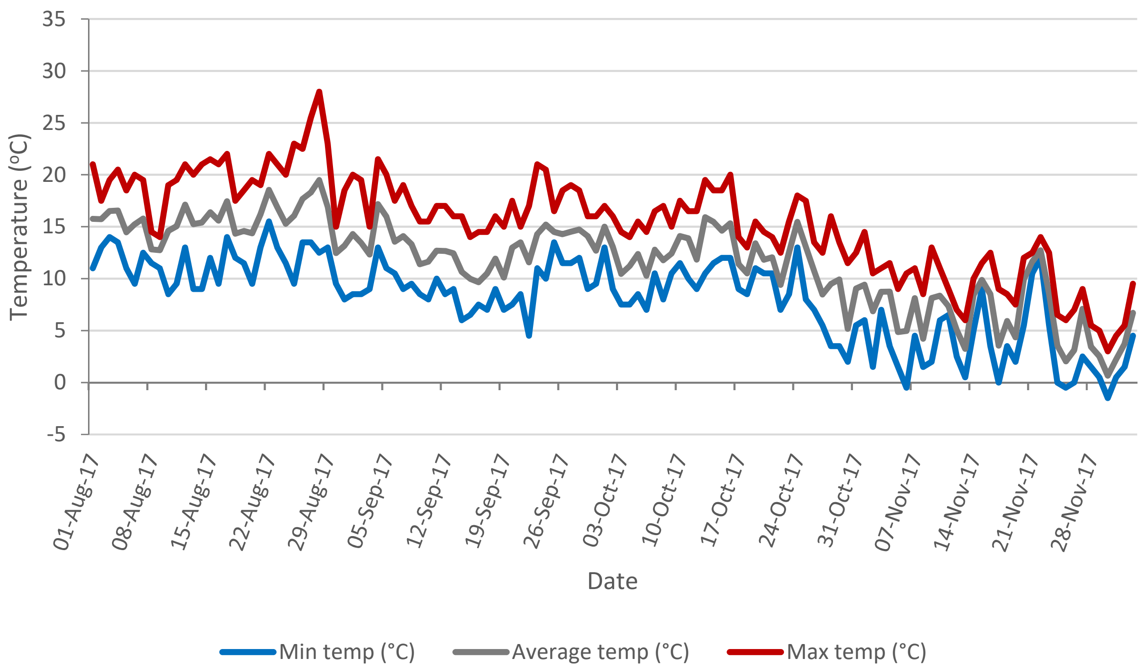

Figure 1.10. Minimum, mean and maximum daily air temperatures in the canopy of the experiment between 1 August and 3 December 2017. Oxfordshire, UK.

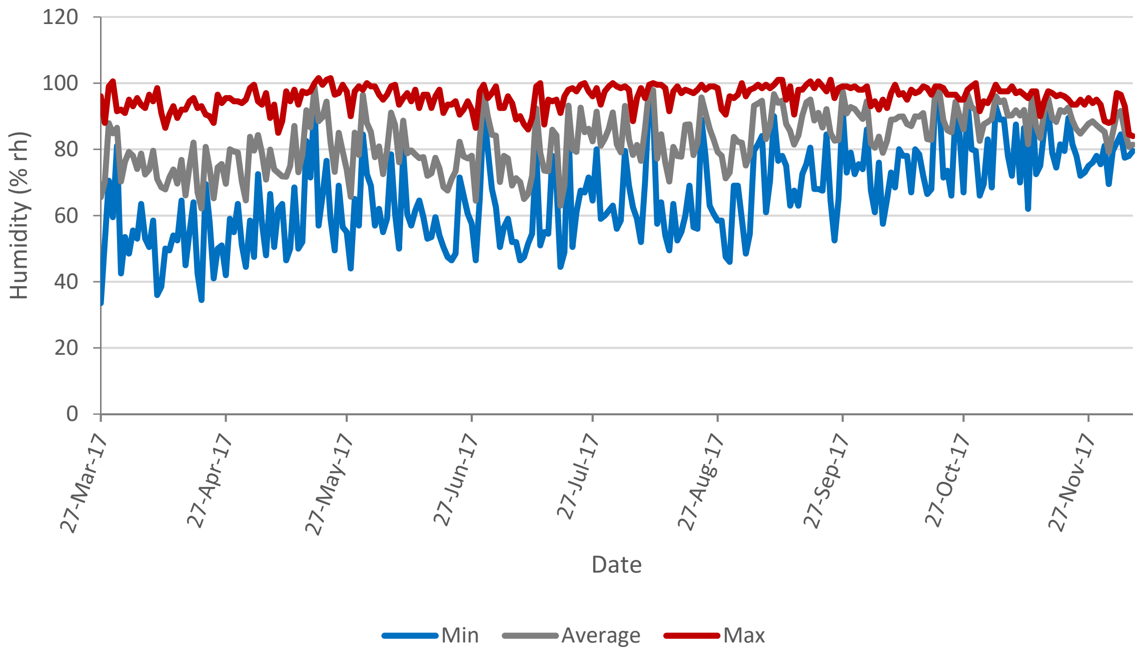

Figure 1.11. Minimum, mean and maximum relative humidity in the canopy of the experiment between 27 March and 3 December, 2017. Oxfordshire.

Temperature (**Figure 1.9** & **1.10**) and relative humidity (**Figure 1.11**) were recorded from 27 March to 3 December 2017. The mean air temperature was 8.5°C on 7 April 2017 when the first application of treatments was made, and the mean relative humidity was 72.4%. On this day, temperatures ranged from 3.5°C to 15.5°C. On 9 May 2017, when the second treatments were applied, the mean air temperature was 8.1°C, which ranged between 3.5°C and 11.5°C. On 9 May 2016 the mean relative humidity was 73.9%. On the final application date, 7 June 2017, temperatures ranged between 9°C and 18°C, with a mean air temperature of 13.3°C and a mean relative humidity of 91.1%. Maximum temperatures of 30°C occurred on the 20 and 21 June at the start of harvest.

Nutrient analysis was carried out following leaf sampling on the 10 July 2017 (**Tables 1.10** and **1.11**). The results are not means so ANOVA was not possible to compare between treatments, but shading has been used in the tables to highlight the range of values for each chemical element. The importance of these ranges to the plant was not known, but comparison can be made with the untreated.

Table 1.10. Analysis of Rectory Farm Raspberry leaf samples taken 10 July 2017. Total values reported on 100% dry matter basis. The darker the shading the higher the value.

Treat-ment	Product	Nitrogen DUMAS % w/w	Phosphorus mg/kg	Potassium mg/kg	Calcium mg/kg	Magnesium mg/kg
1	Untreated	4.301	3973	31609	10485	3475
2	Prestop	4.298	3646	31078	10937	3732
3	HDC F201	4.274	4086	31225	12505	4010
4	Root Grow HYDRO	3.875	3905	33270	10300	2932
5	Serenade ASO	4.327	3932	32638	10129	3439
6	HDC F205	3.929	4638	35333	12558	3651
7	Paraat	4.214	4251	33847	10950	3728

Table 1.11. Analysis of Rectory Farm Raspberry leaf samples taken 10 July 2017. Total values reported on 100% dry matter basis. The darker the shading the higher the value.

Treat-ment	Product	Sulphur mg/kg	Mang-anese mg/kg	Copper mg/kg	Zinc mg/kg	Iron mg/kg	Boron mg/kg
1	Untreated	1739	42.2	2.9	21.7	125	33.9
2	Prestop	1773	41.0	2.6	19.2	117	32.5
3	HDC F201	1817	41.7	2.2	20.0	140	38.1
4	Root Grow HYDRO	1771	36.9	2.8	18.6	135	34.8
5	Serenade ASO	1941	42.4	2.8	21.0	190	36.4
6	HDC F205	1675	60.9	2.9	17.7	130	43.4
7	Paraat	1740	40.4	2.2	20.3	136	36.1

Prestop-treated plants had lower than average phosphorus and potassium, while the chemical HDC F205 had above average of these elements and also of calcium, manganese, copper and boron. Lower potassium was also seen in leaves from the microbial product HDC F201 (last used in 2016), but not from the bacterial biofungicide Serenade ASO. Serenade ASO-treated plant's leaves had below average calcium and above average sulphur and iron. The mycorrhiza product Root Grow HYDRO-treated plants at potting had below average total nitrogen, calcium and manganese than the untreated.

Discussion

Neither foliar disease, cane wilting, nor root rot were seen even in the untreated plots, therefore no conclusions can be drawn regarding product effects on disease incidence or severity.

There were no significant differences recorded either between any of the treatments or between those and the untreated in either plant vigour during crop growth, fruit harvest measurements, or the number and strength of cane produced by January 2018. There were no adverse effects from the treatments, nor any growth promoting characteristics shown from them. There were different levels of nutrients in the leaves in July, but other than the above average levels of phosphorus and potassium present after using product HDC F205, there are no obvious explanations for these, or how the levels might affect plant physiology differently.

At the start of the 2017 growing season, plants treated with Serenade ASO produced significantly more new canes (spawn), than all other treatments. However, five canes per plant maybe in excess of what would be required to be kept as floricane.

When monitoring floricane botrytis pre-treatment in 2017, those treated with HDC F205 had more botrytis than all but the untreated and Root Grow Hydro. This suggests there might be a reduction in resistance to this pathogen. After the final cane botrytis assessment, there were no significant differences between treatments. Pre-season coverage ranged from 5% - 13% whereas post-season winter coverage ranged much higher at 53% - 68%. This indicates the time of year when cane botrytis becomes particularly prevalent.

Conclusions

- No phytotoxic damage arose to the cultivar Tulameen used for the experiment from any of the treatments applied throughout the 2017 growing season.
- Efficacy of the products tested against Phytophthora was not able to be shown as no natural infection of root rot or wilting occurred in any of the plants.
- Serenade ASO treated plants produced significantly more new cane than all other treatments.
- Greater severity of floricane botrytis was observed in spring 2017, following treatment with HDC F205 and Root Grow Hydro. No differences in primocane cane botrytis was observed between treatments in January 2018.

- Neither vigour nor fruit yield differed between any of the experimental treatments, with all being similar to those remaining untreated.

Acknowledgements

We would like to thank Richard Stanley and Paul Clarke for assistance in provision of the site and the crop husbandry for the experiment in Oxfordshire.

Objective 1

Aim

WP 1.2 – To explore *P. rubi* zoospore behaviour with raspberry root exudates.

Introduction

Phytophthora root rot is currently the most economically damaging disease affecting UK raspberries (HDC Factsheet Cane Fruit 02/07). Where raspberries have been grown in the soil *Phytophthora rubi* (previously known as *P. fragariae* var. *rubi*) is almost ubiquitous.

Like other *Phytophthora* species, *P. rubi* zoospores are released by mature sporangia, and swim towards raspberry root tips, before encysting and entering the root. It is thought that zoospores use chemotaxis to target the root, however, compared to other *Phytophthora* spp. relatively little is known about *P. rubi* and its exact mode of infection. Work therefore needs to be done in understanding the behaviour of *P. rubi* zoospores (the infecting part of its lifecycle) to find robust control solutions.

2017 ADAS Boxworth – Pathogenicity and zoospore behaviour

Materials and Methods

Work has been ongoing with the ADAS *Phytophthora rubi* isolate, to observe zoospore behaviour. Images and footage of sporangia releasing zoospores was gathered to ensure the correct species identification (**Figure 2**).

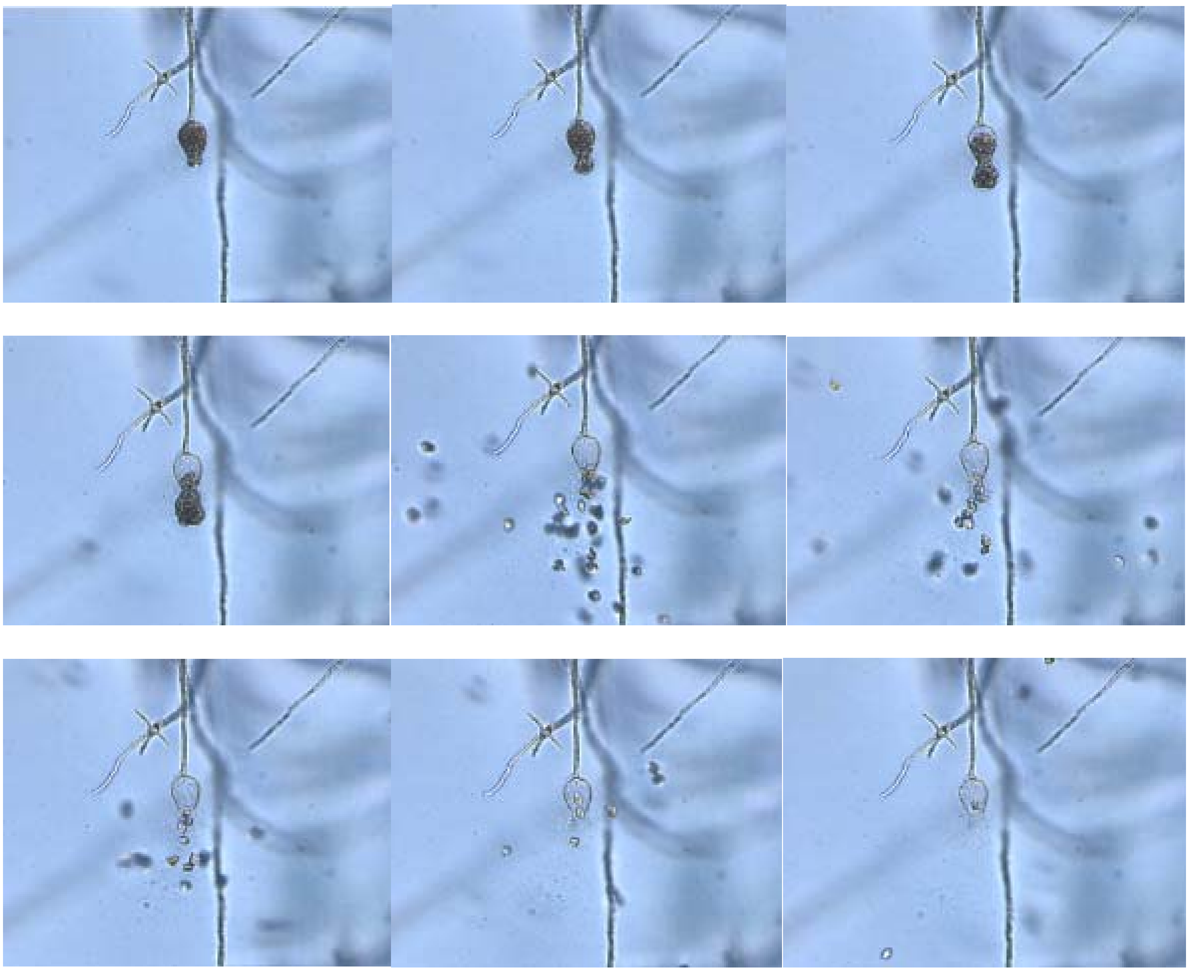

Figure 2. One minute time lapse of *Phytophthora rubi* ovoid sporangium releasing 20-30 zoospores. Copyright ADAS. 2018.

Phytophthora sporangia are often the main differentiating feature between species. In *P. rubi*, like its close relative *P. fragariae*, sporangia are ovoid to obpyriform (**Figure 3**). It was noted that the density of sporangia formed was low, with just two in view (at x400), likely due to the fact that each sporangium is on an individual stalk, and not branched, like other *Phytophthora* spp. With fewer sporangia present, and each only producing 20-30 zoospores, the concentration of zoospores was low in the solution they were in.

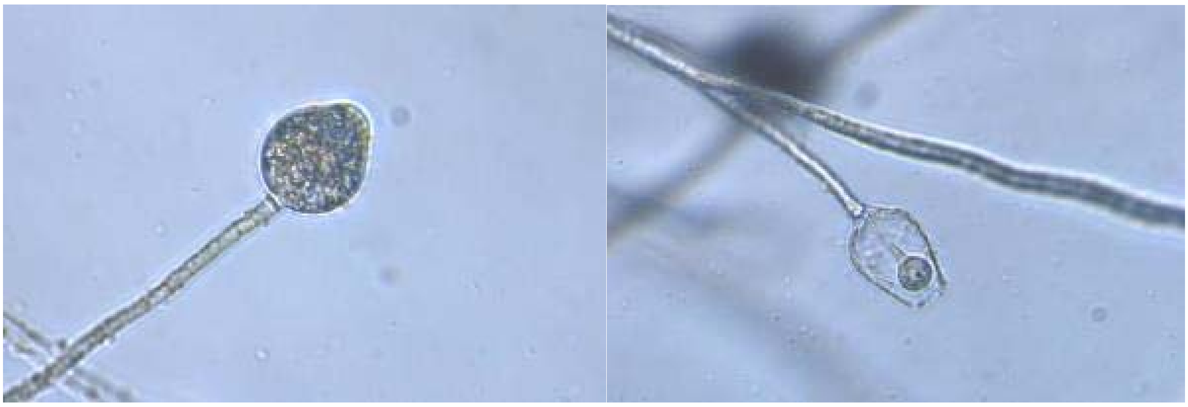

Figure 3. Mature *P. rubi* sporangium (left). Internal proliferation (right), a typical feature of *P. rubi*. Copyright ADAS, 2018.

Zoospore + root exudates:

Once zoospores were observed and confirmed, droplets of them in solution were transferred to a plastic haemocytometer slide for further work on their behaviour, see **Figure 4.**

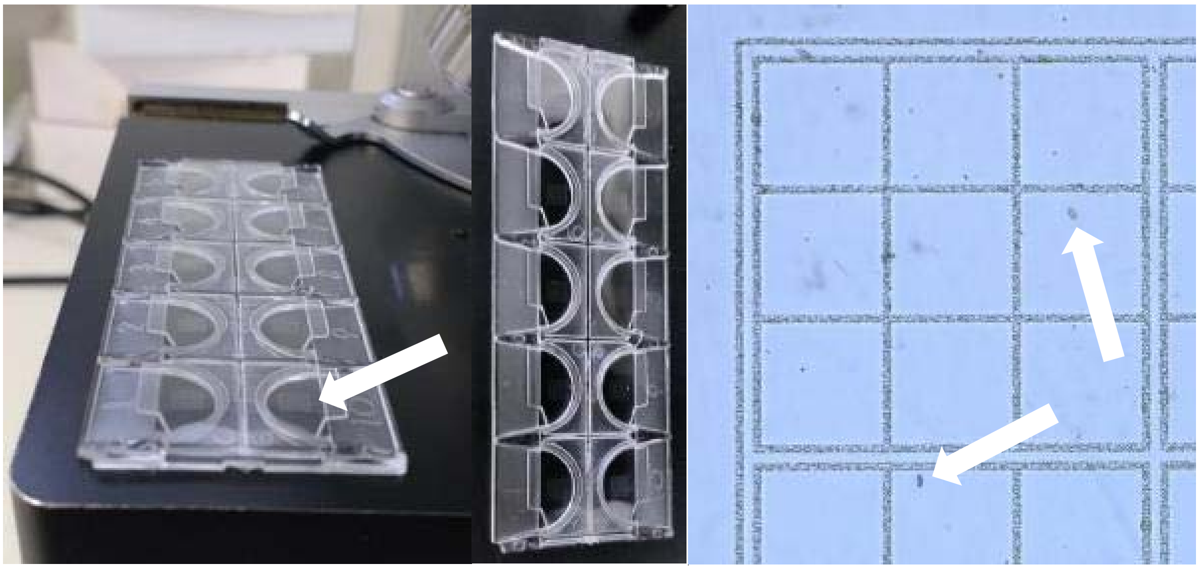

Figure 4. A 10-well, plastic haemocytometer slide (left and middle). Each well can hold 1 ml of solution, and has a 4x4 grid in the centre (left, arrow) that can be used to track, count and monitor zoospores (right, arrows).

Sterile micropipette tips were then able to be placed up against the entrance to the well, to allow fluid in the tip to come in to contact with the solution (containing zoospores) in the well.

To test a possible attractant, V8 juice, known to promote the growth of *Phytophthora* cultures, was placed in the tip, whereby it slowly seeped in to the well. There were zoospores present, but not enough to confirm a sufficient gathering around the V8 juice.

Further to that, a different set-up, involving a plastic tip and water droplet in the centre of a sterile Petri dish, gave a novel approach to observing the zoospores and end of tip in the same frame (**Figure 5**). With just one droplet, the volume of solution is small, so zoospores could be concentrated better than in the 1 ml wells.

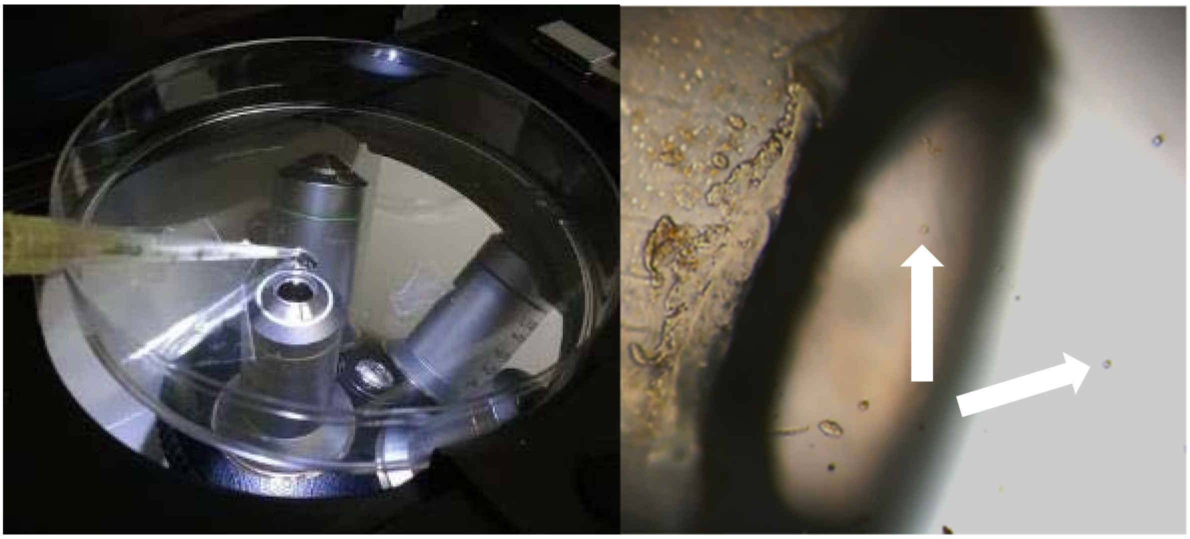

Figure 5. Petri dish set-up (left) with plastic pipette tip secured in water droplet, and raspberry root placed inside tip. The tip is then filled with distilled water, whereby exudates collect. When tip is in contact with water droplet, the zoospores present would then be in contact with the exudate solution. Close-up of pipette tip, and visible zoospores (white arrows).

Root exudate:

A prototype set-up was trialled to collect raspberry root exudates from the susceptible cultivar cv. Glen Moy and resistant cv. Latham. Small 2 ml tubes were attached to the plant pots, at the lip, where a root could be secured inside (**Figure 6**). 1 ml of bottled still spring water was put in each tube, and the root (cleared of debris) dipped inside.

Figure 6. Root exudate collection set-up, cv. Latham (left) and cv. Glen Moy (right). 2 ml micro tubes, with 1 ml bottled still spring water, and root tips dipped in.

Dipped roots were left to exude over 24 hours, after which a sample of the exudate + water solution was used in the set-up mentioned in **Figure 5**. Due to the low number (8-10) of zoospores in the droplet on the Petri dish, observation of mass movement towards the exudate solution was not possible. Further work, with higher concentrations of zoospores would present stronger conclusions.

Pathogenicity testing

With a confirmed isolate of *P. rubi*, tests went ahead to identify its pathogenicity. Pathogenicity tests aim to inoculate healthy plants with the pathogen of concern, and then isolate it back out of the plants. The method used was based on that developed by the Scottish Crops Institute (Graham *et al*., 2011) which involved 12-week old cv. Glen Moy plants being inoculated with four plugs of actively growing *P. rubi* mycelium.

This pathogenicity test observed both stem and root sections for presence of *P. rubi*.

Materials & Methods

Three young plants of cv. Glen Moy were selected, including two young individual plants and one spawn, taken from a larger plant cv. Glen Moy (**Figure 7**). Plants were deemed healthy and free of disease by the sender (The James Hutton Institute, JHI).

Plants were inoculated with eight mycelium plugs of *P. rubi*, four plugs from the outer edge of the *P. rubi* colony, and four taken from the centre. Four holes were made, in a square formation, and two plugs (one from outer, one from inner colony) were placed in each hole (eight plugs total), 10 mm from the stem, 30 mm deep, directly in to the root ball of each plant. Peat growing media was then brushed in to fill the holes above the plugs.

Plants were placed in a growth cabinet at 14°C, under 8:16 hour light: dark light conditions. Plants were watered, twice daily (totalling 35 ml), each time until soil saturation. The plants did not sit in pools of water, as that could have promoted Pythium or the natural rotting of roots that are not aerated.

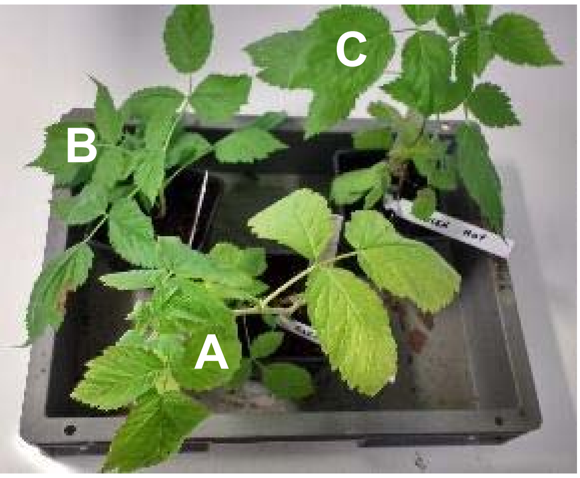

Figure 7. (Top left) three young cv. Glen Moy plants before inoculation. (A) young individual, (B) spawn from larger mother plant and (C) young individual.

Plant A showed early wilting symptoms, so was removed and taken for sampling after 2 weeks. Plants B & C were removed and taken for sampling after 4 weeks.

In communication with Dr David Cooke from the JHI, it was suggested that young susceptible cv. Glen Moy raspberry plants succumb to *P. rubi* within a fortnight, using this method recommended by him.

Root testing:

- Root sections were taken from each plant, cleaned of debris and rinsed in 70% ethanol for 5 seconds. A range of healthy (white) to dying roots (brown) were placed in double filtered soil water, and double filtered sterile (autoclaved) soil water – both have been found to stimulate *Phytophthora* sporangial growth to different extents – in shallow Petri dishes.
- Root isolations, also cleaned of debris and rinsed in 70% ethanol for 5 seconds, were made onto V8 agar and PDA and incubated at 20°C.

Stem testing:

Alongside root isolations, samples of stem were taken, to also look for presence of *P. rubi*. Plant A was removed and sampled after 2 weeks, plant B and C were removed and sampled after 4 weeks.

- Stem isolations, rinsed in 70% ethanol for 5 seconds, were made on to potato dextrose agar (PDA) and V8 juice agar. Four sections of the stem, from the crown up were made. Half of the stem sections had the outer epidermis removed, and half had them remaining. The isolations were incubated at 20^{0}C for 14 days, at which point fungal colonies grew to around 3 cm diameter.
- An LFD was then used, checking the stem sections for presence of Phytophthora.

Results

At the time of removal from the growth cabinet, all three plants were wilting (**Figure 8**), indicative of *Phytophthora* infection. The stem base was brown up to 20 mm on all three plants, and in the roots, browning was seen in every plant to varying extents.

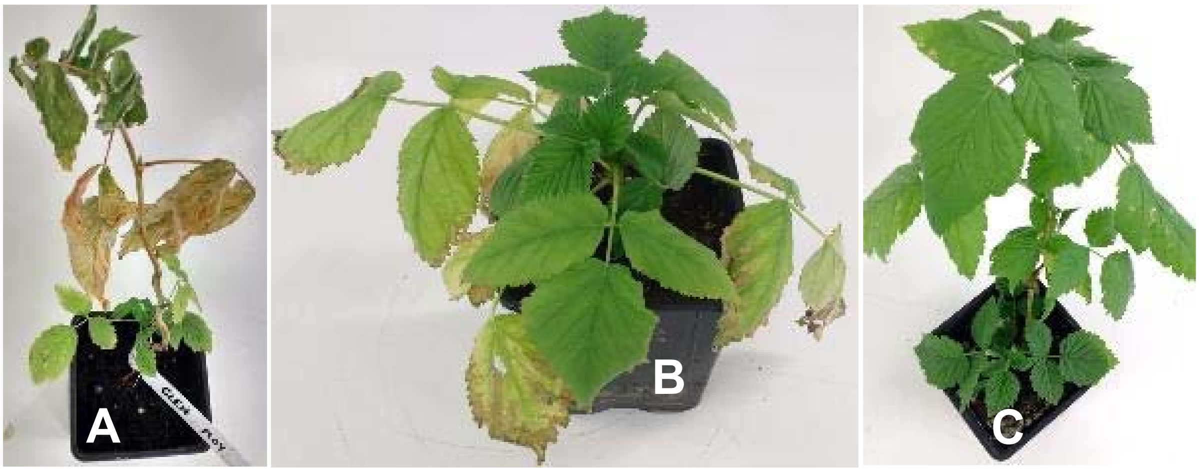

Figure 8. Images of plants on day removed from growth cabinet (left) plant A, (centre) plant B, (right) plant C.

Roots

The roots from plant A, appeared very similar to how they had looked prior to inoculation, no new root growth had taken place, and there was a white: brown percentage ratio of 30:70. New roots from plant B, the very young spawn, were beginning to grow, but the majority (90%) of the root ball was brown. Plant C had a lot of new healthy root growth, growing around the periphery and along the bottom of the pot, but then had a brown centre of the root ball. This area of browning was the site of inoculation.

Root sections: Root isolations were taken from each plant (A, B & C) and placed in soil water. After 2 days at ambient room temperature and natural lighting, zoospores were observed around many of the brown roots from all plants (**Figure 9***).* Two days later, there were far fewer zoospores, and their movement was slower. This is typical of zoospores, as they have finite energy reserves, so will either encyst or die after a few days. This suggests the zoospores observed were newly released.

These zoospores indicate presence of an oomycete, in all three plant root floats. As *P. rubi* was the only oomycete to be placed on the healthy plants, presence of zoospores from the roots, strongly suggest they could only have originated from new sporangia formed in the roots.

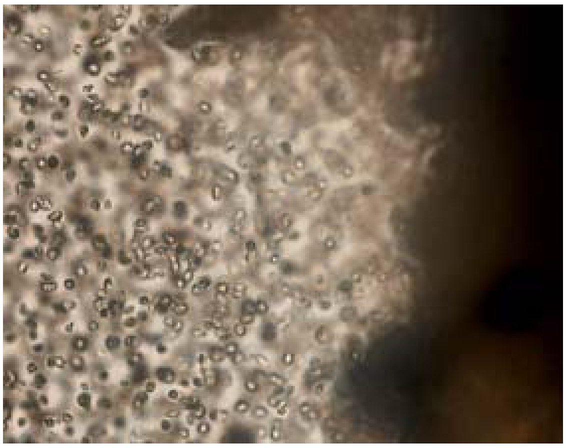

Figure 9. Zoospores (left-side) swarming around the end of a dying raspberry root (right-side brown area).

Root isolations on agar produced some slow-growing, white mycelium, typical of *Phytophthora rubi*, however *Pythium* quickly spread across the plate, so confirmation that it was *P. rubi* was not possible.

Stems

Stem isolations + LFD testing: White mycelial growth, typical of *P. rubi* was observed on 30% of the stem isolation plates for plant B + C, but not plant A. Together, plant B + C stem isolations were selected, and combined with stem samples from plant A to be used in a Phytophthora LFD. The test result was positive (**Figure 10**), and a second LFD test was used to confirm the result.

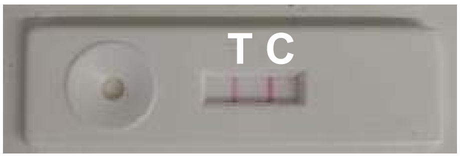

Figure 10. Positive result indicated by red line under 'T' (first) and the control line 'C' (second), in a *Phytophthora* lateral flow device (LFD).

Discussion

The abundance of zoospores seen in every root float, indicate the presence of *Phytophthora*, and most likely *Phytophthora rubi*, as that was the inoculant used on the plant roots.

Two positive LFD test results indicated presence of *Phytophthora* in the stem samples taken from all three cv. Glen Moy raspberry plants. Mycelial plugs with confirmed *Phytophthora rubi* were the sole inoculants to the healthy plants, which suggests that the positive Phytophthora LFD result, was indicative of *P. rubi* infection.

The young cv. Glen Moy raspberry plants sent from the James Hutton Institute were deemed healthy and free of *Phytophthora*. Therefore our results strongly suggest that the selected *P. rubi* isolate is pathogenic and able to infect susceptible raspberry plants.

- Root sections from all inoculated plants showed a strong presence of *Phytophthora* zoospores, but root isolations were inconclusive.
- Stem isolations (from plant B + C) and sections (from plant A) combined, tested positive for *Phytophthora* spp., via LFD testing.
- The imaging and video capacity of ADAS laboratory equipment, alongside a novel observation set-up, enables clear monitoring of zoospore behaviour, for further work on raspberry exudates, chemical fungicides and other solutions.

Objective 1

Aim

WP 1.3 – To explore The effect of cold-storage of long cane raspberries on incidence & severity of *Phytophthora rubi* infection and the potential for protection using biofungicides.

Aim 1: To investigate any effect of cold storage, as opposed to ambient overwinter storage, on long cane raspberry modules and the incidence and severity of root rotting by *Phytophthora rubi*. (Experiments 1 and 2)

Aim 2: To investigation whether a product drench pre or post cold storage can reduce the impact of *P. rubi* infection taking place in spring (Experiments 1 and 2)

Aim 3: To investigate whether a product drench post cold storage can reduce the impact of *P. rubi* infection taking place in late spring (Experiment 2).

Introduction

Around 70% of raspberry material is currently cold-stored at -2°C overwinter between lifting and delivery to the grower. Module plants are grown on outside to produce long cane and can then be cold-stored over winter.

If plants become infested by *Phytophthora* before winter the pathogen will still survive in cold-storage. There is also information from work on strawberry that cold-stored plants may be more susceptible to *Phytophthora* infection post-storage.

Returning of cold-stored infested plants to ambient conditions with recommencement of watering in spring may trigger a mass zoospore release (as produced under laboratory conditions with float dishes) rather than a steady release after ambient storage

Materials and Methods

On 30 July 2017 two adjacent beds of two lines of long-cane plants of cv. Tulameen growing outside at a propagation site in Oxfordshire were selected for evenness of vigour (**Figure 11**). One bed was allocated to each experiment. Each 1.5 L rectangular pot contained two plants of a single cane growing in peat, the pots having legs to allow free drainage onto the woven ground-cover material underneath. Pots were watered through a "leaky hose" run down the bed on top of the pots. Plots were set up with three pots per treatment separated by an untreated discard pot. The ten treatments per experiment (five for cold-storage and five to be kept outside) were randomised into five replicate blocks, a total of 50 plots (300 plants) in each of the two experiments.

Figure 11. Long cane raspberry plants cv. Tulameen on 31 July 2017 showing the selected two rows of plants per bed for Experiment 1 and 2 on adjacent beds. Oxfordshire

A comparison of the schedule of product treatment, overwinter storage and inoculation with *P. rubi* is given in **Table 2**. Products were only applied to Experiment 1 in 2017. Half of the plants from both experiments were taken into overwinter (2017/18) cold-storage, the remainder were left outside (**Table 3**).

Table 2. Comparison of timings of drenching, inoculation with *P. rubi* and storage for Experiments 1 and 2, commencing at a propagators in Oxfordshire in 2017 and moving to a tunnel at ADAS Boxworth in Spring 2018.

Experiment 1 (drenching in 2017 inoculation in 2018)		**Experiment 2** (drenching and inoculation in 2018)	
Drench	Drench	-	-
-	-	-	-
Cold store	Ambient overwinter	Cold store	Ambient overwinter
Pot-up & into tunnel	Pot-up & into tunnel	Pot-up & into tunnel	Pot-up & into tunnel
-	-	Drench	Drench
Inoculate	Inoculate	Inoculate	Inoculate

Table 3. Products and number of applications in either Winter 2017 (Experiment 1) or Spring 2018 (Experiment 2). Inoculation with *P. rubi* in Spring 2018 (except T1) at ADAS Boxworth. Treatments 1-5 with cold storage are shaded in blue.

Experiment 1 (drenching in 2017 inoculation in 2018)		**Experiment 2** (drenching and inoculation in 2018)	
T1 UT no *P. rubi*	Cold Store December 2017 to March 2018	T1 UT no *P. rubi*	Cold Store December 2017 to March 2018
T2 UT		T2 UT	
T3 Prestop x2		T3 Prestop x2	
T4 Serenade x1		T4 Serenade x1	
T5 Paraat x1		T5 Paraat x1	
T6 UT no *P. rubi*	Ambient outdoors December 2017 to March 2018	T6 UT no *P. rubi*	Ambient outdoors December 2017 to March 2018
T7 UT		T7 UT	
T8 Prestop x2		T8 Prestop x2	
T9 Serenade x1		T9 Serenade x1	
T10 Paraat x1		T10 Paraat x1	

Prestop (T3 and T8) is permitted on outdoor cane fruits by Off-label 2773/15. On-label use is permitted for all protected edible and non-edible crops. There is a three week minimum application interval to crops other than strawberry. A total of five applications are permitted per crop (although a maximum of three drenches to outdoor crops). The product information indicates a maximum of 100 L of 0.5% solution per 1000 plants if in they are in 1 L pots. Soaking of the product for 30 minutes is an option and was carried out prior to applications to Experiment 1. Serenade ASO (T4 and T9) drench application is permitted on outdoor raspberry once per year under EAMU 2013/0705 (Drench) in up to1000 L water/ha. On-label advice for Paraat (T5 and T10) on raspberries is to apply immediately after planting in spring/autumn.

Treatment drenches were carried out in Experiment 1 (not Experiment 2), commencing on 28 September 2017 with Prestop only (**Table 4**). On 19 October, Prestop drenching was repeated as permitted, and the single drenches of Serenade ASO and Paraat given (Table 3). Drench applications were made using a gas-assisted Oxford sprayer with a single 02F110 nozzle operating at 2.9 bar pressure, with the nozzle held close to the pots in order to direct the spray over the top of the growing-media in each pot. The 150 ml of spray solution per pot was delivered using a 9 seconds timing.

Table 4. Experiment 1. Treatments applied before either cold-storage (T1-T5, highlighted in blue) or continued standing outdoors (T6-T10) in Oxfordshire, 2017. Treatments other than T1 and T6 are due to be inoculated with *P. rubi* after potting-on in April 2018.

Treatment	Product and [MAPP Number]	Active ingredient	Recommended dose	Product /1.5L pot in 0.15L water (10% by volume)	Application timing/s in 2017-2018
T1	Untreated no *P. rubi*	-	-	-	-
T2	Untreated	-	-	-	-
T3	Prestop [15103]	*Gliocladium catenulatum* strain J1446	5 g/L water (0.5%)	0.75g	28 September 19 October
T4	Serenade ASO [15625]	*Bacillus subtilis* strain QT 713	10 L/ha in 1000 L/ha water (10 ml/L)	1.5 ml	19 October
T5	Paraat [15445]	dimethomorph	1 g per plant	0.75 g	19 October
T6	Untreated no *P. rubi*	-	-	-	-
T7	Untreated	-	-	-	-
T8	Prestop [15103]	*Gliocladium catenulatum* strain J1446	5 g/L water (0.5%)	0.75g	28 September 19 October
T9	Serenade ASO [15625]	*Bacillus subtilis* strain QT 713	10 L/ha in 1000 L/ha water (10ml/L)	1.5 ml	19 October
T10	Paraat [15445]	dimethomorph	1 g/plant	0.75 g	19 October

Plants were examined for any cane disease prior to the product applications, and checks made for any phytotoxicity subsequently. Cane disease and any differences in cane height or width was checked for on the 7 December when the leaf blades had dropped off (**Figure 12**). On the 18 December 2017, as plants were packed into the storage crate, records were made of individual plants with any damage or cane disease. Two untreated pots from the end of the bed discard were removed to the laboratory to examine for any root rot.

Records will be made of poor bud break as a result of any cane diseases after removal to ADAS Boxworth in March 2018. Root assessments will be made on all plants to assess for the occurrence of any root damage over the winter. One pot from each plot (plot = 3x 2-module pots) will have further, destructive root assessments made, by cutting in to the root ball to assess any internal root damage. The remaining four plants per plot will be left to grow to assess vigour, with treatments to be carried out in Experiment 2 prior to the inoculation of *P. rubi* (isolate CC 2106, ex Fera, with confirmed pathogenicity).

Figure 12. Plants in Experiments 1 (near bed) and 2 (next bed) on 7 December 2017 with three pots per plot each with two canes. Most leaf blades had dropped for the winter, often leaving petioles temporarily attached. Oxfordshire.

Figure 13. Plants from Experiment 1 and 2 after packing them in their pots in a storage crate on 18 December 2017. Only occasional apical leaves remained on the plants. Oxfordshire

On 18 December, on a dry day, once the canes had suberized and the leaves and leaf petioles had dropped off the plants, the plants allocated for cold-storage were removed from the beds and, using a standard procedure for the propagator, laid down in their pots in layers in deep wooden slatted crates 2.65 m x 1.22 m x 0.93 m deep (**Figure 13**). Pots were laid on their sides and the next pot rested on the base of the canes of the preceding pot. The canes had been planted towards one long face of the pot and this allowed the next pot to “nest” in space left. Pots were nested to half way up the crate so leaving sufficient head-room for the floricane in the other half of the crate. One side of the crate was allocated to plants from Experiment 1, the other to those of Experiment 2, to be place in cold storage. The four pots per plot were numbered one to four down the beds and kept in sequence in the crate. Pots were kept randomised in the crate in the same order as in the field. Replicates one and two were on the bottom layer, three and four on the middle layer and replicate five formed half a layer on top. Colourless polythene sheet was placed down inside the face of the crate ends and the ends folded in over the top of the final layer of plants to give a loose covering, as standard for the site. The weather was dry and mild during packing and the growing media was moist. The crate was lifted out of the field into the cold store at 14:00 h where it was to be stacked with the grower’s other material at -2 to +2°C.

The plots that had been interspersed within those for cold-storage were left in the field, but gaps left by the removal of plants were closed up by shifting together the plants (plus discard pots) up the beds. The canes were then re-fixed to the supporting wires.

Temperature and humidity loggers were placed in the cold-store crates. The screens at plant pot height in the field were moved to positions within the re-positioned experiments

The plants will remain in cold-storage and outside until early March 2018. The crate and outdoor plants will then be moved to a tunnel at ADAS Boxworth and potted using ericaceous peat growing-media into 5 L pots (one cane per pot). One pot per plot will be taken for root rot assessment, so that there will be four pots per plot in the tunnel. Plants will be arranged in the same order as in the field before December, i.e. with ambient and cold-stored plants randomised within each replicate block. The two experiments will, as before, be kept separate, but adjacent to each other so that they continue to experience similar environmental conditions.

In early and late March 2018 the 5 L pots of Experiment 2 will receive a duplicate product treatment programme as given to Experiment 1 in 2017. It is proposed that Experiment 1 plants are not re-treated, but if this is requested for Experiment 1 then product applications will be made concurrently with those of Experiment 2.

Inoculation (of all treatments, bar the un-inoculated T1 and T6) will be carried out in early April 2018, a month after the plants are potted up and the first treatments made in 2018. Inoculation will be with *P. rubi* isolate CC 2106 using mycelium plugs inserted into the growing-media within the root ball, as used in the pathogenicity testing. This places the pathogen at the precise site of infection. Soil will then be put into the holes made, to cover the plugs. This ensures the *P. rubi* does not dry out, or transfer via splash to other plants. Irrigation by drippers will be set up to keep the water in the pots at field capacity so that a water film is held around the roots by capillary action and any small amount of surplus water is allowed to drain down out of the pots.

Results

No phytotoxicity was visible on the foliage or canes following the treatment applications to the growing-media in September and October 2017. At an assessment on 6 November all pots were given an index score for 9 or vigour (excellent) and none had either phytotoxicity or cane botrytis. Botrytis was observed outside the experimental plots on the leaves of some discard plants at the end of the beds which received wind blast in October. In both Experiment 1 and 2 plants remained of equal good vigour throughout the growing period. Heights ranged at random within and between treatments from 1.5 m to 1.7m by 6 November. By the next assessment on 7 December all the canes had been topped to the required 1.5 m. At this time canes were of uniformly good width of 9 mm. By 18 December, canes had suberized except towards the tops. Botrytis was only seen on a cane in one pot. No other cane diseases (such as cane blight or spur blight) were visible.

The sample untreated discard pots taken for assessment showed a strong root system with roots filling the pot. There was an abundance of white healthy roots, particularly inside the root ball. Around the outer surface of the root-ball some dead roots were present that may have resulted from the temperature extremes in this position. Other brown roots when scraped to remove the surface were confirmed to be healthy inside but with tannin (brown) colouration on the cortex. Results from root floats and isolations are pending.

Conclusions

No adverse or other effects on cane growth resulted from the drenching of either Prestop or Serenade ASO, with no differences in plant vigour between these and either the untreated or Paraat treated plants. Root systems were well developed and mainly healthy in untreated pots.

Objective 2 – Maintaining Integrated Pest Management of two-spotted spider mites whilst controlling spotted wing drosophila

Objective 2.1: To develop and maintain IPM approaches to successfully control two-spotted spider mite whilst controlling SWD and other pests with insecticides.

Aim

To monitor the impacts of a spray programme for SWD on the introduced and naturally occurring predators of two-spotted spider mite (TSSM) and consequently spider mite populations and damage in a commercial raspberry crop.

Introduction

A key current question for growers of soft fruit is how to maintain the successful Integrated Pest Management (IPM) approaches that have been developed over the past 10 years whilst applying insecticides to control spotted wing drosophila (SWD). Two-spotted spider mite (TSSM), *Tetranychus urticae* is a common pest of raspberry crops with severe infestations resulting in complete defoliation (Wood *et al.,* 1994). The pest lays several hundred eggs during its life time which hatch and then go through larval and nymphal stages which can take between 5 to 22 days for completion. A large infestation on raspberry can result in reduced bud survival during the following winter with weaker plants becoming more susceptible to late frosts (Wood, *et al* 1994). This in turn can lead to reduced raspberry yield. The pest has increasingly been identified as a high priority for research by the industry.
Control of TSSM with acaricides requires good spray cover, as most acaricides are contact acting. Effective leaf cover is difficult to achieve in raspberry crops which often have dense canopies. Recent changes in legislation have also meant that there is a limited range of acaricides for use in protected and outdoor raspberries. For example, the new abamectin (Dynamec) EAMU permits its use only on fully protected cane fruit crops, not on those grown in 'Spanish' tunnels or outdoors and the current EAMU for tebufenpyrad (Masai) permits its use on outdoor crops of raspberry and blackberry and has a long (21 day) harvest interval. In addition, abamectin is under threat due to being a potential endocrine disrupter. The difficulties of applying sprays to a raspberry crop and restrictions on the use of insecticides mean that predators of TSSM are essential for effective control of this pest.

Phytoseiid predatory mites are the main natural enemies of TSSM. There are two main naturally occurring, overwintering, species in raspberry (predominantly *Amblyseius andersoni* but *Neoseiulus californicus* is also common). These mites naturally regulate TSSM populations to a greater or lesser extent, but not reliably. In recent years, growers have been successfully introducing *Phytoseiulus persimilis* predatory mites and *Feltiella acarisuga* midges and/or using acaricides for the control of TSSM mite in outdoor/protected raspberry and blackberry crops. However, information on side effects of pesticides on biological control agents and experience in other countries demonstrates that applications of pesticides to control SWD (such as spinosad (Tracer), lambda-cyhalothrin (Hallmark), deltamethrin e.g. Decis) can adversely affect these biological control agents leading to serious outbreaks of TSSM.

Outbreaks of TSSM and other mites, as a result of disruption of biocontrol by naturally occurring and introduced predators by sprays of insecticides for SWD and/or capsid bugs, is an immediate serious threat which the UK cane fruit industry faces. In the first year of this project (2015), ADAS monitored the effects of insecticides applied for control of SWD and capsids on two commercial raspberry crops. The results indicated that naturally-occurring predators are likely to have played an important role in maintaining TSSM control during the spray programmes. The ADAS study in 2017 aimed to build on the work carried out in 2015 to provide more robust information on a commercial crop.

Materials and Methods

Site

The monitoring was done on a commercial tunnel and pot-grown raspberry crop grown in coir and bark substrate (cv. Driscoll Maravilla). *Phytoseiulus persimilis* had been released to the crop by the grower on 29 May 2017 (100,000 in 0.75 ha, equivalent to 13 per m^2). See Appendix 2 for full site details.

Plot layout

Nine replicate plots were selected, three plots in each of the three rows of plants in one polytunnel. The plots were 20 plants long (approximately 7 m) and the mean height of the crop canopy was 1.7 m. The plots were selected to be in areas where there was TSSM damage.

Assessments

An initial visit to was made on 29 June 2017 prior to any fruit ripening and therefore prior to the control programme for SWD control starting. Following the initial preliminary visit, return visits and assessments were made before and after the application of a treatment for control of SWD. On each visit the numbers of TSSM, TSSM eggs, TSSM damage and numbers and species of predatory mites, predatory mite eggs and any other predators of TSSM were recorded. The assessment dates and date of insecticide applications are given in Table 5.

For each assessment 20 trifoliate leaves with symptoms of TSSM damage were selected from each plot, 10 from each of the top and bottom areas of the canopy. Both the upper and lower sides of only the top (terminal) leaflet of the three leaflets on each trifoliate leaf was examined in the laboratory using a low power microscope for the following assessments on both TSSM and predators:

TSSM assessments

The following records were made for each leaflet:

- Numbers of TSSM adults
- Numbers of TSSM juveniles
- Numbers of TSSM eggs
- Percentage leaflet area damaged (i.e. with speckling caused by TSSM feeding)

Predator assessments

The following records were made for each leaflet:

- Numbers of *Phytoseiulus persimilis* adults
- Numbers of *P. persimilis* juveniles
- Numbers of *P. persimilis* eggs
- Numbers and species of naturally-occurring predatory mites (species confirmed after mounting on glass slides and examined using a high power microscope and morphological key)
- Numbers of naturally-occurring predatory mite eggs
- Numbers of any other TSSM predators

SWD assessment

No fruit was assessed prior to the SWD spray as insufficient ripe fruit were available for sampling. Eight days after the SWD spray was applied, 50 ripe fruit were collected from each plot. These were then stored in a ventilated plastic box in a controlled temperature laboratory at 21°C for two weeks. The boxes were checked weekly for the emergence of any SWD adults.

Temperature and relative humidity records

Temperatures and relative humidity were recorded using a USB datalogger in both the top and bottom canopy in the central row of plants.

Statistical analysis

Two-sample t-test (Genstat edition 18.2) was used to compare numbers of TSSM mites (adults and juveniles combined) and eggs and each predator species and eggs (where recorded) on each assessment date with those on the subsequent assessment date. Two-sample t-test was also used to compare numbers of *P. persimilis* mites and eggs with those of *Amblyseius andersoni* on each individual assessment date.

Table 5. Assessment, application and floricane cut back dates

Date	Spray/Visit
29 June	Preliminary visit and leaf assessment
21 July	Pre floricane cut back leaf assessment
27 July	Floricane cut back by grower
2 August (AM)	Pre-spray and post cut back leaf assessment
2 August (PM)	Grower application of tank mix of deltamethrin (Decis at label rate of 500 ml/ha)) for SWD control and thiacloprid (Calypso at EAMU 2139/2014 rate of 250 ml/ha) for blackberry leaf midge control. Hortiboost and Inca were also applied
9 August	Post-spray leaf assessment
17 August	Post-spray assessment on 50 ripe fruit collected from each plot (previous to this date 50 fruit were not available in each plot)
13 October	Final leaf assessment

Results and discussion

Preliminary assessment 29 June 2017

Numbers of both TSSM adults and juveniles, and *P. persimilis* adults and juveniles were recorded using the same approach as that used in the trial at NIAB EMR to ensure consistent records. *Phytoseiulus persimilis* adults will predate TSSM adults, juveniles and eggs whereas *P. persimilis* juveniles only predate eggs and juvenile TSSM. To simplify the analysis, the numbers of adults and juveniles for TSSM, *P. persimilis* and *A. andersoni* were combined. On 29 June the mean number of TSSM (adults and juveniles combined) per leaflet was 39.99, mean numbers of eggs were 215.61 per leaflet and mean leaflet area damage was 35.9% (Table 6). On this preliminary assessment numbers of *P. persimilis* were low with means of 0.14 mites (adults and juveniles combined) and 0.03 eggs per leaflet (Table 6 and Figure 18). Numbers of naturally-occurring predatory mites were significantly higher ($P<0.05$) than those of *P. persimilis*, with mean numbers of 1.36 mites (adults and juveniles combined) and 0.11 eggs per leaflet. High power microscope examination of all the naturally-occurring mites confirmed them to be *Amblyseius andersoni* . The predatory midge *Feltiella acarisuga* pupae (**Error! Reference source not found.**4) and larvae were also found on this date with a mean of 0.93 (pupae and larvae combined) per leaflet. The larvae of *F. acarisuga* are natural predators of TSSM and can also be bought from biological control suppliers for release. Low numbers of *Orius* sp. predatory bugs (mean of 0.03 per leaflet) were recorded: these predate various soft-bodied invertebrates including TSSM. As with *F. acarisuga*, *Orius* spp. can also be bought from commercial suppliers for release in the crop although they were not released at this site.

Figure 144: Adult emerging from predatory midge *Feltiella acarisuga* pupa © ADAS

Second assessment 21 July 2017 (Pre-cut back of floricanes)

This assessment was intended as a pre-SWD spray assessment but after the assessment was completed the grower decided not to apply the spray as originally planned. Mean numbers of TSSM per leaflet were significantly lower than on the previous 29 June assessment, with means of 17.67 mites and 45.99 eggs per leaflet respectively (P <0.05), Table 6 and Figure 18. *Phytoseiulus persimilis* had now established well with means of 3.24

Figure 15: The crop prior to floricane cut-back

mites and 2.22 eggs per leaflet respectively, which were both significantly higher than on the previous assessment date (P<0.05). Mean numbers of naturally-occurring predatory mites (again confirmed as *A. andersoni*) and eggs were similar to those on the previous assessment with means of 1.06 mites and 0.26 eggs per leaflet respectively. On this and subsequent assessments, numbers of *F. acarisuga* larvae and pupae were recorded separately rather than as a combined figure as on the first preliminary assessment. Mean numbers of *F. acarisuga* larvae and pupae per leaflet were 0.44 and 0.71 respectively, with a combined figure of 1.15 per leaflet which was similar to numbers on the previous assessment. Mean numbers of *Orius* sp. per leaflet were 0.08, which was statistically similar to on the previous assessment. In addition the predatory ladybird *Stethorus punctillum* (Figure 16) was also recorded from this assessment onwards, with a mean of 0.53 per leaflet (adults and larvae combined), this was statistically more (P<0.05) than on the previous assessment when no *S. punctillum* were recorded. TSSM mean leaflet damage was recorded at 34.51% which was similar to on the previous assessment. As damage had not increased since the previous assessment this indicated that the TSSM population was probably being regulated by the combination of *P. persimilis* and naturally-occurring predators.

Figure 16: The predatory ladybird *Stethorus punctillum* adult (left) and larva (right) © ADAS

Third assessment 2 August 2017 (Post floricane cut-back, pre- SWD spray)

Following an increase in SWD adults in monitoring traps the grower applied a tank mix of deltamethrin (Decis) for SWD control and thiacloprid (Calypso) for control of blackberry leaf midge on 2 August. The third assessment was done immediately before the SWD spray was applied and 6 days after the floricane was cut back by the grower (Table 1).

On this date, mean numbers of both TSSM mites and eggs had been significantly reduced further (P<0.05) compared with those on the previous assessment, to 0.92 and 2.38 per leaflet respectively, Table 6 and Figure 18. This reduction in numbers of TSSM and eggs is likely to have been due to both predation by the mixture of predators and also to some being removed when the floricane was cut back 6 days previously on 27 July. Mean numbers of *P. persimilis* mites and eggs per leaflet were 1.18 mites and 0.57 eggs per leaflet, both significantly lower than on the previous assessment date (P<0.05). This reduction in numbers is likely to have been due to both reduced numbers of available TSSM prey and also to some being removed when the floricane was cut back. Similarly, mean numbers of naturally-occurring predatory mites (again confirmed as *A. andersoni*) were 0.4 mites and 0.06 eggs per leaflet (both significantly lower than on the previous date, P<0.05). Mean numbers of *F. acarisuga* larvae (0.03 per leaflet) were significantly lower (P<0.05) than on the previous date and mean numbers of pupae (1.13 per leaflet) were statistically similar to those on the previous assessment date. Mean numbers of *Orius* sp. (0.08 per leaflet) were the same as on the previous assessment date and mean numbers of *S. punctillum* (1.07) were significantly higher than on the previous date (P<0.05). Mean percentage leaflet damaged by TSSM was 32.87%, similar to that on both previous assessments. It is important to note that symptoms of TSSM damage remained on the leaves even when numbers of spider mites were reduced. Therefore as leaves with spider mite damage were selected for the assessments, percentage leaf area remaining constant does not indicate that spider mites were not being controlled by the predators.

Figure 17. The crop after floricane cut-back

Fourth assessment 9 August 2017 (post SWD spray)

This assessment was carried out 7 days after the application of the tank mix of Decis and Calypso applied for control of SWD and blackberry leaf midge respectively. On this date, mean numbers of *P. persimilis* mites and eggs were significantly lower (0.2 and 0.01 per leaflet respectively i.e. reductions of 83% and 98% respectively) than on the previous, pre-spray assessment (P<0.05), Table 6 and Figure 18. This reduction in numbers is likely to have been partly due to the adverse effects of the tank mix of deltamethrin (Decis) and thiacloprid (Calypso) applied 7 days earlier. Decis is known to be ‘harmful’ (reduction of over 75%) to both *P. persimilis* mites and eggs and Calypso is known to be ‘moderately harmful’ (reduction of 50-75%) (http://www.biobestgroup.com/en/side-effect-manual, https://www.koppert.com/side-effects/). However, the reduction in numbers of *P. persimilis* may also have been partly due to the very low numbers of TSSM and eggs available as prey by this assessment date (means of 0.09 mites and 0.09 eggs per leaflet which were significantly lower than on the previous, pre-spray assessment date ($P<0.05$). Decis and Calypso do not kill spider mites so this reduction in numbers of TSSM and eggs is likely to have been due to predation by the remaining community of predators rather than to the effect of the spray.

Mean numbers of naturally-occurring predatory mites (again confirmed to be *A. andersoni*) and eggs were also lower than on the previous, pre-spray assessment date (0.18 and 0.02 per leaflet respectively i.e. reductions of 55% and 67% respectively) however these reductions were not statistically significant. Resistance to deltamethrin has been shown in some natural *A. andersoni* populations in French vineyards (Bonafos *et al*. 2007) and it is possible that some natural UK populations may also have developed some resistance. However, a commercial strain of *A. andersoni* was shown in AHDB Horticulture project SF 153 to be susceptible to another pyrethroid insecticide, lambda cyhalothrin (Hallmark), (Fitzgerald, 2017) therefore *A. andersoni* bought from biological control suppliers and released into the crop may not have the same pesticide resistance as natural populations. However, results in SF 153 indicated that a commercial strain of *A. andersoni* had some tolerance or resistance to spinosad (Tracer) and cyantraniliprole (Exirel). Both Tracer and Exirel are used for control of SWD (Tracer has an EAMU for use on both protected and outdoor raspberry and Exirel had an emergency authorisation for use on protected raspberry which expired on 12 January 2018). Unlike *P. persimilis* which is an obligate predator feeding mainly on spider mites and eggs, *A. andersoni* feeds on a range of invertebrate prey and also other food sources including pollen, so is less dependent on spider mite prey for survival and breeding.

Mean numbers of both *F. acarisuga* larvae (0 per leaflet) and pupae (0.63 per leaflet) were significantly lower ($P<0.05$) than on the previous, pre-spray assessment. Both Decis and Calypso are known to be harmful to *F. acarisuga* larvae and adults and as the larvae feed only on spider mites, both the SWD spray and the scarcity of spider mite and egg prey are likely to have contributed to the reductions in numbers of this predator, as with *P. persimilis*.

Mean numbers of *S. punctillum* (0.08 per leaflet) were significantly lower ($P<0.05$) than on the pre-spray assessment. This predator only feeds on spider mites and eggs and the reductions in numbers on this date are likely to have been due to a combination of the potential harmful effects of the SWD spray and to lack of spider mite prey. Mean numbers of *Orius* sp. (0.01 per leaflet) were lower than on the previous, pre-spray assessment but this reduction was not significantly lower. Both Decis and Calypso are known to be harmful to *Orius* sp. which can feed on a range of prey and on pollen.

Mean percentage TSSM damage per leaflet leaf was 32.18% which was still very similar to that in the previous assessments.

Final assessment 13 October 2017

As no more sprays to control SWD were applied to the crop after the spray on 2 August, a final assessment was done to check whether TSSM had built up since the 7-day post spray assessment on 9 August. Mean percentage leaflet area damaged by TSSM had fallen to 15.46% (this significant reduction in damage ($P<0.05$) was likely to have been due to any new leaves developing since the previous assessment showing less damage due to the decrease in numbers of spider mites). Mean numbers of TSSM per leaflet were 0 and 0.19 for mites and eggs respectively. It is likely that in addition to being predated, spider mite adults had entered diapause by this date in response to the decreasing daylength (it is not possible to see spider mites in diapause as they seek sheltered places to spend the winter). Mean numbers of *P. persimilis* were reduced to 0.08 mites and 0.01 eggs per leaflet for mites and eggs respectively (although these reductions were not significantly lower than on the previous date) and this is likely to have been due to scarcity of spider mite prey in addition to the harmful effects of the insecticides applied on 2 August. Mean numbers of all the naturally-occurring predators were also either zero or very low on this final assessment, with numbers of *A. andersoni* being significantly lower ($P<0.05$) than on the previous date.

Table 6. Mean percentage leaflet area with TSSM damage and mean numbers of TSSM, TSSM eggs and predators per leaflet.# Numbers of *Feltiella acarisuga* larvae and pupae were combined on 29 June. * significantly different numbers of *P. persimilis* and *A. andersoni* mites or eggs on each individual assessment date (P<0.05), → statistically similar to the previous assessment, ↓ significantly lower numbers than the previous assessment (P<0.05), ↑ significantly higher numbers than the previous assessment (P<0.05).

	29-Jun	21-Jul	02-Aug	09-Aug	13-Oct
Mean % leaflet area damaged by TSSM	35.9	34.51 →	32.87 →	32.18 →	15.46 ↓
Mean numbers of TSSM (adults plus juveniles)	39.99	17.67 ↓	0.92 ↓	0.09 ↓	0 ↓
Mean numbers of TSSM (Adults)	17.26	11.41	0.72	0.08	0
Mean numbers of TSSM (Juveniles)	22.72	6.26	0.2	0.01	0
Mean numbers of TSSM eggs	215.61	45.99 ↓	2.38 ↓	0.09 ↓	0.19 →
Mean numbers of *P. persimilis* (Adults plus Juveniles)	0.14	3.24* ↑	1.18* ↓	0.2 ↓	0.08 →
Mean numbers of *P. persimilis* (Adults)	0.06	2.22	0.9	0.18	0.03
Mean numbers of *P. persimilis* (Juveniles)	0.08	1.02	0.28	0.02	0.05
Mean numbers of *P. persimilis* eggs	0.03	2.22* ↑	0.57* ↓	0.01 ↓	0.01 →
Mean numbers of *A. andersoni* (adults + juveniles)	1.36*	1.06 →	0.4 ↓	0.18 →	0.017 ↓
Mean numbers of *A. andersoni* (adults)	0.54	0.69	0.36	0.14	0.017
Mean numbers of *A. andersoni* (Juveniles)	0.82	0.37	0.04	0.04	0
Mean numbers of *A. andersoni* eggs	0.11	0.26 →	0.06 ↓	0.02 →	0.02 →
Mean numbers of *F. acarisuga* larvae	0.93#	0.44	0.03 ↓	0 ↓	0.07 ↑
Mean numbers of *F. acarisuga* pupae		0.71	1.13 →	0.63 ↓	0.31 →
Mean numbers of *Orius* sp.	0.03	0.08 →	0.08 →	0.01 →	0 →
Mean numbers of *S. punctillum* (adults plus larvae)	0	0.53 ↑	1.07 ↑	0.08 ↓	0.06 →

Assessment of SWD adults emerging from fruit

No SWD adults emerged from the fruit samples collected 8 days after the SWD spray had been applied.

Temperature and relative humidities

Mean temperature and relative humidities (%) are given in Appendix 2.2.

Conclusions

- High numbers (both in context of the monitoring period and in general) of TSSM and eggs per leaflet recorded on 29 June, when numbers of *Phytoseiulus persimilis* were still low, were significantly reduced by 21 July (P<0.05). This reduction is likely to have been due to predation, not only by *P. persimilis* which had established well by this date but also by four naturally-occurring predator species. The natural predators were the generalist predatory mite *Amblyseius andersoni*, the generalist predatory bug *Orius* sp. and the specialist spider mite predators, the midge *Feltiella acarisuga* and the ladybird *Stethorus punctillum*.

- By 2 August, immediately before the SWD spray was applied, mean numbers of TSSM and eggs per leaflet were significantly reduced still further (P<0.05) and this is likely to have been due to both predation by the predator combination and to some of the floricane being cut back on 27 July. Mean numbers of *P. persimilis* and *A. andersoni* were also significantly lower (P<0.05) on this date than on 21 July and this is likely to have been due to both floricane cut back and to the reduced availability of spider mite prey.
- On 9 August, 7 days after the application of a tank mix of Decis (for SWD control) and Calypso (for blackberry leaf midge control), mean numbers of *P. persimilis* mites and eggs per leaflet were significantly lower (83% and 98% respectively, P<0.05) than on the previous, pre-spray date. This reduction is likely to have been due to both the harmful effects of Decis and Calypso and to the scarcity of TSSM prey. Both TSSM mites and eggs had reached very low numbers by this date which is likely to have been due to predation by the remaining predators.
- On 9 August, mean numbers of *A. andersoni* mites and eggs per leaflet were also lower than on the previous pre-spray date (55% and 67% lower respectively) but this reduction was not statistically significant. This generalist predator seems to be more tolerant of pesticides than *P. persimilis* and it is less dependent on TSSM for food as it will feed on other prey and food sources e.g. pollen.
- On 9 August, mean numbers of *F. acarisuga* and *S. punctillum* were significantly lower (P<0.05) than on the pre-spray assessment and this is likely to have been due to both the effects of the SWD spray and to scarcity of TSSM prey.
- The combination of the spray on 2 August, good site hygiene and the cut back and removal of the floricane seemed to give good control of SWD.
- Both spider mite and predator numbers were very low on the final assessment on 13 October. TSSM damage to leaves did not increase during the monitoring period, but was significantly lower (P<0.05) on the final assessment and this was likely to have been due to any new leaves developing since the previous assessment showing less damage due to the decrease in numbers of spider mites.
- The overall conclusion is that although the SWD spray is likely to have killed many of the released and naturally-occurring predators, due to early good establishment they had controlled the TSSM before the spray was applied and no acaricides were needed.

Future research

Potential strategies for conserving and boosting numbers of TSSM predators before SWD sprays are needed and investigating potential pesticide resistance in naturally-occurring predatory mites would be useful topics for future research.

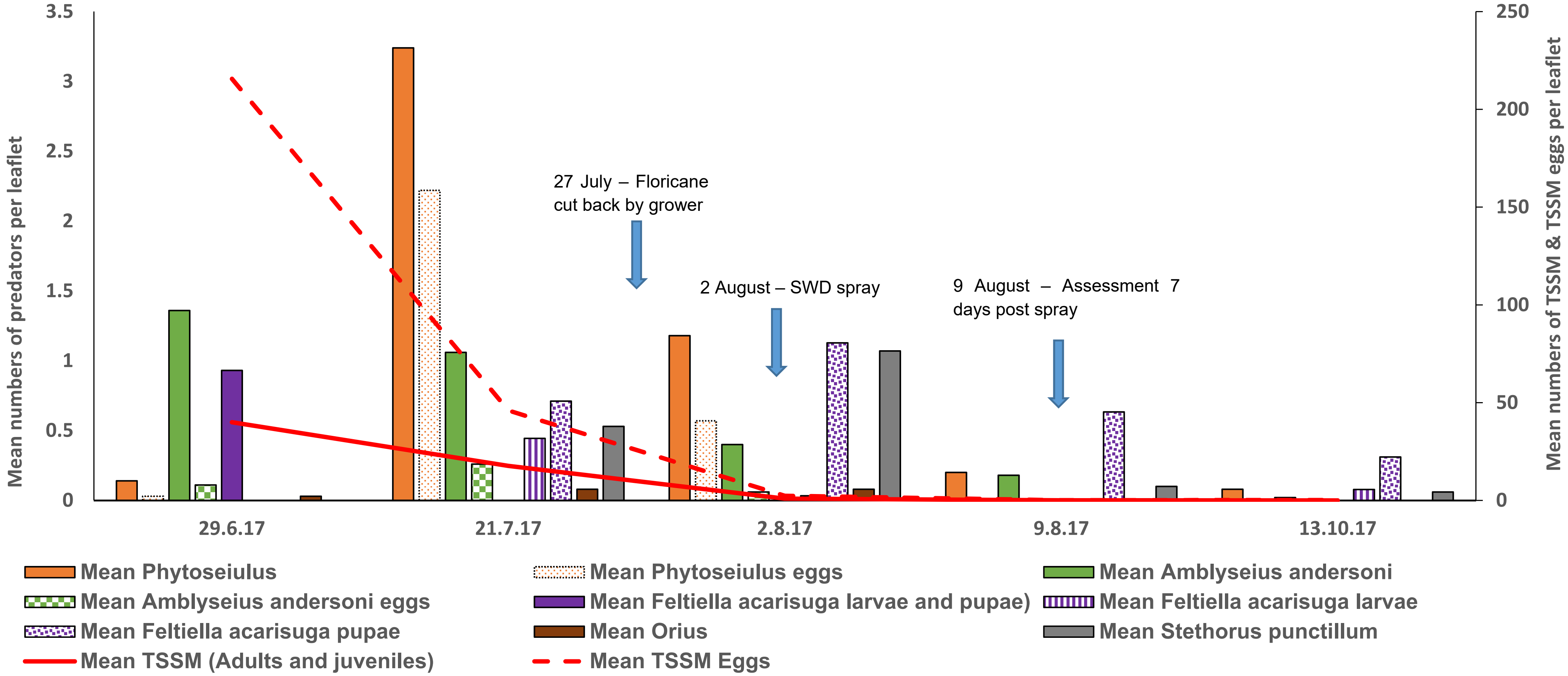

Figure 18. Mean numbers of TSSM predators per leaflet (left hand axis) and mean numbers of TSSM and eggs per leaflet (right hand axis) on each assessment date

Objective 2.2 (NIAB EMR): To develop compatibility strategies for biocontrol of two-spotted spider mites (TSSM) by predatory mites with insecticide sprays for spotted wing drosophila (SWD) and capsids

Aim

To determine the effects of the method of application for a programme of insecticide sprays for the control of SWD on commercially introduced predators (*Phytoseiulus persimilis*) and their ability to regulate TSSM on raspberry.

Task 2.2 To determine the effects of broad canopy spraying versus overhead application of a programme of sprays of deltamethrin and spinosad on SWD and commercially introduced predators (*Phytoseiulus persimilis*) and naturally occurring predators on raspberry and ability to regulate TSSM.

Introduction

Spotted wing drosophila (SWD), *Drosophila suzukii*, has established in the UK and this fruit pest is currently controlled with insecticide spray programmes, coupled with good farm hygiene. Given that much of the control for other pests in raspberry crops, such as the two-spotted spider mite, *Tetranychus urticae*, relies on biological control it is important to develop compatible strategies for biocontrol by predatory mites with insecticide sprays for control of SWD. To maintain control of spider mite within a spray programme for SWD, we are working on the assumption that a spray programme for SWD may negatively affect the biocontrol programme and that leaving unsprayed refuges for commercially introduced and naturally occurring predatory mites may help to maintain the population. Therefore spray application methods which would provide good coverage on the upper leaf surface, but leave the lower leaf surface unsprayed were explored. To determine whether the method of spraying could be important, experiments were done in small purpose-built poly-tunnels to compare the same insecticide spray programme applied by two different spraying methods, pervasive canopy spraying using an air-assisted knapsack sprayer and a system of overhead spraying to give spray deposits mainly on the upper leaf surface.

In 2015, the effects of overall canopy spraying versus overhead misting application of a programme of sprays of deltamethrin (Decis/Bandu), spinosad (Tracer) and chlorpyrifos (Equity) on TSSM and naturally occurring predatory mites were compared using a suite of nine mini tunnels. The effect of date and effect of treatment were significant. In early August, the numbers of natural phytoseiid mites were lower in both of the sprayed treatments, possibly following specific spray applications of spinosad. The numbers of TSSM then rose significantly in the sprayed plots from the 17 August 2015. The numbers of SWD were lower in both of the treated plots.

In 2016, the same system of overhead spraying was used, with different nozzles to give a slightly larger droplet size. This gave less spray on the underside of the leaves in the overhead spray treatment and although the natural phytoseiids were affected by the spray treatments, the effect could be mitigated by spraying from above. TSSM numbers were higher in the sprayed treatments (for all life stages with the knapsack spray). Introduced *P. persimilis* was less affected by the spray programme than anticipated. Both methods of application, boom spraying and knapsack spraying, reduced the number of SWD compared to the control.

The work in 2017 repeated the 2016 experiment to determine if the results could be replicated. In particular to determine the effect of application method on the natural phytoseiids and the effect of the spray programme on the *P. persimilis*. To look at the latter more closely a laboratory spray bioassay using deltamethrin at the recommended field rate was also done.

Materials and Methods

Experimental polytunnels

A dedicated suite of nine mini poly-tunnels (**Figure 19**) (7.3 m wide, 4.5 m long, 3 m tall) in a 3 x 3 array spaced 20 m apart and furnished with drip fertigation were used at NIAB EMR.

Figure 19. The mini-polytunnels at NIAB EMR as used in 2015 -2017

Plants

In each tunnel were 20 raspberry plants, in two rows of ten plants (with one pot every 0.5 m across the width of the tunnel span, rather than down the tunnel length). These were of a proprietary variety, grown from root ball grown in a coir substrate, which yielded approximately 2-3 primocanes per pot (**Figure 20**).

Figure 20. Raspberries in the mini-polytunnel at NIAB EMR in 2017

Experimental booms

Three of the tunnels (one per block) were furnished with overhead hollow cone nozzles (red Albuz ATR 80) spaced 50 cm apart on two 5 m booms (N.P. Seymour, Avon Works, Cranbrook, Kent) (**Figure 21**). There was one boom held approximately 50 cm above each row of raspberry plants. The booms were raised during the experiment, as the plants grew, to maintain the gap between the booms and the upper canopy. The sprays were applied by linking the boom sprayer system to a tractor. The red nozzles were used as in 2016, with the aim of providing coverage of the upper leaf surface, whilst minimising the spray coverage on the lower leaf surface.

Figure 21. Red Albuz ATR 80 nozzles

Treatments

The experiment had three treatments:

1). **Knapsack spray** - A programme of insecticide sprays (**Table 7**) applied to get good cover of the whole canopy using a motorised air-assisted knapsack sprayer, Birchmeier® B 245 with a micron flow restrictor (as used in a rotary atomiser) and with a pressurised tank to maintain a constant flow at a volume of 1000 L/ha

2). **Overhead spray** -The same programme of sprays (**Table 7**) applied by overhead nozzles (red Albuz ATR 80) at 1000 L/ha. The overhead sprays (applied by linking the boom sprayer system to the tractor) were sprayed for 28 seconds at 5.5 bar and 1750 rpm to give 0.053 ml/second for each boom (0.0053 ml/second/nozzle).

3). **Untreated control**

Tables 8 and **9** give further product details and commercial recommendations for use on protected raspberry.

Biological Control

P. persimilis (Koppert UK) was introduced to all plots ahead of spraying on 28 July 2017 (0.75 ml product per plant, approximately 8 to 9 mites per plant, which is just higher than the Koppert curative light recommendation of 6 mites per m^2, but lower than the curative heavy recommendation of 20 to 50 mites per m^2), distributed across three release points per plant. Product quality was checked ahead of release by counting the number of mites in a known volume.

Table 7. Experimental programme of insecticide sprays applied for Treatments 1 and 2 between August and October, Kent 2017

Spray #	**Application date in 2017**	**Active Ingredient**	**Dosage rate product/ L**	**Water volume per plant (ml)**
1	7 Aug	Spinosad	0.2 ml	175
2	17 Aug	Spinosad	0.2 ml	175
3	24 Aug	Deltamethrin	0.5 ml	175
4	31 Aug	Deltamethrin	0.5 ml	175
5	7 Sep	Spinosad	0.2 ml	175
6	14 Sep	Deltamethrin	0.5 ml	175
7	21 Sep	Deltamethrin	0.5 ml	175
8	2 Oct	Exirel*	20 ml	175

*Final spray to extend the control programme whilst assessing SWD numbers, following an EAMU for Exirel.

Table 8. Test products, source, active ingredients and formulation

Active ingredient	Product	Manufacturer	Content of a.i. nominal	Formulation type
Spinosad	Tracer	Dow AgroSciences (marketing company Landseer Ltd)	480 g/L (44.03% w/w)	Suspension concentrate
Deltamethrin	Decis	Bayer	25 g/L (2.8% w/w)	Emulsifiable concentrate
Cyantraniliprole	Exirel 10SE	Du Pont UK Ltd	100 g/L	Suspo-emulsion

Table 9. Commercial recommendations for products for use on protected raspberry

Product	Dose product/ha	Max application	Recommended spray volume	Harvest Interval (days)	Notes
Tracer	200 ml	# sprays - 3 [1]	1000 L water/ha using hydraulic sprayers or hand held equipment	1	EAMU for use against thrips
Decis	500 ml	Total dose – 1500 ml/ha/crop	200-1500 L water/ha as a medium quality spray	7	Full
Exirel 10 SE	900 ml	# sprays - 2 [2]	500 - 1500 L water /ha	3	EAMU No. 2017/1508 Expires 04/12/2017

1 With no more than 2 consecutive sprays, with a 10 day interval between applications, leaving at least 28 days before any further applications of Tracer or any other spinosad containing product and with a maximum of 3 sprays in total

2 With a 10 day interval between applications

In this experimental system, application method, rates, timing of application and number of applications were designed to be similar to the previous experiments to provide reproducible effects between experimental seasons, which may differ from commercial practice i.e. four deltamethrin sprays were used rather than three.

Experimental design

The experiment had three replicates of three treatments in a randomised complete block design. Plots were one tunnel containing the 20 potted raspberry plants in two rows of 10.

Assessments of populations of TSSM, natural phytoseiid predatory mites and P. persimilis

Leaf samples were taken at approximately weekly intervals from late July to September; (20 leaves per plot on 28 Jul, 4, 14, 23 Aug , 40 leaves per plot on 30 Aug, 5, 12, 19, 29 Sep). Twenty leaves were taken per plot across all of the plants, from the main canes (rather than from the later developing lateral shoots), with five leaves from the upper part of the plants (between the 60th to 90th height percentile of the plant) and five leaves from the lower part of the plants (between the 10th to 40th height percentile of the plant), from each of the front and back rows. When the number of TSSM mites decreased, the number of samples were doubled equally across the plants (i.e. the same number from the bottom and the top) (from 30 Aug).

Counts of TSSM eggs, immatures and adults and phytoseiid mite eggs, immatures and adults were made on leaf samples under a binocular microscope. Microscope slides were made of any adult phytoseiid mites that were then identified. Phytoseiid mites were identified to species, i.e. *Phytoseiulus persimilis* and naturally occurring mites such as *Amblyseius andersoni* and *Neoseiulus californicus*.

The experiment was started when numbers of TSSM were confirmed as being suitable for a curative application with *P. persimilis* by an agronomist following a visual inspection, i.e. when motiles were visible on some leaves and signs of damage were apparent. Data were analysed following a square root transformation using snapshot analysis of variance on a date by date basis. This method was used rather than a repeated measures analysis as variance changed over time.

Assessments of Leaf Damage

Each picked leaf was assessed for TSSM damage. The percentage leaf area affected was estimated.

Assessments of Spotted Wing Drosophila

Raspberries were picked as they ripened and were assessed on 4 main occasions: 19, 26 Sep, 02, 09 Oct 2017. They were picked into ventilated Perspex boxes lined with tissue which were held at 20°C and numbers of SWD (and other flies) were counted. Data was analysed using analysis of variance of √N+1 data on each date.

Assessment of Spray Deposition

On completion of the mite assessments, on 12 October 2017, a final spray using a novel tracer was done to assess the spray deposition from the two application methods. These were then analysed using a novel hand-held device. Measurements were taken from three leaves from each of eight areas in each plot, from the back and front rows, and from both the upper and lower leaf side in both the top and bottom canopies.

Following all experimental work on the 19 October 2017, and 17 days after the final pesticide application, the resultant aphid populations on the underside of ten leaves per plot from the top canopy were scored as either none, low (1-10 aphids), medium (11-50 aphids), high (51-100 aphids) or very high (100+ aphids). The data is described.

Husbandry

Plants were drip fertigated using 25 kg/ha/week of either a growing or fruiting product as appropriate (Kristalon Red 12:12:36 Yara UK Ltd)

Temperature and humidity records

EL-USB-2+ data loggers were placed in a Stevenson Screen and were used to record temperature and humidity, placed in the same tunnel (Plot 302). Front doors of the polytunnels were left open during the time period of the experiment to be more representative of temperatures in commercial polytunnels. In addition to the main dataloggers, individual dataloggers were placed in each tunnel in white delta traps to observe the effects of spraying on air temperatures and humidity for selected spray dates.

Pesticide bioassay

A laboratory bioassay was done to observe the effect of deltamethrin on *P. persimilis*. Treatments were sprays of Decis at the recommended field rate applied using a Burkard Table-Top Sprayer. The biological control product was commercially available *P. persimilis* purchased from Koppert UK Ltd. The mites were attached to a 2 cm x 2 cm piece of double-sided sticky tape on a microscope slide; adult female mites were attached on their back, enabling their legs and mouthparts to move (Hoy, 2011). Ten mites were attached per slide, with 10 replicates for each treatment, Decis or control. Slides were held at 20°C for 24 h, and were assessed for mortality under a binocular microscope. The number of mites alive after 24 h were analysed using a GLM with a binomial distribution with a logit link function.

Results

Assessments of populations of TSSM, natural phytoseiid predatory mites and P. persimilis

The effects of spray application method, knapsack, overhead or untreated, on the total √number of the different mite motiles (or in some cases eggs) per leaf are presented below. The distribution within the top and bottom of the raspberry canopy is also presented separately. Combined charts showing the effects of spray application method, both within the top and bottom of the raspberry canopy are shown in **Appendix 3**

Initial mean numbers of TSSM motiles per leaf were low; however there were maximum numbers of up to 29 adults, 102 nymphs and 186 eggs on some leaves. As is typical with this pest there was high variability between leaves. The numbers of TSSM dropped quickly throughout the experiment (**Figure 22**). The introduced *Phytoseiulus persimilis* (**Figure 23**), followed the pattern as for the TSSM with an approximate two-week lag. There was no consistent significant effect of spray treatment application for either of these mite species. There were more TSSM (**Figure 24**) and *P. persimilis* (**Figure 25**) found in the bottom section of the canopy, rather than in the top section of the raspberry canopy.

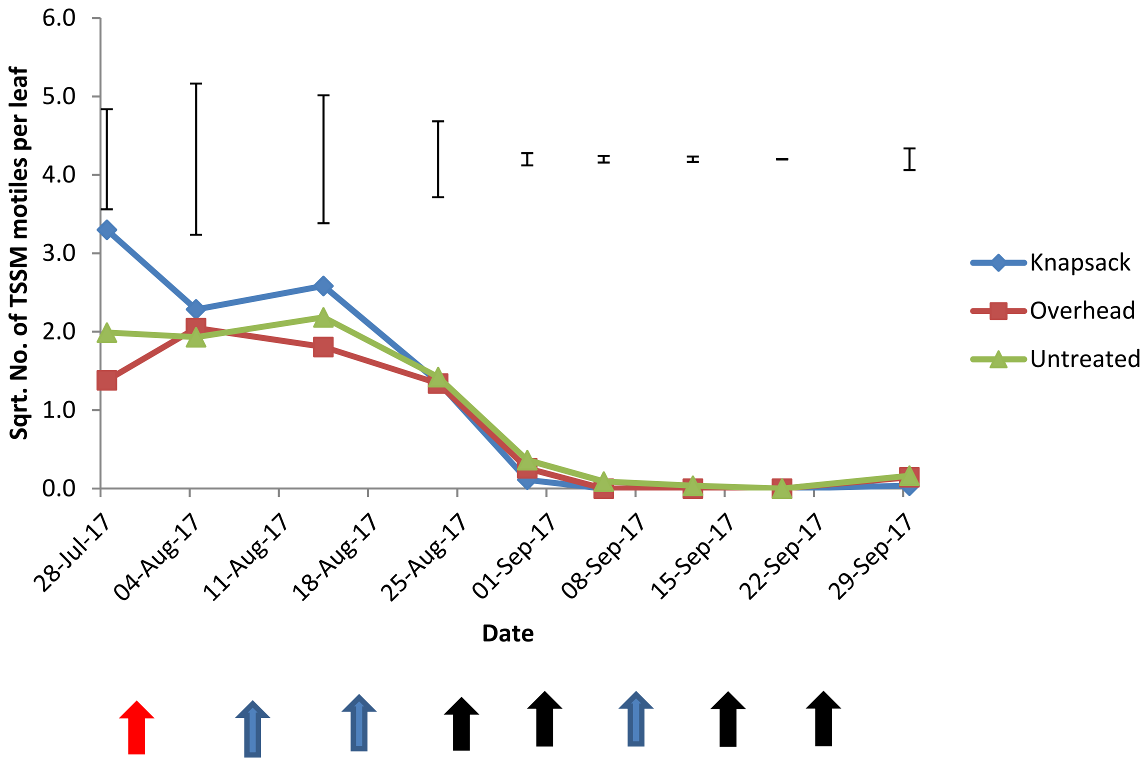

Figure 22. The effect of spray application method, knapsack, overhead or untreated, on the total √number of TSSM motiles per leaf. Error bars showing Treatment LSD. Arrows show introduction of *Phytoseiulus persimilis* (red), spinosad sprays (blue) & deltamethrin sprays (black).

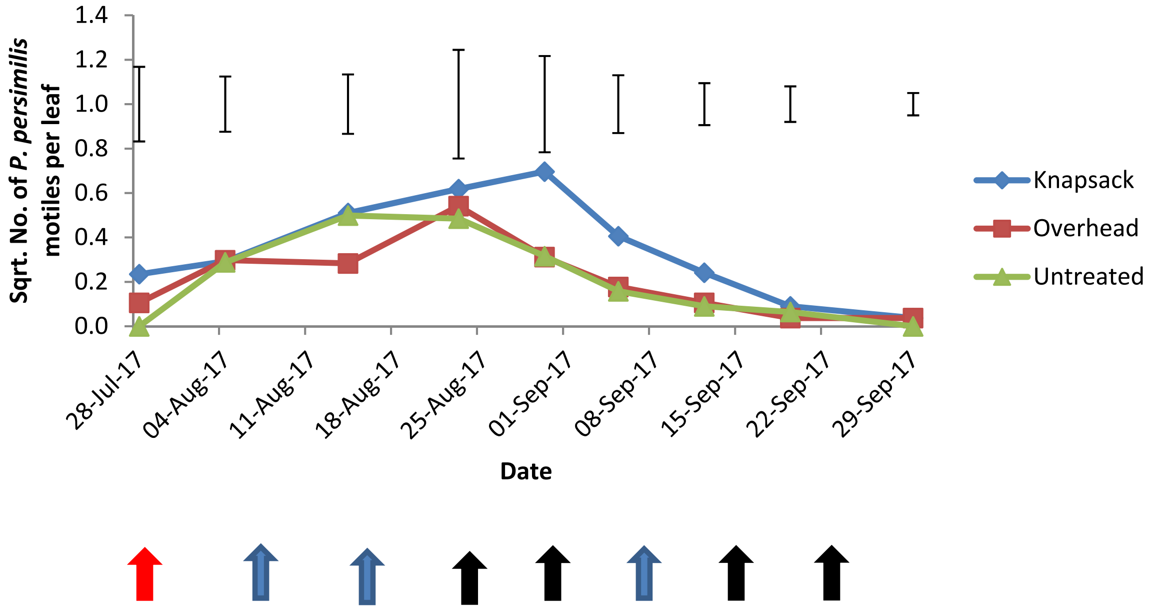

Figure 23. The effect of spray application method, knapsack, overhead or untreated, on the total √number of *Phytoseiulus persimilis* motiles per leaf. Error bars showing Treatment LSD. Arrows show introduction of *Phytoseiulus persimilis* (red), spinosad sprays (blue) & deltamethrin sprays (black).

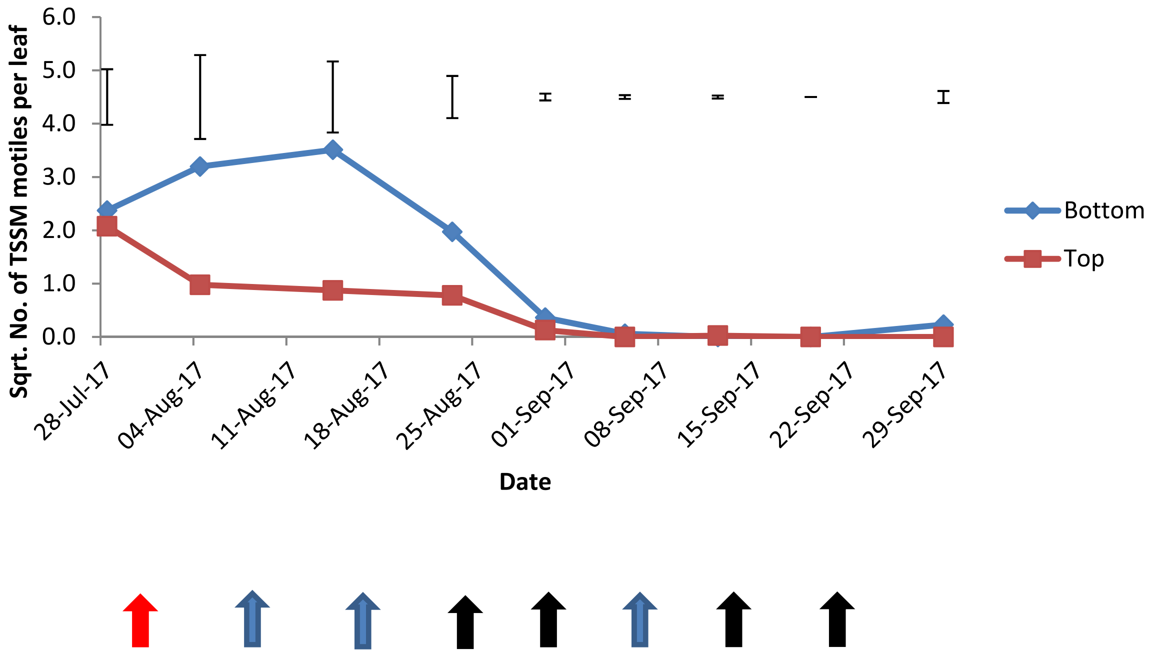

Figure 24. The distribution of TSSM within the raspberry canopy (bottom, or top). Error bars showing Treatment LSD. Arrows show introduction of *Phytoseiulus persimilis* (red), spinosad sprays (blue) & deltamethrin sprays (black).

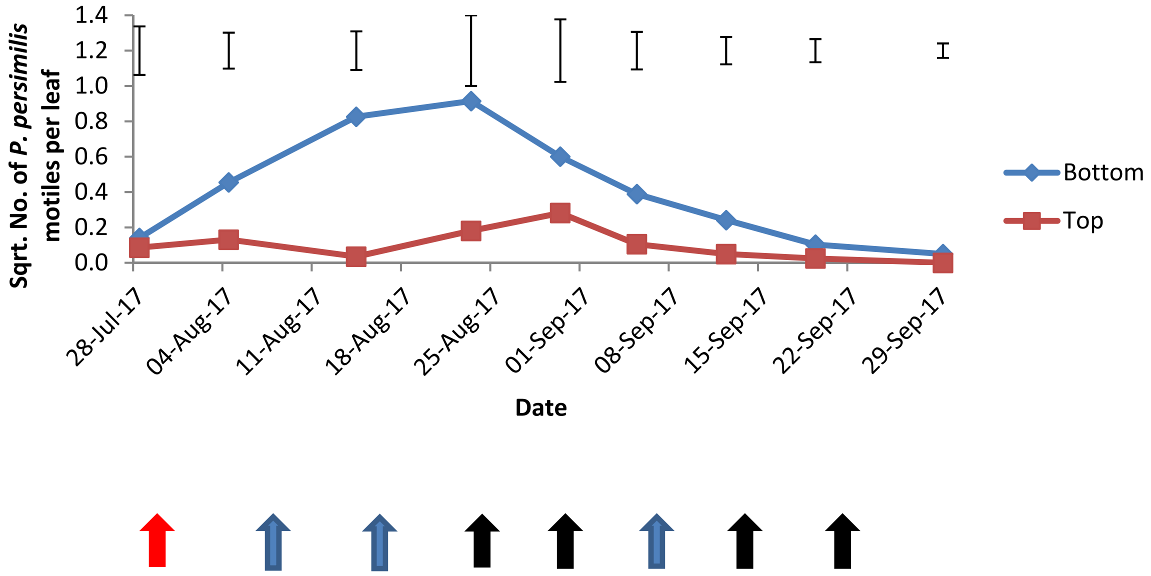

Figure 25. The distribution of *Phytoseiulus persimilis* within the raspberry canopy (bottom, or top). Error bars showing Treatment LSD. Arrows show introduction of *Phytoseiulus persimilis* (red), spinosad sprays (blue) & deltamethrin sprays (black).

As in 2016, the numbers of naturally occurring phytoseiids were higher in the untreated control (**Figure 26**) ($p<0.001$ from 30 August onwards). Although numbers were affected by the spraying this could be mitigated by spraying overhead. The main species present in the crop was *Amblyseius andersoni*, with *Neoseiulus californicus* also found. Similarly the numbers of naturally occurring phytoseiid eggs were low in all plots (**Figure 27**). Although there were treatment significances on 30 Aug, 5, 19 and 29 Sep the pattern was inconsistent. As for the other mite species the naturally occurring phytoseiid motiles and eggs were more prevalent in the lower canopy throughout August (**Figures 28** and **29**).

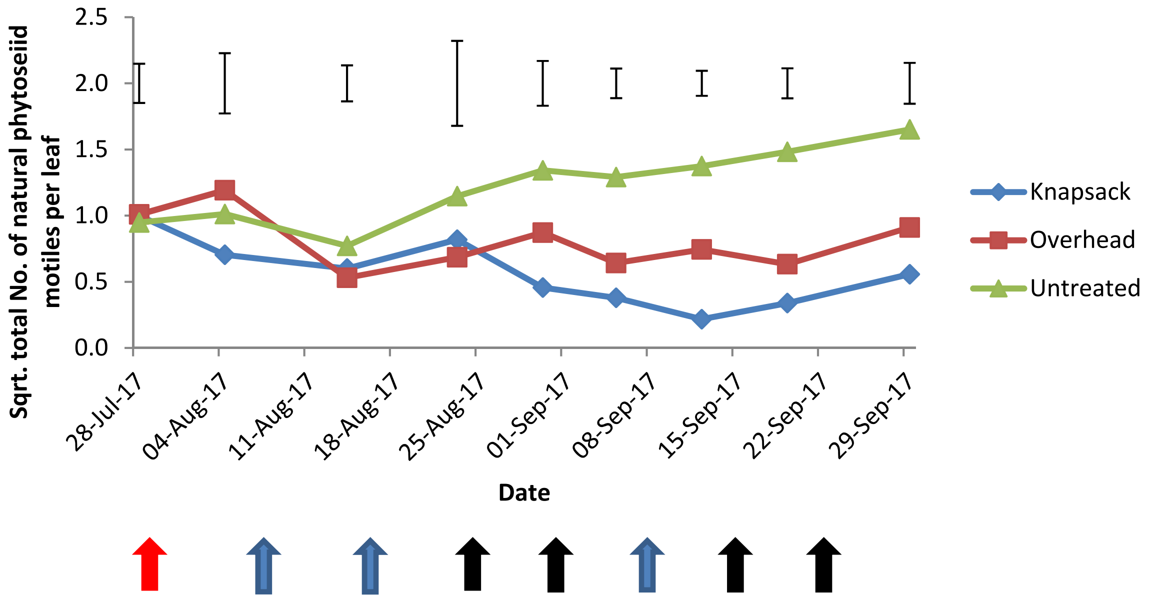

Figure 26. The effect of spray application method, knapsack, overhead or untreated, on the total √number of natural phytoseiid motiles per leaf. Error bars showing Treatment LSD. Arrows show introduction of *Phytoseiulus persimilis* (red), spinosad sprays (blue) & deltamethrin sprays (black).

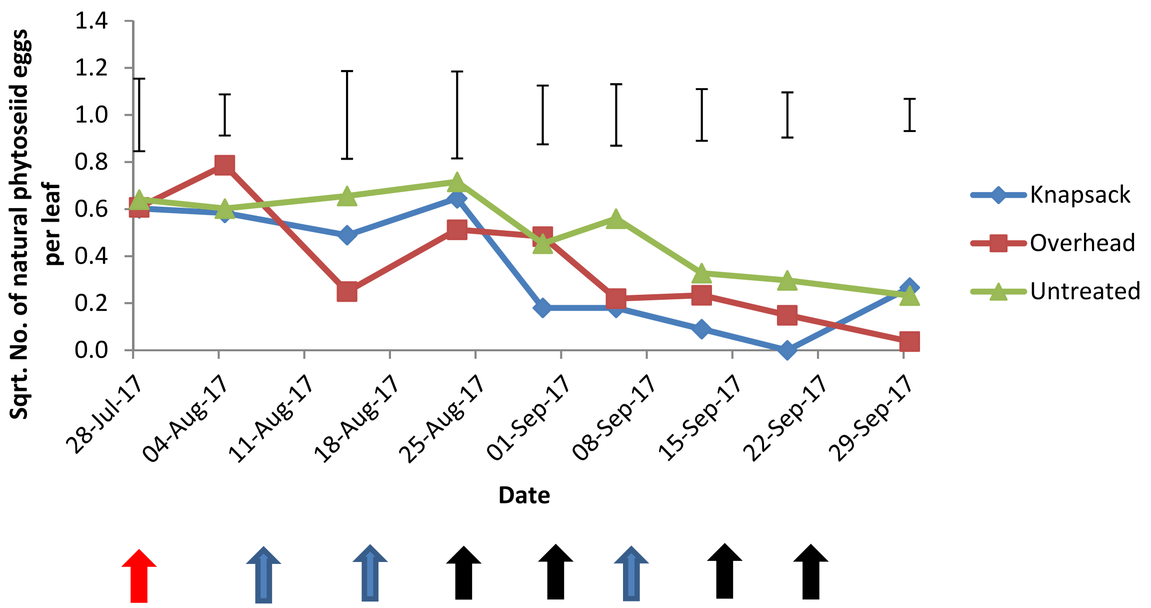

Figure 27. The effect of spray application method, knapsack, overhead or untreated, on the total √number of natural phytoseiid eggs per leaf. Error bars showing Treatment LSD. Arrows show introduction of *Phytoseiulus persimilis* (red), spinosad sprays (blue) & deltamethrin sprays (black).

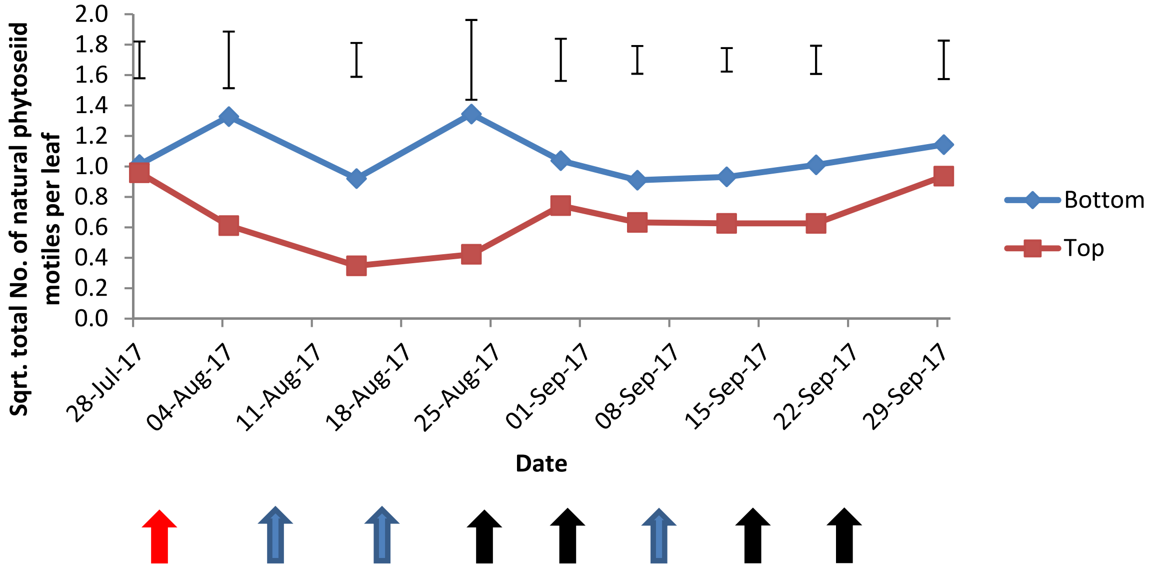

Figure 28. The distribution of natural phytoseiid motiles within the raspberry canopy (bottom, or top). Error bars showing Treatment LSD. Arrows show introduction of *Phytoseiulus persimilis* (red), spinosad sprays (blue) & deltamethrin sprays (black).

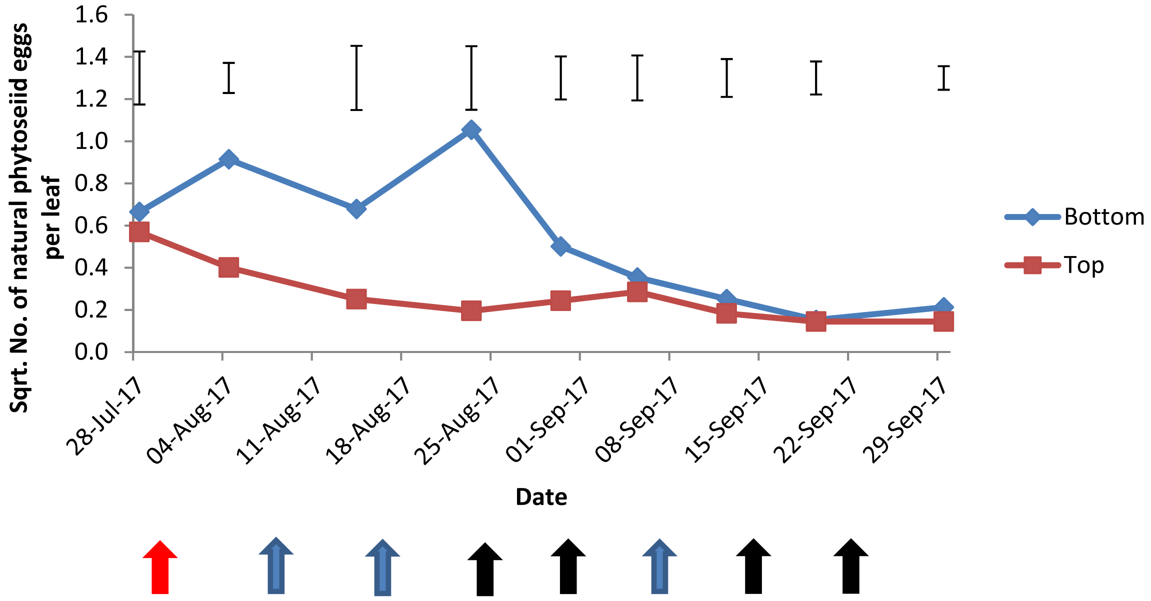

Figure 29. The distribution of naturally occurring phytoseiid eggs within the raspberry canopy (bottom, or top). Error bars showing Treatment LSD. Arrows show introduction of *Phytoseiulus persimilis* (red), spinosad sprays (blue) & deltamethrin sprays (black).

Assessments of Spotted Wing Drosophila

The numbers of fruits picked per plot increased with time, from an average of 20 per plot for picks 1 and 2, 50 per plot for pick 3 and 100 per plot for pick 4. Numbers of SWD were low across all plots, including in the control, with less than one fly per fruit per plot at any pick date (**Figure 30**). In 2016, high numbers of SWD were found in raspberry picks from these tunnels in late September, with a mean number of approximately 30 SWD per fruit; therefore it would be reasonable to assume that we would expect similar numbers of SWD in this experiment. Analysis of variance using transformed data (square-root (n+1)) showed no significant difference between treatments for counts of SWD, however this is possibly due to the very low counts of SWD in 2017. There were also very few *Drosophila* of other species. SWD were found in the nearby SWD monitoring traps in woodland.

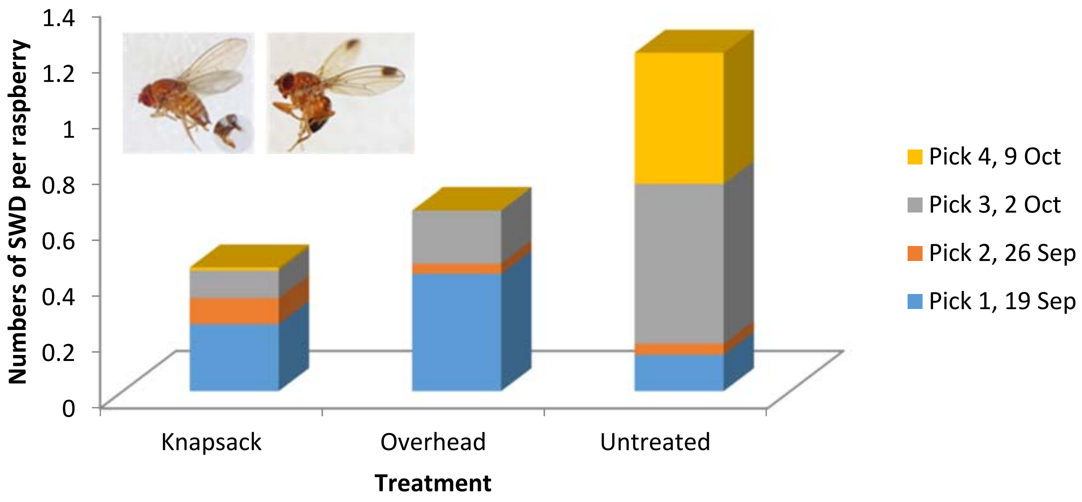

Figure 30. The mean number of SWD per raspberry in 2017.

Assessment of Leaf Damage

Although there was no consistent significant effect of method of application and resultant leaf damage, damage was always higher in the bottom of the raspberry canopy (**Figure 31**), as would be expected from the TSSM distribution data.

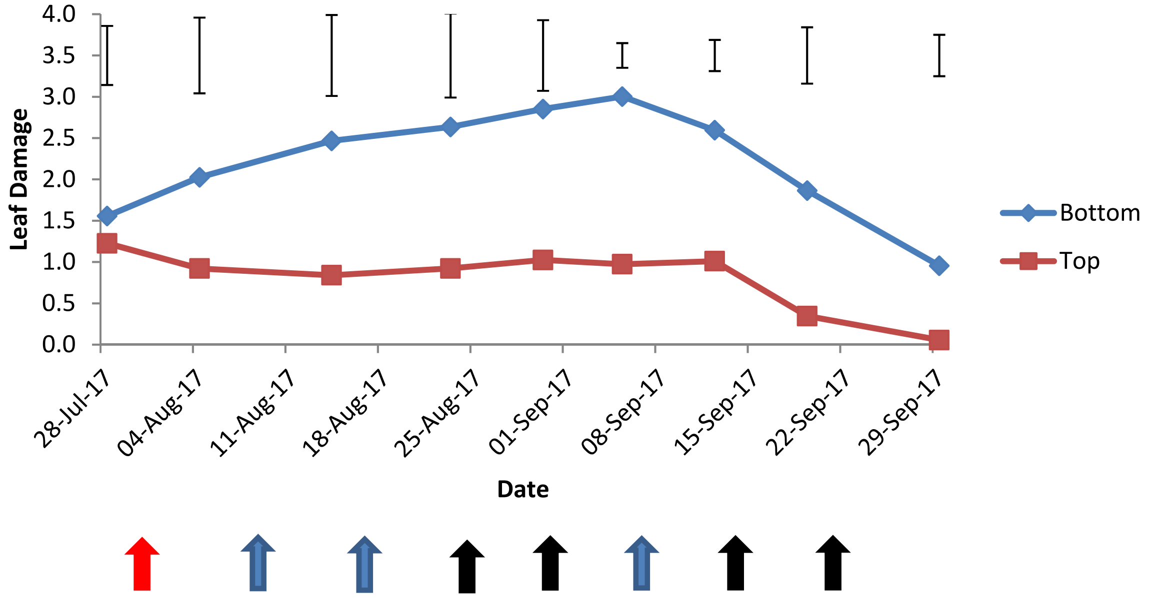

Figure 31. The mean % TSSM damage per leaf at the bottom section and top section of the canopy. Error bars showing Treatment LSD. Arrows show introduction of *Phytoseiulus persimilis* (red), spinosad sprays (blue) & deltamethrin sprays (black).

Assessment of Spray Coverage

Percentage cover

The two spray methods gave very different spray coverage across the plant canopy (**Figure 32**). The overhead boom sprayer gave extremely high coverage on the upper leaf surface particularly at the top of the canopy. The knapsack sprayer provided much greater spray coverage on the lower leaf surface and a generally more even distribution with the exception of the upper leaf surface at the top of the canopy.

Fluorescence / mm^2

Fluorescence is a proxy for quantity of spray. The pattern of the results was similar to that seen for percentage cover, but more pronounced (**Figure 33**). Generally there was much higher fluorescence on the lower leaf side in all canopy sections for the knapsack sprayer compared to the overhead boom sprayer, with the exception of the top section of the canopy.

The overhead boom sprayer gave greater fluorescence on the upper leaf surface at the top of the canopy compared to the knapsack sprayer, but this was still lower than the highest amounts seen on the lower leaf side with knapsack. This was probably because the overhead boom sprayer is spraying to 'run-off'. Once the leaves become saturated, the spray droplets coalesce and run off the leaves, which can greatly reduce the quantity of spray on the leaf.

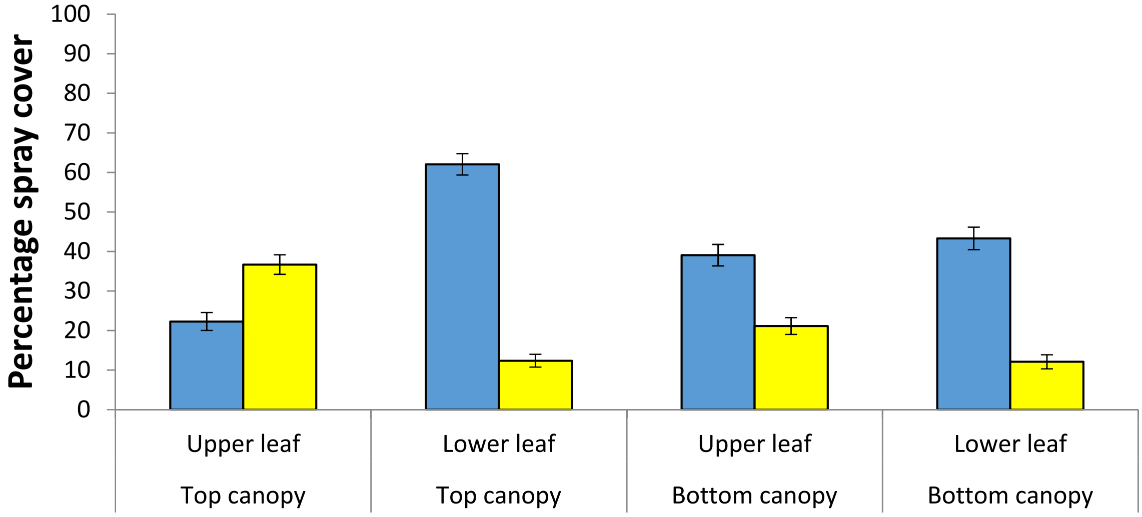

Figure 32. The percentage spray cover obtained in different sections of the canopy and upper or lower leaf sides for either knapsack (blue) or overhead boom (yellow) spraying. Error bars show standard error of the mean.

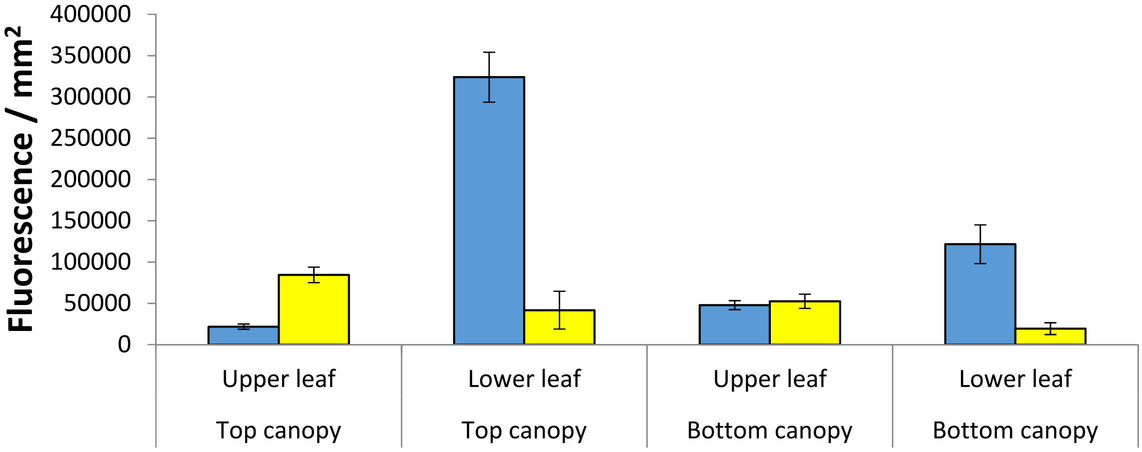

Figure 33. The fluorescence (mm^2) obtained in different sections of the canopy and upper or lower leaf sides for either knapsack (blue) or overhead boom (yellow) spraying. Error bars show standard error of the mean.

Variability

The values of coefficient of variation (CV%) (data not shown) indicate that there is more variation in the spray deposition from the overhead boom spray compared to the knapsack sprayer for both spray coverage and fluorescence / mm^2. This suggests that the overhead boom sprayer gives uneven spraying compared to the knapsack sprayer.

Aphid assessment

No aphids were found on the leaves from the untreated control or knapsack sprayed plots (apart from on one leaf which scored as 'low'). Only three of the 30 leaves from the overhead spray treated plots scored as having no aphids, with the majority of leaves having low or medium scores. This could be remedied by introducing biological control agents and specifically targeting the aphids throughout the season.

Temperature and humidity records

Temperature records can be seen in **Figure 34**. Daytime temperatures were above 35°C on occasion, hence the need to maintain an air flow to prevent scorching of the leaves; humidity was high at night and dropped during the day. Humidity was similar to 2016, increasing at night and decreasing during the day.

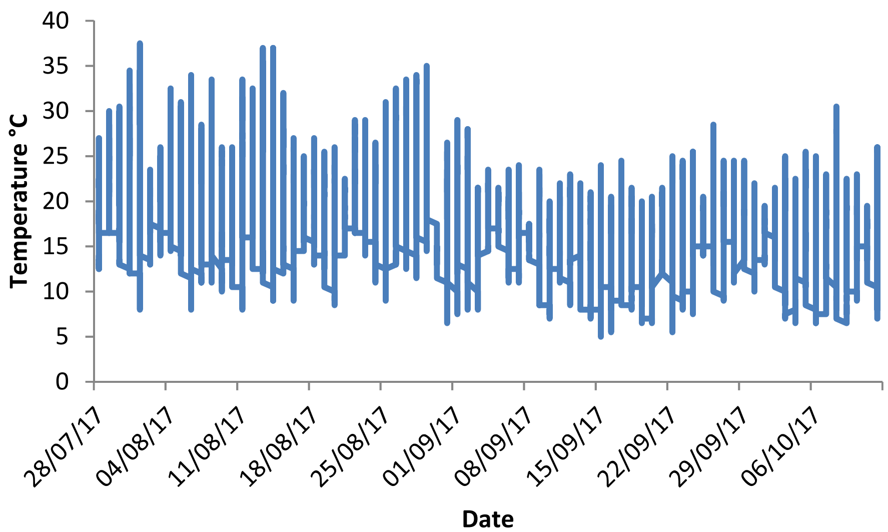

Figure 34. Air temperatures in the mini-polytunnels at NIAB EMR in 2017

Pesticide bioassay

The mortality of *P. persimilis* was significantly higher in the deltamethrin sprayed treatment ($p<.001$). The means were: Control - %Alive = 86% (+/- 4.9%), Decis - %Alive = 4% (+/-

2.8%). Therefore it does not appear that the product from Koppert UK Ltd. is a resistant strain of *P. persimilis*.

Discussion

The effects of overall canopy spraying versus overhead application of a programme of sprays of deltamethrin and spinosad on TSSM and predatory mites, both natural and introduced were assessed; although it was not possible to determine any treatment effects for the TSSM and *P. persimilis* due to the low numbers per leaf, there were treatment effects for the naturally occurring phytoseiids. The sprays affected the natural phytoseiids, however this effect could be mitigated by spraying from above. The assessment of spray deposition showed that there was less spray on the underside of the leaves in the overhead spray treatment, which could provide a refuge for predatory mites. The data also showed that the amount of spray on the underside of leaves was highly variable. Reducing the amount of spray on the underside of leaves may have a knock on effect, reducing control of other pests such as aphids, as found following this experiment. These could be controlled with a biological control agent, such as parasitoid wasps, however this is not a routine treatment in most raspberry crops.

As there were few *P. persimilis* motiles it was not possible to determine the effect of the deltamethrin sprays in the field; the side effects of deltamethrin on the Koppert website quote an effect of '4' i.e. very harmful, with a 75% reduction for the adults, nymphs and eggs, with a 8-12 w persistence, and a moderately harmful effect of spinosad with a 25 – 50% reduction. The bioassay work showed that with direct application of deltamethrin in the laboratory almost all adults were killed within 24 hours, therefore it is not believed that the commercially available strain of *P. persimilis* is resistant. It was also noted that there were more aphids present in the overhead spray treatment by the end of the experiment. This could be controlled by parasitoid introductions during the season as part of an integrated pest management programme.

The numbers of SWD were low in 2017, therefore no significant treatment effects could be determined.

Future work

In 2018 the work from Objective 2, as a whole, will be taken forward in a commercial setting. It may be possible to modify existing commercial spray equipment to achieve a similar effect, by turning off the air-assistance and altering the angles of the nozzles, which can be investigated.

Conclusions

- The natural phytoseiids were affected by the spray treatments, but the effect could be mitigated by spraying from above

- There was less spray on the underside of the leaves in the overhead spray treatment, but with increased variability
- SWD numbers were low in all treatments in 2017

Acknowledgements

We would like to thank Graham Caspell and team for maintenance of the tunnels and irrigation systems. Thanks are also due to Christina Faulder, Celine Silva, Adrian Harris, Gloria Endredi and Charles Whitfield for assistance with the experiments.

Knowledge and Technology Transfer

Jude Bennison and Chantelle Jay presented the results at the AHDB soft fruit day at NIAB EMR on 21 November 2017.

Chantelle Jay showcased the project at the AHDB soft fruit agronomists day on 13 September 2017.

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Appendices

Appendix 1.

WP 1.1: To investigate the effects of a range of novel plant treatments on raspberry growth and their resilience to pests and disease from propagation through to primocane production.

Pesticide applications made by the grower to raspberries cv. Tulameen in the trial during 2017 and reason for application

Date applied	Product	Active ingredient	Rate used / ha	Target pest or disease
11 April 2017	Hallmark with Zeon Technology (EAMU)	Lambda cyhalothrin	100 ml	Aphids
7 May 2017	Decis	deltamethrin	500 ml	Raspberry cane midge (spray directed at bases of primocane)
25 May 2017	Calypso + Signum	Thiacloprid (EAMU) + Boscalid + pyraclostrobin (EAMU)	250 ml + 1.5 kg	Aphids, raspberry beetle Fruit botrytis
16 June 2017	Amistar	Azoxystrobin (EAMU)	1 L	Fruit & cane botrytis, powdery mildew fruit & foliage
22 June 2017	Majestik	maltodextrin	25 ml/L of water	Aphids & TSSM
7 August 2017	Deacon	Tebuconazole (EAMU)	1L	Cane & foliar disease including cane blight & rust
22 August 2017	Signum	Boscalid + pyraclostrobin (EAMU)	1.5kg	Cane & foliar disease including cane blight & rust

Appendix 2

To develop and maintain IPM approaches to successfully control two-spotted spider mite whilst controlling SWD and other pests with insecticides.

Site details

Location: East Anglia

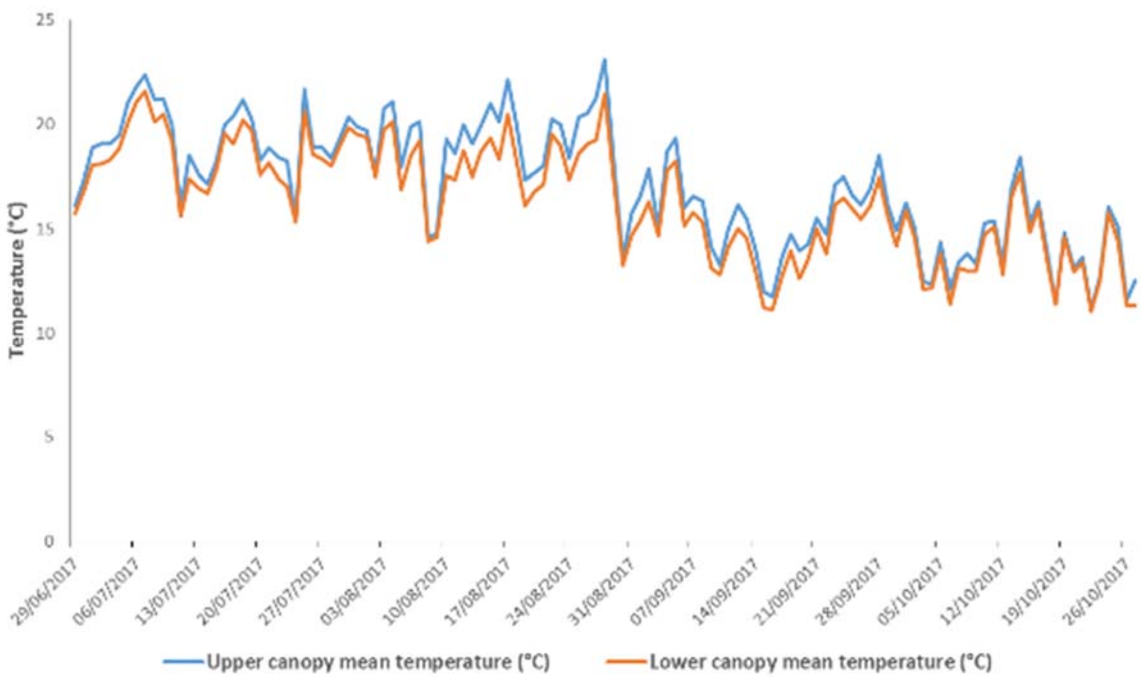
Cultivar: Driscoll Maravilla in polytunnels

Substrate: Coir and bark substrate in pots

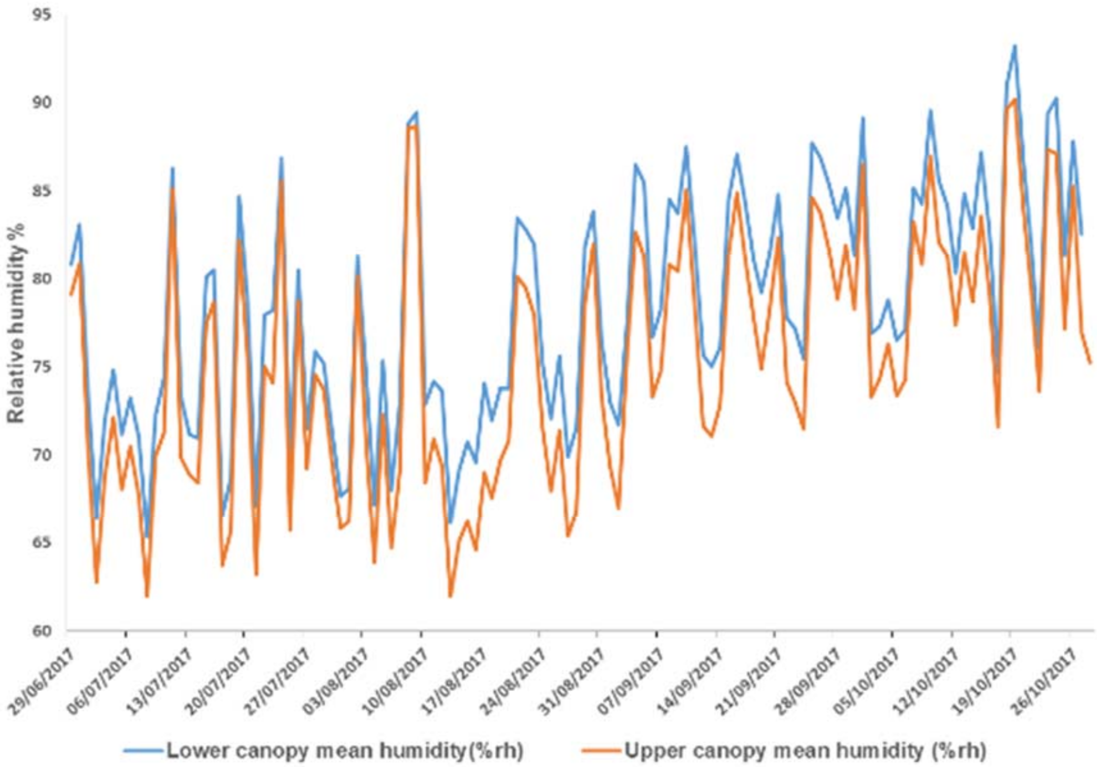
Pesticides applied: deltamethrin (Decis at label rate of 500 ml/ha) for SWD control tank mixed with thiacloprid (Calypso at EAMU 2139/2014 rate of 250 ml/ha) for blackberry leaf midge control on 2 August. No other pesticides applied.

Biological control agents applied: *Phytoseiulus persimilis* released to the crop by the grower on 29 May 2017 (100,000 in 0.75ha, equivalent to 13 per m^2).

Irrigation: Drip irrigation



Mean daily temperatures (°C) in the upper and lower canopy of the raspberry crop used for monitoring the effect of insecticides applied for control of SWD

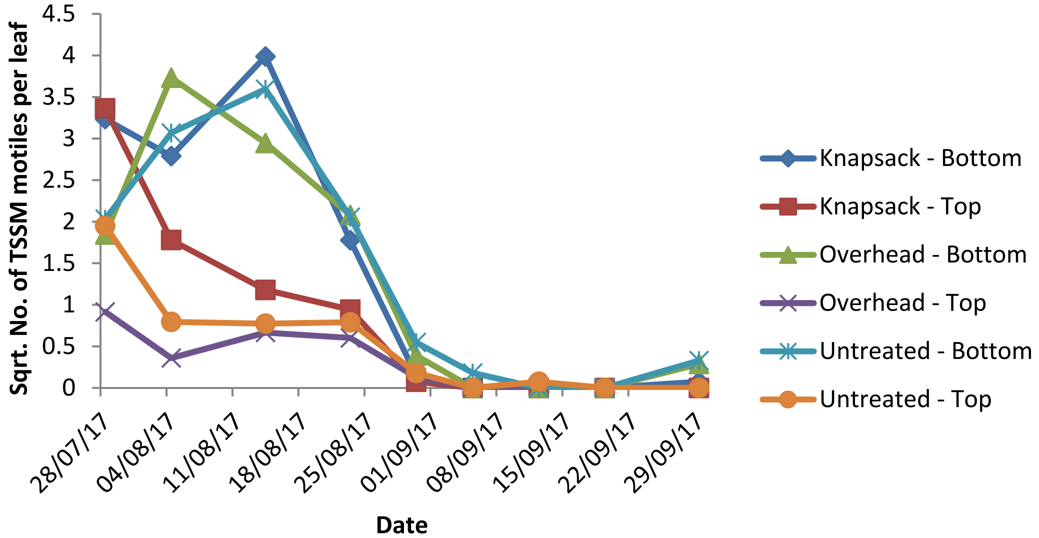


Mean daily relative humidities (%) in the upper and lower canopy of the raspberry crop used for monitoring the effect of insecticides applied for control of SWD

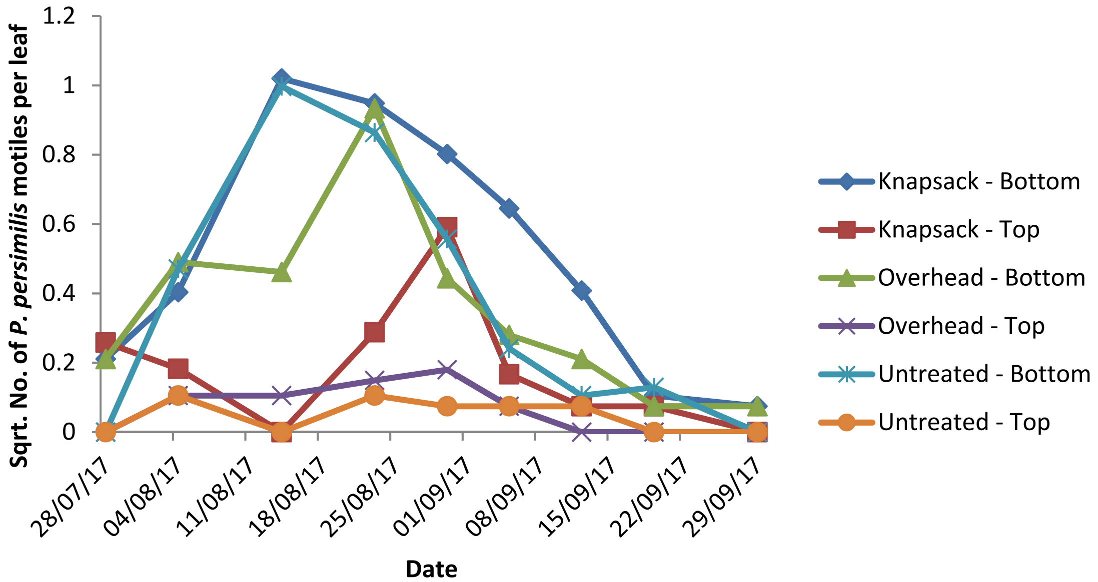
Appendix 3.

Objective 2.2: To develop compatibility strategies for biocontrol of two-spotted spider mites (TSSM) by predatory mites with insecticide sprays for spotted wing drosophila (SWD) and capsids

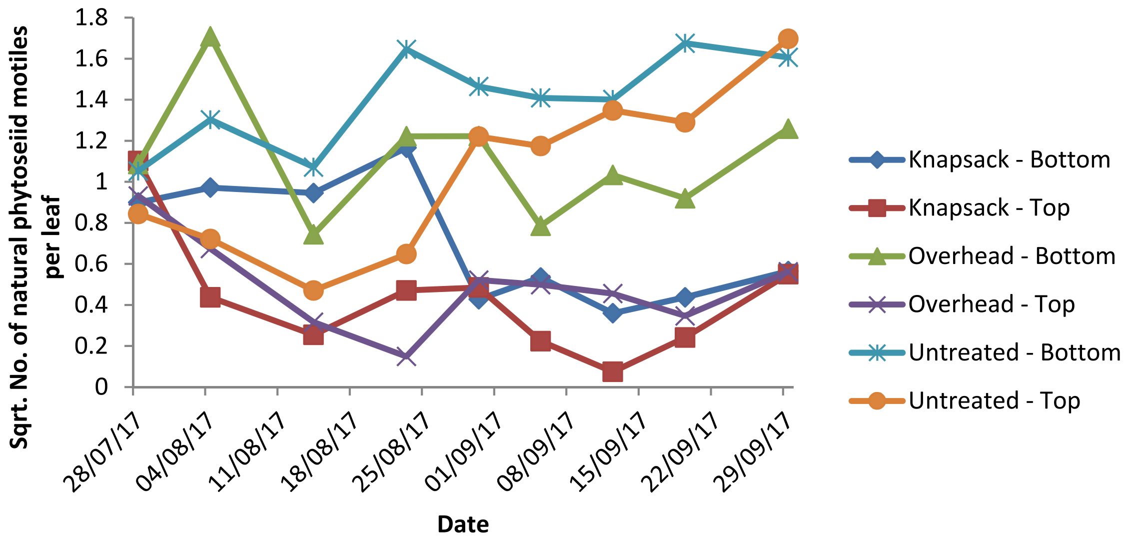
Further charts to show the effect of spray application method, knapsack, overhead or untreated, also highlighting the distribution within the raspberry canopy (bottom or top).



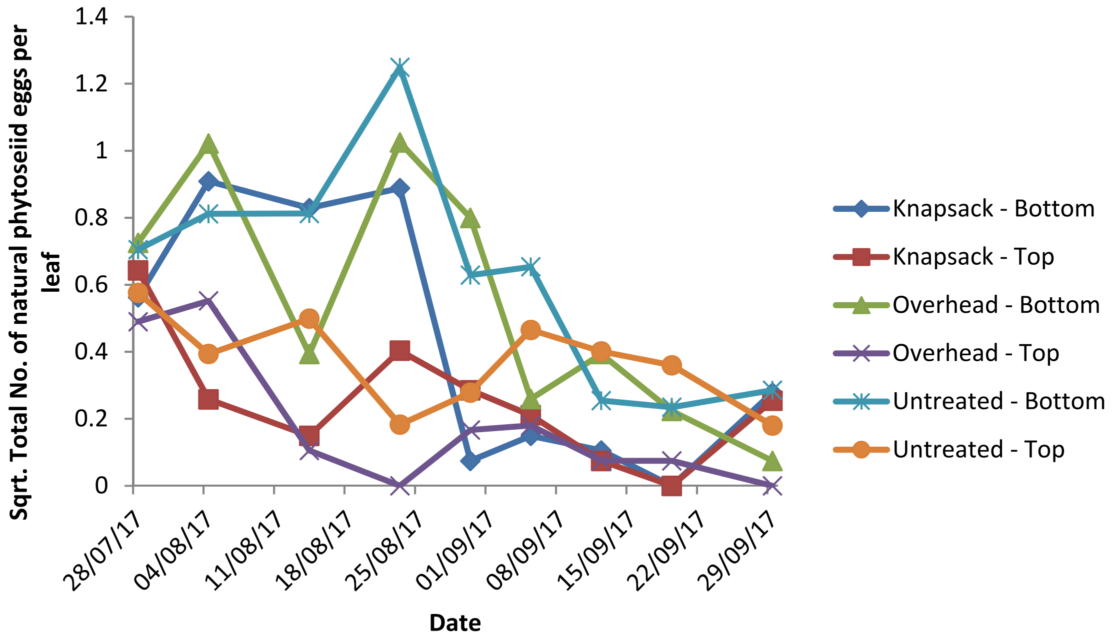
a) The effect of spray application method, knapsack, overhead or untreated, on the total √number of two-spotted spider mite motiles per leaf broken down to show distribution within the raspberry canopy (bottom or top).



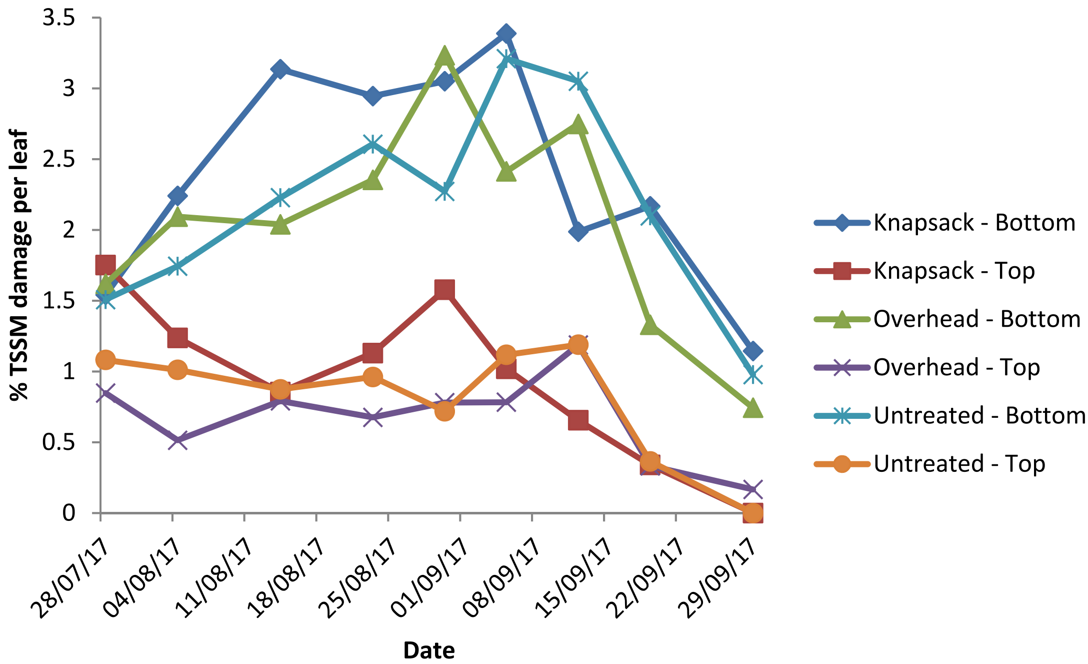
b) The effect of spray application method, knapsack, overhead or untreated, on the total √number of *Phytoseiulus persimilis* motiles per leaf broken down to show distribution within the raspberry canopy (bottom or top).



c) The effect of spray application method, knapsack, overhead or untreated, on the total √number of natural phytoseiid motiles per leaf broken down to show distribution within the raspberry canopy (bottom or top).



d) The effect of spray application method, knapsack, overhead or untreated, on the total √number of natural phytoseiid eggs per leaf broken down to show distribution within the raspberry canopy (bottom or top).



e) The effect of spray application method, knapsack, overhead or untreated, on the % damage per leaf broken down to show damage distribution within the raspberry canopy (bottom or top).