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Project number:	SF 158
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Date project commenced:	1 March 2015
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

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

Dr Erika F. Wedgwood

Plant Pathologist, SF 158 Project Leader

RSK ADAS Horticulture, RSK ADAS Ltd

Signature  Date: 29 March 2019

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Director

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Signature  Date: 29 March 2019

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GROWER SUMMARY

Raspberry root rot

Objective 1 - Investigating the effect of cold storage and biopesticides on Phytophthora root rot in long cane raspberry.

Headline

- Cold storage of substrate raised raspberry canes can increase Phytophthora root rot incidence.

Background and expected deliverables

Phytophthora root rot, principally attributed to *P. rubi* (previously known as *P. fragariae* var. *rubi*) is now the most destructive disease of raspberries worldwide. Outbreaks of this disease across Europe at the same time in traditional raspberry-growing areas suggests that the disease may have spread through the propagation network and has been distributed to farms in new planting material. It therefore arises in both soil (where it forms long-surviving resting spores) and substrate grown crops. Current approaches for Phytophthora control rely on a single fungicide application per year either as a soil-applied drench or through the drip irrigation. A soil drench of Paraat (500 g/kg dimethomorph) is currently used, but resistance developing in pathogens where products have only a single mode of action is a major concern. Biofungicides such as Prestop (*Gliocladium catenulatum* strain J1446) and Serenade ASO (*Bacillus subtilis* strain QT 713) have action against oomycetes such as a *Phytophthora* spp. and certain fungi.

In the UK, cold storage of long cane raspberry propagation material is becoming common practice to guarantee sufficient chilling over winter, with the removal from store timed specifically to allow the programming of fruit harvest. Such plants may be discarded by growers after fruiting, thereby avoiding the carry-over of any pests and diseases into the next cropping year. In strawberry, cold storage of propagation material has been shown to increase susceptibility to *Phytophthora cactorum*, but it is uncertain if increased susceptibility also arises in raspberry long cane with *P. rubi*. This project aims to examine any effect of cold storage on Phytophthora root rot susceptibility in raspberry, and any benefit from biofungicide drench application before or after overwintering.

Summary of the project and main conclusions

Two experiments were set up to investigate whether storing long cane raspberry (cv. Tulameen) over winter affects root rotting and cane infection by *P. rubi* following Spring inoculation. It was also hoped to determine whether there is any benefit from applying protectant fungicide drenches of Prestop, Serenade ASO or Paraat. One experiment was treated in Autumn while the second received drenches in April, prior to inoculation with *P. rubi* a month after potting on. This inoculation timing was chosen to simulate a natural Spring infection, with increased pathogen activity favoured by warming temperatures and free water provided by regular irrigation, allowing dispersal of *Phytophthora* spp. zoospores.

Half the plants were placed in cold store at -1°C and the other half remained outdoors in the field, as ambient stored, over the winter period (December 2017 – March 2018). All plants were then potted up, and placed in a polytunnel with drip irrigation.

A baseline root assessment after winter storage, showed that ambient stored plants, treated in Autumn, had higher levels of root browning than those that were cold stored (Figure i). In these ambient stored plants, black-to-white root discolouration occurred, and was attributed to extreme drop in temperature in February 2018, not encountered by plants in cold storage. A greater root ball surface area of healthy white roots was present in Spring on ambient stored plants drenched with Paraat in Autumn.

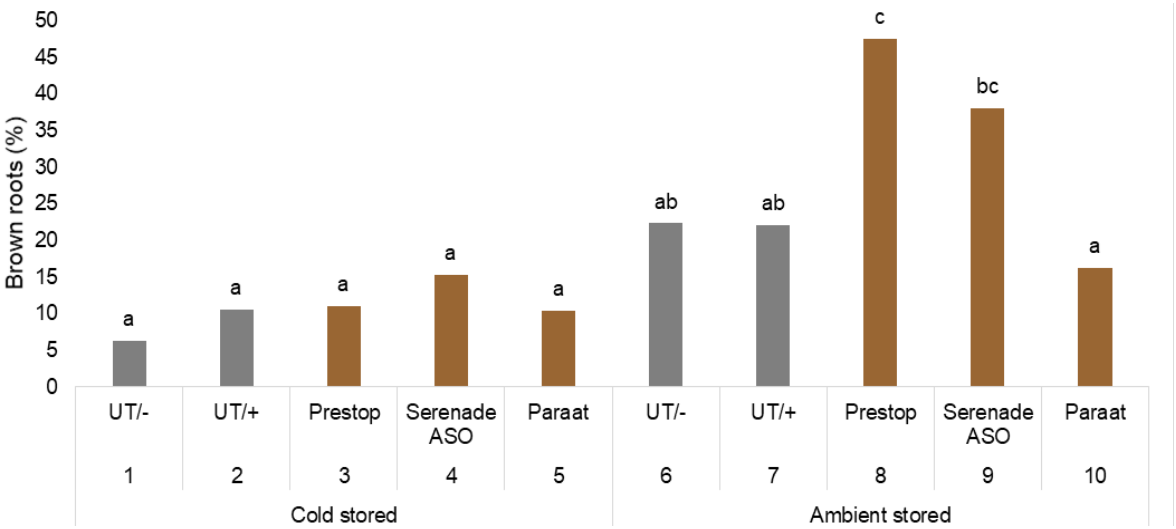

Figure i. Autumn treated plants. Percentage of root ball with brown roots at re-potting in March 2018. Significant differences indicated by differing letters. Brown root surface area in ambient plants includes freeze damaged black roots. No *P. rubi* inoculation had been carried out at this stage.

In contrast to the Autumn treated plants, the ambient stored plants yet to receive Spring drenches showed no more root browning than cold stored plants. This suggested Autumn biofungicide applications to ambient stored plants were linked with increased root browning.

By June, significantly more primocanes had emerged from the Spring treated cold stored plants (mean 3.1) compared with ambient plants (mean 2.0; P<0.001). Wilted canes were present in some uninoculated plants, and molecular testing of a sample plant showed the presence of *Phytophthora idaei* (or less likely *P. cactorum*) but not *P. rubi*.

By October 2018, a mean 46% of cold-stored Spring treated plants displayed symptoms of wilting in canes produced since Winter. This was significantly above the mean 28.3% following ambient storage (**Figure ii;** P<0.05). Autumn treated plants also displayed symptoms of wilting, but there were no storage or treatment differences.

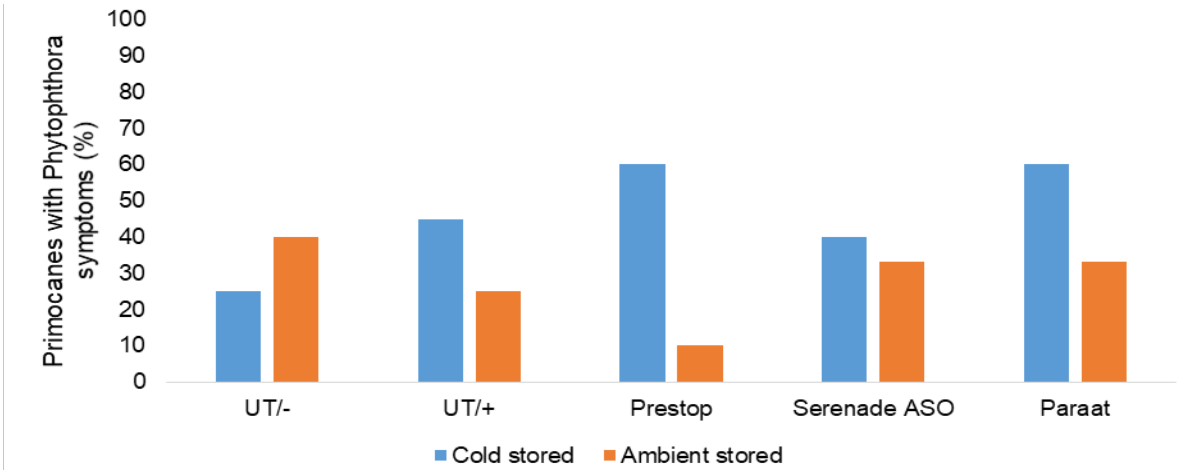

Figure ii. Percentage of primocanes per plant, Spring treated, showing *Phytophthora* spp. symptoms, October 2018. Significant difference (P < 0.05) between storage regimes.

Some Autumn treated plants from both storage regimes had developed red roots, in which *Phytophthora* spp. were detected by LFD test. Significantly more (P<0.05) ex-cold stored plants had this symptom (31.3%) than ambient stored (14.0% incidence) (**Figure iii**).

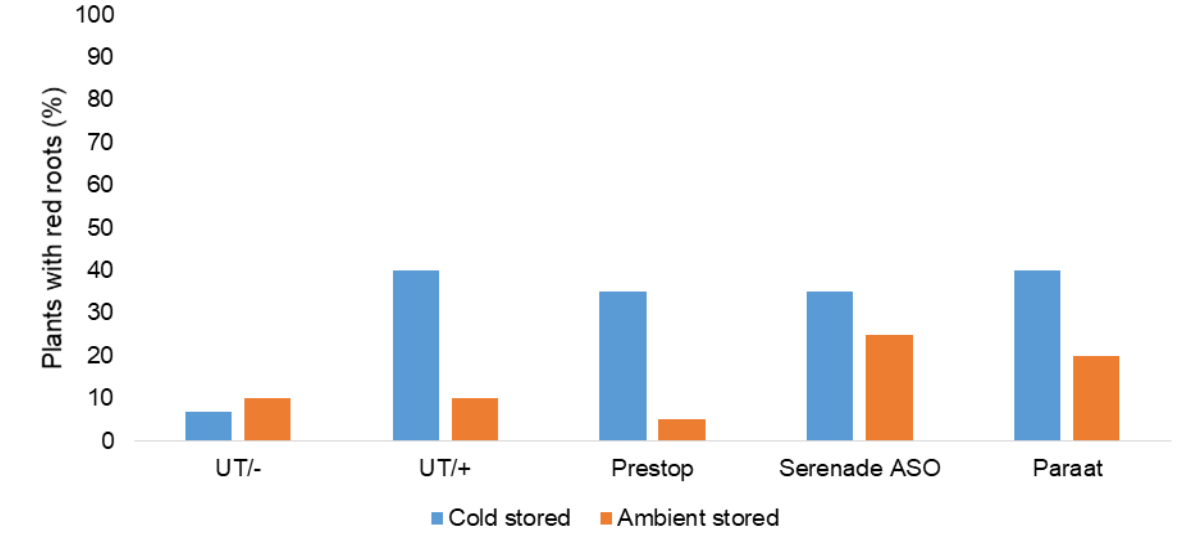

Figure iii. Incidence of red roots (associated with *P. rubi*) in the Autumn treated experiment, October 2018. Untreated UT/- remained uninoculated, all other treatments had *P. rubi* inoculation in April 2018. Significant difference (P<0.05) between storage regimes.

Spring treated plants had similar incidence of red rooted plants in each storage regime, with a mean 14.5% incidence across treated and untreated. Double the incidence of red roots in cold stored Autumn treated plants compared with the other three storage/treatment combinations may indicate either a greater susceptibility to *P. rubi* infection or poorer control.

Some uninoculated plants also had red roots present (mean 8.4%) and as care was taken to reduce any cross-infection from the *P. rubi* inoculation stage, it suggested a *Phytophthora* spp. was in a few of the plants received from the propagator. Limited molecular testing of other plants at the end of the experiment confirmed the presence of *P. rubi*, but *Phytophthora idaei* was also found in some roots.

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

- Be aware that some propagation material may carry *Phytophthora* spp. into a crop, so check for rotted roots when potting-on to indicate the extent of any problem. LFD test kits used to detect Phytophthora spp. will distinguish between the disease and browning caused by freezing that can arise following outdoor overwintering.

- Before being placed in tunnels in Spring/Summer, ensure plants have sufficient time to acclimatise after leaving cold storage. Heat stress may increase the susceptibility of plants to infection.
- In recent Springtimes, hotter than average periods have arisen, so minimise heat stress by venting polytunnels and glasshouses and ensure the correct amount of irrigation is delivered to pots. Be aware that too much free water favours *Phytophthora* spp. infection.
- Be vigilant for early symptoms of Phytophthora such as the characteristic 'shepherd's crook' seen in emerging primocanes. Remove affected plants or pots to stop *P. rubi* spread in run-off water.
- Note that *Phytophthora* spp. are still able to survive in containerised plants when temperatures outside the pot are either below freezing or above 30°C.
- Be alert to any changes in the timing and severity of root rotting as this could indicate the presence of species other than *P. rubi,* such as *P. idaei* which is favoured by warm conditions, and might require preventive treatment at a different time of year.

Two-spotted spider mite

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

Headlines

- Two-spotted spider mite was successfully controlled by and IPM programme before the grower needed to apply a plant protection product for control of spotted wing drosophila.
- Although not consistent on all assessment dates, there was evidence that adding pollen (Nutrimite®) may have led to improved *Amblyseius andersoni* establishment on some dates.

Background and expected deliverables

Two-spotted spider mite (TSSM) is increasingly a common pest of raspberry that can cause severe foliar damage, leading to cane stunting, reduced fruit size and subsequent yiled reduction. The current shortage of acaricides approved for use on outdoor & protected raspberry means that effective biological control of the pest within an Integrated Pest Management (IPM) programme is needed for all stages of the crop's production.

Plant protection products applied for the control of spotted wing drosophila (SWD) and other pests such as aphids and capsids, can have harmful effects on spider mite predators.

Naturally-occurring predatory mites including *Amblyseius andersoni* seem to be more tolerant of spray products than the released predatory mite *Phytoseiulus persimilis*. *A. andersoni* will also feed on pollen, fungal spores, plant sap and other invertebrates as well as on TSSM. In addition to occurring naturally, *A. andersoni* is also commercially available for release. Work on the continent has shown that *Typha* pollen (Nutrimite®) can boost populations of other omniverous predatory mites such as *Amblyseius swirskii* for control of thrips and whiteflies on other protected crops, by providing an alternative food source.

Nutrimite has not yet been tested on cane fruit crops but *A. andersoni* is known to feed on pollen and can be reared on *Typha* pollen in the laboratory. The work in this project aimed to determine whether Nutrimite can boost numbers of *A. andersoni* on a raspberry crop so that higher numbers survive sprays applied for control of SWD or other pests and thus benefit biological control of TSSM.

Summary of the project and main conclusions

This work set out to determine the effect of Nutrimite on numbers of both released *A. andersoni* and any naturally-occurring predatory mites that feed on TSSM on a raspberry crop. It also set out to determine the effect of plant protection products applied for the control of SWD and other pests on spider mite predators and biological control of TSSM on a raspberry crop with or without Nutrimite.

Four different treatments were tested on a commercial second year raspberry crop. Each treatment was applied to a different poly tunnel.

The treatments were:

- An untreated control
- Nutrimite applied to the crop four times every two weeks between 26 April and 7 June at 500g/ha
- *Amblyseius andersoni* applied at one sachet per two linear metres on 26 April and 7 June
- A combination of Nutrimite and *A. andersoni*

The grower released *Phytoseiulus persimilis* on 4 and 8 June and applied spinosad (Tracer) for control of SWD to all tunnels on 31 July, 29 August and 10 September. Assessments on Nutrimite deposition, numbers of TSSM, predatory mites, TSSM and predatory mite eggs, any other TSSM predators and TSSM damage were made on three randomly selected terminal leaflets from both the upper and lower canopies in ten replicate plots per tunnel (60 leaflets per tunnel) on eight dates between 26 April and 17 September.

Nutrimite was detected on both upper and lower leaflet surfaces in the two tunnels where it was applied but significantly more was found on the upper than the lower leaflet surfaces in the tunnel treated with both pollen and *A. andersoni.* This might have influenced the availability of alternative food for *A. andersoni* which lives on the undersides of leaves. *Amblyseius andersoni* were not found in any of the tunnels before they were released. Numbers of naturally-occurring *A. andersoni* were low throughout the trial in the untreated and pollen only tunnels and adding pollen to these tunnels did not increase numbers of the predators. However, adding pollen to the tunnel where *A. andersoni* was released led to significantly higher numbers of the predators than the naturally-occurring population in the control and pollen only tunnels on four assessment dates and led to significantly more than in the *A. andersoni* only tunnel on two dates. Although not consistent on all assessment dates, these results provide some evidence that adding Nutrimite improved the establishment of *A. andersoni* after release on some dates.

There were no significant differences in numbers of TSSM between any of the treatment tunnels. However, on 30 July, 7 August and 5 September, in the tunnel treated with *A. andersoni* and pollen, the mean percentage leaf area damaged by TSSM was significantly higher. This indicated that there had been more TSSM present in these tunnels at some point, possibly in between assessment dates, which could explain the higher numbers of *P. persimilis* in the tunnel treated with *A. andersoni* and pollen. *Phytoseiulus persimilis* established by 30 July by which time the TSSM population had crashed in all tunnels. Therefore the Tracer application programme starting on 31 July for SWD control did not disrupt biological control of TSSM. It is not possible to quantify the control of TSSM provided by *P. persimilis* or *A. andersoni* individually but it is likely that *A. andersoni* supplemented the control offered by *P. persimilis*. The naturally-occurring predators *Feltiella acarisuga*, *Stethorus punctillum* and *Orius* sp. were also found in low numbers and these will also have contributed to TSSM control.

Financial benefits

The estimated value of the UK raspberry crop is £122.2 million (Defra Horticulture Statistics 2018). Accurate figures for crop losses in both fruiting plantations and crops in propagation due to TSSM damage are not available, but even if only a mean of 5% crop losses occurred, annual losses amount to £6.11 million. If biological control of TSSM was disrupted, much higher losses are likely to occur due to the current absence of a ‘fall-back’ acaricide for use on protected & outdoor raspberry. Thus research on reducing the risk of disruption of biological control of TSSM could save the industry significant financial losses.

Action points for growers

- Aim to establish *P. persimilis* as early as possible and be aware of the contribution of naturally-occurring predators to the control of TSSM.
- Consider early release of *A. andersoni* for preventive TSSM control before temperatures are suitable for *P. persimilis* as this predatory mite is more tolerant of low temperatures and could give some control of other pests such as raspberry leaf and bud mite. However, released predators of this species may be less tolerant of certain plant protection products such as pyrethroids, than naturally occurring populations.
- Further work is needed before the use of Nutrimite® to boost numbers of *A. andersoni* for improved control of TSSM on raspberry can be recommended.
- Use IPM-compatible plant protection products or those with the least harmful effects on biological control agents for control of all pests including TSSM and SWD wherever possible.

Spray deposition

Objective 2.2 – To develop and maintain IPM approaches to successfully control two-spotted spider mite whilst controlling SWD and other pests with insecticides.

Headline

- Using very fine spray and half-rate air-assistance may provide slightly better distribution of spray deposition in a raspberry canopy, when sprayed at around 800 L/ha with an air-blast tractor mounted spray machine.

Background and expected deliverables

Restrictions on the use of acaricides in raspberry production means that two-spotted spider mites (TSSM) are primarily controlled using beneficial insects rather than conventional spray products. However, populations of beneficial insects can be adversely affected by product sprays targeting other pests. Previous semi-field trials have shown that overhead spraying provides more spray refuges than air-assisted knapsack spraying, and that plots with more spray refuges had significantly more natural phyotseiids in them, but also more aphids.

On-farm spray trials were undertaken with a commercial tractor mounted air-blast sprayer to assess the effect of two key settings on spray machines that affect spray deposition: air-assistance and spray quality (droplet size). Farm spray machines are often set to generate a

fine spray with air-assistance set to full speed. The trials investigated firstly how spray quality: very fine compared to medium sized droplets, and air-assistance: full rate or half rate, whilst maintaining the same water volume, affects spray deposition throughout the raspberry canopy. Secondly we investigated the effects that these sprayer settings have on the number of refuges for beneficial insects within the raspberry canopy.

Summary of the project and main conclusions

Field trials were done to assess the spray coverage, spray deposition, and distribution of spray throughout the crop canopy. The spray was applied to a raspberry crop in July, using an Ideal Alsazia spray machine at 840 L/ha, with either yellow Albuz ATR 80 nozzles (very fine spray quality) or blue ATR 80 nozzles (medium spray quality), and with the air-assistance set to full rate or half rate. Measurements were taken from the canopy in four zones: top, middle, bottom, and inner canopy (see Figure 1). At each of the zones, the spray deposition was measured on both upper and lower leaf surfaces.

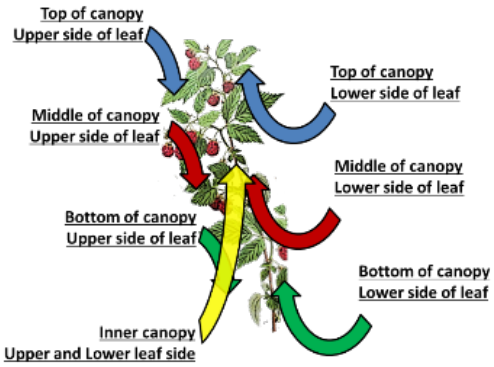

Figure 1: For measuring the spray deposits the raspberry crop canopy was divided into 4 zones: top (blue), middle (red), inner (yellow) and bottom (green). These were approximately 650 mm in height each. The inner zone was at the same height as the middle zone but in the centre of canopy. Within each zone the spray deposition on both sides of leaves was measured. Thus 8 groups of data were collected for each treatment.

Spray deposition (Figure 2) and volume of spray (Figure 30) were highly variable throughout the raspberry canopy. A common trend developed with lots of spray deposited at the top and middle sections of the canopy, much less deposition at the bottom of canopy, and very little deposition at the inner section of the canopy.

The very fine quality spray in combination with half-rate air-assistance spray settings provided a more even distribution of spray throughout the canopy, with significantly more spray coverage and deposition in the bottom and inner canopy sections. The medium quality spray in combination with half-rate air-assistance also partially increased spray deposition at the middle and inner canopy sections.

The percentage of leaves with less than 5 % spray coverage were assessed. Leaves that received less than 5 % coverage could provide a refuge for beneficial insects from product spray. Leaves that received less than 5 % coverage may also benefit pests such as aphids. Previous AHDB funded trials (SF 158, interim report 2018) on raspberry crops which had been sprayed with overhead nozzles to increase refuges for beneficial insects showed that aphid populations also increased.

Greater than 50 % of leaves sampled from the middle canopy section-lower leaf side, inner canopy-both leaf sides, and bottom canopy-lower leaf side received less than 5 % spray coverage, potentially providing many refuges for insects from product sprays. At these canopy-leaf sections, coverage was broadly the same for all of the spray settings assessed.

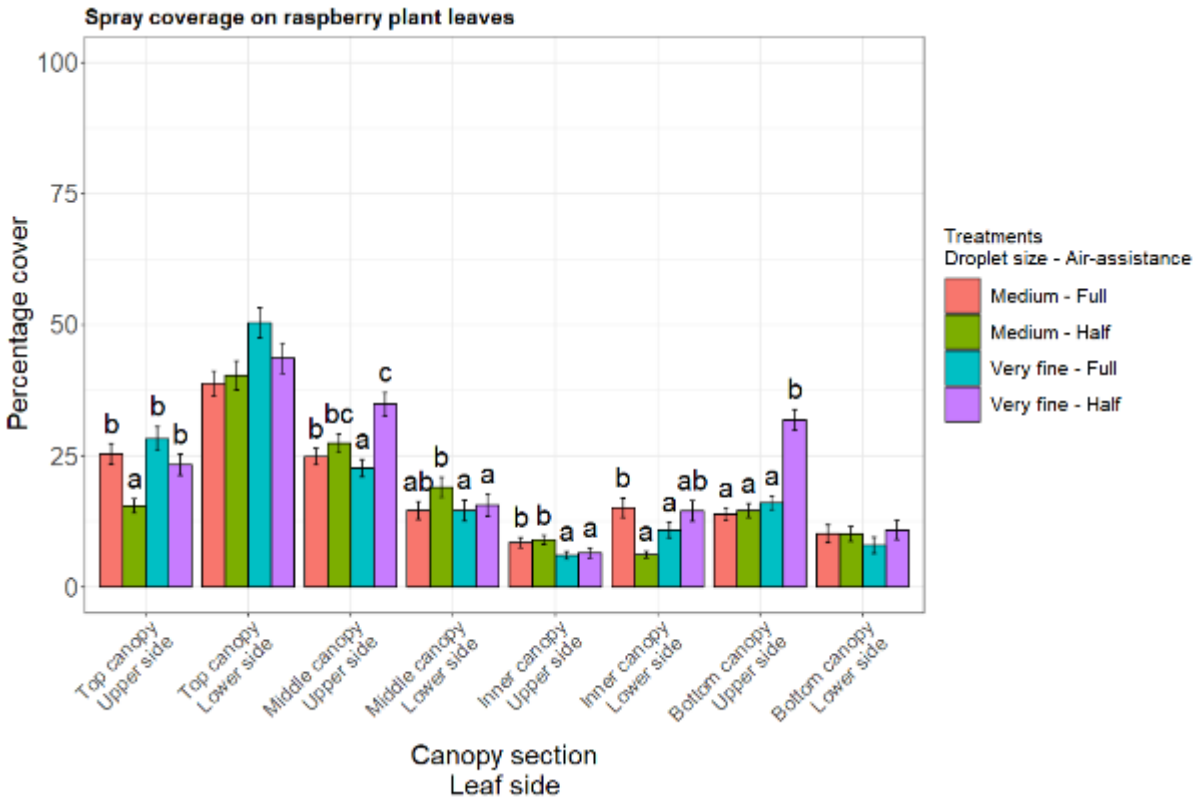

Figure 2: Percentage of leaf area covered with spray deposits at each canopy zone and leaf side, for each of the four spray treatments. The error bars show standard error. Significant differences were identified by GLMER and multiple comparisons Tukey's tests. If significant differences were identified, letter labels denote significant differences between the treatments within each canopy zone/leaf side.

Main conclusions

- Spray deposition was highly variable across the different parts of the raspberry canopy, in particular high coverage and deposition was found at the top and middle sections, whilst the inner and bottom sections of the canopy experienced much lower coverage and deposition.

- All of the spray settings tested provide a high number of leaves with less than 5% spray coverage. It is thought that these leaves could act as refuges for beneficial but also pest insect species.
- The results of this study suggest that using very fine spray and half-rate air-assistance may provide slightly better distribution of spray deposition in a raspberry canopy, when sprayed at around 800 L/ha with an air-blast tractor mounted spray machine.

Financial benefits

The application of plant protective products (PPPs) (fungicides, herbicides, insecticides) in raspberry production can cost between £450 – £1,700 per hectare, depending on the cropping system. With additional costs for labour, fuel, machinery, water, etc. the cost for applying PPPs is substantial. Ensuring PPPs are applied in the most efficient way possible will minimise input costs and maximise returns. Growers should ensure that applied predatory mites are not adversely affected by sprays for other pests, and that the output from spray machines is efficient and hitting the intended target.

Action points for growers

- Minimise the exposure of beneficial mites (natural and released) to crop protection sprays. Even though spray machines are likely to provide a high number of 'spray refuges', *Phytoseiulus persimilis* and other predatory mites are known to be adversely affected by many active ingredients (e.g. spirotetramat, lambda-cyhalothrin, thiacloprid) .
- When product sprays are required, ensure the applications are as efficient as possible. Check the spray deposition produced by the farm's spray machines. Currently Water Sensitive Papers can be used to do this. Pay particular attention to the distribution of the spray deposition throughout the canopy and the location of the target pest or disease within the canopy. Adjust spray to match crop canopy development.
- Consider reducing the fan speed if spray is being blown right through or over the top of plant canopies.

If growers are considering modifying their spray machines to provide more spray refuges for beneficial insects, they must also take into account the risk of providing refuges for other pests, such as aphids. An alternative approach may be to check and optimise the spray output from their spray machines to maximise the effect of sprays, and then modify the timing of spray applications and applications of predatory mites to avoid damaging one with the other.

Cane blight

Objective 3 – To review the current threat posed to the UK raspberry industry by cane blight (*Leptosphaeria coniothyrium*) and identify new control options

Headline

- Control of cane blight in UK raspberries is of increasing importance and requires immediate attention due to the lack of available plant protection products and insufficient control of cane midge

Background and expected deliverables

A literature review was conducted to establish what information was available on the issue of *L. coniothyrium* in UK raspberry. Changes in practice to growing commercial raspberry has resulted in new windows of opportunity for pest and diseases, including *L. coniothyrium*, a relatively weak pathogen, and cane midge, which plays a role in introducing the disease to raspberry canes.

Financial benefits

The estimated value of the UK raspberry crop is £122.2 million (Defra Horticulture Statistics 2018). The levels of crop loss currently being caused by raspberry cane blight in the UK raspberry industry are currently unknown but are believed to be increasing. Potential damage can vary from 1% crop loss through to 90% in very severe cases, although this is very unusual. Some cultivars are more susceptible than others, but if the cultural practices of the day lead to damage of the prmocane rind and the weather conditions favour infection, damage can be very much more serious. Crop losses of as little as 1% would amount to a financial loss of £1.2 million to the industry. Any work that reduces the risk of this level of damage would therefore be very beneficial.

Summary of the project and main conclusions

Cane blight (*L. coniothyrium*) is a relatively weak pathogen and often requires damage to the cane in order to enter the plant. This includes mechanical damage from pruning, strimmer damage to cane base, frost damage, cold injury, hail and pest damage. The poor application of a desiccant (e.g. carfentrazone-ethyl - Shark) for primocane control can also give rise to cane damage which can become infected.

No new information on efficacy testing for *L. coniothyrium* on cane fruit is available, with no relevant Plant Protection Products available for growers in the UK. No work on epidemiology/spray timings/forecasting has occurred since previous AHDB funded work in 2006 (Projects SF 69 and SF 69a).

In other countries where cane blight is a major issue, such as in Canada, primary control is through the use of good crop husbandry and hygiene.

Action points for growers

- Monitor and control raspberry cane midge populations, to limit damage caused to the periderm tissue of primocanes and subsequent development of midge blight disease.
- Pinch off the tips of tender primocanes rather than cutting them, and ensure where possible, to prune when at least 4 days of dry weather is expected.
- Where canes are removed, ensure they are cut close to the ground, to avoid rubbing damage to newly emerging canes, which causes a wound for *L. coniothyrium* to enter.

SCIENCE SECTION

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

Aim

WP 1.3 – Explore the effect of cold storage of long cane raspberries on incidence and severity of *Phytophthora rubi* infection and the potential for protection using biofungicides against infection in Spring.

Aim 1: To investigate effects of cold storage and ambient overwinter storage on long cane raspberry modules and the incidence and severity of root rotting by *P. rubi*.

Aim 2: To investigate whether a plant protection product drench, before or after Winter, can reduce the impact of *P. rubi* infection taking place in the following Spring.

Introduction

Around 70% of raspberry material in the UK is currently cold stored at -1°C overwinter between lifting and delivery to the grower. Cold storage ensures that the plants receive the necessary chilling period for good fruit production in the coming year and is particularly important for long cane which may not be kept for another cropping year.

If plants become infested by *Phytophthora* spp. before winter the pathogen can survive in cold storage. Work on strawberry showed that cold stored plants were more susceptible to *Phytophthora* sp. infection post cold storage (Pettitt & Pegg, 1994).

Returning of cold stored infested plants to ambient conditions with recommencement of watering in Spring may trigger a mass *Phytophthora* spp. zoospore release (as observed under laboratory conditions) rather than a steady release after ambient storage and this may increase the incidence of root infection in cold stored plants.

Materials and Methods

The long cane cv. Tulameen plants used in the trial were selected at six months old while located at the site of a UK propagator. They had a Plant Passport equivalent to Basic 2. The original parent material had been supplied to the propagator from NAKT (Holland) as pre-basic root blocks.

Two experiments were set up, Experiment 1 Autumn treatments and Experiment 2 Spring treatments. A comparison of the schedule of product treatment, overwinter storage conditions and inoculation with *P. rubi* is given in **Table 1**.

Within each experiment there were ten treatments, T1-T5 to be cold stored and T6-T10 to remain outside in ambient conditions. These were randomised into five replicate blocks giving a total of 50 plots (200 plants) in each of the two experiments (**Table 2**).

Table 1. Comparison of timings of Plant Protection Product (PPP) drenches, inoculation with *P. rubi* and storage regime

Timing	**Experiment 1 (Autumn drenched)**		**Experiment 2 (Spring drenched)**	
Autumn 2017	PPP Drenched	PPP Drenched	-	-
	-	-	-	-
Winter 2017/18	Cold stored	Ambient stored	Cold stored	Ambient stored
Spring 2018	Potted-up & in tunnel	Potted-up & in tunnel	Potted-up & in tunnel	Potted-up & in tunnel
Spring 2018	-	-	PPP Drenched	PPP Drenched
Spring 2018	Inoculated	Inoculated	Inoculated	Inoculated

Table 2. Products and number of applications in either Autumn 2017 (Experiment 1) or Spring 2018 (Experiment 2). Inoculation with *P. rubi* in Spring 2018 (except T1 and T6) at ADAS Boxworth. Treatments 1-5 given cold storage are shown shaded in the table.

Experiment 1 (Autumn drenched)		**Experiment 2 (Spring drenched)**	
T1 UT no *P. rubi*	Cold stored December 2017 to March 2018	T1 UT no *P. rubi*	Cold stored 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 outdoor stored December 2017 to March 2018	T6 UT no *P. rubi*	Ambient outdoor stored 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	

Products were applied to Experiment 1 only in Autumn 2017 (**Table 3**) and procedures and assessments were given in full in the previous Annual Report 2018. All plants were topped at 1.5 m height as standard. Half of the plants from both experiments (T1 to T5) were placed in cold storage overwinter (2017/18), and the other half remained in the field, exposed to all weather conditions. Plants in Experiment 2 (**Table 4)** were treated with PPPs in Spring 2018.

Table 3. Experiment 1. Treatments drenched at 10% pot volume before either cold storage (T1-T5) or ambient storage outdoors (T6-T10) in Oxfordshire, 2017. Treatments other than T1 and T6 were inoculated with *P. rubi* on 17 April 2018 after potting-on 16 March 2018.

Treat-ment	Product [MAPP Number]	Active ingredient	Recommended dose	Product /1.5L pot in 0.15L water	Application timing/s in 2017
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

Table 4. Experiment 2. Treatments applied at ADAS Boxworth and timings after either cold storage (T1-T5) or ambient storage outdoors (T6-T10) in Oxfordshire. Treatments other than T1 and T6 were *P. rubi* inoculated on 17 April 2018 after potting-on 16 March 2018.

Treat-ment	Product [MAPP Number]	Active ingredient	Recommended dose	Product /5L pot in 0.5L water (10% by volume)	Application timing/s in 2018
T1	Untreated no *P. rubi*	-	-	-	-
T2	Untreated	-	-	-	-
T3	Prestop [15103]	*Gliocladium catenulatum* strain J1446	5 g/L water (0.5%)	2.5g	19 March 5 April
T4	Serenade ASO [15625]	*Bacillus subtilis* strain QT 713	10 L/ha in 1000 L/ha water (10 ml/L)	5 ml	5 April
T5	Paraat [15445]	Dimethomorph	1 g per plant	1 g	5 April
T6	Untreated no *P. rubi*	-	-	-	-
T7	Untreated	-	-	-	-
T8	Prestop [15103]	*Gliocladium catenulatum* strain J1446	5 g/L water (0.5%)	2.5g	19 March 5 April

T9	Serenade ASO [15625]	*Bacillus subtilis* strain QT 713	10 L/ha in 1000 L/ha water (10ml/L)	5 ml	5 April
T10	Paraat [15445]	Dimethomorph	1 g/plant	1 g	5 April

In Spring (13.03.18) plants from the cold store and ambient field conditions were transferred at ambient via truck to ADAS Boxworth (Cambridgeshire). Plants from the cold store (at -1ºC) were placed in an open fronted shelter for 3 days to acclimatise to ambient conditions. All plants, from both experiments were then re-potted into 5L pots with Ericaceous compost. Each 2-module pot was cut in half, and each raspberry cane planted individually, and put into a polytunnel on 15 March 2018 (**Figure 3**). Of the three 2-module pots per plot, two were potted up, to give four plants in each plot, and the third was taken aside for a more thorough root assessment.

Figure 3. Raspberry plants in Experiment 1 (left 2 rows) and Experiment 2 (right 2 rows) re-potted from 1.5L 2-plant module pots into 5L pots on 15 March 2018, ADAS Boxworth, Cambridgeshire.

Plants were arranged in the same trial layout as in the field before winter, i.e. with ambient and cold store plants randomised within each replicate block. The two experiments were, as before, kept separate, but adjacent to each other so that they continued to experience similar environmental conditions. Data loggers to record temperature and humidity, were placed at root height beside the pots.

Once plants had been placed in the tunnel, bud break and cane health were assessed.

During re-potting, all 1.5L module pot root balls were assessed for brown (rot) and white (new) roots, as a percentage of the total outside root ball surface. Where the module pot root balls

were also cut in half, to separate the two plants, the cut surface was inspected, and any obvious differences in visual root health were recorded.

Experiment 2 treatment applications were made on 19 March 2018, four days after the plants were re-potted, to be in line with typical commercial Spring PPP application timings.

Spring 2018 inoculation with *P. rubi*

Inoculation used the *P. rubi* isolate CC2106 (confirmed by Fera in 2012) which had been confirmed pathogenic in prior testing (SF 158 Annual Report 2018). The isolate was grown on 10% V8-juice agar for 21 days, incubated at 20ºC on a 16:8 hour light:dark cycle, to produce mycelium that nearly filled the 45mm diameter agar plate (**Figure 4**).

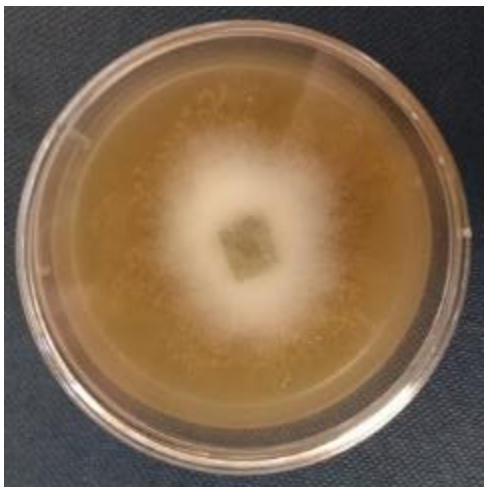

Figure 4. 21-day old *Phytophthora rubi* culture on 10% V8-juice agar, in a 45 mm diameter Petri dish.

Plants were inoculated on 17 April 2018, one month after re-potting and being placed into a polytunnel. Using the same method as for the pathogenicity testing (see SF 158 Annual Report 2018), four holes were made to the root ball (one on each side of the root ball cube) using a dibber, cleaned with IMS between plots. The controls also had identical holes made using a dibber. Agar plugs 8mm wide were cut with a cork borer from the 21 day old *P. rubi* culture plates, with half the plugs from just inside the leading edge of the colony and the other half of the plugs from older mycelium nearer the centre of the dish. Plugs were placed into the holes (2x plugs per hole). Pots each received some older and younger aged mycelium (total 8 plugs per plant). The hole was refilled using the same Ericaceous growing-media as used for potting, and watered by hand.

Irrigation by drippers were set up to keep the water in the pots at field capacity so that a water film was held around the roots by capillary action and any small amount of surplus water allowed to drain out of the pots.

The timing of the inoculation coincided with an abnormally hot week, with most days' temperatures around 25-30°C. Both double doors of the tunnel were permanently open, and a watering schedule was set to nine times per day, to maintain a damp environment around the root ball. Uninoculated pots were placed on clean plastic Ringot pegs, to keep pots off the woven ground-cover material and away from ground sources of infection. Later, as roots developed, these pots were moved onto plastic crates, to form a bigger air barrier above the matting.

Assessments began after potting-on, on 19 April 2018 (**Table 5**). End of season assessment of cane and roots in October included five samples being sent to Fera Science Plant Clinic to undergo analysis. Ten Lateral Flow Devices (LFDs) detecting *Phytophthora* spp. were also used (Neogen – ALERT LF, batch 14-857-21) throughout the trial to check symptomatic roots suspected to contain *Phytophthora* spp.

Table 5. Assessment and treatment application timeline for Experiment 1 (Autumn treated) and Experiment 2 (Spring treated) 2017 to 2018.

Date	**Assessment/Task**	
	Experiment 1 (Autumn treated)	**Experiment 2 (Spring treated)**
2017		
28 September	Treatment drench	
19 October	Treatment drench	
18 December	Half of plants cold stored, half remained as ambient stored.	Half of plants cold stored, half remained as ambient stored.
2018		
13 March	Plants brought out of cold storage and ambient storage and placed in outdoor shelter, Cambridgeshire.	Plants brought out of cold storage and ambient storage and placed in outdoor shelter, Cambridgeshire.
15 March	Root health assessed	Root health assessed
16 March	Re-potted individual canes into 5L pots, and placed under tunnel.	Re-potted individual canes into 5L pots, and placed under tunnel.
19 March	Assessed plant vigour – bud break and vigour recorded.	Assessed plant vigour – bud break and vigour recorded.
19 March		Treatment drench
05 April		Treatment drench
17 April	All plants (except untreated uninoculated, UT/-) inoculated with *P. rubi*.	All plants (except untreated uninoculated, UT/-) inoculated with *P. rubi*.
27 June	Cane vigour assessed – floricanes and primocanes.	Cane vigour assessed – floricanes and primocanes.
15 October	Canes and roots assessed – recording symptomatic primocanes and health of root balls.	Canes and roots assessed – recording symptomatic primocanes and health of root balls.

15 October	Five symptomatic plants sent to Fera Plant Clinic for *Phytophthora* spp. detection.	Five symptomatic plants sent to Fera Plant Clinic for *Phytophthora* spp. detection.

In all root assessments conducted, two classes of roots were recorded: 'brown' and 'white'. Brown roots include those that were unhealthy, damaged, rotted, water soaked or in some cases black-brown (due to freezing damage from ambient storage). The term 'brown' was used as a catch-all, as it was not always possible to relate unhealthy roots to an exact cause (physiological or pathological). White roots were newly formed, healthy, free of disease, often not yet pigmented, were delicate so easily damaged, and often related to a healthy local root environment. Roots that were neither white nor rotted brown, were older, mature healthy tan-coloured. These were not recorded but made up the remainder of the root ball surface area.

During the October 2018 assessments, after recording white and brown roots, it was noted that some plants had obvious patches of red roots. Following a combination of two positive *Phytophthora* spp. specific LFD tests of red roots, and the death of primocanes above extensive red root patches, a separate, additional assessment was made to record incidence of red roots on each plant, as a likely symptom of *Phytophthora* spp. infection. Analysis was of the percentage of plants with red roots (incidence).

Cane assessment on 27 June 2018 assessed all floricanes and primocanes. No primocanes were removed from the plants over the season. The older primocanes displayed *P. rubi* as darkened patches near the stem base, whereas younger canes were caused to wilt, bend over and then die. Floricanes were then cut out at the end of August, once fruiting ceased, so October cane recording assessed remaining primocanes, which would be those continued on to next year's growing season. The October assessment was carried out in advance of leaf senescence, in order to be able to see any leaf wilting.

In every assessment, all four plants per plot were assessed individually and Analysis of Variance (ANOVA) carried out using Genstat Release 18.2 separately comparing storage regimes and treatments and for any interaction between them. Where significant differences occurred, a Duncan's Multiple Range Test was also carried out for use on bar charts, with any means not sharing a letter (a, b or c) being significantly different. As Experiment 1 and 2 were run at the same time and in the same place, a further ANOVA was carried out to compare treatments and storage regimes across the two experiments.

Results

March 2018 – Cane health

Assessment of plants on arrival at ADAS Boxworth after either cold or ambient storage, showed all plants to have excellent vigour (mean index score of 9) and no cane diseases were observed. Ambient stored plants had begun to break bud, cold stored plants had not.

March 2018 – Root health

The pre-potting assessment in March 2018, identified a range in health of root balls (**Figure 5**). Most root balls were healthy and firm, where the majority of root ball was covered in healthy tan coloured roots (**Figure 5**, A). The rest of the root ball either consisted of white healthy roots, or rotted brown roots. The percentage of the root ball covered in white, and rotted brown roots was recorded. Amongst those with healthy white roots, some had stark discolouration on the new outer new roots (**Figure 5**, D - F). Roots here, were blackening, discoloured, soft and deteriorating and were only present on ambient stored plants, not cold stored plants. The discoloration was also just on the outer new roots, with no damage inside the root ball (**Figure 5**, H). The distinct margin was typical of cold damage, and was most likely due to the extreme cold seen at the end of February 2018, when temperatures dropped to -9.5ºC (**Figure 19**). Four samples of roots were tested with a *Phytophthora* spp. LFD. Roots from plants Nos. 138.1 and 145.1 (Untreated) (**Figure 5**, C) and 245.2 (to be Paraat treated) (**Figure 5**, E) were positive on the LFDs, roots from plant No. 240.2 (to be Prestop treated) (**Figure 5**, A) were negative. The positive LFDs indicate that *Phytophthora* spp. was present in these plants prior to inoculation, and coincided with the black discolouration potentially resulting from cold damage.

Figure 5. Range of raspberry root ball health from 1.5L raspberry module plants kept in ambient or cold store over winter. Oxfordshire, 2018. A) Healthy, firm, natural brown roots. B) Healthy, firm, white and light-brown roots (left), dull brown, water-soaked roots (bottom right). C) Damaged, water-soaked brown roots mixed with some healthy brown. D) Underside black discoloration, indicating damage to new white root growth. E) Clear black-to-white discoloration on one side of module root ball. F) Close-up of black-to-white discoloration believed to have followed freezing of ambient stored pots, and damage extending as browning water-soaked roots at black-to-white margin. G) Healthy, undamaged new white roots. H) Inside cross section of root ball showing coir growing media and small, healthy old roots.

Experiment 1 – Autumn 2017 treated

March – Root assessment

At the time of March assessment, treatments had been applied to plants in Autumn, but the Spring inoculation of *P. rubi* had not yet been carried out.

Ambient stored plants had significantly (P<0.001) more brown roots (29.2%) than cold stored plants (10.7%) (**Figure 6** and **Table 6**) with Prestop treated plants having significantly (P<0.001) higher levels of brown root than the controls. Ambient stored Serenade ASO and Prestop plants had significantly (P<0.001) more brown roots than Paraat and all cold stored plants.

Table 6. Autumn treated raspberry plants. Percentage of root ball covered by brown roots (%) at re-potting in March 2018.

	% of root ball with brown roots		
Treatment	**Cold stored**	**Ambient stored**	**Mean**
UT/-	6.3	22.3	14.3
UT/+ *P. rubi*	10.5	22.0	16.3
Prestop	11.0	47.5	29.3
Serenade ASO	15.2	38.0	26.6
Paraat	10.3	16.2	13.3
Mean	10.7	29.2	

ANOVA	**Storage**	**Treatment**	**Storage.Treatment**
P-value	<0.001***	<0.001**	0.116
l.s.d	7.57	16.92	16.92
Df	(1,36)	(9, 36)	(4, 36)

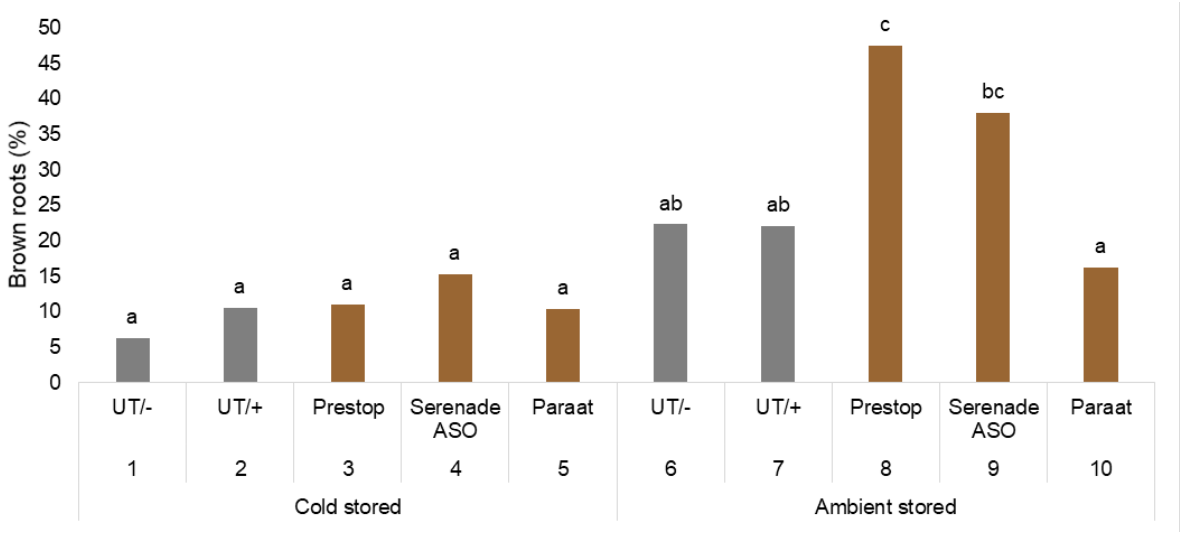

Figure 6. Autumn treated raspberry plants. Percentage of root ball with brown roots at re-potting in March 2018. Cold stored treatments T1-5, ambient stored T6-10. UT remained untreated. Significant differences indicated by differing letters. Brown root surface area in ambient plants includes freeze damaged black roots. No *P. rubi* inoculation had been carried out at this stage.

Differences in the percentage of white root surface area were seen between treatments, but not between storage regimes (**Table 7** & **Figure 7**). Paraat treated plants had the highest proportion of the root ball covered by white roots in ambient stored plants, significantly ($p<0.045$) more than the other treatments. There was no significant difference in the extent of new white growth between any of the untreated control plants and those given either Prestop or Serenade ASO.

Table 7. Percentage surface area coverage of Autumn treated raspberry root balls with white healthy roots. Statistical comparison between all treatments across both storage regimes. March 2018.

	% of root ball with white roots		
Treatment	**Cold stored**	**Ambient stored**	**Mean**
UT/-	14.3	17.4	15.9
UT/+ *P. rubi*	10.3	9.5	9.9
Prestop	18.5	5.5	12.0
Serenade ASO	13.8	12.6	13.2
Paraat	27.2	43.6	35.4
Mean	16.8	17.7	

ANOVA	**Storage**	**Treatment**	**Storage.Treatment**
P-value	0.851	0.045*	0.412
l.s.d	9.49	21.22	21.22
df	(1,36)	(9, 36)	(4, 36)

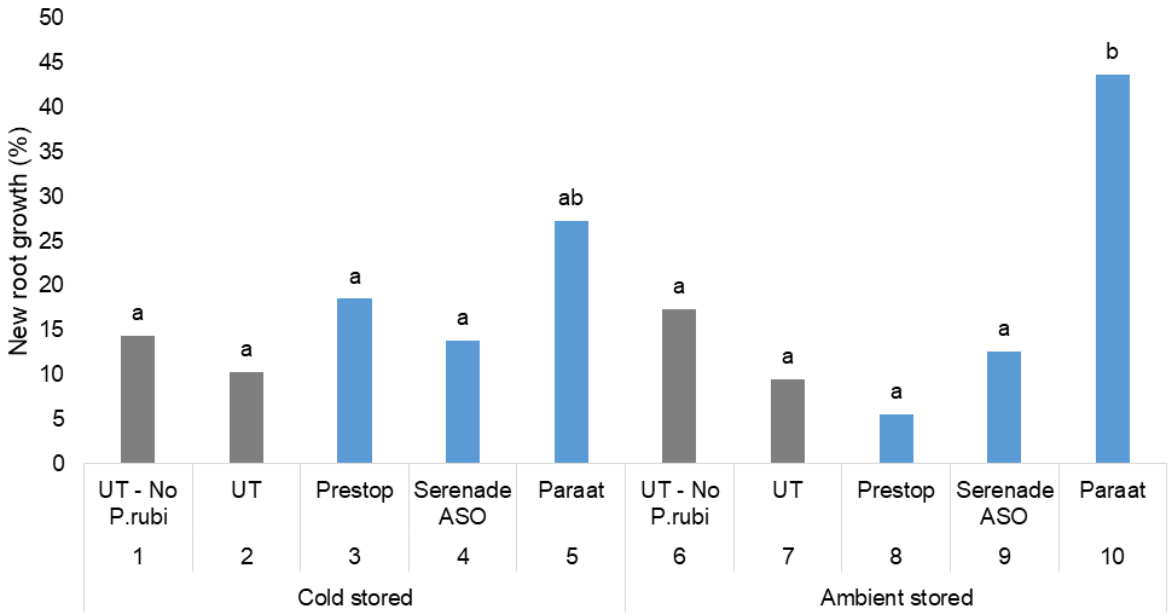

Figure 7. Percentage of root ball surface with new white healthy roots at re-potting of Autumn treated plants in March 2018. Cold stored treatments T1-5, ambient stored T6-10. UT remained untreated. Significant differences indicated by differing letters. No *P. rubi* inoculation had been carried out at this stage.

June – Cane assessment

In June, of the 200 floricanes in Autumn treated plants, 23 showed some form of Phytophthora wilt (**Figure 8**). Due to plot variability there were no significant storage or treatment differences (**T**able **8**).

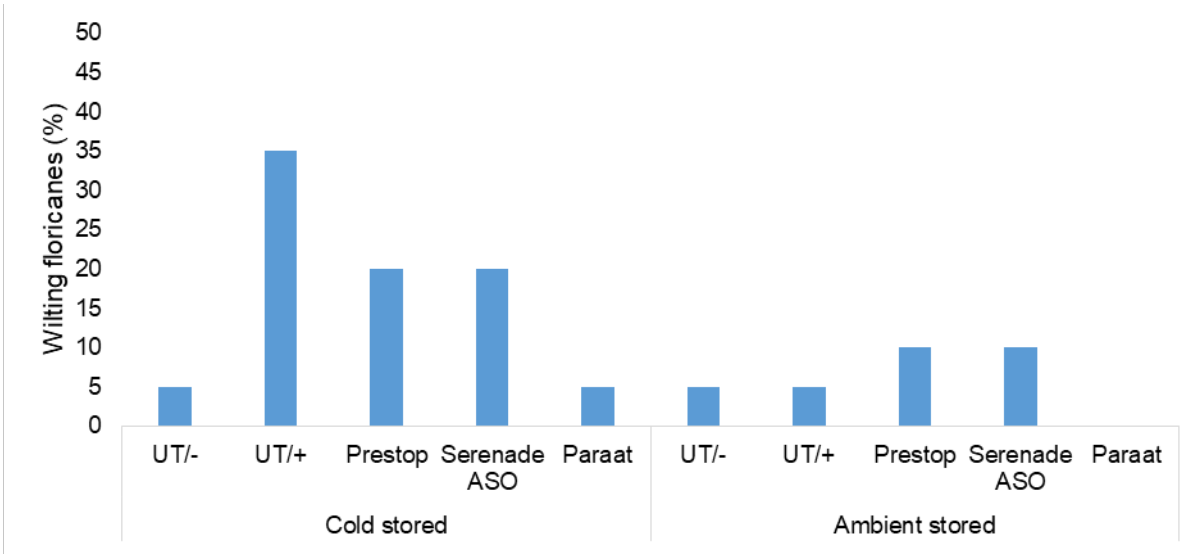

Figure 8. Percentage of floricanes that were wilting on 27 June 2018 in Autumn treated module pots. No significant differences.

Table 8. Percentage of floricanes of Autumn treated plants with Phytophthora wilt on 27 June 2018. All except untreated uninoculated (UT/-) had been inoculated with *P. rubi* on 17 April 2018.

	% of floricane that were wilting		
Treatment	**Cold stored**	**Ambient stored**	**Mean**
UT/-	5.0	5.0	5.0
UT/+ *P. rubi*	35.0	5.0	20.0
Prestop	20.0	10.0	15.0
Serenade ASO	20.0	10.0	15.0
Paraat	5.0	0.0	2.5
Mean	17.0	6.0	

ANOVA	**Storage**	**Treatment**	**Storage.Treatment**
P-value	0.149	0.618	0.759
l.s.d	15.13	33.84	33.84
df	(1,36)	(9, 36)	(4, 36)

By the end of June, following Autumn treatments, most plants had produced on average three primocanes (**Table 9)** with no significant differences between either storage regime treatments. The untreated inoculated ambient stored plants ranked for lowest mean number of primocane per plant, and Paraat and Serenade ASO treated, cold stored plants ranked highest (**Figure 9**). This may have been because primocanes once infected failed to emerge. Wilt was also seen in the uninoculated pots and may have resulted from natural infection of the plants before they were inoculated in April (as suggested by the root analysis in March, and the molecular testing of one uninoculated plant in October).

Table 9. Mean number of primocanes per plant, Autumn treated plants, 27 June 2018.

	Mean number of primocanes per plant		
Treatment	**Cold stored**	**Ambient stored**	**Mean**
UT/-	3.85	3.50	3.68
UT/+	3.15	2.10	2.63
Prestop	3.65	3.15	3.40
Serenade ASO	3.95	3.10	3.53
Paraat	4.00	3.50	3.75
Mean	3.72	3.07	

ANOVA	**Storage**	**Treatment**	**Storage.Treatment**
P-value	0.070	0.426	0.967
l.s.d	0.7068	1.581	1.581
df	(1,36)	(9, 36)	(4, 36)

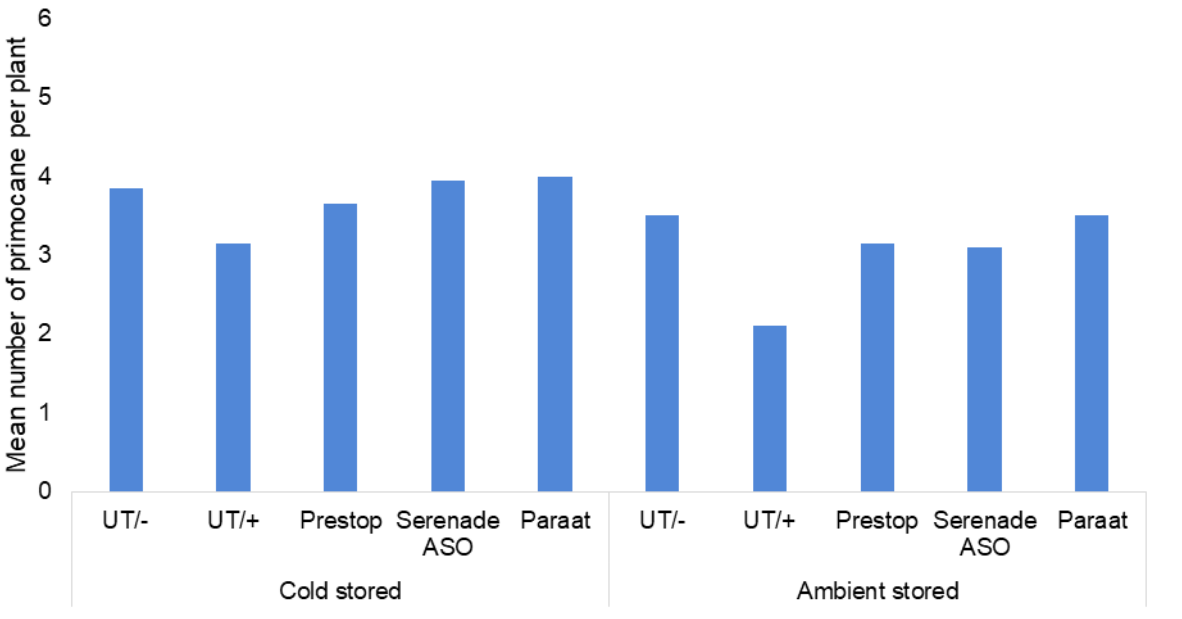

Figure 9. Mean number of primocanes per plant, Autumn treated plants, 27 June 2018. No significant differences.

On average 93.8% of primocanes produced in the Autumn treated experiment were healthy, with no significant differences between either storage regime or treatments (**Table 10**).

Table 10. Percentage of healthy primocanes in Autumn treated plants, 27 June 2018.

	% of primocanes that were healthy		
Treatment	**Cold stored**	**Ambient stored**	**Mean**
UT/-	88.5	98.3	93.4
UT/+ *P. rubi*	93.3	95.0	94.2
Prestop	93.1	91.2	92.2
Serenade ASO	89.8	96.7	93.2
Paraat	91.4	100.0	95.7
Mean	91.2	96.3	

ANOVA	**Storage**	**Treatment**	**Storage.Treatment**
P-value	0.085	0.709	0.661
l.s.d	5.75	12.86	12.86
df	(1,36)	(9, 36)	(4, 36)

Floricanes had been removed in August to allow the primocanes space to grow. October assessment of primocane symptoms included wilting, dark staining and purple lesions at base of cane. Across all inoculated plants, 30-50% of the primocanes exhibited *Phytophthora* spp. symptoms. No differences were seen in cane symptom incidence between treatments or storage regimes (**Table 11**). Primocane symptoms were recorded in the uninoculated untreated plants, but only 5% of canes were affected from ambient stored plants, whereas cold stored plants had similar symptom incidence to the aforementioned inoculated plants. These differences in the uninoculated were mirrored in the root analysis described later.

Table 11. Percentage of symptomatic primocanes in Autumn treated plants, 15 October 2018.

	% of primocanes with *Phytophthora* spp. symptoms		
Treatment	**Cold stored**	**Ambient stored**	**Mean**
UT/-	36.7	5.0	20.9
UT/+ *P. rubi*	40.0	35.0	37.5
Prestop	55.0	35.0	45.0
Serenade ASO	40.0	45.0	42.5
Paraat	50.0	45.0	47.5
Mean	44.3	33.0	

ANOVA	**Storage**	**Treatment**	**Storage.Treatment**
P-value	0.266	0.196	0.797
l.s.d	20.36	40.08	45.53
df	(1,36)	(9, 36)	(4, 36)

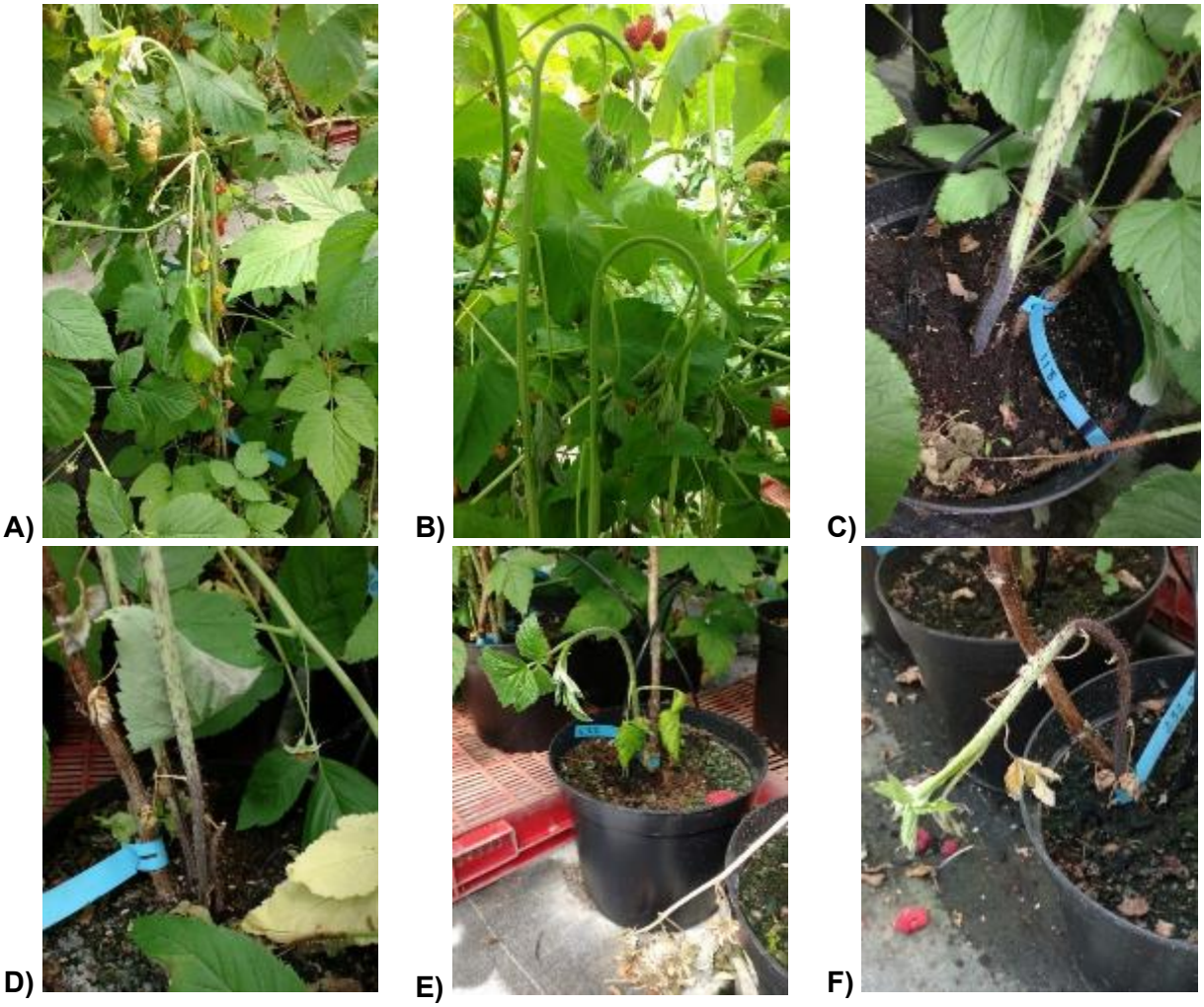

Figure 10. Phytophthora wilt of raspberry (cv. Tulameen) primocanes, seen throughout 2018 A) & B) show a typical shepherd's crook wilting of young primocane. C) & D) show dark staining at primocane base. E) & F) show primocanes beginning to wilt due to *P. rubi* in the stool.

October – primocane and root assessments

By October, new roots had grown out to the edge of the pots and were assessed. A range of roots were observed, including those that were white (healthy, new) or brown (rotted brown), or healthy (tan), as illustrated in **Figure 11**. Red roots (associated with *P. rubi*), as illustrated in **Figure 11** B - F, which were not seen in the Spring assessment before plants were inoculated, were recorded. Two LFD tests of red root samples, taken from **Figure 11** - B and D, indicated the presence of *Phytophthora* spp. on those two plants.

No differences in healthy white root coverage were seen between either treatments or storage regimes (**Table 12**).

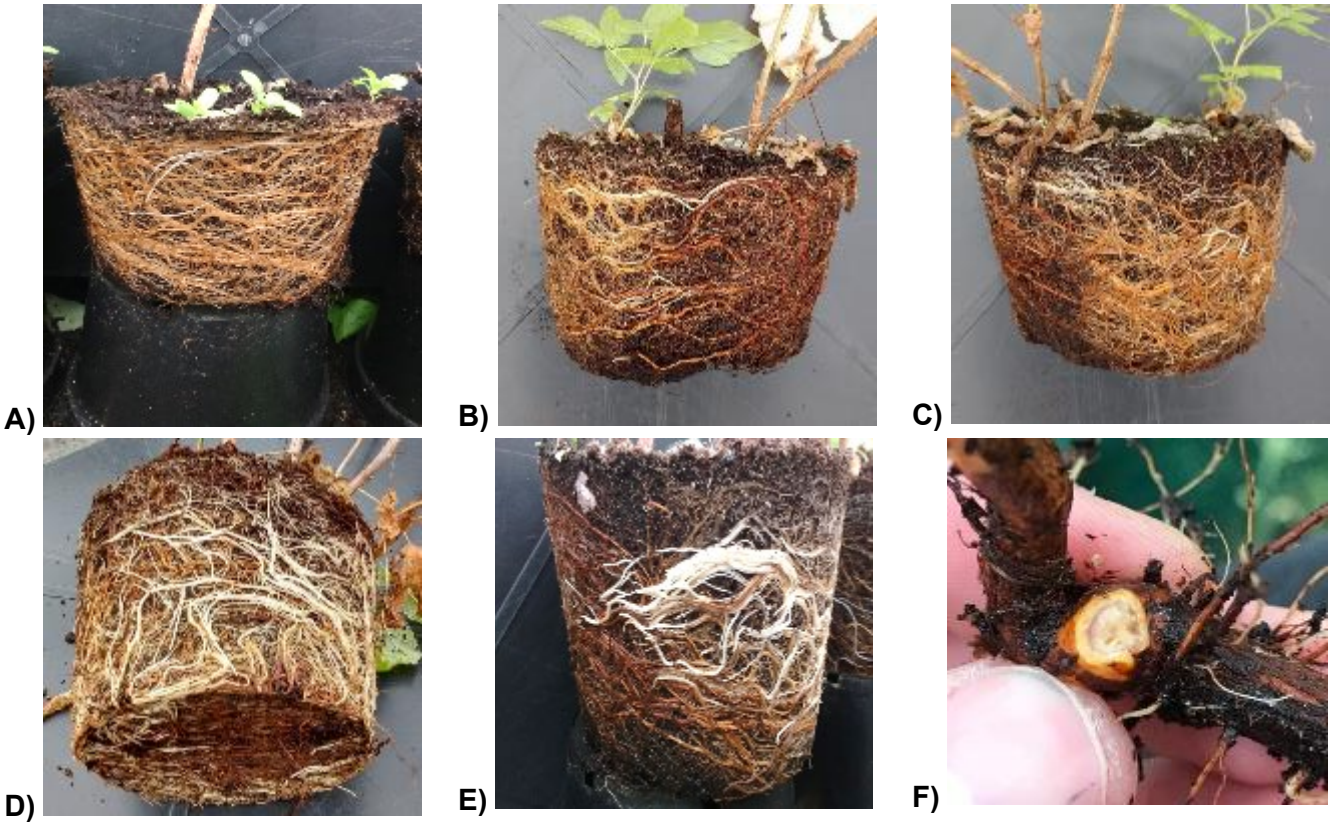

Figure 11. Range of root ball health of long cane cv. Tulameen plants. October, 2018. A) Healthy tan root ball of untreated uninoculated plant, B) red root patches on right, with death of primocane directly above, untreated inoculated plant C) red root (left) with wilted primocane above, next to healthy tan roots (right), untreated inoculated plant, D) healthy root ball with red root patch on underside which tested positive with *Phytophthora* spp. LFD, untreated inoculated plant, E) red roots amongst healthy new white roots, F) red staining on inner cortex of raspberry root.

Table 12. Percentage of root ball covered by healthy white roots in Autumn treated raspberry plants October 2018. Statistical comparison between treatments and each storage regime.

	% of root ball with white roots		
Treatment	**Cold stored**	**Ambient stored**	**Mean**
UT/-	36.3	33.2	34.8
UT/+ *P. rubi*	45.0	24.1	34.5
Prestop	36.0	48.3	42.1
Serenade ASO	35.3	30.0	32.6
Paraat	34.2	50.5	42.3
Mean	37.3	37.2	

ANOVA	**Storage**	**Treatment**	**Storage.Treatment**
P-value	0.981	0.671	0.205
l.s.d	10.83	17.12	24.21
df	(1,36)	(9, 36)	(4, 36)

There were no significant differences between either storage regimes or treatments in the proportion of brown root area on the root ball (**Figure 12** and **Table 13**), although a significant interaction was seen between storage and treatment, with treatments ranking differently between the storage regimes. The uninoculated untreated and the Autumn Paraat treated had more brown root coverage in cold stored plants compared with ambient stored plants. The inoculated untreated had significantly less brown root area (10.2%) in cold stored than in ambient stored plants (23.1%).

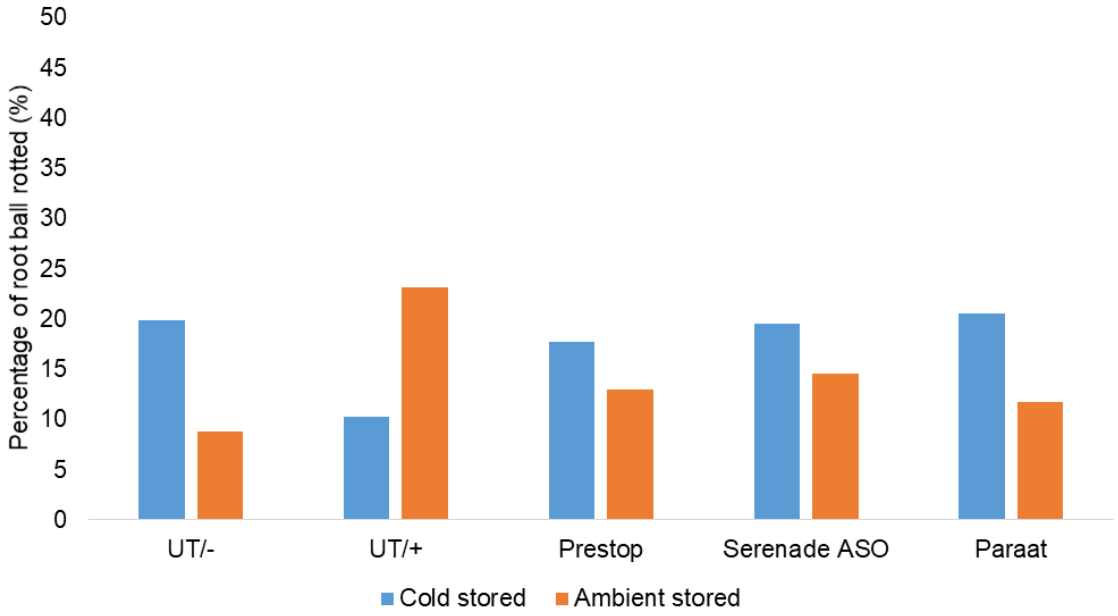

Figure 12. Proportion of Autumn treated raspberry plants' root balls that had brown rotting roots within each of the storage regimes, October 2018.

Table 13. Percentage of root ball covered by brown rotted roots in Autumn treated raspberry plants October 2018. Statistical comparison between treatments and each storage regime.

	% of root ball with brown rotted roots		
Treatment	**Cold stored**	**Ambient stored**	**Mean**
UT/-	19.83	8.75	14.3
UT/+ *P. rubi*	10.25	23.08	16.7
Prestop	17.75	13.00	15.4
Serenade ASO	19.50	14.50	17.0
Paraat	20.50	11.75	16.1
Mean	17.60	14.20	

ANOVA	**Storage**	**Treatment**	**Storage.Treatment**
P-value	0.201	0.205	0.046*
l.s.d	5.212	11.654	11.654
df	(1,36)	(9, 36)	(4, 36)

By October 2018, root reddening was seen on both tanned and new white roots, often in patches and in some pots the primocanes on the same side of the root ball were wilting or had died (**Figure 11**). Significantly (P = 0.019) more Autumn treated plants had red roots (31.3%), (associated with *P. rubi*) after cold storage than ambient storage (14.0%) (**Figure 13** & **Table 14**). The extent of the red areas were not recorded. Few of the cold stored uninoculated plants had red roots.

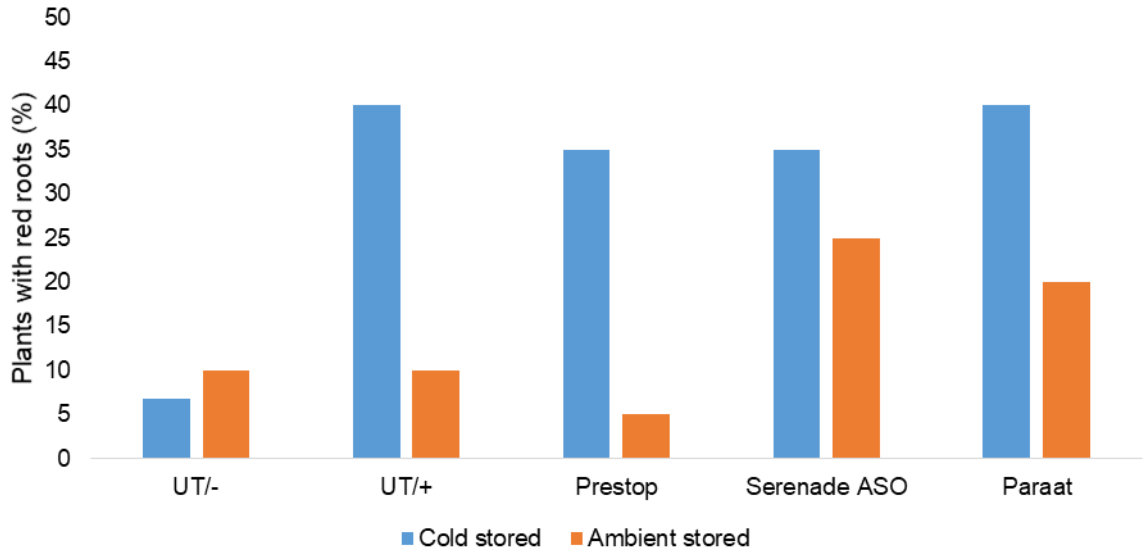

Figure 13. Red roots (associated with *P. rubi*) in the Autumn treated experiment in October 2018 showing significantly higher incidence in cold stored than ambient stored plants. Untreated UT/- remained uninoculated, all other treatments *P. rubi* inoculated in April 2018.

Table 14. Percentage of Autumn treated raspberry plants with red roots, October 2018.

	% of plants with red roots		
Treatment	**Cold stored**	**Ambient stored**	**Mean**
UT/-	6.7	10.0	8.4
UT/+ *P. rubi*	40.0	10.0	25.0
Prestop	35.0	5.0	20.0
Serenade ASO	35.0	25.0	30.0
Paraat	40.0	20.0	30.0
Mean	31.3	14.0	

ANOVA	**Storage**	**Treatment**	**Storage.Treatment**
P-value	0.019*	0.146	0.523
l.s.d	14.28	31.92	31.92
df	(1,36)	(9, 36)	(4, 36)

Experiment 2 – Spring treated raspberry plants

March – Root assessment

On arrival at Boxworth in March 2018 plant roots were assessed, prior to potting-on, drench application and inoculation. There were no significant differences between the proportions of either unhealthy brown roots (**Table 15**), or white new healthy roots (**Table 16**).

Table 15. Spring treated raspberry plants. Percentage of root ball with brown roots (%) at re-potting in March 2018.

	% of plant root ball with brown roots		
Treatment	**Cold stored**	**Ambient stored**	**Mean**
UT/-	9.2	9.6	9.4
UT/+ *P. rubi*	6.5	6.8	6.7
Prestop	10.8	4.6	7.7
Serenade ASO	7.9	8.1	8.0
Paraat	13.5	8.7	11.1
Mean	9.6	7.6	

ANOVA	**Storage**	**Treatment**	**Storage.Treatment**
P-value	0.189	0.409	0.469
l.s.d	3.058	6.838	6.838
df	(1,36)	(9, 36)	(4, 36)

Table 16. Percentage surface area coverage of Spring treated raspberry root balls with white healthy roots. Statistical comparison between all treatments across both storage regimes. March 2018.

	% of plant root ball with white roots		
Treatment	**Cold stored**	**Ambient stored**	**Mean**
UT/-	8.6	10.9	9.8
UT/+ *P. rubi*	13.2	6.0	9.6
Prestop	3.4	11.2	7.3
Serenade ASO	11.2	9.6	10.4
Paraat	5.7	10.8	8.3
Mean	8.4	9.7	

ANOVA	**Storage**	**Treatment**	**Storage.Treatment**
P-value	0.745	0.982	0.757
l.s.d	7.770	17.38	17.38
df	(1,36)	(9, 36)	(4, 36)

June – Cane assessment

In June, of the 200 floricanes in Spring treated plants, 56 plants showed some form of Phytophthora wilt (**Figure 14**), due to plot variability there were no storage or treatment differences (**Table 17**).

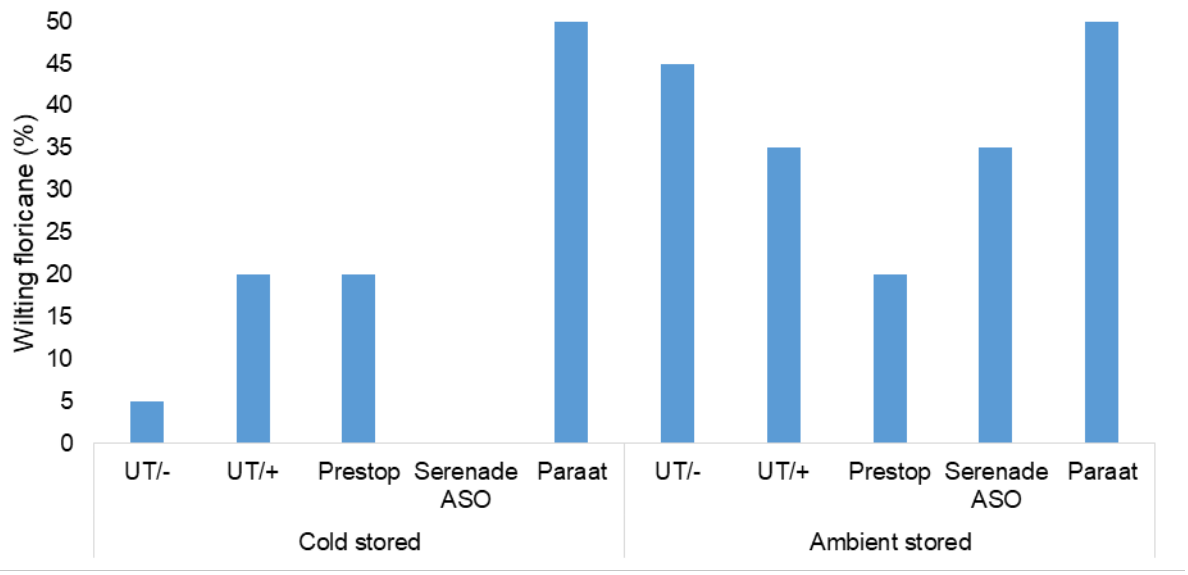

Figure 14. Percentage of floricanes that were wilting on 27 June 2018 in Spring treated module pots. No significant differences.

Table 17. Percentage of floricanes of Spring treated plants with Phytophthora wilt on 27 June 2018. All except untreated uninoculated (UT/-) had inoculation with *P. rubi* on 17 April 2018.

	% of floricane that were wilting		
Treatment	**Cold stored**	**Ambient stored**	**Mean**
UT/-	5.0	45.0	25.0
UT/+ *P. rubi*	20.0	35.0	27.5
Prestop	20.0	20.0	20.0
Serenade ASO	0.0	35.0	17.5
Paraat	50.0	50.0	50.0
Mean	19.0	37.0	

ANOVA	**Storage**	**Treatment**	**Storage.Treatment**
P-value	0.070	0.239	0.552
l.s.d	19.55	43.72	43.72
df	(1,36)	(9, 36)	(4, 36)

By the end of June, following Spring treatments, on average one additional primocane was produced by cold stored plants, resulting in significantly more ($P < 0.001$) than by ambient (**Table 18**). Serenade ASO treated plants had significantly more ($P = 0.045$) primocanes per

plant after cold storage, than after ambient storage. This difference between storage regimes, was not seen in Prestop and Paraat treated plants.

Table 18. Mean number of primocanes per plant, Spring treated, 27 June Cambridge 2018.

	Mean number of primocanes per plant		
Treatment	**Cold stored**	**Ambient stored**	**Mean**
UT/-	3.10	1.70	2.40
UT/+ *P. rubi*	2.95	2.35	2.65
Prestop	3.30	2.15	2.73
Serenade ASO	3.55	1.80	2.68
Paraat	2.35	2.00	2.35
Mean	3.12	2.00	

ANOVA	**Storage**	**Treatment**	**Storage.Treatment**
P-value	<0.001**	0.045*	0.668
l.s.d	0.5642	1.262	1.262
df	(1,36)	(9, 36)	(4, 36)

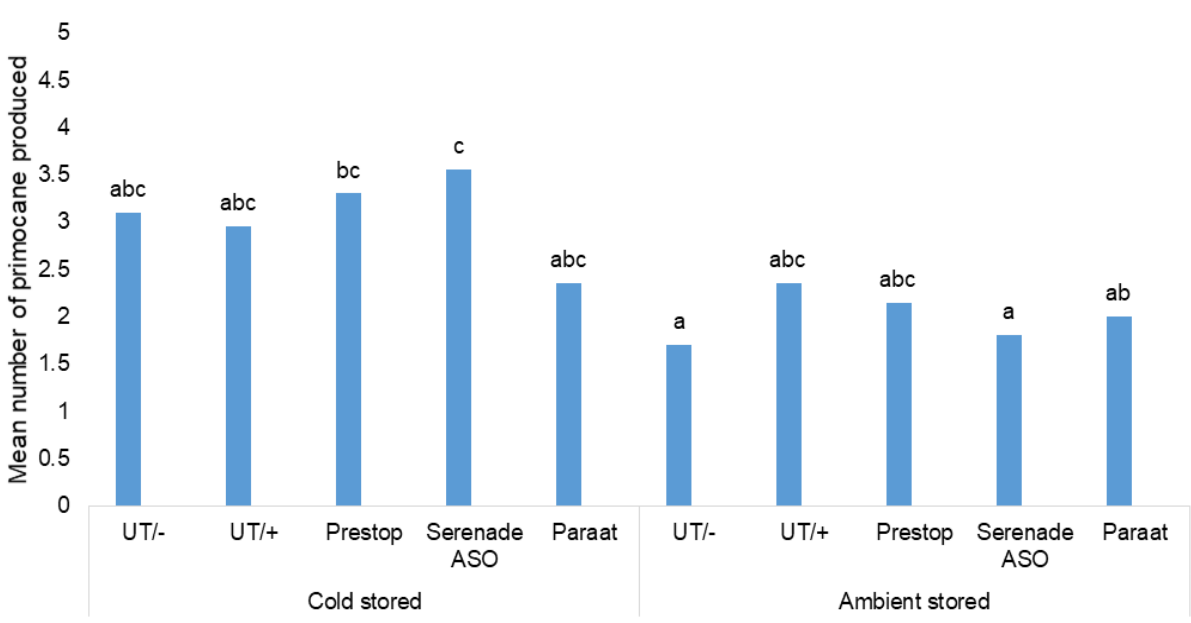

Figure 15. Mean number of primocanes per plant, Spring treated, 27 June Cambridge 2018. Significant differences indicated by differing letters.

Of the total 512 primocanes produced by June, 36 were wilting, of which 21 were from cold stored plants. The majority of primocanes, mean 85%, were not wilting, with no significant differences between storage regimes or treatments (**Table 19**).

Table 19. Percentage of primocanes from Spring treated plants that were healthy on 27 June 2018.

	% of primocanes that were healthy		
Treatment	**Cold stored**	**Ambient stored**	**Mean**
UT/-	98.0	68.3	83.2
UT/+ *P. rubi*	93.0	72.6	82.8
Prestop	95.7	85.0	90.3
Serenade ASO	83.5	85.0	84.2
Paraat	75.0	93.3	84.2
Mean	89.0	80.9	

ANOVA	**Storage**	**Treatment**	**Storage.Treatment**
P-value	0.208	0.430	0.163
l.s.d	12.93	28.91	28.91
df	(1,36)	(9, 36)	(4, 36)

Diagnosis of root rot in June

During cane assessment, plant No. 203.1 was symptomatic of *Phytophthora* sp. (wilting, lack of healthy primocane) and a sample of roots was taken from the edge of the pot. The roots were mainly healthy, but had some browning water-soaked sections. A sub-sample of these roots tested positive in a *Phytophthora* spp. LFD test.

Another sub-sample of these roots were floated in a Petri dish of filtered soil water to induce sporulation. After two days, a number of sporangia were observed on the surface of the roots. Under magnification (**Figure 16**), most appeared like *Phytophthora idaei* in morphology (papillate, predominantly spherical to ovoid), and one sporangia resembled *Phytophthora citricola* (highly variable sporangia, range from ovoid, obclavate and obpyriform, and semi-papillate) rather than *Phytophthora rubi* (non-papillate, ovoid to obpyriform) (Duncan, Cooke, & Young, 2003; Erwin & Ribeiro, 1996; Kennedy & Duncan, 1995).

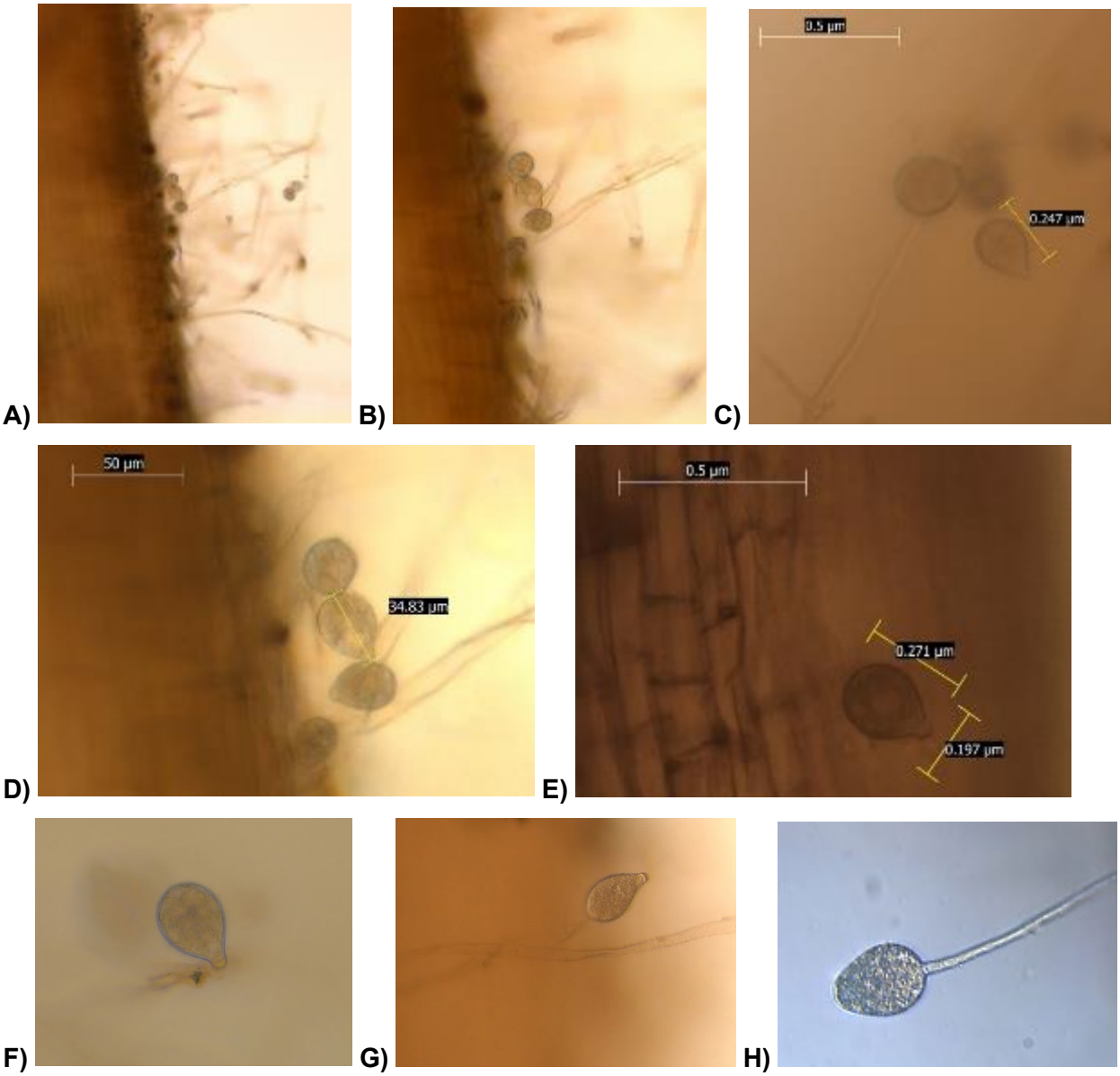

Figure 16. Images (A to G) taken of Phytophthora sporangia on surface of raspberry root, produced after two days in filtered soil water, June, 2018. A, B & F-H at variable scales. A to E show suspected *Phytophthora idaei* sporangia. F & G show obclavate papillate sporangia potentially *Phytophthora citricola*. H shows a non-papillate *Phytophthora rubi* sporangium, on agar plug taken from the pure ADAS culture used to inoculate plants.

October – primocane and root assessments

Floricanes had been removed in August to allow the primocanes space to grow. October assessment of primocane symptoms included wilting, dark staining and purple lesions at base of cane. Significantly more (P = 0.030) Spring treated raspberry primocanes exhibited *Phytophthora* spp. cane symptoms (**Figure 10**) after cold storage (mean 46%) than after ambient storage (mean 28.3%) (**Figure 17** & **Table 20**). There were no significant treatment differences (**Table 20**) in Spring treated plants showing *Phytophthora* spp. symptoms.

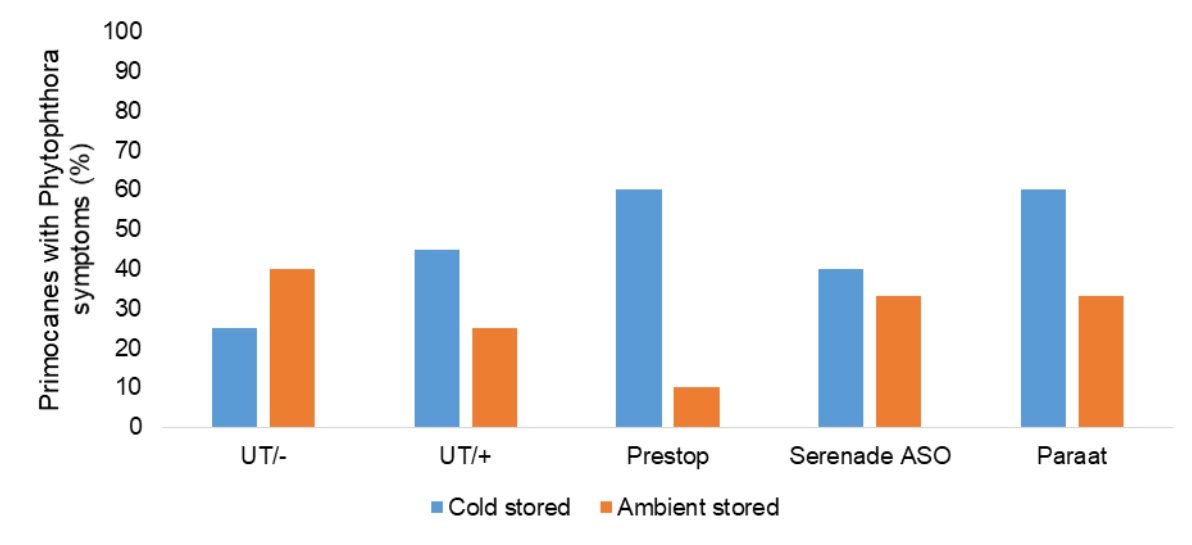

Figure 17. Percentage of primocanes per plant in the Spring treated experiment showing *Phytophthora* spp. symptoms, October 2018. Significant difference (P = 0.030) between storage regimes.

Table 20. Percentage of primocanes with *Phytophthora* spp. symptoms in the Spring treated experiment on 15 October 2018.

	% of primocanes with *Phytophthora* spp. symptoms		
Treatment	**Cold stored**	**Ambient stored**	**Mean**
UT/-	25.0	40.0	32.5
UT/+ *P. rubi*	45.0	25.0	35.0
Prestop	60.0	10.0	35.0
Serenade ASO	40.0	33.3	36.7
Paraat	60.0	33.3	46.7
Mean	46.0	28.3	

ANOVA	**Storage**	**Treatment**	**Storage.Treatment**
P-value	0.030*	0.196	0.131
l.s.d	15.62	40.08	34.94
df	(1,36)	(9, 36)	(4, 36)

By October, new roots had grown out to the edge of the pots, and were assessed. No significant differences occurred in either the percentage of healthy white roots (mean 36.6% of root area)

Table 21) or of brown rotted roots (mean 5.6% of root area) (**Table 22**). Symptoms seen were illustrated in **Figure 11**. The remainder of the root area, not recorded as either healthy white or rotted brown, comprised healthy tanned-brown roots.

Table 21. Percentage of root ball covered by healthy white roots in Spring treated raspberry plants October 2018. Statistical comparison between treatments and each storage regime.

	% of root ball with healthy white roots		
Treatment	**Cold stored**	**Ambient stored**	**Mean**
UT/-	40.3	39.5	39.9
UT/+ *P. rubi*	40.0	30.8	35.4
Prestop	33.5	55.3	44.4
Serenade ASO	25.5	36.8	31.2
Paraat	23.3	41.2	32.3
Mean	32.5	40.7	

ANOVA	**Storage**	**Treatment**	**Storage.Treatment**
P-value	0.142	0.378	0.361
l.s.d	10.94	24.40	24.44
df	(1,36)	(9, 36)	(4, 36)

Table 22. Percentage of root ball covered by brown rotted roots on Spring treated raspberry plants, October 2018. Statistical comparison between treatments and each storage regime.

	% of root ball with brown rotted roots		
Treatment	**Cold stored**	**Ambient stored**	**Mean**
UT/-	4.25	8.50	6.4
UT/+ *P. rubi*	3.50	4.67	4.1
Prestop	3.24	4.25	3.7
Serenade ASO	6.75	6.33	6.5
Paraat	7.50	6.50	7.0
Mean	5.00	6.10	

ANOVA	**Storage**	**Treatment**	**Storage.Treatment**
P-value	0.441	0.633	0.734
l.s.d	2.576	5.750	5.759
df	(1,36)	(9, 36)	(4, 36)

There were no significant differences in the incidence of red roots (a symptom of *P. rubi*) between either treatments or storage regimes by October in the Spring treated experiment. A mean 14.5% of plants had red roots present (**Table 23**).

Table 23. Percentage of Spring treated raspberry plants with red roots, October 2018.

	% of plants with red roots (*P. rubi*)		
Treatment	**Cold stored**	**Ambient stored**	**Mean**
UT/-	15.0	20.0	17.5
UT/+ *P. rubi*	10.0	10.0	10.0
Prestop	5.0	30.0	17.5
Serenade ASO	10.0	5.0	7.5
Paraat	25.0	15.0	20.0
Mean	13.0	16.0	

ANOVA	**Storage**	**Treatment**	**Storage.Treatment**
P-value	0.644	0.734	0.487
l.s.d	12.91	28.82	28.86
df	(1,36)	(9, 36)	(4, 36)

Fera Science Ltd. testing for diagnosis of individual Phytophthora species

In October 2018, five Autumn treated plants, consisting of the lower 100 mm of primocane and the entire root ball were sent to Fera Science Ltd. for *Phytophthora* spp. testing. Plants were chosen at random from across the trial area, to contain a range of red roots and cane symptoms suspected to be *Phytophthora* spp. Analysis included a microscopic visual examination of infected roots, to observe presence or absence of resting structures (oospores) typical of *Phytophthora* species.

A representative subsample was taken from the symptomatic roots all around the root ball, and symptomatic crown tissue when present, and used in real time PCR with *P. rubi* and *P. idaei* / *cactorum* primers. The primers were able to distinguish *P. rubi*, and *P. idaei* or *P. cactorum*, but not distinguish between *P. idaei* and *P. cactorum* (**Table 24**). It was shown that a *Phytophthora* species (most like *P. idaei*) that had not been used in the inoculation, was present in both an uninoculated and an inoculated plant. In one of the plants (No. 114.3) that showed positive for *P. idaei* but not *P. rubi*, typical *Phytophthora* spp. oospores were also seen. *P. rubi* was confirmed in the tissue taken from two of the inoculated plants sent for testing. Its absence in the other two plants may reflect the fact that the small amount of tissue selected for the PCR test was not infected or it could indicate that inoculation of these plants had not succeeded.

Phytophthora spp. LFD (Lateral Flow Device) tests on symptomatic roots from plants No. 101.4 and 126.3 were conducted prior to samples being sent to Fera Science Laboratories. Both LFDs came up as strong positives (clear red line). The positive LFD results for No. 101.4 and 126.3 coincide with the Fera Science analysis, where *Phytophthora* spp. oospores were observed and *P. rubi* and *P. idaei* detected, respectively. This suggests both *Phytophthora* species are picked up by the LFD test.

Table 24. Results of molecular diagnosis by real time PCR from five Fera Science Plant Clinic samples, cv. Tulameen taken from Experiment 1 in October 2018, selected at random to have a range of symptoms attributed to *Phytophthora* spp.

Plot.plant ID code	Treatment	Cold or Ambient Stored	*Phytophthora* spp. oospores (visual)	*P. rubi* detected by PCR	*P. idaei / cactorum* detected by PCR
101.4	Serenade + *P. rubi*	Cold	Yes	Yes	No
114.3	Untreated/-	Cold	Yes	No	Yes
126.3	Untreated + *P. rubi*	Cold	Yes	No	Yes
132.1	Prestop + *P. rubi*	Cold	No	No	No
144.2	Untreated + *P. rubi*	Cold	No	Yes	No

Eight symptomatic canes and their root balls were also sent to Aurélia Bezanger at the James Hutton Institute for isolation. Four cane bases and root balls were kept at ADAS and isolations made onto V8 juice agar. However, neither laboratory was able to isolate *Phytophthora* spp.. Difficulty in isolating a pure culture of *P. rubi* is a known problem to plant pathologists, particularly as rather unusually the mycelium in the agar plate does not form a distinct colony, but is often found to be intermingled/colonised by an unidentified Gliocladium-like fungus. Also it is often isolated with *Pythium* spp. and the slow-growing *P. rubi* often becomes overgrown. Molecular diagnosis now replaces the use of culturing on agar in most laboratories.

Comparison between Autumn and Spring treatment timings (across Experiments 1 and 2)

Analysis combining data from Experiments 1 and 2 either for each of the storage regimes, or for each of the treatments

Table 25) showed that by October there were no significant differences in the proportions primocanes with *Phytophthora* spp. wilt symptoms.

Table 25. Mean percentage of primocanes wilting per plant in October 2018, compared statistically by ANOVA across Autumn (Experiment 1) and Spring (Experiment 2) treated experiments.

Treatment	% of primocanes wilting					
	Cold stored		Mean	Ambient stored		Mean
	Exp.1	Exp.2		Exp.1	Exp.2	
UT/-	36.7	25.0	30.8	5.0	40.0	22.5
UT/+ *P. rubi*	40.0	45.0	42.5	35.0	25.0	30.0
Prestop	55.0	60.0	57.5	35.0	10.0	22.5
Serenade ASO	40.0	40.0	40.0	45.0	33.3	39.2
Paraat	50.0	60.0	55.0	45.0	33.3	39.2
Mean	44.3	46.0		33.0	28.3	

ANOVA	Exp.Storage	Exp.Treatment	Exp.Storage.Treatment
P-value	0.620	0.858	0.342
l.s.d	17.95	28.09	39.84
df	(1,72)	(4, 72)	(4, 72)

Analysis of red roots relating to storage regime (**Table 26**) showed that by October 2018 there were significantly (p = 0.037) more plants with red roots after cold storage (a mean 22.2% of plants with this symptom of *P. rubi*) than in plants that were ambient stored (mean 15.0% of plants). High proportions of plants treated in Autumn before cold storage, then Spring inoculated, had red roots. There was no significant difference in red root incidence between the treatments when combining the results across both the Autumn and Spring treated experiments (**Table 26**).

Table 26. The percentage of plants with red roots recorded in October 2018 by ANOVA across Autumn (Experiment 1) and Spring (Experiment 2) treated experiments.

Treatment	% of plants with red roots					
	Cold stored		Mean	Ambient stored		Mean
	Exp.1	Exp.2		Exp.1	Exp.2	
UT/-	6.7	15.0	10.8	10.0	20.0	15.0
UT/+ *P. rubi*	40.0	10.0	25.0	10.0	10.0	10.0
Prestop	35.0	5.0	20.0	5.0	30.0	17.5
Serenade ASO	35.0	10.0	22.5	25.0	5.0	15.0
Paraat	40.0	25.0	32.5	20.0	15.0	17.5

Mean	31.3	13.0	14.0	16.0

ANOVA	**Exp.Storage**	**Exp.Treatment**	**Exp.Storage.Treatment**
P-value	0.037*	0.282	0.368
l.s.d	11.85	20.20	29.32
df	(1,72)	(4, 72)	(4, 72)

Climatic conditions – Temperature

Abnormal temperature extremes occurred throughout this trial. Following a mild Autumn in 2017 snow fell in the week preceding storage. In 2018, extreme freezing conditions persisted between 22 February and 5 March and temperatures below -5°C could have caused damage to soft root growth on the plants outdoors (**Figure 19**). Plants in cold storage had instead been held just below 0°C (**Figure 18**).

Mild conditions were present for inoculation of *P. rubi* on 17 April 2018 and a mild day followed, but then on the 19 and 20 April, temperatures outdoors in the sun rose to around 25°C and the mean air temperature in the tunnel was 20°C, peaking at a maximum 30°C, near ground level (**Error! Reference source not found.**).

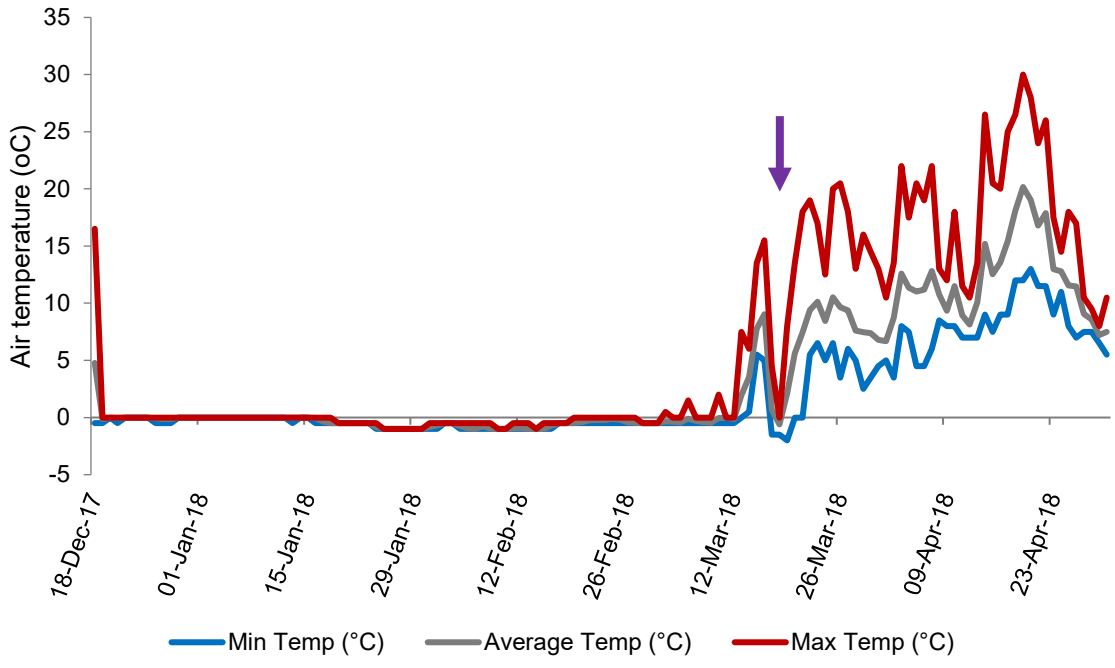

Figure 18. Air temperatures December 2017 to April 2018. Temperatures at pot height for plants in Experiments 1 & 2 moved from field to cold storage on 18 December 2017 and then moved into an open-sided store at Boxworth on 13 March 2018, before being re-potted and grown on (purple arrow) in a tunnel at ADAS Boxworth, Cambridgeshire, 2018.

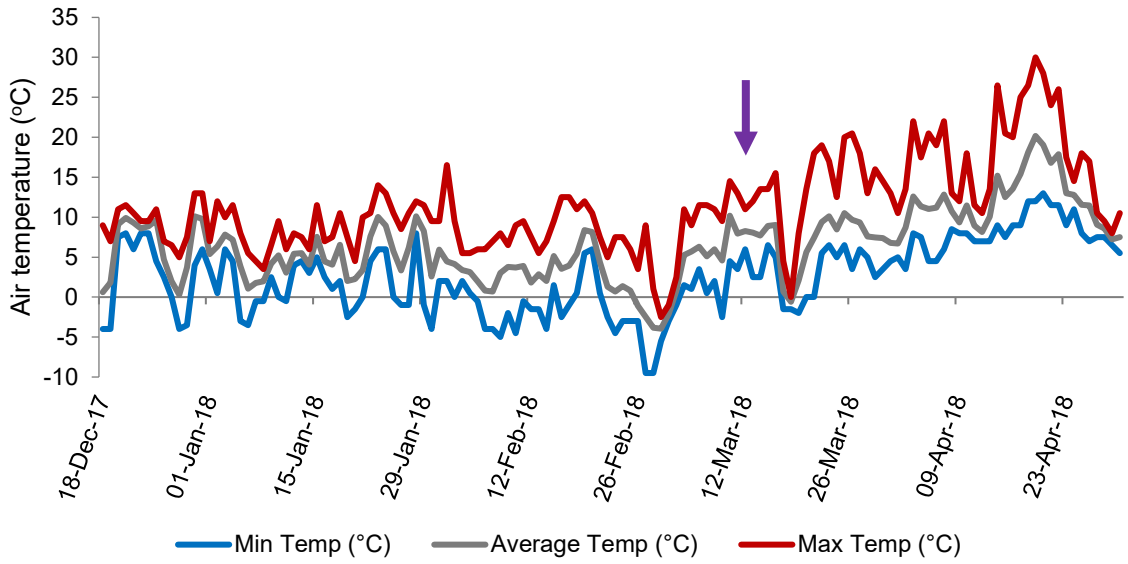

Figure 19. Air temperatures December 2017 to April 2018. Temperatures at pot height for plants in Experiments 1 and 2 remaining in ambient field conditions in Oxfordshire over Winter, then brought to ADAS Boxworth to an open-sided store to before re-potting and growing-on in a tunnel on 16 March 2018 (purple arrow) at ADAS Boxworth.

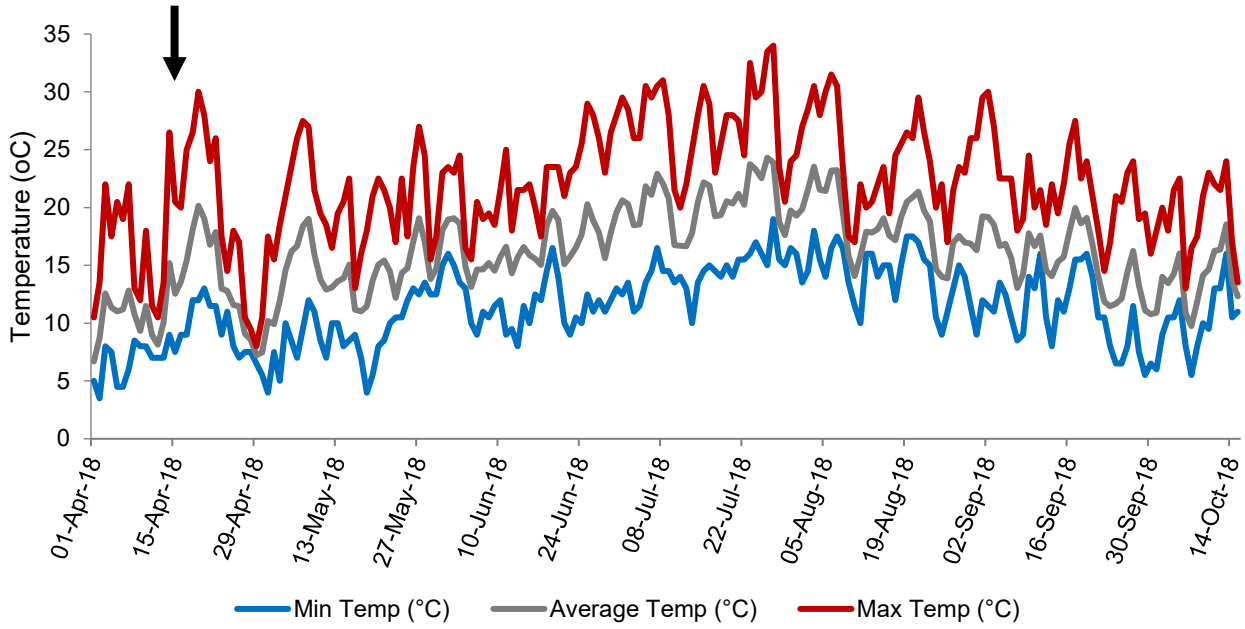

Figure 18. Air temperatures April 2018 to October 2018. Temperatures at pot height for plants taken from both cold and ambient storage in Experiments 1 and 2 growing in a tunnel at ADAS Boxworth. Black arrow indicates time of Spring inoculation with *P. rubi* on 16 April 2018.

Discussion

This trial has highlighted a number of important points around the cold storage of long cane raspberries, and the efficacy of biopesticides.

Unusually hot temperatures occurred throughout the 2018 growing season, and although neither LFD tests nor standard PCR show that an organism is alive (only that it has been detected) from subsequent plant symptoms, it is likely that *Phytophthora* spp. survived in the plant pots from "natural infection" in air temperatures below freezing and when air temperatures peaked above 30°C in Spring following inoculation. How the viability of the *Bacillus subtilis* bacteria in Serenade ASO and the fungus *Gliocladium catenulatum* in Prestop is affected by temperatures in the growing media was not part of the current research but should be determined to gain optimum performance. Serenade ASO is supplied as a liquid concentrate and the product label stipulates the bottle should not be stored below 4°C, and storage above room temperature can reduce shelf life. Prestop is supplied as a powder that the label says should be stored below 4°C. Rehydrated for application Prestop is active from 10°C (ICL, 2018). Replication of this work is needed across future years, or under controlled conditions, to understand more fully the effects of temperature on the tissue structure and infestation rate and the survival of both pathogens and the living organisms in biofungicides.

Baseline root assessments conducted post-winter found plants that received treatment in Autumn and were ambient stored, to have higher levels of root browning that those that were cold stored. In Spring treated plants, there were no differences in root browning between storage regimes. This suggests that an Autumn product application combined with ambient storage, could lead to increased damage to roots in long cane potted raspberry. Black-to-white discolouration of roots due to freeze damage in plants stored at ambient highlights the benefit cold storage provides in protecting canes from fluctuating temperatures. An LFD test of the blackened roots detected *Phytophthora* spp., however, this early season increased root damage in Autumn treated, ambient stored plants did not have a significant impact on plant health later on in the growing season.

A low incidence of floricane wilting occurred in June, which was not unexpected from infection progressing from *P. rubi* inoculation in mid-April. However, two canes did show severe wilting, and subsequent root floats revealed *Phytophthora* sp. sporangia typical of *P. idaei*, not *P. rubi*. This was later confirmed via DNA sequencing to be *P. idaei*, and with the speed of cane decline, was most likely present in the plant prior to the start of the trial.

Spring treated cold stored plants had produced more primocanes than ambient stored plants, at the June assessment. However, at the end of the growing season, Spring treated cold

stored plants had more wilting primocanes, than those plants that had been ambient stored. This indicates that although there might be an enhanced vigour associated with cold storage, there is also an associated increase in symptomatic primocanes. This would have implications for growers wanting to keep plants on another year.

Final assessments conducted at the end of the growing season saw major differences in incidence of red roots, of which were strong indicators of *Phytophthora* spp. infection. Patches of red roots were observed in plants from both storage regimes, but occurred significantly more in cold stored plants than ambient stored plants. In Autumn treated plants only, there was a greater incidence of red roots in cold stored plants, than ambient stored plants. This may indicate that treatments given in Autumn lose their efficacy by the following growing season. Across Spring treated plants, there was a similar incidence of red roots across all treatments. It is interesting to note that where the patch of red roots were extending to the top of the root ball, primocane death occurred in a few instances. On the other hand, where the patch of red root was just on the base of the root ball, no above ground cane symptoms were observed. It is still unknown how much of the root ball needs to be infected by *P. rubi*. before above ground symptoms begin, or how quickly the pathogen progresses once in the plant. Disease progress is likely differ depending on the growing and environmental conditions (including soil or substrate, outdoor or protected plants). Information on symptom development in other *Phytophthora* species, such as *P. idaei*, and their incidence in raspberries is also needed, in order to improve root rot management measures.

Conclusions

Within Autumn treated plants, the post-winter root assessment found Paraat drenching to result in plants with more white roots than either the untreated or the biological treatments. Ambient stored plants that received biofungicide treatments in Autumn, had the highest levels of brown rotted root, including damage caused by exposure to extreme cold damage. By October, more Autumn treated cold stored plants had red roots than ambient stored, but this was not reflected by any difference in primocane wilting.

By October, Spring treated plants that had been cold stored had a greater proportion of primocanes with *Phytophthora* spp. wilt than ambient stored plants, however the proportion of plants with red roots was the same in both cold stored and ambient stored plants.

- Drenching with Paraat (dimethomorph) in Autumn can improve the proportion of healthy new roots after winter, particularly in plants that remain outside rather than being placed in cold storage.

- Autumn drenches of Prestop (*G. catenulatum*) or Serenade ASO (*B. subtilis*) to plants overwintered outdoors resulted in a greater area of the root ball developing brown rotted roots by Spring.
- General root browning observed in Spring on untreated plants was no greater whether plants had been cold or ambient stored, with only around 8% of the root ball surface area damaged.
- Fewer plants overwintered in ambient conditions had red roots (associated with *P. rubi*) by the end of the season compared with cold stored material.
- Paraat, Prestop or Serenade ASO protectant drenches in Autumn to cold stored plants are unlikely to reduce the development of red roots.
- No reduction in the proportion of primocanes with *Phytophthora* spp. symptoms is likely by Autumn drenches before either storage regime.
- The presence of a *Phytophthora* species other than *P. rubi,* was detected using molecular techniques in a limited number of plants.
- LFDs that pick up a range of *Phytophthora* species were shown to be of benefit in the current work and should be used more widely by growers. However, in all diagnostic work a negative result should not be taken as conclusive as only small samples of tissue are taken and the pathogen may cause symptoms beyond where it is located and sampled.
- Limited LFD sampling suggested that one or more *Phytophthora* species might be present in some of the propagation material before overwintering.

Aim

WP 1.4 – Explore the effect of cold storage of long cane raspberries on incidence and severity of *Phytophthora rubi* infection and the potential for protection using biofungicides against infection in Autumn.

Aim 1: To investigate any effect of cold storage and 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 investigate whether a plant protection product drench before or after Winter can reduce the impact of *P. rubi* infection taking place in the winter ahead of storage (Experiments 1 and 2)

Introduction

Around 70% of raspberry material in the UK is currently cold stored at -1°C overwinter between lifting and delivery to the grower. Cold storage ensures that the plants receive the necessary chilling period for even bud break and also allows the programming of harvest. Good yields and profit are needed to compensate for the higher cost of this propagation material, particularly when the plants are not carried forward into a second cropping year partially to minimise pest and disease carry-over. However, if plants become infested by *Phytophthora* spp. before overwintering then the pathogen can survive in cold storage. Work on strawberry has also indicated that cold stored plants can be more susceptible to *Phytophthora* sp. infection post cold storage (Pettitt & Pegg, 1994) and this possibility needs to be considered for raspberry and infection by *P. rubi*.

Under laboratory conditions, the release of zoospores from the sporangia of *Phytophthora* species is synchronised by chilling and then returning of cultures to room temperature. It is thus possible that returning cold stored infested plants to ambient conditions together with recommencement of watering in Spring may trigger a mass *Phytophthora* spp. zoospore release rather than a steady release after ambient storage and this may increase the incidence and severity of root infection in cold stored plants.

Materials and Methods

In 2018, long cane cv. Tulameen plants were selected while growing at the same UK propagator site as the trial in 2017/18. They held a Plant Passport equivalent to Basic 2. Before multiplication they originated from NAKT (Holland) as pre-basic root blocks. On 25 September 2018, two adjacent beds of six month old plants were selected for evenness of vigour (**Figure 20**). One bed was allocated to each experiment. Each 1.5 L rectangular pot contained two plants each 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 (200 plants) in each of the two experiments.

Figure 20. Long cane raspberry plants cv. Tulameen (two plants per module pot) on 25 September 2018 at the time of the first Prestop drench. Oxfordshire.

A comparison of the schedule of product treatment, overwinter storage and inoculation with *P. rubi* is given in **Table 27**. Products were applied only to Experiment 3 in 2018. Plants in both experiments were inoculated with *P. rubi* in Autumn (this differs from the work in 2017/18 when inoculation was carried out in Spring). All plants were topped at 1.5 m, as standard practice by the propagator.

Half of the plants from both experiments were taken into overwinter (2018/19) cold storage at the AHDB Sutton Bridge research station (this differs from work in 2017/18 when all plants were uninoculated and placed into a propagator’s cold store). The remainder of the plants were left outside at ADAS Boxworth, Cambridgeshire over the winter period.

Table 27. Experiments 3 and 4. Timings of drenches with plant protection products (PPP) commencing at a site in Oxfordshire in 2018, followed by inoculation with *P. rubi* and then storage, before plants were moved into tunnel at ADAS Boxworth in Spring 2019.

Timing	**Experiment 3 (Autumn drenched)**		**Experiment 4 (Spring drenched)**	
Autumn 2018	PPP Drenched	PPP Drenched	-	-
Autumn 2018	Inoculate	Inoculate	Inoculate	Inoculate
Winter 2018/19	Cold stored	Ambient stored	Cold stored	Ambient stored
Spring 2019	Potted-up & in tunnel	Potted-up & in tunnel	Potted-up & in tunnel	Potted-up & in tunnel
Spring 2019	-	-	PPP Drenched	PPP Drenched

The PPP treatments applied to modules in Autumn in Experiment 3, and due to be applied to pots in Spring in Experiment 4, are given in **Table 28**. Treatments in Experiment 3 were to be the same as in 2017/18 but an error at inoculation resulted in the Paraat treated plants having to be removed from the trial when the plants were packed for cold storage.

Table 28. Products and number of applications in either Winter 2018 (Experiment 3) or Spring 2019 (Experiment 4). Inoculation with *P. rubi* in Autumn 2018 (except T1 and T6) at ADAS Boxworth. Treatments 1-5 with cold storage are shaded in blue.

Experiment 3 (drenching in 2018 inoculation in 2018)		**Experiment 4** (inoculation in 2018 drenching in 2019)	
T1 UT no P. rubi	Cold Stored December 2018 to March 2019	T1 UT no P. rubi	Cold Stored December 2018 to March 2019
T2 UT		T2 UT	
T3 Prestop x2		T3 Prestop x2	
T4 Serenade x1		T4 Serenade x1	
T5 * missing		T5 Paraat x1	
T6 UT no P. rubi	Ambient storage outdoors December 2018 to March 2019	T6 UT no P. rubi	Ambient storage outdoors December 2018 to March 2019
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 EAMU 2773/15. On-label use is permitted for all protected edible and non-edible crops. 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 3, commencing on 25 September 2018 with Prestop only. On 19 October, Prestop drenching was repeated as permitted, and the single drenches of Serenade ASO and Paraat given. 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 nine seconds timing.

Plants were examined for any cane disease prior to the product applications, and checks made for any phytotoxicity subsequently. On 16 November 2018, all plants were brought to ADAS Boxworth, Cambridge, for inoculation.

Table 29. Treatments applied at 10% pot volume before either cold storage (T1-T5, highlighted in blue) or continued standing outdoors (T6-T10) in Cambridgeshire, 2018. Treatments other than T1 and T6 were inoculated with *P. rubi* in Autumn 2018.

Treat-ment	Product [MAPP Number]	Active ingredient	Recommended dose	Product /1.5L pot in 0.15L water	Application timing/s in 2018
T1	Untreated no *P. rubi*	-	-	-	-
T2	Untreated	-	-	-	-
T3	Prestop [15103]	*Gliocladium catenulatum* strain J1446	5 g/L water (0.5%)	0.75g	25 September 10 October
T4	Serenade ASO [15625]	*Bacillus subtilis* strain QT 713	10 L/ha in 1000 L/ha water (10 ml/L)	1.5 ml	10 October
T5* with-drawn	Paraat [15445]	dimethomorph	1 g per plant	0.75 g	10 October
T6	Untreated no *P. rubi*	-	-	-	-
T7	Untreated	-	-	-	-
T8	Prestop [15103]	*Gliocladium catenulatum* strain J1446	5 g/L water (0.5%)	0.75g	25 September 10 October
T9	Serenade ASO [15625]	*Bacillus subtilis* strain QT 713	10 L/ha in 1000 L/ha water (10ml/L)	1.5 ml	10 October
T10	Paraat [15445]	dimethomorph	1 g/plant	0.75 g	10 October

Autumn inoculation with *Phytophthora rubi*

Plants were inoculated on 19 November 2018. V8-juice agar plugs 8mm wide were cut with a cork borer from 11 day old *P. rubi* plates, where a dense mycelium had formed, with the exception of the untreated uninoculated plots. Inoculation used a field isolate of *P. rubi,* from Aurelia Bezanger of the James Hutton Institute (JHI), which had been isolated from infected raspberry cane earlier in 2018. Pathogenicity testing is underway at JHI. The isolate was grown on 10% V8-juice agar for 11 days, incubated at 20°C on a 8:16 hour light : dark cycle, to produce mycelium that filled the majority of a 45 mm diameter Petri dish.

Three holes were made into the module pot containing two long cane plants, and 16 agar plugs were placed into the holes, in a 5:6:5 formation along the pot. (This gave eight plugs per plant, the same number as used per plant in the 5 L pots in the 2017/18 experiments).The hole was refilled using the same Ericaceous growing-media as used for potting, and thoroughly watered. This placed the *P. rubi* at the natural site of infection.

After inoculation, plants remained under tunnel for 4 weeks to ensure the *P. rubi* infection established, and heavy rainfall did not wash the inoculum out of the 1.5L module pot. Uninoculated plants were kept separate to the inoculated plants in the tunnel.

On 17 December 2018, one month after inoculation, canes had suberized and the leaves and leaf petioles had dropped. 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 a deep wooden slatted crate. As plants had been inoculated, plastic bags were placed on the untreated uninoculated plants, to prevent cross-contamination from neighbouring pots. Plastic bags were left open so the humidity remained the same as un-bagged plants **(Figure 21)**.

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. The weather was dry and mild during packing, and the growing media was moist. The crate was transported to the Sutton Bridge cold stores, where plants were moved into their site specific crates (confirmed clean and dry) and were placed at -1 to +1°C. Temperature and humidity loggers were placed in the cold store crates. Plants were kept upright in the store because the square crate designed to fit into the store did not permit them to be stacked laying down in the same way as in the longer crates used in the propagator's store in 2017/18.

At crate packing, it was noticed that due to a labelling error, T5 and T6 had been switched round. Plants treated with T5 were removed from the trial. Additional plants taken from the same field were taken as replacements for T6. These plants had been in cold storage for four

weeks before being removed and stored outside in Cambridgeshire. Cold storage is thought to affect a plant's susceptibility to *Phytophthora* spp., so although the plants were not kept in cold storage for the usual 12 weeks, their time in cold store will still be noted.

Figure 21. Long cane raspberry plants packed into a crate in the field ready for transfer to cold storage (left) and placed upright in plastic crate at AHDB Sutton Bridge cold stores (right). 17 December 2018.

The plants will remain in cold storage and outside until early March 2019. 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). 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, and this year they will be in adjacent tunnels to give more space for the growth of the laterals than previously.

After removal to ADAS Boxworth in March 2019, observations will be made of bud break and any differences resulting from of any cane diseases. Root assessments will be made on all plants to assess the occurrence any root damage over the winter. Further records will be taken during 2019 of any floricane or primocane cane wilting, and the roots will be examined for any rotting at the destructive assessment in October.

Results

No phytotoxicity was visible on the foliage of the canes following treatments applications to the growing media in September and October 2018. At an assessment on 16 November all pots were given an index score for 9 for vigour (excellent) and none had either phytotoxicity or cane botrytis. In both Experiment 3 and 4 plants remained of equal good vigour throughout the growing period. When plants were brought to Cambridgeshire in November canes were of uniformly good width of 9 mm. By 17 December, as expected, canes had suberized except towards the tops.

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

Aim

WP 2.1 - To develop and maintain IPM approaches to successfully control two-spotted spider mite (TSSM) whilst controlling spotted winged drosophilia (SWD) and other pests with insecticides.

Aim 1: Determine the effect of Nutrimite on numbers of both released *A. andersoni* and any naturally-occurring predatory mites that feed on TSSM on a raspberry crop.

Aim 2: Determine the effect of plant protection products applied for the control of SWD and other pests on spider mite predators and biological control of TSSM on a raspberry crop with or without Nutrimite.

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 plant protection products 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. The pest has increasingly been identified as a high priority for research by the industry.

There is a limited range of acaricides for use in protected and outdoor raspberries. The EAMU for abamectin (Dynamec) MAPP number 13331 for use on protected raspberry (including tunnelled crops) will expire on 31 May 2019. The EAMU for the new MAPP number 13331 permits the use of Dynamec only on fully protected cane fruit crops, not on those grown in 'Spanish' tunnels. The current EAMU for tebufenpyrad (Masai) permits its use only on outdoor crops of raspberry. Therefore biological control of TSSM is key to successful management of TSSM on tunnel-grown raspberry.

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* can also occur. 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, 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. The naturally-occurring predatory mites are regarded as being more tolerant of pesticides than *P. persimilis* as their populations have been exposed to pesticides each season. 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 other pests e.g. aphids, capsids and raspberry beetle is a serious threat which the UK cane fruit industry faces.

In the first year of this project (2015), the effects of insecticides applied for control of SWD and other pests on two commercial raspberry crops were monitored. 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. The 2017 results indicated that after an application of a tank mix of deltamethrin (for SWD control) and thiacloprid (for blackberry leaf midge control) on 2 August, mean numbers of *P. persimilis* mites and eggs were 83% and 98% lower respectively than before the spray and mean numbers of *A. andersoni* mites and eggs were 55% and 67% lower. Although natural populations of *A. andersoni* seem to be more tolerant of pesticides than *P. persimilis*, the reduction in predator numbers may have been due to both some adverse effects of the tank mix applied and also to reduced numbers of TSSM as prey, as by the time the spray was applied, numbers of TSSM had been reduced to very low numbers by a community of predators including *P. persimilis. A. andersoni, Feltiella acarisuga* and *Stethorus punctillum*. Conservation of both released and natural predators is critical for maintaining robust TSSM control.

Typha pollen (Nutrimite™) is being used on the continent to boost populations of released omniverous predatory mite species e.g. *Amblyseius swirskii* for improved control of thrips, spider mites and whiteflies on commercial protected crops e.g. rose, sweet pepper and cucumber. Nutrimite has not yet been used on cane fruit crops but *A. andersoni* is known to feed on pollen and to benefit from Nutrimite (Pijnakker, personal communication) and Nutrimite is now being recommended on protected strawberry to boost numbers of other predatory mite species for thrips control on the continent (http://www.hortidaily.com/article/42717/Bring-a-swirskii-army-at-fighting-strength).

Nutrimite could therefore potentially increase numbers of both natural and released *A. andersoni* on raspberry crops before raspberry pollen is available for food so that higher numbers of the ‘standing army’ survive sprays applied for control of SWD or other pests.

Materials and Methods

Site

The work was done on a second year commercial tunnel and pot-grown raspberry crop grown in coir and bark substrate. The crop was at the same site and in the same field as the crop used in the work done in 2017 and was selected due to the confirmed presence of TSSM and *A. andersoni* in the field during the previous year. Sections of cut back cane left on the ground around the pots were collected on 21 March and placed into Tullgren funnels in the laboratory to extract any overwintering mites sheltering in the canes but no mites were recovered. However, a single *A. andersoni* was confirmed in a leaf debris sample taken from the surface of the growing medium in the pots on 21 March 2018 (Figure 22).

Figure 22. Overwintered raspberry plant at trial site 21 March 2018.

Experimental Treatments

1. Untreated control
2. Pollen (Nutrimite) at 500g/ha at 2-week intervals on 26 April, 9 May, 24 May and 7 June.
3. *A. andersoni* at one sachet per two linear metres on 26 April and 7 June.
4. Pollen (Nutrimite) as in treatment 2 and *A. andersoni* as in treatment 3.

Grower applications of *Phytoseiulus* and sprays for SWD control and cane management

- *Phytoseiulus persimilis* were released by the grower to all the tunnels for control of TSSM on 4 and 8 June at the rate of approximately 24 and 12 per m^2 respectively.
- A tank mix of spinosad (Tracer) and the entomopathogenic fungus *Beauveria bassiana* (Botanigard), both at the recommended rates, was applied to all tunnels using an air-assisted sprayer (Figure 23) for control of SWD and TSSM respectively on 31 July, 29 August and on 10 September.
- The crop was thinned between 11-14 May.

Figure 23. Grower's sprayer used for application of plant protection products.

Trial layout

Four polytunnels were used, one tunnel per treatment in order to reduce contamination of treatment plots with pollen being blown in the wind and with predatory mites walking from plant to plant up the tunnels (Figure 24). As an additional precaution, a 'buffer' tunnel was used on each side of the untreated control tunnel to reduce the risk of pollen or predatory mites moving sidewards between the tunnels. There were three rows of plants in each tunnel and the treatments were only applied to the middle row of plants and samples of leaflets for assessments were only taken from the middle row. Ten replicate assessment plots were marked out in each tunnel, each five metres long and spaced equally up the tunnel. The tunnels varied in length from 80m to 128m but assessment plots were always spaced equally within the tunnel.

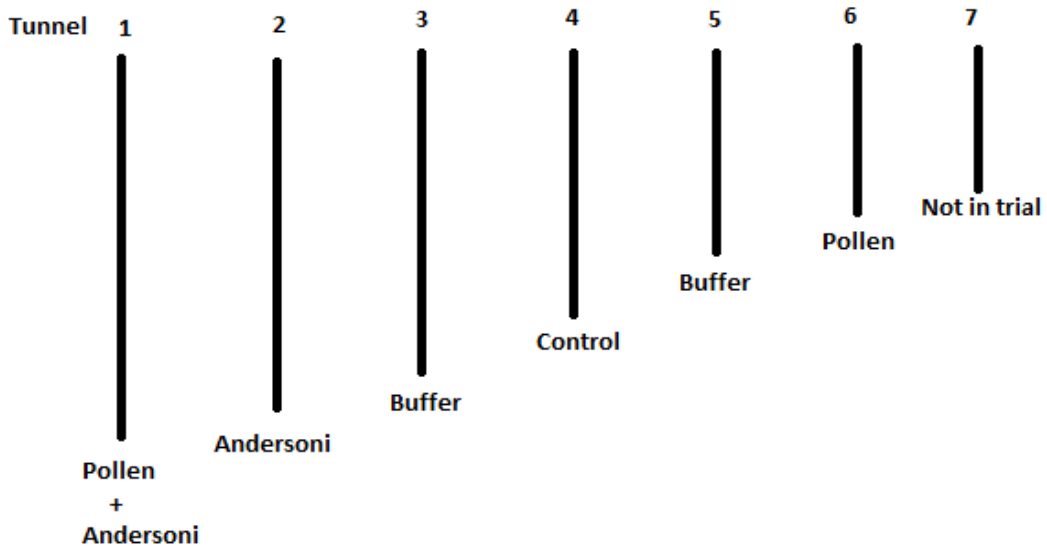

Figure 24. Tunnel and treatment layout.

Amblyseius andersoni application

The *A. andersoni* sachets were hung from the irrigation lines on the first application date on 26 April as the plants were still small and most of the plants were not touching (Figure 25). However, some plants had reached the bottom training string (Figure 26) and the others were estimated to reach the string within two weeks, when it was considered that the predatory mites could use the strings to move from plant to plant. By 24 May the plants were all touching and on the second *A. andersoni* application date on 7 June the sachets were hung from the leaf petioles approximately half way up the plants.

Figure 25. First release of *Amblyseius andersoni* sachets 26 April 2018.

Figure 26. Plants almost reaching the string on the first *A. andersoni* application date.

Pollen application

The pollen was applied on four dates at 2-week intervals (26 April, 9 May, 24 May and 7 June) at the recommended rate of 500g/ha using a Makita blower (Figure 27) after calibration of the blower to calculate walking speed during application. In between application dates the pollen was stored in the freezer. The Nutrimite™ pollen and the Makita blower were supplied by Biobest. A protective face mask and goggles were used during application.

Figure 27. Pollen application using Makita blower.

Pollen deposition assessments

Pollen deposition on the upper and lower surfaces of the leaves was measured on 24 May and 7 June immediately after application. Sticky transparent tape was placed on the upper and lower sides of three similar-size leaflets (Figure 28) in both the top and bottom canopy in each plot in each of the four treatment tunnels (30 leaflets from both the top and bottom canopy in each tunnel) then peeled off and placed onto red card (Figure 29). A small circular quadrat 2cm in diameter was placed in three replicate randomly selected positions on the card and the percentage area covered with pollen when viewed under a binocular microscope was recorded.

Figure 28. Removing pollen from a leaflet for pollen deposition assessment.

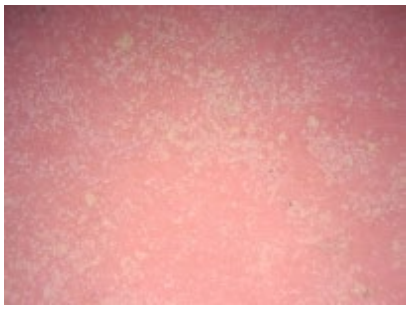

Figure 29. Pollen on sticky tape when placed onto red card for deposition assessment.

Assessments for spider mite and predators

On each assessment date three randomly selected terminal leaflets from the upper canopy and three from the lower canopy were removed the three largest primocanes (these would not be removed during cane thinning) from each plot in each tunnel (a total of 30 leaflets from both the upper and lower canopy per tunnel). The leaflets from each plot were placed in a separate zip-lock plastic bag and returned to the laboratory in a cool box. Assessments were made on the following dates:

- 26 April, immediately before the first *A. andersoni* and pollen application
- 9 May, before the second pollen application
- 24 May, before third pollen application
- 20 June, two weeks after the third pollen application and second *A. andersoni* release
- 30 July, the day before grower application of SWD spray
- 7 August, seven days after the SWD spray
- 5 September, seven days after the second SWD spray
- 17 September, seven days after the third SWD spray days post 3rd SWD spray

Both the upper and lower surfaces of each leaflet were examined under a binocular microscope and the following assessments were made:

TSSM assessments

The following records were made for each leaflet:

- Numbers of TSSM adults and juveniles (combined)
- Numbers of TSSM eggs
- Percentage leaflet area damaged i.e. with speckling caused by TSSM feeding (Figure 30)

Predator assessments

The following records were made for each leaflet:

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

Figure 30. TSSM damage

Temperature records

Temperatures were recorded using a USB datalogger in both the upper and lower canopies in the central row of plants.

Statistical analysis

Anova (Genstat edition 18.2) was used to analyse all data.

Results

Pollen deposition

No pollen was detected on the leaflet surfaces in the control tunnel or the tunnel treated with *A. andersoni* only. Pollen was detected on the upper and lower surfaces of the leaflets in the tunnels treated with pollen only and with pollen plus *A. andersoni*. There was significantly more pollen detected on the upper surfaces of the leaflets than the lower surfaces in the tunnel treated with pollen plus *A. andersoni* on both assessment dates ($P<0.01$, Table 30). In the tunnel treated with pollen only, there were no significant differences in the amount of pollen detected on upper and lower leaflet surfaces.

Table 30. Percentage of 2 cm quadrat covered in pollen on upper and lower surfaces of leaflets. Values not sharing any of the same letters are significantly different ($P<0.05$).

Treatment	24 May 2018		7 June 2018	
	upper surface	lower surface	upper surface	lower surface
Pollen + *A. andersoni*	0.72 b	0.03 a	4.5 c	0.37 ab
A. andersoni	0 a	0 a	0 a	0 a
Untreated Control	0 a	0 a	0 a	0 a

Pollen	0.15 a	0.12 a	2.3 b	0.1 b
P value	0.002	0.002	0.010	0.010
df	3, (108)	3, (108)	3, (108)	3, (108)
sed	0.17	0.1735	0.34	0.34
lsd	0.35	0.3454	0.68	0.68

Amblyseius andersoni numbers

All the predatory mites found on the leaflets except for *Phytoseiulus persimilis* were confirmed as *A. andersoni* except for one individual mite in the untreated control tunnel on 30 July which was confirmed as *Neoseiulus cucumeris*.

Amblyseius andersoni adults and juveniles (combined)

Upper plant canopy:

No *A. andersoni* were recorded on the leaflets in any of the treatment tunnels sampled on 26 April, immediately before the first release of *A. andersoni* sachets. On 9 May and 30 July, significantly more *A. andersoni* were recorded on leaflets in the tunnel treated with pollen and *A. andersoni* than in the tunnel treated with *A. andersoni* only and in the control and pollen only tunnels (P<0.05, Table 31, Figure 31 and Figure 39). The highest mean numbers of *A. andersoni* were recorded on 30 July in all tunnels, with a mean of 0.97 per leaflet in the tunnel treated with pollen and *A. andersoni*.

Table 31. Mean numbers of *A. andersoni* mites (adults and juveniles combined) per leaflet in the upper plant canopy. Values not sharing any of the same letters are significantly different (P<0.05). N.S.= not significant.

Date	Pollen + *A. andersoni*	*A. andersoni*	Untreated control	Pollen	P value	df	sed	lsd
27 April	0	0	0	0				
10 May	0.17 b	0.03 a	0 a	0 a	0.007	116	0.05	0.11
25 May	0.6	0.27	0.03	0				
20 June	0.17	0.2	0	0				
30 July	0.97 b	0.8 ab	0.37 a	0.33 a	0.045	116	0.27	0.53
7 Aug	0.13	0	0	0.03	0.097 (N.S.)	116	0.06	0.12
5 Sep	0	0	0	0				
17 Sep	0	0	0	0				

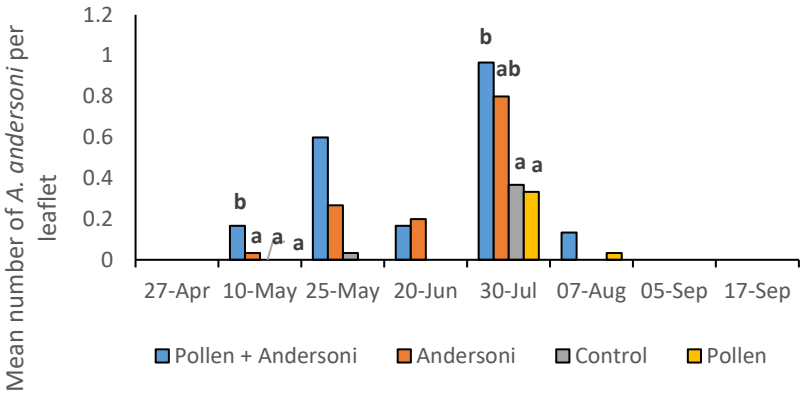

Figure 31. Mean numbers of *A. andersoni* mites (adults and juveniles combined) per leaflet in the upper plant canopy. Values not sharing any of the same letters are significantly different (P<0.05).

Lower plant canopy:

No *A. andersoni* were recorded on the leaflets in any of the treatment tunnels sampled on 26 April, immediately before the first release of *A. andersoni* sachets. On 24 May and 30 July, significantly more *A. andersoni* were recorded on leaflets in the tunnel treated with pollen and *A. andersoni* than in the control and pollen only tunnels (P<0.05, Table 32, Figure 32 and Figure 40) but the numbers of *A. andersoni* were statistically similar in the tunnels treated with *A. andersoni* with or without pollen. On 20 June, significantly more *A. andersoni* were recorded on leaflets in the tunnel treated with pollen and *A. andersoni* than in the other three treatment tunnels. The highest mean numbers of *A. andersoni* were recorded on 30 July in all tunnels, with means of 0.7 and 0.8 per leaflet in the tunnels treated with *A. andersoni* and pollen and *A. andersoni* alone respectively.

Table 32. Mean numbers of *A. andersoni* mites (adults and juveniles combined) per leaflet in the lower plant canopy. Values not sharing any of the same letters are significantly different (P<0.05). N.S.= not significant.

Date	Pollen + *A. andersoni*	*A. andersoni*	Untreated control	Pollen	P value	df	sed	lsd
27 April	0	0	0	0				
10 May	0.23	0.07	0.03	0.03	0.069 (N.S.)	116	0.09	0.17
25 May	0.47 c	0.4 bc	0 a	0.1 ab	0.008	116	0.16	0.31
20 June	0.5 b	0.2 a	0.1 a	0.03 a	0.003	116	0.13	0.26
30 July	0.7 b	0.83 b	0.17 a	0.07 a	0.001	232	0.23	0.45
7 Aug	0.17	0.07	0.13	0	0.181 (N.S.)	232	0.08	0.16
5 Sep	0	0	0	0				

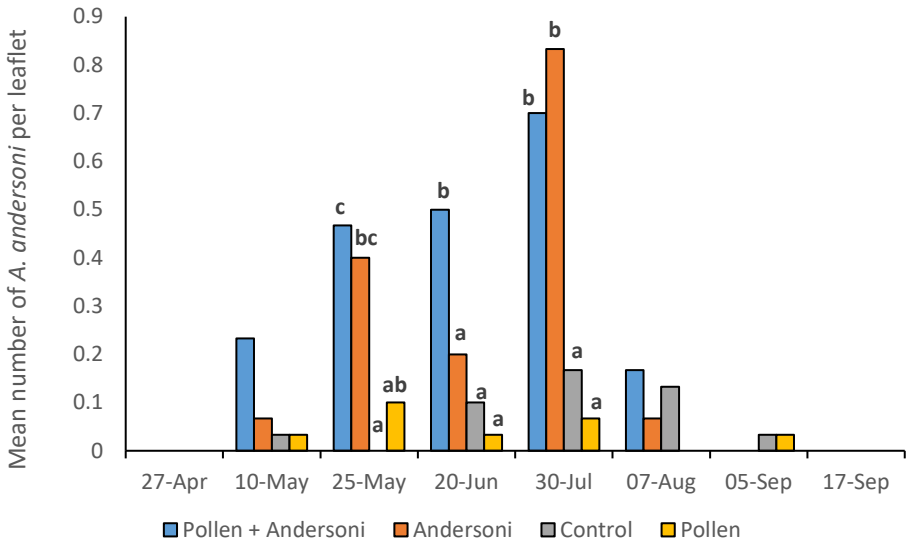

Figure 32. Mean numbers of *A. andersoni* mites (adults and juveniles combined) per leaflet in the lower plant canopy. Values not sharing any of the same letters are significantly different (P<0.05).

Amblyseius andersoni eggs

Upper plant canopy: *Amblyseius andersoni* eggs were only recorded on two dates. On 10 May, they were only found in the tunnel treated with *A. andersoni* and pollen, with a mean of 0.2 per leaflet (Figure 33) On 30 July, they were recorded only in the tunnels treated with *A. andersoni* with or without pollen, with means of 0.1 and 0.03 per leaflet. There were no significant differences between any of the treatments on these two dates.

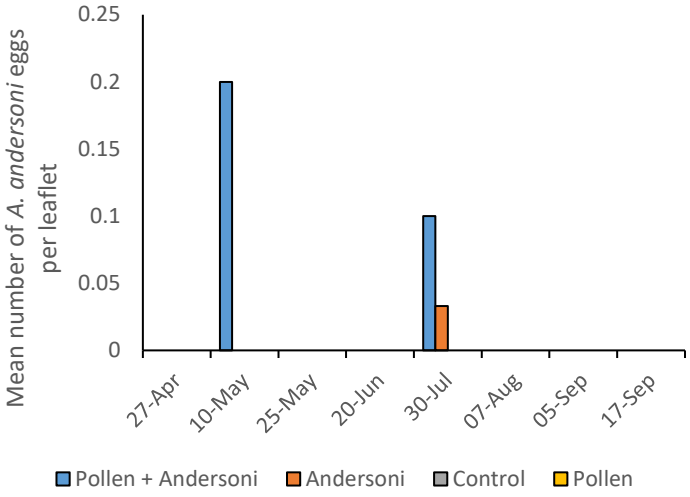

Figure 33. Mean numbers of *A. andersoni* eggs per leaflet in the upper plant canopy.

Lower plant canopy: *Amblyseius andersoni* eggs were recorded on all dates from 10 May. On 10 May, significantly more were recorded in the tunnel treated with *A. andersoni* plus pollen (mean of 0.37 per leaflet) than in the other three treatment tunnels (Figure 34). On the remaining dates, *A. andersoni* were found in all four tunnels but not on every date in all four treatments and mean numbers per leaflet were less than 0.1 per leaflet.

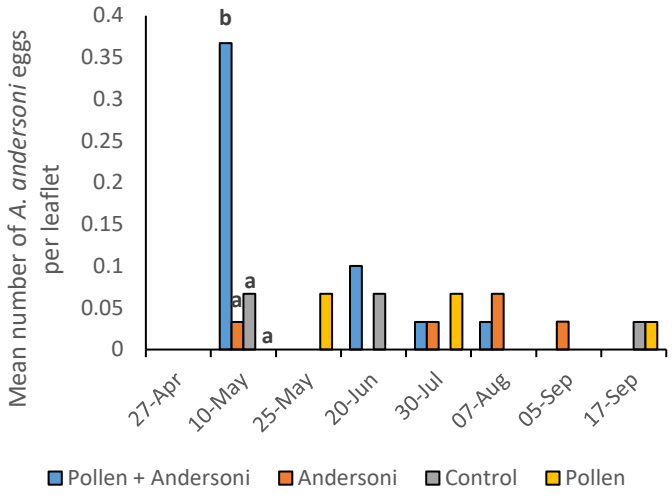

Figure 34. Mean numbers of *A. andersoni* eggs per leaflet in the lower plant canopy. Values not sharing any of the same letters are significantly different (P<0.05).

Phytoseiulus persimilis numbers

Phytoseiulus persimilis adults and juveniles (combined)

Phytoseiulus persimilis adults and juveniles were not recorded on the leaflets until 30 July when there were significantly more (P<0.05) in the tunnel treated with *A. andersoni* and pollen than in the other three tunnels in the top canopy (mean 0.8 per leaflet), Table 33, Figure 41). On the same date, there were significantly more (P<0.05) in the tunnel treated with *A. andersoni* and pollen than in the control and pollen only tunnels in the bottom canopy (mean 3.4 per leaflet), Table 34, Figure 42. No further *P. persimilis* were found in the top canopy during the trial. On 7 August, low numbers of *P. persimilis* were recorded in all four tunnels and on 5 and 17 September very low numbers were recorded in the tunnels treated with *A. andersoni* with/without pollen but there were no significant differences between treatments.

Table 33. Mean numbers of *P. persimilis* mites (adults and juveniles combined) per leaflet in the upper plant canopy on 30 July. Values not sharing any of the same letters are significantly different (P<0.05).

sed	Pollen + *A. andersoni*	*A. andersoni*	Untreated control	Pollen	P value	df	sed	lsd
30 July	0.77 b	0.1 a	0.13 a	0 a	0.047	116	0.29	0.59

Table 34. Mean numbers of *P. persimilis* mites (adults and juveniles combined) per leaflet in the lower plant canopy. Values not sharing any of the same letters are significantly different (P<0.05). N.S.= not significant.

Date	Pollen + *A. andersoni*	*A. andersoni*	Untreated control	Pollen	P value	df	sed	lsd
30 July	3.4 b	2.07 ab	0.4 a	0.13 a	0.048	116	1.31	2.59
7 Aug	0.2	0.1	0.1	0.07	0.589 (N.S.)	116	0.10	0.20
5 Sep	0.03	0.03	0	0	0.574 (N.S.)	116	0.03	0.07
17 Sep	0	0.03	0	0	0.396 (N.S.)	116	0.02	0.05

Phytoseiulus persimilis eggs

No *P. persimilis* eggs were recorded on any date.

Other spider mite predators

Low numbers of three other spider mite predators were recorded from 30 July, with no significant differences between treatments (data not shown). The predatory midge *Feltiella acarisuga* larvae and pupae (up to a mean of 0.2 per leaflet) were recorded on leaflets from the lower canopy in all tunnels from 30 July to 17 September. The predatory ladybird *Stethorus punctillum* (up to a mean of 0.1 per leaflet) were recorded on leaflets from the lower canopy in all tunnels except for the one treated with pollen and *A. andersoni* on 30 July and 7 August. The predatory bug *Orius* sp. (up to a mean of 0.1 per leaflet) were recorded on leaflets from both the lower and upper canopy on 30 July and from the lower canopy on 7 August.

TSSM numbers

TSSM adults and juveniles (combined)

Upper plant canopy:

TSSM adults and juveniles were only recorded on leaflets from the upper plant canopy on 25 May and 20 June, when maximum mean numbers were 0.07 per leaflet on 25 May with no significant differences between treatments on either date (Table 35, Figure 35).

Table 35. Mean numbers of TSSM mites (adults and juveniles combined) per leaflet in the upper plant canopy on 25 May and 20 June. N.S.= not significant.

Date	Pollen + *A. andersoni*	*A. andersoni*	Untreated control	Pollen	P value	df	sed	lsd
25 May	0.03	0.07	0.03	0.03	0.894 (N.S.)	116	0.05	0.10
20 June	0	0.03	0	0.07	0.53 (N.S.)	116	0.05	0.10

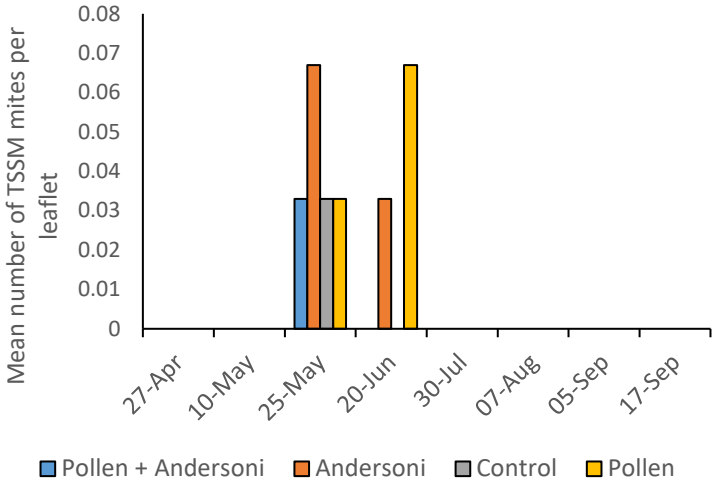

Figure 35. Mean numbers of TSSM per leaflet in the upper plant canopy.

Lower plant canopy:

TSSM adults and juveniles were found on every date except for 7 August and 5 September. Maximum mean numbers per leaflet were recorded on 20 June at between 1.1 and 4.8 per leaflet (Table 36, Figure 36). There were no significant differences between treatments on any date.

Table 36. Mean numbers of TSSM mites (adults and juveniles combined) per leaflet in the lower plant canopy.

Date	Pollen + *A. andersoni*	*A. andersoni*	Untreated control	Pollen	P value (all N.S.)	df	sed	lsd
27 April	0.03	0.17	0	0.1	0.13	116	0.08	0.15
10 May	0.37	0.83	0.07	0.23	0.47	116	0.51	1.01
25 May	1.03	2.0	0.33	0.17	0.06	116	0.73	1.45
20 June	3.37	4.83	3.97	1.13	0.57	116	2.74	5.42
30 July	0.03	0.1	0	0.1	0.56	116	0.08	0.17
7 Aug	0	0	0	0				
5 Sep	0	0	0	0				
17 Sep	0.13	0	0.07	0	0.53	116	0.11	0.21

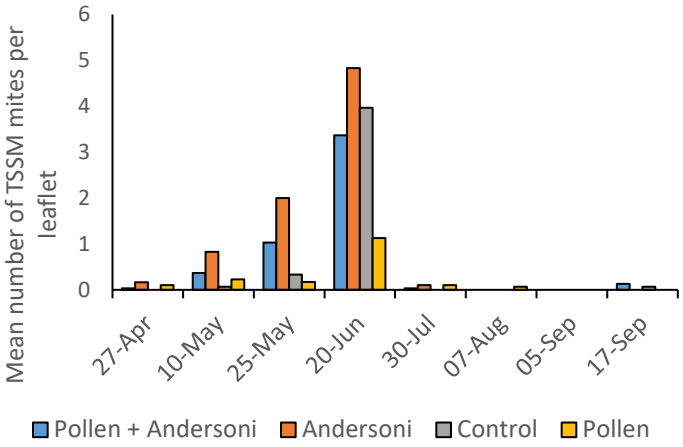

Figure 36. Mean numbers of TSSM per leaflet in the lower plant canopy.

TSSM eggs

Upper plant canopy:

TSSM eggs were only recorded on leaflets from the upper plant canopy on 25 May and 20 June, when maximum mean numbers were 1.8 per leaflet with no significant differences between treatments on either date (Table 37, Figure 37).

Table 37. Mean numbers of TSSM eggs per leaflet in the upper plant canopy on 25 May and 20 June. N.S.= not significant.

Date	Pollen + *A. andersoni*	*A. andersoni*	Untreated control	Pollen	P value (all N.S.)	df	sed	lsd
25 May	1.47	1.83	0.03	0	0.33	116	1.26	2.49
20 June	0	0	1.83	0.77	0.48	116	1.35	2.68

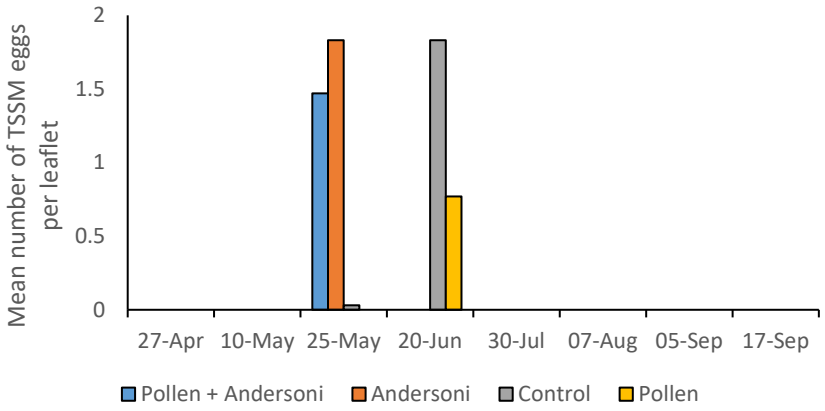

Figure 37. Mean numbers of TSSM eggs per leaflet in the upper plant canopy.

Lower plant canopy:

TSSM eggs were recorded on every date except for 5 September (Table 38, Figure 38). Mean numbers were less than three per leaflet except on 25 May and 20 June. Maximum numbers per leaflet were recorded on 25 May with a mean of 33.1 per leaflet in the tunnel treated with *A. andersoni* only which was significantly higher (P<0.05) than in the other three treatment tunnels.

Table 38. Mean numbers of TSSM eggs per leaflet in the lower plant canopy. Values not sharing any of the same letters are significantly different (P<0.05). N.S.= not significant.

Date	Pollen + *A. andersoni*	*A. andersoni*	Untreated control	Pollen	P value	df	sed	lsd
27 April	1.0	2.8	0	1.3	0.30 (N.S.)	116	1.46	2.89
10 May	0.6	1.4	0.33	1.23	0.85 (N.S.)	116	1.38	2.73
25 May	8.9 a	33.1 b	8.3 a	3.1 a	0.009	116	9.39	18.61
20 June	8.7	9.3	17.8	3.8	0.34	116	7.77	15.39
30 July	0.03	0	0	0.03	0.57 (N.S.)	116	0.03	0.06
7 Aug	0	0	0	0.07	0.40 (N.S.)	116	0.05	0.09
5 Sep	0	0	0	0	0.40	116	0.02	0.05
17 Sep	2.33	0	0	0.7	0.49	116	1.72	3.41

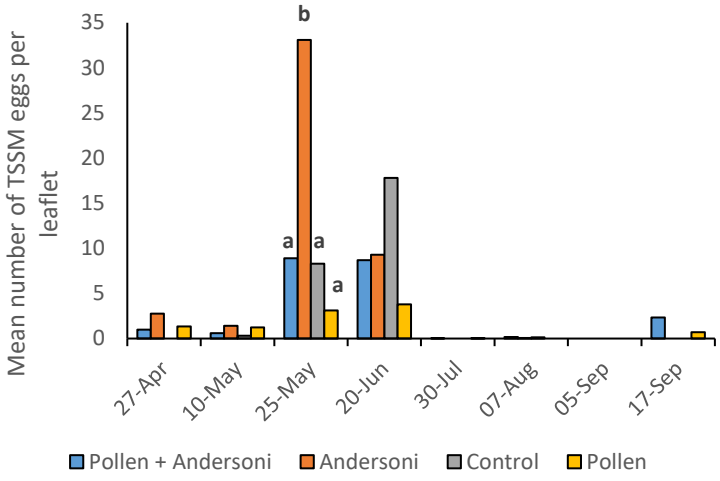

Figure 38. Mean numbers of TSSM eggs per leaflet in the lower plant canopy. Values not sharing any of the same letters are significantly different (P<0.05).

Correlation between numbers of leaflets with TSSM and A. andersoni

The numbers of leaflets in both the upper and lower canopy with TSSM mites and/or eggs and *A. andersoni* were collated to determine whether a correlation could be done on the numbers of leaflets with both the pest and the predator. This was done for two dates, 25 May and 20 June, as these were the only dates when TSSM and/or eggs were recorded on leaflets from the upper canopy and when the highest numbers of TSSM and/or eggs were recorded on leaflets from the lower canopy. Of the 240 leaflets sampled in all the tunnels on 25 May and 20 June, only 38 were infested with TSSM and/or eggs (Table 39). Of these 38 leaflets, only 10 and 7 on each date respectively also had *A. andersoni* present. *Amblyseius andersoni* were also recorded on 24 and 20 leaflets respectively that were not infested with TSSM or eggs.

Table 39. Numbers of leaflets sampled on 25 May and 20 June from all tunnels in both upper and lower leaf canopies with TSSM, TSSM eggs and *A. andersoni* present.

	25 May	**20 June**
Total no. leaflets	240	240
No. leaflets with TSSM and/or eggs but no *A. andersoni*	28	31
No. leaflets with *A. andersoni* but no TSSM or eggs	24	20

No. leaflets with both TSSM and /or eggs and *A. andersoni*	10	7
No. leaflets with neither TSSM or eggs or *A. andersoni*	178	182

As so few of the sample of the 240 randomly selected leaflets had TSSM, *A. andersoni* or both on them it was considered that a meaningful correlation between numbers of leaflets with the pest and predator on would not be possible.

TSSM damage

Upper plant canopy:

Spider mite damage was not recorded on the sampled leaflets until 25 May when it was only found in the tunnel treated with *A. andersoni* (Table 40, Figure 39). From 30 July spider mite damage was recorded in all tunnels. Significantly more damage (P<0.05) was recorded in the tunnel treated with *A. andersoni* plus pollen compared with the other three treatment tunnels on 30 July, 7 August and 5 September, with maximum mean damage recorded on 7 August (19.5% leaflet area).

Table 40. Mean % leaflet area with spider mite damage in the upper plant canopy. Values not sharing any of the same letters are significantly different (P<0.05). N.S= not significant.

Date	Pollen + *A. andersoni*	*A. andersoni*	Untreated control	Pollen	P value	df	Sed	Lsd
27 April	0	0	0	0				
10 May	0	0	0	0				
25 May	0	0.33	0	0	0.40 (N.S.)	116	0.24	0.47
20 June	0	0	0.1	0.13	0.39 (N.S.)	116	0.10	0.19
30 July	16.5 b	1.7 a	0.2 a	0.1 a	0.001	116	3.83	7.59
7 Aug	19.5 b	6.4 a	0.5 a	0.6 a	0.001	116	5.31	10.53
5 Sep	7.6 b	0.2 a	0.4 a	1.1 a	0.02	116	2.65	5.25
17 Sep	0.03	0.5	0.4	0.63	0.08 (N.S.)	116	0.24	0.47

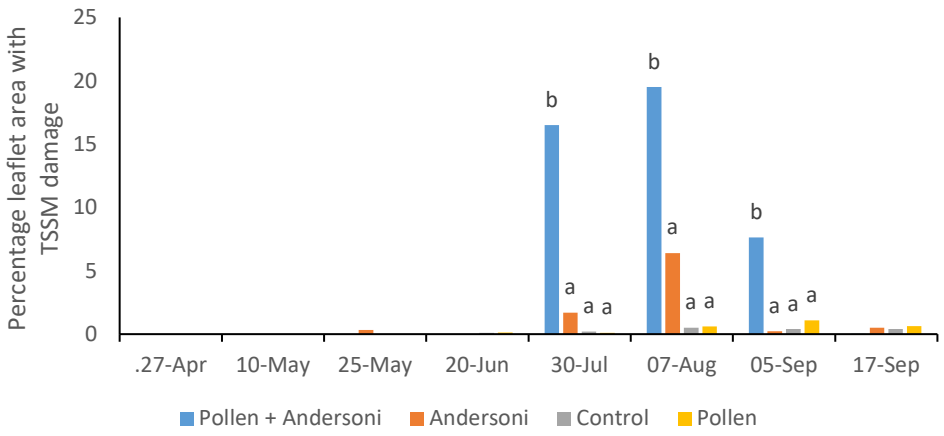

Figure 39. Mean % leaflet area with spider mite damage in the upper plant canopy. Values not sharing any of the same letters are significantly different (P<0.05).

Lower canopy:

Spider mite damage was recorded in all tunnels from 10 May (Table 41, Figure 40). From 30 July to 17 September, significantly more damage (P<0.05) was recorded in the tunnel treated with *A. andersoni* and/or the one treated with pollen and *A. andersoni* than in the control and pollen only tunnels. Maximum damage was recorded on 7 August in the tunnel treated with *A. andersoni* only (mean 50.7% leaflet area damaged).

Table 41. Mean % leaflet area with spider mite damage in the lower plant canopy. Values not sharing any of the same letters are significantly different (P<0.05).

Date	Pollen + *A. andersoni*	*A. andersoni*	Untreated control	Pollen	P value	df	sed	lsd
27 April	0.1	0.22	0	0.09				
10 May	0.13	0.17	0.1	0.1				
25 May	1.53	2.93	1.27	1.67				
20 June	1.37	1.82	2.17	0.5				
30 July	32.6 ab	49.0 b	29.4 a	19.6 a				
7 Aug	38.2 b	50.7 b	12.9 a	13.8 a				
5 Sep	25.5 bc	30.5 c	14.0 ab	3.6 a				
17 Sep	13.6 b	22.4 b	3.32 a	2.4 a				

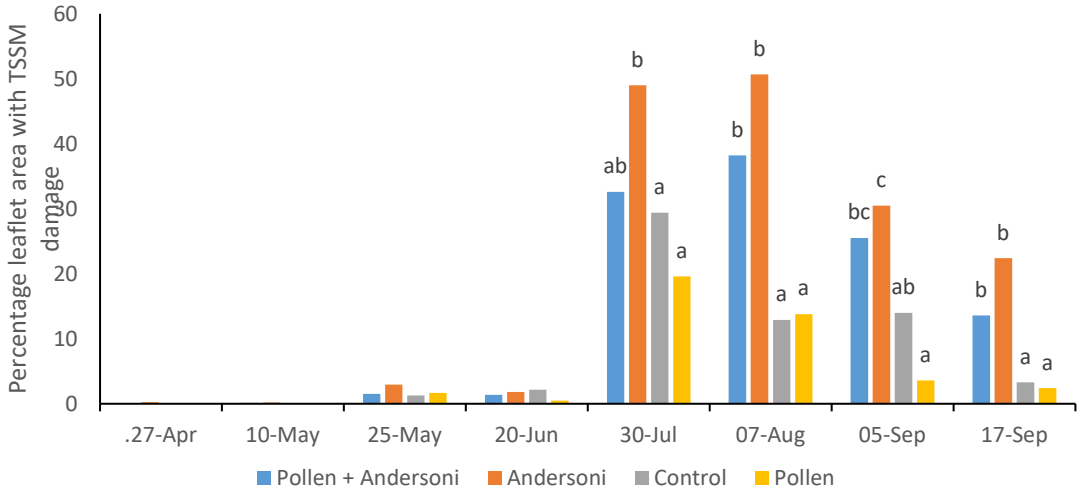

Figure 40. Mean % leaflet area with spider mite damage in the lower plant canopy. Values not sharing any of the same letters are significantly different (P<0.05).

Summary of mean numbers of TSSM, A. andersoni, P. persimilis and IPM programme

A summary of mean numbers of TSSM, *A. andersoni*, *P. persimilis* in the upper and lower plant canopies and release dates of predators and dates of plant protection products applied are shown in Figures 41 and 42.

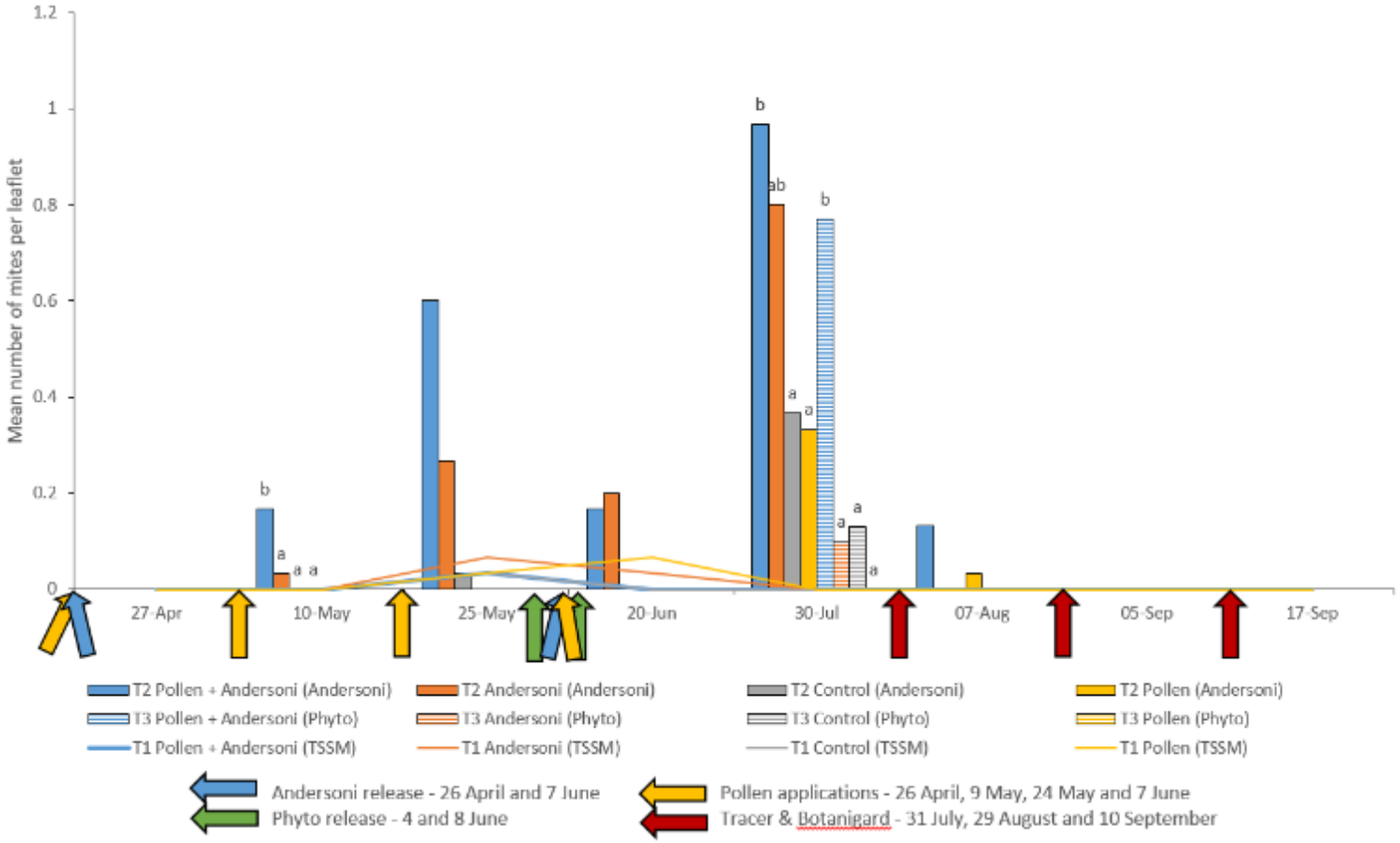

Figure 41. Mean numbers of *A. andersoni*, *P. persimilis* and TSSM per leaflet in the upper canopy.

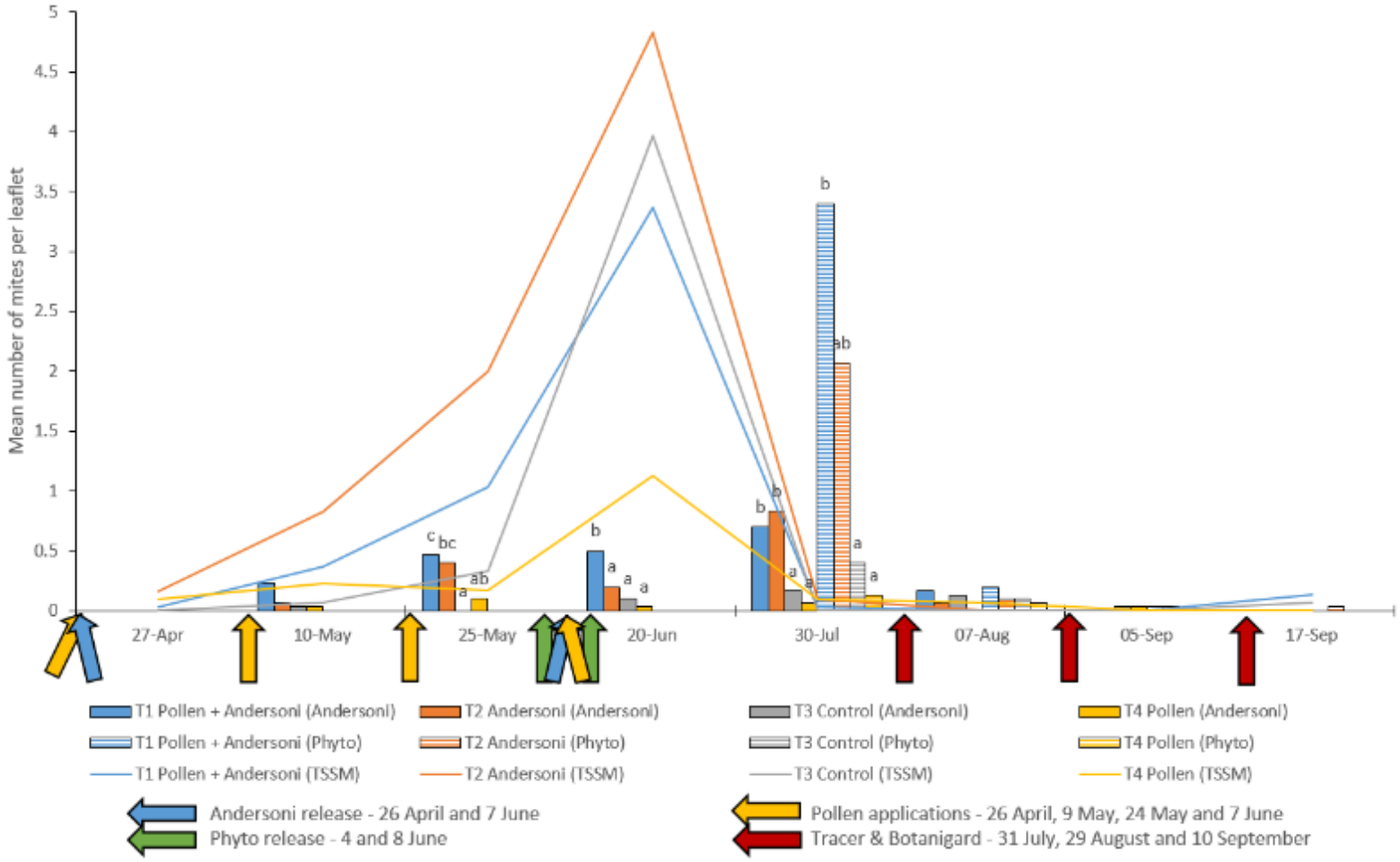

Figure 42. Mean numbers of *A. andersoni*, *P. persimilis* and TSSM per leaflet in the lower canopy

Discussion

Amblyseius andersoni is native to Europe and has a wide range of host plants including many trees and shrubs (van der Linden, 2004). The predator feeds on various prey including TSSM but will also feed on pollen, fungal spores and plant sap. *Amblyseius andersoni* is active at lower temperatures than *Phytoseiulus persimilis* and the naturally-occurring populations seem to be more tolerant of pesticides than *P. persimilis*. *Amblyseius andersoni* is known to overwinter in both leaf litter on the ground e.g. in apple orchards (Szabo & Penzes, 2013) and in crevices in overwintering raspberry canes (Linder *et al*., 2003). This overwintering behaviour is reported to offer potential cultural methods for aiding *A. andersoni* survival over the winter by leaving longer canes in overwintered raspberry crops and for aiding *A. andersoni* establishment in apple orchards by introducing leaf litter from old orchards to new ones.

The crop used for this work was in the same field as that used for the 2017 trial, where *A. andersoni* had been confirmed on a different raspberry crop. The results of sampling discarded cut back canes and leaf litter in the trial crop in March 2018 indicated that very few *A. andersoni* survived the winter of 2017/2018 as none were extracted from the canes and only one was found in leaf litter samples. The canes had been cut back very hard and the research by Linder *et al.* in Switzerland showed that *A. andersoni* overwintered in the bases of the canes when cut back to 40cm tall, therefore insufficient cane may have been left in the trial field for good overwintering populations of *A. andersoni.* This could explain the low incidence of naturally-occurring *A. andersoni* in the control and pollen only tunnels during 2018. The very severe winter of 2017/2018 may also have contributed to poor winter survival of *A. andersoni*.

During the trial, mean numbers of *A. andersoni* per leaflet were statistically equal on every assessment date in the control and pollen only tunnels, which indicated that adding pollen did not boost numbers of naturally-occurring *A. andersoni*. However, mean numbers of *A. andersoni* per leaflet in the tunnel treated with *A. andersoni* and pollen were statistically higher than in the control and pollen only tunnels on four dates; two dates in the upper canopy and three dates in the lower canopy. This result indicated that releasing *A. andersoni* and feeding them with pollen led to higher numbers of *A. andersoni* per leaf than where only natural *A. andersoni* were present.

Mean numbers of *A. andersoni* per leaflet in the tunnel treated with *A. andersoni* but no pollen were significantly higher than the control and pollen only tunnels respectively on only two dates. This result indicated that releasing *A. andersoni* without pollen was not as

effective as releasing the predators with pollen compared within where only natural *A. andersoni* were present.

Adding pollen to the tunnel treated with *A. andersoni* led to significantly higher numbers of the predators than in the tunnel where *A. andersoni* was released without pollen on one date in the upper canopy (10 May) and on one later date (20 June) in the lower canopy. *S*ignificantly more *A. andersoni* eggs were also recorded on leaflets in the tunnel treated with *A. andersoni* and pollen than in the tunnel treated with *A. andersoni* without pollen on 10 May in the lower canopy. Although not consistent on all assessment dates, this provides some evidence that adding pollen may have led to improved *A. andersoni* establishment on some dates.

One factor that is likely to have affected *A. andersoni* establishment after release is that when the first sachets were released on 26 April, the plants were not yet touching and not all had reached the first training string. Thus dispersal of the predators between the plants is likely to have been restricted until all the plants reached the first string a few weeks later. In addition, the crop was thinned between 11 and 14 May which led to some of the first release sachets being dislodged from the plants and falling to the ground. One factor that may have affected the potential benefit of adding pollen was that significantly more pollen was detected on the upper leaflet surfaces than the lower surfaces in the tunnel where *A. andersoni* was released. All the TSSM and predators were found on the lower leaf surfaces so pollen on the upper surfaces may not have been used as food. However, in the tunnel where only pollen was released there were no significant differences in the amount of pollen detected on upper or lower leaf surfaces and it is not known why the pattern of pollen deposition differed in the two tunnels where it was applied.

Active spider mites had been observed on some of the leaflets on 21 March when the crop was visited prior to setting up the trial. Spider mites and eggs were recorded on the randomly selected leaflets in the lower canopy from 27 April to 17 September but only on 25 May and 20 June in the upper canopy where numbers of TSSM were much lower. From 30 July, very few TSSM or eggs were recorded in the lower canopy, indicating that the population had crashed. This was likely to have been due to predation as on 30 July, *Phytoseiulus persimilis* had established after grower release in early June and on the same date, mean numbers of *A. andersoni* per leaflet peaked in both tunnels treated with *A. andersoni*.

It is not possible to quantify the control of TSSM given by *P. persimilis* or *A. andersoni* individually as *P. persimilis* was released to all the treatment tunnels. On 30 July, mean numbers of *P. persimilis* were significantly higher in the tunnel treated with *A. andersoni* and

pollen than in the other three tunnels in both the upper and lower canopies. Mean numbers of TSSM and TSSM eggs had not been higher in this tunnel than in other tunnels on any date. However, mean percentage leaf area damaged by TSSM was significantly higher in the tunnel treated with *A. andersoni* and pollen than in the other three tunnels on 30 July, 7 August and 5 September in the upper canopy and in both tunnels treated with *A. andersoni* than in the control and pollen only tunnels on 7 August and 17 September. This indicated that there had been more TSSM present in these tunnels at some point, possibly in between assessment dates, which could explain the higher numbers of *P. persimilis* in the tunnel treated with *A. andersoni* and pollen. From 30 July onwards, after the TSSM population had crashed, mean numbers of *P. persimilis* were absent in the upper canopy and were very low in the lower canopy. This is typical of *P. persimilis* populations which cannot survive without spider mite prey.

The highest numbers of *A. andersoni* in both tunnels where they had been released were recorded on 30 July in both upper and lower canopies, which coincided with the date when *P. persimilis* established and when mean numbers of TSSM crashed in all tunnels. However, whereas there were more *P. persimilis* in the lower canopy than in the upper canopy, reflecting TSSM distribution on previous assessment dates, mean numbers of *A. andersoni* distribution was not dependent on the presence of TSSM prey. Indeed, on 25 May and 20 June when the highest numbers of TSSM were recorded, only 10 and seven of the 38 leaflets on each date respectively that were infested with TSSM and/or eggs also had *A. andersoni* present. On the same two dates, *A. andersoni* was also present on 24 and 20 leaflets respectively that were not infested with spider mites. This result is consistent with that reported on rose where *A. andersoni* was present on randomly selected leaves both with and without spider mites, reflecting the mixed diet of this predator (van der Linden, 2004). In the research on rose, it was suggested that whereas *P. persimilis* tends to aggregate in TSSM patches and then dies out when no spider mite prey are available, *A. andersoni* has a different distribution in the host crop due to it being a generalist predator, thus the co-existence of the two species could lead to more stable biological control of TSSM. In the work reported here, it is likely that *A. andersoni* supplemented spider mite control by *P. persimilis* although its contribution cannot be quantified. In research in Switzerland, *P. persimilis* was more effective than naturally-occurring *A. andersoni* in controlling TSSM on tunnelled raspberry but in the following spring large numbers of overwintered *A. andersoni* emerged from the canes leading to good establishment on the crop and no TSSM infestation occurred (Linder *et al*, 2003). The other spider mite predators recorded in low numbers i.e. *Feltiella acarisuga*, *Stethorus punctillum* and *Orius* sp. will also have made a contribution to spider mite control.

The grower applied the first spray of spinosad (Tracer) for control of SWD on 31 July, the day after the crash in TSSM numbers on 30 July and repeated the application on 29 August and 10 September, so these sprays did not interrupt biological control of TSSM. The grower tank mixed the Tracer with Botanigard WP for TSSM control but this was unnecessary as by then effective biological control of TSSM had occurred.

Conclusions

- Numbers of naturally-occurring *A. andersoni* were low in both the control and pollen only tunnels.
- Adding Nutrimite® pollen to tunnels where *A. andersoni* was not released did not increase numbers of naturally-occurring *A. andersoni*.
- Releasing *A. andersoni* and adding pollen led to significantly higher numbers of *A. andersoni* than the naturally-occurring population in the control and pollen only tunnels on four dates.
- Releasing *A. andersoni* without pollen led to significantly higher numbers of *A. andersoni* than the naturally-occurring population in the control tunnel on only one date and in the pollen only tunnel on another date.
- Releasing *A. andersoni* and adding pollen led to significantly higher numbers of *A. andersoni* than in the tunnel where *A. andersoni* was released without pollen on two dates and lead to significantly higher numbers of *A. andersoni* eggs on one of these dates. Although not consistent on all assessment dates, this provided some evidence that adding pollen may have led to improved *A. andersoni* establishment on some dates.
- Factors that could have negatively affected *A. andersoni* establishment after the first release included limited dispersal due to the plants not yet being touching on the first release date, some sachets being dislodged during crop thinning and less pollen being deposited on the lower leaf surface where the predators reside than the upper leaf surface in one tunnel.
- *Phytoseiulus persimilis* established by 30 July and by then the TSSM population had crashed in all tunnels. On 30 July there were significantly more *P. persimilis* in the tunnel treated with *A. andersoni* and pollen than in the other three tunnels.
- The highest numbers of *A. andersoni* were recorded on 30 July in tunnels where they were released.
- There were significantly more TSSM eggs in the tunnel treated with *A. andersoni* without pollen on one date.

- There was significantly more spider mite damage to leaflets in tunnels treated with *A. andersoni* with/without pollen than in the control and pollen only tunnels but no significant differences in numbers of TSSM per leaflet in any of the treatment tunnels.
- *Amblyseius andersoni* were found on leaflets with or without spider mites or eggs, indicating that it was using other food sources in addition to TSSM.
- It is not possible to quantify the control of TSSM given by *P. persimilis* or *A. andersoni* individually but it is likely that *A. andersoni* supplemented the biological control of TSSM given by *P. persimilis*.
- *Feltiella acarisuga*, *Stethorus punctillum* and *Orius* sp. were recorded in low numbers and will also have made a contribution to spider mite control.
- The control programme for SWD using Tracer started on 31 July, the day after biological control of TSSM had caused the pest population to crash. Thus the SWD control programme did not interrupt biological control of TSSM.

Aim

WP 2.2 – Investigating the effect of air-assistance and spray quality on spray deposition throughout the canopy of a raspberry crop, and on the prevalence of 'spray refuges' for beneficial insects.

Aim 1: applying spray to a raspberry crop using four different spray machine setups, and quantifying the resultant spray coverage and deposition in four zones of the canopy.

Aim 2: assess the four different spray machine settings for differences in the number and distribution of spray refuges for beneficial insects.

Introduction

Restrictions on the use of acaricides in raspberry production means that two spotted spider mites (TSSM) are primarily controlled using beneficial insects rather than conventional pesticides. However, populations of beneficial insects can be negatively affected by insecticide sprays targeting other pests. In the SF 158 2018 Annual Report, section 'Objective 2.1', it was reported that air-assisted sprays of Decis for control of SWD and Calypso for control of Blackberry leaf midge contributed to a significant reduction in the populations of beneficial *P. persimilis* mites and eggs on sampled leaflets. In addition, the populations of *A. andersoni* were found to be lower, however, not significantly so. Populations of two other beneficial species (*Feltiella acarisuga*, and *Stethorus punctillum*) was also significantly negatively affected by insecticide sprays. In section 'Object 2.2' in the 2018 report, it was reported that overhead spraying greatly reduced the percentage spray deposition on the underside of leaves at the top of the canopy when compared with air-assisted knapsack spraying. This reduction in spray deposition on the underside of leaves is thought to provide a refuge for beneficial insects from insecticides allowing them to survive. Results from the 2017 trials at NIAB EMR found that there were significantly more natural phyotseiids on the leaves sprayed with the overhead system compared to air-assisted knapsack spraying. However, it is important to note that in these 2017 trials, plots with more spray refuges also had more aphids present, presumably able to survive due to the spray refuges.

The use of overhead spraying in commercial raspberry polytunnels is not common, with the majority of growers applying sprays using air-assisted axial or sometimes tower sprayers. Therefore this work was done to assess the spray deposition and distribution through the raspberry canopy with an air-assisted axial sprayer. Spray machines can be set up in different ways to control the output and distribution of the spray. Two readily accessible methods for growers to alter their spray machine set-up is to change the spray quality, specifically, the spray droplet VMD (Volume Median Diameter, i.e. droplet size); and to alter

the fan speed of the air-assistance. Air-assistance has two main effects on spray application: 1) carrying droplets into the crop canopy, 2) ruffling the canopy to expose hidden parts of the leaves and increase spray distribution. Spray operators typically use air-assistance at 100%, making an assumption that 'more and faster air equals better spraying'. This is not necessarily the case, as if air speed and volume are too high, the droplets can be blown off leaf surfaces and also carried through the canopy and blown out the other side or blown up and over the canopy.

With regards to spray quality (droplet size), the highest coverage of a surface from a given volume of sprayed liquid is achieved by spraying droplets which are as small as possible, larger droplets will provide less surface coverage and be more affected by gravity. Therefore we may expect that the highest coverage of the crop may be achieved using very fine spray quality and air-assistance at 100% (as per the assumption that 'more and faster air equals better spraying'). These are the settings many spray operators use. The 'optimum spray coverage' is variable and in most cases it is not known what the optimum spray coverage is for a specific crop, target, and product. Factors affecting optimum spray coverage include, the products being applied (including adjuvants), timing, droplet size (VMD), and droplet distribution. A rough 'rule of thumb' has been suggested that for foliar applications of insecticides and fungicides spray coverage should be between 10 – 15 % across the canopy (Deveau, 2017); others have reported that 50% coverage is considered optimal. It should be noted that both these figures are based on Water Sensitive Papers which are an unrealistic proxy for plant surfaces and may not represent actual spray coverage on the crop. Pesticide labels are, at best, vague on what coverage is required. Pesticide labels may contain statements such as "… Use spray volumes from 500 to 1500 litres of water to give full coverage…", but do not state what coverage is actually required for control. However, if we use the "10 – 15 %" coverage (Deveau, 2017) as a guide we may assume that any coverage below about 5 % leads to insufficient insect control and may provide a suitable refuge for beneficial insects, whilst coverage of over 25 % is not only wasteful of the plant protective product but also may significantly negatively affect beneficial insects.

Here we investigated firstly how spray quality: very fine compared to medium sized droplets, and air-assistance: full rate or half rate, whilst maintaining the same water volume, effects spray deposition throughout the raspberry canopy. Secondly we investigated the effects that these sprayer settings have on the number of refuges for beneficial insects within the raspberry canopy.

The work assessed two commonly available spray machine settings (air-assistance and nozzle type) and the resulting spray deposition. This work did not aim to optimise a spray

machine for a particular target spray coverage, as this would be extremely challenging and may not be transferable to other spray machines and situations.

Materials and Methods

Spray machine

The spray machine used during the trials was an Ideal Alsazia (**Error! Reference source not found.**) mounted onto a tractor. The machine has 16 nozzles (8 on each side). The nozzles were replaced with new ones at the start of the trials. Two sets of Albuz ATR 80 nozzles were used: yellow (for the 'very fine' spray) and blue (for the 'medium' spray). Both sets of nozzles were calibrated prior to the trials, with the yellow nozzles averaging 1.17 L/min/nozzle and the blue nozzles averaging 1.85 L/min/nozzle.

Figure 43: The Ideal Alsazia. A tractor mounted spray machine used during the trials.

In this experiment the water volume was kept constant (840 L/ha) throughout. This water volume was selected as a balance between achievable spray machine settings for the two different nozzles used in the trials, and a realistic water volume that spray operators use to spray raspberries which is anything between about 400 to 1000 L/ha. Two different spray qualities were used: 'very fine' and 'medium'. 'Very fine' spray quality is defined as having a VMD of 50 – 150 microns, whilst 'medium' spray quality is defined as having a VMD of 250 – 350 microns. To achieve these different spray qualities other parameters (pipe pressure, forward speed) had to be altered on the spray machine, as shown in **Error! Reference source not found.**.

Table 41: Spray machine settings for achieving constant water volume of 840 L/ha whilst altering the spray quality and air-speed. The trial had four treatments, which comprised two factors with two levels each in a full factorial design.

No.	Treatment	Nozzles	Pipe pressure (bar)	Spray VMD (microns)	Fan (RPM)	Air speed (m/s)	Forward speed (k/h)
1	Very fine 100% air	Yellow ART 80	10	50 – 150	2500	Top = 8 Middle = 13 Bottom = 25	5.2
2	Very fine 50% air	Yellow ART 80	10	50 – 150	1500	Top = 5 Middle = 9 Bottom = 13	5.2
3	Medium 100% air	Blue ART 80	3	250 – 350	2500	Top = 8 Middle = 13 Bottom = 25	8.1
4	Medium 50% air	Blue ART 80	3	250 – 350	1500	Top = 5 Middle = 9 Bottom = 13	8.1

Trial site

The spray trials were undertaken at Eagle Thorpe 4, Lutton Farm, using the same crop as used in Objective 2, WP 2.1 (**Error! Reference source not found.**). Tunnels labelled 3, 5, and 7 were used for the spray trials. These tunnels were the buffer tunnels for Objective 2, WP 2.1 trials. The dates of the spray trials were: 23rd, 24th, and 25th July 2018.

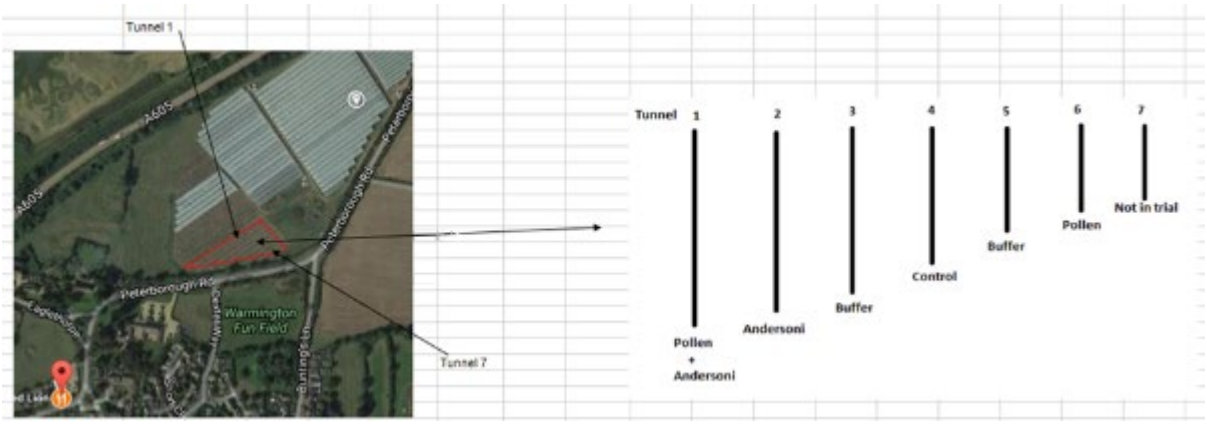

Figure 44: The tunnel layout at the trials site. The spray assessment trials were done in tunnels labelled 3, 5, and 7. Image courtesy of Sam Brown, ADAS, and Google Maps®.

Experiment design

The experiment was set up as cross-factorial, whereby each of the two levels of the factors investigated (spray quality and air-assistance) were tested with each other, as outlined in Table 42. Each of the four treatments was replicated three times over 3 days, and for each replicate, spray deposit was measured by two different handheld imaging fluorometers to provide a pseudo-replicate. The experiment design for one replicate is shown in Table 2**Error! Reference source not found.**; this was repeated on two consecutive days to generate a total of three replicates per treatment. The order of the treatments was varied on each day.

For each replicate of each treatment a 20 m plot in one of the polytunnels was sprayed. Within a polytunnel, each plot was separated by a 10 buffer zone. Spray deposit measurements were only measured on the centre row of polytunnels (both sides of the centre row), and within each plot the first and last metre was avoided. This was to minimise any risk of spray treatments contaminating adjacent treatments (**Error! Reference source not found.**45).

Table 42: Experiment design for one replicate of all the treatments. This was repeated on two consecutive days to provide a total of 3 replicates for each treatment. The order of the treatments was varied for each day.

Treatment No.	Spray date	Replicate (of 3)	Spray quality	Air-assistance	Canopy	Leaf	Number of samples (25 / imaging device)
1	23/07/2018	1	Very fine	100 %	Top	Upper	50
1	23/07/2018	1	Very fine	100 %	Top	Lower	50
1	23/07/2018	1	Very fine	100 %	Middle	Upper	50
1	23/07/2018	1	Very fine	100 %	Middle	Lower	50
1	23/07/2018	1	Very fine	100 %	Inner	Upper	50
1	23/07/2018	1	Very fine	100 %	Inner	Lower	50
1	23/07/2018	1	Very fine	100 %	Bottom	Upper	50
1	23/07/2018	1	Very fine	100 %	Bottom	Lower	50

Treatment No.	Spray date	Replicate (of 3)	Spray quality	Air-assistance	Canopy	Leaf	Number of samples (25 / imaging device)
2	23/07/2018	1	Very fine	50 %	Top	Upper	50
2	23/07/2018	1	Very fine	50 %	Top	Lower	50
2	23/07/2018	1	Very fine	50 %	Middle	Upper	50
2	23/07/2018	1	Very fine	50 %	Middle	Lower	50
2	23/07/2018	1	Very fine	50 %	Inner	Upper	50
2	23/07/2018	1	Very fine	50 %	Inner	Lower	50
2	23/07/2018	1	Very fine	50 %	Bottom	Upper	50
2	23/07/2018	1	Very fine	50 %	Bottom	Lower	50
3	23/07/2018	1	Medium	100 %	Top	Upper	50
3	23/07/2018	1	Medium	100 %	Top	Lower	50
3	23/07/2018	1	Medium	100 %	Middle	Upper	50
3	23/07/2018	1	Medium	100 %	Middle	Lower	50
3	23/07/2018	1	Medium	100 %	Inner	Upper	50
3	23/07/2018	1	Medium	100 %	Inner	Lower	50
3	23/07/2018	1	Medium	100 %	Bottom	Upper	50
3	23/07/2018	1	Medium	100 %	Bottom	Lower	50
4	23/07/2018	1	Medium	50 %	Top	Upper	50
4	23/07/2018	1	Medium	50 %	Top	Lower	50
4	23/07/2018	1	Medium	50 %	Middle	Upper	50
4	23/07/2018	1	Medium	50 %	Middle	Lower	50
4	23/07/2018	1	Medium	50 %	Inner	Upper	50
4	23/07/2018	1	Medium	50 %	Inner	Lower	50
4	23/07/2018	1	Medium	50 %	Bottom	Upper	50
4	23/07/2018	1	Medium	50 %	Bottom	Lower	50

Spraying

The raspberry plants within a plot were sprayed twice, once with the spray machine travelling in one direction and then once from the other side with the spray machine travelling in the other direction (see **Error! Reference source not found.** and **Error! Reference source not found.** and the captions for further details). The spraying method used during the trials was consistent with the farm's spray operator's usual method.

Figure 45: Example of spraying one of the plots. The polytunnels had three rows of plants in, each plot was 20 m in length and was sprayed from both sides (shown in the left and right photos) with both sides of spray machine active for both runs. The spray machine travelled in one direction on the first run, and the opposite direction for the second run. This is consistent with the spray operator's usual spraying method. Spray deposit measurements were only taken from the centre row of plants.

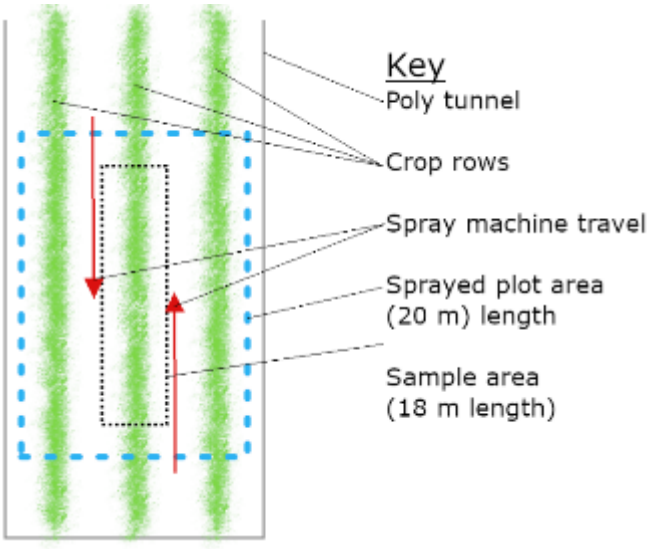

Figure 46: Overhead view of a single plot layout. Not to scale. The distance between the crop rows was approximately 2.6 m. The spray machine sprayed the rows within a plot from both sides travelling first in one direction, then from the other direction. The plots were 20 m in length, and the area that the spray deposits ('sample area') were measured was 1 m from boundaries of the sprayed area and only the centre crop row was measured. There was at least 10 m buffer zone between all plots.

Measuring spray deposits

The canopy of the raspberry plants was divided into four zones: top, middle, inner and bottom. Each zone was approximately 650 mm in height. The inner zone was at the same height as the middle zone, but deeper into the centre of the canopy (**Error! Reference source not found.**47).

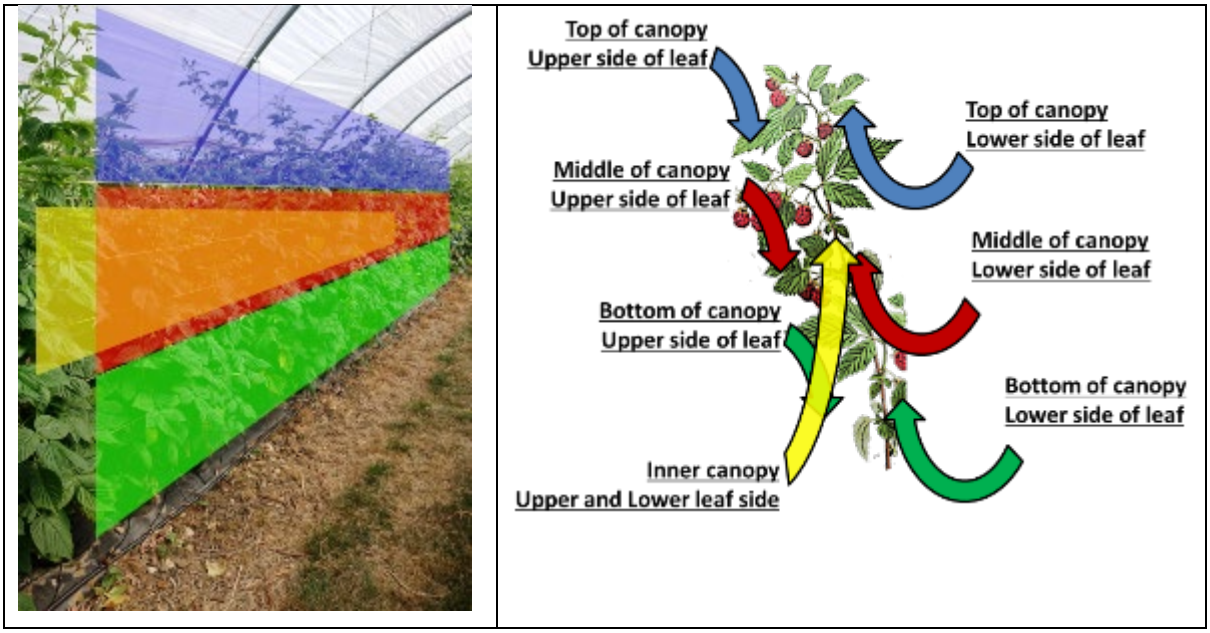

Figure 47: For measuring the spray deposits the raspberry crop canopy was divided into 4 zones: top (blue), middle (red), inner (yellow) and bottom (green). These were approximately 650 mm in height each. The inner zone was at the same height as the middle zone but in the centre of canopy. Within each zone the spray deposition on both sides of leaves was measured. Thus 8 groups of data were collected for each treatment.

Data analysis

The method for analysing the spray coverage and fluorescence data was the same. The data was divided into groups by 'canopy zone' and 'leaf side' to generate eight groups (datasets), one for each of the areas sampled (**Error! Reference source not found.**). For each of the eight, the percent coverage or fluorescence values were analysed by a mixed effect generalised linear model ('GLMER') with a negative binomial distribution. The four spray settings were included in each the GLMERs as a factor with four levels, and the imaging device used to measure the coverage or fluorescence was added as an error term to account for variance in the data due to differences between the two fluorescence imaging devices. Over-dispersion (variance greater than the mean) was assessed and accounted for if necessary by an additional error term (Elston, Moss, Boulinier, Arrowsmith, & Lambin, 2001). The effect of 'treatment' in each GLMER was assessed by a Chi-squared test to check for significance. If 'treatment' was found to be a significant factor, it was followed by a multiple comparison of the means using Tukey's contrasts to identify significant differences between treatments.

The count data was analysed by converting the data into frequency tables and analysing each canopy-leaf side zone with a Chi-squared test.

Data analysis was done using Microsoft Excel (2016), R (v.3.5.1, R Core Team, 2018), and R-Studio (v.1.1.463, 2018). Additional packages used in R were MultComp (Hothorn, Bretz,

& Westfall, 2008), and Ggplot2 (Wickham, 2016), lme4 (Bates, Maechler, Bolker, & Walker, 2015), and car (Fox & Weisberg, 2011).

Aim 1: Assess spray deposition on raspberry cane leaves when sprayed with different air-assistance and spray quality.

To assess the differences in spray deposition throughout the canopy of raspberry plants sprayed with different sprayer settings. The settings assessed were spray quality (droplet size) and amount of air-assistance applied.

Results

The spray deposition on raspberry cane leaves was measured and expressed as the percentage area covered by spray deposits after plots were sprayed with different air-assistance levels (full and half) and spray quality ('Very Fine' and 'Medium' spray droplets). The results are shown in **Error! Reference source not found.**48.

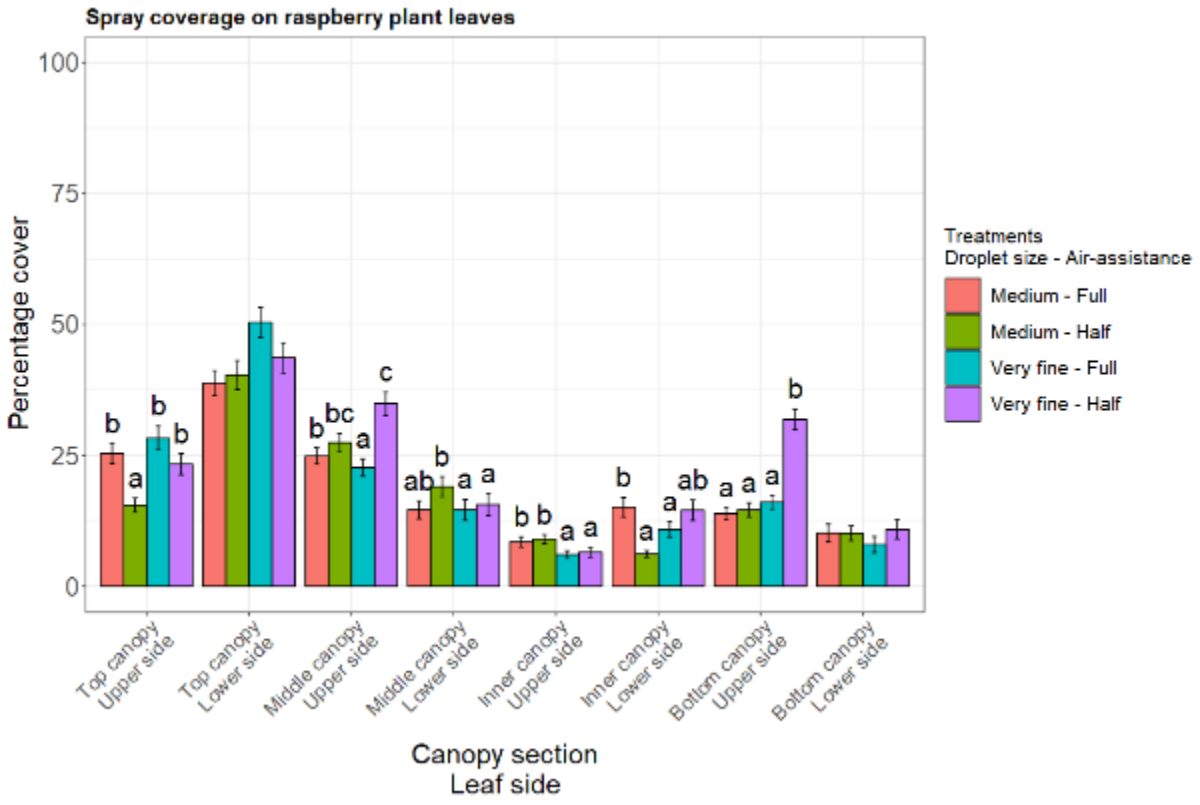

Figure 48: Percentage of leaf area covered with spray deposits at each canopy zone and leaf side, for each of the four spray treatments. The error bars show standard error. Significant differences were identified by GLMER and multiple comparisons Tukey's tests. If significant differences were identified, letter labels denote significant differences between the treatments within each canopy zone/leaf side.

Although the results in **Error! Reference source not found.** show that there are some significant differences between the spray treatments applied, the differences are generally

small. The results show that the spray treatments affect different parts of the raspberry plant's canopy differently.

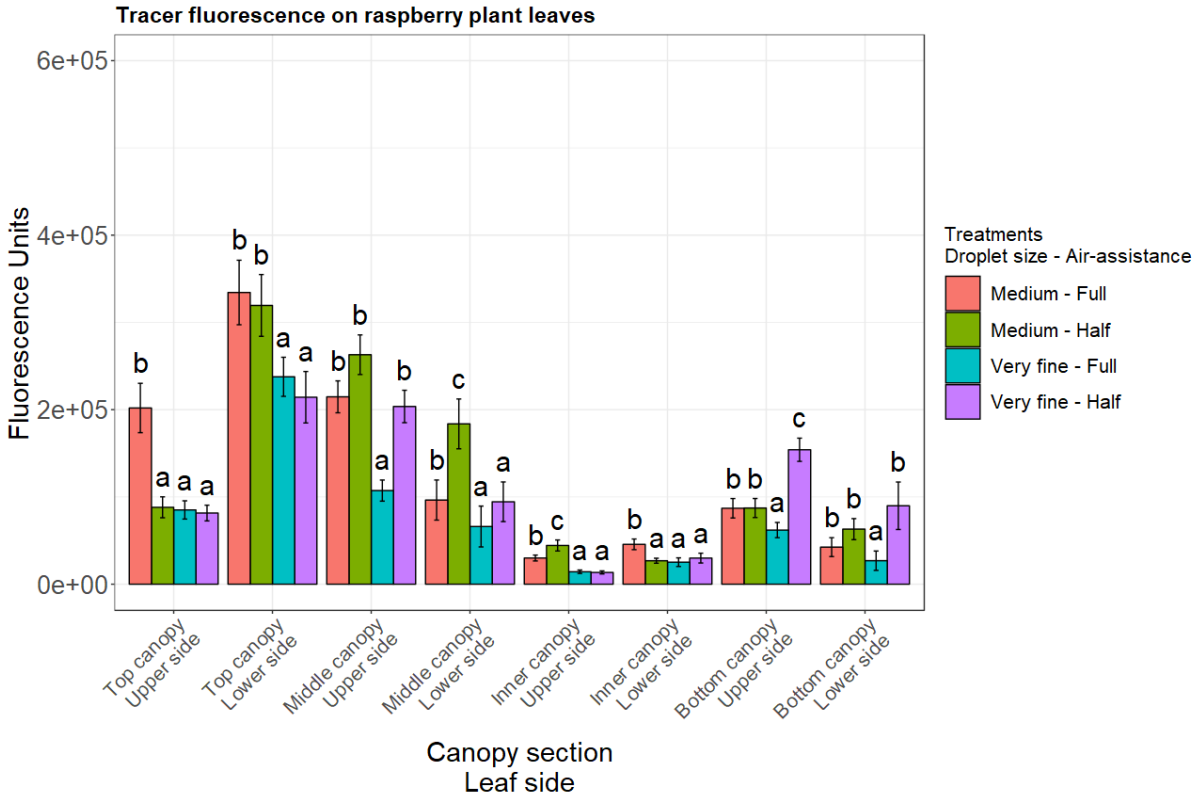

Figure 49: The fluorescence measured on the leaves. The fluorescence of the tracer used to detect the spray deposits is a proxy for the quantity (volume) of sprayed liquid on the leaves. The error bars show standard error. The letter labels denote significant differences between the treatments within each canopy zone/leaf side. Significant differences were identified by GLMER and multiple comparisons Tukey's tests.

The results in **Error! Reference source not found.**48 follow a similar pattern to the percentage cover results shown in **Error! Reference source not found.**49, except that in some instances the differences between treatments are more pronounced for the fluorescence values compared to percentage cover. The fluorescence values shown in **Error! Reference source not found.** are a proxy for the volume of sprayed liquid on the leaf surfaces. A higher fluorescence value indicates that there was more liquid containing the fluorescent tracer present on the leaf.

Spray quality

Two spray qualities were assessed: 'very fine' and 'medium'. See **Error! Reference source not found.**42 for details. Splitting the results of the spray quality by air-assistance shows differences between the spray settings. When air-assistance was set to 'full' (red and teal coloured bars), there were significant differences between the 'medium' and 'very fine' spray

qualities. The medium spray droplets resulted in significantly more fluorescence than the very fine spray droplets at all canopy-leaf zones. At the middle canopy-upper leaf side, inner canopy-upper and lower leaf side the medium spray droplets also resulted in significantly more coverage of the leaf surfaces, although the difference in coverage was small.

When the air-assistance was set to ‘half’ (the green and purple bars), the results were more varied. At the middle canopy-lower leaf side the medium droplets gave significantly more spray coverage and fluorescence than the very fine droplets. Similarly greater fluorescence was recorded for the medium droplets at the top canopy-lower leaf side and inner canopy-upper leaf side. However, at the bottom canopy-upper leaf side the very fine spray droplets resulted in significantly more coverage and fluorescence that the medium droplets, and at the top canopy-upper leaf the coverage was significantly greater for the very fine droplets compared to medium droplets.

Air-assistance

Two air-assistance levels were assessed: ‘full’ and ‘half’ rate. Looking at the air-assistance when the spray quality was medium (red and green bars), there were significant differences between the full and half rate air-assistance at some of the canopy sections. At both the top canopy-upper leaf side and the inner canopy-lower leaf side the full rate provided significantly more spray coverage and fluorescence than the half rate. In the middle canopy-lower leaf side the opposite was found for fluorescence but not spray coverage.

When the spray quality was very fine (teal and purple bars), there were two instances where the half rate air-assistance provided significantly more spray coverage and fluorescence than the full rate air-assistance, which were the middle canopy-upper leaf side and bottom canopy-upper leaf side. In addition, at the bottom canopy-lower leaf side the half rate air-assistance resulted in significantly more fluorescence detected than the full rate air-assistance.

Spray distribution across the canopy

Error! Reference source not found.50 shows the distribution of spray deposits in the raspberry canopy. The pattern of spray deposition is similar for all treatments, with the greatest spray coverage found on the underside of leaves at the top of the canopy, and the least coverage found at the inner and bottom sections of the canopy. This is consistent with previous spray trials. One important, but not statistically significant, difference between the treatments is that with very fine spray quality and air-assistance set to half rate (purple line), the spray coverage is slightly more evenly distributed across the canopy, with less spray deposition at the top of the canopy and more deposition at the bottom of the canopy compared to the other spray settings (**Error! Reference source not found.**).

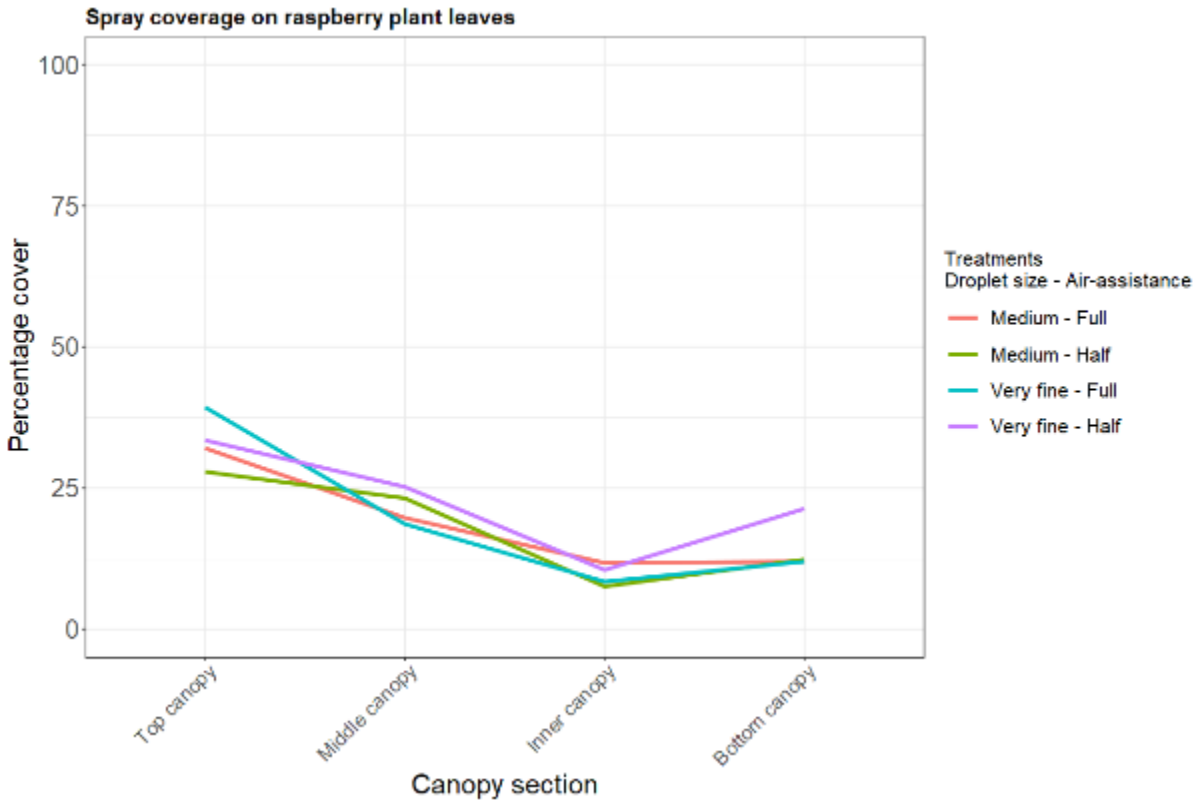

Figure 5023: Spray deposition as percentage of leaf surfaces covered by spray from a spray machine with different spray settings.

Discussion

Air-assistance is generally regarded to improve canopy penetration and improve spray deposition distribution throughout the canopy (van de Zande et al., 2008), but can also increase losses due to drift if the air plume is angled incorrectly or if the volume and speed of air is too high. Larger, heavier droplets carry more momentum as they exit the nozzle orifice compared to smaller lighter droplets, consequently smaller droplets are more susceptible to influences of air-assistance (Nuyttens, Dekeyser, De Schampheleire, Baetens, & Sonck, 2007). Larger droplets are affected more by gravity than smaller droplets so the larger droplets may require a higher air-assistance setting to reach the upper side of the leaves at the top section of the canopy. Evidence for this can be seen in the results presented in **Error! Reference source not found.** and **Error! Reference source not found.** which showed that highest coverage achieved for medium sized droplets was when the air-assistance was set to the full rate. This is likely the result of the full rate air-assistance (faster air currents) pushing more of the medium sized droplets up into the top region of the polytunnel compared to the half rate air-assistance. Gravity then causes the droplets to fall back down to land on the upper surface of the top section of canopy. Similarly at the inner canopy-lower leaf side, the additional air speed of the full rate air-assistance will have

increased the amount of leaf ruffling allowing increased penetration into the canopy and deposition onto the leaves.

It is interesting that the very fine spray droplets with air-assistance set to half rate (purple coloured bars) resulted in significantly more fluorescence and/or coverage in several canopy-leaf zones. The optimal setting for air-assistance is for the air plume to penetrate into the canopy but not much past the other side. Video footage of the very fine sprays with full rate air-assistance showed that the spray was reaching well into the adjacent row, suggesting that the air-assistance at full rate may have been too high. Consequently the very fine water droplets generated by the Albuz ATR 80 yellow nozzles, in conjunction with the reduced air-assistance, caused greater surface coverage and deposition in several parts of the canopy.

More work is required to understand how spray deposition can be improved throughout the raspberry canopy as the plants develop during the growing season. This will be paramount as growers use more biological pesticides which are often much more reliant on good coverage as they may not have any systemic or translaminar effects. The accurate timing of applications is also extremely important.

Aim 2: Assess the differences in refuges for beneficial insects when sprayed with different spray settings.

To establish whether the use of air-assistance or spray quality provides more or fewer refuges for bio-control organisms within the plant canopy.

Results

Percentage of leaves with potential spray refuges for insects

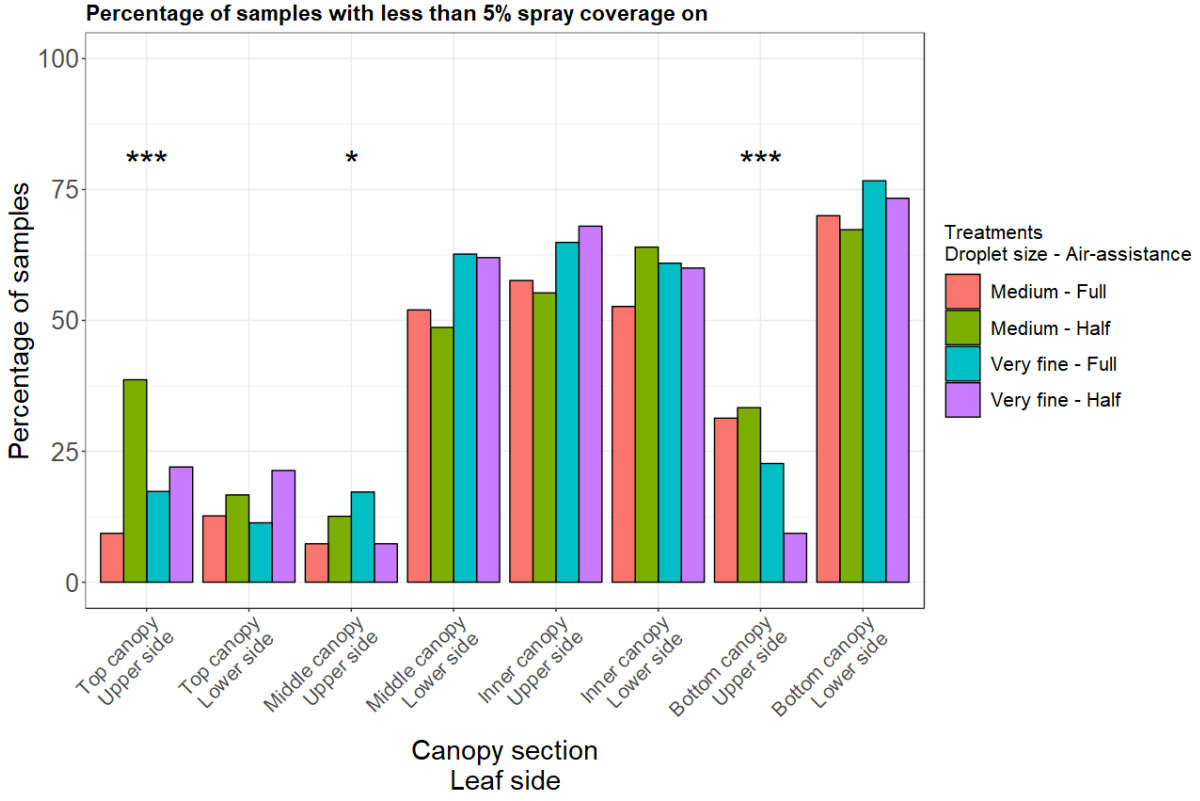

Figure 51: Percentage of leaf samples from each canopy/leaf side zone that had less than 5 % spray coverage for each of the four spray setting treatments. The raw count data from each canopy/lead side zone was analysed by Chi-squared, and the asterisks denote a significant difference between the spray settings treatments (P < 0.05 = *, P < 0.01 = **, P < 0.001 = ***).

The percentage of leaves from each of the canopy/leaf side zones which had less than 5 % coverage on is shown in **Error! Reference source not found.**. The general trend is that there were many more leaves with less than 5 % coverage on at the inner and bottom canopy (except for the bottom canopy-upper leaf side). At the top of the canopy most of the leaves sampled had more than 5 % coverage on.

Significant differences were identified between the spray settings treatments at three zones: top canopy-upper leaf side, middle canopy-upper leaf side, and bottom canopy-upper leaf side. Of these, in both the middle and bottom sections the very fine sprays with half rate air-assistance provided the least number of leaves with less than 5 % coverage on. At the top

canopy-upper leaf side significantly more leaves had less than 5 % coverage when sprayed with medium spray quality and half rate air-assistance.

Percentage of leaves unlikely to be refuges for beneficial insects

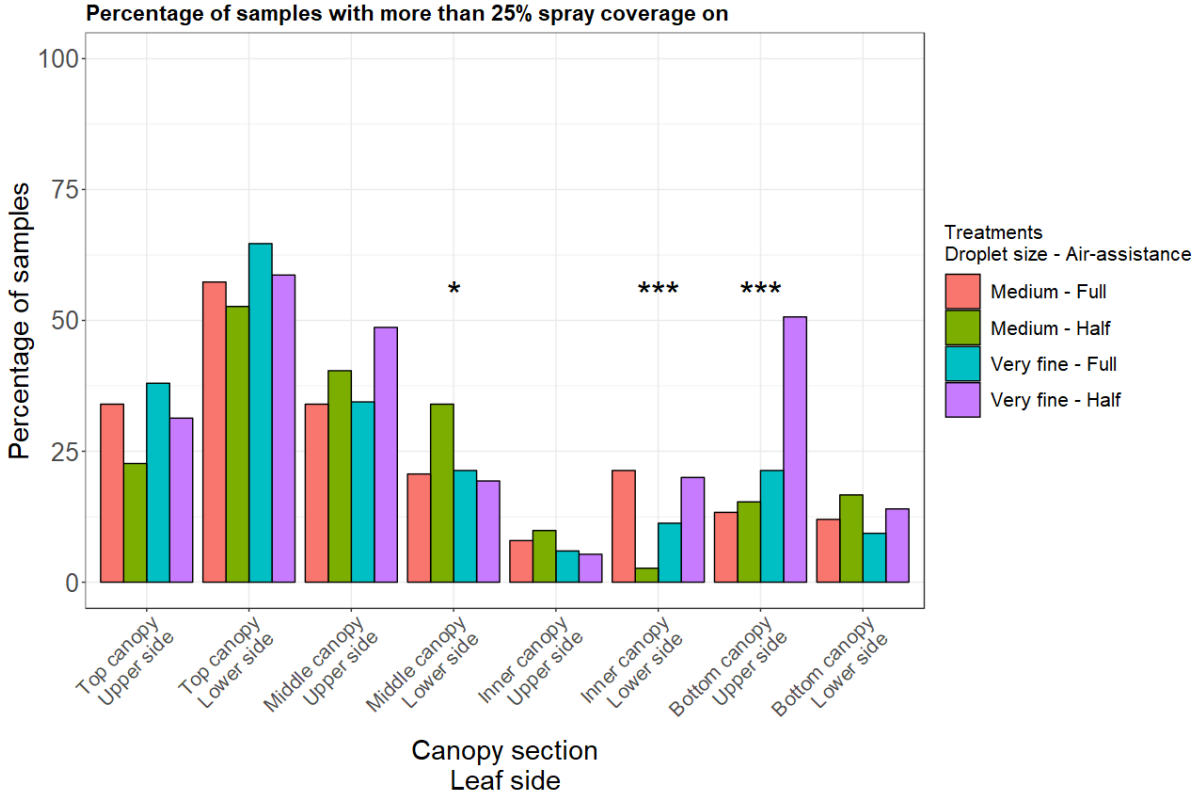

Figure 51: Percentage of leaf samples from each canopy/leaf side zone that had more than 25 % spray coverage for each of the four spray setting treatments. The raw count data from each canopy/lead side zone was analysed by Chi-squared, and the asterisks denote a significant difference between the spray settings treatments (P < 0.05 = *, P < 0.01 = **, P < 0.001 = ***).

The percentage of leaves from each of the canopy/leaf side zones which had more than 25 % coverage on is shown in **Error! Reference source not found.**. The general trend is the direct opposite of that found in **Error! Reference source not found.**, i.e. the greatest percentage of leaves with more than 25 % spray coverage on was found at the top and middle sections of the canopy, with the least at the inner and bottom sections. The exception to this is at bottom canopy-upper leaf side for the very fine spray with half rate air-assistance, which had a very high percentage of leaves with more than 25 % coverage on. Significant differences between the spray treatments was also found at the middle canopy-lower leaf side and inner canopy-lower leaf side. The spray treatment showing a difference at these zones is the medium sized droplets with half rate air-assistance (green bars) which had the highest percentage of leaves with more than 25 % spray coverage at the middle canopy-lower leaf side, but the lower percentage at the inner canopy-lower leaf side.

Discussion

The results of these trials clearly show that there are many areas of the raspberry canopy that receive less than 5 % spray coverage. Areas with less than 5 % coverage may provide refuges for insects, both beneficial insects such as predatory mites but also other pests such as aphids. Leaves with less than 5 % spray coverage are particularly prevalent in the inner and bottom sections of the canopy – with the exception of the very fine spray with half rate air-assistance. This is likely due to the increase in area covered by a given volume of water when a surface is sprayed with smaller diameter droplets compared to larger diameter droplets (Landers, 2004). Moreover, the half rate air-assistance will have reduced the number of droplets passing through or over the canopy allowing more droplets to hit the target.

Comparing the results of these spray deposition trials and the mite assessment trials in Object 2, WP 2.1 it is not possible to confirm or dismiss any negative effects of spray applications on beneficial predatory mites. Applications of Tracer and Botanigard insecticides were applied to the raspberry crop during the mite assessment trials, and populations of *A. andersoni* and *P. persimilis* did greatly reduce after the sprays. However this reduction may have been more related to the large reduction of the predatory mites' food source (*T. urticae*) rather than the applications of pesticides.

The water volume used in the spray deposition trials was 840 L/ha, which is at the higher end of the range that the spray operator at the farm normally uses. The plots used for Objective 2, WP 2.1 investigating the prevalence of *Phytoseiulus persimilis* and *Amblyseius andersoni*, and *Tetranychus urticae* were sprayed at a lower water volume rate of 600 L/ha. The reason 840 L/ha was used in the spray deposition trials rather than 600 L/ha was because it allowed the Albuz ATR 80 yellow nozzles (very fine spray quality) and Albuz ATR 80 blue nozzles (medium spray quality) to have the same water volume output and thus be directly compared. The lower water volume of 600 L/ha used in the mite assessment trials may result in an increase in the number of leaves with less than 5 % coverage, and therefore it is very likely that there were many refuges available to beneficial insects in those trials during any spraying.

Conclusions

- Spray deposition was highly variable across the different parts of the raspberry canopy, in particular high coverage and deposition was found at the top sections, whilst the inner and bottom sections of the canopy experienced much lower coverage and deposition.
- Farms experiencing pest control problems in raspberry should check that the spray is reaching the areas of the canopy where the pests are abundant. This is particularly important for non-systemic products. Water Sensitive Papers are readily available and

can provide some guidance for doing this. The handheld imaging fluorimeter used to measure spray deposition in these trials is expected to be commercially available in 2020. This tool will allow growers and spray operators to measure their spray deposition accurately and quickly, directly on the crop.

- There was minimal differences between the spray settings used in these trials. The very fine droplets in combination with half rate air-assistance provided higher coverage and/or fluorescence in some canopy sections, but not in others. Similarly the medium sized droplets the half rate air-assistance provided higher coverage and/or fluorescence is some canopy sections, but not others.
- The results of this study suggest that using very fine spray and half-rate air-assistance may provide slightly better distribution of spray deposition in a raspberry canopy, when sprayed at around 800 L/ha with an Ideal Alsazia spray machine.
- Overall, spray coverage on the leaves was generally above 10 % for all sprayer settings tested, apart from at the inner canopy section.
- At the middle, inner and bottom sections of the canopy there was a high percentage (greater than 50 %) of leaves measured which had less than 5 % coverage on, indicating that there was likely plenty of refuges for beneficial insects to avoid direct contact with spray deposits.

Objective 3: To review the current threat posed to the UK raspberry industry by Cane blight (*Leptosphaeria coniothyrium*) and identify new control options

Background

UK raspberry growers are beginning to see high levels of infection of cane blight (*Leptosphaeria coniothyrium*). This is in part due to a lack of effective raspberry cane midge control, disease infection of frost damaged tissue (spring, autumn and early winter) and also mechanical damage. Another contributing factor, is the loss of tebuconazole, which has left Signum (pyraclostrobin + boscalid) as the sole product for control of cane blight for which dose rates are low compared to those used in cane blight efficacy trials, especially for tunnelled crops (which are in fact outside during the time of year infection takes place).

A thorough review into cane blight epidemiology was performed in 2006, as well as fungicide efficacy trials (Berrie & Allen, 2006). Information discussed in that review, largely on work conducted in the 1970s and 1980s will not be discussed in this report. Since 2006, cane blight still causes major issues to raspberry growers, UK and abroad. This review sets out to find what further work has been carried out, and where the main gaps in knowledge lie.

The terms 'Leptosphaeria coniothyrium' and 'cane blight' were used in searches in Researchgate, Web of Science, Google scholar, Google search, Wiley online and in advisory notes such as the US extension services. Of the 15 papers/articles related to *L. coniothyrium* in raspberry on Researchgate, six were new since the 2006 review and are discussed in this review (see Appendix 2 for older papers). In addition to scientific publications, extension guides are also discussed, along with personal communication to growers, consultants and cane fruit specialists.

No information on efficacy testing for *L. coniothyrium* on cane fruit was found, over the last 20 years. Further to this, no further work on epidemiology/spray timing/forecasting has occurred since Williamson's work (reviewed in (Berrie & Allen, 2006)).

Cane blight is a major issue in Canadian raspberry plantations, and currently, their primary control method to limit disease is good crop husbandry and hygiene, cutting canes right down to the ground, leaving no stubs (personal communication with a Fruit Crop specialist from OMAFRA). Cane blight is also a major problem in the USA, Germany, Norway, Sweden and so searches were also specifically made here for any literature/conference papers on chemical and cultural control measures.

Personal communication with a large UK grower (South England) found cane blight to be their biggest issue this year and last year on their tunnelled raspberry crop. Symptoms are being seen mainly at the base of canes, but also all over. Where cane is grown for the following season, the grower stated losses of 50% of the crop at times.

Up to 100% crop losses have been seen in plantations in the USA (Brannen & Smith, 2018). Personal communication with a plant pathologist at the University of Georgia identified one student to be working on cane blight disease, but limited information is available for other groups (UK and abroad) working on it currently.

Epidemiology

Work by Berrie and Allen (2006) indicated that infection can take place far later than during and immediately post-harvest (sometimes late into the winter or even the New Year), so any sprays that might be used may well be being applied at the wrong time. This was corroborated by work in North Germany (Neubauer, Heitmann, & Faby, 2010). Infection period is affected by levels of cane maturity, and now, due to most crops fruiting under protection and primocane selection being delayed, the primocane rind is far less mature than when all crops were outside and first or even second flush primocane control was not practiced.

Unlike raspberry spur blight (*Didymella applanata*) and cane botrytis, which weaken or kill individual fruit nodes, cane blight (*Leptosphaeria coniothyrium*) kills floricanes, resulting in year on year decimation of infected plantations. Once *L. coniothyrium* penetrates the cane epidermis, it invades the cortex and then enters the vascular tissue (Madeiras, 2017). This invasion process occurs slowly, but is enhanced by the weakening of a cane, such as during defoliation (Seemüller, Kartte, & Erdel, 2008). Work exploring phenolic content changes within canes that were wounded and infected by *L. coniothyrium*, found that the chemical pathway used in sealing up these wounds competed with the pathway involved in producing antifungal defence compounds (Mikulic-Petkovsek, Schmitzer, Stampar, Veberic, & Koron, 2014). Researchers are also looking at NB-LRR disease resistance genes in *Rubus*, including those associated with gene H conditioned pubescence (van Eck & M. Bradeen, 2018). Graham *et al.* (2006), however, showed that for pathogens causing cane botrytis and spur blight, gene H, has been mapped on to linkage group 2 and shown to be closely associated with resistance to cane botrytis and spur blight but not rust or cane spot.

Figure 52. (Left) cane blight infection showing penetration towards the central pith, (right) orange cane midge larvae underneath split in epidermis.

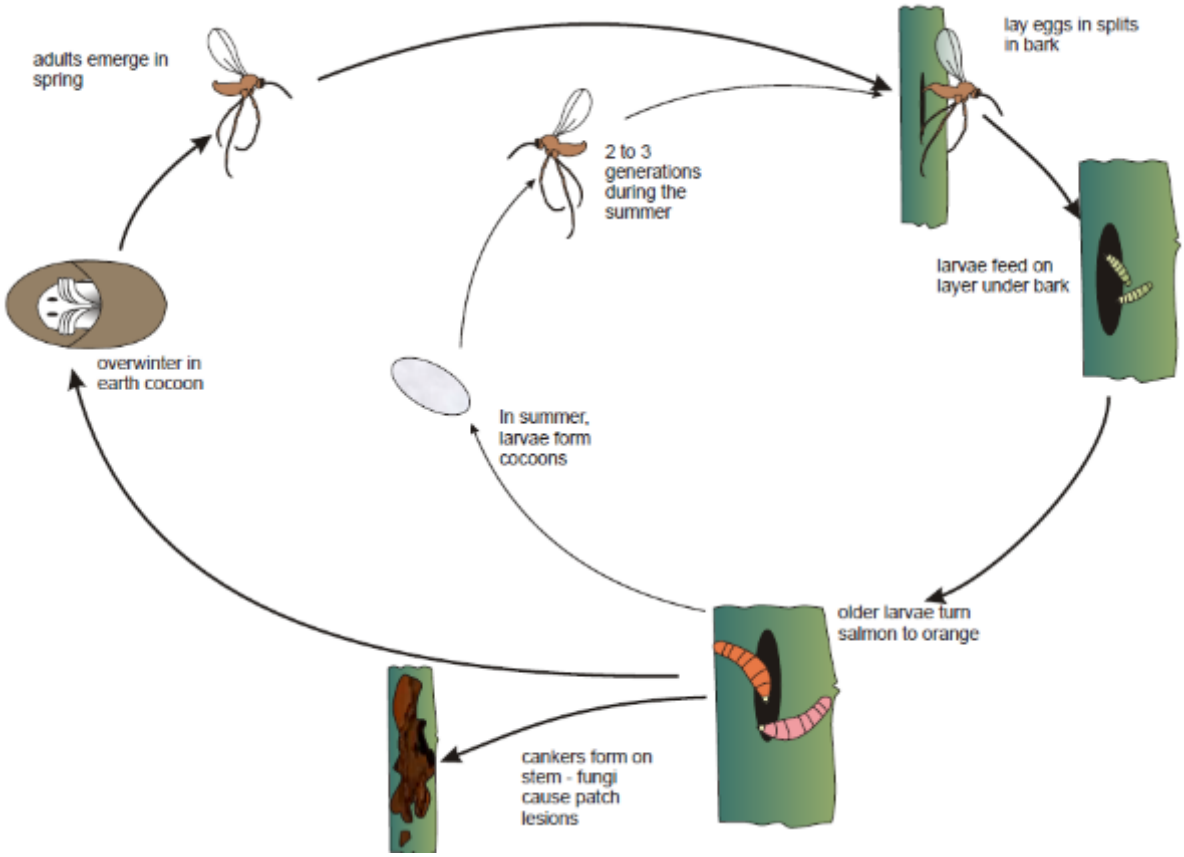

Figure 53. Life cycle of the raspberry cane midge (ADAS, 2017).

Alongside split canes allowing the fungus to invade, the cracked outer epidermis also attracts female cane midge (*Resseliella theobaldi*) to oviposit (Figure 53). More cases of floricane debilitation and death due to cane blight are now being seen in the UK due to growers finding it very difficult and in some cases impossible to effectively control raspberry cane midge (first and following generations) with the pesticides currently at their disposal. Midge damage to cane bases then allows *L. coniothyrium* entry.

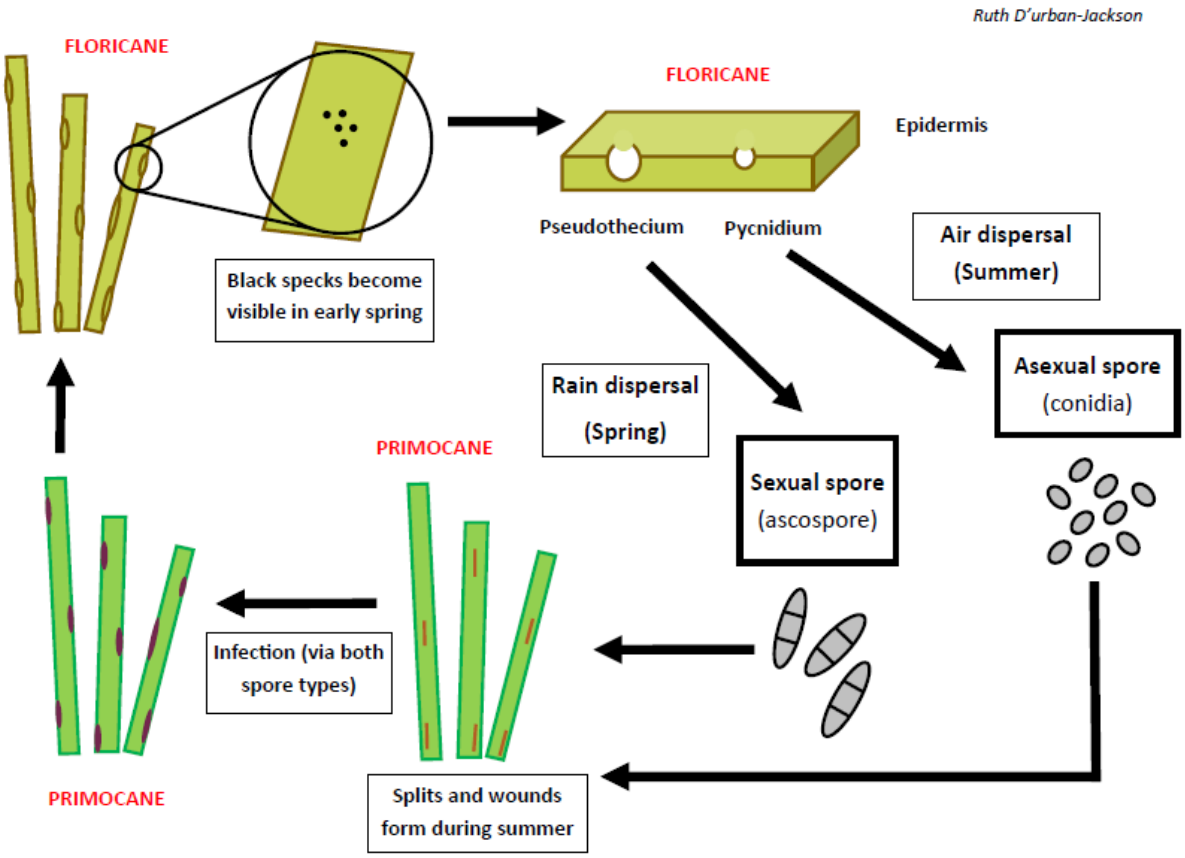

Figure 54. Life cycle of cane blight (*Leptosphaeria coniothyrium*) on raspberry. Illustration by Ruth D'urban-Jackson, adapted from Williamson, E. (1991).

For *L. maculans*, Phoma canker in oilseed rape, a forecasting programme for farmers using weather (principally rainfall, in particular in August) is available from Rothamsted Research to enable protectant sprays to be applied at effective timings. It is possible that this information could be used by cane fruit growers.

Cane blight is infecting plants in propagation and the risk of this happening could be particularly high where long cane is being produced for sale, whether of summer fruiting or primocane fruiting cultivars. Recent observations in outdoor produced long cane in propagation have shown that even with careful tying-in of each single cane per plant to horizontal support wires, autumn wind blowing on tall green leafy canes can cause rubbing and wounding on the wires. Symptoms of cane blight are not able to be seen until significantly later. The risk of infection from debris and cane stubs is less where propagation units are away from crops with spore producing floricane stubs and cane debris, but how important wind-blown ascospores are in addition to locally splash-dispersed conidia is still unclear (Figure 54).

Key Issues:

Physical damage & cultural changes

Cane blight (*L. coniothyrium*) is a relatively weak pathogen, so often requires damage to the cane in order to enter the plant (Williamson, 1991). This includes mechanical damage from:

- Wind rubbing canes on support wires/strings (personal communication)
- Pruning
- Mechanical harvesting (Williamson & Ramsay, 2008)
- Strimmer damage to base of canes
- Frost damage and cold injury (Brennen, 2017)
- Hail (personal communication with OMAFRA)
- Pest damage
- Pesticide damage, where poor application of a desiccant (e.g. Shark herbicide) for primocane control is made.
- Other disease damage, such as by *Fusarium* spp. (Williamson & J. Hargreaves, 1979).

All raspberry varieties split naturally, due to changes in temperature and moisture levels, but some have been seen to be less prone, such as cv. Squamish. Researchers in Germany found that a delay of the emergence of primocanes in spring reduces the occurrence of natural splits and xylem lesions (Neubauer et al., 2010).

Alongside the risk from mechanical damage is the change in crop management, whereby crops are spending more time under protection, later into the autumn (Berrie & Allen, 2006). In fruiting crops, cladding of tunnels into late summer-early autumn (later than the past) and the now widespread removal of the first flush of primocanes, is resulting in very juvenile primocanes in plantations, that are both more susceptible to blight infection, and are susceptible much later into the autumn/winter than in the past.

Cane blight is a major issue in the wet, humid conditions of south-east USA. To reduce infection of cane blight growers are advised to “pinch off” the tip of tender primocanes as opposed to cutting, and to ensure where possible, to prune when at least 4 days of dry weather is expected. Weed free strips under the canopy aid drying and air movement (Brannen & Smith, 2018).

Chemical control

Raspberry cane midge and associated *L. coniothyrium* infection have resulted in cane blight becoming a major threat to raspberry production in the UK since the withdrawal of chlorpyrifos and tebuconazole for their respective control.

Cane fruit crops develop tall dense canopies, particularly when grown under glasshouse or polytunnel protection to the extent that the growing systems used for these protected crops can make spraying pest and disease targets more difficult. The increasing practice of double cropping primocane fruiting raspberries can and often does exacerbate issues as it is difficult to find periods in the growing-cropping season when they can be accessed, as cropping floricane and flowering primocane are present side by side for much of the growing season. This produces often very dense and spreading crop canopies with only very short periods each year when crops are fully dormant.

Control has become more difficult as tebuconazole approval against cane and foliar diseases in outdoor and protected cane fruit crops (as Savannah under EAMU 1812/17) expired on 31 October 2018.

The problem of cane blight is compounded by the loss of the insecticide chlorpyrifos, which was much more effective against cane midge than the remaining products containing either deltamethrin, thiacloprid or lambda-cyhalothrin (even with the addition of wetter). Therefore in the majority of cases some first generation midge survives to produce second, third or more generations and damage sites ideal for colonisation by *L. coniothyrium* and Fusarium in addition to the many more cases seen of floricane debilitation and death due to midge blight.

Signum (boscalid + pyraclostrobin) can be used against cane diseases, however the rate of 1.8 kg/ha was originally tested (in HDC SF 96) and did not give complete control. It is likely that the lower application rates given under current EAMUs of 1.25 kg/ha for protected crops and 1.5 kg/ha for outdoor crops are giving poorer control. Secondly, as a general rule at least two, and in some cases three, applications of a fungicide with activity against cane blight are required to achieve acceptable control, but this is not possible because growers are only permitted three applications of Signum per year for protected and two applications per year for outdoor crops. Consequently, in most cases Signum is used for control of other diseases e.g. fruit and cane botrytis, spur blight, powdery mildew or raspberry rust in both the outdoor and tunnel protected crops and this means it is then not able to be applied again in autumn/winter against cane blight. Even where long cane is being used to produce a single crop, Signum is used for fruit botrytis and powdery mildew control and the timing and foliar application is not right to protect against cane blight.

In south-east USA, a large production area for blackberry and raspberries, growers protect their crop from blight by spraying fungicides on the day of pruning, whilst also potentially treating it with chemical sprays for other fungi such as rust. Most damage takes place when hurricanes occur in August and September (in conjunction with pruning operations) (personal communication with P. Brennan, University of Georgia).

In the USA, chemical control for cane blight includes the following (note that the following active ingredients and products are not all approved for use in the UK; check with your BASIS-registered agronomist or advisor):

Delayed dormant (swollen buds) and green tip

- Copper-based products (FRAC – M1), including copper hydroxide, copper sulphate and others. Apply prior to ¾ inch shoot stage to avoid leaf burn. Copper can cause phytotoxicity on black raspberry cultivars if used with formulated phosphorus products. It is also an occasional problem on red raspberries.

- Calcium polysulfide (FRAC – M2) – Lime sulphur and Sulforix. Apply lime-sulphur at delayed dormant, but before shoots are ¾ inch long. Lime sulphur is dangerous to the applicator, so use caution. Any exposed green tissue will likely be burned. A minimum of 200 gallons of diluted spray is recommended per acre.

Shoots 6 inches long and before blooms open

- Pyraclostrobin (FRAC – 11) 'Cabrio'. No more than two sequential applications of Cabrio should be made before alternating with fungicides of different mode of action. Make no more than four applications of Cabrio or other strobilurins per season.

- Azoxystrobin (FRAC – 11) 'Abound FL'. No more than two sequential applications of azoxystrobin should be made before alternating with fungicides of different mode of action. Make no more than four applications of azoxystrobin or other strobilurins per season.

- Pyraclostrobin + boscalid (FRAC – 11 + 3) 'Pristine WG'. No more than two sequential applications of Pristine should be made before alternating with fungicides of different mode of action. Make no more than 4 applications of Pristine or other strobilurins per season.

- Azoxystrobin + propiconazole (FRAC – 11 + 3) 'Quilt Xcel'. Application should begin prior to disease development and continue on a 14 day schedule. No more than two sequential applications of Quilt Xcel should be made before alternating with fungicides of different mode of action. Make no more than 3 applications of Quilt Xcel or other group 11 fungicides per season.

- Captan (FRAC – M4) 'Captan 80 WG'. Do not apply more than 12.5 lb per acre per season.

See Appendix 2 for more information.

Berrie and Allen (2006) determined that Folicur (tebuconazole) and Signum (pyraclostrobin _ boscalid) could provide some control, but highlighted that in order to cover the potential infection period of cane blight that fungicide application to canes would ideally commence in July during harvest, and that application post-harvest should ideally be done in September as

well as August i.e. much later than when crops were principally grown outdoors. Warmer weather into October followed by milder winters than in recent history was at the same time thought likely to be extending the period of fungal activity.

Leptosphaeria maculans, the causal agent of oilseed rape phoma/stem canker, currently can only be controlled by prothioconazole + tebuconazole (BCPC, 2018), neither of which are available for use on *L. coniothyrium* in raspberry.

Cane management

Much of the research on cane density and components of yield was carried out over 30 years ago and has been captured in HDC factsheet 12/06 (Allen & Raffle, 2006). However there has been very little work in this area on tunnelled production systems and specifically relating cane management to improvements in pest and disease control and spray penetration. Some work on lowering cane density in summer fruiting raspberries to reduce botrytis was carried out in HDC SF 79 and the humidity in the canopy recorded and utilised in forecasting conditions suitable for botrytis infection. In the current work (within SF 158 Cane Fruit IPM), observations of insecticide spraying in a commercial crop have confirmed that when canopies are dense there is less complete spray coverage. Biofungicides, such as Serenade ASO (*Bacillus subtilis*) and Prestop (*Gliocladium catenulatum*) are now available in the UK and require deposition across all surfaces as they do not make the trans-laminar or systemic movement of many chemical products.

As a major cane disease in Ontario (OMAFRA, 2006), the primary way of keeping cane blight infection low in Canada is to ensure canes are pruned right down to the ground, so that the stubs don't rub on new canes and transfer the infection (personal communication with Erica Pate, Food Crop Specialist at OMAFRA - Ontario Ministry of Agriculture, Food & Rural Affairs). See Appendix 3 for extension sources.

Biennial cropping to control cane blight, other diseases and pests

Cane blight is now being seen in double cropping primocane as well as summer fruiting raspberries. There is a potential for a wider range of fungicides and insecticides to be tested for potential EAMUs if there is application to primocanes only (without floricane present) and this would be possible with a conversion into biennial cropping. Biennial cropping also significantly reduces the old to new cane spread of pests and diseases.

Biennial cropping is an approach to cane management which may hold potential as a useful tool for IPM and in particular the control of Spotted Wing Drosophila. In a biennial cropping system, primocanes and floricanes are grown in separate rows. This separation, including the removal of all canes in a row after harvest, disrupts the life cycle of most pests and diseases

by reducing the ease of spread between infested old material and healthy new tissue. Biennial cropping was widely researched historically particularly for machine harvested systems in Scotland as it improved efficiency of harvesting (M. Lawson & S. Wiseman, 1983), and this will also be true for hand harvesting as there is no obstruction by primocane. Reduced labour costs are possible because more berries are produced per floricane in the cropping year, and harvest for some cultivars grown using this system can be expected to start and end earlier than would be the case with growing that cultivar conventionally, which could also be an advantage.

Earlier work on this system of growing identified that it might not be suitable for all cultivars as some displayed a rapid decline in plant vigour after the second time that they had fully cropped. However at that time, crops were grown exclusively in the open, not routinely irrigated or fertigated throughout their growing season and methods of spawn control were less well developed. With current practices involving more sophisticated irrigation and fertigation, and efficient and rapid removal of unwanted primocane, this may not be such an issue. The aim with biennial cropping would be that during the “on” (fruiting) year the canes retained will produce roughly double the amount of marketable fruit per cane than would be possible from annual cropping plants, so making the process economic.

The principal benefits of biennial systems are to expose the primocane to more air and light, (hence improving flower bud initiation) and to generally reduce cane-to-cane competition for water, nutrients etc. so resulting in stronger plants and a less favourable micro-climate for fungal infection, such as of cane blight. There is no need to spray for raspberry beetle and fruit diseases in the non-fruiting year. The system also allows chemistry that is harmful to pollinators, or which lead to unwanted residuals in the fruits, to be applied to primocanes only during the non-cropping year to bring down pest and disease levels which allow more IPM friendly approaches in the fruiting year and reduce residues on fruit. Application of pesticides for raspberry cane midge, aphids, TSSM etc. should be easier to apply as the canopy is thinner and there is the potential for lower water volumes.

Conclusion

In conclusion, the control of cane blight in UK raspberries is of increasing importance, requiring immediate attention. Changes in practice, and the lengthening growing seasons is resulting in immature canes that are susceptible to cold damage and late season cane blight infection. Improper use of desiccants to clear young primocanes leaves cane stubs behind, where cane midge can lay their eggs. Loss of key chemical insecticides are resulting in increased levels of cane midge, with exponential population increases after the first generation, and warming temperatures potentially allowing up to four generations to develop over the season. Chemical fungicides are also becoming limited, and those that are still available primarily target other diseases, with approved rates being lower than in the crop efficacy trials they were studied in.

There is a need for trials to not only test efficacy of new fungicides against cane blight, but also to understand disease life cycle in tunnelled soil & in-substrate plantations. No new work in breeding resistance to *L. coniothyrium* has taken place since 2008. The life cycle of *L. coniothyrium* is still not fully known, with much of the understanding coming from other Leptosphaeria (e.g. *L. maculans*) studies (Bousset, Ermel, & Lebreton, 2018). Both fruiting body structures have been seen on raspberry canes, but the conditions for dispersal and infection, including time of year for spore dispersal (and any changes to this brought about by growing the crop in tunnels for part of the year) need further elucidating.

Work on improving raspberry cane midge control is also required because the increase in damage by this pest is contributing to the increase in infection by cane blight.

Knowledge and Technology Transfer

Ruth D'urban-Jackson presented work at the FARMA & AHDB Growing media workshop in Oxfordshire 19 September 2018.

Ruth D'urban-Jackson and Jude Bennison showcased the project at the AHDB NIAB-EMR Association Soft Fruit Day 21 November 2018.

Exchange of symptomatic canes, *Phytophthora* spp. isolates, and protocols for cane isolation, with Aurelia Bezanger, James Hutton Institute.

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Appendix 1

Extract from the 2018 Southeast Region Caneberries Integrated Management Guide (Brannen & Smith, 2018).

Blackberry and Raspberry (continued)
Delayed Dormant (swollen buds) to Green Tip (continued)

Pest/Problem	Management Options	Amount of Formulation per Acre	Effectiveness or Importance	REI	PHI	Comments
Anthracnose, cane blight, and spur blight	**copper-based products** *FRAC – M1*	See specific product label	P	See label	See label	Multiple copper-based materials are available, including copper hydroxide, copper sulfate, and others. Apply prior to ¾ inch shoot stage to avoid leaf burn. For raspberries only: Copper can cause phytotoxicity on black raspberry cultivars if used with formulated phosphorous acid products (e.g. Aliette). It is also an occasional problem on red raspberries.
	calcium polysulfide *FRAC – M2* (Lime Sulfur, Sulforix and related products)	See specific product label	F	48 hrs	See label	Apply lime-sulfur at delayed dormant, but before shoots are ¾ inch long. Lime sulfur is dangerous to the applicator, so use caution. Any exposed green tissue will likely be burned. A minimum of 200 gallons of diluted spray is recommended per acre. **Follow specific label directions for dilutions, and never use in the undiluted form.**

Blackberry and Raspberry (continued)
Shoots 6 Inches Long and Before Blooms Open (continued)

Pest/Problem	Management Options	Amount of Formulation per Acre	Effectiveness or Importance	REI	PHI	Comments
Powdery mildew (continued)	**pyraclostrobin + boscalid** *FRAC – 11+7* (Pristine WG)	18.5-23 oz	E	12 hrs	0 days	No more than 2 sequential applications of Pristine should be made before alternating with fungicides that have a different mode of action. Make no more than 4 applications of Pristine or other strobilurins per season.
	azoxystrobin + propiconazole *FRAC – 11+3* (Quilt Xcel)	14-21 fl oz	E	12 hrs	30 days	Application should begin prior to disease development and continue on a 14 day schedule. No more than 2 sequential applications of Quilt Xcel should be made before alternating with fungicides that have a different mode of action. Make no more than 3 applications of Quilt Xcel or other group 11 fungicides per season.
Anthracnose, cane blight, spur blight, and leaf spots	**pyraclostrobin** *FRAC – 11* (Cabrio EG)	14 oz	E	12 hrs	0 days	No more than 2 sequential applications of Cabrio should be made before alternating with fungicides of different mode of action. Make no more than 4 applications of Cabrio or other strobilurins per season.
	azoxystrobin *FRAC – 11* (Abound FL)	6-15.5 fl oz	E	4 hrs	0 days	No more than 2 sequential applications of azoxystrobin should be made before alternating with fungicides of a different mode of action. Make no more than 4 applications of azoxystrobin or other strobilurins per season.
	pyraclostrobin + boscalid *FRAC – 11+7* (Pristine WG)	18.5-23 oz	E	12 hrs	0 days	No more than 2 sequential applications of Pristine should be made before alternating with fungicides of a different mode of action. Make no more than 4 applications of Pristine or other strobilurins per season.
	azoxystrobin + propiconazole *FRAC – 11+3* (Quilt Xcel)	14-21 fl oz	E	12 hrs	30 days	Application should begin prior to disease development and continue on a 14 day schedule. No more than 2 sequential applications of Quilt Xcel should be made before alternating with fungicides that have a different mode of action. Make no more than 3 applications of Quilt Xcel or other group 11 fungicides per season.

Blackberry and Raspberry (continued)
Shoots 6 Inches Long and Before Blooms Open (continued)

Pest/Problem	Management Options	Amount of Formulation per Acre	Effectiveness or Importance	REI	PHI	Comments
Anthracnose, cane blight, spur blight, and leaf spots (continued)	**captan** *FRAC – M4*		F	48 hrs	3 days	
	(Captan 80WDG)	2.5 lb				Do not apply more than 12.5 lb per acre per season.
	(Captec 4L)	0.75-1 qt				Do not apply more than 10 lb per acre per season.
	(Captan 50W)	4 lb				Do not apply more than 20 lb per acre per season.
	copper-based products *FRAC – M1*	See specific product label	P	See label	See label	Multiple copper-based materials are available, including copper hydroxide, copper sulfate and others. Apply prior to 3/4inch shoot stage to avoid leaf burn. For raspberries only: Copper can cause phytotoxicity on black raspberry cultivars if used with formulated phosphorous acid products (e.g. Aliette). It is also an occasional problem on red raspberries.

Blackberry and Raspberry (continued)
Pre-Bloom (when flower buds show white) (continued)

Pest/Problem	Management Options	Amount of Formulation per Acre	Effectiveness or Importance	REI	PHI	Comments
Raspberry cane borer	**malathion** *IRAC – 1B* (Malathion 57EC) (Malathion 8F)	 1.5-3 pt 2 pt	 G G	 12 hrs 12 hrs	 1 day 1 day	
Stink bugs	**bifenthrin** *IRAC – 3A* (Brigade 2EC) (Brigade 10WSB) Numerous generics	 3.2-6.4 fl oz 8-16 oz See labels	VG	12 hrs	3 days	
	acetamiprid *IRAC – 4A* (Assail 30SG)	4.5-5.3 oz	G	12 hrs	1 day	
	thiamethoxam *IRAC – 4A* (Actara 25 WDG)	3 oz	G	12 hrs	3 days	
	pyrethrins + azadirachtin *IRAC – 3A* *Unknown but multiple modes of action* (Azera)	2-3 pt	F	12 hrs	0 day	**OMRI-approved**
Anthracnose, cane blight, spur blight, and leaf spots	Same as **Shoots Six Inches Long and Before Blooms Open**					

Blackberry and Raspberry (continued)
Early bloom (5-10%) (continued)

Pest/Problem	Management Options	Amount of Formulation per Acre	Effectiveness or Importance	REI	PHI	Comments
Rosette (Double blossom) (continued)	**azoxystrobin + propiconazole** *FRAC – 11+3* (Quilt Xcel)	14-21 fl oz	E	12 hrs	30 days	Application should begin prior to disease development and continue on a 14 day schedule. No more than 2 sequential applications of Quilt Xcel should be made before alternating with fungicides that have a different mode of action. Make no more than 3 applications of Quilt Xcel or other group 11 fungicides per season.
	cyprodinil + fludioxonil *FRAC – 9+12* (Switch 62.5WG)	11-14 oz	E	12 hrs	0 days	Begin application at early bloom. Do not exceed 56 oz of product/acre/ year. Make no more than two sequential applications before using a fungicide with another mode of action.
	Bordeaux mixture 4-4-50	See note	G	24 hrs	1 day	**Bordeaux recipe: 1.** Fill spray tank to one-half the desired volume of water. **2.** Turn on the agitator. **3.** Dissolve powdered bluestone (copper sulfate) in the spray tank at a rate of 4 lb bluestone/ 50 gallons water. **4.** Make a "milk of lime" suspension by dissolving 4 lb of hydrated lime (calcium hydroxide) in 5 gallons of water in a container, for a rate of 4 lb hydrated lime/ 50 gallons water. **5.** Slowly add the "milk of lime" suspension into the spray tank. **6.** Fill the spray tank to the desired volume of water. **7.** Maintain constant agitation and apply immediately. Do not mix with Topsin-M or Sevin. Bordeaux mixture will cause severe leaf burn if applied on very hot days or if combined with insecticides. Slight phytotoxicity will have relatively minor impact.
Anthracnose, cane blight, spur blight, and leaf spots	**pyraclostrobin** *FRAC – 11* (Cabrio EG)	14 oz	E	12 hrs	0 days	No more than 2 sequential applications of Cabrio should be made before alternating with fungicides of a different mode of action. Make no more than 4 applications of Cabrio or other strobilurins per season.
	azoxystrobin *FRAC – 11* (Abound FL)	6.2-15.4 fl oz	E	4 hrs	0 days	No more than 2 sequential applications of azoxystrobin should be made before alternating with fungicides of different mode of action. Make no more than 4 applications of azoxystrobin or other strobilurins per season.

Pest/Problem	Management Options	Amount of Formulation per Acre	Effectiveness or Importance	REI	PHI	Comments
Anthracnose, cane blight, spur blight, and leaf spots (continued)	**pyraclostrobin + boscalid** *FRAC – 11+7* (Pristine WG)	18.5-23 oz	E	12 hrs	0 days	No more than 2 sequential applications of Pristine should be made before alternating with fungicides that have a different mode of action. Make no more than 4 applications of Pristine or other strobilurins per season.
	azoxystrobin + propiconazole *FRAC – 11+3* (Quilt Xcel)	14-21 fl oz	E	12 hrs	30 days	Application should begin prior to disease development and continue on a 14 day schedule. No more than 2 sequential applications of Quilt Xcel should be made before alternating with fungicides that have a different mode of action. Make no more than 3 applications of Quilt Xcel or other group 11 fungicides per season.
	captan *FRAC – M4* (Captan 80WDG) (Captec 4L) (Captan 50W)	 2.5 lb 0.75-1 qt 4 lb	F	48 hrs	3 days	Do not apply more than 12.5 lb per acre per season. Do not apply more than 10 lb per acre per season. Do not apply more than 20 lb per acre per season.

Full Bloom — Do Not Apply Insecticides During Bloom

Pest/Problem	Management Options	Amount of Formulation per Acre	Effectiveness or Importance	REI	PHI	Comments
Rosette (Double blossom)	Same as **Early Bloom (5-10%)**					
Botrytis gray mold	Same as **Early Bloom (5-10%)**					
Anthracnose, cane blight, spur blight, and leaf spots	Same as **Early Bloom (5-10%)**					

Pest/Problem	Management Options	Amount of Formulation per Acre	Effectiveness or Importance	REI	PHI	Comments
Rose scale	**imidacloprid** *IRAC – 4A* (Admire Pro)	7-14 fl oz (soil application only) 2.8 fl oz (foliar application)	E	12 hrs	7 days	Sporadic problem in Virginia. Do not apply pre-bloom or at bloom. Admire Pro can be applied to the soil or as a foliar treatment. Read the label carefully and exercise caution when making soil applications via irrigation. Foliar applications timed to crawler emergence give best control.
	acetamiprid *IRAC – 4A* (Assail 30SG)	4.0-5.3 oz	G	12 hrs	1 day	Foliar applications timed to crawler emergence give best control.
Botrytis gray mold	Same as **Early Bloom (5-10%)**					
Anthracnose, cane blight, spur blight, and leaf spots	Same as **Early Bloom (5-10%)**					

After-Harvest (after fruit has been harvested)

Cane blight – Cane blight can be a major disease of blackberry in the Southeast, resulting in severe losses – sometimes resulting in the complete destruction of fruiting canes in any given year. It is generally not reported in other states as a major disease of blackberries, except when winter injury occurs on thornless blackberries, and most of the reports are associated with raspberry. However, wet, humid conditions observed in Georgia and other southeastern states allow for significant losses following pruning or other injuries to the primocane. Avoid wounding the primocanes whenever possible. However, pruning is necessary for blackberry production, so wounding will occur through pruning operations. Pruning wounds are the primary site of infection, especially following prolonged rains, such as those observed in tropical storms and hurricanes. Rainfall or overhead irrigation will disperse fungal spores to fresh wound sites and create favorable conditions for infection. Always check the weather forecast before pruning operations. If at all possible, prune when at least four days of dry weather are expected. During the summer, "pinch off" or "tip" tender primocanes when they reach the desired height, as opposed to cutting. Practices which promote quick drying of the canopy will help to decrease infection. A weed-free strip under the canopy will also aid drying and air movement. Strobilurin fungicides (Pristine, Cabrio, Abound) should be applied immediately after each pruning to provide a protective barrier on the wound site until healing can occur. Rally, a DMI fungicide, has also shown efficacy when applied to pruning wounds for cane blight. Alternation of Rally and strobilurins would provide a good method of resistance management for this pathogen. See http://extension.uga.edu/publications/detail.html?number=C894 for additional information.

Seasonal 'at a glance' fungicide spray schedule options for caneberries

Developmental Stage	Delayed Dormant	Shoots 6" long till Pre-Bloom	Early bloom (5-10%)	Full Bloom[a]	Petal Fall	Cover Sprays	Pre-Harvest	Harvest	After Harvest
Disease (Registered fungicide)	**Anthracnose, Spur Blight, Cane Blight** (Lime Sulfur or Copper)	**Anthracnose, Cane Blight, Spur Blight, and Leaf Spots** (Copper, Cabrio, Abound, Pristine, and Captan) **Rusts** (Rally, Abound, Cabrio, Pristine, Tilt) **Powdery Mildew** (Sulfur, Rally, Cabrio, Abound, Pristine, Luna Tranquility,) **Phytophthora Root Rot** (Ridomil, phosphorous acid-based products)	**Botrytis** (Rovral, Nevado, Iprodione, Elevate, Switch, Pristine, Luna Tranquility, Ph-D and Captan) **Rosette** (Switch, Abound, Pristine) **Powdery Mildew** (Rally, Cabrio, Abound, Pristine, Luna Tranquility,) **Rusts** (Rally, Abound, Cabrio, Pristine, Tilt) **Anthracnose, Cane Blight, Spur Blight, and Leaf Spots** (Cabrio, Abound, Pristine, and Captan)	**Botrytis** (Rovral, Nevado, Iprodione, Elevate, Switch, Pristine, Luna Tranquility, Ph-D and Captan) **Rosette** (Switch, Abound, Pristine) **Powdery Mildew** (Rally, Cabrio, Abound, Pristine, Luna Tranquility,) **Rusts** (Rally, Abound, Cabrio, Pristine, Tilt) **Anthracnose, Cane Blight, Spur Blight, and Leaf Spots** (Cabrio, Abound, Pristine, and Captan)	**Botrytis** (Rovral, Nevado, Iprodione, Elevate, Switch, Pristine, Luna Tranqulity, and Ph-D and Captan) **Rosette** (Switch, Abound, Pristine) **Powdery Mildew** (Rally, Cabrio, Abound, Pristine, Luna Tranquility,) **Rusts** (Rally, Abound, Cabrio, Pristine, Tilt) **Anthracnose, Cane Blight, Spur Blight, and Leaf Spots** (Cabrio, Abound, Pristine, and Captan)	**Botrytis** (Rovral, Nevado, Iprodione, Elevate, Switch, Pristine, Luna Tranquility, Ph-D and Captan) **Rosette** (Switch, Abound, Pristine) **Powdery Mildew** (Rally, Cabrio, Abound, Pristine, Luna Tranquility,) **Rusts** (Rally, Abound, Cabrio, Pristine) **Anthracnose, Cane Blight, Spur Blight, and Leaf Spots** (Cabrio, Abound, Pristine, and Captan)	**Botrytis** (Rovral, Nevado, Iprodione, Elevate, Switch, Pristine, Luna Tranquility, Ph-D and Captan) **Rosette** (Switch, Abound, Pristine) **Powdery Mildew** (Rally, Cabrio, Abound, Pristine, Luna Tranquility,) **Rusts** (Rally, Abound, Cabrio, Pristine, Tilt) **Anthracnose, Cane Blight, Spur Blight, and Leaf Spots** (Cabrio, Abound, Pristine, and Captan)	**Botrytis** (Rovral, Nevado, Iprodione, Elevate, Switch, Pristine, Luna Tranquility, Ph-D and Captan) **Rosette** (Switch, Abound, Pristine) **Powdery Mildew** (Rally, Cabrio, Abound, Pristine, Luna Tranquility,) **Rusts** (Rally, Abound, Cabrio, Pristine, Tilt) **Anthracnose, Cane Blight, Spur Blight, and Leaf Spots** (Cabrio, Abound, Pristine, and Captan) **Cane Blight (see notes)**	**Phytophthora Root Rot** (Ridomil, phosphorous acid-based products [Prophyt, etc.]) **Cane Blight** (see notes) **Orange Cane Blotch** (Prophyt) **Powdery Mildew** (Rally, Cabrio, Abound, Pristine, Luna Tranquility,) **Rusts** (Rally, Abound, Cabrio, Pristine, Tilt) **Leaf Spots** (Tilt, Abound, Cabrio, Quilt Xcel, Pristine)

Appendix 2

Table of papers investigated, including those in which the author was asked to be reviewed (but were already discussed in the 2006 work by Berrie and Allen).

Paper	Title	Citation
Mikulic-Petkovsek *et al*. 2014	Changes in phenolic content induced by infection with *Didymella applanata* and *Leptosphaeria coniothyrium,* the causal agents of raspberry spur and cane blight.	Plant Pathology
	Infected raspberry canes were characterized by a significant decrease of total hydroxycinnamic acid derivatives compared to healthy canes. These compounds are involved in the synthesis of chemicals such as lignin and suberin. The biosynthesis of these chemicals involves several steps and the first reactions are shared with the phenylpropanoid pathway, consuming cinnamic acids – thus the decrease of cinnamic acids in infected cane tissue coincides with the deposition of lignin and suberin at the infection site.	
M. Rahman 2013	Improved management of blackberry cane blight caused by *Leptosphaeria coniothyrium*	Journal of Phytopathology
	No abstract available on Resarchgate	
E. Seemüller, Sylvia Kartte, M. Erdel 2008	Penetration of the Periderm of Red Raspberry Canes by *Leptosphaeria coniothyrium*	Journal of Phytopathology
	Protected raspberry canes were inoculated with *Leptosphaeria coniothyrium*. After penetration of the epidermis the fungus invades the cortex. The accumulation of mycelium in this tissue leads to a partial digestion of the middle lamellae of the outermost cork layer within the polyderm. The fungus penetrates this single cell barrier through the separated cells and colonizes the phelloid tissue between the first and second cork layers. After vigorous growth in this non-suberized tissue the fungus penetrates the second cork barrier in the same way as the first layer. This mode of penetration has been continued beyond the peridermal and into the vascular tissues. The invasion process occurred slowly and was enhanced by weakening the canes by defoliation at the time of inoculation.	
D. L. Jennings, E. Brydon 2008	Further studies on resistance to *Leptosphaeria coniothyrium* in the red raspberry and related species	Annals of Applied Biology
Barrie Ia, Johnson CA, Gordon SC 2000	An appraisal of the UK raspberry cane midge prediction system and its application under differing European climates.	Third European Conference on Applied Climatology (ECAC2000) (CD-ROM), Pisa, Italy, 16-20 October, 2000, pp.3.
Williamson 1991	Cane blight. In Compendium of Raspberry and blackberry Diseases and insects	APS Press
	See book	
Williamson and Jennings 1992	Resistance to cane and foliar diseases in red raspberry (*Rubus idaeus*) and related species	

	No abstract available on Researchgate	
Woodford and Gordon 1988	Alternatives to vigour control with dinoseb as a means to control raspberry cane midge and midge blight in red raspberry cv. Glen Clova	J. of Hort. Sci (1988) **63** (4) 587-593
	The contact desiccant dinoseb was used to manage excessive cane growth and to control cane pests and diseases in many Scottish plantations of the raspberry cv. Glen Clova from 1977 to 1987. All further use of dinoseb in the UK was banned in January 1988. This paper describes a trial that compared insecticides to control raspberry cane midge (*Resseliella theobaldi*) and midge blight in plots treated or untreated with dinoseb. Midge populations and damage were greatly reduced where dinoseb had been applied, and insecticide treatment was unnecessary to protect the replacement canes. Only 2.7% of the fruiting canes were found to be dead or wilting in June of the following year compared with 16.8% in completely untreated plots. In plots untreated with dinoseb, two applications in June of fenitrothion at 525 g a.i. or chlorpyrifos at 470 g a.i., both applied in 11001 water/ha, controlled first generation *R. theobaldi* and lesion development. The control of lesion development and subsequent midge blight by chlorpyrifos was as effective as that obtained by vigour control alone. Damage to first-flush canes was decreased to a lesser extent by fenitrothion and by one of two treatments with gamma-HCH. Some alternative methods of controlling *R. theobaldi* without pesticides are discussed.	
Williamson *et al*. 1986	Factors affecting the development of cane blight (*Leptosphaeria*) on red raspberries in Washington, Scotland and Germany	Ann. Appl. Biol. (1986), **108** 33-42
	The effects of cultivar, virulence of isolates of *Leptosphaeria coniothyrium*, cane maturation and wound healing were examined in a series of inoculation experiments carried out over a 3–yr period in three countries in an attempt to explain why cane blight has caused serious yield losses in machine-harvested red raspberries in Europe, but not in the Pacific Northwest of America. Three isolates of *L. coniothyrium* from Puyallup (USA), Dundee (UK) and Dossenheim (FRG) were pathogenic on the three test cultivars Willamette, Malling Jewel and Glen Isla in all the experiments. Isolates and cultivars differed for aggressiveness and susceptibility respectively but their ranking was dependent on the test conditions and the differences were small and unlikely to explain the differences in incidence and severity of cane blight in raspberry fields in Scotland and the Pacific Northwest. Conditions at Dossenheim were most favourable for lesion development. At all sites, canes inoculated in late summer produced shorter lesions than those inoculated earlier. A delay between wounding and inoculation produced shorter lesions than simultaneous wounding and inoculation.	
Lawson and Wiseman 1983	Techniques for the control of cane vigour in red raspberry in Scotland: effect of timing and frequency of cane removal treatments on growth and yield in cv. Glen Clova	J. of Hort. Sci (1983) **58** (2) 247 - 260
	Annual removal of first-flush canes to reduce cane vigour increased the yield of fruit by an average of 38% over a five-year period. Varying the height at which first-flush canes were removed had no effect on cumulative yield, but the later the date of annual treatment the more rapid and severe was the decline in the height and numbers of second-flush canes. This was more than offset by greater productivity per cane except in the final year. Resting plots in alternate years gave a cumulative yield 31% greater than that on wholly untreated plots, but maintained cane production at a higher	

	level than on annually-treated plots, thereby prolonging the potential productive life of the plantation. Increased yield of fruit by second-flush canes in comparison with first-flush canes was associated with a lower incidence of cane death probably due to raspberry cane midge (*Resseliella theobaldi)*, also involved were increases in the number of cropping nodes per cane and the production of more and bigger berries per cropping node. The results are discussed in relation to maximising the beneficial effects of the cane vigour control technique on plantations of cv Glen Clova and exploiting fully the potential yield of this cultivar under Scottish conditions	
Williamson and Hargreaves 1979	Fungi on red raspberry from lesions associated with feeding wounds of cane midge (*Resseliella theobaldi*)	Annals of Applied Biology
	Two types of vascular lesion are described from the base of canes in plantations infested by raspberry cane midge (*Resseliella theobaldi*); (1) brown lobate lesions ('patches') confined to midge feeding areas, (2) brown lesions spreading proximally and distally from the point of infection ('stripes'). Either or both types of lesion may be presented in individual canes. Isolations from (1) produced principally *Fusarium avenaceum*; isolations from tissues where (1) and (2) are contiguous gave *Leptosphaeria coniothyrium* and *F. avenaceum*; isolations from (2) arising from old cane stub wounds in the absence of midge gave *L. coniothyrium*. The important secondary role of fungi in the midge blight complex is confirmed, but the involvement of *L. coniothyrium* in the complex is unclear because it also infects stub wounds on midge-infested canes	
Williamson and Hargreaves 1978	Cane blight (*Leptosphaeria coniothyrium*) in mechanically harvested red raspberry (*Rubus idaeus*)	Ann. Appl. Biol (1978) **88**, 33-43.
	Leptosphaeria coniothyrium, the cane blight pathogen, was the fungus most commonly isolated from vascular lesions developing from mechanical harvester wounds on first-year red raspberry canes. When inoculated to scalpel wounds, it induced similar lesions which were later associated with bud failure and cane death as were infected wounds on machine damaged canes. Cane resistance increased from May until August, inoculations later than July inducing only small lesions and rarely causing bud failure	

Appendix 3

Online extension services/guides to Cane blight control

- http://www.smallfruits.org/ipm-guides.html
- https://hort.uwex.edu/articles/cane-blight/
- https://extension.psu.edu/bramble-disease-cane-blight
- https://ag.umass.edu/fruit/fact-sheets/raspberry-ipm-cane-blight
- http://extension.uga.edu/publications/detail.html?number=C894&title=Cane%20Blight%20of%20Blackberry
- http://www.omafra.gov.on.ca/english/crops/pub360/notes/raspcanebl.htm
- http://ipm.uconn.edu/documents/raw2/Cane%20Diseases%20of%20Brambles/Cane%20Diseases%20of%20Brambles.php?display=print
- https://www.brandonu.ca/hortline/diseases/cane-blight/