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The results and conclusions in this report are based on investigations mainly conducted over one-year periods. 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: 10 September 2020

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GROWER SUMMARY

This summary brings together information on research carried out in this project between 2015 and 2020. The work completed in 2019 and 2020, not covered in previous Annual Reports, is addressed in full in the Science Section.

The diseases and pests selected for study were based on current issues identified by industry intelligence and the need for urgent problem-solving work to assist grower practice. Pathology focussed on the control of Phytophthora root rot and the species involved, the detection of Verticillium wilt and reviews of cane blight and biennial cropping were completed. Entomology examined the effects of spraying for spotted wing drosophila on two-spotted spider mite predators and how predator populations could be boosted, and tested the use of entomopathogenic nematodes for the control of blackberry leaf midge.

Seeking alternative solutions to controlling Phytophthora root rot

Headline

- New information has been gathered on Phytophthora root rot and its control in raspberry.

Background and expected deliverables

Phytophthora rubi is the most serious disease of raspberry, causing root death and die-back of canes. It is soil/substrate-borne and the spores spread in water. Many growers reduce the risk of infection by planting in coir substrate and maintaining control has relied on chemical fungicide drenching in Spring and Autumn.

The work in this objective investigated a range of novel plant treatments for *P. rubi* control. Alongside this, in the last two years, any effect of cold storage of long-canes on the incidence and severity of *P. rubi* infection was studied. In the final year, with wilting being increasingly reported in crops, a country-wide sampling of raspberry plants was undertaken to determine whether *Phytophthora* species other than *P. rubi* might be detected in them.

Summary of work and main conclusions

Years 1 to 3 (2015 - 2017); in-vitro testing of Serenade ASO and Prestop, and use against *P. rubi* on plants during propagation

During the early stages, the project aimed to determine whether products other than conventional chemical fungicides used against Phytophthora root rot, could improve root health and produce propagation material that was less susceptible to root rotting by *P. rubi*.

Agar plate tests (see 2016 Annual Report) showed that *P. rubi* mycelial growth was halted by Paraat (dimethomorph) as well as the metabolites present in Serenade ASO (*Bacillus subtilis* strain QT 713). Mycelial growth was also significantly slowed by Prestop (*Gliocladium catenulatum* strain J1446). Technical difficulties caused abandonment of behaviour observation tests with *P. rubi* zoospores related to finding materials to use as baits.

Raspberry cv. Tulameen in multicellular propagation trays and modules were drenched with biostimulants and growth promoters during 2015. In 2016 they were potted up and received further treatments both at a commercial farm in Oxfordshire and at ADAS Boxworth, where plants received an additional inoculation with *P. rubi*. The treatments did not improve plant vigour or reduce root rotting (see 2016 and 2017 Annual Reports).

Years 3 and 4 (2017 & 2018); efficacy of fungicide application and comparison of plants from cold-storage or outdoor overwintering, with *P. rubi* infestation in Spring

Commencing in September 2017, investigation of root health in mature long cane raspberries and the use of biofungicides continued (see 2018 and 2019 Annual Reports). As there had been UK reports of plant losses following cold-storage (before fruiting), studies investigated the effects of cold-storage on the incidence and severity of *P. rubi* infection. Potted plants in a polytunnel were inoculated with *P. rubi* in the Spring following cold-storage. The plants were drenched either in the preceding Autumn or in the Spring using Paraat (at 1 g per 5 L potted plant), Serenade ASO (at 10 L/ha in 10 ml/L water) or Prestop (at 5 g/L water).

Following *P. rubi* inoculation there were no treatment differences throughout the two crops, with symptoms also found in uninoculated plants. Molecular testing of rotted roots in October confirmed *P. rubi* in inoculated plants, but *P. idaei* was also detected in both *P. rubi* inoculated and uninoculated roots and it was presumed this had infested plants during propagation. There was lower than anticipated *P. rubi* incidence even in the untreated control, probably attributable to the unusually hot weather at the time of artificial inoculation in Spring 2018. Uninoculated untreated cold-stored plants had more rotted roots in Spring than ambient-stored and they produced three rather than two primocanes by June, with a greater proportion of primocanes wilting in October. However, the combination of Autumn treatment with Prestop or Serenade ASO followed by ambient storage resulted in more rotted roots in Spring, but differences in plant health did not persist.

Years 4 and 5 (2018 & 2019); efficacy of fungicide application and comparison of plants from cold-storage or outdoor overwintering, with *P. rubi* infestation in Autumn

Starting in September 2018, a further experiment was commenced in which the modules were inoculated with *P. rubi* during Autumn, rather than Spring, followed by placing half the plants in ambient conditions and half in cold storage (see 2019 and current Annual Reports). Paraat

applied in either Autumn or Spring, significantly reduced root rot, floricane wilting and death and there was good primocane production after fruiting. Treatment with either Prestop or Serenade ASO was not effective. Plants from neither storage regime showed consistently greater severity of all disease symptoms. Further details are provided in the Science Section.

Year 5 (2019/20); sampling survey of crops for wilted plants and the use of lateral flow devices and molecular testing to determine the species of *Phytophthora* present.

A sampling survey of UK raspberry crops was completed in 2019 to determine what species of *Phytophthora* were present in plants showing wilting or root rotting. This arose following concern in the industry that, based on recent samples sent to plant clinics, species other than *P. rubi* could be contributing to plant losses. Within their regions, consultants and growers sampled both partially rotted roots and the lower 100 mm of still-living cane, from symptomatic plants. There was no intention to systematically sample from across the UK. Twenty batches of samples were received between March and September 2019, from approximately 68 plantations, all but one from England. A total of 180 plant tissue samples were tested, from 76 raspberry plants of a range of ages and varieties. Three quarters were containerised rather than soil grown. In most cases one cane and a sample of roots were tested from each plant, resulting in 89 root tissue samples and 79 cane, although 12 raspberry leaves were also tested.

Two experimental lateral flow devices (LFDs) were used, one with the antibody 3H7 which reacts to the presence of a wide range of *Phytophthora* species, and the other with 3C4 which was expected to give a positive test line with *Phytophthora* species only in *Phytophthora* Clade 1 (which includes *P. idaei*), Clade 7 (which includes *P. rubi*) and Clade 8. Subsequently, frozen tissue was re-sampled to extract and amplify DNA of *Phytophthora* spp. by nested PCR to enable sequences to be matched against a database of *Phytophthora* species.

Phytophthora spp. were detected in the majority of samples using the “general” experimental LFD. Tissue which gave positive readings with the 3C4 LFD was later confirmed by DNA sequencing to have successfully detected *P. rubi* and *P. idaei*. The 3C4 LFD also at times was positive for samples later shown to contain DNA of *P. citrophthora*, *P. bishii*, *P. citricola* and *P. plurivora* and, as these are within Clade 2, it was not expected to detect them. It was unclear if this was a true result or whether *P. rubi* had been present only in the tissue sub-sample tested with the LFD. A few instances of *Peronospora sparsa* (downy mildew) DNA were detected and it was unclear whether the positive LFDs for these samples had reacted to the *P. sparsa* or if *Phytophthora* spp. had been present in the material tested by them.

P. rubi was found in 42.8% of samples received. It was isolated from 42.1% of canes and 34.8% of roots. 10.8% of all samples received had no *Phytophthora* spp. DNA detected.

Phytophthora species other than, or in addition, to *P. rubi* were identified from 23.8% of all the samples; principally comprising 8.9% *P. citrophthora*, 6.1% *P. bishii* and 2.8% *P. citricola*. Only 0.5% had *P. idaei.* The extent to which these species contributed to symptom development on raspberry in the UK requires further study. However, *P. bishii*, *P. citrophthora* and *P. citricola* are known as raspberry pathogens in other countries, but only the latter has published records on raspberry in the UK.

A further 16.1% of samples the *Phytophthora* spp. infestation was unable to be defined from the DNA, but probably included *P. rubi* mixed with another species. Some DNA sequences, from nine plants, could not be matched to any *Phytophthora* species currently on the extensive genetic database utilised.

Further details of the survey are given in the Science Section.

Financial benefits

The UK raspberry industry is worth approximately £146.8 million at farmgate prices, from and area of 1,424 ha (Defra Horticulture Statistics, 2019).

Raspberry root rot leads to wilting of crops, and when severe, crops can be lost. Losses before cropping have been reported from long-cane modules that have been cold-stored and in which there has been high investment in order to secure a good fruit yield in the first year of planting. Premature loss in yield of crops normally expected to produce over several years continues to be a problem. Although a drench with Paraat (dimethomorph) is usually used by growers this is not sustainable in the longer-term and more attention will need to be given to identifying where infection is coming from and adopting more plant hygiene measures.

This project has shown that species other than *P. rubi* are present at currently low incidence, and that this includes presence in propagation material. By alerting the industry at this time, action should be able to be taken to find out more about these species and to seek ways to prevent further spread of these and *P. rubi.*

LFDs developed with previous AHDB funding were effective at detecting *P. rubi* and *P. idaei* and other pathogenic *Phytophthora* spp. without including saprophytic species that can mislead disease diagnosis and are detected by the currently commercially available LFDs.

Action points for growers

- Paraat (dimethomorph) has been confirmed to provide control against *Phytophthora rubi*, either when applied in Autumn (pre-infection) or in Spring (post-infection).
- Applications of Prestop (two applications) or Serenade ASO (single application) drenches in Spring to cold stored plants are unlikely to reduce the development of Phytophthora root rot, where plants became infected the previous Autumn.
- Plants coming out of cold-storage can show more roots rotted in Spring than those left outdoors and it is important to facilitate good establishment by allowing thorough thawing before potting-up.
- Free-water in pots is conducive to the spread of *Phytophthora* spp. and so aim to adjust irrigation to the uptake of plants with variations in temperature and growth.
- Be alert to any changes in the extent or timing of wilting due to root rot, as *Phytophthora* species other than *P. rubi* have been confirmed in commercial raspberry plants.
- Novel antibodies used in two lateral flow devices (LFDs) were shown to detect a range of *Phytophthora* species, with one of the pair limiting detection to pathogenic *Phytophthora* spp., but further work will be required before commercial release of these as kits. In the meantime, testing on site with currently available LFDs detecting all *Phytophthora* spp. is recommended if not using a diagnostic laboratory.
- To reduce the probability or re-occurrence in subsequent years, when plants are confirmed to be infected by *Phytophthora* spp. efforts should be made to trace the source of the infection through keeping records of the distribution within the plantation and whether worse in particular fields or propagation batches.
- Ensure that infested irrigation water is not used and other hygiene measures are in place to stop pathogens spreading between plants, particularly in propagation areas.

Sampling raspberry and blackberry plants and soil from crops affected by Verticillium wilt in order to test a newly developed molecular assay

Headline

- Quantitative detection of *V. dahliae* was achieved using qPCR on cane and root tissue and from the soil around roots

Background and expected deliverables

Verticillium dahliae and *Verticillium albo-atrum* are the causal agents of Verticillium wilt in

raspberry and blackberry, resulting in stunted shoots, extensive wilting and ultimately plant death. Crop loss can occur if the canes die before reaching maturity and as plants succumb once established. Severe outbreaks have occurred sporadically in UK cane fruit crops and widespread infection at a lower incidence is suspected. The area of primocane-fruiting raspberries has been increasing in the UK and the cultivars grown tend to be susceptible to Verticillium wilt. *V. dahliae* and *V. albo-atrum* have a wide host range and persist in soils, with the level present in a field thus affected by previous cropping. The relative damage caused to raspberry by each *Verticillium* species is not known.

The Harris test, a wet sieving method, can be carried out on soil samples before planting to enumerate the microsclerotia of *V. dahliae*. However, many growers do not submit samples because the assay takes 6-8 weeks. *V. albo-atrum* is not detected by the Harris test.

In 2015, Real-time, or Quantitative, PCR assays (qPCR) for testing soils for *V. dahliae* had recently been successfully developed in AHDB Project SF 97, providing results within a few days, with detection of *V. dahliae* down to levels correlating with 0.5 microsclerotia / g soil. Observations have suggested a tolerance of up to 50 propagules / g soil for some commercial floricane raspberry cultivars, while some primocane fruiting cultivars may be ten times more susceptible. Records obtained from qPCR of soil before planting a range of cultivars could be used in future to establish microsclerotia thresholds for raspberry and blackberry.

The work in 2015 sought to use qPCR to determine levels of soil and plant tissue infestation by *V. dahliae* and to examine these in relation to the severity of wilting recorded in the crop.

Summary of work and main conclusions

Years 1-2 (2015-2016); qPCR for quantification of *V. dahliae* in cane fruit tissue and soil

Samples associated with 40 mature plants consisting of raspberry, hybrid berry and blackberry were taken in August 2015 from a variety of farms and ranged from asymptomatic to severely infected. Sampling was carried out by excavating the soil beneath and around an affected plant and sections of cane, crown and attached roots were collected. A further 65 samples of symptomless plants in substrate were collected from propagators. The qPCR assay was carried out utilising pre-extraction processing, buffers to remove reaction inhibitors and an automated DNA binding system to capture total DNA to detect *V. dahliae.*

The symptomatic plants with the higher wilt severity indices were those that tested positive for *V. dahliae* DNA in either or both stem bases and roots. Symptomatic plants that were growing in clay loam were more frequently shown to be positive for *V. dahliae* DNA than those from sand. No *V. dahliae* was detected in plants direct from propagators. In all instances the mean levels of DNA of *V. dahliae* detected in the stem base material was greater than in the

roots of raspberry. There was a good correlation between level of *V. dahliae* detected in the stem bases and the roots of each plant.

V. dahliae was detected in almost half of the raspberry plants showing symptoms, but very few of the soils (3 from 22). It was unclear if this was because the assay was less able to detect the pathogen DNA in the soil than it is in plant tissue.

In 2016, the assay was assessed for sensitivity using DNA extracts from pure cultures of *V. dahliae* and detected down to 0.04 pg/ul of extract from *Verticillium* culture.

Survey results were given mainly in the 2016 Annual Report, with an update in the 2017.

Work in 2019 by a PhD student at Fera as part of the AHDB Soil Biology and Soil health project has since reduced soil sample size from 1 kg and is seeking to improve detection sensitivity.

Financial benefits

Verticillium wilt of raspberry and blackberry has become a much greater threat to raspberry and blackberry growers in the past 15 years. Many of the modern primocane raspberry and the recently introduced blackberry cultivars are particularly susceptible to *Verticillium dahliae* (the cause of Verticillium wilt). Plant pathologists and cane fruit growers lack the knowledge of how susceptible different cultivars are to the disease at differing levels of the resting bodies of the pathogen in field soils. This has made it difficult to make management decisions about the safety of a new field destined to host a cane fruit crop.

Molecular quantification of *V. dahliae* in soil has become possible and there is potential for this to replace the much slower Harris test method of extraction of microsclerotia and to be less costly. Comparing the quantities of *V. dahliae* DNA in soil with that in plants of various wilting severities growing in the same soil allows the linking of pathogen levels to host symptom severity and whether there may be plant tolerance to certain soil levels.

The future benefit will be that through improving our knowledge of cultivar susceptibility, with futher work to develop threshold levels of soil inhabiting *Verticillium dahliae* for different cultivars, it will allow growers to make informed decisions about the safety of a new field soil which might be used to establish a new crop, thereby avoiding severe crop losses to the disease in the first two to three years of a plantation’s life span.

Action points for growers

- Caution is needed if intending to plant cane fruit in soil as it can be infested with *Verticillium* spp., with selected cultivars particularly susceptible to infection.

- Soil should still be sent for Harris testing to detect microsclerotia until molecular techniques are validated; allow approximately five weeks for test turnaround.

A review of the current threat posed to the UK raspberry industry by cane blight (*Leptosphaeria coniothyrium*) and identifying new control options

Headline

- Control of cane blight in UK raspberries is of increasing importance and requires renewed attention due to the loss of plant protection products for control of both the pathogen and raspberry cane midge.

Background and review findings

A review of cane blight epidemiology and control was carried out for the 2019 Annual Report. This was carried out because UK raspberry growers are beginning to see high levels of infection of cane blight (*Leptosphaeria coniothyrium*). Cane blight is now being seen in double cropping primocane as well as summer fruiting raspberries. Cane blight is a relatively weak pathogen, so often requires damage to the cane in order to enter the plant. Damage can follow poor control of raspberry cane midge (*Resseliella theobaldi*) (exacerbated by the loss of chlorpyrifos), frost, strimming or poor application of desiccant to control unwanted primocane. With the loss of Folicur (tebuconazole), Signum (pyraclostrobin + boscalid) became the sole product for control of cane blight, but the dose rate permitted is lower than that which gave efficacy in trials.

An extensive review of cane blight epidemiology was performed for the AHDB in 2006, as well as a fungicide efficacy trial. This work indicated that infection can take place far later than when fungicides are normally applied during and immediately post-harvest. 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 previously.

This review for Project SF 158 concluded that there is a need for trials to not only test efficacy of new fungicides against cane blight, but also to understand the disease life cycle in soil and soilless substrate grown crops under protection. 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, in particular the time of year and conditions for spore dispersal and any changes to this brought about by growing the crop in tunnels for part of the year. Knowledge of spore release timing and tissue infection susceptibility is important for decisions on fungicide spray timing. 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. Biennial cropping also significantly reduces the old to new cane spread of pests and diseases and is likely to be of benefit in the control of cane blight.

A review of biennial cropping and annual cropping of long-canes as a means of pest and disease control with a financial comparison of different production systems

Headline

- The yields and gross margins following the first year of biennial cropping were envisaged to be poorer than in the second and third year of traditional systems, in the absence of losses to pests and diseases

Background and review findings

Previous work has demonstrated the benefits of separating the two phases of growth in raspberry in breaking the life-cycles of cane blight, raspberry cane midge and associated midge blight. The purpose of this financial comparison was to assess the impact that this would have on the profitability of raspberry production. A great many assumptions were included in the budget comparisons. These included assumptions on receipts, variable costs of price, variable costs of yield and variable costs based on area.

A table was produced summarising the differences between the systems in each of the three years calculated comparing the yield per hectare for each, the cane management costs for each and the gross margin for each. The traditional Glen Ample (summer fruiting) was used as a baseline against which the annual long-cane Glen Ample and biennial Glen Ample are compared. The traditional Kweli was used as a baseline against which the annual long-cane Kweli is compared.

In Year 1, the yields and gross margins of the annual and biennial systems compare favourably with the traditional summer fruiting and traditional primocane production systems. The annual long-cane system produced over twice the gross margin of the traditional while the biennial system produced over a third more. The annual long-cane primocane system produced almost two and half times the gross margin of the traditional primocane.

In contrast, in Years 2 and 3, the yields and gross margins of the annual (long-cane) and biennial systems compared poorly with the traditional systems and in the majority of cases, these were less than those produced by the traditional Glen Ample and Kweli crops.

In all three years, there were reductions in cane management costs when using annual (long-cane) or biennial cropping compared to traditional.

Although the yields and gross margins were favourable on the first year of production for the annual (long-cane) and biennial systems and less so on the second two years, caution was urged when considering these. The budgets which were constructed took no account of crop loss caused by insect pests or diseases, assuming that optimum yields could be achieved from each system.

The exercise demonstrated the change in finances that may occur should labour availability and loss of crop protection products render the systems of growing more or less viable than at present. Many pests and diseases are becoming increasingly difficult to contain with a diminishing number of crop protection products available. As the availability of these products declines further, raspberry growers may find they have no alternative but to turn to annual cropping of long canes or biennial cropping to achieve satisfactory control.

Developing and maintaining Integrated Pest Management (IPM) to successfully control two-spotted spider mite whilst controlling spotted wing drosophila (SWD) and other pests with conventional sprays

Headlines

- Early releases of *Amblyseius andersoni* led to better control of a late infestation of spider mite compared to a preventative release of *P. persimilis* in a commercial propagation raspberry crop.
- Experimental overhead, rather than crop-face, spraying resulted in less deposition on leaf undersides, so providing refuges for natural predatory mites (mainly *A. andersoni*) thus resulting in their reduced mortality, without any loss of control of SWD.

Background and expected deliverables

Spotted wing drosophila (SWD), *Drosophila suzukii*, has established in the UK and this fruit pest is currently controlled with conventional spray programmes, coupled with good farm hygiene. Given that much of the control for other pests in raspberry crops, such as the two-spotted spider mite, *Tetranychus urticae*, relies on biological control within Integrated Pest Management (IPM) programmes, it is important to develop IPM-compatible strategies for control of SWD. It can be hypothesised that as spray programmes for SWD can negatively affect the biocontrol agents for spider mite, leaving unsprayed refuges on the undersides of leaves for commercially introduced and naturally occurring predatory mites may help to maintain the population of predators. Therefore spray application methods which would provide good coverage on the upper leaf surface, but leave the lower leaf surface unsprayed were explored.

Experiments were carried out (by NIAB-EMR) in small purpose-built poly-tunnels to compare the same spray programme applied by two different spraying methods, pervasive canopy spraying using an air-assisted knapsack sprayer and a system of overhead spraying to give spray deposits mainly on the upper leaf surface.

Options for control of two-spotted spider mite (TSSM) with acaricides are very limited, so biological control of the pest within an IPM programme is key to successful management. However, growers need to know how to maintain their IPM programmes whilst applying control products for SWD. The predatory mite *Phytoseiulus persimilis* can give very effective control of TSSM. However it needs warm temperatures to establish and is very susceptible to plant protection products applied for control of other pests including SWD, aphids and capsids. The native predatory mite *Amblyseius andersoni* occurs naturally on raspberry crops and is also commercially available for release on both protected and outdoor crops. *A. andersoni* is also active at a wider temperature range than *P. persimilis*, can establish earlier in the season, does not need TSSM to survive as it can feed on other food sources such as pollen, fungal spores and certain other pests, and is considered to be more tolerant of conventional spray products than *P. persimilis*.

Investigations were therefore carried out (by ADAS) to monitor populations of natural and released predators on commercial raspberry crops and to investigate the role of *A. andersoni* in the control of TSSM whilst controlling SWD and other pests with sprays of Plant Protection Products. The use of a commercially available pollen to boost *A. andersoni* populations was also studied.

Summary of the project and main conclusions

Year 1 (2015); monitoring effects on predators and other pests of sprays against SWD on commercial raspberry crops

In the first year of this project, ADAS monitored the effects of Plant Protection Product sprays applied for control of SWD and other pests on both released and naturally occurring TSSM predators on two commercial tunnel-grown raspberry crops. At the first site low numbers of released *P. persimilis* and naturally occurring *Amblyseius andersoni* and *Neoseiulus californicus* survived applications of thiacloprid (Calypso) and spinosad (Tracer). At the second site, no *P. persimilis* were released but naturally occurring *A. andersoni* and *N. californicus* survived applications of clofentezine (Apollo), abamectin (Dynamec), Tracer and pyrethrins. The conclusion was that naturally occurring predators are likely to have played an important role in maintaining control of TSSM at both sites.

Year 3 (2017); testing the effect on predators of a spray against SWD on a commercial raspberry crop

The 2017 study by ADAS aimed to build on the 2015 monitoring work to provide more robust information on a commercial tunnel-grown primocane raspberry crop. A high density of TSSM was controlled by late July by a combination of *P. persimilis* (released by the grower) and by four naturally-occurring predators; *A. andersoni*, the predatory midge *Feltiella acarisuga,* the ladybird *Stethorus punctillum* and the predatory bug *Orius* sp. A tank mix of Tracer and deltamethrin (Decis) was then applied in early August for control of SWD and blackberry leaf midge respectively and this timing avoided the disruption of IPM.

Year 4 (2018); testing the use of pollen as a food source to boost predator numbers on a commercial raspberry crop

The 2018 experiment by ADAS tested whether a commercial pollen product (Nutrimite™) could boost numbers of either naturally occurring or released *A. andersoni* and improve control of TSSM on a commercial primocane raspberry crop where *P. persimilis* was also released. Nutrimite™ is being used in Europe and Canada to improve the establishment of other predatory mite species on other horticultural crops but has not previously been tested on raspberry with *A. andersoni.* Numbers of naturally occurring *A. andersoni* were low and adding pollen did not increase their numbers. However, on some of the assessment dates, adding pollen to the plants where *A. andersoni* were released led to significantly higher numbers of these predators. Numbers of TSSM were similar in all treatments and the pest was controlled by late July through a combination of *P. persimilis* and *A. andersoni*, before Tracer was applied on 31 July for control of SWD, this timing thus avoiding disruption of IPM.

Year 5 (2019); testing predator and pollen releases to improve TSSM control

Gaining control of TSSM in raspberry crops during propagation is critical to managing the pest in the fruiting crop the following year. The 2019 experiment by ADAS tested the use of *A. andersoni* compared with and combined with preventive releases of *P. persimilis* to a raspberry propagation crop. The benefits of adding the pollen food supplement Nutrimite™ was tested with *A. andersoni*. Preventive predator and pollen releases started on the young crop in the propagation tunnel and continued after transplant into the field. Early preventive releases of *A. andersoni* led to better control of an unusually late infestation of spider mite compared with preventive releases of *P. persimilis* which disappeared before TSSM was present. There were significantly fewer TSSM, eggs and damage where *A. andersoni* was released with pollen compared to where only *P. persimilis* was released, but no fewer than

where *A. andersoni* was released without pollen. Nutrimite™ boosted the *A. andersoni* population on one date immediately after transplant to the field.

In 2020 the experiment on the propagation crop was followed through winter to compare the survival of *A. andersoni, P. persimilis* and TSSM from the different treatment programmes in cold storage and in ambient conditions. This work showed that *Amblyseius andersoni* and TSSM survived 15 weeks of commercial cold-storage at -1°C in low numbers. The results also showed that significantly more *A. andersoni* eggs were found in the cold-stored primocane buds of plants where *A. andersoni* and Nutrimite™ had been released in the previous season compared with the plants where *A. andersoni* had been released alone or alone with an additional autumn release. In this experiment the additional autumn release of *A. andersoni* on 5 September did not significantly affect the number of overwintered *A. andersoni* or TSSM. The numbers of overwintered TSSM and *A. andersoni* largely reflected the population dynamics seen in each treatment in the previous season. This shows that it is important to gain control of TSSM in a propagation crop before TSSM has a chance to find overwintering positions and also that releasing *A. andersoni* will 'seed' the crop with a reproductively active population of predators for the following spring.

Years 1 - 3 (2015 - 2017); to compare populations of TSSM and predatory mites after either overall canopy spraying or overhead misting application against SWD in small experimental tunnels

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

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

The work in 2017 repeated the 2016 experiment, again to determine the effects of overall canopy spraying verses overhead application of a programme of sprays of deltamethrin and spinosad on TSSM and predatory mites, both commercially introduced and naturally occurring. Although it was not possible to determine any treatment effects for the TSSM and *P. persimilis* due to the low numbers per leaf, there were treatment effects for the naturally occurring predatory mites. As in 2016, the sprays reduced the numbers of natural predatory mites, however this effect could be mitigated by spraying from above. The assessment of spray deposition showed that there was less spray on the underside of the leaves in the overhead spray treatment, which could provide a refuge for predatory mites. The data also showed that the amount of spray deposited on the underside of leaves in the overhead spray treatment was highly variable.

As there were only low numbers of *P. persimilis* present it was not possible to determine the effect of the deltamethrin sprays in the polytunnel experiment in 2017. However, laboratory work showed that direct application of deltamethrin killed almost all *P. persimilis* adults within 24 hours, demonstrating that the commercially available strain of *P. persimilis* was not resistant to pyrethroids. The numbers of SWD were low in 2017 therefore no significant treatment effects could be determined.

Year 4 (2018); to assess and adjust spray deposition throughout a commercial crop canopy

In 2018, a field trial was done to assess the spray coverage, spray deposition, and distribution of spray throughout the crop canopy. The raspberry crop was sprayed with a fluorescent tracer and a handheld imaging fluorometer recorded the fluorescence values that were a proxy for the volume of sprayed liquid on the leaf surfaces. The spray was applied to the crop in July, using an Ideal Alsazia axial fan 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. The plant canopy was divided into three vertical zones: Top third, Middle third, Bottom third, with an additional zone at the middle section but deep in the canopy referred to as the Inner zone. At each of the zones, the spray deposition was measured on both upper and lower leaf surfaces (**Figure 1**).

Spray deposition (coverage and volume of sprayed liquid) was highly variable throughout the raspberry canopy. A common trend was more 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 increased 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 variations in spray product coverage about the calculated means was shown to be important. More than 50% of leaves sampled from the middle canopy section-lower leaf side, inner canopy-both leaf sides, and bottom canopy-lower leaf side had less than 5% spray coverage, potentially providing many refuges for insects and mites from control sprays. At these canopy-leaf sections, it was broadly the same for all of the spray settings assessed.

Further research is needed on the role of *A. andersoni* in TSSM control on raspberry and its best-practice use in an IPM programme.

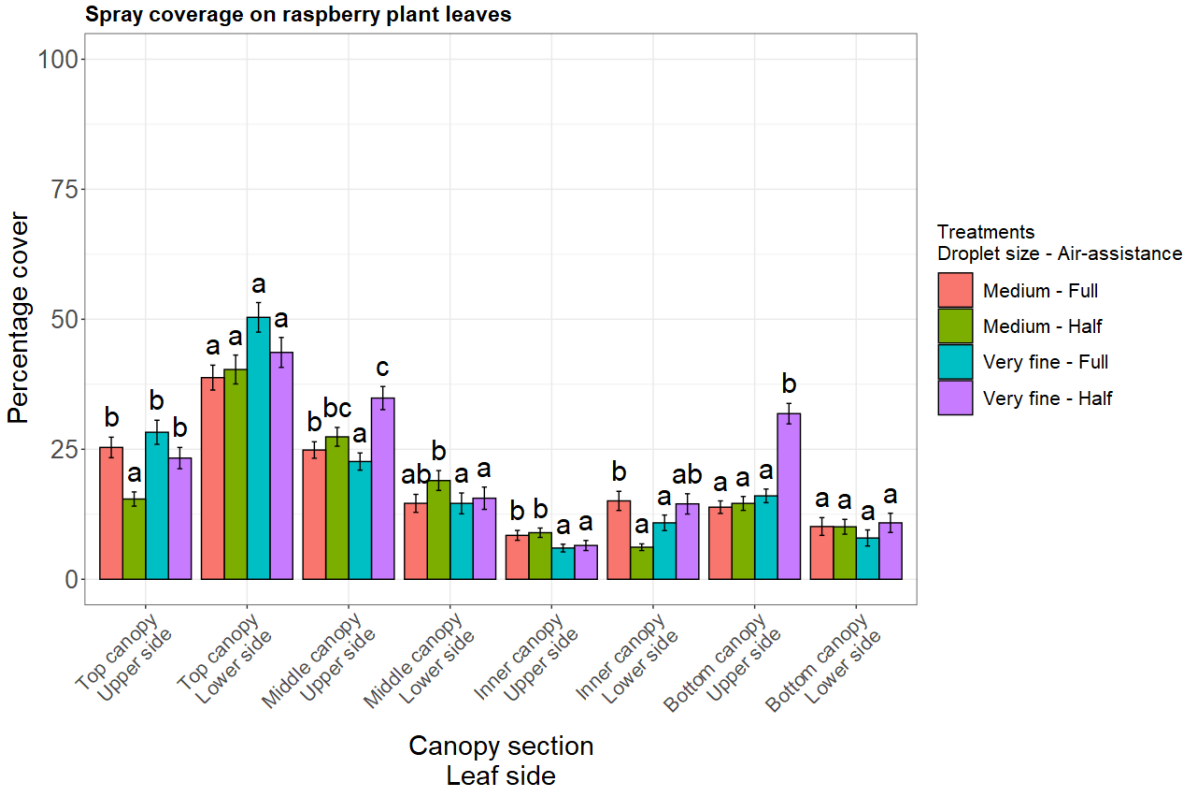


Figure 1. Percentage of leaf area covered with spray deposits at each canopy zone and leaf side, for each of the four spray treatments. The error bars show standard error. Significant differences were identified by GLMER and multiple comparisons Tukey's tests. If significant differences were identified, letter labels denote significant differences between the treatments within each canopy zone / leaf side

Financial benefits

The UK raspberry industry was worth £146.8 million grown over 1,424 ha in 2019 (Defra Horticulture Statistics, 2019). Plant protection products (PPPs) (fungicides, herbicides, insecticides) in raspberry production can cost between £450 and £1,700 per hectare, depending on the cropping system. Application of spray products reduces both natural and

purchased predator populations. Increased grower awareness of, and modifications to, the distribution of spray deposits and the timing of applications could achieve improved pest management by the better integration of both chemical and biological methods.

Figures are not available for the cost of spider mite damage to the raspberry industry. Damage caused to raspberry plants reduces photosynthesis and plant quality. Severe infestations can lead to total defoliation, at great cost to the industry. If an untreated spider mite infestation accounted for only 5% damage to raspberry leaflets and was equivalent to 5% loss in crop value then savings would be made with four biological control treatments, according to the results of the final year of this project (**Table 1**). The cost of six applications of Nutrimite™ (two under protection, four in the field, 26p / pot) was less than one release of *A. andersoni* in the field (31p / pot). Far more *A. andersoni* than *P. persimilis* were released, at a higher cost per pot, but a lower cost per 1,000 mites because they were distributed in breeding sachets rather than released directly in a carrier. Also, since spider mite was not seen in the crop until 5 September, *P. persimilis* was only released three times preventively and once curatively. If TSSM was recorded on earlier assessment dates *P. persimilis* would have been released weekly or fortnightly until established.

Table 1: Costs of the final year treatment programmes compared with potential savings, based on the findings of the experiment. Labour costs have not been included and product costs would be subject to economies of scale. *Estimated savings based on an untreated spider mite infestation causing 5% leaflet damage, equating to 5% loss in crop value.

Treatment	Biocontrol treatment programme	Cost per pot of 3 plants (£)	Total number of mites released	Cost per 1,000 mites	Average % leaflet damage on 16th Oct	Estimated savings to industry* (£ / ha)
T1 *A. andersoni*	4 x release of *A. andersoni*	0.90	1,050,000	£ 0.27	0.325	3,920
T2 *A. andersoni* + pollen	4 x release of *A. andersoni* + 6 applications of Nutrimite™	1.16	1,050,000	£ 0.35	0.062	4,141
T3 *A. andersoni* + *P. persimilis*	4 x release of *A. andersoni* & 4 x release of *P. persimilis* (3 preventive, 1 curative)	1.34	1,080,000	£ 0.40	0.237	3,994
T4 *P. persimilis*	4 x release of *P. persimilis* (3 preventive, 1 curative)	0.45	30,000	£ 4.77	0.587	3,701

Action points for growers

- Aim to establish *P. persimilis* as early as possible and be aware of the contribution of naturally occurring predators in the control of TSSM.

- Consider early, preventive release of *A. andersoni* for TSSM control as soon as temperatures reach 6°C as this predatory mite is more tolerant of low temperatures than *P. persimilis*.
- Use IPM-compatible plant protection products or those with least harmful effects on biological control agents for control of all pests including SWD wherever possible.
- If using plant protection products known to be harmful to biological control agents, some predators may survive in spray refuges' where less deposition occurs, but try to time predator releases to reduce adverse effects.

Developing and combining novel and current IPM approaches to successfully control blackberry leaf midge

Headline

- *Steinernema kraussei* reduced blackberry leaf midge emergence from pupae in laboratory-based coir pot tests but was ineffective in a field trial. More research would be needed to further test potential control in a commercial crop.

Background and expected deliverables

Blackberry leaf midge (*Dasineura plicatrix*) has become an increasing problem on blackberry, hybrid berry and raspberry, with double cropping primocane raspberry being particularly vulnerable to attack. The pest can have up to four generations per year under protection and damages the leaf tips and growing points, which can stunt cane growth, give rise to cane branching and reduce yield. As the midge larvae feed within the leaf tips they are very difficult to target using foliar sprays of plant protection products. Deltamethrin (Decis) can give some control, probably of adults, but is incompatible with IPM. In SF 102 'Biology and integrated control of blackberry leaf midge on blackberry and raspberry', a laboratory pot test showed that using polythene or woven ground-cover matting over the substrate inhibited successful pupation of larvae dropping to the ground, reducing adult midge emergence by 96% and 53% respectively compared with the substrate control. Although covering the entire floor of a polythene tunnel may not be practical, the experiment demonstrated that a ground-based strategy for control of the pest could be effective. Therefore in years 1 and 2, a ground-based strategy was tested using entomopathogenic nematodes to target fully-grown larvae of blackberry leaf midge that drop to the ground to pupate.

Year 1 (2015); testing entomopathogenic nematodes against blackberry leaf midge

In a laboratory pot test, a drench of the entomopathogenic nematode *Steinernema kraussei* (Nemasys® L) significantly reduced mean numbers of blackberry leaf midge adults emerging from treated coir substrate compared with water control pots after adding fully grown midge

larvae to the substrate surface to mimic their natural behaviour of dropping to the ground to pupate. Drenches of two other nematode species (*S. feltiae* and *S. carpocapsae*) were ineffective. Nemasys® L is widely used already as a drench for control of vine weevil on soft fruit crops.

Year 2 (2016); in-crop assessment of nematode drenches against blackberry leaf midge
In a second year, the efficacy was tested of two consecutive drenches of Nemasys® L applied to the soil beneath the canopy of a commercial, soil-grown, tunnelled raspberry crop with a history of blackberry leaf midge, timed to target first and second generation larvae that dropped to the soil. The treatments did not reduce the percentage of leaf tips infested compared with untreated controls. Possible reasons for lack of control were insufficient soil moisture for nematode movement and survival and the short 'window' of opportunity for nematodes to infest the midge larvae before they spin a protective cocoon within which to pupate.

Financial benefits

The blackberry leaf midge is a relatively new pest of raspberry and blackberry in the UK, having assumed greater importance as increasing crop areas have been protected by temporary polythene tunnel structures in the field. It is not uncommon to find that the midge has reduced raspberry yield by 40% and blackberry yield by 10%.

Assuming a typical return for raspberries of £6.49/kg to growers (Defra Basic Horticultural Statistics 2014) and a yield of 14 tonnes/ha, then a 40% crop loss caused by blackberry leaf midge would lead to a financial loss of £36,355/ha. Developing a novel IPM approach will significantly reduce such losses from blackberry leaf midge.

Action points

- No action points were given for blackberry leaf midge control from this piece of work.

SCIENCE SECTION

General Introduction

The research carried out during the five and a half years of this project commenced in 2015 for the advancement and optimisation of integrated pest and disease management (IPM) in cane fruit production systems. There had been significant changes in production style and growing systems including; a) raspberry cropping season extension, including the use of cold-storage and single-cropping of long-canes b) an increasing shift from soil to substrate production due to soil-borne diseases, c) an increase in double cropping primocane raspberry production and autumn (primocane) fruiting varieties of raspberry. These changes presented new challenges for IPM, and more were added with the arrival of spotted wing drosophila (SWD) *Drosophila suzukii* (for which AHDB projects SF 145 and SF/TF 145a were set-up). Research areas were initially based on gaps identified by the AHDB Soft Fruit Panel and the project steering group subsequently directed the course of research to ensure the direction remained relevant to the developing needs of the industry. Delivery was by a consortium of research scientists from ADAS, NIAB EMR, Fera and University of Worcester, with the involvement of UK growers. The project roadmap that developed is shown in **Figure A**.

Research carried out from late 2018 to 2020 is covered in the following sections of this report:

Objective 1) Understanding and managing Phytophthora root rot:

i) To explore the effect of cold-storage of long cane raspberries on *P. rubi* infection and the potential for protection using biopesticides against infestation in Autumn.

ii) To investigate the species of *Phytophthora* present in dying raspberry plants.

Objective 2) Monitoring the benefits of methods for boosting *Amblyseius andersoni* numbers on a commercial raspberry propagation crop for more robust control of *Tetranychus urticae* - two-spotted spider mite (TSSM):

i) A comparison of control by TSSM predators with and without a pollen food supplement.

ii) To study the effect of pollen supplementation on TSSM predator numbers.

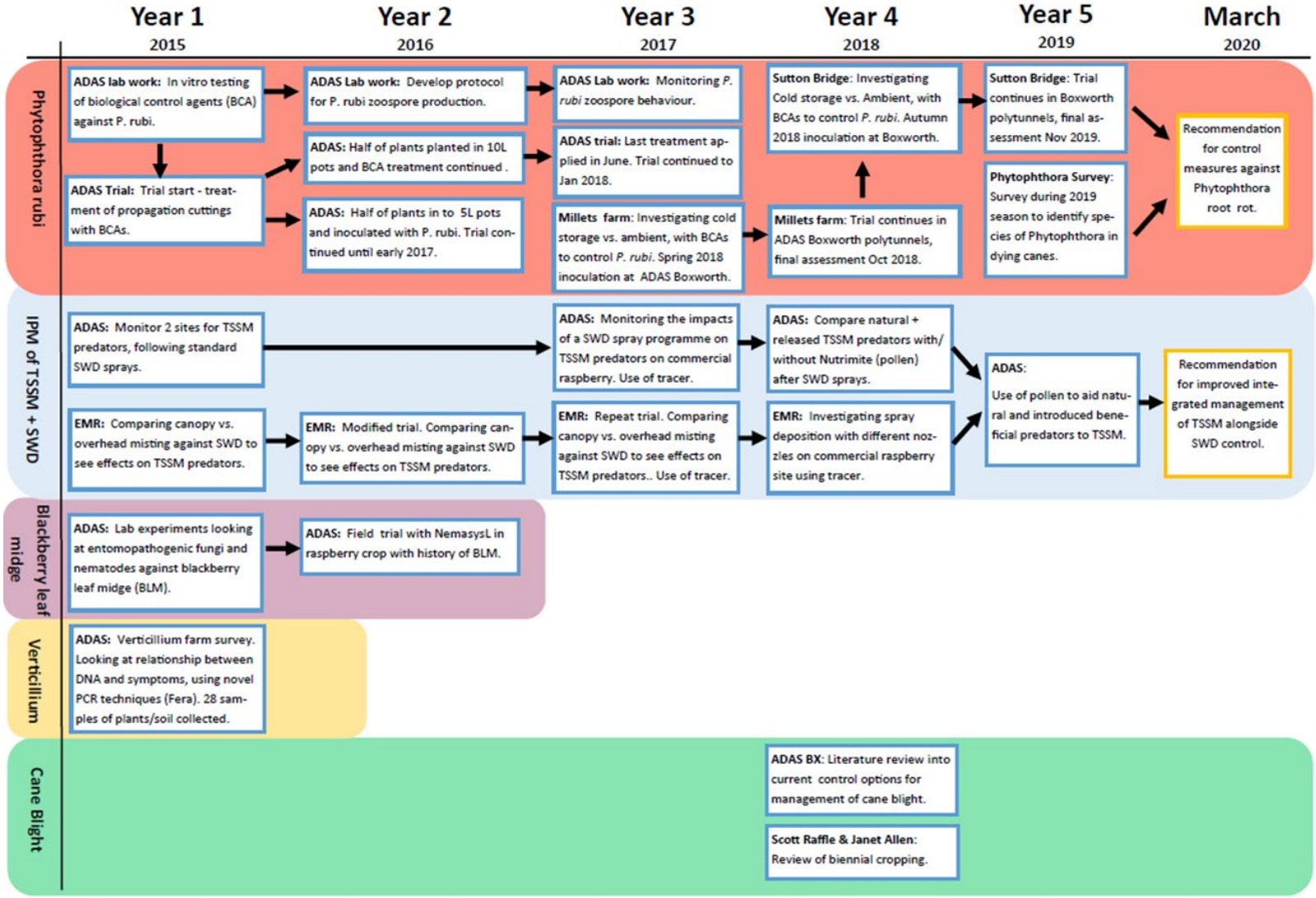
iii) To study the effects on TSSM predator survival of pesticides applied against SWD.

iv) To determine the survival of *A. andersoni* and *P. persimilis* in cold and ambient storage

Objective 3) To review biennial cropping and annual cropping of long-canes as a means of pest and disease control and carry out a financial comparison of different production systems.

Studies in previous years on Phytophthora root rot, Verticillium wilt, cane blight, TSSM and blackberry leaf midge have been outlined in the Grower Summary.

Figure A. Roadmap of the cane fruit research topics covered in AHDB Project SF 158 over the period 2015 to 2020.



Objective 1: Understanding and managing Phytophthora root rot.

Aim 1 : To explore the effect of cold-storage of long cane raspberries on *P. rubi* infection and the potential for protection using biopesticides against infestation in Autumn.

Introduction

Phytophthora root rot, principally attributed to *P. rubi* is now the most destructive disease of raspberries worldwide. It arises in crops grown in both soil (which can contain long-surviving resting spores) and substrate. Current approaches for Phytophthora control rely on a single chemical 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 used currently, 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 QST 713) have multiple modes of action against oomycetes such as a *Phytophthora* spp. and certain fungi.

Around 70% of raspberry material in the UK is currently cold stored at circa -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. Cold storage of strawberry 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*. 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 and/or severity of root infection in cold stored plants.

In previous years of this project, experiments were carried out to investigate the effects of a range of novel plant treatments on raspberry growth and their resilience to pests and disease from propagation through to primocane production. Preliminary work was also carried out to examine *P. rubi* zoospore behaviour with raspberry root exudates.

In the last two years, this project aimed 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 either in a cold-store or outside. The same products Paraat, Prestop and Serenade ASO were tested in successive years on long-cane cv. Tulameen from the same propagation source. In both years the plants were potted-on in April and maintained in a polytunnel at ADAS Boxworth until destructive assessment in October.

In 2017/18 half of the modules to be treated received product applications in Autumn, the other in Spring. After overwintering of the uninoculated plants, the untreated plants had more rotted roots in cold-stored plants than in those from ambient storage. Both sets of applications preceded inoculation of the peat growing medium in the 5 L pots with agar plugs of *P. rubi* mycelium. No significant treatment differences were shown following inoculation, with a low level of root rot in all the inoculated pots including the untreated. There was also root rot in the uninoculated untreated pots, with *P. idaei* identified and believed to have been present in the plants before commencing the experiment.

While infestation by *P. rubi* can occur via e.g. soil-splash or contaminated irrigation water in fruiting plantations, it was recognised that protectant treatment of propagation material before receipt by growers could be beneficial. Work in 2018/19 was therefore proposed to compare module plants inoculated with *P. rubi* in November that were either preventatively treated with fungicide before storage or treated at the standard timing after potting in Spring. The experiment also investigated whether the conditions plants were subjected to over winter after infection (either ambient or cold-storage) affected subsequent Phytophthora root rot development.

Materials and methods

Following on from Experiments 1 and 2 in 2017/18, a further trial was set up in September 2018, with 400 cv. Tulameen long cane raspberry module plants with a Plant Passport equivalent to UK Basic 2. The original NSA Elite motherstock material had been supplied to the propagator from NAKT (Holland) as pre-basic root blocks.

Half the plants received Autumn treatments (September and/or October) in 2018 (Experiment 3) and the other half of the plants received treatments in Spring 2019 (Experiment 4). On 19 November 2018 plants were inoculated by burying agar plugs in the growing-media of the modules using the *P. rubi* isolate SCRP1207[1]. Uninoculated plants received blank agar. In both Experiment 3 and 4, between 17 December 2018 and 11 March 2019, half of each set of plants either remained outside in ambient conditions at ADAS Boxworth stood out on a hard-standing area, or were placed at -1°C standing up in a crate in the AHDB cold store at Sutton Bridge. Temperature and humidity next to the plants were recorded throughout using Lascar Easylog EL-USB-2 loggers. Other than in the storage crates, each logger was shielded within a white, ventilated, screen. Procedures carried out up to the end of Winter 2018/19 are reported fully in the SF 158 Annual report (AHDB, 2019).

[11] Isolate obtained from a dying raspberry plant in 2018 by Aurelia Bezanger, James Hutton Institute and generously shared with ADAS.

The timing of the treatments applied in 2018, or due to be applied in 2019, (**Table 1.1**) and the products applied (**Table 1.2a & Table 1.2b**) are given below.

Table 1.1. Comparison of timings of fungicide drenches in either Autumn 2018 or Spring 2019 in relation to *P. rubi* inoculation timing before overwinter storage. Products applied in Autumn (T1 to T5) were the same as in Spring (T6 to T10), as detailed in the next table.

Dates done	**Experiment 3** (drenched in Autumn 2018)		**Experiment 4** (drenched in Spring 2019)	
	100 plants T1 to T4	100 plants T6 to T10	100 plants T1 to T5	100 plants T6 to T10
25.09.18	Prestop.	Prestop.	-	-
19.10.18	Prestop repeat. Serenade ASO. Paraat.	Prestop repeat. Serenade ASO. Paraat.	-	-
19.11.18	Inoculated with *P. rubi.*	Inoculated with *P. rubi.*	Inoculated with *P. rubi.*	Inoculated with *P. rubi.*
17.12.18	Cold-stored	Ambient stored.	Cold-stored	Ambient stored
11.03.19	Into ambient.	Into ambient.	Into ambient.	Into ambient.
15.03.19	Potted-up into 5L & into tunnel.	Potted-up into 5L & into tunnel.	Potted-up into 5L & into tunnel.	Potted-up into 5L & into tunnel.
19.03.19	-	-	Prestop	Prestop
09.04.19	-	-	Prestop repeat. Serenade ASO. Paraat	Prestop repeat. Serenade ASO. Paraat.

Table 1.2a. Treatments (T1 to T5) applied either in Autumn to 1.5 L module pots before cold-storage (Experiment 3), or in Spring after cold-storage once re-potted into 5 L pots (Experiment 4). All except T1 were inoculated with *P. rubi* on 19 November 2018.

Treat-ment	**Product and [MAPP Number]**	**Active ingredient**	**Label or EAMU dose rate**	**Product /1.5L pot in 0.15L water (10% by volume)**	**Product/5L pot in a 0.5L water (10% by volume)**
				Autumn timing	**Spring timing**
T1	Untreated no *P. rubi*	-	-	-	-
T2	Untreated	-	-	-	-
T3	Prestop [15103]	*Gliocladium catenulatum* strain J1446	5 g/L water (0.5%)	0.75 g	2.5 g
T4	Serenade ASO [15625]	*Bacillus subtilis* strain QT 713	10 L/ha in 1000 L/ha water (10 ml/L)	1.5 ml	5.0 ml
T5*	Paraat [15445]	Dimeth-omorph	1 g per plant	0.75 g**	1.0 g**

*In Experiment 3, T5 was unable to be included (see 2019 Annual Report for further details).

Table 2.2b. Treatments (T6 to T10) applied either in Autumn to 1.5 L module pots before cold-storage (Experiment 3), or in Spring after cold-storage once repotted into 5 L pots (Experiment 4). All except T6 were inoculated with *P. rubi* on 19 November 2018.

Treat-ment	Product and [MAPP Number]	Active ingredient	Label or EAMU dose rate	Product /1.5L pot in 0.15L water (10% by volume)	Product/5L pot in a 0.5L water (10% by volume)
T6	Untreated no *P. rubi*	-	-	-	
T7	Untreated	-	-	-	
T8	Prestop [15103]	*Gliocladium catenulatum* strain J1446	5 g/L water (0.5%)	0.75g	2.5 g
T9	Serenade ASO [15625]	*Bacillus subtilis* strain QT 713	10 L/ha in 1000 L/ha water (10ml/L)	1.5 ml	5 ml
T10	Paraat [15445]	Dimeth-omorph	1 g/plant	0.75 g**	1.0 g**

** Authorisation stipulates a maximum dose of 1 g of Paraat per plant. Although two plants were together in a 1.5 L module container, 0.75 g was deemed appropriate for the small rootball size compared with the 5 L volume to be treated per plant on potting-up in Spring.

On the 11 March 2019, the 200 plants kept in the AHDB Sutton Bridge cold store were transferred, together with their temperature and humidity loggers, to ADAS Boxworth, Cambridgeshire, where they remained in a covered storage area open to the outside for 4 days for the roots to gradually rise to ambient temperature and the stems to acclimatise. Together with the other 200 plants kept at ambient temperature, all plant rootballs were assessed, and all 400 plants potted up into 5 L pots with ericaceous compost on 15 March 2019. 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. All pots were placed on inverted saucers to avoid roots growing down into the woven ground cover (**Figure 1.1**).

The first Spring treatment drenches (of Prestop) were applied 4 days after potting up under tunnel (19 March 2019), and the second application date, when all three products were applied, was 3 weeks later (09 April 2019).

Plants treated in Autumn (Expt. 3) were placed in one polytunnel, and plants treated in Spring (Expt. 4) were placed in the neighbouring tunnel. Both tunnel set-ups were identical, with five randomised blocks and four plants per plot.

Figure 1.1. One of two polytunnels at ADAS Boxworth. Each experiment of five replicates with 200 long cane cv. Tulameen raspberry plants was housed separately, 04 April 2019.

In all root assessments conducted, two classes of roots were recorded: 'brown' and 'white'. Brown roots were all deemed to be infected with *Phytophthora* sp.. They had a red tinge, and appeared unhealthy, rotted, or water soaked. In March, clear patches of brown roots were easily observable on plants kept outside over winter. Samples of these brown roots, from 10 plants, were positive for *Phytophthora* spp. in diagnostic tests by Dr Tim Pettitt (Cornwall Colleges, formerly at University of Worcester).

White roots were newly formed, healthy looking, often not yet pigmented and were delicate so easily damaged, and often related to a healthy local root environment. Roots that were neither white nor rotted brown, were much older, mature healthy tanned (light brown coloured). The tanned roots were not recorded specifically, they made up the remainder of the rootball surface area and so can be calculated by subtraction from the white plus brown.

Cane assessment on 11 June 2019 examined all floricanes and primocanes. No primocane thinning was done over the season in order to monitor wilt symptoms. The older primocanes displayed *P. rubi* as darkened patches near the cane base, whereas infected younger canes were wilted, bent over or dead. A second cane assessment was conducted on 23 July 2019, after noticing a number of canes starting to wilt. Floricanes were cut out at the end of August,

once fruiting ceased, so October cane recording assessed remaining primocanes. The latter would commercially be kept on and selected from to produce next year's floricanes. Prior to natural leaf senescence on 14 October 2019, primocane health was assessed via: cane number, number wilting, and cane vigour in which primocanes were scored (0 = dead, 1 = dying, 2 = good, 3 = excellent). Plant rootballs were also assessed for proportion of white or brown root cover.

In every assessment, all four plants per plot were assessed individually, and plot averages compared by Analysis of Variance (ANOVA), using Genstat Release 18.2, separately comparing storage regimes and treatments and for any interaction between them. In Experiment 3, because there was an experimenter error, there was no Paraat treatment for plants going into cold storage and so analysis used a 4x2 design (as including the ambient Paraat treatment would have made it unbalanced). Statistical comparisons in Experiment 3 therefore exclude Paraat and the mean for the five replicates of Paraat on ambient plants is presented as a stand-alone result. In Experiment 4, with all five treatments across the two storage regimes the 5x2 analysis includes Paraat. Significant differences between treatments or storage regimes enabled comparison of the means across, respectively storage regimes and treatments. Individual treatment effects were noted if significant storage x treatment interactions arose. Duncan's Multiple Range Tests were also carried out on the treatment means across both storage regimes to provide a ranking for use in the results tables for ease of quick reference without calculating from the least significant difference for treatments produced by the ANOVA. However, the analysis provided by the factorial ANOVA was used in commenting on the results and their levels of probability of significant difference.

The trial conducted in this 2019 season was intentionally similar to the trial conducted in 2018, to allow for comparisons. The same cultivar (cv. Tulameen) long cane raspberries, from the same propagator were used, and similar treatment timings were used in Autumn and in Spring. The final location of the trial was in polytunnels at ADAS Boxworth as in 2018. However, the plants were inoculated in Autumn rather than Spring and so overwintering then took place outside at ADAS Boxworth and cold storage was at AHDB Sutton Bridge cold store (rather than remaining at the commercial propagator's locations). A recent virulent isolate of *P. rubi* was used (SCRP1207) in 2019, rather than the SCRP333 isolate used in 2018 (which had been in culture for several years and was not as strongly growing in culture).

The fruit harvest (a period of high water demand for the plants) began the first week of June, and ended mid-August. Yield was not a measure recorded, but all fruit was picked when ripe to reduce sources of oviposition for fruit flies. Plants began to senesce towards the end of October 2019.

Results

Temperature conditions experienced by plants 2018 - 2019

At the trial start, the raspberry plants experienced a mild Autumn and the inoculation (on 19 November 2018) took place with daytime temperatures reaching 10°C (**Figure 1.2**). This was followed by a mild wet winter (December 2018 – March 2019), where no temperature extremes took place.

The half of the plants placed in the AHDB Sutton Bridge cold store, were seen to have been kept at -1°C (**Figure 1.3**). The other half of the plants stood out in ambient conditions (on the hardstanding at ADAS Boxworth), were exposed to natural fluctuations in temperature (**Figure 1.4**) and to rainfall. The daily mean temperature at pot height outside fluctuated between 0°C and 10°C. There was a 3-week period commencing after mid-January when temperatures fell below 0°C. Maximum temperatures above 15°C were seen in a period between 22 and 28 February 2019 and were unusual for the time of year.

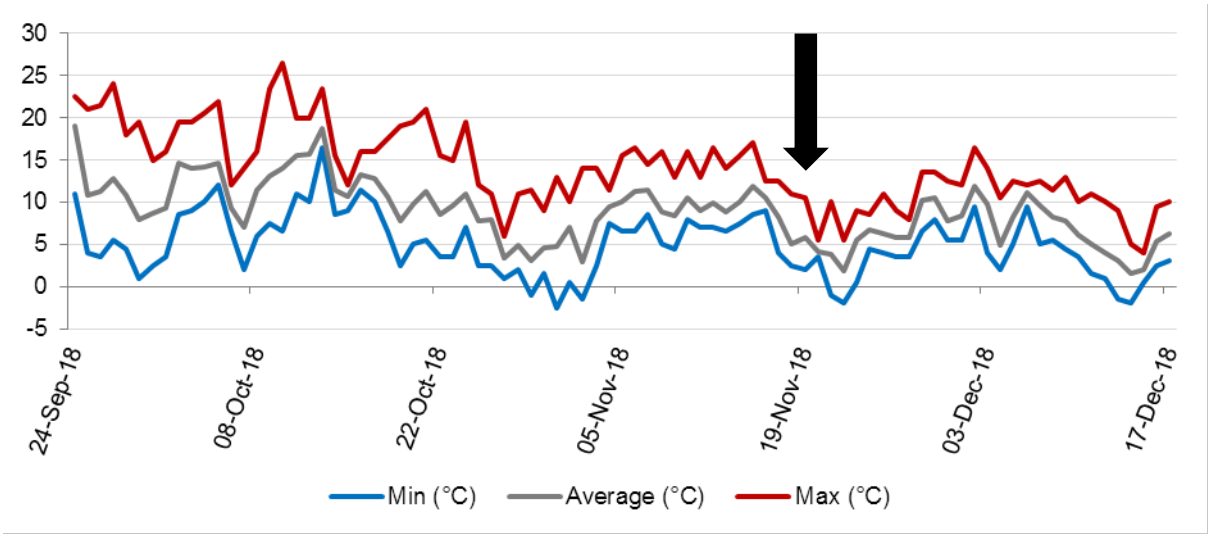


Figure 1.2. Air temperature of raspberry plants in ambient field conditions in Oxfordshire. Plants were taken from the field on 16 November 2018 to ADAS Boxworth. The black arrow indicates the timing of inoculation with *P. rubi* on 19 November at ADAS Boxworth, Cambridgeshire prior to half the plant then being transported away to cold storage.

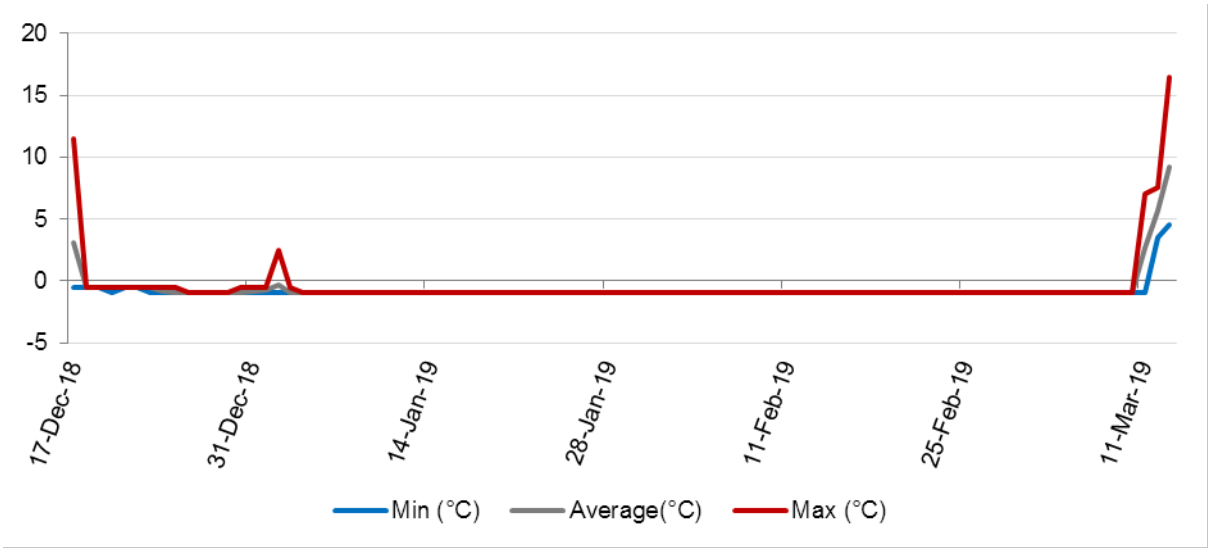


Figure 1.3. Air temperature at -1°C inside an unlidded cold-store crate, where long cane raspberry module plants were stored upright. Sutton Bridge cold stores, December 2018 - March 2019. Plants were removed from the cold-store on 11 March 2019.

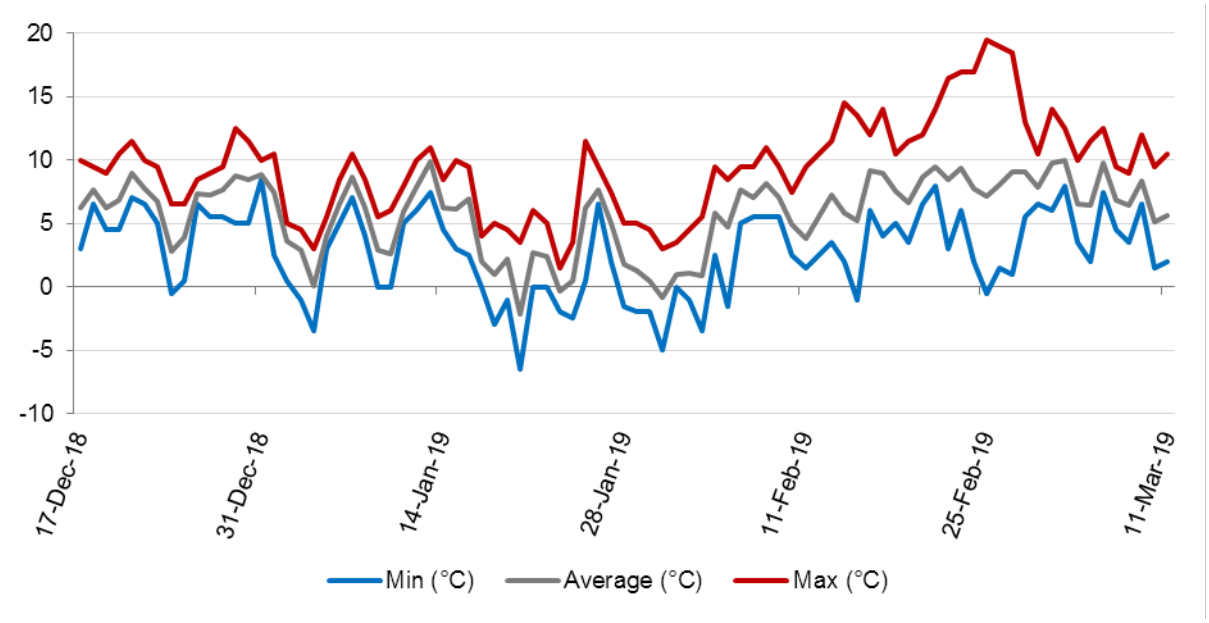


Figure 1.4. Air temperature at pot height of plants kept at ambient, unsheltered on the ADAS Boxworth outdoors hardstanding, Cambridgeshire. December 2018 to March 2019.

After the over-wintering period, from mid-March 2019 all plants were placed inside a polytunnel in Cambridgeshire in which temperatures fluctuated, including days reaching over 25°C between March and September (**Figure 1.5**).

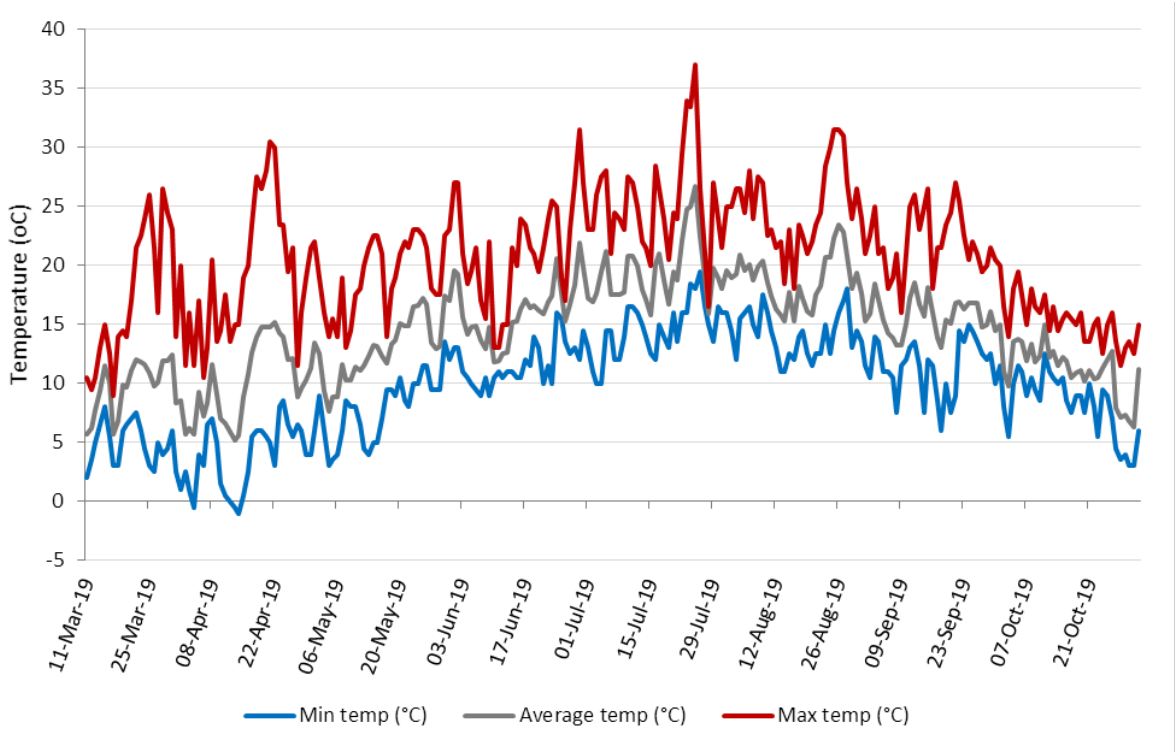


Figure 1.5. Air temperature at pot height of raspberry plants from both storage regimes kept under tunnel, Cambridgeshire. March – October 2019.

Plant assessments

Assessment of plants on arrival at ADAS Boxworth after either cold or ambient storage showed plants to have excellent vigour and no cane diseases. Bud-break had begun on ambient stored plants, but not on cold stored (**Figure 1.6**).

Figure 1.6. Raspberry canes brought in under tunnel, where they were assessed and potted on into 5 L pots, 14 March 2019. Canes from cold-storage (left) and those with buds breaking after outdoor ambient storage (right).

The pre-potting assessment in March 2019 identified a range of root health conditions. Most rootballs were healthy and firm, with the majority of the rootball surface covered in healthy tan coloured roots. The rest of the rootball either consisted of white healthy new roots, or rotted, water-soaked, brown roots (with a reddish tinge) that were identified as Phytophthora root rot. The percentage of the rootball covered in white, and rotted brown roots was recorded. Due to a mild winter, with no extreme cold snap, the frost damage seen in the 2017/2018 trial did not occur to plants outdoors in the 2018/2019 winter.

The rootballs of plants kept in ambient (**Figure 1.7**) were in a similar condition to the rootballs of cold stored plants (**Figure 1.8**). Water soaked, brown roots were observed on plants from both storage regimes, and roots from plants shown in Figure 3: D and Figure 9: F both gave strong positives on *Phytophthora* spp. lateral flow device (LFD) tests (Neogen, batch 14-857). These brown roots, although water soaked and soft, were still intact and held their form when

pulled at slightly. There was a clear difference to roots infected with *Pythium* spp., which are typically pale grey and disintegrate at a light touch.

Rootballs of raspberry modules kept in ambient outdoor storage

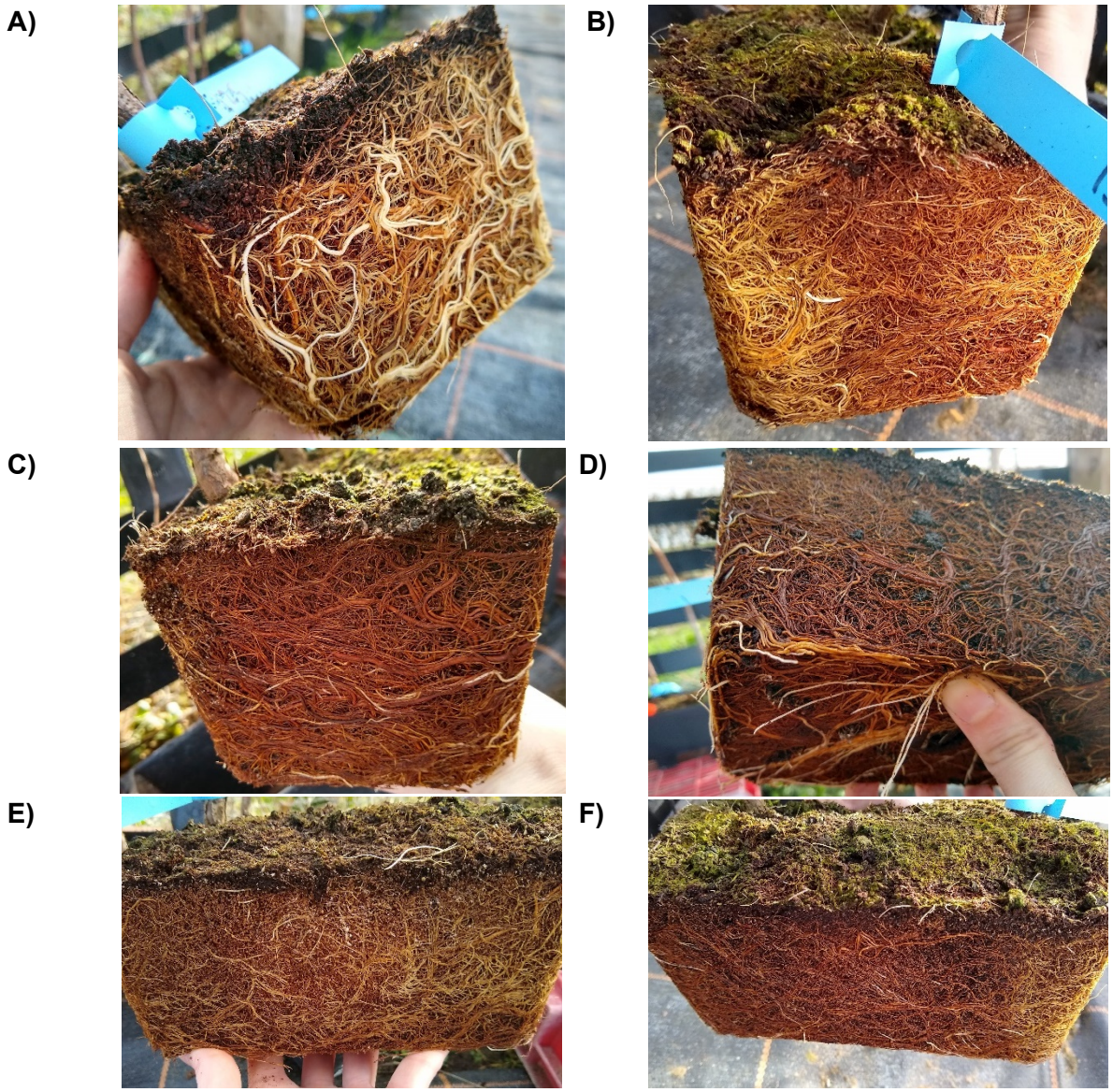


Figure 1.7. A range of raspberry rootball health and disease symptoms of 1.5 L modules (two plants) after removal in March 2019 from the ambient storage held in since December 2018.

A) Healthy white and tanned roots on uninoculated plot.
B) Brown streaking down rootball in centre, between healthy tanned roots either side.
C) Full side of rootball covered in brown roots.
D) Water soaked brown roots, typical of Phytophthora root rot.
E) Old healthy tanned roots, which developed prior to the winter period.
F) Brown discolouration on long side of rootball, with healthy tanned roots down the right hand corner of the module.

Rootballs of raspberry modules kept in cold storage

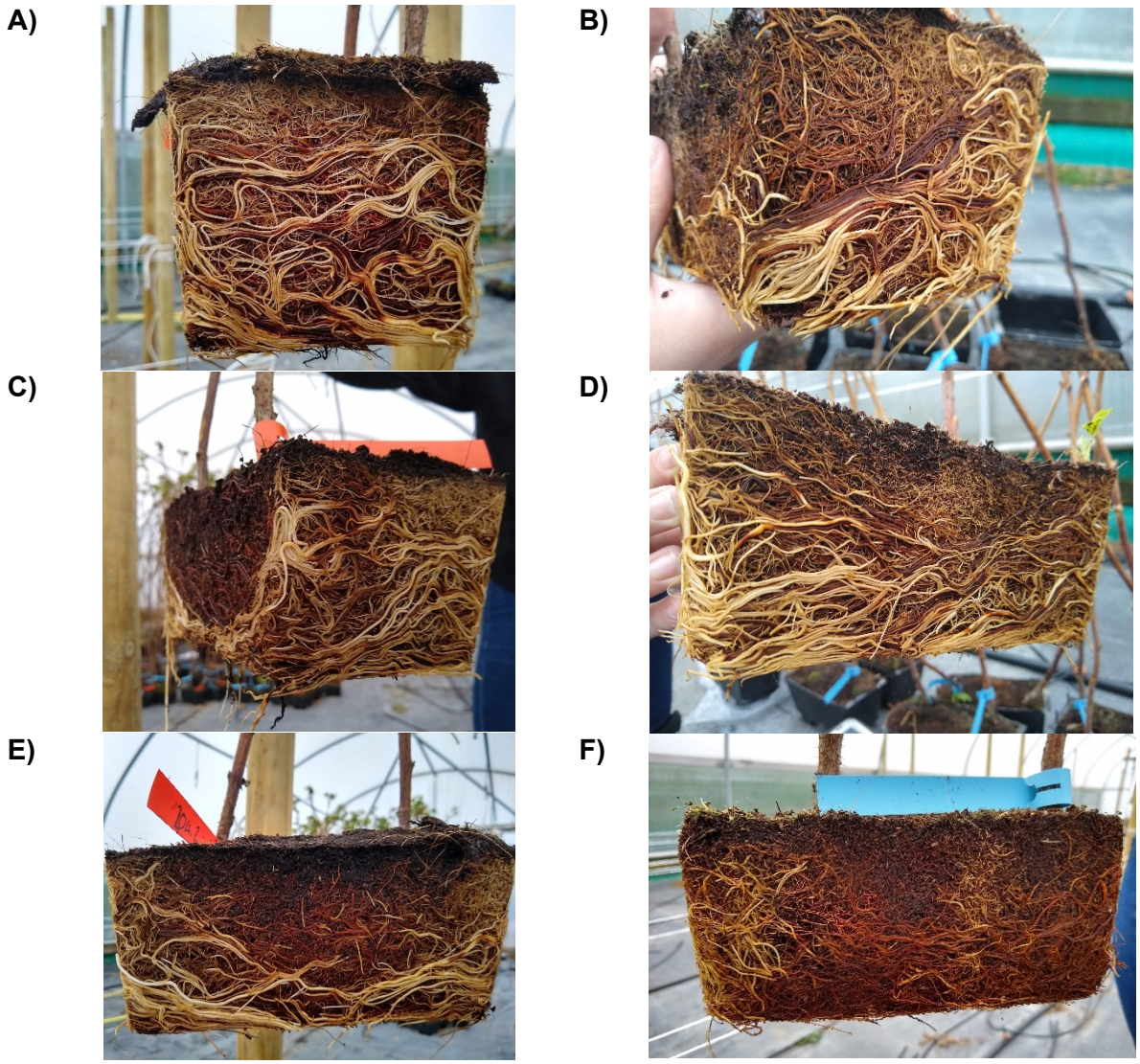


Figure 1.8. A range of raspberry rootball health and disease symptoms on 1.5L module plants (two per pot) after removal in March 2019 from cold-storage over winter from December 2018.

A) Brown stained roots in amongst healthy white roots.
B) Patch of white and tanned healthy roots with water-soaked brown rotted central section.
C) Healthy white roots growing up edges of pot, with distinct brown rotted U-shaped area on long side of rootball.
D) Healthy white roots from uninoculated plot.
E) Mixture of brown rotted roots in centre, and white healthy in corners of rootball.
F) Two columns of brown rotted roots, split by healthy tanned roots indicating *P. rubi* spread within rootball.

Experiment 3 – Autumn 2018 treated trial

The *results* for ambient-stored plants treated in Autumn with Paraat are given underneath the results tables. They were excluded from the factorial analysis since this would have become unbalanced with the absence of cold-stored Paraat treated plants due to experimenter error.

In March 2019 on assessing the modules prior to potting-up, 4 months after inoculation, factorial analysis showed neither of the pre-inoculation treatments had reduced the brown root coverage compared with those inoculated and left untreated. There was no significant difference in brown root coverage between the storage regimes, with on average 11% of the root ball surface rotted (**Table 1.3**. **Figure 1.7** & **Figure 1.8** above).

Table 1.3. Autumn treated raspberries. Percentage of rootball covered by brown roots (%) at re-potting into 5 L containers, 14 March 2019. All *P.rubi* inoculated except one untreated (UT)

	% of rootball with brown roots		
Treatment	**Cold stored**	**Ambient stored**	**Mean & Duncan's**
UT uninoculated	8.50	1.00	4.75 a
UT	8.00	22.00	15.00 b
Prestop	7.00	18.50	12.75 ab
Serenade ASO	11.50	11.50	11.50 ab
Paraat	-	~	-
Mean	8.75	13.25	

Factorial analysis	**Storage**	**Treatment**	**Storage x Treatment**
P-value	0.148	0.120	0.061
l.s.d	6.199	8.767	12.398
d.f.	(1, 28)	(3, 28)	(3, 28)

~ Paraat ambient stored mean 19.50% of rootball with brown roots. †

In March, the proportion of white roots was similar across all treatments, covering on average 8% of root balls, with no significant inoculation, product or storage affects (**Table 1.4**).

Table 1.4. Autumn treated raspberries. Percentage of rootball covered by white healthy roots (%) at re-potting into 5 L containers, 14 March 2019. All *P.rubi* inoculated except one untreated (UT)

	% of rootball with white roots		
Treatment	**Cold stored**	**Ambient stored**	**Mean & Duncan's**
UT uninoculated	8.0	13.0	10.5 a
UT	8.9	6.5	7.7 a
Prestop	8.8	4.2	6.5 a
Serenade ASO	14.5	4.5	9.5 a
Paraat	-	~	-
Mean	10.1	7.05	

Factorial analysis	**Storage**	**Treatment**	**Storage x Treatment**
P-value	0.391	0.846	0.497
l.s.d	14.12	9.98	14.12
d.f.	(1, 28)	(3, 28)	(3, 28)

~ Paraat ambient stored mean 10.0% of rootball with white roots

Further healthy roots in addition to the white roots were present which had become tanned on maturity but, in keeping with previous analysis, not analysed separately. They comprised on average 80% of the root ball surface (i.e. 100% minus (brown roots % + white roots %).

By June 2019, no treatment differences had arisen in the number of primocanes produced (**Table 1.5**) or the very small number of primocanes then wilting (**Table 1.6**).

Table 1.5. Autumn treated raspberries. Mean number of primocanes, 11 June 2019. All *P.rubi* inoculated except one untreated (UT)

	Number of primocanes per plant		
Treatment	**Cold stored**	**Ambient stored**	**Mean & Duncan's**
UT uninoculated	1.05	1.60	1.33 a
UT	1.10	0.60	0.85 a
Prestop	1.05	0.95	1.00 a
Serenade ASO	0.85	1.15	1.00 a
Paraat	-	~	-
Mean	1.01	1.08	

Factorial analysis	**Storage**	**Treatment**	**Storage x Treatment**
P-value	0.758	0.409	0.289
l.s.d	0.411	0.582	0.823
d.f.	(1, 28)	(3, 28)	(3, 28)

~ Paraat ambient stored mean 1.80 primocanes per plant

Table 1.6. Autumn treated raspberries. Mean number of wilting primocanes, 11 June 2019. All *P.rubi* inoculated except one untreated (UT)

	Number of wilting primocanes per plant		
Treatment	**Cold stored**	**Ambient stored**	**Mean & Duncan's**
UT uninoculated	0.00	0.00	0.00 a
UT	0.15	0.10	0.13 b
Prestop	0.05	0.05	0.05 ab
Serenade ASO	0.00	0.10	0.05 ab
Paraat	-	~	-
Mean	0.05	0.06	

Factorial analysis	**Storage**	**Treatment**	**Storage x Treatment**
P-value	0.742	0.157	0.561
l.s.d	0.077	0.109	0.154
d.f.	(1, 28)	(3, 28)	(3, 28)

~ Paraat ambient stored mean 0.00 wilting primocanes per plant

By June, Phytophthora inoculated plants had a noticeable percentage of their floricane foliage wilting (on average 11%). No significant treatment or storage condition differences were shown (**Table 1.7**). The progress of wilting (measured by a leaf death index) varied across the experiment, but no significant treatment or storage differences were found (**Table 1.8**). No floricanes had died in Paraat treated plants, nor was there any foliar wilt.

Table 1.7. Autumn treated raspberries. Percentage of floricane foliage wilting, 11 June 2019. All *P.rubi* inoculated except one untreated (UT)

	% of floricane foliage area wilting		
Treatment	**Cold stored**	**Ambient stored**	**Mean & Duncan's**
UT uninoculated	2.8	4.0	3.4 a
UT	11.0	14.0	12.5 a
Prestop	11.8	16.5	14.1 a
Serenade ASO	5.0	9.0	7.0 a
Paraat	-	~	-
Mean	7.6	10.9	

Factorial analysis	**Storage**	**Treatment**	**Storage x Treatment**
P-value	0.377	0.156	0.988
l.s.d	7.41	10.48	14.82
d.f.	(1, 28)	(3, 28)	(3, 28)

~ Paraat ambient stored mean 0.0% of floricane foliage area wilted

Table 1.8. Autumn treated raspberries. Mean floricane death index; 0 = healthy, 1 = yellowing, 2 = dying, 3 = dead. 11 June 2019. All *P.rubi* inoculated except one untreated (UT)

	Mean floricane death index		
Treatment	**Cold stored**	**Ambient stored**	**Mean & Duncan's**
UT uninoculated	0.05	0.00	0.025 a
UT	0.20	0.40	0.300 a
Prestop	0.25	0.40	0.325 a
Serenade ASO	0.10	0.30	0.200 a
Paraat	-	~	-
Mean	0.15	0.28	

Factorial analysis	**Storage**	**Treatment**	**Storage x Treatment**
P-value	0.277	0.247	0.840
l.s.d	0.231	0.326	0.462
d.f.	(1, 28)	(3, 28)	(3, 28)

~ Paraat ambient stored 0.0 mean floricane death index

Table 1.9. Autumn treated raspberries. Mean floricane death index; 1 = yellowing, 2 = dying, 3 = dead.23 July 2019. All *P.rubi* inoculated except one untreated (UT)

	Mean floricane death index		
Treatment	**Cold stored**	**Ambient stored**	**Mean & Duncan's**
UT uninoculated	1.60	1.55	1.58 a
UT	2.25	1.85	2.05 b
Prestop	2.10	1.60	1.85 ab
Serenade ASO	1.95	1.90	1.93 ab
Paraat	-	~	-
Mean	1.98	1.73	

Factorial analysis	**Storage**	**Treatment**	**Storage x Treatment**
P-value	0.078	0.115	0.540
l.s.d	0.280	0.396	0.560
d.f.	(1, 28)	(3, 28)	(3, 28)

~ Paraat ambient stored mean floricane death index 1.30

By 23 July, during fruiting, floricane leaf death was occurring (as photographed in **Figure 1.10**) but factorial analysis still showed no differences between treatments or storage regimes (**Table 1.9**). Paraat treated plants had the lowest mean floricane death index.

By 23 July, there was a higher proportion of cold-stored plants with a dying or dead floricane compared with ambient-stored plants (**Figure 1.9**). In the ambient treatment, all but the Serenade ASO treatment had 50% or fewer floricanes dead or dying, whereas in cold-stored plants all the inoculated treatments had 80% or more dead or dying. Half of the uninoculated untreated floricanes from both storage regimes were dying or dead during fruiting. Plant stools beneath the growing media could still be alive when the floricane appeared dead. Examples of 'yellowing', 'dying' and 'dead' floricanes are shown in **Figure 1.10**.

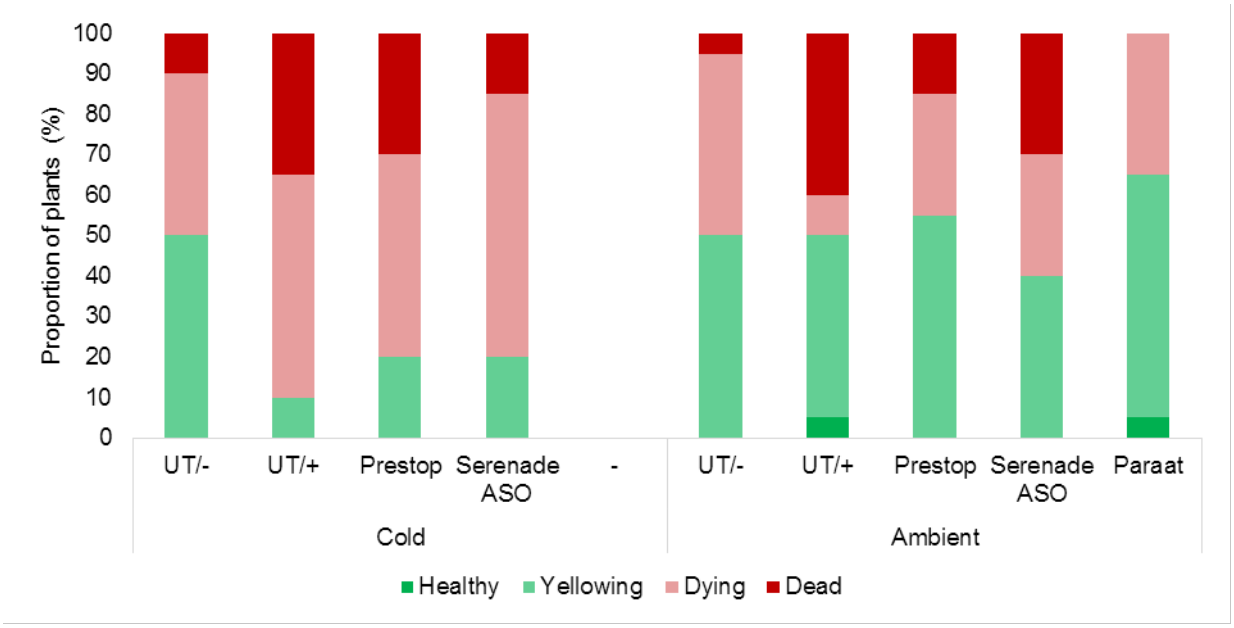


Figure 1.9. Autumn treated raspberries: proportion of plants (each of one floricane) within each floricane death index. Per treatment shown for healthy, yellowing, dying or dead from bottom to top bars (0 -3 indices). More cold stored inoculated plants, whether or not treated, had a dying or dead floricane than ambient stored. 23 July 2019.

Figure 1.10. Raspberry cane symptoms of *Phytophthora rubi* infection. Dead raspberry floricane (A, B, C), with dry necrotic leaves still attached to flowering laterals. Untreated uninoculated plants, with dead floricanes and an absence of primocanes (C). Wilting primocane 'shepherd's crook' (D). Shrivelled, poorly formed berries on wilting lateral, (E). Yellowing plants (F – I). 23 July 2019.

At the end of the growing season in October, root balls were seen to have areas of brown roots caused by Phytophthora rotting that often coincided with the position of the two irrigation drippers (**Figure 1.11**). The percentage of the root ball surface covered by rotted and white roots was assessed. The extent of surface rotting recorded was reflected in the condition of

the roots throughout the root balls. Root hairs were lacking on the brown roots, and staining caused by Phytophthora root rot was visible at the bases of dying canes (**Figure 1.12**).

Figure 1.11. Healthy white and tanned roots covering rootball (left). Patches of dark brown rotted roots under water drippers (centre and right hand picture). 22 October 2019.

Figure 1.12. Comparison between the inner rootballs and stem base section of an untreated inoculated plant (top pictures) and an untreated uninoculated plant (bottom pictures). Fine white root hairs, present on the healthy plant, are absent on the infected root ball and there is staining inside the stem base (top right) indicative of *P. rubi* infection. 07 November 2019.

By October, roots had grown since potting and some had rotted, with (as in March modules post-storage) lower levels of browning in the uninoculated plants, but these levels were now significantly lower (P>0.05) than in inoculated plants. Uninoculated untreated plants had a mean 36% root ball coverage by brown roots. Paraat treated plants kept at ambient also had a relatively low mean 36.5% brown root coverage. There was still no significant benefit from application of either Prestop or Serenade ASO compared with leaving inoculated plants untreated. Storage regime still had had no effect on the extent of brown root rot, with overall around half of the rootball surface area affected (**Table 1.10**).

Table 1.10. Autumn treated raspberries. Percentage of rootball in 5 L pots covered by brown roots (%) at end of season, 22 October 2019. All *P.rubi* inoculated except one untreated (UT)

	% of rootball surface with brown roots		
Treatment	**Cold stored**	**Ambient stored**	**Mean & Duncan's**
UT uninoculated	30.5	42	36.2 a
UT	62	69.2	65.6 b
Prestop	43.5	52.2	47.9 ab
Serenade ASO	51.5	57	54.2 ab
Paraat	-	~	-
Mean	46.9	55.1	

Factorial analysis	**Storage**	**Treatment**	**Storage x Treatment**
P-value	0.237	0.037	0.991
l.s.d	13.98	19.77	27.96
d.f.	(1, 28)	(3, 28)	(3, 28)

~ Paraat ambient stored mean 36.5% of rootball surface with brown roots

Uninoculated plants had the highest (P>0.001) white healthy rootball coverage (mean 48.5%) by October (**Table 1.11**), with Prestop and Serenade ASO autumn treatments resulting in no more healthy roots than present on untreated inoculated plants. Plants treated with Paraat and then ambient stored also had a relatively high proportion, 43%, of healthy roots.

Table 1.11. Autumn treated raspberry plants. Percentage of rootball covered by white roots (%) at end of season, 22 October 2019. All *P.rubi* inoculated except one untreated (UT)

	% of rootball with white roots		
Treatment	**Cold stored**	**Ambient stored**	**Mean & Duncan's**
UT uninoculated	55.5	41.5	48.5 b
UT	22.0	21.0	21.5 a
Prestop	19.5	29.2	24.4 a
Serenade ASO	24.0	20.0	22.0 a
Paraat	-	~	-
Mean	30.2	27.9	

Factorial analysis	**Storage**	**Treatment**	**Storage x Treatment**
P-value	0.608	<0.001	0.328
l.s.d	9.14	12.92	18.27
d.f.	(1, 28)	(3, 28)	(3, 28)

~ Paraat ambient stored mean 43.0% of rootball with white roots

By 22 October, there was a significant difference (P<0.01) between the untreated uninoculated, where no plants were dead and the inoculated, where a proportion of plants were dead with totally rotted roots (**Table 1.12**). There were no significant differences in the proportion of dead plants between the untreated *P. rubi* inoculated (with a mean 42.5% dead) versus Prestop or Serenade ASO treatments, suggesting nil benefit from these biofungicides when applied a month before either storage regime. No plants had died after treatment with Paraat in Autumn (before November inoculation with *P. rubi* prior to ambient storage).

Table 1.12. Autumn treated raspberry plants. Proportion of raspberry plants that were dead at end of season, 22 October 2019. All *P. rubi* inoculated except one untreated (UT)

	% of plants that were dead		
Treatment	**Cold stored**	**Ambient stored**	**Mean & Duncan's**
UT uninoculated	0.0	0.0	0.0 a
UT	40.0	45.0	42.5 b
Prestop	15.0	30.0	22.5 b
Serenade ASO	25.0	40.0	32.5 b
Paraat	-	~	-
Mean	20.0	28.7	

Factorial analysis	**Storage**	**Treatment**	**Storage x Treatment**
P-value	0.238	0.002	0.849
l.s.d	14.87	21.03	29.73
d.f.	(1, 28)	(3, 28)	(3, 28)

~ Paraat ambient stored mean 0.00% of plants were dead

By October, following post-harvest floricane removal, all ambient stored plants had produced significantly more (P<0.05) primocanes than cold-stored. Paraat treated ambient stored plants had a high mean of 6.2 primocanes. However, there was no difference between the untreated inoculated plants and those receiving either Prestop or Serenade ASO, having significantly less than the 5.0 canes of uninoculated plants (**Table 1.13**).

Table 1.13. Autumn treated raspberries. Mean number of primocanes per plant, 22 October 2019. All *P.rubi* inoculated except one untreated (UT)

	Number of primocanes		
Treatment	**Cold stored**	**Ambient stored**	**Mean & Duncan's**
UT uninoculated	5.15	4.85	5.00 b
UT	2.00	4.10	3.05 a
Prestop	1.40	3.55	2.48 a
Serenade ASO	1.65	2.70	2.17 a
Paraat	-	~	-
Mean	2.55	3.80	

Factorial analysis	**Storage**	**Treatment**	**Storage x Treatment**
P-value	0.028	0.004	0.349
l.s.d	1.103	1.560	2.207
d.f.	(1, 28)	(3, 28)	(3, 28)

~ Paraat ambient stored mean 6.25 primocanes per plant

In October, primocane vigour across each pot, encompassing any loss of vigour due to wilting, was significantly (P>0.01) better in the uninoculated treatment, and equally poor in the inoculated treatments whether or not Prestop or Serenade ASO had been applied.There was no difference between the storage regimes (**Table 1.14**). Paraat treated ambient stored plants looked at least as good (mean vigour index above 2) as the uninoculated untreated plants.

Table 1.14. Autumn treated raspberries. Mean primocane vigour index: 0= dead, 1 = dying, 2 = good, 3 = excellent. 22 October 2019. All *P.rubi* inoculated except one untreated (UT)

	Primocane vigour index		
Treatment	**Cold stored**	**Ambient stored**	**Mean & Duncan's**
UT uninoculated	2.40	2.30	2.35 b
UT	1.20	1.20	1.20 a
Prestop	1.15	1.65	1.40 a
Serenade ASO	1.40	1.40	1.40 a
Paraat	-	~	-
Mean	1.54	1.64	

Factorial analysis	**Storage**	**Treatment**	**Storage x Treatment**
P-value	0.654	0.004	0.770
l.s.d	0.452	0.639	0.903
d.f.	(1, 28)	(3, 28)	(3, 28)

~ Paraat ambient stored mean 2.70 index for primocane vigour

At the end of the experiment in November 2019 a sample of floricanes (present as long cane in Autumn 2018) from inoculated and uninoculated pots were cut across at the cane base to look for internal symptoms of Phytophthora rot. Canes lignify with age and this hides the browning by *Phytophthora* spp. from being seen easily externally. Red-brown internal staining associated with *P. rubi* infection was confirmed reaching up to 150 mm above soil level in floricanes from inoculated pots (**Figure 1.13**).

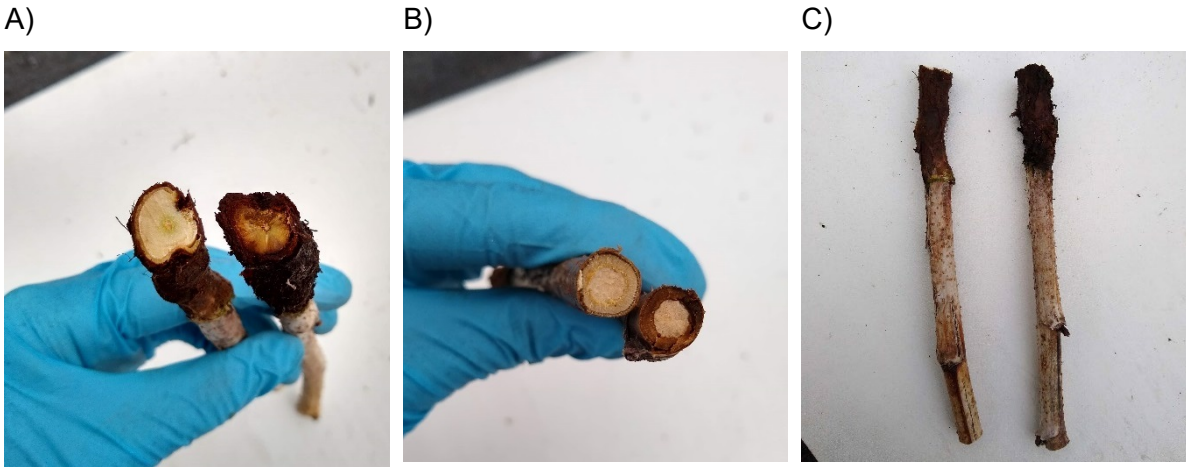


Figure 1.13. Cross sectioned floricanes from uninoculated (left) and inoculated (right) plants A) browning in infected cane base, and B) browning surrounds central pith 150 mm up the cane. C) Side view highlighting a lack of clearly visible external symptoms of *P. rubi* infection on the infected (right) floricane compared with the unininfected. 07 November 2019.

Experiment 4 – Spring 2019 treated trial

At the time of the post-winter root assessment (14 March 2019), plants had been inoculated in Autumn, but treatment drenches had not been applied. Overall, there was a significantly higher proportion of brown roots in cold-stored plants compared with ambient-stored plants (**Table 1.15**). The plants that had not been inoculated (that were to remain untreated) had brown roots; on 6% of the root ball surface area of cold-stored, and 2% of the rootball on ambient-stored plants (data not tabulated).

Table 1.15. Raspberries either due to be treated in Spring or to remain untreated. Mean percentage of rootball covered by brown roots (%) at re-potting across all pots from each storage regime, for combined inoculated and uninoculated plants, 14 March 2019.

	% of rootball with brown roots	
Treatment	**Cold stored**	**Ambient stored**
Mean	8.3	4.4

Factorial analysis	**Storage**
P-value	0.001
l.s.d	2.263
d.f.	(1, 36)

No differences had arisen by March in the proportion of the module rootball covered by healthy white roots when comparing storage regimes (**Table 1.16**). Roots were still largely unaffected by rot, as in addition to the 16% root ball surface covered by white roots that had not had time to tan, around three quarters of the root ball surfaces were covered by more-mature, tanned, healthy roots.

Table 1.16. Raspberries either due to be treated in Spring, or left untreated. Mean percentage of rootball covered by white healthy roots (%) at re-potting across all pots, (both unoculated and uninoculated), from each storage regime, 14 March 2019.

	% of rootball with white roots	
Treatment	**Cold stored**	**Ambient stored**
Mean	18.9	12.7

Factorial analysis	**Storage**
P-value	0.158
l.s.d	8.71
d.f.	(1, 36)

By June 2019, one or two primocanes had begun to emerge from the growing-media. There was no difference between storage regimes (**Table 1.17**). There were significantly more primocanes present in the uninoculated untreated plants compared with Prestop and Serenade ASO treated. There was a significant interaction, with ambient stored Paraat treated plants having more primocanes than those cold-stored and ranking highest of the

ambient treatments, while, conversely untreated uninoculated plants ranked highest after producing more primocanes when plants were cold-stored as opposed to ambient stored.

Table 1.17. Spring treated raspberries. Mean number of primocanes per plant, 11 June 2019. All *P.rubi* inoculated except one untreated (UT)

	Number of primocanes per plant		
Treatment	**Cold stored**	**Ambient stored**	**Mean & Duncan's**
UT uninoculated	1.65	1.00	1.33 b
UT	0.60	1.20	0.90 ab
Prestop	0.60	0.80	0.70 a
Serenade ASO	0.60	0.90	0.75 a
Paraat	0.85	1.50	1.18 ab
Mean	0.86	1.08	

Factorial analysis	**Storage**	**Treatment**	**Storage x Treatment**
P-value	0.123	0.029	0.039
l.s.d	0.281	0.446	0.6308
d.f.	(1, 36)	(4, 36)	(4, 36)

A small number of primocanes had started to wilt by June. With similar cane numbers on average in both storage regimes, cold-stored plants had significantly fewer wilting primocanes on average, than ambient plants (**Table 1.18**), but there were no significant effects of fungicide treatments.

Table 1.18. Spring treated raspberries. Mean number of wilting primocanes, 11 June 2019. All *P.rubi* inoculated except one untreated (UT)

	Number of wilting primocane		
Treatment	**Cold stored**	**Ambient stored**	**Mean**
UT uninoculated	0.00	0.10	0.05 a
UT	0.10	0.25	0.18 a
Prestop	0.10	0.30	0.20 a
Serenade ASO	0.00	0.15	0.08 a
Paraat	0.00	0.00	0.00 a
Mean	0.04	0.16	

Factorial analysis	**Storage**	**Treatment**	**Storage xTreatment**
P-value	0.044	0.163	0.844
l.s.d	0.117	0.184	0.260
d.f.	(1, 36)	(4, 36)	(4, 36)

In June, during fruiting, highly significant differences in the extent of wilting were clearly visible on the lateral shoots growing from the single floricane per plant present before winter, both between treatments and between the storage regimes (**Table 1.19**). Floricane wilting was much greater for ambient-stored plants compared with cold-stored plants. Across storage regimes, Serenade treatments resulted in higher floricane wilting incidence than all other

treatments. Paraat treated plants showed little wilting, similar to that in the uninoculated controls.

Table 1.19. Spring treated raspberries. Percentage of floricane foliage wilting per plant, 11 June 2019. All *P.rubi* inoculated except one untreated (UT)

	% area of floricane foliage wilting		
Treatment	**Cold stored**	**Ambient stored**	**Mean & Duncan's**
UT uninoculated	0.0	5.8	2.9 ab
UT	7.0	19.5	13.2 b
Prestop	3.7	19.2	11.5 b
Serenade ASO	11.2	37.2	24.2 c
Paraat	1.0	0.0	0.5 a
Mean	4.6	16.4	

Factorial analysis	**Storage**	**Treatment**	**Storage x Treatment**
P-value	<0.001	<0.001	0.096
l.s.d	6.33	10.01	14.16
d.f.	(1, 36)	(4, 36)	(4, 36)

In June, as well as differences in the amount of foliage wilting, the progress towards floricane death differed. More floricanes were yellowing in the ambient-stored plants (each plant having one floricane) than in the cold-stored, resulting in a higher floricane death index score (**Table 1.20**). Serenade ASO treated plants had the most developed wilt symptoms, resulting in a highly significant greater mean floricane death index than all other treatments including the untreated inoculated control. Prestop treated plants were no better than the untreated inoculated plants.

Table 1.20. Spring treated raspberries. Mean floricane death index score (0 = healthy, 1= yellowing, 2 = dying, 3 = dead), 11 June 2019. All *P.rubi* inoculated except one untreated (UT)

	Mean floricane death index		
Treatment	**Cold stored**	**Ambient stored**	**Mean & Duncan's**
UT uninoculated	0.00	0.25	0.12 ab
UT	0.30	0.60	0.45 b
Prestop	0.15	0.70	0.42 b
Serenade ASO	0.40	1.35	0.87 c
Paraat	0.05	0.00	0.02 a
Mean	0.18	0.58	

Factorial analysis	**Storage**	**Treatment**	**Storage x Treatment**
P-value	0.002	<0.001	0.112
l.s.d	0.239	0.377	0.534
d.f.	(1, 36)	(4, 36)	(4, 36)

By July, with high water demand for fruiting continuing, leaves were dying, but both the uninoculated and the Paraat treated plants were affected significantly less than all the other

treatments (**Table 1.21**). Since June, the floricane death indices of each of the storage regimes had become similar.

Table 1.21. Spring treated raspberries. Mean floricane death index score (0 = healthy, 1= yellowing, 2 = dying, 3 = dead), 23 July 2019. All *P.rubi* inoculated except one untreated (UT)

	Mean floricane death index		
Treatment	**Cold stored**	**Ambient stored**	**Mean & Duncan's**
UT uninoculated	1.25	1.70	1.47 a
UT	2.20	2.20	2.20 b
Prestop	2.40	2.35	2.37 b
Serenade ASO	2.20	2.55	2.37 b
Paraat	1.30	1.15	1.22 a
Mean	1.87	1.99	

Factorial analysis	**Storage**	**Treatment**	**Storage x Treatment**
P-value	0.376	<0.001	0.548
l.s.d	0.271	0.429	0.607
d.f.	(1, 36)	(4, 36)	(4, 36)

Paraat was the most effective treatment in preventing floricane death, with fewer Paraat treated plants from either storage regime having progressed beyond yellowing by 23 July (close to the condition of uninoculated plants) (**Figure 1.14**). In contrast to the Autumn treated experiment, there was no greater proportion of plants with a dead or dying floricane in the untreated inoculated, Prestop or Serenade ASO treatments following cold-storage compared with the ambient stored.

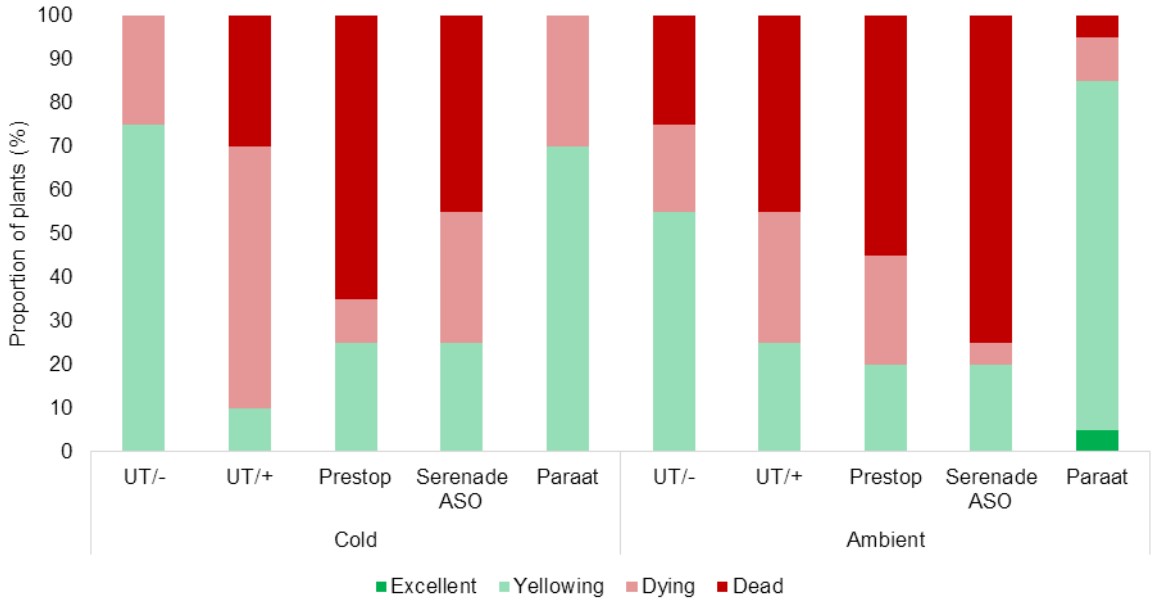


Figure 1.14. Spring treated raspberries: proportion of plants (one floricane per plant) within each floricane death index. For each treatment, proportions of healthy, yellowing, dying and dead (0 - 3 indices) are shown from bottom to top bars, 23 July 2019.

By October 2019, the roots balls in the 5 L pots had higher proportions of brown roots, than when in the modules in March 2019. Overall, cold-stored plants had significantly more root browning than ambient-stored plants (**Table 1.22**). Prestop and Serenade ASO had higher levels of root browning compared with Paraat treated plants and the uninoculated controls. There was not a significant storage/treatment interaction effect.

Table 1.22. Spring treated raspberries. Percentage of rootball covered by brown roots (%) at end of season, 22 October 2019. All *P.rubi* inoculated except one untreated (UT)

	% of rootball with brown roots		
Treatment	**Cold stored**	**Ambient stored**	**Mean & Duncan's**
UT uninoculated	31.5	38.0	34.8 a
UT	65.5	33.5	49.5 ab
Prestop	72.0	61.0	66.5 b
Serenade ASO	65.0	66.0	65.5 b
Paraat	45.5	21.0	33.2 a
Mean	55.9	43.9	

Factorial analysis	**Storage**	**Treatment**	**Storage x Treatment**
P-value	0.043	0.001	0.188
l.s.d	11.61	18.36	25.97
d.f.	(1, 36)	(4, 36)	(4, 36)

In October, the percentage of white roots on the rootball was similar for both storage regimes. Across storage regimes, there were highly significantly greater percentages of healthy roots on the uninoculated controls and Paraat treated plants. Treatments with Paraat or Serenade ASO did not increase white root percentage in comparison with the untreated control. There were no significant storage/treatment interactions. (**Table 1.23**).

Table 1.23. Spring treated raspberries. Percentage of rootball covered by white roots (%) at end of season, 22 October 2019. All *P.rubi* inoculated except one untreated (UT)

	% of rootball with white roots		
Treatment	**Cold stored**	**Ambient stored**	**Mean & Duncan's**
UT uninoculated	50.0	29.5	39.8 b
UT	19.2	26.2	22.8 a
Prestop	10.8	16.0	13.4 a
Serenade ASO	19.5	21.5	20.5 a
Paraat	30.3	45.5	37.9 b
Mean	25.9	27.8	

Factorial analysis	**Storage**	**Treatment**	**Storage x Treatment**
P-value	0.652	<0.001	0.078
l.s.d	8.02	12.68	17.93
d.f.	(1, 36)	(4, 36)	(4, 36)

By the end of the growing season in October, 53 plants out of the 200 in Experiment 4 were dead, with their roots having totally rotted, however no plant losses were recorded in either the uninoculated cold-stored plots, or any of the Paraat Spring treated plots (**Table 1.24**). In the Autumn treated plants of Experiment 3 there had been no plant deaths by October in the uninoculated plants from ambient or from cold-storage. There was only an ambient stored Autumn Paraat treatment in Experiment 3 and in this, similar to the Spring treated experiment, all plants survived. In both storage regimes, approximately half the plants treated in Spring with Serenade ASO or Prestop had died by the end of the season, and this was not significantly different from the proportion of plants dead in the untreated inoculated control.

Table 1.24. Spring treated raspberries. Proportion of raspberry plants that were dead at end of season, 22 October 2019. All *P.rubi* inoculated except one untreated (UT)

	% of plants that were dead		
Treatment	**Cold stored**	**Ambient stored**	**Mean & Duncan's**
UT uninoculated	0.0	15.0	7.5 ab
UT	35.0	20.0	27.5 bc
Prestop	55.0	40.0	47.5 c
Serenade ASO	45.0	55.0	50.0 c
Paraat	0.0	0.0	0.0 a
Mean	27.0	26.0	

Factorial analysis	**Storage**	**Treatment**	**Storage x Treatment**
P-value	0.888	<0.001	0.553
l.s.d	14.36	22.70	32.10
d.f.	(1, 36)	(4, 36)	(4, 36)

More primocanes were produced from plant stools after June, and by October 2019 both the uninoculated untreated plants and the Paraat treated plants still had highly significantly more than all the other treatments (**Table 1.25**). Storage regime over winter had still produced no overall difference in primocane production.

Table 1.25. Spring treated raspberries. Mean number of primocanes per plant, 22 October 2019. All *P.rubi* inoculated except one untreated (UT)

	Number of primocanes per plant		
Treatment	**Cold stored**	**Ambient stored**	**Mean & Duncan's**
UT uninoculated	6.40	3.15	4.77 b
UT	1.65	1.95	1.80 a
Prestop	1.70	2.50	2.10 a
Serenade ASO	1.80	1.65	1.72 a
Paraat	3.80	6.10	4.95 b
Mean	3.07	3.07	

Factorial analysis	**Storage**	**Treatment**	**Storage x Treatment**
P-value	1.000	<0.001	0.007
l.s.d	0.908	1.436	2.031
d.f.	(1,36)	(4, 36)	(4, 36)

Greatest primocane vigour was seen in Paraat treated plants and the uninoculated untreated control (**Table 1.26**). Prestop and Serenade ASO treated plants had poorer vigour similar to the untreated inoculated plants. Poor primocane vigour encompassed cane wilting, browning, presence of purple lesions at the base of the cane, and yellowing or browning leaves. Over all treatments, the strength of growth of the young shoots was unaffected by their overwinter storage treatment.

There was a highly significant interaction between storage and treatment for primocane number and the associated vigour score (**Table 1.25** & **Table 1.26**). More primocanes developed following ambient, rather than cold, storage of Paraat treated plants. The cold-stored untreated uninoculated plants, conversely, produced more primocanes and showed more vigour than those stored at ambient. There was a similar interaction for June primocane counts.

Table 1.26. Spring treated raspberries. Mean primocane vigour score: 0= dead, 1 = dying, 2 = good, 3 = excellent. 22 October 2019. All *P.rubi* inoculated except one untreated (UT)

	Primocane vigour index		
Treatment	**Cold stored**	**Ambient stored**	**Mean & Duncan's**
UT uninoculated	2.90	1.60	2.25 b
UT	1.00	1.35	1.18 a
Prestop	0.90	1.25	1.08 a
Serenade ASO	0.95	1.00	0.98 a
Paraat	2.35	2.80	2.58 b
Mean	1.62	1.60	

Factorial analysis	**Storage**	**Treatment**	**Storage x Treatment**
P-value	0.904	<0.001	0.009
l.s.d	0.334	0.528	0.747
d.f.	(1, 36)	(4, 36)	(4, 36)

Summary of results for Expts 1 & 2 (2017-18) and Expts 3 & 4 (2018-19)

Significant differences between the storage and the fungicide treatments for the year just reported have been summarised in **Table 1.27** alongside results from the year prior to this. Where there is no entry in the table this indicates that no differences were found between either storage regimes or treatments. Some additional cane records were made in 2019 when more plants were wilting and showing a range of wilting severities not seen in 2018. In the table, where a storage regime or a treatment is shown to be significantly more or less than another the first named had the more-favourable results e.g. for Expt. 4 in June the untreated uninoculated UT- had more primocanes (>) than either Prestop or Serenade ASO.

Comparison between the two crop years of the efficacy of the treatments and any differences between the storage regimes in Phytophthora root rot symptoms or plant growth is discussed in the next section, and the conclusions drawn cover both sets of experiments.

Table 1.27. Results comparison across two crop years with *P. rubi* inoculated in April 2018 (Expts 1 & 2) or November 2018 (Expts 3 & 4). Both years had cold and ambient stored plants, either Autumn or Spring fungicide treated. All records are shown where significant differences arose between treatment or storage means based on Factorial Analysis. Key : **<** less than, **>** more than, **=** no significant difference between. **UT +** / **-** untreated plus or minus *P. rubi.* **†** No cold-stored Paraat in Expt. 3 so Paraat excluded from analyses, although ambient results are noted.

Key indicator	**Spring inoculation (Millets Farm 2017-18 winter)**		**Autumn inoculation (Sutton Bridge 2018-19 winter)**	
	Autumn protectant treated (Expt. 1)	**Spring protectant treated (Expt. 2)**	**Autumn protectant treated (Expt. 3) †**	**Spring curative treated (Expt. 4)**
March % brown roots	(UT- = Paraat) < (Prestop = Serenade). Cold < Ambient.	Ambient < Cold Ambient [no treatment yet]		Ambient < Cold [no treatment yet]
March % white roots	Paraat > all other T & UT.			
June no. primocanes		Cold > Ambient.		UT- > (Prestop = Serenade). (UT+ = UT- = all treated). Paraat: Ambient > Cold UT-: Cold > Ambient
June no. primocane wilting				Cold < Ambient
June % floricane foliage wilting	Not recorded.	Not recorded.	Paraat ambient 0 wilting, others wilting.	Paraat < (UT + = Prestop) < Serenade ASO. UT- = UT+. Cold < Ambient.
June % of plants wilting, and floricane death index			Paraat ambient 0 dead, others wilting.	Paraat < (UT + =Prestop) < Serenade ASO. UT- = UT+. Cold < Ambient.
July floricane death index	Not recorded.	Not recorded.	Ambient fewer dying/dead UT+, Prestop, Serenade	(Paraat = UT-) < (UT + = Prestop = Serenade ASO).
October % brown roots			(UT- < UT+) (UT+ = Prestop = Serenade ASO).	(Paraat = UT-) < (Prestop = Serenade ASO). Paraat = UT+ = others. Ambient < Cold
October % white roots			UT- > (UT+ = Prestop = Serenade ASO).	(Paraat = UT-) > (UT + = Prestop = Serenade ASO)
October % dead plants	Not recorded.	Not recorded.	UT- < (UT+ = Prestop = Serenade ASO). UT- & Paraat ambient 0 dead.	(Paraat = UT-) < (UT + = Prestop = Serenade ASO). UT- = UT+
October no. primocanes	Not recorded.	Not recorded.	UT- > (UT+ = Prestop = Serenade ASO). Ambient > Cold	(Paraat = UT-) > (UT + = Prestop = Serenade ASO). Paraat: Ambient.> Cold. UT-: Cold > Ambient.
October primocane vigour		Ambient > Cold (ambient fewer wilting)	UT- > (UT+ = Prestop = Serenade ASO)	(Paraat = UT-) > (UT + = Prestop = Serenade ASO)

Discussion

The experiments on Phytophthora root rot in 2018/19 together with those of 2017/18 compared the health of cv Tulameen plants grown from modules after cold or ambient storage, and examined the efficacy of various fungicide drenches applied preventatively or curatively following Autumn or Spring infestation by *P. rubi*. The findings summarised in **Table 1.27** are discussed below.

Infection by Phytophthora species:

Establishing uniform and realistic disease levels within a trial is crucial for obtaining robust data on treatment efficacy, and so artificial inoculation is necessary. The first crop year aimed to study infection in plants arising once they were potted-up and tunnelled in Spring, but it was found that a significant proportion of plants already contained *Phytophthora* spp. (8.4% of uninoculated plants had red roots diagnostic of *P. rubi*), and inoculation did not increase root rot and wilt severity. The aim in second year's trials was thus to reflect the situation where planting material may be infected in Autumn during propagation not long before it goes into cold store.

In the second year there were some brown roots in the uninoculated plants in October (35% of the rootball surface), and while no floricanes died in cold-stored plants, 15% of ambient stored uninoculated plants died from what may have been "natural" Phytophthora root rot. Precautions taken make this unlikely to have followed cross-contamination from inoculated plants. The infestation of propagation material was not unexpected as any infection can be symptomless / latent in the roots taken from mother plants to produce the rooted cuttings for the modules. With plants grown in the open field and tunnels, infestation by pathogens cannot be avoided and fungicides in propagation that might suppress infection symptoms are discouraged by UK inspectors of material for certification. The *Phytophthora* species present in commercial crops of containerised raspberries from propagation and fruiting crops was investigated as part of this project in 2019 and is presented in the next section of this report.

Artificial inoculation with *P. rubi* in November was more successful than when carried out in the first year's crop in April (with respectively 50% rather than 4% of the rootball surface rotted by October in the untreated inoculated). This may reflect the natural situation, particularly when, as in the first year, a hot spell in Spring may have reduced the success of inoculation. Raspberry plants in propagation were outside in November, with the growing media cool and damp, and this is favourable to *P. rubi* reproduction. *P. rubi* can establish within rootballs before temperatures drop overwinter, *P. rubi* growth being minimal at 4°C or less (Erwin & Ribeiro, 1996). A more recent *P. rubi* isolate was obtained in 2018 for the November inoculation (SCRP 1207, rather than SCRP 3333 used previously) and this could also have

contributed to the greater symptom difference between inoculated and uninoculated plants in the second year. Photographs of the incidence and severity of the Phytophthora wilting seen in the experiments in 2019 were shown to growers and said to be similar to the rapid death being experienced in commercial fruiting crops.

In the 2018/19 crop, although pathogen presence was due to a combination of natural as well as artificial infestation in some plants, there were clear differences between the inoculated and the uninoculated plants throughout the Spring treated experiment and after harvest in the Autumn treated experiment. Comparison between the inoculated plants receiving the various fungicides and those left untreated was possible because it tested product efficacy against the high inoculation level given.

Examination of the rootballs in 5 L pots in both years showed concentrations of root rot directly below the water drippers. *Phytophthora* spp. zoospores require water to be able to swim to infest healthy roots after being released from infected roots. In situations on farms with contaminated irrigation water, zoospores would also be assisted in spread through the root ball by irrigation to field capacity or by poor drainage.

***Storage regimes*:**

Cold-storage of strawberry plants has been shown to increase their susceptibility to *Phytophthora* sp. infection post-storage (Pettitt and Pegg, 1994a & b). Reports of plant losses in commercial raspberry crops within months of removal from cold-storage have been attributed to *Phytophthora* spp. infection before winter, but it is also recognised that where frozen plants are not acclimatised before potting this in itself can cause modules to fail to establish. The current experiments, and those of 2018 (see Annual Report of 2019), were the first to study the relationship between storage temperature and root rot in UK grown long cane raspberry.

In both years, where fungicide treatment was scheduled in Spring, more root rot was seen across cold-stored modules by March, compared with ambient stored plants, suggesting a detrimental effect from cold-storage (irrespective of artificial *Phytophthora* infection). Conversely, in the first year, in the Autumn fungicide-treated experiment, more rotted roots were found after ambient storage. This was primarily associated with plants treated with either Prestop or Serenade ASO. No problems with increased root rot from biofungicide applications in Autumn arose in the second year.

In 2019, where Spring fungicide treatment followed Autumn inoculation, cold-stored plants had fewer primocanes wilting in June, less of the floricane foliage wilting and less severe wilt progress. It is possible that cold-storage (below freezing) after inoculation held back *P. rubi* progress into the plant stool and canes. In March on overwintered module roots there was a

greater rootball coverage of rotted roots in cold-stored rather than ambient stored Spring treated plants, and this was also seen in October on roots produced following potting-up. *P. rubi* inoculum would have entered the roots in the month before storage. In the cold store at -1°C the pathogen and the roots would have been inactive, whereas ambient stored roots which froze for only short periods may at other times have been physiologically active and so developed less rotting. A smaller area of rotted module roots would mean fewer sources of *P. rubi* zoospores, and so after re-potting the ambient plants and watering commencing then there was potentially lower zoospore infestation of the roots that grew.

In 2019, although no significant difference (P = 0.078) between storage regimes was shown in June following Autumn treatments for the mean floricane death index analysis, in July graphical presentation of the index scores for individual plants showed that more plants had a dying or dead floricane in the inoculated untreated, Prestop and Serenade ASO Autumn treated cold-stored plants than in the ambient. If floricane infestation reflected infestation of the plant stool then this could explain why the Autumn treated cold-stored plants then produced one or two fewer primocanes than the ambient by October, a mean 2.5 rather than 3.8 primocanes in the ambient.

In the Spring treated experiment, graphical presentation of the floricane death index scores showed similar high proportions of plants with dead or dying floricanes in the untreated inoculated, Prestop or Serenade ASO treatments in both regimes, resembling the fewer healthy plants following cold rather than ambient storage in the Autumn treated. Both storage regimes of Spring-treated plants produced a mean 3.1 primocanes by October. However, it cannot be concluded that Autumn rather than Spring application of these biofungicides to plants stored in ambient will result in less severe floricane symptoms and more primocanes as for some unknown reason these benefits were also seen in the untreated inoculated.

More primocanes were produced in the cold-stored uninoculated untreated plants (6.4, rather than 3.2 primocanes by October in the ambient) in the Spring-treated experiment, but as plants given the same conditions in the other experiment did not differ (mean 5 canes) this cannot be taken as a general rule. Conversely, cold-stored Spring Paraat treated produced fewer primocanes (3.8 primocanes, rather than 6.1 in the ambient) but still more than other inoculated treatments, but the reason is unclear,

Although floricane foliage was dying in July in both 2018/19 experiments, with the increased water demand by the fruit, these canes were cut off, as standard, after fruiting to allow the primocanes to grow up in their place to potentially be their replacement next year. By October, the storage regimes had no differences in plant death as determined from totally rotted roots.

***Use of bioprotectants and Paraat*:**

Current authorisations for use on raspberry (**Table 1.28**) enable just one application of Serenade ASO, and up to three applications of Prestop for outdoor crops. Two Prestop applications were made experimentally to plants when outdoors in Autumn or protected in Spring to allow comparisons. Paraat is usually applied commercially in Spring to fruiting crops, but is not used on propagation material by the supplier of the plants for these experiments. Current EU certification of raspberry propagation states 'Fungicide treatment that could mask symptoms of Phytophthora root rot are not to be encouraged' (APHA, 2017), but one could argue that protecting clean stock would be worthwhile. Currently the HSE guidance given is that there is no policy specifically limiting the use of bioprotectants during propagation in horticultural crops, as long as their use adheres to label recommendations.

Table 1.28. Approved fungicide products in January 2020 available in the UK that can be applied for control of Phytophthora root rot in raspberry.

Example product & MAPP number	Active ingredient & fungicide group	FRAC code	Approval status for cane fruit & situation	Max. individual dose rate	Max. total appli-cations	Harvest interval	Renewal date of UK product reg-istration
Serenade ASO (16139)	*Bacillus subtilis* (strain QST 713)	44	EAMU 2336/18 Outdoor	10 L/ha	1 / crop	None	31 Jan 2022
Prestop (17223)*	*Gliocladium catenulatum* (strain J1443)	BM 02	EAMU 2843/18 Protected & Outdoor	500 g/100 L water	1 / crop protected 3 / crop outdoors	None	31 Jan 2022
Paraat (15445)	Dimethomorph	40	On label. Protected & Outdoor	3 kg/ha ***	1 / year	90 days	31 Jan 2022†
Previcur Energy (15367)	Fosetyl-aluminium + propamocarb hydrochloride	P 07 + 28	EAMU 2045/12 Protected	3 ml/m^2	2 / crop	365 days	31 Oct 2022

*EAMU issued 10 September 2018, and supersedes EAMU 0706 of 2013.

**EAMU granted 12 October 2018, and supersedes EAMU 2773 of 2015.

*** Authorisation stipulates that for the use on protected raspberries as root drench, the concentration of product must not exceed 5 g product per litre water. Do not exceed 1 g product per plant.

† dimethomorph is currently under review by the EU as a potential endocrine disruptor.

The research over the four experiments did not show any benefit in root or cane assessments from the applications of either Prestop (two close applications) or the single application of Serenade ASO. This was the case if applied preventatively to modules pre-storage or after potting-up, whether plants were inoculated with *P. rubi* pre or post-storage. It should be noted,

however, that in commercial crops if they become contaminated from e.g. infested irrigation water or from soil-splash the challenge from the pathogen could be lower with fewer zoospores arriving in a short period. In competition between the beneficial fungi or bacteria in the products and the pathogen the beneficials would be favoured with lower pathogen numbers. However, there are situations where a zoospore flush could arise naturally. In early Winter rain could stimulate sporangial production on rotted roots and a cold snap trigger synchronised zoospore release. In Spring, plant removal from cold storage followed by irrigation re-commencement could similarly initiate a zoospore flush.

In the first two experiments (2017-2018, Spring inoculated), no significant difference was shown throughout the crop between the inoculated and the uninoculated plants, and this was attributed to “natural” *Phytophthora* spp. infection of the modules before the start of the experiments and the overall lower inoculation success in Spring. By October, brown roots covered 16% of the rootball in inoculated plants in the Autumn-treated and 5% in Spring-treated experiments (including Paraat), compared with 14% and 6% respectively in the uninoculated. Following Autumn treatment with Paraat to uninoculated plants more healthy roots were present prior to Spring *P. rubi* inoculation, suggesting curative activity against the natural infestation. However, once plants in these experiments were potted-up, no reductions in *Phytophthora* spp. root or cane symptoms were shown by any of the treatments from either Autumn or Spring applications.

In the second two experiments (2018-2019, Autumn inoculated) the uninoculated plants had a higher proportion of brown roots by October than in the previous year, but the inoculated (excluding Paraat treated) were significantly more affected. Brown roots covered 55% of the rootball in Autumn-treated and 60% in Spring-treated experiments (excluding Paraat), compared with respectively 36% and 35% in the uninoculated. Paraat applied either as a protectant against the inoculation or curatively was shown to reduce *Phytophthora* spp. symptoms down to levels found in the uninoculated. Paraat was able to reduce the progress of root rotting so that there were sufficient healthy roots to support plant growth. Where Paraat was applied before Autumn inoculation to ambient stored plants neither floricane wilting nor plant death from total root rotting had occurred by June and still no plants had died by October. Similarly, where Paraat was applied curatively in Spring, floricane wilting was significantly less than other inoculated plants (0.5% of floricane foliage wilting for Paraat treated in contrast to 16%) in June, was still less advanced by July, and by October root rot was as low as in uninoculated plants, with equivalent good primocane vigour and no plants were dead. Paraat application was shown to be effective whether applied in Autumn to modules, or at re-potting in Spring when infection occurred in Autumn.

Following April application of Serenade ASO in 2019, greater floricane foliage wilting incidence and severity was seen in June than in the other treatments, which cannot be explained, but subsequently *Phytophthora* spp. symptoms were no worse than for Prestop and the untreated inoculated plants.

At time of study, authorisation of Serenade ASO and Prestop state their use as preventatives. Unlike conventional chemical fungicides with a systemic action, Prestop and Serenade act only on the plant surface. However, *Bacillus subtilis* (the species in Serenade ASO) can stimulate plant growth and the induction of acquired systemic resistance, and enhance stress tolerance in their plant hosts by inducing the expression of stress-response genes, phytohormones and stress-related metabolites (Hashem *et. al.,* 2019). There is an ongoing difficulty in establishing the best way to practically use bioprotectants against root pathogens in a commercial setting. They need to be able to colonise roots before *Phytophthora* spp. zoospores to compete with them. Paraat treated plant in 2019 were often as healthy as uninoculated untreated plots, indicating that this product was able to control the *P. rubi* at the level of inoculation given whereas the Prestop and Serenade ASO were unable to do so. Unlike chemical fungicides, such as Paraat (dimethomorph), products such as Prestop and Serenade ASO are living organisms that have specific mechanisms behind their pathogen control and environmental conditions and the level of competition with pathogens and the environmental conditions will affect performance.

Conclusions

- Experiments completed in two crop years of long-cane raspberry cv. Tulameen examined the efficacy of drench applications of the biofungicides Prestop, (*Gliocladium catenulatum*) and Serenade ASO (*Bacillus subtilis*) and an industry standard product, Paraat (dimethomorph). Comparisons were made between Autumn or Spring applications followed by inoculation of the peat growing media with *Phytophthora rubi* after potting-on in April 2018 (Experiments 1 and 2, respectively). In the following year the products were again applied in autumn or spring (Experiments 3 and 4), but *P. rubi* was inoculated in November 2018, a month before overwinter storage of modules. In both years, comparison was also made between ambient outdoor storage of modules, where temperatures fluctuated, and cold-storage at circa -1°C, with records subsequently made of root rotting and cane wilting.
- Overall, Paraat applied either in Autumn or Spring, significantly reduced root rot, floricane wilting and death, but treatment with either Serenade ASO or Prestop were not effective.

- Comparing plants inoculated with *P. rubi* in both cold-stored and ambient conditions over winter, the health of the plant roots was assessed in the spring and subsequent growing months. Although the results were not conclusive, there was a suggestion that the cold-stored roots may be more prone to developing Phytophthora than those held in ambient conditions.

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Knowledge and Technology Transfer

Findings of this work were presented at:

- AHDB NIAB-EMR Association Soft Fruit day, November 2017, 2018 & 2019.
- FARMA (Farm Retail Association) Meeting (19 September, 2018).

Objective 1: Understanding and managing Phytophthora root rot (continued).

Aim 2: To investigate the species of *Phytophthora* present in dying raspberry plants

Introduction

In the 1980s, Phytophthora root rot emerged as a major problem of raspberry with outbreaks in the UK (Duncan *et al.,* 1987), Scandinavia and Germany (Seemüller *et al.,* 1986). *Phytophthora fragariae* var. *rubi* (Wilcox *et al.,* 1993), now renamed *P. rubi,* was identified as the major causal agent in Europe and North America. It had previously been thought to be a highly pathogenic variant of *P. megasperma* (Wilcox *et al.,* 1993). Phytophthora root rot is now the most destructive disease of raspberries worldwide (Ellis *et al*, 1991). Where raspberries have been grown in the soil, *P. rubi* is almost ubiquitous. Outbreaks of this disease across Europe in traditional raspberry-growing areas suggests that the disease has spread through the propagation network and has been distributed to farms in new planting material.

Although named as root rot, crowns and cane bases (primocane and fruiting cane) can become infested by *P. rubi*. The lesions can spread above soil level for up to 200 mm on primocanes. Affected canes die in the first year of growth or their buds fail to emerge at the start of the second growing season. Alternatively, emerged laterals wilt and die at any time from Spring until late in fruiting (James Hutton Institute web site).

P. idaei was present in over 40% of the Scottish soft fruit plantations surveyed in 2001-2003 and was subsequently shown in a potted field trial in Scotland to be causing root damage without above ground symptoms other than reduced cane height (Young *et al.,* no date). Both *P. idaei* (optimum growth between 20 to 22°C) and *P. citricola* complex (optimum growth between 25-28°C) have from industry knowledge recently been detected in England in raspberry material that was wilting. Various Phytophthora root rot species in addition to *P. rubi* occur in other raspberry-producing regions of the world such as *P. cryptogea* in Australia (Washington, 1988), and *P. citrophthora* and *P. citricola* in Chile (Latorre and Muñoz, 1993, Wilcox and Latorre, 2002). *P. cambivora* isolated from raspberry in Scotland was not classified as pathogenic (Duncan *et al*., 1987, Wilcox *et al.,* 1993).

Species of *Phytophthora* were traditionally grouped by morphology and their form of breeding. However, using the internal transcribed spacer (ITS) regions of rDNA, species have been

now been divided into Clades so that *P. idaei* is in Clade 1, *P. citricola* and *P. citrophthora* are in Clade 2, *P. cryptogea* is in Clade 6 and *P. rubi* is in Clade 7 (Cooke *et al*., 2000).

There have recently been more reports of crops failing within months of planting modules in tunnels. It is possible that *Phytophthora* species well established in warmer climates than that of UK could have started to become of greater prominence here within tunnelled crops and the warmer weather associated with global warming. Determining the *Phytophthora* species present across the UK in a range of crop types and varieties and their prevalence is the first step in understanding the current situation with a view to developing procedures to reduce further impact and work towards eliminating all pathogenic *Phytophthora* species from growing systems. Methods for *Phytophthora* species detection, including immunoassay lateral flow tests and molecular DNA-based assays based on polymerase chain reactions (PCR) were reviewed in AHDB Project CP 126 (Pettitt, McPherson & Wakeham, 2015).

Materials and methods

A survey was conducted in 2019 to investigate the *Phytophthora* species present in dying raspberry canes at both propagator and commercial grower sites in England, collecting plants from a wide range of locations.

Experimental lateral flow devices (LFDs) developed in AHDB project CP 136 and produced by the University of Worcester were utilised. The LFD 3H7 detects “general” *Phytophthora* species, whereas LFD 3C4 was stated to have a narrower detection range comprising “pathogenic” *Phytophthora* species (from Clades 1, 7 & 8). The 3H7 LFD, as well as being broader in its reactivity than 3C4, is more sensitive than 3C4 - being capable of detecting smaller amounts of pathogen (Pettitt & Wakeham, 2018).

The 3H7 and 3C4 LFDs were first tested in the current project for initial detection of *Phytophthora* spp. presence in tissue in March 2019, with roots from seven long cane module plants that had been inoculated with *P. rubi* in November 2018 for the cold storage trial of this project. Samples were taken pre-potting on in March 2019. One uninoculated module was also tested. In addition, at the end of this trial, in November 2019, material from plants with rotted roots was sent from ADAS Boxworth for testing. Canes and root samples were sent from four untreated plants, half having been inoculated with *P. rubi* a year before. Both LFD tests and DNA sequencing was performed.

Before starting a countrywide survey, a test run was carried out in June 2019, separately sampling tissue from various parts of a wilting raspberry plant and sending these 11 samples for testing at the University of Worcester. By doing this is it was possible to finalise the

instructions to growers on the size of samples and what tissue to send. Indication was also obtained on how variable results might be from one plant.
A notification in late July 2019 was sent to fruit agronomist groups, growers and others in the soft fruit industry that samples of plants displaying symptoms of Phytophthora root rot were being sought in order to determine specific species causing infection. A biased survey was intended in order to receive infested plants, not to e.g. evaluate the proportion of infested plants within random collections from across plantations. The last plants sampled were collected in September 2019. Plastic bags and an envelope addressed to the sample laboratory at the University of Worcester were sent out to people wishing to participate in the survey. Each pack also included an instruction sheet with details, including pictures, of how and what to sample and a form requesting details of the incidence of wilting and the nature of the material being sent (Appendix 1.1). Cane tissue was requested from the bottom 100 mm of affected plants, with a separate bag of pinches of root material of various thickness from places around the root ball. In a few instances symptomatic leaves were also sent in a separate bag, and on receipt some leaves adhering to canes were also taken for testing.

Some water samples were also received from irrigation sources. These were put through membrane filters and extracted, and the filters tested with LFDs in the same way as tissue samples.

Samples were tested using a two-phase process, with LFDs used and plant samples accumulated before starting on molecular diagnostics.

Processing of tissue

On receipt of the samples at the University of Worcester they were allocated a batch code and separate accession codes for the roots and the cane per plant and these was held against any identifier provided by the sender. Scans of the sample information forms were sent for collation at ADAS.

Sub-samples were taken separately from the canes and the roots. On a few occasions there were leaves adhering to the canes and these were also taken for a separate test. For stems, the surface material was stripped away with a flame-sterilised scalpel and slivers from the leading edge towards browned tissue collected. The slivers were taken from each end, and midway, along the approximately 100 mm long cane samples received. To sample roots, they were pinched from three locations on the sampled root mass, or the rootball if still intact. They were rinsed under running tap water, blotted to remove excess water, and a subsample taken with flame-sterilised forceps.

Extraction of tissue sap was carried out using Bioreba bags. About 1 g of fresh tissue was used in a bag, one side of the central mesh. When plants not long grown from root cuttings were being tested the whole root or shoot was required. For the immunodiagnostics tests, 3 ml of B2 buffer was added to the sample in the bag. The Bioreba hand-roller was then used to aid tissue mashing. The central fine mesh allowed the extract to be filtered in the bag, with liquid able to be drawn out from the opposite side to the tissue.

After mashing in the Bioreba bag with the buffer solution this yielded about 2 ml of clarified extract. A 100 µl drop was placed one after the other in the test well of each of two LFDs.

One LFD contained the beads coated with the general antibody 3H7UW375 (abbreviated to 3H7) which tests for the presence of a wide range of *Phytophthora* species. The second LFD contained the antibody 3C4UW387 (3C4), which had been shown to bind with antigens from "pathogenic" *Phytophthora* species within Clades 1 (which includes *P. idaei*), Clade 7 (which includes *P. rubi*) and Clade 8. A combination of LFD tests was needed as *Phytophthora* spp. that are not usually considered pathogenic, such as *P. gonapodyides* from within Clade 6, will not cause a positive reading with 3H7. Reading of the test strip was done by a colorimeter 10 minutes after the extract has been adsorbed across the test pad. The fainter the red line produced by each LFD, the more *Phytophthora* spp. antigen was present. The LFDs were frozen after reading them, as the paper test strip inside each LFD holds DNA from the extract which could later be used to detect the species present.

The remainder of the root, cane or leaf extract was collected from the bag and frozen, along with the tissue residue still in the Bioreba bag. The remainder of each sample, not used to create the extract, was also frozen in the grip-seal bag it was sent in. A range of material was frozen for potential later use, because method development was a component of the work and it was possible that results could be improved by following a certain process.

Molecular testing

Preliminary tests were done to determine which of the different types of frozen material would allow the best DNA extraction. Initially, extractions were carried out on the absorbent pads from LFDs following use of the devices with tissue extracts, the solid tissue residue in the Bioreba bags, and the original frozen plant material. In later tests (the majority of samples) frozen plant material left from that taken for LFD testing was used as this was found to give the best DNA extracts. DNA extraction from the LFD pads did not work well. Only the Bioreba

bag residue material from some of the very young propagation plants (which had no remaining unprocessed plant material) worked reasonably well.

Next Generation Sequencing analysis of the internal transcribed spacer (ITS) ribosomal RNA (rRNA) region was carried out on the frozen samples in November 2019. This region of the rRNA (ITS1) was amplified using a nested polymerase chain reaction (PCR). This allowed amplification while reducing non-specific binding in products due to the amplification of unexpected primer binding sites. The process consisted of two rounds of PCR that followed the procedure developed and described by Scibetta *et al*. (2012) with some small adaptations.

The first round of PCR was with the primers used by Scibetta *et al*. (2012) i.e. a 18Ph2F and 28Ph2R reaction mix comprising 12.5 µl Biomix Red, 0.5 µl MgCl2 stock (to give 1.5 mM), 1 µl 18Ph2F, 1 µl 28Ph2R, 9 µl pure water plus 1 µl of DNA extract using reaction conditions of 1 cycle at 95°C for 2 minutes, then 40 cycles of 95°C for 20 sec, 61°C for 25 sec, 72°C for 1 minute and then the last cycle at 72°C for 5 minutes.

The second round of PCR was with the same reaction mix as in the first round except using the primers ITS6 and 5.8-1R and 1 µl of amplicon mix from the first round of PCR (after appropriate dilution if needed).

DNA extractions were carried out using Qiagen DNeasy Plant Pro kits and manufacturer's instructions were followed. Water controls were run alongside.

Once DNA had been extracted from them, the first round of PCR was carried out and a check was done using a sequencing gel to ensure that an amplification product had been obtained. Its concentration was determined by nanodrop. The reaction product was then diluted 1 in 10 if needed (although mostly dilution was unnecessary) and 1 µl of this taken through the second round of PCR. Another gel was carried out to check that amplicons had been produced in a reasonably clean band. Qiagen clean-up was performed using the clean-up kit before measuring the concentration by nanodrop.

The remaining (not used on the gel) reaction product was taken through a clean-up using QIAquick PCR purification kit, then prepared for big dye reaction (using a dilution and adding an aliquot of primer). It was then sent to Birmingham University genomics laboratory for big dye reaction and Sanger sequencing. A file was returned for each sample and a computer program was used to open the sequence files and then “trim” the untidy beginning and ends of the sequence. Each sequence was then taken through a BLAST (Basic Local Alignment

Search Tool) analysis using Chromas software. A free-access USA database was checked and matches with individual *Phytophthora* species automatically searched for.

Results

A total of 180 plant tissue samples were tested, from 76 raspberry plants with wilting and/or root rotting collected around the UK. In most cases one cane and a sample of roots were tested from each plant. Twenty cultivars of raspberry were included within the sampling (these have been coded in Appendix 1.2 to maintain confidentiality), however results are not discussed with reference to variety as sampling was not designed to be used for this purpose. Of the 180 samples, 89 were root tissue, 79 were cane tissue, and 12 were raspberry leaves (**Figure 1.15**). Growers and soft fruit advisors were asked to send in symptomatic raspberry material, but a small number of plant sections received were asymptomatic.

Three quarters of samples were sent in from potted plants rather than soil-grown crops and ranged in age from young 1-month old plugs, to six-year old cane from established soil-grown sites.

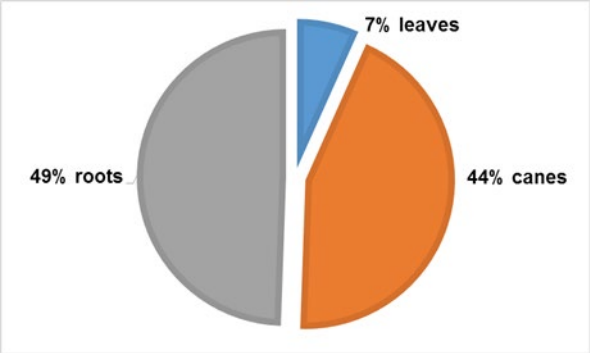

Figure 1.15. Proportion of raspberry plant samples received from UK crops in 2019 that were either root, cane or leaf tissue.

Raspberry plants were sampled from a range of UK counties, predominantly Oxfordshire, Berkshire and Kent (**Figure 1.16**). Samples were received from the principal soft fruit growing areas in England, with from two to 32 plants received from individual senders, with several senders dispatching a number of batches between July and September following site visits. One sender collected material in Scotland. Material was received from around 68 plantations, usually one plant from each.

Map of the British Isles showing counties from which plants were sent for *Phytophthora* spp. diagnosis.

The numbers in the markers show the number of samples of wilting/root rotted raspberry plants submitted for testing between July and September 2019. There was usually one representative plant per variety, or plant age from a plantation. Eight plants in Cambridgeshire assessed from the SF158 biofungicides trial were included, but not included in the full survey results.

Figure 1.16.

The distribution map confirms that raspberries wilting with *Phytophthora* spp. were seen in these counties in summer 2019, but the number of samples from each location can neither be used to indicate the relative frequency of wilted plants in general in those locations, nor to suppose an absence of infested crops in south western and northern England. In addition, in most cases, the propagation material for the planted crops would have come from elsewhere in the UK or abroad.

In addition to samples sent directly to the University of Worcester for testing, some whole plants were processed at ADAS Boxworth resulting in the testing of additional cane or root samples from different locations on the same plant to see how test results compared (sample batch C551). Two farm site visits were also made by ADAS pathologists (sample batches C568 and C585) when the roots of plants that had not wilted were examined and rotted roots sampled.

Phytophthora spp. were present in all ranges of material age, from young propagation material, up to established six year-old plantation material. Of the 180 plant tissue samples, 86% (162) tested positive with the Phytophthora-general antibody (3C4), and of those, 85% (155) also tested positive with the Phytophthora-pathogenic antibody (3H7). All 12 leaf samples tested positive with both antibodies.

As most samples of both roots and canes returned positive LFD results, this indicated that consultants had correctly identified that *Phytophthora* spp. had caused the cane die-back and root rotting of the plants they sampled. For one batch of symptomatic samples received (C591) the sender was able to use a private grower-funded crop disease diagnostic laboratory and *Verticillium* spp. was found in a sub-sample of cane tissue that subsequently gave negative LFDs and subsequently no *Phytophthora* spp. DNA. The plant had been recognised

as being more desiccated than was usual with *Phytophthora* spp.. Other root rot pathogens detected were *Fusarium* spp., *Pythium* spp. and *Ilyonectria* (*Cylindrocarpon*) spp. as well as *P. rubi*. This showed that even in potted plants the roots are likely to be colonised by "soil-borne" species other than *Phytophthora* and so contribute to root loss.

Using the nested PCR it was possible to show a clean amplicon band on the gel in most cases and subsequently one *Phytophthora* species was identified from each individual sample. In some cases, however a cluster of bands was obtained on the gel and if this was because several *Phytophthora* species were present then it was not usually possible to separate their DNA sequences in the output and the term "Gobbledegook" was used in the laboratory record table (Appendix 1.2) to describe these nonsense readings. In addition, some samples had produced positive LFD tests for *Phytophthora* spp., but then the DNA did not amplify and so as with "Gobbledegook" their identity is undefined. Known species, such as *P. rubi,* could be within the undefined samples. *Phytophthora* spp. sequences were obtained from some samples that did not match any on the USA *Phytophthora* spp. genetic database.

Species present in samples and support of LFD results by DNA sequencing

The intensity readings from each LFD test strip was recorded across the full range of detection strengths; with 0 negative, (+) weak positive, but potentially negative, + moderate positive, ++ strong positive and +++ very strong positive detection (**Table 1.29**).

Table 1.29. Showing the number of 3C4 (Clade limited) LFD-tested tissue samples across the whole survey that fell within each of the LFD intensity reading categories, and the species subsequently detected following PCR. Arranged by *Phytophthora* spp. Clade.

	3C4 LFD readings for *Phytophthora* spp. antigen presence				
Species identified from DNA	**0 Negative**	**(+) Weak**	**+ Moderate**	**++ Strong**	**+++ Very strong**
Clade 1:					
P. cactorum	0	0	1	1	1
P. idaei	0	0	0	2	0
Clade 7:					
P. rubi	0	3	13	28	33
P. alni	0	0	0	0	1
Clade 2:					
P. citrophthora	4	3	7	2	0
P. citricola	1	0	4	0	1

P. bishii	1	0	6	3	1
P. plurivora	0	0	0	0	2
“Unknown species”	0	3	13	13	2
Downy mildew species	0	0	3	1	0

In 19 samples, tissue which had given negative readings for both LFDs was also put through PCR. No *Phytophthora* spp. DNA was subsequently found confirming these were true rather than false negatives.

Whenever the general 3H7 LFDs were positive for *Phytophthora* spp. then *Phytophthora* spp. DNA sequences were produced, other than in some cases where the DNA did not amplify (examples for individual samples are given in Appendix 1.2). This supports the use of the 3H7 LFD to reliably detect the presence of *Phytophthora* spp..

With the Clade 1/7/8 specific 3C4 LFD, most gave a strong or very strong positive detection. The 3C4 LFD positive test results were confirmed by subsequent DNA sequencing to be mostly from *P. rubi* (within *Phytophthora* Clade 1), with *P. idaei* from Clade 7 also detected. None of the tissue samples subsequently shown to have Clade 7 *P. rubi* infestation failed to give positive LFDs. Positive tests of moderate or greater strength were shown for tissue later confirmed to be infested by Clade 1 *P. cactorum* / *P. idaei* (for which there is a known issue of not being able to separate their sequences – T. Pettit pers. comm.), none having been missed. DNA sequences for *P. infestans* (Clade 1) and *P. alni* (Clade 7) were also detected by both LFDs from single canes, although these are not usually found on raspberry. It can be concluded that there was confidence in the ability of the 3C4 LFD to detect *Phytophthora* Clades 1 and 7.

Antigens of Clade 8 species such as *P. cryptogea* should also have been capable of detection by the 3C4 LFD, but no such species were named following DNA sequencing. Therefore Clade 8 detection ability by 3C4 was unconfirmed from the DNA results, but such species could have fallen within the proportion of samples with undefined DNA readings or were not present in the samples received.

Although usually both LFDs gave positive results, there were six samples (all but one roots) in which a negative result for the ‘pathogenic’ 3C4 LFD followed a positive reading for *Phytophthora* spp. on the general LFD (Appendix 1.2). The DNA analysis subsequently confirmed *Phytophthora* species other than those from Clade 1, 7 or 8 expected to be detected by 3C4 (plus one where DNA did not amplify enough). They were instead members

of *Phytophthora* Clade 2; *P. citrophthora* (four samples), and *P. citricola* and *P. bishii* with one sample each.

Some detection of DNA for Clade 2 unexpectedly followed positive 3C4 LFDs, as shown in **Table 1.29**. These were both cane and root samples and comprised 12 *P. citrophthora*, five *P. citricola*, ten *P. bishii* and two *P. plurivora*. This was either because the 3C4 LFD can detect these Clade 2 species (perhaps requiring high antigen levels), or potentially a species within Clade 1, 7 or 8 had been present in the tissue used for the LFD, but was not again in the re-sampled tissue used for DNA testing. As the specificity of 3C4 is unclear, in all following results where the 3C4 LFD, gave a positive test result, but only a *Phytophthora* Clade 2 species was then sequenced, the results will be reported as if Clade 2 detection did occur.

The "unknown" species (whose sequences were unable to be matched to *Phytophthora* species in the database) all followed positive 3C4 LFD detection. The LFD readings were commonly within the moderate to strong positive region and so tending to match more closely the pattern of test results for *P. rubi* in Clade 7, as shown in **Table 1.29**.

Information on how individual *Phytophthora* species fall into the different clades of each species is provided as a table by the United States Department of Agriculture (USDA) (Abad *et al*., current update 2019) https://idtools.org/id/phytophthora/tabular_key.php and the species for which DNA was matched in the current report is given in **Table 1.30**. The *Phytophthora* species, other than *P. alni, P. infestans* and *P. plurivora,* have all been reported in the literature on red raspberry and the references to these incidences are given in full in a later section of this report. *Peronospora sparsa* was also sequenced, but is more frequently seen causing blackberry downy mildew in the UK than affecting raspberry.

Table 1.30. *Phytophthora* Clades for the species reported from the DNA sequencing of 180 raspberry root, cane and leaf samples from plants wilting or with root rot from the UK in 2019, and known host range. Information from Cooke *et al.* (2000) and USDA (Abad, 2018).

Clade	*Phytophthora* species	Host range, with any principle host/s noted in the literature
1a	*P. cactorum*	Multiple, mainly woody perennials
1a	*P. idaei*	Single, raspberry
1c	*P. infestans*	Multiple (especially potato, tomato)
2c	*P. citricola*	Multiple, woody perennials (especially citrus)
2a	*P. citrophthora*	Multiple, woody perennials (especially citrus)
2c	*P. plurivora*	Multiple, woody perennials
2d	*P. bishii*	Limited, glasshouse raspberry, rose, strawberry
4	(*Peronospora sparsa*)	Multiple, woody perennials causing downy mildew)
7a	*P. rubi*	Single, raspberry
7a	*P. alni*	Single, alder

Incidence of *Phytophthora* species in different raspberry tissues

Once all samples had been received and tested with the LFDs, the frozen plant material from every sample was put through nested PCR, whether or not they had given a positive test line, and sent for sequencing in November 2019. The distribution of the various *Phytophthora* species between the cane, root and leaf samples is given in **Table 1.31**. The relative proportions of samples of each species compared with the total of those samples tested (whether or not *Phytophthora* DNA was present) is expressed in the form of Pie charts. The Pie charts show the results for individual root, cane and leaf samples from the DNA sequencing with usually a cane and a root sample per plant. All but 10.6% of the total tissue samples were shown by the DNA testing to contain *Phytophthora* spp. (**Figure 1.17**) and these had also been shown to be negative by both LFDs. Fewer cane samples had no *Phytophthora* spp. detected than did root samples (**Figure 1.18** & **Figure 1.19**), with only two negative canes out of the 79 sampled, whereas 17 out of 89 root samples had no *Phytophthora* spp. (**Table 1.31**). However, *P. rubi* infection normally enters via the roots. The chances of sub-sampling *Phytophthora* spp. that enter the canes was greater as the cane base was an easily defined sampling unit, whereas “a few pinches” from a large root ball could miss infested tissue, and sub-sampling then also followed in the laboratory.

Table 1.31. *Phytophthora* species, or *Peronospora sparsa*, identified per sample of cane, root or leaf tissue from their DNA sequence following a positive LFD test.

Species identified by DNA	Number of samples of each tissue per species and the total number of samples with each species			
	Cane	Root	Leaf	Total
P. rubi	38	31	8	77
P. citrophthora	7	9	0	16
P. bishii	2	8	1	11
P. citricola	2	3	0	5
Peronospora sparsa	3	1	0	4
P. cactorum	1	2	0	3
P. plurivora	2	0	0	2
P. alni	1	0	0	1
P. idaei	0	1	0	1
Undefined	16	11	2	29
Unknown	5	6	1	12
No *Phytophthora* sp.	2	17	0	19
TOTAL	79	89	12	180

"Undefined" species comprised samples where either the DNA did not amplify in the PCR, or it was not possible to define the *Phytophthora* species (listed as "Gobbledegook" in subsequent results for individual samples, as in Appendix 1.2) probably because of mixed infections. "Unknown" species comprised samples where there was no match of the DNA sequence to any species recorded on the *Phytophthora* spp. DNA database.

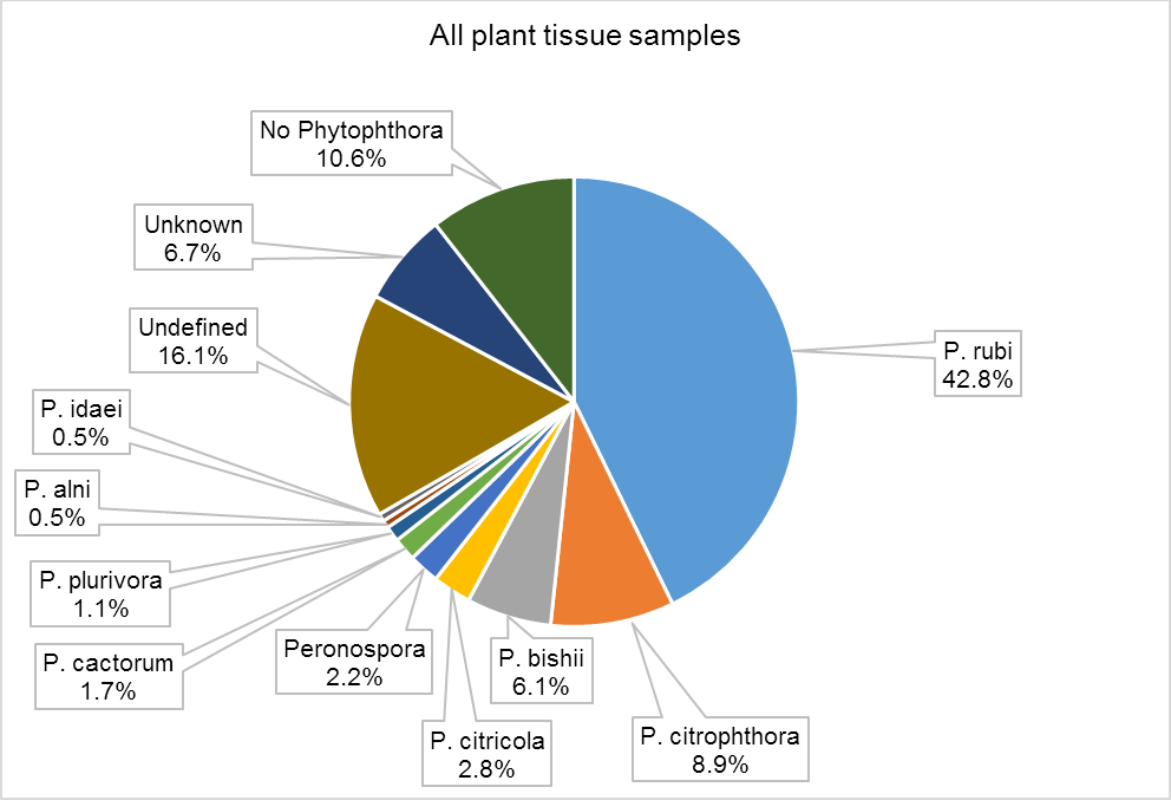

Figure 1.17. *Phytophthora* species either identified, or not identified, by DNA from each of 180 cane, root and leaf samples from symptomatic raspberry plants sampled from UK crops between July and September 2019.

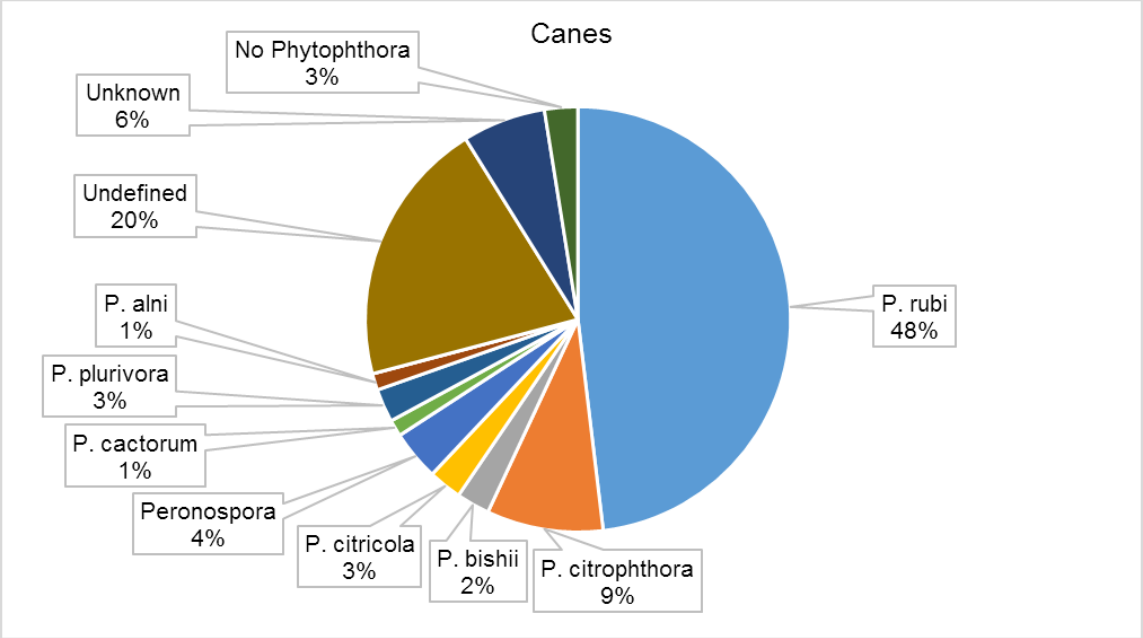

Figure 1.18. *Phytophthora* species either identified, or not identified, by DNA for the 79 cane samples from symptomatic raspberry plants sampled from UK crops between July and September 2019.

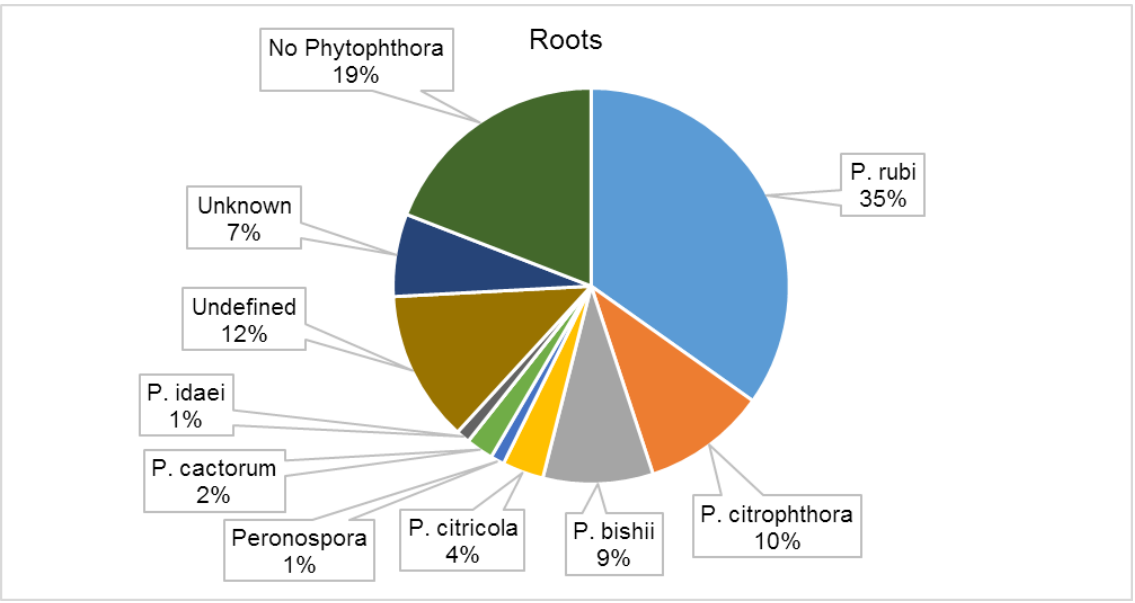

Figure 1.19. *Phytophthora* species either identified, or not identified, by DNA for the 89 root samples from symptomatic raspberry plants sampled from UK crops between July and September 2019.

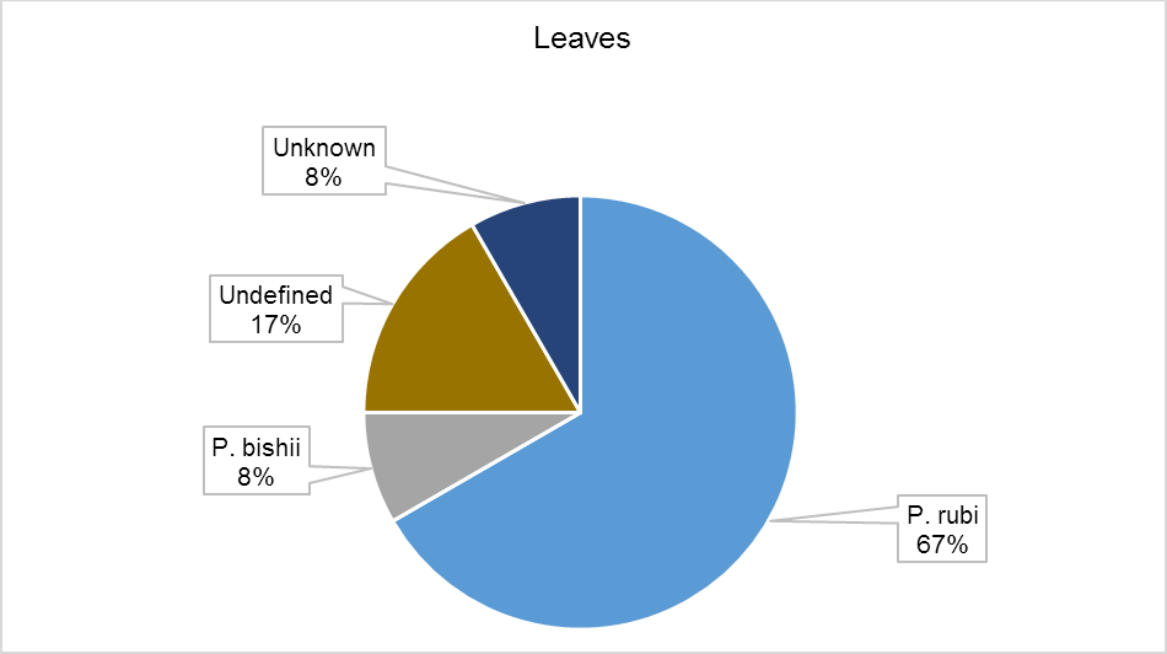

Figure 1.20. *Phytophthora* species identified, or not identified, by DNA for the 12 leaf samples from symptomatic raspberry plant sampled from UK crops between July and September 2019.

P. rubi infested the greatest proportion of tissue (42.8% of all tissue sampled) (**Figure 1.17**). Next most prevalent were *P. citrophthora* (8.9%), followed by *P. bishii* (6.1%) which was more common in roots than canes and *P. citricola* (2.8%). Other species present, from single samples, were *P. plurivora, P. infestans* and *P. alni* (the latter two not being known as raspberry pathogens). *P. idaei* and *P. cactorum* are unable to be separated in the DNA analysis used and combined were only present in four samples (**Table 1.31**).

Unknown / not matched sequences were relatively common, comprising 6.7% of all samples and from cane, root and a shrivelled leaf. They were from five different sites. This may indicate that there are one or more raspberry wilt/root rot pathogen species yet to be identified. In two cane samples the identity was unclear because the sequence appeared to match two different species *P. infestans* / *P. adina* and *P. mengei* / *P. citricola.*

Unknown sequences were distinct from those of the 16.1% of samples undefined, where either the DNA did not amplify or sequences were mixed.

Of all samples, 4% (four instances) contained the DNA of an oomycete other than *Phytophthora*, this was of *Peronospora sparsa* (downy mildew) in root and cane tissue, for which both LFDs had been positive.

Eight out of 12 leaves were infested by *P. rubi* (**Table 1.31**). The only other species identified in leaves was *P. bishii*. One *Phytophthora* sp. sequence from a leaf was unable to be matched to a species on the USA *Phytophthora* spp. genetic database.

***Phytophthora* spp. symptoms in tissue**

Symptoms on leaves:

Leaf discoloration was observed in some samples (**Figure 1.21**). The leaves all gave strong positives when tested for presence of *Phytophthora* spp., and had an aromatic odour, typical to oomycete infection. Most leaves were infested by *P. rubi*, but a young shoot sample from a plant potted in April contained *P. bishii*.

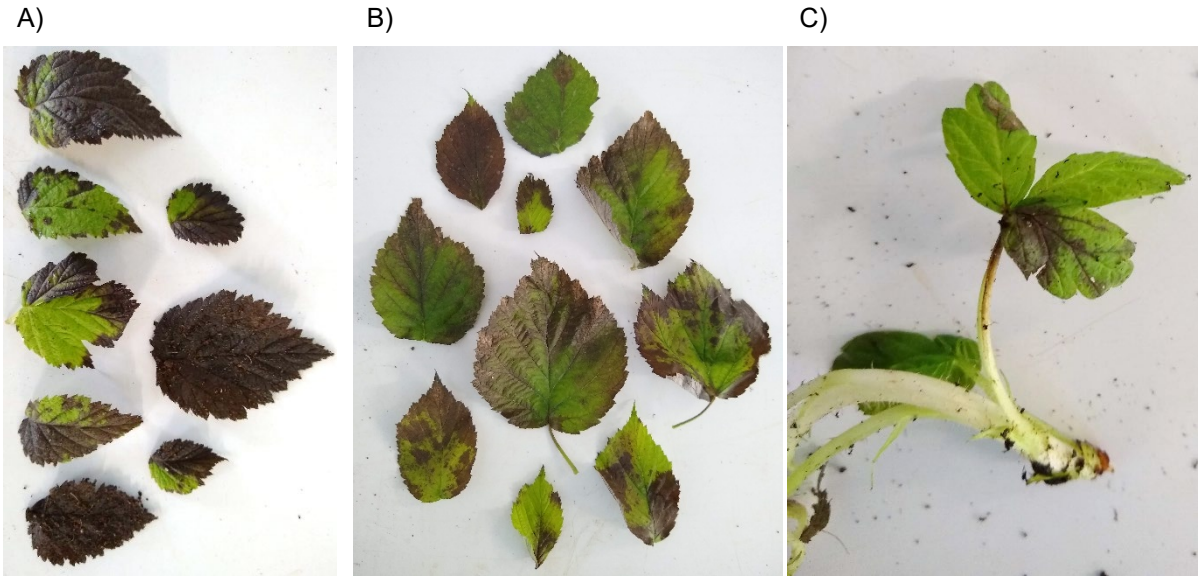

Figure 1.21. Examples of raspberry leaves infected with *Phytophthora* spp.. A) Leaves that were touching the damp compost when the plant arrived at ADAS Boxworth which may have acted as baits to *Phytophthora* species in the substrate. *P. rubi* DNA detected. B) Discoloured leaves that were attached to infected flori- and primocanes. *P. rubi* DNA detected. C) Black staining at the top of the petiole on a young raspberry spawn shoot. July 2019.

Symptoms in canes:

Reddish brown staining was observed on a number of cane samples, both in flori- and primocanes (**Figure 1.22**). The staining was also evident far up the canes, in one instance 300 mm up from the stem base. Often whilst sampling seemingly healthy floricane, a slight darkening was observed on the inner cortex (**Figure 1.22** A). Severe infections where the entire cortical tissue was stained reddish brown coincided with completely dead canes, with no leaves attached. Canes that still had leaves attached, albeit wilting, often just had up to half the cortical tissue stained, with the rest a healthy living green colour.

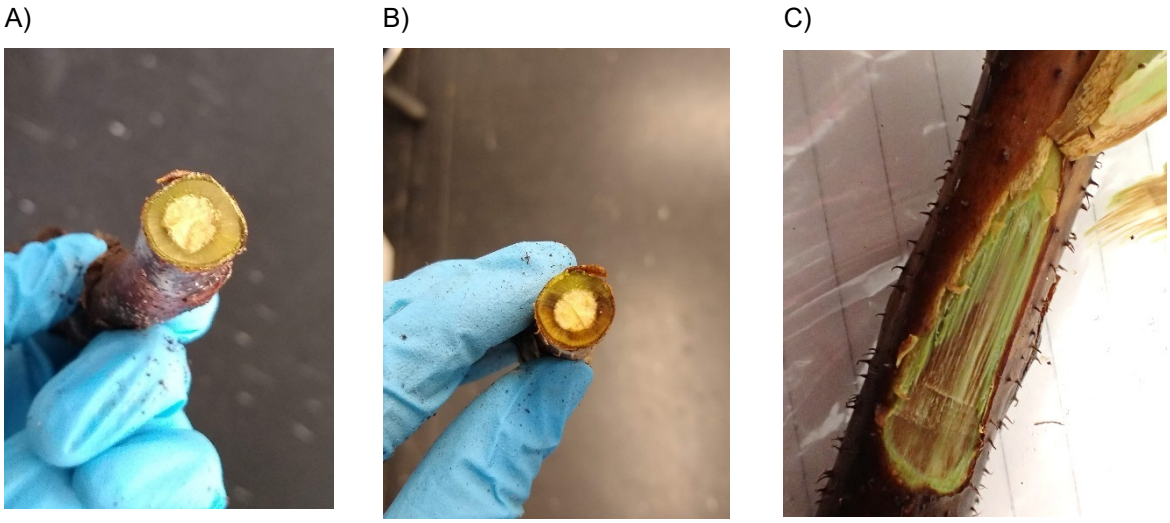

Figure 1.22. Examples of raspberry canes processed during the survey. All tested positive with the *Phytophthora* spp. LFDs. A) Slight staining on inner cortical tissue. B) Obvious staining of cortical tissue around majority of cane. C) Epidermis scraped back to reveal staining in cortex underneath.

Root examination, symptoms and sampling for Phytophthora spp.:

On rootballs of 5 L potted plants that were seemingly healthy (batch C568 that were examined at a propagation site as part of this survey), patches of browning roots were found and sampled. They tested positive for *Phytophthora* spp. with the LFDs. Often these roots were in the corner, or running along the edge of the pot, where water could collect. (**Figure 1.23**).

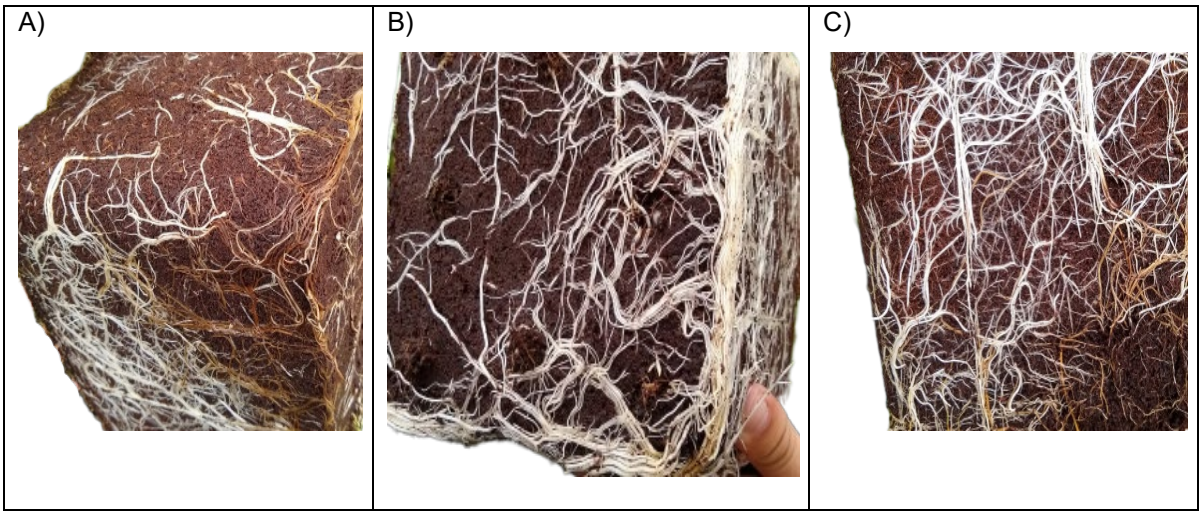

Figure 1.23. White healthy roots with patches of rotting in plants with healthy looking foliage, examined during ADAS survey of a propagation site. A) large patch of brown roots in corner, B) small section of browning beginning in corner of root ball, C) brown and limited root growth in corner of pot. Brown roots tested “moderate positive” for pathogenic *Phytophthora* spp. with 3C4 LFD in July 2019, but DNA did not amplify following PCR so species was not known.

A much greater proportion of rotted roots was present in plants with cane wilting and die-back collected by ADAS in order to establish the survey and testing protocols. Raspberry plants were sampled that had been planted in February 2019 in pots in a glasshouse to obtain an early fruit crop. By mid-June the floricanes were wilting and dying and the roots were rotted throughout the rootball (**Figure 1.24**). *P. rubi* DNA alone was present in the canes and roots.

The dying canes tested positive for *Phytophthora* spp., very strongly on both the “general” 3H7 and the “pathogenic, Clade 1/7/8, 3C4 LFDs.

The root sample was strongly positive for 3H7 and very strongly positive with the 3C4 LFD.

Figure 1.24. A plant with floricanes that had wilted within five months of potting in coir in February. The roots had become totally rotted. *P. rubi* infection was later confirmed. Sample batch C559, LFD tested and found positive for *Phytophthora* spp. 27 July 2019.

Root sample type and Phytophthora spp. detection:

As part of developing the sampling protocols, sub-sampling of the roots of a mature soil-grown plant with Phytophthora root rot was carried out. There was a wide range of root sizes, with tanned and dead material amongst recently rotted roots. Supple roots at the stem base were still alive (with staining visible inside), finer roots still had healthy white areas, but other areas were reddish (a symptom linked with the presence of *P. rubi* earlier in the current project). There were also dried out main and finer roots (see pictures in **Table 1.32**). These were sampled separately and sent for LFD testing and PCR. Dried material did not give positives for *Phytophthora* spp. with the LFDs, nor was *Phytophthora* spp. DNA present. Semi-rotted roots were thus requested from growers. After the survey sampling period, the DNA results obtained for the mature plant pieces showed that *Phytophthora* species differed in the type of source tissue (as shown in **Table 1.32**) *P. citrophthora* was in fine roots and also in the main root at the cane junction. *P. rubi* was present only in the thicker root pieces.

Table 1.32. Sample batch C551: Division of tissue from one bare-root raspberry plant showing the results from the general *Phytophthora* spp. and the Clade-specific *Phytophthora* spp. LFDs on 6 June 2019. Compared with the species subsequently identified following PCR and DNA sequencing for identification using a genetic database. To see whether the location from which a sample was taken on a plant would change the LFD or DNA detection results.

	Tissue type	**LFD 3H7 (general)**	**LFD 3C4 (1,7,8)**	**DNA identity**	**Image**
1	a) fine roots b) main root collar	(+) +	0 (+)	a) *P. citrophthora* b) *P. citrophthora*	
2	Main root collar	++	++	*P. rubi*	
3	Main root collar	+	++	*P. rubi*	
4	Main root with lower cane	+	+	*P. citrophthora*	
5	Thick root piece	+	+	*P. rubi*	
6	Thick root piece	+	++	*P. rubi*	
7	Medium root piece	0	0	Negative	

	Tissue type	LFD 3H7 (general)	LFD 3C4 (1,7,8)	DNA identity	Image
8	Fine roots	0	0	Negative	
9	Fine roots	0	0	Negative	
10	Fine roots	+	+	*P. citrophthora*	
11	Very dead stem collar/ main root	0	0	Negative	

Examples of crops of various ages infected by Phytophthora spp. to compare wilting incidence in the crop, species present and tissue symptoms:

Information on the LFD sample results from the survey and the species detected is given in Appendix 1.2 for individual tissue sections per coded variety and shown within the plant batches received throughout the year. In the 48 plants in which *P. rubi* was detected, 34 plants were only infested by this *Phytophthora* species. *P. citrophthora* affected nine plants, but five also contained *P. rubi*. *P. bishii* was present in nine plants, but six also contained *P. rubi*. In contrast, *P. citricola* was the only named *Phytophthora* species in all four plants it infested. Information from a selection of sample batches has been extracted in the sections below to illustrate results from particular aspects of the survey, in particular where

Phytophthora species other than *P. rubi* were detected following PCR and the results that had been found previously with the two LFDs.

***Phytophthora* spp. present in propagation material, including modules:**

Samples were received of plants that were wilting at various stages in propagation. The incidence and severity of symptoms seen in conjunction with the *Phytophthora* spp. present are described below.

Symptoms associated with *P. citricola* & 3C4 LFD detection of this & *Peronospora sparsa*

Batch C568 (**Table 1.33**) included four plug plants growing in a polytunnel from root cuttings in multicell trays that had been kept back from potting into modules as some were not thrifty. All four shoots gave moderate positives with the 3C4 LFD. *P. citricola* was detected in both the roots and stem of a plug with dying lower leaves and the few roots present mostly rotting.

No *Phytophthora* spp. DNA was detected in the other three plants, but DNA sequences from two of them matched *Peronospora sparsa*. *P. sparsa* is a not uncommon disease of blackberries, particularly in high humidity propagation tunnels, but not usually seen on raspberry. The positive LFDs were unexpected, however *P. sparsa* has been grouped in a "Clade 1 to 8 *Phytophthora – Peronospora* cluster" of evolutionary trends (Cooke *et al.,* 2000). *P. sparsa* was also identified following positive LFDs on modules at another two farms.

At another (outdoors) location for batch C568, symptomless roots of a 2 L module of a further variety also gave *P. citricola.* In this case although the 3H7 LFD gave a moderate positive, the Clade specific 3C4 using the same extract was negative for *Phytophthora* spp. *P. citricola* was also detected in a sample of another variety taken from a small patch of brown roots in the basal corner of otherwise strong white roots in a 5 L mother plant pot stood outdoors (**Table 1.33**, Plant 36). In this case the roots had given a moderate positive 3C4 LFD result.

P. citricola had caused greatest root damage to the immature plants in warmer wet conditions.

Table 1.33. Sample batch C586: Tissue of multicell plugs (plants 29-32), 5 L pot (plants 33 & 34) and 2 L modules (plants 37 & 38) of varieties growing in substrate on the same farm. Results from the 3H7and 3C4 LFDs tests on 21 August 2019, compared with the species subsequently identified following PCR. *P. citricola* is in *Phytophthora* Clade 2.

Tissue type	Cultivar code	Worcester lab. ID code	LFD 3H7 'general'	LFD 3C4 clades 1/7/8	DNA Sequencing results after PCR	Plant
Plug plants:						
Root	A	C568/AZ43	0	0	-	29
Cane	A	C568/AZ44	+	+	Gobbledegook	
Root	A	C568/AZ45	0	0	-	30
Cane	A	C568/AZ46	+	+	*Peronospora sparsa*	
Root	A	C568/AZ47	0	0	Negative	31
Cane	A	C568/AZ48	+	+	*Peronospora sparsa*	
Root	A	C568/AZ49	+	+	*P. citricola*	32
Cane	A	C568/AZ50	++	+	*P. citricola*	
Older plants:						
Root	M	C568/AZ51	+	+	Didn't amplify	33
Root	L	C568/AZ52	+	+	Didn't amplify	34
Root	L	C568/AZ53	(+)	+	Didn't amplify	35
Root	S	C568/AZ54	+	+	*P. citricola*	36
Root	D	C568/AZ55	+	+	Didn't amplify	37
Root	D	C568/AZ56	+	0	*P. citricola*	38

Incidence of wilt across one and two year old plants with *P. rubi* in roots and canes, & presence of *P. bishii*

In batches C577 to C579, from 1% to 7% of one year old module plants of five different varieties in containers in tunnels were seen in August to be wilting throughout the crops (**Table 1.34**). *Phytophthora* spp. were detected in roots and canes by the pathogen specific 3C4 LFD, and subsequently mainly confirmed as *P. rubi.* However, the crop with highest wilt incidence had *P. rubi* in the roots, but DNA of an unidentified *Phytophthora* spp. was found in the canes, and it is not known if the latter was causing the elevated wilting incidence.

Three plants had *P. bishii* in their roots, but two had *P. rubi* in the canes and so it is possible that the wilt seen was caused by the latter species not *P. bishii.* The survey did not seek to identify the initial sources of propagation material of the sampled crops and so it is not known whether the three varieties with *P. bishii* had previously been grown in close proximity.

Two year old module plants of batches C577 to C579 had *P. rubi* in the canes and roots and it was causing wilting in 3% to 9% of the plants. No *P. bishii* was detected, even though it had been found in younger plants of two of the varieties on the same farms.

Table 1.34. Sample batches C557-579: Tissue of one and two year old module plants of six different varieties sampled from across six farms showing the results from the 3H7 and 3C4 LFDs on 4 September 2019. Compared with the species subsequently identified following PCR. *P. bishii* is in *Phytophthora* Clade 2, *P. rubi* in Clade 7.

Tissue type & module age	Cultivar code	Farm coded location	Worcester lab. ID code	LFD 3H7 'general'	LFD 3C4 clades 1/7/8	DNA Sequencing results after PCR	Plant
One year old:							
Root	T	a	C577/AZ89	+	+	*P. bishii*	52
Cane	T	a	C577/AZ90	+	+	*P. rubi*	
Root	K	a	C577/AZ93	(+)	+	*P. rubi*	54
Cane	K	a	C577/AZ94	++	+++	*P. rubi*	
Root	J	b	C578/AZ95	(+)	0	*P. bishii*	55
Cane	J	b	C578/AZ96	(+)	++	Didn't amplify	
Root	coded 8	d	C578/AZ99	++	++	*P. rubi*	57
Cane	coded 8	d	C578/AZ100	+	+	no matches	
Root	H	e	C579/AY1	+	++	*P. bishii*	58
Cane	H	e	C579/AY2	+++	+++	*P. rubi*	
Two years old:							
Root	J	a	C577/AZ91	0	0	Negative	53
Cane	J	a	C577/AZ92	+++	+++	*P. rubi*	
Root	U	b	C578/AZ97	++	++	*P. rubi*	56
Cane	U	b	C578/AZ98	++	++	*P. rubi*	
Root	H	f	C579/AY3	++	++	*P. rubi*	59
Cane	H	f	C579/AY4	+++	+++	*P. rubi*	

Incidence of wilt in plants with *P. citrophthora* in canes plus *P. rubi* in roots or leaves

In sample batch C570, 20% of first year containerised long-cane modules were wilted in August. *P. citrophthora* was detected in the canes of two (**Table 1.35**). *P. rubi* was detected in the roots of one plant and leaves from the other and so it is uncertain whether *P. citrophthora* in the canes was the cause of wilting.

Table 1.35. Sample batch C570: Two first year long cane modules showing the results from the 3H7 and 3C4 LFDs on 21 August 2019. Compared with the species subsequently identified following PCR. *P. citrophthora* is in *Phytophthora* Clade 2. *P. rubi* in Clade 7.

Tissue type	Cultivar code	Worcester lab. ID code	LFD 3H7 'general'	LFD 3C4 clades 1/7/8	DNA Sequencing results after PCR	Plant
Root	B	C570/AZ66	+	+	*Peronospora sparsa*	42
Cane	B	C570/AZ67	+	+	*P. citrophthora*	
Leaves	B	C570/AZ68	+	++	*P. rubi*	
Root	B	C570/AZ69	+	+	*P. rubi*	43
Cane	B	C570/AZ70	++	+	*P. citrophthora*	

Incidence of wilt with *P. rubi* in canes with either *P. citrophthora* or *P. bishii* in the roots

In batch C574 (**Table 1.36**), plants of a variety where around 5% of one year old containerised modules were wilting was collected by a consultant from each of two farms. *P. bishii* was found in the roots of one and *P. citrophthora* in the roots from the other, but how important these species were is still unclear because they both had *P. rubi* in the cane.

From a third farm (as for C570) 20% of the one year containerised modules of the same variety were wilted by August, and this was attributable to the *P. rubi* detected in the roots (which gave a very strong positive 3C4 LFD) although all that was detected from the canes was *Peronospora sparsa* (downy mildew).

Table 1.36. Sample batch C574: One year old modules showing the results from 3H7 and 3C4 LFDs on 2 September 2019. Compared with the species subsequently identified following PCR. *P. citrophthora* & *P. bishii* are in *Phytophthora* Clade 2. *P. rubi* in Clade 7.

Tissue type	Cultivar code	Worcester lab. ID code	LFD 3H7 'general'	LFD 3C4 clades 1/7/8	DNA Sequencing results after PCR	Plant
Root	F	C574/AZ73	(+)	Negative	*P. citrophthora*	45
Cane	F	C574/AZ74	++	+++	Didn't amplify	
Root	B	C574/AZ75	++	+++	*P. rubi*	46
Cane	B	C574/AZ76	++	++	*Peronospora sparsa*	
Root	B	C574/AZ77	+	++	*P. bishii*	47
Cane	B	C574/AZ78	+++	+++	*P. rubi*	
Root	B	C574/AZ79	(+)	(+)	*P. citrophthora*	48
Cane	B	C574/AZ80	++	++	*P. rubi*	

Symptoms from plants with *P. idaei* / *P. cactorum*, *P. citricola & P. rubi*

Sample batch C575, *P. idaei* / *P. cactorum* (molecular separation difficult) was, unexpectedly, the only time this DNA sequence was detected in this survey (**Table 1.37**). It was present in the roots of a young containerised plant, which had green leaves with slight veinal chlorosis in its lower leaves, plus some browning in the stem base and pith. The cane had *P. citricola* (or *P. mengei*, but this is unlikely for this host), with a moderate positive detection in both LFDs. The symptoms resulted from either one or both pathogens in the plant.

A very strong positive 3C4 LCD result was obtained from a cane that had a necrotic stem base and first basal leaf, and *P. cactorum* was later sequenced. However, it is likely this and the records of *P. cactorum* from the roots and stem of another young plant of a different variety from the same site could have been *P. idaei* rather than the *P. cactorum* reported sequenced. A second cane on the same plant had shoot tip dieback which was necrotic but not dry, similar to symptoms of Phytophthora blight in potatoes (*P. infestans*) and *P. rubi* was detected.

Table 1.37. Sample batch C575: Two containerised young plants, both showing stem tissue necrosis, showing the results from the 3H7 and 3C4 LFDs on 2 September 2019. Compared with the species subsequently identified following PCR. *P. idaei* / *P. cactorum* both in *Phytophthora* Clade 1a, *P. rubi* Clade 7.

Tissue type		Cultivar code	Worcester lab. ID code	LFD 3H7 'general'	LFD 3C4 clades 1/7/8	DNA Sequencing results after PCR	Plant
Root		coded 7	C575/AZ81	(+)	++	*P. idaei / P. cactorum*	49
Cane		coded 7	C575/AZ82	+	+	*P. mengei / P. citricola*	
Root		N	C575/AZ83	+	++	*P. cactorum*	50
Cane		N	C575/AZ84	++	+++	*P. cactorum*	
Cane		N	C575/AZ85	+	++	*P. rubi*	

***Phytophthora* spp. within plants potted-up in a crop:**

In addition to the example of a plant only containing *P. rubi* shown in **Figure 1.24**, another wilted plant examined in the ADAS laboratory (**Figure 1.25**) was found to contain other *Phytophthora* spp. in addition to *P. rubi.*

LFD results for Clade 2 *P. bishii* & *P. citrophthora* in separate tissue on a plant with *P. rubi*

From batch C564, a mature, multiple-caned, containerised plant where several canes had died and leaves were wilting and dying on others (**Figure 1.25**) was split apart at the laboratory and three of the canes were sectioned into cane, cane base and roots.

Within this plant, three different *Phytophthora* species were found in the DNA testing (**Table 1.38**). *P. bishii* occurred in one cane base and its roots and in two other canes' roots. *P. rubi* was present in cane bases. *P. citrophthora* was present in two of the canes above the base.

Positive tests were obtained with the Clade 1/7/8 specific 3C4 LFD where Clade 2 *P. bishii* and *P. citrophthora* were later identified (**Table 1.38**). However, *P. rubi* was present in the plant so (as in the above examples from young plants) it could have been in tissue tested with the 3C4 LFD. However for *P. rubi* to have caused the positive LFD not the Clade 2 species in such cases, it would require that, frequently, when the tissue was re-sampled for PCR the section taken instead contained a species other than the *P. rubi.*

Six cane and three root samples from this pot were tested separately with the LFDs. June 2019

P. citrophthora, P. bishii and *P. rubi* DNA was later detected.

Figure 1.25. Plant with canes dead or with wilting leaves. Plant 15 results in **Table 1.38**.

Table 1.38. Sample batch C564: Three samples of roots, and upper and lower cane (within the basal 10 mm) from one containerised raspberry, showing the results from the 3H7 and 3C4 LFDs on 29 July 2019. Compared with the *Phytophthora* species subsequently identified following PCR. *P. citrophthora* and *P. bishii* are in Phytophthora Clade 2, *P. rubi* in Clade 1.

Cane on plant	Tissue	3H7 general LFD	3C4 clades 1/7/8 LFD	DNA sequenced
1	roots	+	Negative	*P. citrophthora*
	cane towards base	++	+	*P. rubi*
	cane	+	(+)	*P. citrophthora*
2	roots	+	+	*P. bishii*
	cane towards base	++	++	*P. rubi*
	cane	++	++	*P. citrophthora / (P. botryosa*)*
3	roots	(+)	+	*P. bishii*
	cane towards base	++	+++	*P. bishii*
	cane	+	+	Gobbledegook

* Unlikely from known host range to infect raspberry and a native of Asia.

Sampling of P. rubi inoculated modules potted for ADAS efficacy experiment:

The March 2019 pre-potting check-testing of the roots of seven modules from the SF158 experiment that had been inoculated with *P. rubi* in November 2018 produced positives with the 3H7 LFD and more importantly with the LFD 3C4 for Clades 1/7/8 that detects *P. rubi.* Both LFDs were negative for the uninoculated module. The PCR set-up for DNA was not in place at this date to confirm the *Phytophthora* spp. was *P. rubi*.

A sample of symptomatic plants taken in November 2019 from the 2018/19 cold storage and biofungicide treatment experiment all produced positive test results for both LFDs, whether or not inoculated with *P. rubi* in November 2018 (**Table 1.39**). DNA testing showed "no matches" from cane tissue of three plants and so potentially a "new species" not listed on the *Phytophthora* spp. molecular sequence database. *P. rubi* was in the roots of an uninoculated plant as well as the inoculated. Infested uninoculated plants had been found in the previous experiment (plants sourced from the same propagator) but *P. idaei* was found at that time.

Table 1.39. LFD and DNA testing of four wilted raspberry plants from the 2018/19 SF158 trial.

Plant inoculation	Tissue	LFD 3H7	LFD 3C4	DNA Sequence
+ *P. rubi*	roots	++	++	*P. rubi*
	cane	+	++	*P. rubi*
+ *P. rubi*	roots	+++	+++	*P. rubi*
	cane	+	++	No matches
no *P. rubi*	roots	++	++	Gobbledegook
	cane	+++	+++	No matches
no *P. rubi*	roots	+++	+++	*P. rubi*
	cane	+++	+++	No matches

Discussion

Crop surveying and sampling technique and interpretation of results

Wilting scattered throughout a containerised crop is more likely to have arisen from infection brought in with the propagation material rather than only plants here and there succumbing to *Phytophthora* spp. carried by contaminated irrigation water. The percentage of plants wilting gave a "snapshot in time" and so may not show the final incidence of infected plants and the speed of plant collapse will vary greatly depending on the plant and its growing conditions and the initial infestation level. *P. rubi* is known to cause wilting in raspberry, and other species detected in the same 14 plants may have been picked up because of this, when alone they may have produced less-obvious symptoms.

The pathogen species present at any one location will have been influenced by factors including the climate (for optimal pathogen species growth), the source of propagation material (for potentially introducing non-native pathogen species), cropping history of the site (particularly when raspberries were soil-grown), the health status of nearby plants, and the cleanliness of irrigation water. Plant age, variety, stress factors and fungicide application will then have been factors influencing whether plants became visibly affected following infestation. Plants in which sufficient root area remained un-rotted so that water uptake was not affected will not have been collected in the current project (nor in any published surveys

looking for wilted shoots at the time of visiting the crop). Sampling could be biased against finding *Phytophthora* species that colonise the host more slowly. This is relevant to the current project as (where the information was supplied) the age of most plants seen with wilt were one or two year old modules or potted crop plants that tend not to be retained for as many years as perhaps soil-grown crops. Soil grown material up to six years old was, however, sampled.

Conversely, sampling of weaker pathogens may have occurred where cold-stored long-cane are not left to defrost on arrival before placing in a warm polytunnel, resulting in failure to establish or thrive (J. Allen, ADAS crop consultant, pers. comm.). More frequent UK extremes of hot weather in recent years, often attributed to climate change, are also liable to cause crop stress, particularly for plants in pots in polytunnels and if irrigation is not adjusted (https://www.metoffice.gov.uk/weather/learn-about/past-uk-weather-events).

With the sampling in the crop there was little chance to miss infested cane tissue when collecting the whole lower 100 mm, particularly as most plants had not long been planted and so there was only a single floricane. In the laboratory a consistent approach to sampling position assisted comparison between samples. However, when root balls were sampled in the crop it would be possible to select browning not caused by *Phytophthora* spp. (e.g. either physiological damage, fungal or *Pythium* spp.). *Phytophthora* spp. root rot was observed to occur in patches such as around dripper pegs or at the base of root balls of containerised plants. Most plants received in the survey were containerised and so the whole root ball could be sampled, however in soil grown crops there was a possibility that peripheral roots (said to be more likely infested by *P. idaei* (D. Cooke of JHI. pers. comm.)) could have been left in the ground. However, most root samples were found to be positive in LFD tests and so sampling techniques in the crop by consultants and then by laboratory diagnosticians were robust.

More cane tissue, taken within the bottom 100 mm, contained *P. rubi* than other species. Staining under the cane epidermis up from the cane base was seen and it is usually assumed that this is caused by the pathogen moving up from the roots, rather than entry into the cane. However, some wilted leaves were tested and *P. rubi* found. It was thought possible that as the leaves were within the bags containing their cane that they acted as baits in the moist conditions and became infested externally. Other *Phytophthora* spp. could also be baited as, unlike *P. rubi*, most are attracted to baits across a range of plant species and tissue types (Erwin & Ribeiro, 1996). This needs further investigation as if *Phytophthora* spp enter leaves growing on the crop they could be a source of infection into the cane and to the roots when they drop. In any future survey, necrotic leaves could be specifically requested to be sent and be put in their own bag, perhaps with some samples of upper as well as lower cane in order

to learn more about the spread of mycelium in the plant. PCR would need to be utilised because of the difficulty in isolating *P. rubi* from tissue.

Extraction methods for different types of material

Given the quite large bulk of most samples received in the post to use in the LFDs (relative to the amount of tissue needed for the tests), extraction of sap from the tissue gave no problems. The standard laboratory procedure developed on the positions to sample and how much tissue was sampled worked well. All plants received for testing had symptoms of root rot and/or wilt on canes, and tissue was able to be taken from where there was browning. Some samples on receipt, having been through the post for several days after sampling, had degraded beyond the stage at which any isolation onto agar would have been attempted, but this was not found to be an issue for extraction.

The extraction of DNA from LFDs detecting *Phytophthora* spp. was a decade ago offered as a service called PDPlus by Forsite Diagnostics in the UK for a short-list of species such as *P. ramorum* and *P. cactorum.* However when using DNA from the pads was tried in the current work, it was disappointing that the pads did not provide good DNA extracts. Otherwise, following on from this project, sample testing by LFD in the field could have been used by growers and then LFDs positive for *Phytophthora* spp. would have been be a convenient type of material to post to a laboratory for PCR and identification to species.

It was unexpected that samples frozen on arrival would give stronger LFD test indications than fresh, but as both the roots from raspberry canes and the canes themselves are quite fibrous it is likely that freezing allowed the tissue to be broken open and the cells fractured more easily.

It was important to get a good tissue extraction, and this is best done with the Bioreba bags to squash the raspberry tissue. The tissue is too hard for effective breaking down by the ball bearings in buffer bottles supplied with commercial LFD kits. With all LFDs there is a shelf life, and the ones used in the present work remain stable for three months.

In a few cases some leaves had found their way into the plastic sample bags and were pressed against rotted roots. They showed darkening and softening and had a distinctive smell and resembled symptoms seen on potato leaves infected with *Phytophthora infestans*, however tests showed *P. rubi* (and one sample with *P. bishii*). Wet necrotic leaf symptoms are not normally seen from *P. rubi*. It was likely that these leaves had acted as “baits” with

perhaps sporulation having developed on the roots in the condensation in bags and the zoospores having transferred to the leaves.

Confidence in interpretation of data from LFDs and gene sequencing

For the LFDs used in this project (recently developed in AHDB project CP 136) the test line on the LFD becomes fainter the more of the pathogen antigen there is present in the sample (with the faintest test line recorded as very strongly positive for *Phytophthora* spp.). The antibody in the beads in the LFD pad binds with the antigen from the sample, so the more intense the colour the less pathogen. This is the opposite way round to commercially available LFDs that detect *Phytophthora* spp. (such as supplied by Adgen) where a faint line follows when less pathogen (antigen) has been put on the LFD. With the project LFDs, it is important to see the fainter lines of high pathogen levels and this is best done following the purchase of an LFD reader (a colorimeter), as visual assessment can otherwise be tricky.

There were issues with the DNA sequence identification in only a few samples. For example, *P. alni* was found; this was an 80% match alongside *P. tenulata* so it was not a confident match. *P. plurivora* also sometimes came out as *P. pini,* but these *Phytophthora* species are not closely related and so the reason for this unclear. This was probably as some plant material received was old, dry, dirty, soggy or decrepit and thus likely to be full of aromatics that might have been inhibitors to the amplification of the DNA and so interfering with the PCR. Either lots of short DNA sequences were then obtained, or not much DNA to sequence, and this meant that sometimes the sequence match could be considered as dubious.

For some samples, no species match was found from the sequencing. These had clean amplicons so the issue was not with the DNA sequencing, just that they are undescribed so unable to be identified by the BLAST analysis. These samples had positive LFD results, so there is confidence that they contained a *Phytophthora* species, but not one that has been documented. With the increasing use of molecular diagnostics over the last thirty or so years there has been a massive increase in the number of *Phytophthora* species identified, and this is ongoing, when formerly there was reliance on mycologists noting differences in morphology.

In general, amplicon bands on the electrophoresis gel separated well so that they could be cut out for DNA sequencing. There was then good confidence in determining in most cases the species. There is however a known issue in not being able to separate out DNA sequences of *P. idaei* from *P. cactorum*, however they are known as pathogens of raspberry and strawberry, respectively.

In a few cases out of the 180 samples, the DNA did not amplify. There can be a number of reasons that the PCR did not work. As it was nested PCR, nothing might be amplified in the first round of PCR, so there would be no products to be amplified in the second round of PCR. It is also possible that the primers may not have matched the *Phytophthora* species in the sample. Alternatively, there could have been inhibitors that stopped the polymerase. With nothing being amplified, then no bands showed up on the gel electrophoresis and so none could be cut out and sequenced. It is possible that a band could be obtained by doing a dilution, this “waters down” the DNA concentration in the sample being put on the gel but can get rid of any DNA inhibitors.

With some samples, there was a cluster of amplicon bands after running the gel and this requires them to be run again to seek to separate them out. When this was not done, because there was not time with some of the later samples, a “clean cut band” could not be sent for sequencing. When these samples were put thorough the BLAST analysis for species identification it was found that it was not possible to separate sequences. This result was suspected to be as a result of mixed infections by more than one *Phytophthora* species. This resulted in the term “Gobbledegook” being used for these 14 samples in this report (as shown in Appendix 1.2). It is thought probable that if re-tested and additional steps performed then separate amplicons could be provided for sequencing.

Where in a few cases species (such as *P. plurivora* and *P. alni*) that are usually considered to only infest roots were unexpectedly found from cane samples then it is possible that they may have been cross contaminated in the bag from roots in the same parcel, or that they had been on the outside of the canes when they were sampled. Efforts were made to remove the epidermis of canes to sample from inside using a sterilised scalpel, but it is possible some DNA from the outside could have contaminated the sample. Disinfection of the tissue sample is not used prior to PCR, and even if killed the pathogen DNA would still be picked up and replicated.

Correspondence of LFD results with PCR/sequencing results

Sequencing and LFD results mainly corresponded well. Wherever no *Phytophthora* spp. DNA was detected both LFDs had been negative. Wherever some *Phytophthora* spp. DNA was found all the LFDs had shown positive. The LFD using antibody 3C4 gave a very good indication of possible 'serious' *Phytophthora* species presence, especially considering the degraded state of samples by the time they reached the laboratory following transit from the field to the courier and then onwards. The 3C4 LFD specific detection was subsequently

supported by the sequencing results showing Clade 1 members *P. idaei* and *P. cactorum* and Clade 7 member *P. rubi*.

Comprehensive cross-reactivity tests were carried out in the development of the 3H7 and 3C4 LFDs in AHDB Project CP 136, covering a wide range of commonly seen fungi and oomycetes. A conclusion that 3C4 did not detect Clade 2 was based on one Clade 2 member, *P. citrophthora* (neither *P. citricola* nor *P. bishii* were tested) (Pettitt & Wakeham, 2018).

In the current project, 3C4 LFD inconsistently picked up Clade 2 species and this was in contrast to the consistent results (that had been anticipated) with Clade 7, although strong positives were obtained for both. In development of the LFD in AHDB Project CP 136, when using an isolate of *P. citrophthora* the antibody used on the 3C4 LFD did not pick it up. Particularly with *P. citrophthora* the survey LFD readings showed a trend towards being less positive than seen for *P. rubi*. There needs to be further work on the use of the 3C4 LFD, as the sample size for all of the Clade 2 species was small and did not provide enough data to accurately determine its capabilities. If isolates of *P. citrophthora*, *P. bishii* and *P. citricola* could be obtained and tested with the 3C4 LFD it could indicate that it can pick up Clade 2 as well as Clades 1, 7 and 8. Further work would be needed to check for pathogenicity to raspberry. If the 3C4 LFD test does not pick up Clade 2 it would seem to suggest that something else in the survey sample was causing a positive test from within the 1, 7 or 8 Clades. Subsequent to completion of this project work package in 2019, some retesting of plant samples was carried out and this time DNA sequences came out as *P. rubi.* So it is possible that the samples contained a mix of both species giving a positive signal using both antibodies; both within the general LFD (all *Phytophthora* spp. Clades) and within the other more specific LFD for pathogenic *Phytophthora* spp. i.e. those in Clades 1, 7 and 8. To further check on the presence of *P. citrophthora* alone in tissue giving positive 3C4 LFD tests*,* different primers could be used in a re-run of the tests.

The 3H7 and 3C4 LFDs when used together were a good combination. The general 3H7 LFD was expected to pick up Clade 2 species and when these were found in the DNA tests there had been a positive test with the 3H7 LFD. If further work determines the 3C4 LFD detection does not actually extend to Clade 2, then members of this Clade will still be picked up by the 3H7 LFD. The 3H7 LFD was found in its development at the University of Worcester to include detection of Clade 6. Clade 6 includes *Phytophthora gonapodyides* (Cooke *et al*., 2000). This species is not uncommonly found in river water samples. It has been isolated from raspberry plants in excessively wet locations, although Phytophthora root and cane damage was not consistently associated with it (Wilcox and Latorre, 2002). The 3C4 LFD would have shown

negative had *P. gonapodyides* been present in the current survey. No DNA of *P. gonapodyides* was recorded following PCR.

There is confidence in the data from the LFDs and DNA sequencing, other than the specific instances highlighted in earlier in this discussion and noted in the table of results. With nested PCR a small amount of external contaminant can affect the results, however a water control was always run and these were always negative.

Commercial opportunity for the experimental LFDs

The 3H7/3C4 combination is a stage away from being ready for use by growers as a tool to determine presence or absence of *Phytophthora* spp. damaging to raspberry. However, the antibodies are the property of AHDB and produced by MoLogic and so commercial production could be set up. It would be possible to put both antibodies on one test strip and have two test lines plus the control line. An increase in confidence of correctly recording the test line intensity is gained by using an LFD reader (costing around £400). Tests would need to be done at the location of the reader, as all LFD tests should be read within 10 minutes or so of adding the extract as the test line can change intensity since the beads forming the test line can move over several hours.

If 3H7 is positive and 3C4 is negative it is 80-90% certain that the detected sample is either a *Phytophthora* Clade 6 (*P. megasperma* or *P. gonapodyides*) or a *Pythium* sp.. The 3H7 LFD also detects a wider range of *Phytophthora* species also picked up by 3C4. From work in its development it seems less likely to pick up *Pythium* spp. (which can be non-pathogenic) than currently available commercial kits (but direct comparisons would need to be done to confirm this). It is the experience of pathologists that if *Pythium* spp. colonies are picked out of culture plates and tested using current commercial *Phytophthora* spp. LFDs that false positives can result, but this has not occurred with the 3C4 LFD.

The current commercial *Phytophthora* spp. LFD kits and the 3H7 LFD can be used to test baits such as apple placed in irrigation water used for a wide range of horticultural plant species, or raspberry leaf baits in water for plantations, to give a warning of infestation. If the 3C4 LFD is used it will not give positives for Clade 6 *Phytophthora* species (non-pathogenic). Currently available commercial kits detect a much wider range of *Phytophthora* species, many of which are not pathogens of raspberry, or likely to cause minimal damage perhaps as secondary invaders.

If LFDs were just required for testing raspberry plant samples (not with baits for testing water or soil to look for the presence of any *Phytophthora* species) then the 3H7 LFD could be dispensed with and only the 1, 7, 8 Clade specific 3C4 used to detect root rotting species such as *P. cactorum* or *P. idaei* (Clade 1), *P. rubi, P. fragariae or P. cinnamomi* (Clade 7). However, both *P. citricola* and *P. citrophthora* are Clade 2. In the present work 3C4 also gave positives when Clade 2 *Phytophthora* species were diagnosed. Further tests are needed determine whether the 3C4 LFD does gives a positive for Clade 2 *Phytophthora* species, particularly if these are confirmed to be causing raspberry crop losses.

The location in the plant from which the sample is taken can have a bearing on the robustness of the results. In the survey a greater number of positive results were found from the canes. This could have been because there is a more defined area of tissue to select from at various stages of sampling, whereas with a root ball and only a “large pinch” of roots to be taken it is easier to miss infected roots. Infection has been seen to develop in patches in rootballs. Other than in cases of recent or minimal root infestation, the cane/root junction would be a good place to sample from.

The time of year of sampling plants might affect the strength of the LFD reading as there may be more pathogen tissue and consequently DNA to detect when the pathogen is active. Similarly good DNA results are more likely to be obtained from fresh material, rather than tissue that has started to deteriorate. The best results were obtained after freezing samples before juice extraction, and so growers might benefit from doing this if feasible for them.

There is unlikely to be a problem with DNA detection following the use of fungicides such as dimethomorph, metalaxyl or phosphite to roots of the crop. The only potential issue might be if the infected treated plants then produce an inhibitor.

Other diagnostic tests are available for detecting oomycetes in soil and water, and these and promising new technologies have been reviewed alongside LFDs for their speed, ease of access and cost effectiveness (Wakeham and Pettitt, 2017).

Phytophthora species identification other than *P. rubi* or *P. idaei* and the importance of these findings

A small number of identifications from sequencing of symptomatic raspberries came from species other than *P. rubi*, *P. rubi* having been identified in 47.8% of the 161 tissue samples. *P. citrophthora* was present in 9.9% of positive samples and *P. citricola* and *P. bishii* combined

were also detected in 9.9%. The high proportion of tissue with *P. rubi* could have been biased by agronomists sending in samples with symptoms they associate with *P. rubi.* In particular there was no speculative uprooting of plants to search for root rot that was not causing wilting (other than on the pathologists' two visits).

P. citrophthora, *P. bishii* and *P. citricola* were present in canes as well as roots, and were found without *P. rubi* in some plants, but too few samples were obtained to define their damage capability in comparison with the known severity of *P. rubi.* Symptoms might differ when more than one *Phytophthora* species is present in a plant. Wilting of 5% and 7% of plants was recorded (in survey batches 575 and 577) where *P. bishii* and *P. rubi* were present, and 20% were wilting (in batch 570) with *P. citrophthora* in canes and *P. rubi* in the roots. *P. citricola* was found in symptomless roots and from a tiny patch of rot in modules (in batch 568), but was within the shoot and root of a dying plug plant. Only a few whole plants were seen in the survey (as the LFD testing laboratory received sampled plant tissue) and at receipt it was not known which *Phytophthora* spp. they were infected by.

Only a low percentage of samples were determined to have *P. idaei* (although not molecularly distinguishable from *P. cactorum*). In the current survey (batch 575), *P. idaei* was detected in roots of a plant with slight lower leaf veinal chlorosis and browning in the stem base, but *P. citricola* was in the cane. Another plant in the same batch had a necrotic stem base and first basal leaf with *P. cactorum* present in the roots and cane. *P. idaei* was present in 40% of raspberry crops sampled in a survey carried out in Scotland by the James Hutton Institute. *P. idaei* in Scotland has been isolated principally from the outer portions of raspberry roots (D. Cooke, James Hutton Institute, pers. comm.). *Phytophthora* spp. with a peripheral location on roots could have been left in the ground when digging up the few soil-grown stools, or in the taking of roots from rootballs to send for testing.

Further work is required to investigate the "no matches" not currently recorded on the USA database. Nine plants from different sites and varieties were affected. This was 11.8% of the plants sampled, and thus not insignificant. All gave positive 3C4 LFDs and so it could be assumed that these are pathogenic species as for the others detected by this device.

Peronospora sparsa (downy mildew) DNA was identified from three shoots and one root, following unexpected positives on the 3C4 LFDs. These samples had been in the sample bag for some time and this result might not have been obtained had they been fresher. Cooke *et al.* (2000) have grouped *P. sparsa* within the phylogenetic tree of 47 "recently evolved" *Phytophthora* taxa worked on as representative of *Phytophthora* Clades 1 to 8. It is mapped as falling evolutionarily on a branch with *Phytophthora* Clades 1 to 5 that have largely papillate

sporangia and an aerial habit (including *P. infestans* in Clade 1, and *P. citrophthora* in Clade 2) although not itself allocated a Clade. It is a common pathogen of blackberry (and rose), but has been recorded on Arctic raspberry and varieties crossed with blackberry (O'Neill, 2010; Kostamo *et al.,* 2015).

P. infestans (potato and tomato blight) and *P. alni* (alder canker) were each identified once from canes by the DNA sequencing. It is possible that the canes might have acted as baits, picking up other *Phytophthora* species normally found infesting other hosts.

No Clade 6 *Phytophthora* species (such as *P. gonapodyides* or *P. megasperma*) were detected from the DNA testing in the present assessment of symptomatic raspberry material.

The importance of *P. citricola*, *P. citrophthora* and *P. bishii* and the "no match" species needs to be elucidated. It is not known whether or not they are of relatively recent appearance and perhaps increasing in raspberry across areas of England, because this is the first countrywide survey carried out here using molecular techniques. Inoculation experiments to determine pathogenicity and further sampling at host sites is needed to look more closely at their distribution and symptoms. Where *P. rubi* was not also present, the possibility of a pathogen other than a *Phytophthora* species, such as *Ilyonectria* spp. (formerly *Cylindrocarpon* spp.), or something else causing wilting should be checked.

P. citricola and *P. citrophthora* both have a moderate temperature range optimum of 22°C to 27°C, whereas *P. rubi* and *P. idaei* have a lower optimum of less than 22°C (Cooke *et al.,* 2000). *P. bishii* has only so far been found in glasshouse crops (Abad, *et al*., 2008). Study is required on whether the predicted global temperature rise with climate change could impact on the pathogenicity of these species in addition to increasing the susceptibility of plants through heat stress.

There may be differences between the *Phytophthora* species in the tissue they colonise, but too few of the "other" species were found for this to be clear. However, of the totals in roots and canes, whereas cane infection comprised 55% of samples (38 out of 69) for *P. rubi*, and 43% (7 out of 16) for *P. citrophthora*, cane infection comprised only 20% of the samples (2 out of 10) with *P. bishii.* The tissue colonised is likely to impact on disease severity, as cane infection damaging the vascular tissue is likely to result in earlier wilting than from root rotting.

In no tissue sample was more than one species identified and this may indicate that they colonised tissue separately, rather than perhaps one being secondary to the other because

it was less pathogenic. This may however have been an artefact of the nested PCR technique as where amplicon band clusters were unable to be separated (as seen on the electrophoresis gels used during PCR) then separate DNA sequences not able to be identified using the BLAST program (“Gobbledegook” readings). With more than one species present in tissue samples this could indicate co-existence, but any need for invasion by one species before the next would require invasion sites to be pin-pointed for sampling.

It should be highlighted that future focus should be put on whether or not any *Phytophthora* spp. at all are present in crops, especially in propagation material, as it indicates weak links in the phytosanitary chain where infection is occurring. If it is possible for a less pathogenic species to be present in a sample, then it may be as likely to be a route that can be taken by a more pathogenic one.

Phytophthora spp. incidence and crop hygiene

In the UK, the 2019 growing season was very hot and dry, leading to many plants wilting when their roots and vascular system became compromised by *Phytophthora* spp.. There was a high interest in this survey, with many growers and advisors actively submitting samples, suggesting the issue of Phytophthora root rot to be important to many growers across the UK. The reporting of wilt in 1% to 20% of crops showed the extent of crop damage being seen in the summer period. The survey deliberately sampled wilting raspberry plants, so the fact that nearly 90% of samples contained *Phytophthora* species, mostly having produced a positive result to the 3C4 LFD detecting pathogenic *Phytophthora* Clades, was not unexpected. However, the results were reported back to the senders of samples and there was concern that *Phytophthora* spp. infestation was more widespread in their crops than they had previously acknowledged. In discussion with senders on avoiding plant infection it was clear that it is difficult to establish whether *Phytophthora* spp. infection arrived on site in the plants, or whether the *Phytophthora* spp. were already present. Either way, *Phytophthora* spp. infection was found in all ages of plant material, so it will be important to establish strict hygiene on site, and to implement strategies to stop the disease spreading, such as filtering and treating water, particularly if sourced from rivers or stored in open reservoirs.

Many plants submitted were from containers, in which it should be more possible than for soil-grown plants to exclude *Phytophthora* spp.. However, it is known that sites with container crops will have *Phytophthora* spp. present on the farm in the soil and is often a reason they have moved into containers. Even if plantations are sited on slopes to aid the free draining of water, in potted plants water can pool in pot corners and facilitate *Phytophthora* spp. zoospore infestation. Infection starting at the corner of a rootball was shown to one grower and they

proposed moving to pots with increased drainage holes. If infested plants are brought onto a site those in particular grown without the confines of a pot may be able to “grow away” from the infestation by producing a vigorous root system, but the pathogen will remain in the tissue and colonise it further if plant growth is checked in any way.

Previous work (Schlenzig and Chard, 2010) highlighted how *P. rubi* can persist in micropropagation. However, this method is likely to have a lower risk than when root cuttings are taken from outdoors grown mother plants which the survey showed can have traces of *Phytophthora* root rot, but the shoots appear healthy. *Phytophthora* spp. infested plug plants for potting into modules were found in the survey. Several infested samples of modules were received that had been potted in spring and were dying-back in the summer, the speed of death suggesting that the plants’ roots had already been infested before arrival on site. Scattered wilted plants were seen throughout crops, such material could have been taken from infested mother plants, and/or become infested as cuttings in multicell modules and/or been infested once in module pots through infested soil splash.

The motto “start clean, stay clean” applies to all crop production. All possible measures need to be taken to ensure that pathogens (and pests) are excluded from propagation areas. This will involve plants being placed on or against surfaces that are washable and new or have been disinfected, taking care not to use contaminated potting media or irrigation water and preventing transmission by mechanical means (Atwood, 2013; Pettitt, 2016a; Pettitt 2016b). Husbandry should seek to reduce plant stresses that lead to an increased susceptibility/reduced plant resistance to infection. Inspecting and identifying pathogens and pests should be carried out as part of integrated crop management. Killing pathogens once inside crops is difficult, even with systemic fungicides. HSE guidance does not prevent such use of preventative fungicides in propagation, the concern has been where chemical fungicides suppress disease symptom expression and can result in certification of diseased material. Biofungicides, and potentially elicitor products, that boost plant defence mechanisms and/or enhance the beneficial microbes in the rhizosphere may have a place in maintaining crop health, but their use may need to be tailored to particular crop situations and used before a pathogen infestation.

Plant health passports are done per batch; keeping track of individual plants and thus disease sources would be very difficult. The experimental LFDs used in this survey were shown to be good at showing *Phytophthora* spp. presence. However, sampling of multicell modules for *Phytophthora* spp. required all the shoot and root tissue for testing and so any use of LFDs could only be done on a few taken from a batch and at a low infestation rate an infested plant would have a high probability of escaping detection. Testing mother plants would similarly not exclude infested roots being taken for propagation, as the vast majority of roots could be

healthy and infested material not sampled. Freshly infested mother plant roots would not show infection and so would be taken for propagation. Thermography, chlorophyll fluorescence and hyperspectral sensors all have potential for non-destructive detection of diseases in plants (Mahlein *et al*., 2012) and should be investigated for use in raspberry propagation and potentially to allow rogueing or treatment of affected plants in fruiting crops.

Review of hosts, symptoms & distribution of *Phytophthora* spp. detected

The survey results from the current project indicated that the predominant species in the UK affecting raspberry showing wilting is *P. rubi* as it accounted for 47.8% of the 161 root, cane and leaf samples in which *Phytophthora* spp. were found (a further 19 samples were negative). The next greatest proportion of 9.9% was for *P. citrophthora*, followed by 6.8% for *P. bishii,* 3.1% for *P. citricola* and 2.5% for *P. idaei and P. cactorum* combined. It should be noted that without pathogenicity testing it cannot be said that an organism found in afflicted tissue has caused the symptoms. All of these six species have been reported previously causing root rot on raspberry, and information on their incidence and severity has been reviewed in the following section of this report. Whereas *P. rubi*, *P. idaei* and *P. cactorum* are known on raspberry in the UK, the presence of *P. citrophthora* and *P. bishii* does not seem to have been reported previously on any UK crop. *P. bishii* may be confined to Rosacea. *P. citricola,* like *P. citrophthora,* has a wide host range, and is reported present in the UK. Industry intelligence indicates that *P. citricola* has recently been detected in UK raspberry samples sent to plant clinics.

P. rubi is acknowledged as being highly pathogenic to raspberry, causing plant wilt and death and (at least in Scotland), *P. idaei* is more often found infesting roots but not causing plant death (D. Cooke, pers. comm., James Hutton Institute). In this survey plants were sampled principally because they were dying-back. Research is required to determine the pathogenicity to raspberry of species that were identified in the survey other than *P. rubi*.

More information on the hosts, symptoms and geographic spread (where known) of *Phytophthora* species detected in the survey is given in the following pages. Invaluable updates and sources of information on the morphology and genetics and symptoms of individual *Phytophthora* species are available online:

https://idtools.org/id/phytophthora/factsheet_index.php homepage for selecting individual *Phytophthora* species on the USA IDphy database: molecular and morphological identification of *Phytophthora* species (Abad *et al.,* 2019).

https://secure.fera.defra.gov.uk/phiw/riskRegister/index.cfm homepage for individual pest and disease species searches on the Defra UK Plant Health Risk Register.

https://planthealthportal.defra.gov.uk/ homepage for Defra Plant Health Portal individual pests and disease information on hosts and current spread in the UK.

Information on the *Phytophthora* clades and their morphological structures is summarised within a table produced by the United States Department of Agriculture (Abad, 2018) at:

http://idtools.org/id/phytophthora/Phytophthora%20Tabular%20Key%20IDphy%20MASTER%20FINAL%20for%20IDphy%207-25.pdf

Phytophthora rubi

P. rubi used to be known as *P. fragariae* var. *rubi,* however it was recorded as a distinct species in 2007. It is in *Phytophthora* Clade 7a. According to IDPhy its host is only *Rubus idaeus* var. *idaeus* (Rosacea), causing root rot and being one of the most important diseases of red raspberries. *Phytophthora rubi* alone of the species identified in the current survey is on the EPPO (European Plant Protection Organisation) List of pests recommended for regulation as quarantine pests (September 2019 version). It is classified as an A2 pest, i.e. pests that are locally present in the EPPO region, on the annually reviewed quarantine list (https://www.eppo.int/ACTIVITIES/plant_quarantine/A2_list).

The EPPO Global Database https://gd.eppo.int/ (updated 2016) lists *P. rubi* as being present with a restricted distribution in England, Scotland and present in Northern Ireland, with no records from the Isle of Man or Jersey, citing Duncan *et al.* (1987). It is reported from most European countries, however Denmark (referencing Thinggaard, 1990) has reported the disease absent, having only found it once in a few plants in 1990 which were then destroyed. *P. rubi* was stated to be most common in cool wet conditions of North America and Northern Europe of the ten *Phytophthora* species implicated in raspberry root rot (Wilcox *et al*., 1999)

A sampling survey similar to the current one was carried out in Washington State between September 2004 and April 2009 when growers collected samples of raspberry roots from a range of varieties pre-planting and from crops up to eight years old that were showing *Phytophthora* spp. wilting symptoms (MacConnell, 2009). Tissue was tested for the presence of *Phytophthora* spp. antibodies using ELISA testing and if positive then PCR was utilised to only look for a *P. rubi* gene. This resulted in half of the 68 positive LFD samples being confirmed as being *P. rubi*, the other half being other species of *Phytophthora.* 21% of

propagation material was found to be infested, with the majority of samples containing *Phytophthora* spp. that did not include *P. rubi* (**Table 1.40**).

Table 1.40. Results of PCR for 199 samples of raspberry roots collected from planting material and crops in Washington State for Whatcom County IPM Project between 2004 and 2009, showing infection by *P. rubi* and in addition *Phytophthora* species that were not *P. rubi.*

	Positive for *Phytophthora rubi*	Positive for *Phytophthora*	Negative	Total Samples
Pre-Plant	2	13	56	71
In-Field	32	21	75	128
TOTAL	34	34	131	199

Key: *P. rubi* = Positive by ELISA, positive *P. rubi* DNA
Other *Phytophthora* spp. = positive by ELISA, negative *P. rubi* DNA
Negative = negative by ELISA (no PCR carried out)

In response, a survey of Phytophthora root rot was carried out on raspberry plants in Washington, Oregon and California to obtain isolates from cane and root material. In addition young raspberry plants were used as baits in water (Stewart *et al.,* 2014). Isolation from cane was the most successful. DNA sequences were obtained using 210 of the 597 isolates and all but one, which was identified as *P. bishii,* were *P. rubi*. The researchers concluded that *P. rubi* was the main species present in raspberry in their sample area. However, it is possible that other species failed to be isolated (*P. bishii* for example is slow growing and known to be hard to isolate) and as fewer isolates were obtained from roots (perhaps because various fungi and other oomycetes are usually present in root isolations so lowering the chance of a clean sub-culture) this reduced the chance of sequencing species mainly invading root tissue.

A review for AHDB found that *P. rubi* is a poorly understood and understudied pathogen (Gilroy *et al.* 2019). EPPO records it as having a cosmopolitan distribution (**Figure 1.26**).

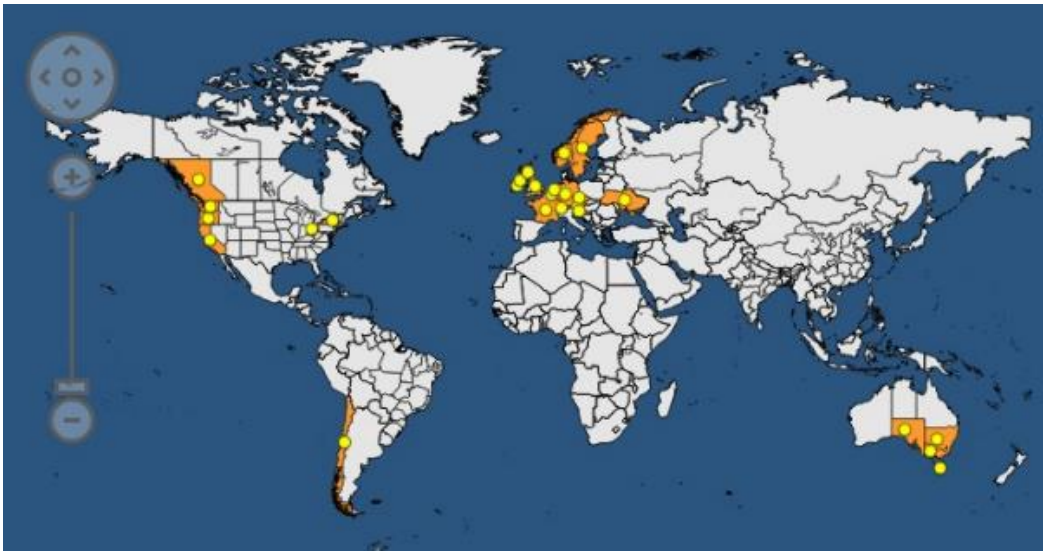

Figure 1.26. Global distribution of *P. rubi* recorded by the European Plant Protection Organisation. The yellow dots show where reports have shown the pathogen to be present, as opposed to transient. EPPO https://gd.eppo.int/taxon/PHYTFU/distribution

The Defra Plant Health Risk Register lists *P. rubi* as widespread in the UK and able to have a significant impact on crops, which is somewhat mitigated by Certification schemes (accessed 1 May 2020) (**Figure 1.27**). However, infested plants not yet symptomatic will pass inspection, as may imported plant material treated with "curative" fungicides.

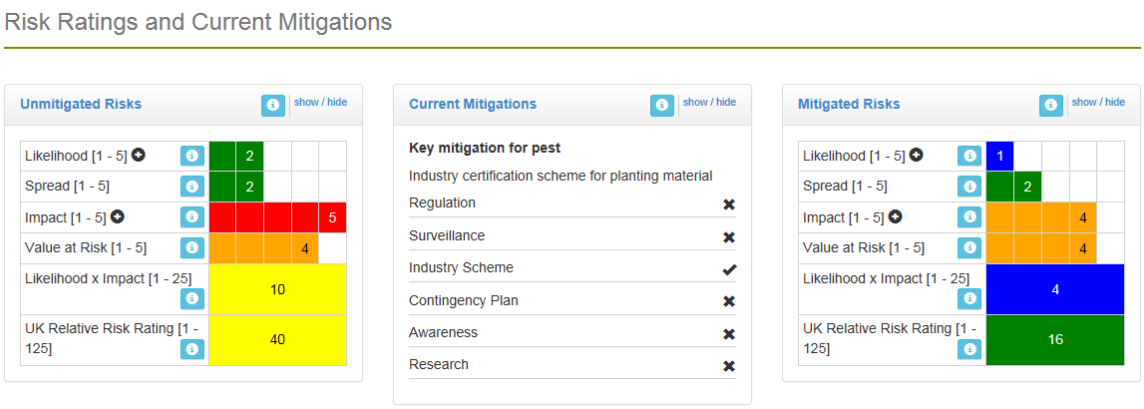

Figure 1.27. Part of the Defra UK Plant Health Risk Register for *P. rubi* showing how the risk of serious impact is considered to be reduced a little by the Certification Scheme for propagation material (viewed May 2020).

With 61% of raspberries in the UK reported to be grown in pots in 2018, rather than the soil, this has reduced the use of fungicides against root rots to 5% of total usage on the crop (11% in 2014), particularly through the use of pots with feet that are stood on woven ground cover away from contaminated soil (Ridley *et al.,* 2018).

The symptoms of infection typically appear on the upper parts of the plant when it is under stress in late spring and summer. Canes in their second year will not break bud, or if they do then fruiting laterals wilt and dry out before or at fruiting. The wood at the base of the canes becomes reddish-brown or brownish-black. Fewer young primocanes grow, and the ones that do appear start to droop (like a shepherd's crook). The foliage bronzes and turns reddish well before the autumn, with black-purple lesions at the base of many of the young canes. The root system becomes rotted with very few white feeder roots, the thicker roots displaying internal discolouration.

P. rubi can be identified based on some key morphological characteristics, it has non-papillate, persistent, ovoid sporangia which originate from simple sympodial or irregular

branched sporangiophores. It has no hyphal swellings or chlamydospores. It is homothallic with smooth walled oogonia which can become golden brown with age (Abad *et al.,* 2019).

Phytophthora citrophthora

P. citrophthora accounted for 8% of all samples tested in the present project, however the Defra plant health portal record records citrus as the major host and gives its distribution status in the UK as absent and there is no record for this species on the Defra plant health risk register. The EPPO pest database does not give a distribution (EPPO, 2020), but the IDphy factsheet by Abad (2019) states a cosmopolitan distribution with hosts in 88 genera and 51 families causing damage to roots, stems and roots. Although it is typically associated with citrus plants. Latorre and Muñoz (1993) were first to report *P. citrophthora* in South America on raspberry, stating that the symptoms included: leaf chlorosis, wilting of primocanes and floricanes, poor growth of floricanes, and poor emergence of primocanes. It was the first record of raspberry root rot in Chile, but a later survey found either *P. rubi* or *P. cryptogea* were causing a high proportion of raspberry root rotting, depending on the region samples, and that *P. citricola* was widespread (Wilcox and Latorre, 2002). It has been found from baiting river water (Nagel *et al.,* 2015).

It is identifiable by its semi-papillate sporangia some which produce two papillae, originating from simple sympodial or irregularly branched sporangiophores. It possesses globose chlamydospores and is absent of hyphal swellings. It is sexual stage it is sterile-heterothallic which rarely produces oospores. (Abad *et al*. 2019)

Phytophthora bishii

P. bishii (originally named *P. bisheria*) was also found in this survey, but no published UK records have been found and the Defra plant health portal (accessed 01/05/2020) states it is unknown whether it is present in the UK. The EPPO Database currently has no records for this species (EPPO, 2020). The Defra Plant Health Risk Register has an entry for *P. bishii* (**Figure 1.28**) recognising that there is a high value crop at risk and the likelihood of infestation being high, but compared with *P. rubi*, with an unmitigated impact high rating of 5, *P. bishii* is only given a rating of 1.

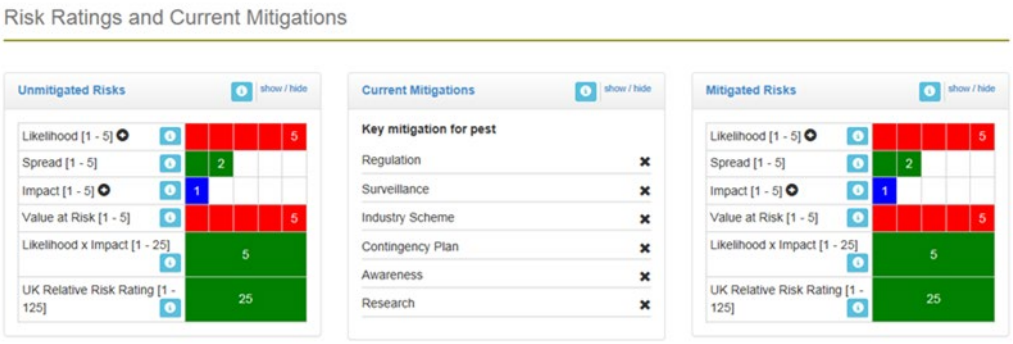

Figure 1.28. Part of the Defra UK Plant Health Risk Register for *P. bishii* showing there being no current mitigation measures, but a relatively low risk rating (viewed May 2020).

P. bishii was isolated in the 1990's in three instances from rotted roots in glasshouse crops; of strawberries (in the USA), rose cuttings (in the Netherlands) and raspberry (in Australia). In Australia, several species of *Phytophthora* had been isolated from a raspberry, *Rubus idaeus*, germplasm collection and, alongside mostly *P. rubi*, a slower growing isolate was initially identified as *P. idaei*. The source plant was wilting and the leaves were becoming chlorotic with necrotic margins. Later, in 2005, DNA sequence data showed the isolate to instead be a new species, then named *P. bishii,* and found to be the same as the other 1990's isolates from strawberry and rose (Abad *et al.* 2008). It is thought likely any other hosts will be within the Rosacea. Inoculation tests with roses in propagation blocks showed new root production ceased, roots rotted and shoots yellowed and then defoliated, older plants developed these symptoms more slowly (Abad *et al.,* 2008). It is a Clade 2 *Phytophthora* species making it closely related to *P. citricola* and *P. citrophthora*. According to Abad *et al.* (2008) it is very difficult to isolate. This makes it likely that it will have been missed in laboratory diagnoses utilising agar plate cultures prior to the wider use of DNA sequencing.

Further work is required to establish the incidence of *P. bishii* in UK raspberry production with records made of the type and severity of disease symptoms. The source/s of the infestations recorded in the current work need to be determined, particularly if it is starting to be introduced into the UK. From the few observations so far (Abad *et al.,* 2008), where strawberries and raspberries are grown in close proximity, or in succession, then transfer of the pathogen between hosts is a possibility.

Phytophthora citricola

P. citricola is recognised as a UK plant pest and considered widespread here according to Defra Plant health portal, but its hosts are not named and it is not named amongst the *Phytophthora* species in the UK plant health risk register. It has a host range across 75 genera

in 38 families (Abad *et al*., 2019). The IMI key of Waterhouse & Waterston (1966) is referenced by Abad *et al*. (2019) which gives *P. citricola* as causing root rot and dieback in raspberry, as well as it being associated with root and crown rots in a wide range of host genera. Waterhouse (1963) first suggested that it is its own species and it was later proved to be different based on its mitochondrial DNA. Kennedy and Duncan (1995) recognised *P. citricola* and *P. syringae* as attacking raspberry. However, According to Abad *et al.* (2019) most isolates identified as *P. citricola* prior to 2009 are most likely to correspond to *P. plurivora* or *P. pini* and other species in the "*P. citricola* complex".

The asexual phase is identified by the semi-papillate and persistent sporangia which originates in single or sympodially branched sporangiophores with hyphal swellings and chlamydospores absent. In the sexual phase it can be identified by paragynous antheridia which are attached near the oogonial stalks with oogonia which have a tapered base (Abad *et al.,* 2019).

In Chile, in a countrywide sampling of raspberry plants with diseased roots or plants that were dead or declining, *P. citricola* was isolated all over the country, but *P. rubi* was the dominant species in the south. However it was not found in the north where *P. cryptogea* was instead dominant. *P. cryptogea* did not occur in south Chile (Wilcox and Latorre, 2002). Work carried out by Laun and Zinkernagel (1993) identified that soil temperature has an impact on the pathogenicity of *P. rubi* and *P. citricola*. *P. rubi* is severe when soil temperatures are between 12 and 20°C whereas *P. citricola* is severe at soil temperatures over 20°C. The average soil temperature at 10 cm depth in southern Britain rises to around 12°C (in July), but temperatures in crops under protection will rise above this in summer and black pots are particularly susceptible to heating in sunshine. The increased use of tunnels and pots in the last 15 years could thus favour *P. citricola* and climate change bringing hotter weather could also add to the problem and so a further understanding of this pathogen could be essential.

Phytophthora idaei

P. idaei was only found in the current survey in the roots of a substrate grown plant from one site in southern England, although it is likely that the root and cane samples identified as containing *P. cactorum* from a second plant from that site are also *P. idaei*. The plant with *P. idaei* in the roots had *P. citricola* in the young shoots and so the symptoms of browning in the stem base and in the pith, with green leaves showing slight veinal chlorosis, may have been caused by the latter pathogen. The plant with *P. cactorum* only in the stem and roots had a necrotic stem base and first basal leaf.

P. idaei was first reported in 1987 in Scotland by the Scottish Crop Research Institute on *Rubus idaeus*, with isolations from roots from crops in England and Scotland. *P. idaei* when

first described was compared with *P. cactorum* and found in contrast to be limited to raspberry and to have more severe symptoms (Kennedy and Duncan, 1995). *P. idaei* was present in over 40% of the Scottish soft fruit plantations surveyed in 2000-2003. It was subsequently shown to be causing root damage without above ground symptoms, other than reduced cane height, in a trial of potted plants outdoors in Scotland. When potted plants in a glasshouse were inoculated with either *P. rubi* or *P. idaei*, while *P. rubi* caused severe root rot and subsequent plant death, plants with *P. idaei* had significant proportions of diseased roots, but no apparent disease symptoms on the stems or foliage (Young *et al.,* no date).

The U.S. Department of Agriculture factsheet for *P. idaei* gives its distribution as only in the UK and exclusively a root rot of raspberries and notes that this species was listed as a species of concern during the 2009 *Phytophthora* prioritization project conducted by USDA (Abad *et al*., 2019). Minnis *et al.* (2020) found that roots became rotted by *P. idaei*, but there were no aerial symptoms expressed in the plants when 20% of the roots were infected. Little else has been published about *P. idaei*, but it is not known whether this is because it has not been credited with causing as much damage as *P. rubi*, and/or whether it has not been found to be widespread in raspberries.

In Scotland it has been reported to be found on roots at the extremity of the root ball which can easily be left in the ground when sampling soil-grown raspberry crops (pers. comm. David Cooke, pathologist at James Hutton Research Institute). The EPPO database no more than names *P. idaei* (EPPO, 2020).

P. idaei resembles *P. cactorum* as their sporangia are nearly spherical and papillate, additionally the antheridia of both are paragynous. However, the sporangia of *P. idaei* are persistent whereas *P. cactorum* are caduceus, also the hyphae of *P. idaei* are wider than that of *P. cactorum* (Abad *et al.,* 2019).

Phytophthora cactorum

In the current survey *P. cactorum* was only identified from the root and shoot tissue of a young plant with a necrotic stem base. *P. cactorum* infects at least 154 genera of plants causing collar rot of trees such as oak, birch, maple and apple. Additionally it causes fruit rot of apple, pear, apricot, strawberry, cucurbits, and eggplant (Waterhouse and Waterston, 1966). It is globally distributed. In the UK, crown and collar rot in apples and crown rot in strawberries are important diseases. *P. cactorum* was isolated from the roots of UK red raspberry plants affected by severe root and crown rot, with associated cane death, along with *Phytophthora* species *P. syringae, P. drechsleri and P. cambivora and P. megasperma* (*P. megasperma* occurring most frequently). Inoculation tests with *P. cactorum* produced small to moderate amounts of root rot on red raspberry (Duncan, *et al.,* 1987). Pathogenic and non-pathogenic

P. megasperma were isolated, and the former was renamed *P. fragariae* var. *rubi* and is now known as *P. rubi* (Wilcox *et al*., 1993).

It can be identified by its papillate sporangia with short pedicels that are borne in simple, close or lax sympodial sporangiophores. Its chlamydospores are terminal and globose, and it is absent of hyphal swelling.

Phytophthora plurivora

Other than once, in a cane from a containerised plant in the current survey, no reports have been found of *P. plurivora* infecting raspberry plants. *P. plurivora* infects woody hosts and is probably responsible, alongside oak decline, for the decline of *Fagus sylvatica* in European forests. It is typically isolated from blighted ornamentals, with rhododendrons being particularly susceptible. It is believed to originate in Europe, probably spreading in plant exports to the USA (Schoebel *et al.,* 2014). It is found also in China, Turkey and South Africa in numerous ecosystems and has been isolated from streams, ponds and forest soils (Jung *et al.,* 2017). There have been two recent first reports of *P. plurivora,* one causing almond seedlings to develop sunken and blackened cankers on above ground parts and root rot (Çiftçi *et al.,* 2016) and another causing canker on wild apple (Liu *et al*., 2018).

Cross-contamination on-farm with infested tissue from nearby woody hosts is possible, however as tissue was cut from under the cane epidermis for testing this should not have occurred. More cane samples producing this DNA sequence are required to aid confirmation of *P. plurivora* presence in raspberry.

P. plurivora was first described by Sawada in 1927, but identified as *P. citricola* based on its morphological and physiological characteristics. Later work carried out using phylogenetic analyses recognised isolates showed a high diversity, (Jung & Burgess, 2009). It is now part of a complex having similar morphological features that includes *P. citricola, P. multivora, P. pini, P. acerina* and *P. pachypleura*. Most isolates identified as *P. citricola* prior to 2009 are most likely *P. plurivora* (Burgess and Abad, 2016).

P. plurivora is in *Phytophthora* Clade 2c. In the asexual phase the sporangia are semi-papillate, persistent and ovoid with two or three papillae being common. The sporangiophores are typically simple sympodia however can be lax sympodia as a result of external proliferation, it is also absent of hyphal swellings and chlamydospores. The sexual phase can be identified by oogonia that are globose with smooth walls of average size, oospores are nearly pleuritic which are globose to sub-globose (Abad *et al*., 2019).

Phytophthora alni

The single cane sample in which *P. alni* was detected in the current survey gave very strong positives for both LFDs showing the presence of high level of antigen (a result most usually given by *P. rubi* in this survey). *P. alni* sub sp*. uniformis* is listed in *Phytophthora* Clade 7a, the same as *P. rubi (*Abad, 2018*).* It is possible there has been misinterpretation of the sequence. No other reports have been found of it infecting the *Rubus* genus.

This species was first discovered in Britain in 1993 on alder trees. It has three subspecies one of which is common *P. alni alni,* the other two, *P. alni uniformis* and *P. alni multiformis,* are less common and the result of recent hybridizations with other species (Brasier, *et al.,* 2004). According to the Defra Plant Health Risk Register it has only been known to infect the *Alnus* genus.

P. alni is distributed around the EU in countries, such as Austria, Belgium, Czech Republic, France, Germany, Hungary, Ireland, Italy, Lithuania, The Netherlands, Poland, Slovakia, Slovenia and Sweden. In the UK it is mostly focused in the southeast of England, however there are heavy losses in other parts of the UK (UK Forestry Commission, 2012). It has been reported in localised areas of the US.

The asexual phase sporangia are non-papillate; persistent; globose, ovoid, pyriform which show nested and extended internal proliferation. Borne from long unbranched or simple sympodial sporangiophores which are absent of both hyphal swellings and chlamydospores. In the sexual phase they are homothallic with globose oogonia and ornamented walls with tapered bases, the antheridia which is predominantly amphigynous of which most are single celled but with a few two-celled.

P. plurivora and *P. alni,* species are more usually associated with roots*,* but were both only found in the canes of each of the raspberry plants sampled in the present survey. As these species are more usually associated with trees, this could be through cross-contamination with inoculum that came in contact with the sampled stem. Alder is seen as a windbreak on English fruit farms and cross-contamination with *P. alni* from there is possible, however only internal cane tissue was taken for the LFD and PCR tests in the laboratory.

Conclusions

- Samples of 76 raspberry plants were obtained in 2019 from crops across England to confirm *Phytophthora* spp. associated with typical symptoms attributed to Phytophthora root rot. *Phytophthora* spp. were detected in the majority of samples.
- Samples included plug plants, modules and plants from fruiting crops and included material from 20 raspberry varieties, resulting in 180 samples of canes, roots and some leaves.

- There was a greater number of root samples that contained no *Phytophthora* spp. DNA than cane samples.
- A two-stage process was used: samples were tested initially by experimental LFDs for *Phytophthora* spp., and subsequently re-sampled for molecular testing using nested PCR followed by sequence matching to a USA *Phytophthora* spp. database.
- Experimental LFDs 3H7 and 3C4 were shown by subsequent DNA analysis of the samples to have successfully detected *Phytophthora* spp. in raspberry tissue.
- The 3C4 LFD was expected to detect *Phytophthora* species within Clades 1, 7 and 8. Tissue which give positive readings was later confirmed by DNA sequencing to have successfully detected *P. rubi* and *P. idaei* from within these Clades.
- The 3C4 LFD also at times was positive for samples later shown to contain DNA of *Phytophthora* species within Clade 2; *P. citrophthora*, P. bishii, *P. citricola* and *P. plurivora.* It was unclear if this was a true result or whether *P. rubi* had been present in the tissue sub-sample tested with the LFD, but was missed in re-samping for PCR and further work is required to clarify the issue.
- A few instances of *Peronospora sparsa* (downy mildew) DNA were detected instead of any *Phytophthora* spp., and it was unclear whether the positive LFDs for these samples had reacted to the *P. sparsa* or whether there had also been *Phytophthora* spp. present in the material tested by them.
- *P. rubi* was found in 42.8% of samples received. It was isolated from 42.1% of canes and 34.8% of roots received for testing.
- 10.8% of all samples received had no *Phytophthora* spp. DNA detected.
- Other *Phytophthora* species were identified from 23.8% of all the samples; principally 8.9% *P. citrophthora*, 6.1% *P. bishii* and 2.8% *P. citricola*. Only 0.5% had *P. idaei.* In around half the plants they were found in there was no *P. rubi*, suggesting they may have been primary pathogens.
- The extent to which these species contributed to symptom development on raspberry in the UK would require further study, however, *P. bishii*, *P. citrophthora* and *P. citricola* are known as raspberry pathogens in other countries. The first two have no published records on raspberry in the UK.
- A further 16.1% of samples the *Phytophthora* spp. infestation was unable to be defined from the DNA, but probably included *P. rubi* mixed with another species.
- Some DNA sequences, from nine plants, could not be matched to any *Phytophthora* species currently on the extensive genetic database utilised. Further work would be required to enable them to be recognised as new species.

Knowledge and Technology Transfer

Preliminary findings of this work were presented at:

IUFRO (International Union of Forest Research Organisations) Phytophthora Congress, Sardinia, Italy, October 2019. (The presented poster is reproduced in Appendix 1.3).

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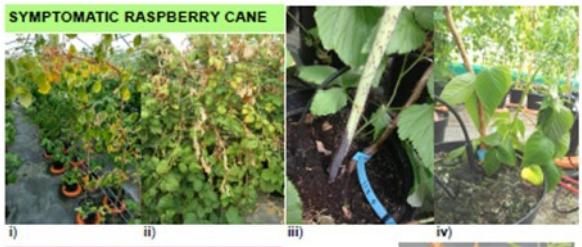
Appendices 1.1 – 1.3. Phytophthora root rot.

Typical *Phytophthora spp.* symptoms in raspberry include:

- Floricane wilting, leaves turning yellow then brown, but remaining attached (i and ii)
- Purple lesions starting at ground level, spreading upwards (iii)
- Young green primocane wilting from the top down, like a Shepherd's crook (iv)
- Small, underdeveloped fruit, or advanced ripening of fruit on wilting cane.
- Red root patches amongst healthy tan roots. Roots still living, and in tact, but stained.

SYMPTOMATIC RASPBERRY CANE



i) ii) iii) iv)

SYMPTOMATIC RASPBERRY ROOT

DO NOT SEND:

- *Whole* plants ~ only symptomatic plant material.
- Completely rotted, disintegrating, soggy roots (v)
- Dry, completely dead, and/or black canes (vi)

v) sodden, unusable root ball (left) next to healthy tan root ball (right).

vi) examples of dry, crispy, dead, blackened primocanes that are unsuitable for testing.

Typical *Phytophthora spp.* symptoms in raspberry include:

- Floricane wilting, leaves turning yellow then brown, but remaining attached (i and ii)
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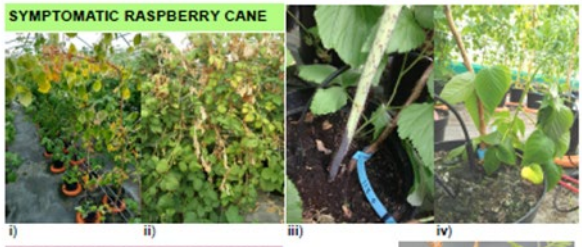
SYMPTOMATIC RASPBERRY CANE



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vi) examples of dry, crispy, dead, blackened primocanes that are unsuitable for testing.

Appendix 1.1

Phytophthora root rot survey form and plant sampling instructions sent to UK growers in July 2019.

Appendix 1.2.

Results of 3H7 and 3C4 LFD tests for *Phytophthora* spp. and DNA later sequenced to identify individual *Phytophthora* sp. from 180 samples of tissue (76 symptomatic raspberry plants) collected from UK crops between March and September 2019. Cultivars have been coded to maintain confidentially. Irrigation water was tested by LFDs from two plant sample sites.

Key: 0 = no *Phytophthora* spp. detected by the LFD (+) weak, + moderate, ++ strong, & +++ very strong indicates increasing *Phytophthora* spp. antigen causing increasingly faint test line.

Tissue type	Cultivar code	Worcester lab. ID code	LFD test date	LFD 3H7 'general'	LFD 3C4 clades 1/7/8	DNA Sequencing results after PCR	Plant
Root	C	C545/1	27/03/2019	0	0	Negative	1
Cane	C	C545/2	27/03/2019	+	0	*P. citrophthora*	
Root	P	C551/1a	06/06/2019	(+)	0	*P. citrophthora*	2
Root	P	C551/1b	06/06/2019	+	(+)	*P. citrophthora*	
Root	P	C551/2	06/06/2019	++	++	*P. rubi*	
Root	P	C551/3	06/06/2019	+	++	*P. rubi*	
Cane	P	C551/4	06/06/2019	+	+	*P. citrophthora*	
Root	P	C551/5	06/06/2019	+	+	*P. rubi*	
Root	P	C551/6	06/06/2019	+	++	*P. rubi*	
Root	P	C551/7	06/06/2019	0	0	Negative	
Root	P	C551/8	06/06/2019	0	0	Negative	
Root	P	C551/9	06/06/2019	0	0	Negative	
Root	P	C551/10	06/06/2019	+	+	*P. citrophthora*	
Root	P	C551/11	06/06/2019	0	0	Negative	
Cane	C	C553/A	17/06/2019	++	++	*P. rubi*	3
Cane	C	C553/B	17/06/2019	++	++	*P. rubi*	
Root	C	C553/C	17/06/2019	+	++	*P. rubi*	
Cane	C	C553/D	17/06/2019	+	+	*P. bishii*	
Root	C	C553/E	17/06/2019	+	+	*P. rubi*	
Roots	G	C554/A	24/06/2019	++	++	*P. rubi*	4
Cane	G	C554/B	24/06/2019	++	++	*P. rubi*	
Root	G	C554/C	24/06/2019	+	+	*P. citrophthora*	5
Cane	G	C554/D	24/06/2019	+	++	*P. rubi*	
Root	G	C554/E	24/06/2019	+	+	*P. rubi*	6
Cane	G	C554/F	24/06/2019	+	+	*P. rubi*	
Cane	C	C558/1	24/07/2019	+++	+++	*P. rubi*	7
Root	C	C558/2	24/07/2019	0	0	Negative	
Leaves	C	C558/3	24/07/2019	+++	+++	*P. rubi*	
Cane	C	C558/4	24/07/2019	+++	+++	*P. rubi*	8
Root	C	C558/5	24/07/2019	+	++	*P. rubi*	
Root	G	C559/8	24/07/2019	0	0	Negative	9
Root	G	C559/9	24/07/2019	0	0	Negative	10
Stem	G	C559/10	24/07/2019	+	+	*P. citrophthora*	
Root	B	C559/11	24/07/2019	++	+++	*P. rubi*	11
Cane	B	C559/12	24/07/2019	+++	+++	*P. rubi*	
Root	B	C559/13	24/07/2019	+	++	*P. citrophthora*	12
Root	coded 1	C564/AZ1	29/07/2019	++	+++	*P. rubi*	13
Cane	coded 1	C564/AZ2	29/07/2019	+++	+++	*P. rubi*	
Cane	coded 3	C564/AZ6	29/07/2019	+++	+++	*P. rubi*	
Root	coded 2	C564/AZ3	29/07/2019	=	(+)	*P. rubi*	14
Cane	coded 2	C564/AZ4	29/07/2019	+++	+++	*P. rubi*	
Cane	coded 2	C564/AZ5	29/07/2019	+++	+++	*P. rubi*	

Tissue type	Cultivar code	Worcester lab. ID code	LFD test date	LFD 3H7 'general'	LFD 3C4 clades 1/7/8	DNA Sequencing results after PCR	Plant
Root	coded 4	C564/AZ7	29/07/2019	+	0	*P. citrophthora*	15
Cane	coded 4	C564/AZ8	29/07/2019	++	+	*P. rubi*	
Cane	coded 4	C564/AZ9	29/07/2019	+	(+)	*P. citrophthora*	
Root	coded 4	C564/AZ10	29/07/2019	+	+	*P. bishii*	
Cane	coded 4	C564/AZ11	29/07/2019	++	++	*P. rubi*	
Cane	coded 4	C564/AZ12	29/07/2019	++	++	*P. citrophthora / P. botryosa*	
Root	coded 4	C564/AZ13	29/07/2019	(+)	+	*P. bishii*	
Cane	coded 4	C564/AZ14	29/07/2019	++	+++	*P. bishii*	
Cane	coded 4	C564/Z15	29/07/2019	+	+	Gobbledegook	
Root	coded 5	C564/AZ16	29/07/2019	+	(+)	Gobbledegook	16
Cane	coded 5	C564/AZ17	29/07/2019	++	++	Gobbledegook	
Cane	coded 5	C564/AZ18	29/07/2019	++	++	Gobbledegook	
Root	coded 6	C564/AZ19		+++	+++	*P. rubi*	17
Cane	coded 6	C564/AZ20		++	+++	*P. rubi*	18
LFD	-	C564/LFD		-	-	Negative	n/a
Root	F	C565/AZ21	12/08/2019	+	+	*P. bishii*	19
Cane	F	C565/AZ22	12/08/2019	++	++	Didn't amplify	
Root	R	C565/AZ23	12/08/2019	+	0	Didn't amplify	20
Cane	R	C565/AZ24	12/08/2019	+	+	Didn't amplify	
Root	E	C565/AZ25	12/08/2019	0	0	Negative	21
Cane	E	C565/AZ26	12/08/2019	++	++	*P. infestans / P. adina*	
Root	E	C565/AZ27	12/08/2019	+	+	*P. cactorum*	22
Cane	E	C565/AZ28	12/08/2019	+	+	Gobbledegook	
Water	-	C565/AZ29	12/08/2019	0	0	Negative	n/a
Water	-	C565/AZ30	12/08/2019	0	0	Negative	
Root	B	C566/AZ31	12/08/2019	+	+	Gobbledegook	23
Cane	B	C566/AZ32	12/08/2019	++	++	*P. rubi*	
Root	D	C566/AZ33	12/08/2019	+++	+++	*P. rubi*	24
Cane	D	C566/AZ34	12/08/2019	+++	++	Didn't amplify	
Root	D	C566/AZ35	12/08/2019	+++	++	*P. rubi*	25
Cane	D	C566/AZ36	12/08/2019	++	++	*P. rubi*	
Root	B	C567/AZ37	12/08/2019	++	++	*P. rubi*	26
Cane	B	C567/AZ38	12/08/2019	+++	+++	*P. rubi*	
Root	D	C567/AZ39	12/08/2019	+	+	*P. citrophthora*	27
Cane	D	C567/AZ40	12/08/2019	++	+++	Didn't amplify	
Root	B	C567/AZ41	12/08/2019	++	++	Gobbledegook	28
Cane	B	C567/AZ42	12/08/2019	++	+++	*P. rubi*	
Root	A	C568/AZ43	21/08/2019	0	0	-	29
Cane	A	C568/AZ44	21/08/2019	+	+	Gobbledegook	
Root	A	C568/AZ45	21/08/2019	0	0	-	30
Cane	A	C568/AZ46	21/08/2019	+	+	*Peronospora sparsa*	
Root	A	C568/AZ47	21/08/2019	0	0	Negative	31
Cane	A	C568/AZ48	21/08/2019	+	+	*Peronospora sparsa*	
Root	A	C568/AZ49	21/08/2019	+	+	*P. citricola*	32
Cane	A	C568/AZ50	21/08/2019	++	+	*P. citricola*	
Root	M	C568/AZ51	21/08/2019	+	+	Didn't amplify	33
Root	L	C568/AZ52	21/08/2019	+	+	Didn't amplify	34

Tissue type	Cultivar code	Worcester lab. ID code	LFD test date	LFD 3H7 'general'	LFD 3C4 clades 1/7/8	DNA Sequencing results after PCR	Plant
Root	L	C568/AZ53	21/08/2019	(+)	+	Didn't amplify	35
Root	S	C568/AZ54	21/08/2019	+	+	*P. citricola*	36
Root	D	C568/AZ55	21/08/2019	+	+	Didn't amplify	37
Root	D	C568/AZ56	21/08/2019	+	0	*P. citricola*	38
Root	A	C569/AZ57	21/08/2019	+	+	Didn't amplify	39
Cane	A	C569/AZ58	21/08/2019	++	+++	*P. rubi*	
Leaves	A	C569/AZ59	21/08/2019	+	++	Gobbledegook	
Water	-	C565/AZ29	12/08/2019	0	0	Negative	n/a
Water	-	C565/AZ30	12/08/2019	0	0	Negative	
Root	Q	C569/AZ60	21/08/2019	+	+	*P. rubi*	40
Cane	Q	C569/AZ61	21/08/2019	(+)	(+)	Didn't amplify	
Leaves	Q	C569/AZ62	21/08/2019	+	+	*P. rubi*	
Root	A	C569/AZ63	21/08/2019	0	0	Negative	41
Cane	A	C569/AZ64	21/08/2019	++	++	Gobbledegook	
Leaves	A	C569/AZ65	21/08/2019	+++	+++	*P. rubi*	
Root	B	C570/AZ66	21/08/2019	+	+	*Peronospora sparsa*	42
Cane	B	C570/AZ67	21/08/2019	+	+	*P. citrophthora*	
Leaves	B	C570/AZ68	21/08/2019	+	++	*P. rubi*	
Root	B	C570/AZ69	21/08/2019	+	+	*P. rubi*	43
Cane	B	C570/AZ70	21/08/2019	++	+	*P. citrophthora*	
Root	A	C571/AZ71	21/08/2019	+	+	*P. rubi*	44
Cane	A	C571/AZ72	21/08/2019	+	(+)	*P. rubi*	
Root	F	C574/AZ73	02/09/2019	(+)	0	*P. citrophthora*	45
Cane	F	C574/AZ74	02/09/2019	++	+++	Didn't amplify	
Root	B	C574/AZ75	02/09/2019	++	+++	*P. rubi*	46
Cane	B	C574/AZ76	02/09/2019	++	++	*Peronospora sparsa*	
Root	B	C574/AZ77	02/09/2019	+	++	*P. bishii*	47
Cane	B	C574/AZ78	02/09/2019	+++	+++	*P. rubi*	
Root	B	C574/AZ79	02/09/2019	(+)	(+)	*P. citrophthora*	48
Cane	B	C574/AZ80	02/09/2019	++	++	*P. rubi*	
Root	coded 7	C575/AZ81	02/09/2019	(+)	++	*P. idaei / P. cactorum*	49
Cane	coded 7	C575/AZ82	02/09/2019	+	+	*P. mengei / P. citricola*	
Root	N	C575/AZ83	02/09/2019	+	++	*P. cactorum*	50
Cane	N	C575/AZ84	02/09/2019	++	+++	*P. cactorum*	
Cane	N	C575/AZ85	02/09/2019	+	++	*P. rubi*	
Root	A	C576/AZ87	04/09/2019	+	++	Didn't amplify	51
Cane	A	C576/AZ88	04/09/2019	+	+	Didn't amplify	
Root	T	C577/AZ89	04/09/2019	+	+	*P. bishii*	52
Cane	T	C577/AZ90	04/09/2019	+	+	*P. rubi*	
Root	J	C577/AZ91	04/09/2019	0	0	-	53
Cane	J	C577/AZ92	04/09/2019	+++	+++	*P. rubi*	
Root	K	C577/AZ93	04/09/2019	(+)	+	*P. rubi*	54
Cane	K	C577/AZ94	04/09/2019	++	+++	*P. rubi*	
Root	J	C578/AZ95	04/09/2019	(+)	0	*P. bishii*	55
Cane	J	C578/AZ96	04/09/2019	(+)	++	Didn't amplify	
Root	U	C578/AZ97	04/09/2019	++	++	*P. rubi*	56
Cane	U	C578/AZ98	04/09/2019	++	++	*P. rubi*	
Root	coded 8	C578/AZ99	04/09/2019	++	++	*P. rubi*	57
Cane	coded 8	C578/AZ100	04/09/2019	+	+	no matches	

Tissue type	Cultivar code	Worcester lab. ID code	LFD test date	LFD 3H7 'general'	LFD 3C4 clades 1/7/8	DNA Sequencing results after PCR	Plant
Root	H	C579/AY1	04/09/2019	+	++	*P. bishii*	58
Cane	H	C579/AY2	04/09/2019	+++	+++	*P. rubi*	
Root	H	C579/AY3	04/09/2019	++	++	*P. rubi*	59
Cane	H	C579/AY4	04/09/2019	+++	+++	*P. rubi*	
Root	H	C581/AY5	11/09/2019	+	+	*P. rubi*	60
Leaves	H	C581/AY6	11/09/2019	++	+	*P. bishii*	
Cane	H	C581/AY7	11/09/2019	++	++	Gobbledegook	
Leaves	H	C581/AY8	11/09/2019	++	++	no matches	
Root	F	C583/AY12	18/09/2019	0	0	Negative	61
Cane	F	C583/AY13	18/09/2019	++	+++	*P. plurivora*	
Root	E	C583/AY14	18/09/2019	+	+	no matches	62
Cane	E	C583/AY15	18/09/2019	++	+++	*P. citricola*	
Leaves	E	C583/AY16	18/09/2019	+	++	Gobbledegook	
Root	E	C583/AY17	18/09/2019	++	+++	*P. rubi*	63
Cane	E	C583/AY18	18/09/2019	+++	+++	*P. plurivora*	
Root	F	C584/AY23	23/09/2019	+	++	*P. bishii*	64
Cane	F	C584/AY24	23/09/2019	+	(+)	Gobbledegook	
Leaves	F	C584/AY25	23/09/2019	+++	+++	*P. rubi*	
Root	E	C584/AY26	23/09/2019	+	+	no matches	65
Cane	E	C584/AY27	23/09/2019	+	++	*P. rubi*	
Leaves	E	C584/AY28	23/09/2019	+++	+++	*P. rubi*	
Root	A	C585/AY29	23/09/2019	+	++	*P. rubi*	66
Cane	A	C585/AY30	23/09/2019	+	++	*P. rubi*	
Cane	A	C585/AY31	23/09/2019	++	++	*P. rubi*	
Root	A	C585/AY32	23/09/2019	+	++	*P. rubi*	67
Cane	A	C585/AY33	23/09/2019	+++	+++	*P. rubi*	
Root	A	C585/AY34	23/09/2019	++	+++	*P. rubi*	68
Cane	A	C585/AY35	23/09/2019	(+)	++	no matches	
Cane	A	C585/AY36	23/09/2019	+	++	*P. rubi*	
Root	A	C585/AY37	23/09/2019	(+)	+	Gobbledegook	69
Cane	A	C585/AY38	23/09/2019	++	+++	*P. rubi*	
Water	n.a.	C586/AY39	24/09/2019	0	0	Negative	n/a
Root	coded 9	C591/AY40	12/09/2019	(+)	+	no matches	70
Cane	coded 9	C591/AY41	27/09/2019	+++	+++	*P. rubi*	
Root	coded 10	C591/AY42	27/09/2019	(+)	+	no matches	71
Cane	coded 10	C591/AY43	27/09/2019	+++	+++	*P. alni*	
Root	V	C591/AY44	27/09/2019	0	0	Negative	72
Cane	V	C591/AY45	27/09/2019	+++	+++	*P. rubi*	
Leaves	V	C591/AY46	27/09/2019	+++	+++	*P. rubi*	
Root	K	C591/AY47	27/09/2019	(+)	+	no matches	73
Cane	K	C591/AY48	27/09/2019	0	0	Negative	
Root	G	C591/AY49	27/09/2019	+	(+)	no matches	
Cane	G	C591/AY50	27/09/2019	+	++	no matches	74
Leaves	G	C591/AY51	27/09/2019	+++	+++	*P. rubi*	
Root	H	C591/AY52	27/09/2019	+	(+)	*P. rubi*	75
Cane	H	C591/AY53	27/09/2019	0	0	Negative	
Root	coded 11	C591/AY54	31/07/2019	0	0	Negative	76
Root	coded 11	C591/AY55	31/07/2019	0	0	Negative	
Root	coded 11	C591/AY56	31/07/2019	(+)	+	*P. rubi*	

Appendix 1.3. Poster presented at IUFRO Phytophthora Congress, Italy, 22 Oct. 2019.

Investigating Phytophthora root rot in raspberry

Ruth D'urban-Jackson[1], Erika Wedgwood[1], Gary Keane[2] and Tim Pettitt[2]

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Background

Raspberry (*Rubus idaeus*) root rot leading to cane wilting and death, is an economically important disease in the UK.

It has been thought to be caused primarily by *Phytophthora rubi*, but with new molecular diagnostic methods, other species of *Phytophthora* are increasingly being detected.

This study set out to establish species of *Phytophthora* present in dying plants, and to further develop lateral flow assays with antibodies 3C4UW387 (3C4) and 3H7UW375 (3H7) for their use in pathogen detection.

Methods

During the 2019 growing season, wilting raspberry plants from across the UK were sampled, testing root, cane and leaf material. Plant extracts were used in prototype competitive immunoassay format LFD tests (**phase 1**) before being frozen for sequencing (**phase 2**).

Phase 1: Test samples via 2 LFD tests.
General *Phytophthora* spp. = 3H7
Pathogenic species= 3C4 (clades 1, 7 & 8)

Plant tissue tested using General LFD.
If negative → no further testing.
If positive → test with Pathogenic LFD.

Phase 2: Using nested PCR, sequencing and BLAST to determine the species present in cane and root tissue.
(Work currently underway).

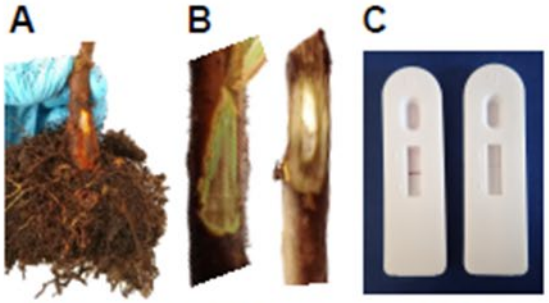
Results

Most of the 166 samples were taken from symptomatic, with a small subset of 12 from asymptomatic raspberry plants. Material was from 73 plants, encompassing 23 different cultivars, from both soil-grown and containerised plantations.

Sites	Plants	Samples			*Phytophthora* species
		Root	Cane	Leaf	
46	73	87	71	8	To be confirmed

Prototype competitive format LFD tests using antibodies 3C4 and 3H7 found:

- Most samples (91%) contained *Phytophthora* spp. and the majority of these (95%) also tested positive for clades 1/7/8.
- Strong presence of *Phytophthora* in above-ground cane material, as well as in root and crown tissue.
- *Phytophthora* spp. detected in some asymptomatic plants.
- *Phytophthora* spp. present in stems of newly propagated mother stock plugs, but not in root of same plant.
- *Phytophthora* infection in all ages of plant, from 2-week old plug plants to 6 year old mature plants.



A) Infected cane base, B) infected cane, and C) LFD test kits

Conclusions

- Together, antibodies 3C4 and 3H7 can be used to determine if certain pathogenic *Phytophthora* species are present in raspberry plant material.
- Antibodies 3C4 and 3H7 may allow users to test more of their plant material, and with a higher sensitivity than many current diagnostic options.

Tests with these antibodies have potential use for detecting *Phytophthora* spp. throughout production systems at very low levels. This requires verification from sequencing work, which is currently underway.

Acknowledgements: This work was funded by the Agriculture and Horticulture Development Board, UK. 2019.
Photo credit: Images B and C - T Pettitt and G Keane, SSE University of Worcester.

Objective 2: Monitoring the benefits of methods for boosting Amblyseius andersoni numbers on a commercial raspberry propagation crop for more robust control of Tetranychus urticae - two-spotted spider mite (TSSM)

Aim 1: Compare the level of TSSM control achieved by *A. andersoni* and *P. persimilis* alone, in combination and when *A. andersoni* is supplemented with pollen.

Aim 2: Determine the effect of Nutrimite™ on numbers of released *A. andersoni* that feed on TSSM on a raspberry propagation crop

Aim 3: Observe the effect of pesticides applied to a commercial raspberry propagation crop on the survival of released predators.

Aim 4: Determine the survival of A. andersoni, TSSM and P. persimilis in commercial cold storage compared with ambient conditions.

Aim 5: Compare the level of mite survival achieved by *A. andersoni* and *P. persimilis* alone, in combination, when *A. andersoni* is supplemented with pollen and when an additional release of *A. andersoni* is made in the autumn.

Introduction

Two-spotted spider mite (TSSM) affects many different crops among its 800 host plant species (Van Leeuwen *et al*., 2010). In the soft fruit industry TSSM is a significant pest and raspberries are particularly vulnerable to feeding damage. The TSSM leave pale yellow flecks as they suck out cell contents from the leaf tissue. This reduces photosynthetic area, leading to reduced berry yield, cane vigour, premature leaf death and fall, and lack of primo/floricane winter hardiness. Weakened plants are more vulnerable to attack by other pests and diseases and severe infestations of TSSM can completely defoliate a plant (Aston, 2017).

There currently is a very limited range of acaricides available for control of TSSM on outdoor and 'Spanish' tunnel grown raspberries. The current EAMU for clofentezine (Apollo 50SC) permits use only in outdoor crops, also abamectin (Dynamec) can now only be used on fully protected cane fruit crops, and is shortly due for withdrawal. Currently one application of Apollo 50SC (use before fruit forms i.e. requires a 26 day harvest interval) and two applications of abamectin (3 day harvest interval) are permitted per year. Some growers also

use products containing maltodextrin e.g. Majestik as spot or overall applications to reduce the population of motile stages of TSSM where the crop is close to, or during harvest (nil day harvest interval). In a review by Van Leeuwen *et al*., 2010 TSSM had developed resistance to the most number of actives of the top ten resistant arthropods, due to its short life-cycle, inbreeding, high fecundity and arrhenotokous reproduction (females can produce males without prior fertilisation). Two-spotted spider mite can complete a life cycle in as few as seven days, producing several generations each year (Osborne *et al*., 1999). Since there are even fewer ovicides available for the control of TSSM eggs, such as clofentezine, (Apollo 50 SC), acaricides are sprayed repeatedly to target the juveniles from hatching eggs.

Integrated Pest Management (IPM) provides an effective means of TSSM control and a solution to acaricide resistance. Growers in the EU are legally required to implement IPM if effective methods are available, under the Sustainable Use Directive (2009/128/EC). There are several biological control agents commercially available for control of TSSM such as Phytoseiid mites: *Phytoseiulus persimilis, Neoseiulus californicus, Amblyseius andersoni* and the gall midge *Feltiella acarisuga. Phytoseiulus persimilis* is most commonly released by growers since it is a specialist predator, which has coevolved to feed on *Tetranychus* species (McMurtry *et al*., 2013). *Phytoseiulus persimilis* can reproduce twice as fast as TSSM in optimum conditions of 20-30°C, >75% RH (Osborne, *et al*., 1999). However, there are limitations to the efficacy of *P. persimilis* in raspberry production. As an obligate predator *P. persimilis* requires re-introduction if the pest is absent and is only effective in a narrow temperature range (15-27°C). At temperatures over 30°C *P. persimilis* is unable to control TSSM and at <60% RH *P. persimilis* eggs are vulnerable to desiccation, whereas the optimum conditions for TSSM are 29-35°C and 20-40% RH (Raworth, 2001). Pesticides used in raspberry cultivation for control of other pests, such as spotted wing drosophila (SWD), aphids and capsids, can be harmful to *P. persimilis.* For example, *s*pinosad (Tracer) and thiacloprid (Calypso) can reduce the *P. persimilis* population by up to 50% and 75% respectively.

The final year of this project has built on the findings of the previous four years. In 2015 ADAS concluded that naturally occurring predators play an important role in maintaining TSSM control in commercial raspberry crops, during spray programmes for SWD and other pests. The ADAS study in 2017 found that pesticides used for SWD and blackberry leaf midge control lowered the population of released *P. persimilis* and to a lesser extent, the naturally occurring predatory mite *A. andersoni*. Naturally occurring *A. andersoni* are considered to be more tolerant of pesticides than *P. persimilis*; a population from the Midi-Pyrenees region of France was found to have resistance to deltamethrin, lamba-cyhalothrin and chlorpyriphos-ethyl (Bonafos *et al*., 2007).

The native generalist predatory mite *A. andersoni* occurs naturally in raspberry crops in the UK and feeds on TSSM along with pollen, fungal spores, honeydew, thrips larvae, fruit tree red spider mite, broad mites, cyclamen mites and russet mites (McMurty *et al*., 2013; Van der Linden, 2004.). *Amblyseius andersoni* is also commercially available and can be released under protection and outdoors in England and Wales. *Amblyseius andersoni* has a wide active temperature range between 6 and 40°C (Bioline Agroscieces Ltd, 2020); it is able to predate at higher temperatures, when *P. persimilis* is ineffective. *Amblyseius andersoni* will overwinter in the crop in leaf litter and overwintered canes, by entering diapause (a period of suspended development) in the autumn. *Amblyseius andersoni* becomes active earlier than TSSM, from January onwards, due to its cold tolerance and omnivorous diet.

In 2018 ADAS monitored the population of naturally occurring and released *A. andersoni* with and without the supplementary food: *Typha angustifolia* L. pollen (Nutrimite™), commercially available from Biobest, for improved biological control of TSSM. Nutrimite™ has been successfully used in Europe to boost other omnivorous predatory mite numbers on other horticultural crops. For example the predatory mites *Amblyseius swirskii* and *Iphiseius degenerans* have been fed with Nutrimite™ for improved establishment of the predators and thus better control of thrips, spider mites and whiteflies in strawberry, rose, poinsettia, sweet pepper and cucumber (Nguyen *et al*., 2015; Pijnakker *et al.,* 2017; Jacob and Pijnakker, 2017). *Amblyseius andersoni* will readily feed and reproduce on *Typha latifolia* in laboratory conditions (Nguyen *et al.,* 2015). The work in this project in 2018 indicated that adding pollen to a fruiting primocane crop led to improved establishment of *A. andersoni* on some assessment dates but concluded that more robust information is required before Nutrimite™ can be recommended on a commercial raspberry crop. The project results in 2018 showed that releasing commercially reared *A. andersoni* significantly boosted the population from the naturally occurring population. It was not possible to quantify the level of control achieved by *P. persimilis* or *A. andersoni* individually in 2018 as both predators were released together. Therefore, further clarification was needed on the level of TSSM control achieved with *A. andersoni*.

The work in this project focussed on fruiting raspberry crops from 2015 to 2018. Gaining control of TSSM in propagated raspberry plants is critical to managing pest populations in the fruiting crop the following year. In 2019 the project focussed on establishment of *A. andersoni* in a raspberry propagation crop in order to provide growers with plants free from TSSM and to ‘seed’ the plants with overwintered predatory mites to provide early protection against TSSM when planted out as a fruiting crop the following spring.

Materials and methods for summer experiment

The work was carried out on a commercial raspberry propagation crop grown in 100% coir. The crop was grown at two sites during the trial.

Figure 2.1 Raspberry cuttings in propagation, height 4 cm.

Site 1 – Under Protection

The trial started with 4 cm raspberry plugs propagated from mother plants situated at the same site. The cuttings were stuck into plugs on 15 May in a commercial propagation Spanish-tunnel (**Figure 2.1**). The trial was situated 2.3 m in on the right-hand side and 3.6 m in on the left-hand side from the North end of the tunnel, to reduce edge effects.

Trial layout

A randomised replicated block design was used, consisting of four treatments and five replicates. Plots measured 90 cm by 2.05 cm containing 12 plug trays of 84 plants each, raised 20 cm from the tunnel floor and separated by a 40 cm gap. Strips of duct tape, 20 cm wide were placed in the gap between the plots and covered in 'Oecotak' glue to prevent the movement of mites between plots (**Figure 2.2**). The plugs were grown under protection for 5 weeks, irrigated by overhead misting. The tunnel was previously cropped with strawberries over winter but was empty from January to May. No plant protection products had been applied to the crop prior to trial commencement.

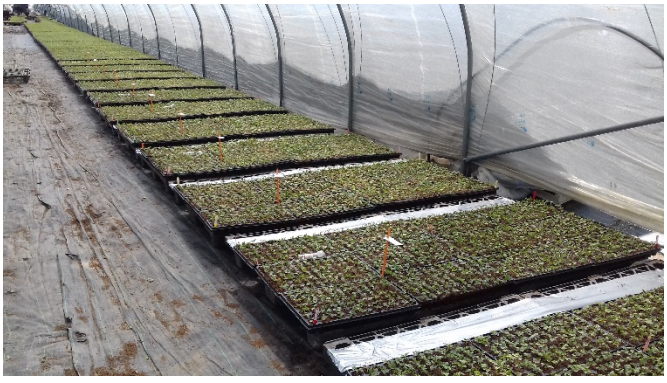


Figure 2.2 Plots in the right-hand side of the propagation tunnel, separated by duct tape and 'Oecotak' glue to prevent movement of mites between plots.

Site 2 - Field

On 26 June the plants were transplanted to a commercial raspberry propagation outdoor field by host farm staff. Plots measured 1.6 m x 50 m, consisting of one double row of raspberry pots. Each plot contained 320 x 4.5 L pots, containing three plants each. Due to grading out, there were too few plants from the trial to fill each plot, therefore the empty pots were filled with propagated plants from the rest of the grower's crop at the north end of the plots. These extra plants were not subsequently sampled after transplant or during the rest of the trial. No biocontrols or pesticides had been applied to these plants. The whole trial covered 10 x 250 m, orientated north to south on a north facing slope. This site was chosen due to having a history of a medium severity spider mite infestation in previous years.

Trial layout

The same randomised replicated block design from the polytunnel experiment was used in the field trial. Plots were arranged in five blocks of four treatments.

Experimental Treatments

Experimental treatments were designed in consultation with biocontrol suppliers (Bioline AgroSciences and Biobest) and the host grower. An untreated control posed too great a risk to the crop, therefore *P. persimilis* was released to match the grower's programme as a control.

T1 - *Amblyseius andersoni*
T2 - *Amblyseius andersoni* + *Typha* pollen (Nutrimite™)
T3 - *Amblyseius andersoni* + *Phytoseiulus persimilis*
T4 - *Phytoseiulus persimilis* control - Grower's IPM programme

Treatment application dates

Treatment application frequency was as per commercial recommendations from Bioline AgroSciences and Biobest. Application dates are summarised in **Table 2.1**.

Table 2.1 Biological control programme treatment application dates.

Treatment	Under Protection		Field				
	15 May	**5 Jun**	**26 Jun**	**10 Jul**	**24 Jul**	**7 Aug**	**17 Sep**
T1	*A. andersoni*	*A. andersoni*	-	*A. andersoni*	-	*A. andersoni*	
T2	*A. andersoni* + Nutrimite™	*A. andersoni* + Nutrimite™	Nutrimite™ only	*A. andersoni* + Nutrimite™	Nutrimite™ only	*A. andersoni* + Nutrimite™	

T3	*A. andersoni* + *P. persimilis*	*A. andersoni* + *P. persimilis*	-	*A. andersoni* + *P. persimilis*	-	*A. andersoni* only	*P. persimilis*
T4	*P. persimilis*	*P. persimilis*	-	*P. persimilis*	-	-	*P. persimilis*

Amblyseius andersoni application rates

Site 1 – Under Protection

Amblyseius andersoni was released twice under protection at a rate of 3.75 / plant, i.e. 3,750 per plot as agreed with Bioline AgroSciences; loose *A. andersoni* is only available in multiples of 25,000. Mites were scattered loose in a vermiculite carrier, distributed evenly by weighing the vermiculite equally into separate containers for each treated plot. All *A. andersoni* was provided by Bioline AgroSciences.

Site 2 - Field

Once the plants had been transplanted into the field, a further application of *A. andersoni* took place on 10 June, followed by the final application on 7 August. *Amblyseius andersoni* was released in Gemini breeding sachets, produced by Bioline. One sachet was hung every two-metre length of row. Each sachet is designed to release up to 2,000 mites in a period of six weeks as the *A. andersoni* feed on prey mites within the sachet and gradually crawl out from tiny holes on the inside of the sachet, protected from rain. This is the equivalent of releasing 100 mites per plant over six weeks. Sachets were hung on the supporting wires and secured from wind displacement with staples on the bottom end of the sachet, ensuring the contents were not pierced. On the first application the plants were small and not yet touching between pots (**Figure 2.3**), however, the supporting wires provided the mites with the ability to move from plant to plant. By the second application on 7 August the plants were all touching and the wires had been moved higher up in the crop (**Figure 2.4**). Therefore, when the sachets were applied they were surrounded by leaves and sheltered from sun, wind and rain.

Figure 2.3 First application of predatory mites on 10 July, after transplant to the field. *Phytoseiulus persimilis* was released loose from tubes of vermiculite. *Amblyseius andersoni* was released in Bioline AgroSciences 'Gemini' breeding sachets.

Figure 2.4 Application of *A. andersoni* in Bioline AgroSciences 'Gemini' breeding sachets on 7 August.

***Phytoseiulus persimilis* application rates**

Site 1 – Under protection

Phytoseiulus persimilis was scattered evenly over the plots in a vermiculite carrier from plastic tubes at a rate of one per plant. The dispensary tube is designed to release five to six mites in every shake. Therefore the tube was shaken once over every second pot, covering six plants. The empty tubes were left open on the plots for any remaining mites to escape from.

Site 2 – Field

After transplant to the field, *P. persimilis* was released at a rate of one mite per plant on 10 July. An additional release of *P. persimilis* was made on 17 September after TSSM was first recorded in the crop. This release was made at a higher rate of three mites per plant, released loose in a vermiculite carrier by shaking the dispenser one and a half times over each pot. All *P. persimilis* was provided by Bioline.

Pollen application

Site 1 – Under protection

Nutrimite™ was applied at the recommended rate of 500 g / ha; 0.1 g per plot was weighed into five beakers, secured with a screw top lid during transport. Each beaker was covered with muslin by a rubber band to apply pollen evenly by shaking onto treated plots (**Figure 2.5**).

Figure 2.5 Typha pollen (Nutrimite™) weighed into muslin-covered beakers to apply evenly to the crop at the label rate of 500 g / ha.

Site 2 - Field

Typha pollen (Nutrimite™) was applied at the recommended rate of 500 g / ha using a Makita blower, both supplied by Biobest (**Figure 2.6**). Output from the blower was calibrated and the correct rate was applied to each plot by calculating the walking speed at 26 seconds per row. When the plants were small the blower could cover both raspberry rows in each plot with pollen using one pass. As the plants grew, the walking speed was doubled to 13 seconds per row in order to cover both rows separately in each plot. Nutrimite™ was stored in the freezer at -17 °C in between application dates according to manufacturers' recommendations and only the amount needed for application was defrosted, no more than 24 h before application and stored in a refrigerator or cool box prior to reaching the site. A protective facemask and goggles were used during application.

Figure 2.6 Nutrimite™ application with a Makita blower, both provided by Biobest.

Pest management by the grower

The grower applied *P. persimilis* at the rate of one per plant to the rest of the crop between 17 and 19 July. One pesticide spray was applied to the crop on 22 June 2019; a tank mix of: Calypso (thiacloprid), Amistar (azoxystrobin) and SP058 (wetter) at recommended rates. Thiacloprid has been shown to have a moderately harmful (50-75% mortality) or harmful (over 75% mortality) effect on *P. persimilis* adults and nymphs (Biobestgroup.com, 2020 and Koppert Biological Systems, 2020 respectively). Azoxystrobin is classed as 'safe' to *P. persimilis* (less than a 25% mortality, Koppert Biological Systems and Biobestgroup.com, 2020). However, there is no data available for the effect of thiacloprid or azoxystrobin on *A. andersoni*. No foliage was trimmed from the crop, so as not to inadvertently remove any mites.

Leaf and leaflet sampling

For the first two assessments (under protection), 24 randomly selected leaves were systematically sampled from each plot by taking two leaves from each of the 12 trays in a plot. These leaves were removed carefully from the plant with scissors to prevent damage to mites. Once transplanted to the field, samples were taken from the first 30 m of raspberry row, starting from the South end of each plot, in order to only sample from the plants that had been subjected to the treatments while under protection. The North end of the plot had been filled with the host grower's plants, which had not been subjected to the treatments while under protection, therefore these plants were excluded. On the third assessment, 24 leaves were randomly sampled from every 12th pot. From the fourth assessment the raspberry leaves had grown and only terminal leaflets were collected, and divided into two categories: randomly selected and targeted samples i.e. leaves with symptoms of possible TSSM damage. Targeted samples were taken to provide certainty as to whether TSSM was present in the crop and to assess whether predatory mites occurred on the same leaves as TSSM. For the random samples, 16 terminal leaflets were systematically sampled from the lower canopy of every 12th pot. Half of the leaves were selected from one side of the plot and the others from the opposite side in order to get an accurate representation of the crop. For the targeted sample, eight terminal leaflets were specifically selected anywhere from the upper or lower canopy of an individual plot with symptoms of possible spider mite damage. The leaflets from each plot were placed in labelled, sealed, plastic bags and returned to the laboratory in a cool box. Leaf/leaflet area approximately doubled at each assessment, as the plants grew, until 7 August, when the final leaflet size had been reached with an average area of 143 cm^2 (**Figure 2.7**).

Leaflets were sampled on the following dates before any treatment applications were applied:

- 15/5/19: whilst under protection, before the first application of all biocontrols and pollen
- 5/6/19: whilst under protection, before the second application of all biocontrols and pollen
- 26/6/19: after transplant to the field and before the application of pollen
- 10/7/19: before application of all biocontrols and pollen
- 24/7/1: before application of pollen only
- 7/8/19: before application of *A. andersoni* only
- 5/9/19: before application of *A. andersoni* and pollen
- 16/10/19: before application of *P. persimilis*

A)  B)  C) 

D)  E)  F) 

Figure 2.7 Plant growth stage representative photos. A) 15 May, height 4 cm. B) 10 July, height, 20 cm. C) 24 July, height 36 cm. D) 8 August, full terminal leaflet size reached: 143 cm^2, height 70 cm. E) 5 September, height 130 cm. F) 16 October, height 230 cm.

Laboratory assessment for two-spotted spider mite and predators

Both the upper and lower surfaces of each leaf or leaflet for every assessment were examined under a binocular microscope and the following assessments were made:

TSSM assessments

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

Predator assessments

- Numbers of *A. andersoni* adults and juveniles (combined)
- Numbers of *A. andersoni* eggs
- Numbers of *P. persimilis* adults and juveniles (combined)
- Number of *P. persimilis* eggs
- Numbers of other predatory mite species
- Numbers of other predatory mite eggs
- Numbers of any other TSSM predators, if occurring (e.g. *Stethorus punctillum*, *Feltiella acarisuga, Orius laevigatus, Chrysoperla carnea*)

Phytoseiid species were confirmed after mites were mounted in a clearing medium on glass slides and examined using a high-powered microscope and dichotomous, morphological key. Eggs could be distinguished between TSSM, *A. andersoni*, and *P. persimilis*. *A. andersoni* eggs could not be distinguished from the eggs of other *Amblyseius* or *Neoseiulus* species.

Temperature records

Temperatures were recorded using two USB data loggers in the centre of each side of the trial when in the propagation tunnel. In the field one logger was placed in a plot at the North end of the trial and the other was placed in a central plot.

Statistical analysis

ANOVA (Genstat edition 18.2) was used to analyse the majority of data. See Appendices 2.1 to 2.8 for tables displaying statistical analysis. Differences between means were compared with Duncan's Multiple Range Test. Analysis of coincidence of TSSM and predatory mites used logit transformation and generalised linear models.

Materials and methods for overwintering experiment

The work was carried out on a commercial raspberry propagation crop, grown in 100% coir. The crop was stored at two sites during the trial, one for ambient storage and one for cold storage.

Ambient storage

The site was a commercial raspberry propagation field, the part of the field used for the summer trial. Treatment plots were 14 m^2 (2 m x 7 m) consisting of 7 m lengths of 1 double row of raspberry pots, with grass paths in between the plots. Pots contain three plants each. Treatments were kept in separate rows. The same trial design was used as for the summer trial. Treatment 1 plots were split in half, North-South so that the North end of the plots could become Treatment 5. Plots consisted of 10 raspberry pots on the right-hand row from the South end of each plot for Treatments 1 – 4 and from the North end of the plots for Treatment 5. These 10 pots from each plot were left *in situ* in the field until assessment (**Figure 2.8).**

Figure 2.8 Raspberry pots marked and left *in situ* in ambient conditions.

Cold storage

The site was a commercial raspberry cold store maintained at -1 °C. On 18 December the field grown raspberry plants went into cold store. The same randomised replicated block trial area was used from the field trial. Treatment 1 plots were split in half, North-South so that the North end of the plots could become Treatment 5. The trial consisted of five treatments and five replicates. Plots for Treatments 2 – 4 measured 1.6 m x 50 m, containing 320 x 4.5 L pots, with three plants in each pot. Plots for Treatments 1 and 5 measured 1.6 m x 25 m, containing 160 x 4.5 L pots, with three plants in each pot. Plots consisted of 20 x 4.5 L

raspberry pots on. Twenty pots from each plot were taken from the left-hand row from the South end of each plot for Treatments 1 – 4 and from the North end of the plots for Treatment 5. The 20 pots were individually labelled according to treatment and block before going into cold store. Pots were put into five separate wooden crates, one for each treatment and loaded into the cold store. Canes were not wrapped to enable the plants to dry and prevent the risk of Botrytis developing. *Amblyseius andersoni* enters diapause between August and September when day length is less than 14.5 h (van Houten & Veenendaal, 1990). Spider mite also begins to enter diapause from September onwards. Mites in diapause are unlikely to be mobile once they have found an overwintering crevice.

Experimental Treatments

Experimental treatments were designed in consultation with biocontrol suppliers (Bioline AgroSciences and Biobest) and the host grower. An untreated control posed too great a risk to the crop, therefore *P. persimilis* was released to match the grower's programme as a control.

T1 - *Amblyseius andersoni*
T2 - *Amblyseius andersoni* + *Typha* pollen (Nutrimite™)
T3 - *Amblyseius andersoni* + *Phytoseilus persimilis*
T4 - *Phytoseilus persimilis* control - Grower's IPM programme
T5 - *Amblyseius andersoni* + Autumn release of *A. andersoni*

Application

Treatment application frequency was as per commercial recommendations from Bioline AgroSciences and Biobest. Application dates are summarised in **Table 2.3**. *Amblyseius andersoni* and *P. persimilis* were released in Treatments 1 – 4 as part of the summer trial, detailed previously. An autumn application of *A. andersoni* was released to the Northern half of Treatment 1 in order to create Treatment 5 on 5 September. *Amblyseius andersoni* was released in Gemini breeding sachets, produced by Bioline. One sachet was hung every two metre length of row. Each sachet is designed to release the equivalent of 100 mites per plant over six weeks. Sachets were hung on the supporting wires, sheltered by the crop canopy and secured from wind displacement with staples on the bottom end of the sachet, ensuring the contents were not pierced.

Table 2.3 Biological control programme application dates by treatment.

Treatment	Under Protection		Field					
	15 May	**5 Jun**	**26 Jun**	**10 Jul**	**24 Jul**	**7 Aug**	**5 Sep**	**17 Sep**
T1	*A. andersoni*	*A. andersoni*	-	*A. andersoni*	-	*A. andersoni*	-	-
T2	*A. andersoni* + Nutrimite™	*A. andersoni* + Nutrimite™	Nutrimite ™ only	*A. andersoni* + Nutrimite™	Nutrimite ™ only	*A. andersoni* + Nutrimite™	-	-
T3	*A. andersoni* *+ P. persimilis*	*A. andersoni* *+ P. persimilis*	-	*A. andersoni* *+ P. persimilis*	-	*A. andersoni* only	-	*P. persimilis*
T4	*P. persimilis*	*P. persimilis*	-	*P. persimilis*	-	-		*P. persimilis*
T5	*A. andersoni*	*A. andersoni*	-	*A. andersoni*	-	*A. andersoni*	*A. andersoni*	

Ambient-stored sampling

The ambient-stored canes were monitored from January through to March for signs of bud break. On 30 March, 50 fully expanded, newly emerged leaves were sampled from the lower canopy of each plot. This was the same day that the cold-stored canes were removed from the cold store. Samples from each plot were stored in sealed, labelled plastic bags and transported to the laboratory in a cool box. Average length of the main leaf vein was 3.07 cm. Where primocane basal buds had emerged these were inspected in-situ with a hand lens for numbers of TSSM, *Amblyseius sp*. predatory mites, *P. persimilis* and eggs.

Cold-stored sampling

The cold stored canes were transported to the field, where the ambient plants were kept, on 30 March. Of the cold stored plants, ten pots from each plot were arranged in a randomised block design in the field. Where primocane basal buds had emerged from the root system this was inspected *in-situ* on 24 April with a hand lens for numbers of TSSM, *Amblyseius sp*. predatory mites, *P. persimilis* and eggs. The crop was monitored regularly for signs of bud-break. On 30 April the leaves had expanded to a similar size as those sampled in the ambient-stored assessment, the average length of the main leaf vein was 3.4 cm. Therefore 50 fully expanded, newly emerged leaves were sampled from the lower canopy of each plot.

Tullgren funnel assessments

One dormant cane from each of the ten pots per plot remaining from the cold store, was cut from the base and stored in a large plastic bag for transport to the laboratory. The bags were

stored in ambient room temperature in the laboratory in order to remain cool and out of direct sunlight before being loaded into Tullgren funnels in order to release any mites sheltering in the canes (**Figure 2.9).** The Tullgren funnels have a maximum capacity of 20 canes every 72 h. Four canes from each treatment were cut into 15 x 10 cm lengths and assessed simultaneously, with one cane in each sieve. Canes one to four were assessed for Blocks one to five subsequently, followed by canes five to eight for blocks one to five and then finally by canes nine and ten for blocks one to five (**Table 2.4).** Canes were left in the Tullgren Funnels for 72 hours for mites to collect in 70% ethanol in universal tubes connected to the bungs. The universal tubes containing ethanol were emptied into 'Doncaster' dishes for counting numbers of adult and juvenile TSSM, *Amblyseius spp*. predatory mites and *P. persimilis* under a binocular microscope. Eggs could not be assessed with this method as they are not extracted in Tullgren funnels. The rest of the canes were assessed every three days (excluding weekends) in batches so that all five treatments were always assessed simultaneously. While the remaining canes were waiting for assessment, they were kept in polythene bags separated by plot (to prevent mites moving between the treatments).

Table 2.4 Details of Tullgren funnel assessment schedule.

Assessment details	**Date assessed**
Assessment 1 Canes 1 -4 (Block 1 T1 - 5)	06/04/2020
Assessment 2 Canes 1 - 4 (Block 2 T1- 5)	09/04/2020
Assessment 3 Canes 1 - 4 (Block 3 T1- 5)	14/04/2020
Assessment 4 Canes 1 - 4 (Block 4 T1 - 5)	17/04/2020
Assessment 5 Canes 1 - 4 (Block 5 T1 - 5)	20/04/2020
Assessment 6 Canes 5 - 8 (Block 1 T1 -5)	23/04/2020
Assessment 7 Canes 5 - 8 (Block 2 T1 - 5)	27/04/2020
Assessment 8 Canes 5 - 8 (Block 3 T1 - 5)	07/05/2020
Assessment 9 Canes 5 - 8 (Block 4 T1 - 5)	14/05/2020
Assessment 10 Canes 5 - 8 (Block 5 T1 - 5)	21/05/2020
Assessment 11 Canes 9 - 10 (Block 1 and 2 T1 - 5)	26/05/2020
Assessment 12 Canes 9 - 10 (Block 3 and 4 T1 - 5)	02/06/2020
Assessment 13 Canes 9 - 10 (Block 5 T1 - 5)	02/06/2020

Figure 2.9 Tullgren Funnels containing raspberry cane material. Heat and light from the bulb causes mites in the canes to move downwards through the sieve and into a collecting tube.

Laboratory leaf Assessments

Both the upper and lower surfaces of each leaf from the ambient-stored and cold-stored assessment were examined under a binocular microscope and the following assessments were made:

TSSM assessments

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

Predator assessments

- Numbers of *A. andersoni* adults and juveniles (combined)
- Numbers of *A. andersoni* eggs
- Numbers of *P. persimilis* adults and juveniles (combined)
- Number of *P. persimilis* eggs
- Numbers of other predatory mite species
- Numbers of other predatory mite eggs

- Numbers of any other TSSM predators, if occurring (e.g. *Stethorus punctillum*, *Feltiella acarisuga, Orius laevigatus, Chrysoperla carnea*)

Phytoseiid species from the leaf and Tullgren funnel assessments were confirmed after mites were mounted in a clearing medium on glass slides and examined using a high-powered microscope and a morphological key. Eggs could be distinguished between TSSM, *A. andersoni*, and *P. persimilis*. *Amblyseius andersoni* eggs could not be distinguished from the eggs of other *Amblyseius* or *Neoseiulus* species.

Temperature records

Temperatures were recorded using two USB data loggers. One was positioned in the centre of the ambient-stored trial and the other was placed inside the middle crate of the stack of crates inside the cold-store and moved to the field when the plants were removed from cold store.

Statistical analysis

ANOVA (Genstat edition 18.2) was used to analyse the data. A log, base 10, transformation was applied to the data for additional clarification of statistical analysis using ANOVA. Differences between means were compared with Duncan's Multiple Range Test. Graphs are presented with back transformed means in order to have relevance for growers.

Results for summer experiment

Spider mite damage – random sample

No two-spotted spider mite damage was seen in the random samples until 10 July. **Figure 2.10** shows that very low numbers were recorded on 10 and 24 July in the *P. persimilis* control plots and 7 August in the *A. andersoni* and *P. persimilis* plots, however these results were not significant (P=0.426). On 16 October there was significantly less TSSM damage found in treatment 2 (*A. andersoni* + pollen) in comparison to treatment 4 (*P. persimilis* control). There was no significant difference in spider mite damage where *A. andersoni* + pollen was applied in comparison to where *A. andersoni* was released alone.

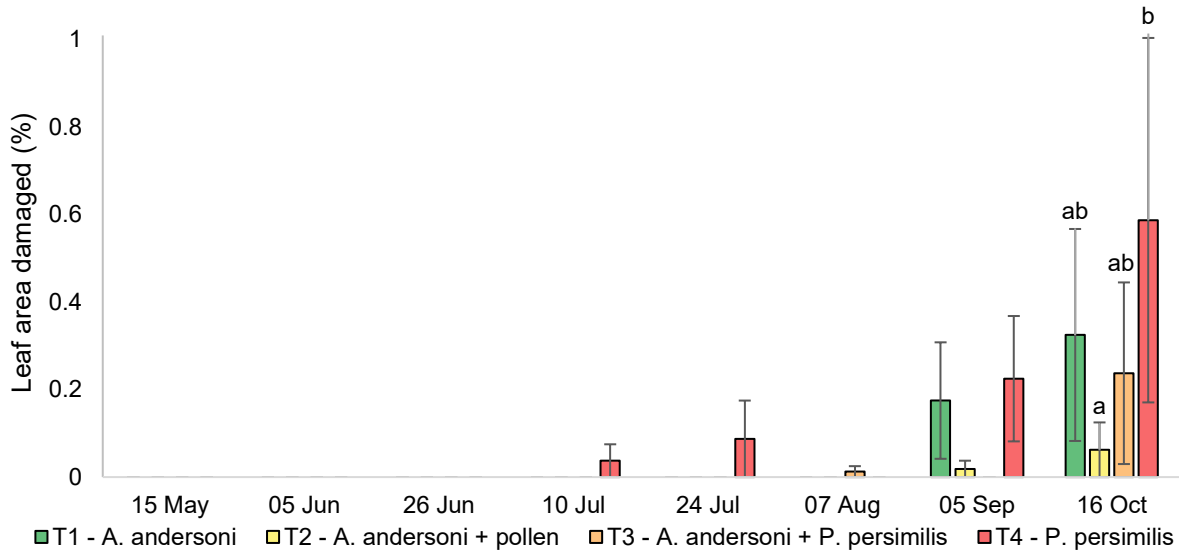


Figure 2.10 Random sample. Mean percentage leaf area damaged by two-spotted spider mite, randomly sampled. Values not sharing the same letter are significantly different (P<0.05).

Spider mite damage – targeted sample

The targeted sample represents spider mite hot spots and shows a similar pattern of results to the random sample (**Figure 2.11**). There was very little damage observed until 5 September. On 16 October there was significantly less damage seen in the *A. andersoni* + pollen plots compared with the *P. persimilis* control.

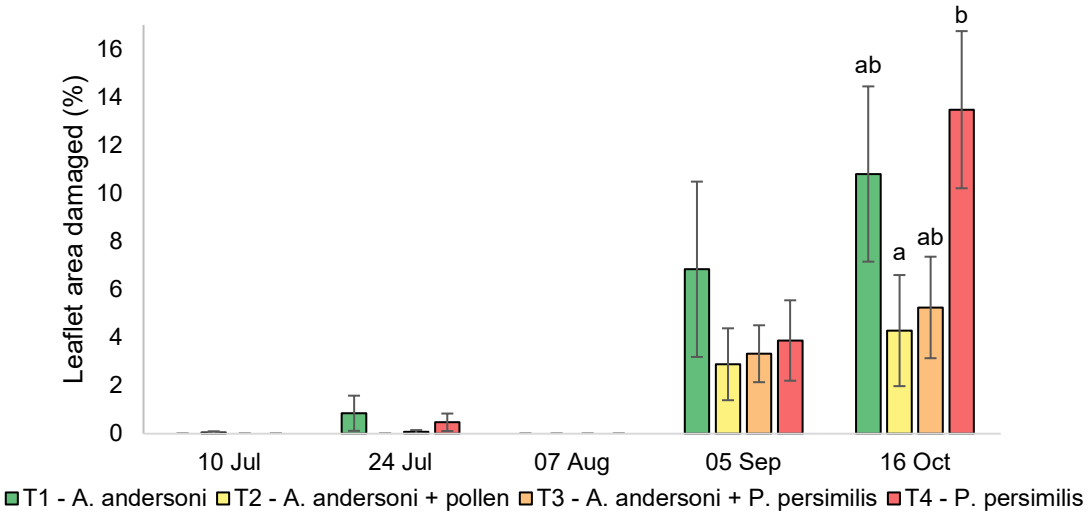


Figure 2.11 Targeted sample. Mean percentage leaf area damaged by two-spotted spider mite from targeted samples (selected as showing possible signs of damage). Values not sharing the same letter are significantly different (P<0.05).

Numbers of mites – random sample

Since TSSM was not recorded in either the targeted or random samples for the first six assessments it was decided that these samples could be combined to increase the replication and improve the confidence in the plot means. Results for 5 September and 16 October are from the random sample only.

TSSM adults/nymphs

As with spider mite damage, TSSM adults and nymphs were not seen in the trial until late in the season, from 7 September (**Figure 2.12**). Despite the presence of TSSM damage in the targeted samples on 24 July, only one two-spotted spider mite was found. Spider mite numbers continued to increase by the final assessment. Despite the additional September release of *P. persimilis*, on 16 October there were significantly fewer spider mite found in treatments 1 and 2, where *A. andersoni* was released without *P. persimilis* compared with treatments 3 and 4 where *P. persimilis* was released, with or without *A. andersoni*

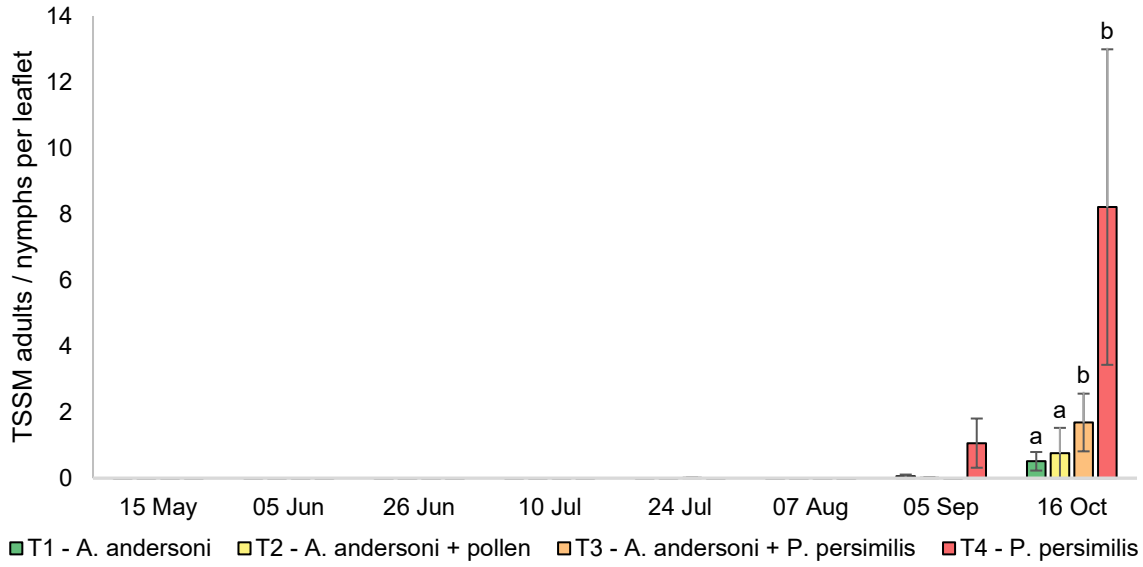


Figure 2.12 Random and targeted samples combined until 7 Aug. Mean numbers of two-spotted spider mite adults / nymphs per leaflet. Values not sharing the same letter are significantly different (P<0.05).

TSSM eggs

Negligible numbers of TSSM eggs were found before 5 September. On 16 October TSSM egg numbers had increased from the previous assessment and there were more eggs than adults/nymphs per leaflet in all treatments except for the *A. andersoni* + pollen treatment, where the mean for both was 0.8 per leaflet (**Figure 2.13**). Duncan's Multiple Range Test revealed the same significant differences as for the leaf area damaged by TSSM; there were significantly fewer TSSM eggs found in plots with *A. andersoni* + pollen treatment in comparison to the *P. persimilis* control.

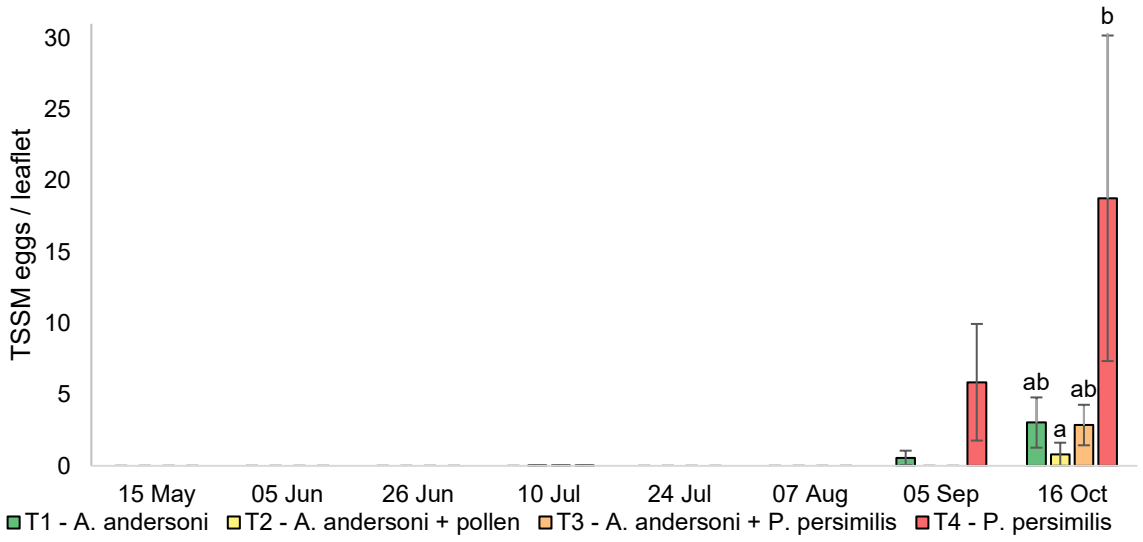


Figure 2.13 Random and targeted samples combined until 7 Aug. Mean numbers of two-spotted spider mite eggs per leaflet. Values not sharing the same letter are significantly different.

***Amblyseius andersoni* adults / nymphs**

The baseline naturally-occurring population of *A. andersoni* (i.e. before any releases) was established at the start of the experiment on 15 May in the propagation tunnel, when only one *A. andersoni* was found on this assessment, in an *A. andersoni* only plot (**Figure 2.14**). The *A. andersoni* population increased by the second assessment on 5 June, following the biocontrol release. A low number of *A. andersoni* were also found in the *P. persimilis* only plots indicative of the natural population. On 5 June the number of *A. andersoni* found was highest in the *A. andersoni* + *P. persimilis* plots although this result was not significant and this trend was observed to a greater extent in the following assessment, on 26 June, immediately after transplant to the field. On this date there were significantly more *A. andersoni* found in the *A. andersoni* + *P. persimilis* plots than in the other treatments (P=0.008).

On 10 July there were 4.4 times more *A. andersoni* found where *A. andersoni* was released with pollen compared to where *A. andersoni* was released alone, this difference was statistically significant. On both 10 July and 24 July there were significantly more *A. andersoni* found where *A. andersoni* + pollen was released compared with the *P. persimilis* control. On 7 August mean *A. andersoni* numbers per leaflet were significantly higher in the *A. andersoni* + *P. persimilis* plots compared with the *P. persimilis* control but not higher than in the *A. andersoni* alone or with pollen treatments. There were no significant differences between mean numbers of *A. andersoni* per leaflet on the final two assessment dates on 5 September and 16 October.

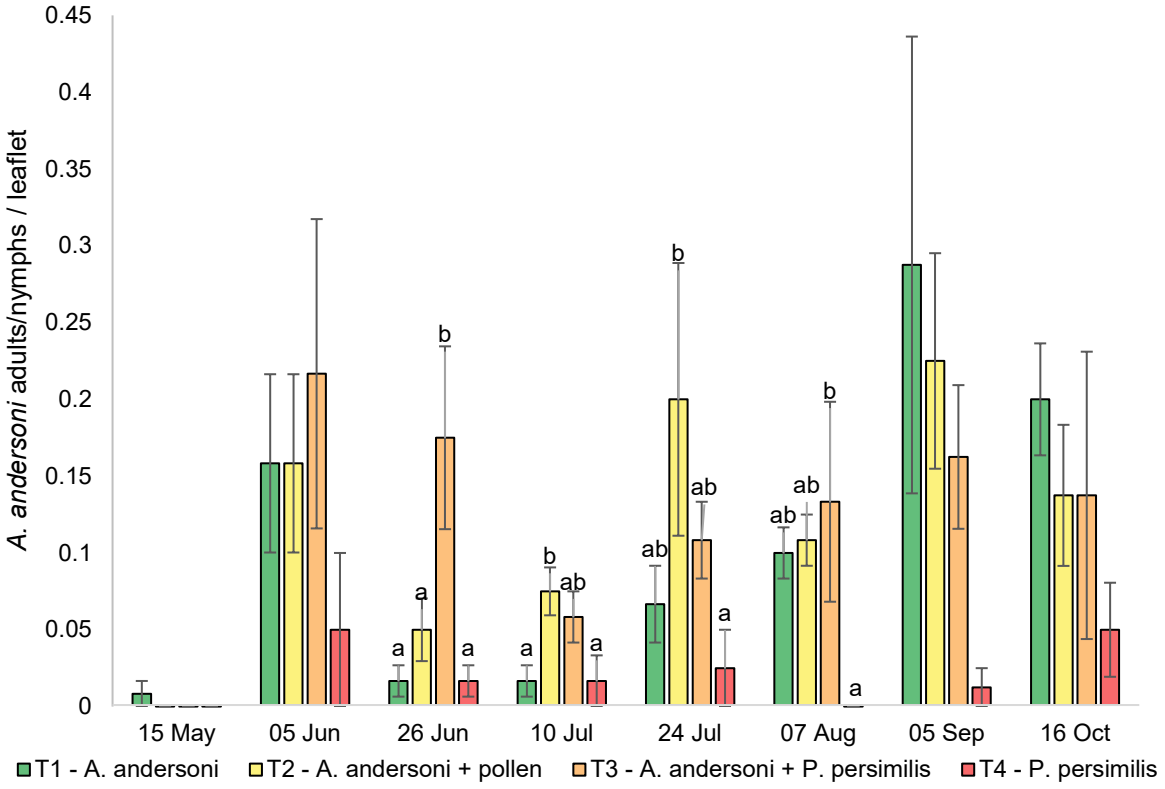


Figure 2.14 Random and targeted samples combined until 7 Aug. Mean *A. andersoni* adult/nymph numbers per leaflet. Values not sharing the same letter are significantly different (P<0.05).

Amblyseius andersoni eggs

Mean numbers of *Amblyseius sp.* eggs show similarity to the number of adults / nymphs found on each assessment. Greater mean numbers of eggs were found in the *A. andersoni* + *P. persimilis* treatment than the other treatments, on 5 and 26 June, before the crop had spent time growing in the field (**Figure 2.15**). On 5 June there were significantly more eggs found where *A. andersoni* had been released with *P. persimilis* compared to where *A. andersoni* was released with pollen (P<0.05). On 26 June, eggs were only found in plots where *A. andersoni* had been released with pollen and this difference was significant (P<0.05). Low numbers of eggs were found between 24 July and 7 August; treatment results did not significantly differ. No eggs were found in the *P. persimilis* control throughout the experiment, except on the 5 June. No eggs were found in any treatment on 16 October.

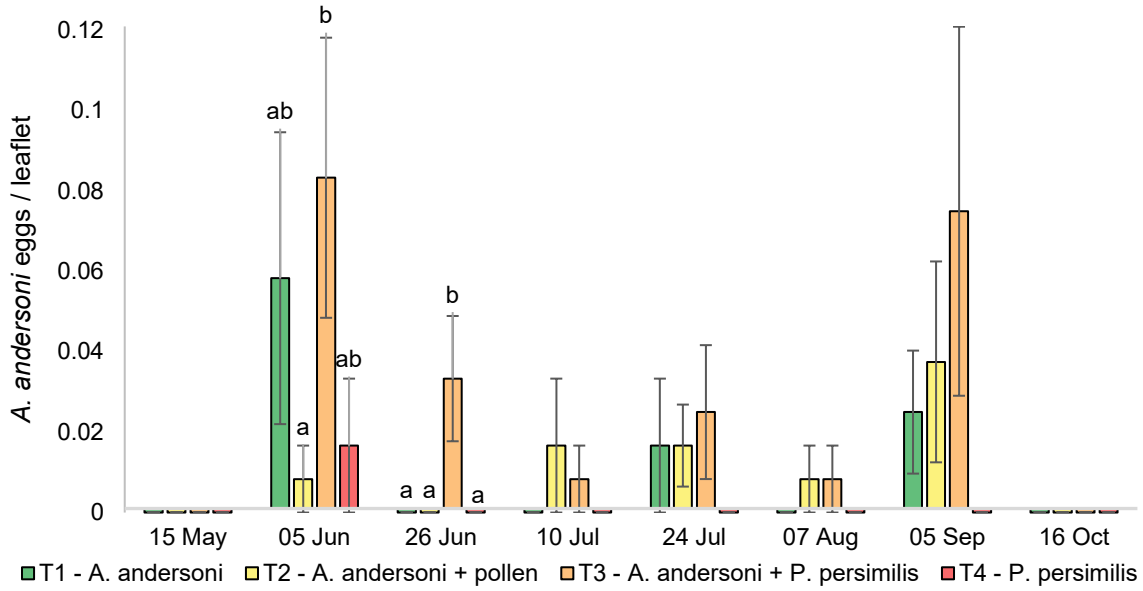


Figure 2.15 Random and targeted samples combined until 7 Aug. Mean numbers of *Amblyseius sp*. eggs per leaflet. Standard error bars. Random samples only for 5th Sep and 16th Oct. Values not sharing the same letter are significantly different (P<0.05).

Phytoseiulus persimilis adults / nymphs

Phytoseiulus persimilis was found in the trial on only two dates: 5 June and 16 October (**Figure 2.16**). Only one *P. persimilis* was found on 5 June, in plots treated with *A. andersoni* plus *P. persimilis*. On 16 October *P. persimilis* was found in all four treatments, despite being released in only treatments 3 and 4. Although the mean number of *P. persimilis* was higher where *P. persimilis* was released, and where *P. persimilis* was released alone the mean number of mites was 3.4 times greater than where *P. persimilis* was released with *A. andersoni*, the differences between treatments were not significant with or without transformation.

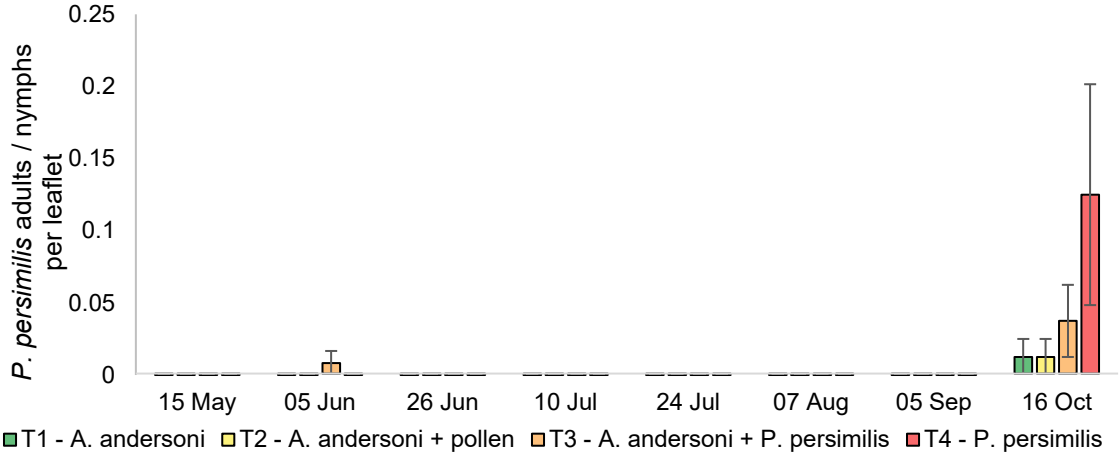


Figure 2.16 Random and targeted samples combined until 7 Aug. Mean numbers of *P. persimilis* per sampled leaflet. Values not sharing the same letter are significantly different (P<0.05).

Phytoseiulus persimilis eggs

Phytoseiulus persimilis eggs were found only on 16 October in *P. persimilis* control plots (**Figure 2.17**). Although the mean number per leaflet was high (1.71), the difference between treatment means was not significant.

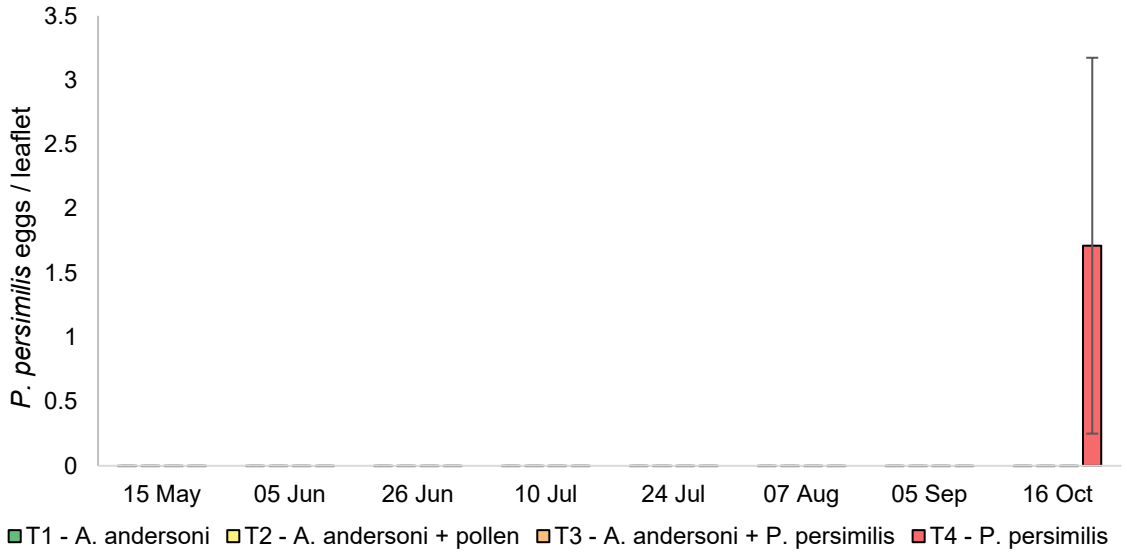


Figure 2.17 Random and targeted samples combined until 7 Aug. Mean numbers of *P. persimilis* eggs per leaflet. Values not sharing the same letter are significantly different (P<0.05).

Targeted Leaf Samples – Hot spots

Targeted leaflet samples were analysed for 16 October, since the highest numbers of TSSM were recorded on this date; 111 out of 160 targeted leaf samples (69%) showed signs of TSSM damage when assessed under the binocular microscope (**Table 2.5**). Two-spotted spider mite adults / nymphs and eggs were found on 98% and 94% of the damaged leaves, respectively. *Amblyseius andersoni* was found on 35% of damaged leaves, whereas *P. persimilis* was found on 50% of the damaged leaves. *Amblyseius* sp*. and P. persimilis* eggs, combined, were found on 33% of the damaged leaves.

Table 2.5: Numbers of leaflets with presence of two-spotted spider mite (adults, nymphs or eggs), *A. andersoni* (adults / nymphs) or *P. persimilis* (adults / nymphs) out of 160.

Treatment	TSSM (eggs / adults / nymphs)	*A. andersoni*	*P. persimilis*	Phytoseiid eggs	Damaged leaflets
Total	114	39	56	37	111

Comparison of mite presence by treatment shows that the *A. andersoni* + pollen treatment had the greatest proportion of leaflets with no mites on (**Figure 2.18**). All treatments had a similar proportion of leaflets found with TSSM only on. Treatments 1-3 showed the same number of leaflets with *A. andersoni* found on its own. There were more *P. persimilis* found on their own where *P. persimilis* was released by itself compared to where it was released with *A. andersoni*. All the *A. andersoni* with TSSM found in treatment 2 were found on leaves with *P. persimilis* also, although *P. persimilis* was not released in this treatment.

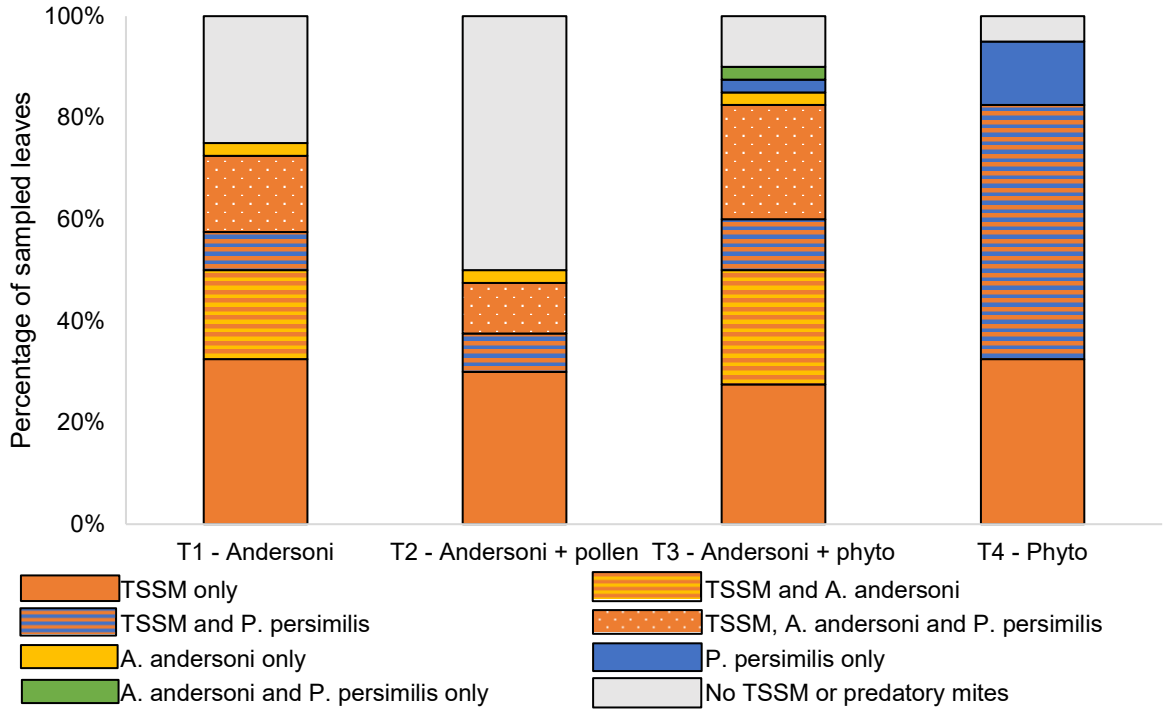


Figure 2. 18 Leaflets with presence of two-spotted spider mite (adults, nymphs or eggs), *A. andersoni* (adults / nymphs) or *P. persimilis* (adults / nymphs) represented as a percentage of the total number of leaflets sampled.

There was no significant difference in the number of leaves with coincidence of *A. andersoni* and TSSM as a percentage of the total number of leaves with TSSM when *A. andersoni* was released alone compared with release with pollen or with *P. persimilis* (**Table 2.6**). All treatments where *A. andersoni* was released significantly differed from the *P. persimilis* only treatment since no *A. andersoni* was found in this treatment there was no coincidence with TSSM. When *A. andersoni* was released with pollen *A. andersoni* was only found on 21 % of the leaves with TSSM, which significantly differed from release of *A. andersoni* with *P. persimilis* where *A. andersoni* was found on 55 % of the leaves with TSSM. Only 80 % of the total *A. andersoni* found in treatment 2 coincided with TSSM on the same leaflet. Whereas approximately 90% of the *A. andersoni* found in treatments 1 and 3 were found on leaflets

with TSSM, there was no significant difference between treatments. All of the *P. persimilis* found in treatments 1 and 2, where *P. persimilis* was not released, were found on leaves where TSSM was also found. Where *P. persimilis* was released (Treatments 3 and 4) a lower percentage of the total number of *P. persimilis* were found on leaves with TSSM compared to where *P. persimilis* had occurred naturally, although this difference was not significant. Where *P. persimilis* was released with *A. andersoni* (Treatment 3), *A. andersoni* occurred on a greater percentage of leaves with TSSM presence than *P. persimilis* did. Where *P. persimilis* was released by itself the greatest level of coincidence with TSSM was observed, although there was no significant difference between the treatments. *Amblyseius andersoni* was found with TSSM more than 80 % of the time in all treatments except T4 where no *A. andersoni* was found. Whereas *P. persimilis* was found with TSSM more than 80 % of the time in all treatments.

Table 2.6: Numbers of targeted leaflets per treatment with presence of two-spotted spider mite, *A. andersoni* or *P. persimilis* represented as a percentage of the total number of leaflets with presence of two-spotted spider mite, *A. andersoni* or *P. persimilis*. Values not sharing the same letter are significantly different, * denotes a missing value. S.e. denotes standard error.

Treatment	Leaves with *A. andersoni* and TSSM		Leaves with *P. persimilis* and TSSM	
	% of total leaves with TSSM	% of total leaves with *A. andersoni*	% of total leaves with TSSM	% of total leaves with *P. persimilis*
T1 - *A. andersoni*	45bc	93a	31a	100a
s.e.	9.52	7.86	16.20	0.16
T2 - *A. andersoni* + pollen	21b	80a	37a	100a
s.e.	9.64	20.56	20.87	0.18
T3 – *A. andersoni* + *P. persimilis*	55c	90a	39a	87a
s.e.	8.93	7.70	16.04	12.25
T4 - *P. persimilis*	0a	no *A. andersoni*	61a	80a
s.e.	0.03	*	16.04	11.16
P value	<0.001	0.807	0.627	0.464

Other invertebrates

No other spider mite predators were found during the trial except for the occasional lacewing egg or larva; a generalist predator. Other invertebrates identified in low numbers included: springtail, blackberry leaf midge, *Neoseiulus cucumeris* and large raspberry aphid.

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

A summary of mean numbers of TSSM, TSSM damage, *A. andersoni* and *P. persimilis* and release dates of treatment programmes are shown in **Figures 2.19 and 2.20**.

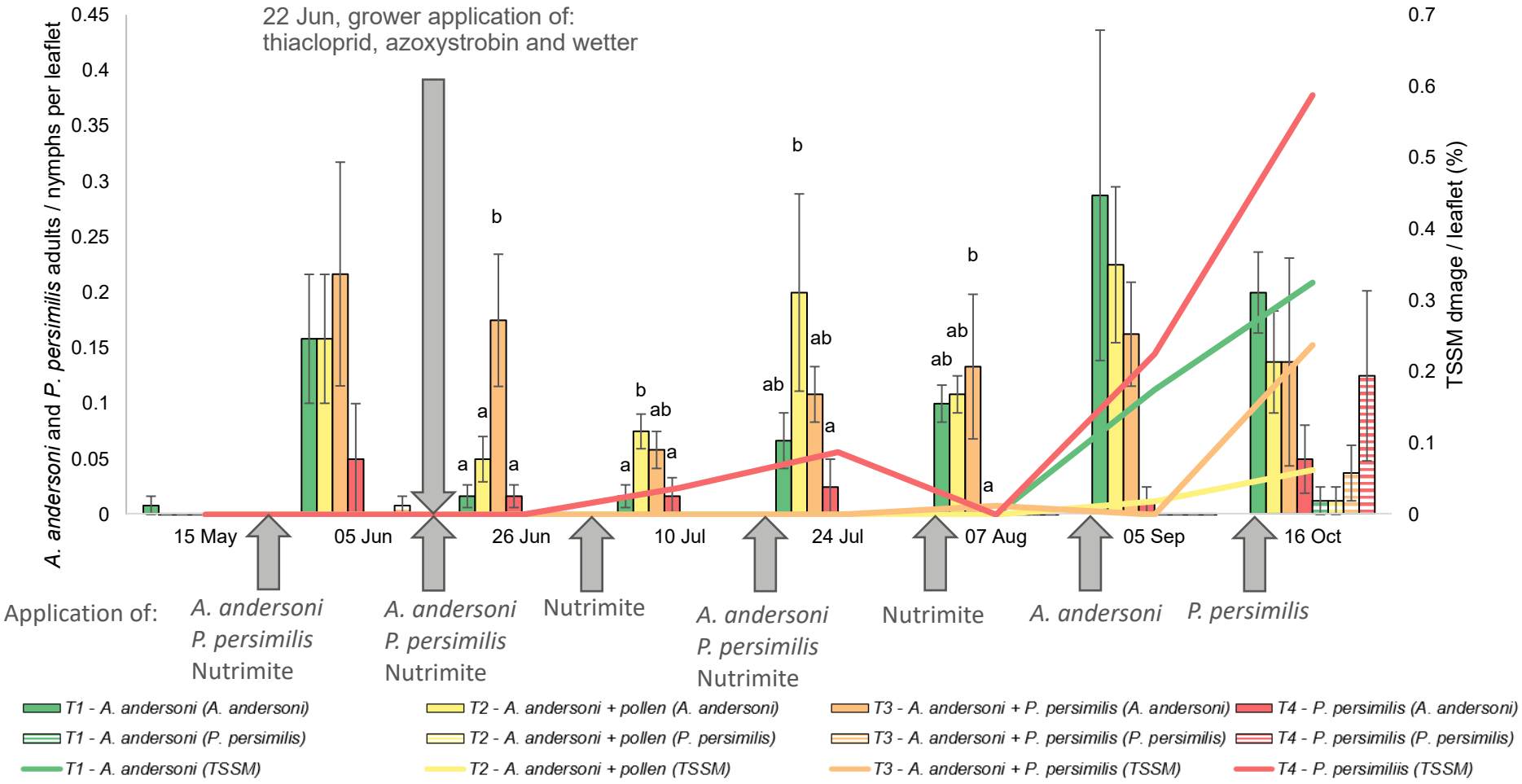


Figure 2.19 Mean numbers of *A. andersoni, P. persimilis* and percentage two-spotted spider mite damage from random samples. Error bars represent standard error. Values with none of the same letters are significantly different (P<0.05).

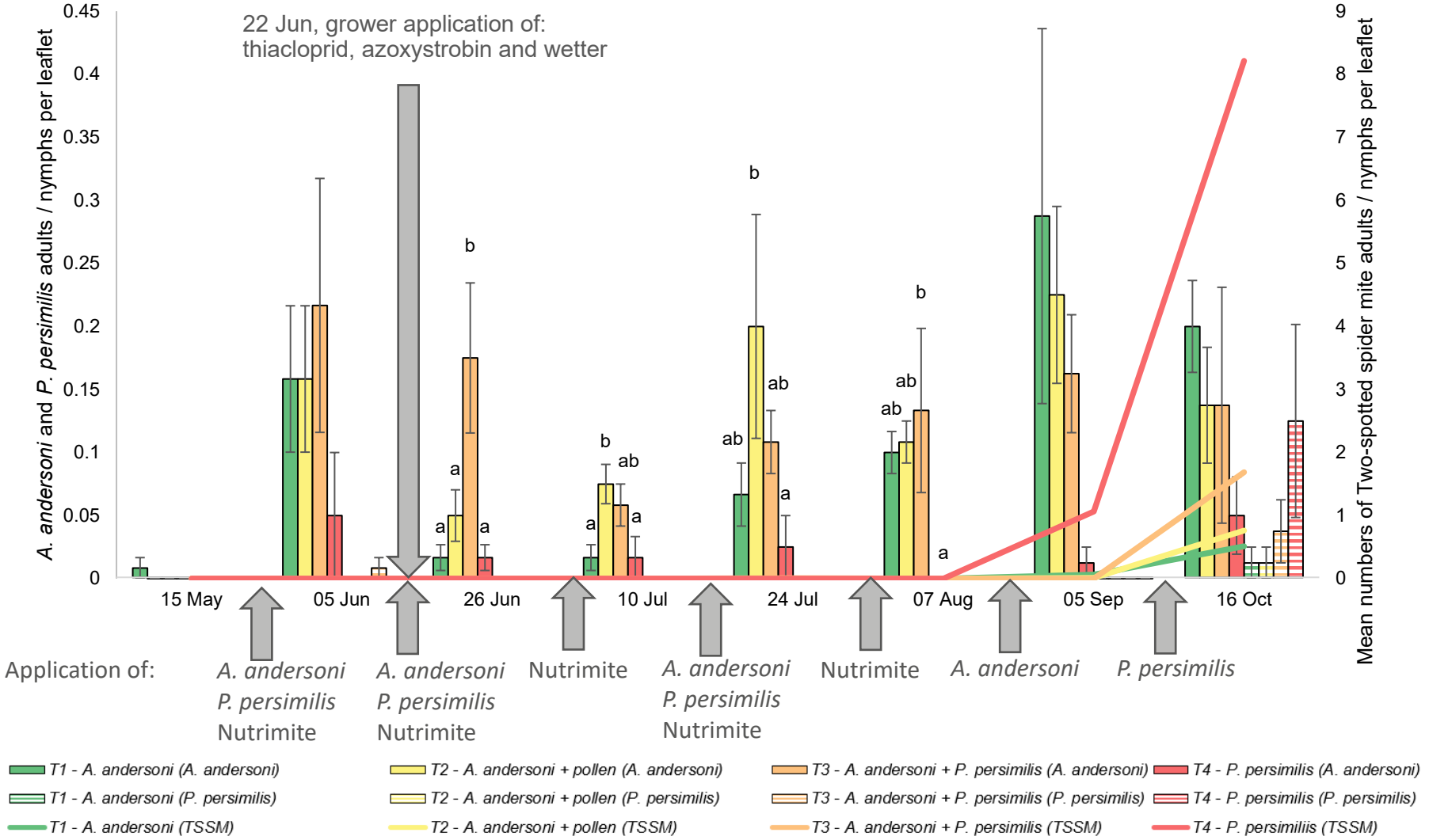


Figure 2.20 Mean numbers of *A. andersoni, P. persimilis* and two-spotted spider mite from random samples. Error bars represent standard error. Values with none of the same letters are significantly different (P<0.05).

Temperature records

Unfortunately one data logger was lost and the other failed to record after the first month. Temperature and humidity data recorded under protection from 15 May to 16 June has been given (**Figures 2.21 & 2.22**). Satellite meteorological data has been used for the period 17 June to 17 October for temperature and rainfall (**Figures 2.23 & 2.24**).

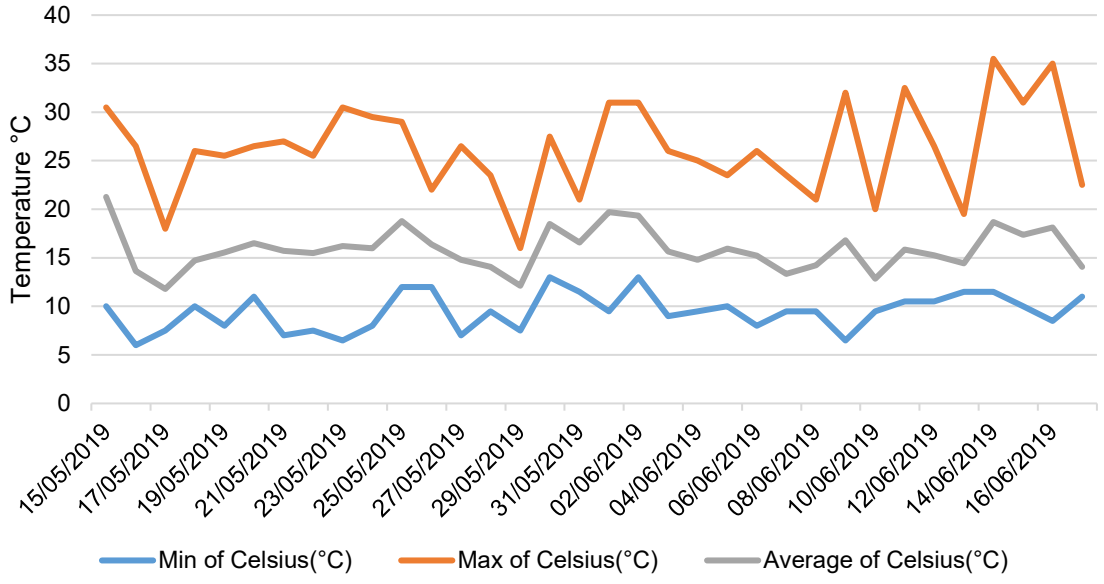


Figure 2.21 Daily minimum, maximum and average temperature, under protection, from 15 May to 17 June.

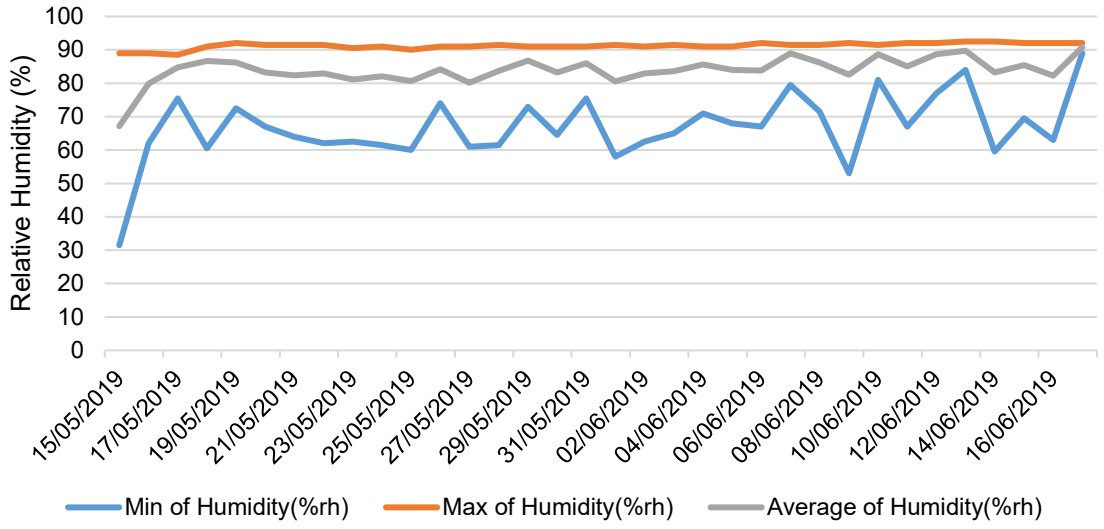


Figure 2.22 Daily minimum, maximum and average humidity under protection, from 15 May to 17 June.

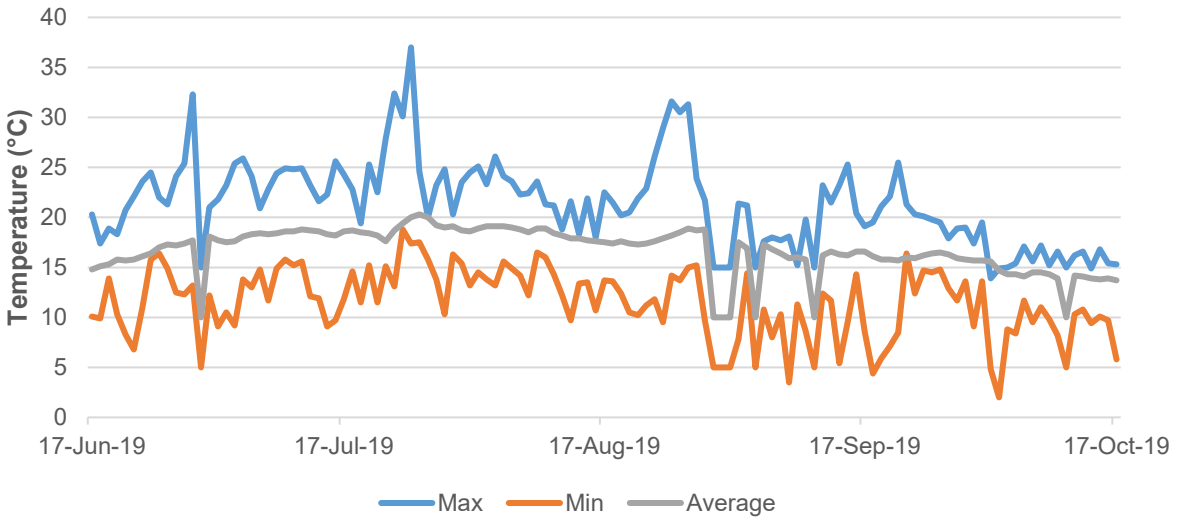


Figure 2.23 Daily minimum, maximum and average temperature from 17 June to 17 October using satellite data accurate to 100 m from the field trial, grid reference SU770612. Data obtained from ADAS Irriguide model.

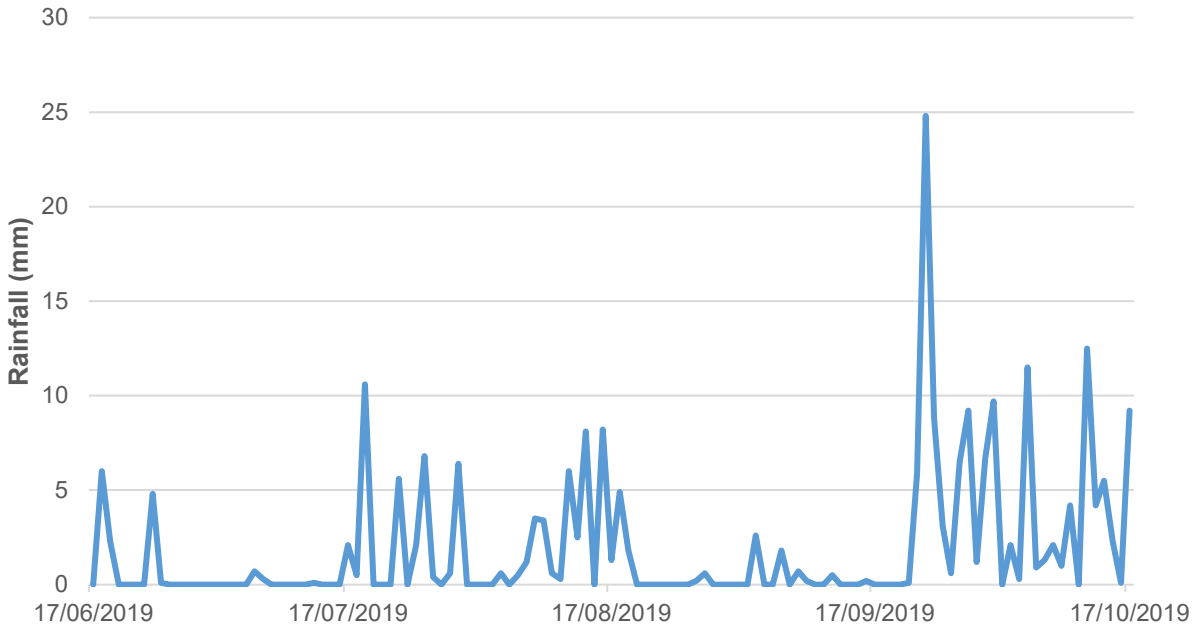


Figure 2.24 Daily rainfall from 17 June to 17 October using satellite data accurate to 100 m from the field trial, grid reference SU770612. Data obtained from ADAS Irriguide model.

Discussion for summer experiment

Overall, all biological control programmes were successful, including the *P. persimilis* only treatment, the TSSM infestation was low and less than a mean of 1% damage was caused per leaflet in the random samples. There were no adult TSSM found in the trial until 5 September, either in the random samples or targeted samples. This could be considered a late infestation compared to the commercial fruiting crop studied in 2018 for the project where spider mite was observed as early as March and the population had peaked and crashed by 30 July. It is unlikely that the sampling technique missed the TSSM since both random and specifically targeted samples were taken. Also, the lower canopy was sampled since the work in this project in 2018 showed that spider mite numbers were much lower in the upper canopy of a raspberry crop. Under protection the maximum temperature climbed above 30°C on a few days, but the average temperature stayed below 20°C under protection and in the field, sub-optimal conditions for TSSM reproduction, which is 29 – 34°C. It is also likely that the good hygiene standards of the site and use of cuttings from mother stock free from TSSM, prevented an early infestation of TSSM. The minimum temperature in the polytunnel at the start of the trial remained above 6°C, which is the minimum temperature for *A. andersoni* to be active (Bioline Agroscience Ltd, 2020) and the minimum relative humidity only dropped below 60% on two occasions under protection, by less than 10%, providing suitable conditions for *P. persimilis* to establish. However, a dry period from late June to midJuly could have inhibited *P. persimilis* from establishing in the field.

Discussion of Aim 1: Compare the level of TSSM control achieved by *A. andersoni* and *P. persimilis* alone, in combination and when *A. andersoni* is supplemented with pollen.

In this experiment *A. andersoni* contributed to improved biocontrol of TSSM. It is especially important to control TSSM numbers by the end of the season in a propagation crop, in order to prevent TSSM from overwintering in the crop and being a source of the pest when planted out as a fruiting crop the following year. The treatments in which *A. andersoni* was released (1-3) achieved greater control than where *P. persimilis* was released alone, in terms of damage caused and numbers of TSSM adults / nymphs and eggs. Unlike *A. andersoni,* which can survive on alternative food to TSSM, the released *P. persimilis* was unable to establish until after the 17 September release, when TSSM was present in the crop. As a specialist predator *P. persimilis* needs to eat TSSM to reproduce, without TSSM the population will have died out (McMurtry *et al*., 2013). However, once TSSM was present in the crop *P. persimilis* was able to produce eggs, as late as 16 October and become established, even as

temperatures declined. There was no untreated control in the experiment for comparison, due to the crop risk.

Some *P. persimilis* was found in the targeted samples from plots where it had not been released, these mites could have been transported by workers or machinery. These predators were found exclusively on leaves with spider mite, demonstrating the ability of *P. persimilis* to locate and move towards spider mite. It is also possible that *P. persimilis* could have moved from adjacent rows as they travel in a straight line towards TSSM, by detecting plant volatiles, when starved (Bernstein, 1983). However, Casey and Parrella (2005) showed that three times more *P. persimilis* will move to plants connected by bridges or foliage, so they would have been most likely to move towards connected TSSM patches within plots.

Raspberry leaves are very vulnerable to TSSM feeding damage and the symptoms show more easily than on some other crops such as strawberry. Leaf damage is permanent and can be indicative of spider mite presence after the pest has gone. There was significantly less damage found where *A. andersoni* was released with pollen, in comparison to the *P. persimilis* control. Damage caused by the same number of TSSM becomes visible sooner when the mites are concentrated as opposed to when they are dispersed (Alatawi *et al*., 2011). If the TSSM population was prevented from forming concentrated patches where *A. andersoni* and pollen was applied this could account for the observed reduction in damage. There were also significantly fewer TSSM adults / nymphs and eggs in comparison to the *P. persimilis* control on 16 October, despite the *A. andersoni* population not significantly differing from the other treatments on this date. This suggests that the *A. andersoni* may have been better distributed where pollen was applied in order to gain control of the TSSM more quickly, after infestation. Provision of Nutrimite™ may also have encouraged *A. andersoni* to remain in the crop when other food sources were scarce, since a lack of food encourages predatory mites to disperse in search of food (Schausberger & Croft, 1999). *Phytoseiulus persimilis* is able to locate TSSM by detecting plant volatiles, released as a response to plant feeding (Nachappa *et al*., 2006). Therefore, the response of *P. persimilis* is limited by the level of plant damage. Alatawi *et al*. (2011) found that generalist predatory mites suppressed tetranychid mites more effectively when they were distributed evenly over multiple leaves as opposed to being concentrated on one leaf. Therefore *A. andersoni* could have been more effective at controlling a very low density of widely distributed TSSM.

Amblyseius andersoni was present on leaves with TSSM on the majority (>80%) of the targeted leaf samples, which represent the hot spots of TSSM activity. This contrasts to the findings in the previous year of this project's work and work done on rose, which found *A. andersoni* coinciding with TSSM in only 50% of cases (van der Linden, 2004). These results suggest that *A. andersoni* was attracted to TSSM and not found randomly throughout the

propagated raspberry crop, where there may be fewer sources of other food. The incidence of *A. andersoni* coinciding with TSSM was lowest where pollen was applied, suggesting that the pollen could have provided a distraction from the TSSM although this was not a significant difference. The high number of targeted leaves with no mites found, in treatment 2 (*A. andersoni* plus pollen), suggests that TSSM infestation had been controlled at an earlier stage. There was no significant difference in the percentage of damaged leaf area or numbers of TSSM achieved whether *A. andersoni* was released with or without Nutrimite™. Therefore, the improvement in TSSM control cannot be directly attributed to the addition of pollen. However, reduction of TSSM damage was significantly better in the *A. andersoni* plus pollen treatment than in the *P. persimilis* control plots on the final assessment date but not in the *A. andersoni* alone plots.

When comparing the interaction of *A. andersoni* with *P. persimilis* under protection, releasing both predators together led to increased numbers of *A. andersoni* and eggs compared with the other treatments. Schausberger and Croft (1999) have shown that adult female *A. andersoni* will eat the eggs and larvae of other species of Phytoseiidae preferentially to cannibalising their own eggs or larvae. This indicates that the *A. andersoni* could have predated *P. persimilis* or their eggs. It is unlikely that *P. persimilis* had produced eggs, without TSSM to feed on. This experiment shows that *A. andersoni* may have benefited from the release of *P. persimilis*, whereas research in a Swiss raspberry crop found that releasing *P. persimilis* had a negative effect on the establishment of *A. andersoni* the following year, possibly due to competition for spider mite (Linder *et al*., 2003). In the hot spots *P. persimilis* coincided with TSSM on a lower percentage of leaves where released with *A. andersoni* than without. Schausberger and Walzer (2001) conducted a study of competition between *P. persimilis* and *N. californicus*, which showed that when prey was abundant *P. persimilis* was more affected by intraspecific competition (competition within a species) than competition with *N. californicus*. Whereas *N. californicus* was more affected by competition with *P. persimilis* than by intraspecific competition, due to microhabitat and prey-stage preference. Thus is it unlikely that *A. andersoni* was outcompeting *P. persimilis* for TSSM, but, by helping to reduce the population of TSSM, this may have slowed reproduction of *P. persimilis*. However, the coincidence of *A. andersoni* with TSSM was greater when released with *P. persimilis* compared to where *A. andersoni* was released alone, which could suggest that *A. andersoni* was better able to locate TSSM in the presence of *P. persimilis*. Further work is required on the interaction of these two species.

Discussion of Aim 2: Determine the effect of Nutrimite™ on numbers of released *A. andersoni* that feed on TSSM on a raspberry propagation crop

Nutrimite™ significantly boosted the population of released *A. andersoni* in comparison to where *A. andersoni* was released alone, on one assessment date: 10 July, after transplant to the field. Only Nutrimite™ had been applied on the previous assessment on 26 June when the plants were too small and widely spaced for a release of predatory mites to be suitable. Flowering is discouraged in raspberry propagation since any cane that flowers in the first year will not flower the following year, in the fruiting season. Therefore, in a propagated raspberry monoculture there is little natural pollen available for mites to feed on. Nutrimite™ was shown to boost numbers of the predatory mite *Iphiseius degenerans* before availability of natural pollen in a sweet pepper crop (Pijnakker *et al*., 2017). Juvenile development of generalist predatory mites can benefit from a diet of varied prey species. Messelink *et al.* (2008) reported that *A. swirskii* developed faster when fed a diet of thrips and whiteflies than when fed on a diet of thrips or whiteflies alone. Providing Nutrimite™ when few other food sources were available could have increased the rate of development of *A. andersoni* and thus, reproductive rate. Results indicate that Nutrimite™ was a suitable food supplement at this stage in cropping due to the lack of natural pollen and lack of foliar bridges for mites to disperse.

However, Nutrimite™ did not augment the *A. andersoni* population on any other assessment dates. The assessment on 7 August followed an application of only Nutrimite™, on 24 July, but the *A. andersoni* population was no different from in the other treatments. This suggests that there could have been enough other sources of food for *A. andersoni* to negate the benefit of the Nutrimite™ on the crop at this stage. Nutrimite™ did not significantly augment the *A. andersoni* population during the early phase of the trial under protection, whereas releasing *A. andersoni* with *P. persimilis* significantly increased numbers of *A. andersoni* and eggs. The response of *A. andersoni* to Nutrimite™ may have been limited because pollen was applied onto the tops of leaves but the majority of mites are found on the underside of leaves. However, Nutrimite was found on both upper and lower leaf surfaces in the previous year's trial in this project. According to Biobest user guidelines *A. andersoni* benefits less from Nutrimite™ than some other predatory mite species such as *A. swirskii*, therefore *A. andersoni* might respond better to a pollen species more similar to its native host plants. Nutrimite™ is *Typha* (cattail or lesser bulrush) pollen due to its abundance, hypoallergenic properties, comparative lack of response from thrips and resistance to mould.

Natural *A. andersoni* numbers were very low throughout the experiment; only one *A. andersoni* was found before any biocontrols were released. *A. andersoni* overwinter in cracks and crevices in host plants or in ground litter, this mite may have migrated from a host tree or

shrub species in the nearby hedgerow or it may have overwintered in the polytunnel structure itself (van der Linden, 2004). In propagation any leaf litter from the previous crop is removed; thus the *A. andersoni* is unlikely to have originated from here although it may have been transferred from the mother plant. The natural population of *A. andersoni* remained at a low density and did not increase during the experiment. This corroborates the work done in this project in 2018 that without additional releases the natural *A. andersoni* population stayed at a low density. This could be because *P. persimilis* was released in both experiments and may have outcompeted the natural *A. andersoni* for spider mite. Although in this year's work, the *P. persimilis* was absent until the end of the season, where *A. andersoni* was released the population grew throughout the trial, as was found in the work completed for the project in 2018.

Discussion of Aim 3: Observe the effect of pesticides applied to a commercial raspberry propagation crop on the survival of released predators.

Only one plant protection application was made (a tank-mix of Calypso, Amistar and SP058), between the last assessment under protection and transplant to the field. The host grower limited applications of plant protection products in order to preserve the predatory mites. Therefore it cannot be inferred whether any effects on the population of *A. andersoni* between these assessments were caused by the pesticide or by the process of transplantation.

Results for overwintering experiment

Ambient storage conditions

There were no mites or mite eggs found in the leaf samples of the ambient-stored plants. There were no *A. andersoni, P. persimilis* or predatory mite eggs found in the bud samples of the ambient stored plants. There were some TSSM and eggs found in the buds of the ambient stored canes but there were no significant differences between treatments (**Table 2.7**). However, it is noticeable in **Figure 2.25** that no TSSM or eggs were found in Treatment 2 (*A. andersoni* + pollen).

Table 2.7 Mean number of Two-spotted spider mite adults / nymphs and eggs found in buds, stored in ambient conditions. Values not sharing the same letter are statistically different, P>0.05. P values, s.e.d and l.s.d refer to ANOVA. Transformation: log10(x+1).

Treatment	TSSM adults	TSSM eggs
T1 (*A. andersoni*)	0.008a	0.029a
T2 (*A. andersoni* + pollen)	0.000a	0.000a
T3 (*A. andersoni* + *P. persimilis*)	0.000a	0.008a
T4 (*P. persimilis*)	0.010a	0.020a
s.e.d	0.00764	0.02235
l.s.d	0.01665	0.04869
P	0.426	0.587

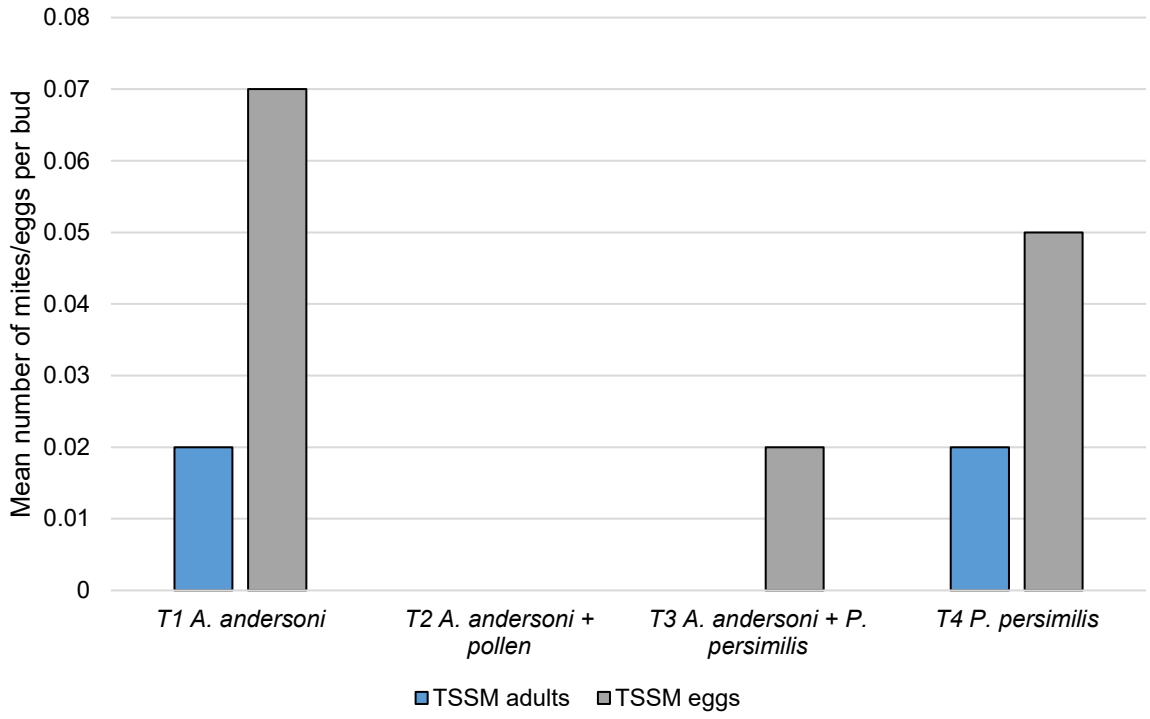


Figure 2.25 Back transformed mean numbers of Two-spotted spider mite adults / nymphs and eggs per treatment from bud samples stored in ambient conditions.

Cold-storage conditions (*Amblyseius andersoni*)

Amblyseius andersoni adults and nymphs were found in cane, bud and leaf samples of cold-stored plants in low numbers and *Amblyseius sp.* eggs were found in the buds. There were no statistical differences in the number of *A. andersoni* adults or nymphs found in the cane, bud or leaf samples **(Table 2.8)**. *Amblyseius andersoni* was the only species of predatory mite identified. Therefore, it is reasonable to assume that the *Amblyseius sp.* eggs found were *A. andersoni* eggs. There were significantly more *A. andersoni* eggs found in Treatment 2, where Nutrimite™ had been applied with released *A. andersoni* compared with Treatment 1 and Treatment 5, where *A. andersoni* had been released alone and alone with an additional autumn release, respectively. However, there were not significantly more *A. andersoni* eggs found in Treatment 2 when compared with Treatment 3 (*A. andersoni* released with *P. persimilis*) and Treatment 4 (*P. persimilis* released alone). Treatments 1, 3, 4, and 5 did not significantly differ. The most mites were found in the cane samples but more mites were found in the buds than the leaves **(Figure 2.26)**.

Table 2.8 Mean numbers of *A. andersoni* adults / nymphs and eggs found in cane, bud and leaf samples of cold-stored plants. Values not sharing the same letter are statistically different, P>0.05. P values, s.e.d and l.s.d refer to ANOVA. Transformation: log10(x+1).

Treatment	Cane	Bud		Leaf
	***A. andersoni* adults**	***A. andersoni* adults**	***A. andersoni* eggs**	***A. andersoni* adults**
T1 (*A. andersoni*)	0.08a	0.000a	0.000a	0.008a
T2 (*A. andersoni* + pollen)	0.10a	0.025a	0.054b	0.002a
T3 (*A. andersoni* + *P. persimilis*)	0.25a	0.035a	0.010ab	0.000a
T4 (*P. persimilis*)	0.02a	0.012a	0.013ab	0.002a
T5 (*A. andersoni* + autumn release)	0.02a	0.019a	0.000a	0.002a
s.e.d.	0.1058	0.03040	0.02320	0.00548
l.s.d.	0.2243	0.06445	0.04918	0.01161
P	P 0.252	0.816	0.165	P 0.610

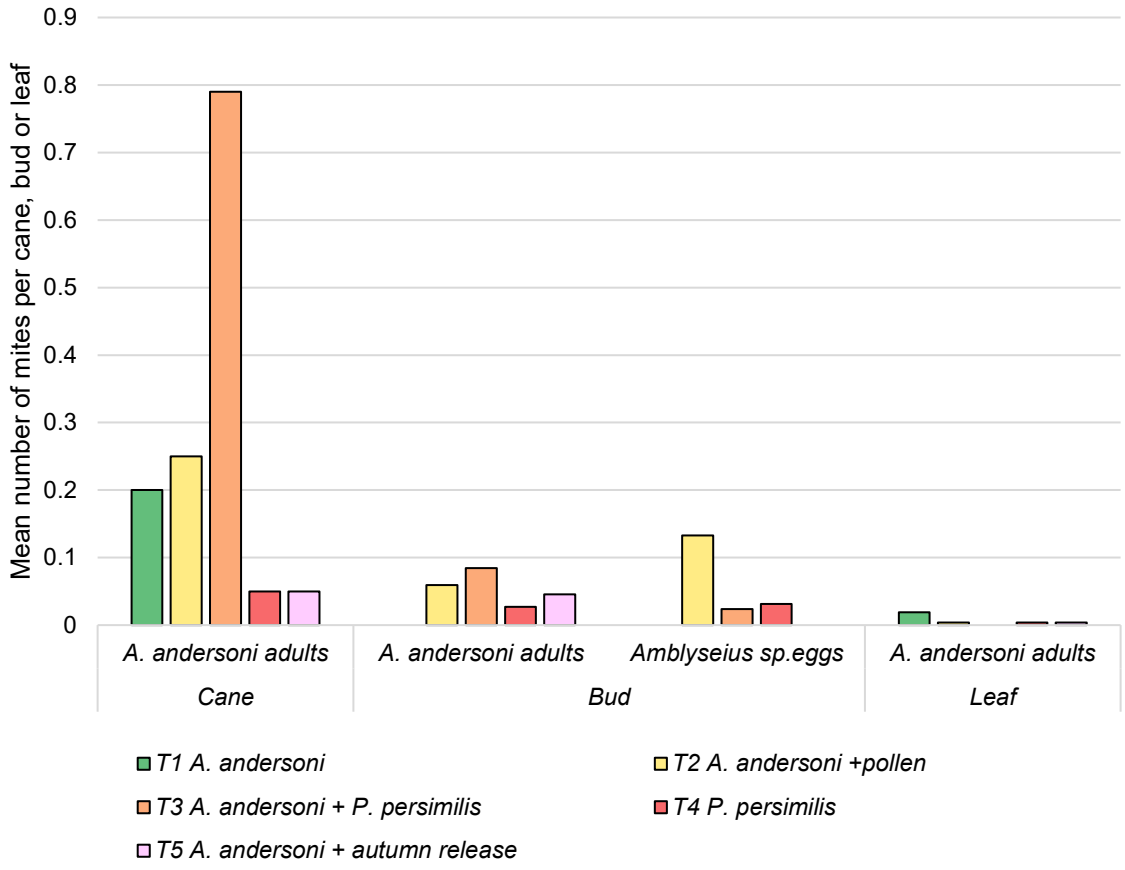


Figure 2.26 Back transformed mean numbers of *A. andersoni* adults / nymphs or *Amblyseius sp.* eggs found in cane, bud and leaf samples from cold stored plants.

Cold-storage conditions (TSSM)

Two-spotted spider mite adults and nymphs were found in the cane, bud and leaf samples of the cold-stored plants at a similar density to the *A. andersoni* adults and nymphs. Two-spotted spider mite eggs were found in high numbers on a small number of buds in Treatments 1 and 4, representing hot spots. However, there were no significant differences between treatments **(Table 2.9)**. However it can be noted that no TSSM adults or nymphs were found in Treatment 2 of the cane samples, no TSSM eggs were found in Treatment 2 of bud samples and the lowest mean number of TSSM adults or nymphs was found in Treatment 2 of the bud samples compared to the other treatments where greater mean numbers of TSSM were found **(Figure 2.27).** Of the leaf samples, there was only one TSSM found out of 1,250 leaves sampled, which was found in Treatment 4, where *P. persimilis* alone had been released.

Table 2.9 Mean numbers of Two-spotted spider mite adults / nymphs and eggs found in cane, bud and leaf samples stored in cold-storage. Values not sharing the same letter are statistically different, P>0.05. P values, s.e.d and l.s.d refer to ANOVA. Transformation: log10(x+1).

Treatment	Cane	Bud		Leaf
	TSSM adults	TSSM adults	TSSM eggs	TSSM adults
T1 (*A. andersoni*)	0.08a	0.06a	0.21a	0.000a
T2 (*A. andersoni* + pollen)	0.00a	0.01a	0.00a	0.000a
T3 (*A. andersoni* + *P. persimilis*)	0.07a	0.03a	0.04a	0.000a
T4 (*P. persimilis*)	0.04a	0.16a	0.35a	0.002a
T5 (*A. andersoni* + autumn release)	0.08a	0.03a	0.04a	0.000a
s.e.d	0.0781	0.0758	2.055	0.001055
l.s.d	0.1655	0.1608	0.4357	0.002306
P	0.794	0.360	0.416	0.436

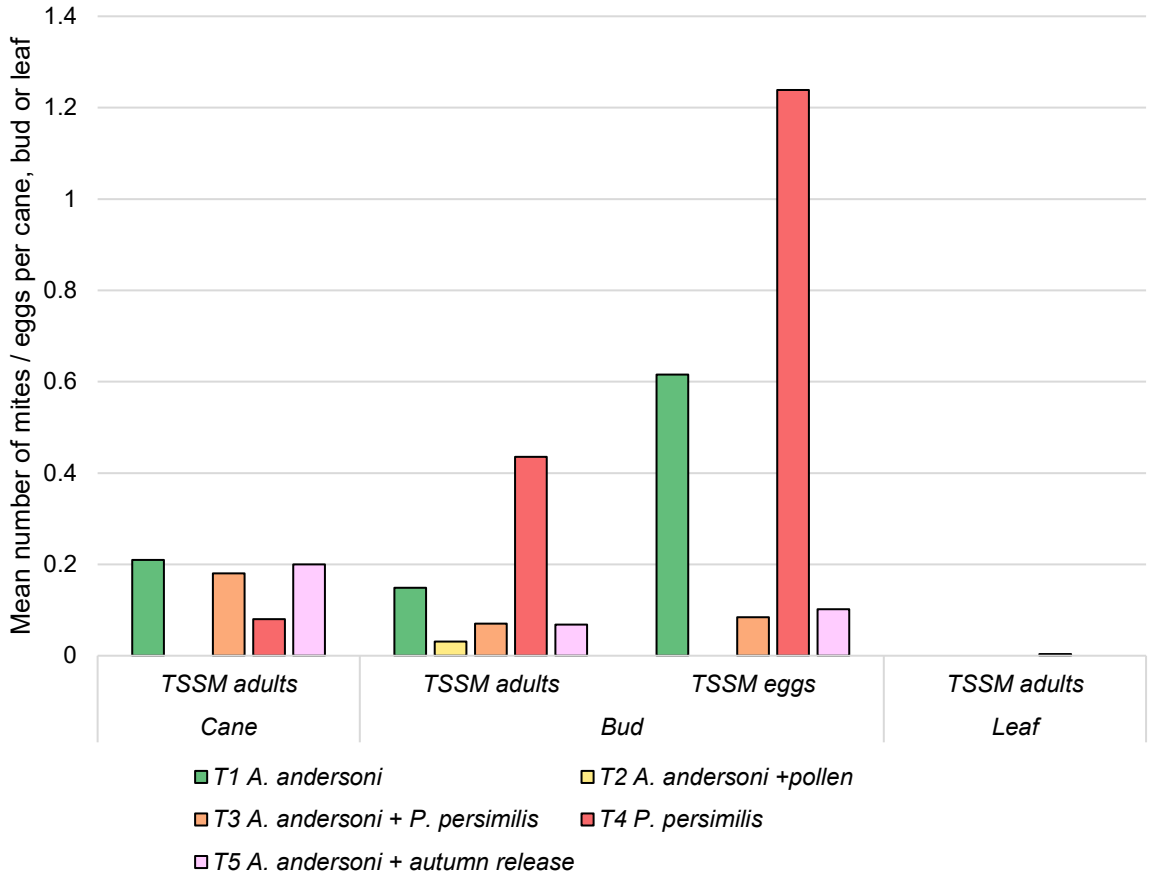


Figure 2.27 Back transformed mean numbers of Two-spotted spider mite adults / nymphs and eggs in cane, bud and leaf samples from cold stored plants.

Weather data

Average temperature in ambient storage ranged from -1 to 11°C between December and March, then started rising in April **(Figure 2.28).** Minimum temperature dropped to -4.5°C on 20 January. The last frost before sampling was on 25 March.

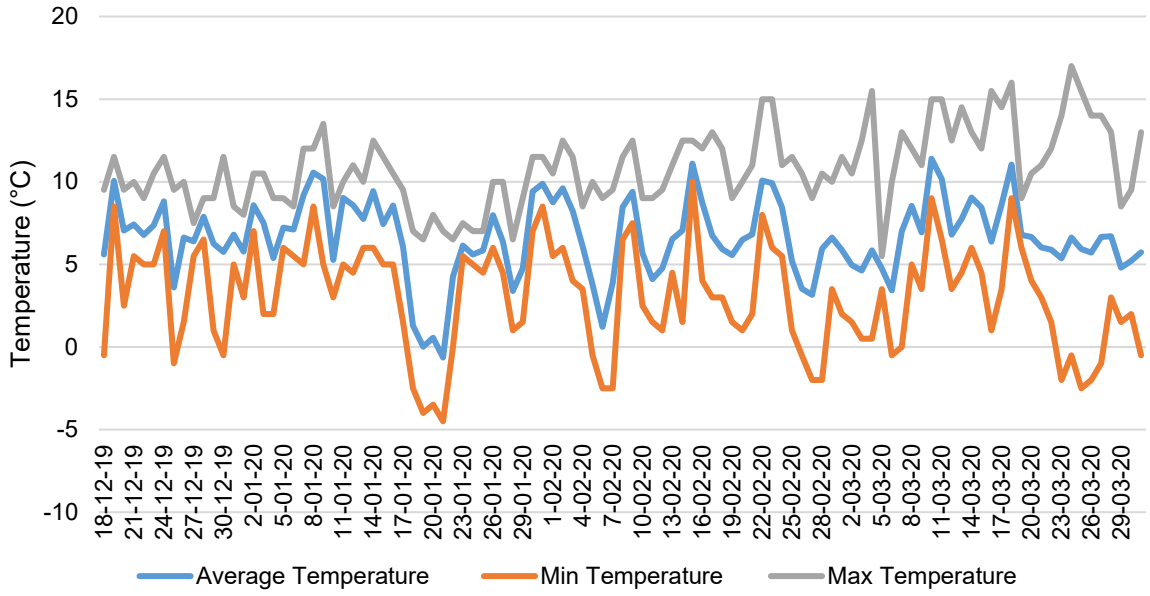


Figure 2.28 Minimum, maximum and average temperature in ambient storage between 18 December 2019 and 30 March 2020.

Average humidity in ambient storage remained between 90 and 100% between December and February, however humidity fluctuated more in March **(Figure 2.29).** There was a large decrease in humidity in the two weeks before sampling and great variability in the days before sampling, representing strong winds which were observed at that time.

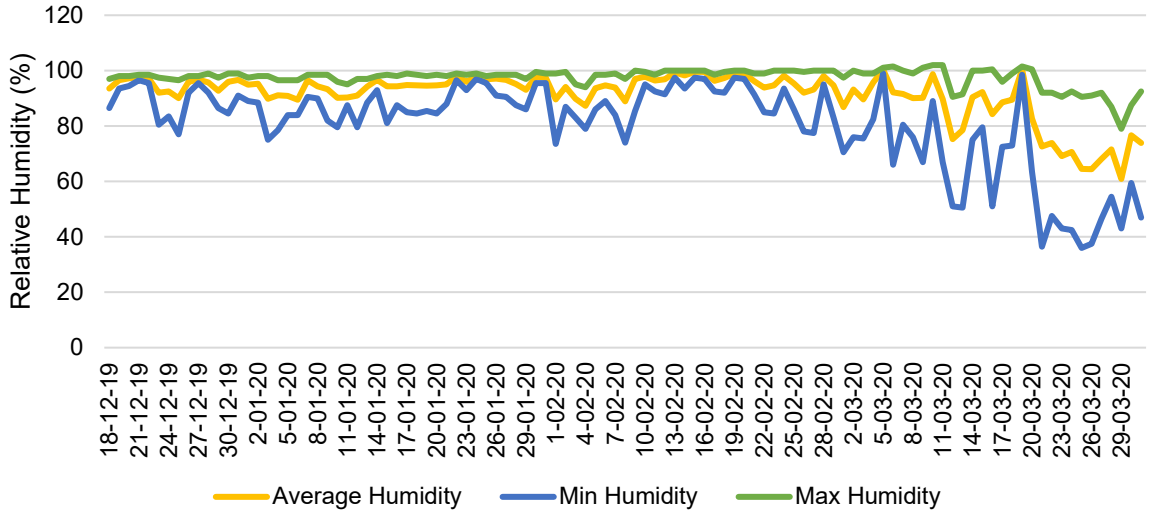


Figure 2.29 Minimum, maximum and average relative humidity in ambient storage between 18 December 2019 and 30 March 2020.

Average temperature in the cold store remained at -1°C. The maximum temperature fluctuated between 0 and 7°C, which could be due to opening doors but the minimum did not drop below -2°C **(Figure 2.30**). In the first day of cold storage the temperature dropped from 8 to 0°C and then reached the desired temperature. The plants remained in cold store for 15 weeks. When the plants were removed from cold store the temperature rose sharply from -0.5 to 13.5°C in two hours. Average temperature rose in April but maximum and minimum temperatures varied on a daily basis. There was a frost of -2.5°C on 1st April.

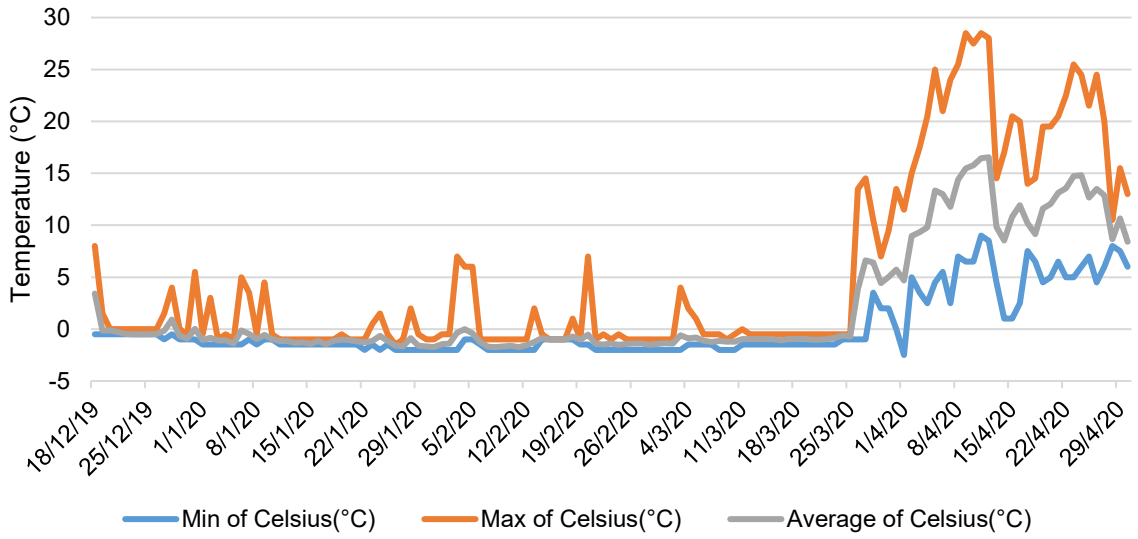


Figure 2.30 Minimum, maximum and average temperature in cold storage between 18 December 2019 and 30 March 2020.

Average humidity was maintained at or near 100% in the cold store **(Figure 2.31).** Average relative humidity rose from 83% to 96% in the first day of cold storage and dropped sharply from 102% to 51.5% in three hours. After removal from cold-store average humidity remained between 60% and 80% except during two rainfall events where it rose above 80%.

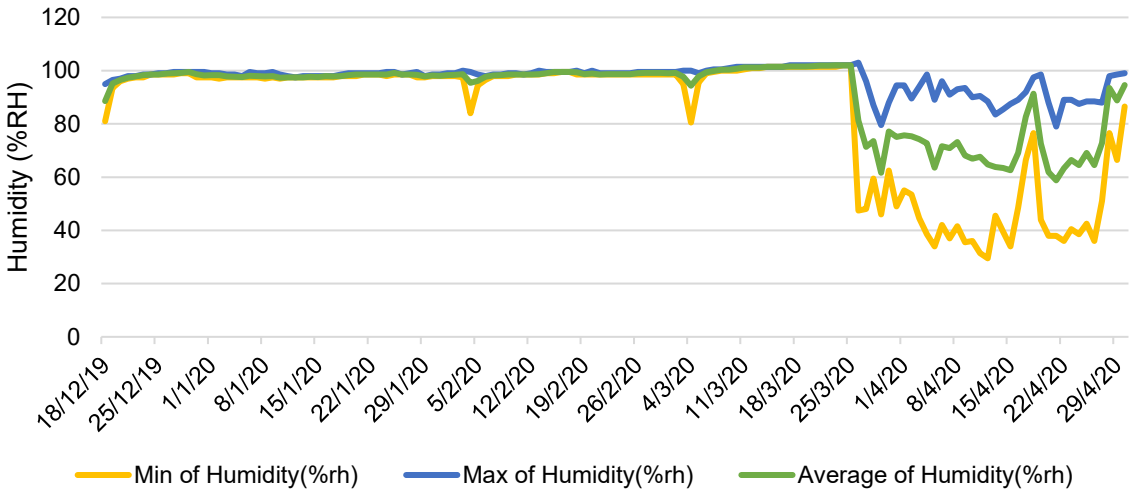


Figure 2.31 Minimum, maximum and average relative humidity in cold storage between 18 December 2019 and 30 March 2020.

Discussion for overwintering experiment

Discussion of Aim 4: Determine the survival of A. andersoni, TSSM and P. persimilis in commercial cold storage compared with ambient conditions.

Ambient-storage mite survival

Amblyseius andersoni was not found in the ambient-stored bud or leaf samples which could indicate that *A. andersoni* had not survived the conditions. However, Irving *et al.,* 2012 found *A. andersoni* in UK raspberry crops having successfully overwintered the cold winter of 2009/2010. Van der Geest *et al.,* 1991 showed that *A. andersoni* mortality is 50% at -5°C, however in that experiment, survival time greatly increased when mites were previously acclimated to 4°C. A temperature of -4.5°C was reached on 20 January, which could have reduced the surviving population, however in ambient conditions the mites were acclimated to increasingly lower temperatures, which is likely to have increased their cold-hardiness. *Amblyseius andersoni* has been recorded overwintering in the cold winters of Switzerland and Hungary in ground litter, crevices, cracks in tree trunks, dried weeds and in raspberry canes, particularly in the lower 40 cm since this is where the largest number of growth niches are found (Linder *et al*., 2003; Szabó & Pénzes, 2013). These sites provide shelter and it is likely that the temperatures that the *A. andersoni* were exposed to in these niches were higher than those recorded by the data logger. Since *A. andersoni* were found in the cane and bud samples of the cold-stored plants it is likely that they would have also been present in the cane and buds of the ambient stored plants, however they may have dispersed in search of a food source, becoming active before the sampling was carried out. It is also possible that the strong winds and low humidity may have caused *A. andersoni* to seek shelter in the leaf litter and weeds, leaving the crop since the newly emerged foliage was badly damaged by the wind.

Two-spotted spider mite was found in the buds of the ambient stored plants along with eggs, showing that TSSM was able to successfully overwinter in the crop in ambient conditions and had already started to reproduce by 30 March, when the average temperature had not yet exceeded 12°C. Diapause is initially controlled by photoperiod in TSSM in order to prevent high autumn temperatures from terminating diapause prematurely, however photoperiodic sensitivity is lost after 16 weeks of chilling (Koveos *et al.,* 1993). TSSM can remain in a dormant state called post-diapause quiescence until rising spring temperatures terminate diapause (Kroon *et al.,* 1998). The rising maximum temperatures in late February and March,

including a high of 17°C on 24 March may have activated oviposition in TSSM. At 10°C TSSM eggs develop into larvae in 19 days (Purdue University, 2009), which suggests that the large number of eggs discovered could have been laid between one and 18 days previously.

Cold-storage mite survival

Amblyseius andersoni was able to survive commercial cold storage conditions at -1°C despite the sudden drop in temperature when entering cold store and the sudden rise in temperature when exiting the cold store. *A. andersoni* had been able to acclimate to an outdoor temperature of 8°C prior to entering cold store, which may have impacted survival. *Amblyseius andersoni* eggs were present in the buds, which indicates that the *A. andersoni* were fecund after cold-storage and that temperature had risen sufficiently for oviposition. Gambaro (1990) found that low temperature inhibits oviposition in *A. andersoni* but oviposition was resumed in the field in the Po Valley of Italy towards the end of April, as seen in the present experiment.

The cane samples contained the most *A. andersoni* suggesting that when the samples were collected on 30 March *A. andersoni* were still in overwintering positions, however there is also greater surface area in a cane to contain mites compared with a single leaf or bud. *Amblyseius andersoni* were found in the primocane buds on 24 April which indicates that the *A. andersoni* may have come out of overwintering positions in the canes and migrated to the newly developing buds in search of food, since TSSM were also found there and producing eggs. There were more *A. andersoni* found in the buds than the leaves, which indicates that the majority of mites had not moved up the plant into the foliage by 30 April.

Only canes one to four for each replicate contained mites, showing that the maximum time the mites could survive in the cut canes before entering the Tullgren funnels was 17 days. Gambaro (1990) found that more than 20% of diapausing female *A. andersoni* were able to survive starvation for 40 days and some females could continue to survive starvation once diapause had ended. Diapause can be maintained in TSSM for several months under short day conditions (Veerman, 1977). However, Koveos et al., (1993) found that the longer TSSM spends in cold storage the shorter the critical daylength for ending diapause becomes until photoperiodic sensitivity is lost altogether. After 15 weeks in cold storage it is likely that TSSM would have terminated diapause upon leaving cold storage, after being exposed to rising temperatures despite being kept in black bags, which exclude light, for the Tullgren Funnel experiment. Therefore it is possible that the mites survived 17 days of starvation after diapause had terminated.

This experiment has shown that TSSM can survive commercial cold storage conditions at -1°C and the adult females are fecund after leaving cold storage. Two-spotted spider mite was successfully extracted from overwintering crevices in the canes collected at the end of March. However, by the end of April a greater mean number of TSSM was present on the primocane buds compared with the newly expanded leaves, where only one TSSM was found. This could suggest that the overwintered TSSM in the canes are more likely to move down the cane towards the growing primocane buds than up the cane to the buds breaking on the floricane, where the uppermost buds break first. Slightly more *A. andersoni* than TSSM were found in the leaf samples, as an indicator of their greater mobility in search of food. The high mean numbers of eggs found in the buds shows that TSSM was reproducing before moving into the foliage. Leaf damage is often the first visible sign of an infestation, however this experiment suggests that there could be a significant infestation in the buds before any leaf damage is seen.

***Phytoseiulus persimilis* survival**

No *P. persimilis* were found, indicating that they did not survive cold storage or the 2019-2020 UK winter, in ambient conditions. *Phytoseiulus persimilis*, subtropical in origin does not enter diapause, which is a mechanism by which many species of arthropods successfully overwinter in climates with cold winters (Morewood, 1993). Cold-hardiness can be enhanced by diapause but may also occur independently, *P. persimilis* has been shown to be more cold-hardy than non-diapausing *Neoseiulus cucumeris* without a reduction in fecundity or longevity (Morewood, 1993). Hamamura et al., (1976) found that adult female *P. persimilis* could survive four days at 0°C and two days at -5°C in Japan. This indicates that *P. persimilis* could have survived the subzero events in ambient conditions since they did not last for more than one night, whereas the prolonged subzero temperature in cold-storage is likely to have killed the *P. persimilis*. However since *P. persimilis* does not enter diapause, which causes the body to accumulate lipid reserves it is likely that any surviving *P. persimilis* would have died of starvation since (Morewood, 1993). TSSM would have been hidden in crevices and *P. persimilis* locate TSSM using plant volatiles as a result of feeding damage and diapausing TSSM do not feed (Nachappa *et al*., 2006). If any *P. persimilis* did survive cold storage or ambient conditions in winter then they would have been in too low numbers to be detected by the sample size.

Discussion of Aim 5: Compare the level of mite survival achieved by *A. andersoni* and *P. persimilis* alone, in combination, when *A. andersoni* is supplemented with pollen and when an additional release of *A. andersoni* is made in the autumn.

There were significantly more *A. andersoni* eggs in cold-stored bud samples where *A. andersoni* had been released with Nutrimite™ compared with releasing *A. andersoni* alone or with an additional autumn release. Although the results were not statistically significant TSSM was notably absent from the plots where *A. andersoni* had been released with Nutrimite™. The Nutrimite would have degraded around two weeks after the last application in August and therefore will not have had a direct effect on the overwintering population. However this shows the importance of early control of TSSM, since applications of Nutrimite™ boosted the *A. andersoni* population early in the season, which led to significantly fewer TSSM and eggs compared with the control and therefore fewer overwintering TSSM. A much larger trial would be needed in order to show statistically significant results between treatments due to the low number of mites and eggs found in the samples.

The results suggest that there was no benefit to the autumn release of *A. andersoni*. Only diapausing females survive the winter by going into diapause, which is induced in female juveniles developing from eggs laid between the end of August and beginning of September when the photoperiod is 13 hours (Gambaro, 1990), or given a critical photoperiod of 14.5 h (van Houten & Veenendaal 1990). The autumn release of *A. andersoni* was made on 5 September which may have been too late for the released mites to lay eggs and receive exposure to diapause inducing conditions at the critical life stage. Most phytoseiids must be exposed as juveniles in order for diapause to be expressed after mating, whereas *A. andersoni* female adults remain sensitive to diapause inducing conditions and can enter diapause as adults (Morewood, 1993; van Houten, 1989). This indicates that the autumn released *A. andersoni* adults should have been able to enter diapause, although this may have made more of a difference on the overwintered population if the release had been made earlier.

Conclusions

- Biological control in all four treatment programmes was used successfully for TSSM in a propagated raspberry crop.
- Early releases of *A. andersoni* led to better control of a late infestation of spider mite compared to a preventative release of *P. persimilis.*
- Significantly fewer TSSM, eggs and leaf damage were recorded where *A. andersoni* was released with pollen compared to the *P. persimilis* control, but not significantly fewer than where *A. andersoni* was released without pollen.
- Released *P. persimilis* disappeared before spider mite was present.
- A late *Phytoseiulus persimilis* release in warm sunny September led to good establishment but did not control spider mite by October.
- Nutrimite™ boosted the *A. andersoni* population on one date, shortly after transplant to the field.
- Further research is needed on best practice use of *A. andersoni* for TSSM control in raspberry within an IPM programme.
- *Amblyseius andersoni* and TSSM survived 15 weeks of commercial cold-storage at -1°C in low numbers.
- Two-spotted spider mite was found in the ambient-stored primocane buds at the end of March but *A. andersoni* was not.
- Significantly more *A. andersoni* eggs were found in the cold-stored primocane buds of plants where *A. andersoni* and Nutrimite™ had been released in the previous season compared with the plants where *A. andersoni* had been released alone or alone with an additional autumn release.
- Making an additional autumn release of *A. andersoni* on 5 September did not significantly affect the number of overwintered *A. andersoni* or TSSM.

Knowledge and Technology Transfer

A workshop presenting interim results was presented at AHDB Horticulture Fruit Agronomist's Day. 11th September 2019 at NIAB EMR, East Malling, Kent.

A results summary was presented at AHDB Horticulture Soft Fruit Day. 20th November 2019 at NIAB EMR, East Malling, Kent.

A results summary was presented at AAB conference – Advances in Biological Control and IPM 2019: Addressing the Innovation Crisis. 21st November at Olde Barn Hotel, Lincolnshire.

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Appendices

Appendix 2.1 Random sample mean % TSSM damage per leaflet. Means followed by the same letter are not significantly different, P>0.05. P values, s.e.d and l.s.d refer to ANOVA.

Date	T1	T2	T3	T4	P value	D.f	S.e.d	l.s.d
10 Jul	0a	0a	0a	0.038a	0.426	12	0.02652	0.05777
24 Jul	0a	0a	0a	0.088a	0.426	12	0.0619	0.1348
07 Aug	0a	0a	0.013a	0a	0.426	12	0.00884	0.01926
05 Sep	0.175a	0.019a	0a	0.225a	0.348	12	0.1442	0.3141
16 Oct	0.325ab	0.062a	0.237ab	0.587b	0.155	12	0.2139	0.4661

Appendix 2.2 Target sample mean % TSSM damage per leaflet. Means followed by the same letter are not significantly different, P>0.05. P values, s.e.d and l.s.d refer to ANOVA.

Date	T1	T2	T3	T4	P value	D.f	S.e.d	l.s.d
10 Jul	0a	0.05a	0a	0a	0.426	12	0.0354	0.077
24 Jul	0.85a	0a	0.075a	0.475a	0.509	12	0.615	1.34
05 Sep	6.838a	2.888a	3.325a	3.875a	0.357	12	2.319	5.054
16 Oct	10.8ab	4.287a	5.250ab	13.475b	0.098	12	3.85	8.38

Appendix 2.3 Random sample mean numbers of TSSM adults/nymphs per leaflet. Means followed by the same letter are not significantly different, P>0.05. P values, s.e.d and l.s.d refer to ANOVA.

Date	T1	T2	T3	T4	P value	D.f	S.e.d	l.s.d
24 Jul	0a	0a	0.008a	0a	0.426	12	0.00589	0.01284
05 Sep	0.063a	0.013a	0a	1.063a	0.175	12	0.526	1.145
16 Oct	0.5a	0.8a	1.7b	8.2b	0.084	12	3.08	6.7

Appendix 2.4 Mean numbers of TSSM eggs per leaflet. Means followed by the same letter are not significantly different, P>0.05. P values, s.e.d and l.s.d refer to ANOVA.

Date	T1	T2	T3	T4	P value	D.f	S.e.d	l.s.d
10 Jul	0a	0.008a	0.008a	0.008a	0.773	12	0.00962	0.02097
05 Sep	0.562a	0a	0a	5.862a	0.185	12	2.93	6.39
16 Oct	3.0ab	0.8a	2.9ab	18.8b	0.109	12	7.45	16.23

Appendix 2.5 Mean numbers of *A. andersoni* per leaflet. Means followed by the same letter are not significantly different, P>0.05. P values, s.e.d and l.s.d refer to ANOVA.

Date	T1	T2	T3	T4	P value	D.f	S.e.d	l.s.d
15 May	0.008a	0 a	0 a	0 a	0.426	12	0.00589	0.01284
05 Jun	0.158a	0.158a	0.217a	0.050a	0.233	12	0.0769	0.1676
26 Jun	0.017a	0.050a	0.175b	0.017a	0.008	12	0.0426	0.0927
10 Jul	0.017a	0.075b	0.058ab	0.017a	0.061	12	0.02332	0.05082
24 Jul	0.067ab	0.200b	0.108ab	0.025a	0.125	12	0.0691	0.1506
07 Aug	0.100ab	0.108ab	0.133b	0a	0.081	12	0.0490	0.1068
05 Sep	0.287a	0.225a	0.163a	0.013a	0.171	12	0.1185	0.2583
16 Oct	0.2a	0.138a	0.138a	0.050a	0.362	12	0.0806	0.1756

Appendix 2.6 Mean numbers of *A. andersoni* eggs per leaflet. Means followed by the same letter are not significantly different, P>0.05. P values, s.e.d and l.s.d refer to ANOVA.

Date	T1	T2	T3	T4	P value	D.f	S.e.d	l.s.d
05 Jun	0.058ab	0.008a	0.083b	0.017ab	0.089	12	0.0301	0.0657
26 Jun	0a	0a	0.033b	0a	0.023	12	0.011	0.024
10 Jul	0a	0.017a	0.008a	0a	0.577	12	0.01361	0.02965
24 Jul	0.017a	0.017a	0.025a	0a	0.561	12	0.01751	0.03816
07 Aug	0a	0.008a	0.008a	0a	0.618	12	0.00867	0.01890
05 Sep	0.025a	0.038a	0.075a	0a	0.227	12	0.0342	0.0746

Appendix 2.7 Mean numbers of *P. persimilis* per leaflet. Means followed by the same letter are not significantly different, P>0.05. P values, s.e.d and l.s.d refer to ANOVA.

Date	T1	T2	T3	T4	P value	D.f	S.e.d	l.s.d
05 Jun	0a	0a	0.008a	0a	0.426	12	0.00589	0.01284
16 Oct	0.012a	0.012a	0.037a	0.125a	0.214	12	0.0574	0.1251

Appendix 2.8 Mean numbers of *P. persimilis* eggs per leaflet. Means followed by the same letter are not significantly different, P>0.05. P values, s.e.d and l.s.d refer to ANOVA.

Date	T1	T2	T3	T4	P value	D.f	S.e.d	l.s.d
16 Oct	0a	0a	0a	1.71a	0.298	12	1.03	2.25

Objective 3: To review biennial cropping and annual cropping of long-canes as a means of pest and disease control and carry out a financial comparison of different production systems

Background to observations of increased damage by cane and midge blight

In recent times, raspberry cane blight and midge blight have become more prevalent in the UK raspberry industry, leading to some significant cane dieback or death and subsequent crop losses. This is not a new problem, but one which has periodically appeared over the last 40-50 years in commercial UK plantations. The use of midge and blight susceptible cultivars and the lack of effective measures for control have exacerbated the problem. The opportunistic fungal pathogens responsible for midge blight can vary and can include *Leptosphaeria coniothyrium* and *Fusarium* species.

Leptosphaeria coniothyrium is the primary pathogen causing cane blight. Infection of primocane occurs during the growing season, usually but not exclusively at the site of a wound caused by mechanical or physical injury, such as that caused by frost. The use of mechanical harvesters has often been attributed to such damage, but poor support and management of primocane can also create wound sites. Leaving cane stubs at ground level during the task of spent floricane removal is an example of this. Midge blight is an associated disease which can be caused by various pathogens, which infect primocane on the periderm of the cane where raspberry cane midge larvae (*Resseliella theobaldi*) have been feeding. Varieties with rind that splits readily on primocane, are more susceptible, as the raspberry cane midge adult female is able to lay its eggs underneath these splits. The resulting larvae can remain undetected whilst they feed on the periderm. Various pathogens can gain entry to the periderm and infect the water conducting tissues giving rise to midge blight.

The pathogens contributing to cane and midge blight overwinter in the primocane. The resulting fruiting floricane fail to break bud, or wilt the following spring at any stage from bud break until the fruit begins to ripen.

To gain control, growers aim to improve the management of their primocane to prevent mechanical or physical injury, thereby reducing sites which allow infection by cane blight. They also monitor for the presence of raspberry cane midge to allow them to implement chemical control measures of the pest at the optimum time to reduce feeding on primocane and avoid further fungal infection which sets up midge blight. However, they have also resorted to the use of fungicidal sprays to gain control of the pathogens that cause the blight. There are currently no reliable spray products available in the UK for control of raspberry cane midge and only one fungicide product (with limitations on use) for cane blight control.

With the continuing loss of available control products for these pathogens and the unlikely release of viable alternatives, the industry needs to develop alternative ways of reducing or avoiding cane midge damage and associated disease infection. One way of achieving this is to separate the two phases of raspberry growth – the vegetative phase (primocane) and the fruiting phase (fruiting canes or floricane).

Background to biennial and annual cropping techniques

Biennial cropping

Biennial cropping is a system of production which achieves this separation in summer fruiting (floricane) varieties. It was experimented with at research stations in the UK in the 1970s and is still practiced in some countries today. Biennial crops are established like any summer fruiting raspberry plantation that would be retained to crop for the following 6-10 years. Once established, the plantation is divided into two.

In half of the crop, the primocane is removed several times during the growing season, so that only fruiting (floricane) is retained to develop and produce fruit. Primocane is removed until the middle or end of harvest, a process which involves directed application of a herbicide/desiccant as soon as possible after its emergence from the soil or substrate surface. This prevents competition between primocane and floricane for light, water and nutrients, helping to reduce required inputs whilst improving fruit size, quality and evenness of ripening. It can also advance the onset of harvest, improve the presentation of the fruit to the pickers and reduce both picking costs and the labour costs associated with cane management. That part of the crop that is fruiting is termed the ‘on-year’. Because of the separation of the two phases of growth, it is possible to retain a higher number of fruiting canes in the ‘on-year’ thereby increasing the picked yield per unit area of crop.

In the other half of the biennial cropping plantation, all fruiting (flori) cane is removed after harvest and the following year, only primocane develop to generate a new stand of fruiting canes for the year after. As the two phases of growth have been separated, a greater number of canes can be retained than normal in a traditional growing system. The part of the crop which develops only primocane is termed the ‘off-year’. A major advantage of growing only primocane in this year is that they can be trained into their final fruiting position unhindered, without being damaged by pickers, tractors or other machinery, which can lead to infection by cane blight. Similarly, should the primocane rind split, allowing invasion by raspberry cane midge adults, the application of any control products is more effective, as access is improved due to the loss of competition with fruiting canes. Furthermore, there are some control products which have a 365-day harvest interval which could not be used in a fruiting crop, but

which can be used in the ‘off-phase’ of production. By separating the two phases of growth, less cross-infection from fruiting canes to new primocane occurs, creating another benefit.

Apart from separating the two phases of growth and potentially breaking the life-cycle of diseases and insect pests, there are other advantages to be had, primarily in the cane management costs. The training and support of canes is made easier and simpler, while cutting out and tying in new canes can be done more quickly and even mechanised in some cases. There are therefore associated labour savings. Through a combination of higher yields per unit area in the ‘on-phase’, reduced production and picking costs overall and improved pest and disease control, researchers found that biennial cropping could provide equal or better gross margins to those achieved using traditional cropping systems.

Despite these considerable advantages for pest and disease control and reduction in labour and management costs, biennial cropping has not been adopted on a wide scale in the UK for a number of reasons. In the 1970s and 1980s, the cultivars of the day did not lend themselves to the system. Many of them did not have sufficient vigour, while some proved to be susceptible to aphid or mite-borne viruses and soil borne disease (notably *Phytophthora rubi*). As a result, many crops converted to biennial systems on commercial sites were unable to cope with the demands imposed on them so lost vigour very early in their lives. With the development of new and improved cropping systems, use of cultivars with improved vigour in containerised production, irrigation/feed through drip irrigation systems and protected cropping with associated increase in air temperatures, it is believed that biennial cropping could be more viable today than it was before.

Annual cropping of long-canes

Another system that achieves separation of the two phases of growth is ‘long-cane’ material in annual cropping systems. The term ‘long-cane’ refers to a single primocane grown in a propagation site, separate from a fruiting plantation. The cane grows in a field soil, is lifted with its roots intact at the end of the season then transferred to its final fruiting site. Alternatively and more commonly in commercial production in 2020, it is grown in a module or pot in a soilless substrate, then transplanted and grown on in the same container or new container in its final fruiting position.

To avoid the risk of *Phytophthora rubi* infection, which is commonly present in field soils in fruit growing regions and which can cause devastating crop losses, the majority of UK growers now establish summer-fruiting (floricane) cultivars using long-cane material planted into pots containing coconut fibre (coir). Establishing these long-canes and cropping for one year only, effectively offers a similar alternative to biennial cropping, as both phases of growth are separate, offering the same advantages in control of cane blight and midge blight.

Unlike biennial cropping however, use of annual cropping of long canes allows the timing of crop production to be scheduled to suit the grower. Long canes generally crop some 90 days after planting and establishment, so to delay cropping until the late summer and autumn months, cane can be held in cold store over the winter and spring months and removed for planting in early summer to crop in the autumn months. Although cold-storage of long canes incurs significant costs, one advantage is that the cane receives adequate chilling to ensure full bud break down the length of the cane allowing it to achieve its maximum yield potential. With the ever-warmer winters experienced in the UK, over-wintered crops do not always achieve adequate winter chill to produce a full crop the following season.

Financial comparison of different production systems

Given the knowledge that separating the two phases of growth of summer fruiting raspberries (floricane varieties) offers significant advantages in crop protection and labour management costs, the project steering group decided to make financial comparisons between different production systems, to allow growers to weigh up the advantages and shortcomings of each system.

Salih Hodzhov (industry representative for Project SF 158) agreed to help by providing financial information from the raspberry production systems employed by W.B. Chambers Farms Ltd in Kent. Salih worked with Scott Raffle (AHDB) and Janet Allen (ADAS) to construct typical gross margin budgets on a spreadsheet to compare the following production systems:

- **Traditional** cropping of the summer fruiting raspberry Glen Ample
- **Annual** cropping of the summer fruiting raspberry Glen Ample
- **Biennial** cropping of the summer fruiting raspberry Glen Ample
- **Annual** cropping of the **primocane** raspberry Kweli
- **Traditional** cropping of the **primocane** raspberry Kweli

Technical details of the systems compared

The costs, returns and margins for each were compared, using the typical production systems, yields, prices and costs experienced each year by W.B. Chambers Farms Ltd. The following assumptions are made:

Glen Ample raspberry is grown as:

1. Traditional cropping system growing fruiting canes and vegetative canes simultaneously
2. Annual system planting fresh cold-stored long-canes every year

3. Biennial system, where the fruiting and vegetative phases of growth are separate.

Each of the above growing systems is established in Year 1 using long-cane plants. These plants are often produced by specialist propagators in modules, to a standard specified by the grower customer. They are cold-stored until the required planting time, usually in May, and crop in August and September.

Kweli raspberry is grown as:

1. Planted in Year 1 in March using tip plants and allowed to develop into a single primocane to crop from August onwards
2. Planted as cold-stored long-canes planted in May and cropped in August and September.

Like Glen Ample, the canes are often produced by specialist propagators in modules to a standard specified by the customer.

All of the crops covered by this exercise are grown under protective polythene tunnel structures.

The Glen Ample canes are planted into 7.5 litre rigid plastic pots of coir substrate and laid on raised beds, which are covered by polythene mulch. Canes are supported using an ADAS moveable wire support trellis and protected by a moveable Spanish style tunnel clad with polythene. The Glen Ample canes are planted at a spacing of two per 7.5 litre pot (density of 2.5 pots per metre) and 8' (2.44 m) between rows with a final cane density of 20,000 canes per hectare (10,000 pots/ha using 75,000 litres or 75 m^3 of substrate) with two spaghetti irrigation drippers per pot.

The long-cane primocane system uses the variety Kweli, planted at a spacing of two long canes per 7.5 litre pot (density of two pots per metre) and 8' (2.44 m) between rows with a final cane density of 16,000 per hectare (8,000 pots/ha using 60,000 litres or 60 m^3 of substrate). The traditional primocane system uses Kweli tips, which are planted at a spacing of two per 7.5 litre pot (two pots per metre) and 8' (2.44m) between rows with a final cane density of 16,000 tips per hectare (8,000 pots/ha using 60,000 litres or 60 m^3 of substrate).

At the end of Year 1, the systems are managed differently. For the Glen Ample in the traditional system, fruiting canes are cut out and removed and primocane (vegetative) are tied in to their final fruiting position at a density of seven canes per metre (28,000 canes/ha). Thereafter, primocane are allowed to develop each year but thinned by hand during the growing season to 7 per metre for tying in to fruit the following year after cutting out the old fruiting canes.

In the annual system, the canes and pots are removed and new canes are planted into new pots in a repeat of the first year.

In the biennial system, the area of canes is split into two halves. In one half, all canes (fruiting and vegetative) are cut out and in Year 2, primocane are allowed to develop and grow to achieve a density of 7.5 primocane per metre or 30,000 canes per ha (Vegetative phase). In the other half, 7.5 primocane per metre are retained (30,000 canes per ha) to fruit in Year 2 (Fruiting phase). During Year 2, as new primocane develop, they are removed completely twice in the season using a directed spray of the desiccant carfentrazone-ethyl (Shark). Thereafter, any developing primocane is removed by hand. From Year 3 onwards, the system is reversed each year so that the Vegetative Phase becomes the fruiting phase and so on.

For the Kweli long-cane system, the canes and pots are removed and new canes are planted into new pots in a repeat of the first year.

For the traditional Kweli primocane system, the primocane which were planted in Year 1 are retained as floricane at two canes per pot (16,000 canes per ha), to overwinter and crop in the spring of Year 2. During their spring cropping, four primocane are allowed to develop per pot (32,000 canes per ha) to fruit in the autumn. In Year 3, all canes are cut out and four primocane allowed to develop per pot (32,000 canes per ha) for a single autumn crop.

Budget assumptions

A great many assumptions were included in the budget comparisons. These included assumptions on receipts, variable costs of price, variable costs of yield and variable costs based on area. These are broken down as follows:

Receipts

- Yield per cane (kg) and hectare (tonnes)
- Price

Variable costs of price

- Commission

Variable costs of yield

- Transport costs per tonne
- Packhouse labour costs per tonne
- Packaging per tonne
- Picking per tonne

Variable costs of area

- Canes
- Planting per thousand canes
- Pots
- Drippers
- Substrate
- Water
- Feed
- Crop protection (herbicide, fungicide, insecticide, biocontrol)
- Lateral training labour costs
- Primocane removal labour costs
- Cutting-out labour costs
- Tying-in labour costs

Costs not included in the budgets constructed

- The cost of tunnel structures and polythene covers
- The cost of erecting tunnel structures and subsequent venting
- The cost of in the irrigation infrastructure, only spaghetti drippers

Other assumptions

It should also be noted that a single price was used for all of the budget comparisons, irrespective of the time of year. For most growers, prices would vary depend on time of year and demand for fruit, but it was felt that it would be simpler to keep the same price for all weeks of the marketing season.

The budgets were created, not to show how much margin could be made from each system, but to compare each on a like for like basis, to illustrate if growers would be disadvantaged by employing biennial cropping or annual cropping of long-canes to provide a novel way of reducing pest and disease attack and yield loss. The budgets created also assumed that each crop is healthy and has not been adversely affected by pest or disease attack.

Results of budget comparisons

After compiling budgets for the five production systems, a gross margin figure was revealed for each. The budgets were compiled on a spreadsheet. They were assessed by Salih

Hodzhov, in consultation with Scott Raffle and Janet Allen, to ensure that they were representative of production at W.B. Chambers Farms Ltd.

For reasons of commercial confidence, the AHDB soft fruit panel members and W.B. Chambers Farms Ltd agreed that the returns and costs that were laid out in the spreadsheet, should not be made publicly available in this report. Instead, it was agreed that the relative returns and costs for Glen Ample (summer fruiting raspberry) and Kweli (primocane raspberry) should be presented for each system as a percentage of the traditional systems employed.

Table 3.1 summarises the differences between the systems in each of the three years calculated comparing the yield per hectare for each, the cane management costs for each and the gross margin for each. The traditional Glen Ample (summer fruiting) is used as a baseline against which the annual long-cane Glen Ample and biennial Glen Ample are compared. The traditional Kweli is used as a baseline against which the annual long-cane Kweli is compared.

In Year 1, the yields and gross margins of the annual and biennial systems compare favourably with the traditional summer fruiting and traditional primocane production systems. The annual long-cane system produced over twice the gross margin of the traditional while the biennial system produced over a third more. The annual long-cane primocane system produced almost two and half times the gross margin of the traditional primocane.

In contrast, in Years 2 and 3, the yields and gross margins of the annual and biennial systems compared poorly with the traditional systems and in the majority of cases, these were less than those produced by the traditional Glen Ample and Kweli crops.

In all three years, there were reductions in cane management costs when using annual or biennial cropping compared to traditional.

Interpreting the results

Although the results look favourably on the first year of production and less so on the second two years, caution should be exercised when considering these. The budgets which were constructed took no account of crop loss caused by insect pests or diseases, assuming that optimum yields could be achieved from each system. The main purpose of undertaking this project was to identify new and alternative measures for controlling insect pests and diseases of commercial raspberry crops. Previous work has demonstrated the benefits of separating the two phases of growth in raspberry in breaking the life-cycles of cane blight, raspberry

cane midge and associated midge blight. The purpose of this financial comparison was to assess the impact that this would have on the profitability of raspberry production.

In practice, it is likely that raspberry growers will continue to use all five of these production systems to spread the season of production and fill gaps in the season to improve the yield profile to maintain continuous sales of fruit.

However, the exercise has demonstrated the change in finances that may occur should labour availability and loss of crop protection products render the systems of growing more or less viable than at present. Many pests and diseases are becoming increasingly difficult to contain with a diminishing number of crop protection products available. As the availability of these products declines further, raspberry growers may find they have no alternative but to turn to annual cropping of long canes or biennial cropping to achieve satisfactory control.

Table 3.1. Relative differences (expressed as %) between parameters within the budgets for the five growing systems analysed over three years.

For Glen Ample (summer fruiting), the annual and biennial crops are compared to the traditional.

For Kweli, the long cane primocane is compared to traditional primocane.

	Year 1					Year 2					Year 3				
	Traditional	Annual	Biennial	Traditional primocane	Long cane primocane	Traditional	Annual	Biennial	Traditional primocane	Long cane primocane	Traditional	Annual	Biennial	Traditional primocane	Long cane primocane
Yield / ha	0	+17	+6	0	+79	0	-11	-40	0	-20	0	-11	-40	0	+11
Cane management costs/ha	0	-87	-22	0	-21	0	-87	-22	0	-87	0	-87	-22	0	-57
Gross margin	0	+205	+137	0	+249	0	-40	-33	0	-44	0	-39	-32	0	-36

