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

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AUTHENTICATION

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

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GROWER SUMMARY

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

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

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

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

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

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

For ease of reading, this Grower Summary report is split into sections for each of the objectives (pests & diseases) being worked upon. The first year's work concentrated on Objectives 1 to 4, so only these are reported on in this first annual report.

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Raspberry root rot

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

Headline

- Prestop and three coded products showed some promise in controlling *Phytophthora rubi* when compared to Paraat treated plants and an untreated control.

Background and expected deliverables

Phytophthora root rot is now the most destructive disease of raspberries worldwide. Where raspberries have been grown in the soil *Phytophthora rubi* (previously known as *P. fragariae* var. *rubi*) is almost ubiquitous. Outbreaks of this disease across Europe at the same time in traditional raspberry-growing areas suggests that the disease has spread through the propagation network and has been distributed to farms in new planting material (Graham et al. 2011). Current approaches for Phytophthora control rely on fungicide applications twice per year either as a soil-applied drench or through the drip irrigation. SL567A (44.7% w/w metalaxyl-M) and Paraat (500 g/kg dimethomorph) can be used, although resistance developing in pathogens where products have only a single mode of action is a major concern. To reduce the risk of this occurring, agrochemical companies are developing co-formulated products such as Fenomenal (fenamidone and fosetyl-aluminium) which should be gaining approval for cane fruit. Although this will help, none of these types of products can completely control this disease.

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

Task 1.1: To elucidate whether more-susceptible varieties cause greater attraction of *P. rubi* zoospores than more-resistant varieties.

Task 1.2: To determine if selected elicitors and nutrients have a detrimental effect on *P. rubi* infection.

Task 1.3: To determine if or how selected fungal and bacterial supplements or microbial plant protection products have a detrimental effect on *P. rubi* infection.

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Task 1.4: To determine whether non-conventional products (microbial and plant stimulants) have the potential to maintain and potentially improve plant establishment and root health of raspberry plants when applied from propagation onwards.

Summary of the project and main conclusions in year 2

In 2015, Tulameen plants in growing media had been treated with biostimulants and growth promoters following propagation. In 2016, these were potted up at two separate sites - a commercial farm in Oxfordshire and ADAS Boxworth. The plants then received further treatments at each of the sites according to the product labels. Treatments included Prestop (*Gliocladium catenulatum*) as well as products without approval for use in growing media in cane fruit. They were compared with a single dench of Paraat (dimethomorph).

The ability of the products to improve crop performance and vigour was investigated at the Oxfordshire site, but no differences were found between any of the treatments and the untreated control.

At ADAS Boxworth, plants were treated with preventive applications of products before inoculation with *P. rubi*. In some cases, curative applications were also made. No collapse of primocanes occurred during 2016 as a result of root rot, but destructive assessments were made for root rot in early 2017 and the results will be reported later.

Prestop and three of the coded products had slightly, but significantly, increased the number of primocanes compared with the untreated control and Paraat treated plants. No phytotoxicity was caused by any of the products at either site. The Oxfordshire site is planned to continue for a second cropping year with further treatment applications.

Financial benefits

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

Assuming a typical return for raspberries of £6.49/kg to growers (Defra Basic Horticultural Statistics 2014) and a yield of 14 tonnes/ha, then 75% crop loss would lead to a financial

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loss of £68,166/ha. Increasing the health of propagation material and providing material that is more resistant to the disease would not only significantly reduce such losses but lengthen the life expectancy of a raspberry plantation, thereby reducing the additional costs of re-establishing new plantations on a frequent basis.

Action points for growers

- Consider biological alternatives to plant protection products for the control of *P. rubi.*

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Two-spotted spider mite

Objective 2 – To develop and maintain IPM approaches to successfully control two-spotted spider mite whilst controlling spotted wing drosophila (SWD) and capsids with insecticides

Headline

- The use of overhead mist spraying using large droplet size reduces spray coverage on the undersides of leaves in the crop canopy, offering more refuge to predatory mites which control two-spotted spider mite.

Background and expected deliverables

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

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

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

This study aims to address this problem through two specific work packages:

Task 2.1: To investigate consequences of SWD control strategies on two-spotted spider mite populations on a commercial holding already dealing with the pest.
Task 2.2: To develop compatibility strategies for biocontrol of two-spotted spider mite by predatory mites with insecticide sprays for SWD and capsids.

Summary of the project and main conclusions in year 2

In 2015, the effects of overall canopy spraying verses 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 both the overall effect of date and overall effect of treatment were significant. In early August, the numbers of natural phytoseiid mites were lower in both of the sprayed treatments, possibly following specific spray applications of spinosad. The numbers of TSSM then rose significantly in the sprayed plots from the 17 August 2015. The numbers of SWD were lower in both of the treated plots (spraying and overhead misting).

In 2016, the effects on raspberries in tunnels of overall canopy spraying verses overhead application of a programme of sprays of Decis (deltamethrin) and Tracer (spinosad) on TSSM**,** naturally occurring predatory mites and introduced *Phytoseiulus persimilis* were compared**.** Nozzles were used that gave a slightly larger droplet size than in 2015 in order to determine whether this would give less spray on the underside of the leaves, so providing refuge for the predatory mites and therefore improved control of TSSM. The results showed that both treatments reduced the number of SWD compared with the untreated. There was less spray on the underside of the leaves in the overhead spray treatment. TSSM numbers were higher in the sprayed treatments (for all life stages with the knapsack spray). The natural phytoseiids were affected by the spray treatments, but the effect could be mitigated by spraying from above. Introduced *P. persimilis* was less affected by the spray programme than anticipated.

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Financial benefits

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

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

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

Action points for growers

- Be aware of the contribution of natural predators in the control of two-spotted spider mite.
- Using spray coverage that allows predators to shelter from control products intended for SWD is likely to preserve natural and introduced predators.

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Blackberry leaf midge

Objective 3 – Develop and combine novel and current IPM approaches to successfully control blackberry leaf midge

Headline

- *Steinernema kraussei* can reduce blackberry leaf midge emergence from pupae in the ground.

Background and expected deliverables

The blackberry leaf midge (*Dasineura plicatrix)* has become an increasing problem on blackberry, hybrid berry and increasingly raspberry, with double cropping primocane raspberries being particularly vulnerable to attack by this pest. The blackberry leaf midge can have up to four generations per year under protection and causes damage to the leaves and the growing points of plants. This can result in the stunting of cane growth leading to loss of yield. It has been estimated that the midge could reduce raspberry yield by 40% and blackberry yield by 10% (Fountain, 2013). This pest has increasingly been identified as a high priority by the industry, particularly in primocane systems.

Project SF 102 'Biology and integrated control of blackberry leaf midge on blackberry and raspberry' (Bennison, 2011) assessed alternative ground-based methods of control for this pest. Treatments included cultural controls, ground-based predator introductions and entomopathogenic fungi applications to soil/substrate. Promising approaches were identified in laboratory tests, particularly covering the soil surfaces with polythene. Another promising approach included introducing the predatory mite *Macrocheles robustulus*. The entomopathogenic fungus *Beauveria bassiana* (Naturalis-L) was not effective when applied as a soil drench or as a foliar spray.

Since completion of SF 102, another entomopathogenic fungus, *Metarhizium anisopliae* (Met 52) (recommended for vine weevil control) has gained EAMUs for use on rubus hybrids either pre-planting as a growing media-incorporated treatment or as a post-planting mulch for control of midges with a pupal stage in the soil and warrants testing against blackberry leaf midge. A liquid formulation of Met52 (Met52 OD) is now approved in the UK as a foliar spray on various crops including protected strawberry for control of thrips and mites. The product is not currently approved on cane fruit or for use as a drench but it is possible that an EAMU could be sought if shown to be more effective than Met52 granular, as a substrate drench would be more practical than incorporation for soft fruit growers.

Entomopathogenic nematodes were not tested as a ground drench in SF 102 as recent

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work had been carried out at Wageningen investigating three species of nematodes (*Steinernema feltiae*, *S. carpocapsae* and *Heterorhabditis* sp.) but none were found to be effective against blackberry leaf midge larvae (Wenneker, 2008 and personal communication). *Steinernema kraussei* is widely used for vine weevil control on soft fruit but has not yet been tested against blackberry leaf midge.

Spinosad (Tracer) is used as a drench to brassica modules for control of cabbage root fly and could have potential for control of blackberry leaf midge larvae when they drop to the ground to pupate. Tracer has EAMUs for use as a foliar spray on protected and outdoor crops of raspberry for control of thrips so if it was shown to be effective as a ground drench for control of blackberry leaf midge, an EAMU application could be made for use as a drench.

Project SF 141 'Efficacy of insecticides, timed use of the blackberry leaf midge sex pheromone trap, to control the pest on raspberry' provided a strategy for the control of blackberry leaf midge. However the insecticides used at present are not IPM-compatible and the strategy has practical limitations. Problems with spray coverage and timing mean that other tools are required to limit the threat that this pest poses.

Summary of the project and main conclusions in year 2

The results of a laboratory test in the first year of the project showed that a drench of the entomopathogenic nematode, *Steinernema kraussei* (Nemasys® L) to coir substrate in pots significantly reduced the numbers of adult blackberry leaf midges emerging compared with water controls after adding fully grown midge larvae to the treated substrate surface to mimic them dropping from infested leaf tips to the ground to pupate.

In Year 2, a field trial was done to test drenches of Nemasys® L applied to the soil beneath the crop canopy of a commercial soil-grown raspberry crop under a polythene tunnel. The crop had a history of blackberry leaf midge. Nemasys® L is already widely used by soft fruit growers for control of vine weevil. Two consecutive drenches were made to replicate plots by the host grower, the first on 5 May following the first midge larvae being recorded in leaf tips on 27 April and the second on 6 June following the second generation of midge larvae being recorded in leaf tips on 31 May. Assessments were made of numbers of twisted leaf tips, infested leaf tips and numbers of midge larvae in infested leaf tips every two weeks from 5 May to 4 July.

The mean percentage of twisted and infested leaf tips and mean numbers of midge larvae in infested leaf tips were not reduced in Nemasys ® L-treated plots compared with those in untreated plots on any assessment date. On 5 May when first generation midge larvae

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were active in leaf tips, there was a mean of 20% and 23% leaf tips infested in untreated and nematode-treated plots respectively. By 4 July, the percentage leaf tips infested had increased to 100% and 93% in untreated and nematode-treated plots respectively.

Possible reasons for the lack of control by Nemasys ® L was insufficient soil moisture to allow nematode movement and survival and the short 'window' of opportunity for nematodes to infest the midge larvae before they spin a protective cocoon in 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 for growers

- No action points have been developed for blackberry leaf midge at this early stage of the project.

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Verticillium wilt

Objective 4 – To investigate strains of Verticillium spp. present in UK cane fruit plantations and the thresholds for infection in blackberry and raspberry

Headline

- Molecular assays for *Verticillium dahliae* allow pathogen presence determination.

Background and expected deliverables

Verticillium dahliae and *Verticillium albo-atrum* are the causal agents of Verticillium wilt in raspberry and blackberry. These fungi have a wide host range of over 300 woody and herbaceous plants. In raspberry and blackberry, the disease can be very destructive 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.

Characteristic symptoms in raspberry include: Leaves turning pale and wilting; premature drop of leaves from the bottom up, occasionally leaving only a tuft of leaves at the top of canes. In severe cases, infected primocanes are stunted and develop a blue colour on one side of the cane. Symptoms differ from root rot in that wilting occurs from the top down in root rot, with the characteristic shepherds crook on spawn and suppression of spawn growth.

In blackberry, the infected canes wilt and the leaves turn yellow and become brown and necrotic similar to raspberry. However you do not see the same characteristic blue cane staining.

Severe outbreaks have occurred sporadically in UK cane fruit crops and widespread infection as a lower incidence is suspected. The causal fungi are difficult to isolate from infected canes so diagnosis is often presumptive (i.e. bases on symptoms). Some of the newer raspberry and blackberry varieties being planted by growers are derived from USA breeding lines with known high susceptibility to verticillium wilt.

Verticillium dahliae is considered the primary pathogen and it can survive in the soil for many years. Once susceptible plants are placed in infested ground, the fungus can grow into the xylem and colonise the whole plant. Therefore knowledge of whether the soil is infested with the fungus prior to planting is useful to aid planting decisions.

The expected minimum five year life of raspberry and blackberry plantations can be severely shortened when roots become infected by *Verticillium* species and / or *P. rubi* leading to cane death. Symptoms often do not clearly distinguish between verticillium wilt and phytophthora root rot. The area of primocane-fruiting raspberries has been increasing

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in the UK and the varieties grown tend to be susceptible to Verticillium wilt. *V. dahliae* and *V. albo-atrum* have a wide host range and persist in many soils. 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.

Real-time, or Quantitative, PCR assays (QPCR) for testing soils prior to planting for specific soil-borne *Verticillium* species using DNA extracted from large volumes (up to 1 kg) of soil have now been successfully developed (project SF 97). The techniques were initially developed during Potato Council-funded potato diagnostics research (Project R253) and utilize pre-extraction processing, buffers to remove reaction inhibitors and an automated DNA binding system to capture total DNA. These tests provide results within a few days (rather than 6-8 weeks for *V. dahliae* microsclerotia). PCR detects DNA in both dead and live cells, but microbe DNA deteriorates quickly in normal conditions and so fungicide/fumigant killed pathogens would rarely be detected. Detection of *V. dahliae* using QPCR has been achieved down to levels correlating with 0.5 microsclerotia / g soil and the assay is still being improved in order to attain a detection of <0.5 microsclerotia / g soil. It is already possible to detect below one microsclerotia by testing multiple soil extractions, but this increases the cost of the test. Low detection rates may not, however, be as important for raspberries as it is for strawberries.

Strawberry growers benefit from information on the relative susceptibility of strawberry varieties to certain levels of *V. dahliae* microsclerotia (e.g. 5-9 microsclerotia / g soil can cause 50% loss in variety x) and can base their variety selection on this information. However, the threshold for damage in *Rubus* is not known. Observations suggest 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.

Current *Rubus* varieties come from a number of breeding lines and so differences in susceptibility to *Verticillium* and *Phytophthora* are likely. The James Hutton Institute has a programme of screening lines for *Phytophthora* resistance using molecular markers. Two regions of the genome influence susceptibility to the disease. Molecular markers to aid *Verticillium* resistance breeding have not been found although variable resistance between *Rubus* species has been noted (with black raspberry very susceptible) and so there is potential for variety rankings and the production of thresholds for V*erticillium* spp. in the soil for different varieties.

V. dahliae is also a very diverse fungus with different strains reported that may differ in

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pathogenicity. Relatively little is known about verticillium in UK blackberry and raspberry crops. Nothing is known about which strains are present, their pathogenicity to individual species, the importance of soil-borne inoculum, thresholds for causing disease, and disease development in container grown crops. Work on verticillium wilt in strawberry in Project SF 97a resulted in the development of a molecular 'tool box' of real-time PCR assays for the detection of various verticillium strains directly in soil and also showed there was some diversity in the UK of *V. dahliae* strains present.

In this project, these new molecular tools will be used to determine which strains of *V. dahliae* are present in UK cane fruit crops and work towards determining the threshold for causing disease in raspberry and blackberry.

Summary of the project and main conclusions in years 1 and 2

In Year 1, *Verticillium dahliae* was detected in raspberry and blackberry plant material from the field but was not detected in young plants from three propagators. There was a relationship between DNA of *V. dahliae* in the stems and in the roots although DNA of the pathogen in stem bases was considerably higher. This will inform future sampling strategies. Little *V. dahliae* DNA was recovered from the soil under plants with *V. dahliae* DNA and the reasons are unclear.

A range of fungi were isolated from the plants including Fusarium, Alternaria and Pythium. Interestingly, Ilyonectria species were isolated from two plants and this was confirmed by DNA sequencing of the rDNA region. Ilyonectria is a known pathogen of raspberry and may also be causing plant death. Further work in this project will attempt to characterise these isolates further by sequencing additional loci.

A novel sequence from the *V. albo-atrum* EF region has been identified and four TaqMan assays have been designed and tested for specificity. Although the TaqMan assays detected *V. albo-atrum* and not *V. dahliae*, they did cross react with *V. tricorpus*. An alternative assay site has been identified. To validate the VCG1 assay, isolates belonging to different VCGs are in the process of being collected. IGS primers suitable for determining VCG through IGS sequencing have been identified and will be used once this isolate collection is complete.

Further work conducted by Fera validated a DD assay demonstrating that it is able to detect *V. dahliae* and *V. longisporum* to an acceptable level. The assay could therefore be used to detect infection of plant material by these fungi and assess infection levels in soils. The

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assay was used in 2015 for the survey of raspberry and blackberry plants, planting material and soils.

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). Since it began to cause crop loss, plant pathologists and cane fruit growers have been lacking in the knowledge of how susceptible different cultivars are to the disease at differing levels of the pathogen in field soils. This has made it difficult to make management decisions about the safety of a new field soil which has never before hosted a cane fruit crop.

By improving our knowledge of this and developing 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

- Note that stem necrosis is a better determinant of the presence of Verticillium causing wilt than leaf wilting alone.

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SCIENCE SECTION

General Introduction

This work follows on from a five-year project on integrated crop management SF 74 completed in 2011 in which foliar and cane diseases (in particular botrytis and powdery mildew) and fruit and cane pests (in particular raspberry beetle, raspberry cane midge and aphids) were researched. Since then there have been losses of conventional plant protection products including soil sterilants through EU legislation and development of products containing beneficial microbes and plant stimulants to aid plant health.

There are now longer cropping periods, more crops being grown under cover, and more crops grown in containers. All these have changed the crop environment and have allowed certain pests and diseases to become more important. Further changes in grower practice have been brought about by the arrival of spotted wing drosophila (SWD) which has meant the use of pesticides that have the potential to disrupt fruit crop control programmes using macro-biologicals. The increased cost of labour means that time cannot be wasted picking unmarketable fruit and so pest and disease control programmes need to be as good as they can be.

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

Aim

Objective 1: To investigate the effects of a range of novel plant treatments on raspberry growth and their resilience to pests and disease from propagation through to primocane production.

Introduction

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

There is interest in the use of nutrients, elicitors and microbial products against pathogens

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such as *Phytophthora* spp. and also pests. Phosphites and silicon for example have been shown to prime plant defence responses and other products can stimulate systemic acquired resistance (SAR) whereby plants accumulate proteins which aid defence (Fauteux *et al.,* 2005). Microbes in biological products such as Prestop (*Gliocladium catenulatum*) as well as competing with pathogens can also induce plant defences, and have been shown to improve efficacy when used in combination with conventional fungicides.

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

Half of the plants from year one remained at the propagation nursery and half of the plants were relocated to ADAS Boxworth. The plants that were moved to Boxworth were left in the tunnel after their leaves had dropped to be cut back before potting-on in spring 2016.The plants remaining at the propagation nursery were put into cold-storage over winter (2015/16) as they were required for long cane production in 2016.

Treatment with the microbial products in 2015 should have allowed any plant defence stimulation claimed by the products, or protective colonisation of the plants by the beneficial microbes to have had time to take place. Thus, in 2016, investigations focused on whether, once plants were potted for production and given further treatment with the same products, their growth was improved and the effects of any pest or disease reduced. At the Boxworth site, artificial inoculation by *Phytophthora rubi* was carried out. The first section of this report on 2016 deals the plants put into cold-storage for long cane production in Oxfordshire. The plants moved to Boxworth for inoculation are reported on in the following section.

Materials and Methods

2016 Oxfordshire

The cv. Tulameen module plants were taken out of cold-storage in April 2016 and potted in to 10 L pots at a commercial farm in Oxfordshire on 20 April 2016. The trial was set up as a fully randomised block design, set up as a single row of plants (**Figure 1.1**). Each pot

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contained one cane, split from the original two plant modules that went into storage, with four individual pots per plot and one empty pot separating the plots. The 10 L pots were stood on a woven ground cover covered soil ridge. The plantation left as an outdoor crop, not tunneled.

Figure 1.1. Setting up the biopesticide trial on a commercial farm in Oxfordshire, April 2016.

The on-farm trial in 2016 consisted of six treatments and an untreated control and the treatments were replicated four times (**Table 1.1**). No pots were inoculated with *P. rubi*. The module plants established in the 2015 trial were placed within a corresponding replicate of the 2016 experiment so as to receive the same treatment as they received in 2015. Two experimental treatments used in 2015 (HDC F202 and F203) were withdrawn from the 2016 trial by the manufacturers pending decisions on their future UK availability.

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Table 1.1. Summary of products and application timings made in 2015 at the propagators in multicell trays and modules, and application timings after the same plants were moved to be potted at the Oxfordshire site in 2016

Trt. no.	Treatment	Active ingredient	Application months 2015*	Application timings 2016**	Approval status
1	Untreated	(tap water)	1) April 2) May 3) July 4) October	5) 20 April 6) 26 May 7) 24 June	Not applicable
2	Prestop [MAPP 15103]	*Gliocladium catenulatum*	1) April 2) May 3) July 4) October	5) 20 April 6) 26 May 7) 24 June	EAMU 2015/2773 outdoor crops On label protected edible and non-edible crops
3	HDC F201	microbial	1) April 2) May 3) July 4) October	5) 20 April 6) 24 June	EAMU permanently protected fruit
4	Root Grow HYDRO	microbial	1) April 2) May	3) 20 April	Not currently registered as a pesticide
5	HDC F228	microbial	1) April 2) May 3) July 4) October	6) 20 April	EAMU outdoor crops only as drench, one per crop
6	HDC F205	chemical	1) April 2) May 3) July 4) October	5) 20 April 6) 26 May 7) 24 June	Not currently registered as a pesticide
7	Paraat [MAPP 15445]	Di-methomorph	1) April 2) May	3) 20 April	Label approval for one drench

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* April 2015 applications to tray plants two weeks after cuttings were taken, May 2015 applications to rooted cuttings when potted into modules. ** April 2016 applications made when modules were planted into 10 L pots

Table 1.2. Summary of products and dose rates used at the Oxfordshire site in 2016

Trt. no.	Treatment	Dose	Product per 10 L growing media
1	Untreated	-	Water only
2	Prestop [MAPP 15103]	5 g/L water (0.5%)	5.00 g
3	HDC F201	Not disclosed	Not disclosed
4	Root Grow HYDRO	7 g/1L growing media for 10 L pots	70.0 g
5	HDC F228	Not disclosed	Not disclosed
6	HDC F205	Not disclosed	Not disclosed
7	Paraat [MAPP 15445]	1.0 g/plant	1.0 g

All treatments except Root Grow HYDRO were applied to plots as drenches using a 1 L calibrated watering can with rose, applying 1 L of diluted product per pot to give a drench of 10% of pot volume per 10 L pot. Root Grow HYDRO was a granular product and applied according to label instructions by sprinkling the granules on the growing media in the planting hole before placing the plant in. Treatments were applied up to three times following the treatment manufacturers' instructions (**Table 1.1**). Dose rates can be found in **Table 1.2**. Prestop was applied at the standard 0.5% concentration (5 g/L water). Each plant in Treatment 7 received a drench of 1 g Paraat / 1 L of water (based on the label recommendation for raspberries of 1 g per plant in a minimum of 200 ml water). The application rates for products under experimental use are not able to be presented, in order to maintain confidentiality, but were according to label specifications. Where label product rates were given per hectare the dose was calculated based on the surface area of growing media across the top of the pot.

The crop was managed by the grower in the same way as other raspberry crops in the plantation, with all insecticides and preventative fungicides applications carried out as

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standard except for withholding the normal application of Paraat to the plants in the experiment.

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

Four weeks after each treatment application a more detailed assessment was carried out to assess the vigour of the plants and also to assess for the presence of key pests and diseases. These detailed assessments were carried out four times during the trial period. Vigour was recorded by measuring variables such as cane height, leaf size and the number of new primocane produced by each plant. A vigour score was awarded for each plant based on a visual assessment taking these variables into account. Plants were scored on a scale of 0 to 10, with 0 being dead and 10 being excellent vigour. At the final assessment, on 24 November 2016, vigour was assessed by measuring the height of the plants, recording the numbers of old cane in each plot, the numbers of old cane in each plot that had branched and by recording the number of new cane in each plot. In addition, the width of canes in each plot were assessed, as another indicator of vigour, by categorising canes as either stout, medium or thin and recording the number of canes in each category.

Disease was assessed by examining the plants for any signs of disease, in particular wilting. Pests were assessed by looking for key pests, or damage resulting from them. The only pests observed during the trial were aphids and capsids. Aphids were assessed by counting the number of aphids on 10 leaves at random in each plot and scored on a scale of 0 to 4 (**Table 1.3**). Capsid damage was assessed by looking for damage on 10 leaves at random in each plot and was recorded on a scale of 0 to 3 (**Table 1.3**). No macro-biological controls (parasitoids/predators) are used on this farm and no assessment was made of the natural fauna in the trial area.

Table 1.3. Indices for aphid numbers and capsid damage based on the total number of aphids or total number of capsid-damaged leaves per 10 leaves examined per plot in the on-farm biopesticide trial in Oxfordshire, 2016.

Number of aphids on 10 leaves	**Number of leaves with capsid damage**	**Index**
0	0	0
1 to 10	1 or 2	1
11 to 20	3 or 4	2

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21 to 30	5 or 6	3
31 +	7 or 8 (not attained)	4

Fruit was picked, as commercially, three times a week, by farm staff over the harvest period, which commenced on 18 July 2016 and finished on 8 August 2016. Fruit picked was split into two classes: class 1 (marketable fruit) and waste fruit. Researchers recorded mean berry weight per plot and noted any recurring problems with the fruit in each plot such as over-softness, sun scorch or small size.

Results

No significant differences were seen in phytotoxicity scores between the treatments at any of the assessments that were made of the trial throughout the treatment application period (**Table 1.4**). All of the treatments scored a mean index of seven or above, meaning that all the plants were considered commercially acceptable after each treatment application was made.

Table 1.4. Mean phytotoxicity indices (0 = dead, 9 = no reduction in leaf quality) for raspberry plants throughout the treatment application period in Oxfordshire in 2016.* Treatment means were calculated from individual plant scores.

Treatment	**Product**	**Mean Phytotoxicity index**		
		12 May 2016	**6 June 2016**	**8 July 2016**
1	Untreated	7.9	8.7	8.2
2	Prestop	8.0	8.3	8.3
3	HDC F201	8.2	8.4	8.4
4	Root Grow HYDRO	8.0	8.7	8.1
5	HDC F228	8.0	8.5	8.2
6	HDC F205	8.1	9.0	8.2
7	Paraat	8.1	8.6	8.3
P value		0.940	0.346	0.604
L.S.D (d.f. 18)		0.5697	0.6381	0.4132

*Plants had previously received treatment in 2015 at the propagators in both multicell trays and modules; the plants were then put into cold store before being planted out at the Oxfordshire site in 2016.

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No significant differences were seen in plant vigour between the treatments over the application period when scored on a scale of 0 to 10, with 0 being dead and 10 being very vigorous, all having good mean vigour (**Table 1.5**). There were no significant differences in plant height (mean 2.69 m) between the treatments when the plants were assessed on 24 November, nor between treatments in the total number of 2016 primocane in a plot (mean 4.39), the total number of branched primocane that was produced in 2016 in a plot (mean 1.7) or the number of new primocane in a plot (mean 5.61) (**Table 1.6**). The branched primocanes were canes which had stopped temporarily in growth in summer 2016, and as a consequence the terminal bud of the cane was lost or weakened permitting branching. Branched canes are likely to have consequences on the cropping height of the cane, which is likely to be reduced after tipping back as one branch will be retained. On 24 November there were also no significant treatment differences in the number of stout canes (mean 2.61), number of medium canes (mean 2.56) and the number of thin canes (mean 0.9) in a plot (**Table 1.7**).

Table 1.5. Mean plant vigour index (0 = dead, 10 = excellent vigour) for raspberry plants throughout the treatment application period, Oxfordshire 2016

Treatment	**Product**	**Plant vigour index**		
		26 May 2016	**24 June 2016**	**8 August 2016**
1	Untreated	7.3	7.8	8.0
2	Prestop	7.2	7.5	7.8
3	HDC F201	7.1	7.8	8.0
4	Root Grow HYDRO	7.4	7.5	7.6
5	HDC F228	7.5	7.6	7.8
6	HDC F205	7.2	8.8	9.0
7	Paraat	7.3	8.6	8.8
P value		0.351 (n.s.)	0.252 (n.s.)	0.138 (n.s.)
L.S.D (d.f. 18)		0.3772	1.342	1.157

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Table 1.6. Vigour assessments carried out on 24 November 2016 on the four plants per plot; cane height, total number of 2016 primocanes per plot, total number of branched primocane* produced in 2016 per plot and the total number of new primocane in a plot (produced in summer/autumn 2016), Oxfordshire 2016.

Treatment	Product	Mean plant height (m)	Mean total primocane	Mean total branched primocane	Mean total branched new primocane
1	Untreated	2.5	3.3	2.0	4.5
2	Prestop	2.8	5.0	2.0	3.8
3	HDC F201	2.6	3.8	1.3	5.3
4	Root Grow HYDRO	2.5	4.3	2.0	5.8
5	HDC F228	2.8	4.8	0.8	11.3
6	HDC F205	2.7	5.5	1.5	4.8
7	Paraat	2.9	4.0	2.3	3.8
P value		0.166 (n.s.)	0.873 (n.s.)	0.557 (n.s.)	0.283 (n.s.)
L.S.D (d.f. 18)		0.3263	3.670	1.736	6.644

*Branched primocanes had stopped temporarily in growth in summer 2016 and as a consequence the terminal bud of the cane was lost or weakened permitting branching.

Table 1.7. Cane width assessments carried out on 24 November 2016. Mean total number of stout canes per plot, mean total number of medium canes per plot and the mean total number of thin canes per plot, Oxfordshire 2016.

Treatment	Product	Total stout canes	Total medium canes	Total thin canes
1	Untreated	2.0	2.8	0.3
2	Prestop	2.3	2.5	1.8
3	HDC F201	2.5	2.5	0.3
4	Root Grow HYDRO	3.0	1.8	1.5
5	HDC F228	2.0	2.3	1.3
6	HDC F205	3.5	3.0	0.8
7	Paraat	3.0	3.0	0.3
P value		0.769 (n.s.)	0.985 (n.s.)	0.873 (n.s.)
L.S.D (d.f. 18)		2.317	3.314	3.670

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No foliar diseases nor above-ground symptoms of root rot were seen throughout the trial period. Aphids were only present at two of the assessments. There were no significant differences between the numbers of aphids recorded in either May or June (mean 10 or less aphids per 10 leaves) (**Table 1.8**). The amount of capsid damage on 26 May to plants treated with either the biofungicide HDC F F228 or Paraat was statistically significantly higher compared with the untreated plots, scoring a mean of 1.3 (around two out of 10 leaves damaged) compared to 0.3 (one or less leaves damaged) in the untreated plots (**Table 1.8**).

Table 1.8. Mean aphid and capsid damage indices on the raspberries throughout the trial period, Oxfordshire 2016. Using 0 to 4 indices for 10 leaves/plot ranging from 0 to over 31 aphids and 0 to 8 leaves with capsid damage as detailed in **Table 1.3**.

Treatment	**Product**	**Mean aphid index 26 May 2016**	**Mean capsid damage index 26 May 2016**	**Mean aphid index 24 June 2016**
1	Untreated	0.3	0.3	1.0
2	Prestop	0.0	0.8	0.8
3	HDC F201	0.5	0.5	0.8
4	Root Grow HYDRO	0.5	0.5	1.0
5	HDC F228	1.3	**1.3**	1.3
6	HDC F205	0.0	0.5	1.0
7	Paraat	0.0	**1.3**	0.3
P value		0.183 (n.s.)	**0.017**	0.576 (n.s.)
L.S.D (d.f. 18)		1.038	**0.6208**	1.050

On completion of nine harvests there were no significant differences between treatments in either the mean total marketable yield per plot of four plants (mean 1.66 kg), the mean total waste yield (mean 0.96 kg) or the mean berry weight (mean 5.96 g) (**Table 1.9**).

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Table 1.9. Fruit assessments over all harvests including total marketable yield, total waste yield and average berry weight, Oxfordshire 2016.

Treatment	Product	Over-all harvest records		
		Mean total marketable yield (g)	**Mean total waste yield (g)**	**Mean berry weight (g)**
1	Untreated	1684	888	6.0
2	Prestop	1576	874	5.8
3	HDC F201	1504	918	5.9
4	Root Grow HYDRO	1812	944	5.9
5	HDC F228	1729	948	6.2
6	HDC F205	1668	1008	5.9
7	Paraat	1653	1124	6.0
P value		0.878 (n.s.)	0.648 (n.s.)	0.610 (n.s.)
L.S.D (d.f. 18)		478.0	302.3	0.4114

Environmental conditions

The mean air temperature was 12.2°C on 20 April 2016 when the first application of treatments was made and the mean relative humidity was 63.3% (**Figure 1.1 and Figure 1.3**). On this date temperatures ranged from 5.5°C to 18°C. On 26 May 2016, when the second treatments were applied, the mean air temperature was 12.2°C, which ranged between 8.5°C and 17°C. On 26 May 2016 the mean relative humidity was 78.8%. On the final application date, 24 June 2016, temperatures ranged between 10.5°C and 14.4°C, with a mean air temperature of 14.4°C and a mean relative humidity of 91.7%. Temperature and humidity data from 1 September to 31 December for the trial can be found in **Figure 1.2** and **1.4**

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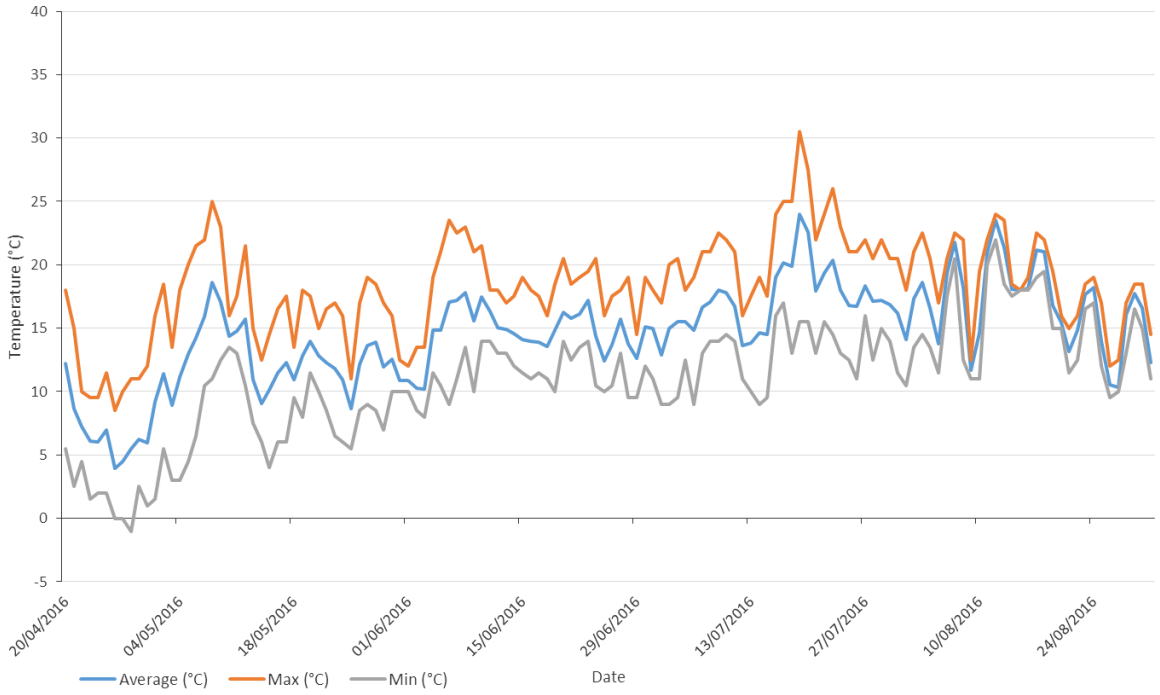

Figure 1.1. Minimum, maximum and mean daily air temperatures between 20 April and 31 August 2016, Oxfordshire.

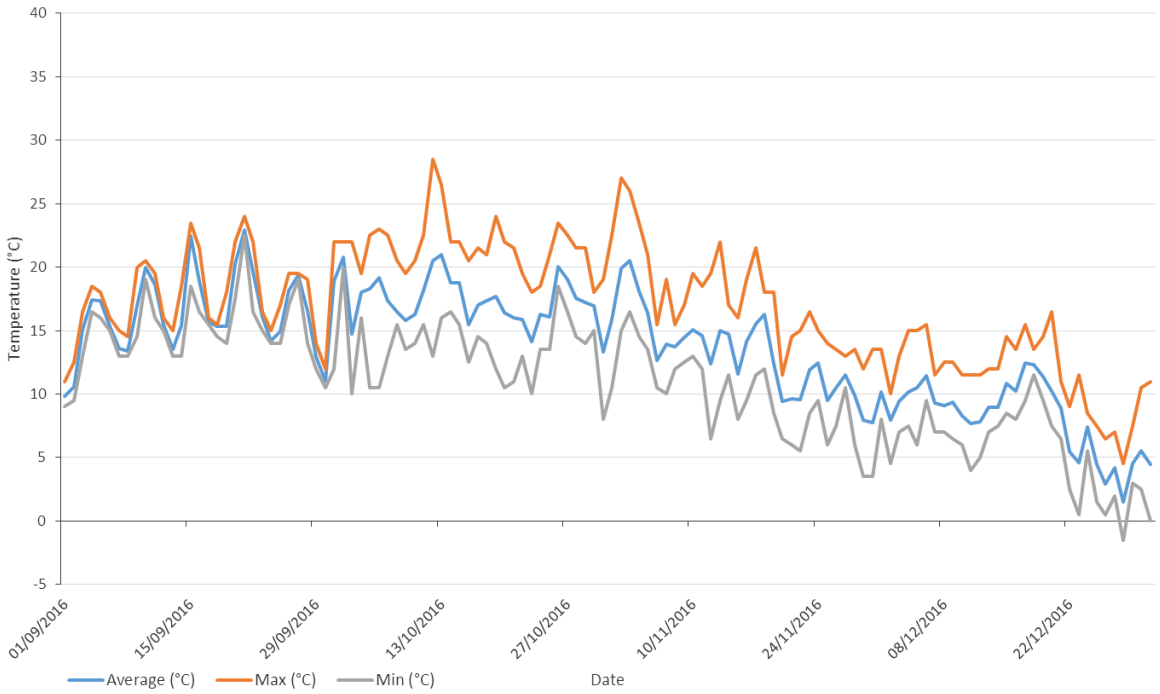

Figure 1.2. Minimum, maximum and mean daily air temperatures between 1 September and 31 December 2016, Oxfordshire.

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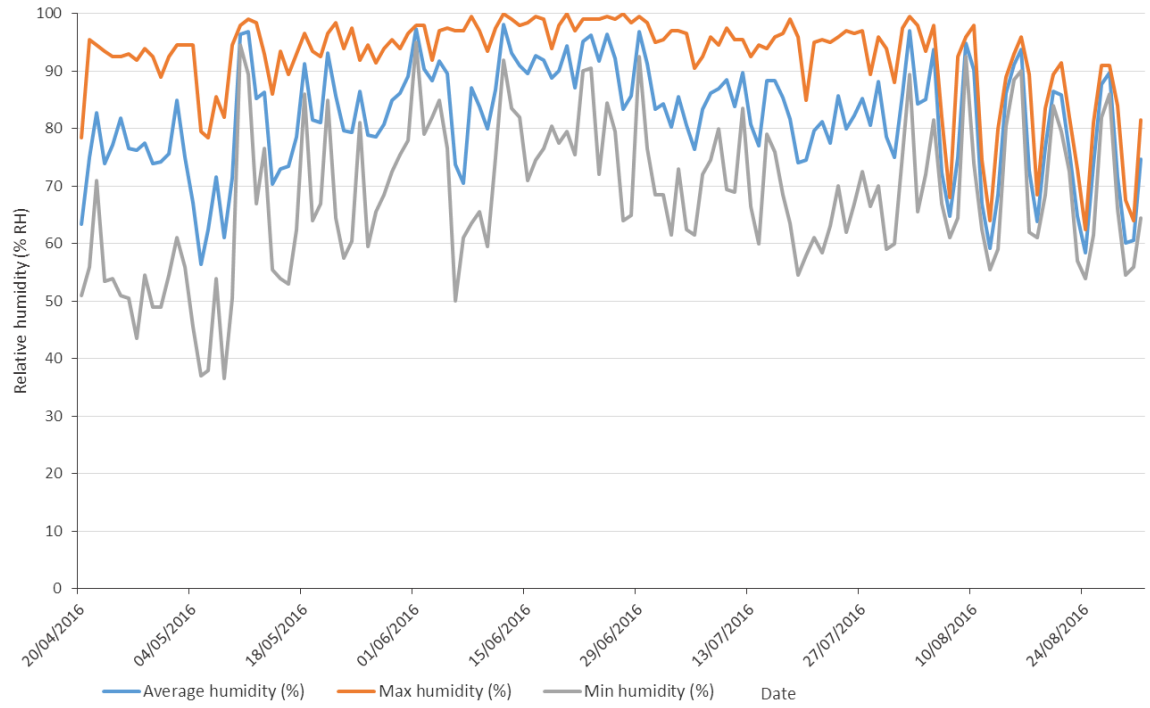

Figure 1.3. Minimum, maximum and mean daily relative humidity between 20 April 2016 and 31 December 2016, Oxfordshire.

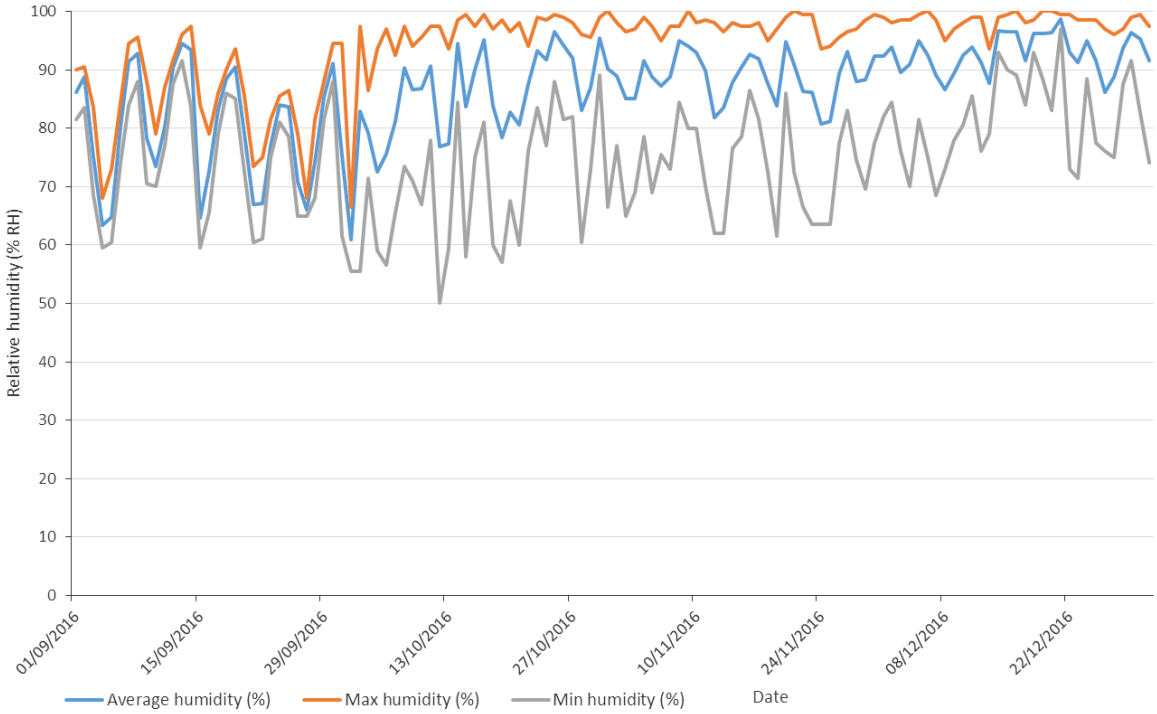

Figure 1.4. Minimum, maximum and mean daily relative humidity between 20 April 2016 and 31 December 2016, Oxfordshire.

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Discussion

Neither foliar disease nor symptoms of root rot such as wilting were seen even in the untreated plots, therefore no conclusions can be drawn regarding product effects on disease incidence or severity. Waste fruit yields were relatively high for all the treatments in this trial which was mainly the result of solar damage to the fruit during exceptionally sunny weather in August.

As there were no differences recorded either between any of the treatments or between these and the untreated in either plant vigour during crop growth, fruit harvest measurements, or the number and strength of cane produced by the end of November then neither any adverse effects from the treatments nor growth promoting characteristics were shown from them. The beneficial microbes in biofungicides are said to stimulate plant defence responses to attack by a wide range of organisms and so if there had been any disease developing in the roots or non-symptomatic foliar disease then plant growth might have been expected to have been better in these treatments than in the untreated.

It is unclear why drenches of either HDC F228 or Paraat might increase the level of capsid damage compared with the other treatments and the untreated.

Phytophthora root rot can take more than a year to cause cane wilting and death and so continuing the experiment for a further year could allow differences to show. Natural infection by soil splash of resting spores by rain is most likely to occur over the 2016/17 winter and the cooler, wetter, conditions experienced by the plants will be favourable to root rotting.

Conclusions

- No phytotoxic damage arose to the cultivar Tulameen used for the experiment from any of the treatments applied at potting or applied throughout the growing season
- Efficacy of the products tested was not able to be shown as no natural infection of root rot or wilting occurred in any of the plants
- Neither vigour nor fruit yield differed between any of the experimental treatments, with all being similar to those remaining untreated
- Treatments of HDC F228 and Paraat had significantly higher levels of capsid damage compared with the rest of the treatments.

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Materials and Methods

2016 Boxworth

The module plants of cv. Tulameen treated and brought to Boxworth in 2015 to be used in 2016 were potted up into 5 L pots of ericaceous peat-based growing media on the 28 April 2016. One plant, each with a single overwintered cane, was planted per pot, with three plants per plot. The trial consisted of eight treatments, including two untreated controls one of which was uninoculated and the other was inoculated. Treatment plots were randomised in four replicate blocks. The pots were stood in pot saucers in four single lines (blocks) in a polytunnel with netted lower side walls (**Figure 1.5**). Each pot was fitted with an irrigation dipper and received Sangral Select fertiliser (N:P:K 3:2:6) via an injection pump at 1:100 dilution during fruiting, but at 1:150 at other times. The watering was adjusted manually as water demand changed, according to the temperature and increased plant growth, aiming to keep the root ball moist to facilitate root rotting. A temperature logger was buried at a depth of 50 to 80 mm in one of the pots. Another logger was placed 1 m from the ground, within the canopy, with a white cover to shield it from direct sunlight.

Figure 1.5. Layout of plants in the polytunnel at ADAS Boxworth in four replicate lines of randomised treatments. New canes growing above overwintered canes, 2 August 2016.

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Treatment application

The products which were applied, the doses and application intervals were identical to the Oxfordshire site (**Table 1.1**). Drenches were made as 10% of pot volume. Applications were made using measuring cylinders, ensuring even coverage over pot surfaces and taking care to prevent any cross-contamination. Prestop was applied at the standard 0.5% concentration (2.5 g/0.5L water/pot). Each plant in Treatment 7 received a drench of 1 g Paraat in 0.5 L of water (based on the label recommendation for raspberries of 1 g per plant in a minimum of 200 ml water). The application rates for products under experimental use are not able to be presented, in order to maintain confidentiality, but were according to label specifications. Where label product rates were given per hectare the dose was calculated based on the surface area of growing media across the top of the pot.

Applications of products at Boxworth on 28 April, and for some products also on 10 June 2016 if permitted by Approval regulations, were made preventatively. Where product directions required an application interval that resulted in application on 21 July 2016 then this was done after *P. rubi* inoculations made on the 7 July and 8 July 2016 (**Table 1.10**). Treatment rates are detailed in **Table 1.11**. T1 to T7 all received inoculation with *P. rubi*. Extra untreated modules had been produced and brought to Boxworth in 2015 and only these were left uninoculated, as T8.

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Table 1.10. Summary of products, application mode and timings made in 2015 at the propagators in multicell trays and modules, and application timings after the same plants were potted at the Boxworth site in 2016

Trt. no.	Treatment	Active ingredient	Application months 2015*	Application timings 2016**	Approval status
1	Untreated	(tap water)	1) April 2) May 3) July 4) September	4) 28 April 5) 10 June 6) 21 July	Not applicable
2	Prestop [MAPP 15103]	*Gliocladium catenulatum*	1) April 2) May 3) July 4) September	4) 28 April 5) 10 June 6) 21 July	EAMU 2015/2773 outdoor crops On label protected edible and non-edible crops
3	HDC F201	microbial	1) April 2) May 3) July 4) September	5) 28 April 6) 21 July	EAMU permanently protected fruit
4	Root Grow HYDRO	microbial	1) April 2) May	3) 28 April	Not currently registered as a pesticide
5	HDC F228	microbial	1) April 2) May 3) July 4) September	6) 28 April	EAMU outdoor crops only as drench
6	HDC F205	chemical	1) April 2) May 2) July 3) September	4) 28 April 5) 10 June 6) 21 July	Not currently registered as a pesticide
7	Paraat [MAPP 15445]	Di-methomorph	1) April 2) May	3) 28 April	Label approval for one drench
8	Untreated Not inoculated	(tap water)	1) April 2) May 3) July 4) September	4) 28 April 5) 10 June 6) 21 July	Not applicable

*April 2015 applications to tray plants two weeks after cuttings were taken, May 2015 applications to rooted cuttings when potted into modules. ** April 2016 applications made when modules were planted into 5 L pots

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Table 1.11 Summary of treatment products and dose rates, ADAS Boxworth in 2016

Trt. no.	Treatment	Dose	Product per 5 L growing media per pot
1	Untreated	-	-
2	Prestop [MAPP 15103]	5 g/L water (0.5%)	2.5 g
3	HDC F201	Not disclosed	Not disclosed
4	Root Grow HYDRO	40.0 g per 5 L pot according to label table	40.0 g
5	HDC F228	Not disclosed	Not disclosed
6	HDC F205	Not disclosed	Not disclosed
7	Paraat [MAPP 15445]	1.0 g/plant	1.0 g
8	Untreated	-	-

Inoculation

Inoculum of *P. rubi* was produced using an isolate from raspberry obtained from Fera (CC2106) whose identity had been confirmed from its DNA. The isolate of SCRP333 that had been used in 2015 was not used in 2016 because (as described in the 2015 report) it had not been possible to get good production of zoospores. The procedures used for sporangia and zoospore product in 2016 are given in **Appendix 1**. For inoculation of the plants the sporangia were able to be stimulated to be produced in water collected from a soil suspension which was not sterile (i.e. not autoclaved). However, for future observations of zoospore behaviour without the presence of protists a technique of partial sterilisation was developed later in 2016 in which the "soil water" was microwaved for 40 seconds and preserved the substance required to stimulate sporangia production but reduced contaminants.

Two methods of inoculation were used; zoospores to allow immediate root infection and also mycelium which could produce sporangia when stimulated to do so in the pot. On 7 July, the mycelium was cut as plugs (borer size 4) from *P. rubi* cultures and two plugs dropped down each of four dibbed holes about 100 mm deep spaced around the pot in the zone of new root growth beyond the module root ball. The holes were then filled with moist

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growing media. On 8 July, two Petri dishes of *P. rubi* zoospores (total 8 ml) were gently poured onto the moist growing media surface of each pot, encircling the stems. There were 5 x 10^4 zoospores per ml. To wash the zoospores into each pot 50 ml of water was then poured around the surface of each pot. Plants in T8 received blank agar plugs and soil water instead of *P. rubi* inoculum on the same days as T1 to T7 were inoculated. Inoculations were made in the late afternoon.

Assessments

Observations of the plants were made throughout the growing period from April 2016 to January 2017, comparing the treated plots with both the inoculated and uninoculated untreated plots. Observations included phytotoxicity, vigour and survival of the old cane and establishment and survival of the new cane. Assessments of individual plants were made when particular differences were observed which might have been treatment related. Vigour was assessed using a 1-9 index, where 1=no bud break on old cane; 5=several buds open, no new cane; 9=new shoots on old cane and new cane growing well. Lack of greenness of the leaves was recorded separately on one date with 4=severe yellowing and 6=little yellowing. The plants were grown to produce new cane in 2016 that would be susceptible to *P. rubi* infection. The plants were not cold stored to initiate fruit production on the old canes, as cane death was the principal symptom of infection to be recorded. Primocane height (as and index of 1=just emerged, 2=grown 50 mm to 120 mm and 3=120 mm to 0.5 m) and any death pre-inoculation was recorded on 8 July 2016. Final primocane numbers and the number of any dead primocanes were recorded on the 15 November 2016 before the leaves dropped overwinter. An assessment to evaluate the extent of root rotting will be made in February 2017.

Results

The overwintered cane was assessed for vigour as it broke bud and developed shoots prior to inoculation and pruning back at the end of June to encourage new cane development (**Table 1.12**). There were no significant differences between the untreated in T1 and T8 and those treated on 28 April 2016 and those further treated on 10 June. On average, vigour increased from a mean index of 4 (some bud break) to a mean index of 6 (bud break and new primocane emerging). Only three plants failed to break bud, one each of T1, T5 and T6 and so their death was probably unrelated to earlier treatments.

Some yellowing was observed on the plants on 23 June, particularly on the primocanes, following the treatment applications on 10 June to T2 and T6. However, no significant differences were seen between treatments (**Table 1.12**), with plants on average having a mean index of 6.5 indicating little yellowing present.

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Table 1.12 Vigour assessment 1 to 9 on overwintered canes and emerging primocanes, where 1 = no bud break on old cane; 9 = new shoots on old cane and strong new cane. Greenness index where 4 = most severe yellowing and 6 = little yellowing, Boxworth 2016.

Treatment	Product	Mean cane vigour index			Greenness index
		9 May 2016	9 June 2016	17 June 2016	23 June 2016
T1	Untreated	3.17	5.67	5.58	6.58
T2	Prestop	3.08	5.83	5.75	5.75
T3	HDC F201	5.75	7.00	6.67	6.42
T4	Root Grow HYDRO	3.92	6.08	5.83	6.75
T5	HDC F228	5.75	6.17	5.92	6.50
T6	HDC F205	3.17	6.50	6.33	7.29
T7	Paraat	5.17	6.00	5.83	6.33
T8	Untreated Uninoculated	2.83	6.08	6.25	6.67
P value		0.393	0.893	0.910	0.116
LSD (21 d.f.)		3.50	1.94	1.75	0.92

On 9 June 2106 new canes were emerging in 80% of the pots, with at most three canes, but mainly one cane per pot (mean 1.1 new canes/plant). The speed of new cane emergence did not differ between treatments (**Table 1.13**), with all treatments having between one and four pots without new growth.

After pruning the old cane on 27 June 2016 to encourage more new cane to emerge, assessment on the new cane on 8 July still showed a significant difference (P=0.006) in the number of canes grown (mean 1.8 new canes/plant), with more in T3, T4, T5 and T7 (Paraat) than both the untreated and T6 and T2 (Prestop) . There was no significant difference in growth height of the new canes (mean height index 2.5, indicating canes had grown up a few centimetres above the stool) (**Table 1.13**). No new canes had grown in two pots of T1 (untreated) and one of T5 prior to *P. rubi* inoculation.

By the assessment on the 15 November a few more primocanes had grown, giving a mean 2.0 canes/plant, with at this time T2 (Prestop) and again T4 and T5 having significantly more canes than the other treatments. There was no difference between the inoculated and the uninoculated untreated plants, indicating that any *P. rubi* infection was not affecting

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cane numbers (**Table 1.13**). A small number of primocanes present on 8 July had died, with one in each of three pots of T6, one in T7 (Paraat) and one each in two pots of T8 (untreated uninoculated) and thus not consistently across any particular treatment. At no time was the typical "shepherd's crook" symptom of wilted primocane growth seen following inoculation with *P. rubi.*

Table 1.13. Mean number of primocanes canes per pot and 1 to 3 growth index from emergence to up to 0.5 m, Boxworth 2016.

Treatment	**Product**	**Mean number new canes/pot 9 June 2016**	**Mean number new canes/pot 8 July 2016**	**Mean cane height index 8 July 2016**	**Mean number new canes/pot 15 Nov 2016**
T1	Untreated	1.00	1.25	2.88	1.25
T2	Prestop	1.08	1.42	2.71	**2.75**
T3	HDC F201	1.08	1.92	2.74	2.08
T4	Root Grow HYDRO	1.17	**2.75**	2.23	**3.33**
T5	HDC F228	1.17	**2.50**	1.98	**2.42**
T6	HDC F205	0.92	1.50	2.62	1.25
T7	Paraat	1.17	1.92	2.46	1.92
T8	Untreated Uninoculated	0.83	1.17	2.75	1.42
P value		0.983 (n.s.)	**0.006**	0.080 (n.s.)	**0.005**
LSD (21 d.f.)		0.84	**0.85**	0.61	**1.08**

Aphids, mainly Raspberry aphid, multiplied in the crop in May and were brought under control by application of Chess WG (pymetrozine) on 19 May 2016 followed by releases of Aphidius and a natural infestation of Harlequin ladybirds. An assessment of the proportion of leaves with aphids in each plot did not show any significant differences between any of the treatments, with a mean 40% of leaves examined having aphids present (**Table 1.14**).

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Table 1.14. Percentage of leaves with raspberry aphid on 18 May 2016, based on the number of leaves out of 10 per plot (all 3 plants) with aphids.

Treatment	Product	Mean % of leaves with aphids
T1	Untreated	30
T2	Prestop	20
T3	HDC F201	40
T4	Root Grow HYDRO	38
T5	HDC F228	65
T6	HDC F205	42
T7	Paraat	48
T8	Untreated Uninoculated	38
P value		0.521 (n.s.)
LSD (21 d.f.)		40.46

Environmental conditions

The mean air temperature was 20°C on both inoculation days on 7 and 8 July 2016, however on 19 July, 11 days after the second inoculation, the mean air temperature in the tunnel was the highest of the year of 30°C, with temperature peaking at 45.5°C on 23 August (**Figure 1.6**). Another high maximum temperature occurred on 13 September (**Figure 1.7**) The temperatures in the root ball were slightly lower, with a mean on 19 July of 27°C and maximum of 36.4°C (**Figure 1.10**). Daily mean relative humidity was on average over 60% in the tunnel in summer, rising to over 80% in winter (**Figures 1.8 and 1.9**).

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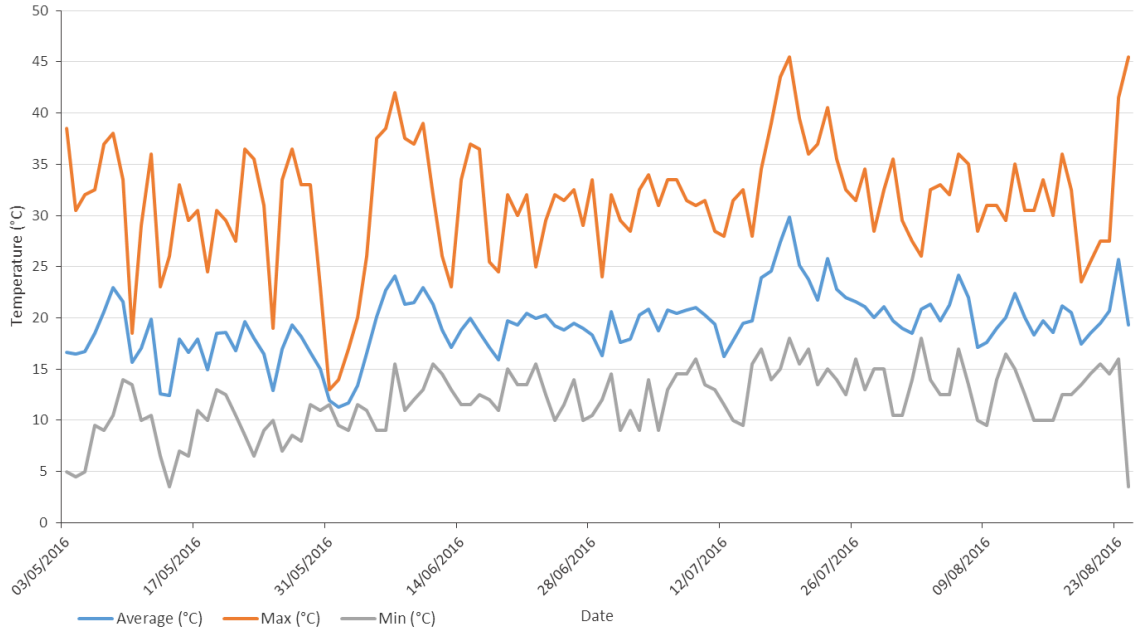

Figure 1.6. Minimum, maximum and mean daily air temperatures between 3 May and 23 August 2016 in the trial polytunnel, ADAS Boxworth.

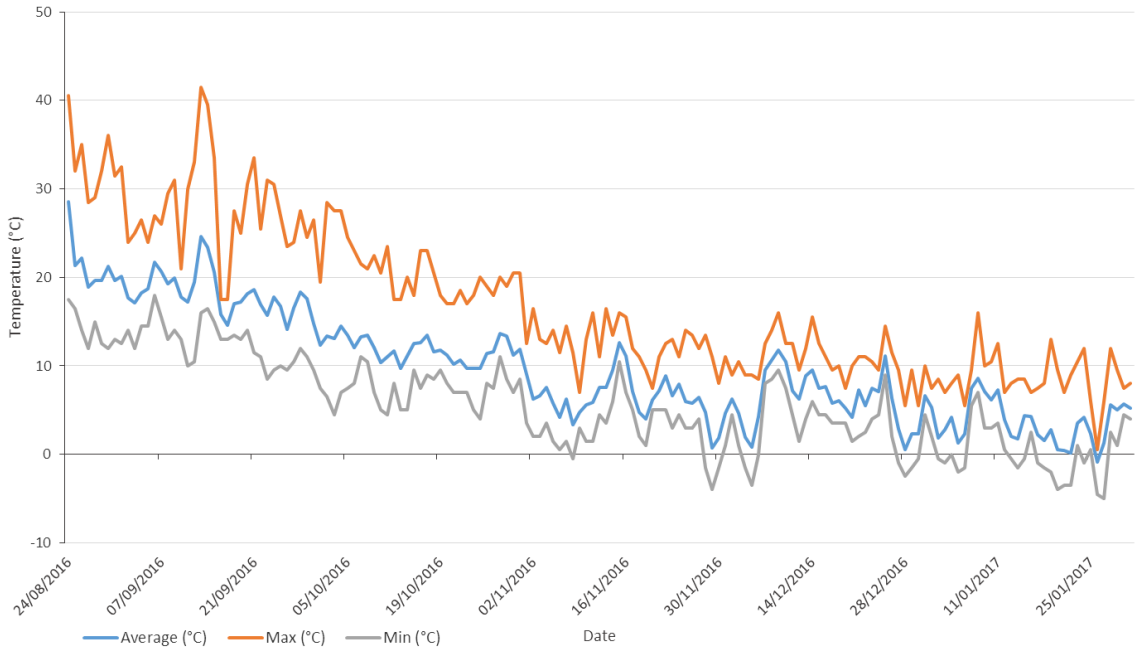

Figure 1.7. Minimum, maximum and mean daily air temperatures between 24 August 2016 and 31 January 2017 in the trial polytunnel, ADAS Boxworth.

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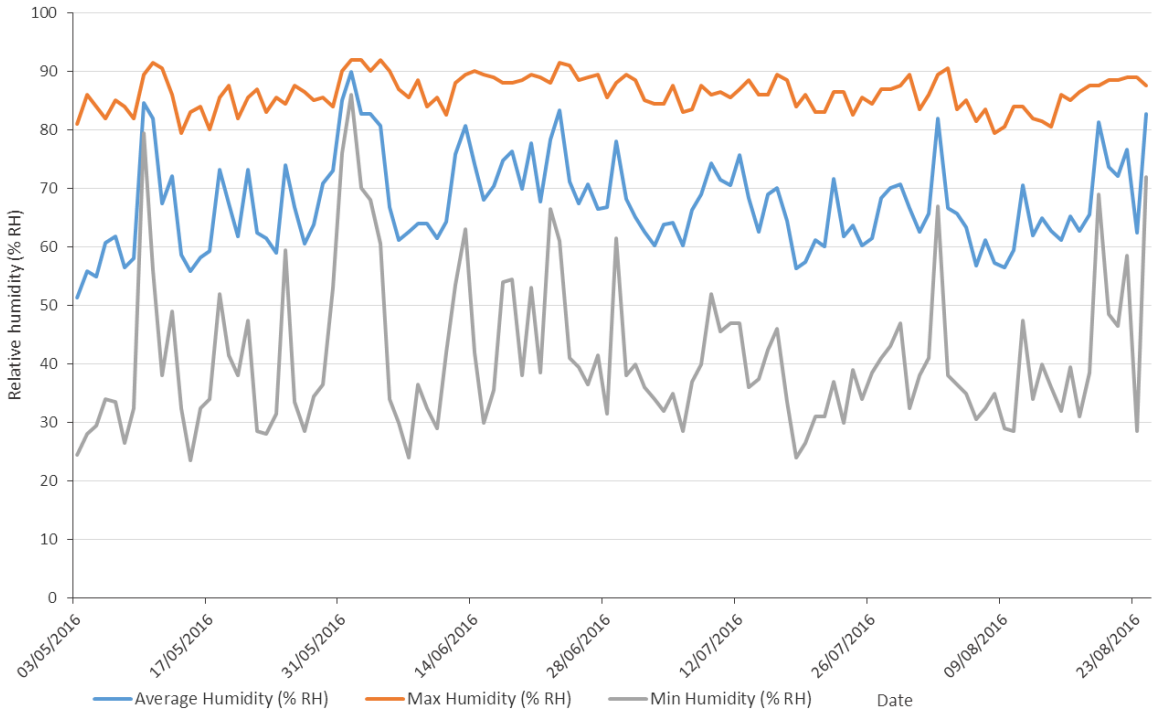

Figure 1.8. Minimum, maximum and mean daily relative humidity between 3 May 2016 and 23 August 2016 in the polytunnel, ADAS Boxworth.

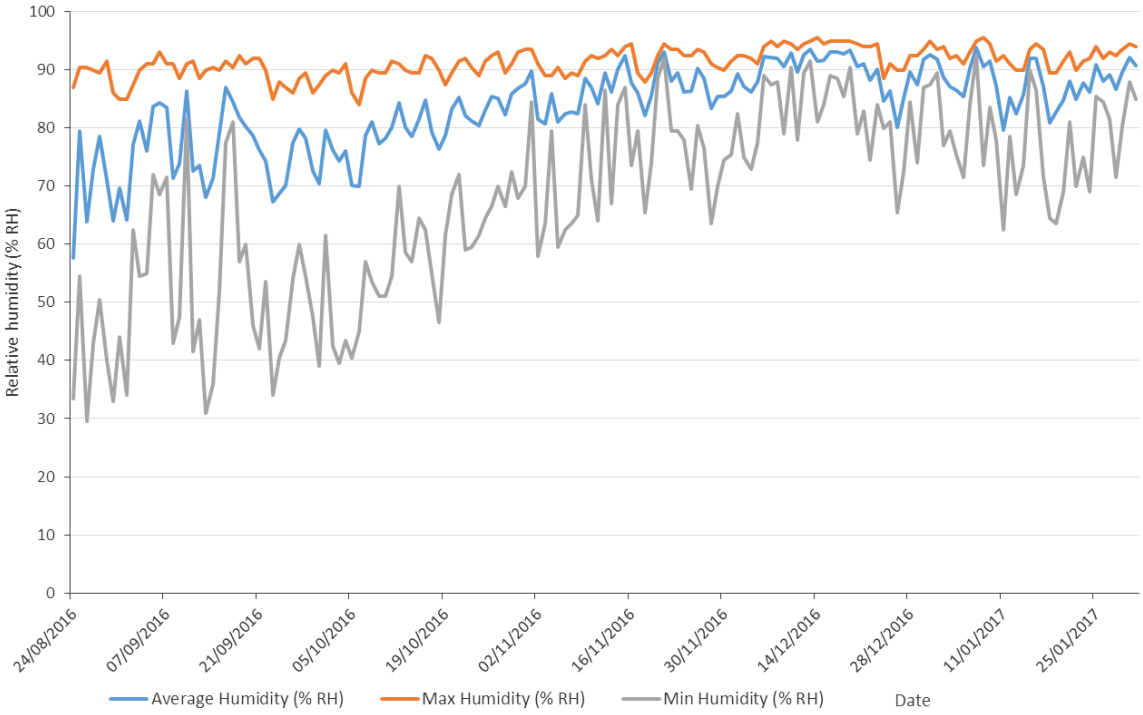

Figure 1.9. Minimum, maximum and mean daily relative humidity between 24 August 2016 and 31 January 2017 in the polytunnel, ADAS Boxworth.

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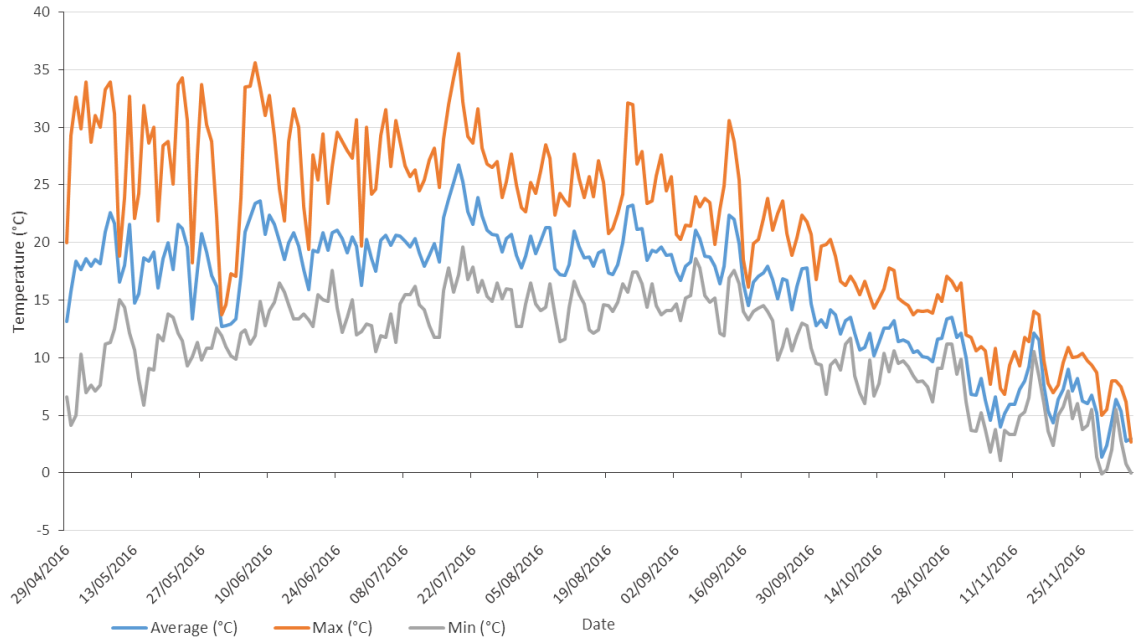

Figure 1.10. Mean, minimum and maximum daily temperatures between 50 to 80 mm deep from 29 April to 5 December 2016 in a potted raspberry in the polytunnel, ADAS Boxworth.

Discussion

Although the temperature in the pots peaked at 36°C on the 21 July (by which time root infection should have taken place), and the maximum temperatures attained from May to August was in the region of 30°C, this would not be expected to eradicate the *Phytophthora* spp.. Information on survival gained from work on composting of green waste (Defra, 2004) has shown that temperatures of 58°C for seven days are required to eradicate *P. nicotianae*, 55°C for 21 days for *P. infestans*, 55°C for over 14 days for *P. ramorum* and 60°C for 21 days for *P. cryptogea*. The lowest temperature for complete eradication given was for *P. cinnamomi* at 40°C, but this required this temperature to be maintained over a period of seven days. Mycelial growth (as opposed to survival) for the closely related *P. fragariae* var. *fragariae* is seen between 3°C and 30°C (Erwin and Ribeiro, 1996). To achieve death of a susceptible raspberry variety (Glen Moy) within two weeks of inoculation a technique in which young plants (100 mm tall) are watered heavily twice and day and kept at under 15°C for a fortnight following mycelial plug infection with *P. rubi* can be used (David Cooke, Pathologist at the James Hutton Institute, pers. comm.). This low temperature is favourable to mycelial growth. In the current work zoospores were produced following growth of the mycelium at 15°C in the laboratory and a quantified amount of zoospores applied to wet pots. The aim was to achieve a gradual increase in root damage over the year where treatments were unable to achieve control.

No obvious infection by *P. rubi* was seen in the plants. However, the root balls of the modules were substantial and destruction of the roots in advance of new root production

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can take place naturally over a period of years, particularly in cooler conditions when root growth ceases. The experiment was given steady irrigation to maintain moist compost (aiming to have water in the plant saucer at the end of each irrigation burst which was then taken back up by the plants before the next irrigation period) in order to aid zoospore infection as these flagellate spores need to be able to swim, however this also meant that the plants would be able to survive without showing water stress from a reduced root system. Assessment of roots in SF123 showed that substantial root rotting had occurred without above-ground symptoms and so destructive assessment of the plants in February 2017 will determine the situation in the current project.

Further work

Laboratory experiments will be carried out in 2017 using young plants of a cultivar susceptible to *P. rubi* under controlled environment conditions to check on the pathogenicity of the isolate CC2106 used in the experiment.

Laboratory work in 2016 developed a microscope viewing chamber for zoospores that allows substances to be introduced, using a micropipette tip, into the water with zoospores. It was also determined that zoospore observations were complicated by the presence of other micro-organisms in the soil water essential for the production of sporangia. Following the development in 2016 of a microwave treatment to destroy most of these other micro-organisms in soil water, work will be re-commenced with the viewing chambers. The use of this semi-sterilisation technique will need to be investigated with non-sterile root exudates collected from susceptible and resistant raspberry cultivars in order to allow the viewing of zoospore behaviour towards them in order to determine whether exudates are a factor in susceptibility. Exudates may be produced naturally when plants take their nutrients down into the stool in autumn and also when new root tips are produced in spring as these tend to be leaky. If exudates are important to zoospore infection, then targeting treatment for when plants are most attractive and using beneficial fungi and bacteria to compete for these nutrients could improve control measures.

Conclusions

- No phytotoxic damage arose to the cultivar Tulameen used for the experiment from any of the treatments applied at potting or applied throughout the growing season
- No vigour differences were seen on the overwintered cane following one or two applications of product in 2016
- Efficacy of the products tested against *P. rubi* was not able to be shown as no symptoms of primocane death occurred in any of the inoculated plants

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- A small increase in primocane production arose with the use of all the biofungicides (Prestop, HDC F201, Root Grow Hydro and F228) compared with pots left untreated or given Paraat or the chemical HDC F205.
- None of the product treatments in April 2016 caused any differences in the incidence of aphid infestation of the leaves in May compared with untreated plants.

Acknowledgements

We would like to thank Slavey Slavchev of EU Plants Ltd for the supply, growing and then cold-storage of the module plants used in the experiments. We also thank Richard Stanley and Paul Clarke for hosting the experiment in Oxfordshire and providing the crop husbandry.

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

Aim

Objective 2: To develop compatibility strategies for biocontrol of two-spotted spider mites (TSSM) by predatory mites with insecticide sprays for spotted wing drosophila (SWD) and capsids: To determine the effects of method of application of a programme of insecticide sprays for SWD on commercially introduced predators (*Phytoseiulus persimilis*) and their ability to regulate TSSM on raspberry.

Task 2.2

To determine the effects of overall canopy spraying verses overhead application of a programme of sprays of deltamethrin and spinosad on SWD and commercially introduced predators (*Phytoseiulus persimilis*) on raspberry and ability to regulate TSSM.

Introduction

In 2015, the effects of overall canopy spraying verses 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 both the overall effect of date and overall effect of treatment were significant. In early August, the numbers of natural phytoseiid mites were lower in both of the sprayed treatments, possibly following specific spray applications of spinosad. The numbers of TSSM then rose significantly in the sprayed plots from the 17 August 2015. The numbers of SWD were lower in both of the treated plots.

In this work in 2016, the same system of overhead spraying was used, with different nozzles to give a slightly larger droplet size to determine whether this will give less spray on the underside of the leaves, so providing refuge for the predatory mites and therefore improved control of TSSM. This work will also study the effects on biocontrol agents such as *P. persimilis* which are introduced in commercial systems.

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Materials and Methods

Experimental polytunnels

A dedicated suite of 9 mini polytunnels (7.3 m wide, 4.5 m long, 3 m tall) in a 3 x 3 array spaced 20 m apart and furnished with drip fertigation were used at NIAB EMR (**Figure 2.2.1**). Isolation of tunnels was necessary because TSSM and predatory mites are highly dispersive. This enabled a randomised block experiment with three replicates of three treatments, with the nine tunnels as plots, to be done.

Plants

In each tunnel were 20 second year cv. Kweli raspberry plants (from W. B. Chambers, Maidstone, UK), in two rows of ten plants. The raspberry plants had the main cane cut back to encourage a more dense habit to encourage spider mite population growth.

Experimental booms

Three of the tunnels (one per block) were furnished with overhead hollow cone nozzles (red Albuz ATR 80) spaced 50 cm apart on two 5 m booms (N.P. Seymour, Avon Works, Cranbrook, Kent) (**Figure 2.2.2**). There was one boom above each row of raspberry plants and the nozzles were always at least 0.5 m above the crop, necessitating one trimming of the cane tops to maintain the separation distance. The sprays were applied by linking the boom sprayer system to a tractor. The red nozzles were used to provide a larger droplet spectrum than the nozzles used in 2015, with the aim of providing coverage of the upper leaf surface, whilst minimizing the spray coverage on the lower leaf surface.

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Figure 2.2.1. Mini-polytunnel at East Malling Research, as used in 2015 and 2016

Figure 2.2.2. Red Albuz ATR 80 nozzle (with constant pressure valve visible to one side).

Treatments

The experiment had three treatments:

1). **Knapsack spray** - A programme of insecticide sprays (**Table 2.2.1**) applied to get good cover of the whole canopy using a motorised air-assisted knapsack sprayer, Birchmeier® B 245 with a micron flow restrictor (as used in a rotary atomiser) and with a pressurised tank to maintain a constant flow at a volume of 1000 L/ha

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2). **Overhead spray** -The same programme of sprays (**Table 2.2.1**) applied by overhead nozzles (red Albuz ATR 80) at 1000 L/ha. The overhead sprays (applied by linking the boom sprayer system to the tractor) were sprayed for 28 seconds at 5.5 bar and 1750 rpm to give 0.053 ml/second for each boom (0.0053 ml/second/nozzle).
3). **Untreated control**

In this experimental system, application method, rates, timing of application and number of applications were designed to provide reproducible effects between experimental seasons, which may differ from commercial practice i.e. four deltamethrin sprays were used rather than three, the boom system is currently not being used in a polytunnel setting but provides an overhead spray system.

Biological Control
P. persimilis (Koppert UK) was introduced to all plots on 22 July and 17 August 2016 at a light curative (6 /m^2) and heavy curative (20 – 50 /m^2) rate (Koppert UK) respectively. Product quality was checked ahead of release by counting the number of mites in a known volume.

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Table 2.2.1. Test products, source, active ingredients and formulation

Active ingredient	Product	Manufacturer	Content of a.i. nominal	Formulation type
Spinosad	Tracer	Dow AgroSciences (marketing company Landseer Ltd)	480 g/L (44.03% w/w)	Suspension concentrate
Deltamethrin	Decis; Bandu	Bayer; Headland Agrochemicals	25 g/L (2.8% w/w)	Emulsifiable concentrate

Table 2.2.1. Experimental programme of insecticide sprays applied for Treatments 1 and 2 between July and September, Kent 2016.

Spray #	Application date in 2016	Active Ingredient	Dosage rate product/ L	Dosage rate a.i. (g)	Water volume per plant (ml)	Amount a.i./ plant (g)
1	26 Jul	Spinosad	0.2 ml	0.096	175	0.0168
2	03 Aug	Spinosad	0.2 ml	0.096	175	0.0168
3	11 Aug	Deltamethrin	0.6 ml	0.015	175	0.0026
4	22 Aug	Deltamethrin	0.6 ml	0.015	175	0.0026
5	30 Aug	Spinosad	0.2 ml	0.096	175	0.0168
6	06 Sep	Deltamethrin	0.6 ml	0.015	175	0.0026
7	14 Sep	Deltamethrin	0.6 ml	0.015	175	0.0026

Table 2.2.2. Commercial recommendations for products for use on protected raspberry

Product	Dose product/ha	Max application	Recommended spray volume	Harvest Interval (days)	Notes
Tracer	200 ml	# sprays - 3 [1]	1000 L water/ha using hydraulic sprayers or hand held equipment	1	EAMU for use against thrips
Decis/Bandu	500 ml [2]	Total dose – 1500 ml/ha/crop	200-1500 L water/ha as a medium quality spray	7	Full

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

2 The amount of product/ha has now changed for Bandu for control of raspberry beetle from 600 to 500 ml; the 600 ml rate of deltamethrin was used experimentally in 2016.

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Experimental design

The experiment had three replicates of three treatments in a randomised complete block design (**Table 2.2.2**). Plots were one tunnel containing the 20 potted raspberry plants in two rows of 10.

Table 2.2.2. Randomisation of treatments to plots at NIAB EMR

Plot	Row	Column	Treatment
101	1	1	3
102	1	2	2
103	1	3	1
201	2	1	2
202	2	2	1
203	2	3	3
301	3	1	1
302	3	2	3
303	3	3	2

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

A pre-treatment leaf sample was taken ahead of the first spray on 25 July. Post-treatment leaf samples were taken at approximately weekly intervals on 1, 10, 16, 22, 30 August and 5, 12 and 21 September. Twenty leaves were taken per plot across all of the plants, from the main canes (rather than from the later developing lateral shoots), with 10 from the front and 10 from the back rows. As the experiment progressed the sampling was further split into two samples: five leaves from the upper part of the plants (between the 60th to 90th height percentile of the plant) and five leaves from the lower part of the plants (between the 10th to 40th height percentile of the plant), from each of the front and back rows.

Counts of TSSM eggs, immatures and adults and Phytoseiid mite eggs, immatures and adults were made on leaf samples under a binocular microscope. Microscope slides were made of any adult phytoseiid mites that were then identified. Phytoseiid mites were identified to species, i.e. *Phytoseiulus persimilis* and naturally occurring mites such as *Amblyseius andersoni* and *Neoseiulus californicus*. Additional pests/predators were noted and placed in 70 % ethanol in an Eppendorf tube.

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Assessments of Leaf Damage

Each picked leaf was assessed for TSSM damage and recorded on a score from 0 to 4, where damage was recorded as leaf area affected 0 = no damage, 1 = 1-25%, 2 = 26-50%, 3 = 51-75% and 4 = 76-100% (**Figure 2.2.3**).

Figure 2.2.3. A raspberry leaf var. Kweli showing two-spotted spider mite (TSSM) damage.

Assessments of Spotted Wing Drosophila

Fifty raspberries were picked on four occasions: 6, 12, 19 and 27 September 2016. They were picked into ventilated Perspex boxes lined with tissue which were held at 20°C and numbers of SWD (and other flies) were counted weekly for two weeks.

Assessment of Spray Deposition

On completion of the experiment, on 13 October 2016, a final spray using a novel tracer was done to assess the spray deposition. Samples were taken from three leaves from each of eight areas in each plot, from the back and front rows, and from both the upper and lower leaf in both the top and bottom canopies. In addition 10 samples were assessed from the untreated plots with leaves taken randomly between the eight areas. These were then analysed using a novel hand-held device, and a leaf-wash off method.

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For the leaf wash-off method, tubes were pre-prepared by adding 6 ml of deionised water to Sarstedt 60.541.685 tubes (13 ml, with 7 mm radius) wrapped in aluminium foil and capped. To wash the spray deposits from the leaves, the rim of the tube was placed firmly against the leaf and the tube was shaken five times. This was repeated twice, giving a total sample area per leaf of 308 mm^2. To assess, 4 ml of each sample was pipetted into a cuvette and the Relative Fluorescence Unit (RFU) was measured using a benchtop fluorimeter. The process was repeated to give 10, 100 and 1000-fold dilutions. The excitation and emission filter were optimised for the fluorescent tracer used during the study. The RFU values were converted into concentration of the novel tracer using a calibration equation. The following equation was used to calculate spray in l/ha on a leaf from the percentage of the tracer as calculated from a calibration equation from RFU.

$$\frac{1.0 \times 10^7 \times T \times v}{s \times a} = l/ha$$

Where, *T* = percentage of the novel tracer as calculated from the RFU by the calibration equation; *v* = volume of water in the sample tube (ml); *s* = the tracer percentage (v/v) used in the spray tank; *a* = area sampled for the wash-off (mm^2).

Husbandry

Plants were drip fertigated using 25kg/ha/week of either a growing or fruiting product as appropriate.

Temperature and humidity records

Two EL-USB-2+ data loggers were used to record temperature and humidity, placed in the same tunnel (Plot 302). Front doors of the polytunnels were left open during the time period of the experiment to be more representative of temperatures in commercial polytunnels. Daytime temperatures reached 35°C on occasion (**Figure 2.2.4**), hence the need to maintain an air flow to prevent scorching of the leaves; humidity was high at night and dropped during the day (**Figure 2.2.5**).

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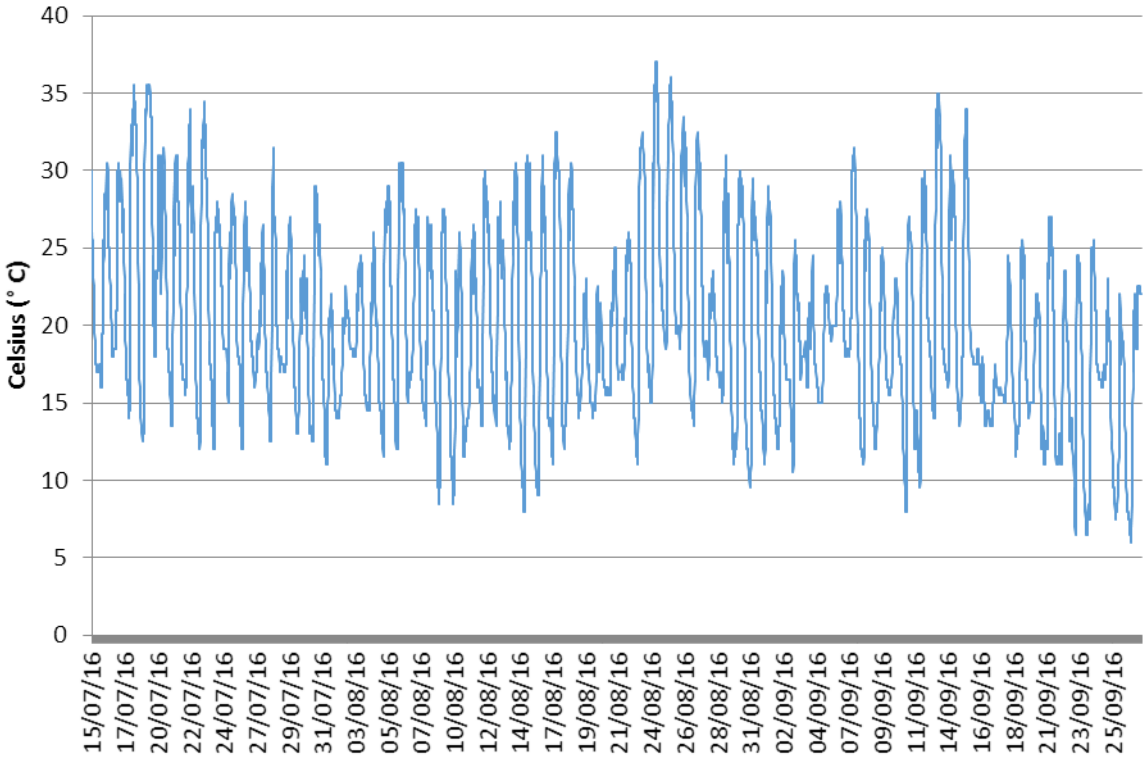

Figure 2.2.4. Air temperatures July to September 2016 in the mini-polytunnels, NIAB EMR

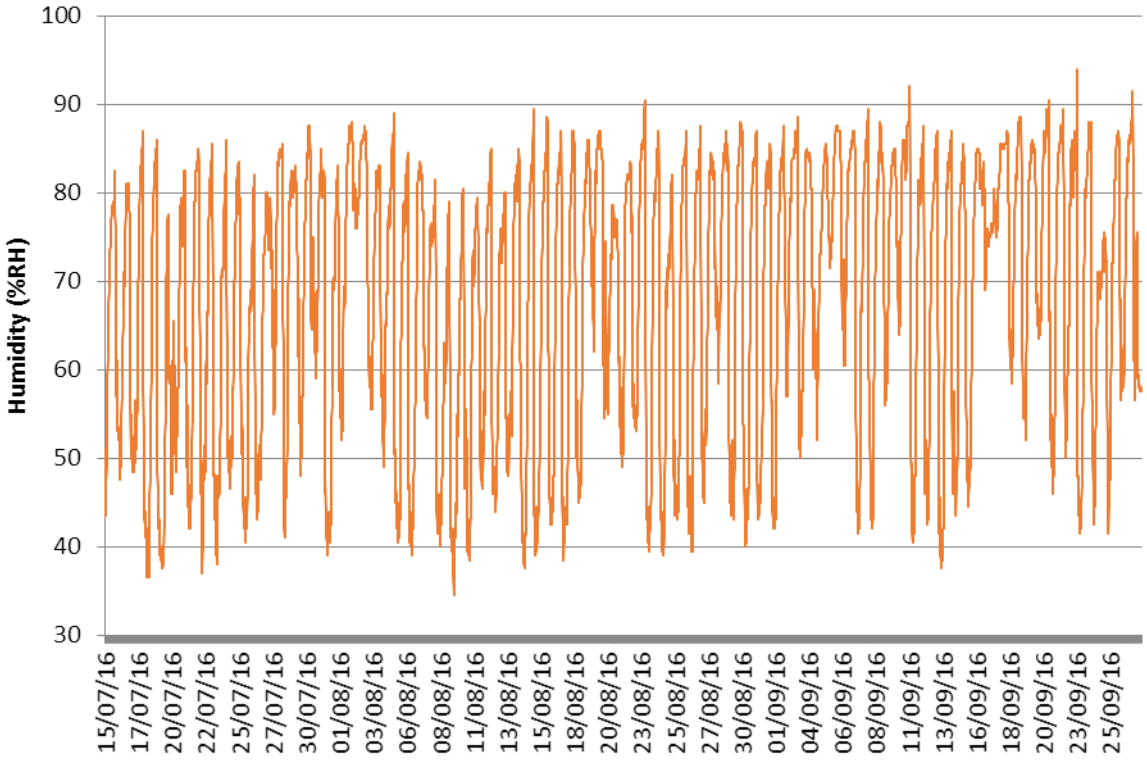

Figure 2.2.5. Relative humidity July to September 2016 in the mini-polytunnels, NIAB EMR

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Results

Assessments of populations of TSSM, natural phytoseiid predatory mites and P. persimilis
Data were analysed following a square root transformation, to look at date and treatment effects with repeated measures. For all stages (adults, immatures, eggs) of TSSM, natural phytoseiids and *Phytoseiulus persimilis* there was generally a date effect and a treatment effect **(Table 2.2.3)**, but no date by treatment interaction.

Table 2.2.3. The effect of method of spray application on the mean square root numbers of two spotted spider mite (TSSM), naturally occurring phytoseiids and introduced *Phytoseiulus persimilis* per leaf, Kent 2016. Data presented shows the treatment effect, following square root transformation and repeated measures analysis

Mite	Life stage	Control		Overhead spray		Knapsack spray		s.e.d.	l.s.d.	p
TSSM	adults	3.19	a	4.1	ab	4.96	b	0.336	0.934	0.016
	immatures	3.03	a	4.38	b	5.24	b	0.227	0.629	0.002
	eggs	4.46	a	5.49	b	7.74	b	0.351	0.975	0.002
Natural phyto-seiids	adults	2.01	a	1.558	b	0.799	c	0.4177	0.4101	0.003
	immatures	1.329	a	0.952	b	0.492	c	0.0882	0.2449	0.002
	eggs	1.021	a	0.833	a	0.35	b	0.0742	0.2061	0.002
P. persimilis	adults	0.701	a	1.164	b	1.252	b	0.1084	0.3011	0.014
	immatures	0.584	a	0.986	b	1.012	b	0.1382	0.3838	0.062
	eggs	0.62	a	0.99	b	1.165	b	0.1132	0.3143	0.02

The numbers of TSSM were higher in the treated plots, all life stages were significantly higher than the control in the knapsack sprayed plots, and the immatures and eggs were also significantly higher than the control in the overhead sprayed plots. These effects were clearer in late August and early September. The two application methods were not significantly different.

The effect of treatment application on the numbers of natural phytoseiid motiles (immatures + adults) showed that the numbers were reduced where a spray was applied, but there was also a significant difference between the two treatments, with the knapsack spray having fewer natural phytoseiids than the overhead spray. This effect was also seen for the eggs, where the numbers were significantly reduced in the knapsack sprayed plots compared to the control or overhead spray. The species of predatory mites were assessed throughout the season. Almost all natural phytoseiids were *A. andersoni*, with other phytoseiids such as *N. californicus* being found in the latter dates.

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Conversely, the spray programme did not negatively affect *P. persimilis* which significantly increased in the sprayed treatments compared to the control.

The square-root transformed data, which links to Table 2.2.3 is presented in **Figures 2.2.6 to 2.2.8.** The back-transformed mean data to compare the different spray application methods is presented in **Figures 2.2.9 and 2.2.10**. The same back-transformed mean data is also presented within each spray system in **Figures 2.2.11.** The population development of *P. persimilis* follows that of the TSSM, albeit on a different numerical scale, which can be clearly seen in the knapsack sprayed treatment.

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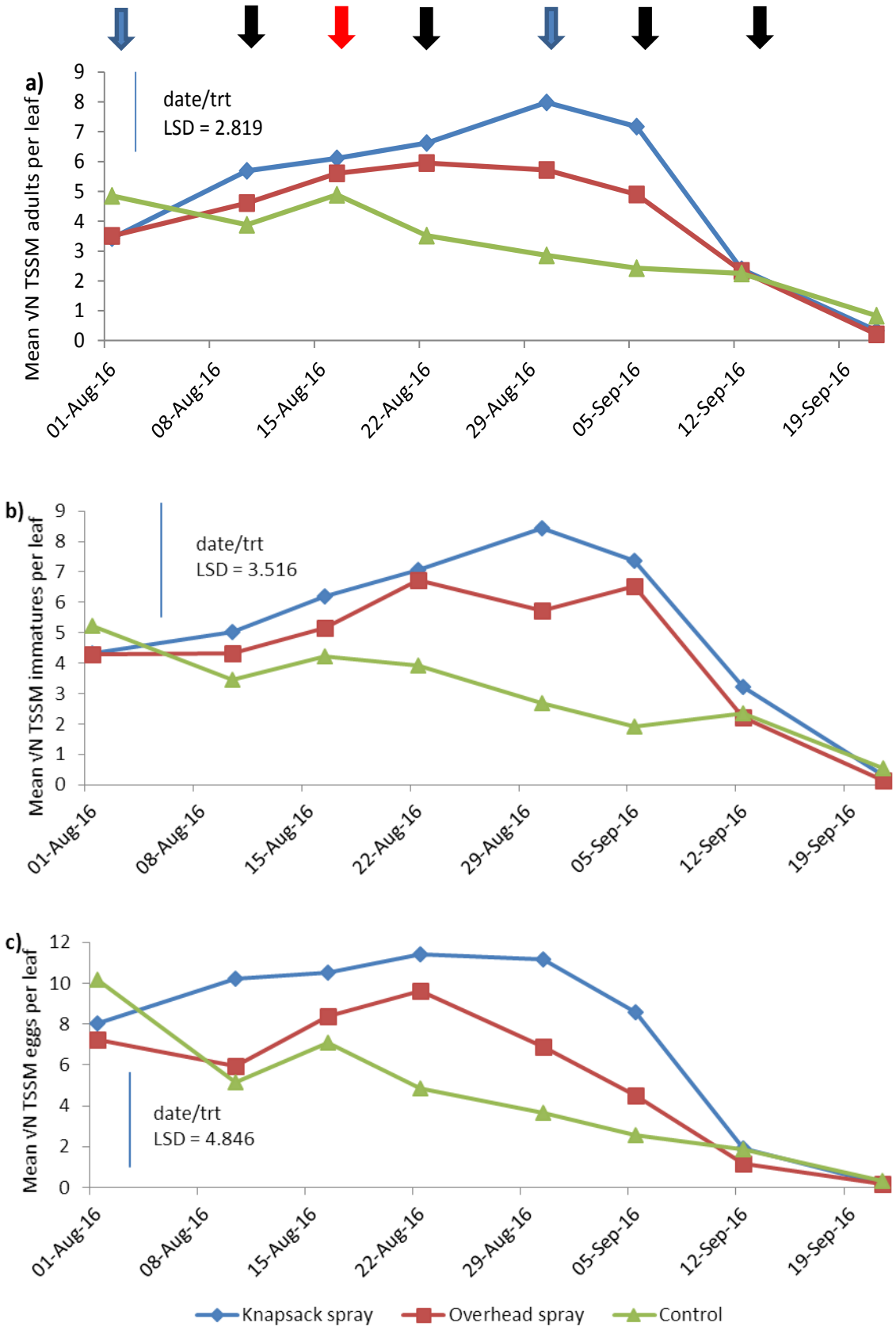

Figures. 2.2.6. a-c Two Spotted Spider Mite: The effect of spray application method on mean √N TSSM a) adults, b) immatures and c) eggs per leaf per plot in 2016. First *P. persimlis* introduction was on 22 July, first spray of spinosad was on 26 July. Arrows show additional introduction of *P. persimilis* (red), spinosad sprays (blue) & deltamethrin sprays (black). Treatment/date LSD shown.

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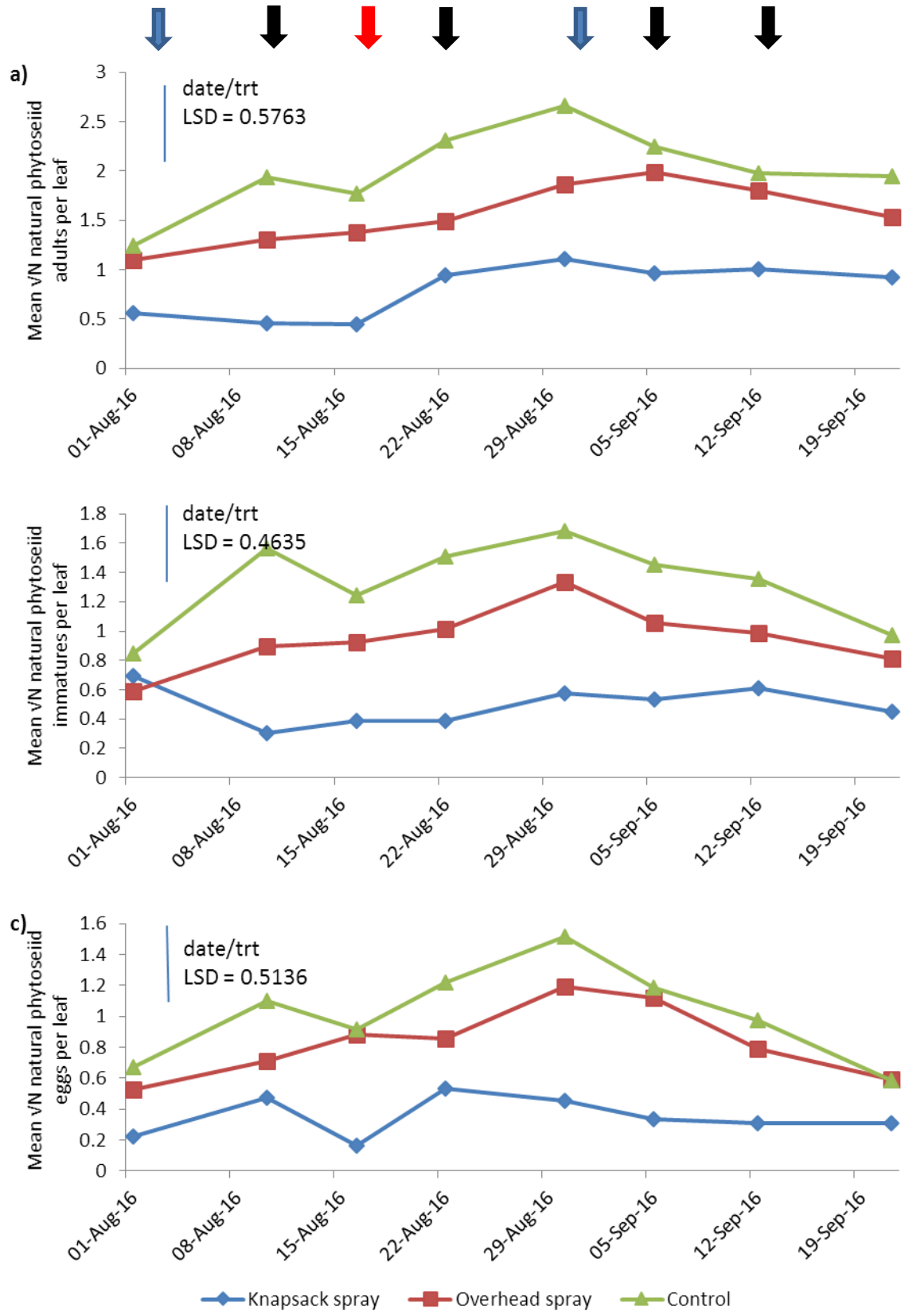

Figures 2.2.7. a-c Phytoseiids. The effect of spray application method on mean √N natural phytoseiid a) adults, b) immatures and c) eggs per leaf per plot in 2016. First *P. persimlis* introduction was on 22 July, first spray of spinosad was on 26 July. Arrows show additional introduction of *P. persimilis* (red), spinosad sprays (blue) and deltamethrin sprays (black). Treatment/date LSD shown.

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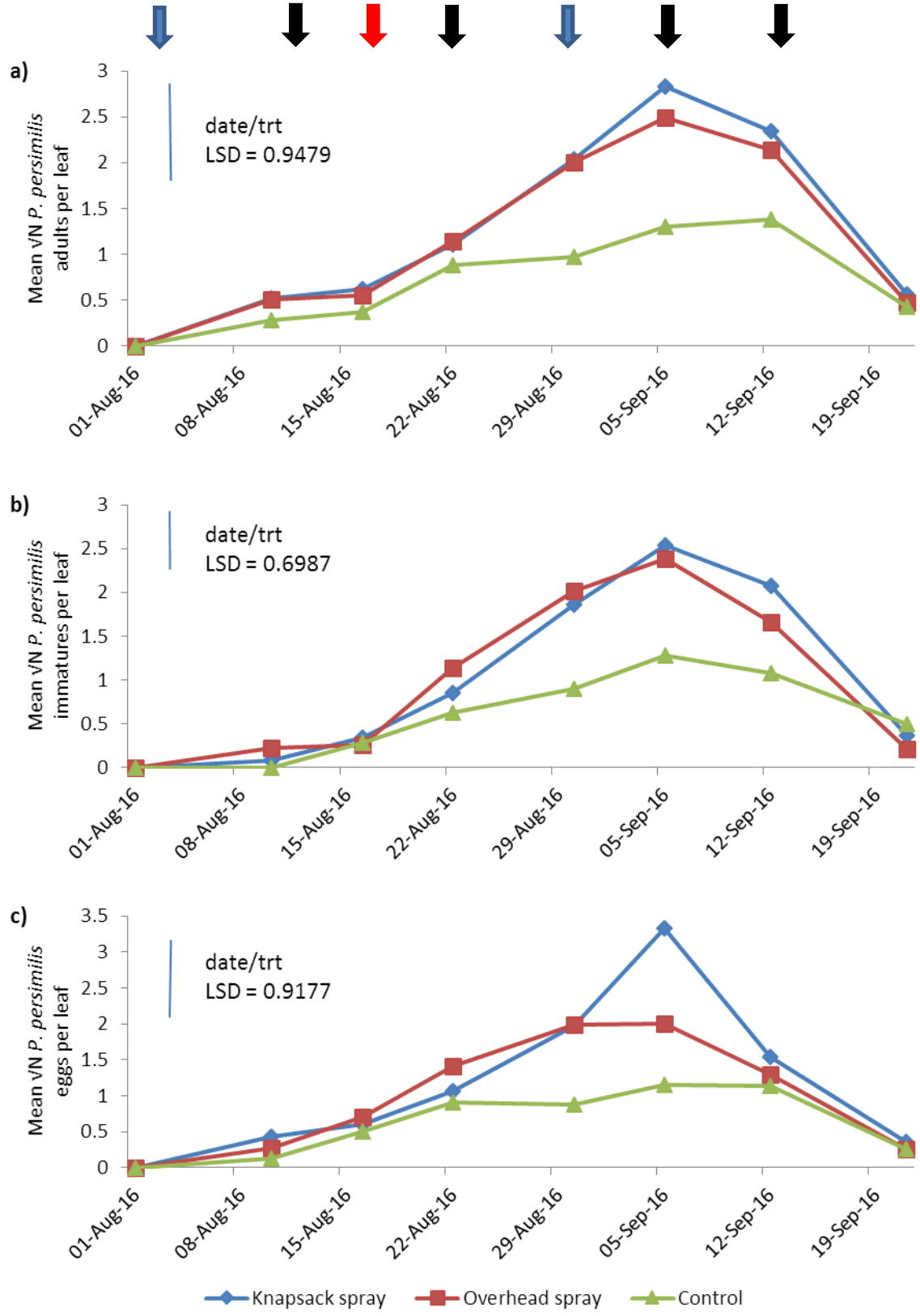

Figures 2.2.8. a-c *Phytoseiulus persimilis.:* The effect of spray application method on mean √N *P. persimilis* a) adults, b) immatures and c) eggs per leaf per plot in 2016. First *P. persimlis* introduction was on 22 July, first spray of spinosad was on 26 July. Arrows show additional introduction of *P. persimilis* (red), spinosad sprays (blue) and deltamethrin sprays (black). Treatment/date LSD shown.

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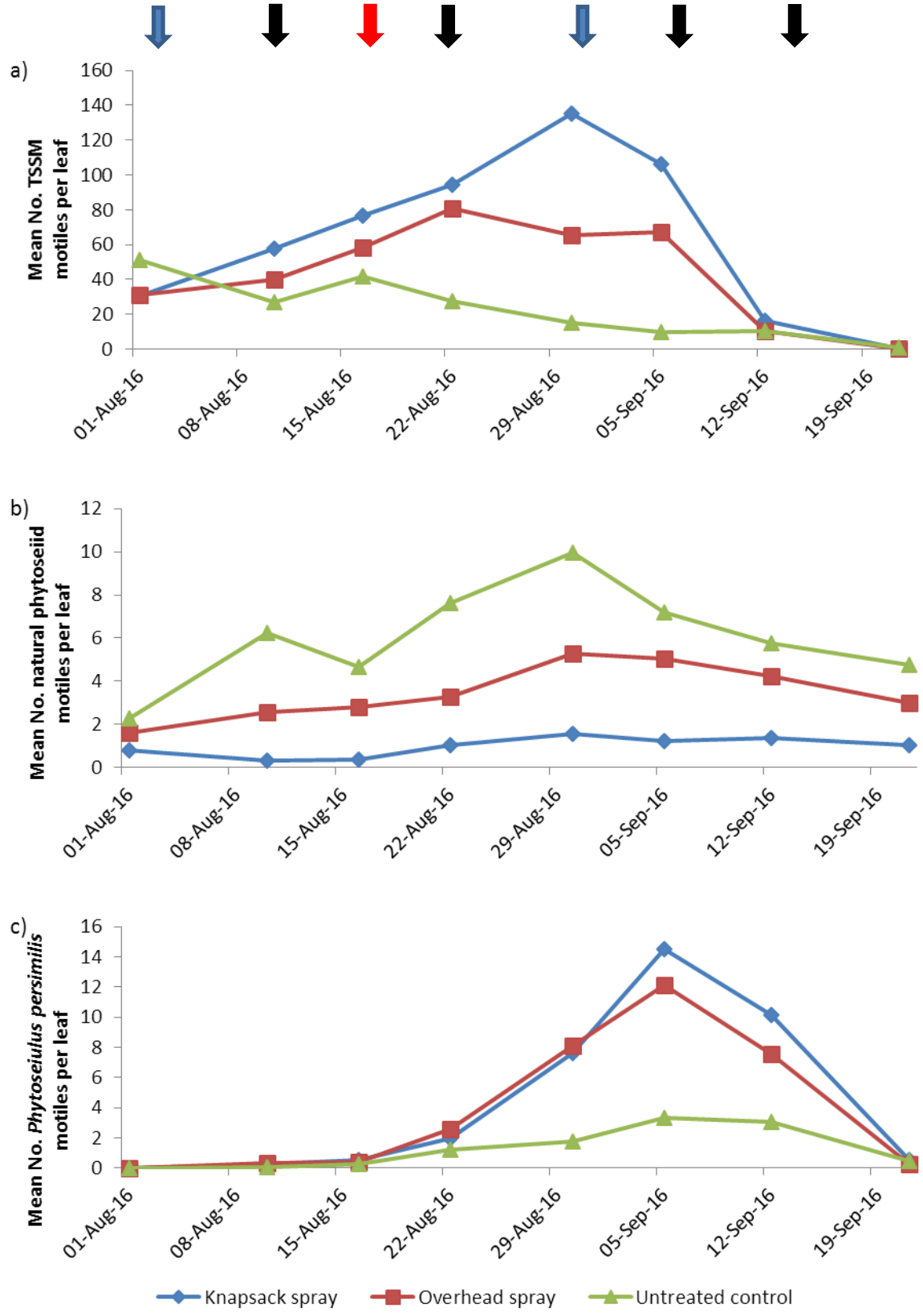

Figures 2.2.9. a-c The effect of method of application of spray treatments on the backtransformed mean number of a) TSSM, b) natural phytoseiids and c) *Phytoseiulus persimilis* motiles per leaf per plot in 2016. First *P. persimlis* introduction was on 22 July, first spray of spinosad was on 26 July. Arrows show additional introduction of *P. persimilis* (red), spinosad sprays (blue) and deltamethrin sprays (black).

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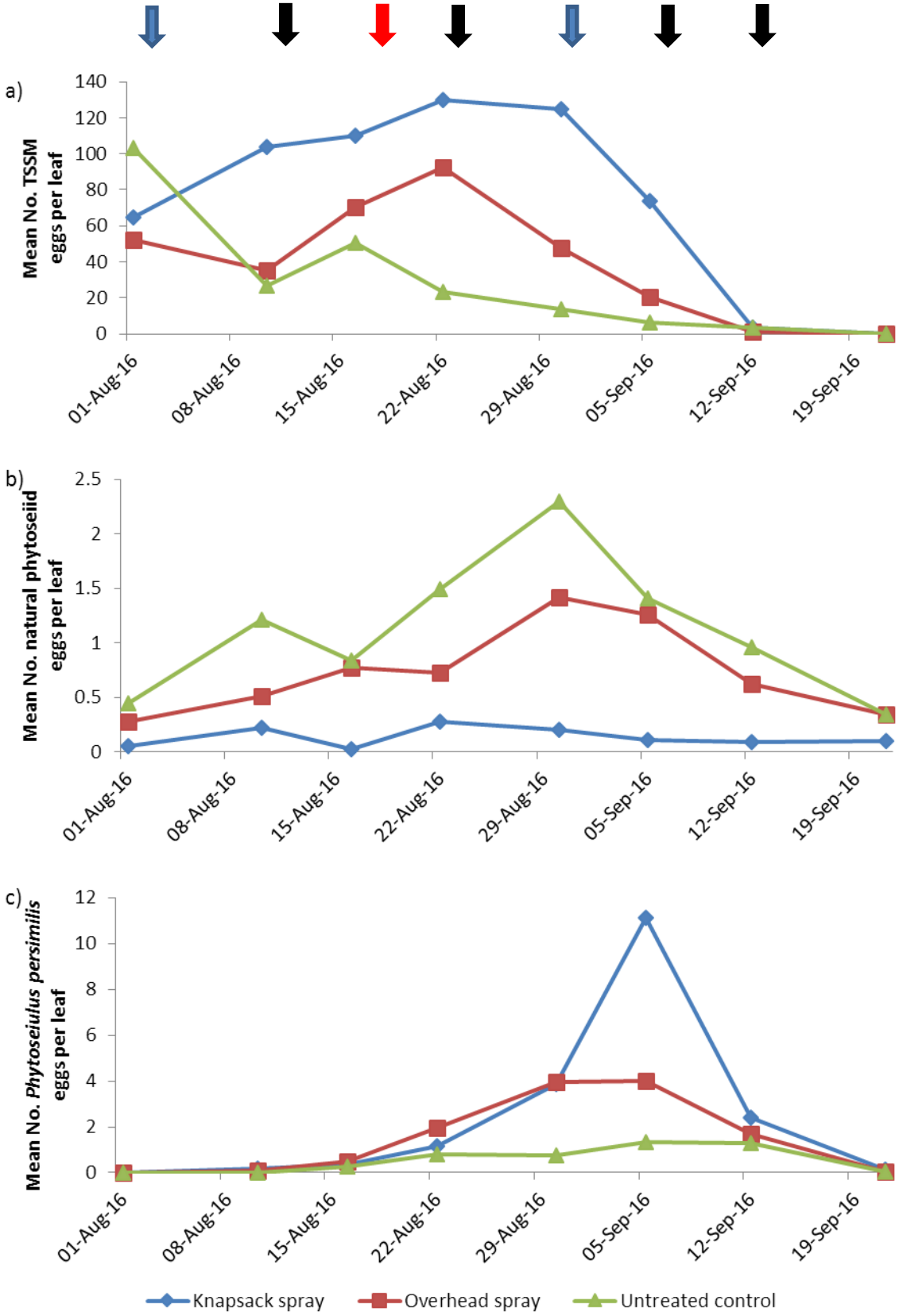

Figures 2.2.10. a-c The effect of method of application of spray treatments on the backtransformed mean number of a) TSSM, b) natural phytoseiids and c) *Phytoseiulus persimilis* eggs per leaf per plot in 2016. First *P.persimlis* introduction was on 22 July, first spray of spinosad was on 26 July. Arrows show additional introduction of *P. persimilis* (red), spinosad sprays (blue) and deltamethrin sprays (black).

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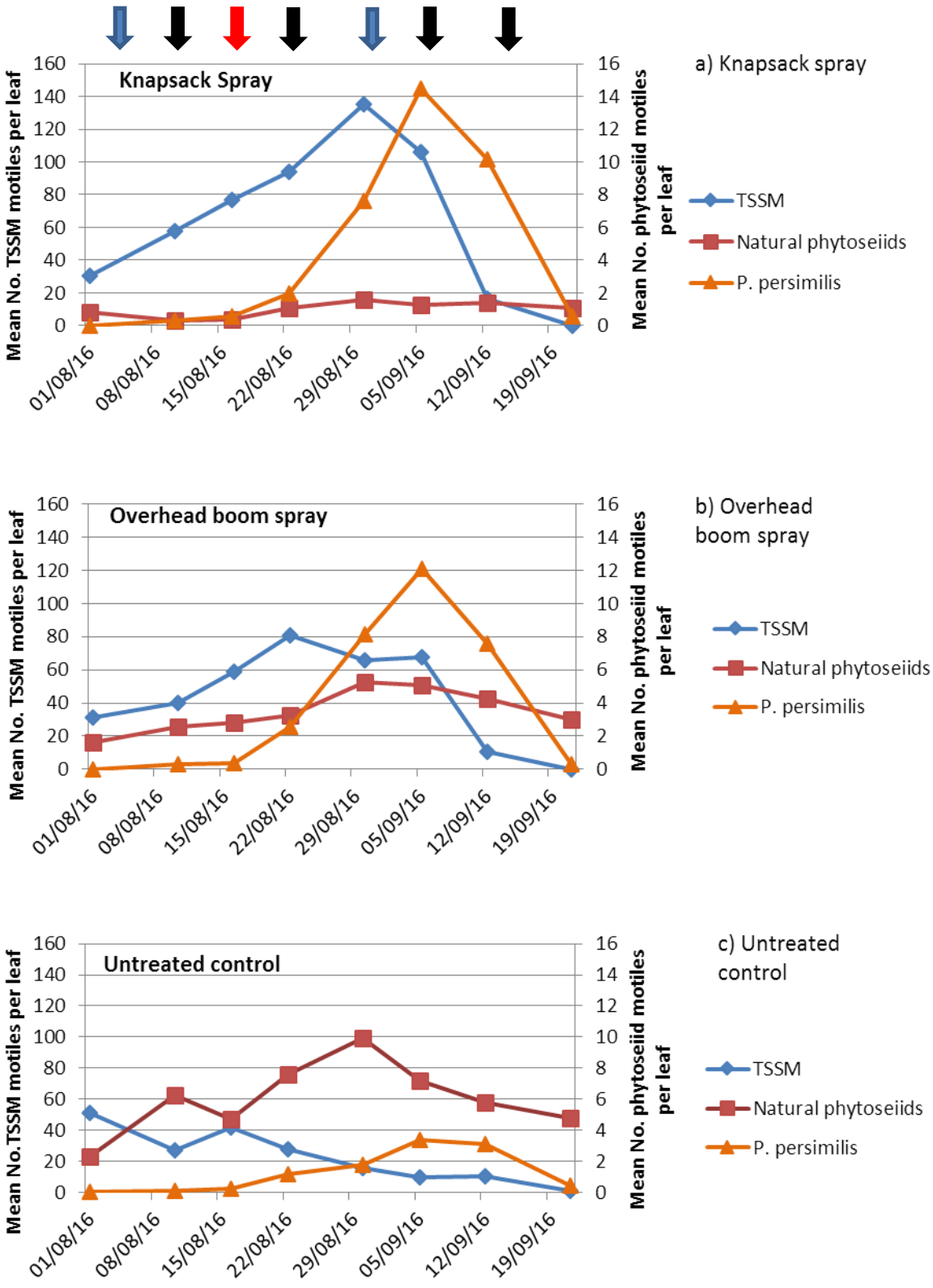

Figures 2.2.11. a-c The effect of spray treatment system a) knapsack spray, b) overhead spray boom and c) untreated control, on the backtransformed mean number of TSSM, natural phytoseiids and *Phytoseiulus persimilis* motiles per leaf per plot in 2016. First *P. persimlis* introduction was on 22 July, first spray of spinosad was on 26 July. Arrows show additional introduction of *P. persimilis* (red), spinosad sprays (blue) and deltamethrin sprays (black).

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Assessments of Spotted Wing Drosophila

The appearance of the fruit after two weeks at 20°C was drastically different between the untreated control and the sprayed treatments. The fruit from untreated control plots had high numbers of SWD pupae and the fruit had transformed to a layer of pulp, as opposed to sprayed fruit, which albeit mouldy, had retained their structure.

The overall numbers of SWD were significantly lower in both of the treated plots (**Figure 2.2.12.**), but not significantly different between the two treatments. Following a $\log^e$ transformation, and repeated measures analysis there was a treatment mean per plot per pick (50 fruits) of 3.86, 4.13 and 5.82 (back-transformed numbers 47, 62 and 337) for the knapsack spray, overhead spray and untreated control respectively, *p* for treatment effect = 0.014, l.s.d. = 1.081, d.f. = 4. There was no significant interaction, which implies that the relative difference between treatments were the same on all dates. Other flies were also found, mainly *Drosophila spp.*, albeit in low numbers, therefore these are not presented.

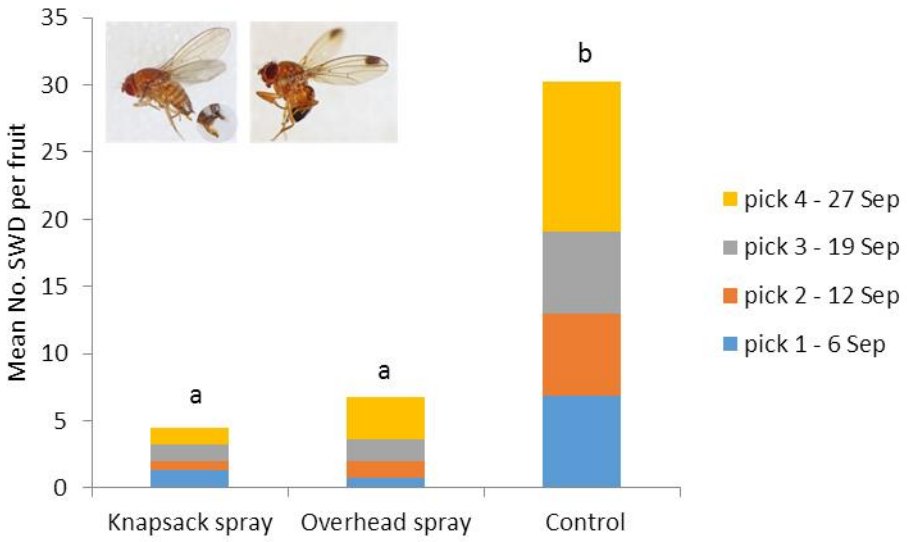

Figure 2.2.12. Mean number of SWD per raspberry fruit.

A spinosad spray was applied on 30 August and deltamethrin sprays were applied on 6 and 14 September (following picking).

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Assessment of Leaf Damage

Leaf damage was not significantly different between treatments, although the mean damage score per leaf appeared to be higher in the sprayed plots in late August and early September **(Figure 2.2.13)**.

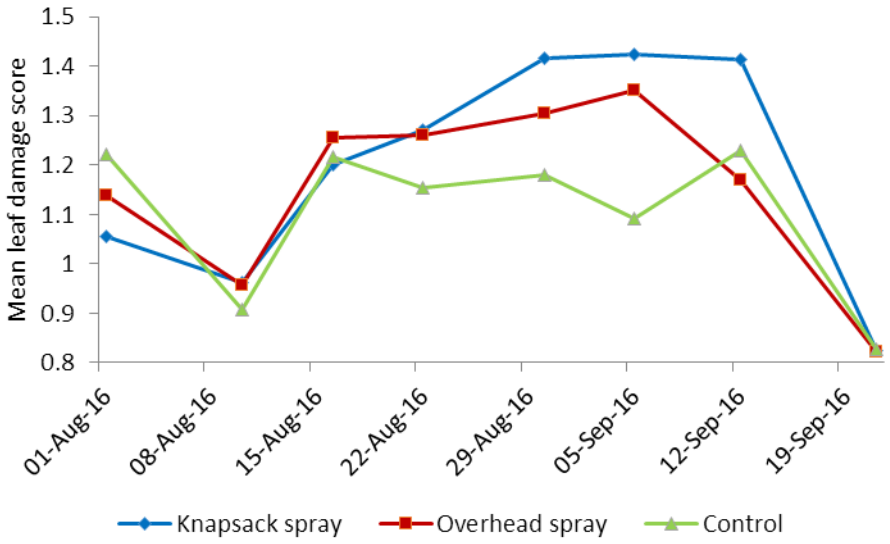

Figure 2.2.13. The mean damage score per leaf, recorded on a score from 0 to 4, where damage was recorded as leaf area affected. 0 = no damage, 1 = 1-25%, 2 = 26-50%, 3 = 51-75% and 4 = 76-100%.

Assessment of Spray Deposition

For the spray deposition experiment, the percentage cover data from image analysis of leaves sprayed by the two treatments was analysed using two methods. Initially using a generalised linear model, assuming that the coverage is distributed as a binomial distribution. Also transforming the percentage cover data by arcsine transformation ($\sin\sqrt{y} \div 100$) and analysing by a generalised linear model with a normal distribution. The models were assessed for goodness of fit using the Hosmer-Lemeshow test. Significant factors in predicting the spray coverage were found to be 'treatment', 'leaf side', and the interactions 'treatment:leaf.side' and 'treatment:canopy:leaf.side' **(Table 2.2.4.).** There was higher spray coverage in the knapsack sprayed treatment, compared to the overhead sprayed treatment (**Figure 2.2.14.**). There was also less spray on the lower leaf side of the overhead sprayed treatment. There is also more variability in the amount of spray on the lower leaf surfaces, especially in the overhead spray treatment, i.e. some leaves will have very little spray, and other leaves may have more coverage. This may be partly due to inherent variability in plant architecture and leaf angle.

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Table 2.2.4. Percentage cover data from image analysis of leaves sprayed by the two treatment methods.

Treatment	Canopy	Leaf side	N	% Cover	s.d.	Max.	Min.	s.e.	c.i.	%C.V.
Overhead	Top	Upper	24	50.74	30.89	98.50	0.64	6.31	13.04	60.9
Overhead	Top	Lower	24	3.72	7.96	35.54	0.00	1.62	3.36	213.9
Overhead	Bottom	Upper	24	40.16	32.01	94.33	0.00	6.53	13.52	79.7
Overhead	Bottom	Lower	24	13.73	22.32	71.62	0.00	4.56	9.42	162.6
Knapsack	Top	Upper	25	55.18	32.94	96.66	3.93	6.59	13.60	59.7
Knapsack	Top	Lower	24	55.71	39.56	100.00	0.00	8.08	16.70	71.0
Knapsack	Bottom	Upper	24	72.16	30.83	99.66	6.94	6.29	13.02	42.7
Knapsack	Bottom	Lower	24	37.37	30.67	98.96	0.00	6.26	12.95	82.1

Table 2.2.5. Percentage of spray in L/ha as a percentage of the application rate (1000 L/ha) found on leaves sprayed by the two treatment methods.

Treat	Canopy	Leaf side	N	% L/ha from application rate	s.d.	Max.	Min.	s.e.	c.i.	%C.V.
Overhead	Top	Upper	18	23.39	22.40	77.97	5.27	5.28	11.14	95.8
Overhead	Top	Lower	18	1.33	4.45	18.96	0.00	1.05	2.21	333.7
Overhead	Bottom	Upper	18	15.10	16.81	61.78	0.38	3.96	8.36	111.3
Overhead	Bottom	Lower	18	0.00	0.02	0.07	0.00	0.00	0.01	424.3
Knapsack	Top	Upper	18	13.76	10.49	43.66	3.36	2.47	5.21	76.2
Knapsack	Top	Lower	18	5.16	3.55	10.82	0.00	0.84	1.77	68.8
Knapsack	Bottom	Upper	18	13.39	7.37	30.49	5.53	1.74	3.66	55.0
Knapsack	Bottom	Lower	18	2.73	5.51	23.90	0.00	1.30	2.74	201.9

The amount of the novel tracer on the sampled leaves is shown in **Table 2.2.5.** The amount of the tracer on sampled leaves was analysed in two parts, in a hurdle model: percentage of leaves with the tracer detected counts and the amount of the tracer on those leaves with the tracer detected. The first model part is a logistic model [GLM assuming binomial distribution] for % leaves with the tracer detected. The second part (amount of the tracer) was modelled as GLM assuming the distribution follows either a negative binomial distribution (treating the tracer as a counts variable) or gamma distribution (hence a continuous variable). The

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percentage leaves with the tracer detected was influenced by canopy, all leaves without the tracer detected were from the 'lower leaf side' treatment. The amount of the tracer on those leaves with the tracer detected is affected by row, treatment and leaf side. The yellow treatment had a higher amount of the tracer (as seen on the upper leaf side) and the upper leaf side had more of the tracer than the lower leaf side.

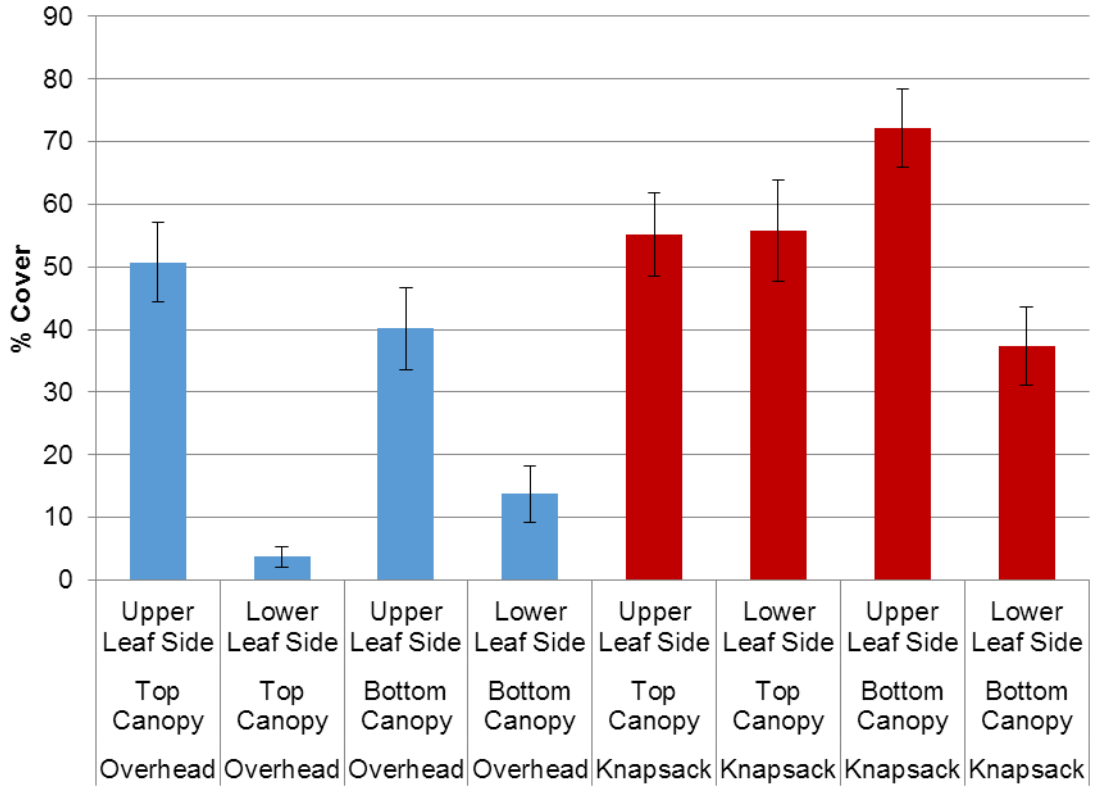

Figure 2.2.14. Mean percentage cover from image analysis of sprayed leaves with either an overhead boom spray or knapsack spray treatment, +/- SE.

Discussion and Conclusions

The effects of overall canopy spraying verses overhead application of a programme of sprays of deltamethrin and spinosad on TSSM and predatory mites, both natural and introduced, were compared and both the overall effect of date and overall effect of treatment were significant. The numbers of TSSM were higher for all lifestages in the knapsack sprayed plots, and also in the overhead plots for the immatures and eggs, especially in late August/early September. Although there were significantly less natural phytoseiids in these treatments throughout the season, the numbers of *Phytoseiulus persimilis* motiles were not reduced by the sprays, nor were there less *P. persimlis* eggs laid. *P. persimilis* was less affected by the deltamethrin sprays than expected; the side effects of deltamethrin on the Koppert website quote an effect of '4' i.e. very harmful, with a 75% reduction for the adults, nymphs and eggs, with an 8-12 week persistence, and a

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moderately harmful effect of spinosad with a 25 – 50% reduction. The sprays affected the natural phytoseiid, *A. andersoni*, 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. This also showed that the amount of spray on the underside of leaves was variable.

Both treatments significantly reduced the number of SWD per fruit compared to the control. Although there were some SWD in these treatments, this experiment had small isolated units and was in a high pressure area (i.e. near to woodland, in which results from weekly trap catches showed a mean of 100 SWD per trap, from SF 145, and adjacent to fruit crops). The last picking date had a higher number of SWD than the initial picking dates, perhaps due to the two week timing between the last spray on the 14 September and the final pick on 27 September.

Future work

In 2017 it is proposed to use the same system of overhead spraying, to determine whether this will again give less spray on the underside of the leaves, so providing refuge for the predatory mites and therefore improved control of TSSM. Biocontrol agents such as *P. persimilis* will again be introduced on two occasions.

Conclusions

- TSSM numbers were higher in the sprayed treatments (for all life stages with the knapsack spray)
- The natural phytoseiids were affected by the spray treatments, but the effect could be mitigated by spraying from above
- Introduced *P. persimilis* was less affected by the spray programme than anticipated
- There was less spray on the underside of the leaves in the overhead spray treatment
- Both methods of application, the boom spray and the knapsack spray, reduced the number of SWD compared to the control

Acknowledgements

We would like to thank Salih Hodzhov of W. B. Chambers for provision of plants. We would also like to thank Graham Caspell and team for maintenance of the tunnels and irrigation systems. Thanks are also due to Zeus Mateos Fierro and Adrian Harris for assistance with the experiments.

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Objective 3: To develop and combine novel and current IPM approaches to successfully control blackberry leaf midge

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. This damage can stunt cane growth leading to yield loss. It has been estimated that the midge could reduce raspberry yield by 40% and blackberry yield by 10% resulting in losses of £12,000 and £3,000 per ha respectively (Fountain, 2013). The pest has increasingly been identified as a high priority for research by the industry, particularly in primocane systems.

As the midge larvae feed within the leaf tips they are very difficult to target using foliar sprays of plant protection products. In SF 102 'Biology and integrated control of blackberry leaf midge on blackberry and raspberry' (Bennison, 2011), the only effective foliar spray in a trial on a commercial blackberry crop was chlorpyrifos, but this insecticide is no longer approved for use in the UK and in any case is not compatible with IPM. Sprays of thiacloprid, abamectin and the entomopathogenic fungus, *Beauveria bassiana* (Naturalis-L) were ineffective. In SF 141 'Efficacy of insecticides, timed using the blackberry leaf midge pheromone trap, to control the pest on raspberry 2014' (Fountain, 2014), deltamethrin reduced damage and midge numbers on a commercial raspberry crop but this insecticide is incompatible with IPM. In SF 102, in laboratory tests, application of Naturalis-L to substrate in pots did not reduce the numbers of midge larvae completing their development and emerging as adults. However, in the same project, a pot experiment 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.

In the first year of this current project, SF 158, a laboratory pot test demonstrated that the entomopathogenic nematode *Steinernema kraussei* (Nemasys® L) significantly reduced mean numbers of blackberry leaf midge adults (mean 0.8) emerging from treated substrate compared with control pots (mean 2.3) after adding eight fully grown midge larvae to the substrate surface. Nemasys® L is widely used already as a drench for control of vine weevil on soft fruit crops.

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Aim

Objective 3: To evaluate the efficacy of drenches of *Steinernema kraussei* (Nemasys® L for the control of blackberry leaf midge in a commercial raspberry crop.

Materials and Methods

Site

The trial was carried out in 2016 on a commercial soil-grown polythene tunnelled raspberry crop (cv. Amara) on a farm in Cambridgeshire with a history of blackberry leaf midge damage. The crop was managed by the grower according to commercial practice with no restrictions on plant protection products used. The crop was watered using drip irrigation with one irrigation line per row of plants.

Treatments

Nemasys® L was used at the rate and water volume recommended for vine weevil control in soil-grown crops (**Table 3.1**).

Table 3.1. Treatments used in the field trial

Treatment No.	Treatment	Rate	Timings
1	Untreated	-	-
2	*Steinernema kraussei* (Nemasys® L)	1,000,000 /m^2 at 4 L/m^2 application volume	First sign of larvae in leaf tips for each of the first two generations

Trial design

- One tunnel was used for the trial, this was 159 m long and 8.2 m wide with three rows of raspberry plants (**Figure 3.1**).
- There were four replicate plots of each treatment with each plot being 15 m long and three rows wide.
- The plots were not randomised; the treated plots were in one half of the length of the tunnel and the untreated plots were in the other half. This was to allow easy application of Nemasys® L by the host grower.
- There were three buffer areas; four metres at each end of the tunnel and 31 m in between the two halves of the tunnel, in order to reduce the risk of adult midge immigration from untreated plots and from adjacent tunnels.
- All three rows of raspberries were treated with treatment 2 but only the middle row was assessed, again to reduce the risk of adult midge immigration from adjacent tunnels.

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Figure 3.1. Commercial crop in tunnel used for trial with three rows of raspberry plants

Treatment timings

The first nematode treatment was targeted against the first generation of midge larvae dropping to the ground to pupate. The aim was to apply the nematodes before the larvae dropped to the ground so that they were present in the soil when larval drop occurred, to allow them to infest the larvae in the soil before they spun a protective cocoon in which to pupate. Therefore the nematodes were applied as soon as possible after the first larvae were seen in the leaf tips. The nematodes were not applied before this date as there were concerns that they may not survive long in the soil after application. Blackberry leaf midge pheromone traps were used to monitor the first adult males becoming active in the crop and leaf tips were monitored for the first larvae in order to trigger the first nematode application.

Two Agralan blackberry leaf midge delta traps were used to monitor for the first adult males. These were fitted with pheromone lures and placed out into the crop one week ahead of the predicted date of first adult emergence using the ADAS met office prediction service for blackberry leaf midge at this particular farm. The traps were placed at opposite ends of the block of tunnels diagonally from each other, with one trap located in the cv. Amara crop and one in the cv. Kweli crop which was adjacent to the trial tunnel. The traps were monitored weekly until the first male adult had been recorded in the trap. From this point on the traps were monitored fortnightly to help determine when the second generation occurred.

Following the first adult males caught in the pheromone traps the trial tunnel was monitored weekly for symptoms of blackberry leaf midge damage. Leaf tips were gently opened without removing them from the plants to confirm presence of larvae to trigger the first

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application date. The second application date was timed when leaf tip assessments on ten leaf tips per plot indicated that second generation midge larvae were present.

Treatment application

The Nemasys® L was applied by the host grower using his tractor-mounted spray equipment. It was not possible to apply the nematodes through the drip irrigation system as the irrigation lines in the trial tunnel could not be isolated from those in adjacent tunnels. The pack of nematodes was mixed with a small amount of water in a bucket to create a 'paste'. This was then added to the larger volume of water in the tank to achieve the desired concentration. The nematode suspension was then applied as a drench to the soil beneath the crop canopy whilst maintaining agitation in the tank to prevent the nematodes settling out. The area drenched was 60 m long i.e. the total length of the four treated plots, and 0.6 m to 0.64 m wide on each side of each of the three rows of plants in the trial tunnel (**Figure 3.2**). This ensured that all the soil beneath the infested leaf tips was treated. Immediately following application an additional irrigation cycle was used to help to wash the nematodes into the soil and to increase soil moisture in order to aid nematode movement and survival.

Figure 3.2. Area of soil over plastic mulch under raspberry canopy drenched with Nemasys® L.

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Checking nematode viability and numbers applied

At the time of nematode application four replicate 10ml samples were taken from the nematode suspension in the tank. An additional four replicate samples were collected in Petri dishes placed onto the soil beneath the crop canopy to check nematode viability and numbers applied. The samples were transferred to screw-top tubes in order to take them to the laboratory for checking. In the laboratory, the nematode suspension in each tube was agitated and three replicate 1ml samples were taken from each tube and placed into a Hauxley haemocytometer counting chamber. Numbers of live nematodes per ml were recorded after examination under a low power microscope.

Monitoring larvae in leaf tips to assess treatment efficacy

Once the first larvae had been detected in the leaf tips in the field, leaf tip assessments were carried out fortnightly with the first assessment carried out on the same date as the first nematode application. Ten plants were randomly selected in the middle of the three rows of plants in each plot and a lateral on each selected plant was marked. One leaf tip per marked lateral was sampled at each assessment, giving 10 leaf tips per plot. Each leaf tip consisted of a group of leaves at the growing point. These were carefully examined and dissected under a low power microscope and the numbers of twisted leaf tips (**Figure 3.3**) and the presence and numbers of midge larvae recorded (**Figure 3.4**).

Figure 3.3. Raspberry leaf tip twisted by blackberry leaf midge

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Figure 3.4. Blackberry leaf midge larvae inside twisted leaf tip

Soil temperature records

Soil temperatures to 30 mm depth were recorded in the area treated with nematodes, using a data logger. The 30 mm depth was selected as blackberry leaf midge larvae and cocoons were found at this depth when sampling the soil in a blackberry crop to determine the pest's life cycle in project SF 102 (Bennison, 2011).

Data analysis

The percentage twisted leaf tips, percentage infested leaf tips and numbers of larvae per leaf tip were subjected to Analysis of Variance.

Results

Treatment timings

The blackberry leaf midge pheromone traps were placed in the crop on 21 April 2016, one week prior to the ADAS forecast based on meteorological data for the first adult emergence at this farm on 29 April. The first adult males were found in the pheromone traps on 27 April, when 13 males were found in the trap in the trial raspberry crop cv. Amara and seven males were found in the adjacent raspberry cv. Kweli crop (**Table 3.2** and **Figure 3.5**). Blackberry leaf midge larvae were also found in the leaf tips on 27 April, therefore the Nemasys® L was applied by the host grower as soon as possible after this date, on 5 May.

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Table 3.2. Numbers of adult male blackberry midge adults in pheromone traps in the trial crop cv. Amara and the adjacent crop cv. Kweli

Date	Numbers of males per trap in cv. Amara	Numbers of males per trap in cv. Kweli
27 April	13	7
5 May	3	1
19 May	1	1
31 May	1	4
22 June	4	2
4 July	3	2

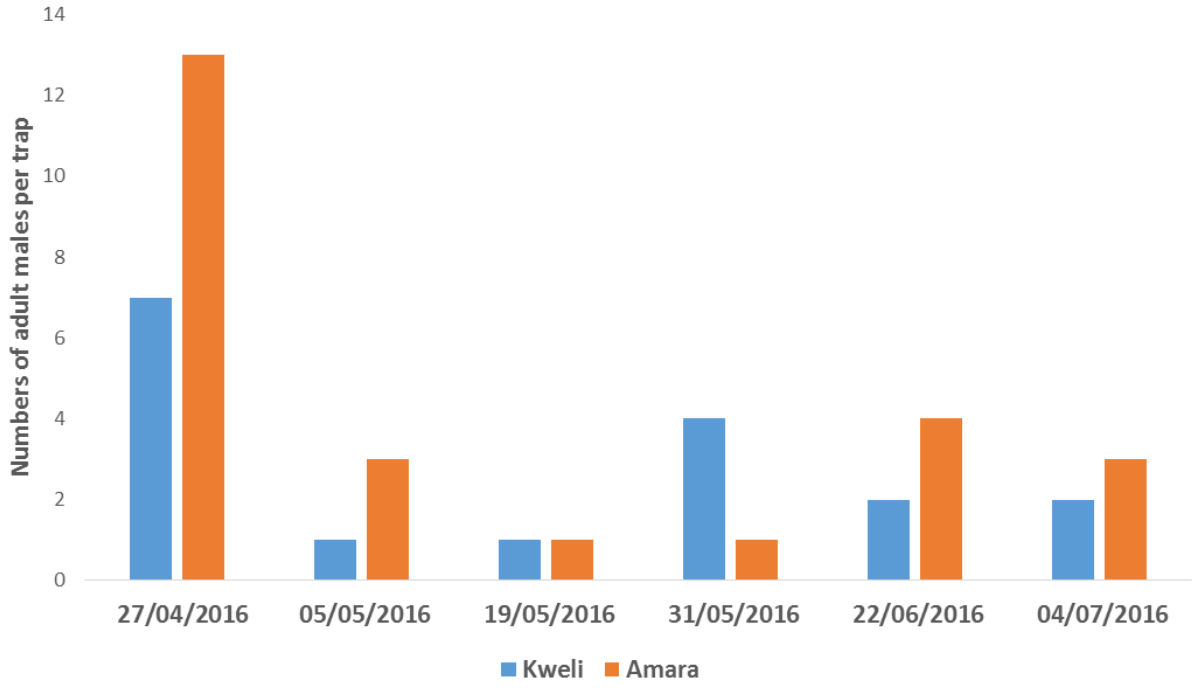

Figure 3.5. Numbers of adult male blackberry leaf midge adults in pheromone traps in the trial raspberry crop cv. Amara and the adjacent crop cv. Kweli. Nematode drenches applied on 5 May and 6 June 2016.

The second application of Nemasys® L was applied on 6 June, as soon as possible after leaf tip assessments on 31 May indicated that the second generation larvae were present in the leaves (**Table 3.3** and **Figure 3.6**).

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Table 3.3. Mean numbers of blackberry leaf midge larvae per leaf tip in untreated and Nemasys® L-treated plots. (N.S.) = not significantly different (P<0.05). 2016.

Assessment Date	Mean numbers of larvae per leaf tip untreated	Mean numbers of larvae per leaf tip Nemasys® L	Statistical analysis
5 May	0.35	0.47	(N.S.)
19 May	0	0.13	(N.S.)
31 May	8.2	6.6	(N.S.)
6 June	8.5	15.1	(N.S.)
22 June	0.75	0.12	(N.S.)
4 July	11.4	15.3	(N.S.)

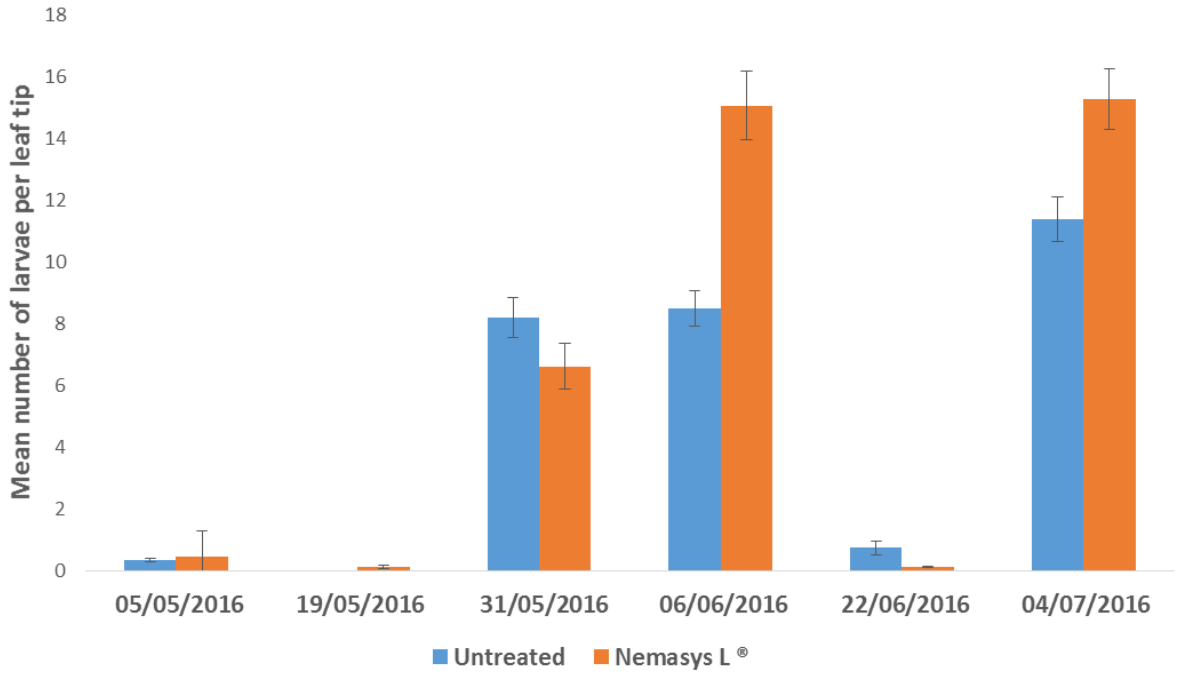

Figure 3.6. Mean numbers of blackberry leaf midge larvae per leaf tip in untreated and Nemasys® L-treated plots (error bars show standard error). Nematode drenches applied 5 May and 6 June 2016.

Nematode viability and numbers applied

As the Nemasys® L was applied at 1,000,000 per m^2 in 4L water per m^2 the expected number of live *Steinernema kraussei* in the spray tank and in the Petri dishes used to collect nematode samples on the ground was 250 per ml. Mean numbers of live nematodes per ml in the samples collected from the spray tank and in the Petri dishes used to collect nematodes applied to the soil are shown in **Table 3.4**. Mean numbers of live nematodes per ml exceeded the expected 250 per ml in all samples except for the Petri dish samples

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on the soil on the second application date, when mean numbers were slightly lower than expected, at 224 per ml.

Table 3.4. Mean numbers of live *Steinernema kraussei* per ml in samples taken from the spray tank and the Petri dishes placed on the soil. 2016.

Nematode treatment application dates	Mean numbers of nematodes per ml in spray tank	Mean numbers of nematodes per ml in Petri dishes on soil
5 May	257	279
6 June	281	224

Monitoring leaf tip damage and larvae to assess treatment efficacy

Percentage twisted and infested leaf tips

The percentage twisted and infested leaf tips in untreated and Nemasys® L-treated raspberry plots are shown in **Table 3.5** and the percentage infested leaf tips are shown in **Figure 3.7**. There were no significant differences between the mean percentage twisted or infested leaf tips in untreated and Nemasys® L-treated plots (P<0.05).

Table 3.5. Percentage twisted and infested leaf tips in untreated and Nemasys® L-treated plots in 2016 soil-grown raspberry. (N.S.) = not significantly different (P<0.05).

Assessment Date	Untreated		Nemasys® L-treated		Statistical analysis
	Mean % twisted leaf tips	Mean % infested leaf tips	Mean % twisted leaf tips	Mean % infested leaf tips	For both % twisted and % infested tips
5 May	32.5	20.0	45.0	22.5	(N.S)
19 May	30.0	0.0	18.0	2.5	(N.S)
31 May	72.5	77.5	50.0	67.5	(N.S)
6 June	90.0	95.0	85.0	90.0	(N.S)
22 June	12.5	15.0	5.0	12.5	(N.S)
4 July	8.0	100	90.0	92.5	(N.S)

The mean percentage of infested leaf tips was 20% and 22.5% in untreated and treated plots respectively, when the first blackberry leaf midge larvae were recorded. Following a reduction in mean percentage of infested leaf tips on 19 May after the grower had cut back the canes and when most of the larvae in twisted leaf tips are likely to have dropped to the ground to pupate, mean percentage infested leaf tips had increased on 31 May to 77.5%

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and 67.5% in untreated and treated plots respectively, indicating that the second generation larvae were active. Mean percentage infested leaf tips in the second generation peaked on 6 June when the second application of Nemasys® L was made, with means of 95% and 90% infested leaf tips in untreated and Nemasys® L-treated plots respectively. Following another reduction on 22 June, the percentage infested leaf tips increased on 4 July to 100% and 92.5% respectively in untreated and treated plots, indicating activity of the third generation midge larvae. As there were no significant differences between percentage infested leaf tips in untreated and Nemasys® L-treated plots on any assessment date ($P<0.05$), no further applications of Nemasys® L were made.

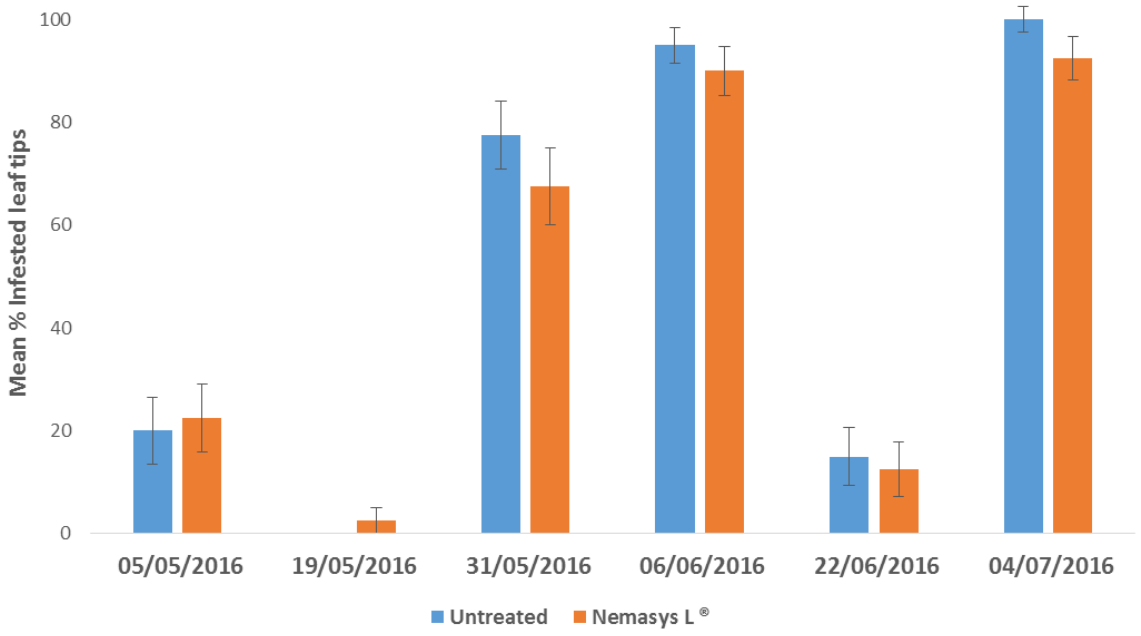

Figure 3.7. Mean percentage infested leaf tips in untreated and Nemasys® L-treated plots (error bars show standard error) in May, June and July. Nematodes applied on 5 May and 6 June 2016.

Mean numbers of larvae per leaf tip

The mean numbers of larvae per leaf tip are shown in **Table 3.3**, **Figure 3.6** and demonstrate that there was no significant difference between numbers in untreated and Nemasys® L-treated plots on any assessment date.

Soil temperature records

Mean, maximum and minimum soil temperatures at 30 mm depth during the trial period are shown in **Figure 3.8**. Temperatures remained within the recommended temperature range for Nemasys® L (5-30°C) throughout the trial period.

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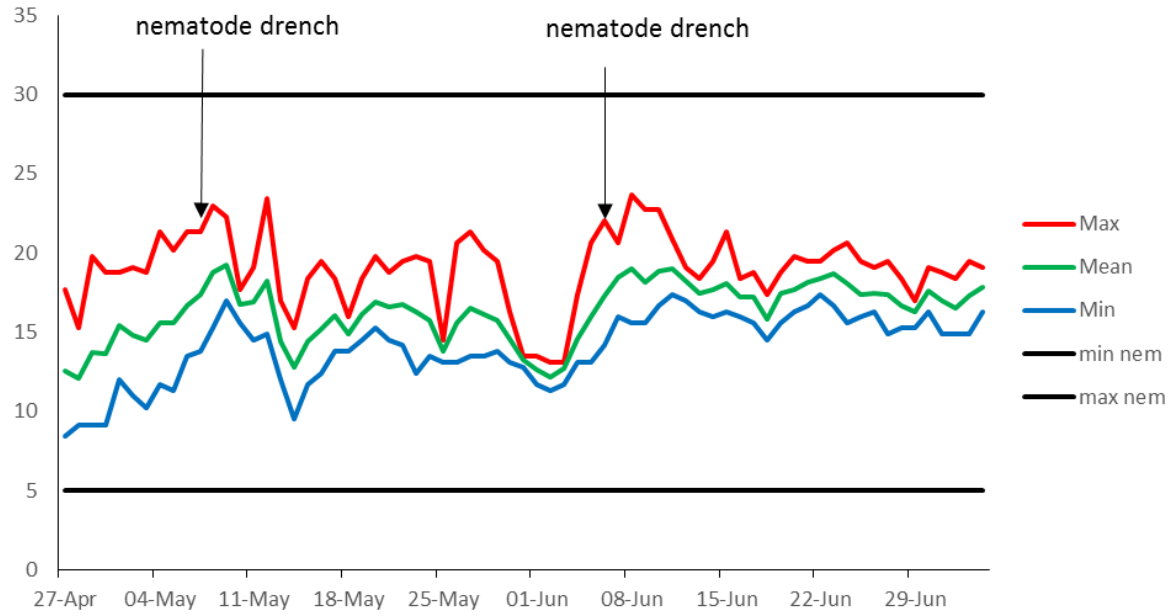

Figure 3.8. Mean, maximum and minimum soil temperatures at 30 mm depth beneath the crop canopy in 2016 from the end of April to early July, and the minimum and maximum temperature range recommended for the efficacy of Nemasys® L.

Discussion

The results of this trial showed that although Nemasys® L gave promising results in a laboratory test in 2015, reducing numbers of adult blackberry leaf midge adults emerging from treated coir substrate in pots compared with those in control pots, it was not effective in controlling the pest in this soil-grown raspberry crop. Soil temperatures were within the recommended range for efficacy of Nemasys® L (5-30°C) throughout the trial period, so these will not have limited nematode efficacy.

Although a proportion of entomopathogenic nematodes including *Steinernema kraussei* can survive for four weeks after application, this depends on the soil or substrate moisture being sufficient. It is known that entomopathogenic nematodes can be less effective for vine weevil control in soil-grown crops than in substrate crops if irrigation is not adequate to maintain sufficient soil moisture to enable the nematodes to move to find the target insect host and to survive. In this field trial, an extra irrigation cycle was added immediately after drenching the nematodes on each application date in order to help wash the nematodes into the soil and aid their movement and survival, but this may not have been sufficient. It was observed on the first application date on 5 May that the soil surface dried out soon after application, but on the second application on 6 June the soil surface remained wet. A 'soil conditioner' has been evaluated for improving soil moisture and thus control of vine weevil by entomopathogenic nematodes in soil-grown strawberry (Ansari, personal

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communication) and it is possible that this could also improve control of blackberry leaf midge by Nemasys® L.

In addition to soil moisture being a possible factor affecting nematode efficacy, the biology of blackberry leaf midge made it difficult to time the nematode drenches for best effect. The midge larvae are known to spin a protective cocoon in which to pupate shortly after dropping to the ground, although it is not known how long after dropping this occurs. For nematodes to have optimum effect, they need to be in the soil in sufficient numbers to infest the larvae soon after dropping before the cocoon develops.

On 5 May when the first application of Nemasys® L was made, the mean percentage infested leaf tips was lower than the mean percentage infested leaf tips in both untreated and Nemasys® L-treated plots. This indicated that some of the larvae in the twisted leaf tips had already dropped to the ground to pupate before the first nematode application was made, eight days after the first larvae were recorded in leaf tips on 27 April.

As control of the first generation of blackberry leaf midge is key to preventing or reducing subsequent generation populations, it is possible that a 'little and often' system for nematode application through the drip irrigation may offer improved but cost-effective control, starting with a preventive application even before the first generation adults are found in pheromone traps. This system is being investigated by ADAS in the current AHDB Horticulture project HNS 195, 'Improving control of vine weevil in hardy nursery stock'.

Conclusions

- Although *Steinernema kraussei* (Nemasys® L) gave promising results when used as a drench in pots of coir in a laboratory experiment in this project in 2015 when compared with control pots, it did not control the first two generations of blackberry leaf midge when applied as two consecutive drenches to a soil-grown raspberry crop in this field trial.
- Further research is needed to identify an effective IPM-compatible control strategy for blackberry leaf midge in both substrate and soil-grown crops and to determine the potential role of Nemasys® L.
- Gaps in knowledge of blackberry leaf midge biology need filling, including how long after dropping to the ground that the larvae spin protective cocoons and what soil or substrate moisture is needed for successful adult emergence.

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Acknowledgements

Thanks to the host grower for providing the trial site and for applying the nematodes, Agralan for providing the pheromone traps and BASF for providing the Nemasys® L.

Objective 4: To investigate strains of *Verticillium* spp. present in UK cane fruit plantations and the thresholds for infection in blackberry and raspberry

Introduction

No further work on this objective was scheduled for 2016, however work by Fera is detailed here that was not previously reported but which adds to the information gained during the 2015 work programme in which propagation material and plants from 28 cane fruit crops with and without wilt symptoms (together with their soil) were taken for molecular diagnosis. *Verticillium dahliae* was not detected in the propagation material, but was found in the roots and stems of a proportion of the wilting soil-grown raspberry and blackberry crops (although little DNA was recovered from the soil taken from beneath plants).

Real-time, or Quantitative, PCR assays (QPCR) for testing soils prior to planting for specific soil-borne Verticillium species using DNA extracted from large volumes (up to 1 kg) of soil were successfully developed (project SF 97). Detection of *V. dahliae* using QPCR has been achieved down to levels correlating with 0.5 microsclerotia / g soil and the assay is still being improved in order to attain a detection of <0.5 microsclerotia / g soil. The DD fragment used for design of the QPCR test is totally specific for *V. dahliae* (and *Verticllium longisporum*) and uses a sequence-characterized amplified region (SCAR). Specific primers were designed for use in PCR detection assays, and amplified a unique DNA fragment in all isolates of *V. dahliae* and *V. longisporum*, but not in the related pathogens. The DD fragment was used in conjunction with the IGS primers (Bilodeau) as a check, as Bilodeau is sensitive but not specific whilst the DD assay is 10-100 times less sensitive.

Aims

To carry out laboratory validation of the DD assay for *Verticillium dahliae* to confirm specificity and sensitivity

Materials and Methods

The assay was assessed for specificity using a panel of *V. dahliae* and *V. longisporum* isolates held at the Fera Sand Hutton laboratory (**Table 4.1**).

Results

The assay detected both *Verticillium dahliae* and its hybrid *V. longisporum* but not *V. albo-atrum* (**Table 4.1**).

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Table 4.1. Ct values obtained in RT PCR reactions against a panel of Verticillium isolates – *V. dahliae* (Vd), *V. albo-atrum* (Vaa) and *V. longisporum* (Vd). Values of 40.00 show no detection of Vaa (equivalent to H2O).

Isolate species	Isolate code, or other test material	Ct values			Mean Ct values
Vd	V86	18.23	18.37	18.32	**18.31**
Vd	V116	17.96	18.10	17.96	**18.01**
Vd	Vd 20609	23.27	23.12	22.79	**23.06**
Vd	Vd VCG 2A	23.61	23.71	24.00	**23.78**
Vd	VCR 2A	19.58	19.22	19.26	**19.35**
Vaa	CC630	40.00	40.00	40.00	**40.00**
Vd	12973	40.00	35.79	35.90	**37.23**
Vaa	CC630Vaa	40.00	40.00	40.00	**40.00**
VL	VL 143	21.71	21.82	21.98	**21.84**
VL	VL WLV3	20.64	20.68	20.25	**20.53**
VL	VL OSR	33.35	35.06	33.81	**34.07**
Vd	20609	19.71	20.97	20.10	**20.26**
Vaa	Vaa 1871	36.84	40.00	40.00	**38.95**
Vd	Vda 22268	19.04	19.50	19.47	**19.34**
VL	VL PF1	21.43	21.41	21.54	**21.46**
	H20	40.00	40.00	40.00	**40.00**
	Control	20.08	19.82	19.88	**19.93**

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 (**Figure 4.2**)

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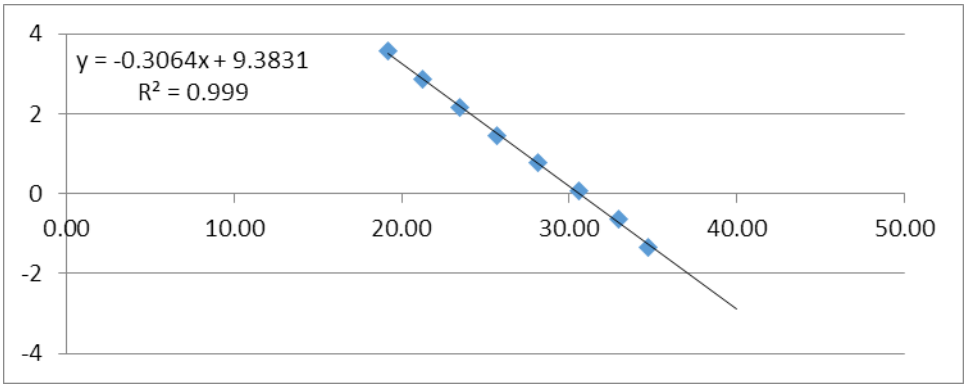

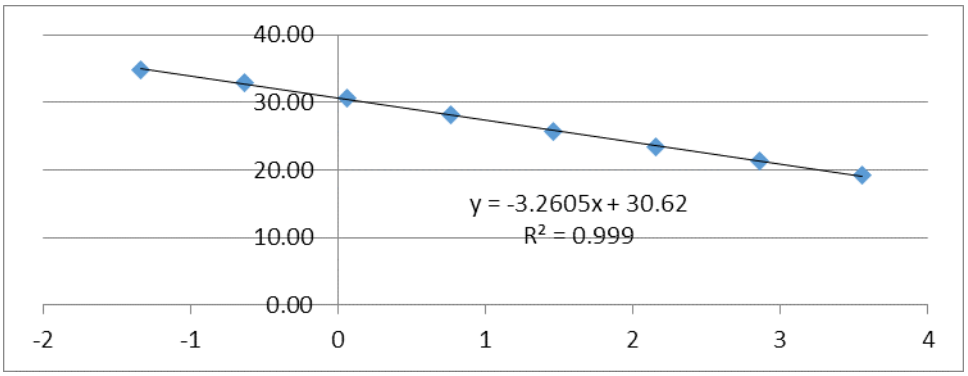

Efficiency	
y=	-3.261
-1/	0.307
10^	2.026
x-1	1.026
x*100	102.6
%E	103%

Figure 4.2. Detection of a dilution series of *V. dahliae* DNA extracts using DD assay

Discussion

The DD molecular assay was shown to be able to record the presence of *V. dahliae* without picking up any *V. albo-atrum* as well. However, the surveys carried out in 2015 indicate that *V. dahliae* may not be the only cause of wilting in raspberry and blackberry. *Phytophthora rubi* was detected in some of the samples. In addition, two *Ilyonectria* species were isolated from a subsample of infected canes. An experiment where wounded canes were inoculated with agar plugs of Ilyonectria showed that these species were highly aggressive and colonized the vascular tissue several cm in each direction of the wounding site.

Some plants in the 2015 farm samples were symptomatic with wilting and did not have any *Ilyonectria* species or *V. dahliae* present. A likely explanation for this is that *Verticillium albo-atrum* was causing the disease in this instance. At this time no assay exists with known specificity for *V. albo-atrum*. It is important to resolve whether these fungi are involved in wilting symptoms. Suggested future work would further characterize the Ilyonectria samples

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associated with cane fruit and determine their prevalence in DNA samples collected in 2015 for this project. It will also seek to determine the prevalence of *V. albo-atrum*.

Conclusion

The validation of the DD assay demonstrated that it is able to detect *V. dahliae* and *V. longisporum* to an acceptable level. The assay could therefore be used to detect infection of plant material by these fungi and assess infection levels in soils. The assay was used in 2015 for the survey of raspberry and blackberry plants, planting material and soils.

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Activities and Milestones (Item marked * is a milestone which substituted partially in those years for others within the same Work Package (WP)).

WP	Description	Research lead	YEAR 1 (2015/16)				YEAR 2 (2016/17)				Milestone dd/mm/yyyy
			Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	
1	***Phytophthora rubi* research**										
1.1	In vitro *response of zoospores to varieties*	*ADAS*									*30/06/2015*
1.2& 1.3	In vitro *effect on* P. rubi *of biofungicides & biostimulants*	*ADAS*									*31/03/2016*
1.4	In vivo *response of raspberries infested with* P. rubi *to biofungicides & bio stimulants*	*ADAS*									*30/10/2016*
*1.5**	*Effect on raspberries of biofungicides and biostimulants*										*31/03/2017*
2	**Optimising IPM in the light of SWD**										
2.1	*Complete monitoring of selected site*	*ADAS*									*31/10/2015*
2.2.1	*Set up modular tunnel trial site*	*EMR*									*30/06/2015*
	Complete BCA compatibility trial 1	*EMR*									*31/03/2016*
2.2.2	*Complete BCA compatibility trial 2*	*EMR*									*31/03/2017*
3	***Blackberry leaf midge***										
3.1	*Complete assessments of alternative control options - lab*	*ADAS*									*31/03/2016*
3.2	*Complete assessments of alternative control options - field*	*ADAS*									*31/03/2017*
4	***Verticillium wilt***										
4.1	*Complete field sampling*	*ADAS*									*31/11/2015*
4.2	*Complete laboratory analysis*	*Fera*									*31/03/2016*

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5	***Knowledge transfer***	*All*									
5.1	*1st annual report*	*All*									*31/03/ 2016*
5.2	*Speak at AHDB event / AHDB News article*	*All*									*31/03/ 2016*
5.3	*2nd annual report*	*All*									*31/03/ 2017*
5.4	*Speak at AHDB event / AHDB News article*	*All*									*31/03/ 2017*

Achievement of milestones

Research *in vitro* with *P. rubi* under Objective 1 testing biofungicides and chemical stimulants against was detailed in the 2015 annual report. Longer than expected was required to achieve zoospore production in 2015 and so work on zoospore response was delayed into 2016. The objective to use zoospores produced in non-sterile soil water for the inoculation of plants for work under a modified Task 1.4 (using single products not programmes) was completed. However, for *in vitro* work further experimentation was required in 2016 to obtain zoospore production in a soil-water treated to kill protists (which were confounding behaviour observation), without destruction of the unknown stimulant for zoospore production. It was subsequently agreed with the PMG that more-detailed zoospore behaviour observations could be recorded using video equipment, but the purchase of this has been delayed by the take-over of ADAS by RSK Ltd. Work on zoospore behaviour in response to biofungicides and to root exudates from a resistant and a susceptible raspberry variety has been moved into Year 3. The additional work on health and strength effects of biofungicides and stimulants on raspberries from propagation through to fruiting (Task 1.5) carried out *in lieu* of some of the *in vitro* work in 2016 was completed and the trial remains in place for further work agreed for Year 2.

All other objectives 2, 3, 4 and 5 have been fully met.

Knowledge transfer

Work within SF 158 on two-spotted mite, blackberry leaf midge, Phytophthora root rot and Verticillium wilt was reported in the AHDB Grower Soft Fruit Review 2016/17 pp. 16-17.

Presentations were given at the East Malling Research Association/AHDB Soft Fruit Day on 23 November 2016 at NIAB EMR, Kent by Jude Bennison on both “The effect of SWD control on two-spotted spider mite in raspberry” and “New research into blackberry leaf midge”. Erika Wedgwood presented “New studies into raspberry root rot”. Chantelle Jay presented “Integrating sprays with biocontrol of spider mite in raspberry”.

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Jude Bennison and Sam Brown wrote an article about the experiment for AHDB Horticulture Grower in February 2016, due to be published in the March edition.

Glossary

Entomopathogenic – capable of causing disease or death in insects

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Fountain, . (2015). Efficacy of insecticides, timed using the blackberry leaf midge sex pheromone trap, to control the pest on raspberry. Final report to AHDB Horticulture on project SF 141.

Appendices

P. rubi culturing and zoospore production

Sporangium production

- Aseptically subculture *P. rubi* (Vi 5 CC2106) onto fresh V8 agar plates (see directions below for recipe) and incubate at 20^{o}C on a 16:8 hour light:dark cycle for 15-18 days, until colony growth is about half way across the 90 mm diameter Petri dish. Move to 15-17^{o}C in the dark, once grown if not needed straight away.
- Aseptically remove agar plugs (up to 10 possible), using a size 3 corer, from inside the periphery of the growing colony and place mycelium uppermost in a 45mm diameter Petri dish.
- Add 4 ml non-sterile soil water ideally 3-7 days old (see below for recipe) to just completely cover all the plugs (too much liquid will inhibit sporangium production) and place in and incubator at 20^{o}C on a 16:8 hour light:dark cycle.
- After 12-24 hours examine the plates for the first flush of mature sporangia.
- If no sporangia have formed after 24 hours then leave for a further 24 hours at 20^{o}C on a 16:8 hour light:dark cycle before examining again.
- If sporangia still have not formed then carefully remove the soil water using a 5ml syringe and replace with 4 ml of sterile distilled soil water. Leave the plugs in the incubator at 20^{o}C on a 16:8 hour light:dark for 24-48 hours until sporangia formation.

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Zoospore release

- Place the water dishes containing mature sporangia into a fridge for one hour at 4ºC (no warmer). Air should be free to flow around the plates to ensure the plates chill quickly.
- Remove the plates from the refrigerator back to the laboratory, to warm up to 20ºC as soon as possible
- Zoospore release will occur between 15 minutes and 2 hours after removal from the refrigerator.
- Zoospore concentration can be determined using a haemocytometer. It is important to very gently swill the dishes to disperse the zoospores through the water (they tend to collect at the water surface) before pipetting up and immediately gently filling up the haemocytometer.
- When moving the zoospores ensure that they are not given any shocks such as banging or rapid temperature change as this can cause them to encyst. Ensure that any containers used have no traces of detergent as this can cause them to explode.

10% V8 agar recipe

- V8 juice for the production of V8 agar should be used within three days of opening the carton and the opened carton should be kept in a refrigerator. Shake the carton well before opening. Opened V8 juice cartons can be dispensed after opening into 100 ml batches and frozen in plastic bags for future use.
- Mix 1g calcium carbonate ($CaCO_3$), 20g Agar technical number 3, 100ml V8 juice and 900ml distilled water together.
- Add 10ml Streptomycin suspension.
- Autoclave and pour into Petri dishes.
- Once set, store upside down in a refrigerator for up to three weeks if to be used for Phytophthora or Pythium (as oomycetes require a moist agar surface).

Soil water production

- Loamy/silty top soil should be collected from an area where plants are growing nearby. The site should not have a history of Phytophthora.
- Mix the soil and 5 mm sieved to remove any large debris.
- Add 100g of soil to 1 litre distilled water and mix using a magnetic stirrer for 4 hours.
- Allow the suspension to settle for at least 4 hours, preferably overnight.
- Decant the supernatant and centrifuge it in bottles for 10 mins at 4600rpm
- Decant and retain supernatant from the centrifuge bottles. If required for zoospore observation work the supernatant should be filtered through two layers of Whatman No. 1 filter paper under vacuum to remove further any fine particulates.
- Store in a dated and labelled clean glass bottle at 4ºC in the dark.
- Soil water is best when aged for 3-7 days but can be used after several months.
- If the soil water is to be used in zoospore observation work then protists (e.g. *Colpoda*, *Paramecium*, Amoebae) which will be in the water need to be reduced in number. If the soil water is autoclaved it loses the chemical properties that cause sporangia to be formed. Therefore a partial sterilisation should be carried out by microwaving the soil water for 40 seconds.

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