Project title:	Improving integrated disease management in strawberry
Project number:	SF157
Project leader:	Prof Xiangming Xu NIAB EMR
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Industry Representative:	Marion Regan
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

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

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Contents

GROWER SUMMARY

For ease of reading, this Grower Summary report is split into sections for each of the diseases being worked upon in the project.

Crown rot and red-core caused by Phytophthora spp.

Headline

- The level of bare-root runners with *Phytophthora fragariae* (red-core) DNA detected is very low; however the level of *P. cactorum* DNA detected could reach 30%.

Background and expected deliverables

Adopting a clean propagation system is the first line of defence against crown rot and red-core diseases. This strategy has been working for many years until recent times. Currently, crown rot and red-core can cause significant damage in strawberry even in substrate production. The most likely cause is asymptomatic infection in planting materials. Frequent application of fungicides, alleged to have occurred in overseas nurseries, may delay the onset of symptom development until post-transplanting. Subsequent disease spread is likely to occur because of over-irrigation or rain-splash. Currently, NIAB EMR is testing fungicides and alternatives (in the SCEPTRE project) against crown rot and has identified effective products against this disease. Recent research on *Phytophthora* spp. has concentrated on detecting the pathogens and seeking products to reduce root rotting. Two AHDB Horticulture projects have just been completed; SF 130 focussed on fungal molecular quantification and an assay was developed that detected *Phytophthora rubi*, although it was not as sensitive as the *Phytophthora fragariae* assay (which however detects both pathogens); SF 123 investigate alternative products against *P. rubi* on raspberry where one novel chemical product gave reduction. Red-core is more difficult to control and currently there is no work on controlling this disease. Note that BBSRC is funding NIAB EMR to manage a five-year project to identify Phytophthora virulence factors against strawberry. More research is required to assist growers to be able to plant disease-free propagation material in order to reduce crop protection product use and crop losses.

The aim of this project on Phytophthora is to quantify the extent of hidden infection in initial planting materials and identify treatments to reduce plant losses due to these hidden infections.

Summary of the project and main conclusions in Year 1

A survey was conducted along with a molecular screening of bare-rooted runners for the presence of *Phytophthora* spp. Results suggested that the level of runners with contamination of *P. fragariae* (causal agent of red core) is very low; however, the level of contamination of *P. cactorum* (causal agent of crown rot) could reach 25-30% (although only up to 5% of runners may have symptoms of crown rot at the time of plantings). Further sampling and screening is needed, focusing on *P. cactorum*.

Small-scale experiments (as constrained by the nature of *Phytophthora* spp. as quarantine pathogens) were conducted at NIAB EMR to determine whether separate or joint use of AMF (arbuscular mycorrhizal fungi) and PGPR (Plant growth promoting bacteria) could reduce Phytophthora development. Results suggested that amendment of compost with both AMF and PGPR together can reduce severity of red-core development. Further experiments will be conducted to test the effect of AMF and PGPR on Phytophthora development, particularly with crown rot.

Financial benefits

The results are from only the first year and hence it is too early to quantify benefits to growers.

Action points for growers

- At this stage of the project, there are no action points to recommend to growers.

Powdery mildew

Headline

- Weekly application of silicon through fertigation can lead to reduced mildew development and higher pollen viability.

Background and expected deliverables

Projects SF 62, SF 62a and SF 94 (Defra Horticulture LINK HL0191) focussed on development, implementation and use of a strawberry powdery mildew prediction system. The prediction system was based on the one developed at the University of Hertfordshire. The project clearly demonstrated the benefit of using the system for early crops where initial mildew inoculum is low. Recent research in UK and Norway showed the importance of chasmothecia as a source inoculum, particularly for perennial cropping systems, and indicated the importance of removing debris from previous crops. Recent research in Norway also suggested young leaves and fruit are most susceptible to mildew infection. An EU-interreg funded project at NIAB EMR demonstrated a small reduction of powdery mildew under a deficit irrigation regime. A pilot study at the University of Hertfordshire showed that application of silicon nutrients changed plant morphology and delayed mildew development by 8-10 days on several cultivars. A TSB-funded project at NIAB EMR identified several QTL for resistance to powdery mildew. Another TSB project at EMR is investigating whether we could develop imaging tools to detect mildew infection before visual symptoms.

The central aim of this project is to optimise and integrate non-fungicide alternatives with conventional fungicides in the control of powdery mildew, particularly integrating nutrients and resistance inducers.

Summary of the project and main conclusions in Year 1

Two trials were conducted (one at NIAB EMR and one at ADAS, Cambridge) to study the effects of combining alternative products with reduced fungicide input on powdery mildew development. At both sites, the level of pre-harvest powdery mildew was low. Subsequently, at NIAB EMR the trial continued on the post-harvest regrowth for which severe mildew epidemics developed. Results suggested that combining certain alternative products with reduced fungicide input could be effective in controlling mildew, particularly when the level of inoculum is relatively low. In the coming season, further trials will be conducted, focusing on a few specific combinations of alternative products and fungicides.

A trial was conducted at a grower's site to investigate the effect of applying silicon through fertigation on strawberry powdery mildew [note - this work was funded by a private company who kindly agreed to share the results]. Results showed that applying the concentration of silicon (0.017%) once a week to the plants led to reduced development of powdery mildew, a lower level of two-spotted spider mite infestation and a higher level of pollen viability. It demonstrated that the silicon nutrient needs to be applied via the fertigation system on a weekly basis throughout the life of the plant. Brix levels in petioles in a commercial crop using Si nutrient were consistently higher than those in petioles from plants not receiving Si nutrient. There were no adverse effects at the Silicon concentration used. Full details of the rates used are included the Science Section of this report.

Financial benefits

The results are from only the first year and hence it is too early to quantify benefits to growers.

Action points for growers

- At low mildew levels, preventative fungicide programmes using biofungicides alone or alternated with standard fungicides are as effective as weekly standard applications, without increasing post-harvest rot incidence.
- Weekly application of silicon through fertigation can lead to reduced mildew development and higher pollen viability.

Fruit rot complex

Recent evidence in the UK and New Zealand has shown that Botrytis is not the only pathogen causing fruit rot, and that the importance of *B. cinerea* in strawberry may have been over-stated because of similar morphological characteristics of Botrytis fungal morphology with two other rotting fungi – Mucor and Rhizopus spp. The relative importance of these three pathogens may vary greatly with time and location. Although the overall direct loss to these pathogens may be relatively small compared with other diseases, the consequence (e.g. rejection of a consignment by retailers) of fruit rot is much more serious.

Botrytis cinerea, causing grey mould, is the most-studied disease in strawberry worldwide. Infection at flowering stages leads to the establishment of latent infection, which becomes active during fruit

ripening. Direct infection of fruit by conidia during ripening is also possible, which may account for a high proportion of post-harvest rot. Previous work (Project SF 94, Defra Horticulture LINK HL0191) has shown that it is possible not to use fungicides against Botrytis for early-covered June-bearers. Controlling Botrytis in late season strawberry, particularly ever-bearers, is problematic. Use of bees to deliver biocontrol agents to flowers gave the same level of Botrytis control as a fungicide programme on one strawberry farm. There is an on-going European core organic project on using bees to deliver biocontrol agents to strawberry flowers. However, it should be noted that using bees to deliver biocontrol products may face registration hurdles or even negative public responses. Because of the spotted wing drosophila (SWD) risk, growers are now implementing strict hygiene measures by removing all old, damaged or diseased fruit from the plantation during and after harvest. This may help to reduce Botrytis risk in late season crops.

Projects SF 74 (Defra Horticulture LINK HL0175) and SF 94 (Defra Horticulture LINK HL0191) suggested that in raspberry and strawberry, rapid post-harvest cooling to storage at 2°C is effective in delaying Botrytis development. However, such cooling treatment is not effective against Mucor as it can develop in cold conditions. In Project SF 98, NIAB EMR identified a few fungicides that can control Mucor effectively. Currently, Berry Gardens Growers is funding a PhD project at NIAB EMR on the epidemiology and management of Mucor *and* Rhizopus rot in strawberry. We have made Significant progress has been made in this project but due to commercial confidentiality the findings cannot be disclosed in this report.

For fruit rot complex in this project, the integration of biocontrol products with reduced fungicides will be investigated, along with post-harvest handling to reduce fruit rot and/or delay rot development.

Work to understand the epidemiology of fruit rot complex and to develop management strategies will start in Year 3 of the project.

Verticillium wilt

Headline

- Alternative biofumigation-derived products can significantly reduce the level of *Verticillium dahliae* inoculum in the field.

Background and expected deliverables

Recent withdrawal of methyl bromide and other soil fumigants has instigated new research seeking alternative soil treatments against Verticillium. Disappointingly, a new microencapsulated product did not have sufficient efficacy to have any commercial future (a TSB funded project which ended in December 2014). AHDB Horticulture is funding a PhD studentship project on pre-colonising strawberry runners or tipping plants to manage wilt. With AHDB funding, Fera developed a molecular diagnostic tool to quantify soil inoculum and currently ADAS is using this tool to investigate the relationship of wilt development in relation to nematodes. Separately, EMR (in collaboration with

Chinese researchers) has developed a more sensitive qPCR tool for quantifying Verticillium inoculum in soils. In an on-going TSB project, significant yield reduction associated with stunted strawberry growth has been observed that is apparently not associated with *Verticillium*. Further metagenomics research suggested several candidate organisms are responsible for this stunted growth (though further research is needed to confirm this), including two fungal pathogens *Ilyonectria robusta* and *I. coprosmae* (former *Cylindrocarpon* spp.) and the suppressive effects by *Bacillus* and *Pseudomonas* species. A new AgriTech proposal is currently under development to tackle these issues related to the *Ilyonectria* pathogens.

For wilt control, the emphasis in this project is on the use of anaerobic soil disinfestation and addition of beneficial bacteria to improve soil health.

Summary of the project and main conclusions in Year 1

Combined use of three alternative products (microencapsulated terpenes, liquid BioFence^{TM} and digestate [by product of anaerobic process]) have been tested in small-plot filed trials at NIAB EMR against wilt. The three tested alternative products can reduce *V. dahliae* inoculum by more than 50% when used individually. However, the combined use of these products in most cases did not result in efficacies as great as expected on the assumption of independent actions. Whether these products can be used in commercial agriculture depends on the level of inoculum and the inoculum threshold for causing economic damage. For crops like strawberry with a very low wilt threshold (0.5 – 1.0 CFU g^{-1} of soil (Harris and Yang, 1996), their use is not likely to be commercially viable for highly susceptible cultivars in fields with moderate to high inoculum levels. The value of combining these treatments is also questionable and needs to be further studied in different types of soils and cropping systems.

Anaerobic soil disinfestation was carried out on soil collected from a soft fruit farm that had a natural infestation of 2.3 microsclerotia of Verticillium per gram of soil. The soil was collected into replicated pots treated with either one or two products that provided nutrition to encourage the activity of the anaerobic bacteria present in the soil. The pots were sealed for eight weeks and the metabolites were anticipated to reduce the viability of the microsclerotia. Significant reduction in propagule viability occurred after the incorporation of Herbie 82 in four treatments, to give a mean 0.28 microsclerotia/g of soil, with four out of sixteen pots having zero.

Financial benefits

The results are from only the first year and hence it is too early to quantify benefits to growers.

Action points for growers

- If the level of wilt inoculum is not two high < 1.5 CFU per gram of soil, treating soils with alternative products can be effective in suppressing wilt development

SCIENCE SECTION

Introduction

Strawberry is attacked by several pathogens, including *Botrytis cinerea,* powdery mildew and *Phytophthora* spp. A recently completed Hort-LINK project focussed on botrytis and powdery mildew. In recent years, *Phytophthora* species have gradually increased in their prevalence. Other fungal fruit rot pathogens have also become more prevalent but have not received sufficient research attention. IPM best practice involves using biopesticides in combination with the remaining synthetic pesticides and other cultural and manipulative measures including the use of clean (certified) planting materials, resistant cultivars, semiochemicals, biocontrol agents, disease forecasting and other IPM tools to achieve commercially acceptable control of pests, diseases and weeds.

Crown rot and red-core caused by Phytophthora spp.

Adopting a clean propagation system is the first line of defence against crown rot and red-core diseases. This strategy has been working for many years until recent times. Currently, crown rot and red-core can cause significant damage in strawberry even in substrate production. The most likely cause is asymptomatic infection in planting materials. Frequent application of fungicides, alleged to have occurred in overseas nurseries, may delay the onset of symptom development until post-transplanting. Subsequent disease spread is likely to occur because of over-irrigation or rain-splash. Currently, EMR is testing fungicides and alternatives (in the SCEPTRE project) against crown rot and has identified effective products against this disease. Recent research on *Phytophthora* spp. has concentrated on detecting the pathogens and seeking products to reduce root rotting. Two HDC projects have just been completed; SF 130 focussed on fungal molecular quantification and an assay was developed that detected *Phytophthora rubi*, although it was not as sensitive as the *Phytophthora fragariae* assay (which however detects both pathogens) and SF 123 on alternative products against *P. rubi* on raspberry where one novel chemical product gave reduction. Red-core is more difficult to control and currently there is no work on controlling this disease. Note that BBSRC is funding EMR to conduct a five-year project to identify Phytophthora virulence factors against strawberry. More research is required to assist growers to be able to plant disease-free propagation material in order to reduce pesticide use and crop losses.

Powdery mildew

The Hort-LINK project focussed on development, implementation and use of a strawberry mildew prediction system. The prediction system was based on the one developed at the University of Hertfordshire. The project clearly demonstrated the benefit of using the system for early crops where initial mildew inoculum is low. Recent research in UK and Norway showed the importance of chasmothecia as a source inoculum, particularly for perennial cropping systems, and indicated the importance of removing debris of previous crops. Recent research in Norway also suggested young

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Fruit rot complex: Botrytis cinerea, Mucor and Rhizopus

Recent evidence in the UK and New Zealand has shown that Botrytis is not the only pathogen causing fruit rot, and that the importance of *B. cinerea* in strawberry may have been over-stated because of similar morphological characteristics of Botrytis fungal morphology with two other rotting fungi – Mucor and Rhizopus spp. The relative importance of these three pathogens may vary greatly with time and location. Although the overall direct loss to these pathogens may be relatively small compared with other diseases, the consequence (e.g. rejection of a consignment by retailers) of fruit rot is much more serious.

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For fruit rot complex in this project, the integration of biocontrol products with reduced fungicides will be investigated, along with post-harvest handling to reduce fruit rot and/or delay rot development.

Verticillium wilt

Recent withdrawal of methyl bromide and impending withdrawal of chloropicrin as soil fumigants have focussed the industry on searching for alternative soil treatment against this pathogen. Disappointingly, a new microencapsulated product did not have sufficient efficacy to have any commercial future (an on-going TSB project to end in December 2014). AHDB Horticulture is funding a project at EMR on pre-colonising strawberry runners or tipping plants to manage wilt. At present, this project is only in the first year and the main disease management results will be available next year. With HDC funding, Fera developed a molecular diagnostic tool to quantify soil inoculum and currently ADAS is using this tool to investigate the relationship of wilt development in relation to nematodes. Separately, EMR (in collaboration with Chinese researchers) has developed a more sensitive qPCR tool for quantifying Verticillium inoculum in soils. In an on-going TSB project, we have observed significant yield reduction associated with stunted strawberry growth that is apparently not associated with *Verticillium*. Further metagenomics research suggested several candidate organisms responsible for this stunted growth (though further research is needed to confirm this), including two fungal pathogens *Ilyonectria robusta* and *I. coprosmae* (former *Cylindrocarpon* spp.) and the suppressive effects by *Bacillus* and *Pseudomonas* species. A new AgriTech proposal is currently under development to tackle these issues related to the *Ilyonectria* pathogens.

Objective 1: Phytophthora

To quantify the extent of asymptomatic *Phytophthora* infections in relation to nursery sources and cultivars, and to develop alternative methods to reduce the losses due to *Phytophthora* infections

1.1 Survey

1.1.1 Materials and methods

Five different sites (Table 1.1) were visited across the South East England in spring 2015 to sample a total of six consignments of bare-root runners (4 different cultivars) prior to planting. Runners were taken from 2-5 boxes from each of 4-10 pallets (depending on availability); 100 runners in total were sampled, with no more than 10 runners per box. All runners were cut in half with a knife across the crown on site to examine for symptoms of *P. cactorum*; the knife was sterilised with 70% ethanol between two samples. Crown and root material from each runner were placed in a sealable bag and placed at 4°C on return to EMR for DNA extraction.

Approximately 100 to 250 mg of crown material was cut into small pieces with a scalpel and placed into a 2 ml microtube with two 4 mm ball bearings. Roots from the middle $^2/_3$ of the root system were also placed into a 2 ml microtube with two 4 mm ball bearings. DNA was extracted from 50 crown

samples per consignment (randomly selected from the 100 samples) and 24 root samples using a DNeasy Plant Mini Kit (Qiagen) following the manufacturer's instructions with all optional steps. Not all samples were subjected to DNA screening because of the budget constraint. DNA was quantified and quality-checked using a Nanodrop 1000 spectrophotometer and stored at -20°C. DNA from root was cleaned through Polyvinylpolypyrrolidone (PVPP) in a polypropylene spin column due to the high level of carryover of contaminants from DNeasy kit extraction.

To test for *Phytophthora* DNA in each sample, extracted DNA was tested in a PCR with *Phytophthora* species-specific primers. PCR was performed with 1x buffer, 2mM $MgCl_2$, 0.2mM dNTPs, 0.25 U Taq and 0.2µM of each primer in a total volume of 12.5µl. All PCRs were performed on a thermal cycler using the following touchdown cycle: an initial 95°C for 3 min, followed by 35 cycles of 95°C for 30 s, 60°C for 60 s (decreasing 0.5°C per cycle until 58°C) and 72°C for 60 s, followed by a final extension at 72°C for 5 min.

Crown DNA was run in a PCR with FaEF primers as a control for strawberry DNA to indicate whether DNA extraction was successful and in a nested PCR using Yph (*Phytophthora* Spp.) primer set in the first PCR and then Ycac (*P. cactorum* specific) primers (Schena *et al.*, 2008) in the second PCR with 1/10 dilutions of the amplicons from the 1st (YPh) PCR. DNA from roots was run with FaEF primers as a control for strawberry DNA and in a nested PCR using *P. fragariae* specific TRP-PFF309a9 primer set (Ioos *et al.*, 2006) in two rounds of PCR with 1/10 dilutions of the amplicons from the 1st PCR used in the second. Table 1.2 shows sequences of all primers used. PCR amplicons were run by gel electrophoresis on a 1.5% agerose gel with Gel Red at 100V and viewed under UV light on a GelDoc XR+.

Table 1.1 Sites visited for screening of runners prior to planting for *Phytophthora* spp.

Organisation	**Location**	**Cultivar**	**Sampling date**
Site 1	Kent	V1 & V2	30/03/2015
Site 2	Berkshire	V3	10/04/2015
Site 3	Kent	V2	20/04/2015
Site 4	Hampshire	V3	30/04/2015
Site 5	Kent	V4	15/05/2015

Table 1.2 Sequences (5'-3') for primer pairs used to screen strawberry runners

Primer set	Target	Forward primer	Reverse Primer
Yph[a]	*Phytophthora* spp.	CGACCATKGGTGTGGACTTT	ACGTTCTCMCAGGCGTATCT
Ycac[a]	*P. cactorum*	CCATACAAAATTCTGCGCTAGG	AGACACACAAGTGGACCGTTAG
TRP-PFF309a9[b]	*P. fragariae*	CTACCTCCCTAAGCTTATCA	ACGCAGCATCATAGAAAAT
FaEF1a	*Fragaria*	TGGATTTGAGGGTGACAACATGA	GTATACATCCTGAAGTGGTAGACGGAGG

[a]Schena *et al.*, 2008

[b]Ioos *et al.*, 2006

1.1.2 Results

There were a total of 12 runners from the 600 sampled with *P. cactorum* symptoms when assessed during sampling. Molecular testing of all the successful DNA extractions from these symptomatic samples confirmed the presence of *P. cactorum* DNA. There were also 22 samples, out of the 196 samples amplified with FaEF primers (i.e. DNA successfully extracted from crown material), that were amplified with the Ycac primers in a nested PCR (i.e. positive for *P. Cactorum*), that had no obvious symptoms when the crowns were split in the field (i.e. asymptomatic samples) (Table 1.3). The incidence of positive detection results of *P. cactorum* ranging from 5.1% to 37.5%. The lowest incidence (5.1%) is significantly less than the maximum (37.5%; $P < 0.001$).

DNA was extracted from root more successfully than from crown – predominantly due to the difficulty in tissue disruption of crown. There were no positives in the molecular screening of root material for *P. fragariae* (Table 1.4).

Table 1.3 Screening of strawberry crown for *P. cactorum*

Farm	**Cultivar**	**Symptomatic (in 100)**	**Number extracted**	**Successful extractions**	**Ycac positives**	**Asymptomatic positives**
F1	V1	0	50	37	7	7
F1	V2	0	50	24	9	9
F2	V3	4	50	23	3	1
F3	V2	5	50	29	3	1
F4	V3	2	50	44	3	3
F5	V4	1	50	39	2	1

Table 1.4 Number of positives with *P. fragariae* specific primers from extracted DNA of 24 root samples

Farm	Cultivar	Successful extractions	Number of positives
F1	V1	22	0
F1	V2	21	0
F2	V3	23	0
F3	V2	21	0
F4	V3	16	0
F5	V4	22	0

1.2 Use of PGPR and AMF to manage Phytophthora spp.

1.2.1 Materials and methods

Two experiments were carried out in 2015-2016 to study the protective effect of beneficial microbes against *P. fragariae*, the causal agent of strawberry red core disease. The experiments had respectively duration of five and six weeks and they were both conducted in growth cabinet at NIAB EMR, UK. Experiment 1 was carried out in the middle of the summer, while Experiment 2 was conducted during the winter. Table 1.5 shows the microbial and pathogen treatments and duration of both experiments.

AMF inoculum and PGPR inoculum used in both experiments were provided by PlantWorks Ltd, Sittingbourne, UK. AMF inoculum was provided as attapulgite clay/pumice/zeolite mix containing spores, mycelium, and colonised host plants root fragments of 5 different AMF species known as Rootgrow™ (Table 1.5). PGPR was provided as fine grade (0.5-1 mm) pumice inoculated with 6 different bacteria species (Table 1.5). AMF and PGPR were incorporated with the potting substrate just before planting at the rate of 1.9 % (v/v). In addition, the mycorrhizal status of the pre-inoculated strawberry plugs 'Malling centenary' (Experiment 2) was determined after staining (as Kormanik & McGraw, 1982 but omitting phenol) using the magnified intersection method (McGonigle et al., 1990). The result showed that 2.9 % of the root system of each plant was colonised by AMF.

Pre-inoculation of runner tips derived plants: AMF inoculated and non-inoculated strawberry plug were produced at R W Walpole Ltd, Norfolk, UK. Plastic trays (40 cells, ~132 cm^3 per cell; PG Horticulture Ltd, Northampton, UK) were filled up with a peat/perlite/coir mix (7:2:1, v/v; fertilised by the supplier with Osmocote® [15N-9P_2O_5-11K added at 3 kg m^3] and Micromax® premium [trace elements and magnesium fertiliser added at 0.3 kg m^3]; Legro Beheer b.v., Asten, The Netherlands) using an automatic Javo tray filler (Javo b.v., Noordwijkerhout, The Netherlands). For the AMF inoculated plants, Rootgrow™ (PlantWorks Ltd, Sittingbourne, UK) was mixed homogeneously with the potting mix at the rate of 10 % (v/v). On 7th July 2015 uniform runner tips of similar size of 'Malling

Centenary' (propagated by R W Walpole Ltd, Norfolk, UK) were harvested and then pinned-down under glasshouse conditions. The plantlets were firstly weaned (using a misting system spraying water every hour for the first five days aiming for RH 80 % and then every three hours for the next five days, while plants were protected from direct sunlight). The misting system was then switch off. The plants were irrigated as needed, no additional fertiliser was added and direct sunlight was progressively introduced. The plants were grown for seven weeks after transplanting. On 27th August 2015 (seven weeks post transplantation) plug plants were sent to EMR glasshouse facilities, where they were cold stored at 2 °C for several months until the plants were used for the Experiment 2.

Table 1.5 Microorganisms used and duration of experiments

Treatments	Experiment	
	1	**2**
Untreated	x	x
AMF[a]	x	x
PGPR[b]	x	x
AMF + PGPR	x	x
AMF + PreAMF[a] + PGPR		x
AMF + PreAMF		x
PreAMF + PGPR		x
PreAMF		x
Pathogen inoculated[b]	x	x
Strawberry cultivar	'Vibrant'	'Malling centenary'
Starting day	20/07	18/12
Year	2015	2015-2016
Duration	5 weeks	6 weeks

[a]AMF: commercial inoculum Rootgrow™ containing *Funneliformis mosseae*, *Rhizophagus irregularis*, *Claroideoglomus claroideum*, *Glomus microagregatum*, *Funneliformis geosporum* (disclosed by PlantWorks Ltd, Sittingbourne, UK)

[b]PGPR: preparation containing *Azospirillum brazilense* 245, *Rhizobium* (Agrobacterium) strain IRBG74, *Gluconacetobacter diazotrophicus* PAL5, *Burkholderia phymatum* sp. nov., *Bacillus megaterium* and *Bacillus amyloliquefaciens* (disclosed by PlantWorks Ltd, Sittingbourne, UK)

[c]PreAMF: plug plant pre-inoculated with Rootgrow™ during the weaning stage

[d]All the plants are inoculated with the pathogen *P. fragariae*

Applying treatments: Experiment 1 was carried out in 2015 at EMR. There were four inoculation treatments (three beneficial microbial treatments + one untreated control; Table 1.5), each with 20 replicates, giving a total of 80 plants. A randomised block design with four blocks (four shelves) was used. About three weeks prior the start of the experiment, 80 plug plants of 'Vibrant' (propagated

and cold stored at -2 °C for several months by the glasshouse facilities of NIAB EMR, UK) were transferred to a poly-tunnel with natural temperature and light to induce plant re-growth. Plants were watered once a day with tap water and no additional fertiliser was added. On 20th July 2015 (the start of the experiment) plugs' roots were gently washed with tap water to remove weaning substrates particles. All the plants were then inoculated with *P. fragariae* by dipping the roots in the slurry. Immediately following pathogen inoculation, the plants were transplanted in 500 mL plastic pots (9 x 9 x 10 cm, Desch Plantpak Ltd, Essex, UK) beforehand filled up with about 400 mL of autoclaved (two cycles at 121 °C for 20 min with 4 d between cycles) potting mix and the microorganisms. The potting mix consisted of one part of sand (Sinclair horticulture Ltd, Lincoln, UK) and one part of sieved compost (reduced peat mix with added grit from Fargro Ltd, West Sussex, UK). Pots were placed in a growth cabinet (Meridian Refrigeration Ltd, Croydon, UK; day and night 15 °C, ~72 % RH, light: dark 16h / 8 h, photosynthetically active radiation (PAR) of 40 µmol m^{-2} s^{-1}); such conditions were known to favour *P. fragariae* infection and development. Each plant was placed in a saucer with a shallow layer of water (2-7 mm) during the entire experiment. The experiment was terminated five weeks after transplanting when severe symptoms had developed in the control treatment.

Experiment 2 was undertaken in 2015-2016 at EMR. There were eight inoculation treatments (eight beneficial microbial treatments + one untreated control; Table 1.5, each with 16 replicates, giving a total of 128 plants. A randomised block design with four blocks (four shelves) was used. About two weeks prior the start the experiment, about 320 cold stored plug plants of 'Malling Centenary' were transferred from a cold store to a growth cabinet (Meridian Refrigeration Ltd, Croydon, UK; day and night 15 °C, ~72 % RH, light: dark 16h / 8 h, photosynthetically active radiation (PAR) of 35 µmol m^{-2} s^{-1}); such conditions were known to favour *P. fragariae* infection and development. Plants were watered once a week with tap water and no additional fertiliser was added. On 20th July 2015 (the start of the experiment) plugs' roots were inoculated and plant treated as in Experiment 1. The experiment ran for six weeks.

Pathogens inoculation: *P. fragariae* isolate BC-1, isolate BC-16 and isolate Nov-9 derived from the pathogen collection of NIAB EMR, UK. The stock cultures of the three pathogen isolates were cultured in Petri dishes on a sterile modified kidney bean agar (KBA) (Maas, 1972) for 1-2 months in the dark at 18 ± 1 °C. Then one piece (1-4 mm^2) of colonised KBA (from stock cultures) was transferred to Petri dishes containing fresh KBA. The mycelium from the pathogens was then harvested after an incubation period of ~30-60 days (because of the slow-growing nature of this pathogen) at 18 ± 1 °C in the dark. Inoculation procedures were based on Maas (1970) and Wynn (1968). The excised colonies consisting of mycelium and the agar beneath it were put into a blender with ice H_2O (1 g culture: 1 g ice H_2O) and blended twice for 2 seconds (19 x 103 rpm). The resulting slurry was transferred to a cooled beaker with inoculum adjusted to 10^3 zoospore.mL^{-1}. The inoculum was kept on ice during the entire inoculation procedure. Plants were inoculated by dipping their roots into the slurry for 2 second, and then planted back into plastic pots containing autoclaved

compost/sand mix. This inoculation procedure ensure approximately equal dose of inoculum per unit area of roots.

Disease assessment and data analysis: Plants were assessed once a week on their above ground symptoms on a rating scale from 1 to 3: 1 – no symptoms, 2 – 'floppy' foliage, 3 – totally collapsed/ dead (Photo 1.1). Since the experiments were conducted within a confined area because of the nature of the pathogen, it was not possible to assess fruit production due to inadequate pollination. All data were analysed using GenStat 13th edition (VSN International Ltd, Hemel Hempstead, UK). In both experiments the disease incidence was tested using nonparametric Kruskal-Wallis test. Post hoc tests were conducted by Dunn test adjusted with Bonferroni method.

Photo 1.1. Disease assessment keys on strawberry plants inoculated with Phytophthora spp.: 1 – healthy, 2 – floppy foliage and 3 – dead.

1.2.2. Results

In Experiment 1, there was a significant reduction of the disease score of 'Vibrant' with the dual inoculation AMF + PGPR ($\chi^2 = 8.3$; df = 3; P = 0.041). Addition of both AMF and PGPR led to reduced disease development when compared with the untreated (Fig. 1.1). Whether such a reduction in disease development (achieved by the joint use of AMF and PGPR) could have impact in commercial production is likely dependent on other factors. For instance, if the cultivar is more tolerant to the pathogen, any additional increase in tolerance is likely to be beneficial. For the same reason, an increase in plant tolerance could be of commercial advantage when the level of inoculum is low. Conversely, for highly susceptible cultivars and high disease pressure situation, such a reduction in disease development is unlikely to result in any commercial benefit.

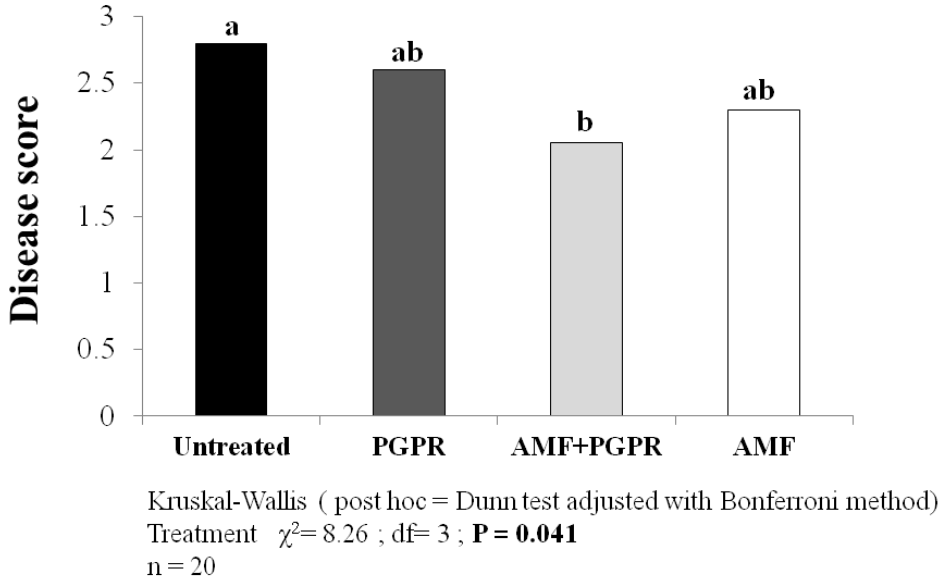

Figure 1.1. Effect of inoculation with arbuscular mycorrhiza (AMF) and/or plant growth promoting bacteria (PGPR) 35 days post inoculation on the disease score of *Phytophthora fragariae* of the strawberry 'Vibrant'.

In Experiment 2, there was no effect of the beneficial microbes on disease score 'Malling Centenary'. Indeed, all the plants in the Experiment 2 appeared not to suffer from visual red core symptoms. We cannot identify any apparent reason for the lack of disease development in this experiment since all the experimental conditions were the same as in the Experiment 1 except the cultivar. However, 'Malling Centenary' is not known to be resistant against red-core (that is the reason we used this cultivar in addition to its increasing popularity).

Objective 2: Powdery mildew

To develop programmes to effectively manage powdery mildew, integrate alternative methods with reduced fungicide input

The mildew work was conducted by three organisations: NIAB EMR, ADS and University of Hertfordshire (UoH). NIAB EMR and ADAS focused on the integration of alternative products with reduced fungicides; both sites shared several comment mildew control treatments. UoH focused on the use of a silicon nutrient product in the fertigation system to reduce the severity of strawberry powdery mildew epidemics in commercial strawberry tunnels.

2.1 Integration of alternative products with reduced fungicides

2.1.1 Materials and methods

This experiment was conducted at both EMR and ADAS in Cambridge. At EMR, the trial was conducted on raised-bed June-bearers planted in 2014 whereas at Cambridge it was on table-top plants using cold-stored runners. The difference in the planting systems between the two sites led to differences in crop managements/experimental procedures.

Plants

EMR: A plantation of the June-bearer variety cv Elsanta planted in July 2014 was used (Photo 2.1). This consisted of 6 plastic mulched raised beds with trickle irrigation, 3 beds per tunnel, each sufficient in length for up to 16 plots. The soil area was sterilised with chloropicrin prior to planting. The area is covered by two Spanish tunnels. The outer two beds in each tunnel were used as plots, with the middle bed as a guard row. Each plot consisted of a double row of 15 plants, 30 plants in total (approximately 4.5 metres in length). Plots were separated in the row by 1.5 metres. The plants were cleaned up in spring and crop debris left in alley ways as a source of inoculum for the trial. Plots were covered on 20 April. Plastic covers were removed 4-6 May due to strong winds and then replaced.

Photo 2.1 Trial layout, NIAB EMR, 2015

ADAS: Strawberries cv. Sonata as cold stored heavy waiting bed plants were planted in coir grow-bags in a Spanish tunnel with netted sides at ADAS Boxworth on 26 May 2015. The plants were misted for five days after planting and trickle fertigated throughout the trial. Plots consisted of two grow-bags of eight plants, replicated four times within randomised blocks, without discards between the plots (Photo 2.2).

Photo 2.2 Trial layout, ADAS Boxworth 2015

Treatments

EMR: All plots received a standard programme for pest and disease as required up to the start of the trial. Treatments were started in April at the onset of growth. The treatments in Table 2.1 were applied to the plots as the programmes in Table 2.2. Treatments at the start of the programme were targeted at mildew only. Products for Botrytis control were included from flowering onwards. Treatments for pests were applied to all plots. Each treatment was applied at 7 to 14 day intervals as specified up to harvest. As insufficient powdery mildew developed in the plots prior to harvest the plots were mown off at the end of harvest and the programmes in Table 2.3, targeted only at mildew, were applied to the regrowth. Treatments were applied using a CP knapsack sprayer at 1000 L/ha.

Table 2.1 Details of products used in powdery mildew control in strawberry at NIAB EMR in 2015

Product*	**Active ingredient**	**Rate of product / ha**	**Other information**	**Product type**
Systhane	myclobutanil	450 ml		Fungicide
Fortress	quinomethionate	0.25 L		Fungicide
Amistar	azoxystrobin	1.0 L		Fungicide
Nimrod	bupirimate	1.4 L		Fungicide
Signum	pyroxystrobin + boscalid	1.8 kg	2 sprays max	Fungicide
Switch	cyprodinil + fludioxonil	1.0 kg	2 sprays max	Fungicide
Rovral	iprodione	1.0 kg	4 sprays max	Fungicide
HDC F208	Coded product	5 L		BCA
SP057	Coded product	2.5 L		Resistance promoter
AQ 10 + SP057	*Ampelomyces quisqualis*	70 g + 0.05%		BCA
HDC F207	Coded product	250 ml	21 day harvest interval	Growth promoter
HDC F209	Coded product	60-80 g		Resistance promoter
*: no products (or water) was applied to the untreated control				

Table 2.2 Programmes applied for control of powdery mildew and rots up to start of harvest on June 11 at NIAB EMR in 2015

Programme	Date treatment applied						
	22 April	**28 April**	**8 May**	**15 May**	**22 May**	**29 May**	**5 June**
	BBCH 55	**BBCH 57: Flower buds**	**BBCH 60: 1st Flower**	**BBCH 64**	**BBCH 66: Full flower**	**BBCH 67: Flowers fading**	**BBCH 85: Fruit colouring**
P1: Untreated	-	-	-	-	-	-	-
P2: Standard fungicide 7-10 days	Fortress	Systhane	Rovral+Nimrod	Signum	Switch	Signum	Switch+Systhane
P3: Fungicide 14 days	Fortress	-		Rovral+Nimrod	-	Switch	
P4: Reduced fungicide	Fortress	Systhane	Rovral+Nimrod	Signum	-	-	
P5: HDC F207	HDC F207	-	-	HDC F207	-	-	HDC F207
P6: HDC F207+P3	HDC F207+Fortress	-	-	HDC F207+Rovral+Nimrod	-	Switch	HDC F207+Switch
P7: HDC F208	Fortress	HDC F208+SP057	HDC F208+SP057	HDC F208+SP057	HDC F208+SP057	HDC F208+SP057	HDC F208+SP057
P8: HDC F208+P4	Fortress	Systhane	Rovral+Nimrod	Signum	HDC F208+SP057	HDC F208+SP057	HDC F208+SP057
P9: AQ 10+SP057	AQ 10+SP057	AQ 10+SP057	AQ 10+SP057	AQ 10+SP057	AQ 10+SP057	AQ 10+SP057	AQ 10+SP057
P10: AQ 10+P3	Fortress	AQ 10+SP057	AQ 10+SP057	Rovral+Nimrod	AQ 10+SP057	Switch	AQ 10+SP057
P11: HDC F209	HDC F207	HDC F209	-	HDC F209	-	-	HDC F209
P12: HDC F209+P3	HDC F207+Fortress	HDC F209	-	HDC F209+Rovral+Nimrod	-	Switch	HDC F209+Switch
P13: HDC F210	HDC F210	HDC F210	HDC F210	HDC F210	HDC F210	HDC F210	HDC F210
P14: HDC F210+P4	Fortress	Systhane	Rovral+Nimrod	Signum	HDC F210	HDC F210	HDC F210

Table 2.3 Programmes applied for control of powdery mildew post-harvest to regrowth at NIAB EMR in 2015

Programme	Date treatment applied						
	19 August	**26 August**	**2 September**	**10 September**	**16 September**	**23 September**	**1 October**
P1: Untreated	-	-	-	-	-	-	-
P2: Standard fungicide 7-10 days	Fortress	Systhane	Nimrod	Fortress	Systhane	Nimrod	Systhane
P3: Fungicide 14 days	Fortress	-	Systhane		Nimrod		Fortress
P4: Reduced fungicide	Fortress	Systhane	Nimrod	Fortress			
P5: HDC F207	HDC F207	-	-		HDC F207		HDC F207
P6: HDC F207+P3	HDC F207+Fortress	-	Systhane		HDC F207+Nimrod		HDC F207+Fortress
P7: HDC F208	HDC F208+SP057	HDC F208+SP057	HDC F208+SP057	HDC F208+SP057	HDC F208+SP057	HDC F208+SP057	HDC F208+SP057
P8: HDC F208+P4	Fortress	Systhane	Nimrod	Fortress	HDC F208+SP057	HDC F208+SP057	HDC F208+SP057
P9: AQ 10+SP057	AQ 10+SP057	AQ 10+SP057	AQ 10+SP057	AQ 10+SP057	AQ 10+SP057	AQ 10+SP057	AQ 10+SP057
P10: AQ 10+P3	Fortress	AQ 10+SP057	Systhane	AQ 10+SP057	Nimrod	AQ 10+SP057	Fortress
P11: HDC F209	HDC F209		-		HDC F209		HDC F209
P12: HDC F209+P3	HDC F209+Fortress		Systhane		HDC F209+Nimrod		HDC F209+Fortress
P13: HDC F210	HDC F210	HDC F210	HDC F210	HDC F210	HDC F210	HDC F210	HDC F210
P14: HDC F210+P4	Fortress	Systhane	Nimrod	Fortress	HDC F210	HDC F210	HDC F210

ADAS: Treatments were applied according to the treatment schedule in Table 2.4 at the rates detailed in Table 2.5. HDC F207 was applied as a pre-planting dip; all plants were fully submerged in the HDC F207 solution for 15 minutes, removed and allowed to drip dry before planting. Spray treatments were applied to each plot at 1000 L water/ha using an air assisted knapsack (Oxford Precision) and hand lance with a 02 F110 nozzle at medium pressure (3 bar) and quality. Spray guard boards were used between plots to avoid any drift. The untreated control received a spray of tap water at the same volume. The first spray treatments were applied on 2 June 2015, with the grower standard and three other programmes being applied at seven day intervals and the other three programmes using a reduced number of applications over the same time period. Once flowers were present, the youngest three per plot were marked at intervals with wool, changing the wool colour between dates, in order to be able to indicate when fruit was picked from that flower opening date.

Table 2.4 Powdery mildew treatment application schedule – ADAS Boxworth 2015

	Dip	**Spray 1**	**Spray 2**	**Spray 3**	**Spray 4**	**Spray 5**	**Spray 6**	**Spray 7***
Programme (P1 to P8)	**Prior to planting**	**Just after start of growth**	**7 days later**	**7 days after Spray 2**	**7 days after Spray 3**	**7 days after Spray 4**	**7 days after Spray 5**	**7 days after Spray 6**
P1: Untreated control		Water	Water	Water	Water	Water	Water	Water
P2: Standard fungicide		Fortress	Systhane	Rovral + Nimrod	Signum	Switch	Signum	Switch
P3: Fungicides at 14 days		Fortress		Systhane		Rovral + Nimrod		Signum
P4: HDC F207	HDC F207 dip			HDC F207			HDC F207	
P5: HDC F207 + P3	HDC F207 dip	Fortress		Systhane HDC F207		Rovral + Nimrod	HDC F207	Signum
P6: AQ 10		AQ 10 + Silwett	AQ 10 + Silwett	AQ 10 + Silwett	AQ 10 + Silwett	AQ 10 + Silwett	AQ 10 + Silwett	AQ 10 + Silwett
P7: AQ 10 + P3		Fortress	AQ 10 + Silwett	Systhane	AQ 10 + Silwett	Rovral + Nimrod	AQ 10 + Silwett	Signum
P8: HDC F210 + reduced P2		Fortress	Systhane	Rovral + Nimrod	Signum	HDC F210	HDC F210	HDC F210

* No Timing 7 treatments were carried out due to the 3 day harvest interval for Signum		
Table 2.5 Products used within treatment programmes against powdery mildew – ADAS Boxworth 2015		
Product	**Active ingredient**	**Dose rate**
Untreated	water	-
Fortress	quinomethionate	0.25 L/ha
Systhane 20 EW	myclobutanil	450 mL/ha
Rovral	iprodione	1 kg/ha
Nimrod	buprimate	1.4 L/ha
Signum	pyroxystrobin + boscalid	1.8 kg/ha
Switch	cyprodinil + fludioxonil	1 kg/ha
HDC F207	Coded product	Drench 50 mL / 100L water
		Spray 250 mL/ha
AQ 10 + Silwett L77	*Ampelomyces quisqualis* + silicon wetter	70g/ha + 0.05%
HDC F210	Coded product	2.5 L/ha

Assessments

***EMR*:** The plots were regularly inspected for mildew and the incidence on foliage assessed as percentage leaf area infected on the youngest five expanded leaves on each of five plants per plot as needed using a standard key supplied by Dr Avice Hall. No mildew was seen. Mildew inoculum was introduced into the adjacent trial on 21 May and 26 May (only a few leaves as very little mildew inoculum available). The first mildew was seen in that plot on 28 May but none was visible in this trial until 18 June. Mildew was assessed on 28-30 July (three mature leaves + two young leaves). Powdery mildew on post-harvest regrowth was assessed on 8 September, 22 September and 5 November. Assessments were also made for other diseases (e.g. leaf spots) as needed. Assessments for fungal rots were made at harvest and in post- harvest tests.

Fruit was harvested from the plots twice weekly starting on 11 June. The sixth and final harvest was on 30 June. Fruit number and weight was recorded at each harvest. Rots, including powdery mildew, were identified and recorded. A sample of 50 fruit was taken from each plot at each harvest and incubated at high humidity in plastic module trays where each fruit occupied an individual module separating it from adjacent fruit. The fruit were incubated at ambient temperature for seven days after which the rots were identified and incidence recorded.

Phytotoxicity was assessed after each spray by visual assessment of % leaf area with necrosis / chlorosis, leaf drop, growth regulatory effects (EPPO Guideline PP 1/135 (3)). Records of daily

maximum and minimum temperature and rainfall were taken from a weather station located at the NIAB EMR main site, approximately 500m south west of the trial.

A summary of strawberry treatments and assessment timings are shown in Table 2.6.

Table 2.6 Summary of strawberry treatment and assessment timings – NIAB EMR 2015

Activity	**Date**
Plants cleaned up. Crop debris left in alley way	Spring 2015
Plots covered	20 April
First spray	22 April
Second spray	28 April
Third spray	8 May
Fourth spray	15 May
Mildew introduced to adjacent trial	21 & 26 May
Fifth spray	22 May
First mildew in adjacent trial. None in this trial	28 May
Sixth spray	29 May
Seventh spray	5 June
First harvest	11 June
First mildew in trial	18 June
Final harvest	30 June
Mildew assessed	28 July
Plots mowed off	3 August
Regrowth and mildew	11 August
First post-harvest spray	19 August
Second post-harvest spray	26 August
Third post-harvest spray	2 September
First mildew assessment	8 September
Fourth post-harvest spray	10 September
Fifth post-harvest spray	16 September
Second mildew assessment	22 September
Sixth post-harvest spray	23 September
Seventh post-harvest spray	1 October
Final mildew assessment	5 November

ADAS: Powdery mildew infection was anticipated to arrive naturally in the crop. However, when symptoms were still not seen at the time of the third spray timing (Growth stage 57-59) then 12 pots of naturally infested plants of cv. Elsanta (sourced from a garden centre) were spaced evenly around the trial on the 17 June, moving them at intervals. The floor of the tunnel was hosed and the doors

closed to increase humidity in order to favour the germination of mildew spores on the plants. To further increase the amount of inoculum present, infected leaves of cv. Elsanta collected from a commercial crop were scattered one per grow bag on 26 June when no infection was obvious, although symptoms developed soon afterwards.

Plants were monitored weekly just before any spray for any evidence of phytotoxicity or powdery mildew infection. Once the first mildew symptoms were seen on the leaves on 30 June (at spray Timing 5) the severity was assessed fortnightly until the end of the experiment. Powdery mildew symptoms were assessed on the youngest five expanded leaves on each of five plants per plot as the percentage leaf area covered by mycelium and any purpling, using a standard key. Fruit harvesting commenced on 9 July 2015 and finished on 14 August 2015. Fruit was harvested twice a week, picking all those which were ripe and grading these into marketable and waste fruit. The number and weight of healthy and waste fruit per plot were recorded. The waste fruit was also assessed for defects including powdery mildew, botrytis, Mucor spp., misshapen fruit, split fruit, pest damage etc. recording the number of fruit per plot in each of these categories. Small fruit were included in the marketable grade.

On three occasions, at the beginning (when there was enough fruit/plot), middle and end of harvest, 45 healthy fruit per plot were sampled and incubated individually at high humidity in plastic module trays so that each fruit occupied an individual module cell separating it from adjacent fruit. The fruit was incubated at ambient temperature for seven days, after which the rot types were identified and the number of marketable and rotten fruit recorded. This was done because there was not expected to be any botrytis visible on fruit in the crop as they were picked promptly to avoid any problems with spotted wing drosophila. This plant protection products tested in this experiment were primarily focussed on powdery mildew treatment and so no every harvest was taken for post-harvest assessment.

SPAD (Single Photon Avalanche Diode) measurements were taken on replicates 3 and 4 after the final harvest, on 8 August 2015. This machine records the chlorophyll content of leaves. All the plants in a plot were assessed taking readings from the three youngest fully expanded leaves per plant.

2.1.2 Results

EMR – Powdery mildew

Pre-harvest: The incidence of powdery mildew was very low with only 7% leaf area mildewed on untreated plots. All treatments (Table 2.7, Figure 2.1) reduced the incidence of powdery mildew. The least mildew was recorded on plots receiving the standard fungicide programme and programmes based on HDC F208 or HDC F210.

Post-harvest: The weather conditions post-harvest were very favourable for mildew development and the incidence on untreated plots rapidly increased to around 30% leaf area mildewed. The mildew incidence recorded on 8 September, after 3 treatment applications, was in general lower on

treated plots than on untreated plots. By the second assessment the incidence of mildew on treated plots had reduced considerably compared to the untreated plots. At the final assessment on 5 November, after all the treatment programmes had been applied, the mildew on plots receiving the full fungicide programme was reduced to around 11% leaf area mildewed. All treated plots had significantly less mildew than the untreated control. Plots receiving the standard fungicide programme and on plots based on HDC F208 and HDC F210 (Figure 2.2) had significantly less mildew than the reduced fungicide programmes.

Typical plant growth was shown in Photo 2.3 for each treatment (post-harvest trial).

Table 2.7 % leaf area mildewed on Strawberry cv. Elsanta on 28 July following treatment with various programmes of fungicides, elicitors and biofungicides applied pre-harvest and on 8 September, 22 September and 5 November following programmes applied post-harvest after mowing off at NIAB EMR 2015

Treatment	**Programme**	**28 July**	**8 Sep**	**22 Sep**	**5 Nov**
P1	Untreated	15.6 (7.3)	35.5 (33.8)	31.4 (27.1)	35.1 (33.1)
P2	Standard fungicide 7-10 days	8.8 (2.3)	29.4 (24.2)	17.0 (8.5)	19.2 (10.8)
P3	Fungicide 14 days	10.8 (3.5)	31.0 (26.6)	25.3 (18.2)	26.8 (20.3)
P4	Reduced fungicide	10.8 (3.5)	29.7 (24.6)	19.3 (10.9)	24.7 (17.5)
P5	HDC F207	12.0 (4.3)	31.4 (27.2)	24.3 (17.0)	27.4 (21.2)
P6	HDC F207 + P3	14.2 (6.0)	31.2 (26.8)	24.2 (16.8)	26.0 (19.2)
P7	HDC F208	9.2 (2.6)	32.1 (28.3)	22.0 (14.0)	23.0 (15.3)
P8	HDC F208 + P4	9.6 (2.8)	30.9 (26.3)	18.8 (10.4)	20.9 (12.8)
P9	AQ 10 + SP057	10.4 (3.3)	32.6 (29.0)	23.7 (16.2)	22.4 (14.5)
P10	AQ 10 + P3	9.2 (2.6)	31.9 (28.0)	23.1 (15.4)	22.5 (14.6)
P11	HDC F209	11.0 (3.6)	32.7 (29.2)	24.9 (17.7)	28.5 (22.8)
P12	HDC F209 + P3	11.4 (3.9)	33.4 (30.3)	23.5 (15.8)	24.3 (16.9)
P13	HDC F210	11.3 (3.8)	30.8 (26.2)	19.6 (11.2)	22.3 (14.4)
P14	HDC F210 + P4	9.5 (2.7)	30.5 (25.7)	18.1 (9.6)	21.4 (13.3)
	F Prob	<0.01	0.004	<0.001	<0.001
	SED (39)	1.443	1.29	1.216	1.37
	LSD (P=0.05)	2.918	2.609	2.46	2.772

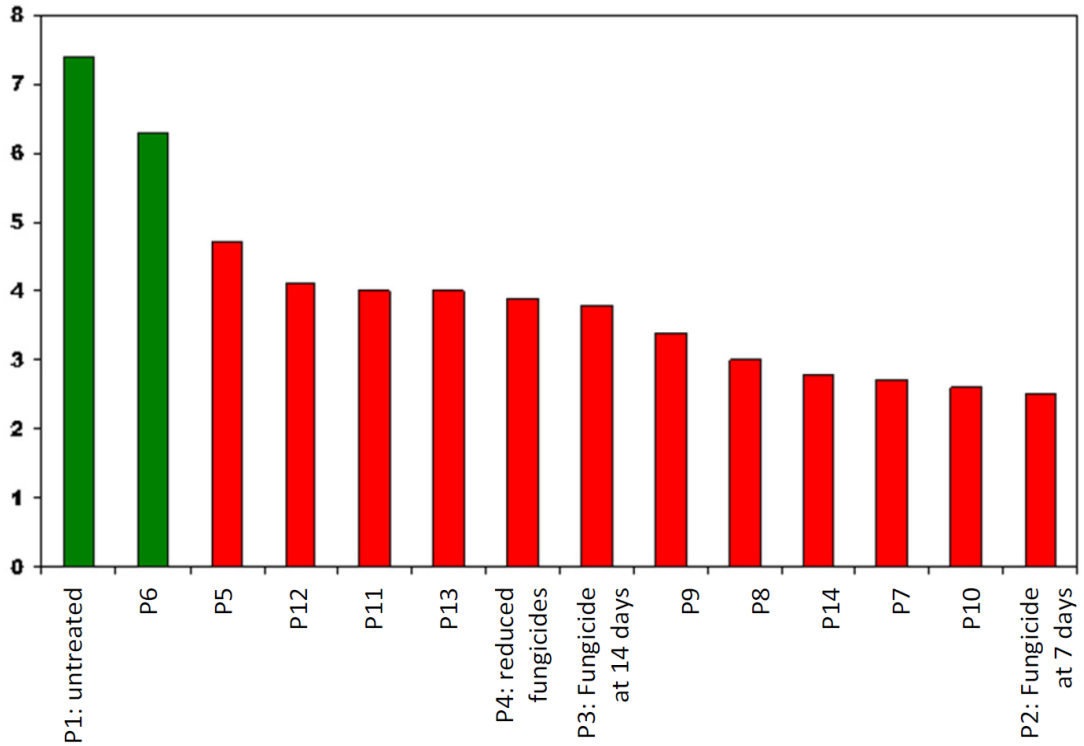

Figure 2.1 % leaf area mildewed on Strawberry cv. Elsanta 28 July following treatment with various programmes of fungicides, elicitors and biofungicides at NIAB EMR in 2015. Figures in red are significantly different from the untreated

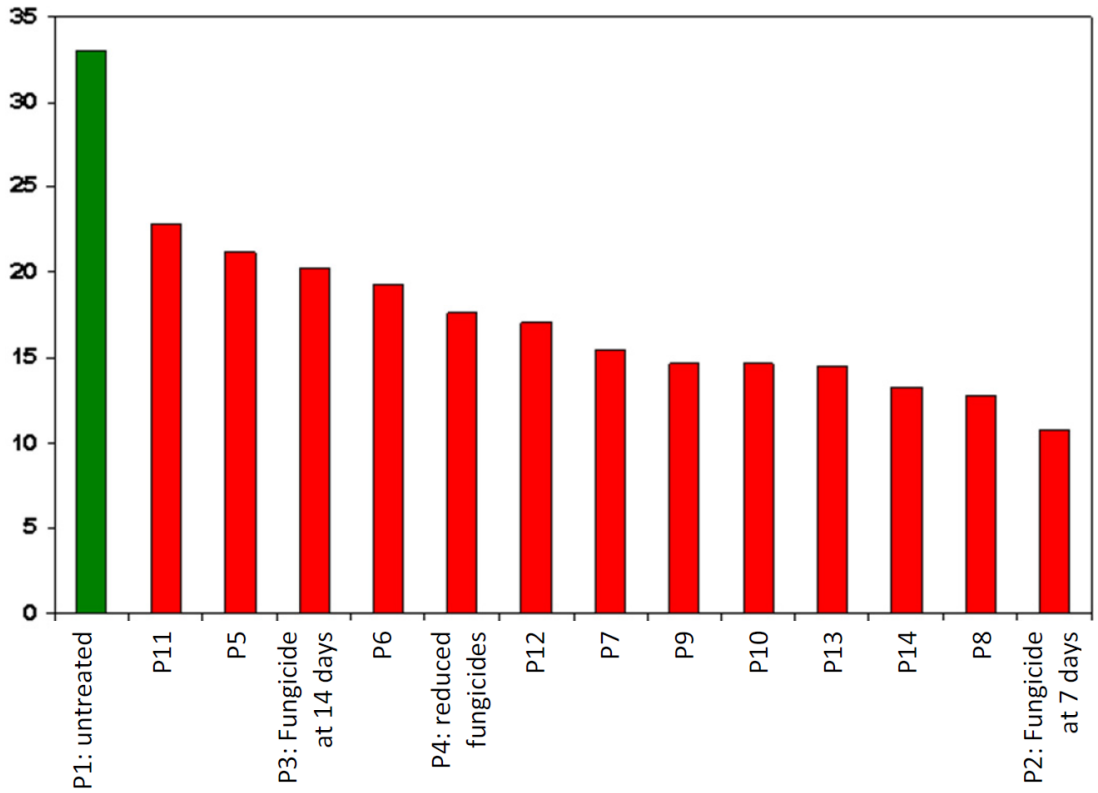

Figure 2.2 % leaf area mildewed on Strawberry cv. Elsanta 5 November following treatment on post-harvest regrowth with various programmes of fungicides, elicitors and biofungicides at NIAB EMR in 2015. Figures in red are significantly different from the untreated.

East Malling – Yield and fruit rots

There were no effects of treatments on yield or fruit number per plot (Table 2.8). No phytotoxic effects of treatments were observed. The incidence of fruit rots at harvest was negligible and the data is not shown here. The overall means of the fruit rots in post-harvest tests over six harvests are shown in Table 2.9. The incidence of Botrytis rot was high. There were no effects of treatments on overall means of *Penicillium* or *Mucor* rots. For Botrytis and total fruit rot the standard fungicide programme, Programme 6 (HDC F207 + P3) and Programme 12 (HDC F209 + P3) all significantly reduced rots compared to the untreated control. None of the other treatments had any significant effect.

Treatment 1 - Untreated

P2: Standard fungicide 7-10 days

P3: Fungicide 14 days

P4: Reduced fungicide

P5: HDC F207

P6: HDC F207 + P3

P7: HDC F208

P8: HDC F208 + P4

P9: AQ 10 + SP057

P10: AQ 10 + P3

P11: HDC F209

P12: HDC F209 + P3

P13: HDC F210 **P14: HDC F210 + P4**

Photo 2.3. Photos of typical plant growth for each treatment (post-harvest powdery mildew trial) at NIAB EMR in 2015.

Table 2.8. Total fruit yield kg and fruit number per plot on Strawberry cv. Elsanta following treatment with various programmes of fungicides, elicitors and biofungicides at NIAB EMR in 2015

Treatment	**Programme**	**Total yield (kg)**	**Total number of fruit**
P1	Untreated	24.8	1589
P2	Standard fungicide 7-10 days	23.4	1544
P3	Fungicide 14 days	24.4	1533
P4	Reduced fungicide	25.0	1587
P5	HDC F207	25.1	1643
P6	HDC F207 + P3	25.4	1626
P7	HDC F208	23.5	1612
P8	HDC F208 + P4	24.4	1520
P9	AQ 10 + SP057	21.5	1389
P10	AQ 10 + P3	25.8	1647
P11	HDC F209	23.6	1482
P12	HDC F209 + P3	22.6	1444
P13	HDC F210	23.4	1545
P14	HDC F210 + P4	20.6	1367
F Probability		0.27	0.40
SED (39 df)		1.86	122.3
LSD (P= 0.05)		3.76	247.4

Table 2.9. Average % fruit rots over six harvests on Strawberry cv. Elsanta following treatment with various programmes of fungicides, elicitors and biofungicides at NIAB EMR in 2015. (values in brackets are back transformed means, and values in bold are significantly different from the untreated)

Treatment	Programme	% Botrytis	% Penicillium	% Mucor	% Total rots
P1	Untreated	39.2 (40.0)	23.2 (15.5)	20.6 (12.4)	50.2 (59.0)
P2	Standard fungicide 7-10 days	**33.2** (30.0)	22.7 (14.9)	18.1 (9.7)	**42.9** (46.4)
P3	Fungicide 14 days	36.0 (34.6)	25.5 (18.5)	21.9 (14.0)	49.9 (58.5)
P4	Reduced fungicide	38.4 (38.5)	23.9 (16.4)	22.6 (14.7)	50.9 (60.2)
P5	HDC F207	35.9 (34.3)	26.0 (19.2)	24.4 (17.0)	50.9 (60.2)
P6	HDC F207 + P3	**32.8** (29.4)	24.7 (17.5)	20.1 (11.8)	**44.9** (49.8)
P7	HDC F208	44.7 (49.5)	25.7 (18.7)	22.1 (14.2)	56.0 (68.7)
P8	HDC F208 + P4	36.6 (35.6)	24.4 (17.0)	19.8 (11.5)	48.7 (56.4)
P9	AQ 10 + SP057	35.6 (34.0)	26.1 (19.3)	22.1 (14.2)	49.5 (57.9)
P10	AQ 10 + P3	34.6 (32.3)	23.8 (16.3)	22.7 (14.9)	48.9 (56.8)
P11	HDC F209	42.7 (46.1)	25.1 (17.9)	19.5 (11.1)	50.4 (59.4)
P12	HDC F209 + P3	**31.6** (27.5)	24.6 (17.3)	21.7 (13.7)	**45.6** (51.0)
P13	HDC F210	41.7 (44.2)	27.4 (21.1)	23.0 (15.3)	51.3 (60.9)
P14	HDC F210 + P4	39.2 (39.9)	27.3 (21.0)	19.6 (11.3)	49.8 (58.4)
F Probability		<0.001	0.34	0.618	<0.001
SED (39 df)		2.718	1.837	2.647	2.295
LSD (P= 0.05)		5.498	3.715	5.353	4.641

ADAS

Powdery mildew was first seen and recorded in the crop on 30th June 2015, before the final spray date. At this time temperatures were high in the polytunnel, rising to around 40 °C towards the end of June/early July. The seventh spray applications did not go ahead as the crop was ripening rapidly and it was not possible to allow the 3 day harvest interval for Signum. The final applications on 7 July to go on the crop included the last proposed applications of HDC F207 (Table 2.10).

Spray applications to the experiment on each date commenced with an application of water to the untreated plots as a control for the application of liquid to the treated plots. However,

before the first application, it was found that the equipment had been insufficiently rinsed out following herbicide application to another trial, and so leaves in the untreated plots in the present trial started to show necrosis. Symptoms decreased from Replicate 1 through to 4 and the fourth replicate appeared to recover fully. Assessments are presented for the untreated plants in replicate four, but analysis has not included this plot.

The crop was assessed before each of the six spray applications and when the seventh would have been given and no phytotoxicity was observed resulting from the fungicide programmes. The powdery mildew was slow to develop and so records were made fortnightly from 30 June to 10 August, with visible infection on the five youngest expanded leaves reaching a maximum of 5 % where the standard programme was used (Table 2.11). Symptoms including leaf curling as a result of powdery mildew were visible on the untreated plants in replicate 4, with 8 % mildew cover (Photo 2.4). The mildew risk was relatively low in the early season, except during the mid-July period (Figure 2.4), with increased risks in the late season, which may explain the moderate level of mildew observed on 28th July (Figure 2.3).

Table 2.10 Summary of strawberry treatment and assessment timings – ADAS Boxworth 2015

Activity	**Date**
HDC F207 root dip given to plants in Treatments 4 and 5 All strawberry plants planted, eight plants to a grow-bag	26/05/2015
Assessed plants then applied spray 1 (Treatments 1, 2, 3, 5, 6, 7, 8)	02/06/2015
Assessed plants then applied spray 2 (Treatments 1, 2, 6, 7, 8)	09/06/2015
Assessed plants then applied spray 3 (Treatments 1-8)	16/06/2015
Assessed plants then applied spray 4 (Treatments 1, 2, 6, 7, 8)	23/06/2015
Assessed plants then applied spray 5 (Treatments 1-8)	30/06/2015
Powdery mildew first seen. Powdery mildew assessment 1	30/06/2015
Assessed plants then applied spray 6 (Treatments 1-8)	07/07/2015
Harvest assessment 1	09/07/2015
Harvest assessment 2	13/07/2015
Assessed plants. No spray 7 (Treatments 1-8) given on 14/07/2015 as crop more advanced with fruiting than anticipated	
Powdery mildew assessment 2	14/07/2015
Harvest assessment 3	16/07/2015
Harvest assessment 4	21/07/2015
Post-storage botrytis assessment 1 of fruit from Harvest 3	23/07/2015
Harvest assessment 5	24/07/2015
Harvest assessment 6	27/07/2015
Powdery mildew assessment 3	28/07/2015
Post-storage botrytis assessment 2 of fruit from Harvest 5	31/07/2015
Harvest assessment 7	31/07/2015
Post-storage botrytis assessment 3 of fruit from Harvest 7	07/08/2015
Harvest assessment 8	07/08/2015
Powdery mildew assessment 4	10/08/2015
Harvest assessment 9	14/08/2015
Trial ended and cleared away	26/08/2015

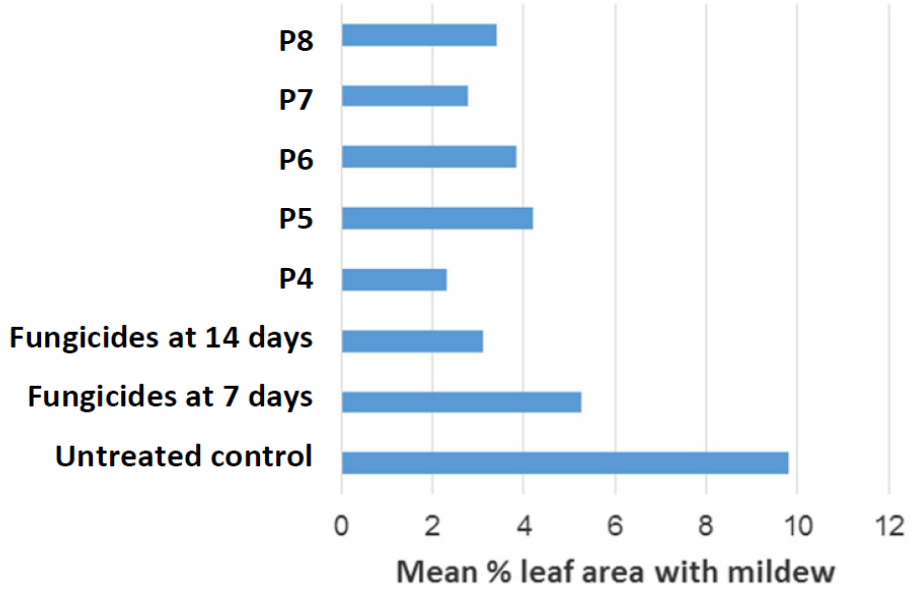

Figure 2.3 % powdery mildew on youngest leaves 28th July (mean of UT in Reps 3 & 4)

Table 2.11 Average leaf area with powdery mildew assessed on four dates between June and August, statistical analysis excludes untreated control due to plot damage – ADAS Boxworth 2015

Programme	**Treatments and interval between applications**	**30 June 2015**	**14 July 2015**	**28 July 2015**	**10 Aug 2015**
P1	(Untreated control 1 plot)	(0.000)	(0.78)	(8.06)	(4.31)
P2	Standard fungicide at 7 days	0.038	1.41	5.22	2.00
P3	Fungicides at 14 days	0.035	0.89	3.12	1.54
P4	HDC F207	0.017	0.98	2.30	1.05
P5	HDC F207 & Fungicides at 14 days	0.056	0.98	4.20	2.33
P6	AQ 10 at 7 days	0.010	1.18	4.06	2.56
P7	AQ 10 + fungicides at 14 days	0.019	0.59	2.76	1.36
P8	Fungicides at 7 days then HDC F210	0.025	0.82	3.41	1.56
	P value (excluding UT)	N.S. (0.766)	N.S. (0.359)	N.S. (0.082)	N.S (0.409)
	LSD (df = 18)	0.0637	0.712	1.945	1.556

Untreated control HDC F210 + reduced fungicide program

Photo 2.4 Untreated plot showing leaf curling that was associated with powdery mildew.

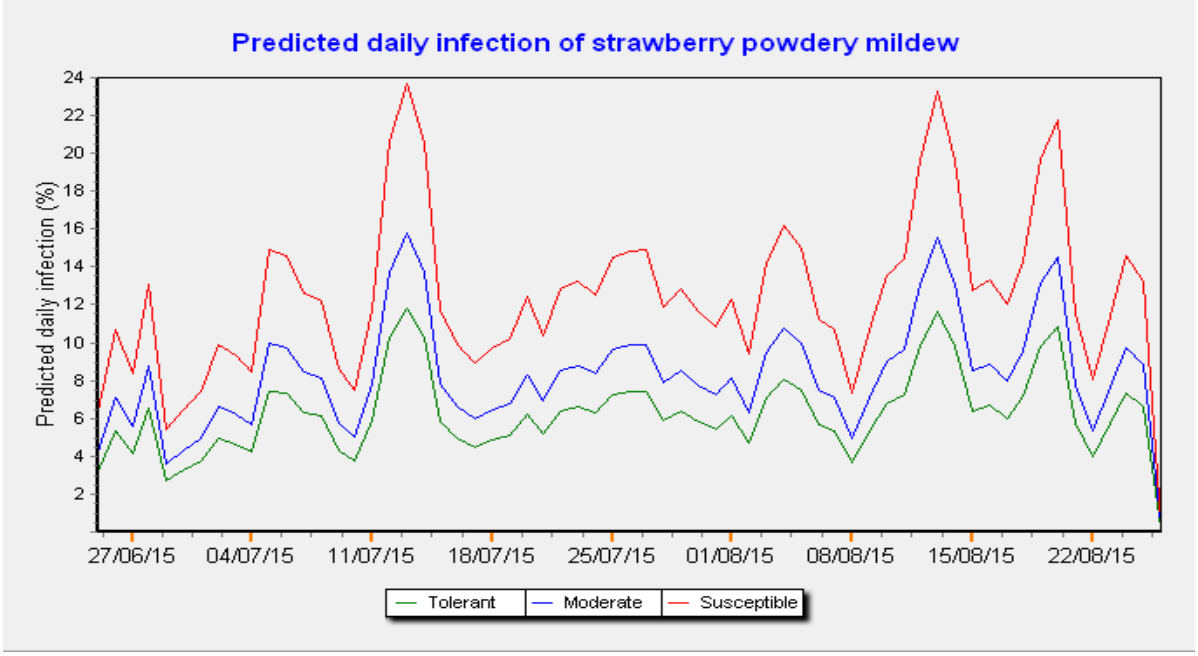

Figure 2.4 Predicted daily risks of powdery mildew for the ADAS site in 2012; the predictions were given by the EMR model where a period of consecutive four days with risks > 10% is considered to need growers' intervention with a moderate to high level of inoculum.

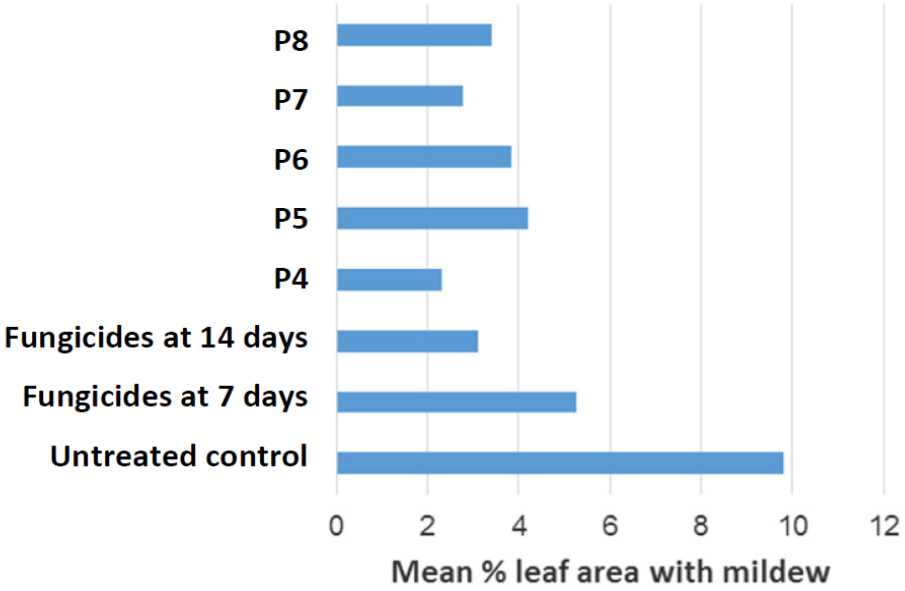

Figure 2.5 % powdery mildew on youngest leaves 28th July (mean of UT in Reps 3 & 4)

The first powdery mildew assessment followed a week after Timing 4; programmes in which Signum (P2 and P8) and AQ 10 + wetter (P6 and P7) were used. The second assessment was a fortnight after the last applications at Timing 6; application of Signum (P2), HDC F207 (P4 & P5), AQ 10 + wetter (P6 & P7) and HDC F210 (P8) (Table 2.12). There were no significant differences between any of the treatment programmes (Table 2.13), indicating that at the relatively low level of symptoms (up to 5% cover on the youngest leaves) each programme had given equal control. All treatments had fewer (not analysed) fruits with botrytis than in the untreated plots where plants had survived the herbicide damage (Figure 2.6).

Table 2.12. Mean plot yields and proportion of marketable fruit per plant over all harvests 9th July to 7 August. Statistical analysis excludes untreated control due to plot damage – ADAS Boxworth 2015

Programme	Treatments	Total marketable plant (g)	Waste plant (g)	% Market-able	Number fruit/plant
P1	(Untreated control 1 plot)	(89.6)	(20.2)	(81.6)	(14.1)
P2	Standard fungicide	145.6	15.9	90.6	22.2
P3	Fungicides at 14 days	137.2	17.3	88.6	23.9
P4	HDC F207	144.9	17.1	89.5	23.6
P5	HDC F207 + Fungicides at 14 days	153.9	15.8	90.8	24.2
P6	AQ 10	160.6	17.4	90.5	26.7
P7	AQ 10 + Fungicides at 14 days	156.2	17.7	89.7	26.5
P8	HDC F210 + reduced standard fungicides	147.9	14.7	90.8	23.5
	P value (excluding UTC)	N.S. (0.892)	N.S. (0.919)	N.S. (0.791)	N.S. (0.079)
	LSD (18df)	39.0800	5.795	3.548	3.254

Table 2.13 Post-harvest storage assessments 7 days after Harvest 3, including % marketable fruit per plant, % fruit with botrytis and % fruit with other rots and insect damage. Statistical analysis excludes untreated control due to unrelated plot damage – ADAS Boxworth 2015

Prog-ramme	Treatments	% Market-able	% Botrytis	% Mucor	% Tricho-derma	% Clado-sporium	% insect damage
P1	(Untreated control 1 plot)	(20.0)	(70.0)	(10.0)	(40.0)	(0.0)	(0.0)
P2	Standard fungicide	39.2	51.8	7.7	17.6	3.4	3.6
P3	Fungicides at 14 days	43.3	44.5	15.4	30.2	5.1	5.6
P4	HDC F207	41.4	51.1	13.8	34.1	2.5	2.9
P5	HDC F207 + Fungicides at 14 days	31.2	52.1	26.0	44.1	0.7	0.6
P6	AQ 10	33.2	41.2	25.7	41.7	6.3	2.1
P7	AQ 10 + Fungicide at 14 days	40.2	42.9	19.7	34.5	4.9	3.4
P8	HDC F210 + reduced standard fungicide	29.2	52.2	41.1	45.0	1.9	0.9
	P value (excluding UTC)	N.S. (0.460)	N.S. (0.385)	N.S. (0.506)	N.S. (0.200)	N.S. (0.679)	N.S. (0.540)
	LSD (18df)	16.51	13.65	33.77	22.41	7.17	5.46

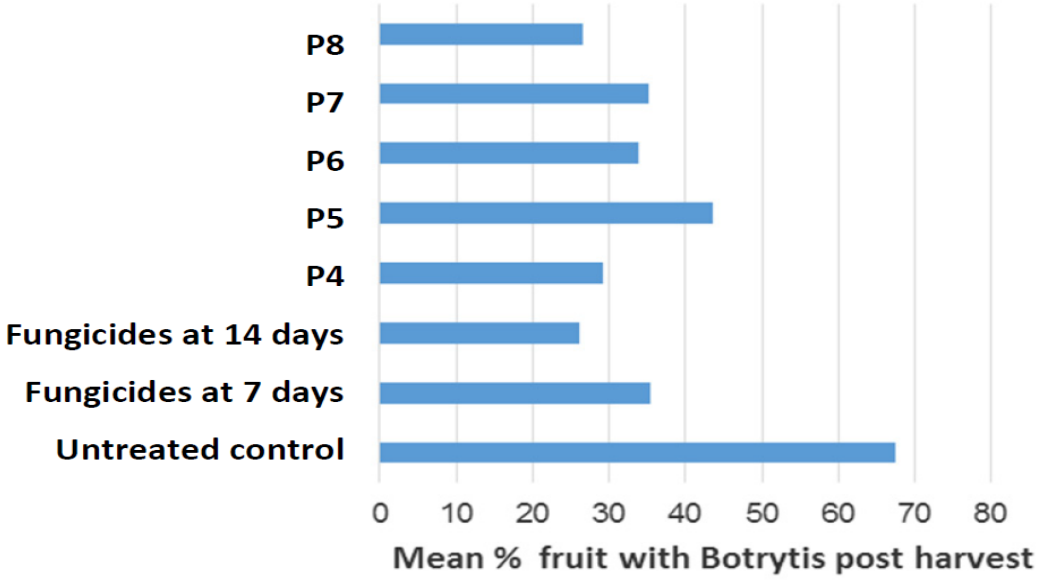

Figure 2.6 Amount of fruit with botrytis (%) harvested 24 July 2015 (Harvest 5) after storage (mean of the untreated in Reps 3 & 4)

There were no significant differences between the treatment programmes in either the mean marketable weight of fruit per plant (between 137g and 160g) or the proportion of the fruit that were marketable (between 89 to 91%) across all the treated plots and over all harvests. The mean number of fruit per plant was between 22 and 27, with no treatment differences. There were fewer fruit and slightly fewer were marketable in the untreated plot, but this could not be linked to disease as the unintended herbicide use could also have caused some damage (Table 2.12). Fruit in the waste category were principally very small and desiccated, with others having been damaged by undetermined pests.

There were no significant differences between the treatment programmes in any of the fruit rots after post-harvest storage (Tables 2.13, 2.14 and 2.15). All treatments had fewer (not analysed) fruits with botrytis than in the untreated plots where plants had survived the herbicide damage (Photo 2.4).

Table 2.14 Post-harvest storage assessments seven days after Harvest 5, including % marketable fruit per plant, % fruit with botrytis and % fruit with other rots and insect damage. Statistical analysis excludes untreated control due to unrelated plot damage – ADAS Boxworth 2015

Programme	Treatment	% Market-able	% Botrytis	% Mucor	% Tricho-derma	%Clado-sporium	% insect damage
P1	(Untreated control 1 plot)	(20.9)	(67.4)	(14.0)	(30.2)	(4.7)	(9.3)
P2	Standard fungicide	26.1	34.8	23.3	29.0	4.0	13.4
P3	Fungicides at 14 days	26.2	26.3	37.5	23.4	1.1	8.7
P4	HDC F207	33.7	29.5	25.5	18.6	2.0	9.6
P5	HDC F207 + Fungicides at 14 days	17.3	43.4	35.1	24.4	0.6	6.0
P6	AQ 10	20.8	30.7	39.0	36.3	4.1	3.8
P7	AQ 10 + fungicide at 14 days	31.7	35.4	23.5	20.0	1.1	8.9
P8	HDC F210 + reduced standard fungicide	34.0	26.3	27.7	14.1	1.2	13.0
	P value (excluding UTC)	* (0.044)	N.S. (0.079)	N.S. (0.160)	N.S. (0.150)	N.S. (0.209)	N.S. (0.103)
	LSD (18df)	11.520	11.880	15.120	16.050	3.449	7.092

Table 2.15 Post-harvest storage assessments 7 days after Harvest 7, including % marketable fruit per plant, % fruit with botrytis and % fruit with other rots and insect damage. Statistical analysis excludes untreated control due to unrelated plot damage – ADAS Boxworth 2015

Programme	Treatment	% Market-able	% Botrytis	% Mucor	% Tricho-derma	% Clado-sporium
P1	(Untreated control 1 plot)	(47.4)	(31.6)	(26.3)	(26.3)	(0.0)
P2	Standard fungicide	32.4	38.3	27.6	8.2	1.4
P3	Fungicides at 14 days	25.9	38.3	45.3	27.0	2.2
P4	HDC F207	42.1	31.8	22.6	25.8	0.0
P5	HDC F207 + fungicides at 14 days	22.7	49.5	31.1	20.6	3.8
P6	AQ 10	28.1	48.2	18.8	22.8	4.4
P7	AQ 10 + Fungicides at 14 days	41.4	32.5	33.9	17.6	4.7
P8	HDC F210 + reduced standard fungicide	32.1	25.1	43.8	17.8	2.6
	P value (excluding UTC)	N.S. (0.261)	N.S. (0.153)	N.S. (0.217)	N.S. (0.454)	N.S. (0.621)
	LSD (18df)	18.48	19.52	23.87	18.81	5.84

The fruit picked at harvest 3 mainly had yellow wool attached and so their flowers were open when the 23 June treatments were given. There were no significant differences between any of the treatments for either the marketable yield, or post-harvest rots, or insect damage at harvest 3 (Table 2.14). Between the treatments there was an average marketable yield of 36.8%, with a 14 day spray programme of standard fungicides showing no difference to the 7 day spray programme. Botrytis was the most prolific post-harvest infection, with an average 50% of fruit being affected over all treatments.

The fruit picked at Harvest 5 had been mainly open flowers at the time of the 30 June spray applications. Across the treatments in Harvest 5 there was an average marketable yield of 27.1%, considerably lower than the previous assessment; fruit affected by *Botrytis* and *Trichoderma* are on average lower than the previous assessment but levels of *Mucor* and insect damage appear higher. There was a significant difference in the marketable yield between the 14 day spray programme with standard fungicides combined with HDC F207

and HDC F207 on its own. Integrating HDC F207 applications within a 14 day programme of standard fungicides gave a reduction in marketable yield of 16.4% compared to treatment solely with HDC F207. There is no difference in the marketable yield obtained between treatments with the 14 day spray programme on its own and HDC F207 on its own. The programme combining HDC F207 with the standard fungicides gave the lowest marketable yield of all treatments and although not significant, the levels of botrytis infection appeared to be higher. Treatments with HDC F210 integrated into a programme of reduced standard fungicides gave the highest marketable yield and this was statistically higher than treatments with AQ 10 on its own and HDC F207 integrated within the 14 day standard programme (Table 2.15).

The fruit picked at Harvest 7 were flowers when the 7 July spray applications were applied. There were no significant differences between the treatments on any of the parameters at Harvest 7. However the programme combining HDC F207 with the 14 day programme of standard fungicides (as at Harvest 5) gave the lowest marketable yield and had the highest level of botrytis. The average marketable yield between all treatments was 32.1% (Table 2.16).

Table 2.16 Post-harvest mean SPAD readings on 10 August from Replicate Blocks 3 and 4. Two statistical analyses including and excluding untreated control plots with accidental herbicide spray – ADAS Boxworth 2015

Programme	**Treatments**	**Mean SPAD reading**	**Mean SPAD reading (excl. UT)**
P1	Untreated control	33.48	-
P2	Standard fungicide	38.94	38.94
P3	Fungicides at 14 days	37.50	37.50
P4	HDC F207	37.53	37.53
P5	HDC F207 + Fungicides at 14 days	37.90	37.90
P6	AQ 10	37.04	37.04
P7	AQ 10 + Fungicides at 14 days	38.84	38.84
P8	HDC F210 + standard fungicides	36.12	36.12
	P value	0.015	0.214 (N.S)
	LSD (7df)	2.386	2.436

When the untreated control was included in statistical analyses, the programme ending with HDC F210 applications had a statistically lower average SPAD reading than the standard fungicide programme and the programme of AQ 10 incorporated into a 14 day standard fungicide programme. The untreated control had the lowest average SPAD readings and was statistically significant from all other treatments. When the untreated control was excluded, there was no significant difference between the treatments (Table 2.16). No phytotoxicity to the leaves, flowers or fruit was recorded throughout the experiment.

2.1.3 Discussion

This is the only the results of the first year. We need at least one more season before we arrive at any conclusions regarding the integration of alternative products and reduced fungicides.

2.2 Silicon fertigation trial

The aims of the trial were to assess the use of the silicon nutrient in the fertigation system to reduce the severity of strawberry powdery mildew epidemics in commercial strawberry tunnels. UoH carried out this experiment with funding from a commercial funding who agreed to share the results with AHDB.

2.2.1 Materials and methods

The silicon fertigation trial was set up in two commercial strawberry polythene tunnels in Pheasant Field at Maltmas Farm, Wisbech, in April, 2015. There are six treatments carried out in two tunnels. Each tunnel is 180m long and has five beds of strawberry cultivar 'V1'. The silicon nutrient used was Sirius, applied weekly to the plants through the fertigation tubes. Application started on 22/04/15.

There were six treatments: T1: No Si no Fungicides; T2: Fungicides only; T3: 0.017% Si single application once a week; T4: 0.017% Si single application once a week plus fungicides; T5: 0.017% Si double applications once a week; and T6: 0.017% Si double applications once a week plus fungicides.

Tunnel 1 received no silicon treatment. The first 15m of each bed from the north end also received no fungicide applications, while the rest of the tunnel received normal fungicide applications in accordance with commercial practice. Tunnel 3 is divided into two parts from the central roadway. Each part is 90m long. The half tunnel from the south end received 0.017% Sirius application once a week, the first 15m of each bed received no fungicide application and the rest received commercial fungicide applications. The half tunnel from the north end received double applications of 0.017% Sirius once a week. The first 0.017% application is followed immediately by the second one at the same time. The first 15m of each

bed received no fungicide applications and the rest received commercial fungicide applications.

In each treatment, 15 leaves were collected from each 15m sampling stretch of the five strawberry beds. 75 leaf samples are collected fortnightly from each treatment. Replications are provided by five beds in the tunnel. Collected samples are placed in the sample bags and brought back to the laboratory. Samples are stored in a cold room (+ 4°C) and be assessed immediately. Each leaflet of sampled leaves was placed under a dissecting microscope at ×100 and ×400 magnifications to assess disease level. The disease level was expressed as % cover of colonies (amount of mycelium) per leaf. Numbers of two spotted spider mite per leaf were also counted.

Silicon content measurement: 3 mature leaves and 3 newly expanded new leaves were sampled from 3 individual strawberry plants per strawberry bed, 3 beds were sampled per treatment. Leaves were then brought back to the lab for Si extraction. Leaf silicon measurement was done once a month. Six flowers were sampled per strawberry bed, 3 beds were sampled per treatment. Samples were then brought back to the lab for Si extraction. Flower silicon measurement was done once a month.

2.2.2 Results

Silicon single dosage plus fungicides, silicon double dosage plus fungicides and fungicides only have shown lower disease level compared to untreated (Figure 2.6). Disease level on untreated leaves increased from 11/08/15 then reached the highest on 25/08/15; while during the same period, level of disease in silicon single dosage treatment and double dosage treatment have seen a slow decrease. Treatments with silicon and fungicides had lower disease level throughout the trial period, silicon single dosage plus fungicides treatment also helped to delay the epidemic build-up.

On the last sampling date 29/09/15, both silicon single dosage treatment and double dosage treatment had higher disease level than control. This could be explained later in combination with the silicon concentration level tested in the leaves.

The area under the disease progress curve (AUDPC) shows that the silicon single dosage plus fungicides treatment and silicon double dosage plus fungicides have given the best disease suppression during the trial period. They had the lowest AUDPC throughout the trial. The AUDPC of silicon single dosage only treatment was higher than those of three fungicide treatments, but lower than no silicon & no fungicides treatment. Oddly, silicon double dosage only treatment had even higher AUDPC than control, this shall be explained in combination with the silicon concentration results later.

There were higher numbers of two-spotted spider mites present on leaves without silicon treatments (No silicon no fungicides & Fungicides only) throughout the trial period. The average number of spider mites per leaf steadily increased from 21/04/15 then reached the highest point on 30/06/15. Silicon once per week and silicon once per week plus fungicides treatments showed better resistance level to spider mites. All six treatments showed similar trend with the number of spider mite per leaf increased from less than five on 21/04/15 to a peak on 02/06/15 and then gradually decreased (apart from no silicon no fungicides treatment) to nearly zero to the end of July.

The average number of non-viable pollens showed no big difference between treatments. Compare to other sampling dates, pollens from 29/09/15 showed relatively higher level of non-viability, especially from control, silicon once per week and silicon double dosage per week treatments. In connection with flower silicon concentration, these three treatments also showed lower level of silicon content.

On the other hand, silicon once per week treatments (with and without fungicides) had higher level of viable pollens throughout the trial than other treatments, with an overall monthly average 105.2 and 96 viable pollen grains per microscopic field respectively. Silicon double dosage treatments (with and without fungicides) had lower pollen viability than control, with average 75.2 and 79.3 viable pollen grains respectively compared to 82.8 per microscopic field from control. The ratio of non-viable pollens against viable pollens showed the lowest value from treatments silicon once per week with and without fungicides treatments (0.194 and 0.245) and the highest from treatments fungicides only and silicon double dosage per week (0.289 and 0.287).

2.2.3 Discussion

This trial has further proved that the silicon nutrient needs to be applied via the fertigation system at a weekly basis throughout the life of the plant, therefore ensuring that the silicon is efficiently taken up by the plant root systems, and so provides a continuous supply of silicon to the growing plants throughout the season.

Nevertheless, there were some issues which occurred in this trial in relation to the silicon double dosage treatments, which led to some unexpected results. Based on the silicon concentration results, it was suggested that the plants from the double dosage treatments may not have received the expected silicon dosage in August and September, thereby showing higher susceptibility to disease and pest. This could be caused by several reasons: for example, it could be the problems with the delivery of the silicon nutrient via the fertigation system, or could be the incorrect amount of the silicon be put into the fertigation system etc.

There is evidence elsewhere on the farm that there were problems at this time. All of these need to be further investigated.

Objective 3: Fruit rot complex

To understand epidemiology of fruit rot complex and to develop management strategies (to start in Year 3).

Objective 4: Wilt

To develop alternative methods to reduce wilt in soils.

This objective includes two aspects: (1) evaluating combined use of alternative products (EMR), and (2) evaluating the efficacy of ASD products (ADAS). The EMR trial was a continuation of a field trial initiated in a recently completed Innovate UK project on biofumigation to control wilt.

4.1. Combined use of alternative products

Two experiments were carried out: one in the lab and one in the field. The lab study focussed on the effects of different concentrations of an experimental turpene product whereas the field test focussed on the individual and combined use of three alternative products. Most of the funding for the work was from Innovate UK; AHDB funding enabled the quantification of viable wilt inoculum of field samples. Results from the initial lab study determined whether we need to carry out the field test (i.e. whether there is a sufficient reduction in wilt inoculum viability for these selected alternative products), and also the appropriate rate of the terpene product in the field test.

4.1.1 Material and methods

***Products*:** Four products were tested: an experimental product of microencapsulated terpenes, a pellet formulation of defatted seedmeal of *Brassica carinata* (BioFence™), pelletised lavender waste, and liquid digestate. The first three products were used in the lab test and all four were used in the field study.

The terpene product was manufactured by Eden Research (Witney, Oxfordshire, England) using its patented technology; this product contained 9.9 % cineole, 3.3% camphor and 3.3% borneol – these terpenes were identified as key compounds in lavender waste responsible for the observed biocidal effect against *V. dahliae* (Yohalem and Passey, 2011). In the field test, a liquid formulation of BioFence™ was used for the ease of application. The liquid formulation was not available when the initial lab study was carried out – otherwise the same formulation would have been used in both tests. Lavender waste was collected from a commercial farm after the oil had been extracted and pelletised by a commercial company.

Liquid digestate was obtained from St Nicholas Court Farms (Birchington, Kent, UK); feedstock was 5% chicken manure, 5% fruit waste, 75% maize silage, 15% grass silage; retention time in the digesters is roughly 90 days.

The rate of using the terpene product was recommended by the manufacturers based on other terpene products manufactured by the company (Edmonds of Eden Research – pers. com.). Before use, this product was diluted with water to a ratio of 1:20 (product:water, ca. 4.8%). For other products, the rate used was the maximum permissible rate under the UK NVZ (nitrate vulnerable zones) regulations given the estimated N concentration in each product.

Quantifying V. dahliae inoculum density: The density of *V*. *dahliae* inoculum in each soil sample was estimated using a well-established wet-sieving and plating method (Harris et al., 1993) with several modifications. In short, soil samples were first air-dried for three weeks to kill conidia and the mycelial fragments of *V*. *dahliae* before sieving (2 mm mesh). A sample of 10 g of soil was placed into a screw-cap bottle (200 mL) and distilled water was added to a volume of 40 mL. The bottle was shaken vigorously for 1 h on a reciprocating shaker (Edmund Buhler, 7400 Tubingen Shaker-SM 25, Germany) at 175 rpm to break-down soil clumps. Then the suspension was washed through nested 160 and 20 µm sieves with tap water. The material on the 20 µm sieve was recovered into the original bottle and made up to 20 mL with distilled water. Aliquots of 1 mL soil suspension were transferred individually with a pipette to each of 20 Petri dishes (9-cm diameter) of semi-selective medium. The suspension was stirred while withdrawing each aliquot. The semi-selective medium contained 2 g $NaNO_3$, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.01 g $FeSO_4 \cdot 7H_2O$, 2 g PGA, 1 mL tergitol NPX (Sigma, UK) and 15 g agar (Fluka, UK) per litre water, adjusted to pH 6.4 with 1M KOH prior to addition of the agar. After autoclaving, the basal medium was held at 55°C and 10% by volume sterile filtered antibiotic solution added (containing 6 mg streptomycin, 6 mg chloramphenicol, 6 mg chlortetracycline, 0.6 mg biotin per 10 mL water). Soil plates were incubated at 22 °C for 4 weeks, before soil particles were washed away with tap water. The plates were scanned for colonies of *V*. *dahliae* with a dissecting microscope (Olympus SZ-PT, Japan) at 24× magnification. Each CFU (colony forming unit) was assessed as one germinated microsclerotium. The method gives a detection threshold of 0.1 CFU g^{-1} of soil. For each field sample, the procedure was the same except that (1) the material on the 20 µm sieve after sieving 50 g of soil was made up to 100 mL with distilled water and (2) aliquots of 1 mL soil suspension were transferred individually with a pipette to each of 10 Petri dishes, giving the detection threshold of 0.2 CFU g^{-1} of soil.

Laboratory test: naturally infested soil (free-draining, brown earth of the Marlow series) to a depth of 15 cm was collected from an experimental field at NIAB EMR, UK, which is known to contain a high level of *V. dahliae* inoculum (i.e. high colony forming unit, CFU, per gram of soil). Soil was air dried for 3 weeks until sieving (2 mm mesh) was possible. There were six product treatments: three rates (17, 50 or 150 µl per kg of soil) of the diluted terpene product, BioFence™ pellet (1.6 g kg^{-1} of soil), lavender waste pellet (6.5 g kg^{-1} of soil), and untreated, each at two levels of soil moisture (80 and 100% of soil water capacity). For the terpene product, 17 µl per kg of soil is the recommended rate by the manufacturer. A completely randomised block design (with three blocks) was used. Within each block, there was a single replicate (a glass jar of 350 mL) for each of the 12 treatments. The experiment was conducted in 2011. Aliquots of 200 g dry weight of soil were transferred individually to 36 glass jars. To keep a good soil structure, the amount of water to be added was divided into four equal portions (a single portion of 10.5 mL and 8.4 mL for the 100% and 80% soil water capacity, respectively, based on the estimated water capacity of 21%) and added to each jar at an interval of two hours. Before adding the final portion of water, the product was inserted into the center of the jar and covered with the soil. All 36 jars were sealed with black polythene (100% LDPE - low density polyethylene, including UV stabilisation) and incubated at 25°C for four weeks. Viable microsclerotium density was estimated for each jar as described above.

Field experimental design and treatments: Field experiments were conducted to evaluate the efficacy of three products (liquid formulation of BioFence™, microencapsulated terpene, and liquid digestate) at NIAB EMR, UK, from September to November 2014. Strawberry was grown in the field (free-draining, brown earth of the Marlow series) in the preceding two years and the level of wilt disease varied considerably. Eight blocks (ca. 10 m long) of raised beds were selected since they had high levels of wilt disease development on strawberry, ranging from 58% to 89%. Each block was divided into nine mini-plots 40 cm long × 40 cm wide with at least 40 cm gap between two neighbouring mini-plots. Each of the nine mini-plots was randomly assigned to one of the nine treatments given in Table 4.1. The volume of applied for each product (50 ml per plot) was calculated from the commercial application volume of chloropicrin. Treatments were applied on four different days (two blocks per day). A depth of 20 cm soil in each mini-plot was removed first and any large stones or plant tissue removed. The bottom layer of soil was sprayed with 10 mL treatment via a hand held sprayer, as were four subsequent layers each followed by re-filling soil 5-cm deep to give 5 layers of soil. Each plot was covered with blue polythene (100% LDPE - low density polyethylene, including UV stabilisation) after treatment. For combined treatments, each individual product was applied at the same volume as for single product treatments (i.e. 20 mL total volume for two products, 30 mL for three). There is always a debate point comparing combined use of two liquid

treatments against individual products – whether to use the same volume as each individual treatment (i.e. 100 ml instead of 50 ml – since each product was separately applied) or to use the same total volume (i.e. 25 ml for each product to give 50 ml for both products). In this study, we adopted the first method, i.e. each product was applied in the same volume between the single or combined treatments since this on balance would give better basis for statistical analysis of independence.

Five soil cores (2.5 cm in diameter) from just below the surface to a depth of 15 cm on two diagonals were collected from each mini-plot immediately before treatment and at 10 weeks after, and bulked into a single sample for each mini-plot for assessing the inoculum density.

Table 4.1 Details of nine biofumigation treatments applied to individual plots (0.16 m^2) to a depth of 20 cm to reduce the level of *Verticillium dahliae* inoculum at NIAB EMR

Treatment ID	Products and application rates	Total volume (mL/plot)
B	50 mL 4% BioFence™	50
T	50 mL 4.8% terpene	50
D	50 mL 12.5% digestate	50
BT	50 mL 4% BioFence™, 50 ml 12.5% terpene	100
BD	50 mL 4% BioFence™, 50 ml 12.5% digestate	100
TD	50 mL 4.8% terpene, 50 ml 12.5% digestate	100
BTD	50 mL 4% BioFence™, 50 ml 12.5% terpene, 50 ml 12.5% digestate	150
W	50 mL sterile distilled water	50
U	Untreated control	0

Statistical analysis: Analysis of variance (ANOVA) was applied to the laboratory data with a randomised block design with two factors (moisture and treatment). Standard residual plots were used to check whether there was large deviation from the normal distribution assumption. When there were significant treatment differences, the LSD test at P = 0.05 was used to compare treatments. For the field trials, as there were many plots with very low (often zero) CFU counts, which may lead to greater differences in control efficacy (as efficacy was measured as a ratio), two blocks of treatments applied on the same day were combined together. Thus, in ANOVA a randomised block design was used with four blocks (four treatment days). As there were virtually no differences in CFU counts between the untreated

and the water treatments in both occasions, these two were combined to estimate the inoculum density for the control. A control efficacy (E) for a given treatment was estimated as $E = \frac{CFU_0 - CFU_{10}}{CFU_0}$, where CFU0 and CFU10 was the CFU counts on day 0 and 10 weeks after treatment, respectively. ANOVA was applied to the estimated efficacy to assess treatment effects.

We used several statistical methods (including analysis of variance – ANOVA) to assess the differences between, single-, two- and three-product treatments. Only when there were overall significant treatment differences, the LSD test was applied to individual pairs of treatments. None of variables needs transformation as the residuals were close to be normally-distributed. All analysis was carried out in Genstat (version 13.1, VSN International, England).

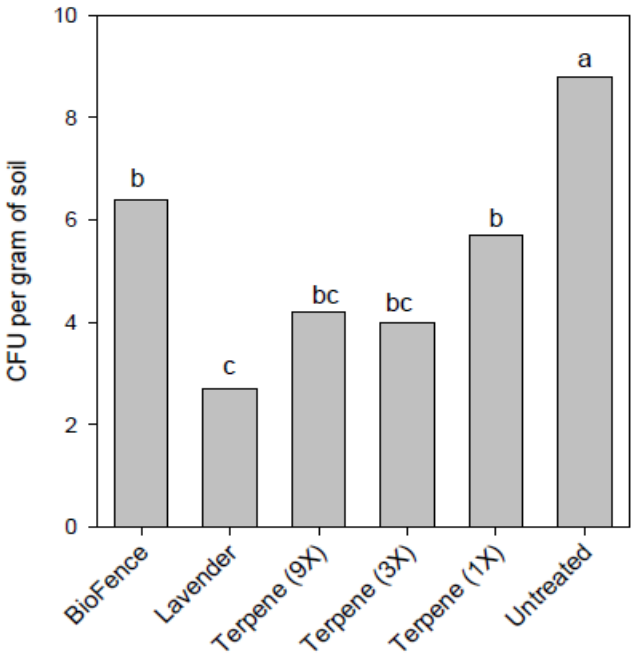

Figure 4.1 Average *Verticillium dahliae* CFU (colony forming units) counts per gram of soil after treatment. The experiment was conducted in honey jars at two moisture levels; there were no significant differences in the CFU counts between the two moisture levels. Treatments containing at least one common letter (above the bar) are not statistically different based on the LSD test at the level of 5%.

4.1.2 Results

Laboratory test: There were significant (P < 0.001) differences among the six treatments; 61% of the total variability (with the block differences removed) was due to the differences among treatments. All treatments led to significant reductions in CFU counts, compared to the untreated control (Fig. 4.1). Biofence™ and lavender waste were the least and most effective (Fig. 4.1), respectively, with the respective efficacy of 27% and 69%. Lavender waste reduced *V. dahliae* CFU from 8.8 to 2.7 g^{-1} of soil, significantly greater than the 1X terpene (35%, P < 0.05) and the BioFence™ (P < 0.01). Although there were no statistical significant differences among three terpene treatments, the 1X treatment resulted in a much lower control efficacy (35%) than the 3X (55%) and 9X (53%) treatments. The differences between BioFence™ and the 3X and 9X terpene treatments were close to statistical significance at the 5% level.

Field mini-plot trials: For the field mini-plot trial, CFU counts per gram of soil varied greatly from plot to plot, ranging from zero to 7.2 g^{-1} of soil (Figure 4.2), with 67 out of the 144 CFU values less than 0.8 g^{-1} of soil. CFU values were generally low for two blocks treated on the same day. There was little difference in the CFU values between day 0 and 10 weeks after treatment for the untreated and the water-treated plots (Figure 4.2, Figure 4.3). In contrast, there were considerable reductions in CFU counts following treatment of soils (Figure 4.2, Figure 4.3).

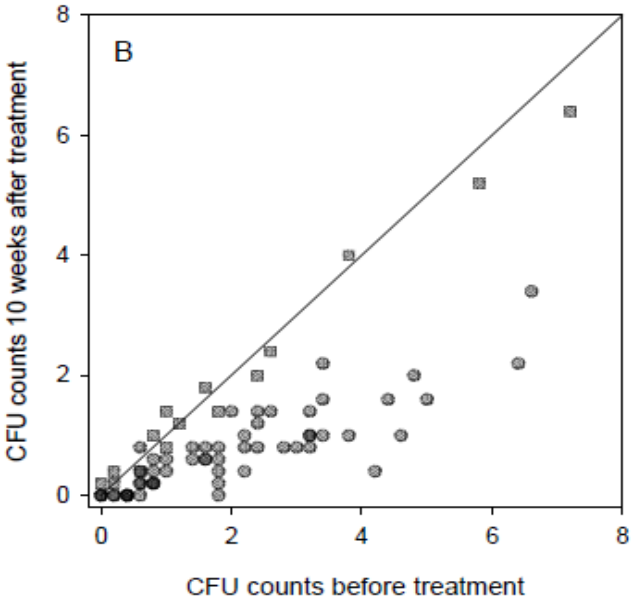

Figure 4.2 Observed *Verticillium dahliae* CFU counts per gram of soil in each plot before treatment plotted against those values 10 weeks after treatment with square symbols representing the untreated or water treated plots.

There were significant differences in the efficacy among the eight treatments (the water and untreated were combined as the control) (Figure 4.3): the differences accounted for 85% of the total variability (after blocking differences, e.g. four treatment times, were removed). As expected, the efficacy for the control treatment was close to zero (4%). All treatments led to significant reductions ($P < 0.001$) in CFU counts. Efficacy ranged from 50% (digestate) to 78% (three-product treatment) (Figure 4.3). Of all pairwise comparisons among the seven treatments, only the difference between digestate and the three-product treatment differed significantly ($P < 0.01$).

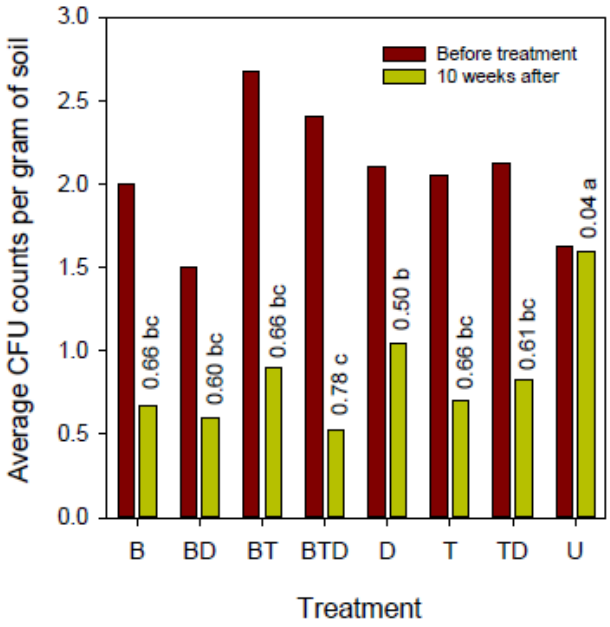

Figure 4.3 Average of observed *Verticillium dahliae* CFU counts per gram of soil before and 10 weeks after treatment for all eight treatments. Figures above the bars are the average efficacy for the treatment concerned; treatments containing at least one common letter (above the bar) are not statistically different.

As a group, the three single product treatments did not differ significantly in their efficacy from the three two-product treatments (61% versus 62%), but were treatment (61% versus 78%). All observed efficacies were less than the expected under the assumption of Bliss independence except for three observations (Figure 4.4), which came from the blocks where CFU counts were generally low for all treatments. This overall difference was significant ($P < 0.001$): 73% (observed) versus 88% (expected), consistent over the four treatments (Figure 4.4). For the combined treatments, there were no significant differences between the observed efficacy and the best single component efficacy (Figure 4.5).

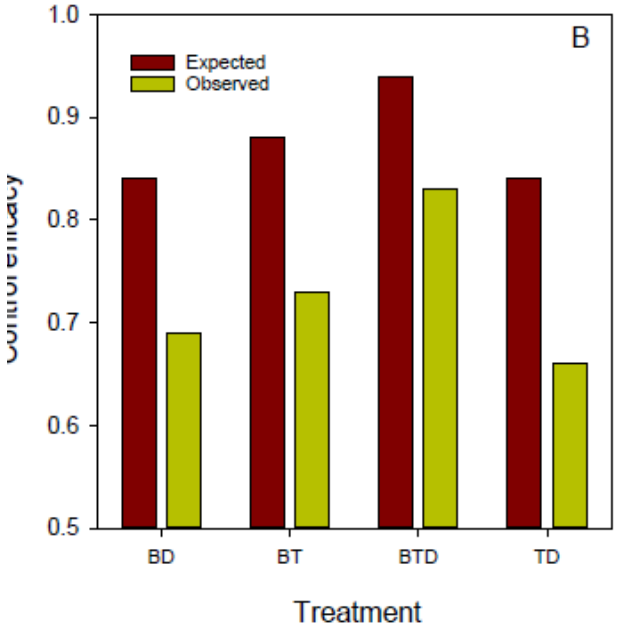

Figure 4.4 Average observed and expected values for all four combined treatments (the expected efficacies of combined product treatments were calculated from the efficacies of single product treatments in the field mini-plot trials.

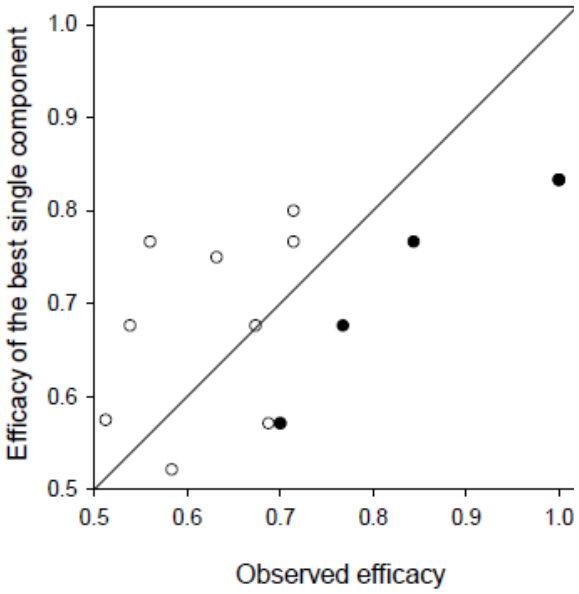

Figure 4.5 Observed efficacy in reducing *Verticillium dahliae* CFU counts for two- or three-product treatments plotted against the best single product efficacy; the line represents the 1:1 between the expected and observed values. Each point represents a treatment on a single treatment day; the filled points are for the three-product treatment.

4.1.3 Discussion

Both laboratory and field trials demonstrated that the tested alternative products could reduce viable wilt *V. dahliae* inoculum in the soil by up to two thirds when applied alone. However, combined use of two products did not lead to significant improvement in the control efficacy over the single product. Only the combined use of all three-product appeared to improve the efficacy over the single products, but still less than the expected under the assumption of Bliss independence. Two types of BioFence™ formulation were used: pellet in the laboratory and liquid form in the field, which may explain the large difference in the resulting efficacies: 67% (field) versus 27% (laboratory). The liquid formulation of BioFence™ may be more efficient in the release of the ITC than the solid version, leading to greater dispersion of the active ingredient through the soil. BioFence™, derived from defatted seedmeal of *B. carinata*, contains the glucosinolate sinegrin, which decomposes into allyl ITC upon hydrolysis in the presence of myrosinase (Lazzeri et al., 2004.). The control efficacy of BioFence™ observed in the present study agrees with previous findings. For example, broccoli green manure treatment reduced the density of *V. dahliae* inoculum by 50%, leading to reduced severity of wilt (Ochiai et al., 2007). The potential of 19 cultivars of *Brassica juncea*, *Raphanus sativus* and *Sinapis alba* as a biofumigant was evaluated and only *B. juncea* shoot tissue was shown

to reduce the viable microsclerotia significantly (Neubauer et al., 2014). The seed meal of six genotypes of *B. juncea* achieved 100% efficacy in sterilised quartz soil against *V*. *dahliae* microsclerotia whereas three genotypes reduced microsclerotium viability by more than 92% (Neubauer et al., 2015). However, in six samples of different unsterilized soil, the control efficacy achieved by the seedmeal of a particular *B. juncea* genotype ('Energy') increased with application rate and varied greatly with the soil samples. For instance at the rate 0.2% (vol/vol), the efficacy ranged from 0 to 51.5%, compared to 14.7% to 81.4% at the rate of 0.4% (vol/vol). This result indicates that the control efficacy is also affected by soil characteristics (including microbial population composition), which may also explain the large variability between replicate mini-plots in the present study. Another factor may also account for the difference in BioFence™ efficacy, namely the difference in the length of treatment time: 4 weeks in the laboratory test compared with 10 weeks in the field test.

The present study confirmed previous findings (Yohalem and Passey, 2011) that lavender waste amendment significantly reduces *V*. *dahliae* microsclerotia in soils and three terpenes (cineole, camphor and borneol) are mainly responsible for the observed reduction. The control efficacy (69%) achieved by lavender waste in the present laboratory test is less than previously observed (> 99%) (Yohalem and Passey, 2011). This difference is likely to be explained by the difference in the application rates: 6.5 g (present study) versus 53 g (previous study) kg^{-1} of soil. In addition, the differences in the quality of lavender waste and the formulation (pellet versus unprocessed) in terms of the concentration of terpenes (and/or other chemicals) may also have contributed to this difference in efficacies. Different batches of lavender waste varied greatly in their concentration of the three terpenes (David Hall of NRI, University of Greenwich, per. comm.). Lavender waste also had higher efficacy than the three terpene treatments (particularly the 1X rate); this could arise from the difference in the concentration of terpenes released and/or other chemicals in the waste also contributing to the control. Terpenes have been demonstrated to have inhibiting effects against plant pathogens (Isman, 2000; Rodriguez et al., 2014).

ASD has been shown to be able to reduce the density of *V. dahliae* microsclerotia in soil and the incidence of Verticillium wilt (Blok et al., 2000; Goud et al., 2004; Runia et al., 2012; Korthals et al., 2014). A 50-60% reduction in microsclerotia by ASD was obtained (Korthals et al., 2014). ASD treatment using molasses as the carbon source paired with solarisation can be an effective strategy to maintain crop yields in the absence of soil fumigants (Butler et al., 2014). A few studies have shown that plant pathogens cannot survive the AD process (Haraldsson, 2008; Zetterstrom, 2008). However, there have been no published studies to show that digestate (from the AD process) can itself inhibit plant pathogens. This study showed for the first time that the specific liquid disgestate used is able to kill about 50% of *V.*

dahliae microsclerotia after 10 weeks. Liquid disgestate is known to be high in nutrients such as ammonia and phosphates and hence can be used to fertilise plants. Further studies are needed to confirm whether the controlling effect varies greatly with soil of different characteristics and different feed-materials used in the AD process.

There has been increasing research into combining alternative control methods against plant diseases in order to increase control efficacies and exploit potential synergy among different control measures (Xu et al., 2011), such as combining biocontrol agents with soil solarization to control soilborne pathogens. For instance, organic amendment combined with biocontrol bacteria could strongly suppress *V*. *dahliae* (Vitullo et al., 2013); ASD combined with soil solarisation can maintain crop yield in the absence of soil chemical fumigants (Butler et al., 2014). The present study shows that efficacies of all combined treatments were not only much less than the expected based on the assumption of Bliss independence but also not significantly greater than the best single component product, indicating the lack of additivity not to mention synergy. This observation agrees with most previous studies on the combined use of biocontrol agents to manage plant diseases (Xu et al., 2011). This could be due to the fact that there are a considerable proportion of larger and melanized microsclerotia that are recalcitrant to the treatments. Large microsclerotia probably contain more layers of cells; killing outside layers of cells of a single microsclerotium is probably insufficient to kill the whole microsclerotium. Microsclerotia of *V. dahliae* in naturally infested soil vary greatly in their size (diameter), ranging between 11 and 125 µm, and larger aggregates may occur (Ashworth et al., 1972; DeVay et al., 1974). Fully melanised and large (> 75 µm) microsclerotia are likely to be more tolerant to harsh external conditions (Hawke and Lazarovits, 1994). Even fumigation with methyl bromide + chloropicrin (2:1) significantly reduced but did not eliminate microsclerotia below 2 CFU g^{-1} of soil (Subbarao et al., 2015), which may partly contribute to survival of large microsclerotia. The increased efficacy in the three-product treatment may also be due to increased volume of application; further research is needed to investigate the efficacy of combined use of products in relation to microsclerotium size, concentration of active ingredients, and application volume.

4.2 Use of Anaerobic soil disinfestation to control wilt

4.2.1 Materials and methods

Treatments; ca. 400 L of soil was collected up to a depth of 250mm in May 2015 from a grower holding in Essex known to have *V. dahlia* problem. The soil was evenly mixed and clumps broken up and on 6 May 2015 a sample of this soil was sent for evaluation of *V. dahliae* infestation. The remaining soil was stored in sheeted-over bulk bag under cover in an unheated barn until the start of the experiment to ensure that the microbial flora and moisture

content changed little after its collection. On 10 September 2015, prior to the start of the trial a 1 kg soil sample was analysed for to pH, % organic matter and nutrient analysis with micronutrient to include sulphate and textural classification. Another 1 kg was analysed for moisture content. A further 1 kg was taken for moisture analysis once pots had been filled, sampling from a spare container at 250 mm depth.

There were 10 treatments (Table 4.2), and four replicates per treatment. These included pots treated with Herbie 67P only, Herbie 82 only, Herbie 67P and Herbie 82, as well as four untreated (one pair sealed and one pair unsealed) all at two different water regimes.

Treatment application: A further 10 L of wet soil from the grower holding previously sampled was collected at a depth greater than 200 mm in order to obtain soil that was expected to have a higher population of anaerobic bacteria. This was collected just before it was required in the production of a “starter treatment” (to boost the population of anaerobic bacteria). The starter treatment was prepared on 24 August 2015, nine days before the main experiment was set up on the 2 September 2015. A 20 mm layer of this soil was spread in the base of a 10 L translucent container, followed by 10 g of Herbie 67P and another 20 mm layer of soil, this was compressed to remove air gaps. This layering process was repeated to use 100 g of product in approximately 8 L of soil until leaving an air space of only 25 mm once the lid was fitted. Before sealing, 1600 ml of tap water was added to the container to evenly wet the surface and so that water permeated and could be seen through the sides of the tub to be held around the soil clumps, so that the soil was wet throughout but that there was air still present. The container was sealed with tape and wrapped in black polythene and left in a dark place on the laboratory floor for nine days.

On the 2 September 2015 after incubation with Herbie 67P, the starter soil was mixed with sieved, untreated infested soil which had been collected in May. This allowed 67 ml of starter soil per litre of soil (8 L starter soil mixed with 160 L untreated infested soil) and this was then used immediately for the treatments that were to contain Herbie 67P. The containers treated with Herbie 67P only (T5 and T6) were filled with the starter plus sieved soil to within 20 mm of the rim and compacted. The starter and untreated infested soil mix was also used for the Herbie 67P + Herbie 82 treatments (T9 and T10). Each container had 110 g of Herbie 82 added per 10 L of soil mix and was thoroughly combined before the soil was added to the container. In Herbie 82only treatments (T7 and T8), the untreated infested soil was thoroughly mixed with Herbie 82 at a rate of 110 g Herbie 82 per 10 L of untreated soil. The containers were filled with the soil and compacted, the watering regimes were applied and the containers sealed immediately as before.

Table 4.2 Treatments used in pots of field soil with viable Verticillium microsclerotia, Boxworth September 2015. All plots with Herbie treatments were air-tight sealed.

No.	Treatment	Herbie 82 treatment rates per L soil in each pot	Starter Product	Starter soil volume per L soil in pot	Depth of water added
T1	Untreated, unsealed	-	-	-	5 mm
T2	Untreated, unsealed	-	-	-	10 mm
T3	Untreated, sealed	-	-	-	5 mm
T4	Untreated, sealed	-	-	-	10 mm
T5	Starter only, sealed	-	Herbie 67P	67 ml/L	5 mm
T6	Starter only, sealed	-	Herbie 67P	67 ml/L	10 mm
T7	Herbie 82, sealed	11 g	-	-	5 mm
T8	Herbie 82, sealed	11 g	-	-	10 mm
T9	Herbie 82 + 67 P, sealed	11 g	Herbie 67P	67 ml/L	5 mm
T10	Herbie 82 + 67 P, sealed	11 g	Herbie 67P	67 ml/L	10 mm

The containers were filled with soil to within 20mm of the rim and compacted. The volume of water needed for the 5 mm and the 10 mm depth watering regimes given to each pot (Table 1) was calculated and the water applied evenly across the soil surface of the relevant container once any treatments had been mixed into the soil. For the containers that were to be sealed, this was done immediately with black Gas Stop TIF film to exclude oxygen immediately after each had been filled with the appropriate soil and products and a Delta T logger probe inserted to about 200 mm below the surface. The loggers in each pot recorded temperatures at half hourly intervals, with a separate probe recording the temperature outside the pots. Each treatment had four replicates which were arranged in randomised blocks and left to incubate for eight weeks in a polythene tunnel at Boxworth.

Assessments: After eight weeks, on the 30 October 2015, samples from each container were analysed for pH, percentage organic matter, nutrient and moisture content. A 2 kg sample from each container was assessed for the amount of *Verticillium* microsclerotia per gram of soil. The data were analysed using analysis of variance (ANOVA).

4.2.2 Results

Tables 4.3 and 4.4 show a comparison of soil analysis pre and post-ASD treatment. All of the Herbie treatments (T5-T10) show a marked increase in the levels of sulphate compared to the untreated controls (Table 4.4). Both sealed and unsealed untreated pots had similar sulphate content to the soil when it was originally sampled (Table 4.3). T9 and T10 with both Herbie 82 and Herbie 67P showing the highest levels. In general, potassium levels were higher in the treated pots compared to the untreated (Table 4.4) and the pre-experiment analysis (Table 4.3). Phosphorous, pH, and Magnesium remain relatively consistent both between treatments (Table 3), and between pre and post experiment analysis (Tables 4.3 and 4.4). Treatments involving the incorporation of the Herbie 82 product show an increase in organic matter content (Table 4.4). There was no consistent trend in the data that could be attributed to either the high or low amounts of water added.

Table 4.3 Analysis of the field-collected soil before the start of the experiment in September 2015 (sample sent for analysis 10.09.15)

% soil moisture	organic matter WB % w/w	pH	P mg/l (index)	K mg/l (index)	Mg mg/l (index)	Sulphate mg/l	Sand:Silt:Clay %
13.53	2.7	6.7	32.2	234	109	77.9	44:42:14

Table 4.4 Analysis of the soil after the completion of the experiment in October 2015 (sample sent 30.10.15)

Treatment	% soil moisture	organic %	pH	P mg/l (index)	K mg/l (index)	Mg mg/l (index)	Sulphate mg/l	Sand:silt:clay %
T1 untreated, unsealed (5mm)	6.97	2.9	6.3	33.0	216	101	73.5	50:39:11
T2 untreated, unsealed (10mm)	7.82	2.9	6.5	33.8	236	110	76.4	51:37:12
T3 untreated, sealed (5mm)	11.13	2.9	6.5	29.4	204	105	72.4	47:41:12
T4 untreated, sealed (10mm)	11.81	2.8	6.4	31.6	215	107	77.1	49:39:12
T5 Herbie 67P (5mm)	12.79	2.9	6.4	32.0	250	112	90.4	48:40:12

Table 4.4 Analysis of the soil after the completion of the experiment in October 2015 (sample sent 30.10.15)

Treatment	% soil moisture	% organic	pH	P mg/l (index)	K mg/l (index)	Mg mg/l (index)	Sulphate mg/l	Sand:silt: clay %
T6 Herbie 67P (10mm)	12.27	2.9	6.4	34.2	244	97	108.4	49:39:12
T7 Herbie 82 (5mm)	10.48	3.4	5.8	43.6	291	127	104.2	53:36:11
T8 Herbie 82 (10mm)	12.94	3.1	6.1	32.8	249	110	95.1	58:32:10
T9 Herbie 82 + Herbie 67P (5mm)	13.77	3.3	6.3	35.8	280	124	130.4	56:33:11
T10 Herbie 82 + Herbie 67P (10mm)	13.95	3.4	6.0	37.2	282	124	135.2	51:38:11

Table 4.5 shows a comparison between treatments of the highest, lowest, and average temperatures over the duration of the experiment. There is limited difference in temperature values between any of the treatments, with the trend following that of the “no pot” probe monitoring the tunnel environment (Fig. 4.6). Temperatures were not recorded over the full eight week duration of the experiment because the loggers hit capacity after five weeks

Table 4.5 Mean temperature probe data from 02 September to 3 October 2015 inside the four replicates of pots in a polytunnel showing lowest, highest, and mean temperatures

Treatment	Mean Lowest	Mean Highest	Overall Mean
T1 untreated, unsealed, low water (5mm)	9.51	28.13	16.55
T2 untreated, unsealed, high water (10mm)	9.30	28.12	16.48
T3 untreated, sealed, low water (5mm)	9.23	30.54	16.82
T4 untreated, sealed, high water (10mm)	9.39	29.80	16.80
T5 Herbie 67P, low water (5mm)	9.13	28.87	16.52
T6 Herbie 67P, high water (10mm) *	9.61	28.31	16.68
T7 Herbie 82, low water (5mm) **	9.82	27.14	16.53
T8 Herbie 82, high water (10mm) *	9.26	29.18	16.70
T9 Herbie 82 + Herbie 67P, low water (5mm) *	9.83	28.08	16.74
T10 Herbie 82 + Herbie 67P, high water (10mm) *	9.58	27.06	16.33

Table 4.5 Mean temperature probe data from 02 September to 3 October 2015 inside the four replicates of pots in a polytunnel showing lowest, highest, and mean temperatures

Treatment	Mean Lowest	Mean Highest	Overall Mean
Outside temperature	9.07	29.58	16.52
* Results from one replicate excluded; ** Results from two replicates excluded because of erroneous probe recordings			

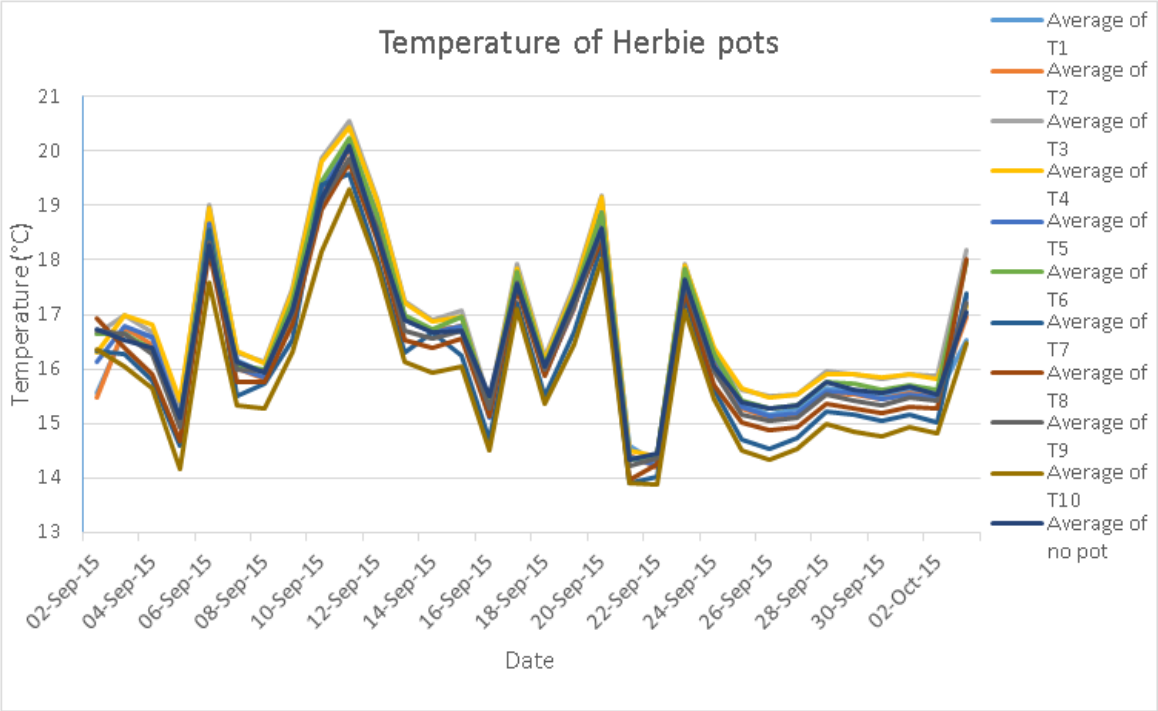

Figure 4.6 Temperature probe data from inside all soil pots. T1 to T10 refer to the ten treatments as given in Table 4.2.

The soil bulk sampled in May 2015 and used to fill the pots in September contained 2.3 viable microsclerotia using the Harris test. The same test showed highly significant differences between treatments following eight weeks in the pots (Table 4.6). A slightly greater number of propagules were extracted in the untreated pots by the second Harris test. The act of sealing the pots (without the use of Herbie products) was shown not to have caused any reduction in microsclerotia viability. All four treatments with Herbie 82, whether or not with the Herbie 67P and regardless of the amount of water added, had 0.35 or fewer propagules per gram of soil than any other treatment (Fig. 4.7). One of the four pots in each of these treatments had zero propagules, indicating complete control that was not seen in any of the treatments which did not use Herbie 82.

After 1 kg of soil from each of the pots at the end of the experiment was dried, except for T7 the pots which received the wet “starter” soil containing Herbie 67P had more moisture, and those left open were confirmed to be the driest (Table 4.6). There were no other trends, with covered pots having between 10.5% and 14% moisture content, compared with 13.5% in the bulk before filling pots.

Table 4.6 Viable microsclerotia of *Verticillium* after eight weeks, on 30 October 2015, and dry weight of 1 kg samples of soil and ANOVAR results. Similar letters against each result show no significant difference between treatments as determined by Duncan’s multiple range test

Treatment	***Verticillium* microsclerotia per g soil after 8 weeks**	**Dry weight of 1 kg of soil after 8 weeks**
T1 untreated, unsealed, low water (5mm)	3.27 bc	930.27 g
T2 untreated, unsealed, high water (10mm)	3.22 bc	921.85 f
T3 untreated, sealed, low water (5mm)	4.22 c	888.75 de
T4 untreated, sealed, high water (10mm)	3.47 bc	881.95 cd
T5 Herbie 67P, low water (5mm)	2.87 b	872.12 b
T6 Herbie 67P, high water (10mm)	3.45 bc	877.32 bc
T7 Herbie 82, low water (5mm)	0.22 a	895.25 e
T8 Herbie 82, high water (10mm)	0.30 a	870.60 b
T9 Herbie 82 + Herbie 67P, low water (5mm)	0.27 a	862.35 a
T10 Herbie 82 + Herbie 67P, high water (10mm)	0.35 a	860.55 a
Mean	2.17	886.10
LSD	1.138	8.042
d.f.	27	27
F Probability	<0.001	<0.001

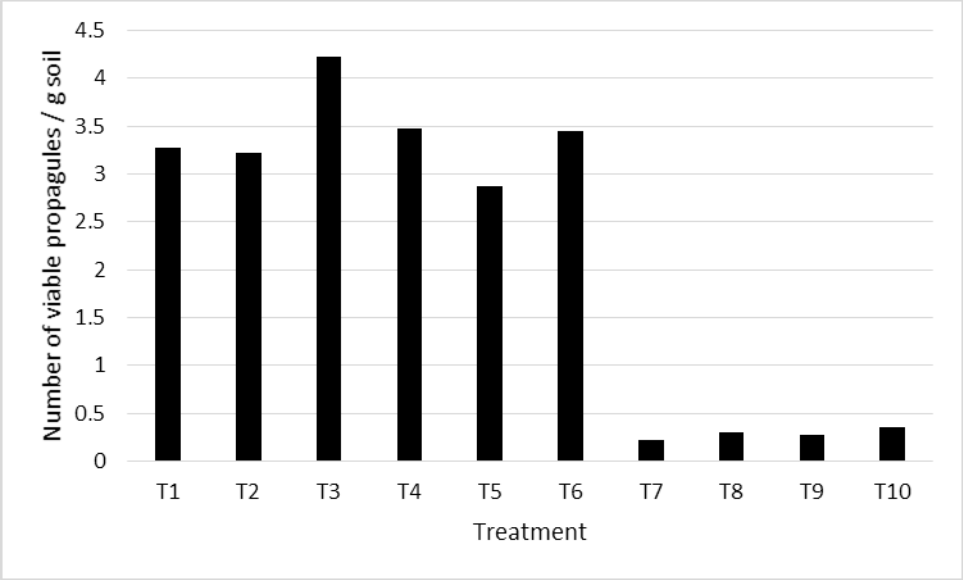

Figure 4.7 Number of viable Verticillium propagules in soil after recovery from pots of soil on 30 October 2015 following eight weeks of treatment, showing reduction only following sealing after incorporation of Herbie 82 in treatments T7 to T10 compared with Untreated T3 and T4.

4.2.3 Discussion

As there was no analysis of the Herbie products, it remains uncertain if their incorporation into the soil contributed to the rise in potassium levels.

The different water amounts were suggested by the supplier of the product, and the highest was not intended to saturate the soil. Increasing the water further might assist the bacteria, but this might however in a commercial soil-grown crop then make the soil less suitable for the planting of strawberries through the membrane after treatment.

In summary, Incorporation of Herbie 82 into sandy silt soil at around 14% moisture naturally infested with Verticillium sclerotia resulted in the reduction in viability of a proportion of the microsclerotia after sealing it in pots for eight weeks between September and October when temperatures were on average 16°C.

Knowledge and Technology Transfer

1. Feb 2016: Presenting the overview of the project at the AHDB agronomist day at East Malling.
2. Feb 2016: Verticillium work was briefly introduced by James Woodhall at the Soft Fruit Agronomists' Day on 11 February 2016 "emerging soil-borne plant pathogens"

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