Project title:	**Improving integrated disease management in strawberry**
Project number:	SF 157
Project leader:	Prof Xiangming Xu NIAB EMR
Report:	Annual report, March 2018
Previous report:	Annual report, March 2017
Key staff:	Erika Wedgwood (ADAS) Angela Berrie (NIAB EMR) Rob Saville (NIAB EMR) Avice Hall (UoH) Tom Passey (NIAB EMR)
Location of project:	East Malling; Cambridge; Wisbech
Industry Representative:	Louise Sutherland
Date project commenced:	1 March 2015
Date project completed (or expected completion date):	31 March 2020

DISCLAIMER

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

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.

Xiangming Xu
Head of Pest & Pathogen Ecology Department
NIAB EMR

Signature .....  ..... Date ...28 March 2018.....

Erika Wedgwood
Senior Plant Pathologist
ADAS

Signature ...  Date ...28 March 2018...

Report authorised by:
Xiangming Xu
Head of Pest & Pathogen Ecology Department
NIAB EMR

Signature .....  Date .....29 March 2018.....

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

- Research is ongoing to assess if plants treated with fungicides and bio-fungicides before planting have increased tolerance to latent infection by *Phytophthora cactorum*.

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 material. 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. Alternative products for control of crown rot (both fungicides and biocontrol products) were identified in trials conducted by NIAB EMR as part of the SCEPTRE project. 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 investigated 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 material and identify treatments to reduce plant losses due to these hidden infections.

Summary of the project and main conclusions in Year 3

Pre-inoculation of plants with arbuscular mycorrhizal fungi (AMF) and/or plant growth promoting rhizobacteria (PGPR) did not reduce infection of strawberry crowns by *P. cactorum*.

However, it is not clear whether such treatment would improve plant tolerance to latent pathogen infection, which may enhance fruit production compared to untreated plants. This is to be investigated in a large experiment initiated in year 3 and completed in Year 4. The pathology team at NIAB EMR developed protocols for this large experiment to mimic commercial practice in which infected plants are held in cold store before planting. Plants are inoculated with *P. cactorum* prior to cold storage and those plants without visual symptoms at planting time will be transplanted and treated with a number of products. In addition to plant growth, fruit production will be assessed.

Financial benefits

Potential loss of plants due to *P. cactorum* could reach 20-30%. In 2016, 90,000 tonnes of strawberries were sold in the UK season with the market valued at £386 million (Data from Kantar). Should 25% of plant losses occur in the UK as a result of crown rot, the volume of fruit sold could be reduced by up to 22,500 tonnes, representing a value of £96 million. Techniques and measures to control *P. cactorum* could therefore save such potential losses.

Action points for growers

- As this project is still in its infancy, growers should continue their current commercial practice of treating runners with an approved fungicide soon after planting to suppress and control *P. cactorum* and *P. fragariae*.

Strawberry powdery mildew (SPM)

Headlines

- Managed mildew programmes employing predominantly biological control agents provided equal control of powdery mildew to routine fungicide programmes.
- Three products with relatively new approval on protected strawberry provided useful curative and protective action against strawberry powdery mildew.

Background and expected deliverables

Trials in 2015 demonstrated how supplementing a reduced fungicide spray programme with alternative products could effectively control powdery mildew in strawberry, particularly when the level of inoculum is relatively low. Further trials were conducted in 2016 where two biocontrol products, one coded HDC F208 and *Ampelomyces quisqualis* (AQ10) were combined in control programmes with a plant strengthener (Cultigrow), both with or without a reduced fungicide programme. The mildew risk was much greater in 2016 but the results

showed that the biocontrol products were as effective in controlling powdery mildew as the standard fungicide programme, particularly when applied alone in a programme. Having identified effective alternative products, the next step is to combine their use in programmes, incorporating other factors such as disease risk, growth stage and type of fungicide (curative, protectant, antisporulant) in order to develop a decision-based management programme for growers.

Work by the University of Hertfordshire has shown that the use of weekly delivery of silicon through fertigation in strawberries from early in the season can delay the development of powdery mildew. Silicon is known to strengthen plants against abiotic and biotic stress and effects against both pests and diseases are reported in the literature. Work at the University has shown differences in the structure of leaf surface wax after silicon treatment.

In 2017, a trial was set up at NIAB EMR to compare the mildew control achieved in three managed programmes based on biological control agents (BCAs) and alternative chemicals compared to that achieved by a routine fungicide-only programme.

In addition, further work was undertaken in 2017 to assess the efficacy and mode of action of several mildew fungicide products, which are relatively recent approvals on strawberry. The trial included the fungicide products Takumi (cyflufenamid), Luna Sensation (fluopyram + trifloxystrobin) and Talius (proquinazid). These were assessed for both their curative and protectant properties. Two biological control agents (BCAs) including the coded product HDC F208 and AQ10 (*Ampelomyces quisqualis*) were also assessed for their protectant properties.

Summary of the project and main conclusions in Year 3

In the work to compare routine fungicide programmes with managed programmes, a fully replicated trial took place in a Spanish tunnel at NIAB EMR using an everbearer variety kindly supplied by Berry Gardens Growers. The plants grew in coir bags with a drip irrigation and fertigation system supplied. Treatments for Botrytis were the same for all plots. Similarly, control of aphids and capsids was the same across all plots. *Phytoseiulus persimilis* (for two-spotted spider mite control) and *Neoseiulus cucumeris* (for western flower thrips and tarsonemid mite control) were introduced to all plots as necessary throughout the season.

Five treatments were set up to compare powdery mildew control. These included an untreated control, a routine fungicide programme and three managed programmes which employed BCAs as the initial choice of product, but if risk of infection increased, then the programme switched to a fungicide or alternative BCA (AQ10 – which was shown to offer similar control to standard fungicides in the previous year's work).

The routine fungicide programme and fungicides employed in the managed programmes were

drawn from a list of 12 products including Systhane (myclobutanil), Fortress (quinoxyfen), Nimrod (bupirimate), Amistar (azoxystrobin), Karma (potassium bicarbonate), Potassium bicarbonate (commodity product), Luna Sensation (fluopyram/trifloxystrobin), Stroby (kresoxim-methyl), Takumi (cyflufenamid), Kumulus (sulphur), Topas (penconazole) and Talius (proquinazid). Full details are included in the Science Section of the report. The biological control agents included were a coded product HDC F208 + Silwet (*Bacillus pumilis*) and AQ10 + Silwet (*Ampelomyces quisqualis*). Other products applied were Cultigrow B204 (flavonoids) and Sirius (silicon).

The managed programmes employed BCAs and fungicides (where risk of infection was high). In one managed programme treatment, Cultigrow was applied monthly from start of growth and in another treatment Sirius (silicon) was applied weekly from the start of growth.

The five treatments are summarised in the table below:

Treatment programmes evaluated at NIAB EMR in 2017

Treatment	Type	Plant protection products	Other
T1	Untreated	-	-
T2	Routine	Fungicides	None
T3	Managed	Fungicides, BCAs,	Cultigrow B204 applied monthly from start of growth
T4	Managed	Fungicides, BCAs,	Sirius applied weekly from start of growth
T5	Managed	Fungicides, BCAs	None

Management decisions on product choice in the managed treatment programmes were based on mildew incidence (monitored weekly), the growth stage of the strawberries and the environmental risk produced by the powdery mildew prediction model which was run using humidity and temperature data collected from data loggers in the tunnels (see table below).

Conditions were very favourable for powdery mildew development throughout the trial, but despite this, the level of mildew on the leaves was very low throughout, even on untreated plots. However, in contrast, levels of mildew on the fruit in untreated plots rose rapidly from 2% on the first pick (28th July) to more than 90% at the sixth pick (21st August). The level of mildew on the routine fungicide plot and all three managed plots remained low throughout the trial on leaves, flowers and fruits. In the managed plots which relied primarily on BCAs, fungicide intervention for mildew was needed only once in early July. The BCA used throughout was the coded product HDC F208, with no obvious reason to switch to AQ10. There was a suggestion that the programmes that included Cultigrow or Sirius had less mildew than the HDC F208 only programme, but this difference was not statistically significant. There

were no significant differences in marketable yield between the managed programmes and the routine fungicide programme, but all treated plots had a significantly higher total and marketable yield than the untreated control.

Criteria for powdery mildew management decisions

Item	How determined	Risk	Management options
Disease risk	Determined from input of humidity and temperature from logger in tunnel to disease risk model (see below) and forward weather forecast from internet	More than 4 days with risk above 10% requires action	**Product choice** – Fungicide (antisporulant or protectant), BCA
Growth stage and rate of growth	Inspections 1-2 times per week	Rapid leaf production, start of flowering/ fruiting indicates increased risk and possible change of product	**Spray interval** – 7 or 14 days **Tunnel ventilation**
Mildew monitoring	Inspections 1-2 times per week on youngest leaves on 5 plants per plot. Plants will be selected at random for each inspection	Scored 0-5, where 0 = no mildew	

The trial therefore demonstrated that the use of biological control agents, with or without alternative chemicals, offered good control of powdery mildew in strawberry compared to a routine fungicide only programme. In future, it will be important to explore how this approach for managing powdery mildew can be integrated with control of Botrytis and other fruit rots.

In the trial to assess the efficacy and mode of action of new fungicide products and biological control agents, the three new fungicides (Luna Sensation, Takumi and Talius) displayed useful curative properties. They could prevent young incubating colonies from becoming visible lesions, even up to three days after the strawberry leaves were infected.

All three products also offered protectant activity. Talius reduced the incidence of powdery mildew lesions developing up to 7-10 days after treatment, while Luna Sensation and Takumi could reduce incidence up to 4-7 days after treatment. Of the two biocontrol agents tested for protectant activity, only the coded product HDC F208 reduced mildew development, within 4 days of application.

Financial benefits

Powdery mildew can result in yield losses of between 20-70% of crop potential. In 2016, 90,000 tonnes of strawberries were sold in the UK season with the market valued at £386 million (Data from Kantar). At 20% losses, using these figures, this could contribute to an

industry volume of 18,000 tonnes at a value of £77.2 million. Providing effective control can therefore offer enormous financial benefits.

The results of the powdery mildew research in 2017 suggest that managed programmes could result in the reduction in use of traditional fungicide products, which may help to reduce the number of spray applications made whilst also reducing the risk of incurring residues in fruit. The availability of new and improved fungicide products with longer lasting action would have a similar effect.

Action points for growers

- Luna Sensation, Takumi and Talius offer useful new products to improve the control of strawberry powdery mildew when integrated within routine fungicide programmes.
- Managed programmes based on biofungicides with or without Cultigrow or silicon alone are as effective as weekly standard fungicide applications and offer an alternative for strawberry powdery mildew control to growers. However, it is important to ensure early control using this technique.

Fruit rot complex

Headline

- The species of *Pestalotiopsis* present in the UK and found on strawberry is *Pestalotiopsis clavispora*.

Background and expected deliverables

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.

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 offer partial control of *Mucor*. Berry Gardens Growers (BGG) recently funded a PhD project at NIAB EMR on the epidemiology and management of *Mucor* and *Rhizopus* rot in strawberry; significant progress has been made in this project but due to

commercial confidentiality the findings cannot be disclosed in this report. BGG continues to fund work on the control of fruit rotting at NIAB EMR.

Towards the end of Year 2 of this project, there were increasing reports on the occurrence of a new pathogen (*Pestalotiopsis* spp.) isolated from the crowns of wilting plants. In addition, this pathogen was shown to cause fruit rot on strawberry in Egypt. In Year 3, the pathology team carried out preliminary work on this new pathogen of strawberry to determine the importance of this disease to the UK industry.

Summary of the project and main conclusions in Year 3

Using a collection of *Pestalotiopsis* isolates collected from strawberry plants that were not of high health status the pathology team at NIAB EMR molecularly characterised representative isolates to species level which identified *Pestalotiopsis clavispora* as the species that is present in the UK. A series of pathogenicity tests were developed to: 1) prove if *Pestalotiopsis* is pathogenic against popular commercial strawberry cultivars and hence can be a primary pathogen and 2) determine how widespread the pathogen is in the UK industry. Using a detached fruit and leaf pathogenicity test, the team demonstrated that all the *Pestalotiopsis* isolates tested can establish infection and colonise the host tissue. The pathogen was also able to cause a post-harvest rot following inoculation during fruit development. However, it could not be proved that the isolates tested were able to cause a disease in the crown. Plant leaves and crown were inoculated with the *Pestalotiopsis* spore and mycelium inoculum and despite providing highly favourable conditions, only a background level of disease was recorded. Based on the findings and the literature it can be concluded that *Pestalotiopsis* is a weak pathogen which is able to infect the plant when it is under other stresses. To determine the presence of this disease in the UK industry, molecular primers are currently being validated for detecting this new pathogen and will be used to determine the incidence of *Pestalotiopsis* in the DNA samples collected from crown tissue of >2000 nursery strawberry plants in years 1 and 2 of this project.

Financial benefits

- It is too soon to speculate on the financial benefits of this specific work. This will become clearer once it has been demonstrated how widespread the fungus is following the molecular based survey.

Action points for growers

- Current results are insufficient for making any recommendations.

Verticillium wilt

Headline

- Some early trends are developing in a trial to compare three biocontrol methods for Verticillium wilt in soil-grown strawberry.

Background and expected deliverables

Verticillium wilt of strawberry, caused by *Verticillium dahliae* is a soil borne disease which causes plant wilting and death. Depending upon the population levels of the pathogen in a field soil and the susceptibility of the strawberry variety being grown, plant losses can vary between 5-90%, so very significant yield loss occurs.

In the past, strawberry growers relied on the use of a range of chemical biocides to fumigate soils before planting strawberry crops, to reduce the soil inhabiting *V. dahliae* populations to a level which would not adversely affect the crop. The availability and approvals for such fumigants have declined over the past 20 years, so growers wishing to grow crops in soil need alternative methods of treating the pathogen.

Previous research has identified and tested a number of alternatives to chemical fumigants including plant derived materials which, when incorporated into soils, create biofumigation. Biofumigation is the suppression of soil-borne pathogens and pests by naturally occurring compounds. Not all materials tested have been sufficiently effective to require development. However, a number have been worthy of further investigation.

Bio-Fence is one such material which is a granular product incorporated into field soils and releases chemicals called isothiocyanates, which are known to reduce *V. dahliae* inoculum and the viability of its spores. Anaerobic digestate is another material which is organic in nature and may be able to suppress plant pathogens by encouraging the build-up of beneficial microbial populations. The fungicide Serenade ASO which is composed of a strain of the bacterium *Bacillus subtilis* is another material which has successfully controlled plant pathogens such as white rot sclerotia and is thought to have potential activity against *V. dahliae*.

These three materials will be assessed in the project in a field trial on a farm in Oxfordshire with an existing population of V. dahliae spores. Their efficacy at controlling the pathogen in the soil will be compared using the moderately susceptible strawberry variety Symphony. This

variety was chosen because it is known to become infected and display wilt symptoms whilst still producing fruit, whereas a more susceptible variety would die completely.

Summary of the project and main conclusions in Year 3

The trial was set up in a field soil at Rectory Farm, Stanton St. John, Oxfordshire, by kind permission of Richard Stanley. The soil had previously grown barley and when tested for existing levels of *V. dahliae*, was shown to have between 2.6 and 5.6 propagules per gram of soil (depending on the area of the trial sampled). The soil was also sampled for nutrition, a base dressing of fertiliser applied, then re-tested. The trial was established within a commercial field-soil strawberry crop, which was grown on raised beds covered with blue polythene mulch. All beds except the trial area were fumigated with chloropicrin before planting. The trial plots received one or two of three alternative soil treatments, with the five treatments in five replicated plots in a Latin Square design. The treatments are summarised in the table below:

Materials applied to plots before and after planting cv. Symphony cold-stored strawberry runners on 6 June 2017 in a Verticillium infested field in Oxfordshire

Code	Product	Ingredients	Rate per ha	Application method
T1	None	N/a		
T2	Anaerobic digestate solids (pasteurised PAS 110)	Chopped maize and vegetable crop waste	50 tonnes	Spread then incorporated up to 150 mm depth then covered
T3	Bio-Fence pellets	*Brassica carinata* meal	2,000 kg	Spread then incorporated up to 150 mm depth, irrigated then covered directly with polythene
T4	Serenade ASO*	*Bacillus subtilis* strain QST 713	10 L in 1,000 L water	Single nozzle directed 40 ml over each plant (0.4 ml concentrate)
T5	Bio-Fence pellets	*Brassica carinata*	2,000 kg	As for T3 and T4 combined; pre-planting incorporation of Bio-Fence then plant drench with Serenade ASO
	Serenade ASO	*Bacillus subtilis*	10 L in 1000 L water	

* Applied as an over-plant drench under experimental permit COP 2016/00922. EAMU 0706 of 2013 permits the same 10 L /ha in 1,000 L/ha water as a spray to outdoor strawberries

The soil was formed into raised beds and marked into 7m long plots. Treatments were applied to the central 6m. On 24 May 2017, anaerobic digestate solids were applied to Treatment 2 and incorporated to 150mm by rotavation into the soil. Nutritional analysis of the anaerobic digestate was also carried out. Bio-Fence granules were applied to Treatments 3 and 5 on the same day and also incorporated by rotavation into the soil. Two lines of trickle irrigation were laid on each bed. The Bio-Fence treatments were irrigated on 26 May and all 25 plots were .

then covered straight away with blue polythene mulch over the raised beds. Seven days later, all plots were ventilated by making planting holes in the polythene. Five days later, on 6 June 2017, cold stored bare-root Symphony plants were planted. Six days after planting, on 12 June 2017, the 27 plants in Treatments 4 and 5 in the central 6m of each plot were drenched with Serenade ASO through the planting holes in the polythene mulch.

Both plant phytotoxicity and plant establishment were assessed and recorded three times after planting, in June 2017. Numbers of fruits were recorded on each plant prior to the first two picks. In September 2017, the percentage of plants wilted or totally collapsed were recorded. In October, the percentage of plants wilting and total percentage of plants still alive were recorded. Observations on foliage growth were made in January 2018. Fruit yield and berry size are to be recorded in June 2018 both within the 25 trial plots and five plots marked out in an adjacent chloropicrin treated bed.

Results

In one area of the trial, plant establishment was poor, particularly in two adjacent plots. This may have been a result of very hot, dry weather conditions in June 2017 following planting, coupled with stony areas of the field where root contact with the field soil was reduced.

Plants throughout the trial displayed symptoms of leaf scorch, but the incidence of this was greater in those plots treated with Bio-Fence or anaerobic digestate solids. It is possible that the incorporation of these materials into the soil beds, created more of an open structure to the soil, which resulted in more rapid soil drying. It is also possible that during the hot soil conditions which followed planting, more rapid chemical release from these treatments may have resulted in plant scorch, particularly as the chemicals tend to escape through the polythene mulch via the planting holes. It is also possible that the interval of 10 full days between treatment and planting may have been insufficient to allow such chemicals to dissipate, but this timing was chosen to reflect the practice employed in the commercial crop surrounded by the trial which were treated with chloropricrin.

Verticillium wilt often manifests itself following the stress of fruiting, and symptoms of wilting were recorded during plant assessments made during September. The typical symptoms of wilt on one side of the plant coupled with leaf collapse were more apparent by the October assessment, but there were no significant differences between treatments. However, a trend did appear to show that Treatments 3 and 5 which had been treated with Bio-Fence, had a

lower proportion of wilting plants (5%) compared with the other treatments (mean 10%). Plants in the anerobic digestate treated plots had fewer fruits at the first pick, but it is possible that fruit formation was delayed until later. Further plant assessments will be made in Spring 2018 by which time winter stress may have increased the incidence of wilting.

These treatments are not expected to eliminate Verticillium in the soil like chloropicrin, but either reduce the level and so reduce infestation severity (in the case of Bio-Fence) or increase the resilience of the plants (in the case of anaerobic digestates or Serenade ASO).

Further assessments in 2018 will determine if the higher ranking incidence of wilting in the untreated and Serenade ASO only treated plots, is a trend that continues.

Financial benefits

Potential loss of plants due to *V. dahliae* in soil grown crops can vary between 5-90%. In 2016, 90,000 tonnes of strawberries were sold in the UK season with the market valued at £386 million (Data from Kantar). At present, it is estimated that 30% of the UK strawberry crop is grown in field soils, equating to £115 million. Should 25% of plant losses occur in the UK as a result of Verticillium wilt, this would represent lost revenue of £29 million. Techniques and measures to control Verticillium wilt could therefore save such potential losses.

Action points for growers

- Sample soil for Verticillium, allowing at least six weeks for Harris testing results.
- Incorporation of materials that increase soil organic matter should improve soil health.
- Consider the use of bio-fumigants before polythene covering beds.
- After adding organic materials, check if watering should be adjusted.
- When possible, use strawberry varieties with some resistance to Verticillium.

SCIENCE SECTION

Introduction

Strawberry is attacked by several pathogens, including *Botrytis cinerea,* strawberry powdery mildew (SPM) and *Phytophthora* spp. A recently completed Hort-LINK project focussed on *Botrytis* and SPM. 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, semio-chemicals, 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 *Phytophthor*a 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. Alternative products for control of crown rot (both fungicides and biocontrols) were identified in trials conducted by NIAB EMR as part of the SCEPTRE project. Recent research on *Phytophthora* spp. has concentrated on detecting the pathogens and seeking products to reduce root rotting. Recent HDC projects are relevant; SF 130 focussed on fungal molecular quantification and an assay was developed that detected *P. rubi*, although it was not as sensitive as the *P. fragariae* assay (which however detects both pathogens) and SF 123 on alternative products against *P. rubi* on raspberry where one novel chemical product gave disease 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 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.

Strawberry powdery mildew (SPM)

The Hort-LINK project focussed on development, implementation and use of a SPM 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 SPM 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 leaves and fruit are most susceptible to SPM infection. An EU-interreg funded project at NIAB EMR suggested a small reduction of SPM under a deficit irrigation regime. A pilot study at the University of Hertfordshire showed that application of silicon nutrients changed plant morphology and delayed SPM development by 8-10 days on several cultivars. A TSB-funded project at NIAB EMR identified several QTL for resistance to SPM. Another TSB project at NIAB EMR is investigating whether we could develop imaging tools to detect SPM infection before visual symptoms.

Work in a recent AHDB project on edible crops highlighted the efficacy of at least three biological plant protection products against powdery mildews on crops other than strawberries. These biofungicides could gain approval for use on strawberry; however work was required to determine how these might be integrated into crop protection programmes used against SPM.

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 rot causing 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. However, controlling *Botrytis* in late season strawberry, particularly ever-bearers, is problematic. The 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. Due to the risk of spotted wing drosophila (SWD), 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 give partial control of *Mucor*. Recently Berry Gardens Growers (BGG) funded a PhD project at NIAB EMR on the epidemiology and management of *Mucor* and *Rhizopus* rot in strawberry; significant progress has been made in this project but due to commercial confidentiality the findings cannot be disclosed in this report. BGG continues to fund work on the control of fruit rotting at NIAB EMR.

Verticillium wilt

Recent withdrawal of methyl bromide and recent withdrawal of chloropicrin as soil fumigants have focussed the industry on searching for alternative soil treatments against this pathogen. Disappointingly, a new microencapsulated product did not have sufficient efficacy to have any commercial future (TSB project ended December 2014). AHDB Horticulture is funding a project at NIAB EMR on pre-colonising strawberry runners or tipping plants to manage wilt and results showed that pre-colonising strawberry plants did not help plants to reduce wilt development. 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, NIAB EMR (in collaboration with Chinese researchers) has developed another qPCR tool for quantifying *Verticillium* inoculum in soils. However, neither of these two methods is sensitive enough to quantify inoculum below 0.5 CFU per gram of soils, at which level wilt can still be caused on susceptible strawberry cultivars.

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.

Objective 1: *Phytophthora*

In year 1, we demonstrated that joint use of Arbuscular mycorrhizal fungi (AMF) and plant growth promoting bacteria (PGPR) can reduce the development of red core (*P. fragariae*) on roots that were dipped into spore suspensions; however, field survey work in year 1 suggested that *P. cactorum* is more important than *P. fragariae*. Most *P. cactorum* detected in plant materials in years 1 and 2 was latent. Indeed, most of these latent infections failed to develop into visual symptoms after planting in the field. Thus, plants may grow out of the latent infection and/or some of these positive detections based on the nested PCR technique could be due to non-viable microbial DNA present in the crown material.

Thus, in year 2, we conducted experiments to study the effects of AMF and PGPR on *P. cactorum* when plants were inoculated at the time of planting (post cold storage) using a standard inoculation method. This inoculation method was originally developed primarily for the purpose of evaluating host resistance and pesticides; hence the disease pressure is very high. In these experiments, we demonstrated that neither individual nor joint use of AMF and PGPR significantly reduced *P. cactorum* development on inoculated plants. This may not be surprising because under high disease pressures the curative effect [killing young developing infection] of AMF and PGPR is unlikely to be observed. Nevertheless, a recent Finnish study suggested that combined use of AMF and PGPR may lead to some reduction in crown rot development.

In Year 3, our efforts centred on two aspects. First, we completed the assessment of a previous experiment, initiated in Year 2. The objective of this experiment was to study the effect of individual and combined use of AMF and PGPR on *P. cactorum* development. Second, we initiated a new experiment to evaluate the effects of post-cold-storage (prior to planting) handling and selected products on the development of latent infection of *P. cactorum*. In this new experiment, we aim to assess the effect of treatments at and post the planting time on symptom development, plant vigour and fruit production. These treatments will be applied in the spring 2018.

1.1 Controlling *Phytophthora* spp.

1.1.1 Materials and methods for an experiment initiated in year 2

This experiment was to study the effect of AMF and PGPR against *P. cactorum* development (not fruit production as the experiment was initiated in winter) when inoculated onto wounded or non-wounded plants. Study details were given in the Year 2 annual report and Table 1.1 gives the dates of key experimental tasks.

Table 1.1 Dates of key tasks in an experiment to assess whether pre-inoculation with AMF and PGPR could reduce the effects of *P. cactorum* on development of potted strawberry plants (cv. "Malling Centenary" in an Irish dark peat and 2-5 mm perlite mix (7:3))

Date	Tasks
14/11/2016	Inoculating plants with AMF and/or PGPR (i.e. potting up plug plants)
01/12/2016	Inoculating healthy or wounded crown tissues with *P. cactorum* spore suspensions
02/12/2016	Inoculating plants with *P. cactorum* spore suspensions again
03/02/2017	First assessment of plant development
04/02/2017	Stopped watering plants
08/02/2017	Second assessment of plant development
14/02/2017	Final assessment of plant development and crown tissues; samples taken for molecular detection of *P. cactorum* DNA in crown tissues

There were eight treatments: (wound, healthy) x (AMF, control) x (PGPR, control) (Table 1.2.1), each with 10 replicate plants. Cold stored (-2°C) cv. "Malling Centenary" plugs were used. The experiment was repeated once, giving a total of 160 plants: 80 plants were inoculated with *P. cactorum* in the morning, and the other 80 in the afternoon.

Table 1.2.1 Summary for AMF & PGPR treatments used in Year 2 to manage *Phytophthora cactorum,* which was inoculated onto wounded or non-wounded plants

Treatment	Wounded?	AMF	PGPR
1	Y	Y	Y
2	Y	Y	N
3	Y	N	Y
4	Y	N	N
5	N	Y	Y
6	N	Y	N
7	N	N	Y
8	N	N	N

Inoculum of AMF was provided by PlantWorks Ltd, Kent. At the time of transplanting, each pot received 25 ml of granular AMF inoculum (recommended by the manufacturer, mixture of *Rhizophagus irregularis*, *Glomus microagregatum*, *Funneliformis mosseae*, *Funneliformis geosporus* and *Claroideoglomus claroideum*) before planting plugs. A formulated PGPR experimental product contained 108 CFU ml-1 (PlantWorks Ltd) of four rhizobacterial species (*Bacillus amyloliquefaciens*, *Bacillus megaterium*, *Azospirillum brassilense*, Rhizobacterium strain IRBG74); each plant received 7.6 ml of PGPR.

"Malling Centenary" plugs were transplanted into 1 L plastic pots filled up with ca. 450 ml of Irish dark peat and 2-5 mm perlite mix (7:3) inoculated with AMF and/or PGPR as appropriate in November 2016. Potted plants were maintained in a polytunnel under natural temperature

and light conditions for three weeks to ensure AMF colonisation. All plants were then inoculated with one *P. cactorum* isolate (P414; known to be pathogenic against "Malling Centenary") on December 1st 2016. A suspension of 10^2 zoospores ml^{-1} was produced following a previously published method [this low inoculum dose was used as we did not want to kill those wounded plants too quickly]. A vertical slit (ca. 10 mm long) was made using a scalpel blade at the base of an internal leaf (close to the crown); 5 ml *P. cactorum* inoculum was then immediately directly pipetted onto the wounded or corresponding healthy area. Inoculated plants were placed into a glasshouse compartment with heating to maintain > 10°C until the experiment was terminated in February 2017. Each plant was re-inoculated again the next day to increase disease pressure.

Plants were individually watered manually once a day with tap water; cross-contamination was avoided since plants were on a metal wire bench and any overflow (though unlikely) would drip to the concrete floor. To speed up disease development, plants were not watered from early February 2017 for 10 days before final assessment and sampling crown tissues.

Plants were assessed once a week for foliar symptoms for presence or absence of wilting symptoms (without recording wilting severity): healthy (no wilting) and wilting (including dead ones) (Photo 1.1). After the final foliar disease assessment (14th Feb 2017), the crowns were cut longitudinally and assessed for presence or absence of internal necrosis (browning). Crown samples were then taken from each plant for detecting *P. Cactorum* DNA by nested PCR.

Photo 1.1 Visual plant assessment keys on strawberry plants inoculated with *Phytophthora* spp. from left to right: healthy, wilting, and severe wilting (dead).

1.1.2 Controlling latent infection of *P. cactorum* for fruit production

In 2017, we initiated an experiment to evaluate the effects of post-cold-storage (prior to planting) handling and selected products on the development of latent infection by *P. cactorum,* in terms of symptom development, plant vigour and fruit production. Latent infection is when a pathogen has successfully penetrated (infected) plants but without any obvious visual symptoms for a long period of time. Plants were inoculated with *P. cactorum* prior to cold storage in order to mimic the commercial situation. Previous work suggested that infection

at this stage is most likely to lead to a large proportion of surviving plants with latent infection (Pettitt and Pegg, 1994).

Before we started this new experiment, we developed and finalised experimental protocols. First, we evaluated whether it is possible to develop a propidium monoazide (PMA™) based qPCR method to quantify viable *P. cactorum* biomass in plant tissue. After several discussions with one PhD student working on this subject at NIAB EMR, we decided not to proceed with this avenue. This is because the application of PMA within solid crown material (if at all possible) and the presence of a large amount of DNA (mostly plant DNA) requires a considerable amount of PMA to make this approach work – which is prohibitively expensive for this project [estimated to cost £75 to £375 per sample]..

We then carried out a preliminary experiment to assess whether we need to inoculate both healthy and wounded tissues with *P. cactorum* prior to cold storage. A total of 160 bare-rooted runner plants of cv. "Malling Centenary" were potted up in mid-May and allowed to grow in glasshouse conditions for 3 weeks. Of these plants, six failed to grow/died and 52 were used as un-inoculated controls (including no artificial wounding). The remaining 102 plants were wound-inoculated with *P. cactorum* as in the Year 2 experiment. A suspension of *P. cactorum* zoospores was produced at a concentration of $3.2x10^4$ spores ml^{-1} [this high dose of inoculum was used to maximise the chance of obtaining a high level of latent infection]. The inoculated plants and un-inoculated plants were left on separate benching (to avoid water splash contamination) in the glasshouse for a further week. Plants were then placed into trays lined with black plastic, 6 pots per tray; and un-inoculated controls were kept in separate trays to avoid cross-contamination. The trays were placed inside a polythene bag and moved to a 2 $^{\circ}C$ cold store for 10 weeks. On removal from the cold store the plants were moved back to the glasshouse. Of the 102 inoculated plants, 25 were still alive while 77 had died; only 7 of the 52 un-inoculated control plants had died during cold storage. Visual assessment of these plants indicated the majority of plants that had died were infested with *Botrytis cinerea*. It is possible that the higher level of botrytis in the inoculated plants was due to the artificial wounds (un-inoculated plants were not wounded), the weakened plant due to crown rot infection or a combination of the two.

Throughout the summer, we have been discussing with Tim Pettitt about the experimental protocols. Indeed he visited NIAB EMR in September 2017 to finalise the experiment protocols. So the experimental protocols were developed based on the following considerations and results:

(1) Wound-inoculation leads to much higher mortality

(2) High moisture prior to cold storage would increase the risk of *B. cinerea* (particularly on wounded plants)

(3) Inoculation of healthy crown tissues led to 50% latent infection (year 2 experiment, see results below)

(4) Pre-cold-storage inoculation is likely to lead to high mortality of plants during storage (Pettitt and Pegg, 1994)

(5) There is large variability in the proportion of latent infection resulting from inoculation and plants surviving the cold storage period (Tim Pettitt, pers.comm.)

(6) We speculated that certain treatments may not be able to cure plants [i.e. reduce the incidence of pathogen infection] but able to improve plant tolerance to the disease. Thus we need to assess fruit production throughout the trial period.

This was a very large experiment: c. 2000 Petri plates were used to produce sufficient amount of inoculum for inoculation. Table 1.2.2 gives dates for key tasks performed so far.

1.1.2.1 Plants, pathogen and inoculation

Fresh tray plants of cv. “Malling Centenary” were obtained from BerryPlants Ltd and delivered to NIAB EMR (Photo 1.2). Tray plants (instead of runners) were used as we would like to minimise the extent of natural infection from nursery (particularly soil). Because of the expected high mortality of inoculated plants (ca. 30-50%) in cold store, we ordered 3000 plants for this experiment.

Two *P. cactorum* isolates (P 404 and P414; known to be pathogenic against “Malling Centenary”) were used. A suspension of 10^5 zoospores ml^{-1} was produced following a previously published method. Each crown was inoculated without wounding by directly pipetting 3 ml inoculum onto the crown. Inoculated plants were placed into a polytunnel for 3-5 weeks to allow infection to take place and to harden before cold storage. Because of the large variability in the incidence of latent infection following inoculation, we divided the plants into three groups, each with 850 plants for inoculation; the remaining 350 plants as un-inoculated control. The first group of plants were inoculated once, the second twice, and the third three times. There was an interval of a week between consecutive inoculations. This inoculation schedule was used to increase the probability of more plants with latent infection and at the same time to ensure we have a sufficient number of inoculated plants surviving the cold storage for treatment application at planting.

Table 1.2.2 Dates of key tasks in an experiment to assess effects of treatments at planting on strawberry plants inoculated with *P. cactorum* prior to cold storage (cv. "Malling Centenary")

Date	Tasks
04/10/2017	3000 fresh tray plants delivered and maintained in trays in a polytunnel
08-09/11/2017	Inoculating healthy crown tissues of all plants (except those allocated to the control) with *P. cactorum* spore suspensions
15-16/11/2017	Inoculating plants (allocated to receive 2nd and 3rd inoculations) with *P. cactorum* spore suspensions
22-23/11/2017	Inoculating plants (allocated to receive 3rd inoculation) with *P. cactorum* spore suspensions
18/12/2017	Moving plants to -2°C cold store
Late April to mid May 2018	Starting post-storage planting treatments (exact time may depend on weather conditions – we would like to establish the trial under host/stressful conditions to induce *Phytophthora* development)

1.1.2.2 Treatments and their application

The single main experimental treatment factor is the PRODUCT as shown in Table 1.2.3. Full details of product applications, crop management, experimental design and assessments will be included together with experiment results in the next annual report.

Table 1.2.3 Products for crown rot control in strawberry

Product	Active ingredient	Rate (g/L)	Application method	Note
Fenomenal	fosetyl-Al + fenamidone	1.5	Pre-plant dip 15 mins	
		0.75	Drench 100 ml/plant	
New product	-		Application method to be confirmed	Need to apply for permit
Prestop	*Gliocladium catenlanum*	5	Pre-plant dip 15 mins	
		5	Drench 100 ml/plant	
T34	*Trichoderma asperellum*	0.1	Pre-plant dip 15 mins?	EMR has for permit for use &34 in open field until 2019
		0.25	Drench 100 ml/plant	
Amylo-X	*Bacillus amyloliquefaciens* subsp*. plantarum*	197	Dip for 10-15 seconds (may need longer since we use tray plants)	Experimental permit: COP 2017/02721; crop destruction.
		0.07-1.0	Drench 100 ml/plant	

1.1.2.3 Data analysis

All data were analysed using R (version 3.2). Only significant ($P < 0.05$) or close-to-significant ($P < 0.1$) [this is now recommended as a good practice in data presentation] differences are

reported in the text. The disease data (visual scores) were analysed using generalised linear models (GLM) with residual errors assumed to follow a binomial distribution. Because of the nature of GLM, significance of treatment differences is not directly based on the standard errors on the original measurement scale; thus we did not present error bars on the original scale in graphs. Individual experiments conducted at different times were treated as a blocking factor.

1.2 Results

1.2.1 AMF & PGPR on *P. cactorum* (Year 2 experiment)

All 160 plants were inoculated with *P. cactorum*. Visual assessment was completed on 14th February 2017. Eight plants died before the imposed drought on 4th February 2017, without any obvious relationship to treatment factors. On 14th February, only 25 had healthy crown tissues (i.e. with browning score of 0). Of the 160 plants, 32 plants and 108 plants showed wilting symptoms 4 and 10 days after watering was terminated, respectively.

We successfully extracted DNA from 153 of 160 plants (including dead ones). Of the 153 samples, 79 and 74 were from non-wounded and wound-inoculated plants, respectively. Overall, *P. cactorum* DNA was detected in 50% of samples (note: all plants were inoculated). The effect of wounding on the incidence of *P. cactorum* DNA detection was close to statistical significance ($P < 0.1$): 43% (of non-wounded plants) vs. 57% (of wounded plants) (Fig. 1.1A). Incidence of *P. cactorum* DNA detection was also greater ($P < 0.05$) on plants with necrotic crown tissues than those without (Fig. 1.1B). However, there were also many plants with without detectable *P. cactorum* DNA, suggesting that *P. cactorum* may not be the only reason for crown tissue browning.

AMF and PGPR treatments did not have significant effects on the incidence of *P. cactorum* DNA detection.

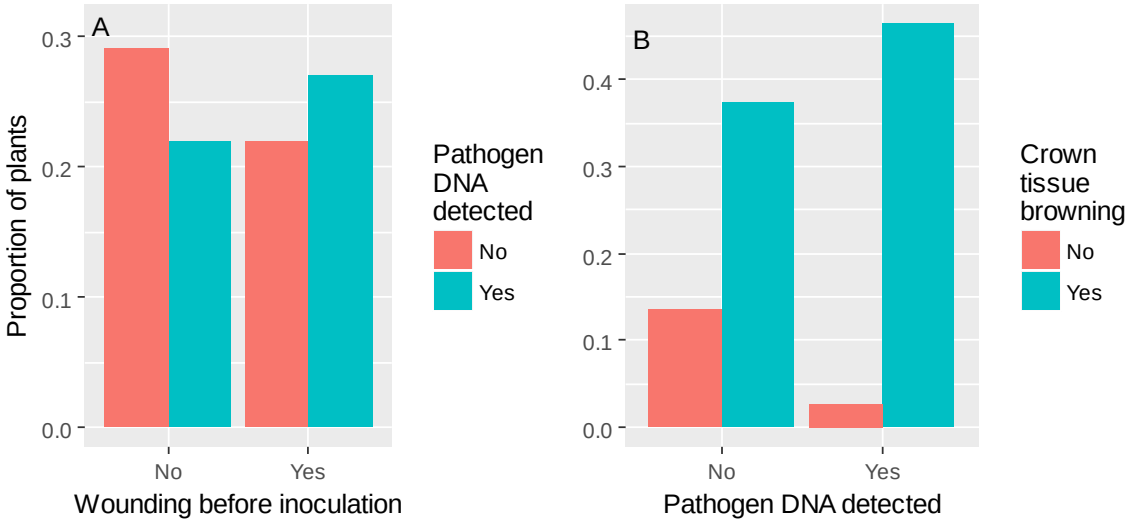


Figure 1.1 Proportion of plants with or without *P. cactorum* DNA detected in relation to wounding before inoculation (A) and crown tissue browning (B).

The incidence of plant wilting 4 days after watering was stopped was significantly ($P < 0.01$) affected by the joint effect of PGPR and AMF treatment – this interaction is due to the fact that plants treated with PGPR only, had a higher incidence of plant wilting irrespective of wounding (Fig. 1.2). It is not clear why applying PGPR led to a high incidence of plant wilting.

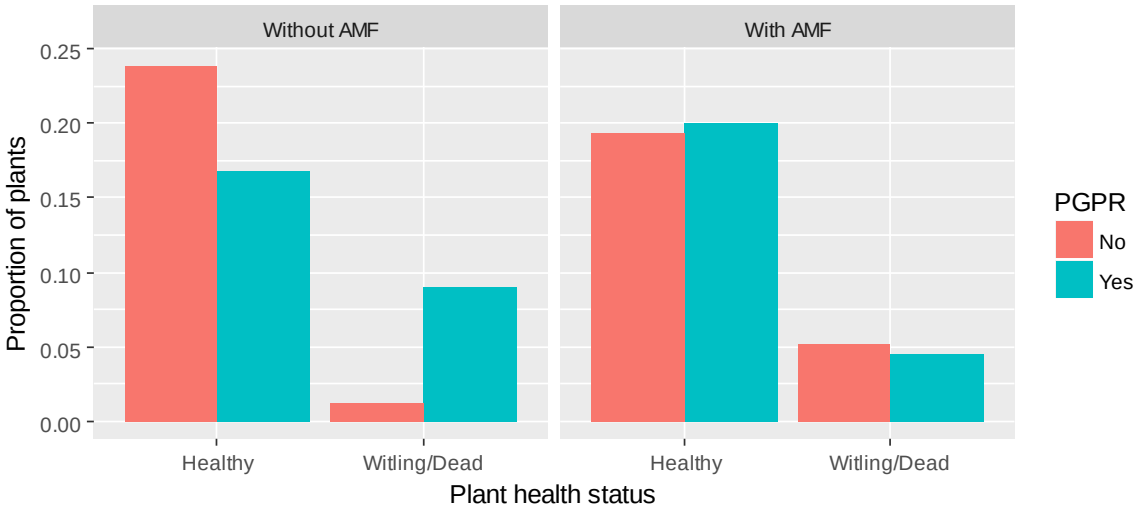


Figure 1.2 Proportion of "Malling Centenary" plants that were either healthy or wilting (including dead) 4 days after stopping watering, in relation to PGPR treatments and AMF treatments; all plants were inoculated with *P. cactorum* three weeks post-inoculation with AMF and/or PGPR.

The incidence of plants with wilting symptoms (including dead ones) 4 days after watering was stopped was higher ($P < 0.05$) for wound-inoculated plants than for non-wound-inoculated plants (Fig. 1.3A). Similarly, the incidence of wilting/dead plants were much higher ($P < 0.001$) in those plants with necrotic crown tissues than in non-necrotic tissues (Fig. 1.3B), and higher ($P < 0.001$) in those plants with *P. cactorum* DNA detected than in those plants without *P. cactorum* DNA detected (Fig. 1.3C).

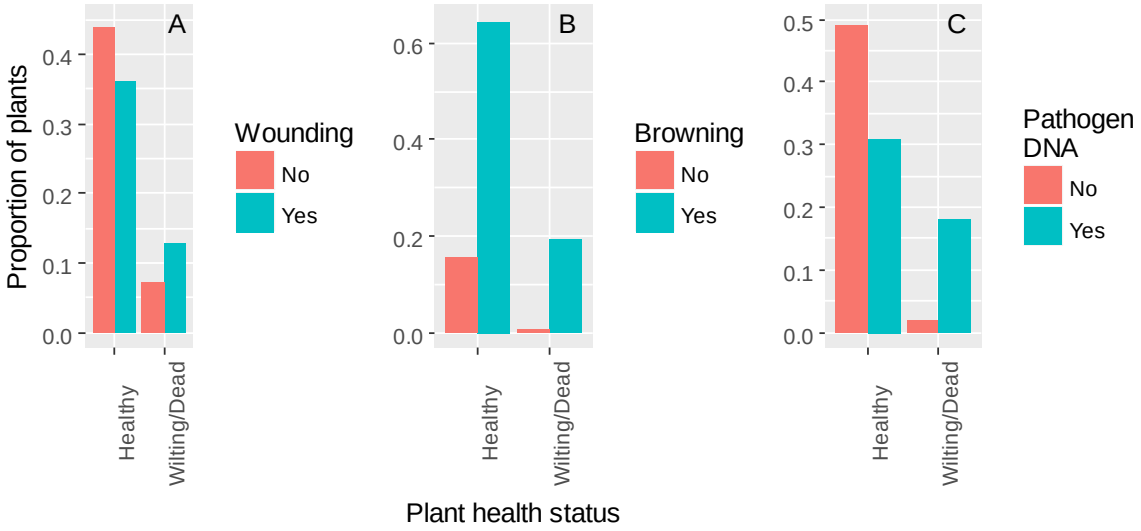


Figure 1.3 Proportion of "Malling Centenary" plants that were either healthy or wilting (including dead) 4 days after stopping watering, in relation to wounding treatment (A) before pathogen inoculation, crown tissue browning (B), and presence of pathogen DNA in crown tissues (C); all plants were inoculated with *P. cactorum* three weeks post-inoculation with AMF and/or PGPR.

Plant wilting when assessed 10 days after watering was stopped was not significantly affected by any of treatment factors (Fig. 1.4).

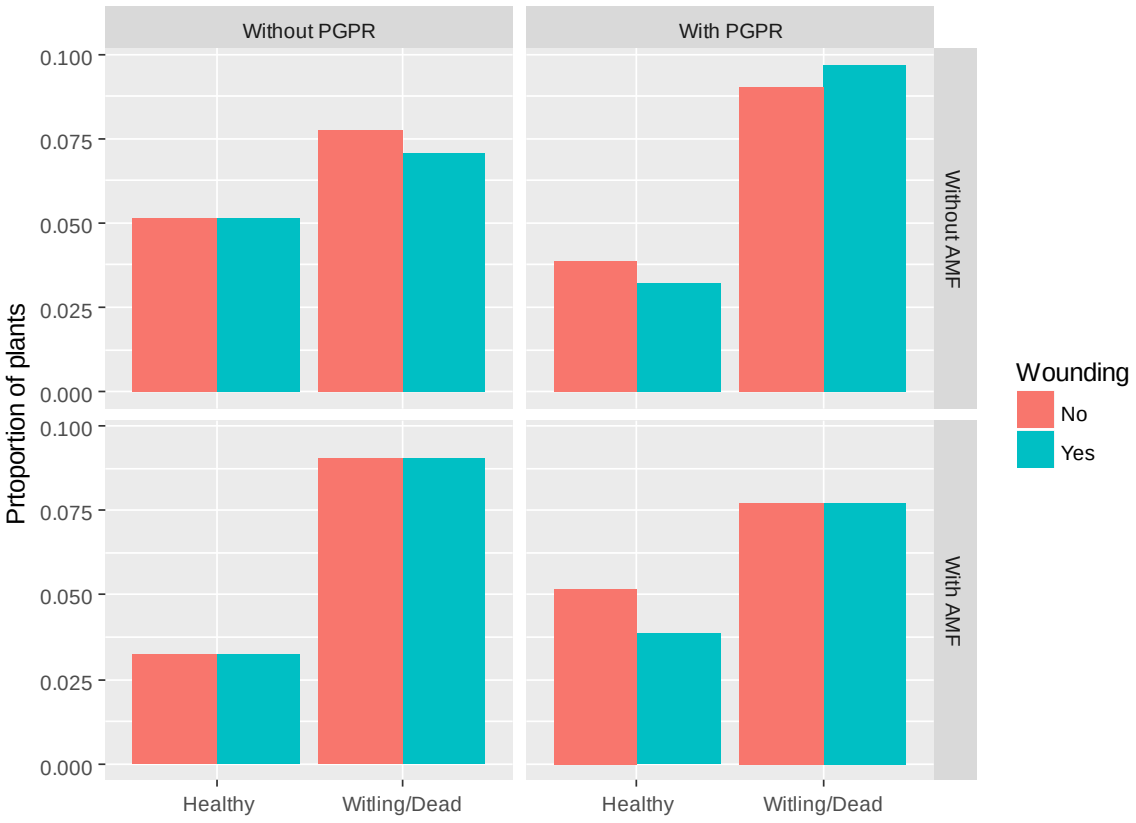


Figure 1.4 Proportion of "Malling Centenary" plants that were either healthy or wilting (including dead) 10 days after termination of watering in relation to wounding before pathogen inoculation, AMF and PGPR treatments.

1.3 Discussion

The experiment showed that pre-inoculation of plants with AMF and/or PGPR does not have the ability to reduce *P. cactorum* in strawberry crowns. However, it is not clear whether such treatment would have effects of improving plant tolerance to latent pathogen infection and hence improved fruit production over those untreated. However, fruit production in the year 2 experiment was not possible because of the seasonality.

This experiment showed that inoculation of healthy crowns with *P. cactorum* spore suspension is sufficient to result in ca. 50% of latent infections (i.e. with *P. cactorum* DNA detected in the crown tissue). This high success rate of inoculation of healthy crowns has contributed to the design of the large experiment in Year 3 as outlined in the "Materials and Methods" section.

Nearly 95% of plants with *P. cactorum* DNA detected were from those plants with necrotic crown tissue browning. Thus, in the future, it would be more efficient to use molecular detection to assess pathogen presence in those plant tissues with necrotic crown tissue. However, using the necrotic crown tissue itself would considerably overestimate the presence of *P. cactorum.* In addition, presence of *P. cactorum* DNA in crown tissue also resulted in more

rapid wilting following the drought treatment, suggesting that latent infection may predispose plants to abiotic stress events (Fig 1.3C).

Objective 2: Strawberry powdery mildew (SPM)

2.1: Epidemiological mode of action of new products against SPM

2.1.1 Background

Fungicides are often sprayed at regular intervals throughout a growing season to manage SPM. Usually, field trials are conducted to evaluate the effect of fungicide doses and application intervals on their mildew control efficacy. This approach of using fungicides based on the application dose and interval does not fully exploit the different characteristics conferred by modern fungicides, targeting different aspects of pathogen life cycles. This epidemiological mode of action against the pathogen life cycle differs from those molecular mechanisms of the fungicides in killing pathogens given by manufacturers. The epidemiological mode of action is usually defined as

- Protectant: the ability of fungicides in preventing newly arrived inoculum from germinating and infecting host tissues - fungicides applied before infection;
- Curative: the ability of fungicides in killing young developing (non-symptomatic) colonies – fungicides applied after infection;
- Anti-sporulant: the ability of fungicides in suppressing inoculum production – fungicides usually applied directly onto actively sporulating colonies.

For a given product, the key information is the length of time for which each mode of action remains effective. For several new powdery mildew fungicides, there is no information on their modes of actions, preventing their effective use in management programmes within the framework of disease predictions.

Understanding fungicide mode of action will help growers in selecting fungicides in response to disease risks. NIAB EMR has developed a forecasting model for SPM, predicting daily infection risks taking into account the effects of weather conditions and past management practice (i.e. treatment application) in the context of the pathogen life cycles (i.e. sporulation and infection). For instance

- If there are high risks of infection over the last few days, you would need to choose a fungicide with good curative efficacy to kill these young developing colonies
- If high risks of infection are anticipated based on weather forecasts (particularly over a long bank holiday weekend), you would choose a fungicide with good protectant ability to protect tissues from infection
- If the level of [fresh, i.e., sporulating] visual mildew is moderate to high [indicating failure of mildew control in the recent past], you would choose a fungicide with good anti-sporulant efficacy.

2.1.2 Materials and method

2.1.2.1 General procedure

Table 2.1 gives the products tested and their rate of use. Serenade ASO was not tested because AQ10 and HDC F208 were shown to be better in controlling SPM in recent studies conducted at NIAB EMR [SCEPTRE project]. All products were applied at the recommended dose to run-off (unless otherwise specified by the manufacturers) – spray to run-off is necessary to avoid potential differences in spray coverages between leaves and between treatments over time.

Location and plants: Tray plants of cv. “Malling Centenary” were used. This work was done in a glasshouse. A key requirement for this experiment was to keep batches of plants free from external SPM before the exposure of treated plants to SPM inoculum. A glasshouse compartment was used as a ‘clean’ area with ‘restricted’ entry and plants in this area were checked at least twice weekly for SPM. If SPM was found, the infected leaves were removed and all plants sprayed with a standard powdery mildew fungicide. Plants were only used at least 10 days after such a spray was applied. This ‘clean’ glasshouse compartment was at least 20 metres away from the polytunnel where SPM inoculum (plants with fresh SPM colonies) was kept.

Inoculation: During the exposure period, treated plants were moved to the polytunnel and the two youngest leaves on each treated plant were then inoculated via a paintbrush transferring inoculum from fresh SPM colonies to the two youngest leaves that are susceptible to SPM: one still curled, and the other one just fully/nearly unrolled. To ensure continuing dispersal of SPM conidia during the exposure period, we placed individual potted ‘SPM spreader’ plants slightly higher than the experimental plants: one spreader to every four treated plants. After the exposure period, plants were moved to another location (free from mildew) to incubate before assessment.

Environmental conditions: We did not control or record temperature/humidity as climatic conditions are in general suitable for mildew infection from spring to autumn in the UK (in addition to cost consideration). For every single study we included an appropriate untreated (but inoculated) control – treatments were only compared against the control for the same exposure (inoculation) period (hence not over time).

Experimental design and assessment: In all experiments, a completely randomised design was used; each treatment had five replicate plants. Each type of experiment was repeated once. Number of lesions on each inoculated leaflets was recorded 8-10 days after inoculation. In a few cases, where counting lesions was not possible, we estimated the % of leaf areas

with powdery mildew. Statistical comparisons are between treated and untreated controls from the same experimental run [hence subjected to the same climatic conditions].

Table 2.1. Rate of application and preparations for each product (assuming spray volume of 500 L per ha)

Product	Active ingredient	Rate (/ha)	Stock concentration	Test
Takumi	Cyflufenamid	0.15 L (300 ppm)	30000 ppm	Curative, protectant and antisporulant
Luna Sensation	Fluopyram + trifloxystrobin	0.8 L (1600 ppm)	160000 ppm	Curative, protectant and antisporulant
Talius	Proquinazid	0.25 L (500 ppm)	50000 ppm	Curative, protectant and antisporulant
AQ10	*Ampelomyces quisqualis* strain AQ10	75 g (150 ppm)	15000 ppm	Protectant and antisporulant
HDC F208	-	5 L (10000 ppm)	100000 ppm	Protectant and antisporulant

2.1.2.2 Curative test

Three products (Takumi, Talius and Luna Sensation) were included for curative tests. Usually, we do not expect that any product could kill young developing colonies that resulted from infections 96 hours ago [indeed, under optimum conditions, SPM only takes 4-5 days from infection to visual symptoms]. Thus, we tested three inoculation times: 1, 2 and 3 days before treatment – plants allocated for 3-day treatments were inoculated and exposed to SPM inoculum for three days before treatment application, etc. In total there were 12 treatment combinations [3 inoculation times x 4 products (or control)], each with five replicate plants. The two replicate experiments were conducted in June-July 2017.

2.1.2.3 Protectant test

Five products (Takumi, Talius and Luna Sensation, AQ10 and HDC F208) were included for this test. There were four inoculation (exposure) times: 1, 2, 4 and 7 days after treatment. For each inoculation, plants were inoculated and exposed to SPM inoculum for 3 days. In total there were 24 treatment combinations [4 inoculation times x 6 products (or control)], each with five replicate plants. The two replicate experiments were conducted in August-October 2017.

2.1.2.4 Data analysis

Data were analysed separately for each inoculation period to compare the treatments with the control. Generalised Linear Model (GLM) was used to assess the incidence of leaflets with visible SPM lesions, assuming a quasi-binomial distribution for residual errors. Similarly, when comparing SPM lesion densities, Generalised Linear Model (GLM) was used, assuming a quasi-Poisson distribution for residual errors. Because of the nature of GLM, significance of treatment differences is not directly based on the standard errors on the original measurement scale; thus we did not present error bars on the original scale in graphs. Individual experiments conducted at different times were treated as a blocking factor.

2.1.3. Results

2.1.3.1 Curative tests

The incidence of leaflets with SPM was 9.8% (50 out of 509 leaflets) and 9.1% (49 out of 537 leaflets) for the two repeat curative experiments; 80 out of the 99 infected leaflets were from the control treatment. None of the inoculated leaflets treated with Luna Sensation had visible SM lesions. The average number of SPM lesions was 0.7 and 1.1 per inoculated leaflet for the two repeat tests; the corresponding values on the infected leaflets (i.e. conditional lesion density – number of lesions on the infected leaves only) were 7.5 and 11.9.

Fig. 2.1 shows the incidence of mildewed leaflets and number of lesions as % of the corresponding values for control treatment; the actual incidence and lesion values are given in Table A1 in the appendix. The overall incidence of leaflets was significantly ($P < 0.001$) higher for the control than for the three fungicide treatments for all three exposure time periods. The three fungicides did not differ significantly in the incidence of leaflets with SPM for all three exposure periods: their incidences were all less than 10% of the SPM incidence on the control plants (Fig. 2.1A).

Number of lesions per plant was much greater ($P < 0.001$) on the control than the fungicide-treated plant. Although there was large variability in the number of lesions on those infected leaflets, most of the infected leaflets had fewer than 18 lesions (Fig. 2.2). The conditional lesion density was 9.9, 2.5 and 10.8 for the control, Takumi and Talius, respectively [note: none of the leaflets in the Luna Sensation treatment had developed visible SPM lesions]. As for the incidence data, no significant differences were observed between the three fungicides but there were significantly ($P < 0.01$) fewer lesions on fungicide-treated plants than for the control (Fig. 2.1B). Summarised over all three exposure periods, the average number of SPM lesions on each plant was 29.7, 0.0, 0.3 and 1.8 for the control, Luna Sensation, Takumi and Talius, respectively.

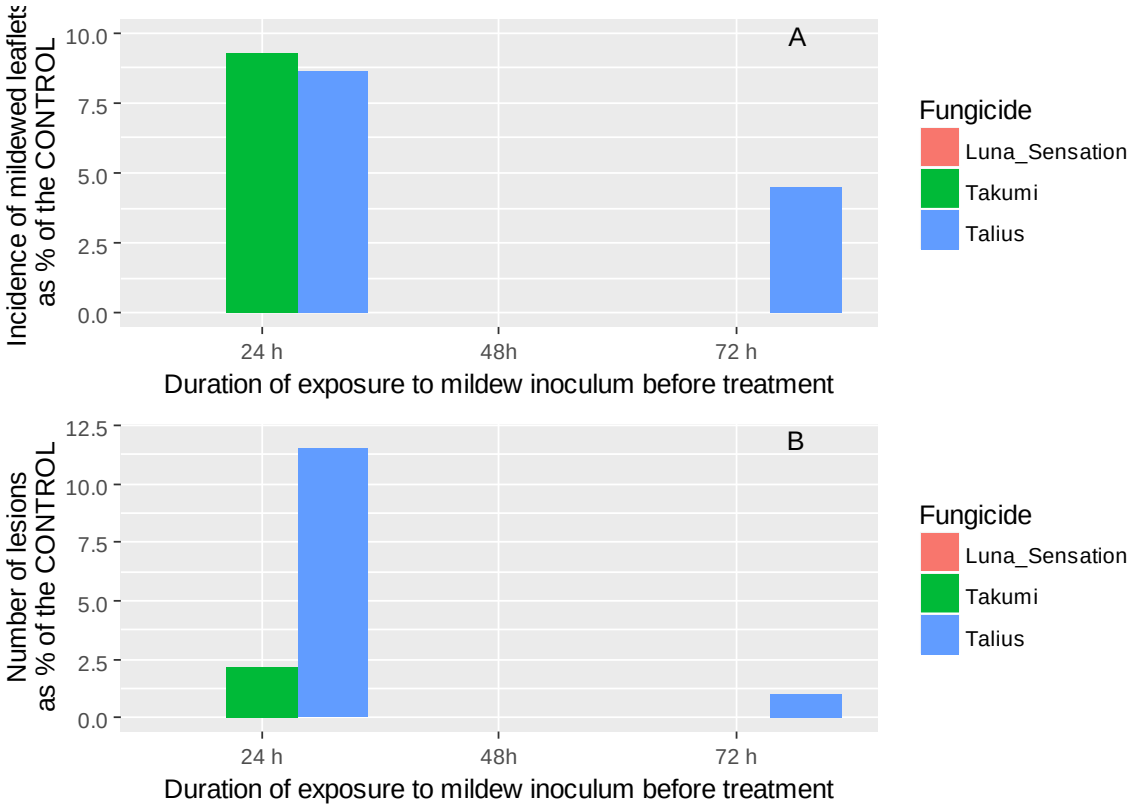


Figure 2.1 Incidence of inoculated leaflets with visible SPM lesions over the two replicate experiments (A) and number of SPM lesions (B) expressed as a percentage of the CONTROL treatment in the experiments testing for curative efficacy of three new fungicides against SPM at NIAB EMR in 2017. SPM was significantly less on the treated plants than on the control but no significant differences between fungicides. Table 2.1 gives the details of products used. Table A1 in the appendix gives the mean values of SPM incidence and lesion density. Plants of cv. "Malling Centenary" were used.

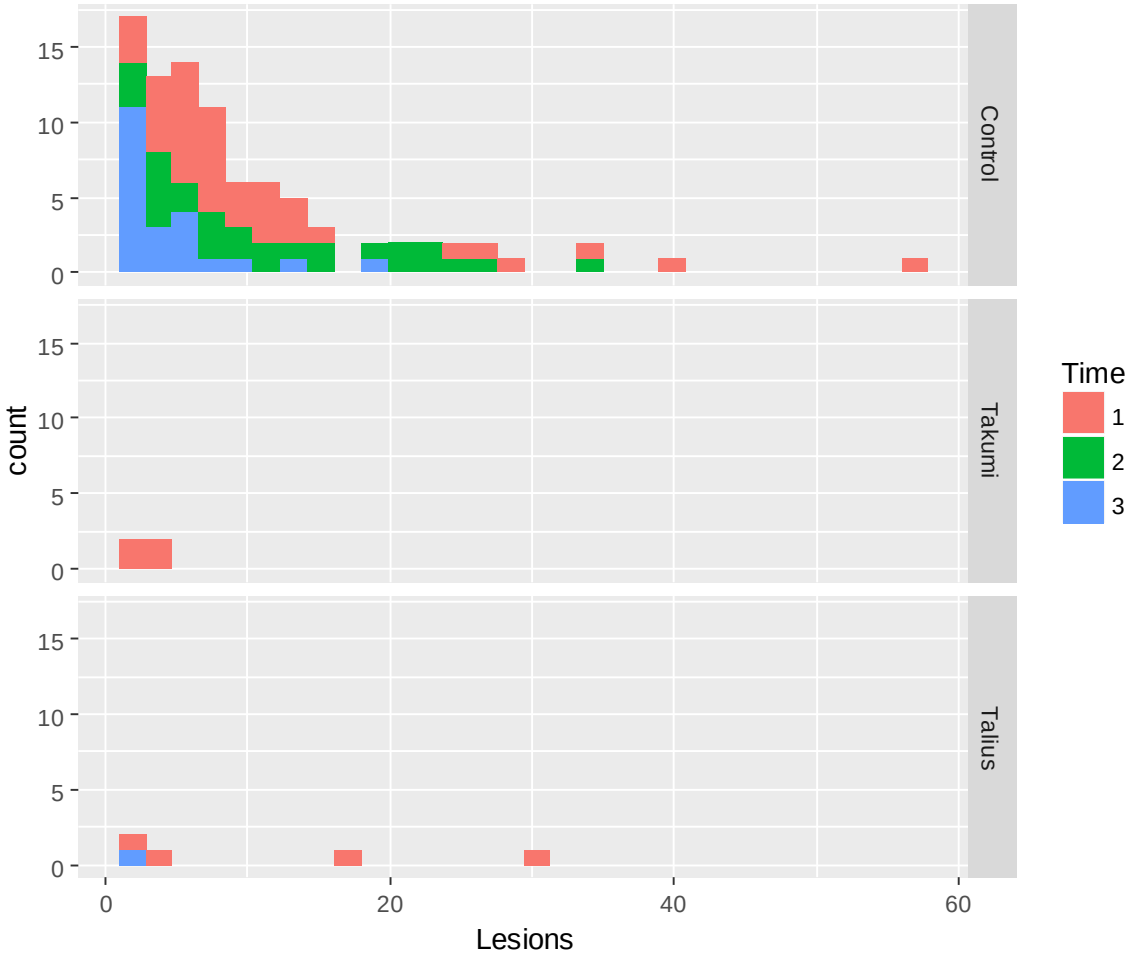


Figure 2.2 Histogram of the number of SPM lesions on each of those leaflets with visible lesions over the two replicate experiments; these leaflets were inoculated 1, 2 or 3 days before fungicide treatment (i.e. testing for curative effect) at NIAB EMR in 2017; no lesions were observed on the plants treated with Luna Sensation. Plants of cv. "Malling Centenary" were used.

2.1.3.2 Protectant tests

The overall mildew level was much lower in the first experiment than in the second repeat test: 5.4% (39 out of 719 leaflets) vs. 32.0% (228 out of 712 leaflets). Unlike in the curative tests, these infected leaves were more evenly distributed among all treatments (Fig. 2.3A). The overall incidence of leaflets with SPM was 22.6%, 15.0%, 24.0%, 15.4%, 7.5% and 27.8 for AQ10, Luna Sensation, HDC F208, Takumi, Talius and the control, respectively. The average number of SPM lesions was 0.3 and 2.7 per inoculated leaflet for the two repeat tests; the corresponding values on the infected leaflets were 4.7 and 8.5.

Fig. 2.3 shows the incidence of mildewed leaflets and number of lesions as a percentage of the corresponding values for control treatment; the actual incidence and lesion values are given in Table A2 in the appendix. The incidence of leaflet infection appeared to be lower for all treatments than the control for exposure period 1 (24-96 h after treatment) (Fig. 2.3A) but the difference was statistically significant ($P < 0.05$) for HDC F208 and Talius only. For

exposure period 2 (28-120 h after treatment), all three fungicides had lower ($P < 0.05$ for Luna Sensation, and $P < 0.01$ for Takumi and Talius) incidence than the control (Fig. 2.3A). For exposure period 3 (96-168 h after treatment), only Talius-treated plants had lower ($P < 0.05$) incidence than the control (Fig. 2.3A). For exposure period 4 (168-240 h after treatment), none of treatments led to significant reductions in SPM incidence. Within all four exposure periods, there were no significant differences between the three fungicides.

Number of lesions per plant varied greatly within each treatment as indicated by the relatively large values of standard error (Table A2 in appendix). Most of those infected leaflets had fewer than 18 lesions (Fig. 2.4). The conditional lesion density was 5.9, 8.9, 10.6, 7.1, 4.9 and 7.9 for AQ10, Luna Sensation, HDC F208, Takumi, Talius and the control, respectively.

For exposure period 1, there were fewer lesions on plants treated with HDC F208 ($P < 0.05$), Takumi ($P < 0.07$) and Talius ($P < 0.05$) than the control. For exposure period 2, all treatments, other than HDC F208, led to fewer lesions ($P < 0.05$) than the control. For exposure period 3, only Talius led to fewer lesions ($P < 0.05$) than the control. For exposure period 4, plants treated with HDC F208 had more lesions ($P < 0.05$) than the control. Within all four exposure periods, there were no significant differences between the three fungicides.

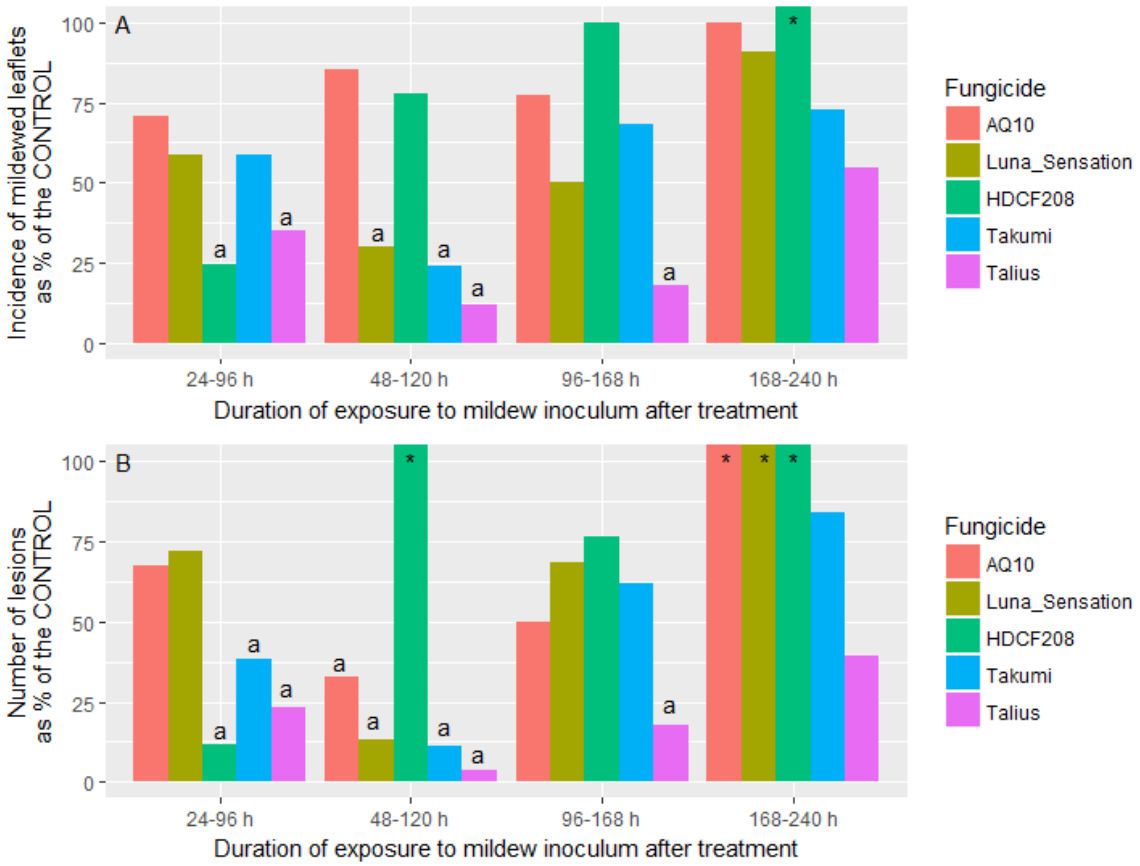


Figure 2.3 Overall percentages of inoculated strawberry leaflets with visible SPM lesions (A) and average number of SPM lesions per plant (B) in the two experiments testing for protectant effects of five products against SPM at NIAB EMR in 2017. Table 2.1 gives the details of products used. The error bars represent one standard error. Treatments with 'a' above the bar had significantly less SPM than the control. Treatments with '*' are those with values > 100%. Table A2 in the appendix

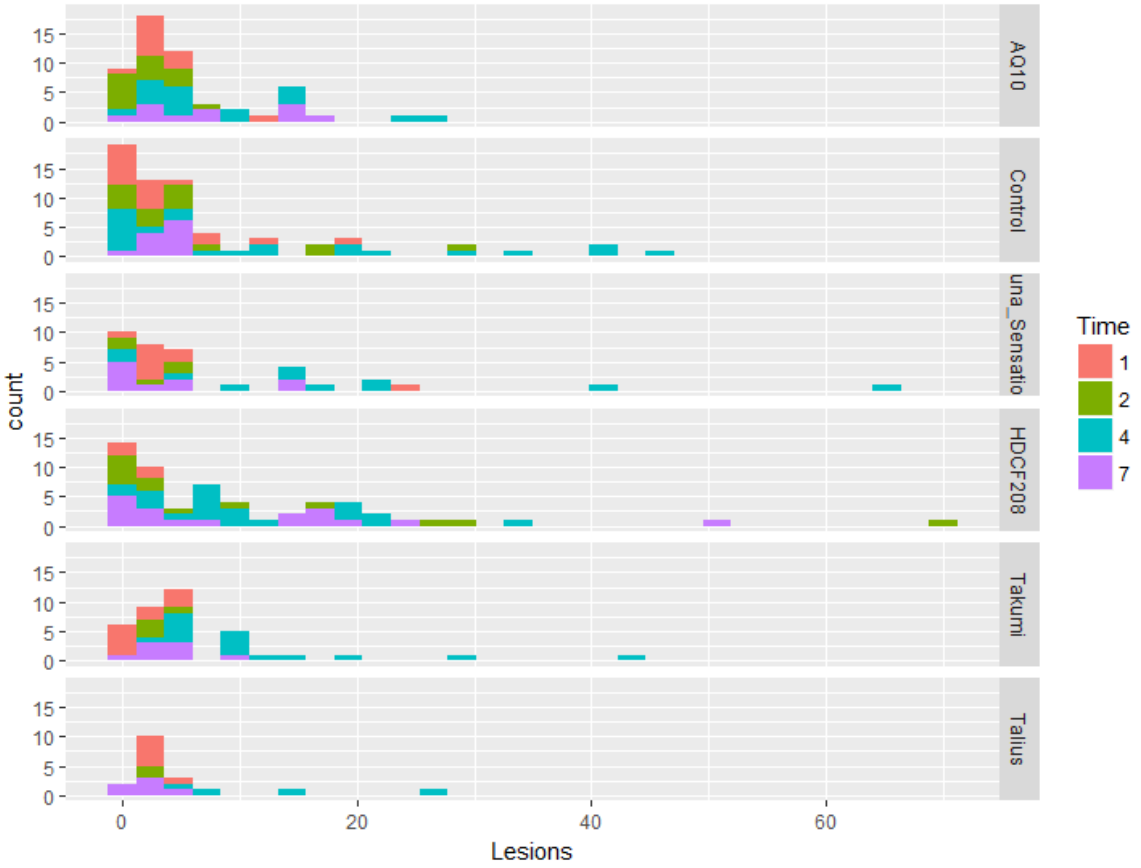


Figure 2.4 Histogram of the number of SPM lesions on those leaflets with visible lesions; these leaflets were inoculated/exposed to SPM 1, 2, 4 or 7 days after treatment for consecutive 72 hours (i.e. testing for protectant effect) at NIAB EMR in 2017. Plants of cv. "Malling Centenary" were used.

2.1.4 Discussion

Curative test: The results demonstrated that all three chemicals have excellent efficacy in preventing young incubating colonies from becoming visible lesions. Even for those leaflets inoculated 3 days before fungicide application, SPM lesions were only observed on a few leaves. This is very efficacious considering the fact that under favourable conditions it would only take 5 days from inoculation to visible lesions for SPM. The present experiments were conducted in the June and July period under tunnel conductions favouring both SPM and plant growth. Thus, under cooler conditions, the effective period for these fungicides when used as a curative treatment may be even greater than 3 days.

Protectant test: In contrast to the curative tests, more leaflets developed SPM lesions on treated plants. In addition to the efficacy issue, spray coverage in relation to growth (commonly called growth dilution effect) also needs to be considered. Since products were applied before inoculation, plant growth would have led to larger new leaf areas without product cover, hence susceptible to SPM infection. The longer the interval between treatment and inoculation is, the

greater the new leaf area. Unless the products are truly systemic and effective at a very low dose, these new leaf areas are expected to be susceptible to SPM. Thus, in practice the growth rate has to be taken into account.

Of all the products tested, Talius appears to be most effective: it can reduce the incidence of leaflets with SPM lesions even when plants were inoculated 7-10 days after treatment. For Luna Sensation and Takumi, the effective protectant period is 4-7 days post-treatment. Although AQ10 led to fewer leaflets infected than the control for inoculation during the period of 1-10 days post treatment, the difference was not statistically significant. Based on the biocontrol mechanism (myco-parasitism) of AQ10, we would expect that AQ10 is least effective in reducing SPM sporulation when used as a protectant treatment. HDC F208 led to reduced incidence of leaflet infection only during the period of 1-4 days post treatment. HDC F208 is a bacterium biocontrol organism and antibiosis is likely to be the main mechanism. The lack of control in the later inoculation periods implies that this bacterium failed to multiply and disperse sufficiently to new leaf tissue.

2.2 Evaluating SPM management programmes (ORETO Trial 17/007)

2.2.0 Introduction

Trials in 2015 showed that combining certain alternative products with reduced fungicide input could be effective in controlling powdery mildew, particularly when the level of inoculum was relatively low. In 2016 further trials were conducted at two sites in which programmes were evaluated for control of powdery mildew where biofungicides HDC F208 or AQ10 were combined in programmes with a plant strengthener (Cultigrow) with and without a reduced fungicide programme. The mildew risk was much greater in 2016 but the results showed that the BCAs were as effective in controlling mildew as the standard fungicide programme, particularly when applied alone in a programme. Having identified effective alternative products, the next step is combine their use in programmes and incorporating other factors such as disease risk, growth stage, type of fungicide (curative, protectant, anti-sporulant) in order to develop a decision-based management programme for growers.

Work by the University of Hertfordshire showed that the use of weekly silicon fertigation in strawberries from early in the crop can delay the development of powdery mildew i.e. the area under the disease progress curve was lower with silicon alone compared to untreated plots. Silicon is known to strengthen plants against abiotic and biotic stress and effects against both pests and diseases are reported in the literature - work at the University has shown differences in the structure of leaf surface wax after silicon treatment. In 2017, potential programmes / management systems were tested at NIAB EMR only, with the specific aim to compare the

disease control achieved by managed programmes of fungicides and biofungicides with and without routine applications of silicon or Cultigrow with that achieved by a routine fungicide programme and an untreated control.

2.2.1 Objectives

To compare the disease control achieved by managed programmes of fungicides and biofungicides with and without routine applications of silicon or Cultigrow with that achieved by a routine fungicide programme and an untreated control.

2.2.2 Materials and methods

Everbearer strawberry module plants (cv. code AM), supplied by Berry Garden Growers Ltd (BGG) were planted on 28 March. Unfortunately, the plants failed to establish and grow adequately: half of the plants were stunted and displayed symptoms of yellow leaves by mid-May. There was no obvious spatial pattern in normal or abnormal plants [hence ruling out possible systematic faults in the irrigation system, which was confirmed by subsequent lab analysis of irrigation water]. Analysis of leaf samples from healthy and abnormal plants showed that yellow leaves were deficient in nitrogen and phosphate. However, it is not clear what factor(s) caused the observed nutrient deficiencies. We had been discussing this issue with the PMG when we visited Sandy Booth in early May; Sandy kindly visited the trial in mid-May and suggested several measures to improve drainage. We suspected two possible causes that may have acted together to the detriment of root development: initial excessive irrigation and a prolonged cold period in April.

This trial was scrapped in mid-June and, with help from the PMG and BGG, a new trial was established with AM plants (already planted in peat/coir bags for 3-4 weeks prior to move from commercial production), sourced from BGG. Several changes were made to the experimental setup, particularly the watering / feed regime and to the drainage of the bags, in order to improve plant establishment. In addition, Scott Raffle (AHDB) and BGG advisors regularly visited the new trial, ensuring that plants were growing satisfactorily. We are most grateful for their assistance.

The remaining part of this section refers to the new trial.

Photo 2.1 Picture of cv. AM plants in late June before the mildew trial commenced in early July 2017.

2.2.2.1 Study design

Site: NIAB EMR East Malling, Kent.

Strawberry planting

A new plantation of the ever-bearer variety AM, which is very susceptible to powdery mildew, was used. The plantation consisted of two Spanish tunnels with 3 mypex covered raised beds in each. To minimise the risk of waterlogging, plastic boxes (with holes to allow water through) were laid directly onto the mypex. Plants (grown in peat/coir bags for 3-4 weeks) were delivered to NIAB EMR on 21 June and were immediately placed onto the boxes (Photo 2.1). Each bag contained five plants, staggered in the bag, irrigated with two sub-drippers with trickle irrigation, located in the mid end section of each bag. Plants were fertigated with a 6 L per hour dripper (with four sub-drippers) shared between two bags, i.e. 3 L per hour per bag. There were 14 bags per plot giving a total of 70 plants per plot. Each plot was 10 m in length and separated in the row by 2 m.

Treatments: The programmes evaluated are given in Table 2.2. Details of the fungicides, biofungicides, plant strengtheners and nutrients used in the programmes are given in Tables 2.3 and 2.4. All products received for inclusion in the trial were stored, handled and applied according to the manufacturer's instructions on the product label. All were applied as foliar sprays. All plots were sprayed with Amistar on 23 June and Fortress on 3 July to control the low incidence of powdery mildew present on most plants when they were delivered. The trial treatments were then started on 10 July. Decisions on spray applications to treatments 3-5 were based on the criteria given below in Tables 2.5 and 2.6. All management decisions were recorded (Tables 2.7 and 2.8). These treatments were compared with a routine fungicide programme applied every 7 days (Treatment 2) and with an untreated control (Treatment 1). Details of the programmes applied are given in Table 2.7.

Brief description of the NIAB EMR powdery mildew model: This model estimates the favourability of weather conditions during a 24 h period (from 9:00 am to 8:59 am next day) on mildew development. The model framework was adapted from the apple powdery mildew model developed at East Malling, which was shown to be able to predict apple powdery mildew epidemics satisfactorily. Recent work at BGG (commercially confidential) suggested that predictions by the strawberry mildew model can satisfactorily explain the observed outbreaks at a number of sites where the model was used.

Conidia from external sources and lesions/spores coming with planting materials disperse in air and initiate many cycles of 'secondary mildew' epidemics, which cause crop losses. The EMR model uses weather data (recorded at an interval <= 30 minutes) to quantify the favourability of daily weather for sporulation, and infection of young strawberry leaves by

conidia, taking into account the effects of temperature and relative humidity on the early development of colonies and on the mortality of conidia. The daily mildew risk is then forecast by combining the above two weather indices (sporulation and infection). The model assumes the same infection conditions for fruit and leaves.

It should be noted that only young leaves (until fully unrolled) and young fruitlets are susceptible to infection by powdery mildew conidia. Although lesions usually appear on old leaves, actual infection occurred much earlier because (1) it usually takes 5-10 days from infection to visible lesions depending on temperature, (2) it is not easy to spot mildew lesions in the early phase (before leaves become curled or lesions begin to turn purple). Thus, presence of young tissues is one of the key factors determining the magnitude of mildew risks, and hence growth stage (rate) is a key component of decision making in managing powdery mildew diseases in general.

Spray application: Treatments were applied using a CP20 knapsack sprayer with a Albuz hollow cone red nozzle at 1000 L/ha following SOP 724. The sprayer lance was used to ruffle the strawberry plants to ensure spray penetration to the centre of the plant, the youngest leaves and to the leaf undersides. Details of each application are given in Tables A3-A4 (Appendix). All treatments were applied using the same sprayer for the fungicides and for HDC F208 as it is compatible with all fungicides.

Other treatments: Treatments for control of botrytis fruit rot and other rots were applied routinely across all treatments (except the untreated control) and were not part of the management system. Control of botrytis fruit rot was based on Rovral (iprodione), Signum (pyraclostrobin + boscalid), Switch (cyprodonil + fludioxonil), Luna sensation (fluopyram + trifloxystrobin), Amistar (azoxystrobin), Frupica (mepanipyrim), Scala (pyrimethanil) and Teldor (fenhexamid). Where these products also controlled powdery mildew such as Luna Sensation or Amistar then this was taken into account in the powdery mildew management decisions.

Pests were monitored during the weekly inspection. Where pests were found an entomologist was consulted regarding treatment. Insecticides were applied to all plots including the untreated. Where checks showed that the treatments were affecting pest incidence, such as mites, then an entomologist was advised and a formal assessment done on mite numbers. Biological control was used for pest management where appropriate. Treatments were applied (primarily using predators) during the first month for two-spotted spider mites, thrips and capsids (Calypso).

All plots received a standard nutrient programme via the irrigation for cv. AM (pre and post-flowering), supplied by BGG. The amount of irrigation provided varied from time to time, depending on the substrate moisture level and advice from BGG advisors and Scott Raffle.

Experimental Design: The experiment was conducted with a randomised block design with four blocks (i.e. rows). Within each block there were five plots, each randomly assigned to one of the five treatments. Within each plot, there were 14 bags (i.e. 70 plants). Plots were separated in the row by 2 metres.

Table 2.2. Treatment programmes evaluated at NIAB EMR in 2017

Treatment	Type	Plant Protection Products	Other
T1	Untreated	-	-
T2	Routine	Fungicides	None
T3	Managed	Fungicides, BCAs	Cultigrow applied monthly from start of growth
T4	Managed	Fungicides, BCAs	Sirius applied weekly from start of growth
T5	Managed	Fungicides, BCAs	None

Table 2.3. Fungicide products for powdery mildew control on strawberry

Product	Active ingredient	Rate (/ha)	Product type	Other information	Harvest interval days	Chemical group
Systhane	myclobutanil	450 ml	AS*, P	Max 6 sprays. Not after Sept 30 2017	3	DMI
Fortress	quinoxyfen	0.25 L	P	Max 2 sprays	14	Aza naphthalenes
Nimrod	bupirimate	1.4 L	AS, P	Max 3 sprays	1	Hydroxyl-pyrimidine
Amistar	azoxystrobin	1.0 L	P	Max 4 sprays	7	QoI
Karma	potassium bicarbonate	3 kg	AS	Max 8 sprays	1	Inorganic
Luna Sensation	trifloxystrobin + fluopyram	0.8 L	AS, P	Max 2 sprays	1	SDHI + QoI
	Potassium bicarbonate	20 kg	AS	Max total dose of 60 kg/ha	0	Inorganic
Stroby	Kresoxim-methyl	0.3 kg	P	Max 3 sprays	14	QoI
Takumi	cyflufenamid	150 ml	P	Max 2 sprays	3	Phenyl-acetamide
Kumulus	sulphur	200g/100 L	P	No limit	0	Inorganic
Topas	penconazole	0.5 L	AS, P	Max 4 sprays	3	DMI
Talius	proquinazid	190 ml	P	1	3	Aza-naphthalenes

***:** AS = Antisporulant, P= protectant. In addition, BCAs and other products are shown in Table 2.4.

Table 2.4. BCAs and other products applied as foliar sprays

Product	Active ingredient	Rate (/ha)	Maximum number of sprays	Product type
HDC F208 + Silwet	*Bacillus pumilis* QRD2808	5 L + 0.05%	Not specified	BCA
AQ10 + Silwet	*Ampelomyces quisqualis*	70 g + 0.05%	12	BCA
Cultigrow B204	flavonoids	250 ml	5 at 28 day intervals. Last spray no later than one month before end of cycle	Plant strengthener
Sirius	silicon	0.05-0.1%	2-6 at 10-14 day intervals	Nutrient

Table 2.5. Criteria for powdery mildew management decisions

Item	How determined	Risk	Management options
Disease risk	Determined from input of humidity and temperature from logger in tunnel to disease risk model (see below) and forward weather forecast from internet	More than 4 days with risk above 10% requires action	**Product choice** – Fungicide (anti-sporulant or protectant), BCA
Growth stage and rate of growth	Inspections 1-2 times per week	Rapid leaf production, start of flowering/ fruiting indicates increased risk and possible change of product	**Spray interval** – 7 or 14 days **Tunnel ventilation**
Mildew monitoring	Inspections 1-2 times per week on youngest leaves on 5 plants per plot. Plants will be selected at random for each inspection	Scored 0-5, where 0 = no mildew	

2.2.2.2 Assessment

Powdery mildew

Plots were inspected for mildew twice weekly for management decisions. A full assessment for powdery mildew on leaves as percentage leaf area infected on the youngest five expanded leaves on each of ten plants per plot were assessed at an interval of three weeks using a standard key (Anonymous, 1976) [also shown in Appendix F1]. This interval of assessment was used initially to allow for six assessments by late September. However, no new leaves were produced after early August. Thus only three leaf assessments were completed.

Other diseases

Assessments were made for other diseases (e.g. leaf spots) as needed. Assessments for fungal rots were made at harvest.

Harvest

All fruit was picked and assessed for the presence of powdery mildew and other defects. For each plot at each pick, total yield, total number of fruit, total number of Class 1 fruit, and number of mildewed fruit and number of fruit with rots were recorded. The first pick was on 28th July and the last pick was on 27th September; a total of 14 picks.

Plant vigour

If during the trial differences in plant vigour become apparent between the treatments then formal assessments were made by measuring the height and spread of 10 plants per plot.

Phytotoxicity

Phytotoxicity was assessed 7 days after each spray by visual assessment of % leaf area with necrosis / chlorosis, leaf drop, growth regulatory effects (EPPO Guideline PP 1/135 (4)). Any effects were recorded.

Meteorological records

A data logger (USB-502) was placed at crop height in each tunnel to monitor temperature and humidity. This was downloaded 1-2 times weekly and the data input to the mildew model for disease risk determination. Records of daily maximum and minimum temperature and rainfall were also taken from a weather station located at East Malling main site, approximately 500 m east of the trial.

Table 2.6. Decision making criteria for selecting powdery mildew treatments

Predicted risk		Current		Decisions (& product type)*			
Last 2 days	Last 7-10 days	Growth rate	disease level	Curative	Anti-sporulant	Protectant	Biocontrol*
Low	Low	Low	Low			X	X
Low	Low	Low	High		X	X	X (AQ^{+})
Low	Low	High	Low			X	X
Low	Low	High	High	X	X	X	X (AQ)
Low	High	Low	Low	X			X
Low	High	Low	High	X	X	X	X (AQ)
Low	High	High	Low	X			X
Low	High	High	High	X	X	X	X (AQ)
High	Low	Low	Low				X
High	Low	Low	High		X	X	X (AQ)
High	Low	High	Low			X	X
High	Low	High	High		X	X	X (AQ)
High	High	Low	Low	X	X		X
High	High	Low	High	X	X	X	X (AQ)
High	High	High	Low	X	X	X	X
High	High	High	High	X	X	X	X (AQ)

*During April and May product choice will mainly focus on fungicides. From June onwards BCAs will be used as blocks of treatments as all our current experience with these products is on their use in trials from June onwards when weather conditions are generally warmer. Also experience from 2015 and 2016 suggest performance is better as blocks of treatment rather than alternating sprays.

$^{+}$: AQ10

2.2.2.3 Statistical analysis

The data were analysed using a repeated measures ANOVA, combining data recorded over time for each type of variable. This takes account of the correlations between successive measurements from the same plot. All percentage figures were transformed to the angular

scale before analysis. In addition mean yield per plot for the fourteen harvests was also included. Fruit number was square root transformed and fruit size log transformed prior to analysis.

2.2.3 Results

2.2.3.1 General

After plot establishment on 21st June, the plants resumed growth and started flower production. Flowers were removed until 5th July. Plant growth was good and at the level commercially acceptable (Photo 2.2A; BGG advisors, pers. comm.) There were no obvious phytotoxic symptoms observed on foliage or fruit in any of the plots following the spray treatments. There were also no obvious differences in plant vigour (height and spread) between the plots.

2.2.3.2 Powdery mildew

Mildew risk

The weather conditions (warm temperatures coupled with high humidity) were very conducive to SPM development throughout the trial period, particularly in the mid-to-late July period which was confirmed by the high risk (consecutive days with risk > 10%) shown by the mildew risk model (Fig. 2.5). The programmes applied to all treatments are given in Table 2.7. The trial activities, disease monitoring and assessments together with the decisions in response to the predicted risks, based on mildew monitoring in the crop and the model, are shown in Table 2.8. Spray decisions are highlighted. For treatments 3-5 the principle was to apply HDC F208 as the basic treatment. Sirius or Cultigrow were applied routinely. If the incidence of SPM increased or was predicted to increase then the option was to switch to a fungicide or to change to AQ10 as an alternative BCA. However, the SPM on leaves and fruit remained very low on all the treated plots and there was only one occasion (14th August, Table 2.7) where intervention with fungicide was needed due to a high risk identified by the model and the appearance of new mildew colonies on leaves and flower stalks at a low incidence. On this basis HDC F208 remained as the basic BCA treatment in programmes 3-5. As the weather conditions remained conducive to SPA throughout the trial period (shown as a continued risk > 10% by the mildew model (Fig. 2.5) there was little opportunity for omitting treatments or extending the spray interval in the managed plots.

Mildew incidence

Despite the high risk of SPM development, the level of SPM on leaves was very low; only 2% and 7% of leaf area were mildewed on untreated plots when assessed on 27th July and 15th August, respectively. There was virtually no SPM observed on all other treated plots. On the

day when the fourth mildew assessment was originally planned (5th September), there were no more new leaves since the last assessment and hence no further leaf assessments were conducted.

By contrast, SPM mildew on fruit rose rapidly from 2% for the first pick (28th July) to > 90% for the sixth pick (21st August) on untreated plots; the percentage of mildewed fruit remained > 90% thereafter for untreated plots (Fig. 2.6). On treated plots percentage of fruit with SPM did not rise above 3% with programmes based on BCAs performing as well as the routine fungicide programme. Photo 2.2BC illustrates the differences in SPM on fruit between untreated and treated plots.

Fig. 2.7 gives the summary of SPM development and fruit production for all five programmes. The untreated control had lower yield than the other treatments (Fig. 2.7A), which was due to reduced yield from late August (Fig. 2.8A). Because of SPM on fruit, the overall Class I yield for the control was less than 30% of the other treatments (Fig. 2.7B and Fig. 2.8B). Across all picks, nearly 81% of fruit had visible SPM lesions at harvest for untreated plots (Fig. 2.7C), which was significantly ($P < 0.001$) greater than other treatments. The other four programmes did not differ significantly among each other.

2.2.3.3 Other diseases

An unknown fungus was found colonising the stigmas on flowers at a moderate incidence in most plots in assessments on 14 August (Photo 2.3). This may have been responsible for causing abnormal fruit development in the trial. The mis-shaped fruit could also be caused by capsids which were also present in the trial. We are currently culturing the slow-growing fungus and trying to identify it by molecular means. As a result of the presence of this flower fungus a spray of Luna Sensation (activity against a wide spectrum of fungi) was applied to treated plots on 14 August. After this treatment the incidence of the disease appeared to decrease in the treated plots, but remained obvious in the untreated plots. No further sprays were applied for this problem.

2.2.3.4 Harvest

Fruit was harvested weekly or twice weekly from 28 July to 27 September, a total of 14 harvests. There were no significant differences in yield and marketable yield between the managed programmes and the routine fungicide programme. However, all treated plots had a significantly higher ($P < 0.001$) total yield and marketable yield than the untreated control (Figs. 2.7 and 2.8). Most of the unmarketable fruit was due to infection with powdery mildew. The incidence of rots, primarily Botrytis was very low and similar in all treated plots.

Photo 2.2 Examples of strawberry plant growth (A) and severely mildewed fruits in the untreated plots (B) in comparison with those fruit from treated plots (C).

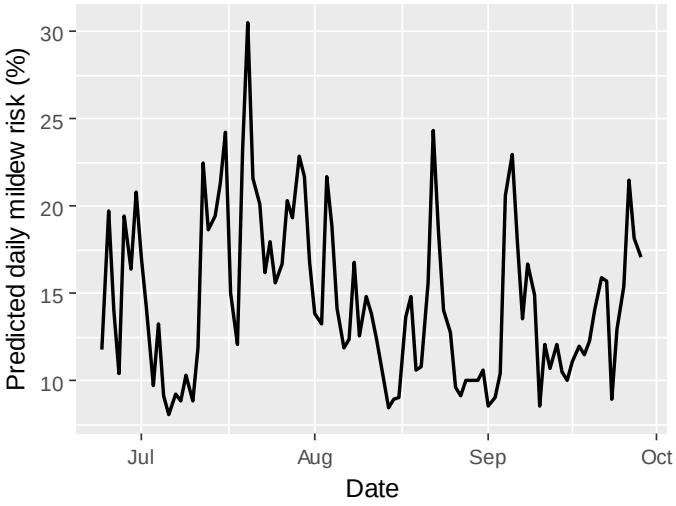


Figure 2.5 Predicted daily risk of SPM on susceptible cultivars for the NIAB EMR site in 2017. The predictions were given by the NIAB EMR model where a period of four (or more) consecutive days with risks > 10% is considered to need growers' intervention with a moderate to high level of inoculum (usually when the incidence of leaves with SPM is above 5%).

Table 2.7 Details of treatments applied in managed and routine programmes for control of SPM at NIAB EMR in 2017

Programme	Date treatment applied										
	10 Jul	17 Jul	24 Jul	31 Jul	7 Aug	14 Aug	21 Aug	28 Aug	4 Sep	11 Sep	18 Sep
1 - Untreated											
2 - Routine fungicide	Systhane + Frupica	Luna Sensation	Nimrod + Switch	Topas + Frupica	Nimrod + Switch	Luna Sensation	Topas + Scala	Nimrod + Signum	Takumi + Scala	Topas + Signum	Takumi + Rovral
3 – HDC F208 + Cultigrow	HDC F208 + Frupica + Cultigrow	Luna Sensation	HDC F208 + Switch	HDC F208 + Frupica + Cultigrow	HDC F208 + Switch	Luna Sensation	HDC F208 + Scala	HDC F208 + Signum + Cultigrow	HDC F208 + Scala	HDC F208 + Signum	HDC F208 + Rovral
4 - HDC F208 + Sirius	HDC F208 + Frupica + Sirius	Luna Sensation	HDC F208 + Switch + Sirius	HDC F208 + Frupica	HDC F208 + Switch + Sirius	Luna Sensation	HDC F208 + Scala + Sirius	HDC F208 + Signum	HDC F208 + Scala + Sirius	HDC F208 + Signum	HDC F208 + Rovral + Sirius
5 - HDC F208 only	HDC F208 + Frupica	Luna Sensation	HDC F208 + Switch	HDC F208 + Frupica	HDC F208 + Switch	Luna Sensation	HDC F208 + Scala	HDC F208 + Signum	HDC F208 + Scala	HDC F208 + Signum	HDC F208 + Rovral
Management decisions. See also Table 2.8	First trial treatments following fungicide sprays	Low incidence mildew in flowers + high risk	Mildew incidence= 1. Model above threshold. Continue HDC F208	Mildew incidence= 1. Model above threshold. Continue HDC F208	Mildew incidence= 1. Model above threshold. Continue HDC F208	Unidentified fungus infecting flowers	Mildew incidence =1. Model above threshold. Continue HDC F208	Mildew incidence= 1. Model above threshold. Continue HDC F208	Mildew incidence= 1. Model above threshold. Continue HDC F208	Mildew incidence= 1. Model above threshold. Continue HDC F208	Mildew incidence= 1. Model above threshold. Continue HDC F208

Table 2.8 Summary of strawberry treatments, assessments and management decisions in powdery mildew management trial – NIAB EMR 2017

Activity	Date
Plants delivered in bags and laid out in plots on crates, 5 plants per bag, 14 bags per plot = 70 plants per plot	21 June
Each bag was irrigated with two sub-drippers with trickle irrigation, located in the mid end section of each bag. Plants were fertigated with a 6 L per hour dripper (with four sub-drippers) shared between two bags, i.e. 3 L per hour per bag.	
Amistar applied to all plots	23 June
Calypso applied for capsid control	
Fortress applied to all plots	3 July
Thrips in flowers and Two Spot spider mite present. Predators ordered	
Full mildew assessment (5 leaves x 10 plants per plot). Mildew incidence very low on all plot score 0-1. Model risk, 10%, rapid leaf growth. Start trial with HDC F208 (sprayed 10 July) on T3-T5	5 July
First trial sprays applied	10 July
Inspect strawberries. Low incidence mildew on flowers in managed plots. Trace on leaves, Score 1 but model high = change programme to eradicant fungicide – Luna Sensation on T2-T5	13 July
Second spray High risk Luna Sensation all treated plots	17 July
Check plots. Mildew incidence 1, model risk above threshold revert to Sonata on T3-T5	19 July
Third spray Revert to HDC F208 programme	24 July
Full mildew assessment. Very low on leaves and flowers on managed plots. Score 1, Model above threshold – Continue with HDC F208 programme on T3-T5	26 July
First harvest	28 July
Fourth spray, Continue with HDC F208 programmes	31 July
Check plots. Mildew incidence 1, model risk above threshold. Second harvest – Maintain HDC F208 programme on T3-T5	2 August
Fifth spray. Continue HDC F208 programme Third harvest	7 August
Check plots. Mildew incidence 1, model risk above threshold. UTC-Obvious mildew on young leaves and flowers, stalks and fruit. Very obvious fungal colonisation of flowers and young developing fruit. Grey/purple growth not mildew, confined to top of stigma oval spores. Cultured to identify. Moderate incidence of problem on all plots. Some dried fruit in previous harvest. Possible distorted fruit??	10 August
Fourth harvest	11 August

Sixth spray. Applied Luna Sensation as broad spectrum fungicide to T2-5 to control flower fungus	14 August
Fifth harvest	16 August
Third full mildew assessment. Luna S seems to have given some control of flower infection problem. A lot of shrivelled young fruit in untreated plots. Mildew incidence very low score 0-1 in Managed plots. Model above threshold. Revert to HDC F208 on T3-T5	17 August
Seventh spray. Continue HDC F208 programme Sixth harvest	21 August
Check plots. Flower fungus still present, fresh infection on new flowers in UTC. Mildew incidence on Managed plots very low Score 1. UTC Very high mildew on Flowers and fruits. Very little new leaf growth. Mildew model above threshold. Continue with HDC F208 programme on T3-T5	24 August
Seventh harvest	25 August
Eighth spray. Continue HDC F208 programme	28 August
No new leaf growth. Very low mildew incidence in managed plots. Score 0-1 High inoculum from UTC plots. Moderate weather risk. Mildew model above threshold. Continue with HDC F208 programme on T3-T5 Eighth harvest	30 August
Ninth spray. Continue with HDC F208 Programme Ninth harvest	4 September
Fourth full mildew assessment due but no new leaf growth so not done. Fungus attacking Stigmas still present, possibly associated with distorted fruit?? Very low mildew incidence in managed plots. High inoculum from UTC plots. Moderate weather risk. Mildew model above threshold. Continue with HDC F208 programme on T3-T5	5 September
Tenth harvest	8 September
Tenth spray. Continue HDC F208 programme	11 September
Very low mildew incidence in managed plots. High inoculum from UTC plots. Moderate weather risk. Mildew model above threshold. Continue with HDC F208 on T3-T5 Eleventh harvest	14 September
Eleventh spray. Continue with HDC F208 programme Twelfth harvest	18 September
Thirteenth harvest	22 September
Fourteenth harvest	27 September

Photo 2.3 Examples of unidentified fungal problem on flowers fruit, possibly resulting in small and unmarketable fruit in the SPM trial in 2017.

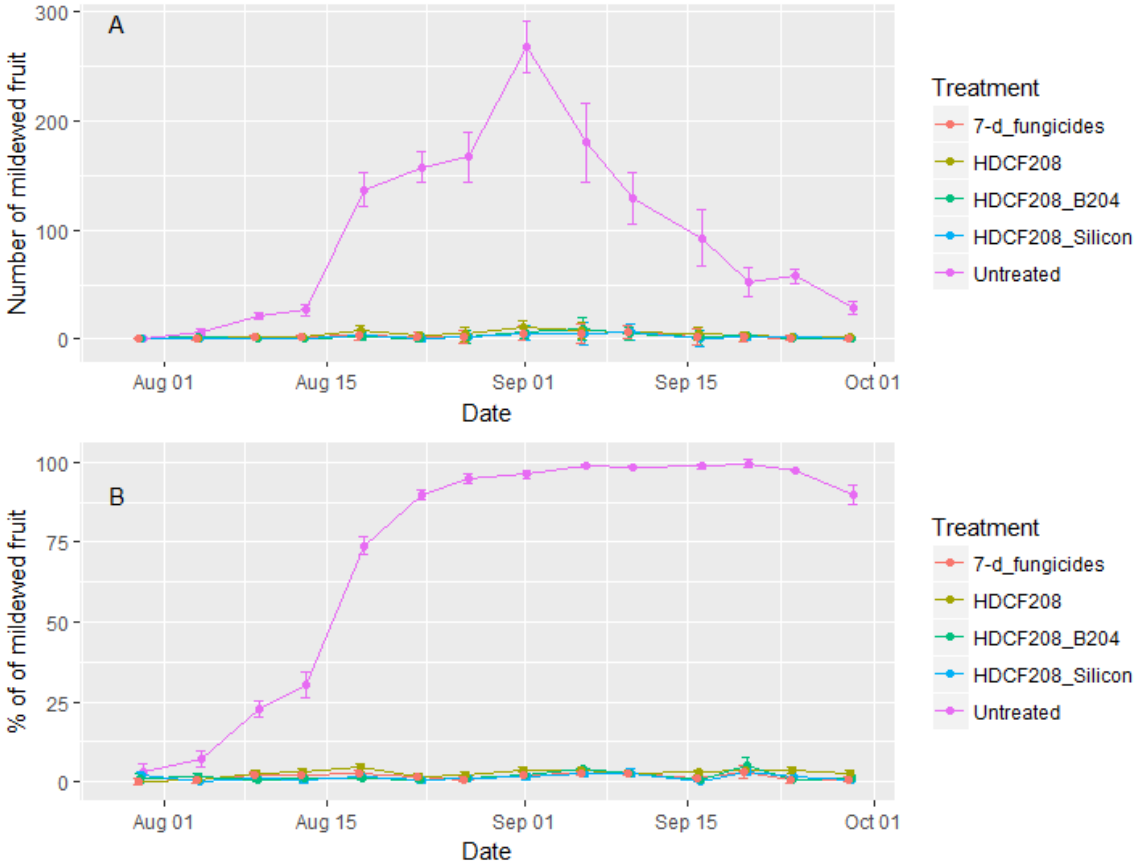


Figure 2.6 Average number of fruit with mildew (A) and incidence of fruit with mildew at each pick for five management programmes against SPM at NIAB EMR, 2017. There were 70 plants of cv. "AM" within each plot. Tables 2.1-2.4 give the details of each management programme and products used. The error bars represent one standard error.

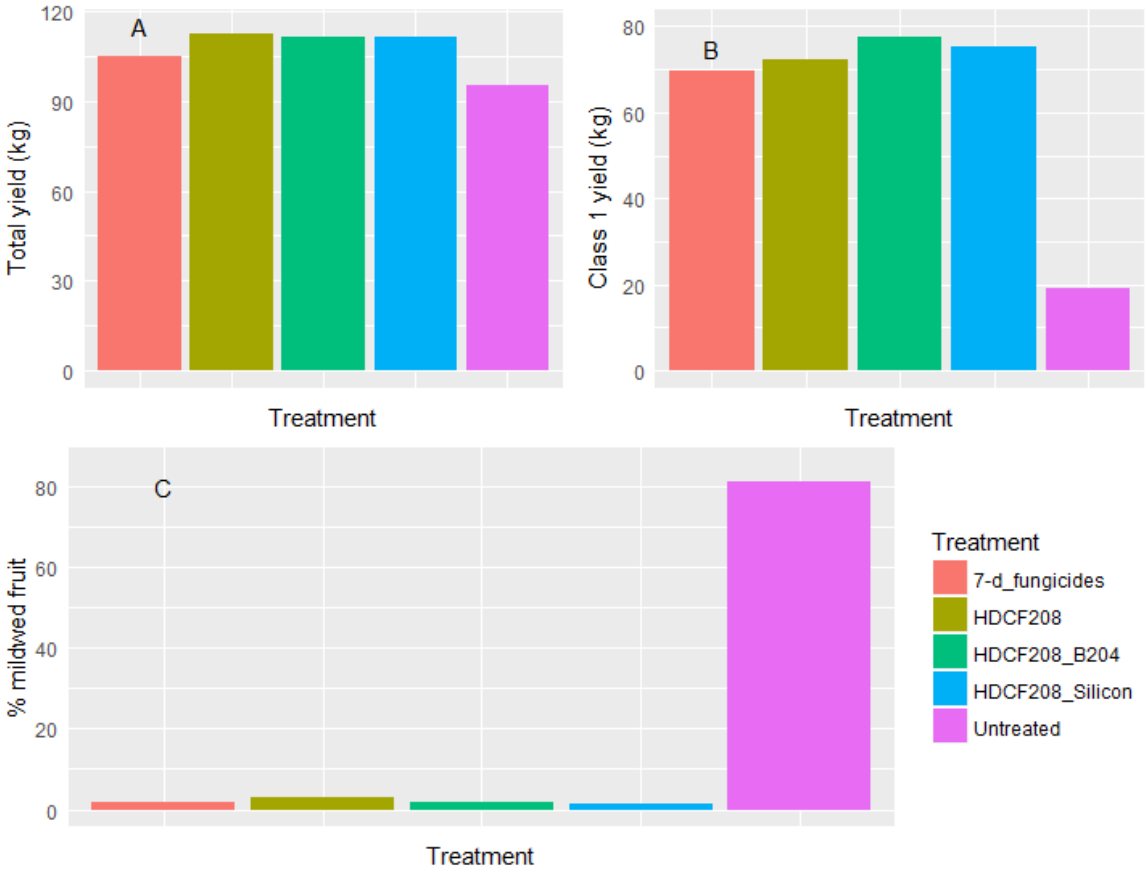


Figure 2.7 Total yield (A), total Class 1 yield (B) and overall percentage of fruit with SPM (C) overall picks for five management programmes against SPM at NIAB EMR, 2017. There were 264 plants of cv. AM within each plot. Tables 2.1-2.4 give the details of each management programme and products used.

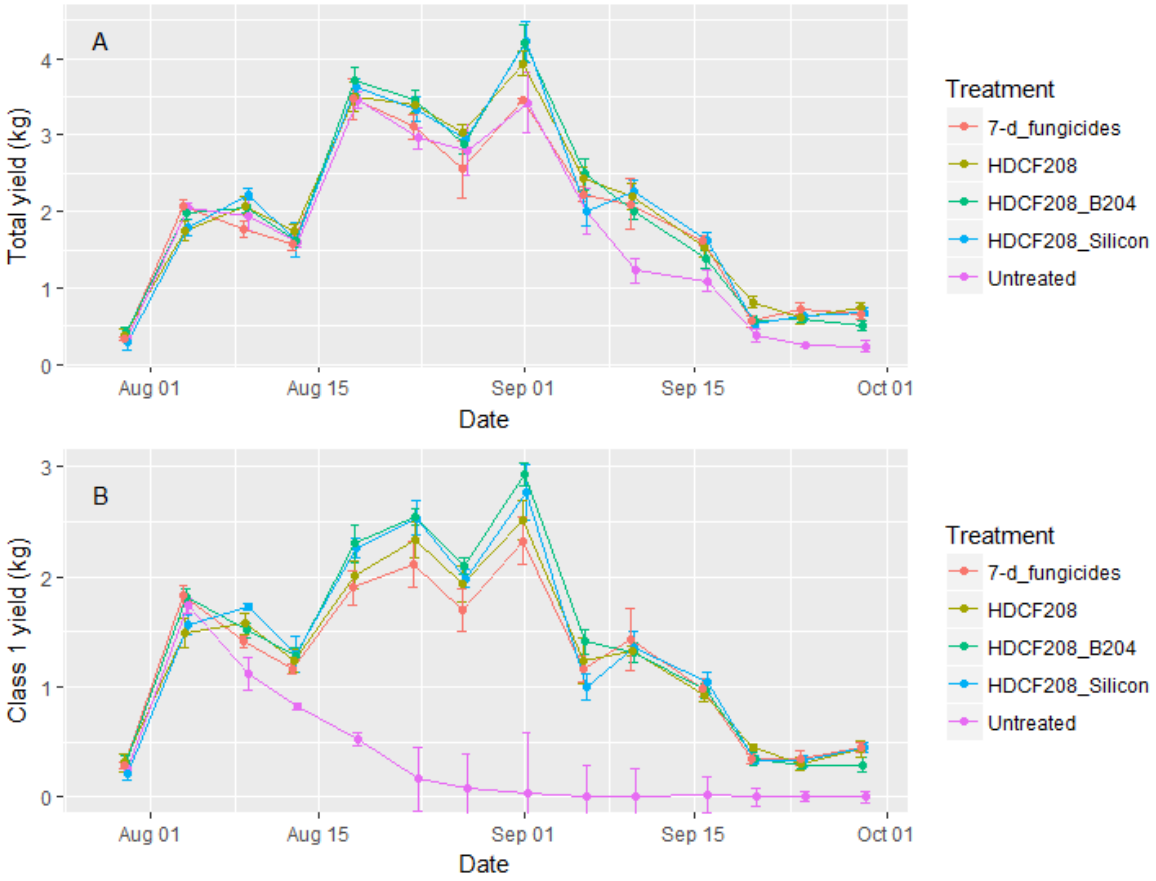


Figure 2.8 Average total yield (A) and Class 1 yield (B) per plot at each pick for five management programmes against SPM at NIAB EMR, 2017. There were 70 plants of cv. AM within each plot. Tables 2.1-2.4 give the details of each management programme and products used. The error bars represent one standard error.

2.2.4 Discussion

The objective of this trial was to compare the mildew control achieved in three managed programmes, based on BCAs and alternative chemicals compared to that achieved by a routine fungicide-only programme. Two of the managed programmes included routine use of either a silicon-based product (Sirius) of Cultigrow both of which from previous work are known to increase the plant resistance to powdery mildew. The BCA HDC F208 was applied in addition to these products as the initial choice, based on the results from 2015 and 2016 and, as a bacterial BCA, there would be no risk of problems when mixed with the fungicide used for Botrytis control. If the mildew risk increased (as measured by increasing mildew incidence in the crop and / or high risk as determined by the model) then the option was to switch to a fungicide or change to an alternative BCA (AQ10). Increasing the concentration of Sirius or going to weekly sprays was an additional option in Treatment 4.

Conditions were exceptionally favourable for SPM from the start of the trial in early July, and were particularly favourable during the period from mid to late July as shown by the mildew

model output in Fig. 2.1. Thus there was little scope in the managed plots for extending the spray interval or reducing sprays. However, despite this high risk, the actual mildew incidence in all the managed plots remained very low on leaves and flowers and fruits in all the monitoring and fungicide intervention for mildew was only needed once in early July. HDC F208 remained as the BCA used with no obvious reason to change to AQ10. The everbearer used in the trial appears to have low susceptibility to mildew on the leaves, which may have contributed to the success of the control in the BCA-based sprays in the managed treatments. The cultivar is very susceptible to mildew on the flowers and fruit, demonstrated by the high incidence of the disease in untreated plots. The three managed programmes also gave excellent control on flowers and fruits. There was a hint that the programmes that included Sirius or Cultigrow had less mildew than the HDC F208 only programme, but the overall mildew incidence was too low for any differences to be significant.

This trial has demonstrated that use of BCAs, with or without alternative chemicals, gave good control of mildew in strawberry comparable to a fungicide-based programme. It would be interesting to see whether the result would be as good using an everbearer cultivar more susceptible to foliar mildew. The trial was conducted from late June to September, a time of year when weather conditions are usually very favourable to mildew, giving few opportunities to omit sprays. If the trial had been started in March, then there would have been more opportunities to manage the mildew during the period up to June when mildew risks are generally much lower. It is important now to explore how the approach for managing mildew can be integrated with control of botrytis and other fruit rots.

2.2.5 Summary and conclusions

- Weather conditions were very favourable for development of SPM throughout the trial period to, particularly in the mid-to-late July period which was confirmed by the high risk (consecutive days with risk > 10%) shown by the mildew risk model
- However, the SPM on leaves and fruit remained very low on all the treated plots and there was only one occasion where intervention with fungicide was needed due to a high risk identified by the model and the appearance of new mildew colonies on leaves and flower stalks at a low incidence
- Despite the high risk of SPM development, the level of SPM on leaves was very low; only 2% and 7% of leaf area were mildewed on untreated plots when assessed on 27th July and 15th August, respectively. There was virtually no SPM observed on all other treated plots

- By contrast, SPM mildew on fruit rose rapidly from 2% for the first pick (28th July) to > 90% for the sixth pick (21st August) on untreated plots. On treated plots percentage of fruit with SPM did not rise above 3% with programmes based on BCAs performing as well as routine fungicide programme.
- There were no significant differences in yield and marketable yield between the managed programmes and the routine fungicide programme. However, all treated plots had a significantly higher total yield and marketable yield than the untreated control. Most of the unmarketable fruit was due to infection with powdery mildew. The incidence of rots, primarily Botrytis was very low and similar in all treated plots.
- There were no obvious phytotoxic symptoms observed on foliage or fruit in any of the plots following the spray treatments
- There were no obvious differences in plant vigour (height and spread) between the plots

2.2.6 References

Anon, 1976. Strawberry powdery mildew ADAS Key No 8.1.1. MAFF, Plant Pathology Laboratory, Harpenden, Herts.

Objective 3: Fruit rot complex: Strawberry fruit rot caused by *Pestalotiopsis*

3.1 Background

The fungus *Pestalotiopsis longisetula* Guba can cause strawberry leaf spot and has become a major disease affecting strawberry production in Brazil (Rodrigues et al., 2014). This fungus is believed to also cause fruit rot in Egypt (Embaby, 2007). More recently, research showed that root and crown rot can also be caused by *P. clavispora* (recently renamed as *Neopestalotiopsis clavispora*) in Spain (Chamorro et al., 2016) and by *P. longisetula* in Florida. The crown rot symptoms caused by *Pestalotiopsis* spp. are similar to those caused by *Phytophthora cactorum*. The incidence of *Pestalotiopsis* spp. in strawberry has recently been increasing in Europe and the pathogens are associated with plant mortality after transplanting. In some cases both *Pestalotiopsis* spp. and *P. cactorum* can be detected from the same crown sample, suggesting the potential for a disease complex. The NIAB EMR plant clinic has received numerous samples infected with *Pestalotiopsis* spp. over the last two years and have been curating an isolate collection.

Before we embark on developing diagnostic tools for the new pathogens, we need to prove that they are pathogenic against popular commercial strawberry cultivars and hence can be

a primary pathogen. We report here the pathogenicity tests for several *Pestalotiopsis* isolates on detached leaves and fruit inoculated with either spore suspension or mycelial plugs and *in vivo* tests on whole strawberry plants and attached fruit.

In the survey for *Phytophthora* spp. in year 1 and 2 (SF 157), we observed typical crown rot symptoms in a number of samples but failed to detect *P. cactorum*. These symptoms could be due to frost damage or infection by other pathogens, such as *Pestalotiopsis* spp. Further work is needed to assess the importance of *Pestalotiopsis* spp. in the UK, and this material provides a great opportunity to maximise the value for AHDB funding.

3.2 Materials and Methods

3.2.1 Isolate collection

An isolate collection of *Pestalotiopsis* spp. has been curated at NIAB EMR and represents a diverse range of isolates (Table 3.1) mostly collected through the activities of the NIAB EMR plant clinic. The collection consists of *P. clavispora* as determined by sequencing phylogenetically informative regions of the genome of two representative isolates in the collection (PC26/16_1 and PC26/16_2, Table 3.1).

3.2.2 Detached fruit and leaves

3.2.2.1 Inoculum

Five isolates from the NIAB EMR isolate collection were used (Table 3.1). Fungal cultures were grown on potato dextrose agar (PDA) media and used after 15 days of growth. Both mycelial discs and conidial suspension were used to inoculate fruit and leaves. For the conidial suspension, spore concentration was adjusted using a haemocytometer to a final concentration of $1x10^5$ conidia ml^{-1} with sterile distilled water containing 0.05% Tween 20.

3.2.2.2 Plant material

Strawberry fruit cv. “Redeva Gold” (produce of Egypt, ex. Tesco supermarket) and leaves (cv. “Malling Centenary” grown at NIAB EMR in a glasshouse with no fungicide inputs) were used. Although not tested the strawberry fruit likely had a high number of fungicide residues present and although washing steps were performed some residue would still have been present at the point of inoculation. Plant material was washed in running water and surface disinfected by immersion for 1 min in 95% ethanol and followed by 15 min in a 0.5% sodium hypochlorite solution as per Mouden *et al* (2014). Plant material was then rinsed three times in sterile distilled water and dried in sterile conditions.

Table 3.1 *Pestalotiopsis* isolate collection at NIAB EMR and the isolates used in each experiment.

Isolate	Year Collected	Host Species	Isolates used for			
			Species identity	Detached tissue	Whole plant assay	Attached fruit assay
PC26/16_1	2016	Strawberry	Y	Y		
PC26/16_2	2016	Strawberry	Y	Y	Y	
PC29/16	2016	Strawberry		Y	Y	
PC36/16	2016	Strawberry		Y	Y	
PC38/16_a	2016	Strawberry		Y	Y	
PC38/16_b	2016	Strawberry			Y	Y
R17/17*	2017	Pear				
R71/17*	2017	Strawberry				
PC30/17*	2017	Blueberry				

*These were collected after the experiments were planned/conducted, and hence were not used in experimental studies; The table was to illustrate the collection that is held at NIAB EMR.

3.2.2.3 Inoculation with mycelial disc

The detached fruits and leaves were inoculated with 5 mm mycelial discs of the fungus placed in the middle of the intact, unwounded fruits and leaves. The control treatment was 'mock'-inoculated with PDA medium discs. The inoculated plant tissue was placed aseptically in Perspex containers (16 cm × 28 cm), three fruits or leaves per container. Lids were placed loosely on the container to avoid excessive moisture and to maintain sterile conditions. The containers were incubated at ambient temperature in the laboratory. There were three replicate fruits and leaves for each isolate.

3.2.2.4 Inoculation with spore suspension

The spore suspension prepared in 3.2.2.1 was sprayed onto intact, unwounded fruits and leaves. The control treatment was 'mock'-inoculated with sterilised distilled water. The inoculated plant tissue was placed aseptically in Perspex containers (16 cm × 28cm), three fruits or leaves per container. Lids were placed loosely on the container to avoid excessive moisture and to maintain sterile conditions. The containers were incubated at ambient temperature in the laboratory. There were three replicate fruits and leaves for each isolate.

3.2.2.5 Assessment

Observations were made on the development of disease symptoms. After 15 days, spores from diseased fruits and leaves were aseptically transferred onto PDA plates. The resultant cultures were checked for colony and spore morphology to confirm Koch's postulates.

3.2.3 Inoculation of whole plants

3.2.3.1 Inoculum

Five isolates from the NIAB EMR isolate collection were used (Table 3.1). Four of the five isolates were common with those used in the detached assays (3.2.2) whilst Isolate PC26/16_1 was substituted with PC38/16_b because the latter isolate was found to be a prolific producer of spores in culture required to produce sufficient spores for inoculating whole plants. Fungal cultures were grown on potato dextrose agar (PDA) media. Cultures were placed under black light (UV) following 15 days incubation in white light because there were too few acervuli (spore producing structures). Following a further 15 days under black light only 1 isolate (PC38/16_b) had sufficient acervuli to prepare a spore suspension. Spore suspension was prepared on the day of inoculation; the concentration was adjusted following quantification of the spores using a haemocytometer to a final concentration of $1x10^5$ conidia ml^{-1} with sterile distilled water containing 0.05% Tween 20. A germination test of the suspension showed 65% of spores germinated within 24 hours. The mycelial disk method of inoculation was used for the other 4 isolates – this is because we aimed to study isolate differences. In addition, despite every effort was made to promote sporulation (as described in the text) four of the five isolates did not form sufficient acervuli.

3.2.3.2 Plant material

Strawberry cv. Malling Centenary plants (bare rooted runners) were planted in FP9 pots (0.5 Lt. compost) in a compost mix (Sinclair Pro - Medium Peat: 50%, Medium/Coarse Peat : 25%, Coarse Peat: 15%, and Container bark: 10%) containing Osmocote® on the 13/07/17. Irrigation was delivered via drippers 2 x 30 sec/day. Temperature was not controlled in the glasshouse compartment (i.e. no chillers) but was recorded using EasyLog™ USB loggers. No supplementary light was provided.

3.2.3.3 Inoculation

Inoculations took place on the 14th August. Ten plants were inoculated per isolate, half were wounded and the other half unwounded. Eighteen plants were 'mock'-inoculated half with wounding and the other half without. Wounds were administered by using a scalpel to make a c. 1 cm surface cut at the base of the petiole near the crown. Inoculum (as described in 3.2.2.1) was applied as follows: i) 5 ml of spore suspension was pipetted on and around the crown (ensuring the wound is covered if present), ii) a 5 mm mycelial disk is placed at the wound site (or equivalent position on unwounded plants). [note a non-sterile scalpel was used because the plant tissue was not sterile and neither the environment in which they are grown this is not deemed necessary]. The pots representing different treatments were positioned in a randomised block design across one bench. Immediately after inoculation misting was switched on for 14 h to maintain high humidity following 10 hours without misting on the evening of 15th August misting was switched on again for a further 14 h.

Following 6 weeks (two weeks prior to assessment) of incubation in conditions conducive to healthy plant growth the irrigation was reduced and supplementary heat was applied in order to apply abiotic stress to the plants to facilitate disease expression.

3.2.3.4 Assessment

Eight weeks following inoculation the following assessments were carried out; (i) plant health (of foliage and roots), (ii) crowns cut longitudinally to assess rot symptoms which was assessed binomially (present or absent) and descriptively, (iii) tissue was excised from crown at the leading edge of symptomatic tissue (if present) to be (a) plated on to PDA and (b) frozen for subsequent DNA extraction. Plated tissue sections were incubated at 20°C for up to 14 days. Plates were checked regularly and cultures were identified to the genus level based on morphology.

3.2.4 Inoculation of attached fruit

3.2.4.1 Inoculum

A single isolate from the NIAB EMR isolate collection was used (Table 3.1). PC38/16_b was used because it has a propensity to produce acervuli in culture. The isolate was bulked up on potato dextrose agar (PDA) media for 14 days. Spore suspension was prepared on day of inoculation; the concentration was adjusted using a haemocytometer to a final concentration of $1x10^5$ conidia ml^{-1} with sterile distilled water containing 0.05% Tween 20. A germination test of the suspension showed 71% of spores germinated within 24 hours.

3.2.4.2 Plant material

Plug plants of cultivar BAM (a coded everbearer cultivar) were planted in June and grown in peat/coir bags. This cultivar was used because the experiment was carried out at the end of the season (see section below for dates) and they were producing all the phenological stages (flowers, green fruit and red fruit) at the time of the trial. The crop was grown as per untreated controls described in 2.2.2.1.

3.2.3.3 Inoculation

All inoculations took place on the 6th September. Fruit at each stage was marked (as red, pink and green berries and flowers). Control and inoculated fruit were paired ensuring a common developmental stage. Half of the marked berries/flowers were inoculated with a spray bottle to run-off with inoculum prepared in 3.2.3.1. Immediately after inoculation berries/flowers were bagged to maintain humidity (Photo 3.1). Control fruit was 'mock'-inoculated with sterile water and bagged as for inoculated fruit. Bags were removed after 24 hours.

Photo 3.1 Experimental setup for attached fruit assay showing the marked flower prior to inoculation/ mock inoculation and an inoculated fruit following inoculation with the bag fastened to maintain humidity.

3.2.4.4 Assessment

Fruit was harvested when ripe and incubated in individual moisture chambers for 7 days. The rots that developed were identified by morphology and recorded.

3.3 Results

3.3.1 Detached fruit and leaves

All five *Pestalotiopsis* isolates caused symptoms on inoculated strawberry fruit and leaves. Re- isolations from the infected tissue proved that symptoms were caused by *Pestalotiopsis* thus satisfying Koch's postulates. Despite thorough surface sterilisation prior to inoculation

and incidental fungicide residues being present on the detached fruit used in these assays, Botrytis and *Pestalotiopsis* often occurred together (Photo 3.2) suggesting a high incidence of latent Botrytis infection in the fruit, however, *Pestalotiopsis* was never identified on the control fruit.

Circular, brown and slightly sunken spots appeared on the fruits inoculated with mycelial discs and conidial suspension seven days after inoculation. The infected tissues were initially, discoloured, showing pale brownish colour and softening, then were covered with dense aerial mycelium within 10 days of inoculation.

Irregular dark brown zones developed on the surface of inoculated leaves within 15 days of inoculation and mycelium became evident, in some cases covering the whole leaf.

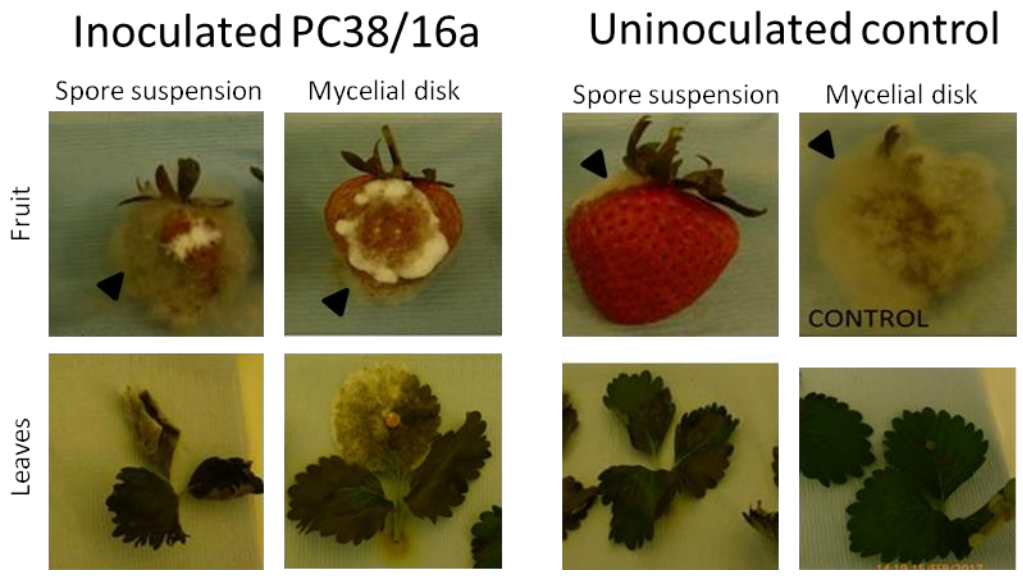


Photo 3.2 Representative pictures of artificial inoculations of detached fruit (top) and leaves (bottom) using either a spore suspension (left) or mycelial disk (right) infection assay. Figure shows one of the five isolates, PC38/16a (left panel) compared to the un-inoculated control (right panel). Botrytis (Black arrow head) is evident on the incubated fruit.

3.3.2 Inoculation of whole plants

Assessments were conducted 8 weeks following inoculation. The health status of the foliage, roots and crowns were described (Table 3.2). Foliar symptoms were described as healthy (turgid leaves) or unhealthy (wilted) and were measured to determine the health status of the crown. Sixty percent of the plants were wilted but there was no correlation between wilting and either treatment (inoculated vs control) or crown health. All samples had a healthy root system. When the crowns were cut longitudinally they were assessed for rot symptoms; 83% of the plants exhibited a rot. Crown rots were dark brown and well defined, usually confined

to the centre of the crown but sometimes spreading towards roots or petioles. Isolations on to PDA were made for a subset of the rots; because of the cost issue only three of five replicates for each inoculated treatment and all eight control treatments. Overall *Pestalotiopsis* was isolated from only 12% of the samples (four inoculated and three control plants). *Fusarium* was the most common fungus to be isolated with 43% of the samples positive for *Fusarium* overall (23 inoculated and three control plants). 'Secondaries' (such as yeasts and *Penicillium* which alone cannot cause crown rot but do colonise dead tissue accounted for 13% of the isolations from rotten crown samples overall (four inoculated and four control plants). A low level of *Phytophthora* was recorded by LFD but none was isolated.

3.3.3 Inoculation of attached fruit

The majority of fruit rots were caused by *Botrytis* (71%), followed by soft rots (*Mucor* and *Rhizopus*; 20%) and *Pestalotiopsis* (10%, Table 3.3). *Pestalotiopsis* was not recorded on control fruit so despite the low levels of *Pestalotiopsis* recorded on inoculated fruit the fact that none was recorded on control fruit suggests that there was a very low/no background level of *Pestalotiopsis* and that the *Pestalotiopsis* that was recorded was a result of inoculation. Of the flowers that were inoculated that went on to produce fruit (n=4), one of the fruit developed a *Pestalotiopsis* fruit rot. Fruit inoculated at green and pink stages resulted in one and two fruit recorded as *Pestalotiopsis* fruit rot respectively. No *Pestalotiopsis* fruit rot was recorded on fruit inoculated when ripe (red).

Table 3.2 Plant health assessment and isolation results of the whole plant pathogenicity assay.

Isolate	Wounded or unwounded	Replicate no.	Inoculation Method	Foliage (h/u)[1]	Roots (h/u)[1]	Crown (h/u)[1]	Isolation result
PC38/16	W	1	Spore suspension	u	h	u	Secondaries
PC38/16	W	2	Spore suspension	u	h	u	*Fusarium* Sp.
PC38/16	W	3	Spore suspension	u	h	u	*Fusarium* Sp.
PC38/16	W	4	Spore suspension	h	h	u	~
PC38/16	W	5	Spore suspension	u	h	u	~
PC38/16	U	1	Spore suspension	u	h	u	Secondaries
PC38/16	U	2	Spore suspension	u	h	u	*Fusarium* Sp.
PC38/16	U	3	Spore suspension	h	h	u	Secondaries
PC38/16	U	4	Spore suspension	u	h	u	~
PC38/16	U	5	Spore suspension	h	h	u	~
PC26/16/2	W	1	Mycelial disc	u	h	u	*Fusarium* Sp.
PC26/16/2	W	2	Mycelial disc	u	h	u	*Pestalotiopsis* Sp. and *Fusarium* Sp.
PC26/16/2	W	3	Mycelial disc	u	h	u	*Fusarium* Sp.
PC26/16/2	W	4	Mycelial disc	h	h	u	~
PC26/16/2	W	5	Mycelial disc	u	h	u	~
PC26/16/2	U	1	Mycelial disc	h	h	u	*Pestalotiopsis* Sp.
PC26/16/2	U	2	Mycelial disc	h	h	u	*Fusarium* Sp.
PC26/16/2	U	3	Mycelial disc	u	h	u	*Fusarium* Sp.
PC26/16/2	U	4	Mycelial disc	u	h	u	~
PC26/16/2	U	5	Mycelial disc	u	h	u	~
PC29/16/1	W	1	Mycelial disc	h	h	u	Secondaries
PC29/16/1	W	2	Mycelial disc	u	h	u	*Fusarium* Sp.
PC29/16/1	W	3	Mycelial disc	u	h	u	*Fusarium* Sp.
PC29/16/1	W	4	Mycelial disc	h	h	u	~
PC29/16/1	W	5	Mycelial disc	h	h	u	~
PC29/16/1	U	1	Mycelial disc	h	h	h	*Pestalotiopsis* Sp.
PC29/16/1	U	2	Mycelial disc	u	h	h	*Pestalotiopsis* Sp.
PC29/16/1	U	3	Mycelial disc	h	h	u	*Fusarium* Sp.
PC29/16/1	U	4	Mycelial disc	u	h	u	~
PC29/16/1	U	5	Mycelial disc	u	h	u	~
PC36/16	W	1	Mycelial disc	h	h	u	*Fusarium* Sp.
PC36/16	W	2	Mycelial disc	h	h	u	*Fusarium* Sp.
PC36/16	W	3	Mycelial disc	h	h	u	*Fusarium* Sp.
PC36/16	W	4	Mycelial disc	u	h	u	~
PC36/16	W	5	Mycelial disc	u	h	u	~
PC36/16	U	1	Mycelial disc	h	h	u	*Fusarium* Sp.
PC36/16	U	2	Mycelial disc	u	h	u	*Fusarium* Sp.
PC36/16	U	3	Mycelial disc	u	h	u	*Fusarium* Sp.
PC36/16	U	4	Mycelial disc	h	h	h	~
PC36/16	U	5	Mycelial disc	h	h	h	~
PC38/16	W	1	Mycelial disc	h	h	u	*Fusarium* Sp.
PC38/16	W	2	Mycelial disc	h	h	h	*Fusarium* Sp.
PC38/16	W	3	Mycelial disc	u	h	u	*Fusarium* Sp.
PC38/16	W	4	Mycelial disc	u	h	u	
PC38/16	W	5	Mycelial disc	u	h	u	~
PC38/16	U	1	Mycelial disc	h	h	u	*Fusarium* Sp.
PC38/16	U	2	Mycelial disc	h	h	u	*Fusarium* Sp.
PC38/16	U	3	Mycelial disc	u	h	h	*Fusarium* Sp.
PC38/16	U	4	Mycelial disc	u	h	h	~
PC38/16	U	5	Mycelial disc	u	h	u	~
Uninoculated	W	1	Sterile Water (Control)	h	h	u	*Pestalotiopsis* Sp.
Uninoculated	W	2	Sterile Water (Control)	u	h	u	Secondaries
Uninoculated	W	3	Sterile Water (Control)	h	h	h	Secondaries
Uninoculated	W	4	Sterile Water (Control)	u	h	u	*Pestalotiopsis* Sp.
Uninoculated	W	5	Sterile Water (Control)	u	h	u	Secondaries
Uninoculated	U	1	Sterile Water (Control)	u	h	u	*Fusarium* Sp.
Uninoculated	U	2	Sterile Water (Control)	u	h	h	Secondaries
Uninoculated	U	3	Sterile Water (Control)	u	h	h	*Pestalotiopsis* Sp.
Uninoculated	U	4	Sterile Water (Control)	u	h	u	*Fusarium* Sp.
Uninoculated	U	5	Sterile Water (Control)	h	h	u	*Fusarium* Sp.

[1] An assessment of tissue health was made for each plant and recorded as healthy (h) or unhealthy (u).

Table 3.3 Post-harvest rots that developed following the attached fruit pathogenicity assay.

Inoculation	Stage at which inoculated	Total	Post-harvest rots (7 days post-harvest)		
			Botrytis	Soft rots	*Pestalotiopsis*
Control	Flower	5	4	0	0
	Green	7	6	0	0
	Pick	5	3	2	0
	Red	2	1	2	0
Inoculated	Flower	4	3	0	1
	Green	8	7	0	1
	Pick	8	5	2	2
	Red	2	0	2	0

Table 3.4 A summary of the pathogenicity tests conducted and the incidence of infection.

Assay	% inoculated samples infected with *Pestalotiopsis*	% control samples infected with *Pestalotiopsis*
Detached fruit	67%	0%
Detached leaf	86%	0%
Whole plant	8%	30%
Attached fruit	10%	0%

3.4 Discussion

The genus of *Pestalotia* contains many species which occur as pathogens, endophytes and saprophytes across the plant kingdom (Jeewon et al., 2004). Recently there have been reports of a *Pestalotiopsis* species affecting strawberry crops in Europe causing root and crown diseases which have been associated with poor plant establishment and mortality following transplanting. Isolates were collected and identified using molecular techniques to species level as *P. clavispora.* Pathogenicity tests were then conducted to 1) determine the importance of this disease to the UK strawberry industry and if found to be important 2) provide standard controlled environment protocols which could be used to further our understanding of this disease (e.g. cultivar susceptibility, pathogen population structure including virulence, fungicide choice and agronomic practices which may lead to susceptibility).

A range of pathogenicity tests that were conducted in this study (Table 3.4) show that the *Pestalotiopsis* isolates that were used cannot be considered as virulent plant pathogens. It may be speculated that the isolates colonise the plant as either weak pathogens (an organism that requires a compromised host, either by abiotic or biotic factors, in order to establish infection) or a saprophyte (an organism that derives its nutrition from dead or dying

(senescing) tissue). This is based on; 1) the detached leaf and fruit tests which show that *Pestalotiopisis* is capable of colonising host tissue when the conditions are favourable (detached sensescing tissue and optimum humidity and temperature); 2) the attached fruit assay and whole plant assay which despite favourable conditions (high inoculum applied to host tissue and optimum humidity and temperature provided to establish infection), low levels of infection were recorded. This may suggest the fungus is less successful at colonising living host tissue which can defend itself.

It was surprising that we were able to isolate *Pestalotiopisis* from 30% of control 'whole' plants (Table 3.4). Control plants were separated from the inoculated plants to ensure that the inoculum would not contaminate the control plants. The plant material used was bare rooted strawberry plants and as such was not classified as high health status. Using runner plants it would be expected that a background level of incoulfection and controls are used to determine background levels of disease. It would however be anticipated that inoculated plants would have greater levels of infection. At this moment, it is difficult to explain why less infection was observed in the inoculated plants than in the control.

The whole plant pathogenicity test conducted in the current study did not result in high enough infection rates to draw any conclusions on relative isolate virulence and the relative success of inoculation method (wounded vs. unwounded and spores vs. mycelial disks). The low incidence of disease establishment in inoculated plants may have been a result of the plants being actively growing at the point of inoculation (plants were potted 2 weeks prior to inoculation) and being too healthy during the initial infection period enabling the host to successfully defend itself against the weakly pathogenic fungus. The wounding used in the current pathogenicity test was at the base of the petioles, based on our experience with other crown rot pathogens, whilst the Belgian group directly wounded the crown which may explain why infection was more successful in their study. The occurrence of *Pestalotiopsis* in the control plants suggest that there could have been background levels of the disease in the planting material used in the experiment as the possibility of cross contamination of inoculum from the inoculated plants is ruled out due to the experimental design. There was a high level of crown rot, mostly attributed to *Fusarium* rather than *Pestalotiopsis*, recorded across the experiment. *Fusarium* species are ubiquitous in strawberry tissue and often isolated from non-symptomatic tissue and assumed to be an endophyte/latent saprophyte. The rot symptoms may have been caused by a more pathogenic species/isolate of *Fusarium* or may have resulted from another biotic/abiotic factor leading to crown rot and subsequent colonisation by the *Fusarium* e.g. frost/cold damage pre/during cold storage.

From the studies carried out by other groups, together with the results we have obtained above, the *Pestalotiopsis* genus does have the capacity to be pathogenic, albeit weakly, on

strawberry. The reasons for the reduction in successful infection that we experienced in whole plants can be summarised as: 1) virulence of **isolates** used in this study, although several isolates were used from a wide range of sources which are known to have caused disease (i.e. they were isolated from diseased plants). 2) other studies used different **cultivars** (widely grown cultivars from their region), we used Malling Centenary in the whole plant inoculation because of the availability of this variety and because we know that it has been susceptible to disease attributed to *Pestalotiopsis* in the field, 3) lack of appropriate wounding sites and 4) health of plants seems like the most likely discrepancy between the assay described here and that in other studies (e.g. Ceustermans *et al.* 2016) in which wounded plants were potted up straight from the cold store and inoculated, giving the fungus a window of infection during which the host's defences are compromised.

3.5 Conclusions

- The species present in the UK is *Pestalotiopsis clavispora*
- A detached fruit and leaf pathogenicity assay has been established and demonstrates that the fungus is weakly pathogenic, corroborating studies from other groups.
- An attached fruit pathogenicity assay showed that infection was possible at various developmental stages including flower infection
- A whole plant pathogenicity assay failed to achieve sufficient levels of infection; *Pestalotiopsis* was isolated from 30% of control plants. The plants used were not of the highest health status therefore it is not possible to draw meaningful conclusions from this particular assay.

3.6 Ongoing research

Currently we continue to conduct studies on (1) validating molecular primers for *Pestalotiopsis*, (2) once validated, using the primers to screen the DNA from the survey samples from 2015 and 2016, and (3) carrying out a cold storage experiment [similar to the ongoing *P. cactorum* experiment] .

Objective 4: To evaluate the effects of individual and combined use of alternative products against *Verticillium* wilt of strawberry

4.1 Introduction

Managing strawberry wilt (*Verticillium dahliae*), which persists in soil and can reduce yields by 75%, using biofumigants crops has been investigated previously in AHDB SF 77 at East Malling Research. Biofumigation refers to the suppression of soil-borne pests and pathogens by naturally occurring compounds. Bio-Fence (which releases isothiocyanates) has been shown to reduce levels of Verticillium wilt inoculum and the viability of spores. The research proposed will utilise the Bio-Fence granules product on a commercial crop of strawberries to see both the effect on the development of plant wilt and any phytotoxicity that might arise.

Other work has reviewed the benefits of incorporating organic material into the soil for the suppression of plant pathogens by encouraging the build-up of beneficial microbes. Various AHDB projects are assessing the benefits of the incorporation of organic material on soil health in vegetable and arable crops. Anaerobic digestate solids is one source of organic material that has become widely available across the UK using feedstock of vegetable wastes or maize crop and it is proposed to use a maize and vegetable crop-based stock in the current work. This will have been pasteurised and be certified to PAS110 standards in order to fall within the recommendations for Red Tractor Fresh Produce Assured strawberries.

Work with various biopesticides showed there could be a 77% reduction in white rot sclerotia by a Serenade ASO soil drench and it is hypothesised that it might thus have activity against Verticillium microsclerotia. This is supported by USA label recommendation of this product to be applied to newly rooting plants against Verticillium and other soil-borne pathogens in strawberries and the UK suppliers (Bayer CropScience Ltd.) agree to its inclusion in this work. The product has UK approval for foliar application against Botrytis on protected strawberry crops and EAMU 0706 of 2013 on outdoor strawberries as an overhead spray at the same rate of 10 L/ha in up to 1000 L of water, with up to twenty applications a year. EAMU 0705 of 2013 permits a single drench on outdoor crops including bush and cane fruit at 10 L/ha by hydraulic sprayer or drip irrigation in up to 1000 L of water.

The product will also be tested following the use of Bio-Fence as various publications have shown that there can be a benefit in the microbes lost following soil sterilisation being replaced with beneficial microbes.

4.2 Methods and Materials

An experiment was set up at a strawberry farm in Oxfordshire to compare the use of anaerobic digestate solids, a potential bio-fumigant and a biofungicide in maintaining the plant growth and yield of an outdoor crop of the moderately susceptible strawberry variety, Symphony, in a field with a known history of Verticillium wilt, and to assess any phytotoxicity resulting from these soil treatments. Work commenced in September 2016 and assessment will finish in September 2018 (Table 4.1).

Table 4.1. Planting, sampling, treatment application and assessment dates at the Oxfordshire outdoor soil-grown strawberry site.

Date	Activity
15.09.16	Soil sampled for Verticilium and nutrients.
06.06.17	Strawberry plants planted.
27.04.17	Soil re-sampled for nutrient analysis after base dressing.
23.05.17	Soil sampled in all 24 plots and stored in case of a need for Verticillium.
23.05.17	Anaerobic digestate solids spread over beds on T2 plots.
24.05.17	Bio-Fence pellets applied to T3 and T5 plots. All beds rotavated.
26.05.17	Water sprayed onto Bio-Fence plots. All beds sealed in plastic sheet.
01.06.17	All plots ventilated by making planting holes in sheeting.
06.06.17	Cold-stored bare-root runners of cv. Symphony planted. Irrigation
12.06.17	Phytotoxicity assessed. Serenade ASO drenched over plants of T4 and
16.06.17	Phytotoxicity and plant establishment assessed
29.06.17	Phytotoxicity and plant establishment assessed. Soil in some plots
18.07.17	Fruit numbers per plant noted prior to first of two picks.
06.09.17	% of plants with wilted or totally collapsed foliage.
11.10.17	% of plants wilting (Verticillium) and total plants alive.
15.01.18	Observation of foliage growth.

4.2.1 Experimental design and treatment

A Latin square design was used (**Figure 4.1**), to allow for potential variability in the soil and Verticillium microsclerotia numbers down the beds and across the field, with five replicate blocks (each bed forming a block). The trial area was within a commercial strawberry crop of the same variety, planted at the same time.

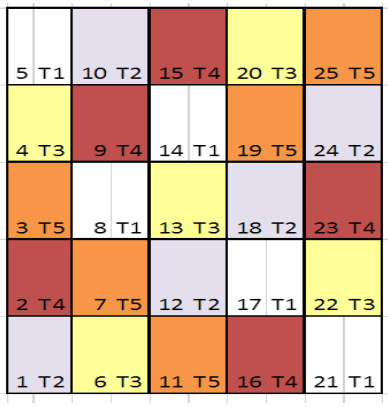


Figure 4.1. Oxfordshire 2017: Layout of 6 m long plots, with beds running plot 1 to 5 etc., each with two rows of strawberries. In this Latin Square design each treatment is present in each bed and in each 6 m band up the beds.

Four treatments were carried out and a plot in each replicate left untreated (**Table 4.2**). The experiment was conducted at Stanton St John in Oxfordshire where the soil after harvest of the preceding barley crop in 2016 contained 2.6 to 5.6 *Verticillium* propagules/g soil. Figs. 4.2-4.10 show treatment application, crop development, and wilt.

Table 4.2. Materials applied to plots before and after planting cv. Symphony cold-stored strawberry runners on 6 June 2017 in a Verticillium infested field in Oxfordshire

Code	Product	Ingredients	Rate per ha	Application method
T1	None	N/a		
T2	Anaerobic digestate solids (pasteurised PAS 110)	Chopped maize and vegetable crop waste	50 tonnes	Spread then incorporated up to 150 mm depth then covered
T3	Bio-Fence pellets	*Brassica carinata* meal	2000 kg	Spread then incorporated up to 150 mm depth, irrigated then covered
T4	Serenade ASO*	*Bacillus subtilis* strain QST 713	10 L in 1000 L water	Single nozzle directed 40 ml over each plant (0.4 ml concentrate)
T5	Bio-Fence pellets Serenade ASO	*Brassica carinata* *Bacillus subtilis*	2000 kg 10 L in 1000 L water	As for T3 and T4; pre-planting incorporation then plant drench

* Applied as an over-plant drench under experimental permit COP 2016/00922. EAMU 0706 of 2013 permits the same 10 L /ha in 1000 L/ha water as a spray to outdoor strawberries

Figure 4.2 23 May 2017; adding soil amendments.

Figure 4.3 23 May; rotavating the soil to incorporate soil amendment.

Figure 4.4 Laying down irrigation piping.

Figure 4.5 Strawberry beds on 12 June 2017 (planted 6 June).

Figure 4.6 Straw addition during fruiting.

Figure 4.7 29 June; scorch damage: temperatures > 30° C after planting.

Figure 4.8 18 July 2017 fruiting.

Figure 4.9 11 October 2017; beginning of Verticillium wilt symptoms.

Figure 4.10 11 October; Verticillium wilt early symptoms: close-up.

4.2.2 Soil sampling for Verticillium

The trial area was sampled for viable *Verticillium dahliae* microsclerotia in August 2016 after the harvest of a barley crop. Core samples were taken at 150 mm depth according to a standard procedure, dividing the potential location of the experiment into three sampling bands up from the grass headland with 1 kg of soil tested from each. Harris tests were performed to confirm the level of viable inoculum in the soil before siting the experiment in this field.

Verticillium propagule density differs across fields and this was why a Latin Square design was used so that each of five beds had all five treatments, but that each treatment never had the same position down the beds. Plot soil samples were taken pre-treatment to allow potential quantification of Verticillium levels in the soil if required to compare with those that could be carried out at experiment termination in September 2018. Soil sampling was carried out in each plot immediately prior to any treatment application on 24 May 2017. Between 30 and 40 cores were taken per plot until the required sample weight of at least one kilogramme was obtained. The samples are being cold-stored at ADAS Boxworth to potentially allow the

calculation of viable propagule numbers by Harris test to add to the information to be gained from the visible symptoms of Verticillium in the plants.

4.2.3 Nutrient analysis of soil and digestate

The soil was sampled across the trial area at three depths; 0-300 mm, 300-600 mm and 600-900 mm in September 2016. Analysis was carried out of dry matter %, nitrate, ammonium and available Nitrogen (N), pH and Phosphate (P), Potassium (K) and Magnesium (Mg) index and available P, K and Mg.

A further set of samples to determine the soil nitrogen status was taken on 27 April 2017 just after the grower had applied base dressing of 800 kg per hectare of 9:20:30 N:P:K i.e. 72 kg/ha Nitrogen, 160 kg/ha P and 240 kg/ha of K. Sampling was carried out taking from 0-300 mm depth i.e. the soil layer where over-winter depletion and then spring application of fertiliser would have produced changes. The use of base dressing meant that it was not necessary to fertigate the strawberry crop and trial area during the first year. Although nitric acid is in use on the host farm for water destined for dripper irrigation (in order to prevent limescale build up) it is not used for water sent to the drip tape in use on the strawberry and other field crops.

A sample of the anaerobic digestate solids obtained from G's Growers' May Farm digester in Cambridgeshire was taken on delivery of the load on 18 April 2017 and received at the analytical laboratory on 3 May 2017. It was analysed for Total N, P, K Mg and sulphur, pH, dry matter %, ammonium nitrogen and organic carbon. The digester operates to produce material to pasteurised PAS 110 standards, with testing carried out to certify that in particular potentially toxic elements, physical contaminants and human and animal indicator species (Salmonella and *E.coli*) are absent or below upper limits. The digestate was moved to an open crate in a barn at the farm where the experiment was to be sited.

On 29 June 2017, after a number of plants in two plots of different treatments were seen with leaf scorch at the headland end of the field, further soil samples were taken from all plots across the headland end for analysis of Phosphorus, Potassium and Magnesium levels as excess of any of these had the potential to scorch plants. It was considered that one explanation of the scorch could have been uneven spreading of base dressing by the farm.

4.2.4 Bed set-up & maintenance

Beds were formed by the host grower using damp soil in the week before the first experimental treatment applications on 23 May 2017. Each bed was 1.7 m from wheeling furrow centre to wheeling furrow centre, with beds raised 0.25 m above the 0.4 m wide furrow. Bed tops were 0.55 m wide.

Before planting on 6 June 2017, the farm-crop beds received chloropicrin treatment on 25 May 2017 through the drip tapes under a polythene weed-suppression mulch sealed over the bed ridges. However, this soil sterilisation treatment was withheld along the whole length of the five beds to be used for the experiment i.e. including before and after the experiment plots in each bed.

Prior to planting, cuts were made in the polythene mulch in two rows at 0.46 m spacing (18 inch) (Figure 4.5). Bare root cold-stored runners of cv. Symphony were planted singly in each hole, followed by the standard practice of fine-droplet overhead irrigation for a week until the plants became established in addition to irrigation through plastic tapes under the polythene mulch. Planting was continuous across the treatment plots and guard strip, without any solid barriers between plots. Plants in the experiment and the farm crop were planted by the farm staff through holes made in the polythene. The experimental treatment applications were timed so that they preceded the chloropicrin treatment of the farm-crop so that the trial plots could be planted at the same time as the commercial crop.

Subsequent husbandry (Appendix 1), including chemical application as required against pests and foliar diseases, hand-weeding, strawing to protect the fruit (**Figure 4.6**) fruit harvesting and runner removal were carried out across the whole field at the same time.

4.2.5 Experimental product application

The five plots in each bed were marked out as 7 m long, with 6 m to be treated, leaving 0.5 m as untreated guards at either end (thus 1 m between neighbouring plots in a bed). Treatment calculations were based on 1.7 m width furrow to furrow by 6 m long to give a surface area of 10.2 m². This was based on the assumption that under commercial practice the incorporated materials (digestate and Bio-Fence granules) would be spread over the whole field surface before ridging-up, rather than experimentally having to keep the materials confined within plots.

4.2.5.1 Digestate solids

Following base dressing the trial site already had 266 kg available N in the top 300 mm in a sample taken on 27 April 2017. The host farm is not subject to Nitrate Vulnerable Zone (NVZ) regulations. Application of digestate solids for Treatment 2 at a standard rate of 50 tonnes/hectare (5 kg/m²) would be expected to provide in the region of 250 total N. At 5 kg/m² application rate, each plot treatment area of 10.2 m² required 51 kg of digestate across the 6 m length of each plot area to be treated. The digestate looked like moist slightly composted coarse chopped straw and smelt pleasantly of straw. On 23 May 2017, the required mass of digestate solids was scattered as evenly as possible across the bed width, so that loosely

spread it was on average 50 mm deep. For the purposes of the experiment it was flattened down by hand to stop it falling off the bed, so resulting in a minimum depth of 38 mm (**Figure 4.2**). Incorporation was made the next day (18 hours after spreading) as detailed below. This interval before incorporation was due to logistics in order that the farm staff could polythene cover all plots at the same time i.e. directly after the BioFence had been irrigated (see below).

4.2.5.2 Bio-Fence

The Bio-Fence application rate is recommended to fall within 2000 to 3000 kg per ha (200 to 300 g/m²) if worked in to 150 mm (Tozer seeds, Alec Roberts, pers. comm.). The field had a high level of available nitrogen already and so the lower rate was selected. Based on the 6% Nitrogen value stated by the Bio-Fence supplier 200 g/m² would be expected to provide 120 kg N per ha. Therefore for Treatments 3 and 5 taking the area to be treated as 10.2 m², calculated wheeling to wheeling furrow, each plot required 2.04 kg of Bio-Fence granules. On 24 May 2017 the Bio-Fence pellets were scattered by (gloved) hand across the width of the central 6 m length of each bed to be treated. This gave a ca. 40% coverage of Bio-Fence pellets of the top of the bed. On the same day the pellets were then rotavated into the soil. Incorporation was achieved to a depth of around 150 mm using a hand-guided self-propelled 2-spindle Honda F220 rotavator with 170 mm blade lengths from spindle shaft (**Figure 4.3**).

All plots of the five treatments (including the untreated) and the between-plot guards were cultivated at the same time by running the rotavator along the top of each bed length. A record was made of the direction of travel of the rotavator and checks made to determine the extent of any movement of products beyond the intended treated area. Plot ends were marked outside the area of rotavation and the markers re-instated afterwards.

Two lengths of “leaky hose” flat irrigation pipe were laid out down each bed to supply the two lines of strawberries when planted (**Figure 4.4**). They were later covered by the polythene mulch.

On 26 May 2017 the farm staff used a sprayer to apply 150 mm depth of irrigation water to activate the Bio-Fence plots before covering them straight away. It had not been possible to cover the plots with polythene until the morning of 26 May 2017 (the supplier had failed to deliver the farm supply on schedule). There was no rain in the period before covering and the soil was dry when the pellets were applied. Polythene sheet was used to cover the beds as standard practice in order to supress weeds and reduce future soil contamination of fruit. It was fixed into the bed ridge sides using the soil from the bottom of the ridge. All plots in the experiment were covered at the same time, with one continuous sheet down each bed, dug into the soil at the top and end of each bed.

On 1 June 2017, on the seventh day after activation and covering, the Bio-Fence treated plots and all the other plots were ventilated by making planting slits in the polythene. The farm

chloropicrin-treated beds were also ventilated. On 6 June the whole field together with the experiment area was planted and irrigated (as detailed above).

4.2.5.3 Serenade ASO drench

On 12 June 2017, once the strawberry plants were becoming established, application was made of the Serenade ASO drench for Treatments 4 and 5. This was to enable colonisation of *Bacillus* on roots that had developed after planting following cold storage of the plants (as suggested by the product suppliers, Bayer CropScience, Dorin Pop, pers. comm.).

Application rates were based on the 10 L/ha rate and the maximum water volume of 1000 L/ha given under EAMU 0705 of 2013 for an outdoor cane and bush fruit drench of Serenade ASO. The volume of diluted product that could be applied across the whole bed was shared between the 27 plants within the centre 6 m plot length, resulting in 0.37 ml Serenade ASO per plant within 40 ml of spray volume per plant (delivered using a calibrated eight seconds of delivery time per plant). Application was by using an Oxford gas-assisted hand sprayer with a single nozzle lance directed over and around the crown of each plant in order to reach the soil beneath the slit in the polythene mulch. Further movement of the product into the soil around the roots was facilitated by the leaky-hose irrigation in operation under the polythene mulch across the field.

4.2.6 Environmental monitoring

A temperature and humidity logger within a ventilated white screen was set out on the bed beside the experimental plots on 23 May before application of the digestate at 14:00 h on 23 May and after completion of rotavation it was moved to the centre of plot 13.

Soil temperature data loggers were placed in the trial area at a depth of 100 mm at 12.10 h after rotavation of the beds commenced at 11:10 h on the 24 May 2017. Loggers were placed in the middle of the experiment area in the centre of the bed width of plots No. 8, 13 and 18 in order to record within the Untreated, Bio-Fence and Anaerobic Digestate (A.D.) treatments, respectively. They were each attached to a coloured string to aid location and retrieval.

Records were also made of the weather during product applications.

4.2.7 Plant assessments

The plants in the centre 6 m of each plot (two rows) were assessed for wilt development (Table 4.3). The assessed plants were five plants in from the start of each treated area (i.e. three and two plants per row), which (according to how the dividing line fell) gave 27 or 28 plants to assess. Photographs were taken at each visit of plants representative of those being

assessed. Regression analysis was used to compare the proportion of plants that were damaged or poorly established in each treatment.

Table 4.3 Assessments of plant establishment, phytotoxicity and development of Verticillium wilt

Date	Assessment
16.06.2017	Phytotoxicity & establishment (No. plants wilting or scorched) 10 days after planting
29.06.2017	Phytotoxicity & establishment (No. plants scorched or dying) 14 days after drench
18.07.2017	Average (modal) number and mean highest number of fruit per plant (visual)
06.09.2017	Establishment & first signs Verticillium wilt (No. plants weak, wilting or dead)
11.10.2017	Verticillium wilt (No. plants with any wilt) & Total plants established

The bare-root cold-stored plants were not expected to produce many fruit in their first year, and plants were likely to be naturally highly variable, and so plot yields were not intended to be recorded to compare between treatments.

4.3 Results

4.3.1 Soil nutrient analysis

Soil samples taken on 15 September 2016 dividing the proposed experiment area into three bands at increasing distance from the headland (progressing in the direction of future beds) showed that a high level of viable *V. dahliae* propagules were present. The Harris tests showed that the levels decreased into the field, with row 1 having 5.6 propagules/g soil, row 2 having 3.6 propagules/g soil and row 3 having 2.6 propagules/g soil. In soil with a level of 2.1 to 5.0 propagules/g of soil a susceptible strawberry variety would have a high chance, and a moderately susceptible variety a medium chance, of wilt occurring at economically significant levels.

Soil sampled on 15 September 2016 was analysed for nitrogen and dry matter (**Table 4.4**). The soil was confirmed to be a sandy loam comprised of 72% sand, 13% silt and 15% clay. In the field there was a noticeable scattering of large stones. The soil pH was determined as 7.8. There was 19.2 mg/L Phosphorus, 186 mg/ L Potassium and 41 mg/ L Magnesium available (equivalent to indices of 2 P, 2+ K and 1 Mg).

Table 4.4. Analysis of soil cores taken at 300 mm deep intervals down to 900 mm on 15 September 2016 from the Oxfordshire site proposed for the experiment in 2017. Result are calculated on a “dry matter” basis.

Sample depth	Dry matter % w/w	Nitrate N (+) mg / kg	Ammonium N (+) mg / kg	Available N (+) 30 cm profile kg N / ha
0 - 300 mm	87.9	28.12	0.79	108.4
300–600 mm	88.9	12.47	0.50	48.6
600–900 mm	91.3	12.81	0.52	50.0

After application of fertiliser base dressing across the field by the grower the 0 to 300 mm of soil when re-sampled on 27 April 2017 showed that available nitrogen had increased (**Table 4.5**) and it would not be necessary to apply fertigation through the irrigation lines during the first year of cropping.

Table 4.5 Analysis of soil sample to 300 mm deep on 27 April 2017 prior to treatment application and strawberry planting at the Oxfordshire site

Sample depth	Dry matter % w/w	Nitrate N (+) mg / kg	Ammonium N (+) mg / kg	Available N (+) 30 cm profile kg N / ha
0 - 300 mm	89.2	62.35	8.75	266.6

The soil analysis carried out from samples taken on the 29 June 2017 following concerns of scorch showed no appreciable difference in potassium (K) or magnesium (Mg) levels between the plots of the same experimental treatments in nearby plots with leaf scorch (plots 1 and 6) and without (plots 12 and 22), but the Phosphorus (P) index was 4 rather than index 3 (**Table 4.6**). The nutrient levels in the Serenade ASO treated plot (T4) were similar to those in the untreated plot (T1). The two Bio-Fence plots (T3) had higher amounts of the three elements than the untreated, with no difference resulting from the subsequent use of Serenade ASO in the T5 plot. Digestate solids (T2) application had resulted in the greatest increase in all three elements.

Table 4.6 Analysis of top 300 mm of soil for pH, Phosphorus (P), Potassium (K) and Magnesium (Mg) following sampling on 29 June 2017. *Plots 1 + 6 showed signs of scorch.

Plot	Experimental Treatment	pH	Index			Mg / L available		
			P	K	Mg	P	K	Mg
21	Untreated (T1)	7.5	3	3	1	35	269	38
1*	Digestate solids (T2)	7.4	4	4	2	53	533	62
12	Digestate solids (T2)	7.8	3	4	2	42	536	70
6*	Bio-Fence (T3)	7.1	4	3	2	46	352	66
22	Bio-Fence (T3)	7.0	3	3	2	41	354	56
16	Serenade ASO (T4)	7.6	3	3	1	32	283	42
11	Bio-Fence then Serenade ASO (T5)	7.2	3	3	2	44	330	51

After a further visit to the field on 31 July 2017 it was seen that there were some lengths of row throughout the farm crop, not just in the experiment area that had failed to establish. The planting day and following days had been very hot and it was probable that, even though irrigation had been given by the grower, where plants had been put into a more stony area of land that roots had failed to establish (**Figure 4.11**)

4.3.2 Digestate analysis

The pH of the fresh digestate was 8.97 (alkaline). Total carbon was 45.3% w/w on a dry matter basis. It was 23.0% dry matter (as determined following oven heating), similar to the “standard” 24% present in farm-sourced separated fibre and the 25% present in either cattle farmyard manure or digested cake biosolids reported in Defra’s Fertiliser Manual 2017 (RB209). Comparisons for nitrogen, phosphate, potash, sulphur and magnesium in the digestate with “standard” farm-sourced separated fibre show they are similar (**Table 4.7)**.

Table 4.7 Analysis of maize and vegetable anaerobic digestate solids sampled 18 April 2017 and compared (where figures are available) with a "standard" farm-sourced separated fibre.

Analysis on dry matter basis			Analysis on fresh matter basis		
Analysis	**Units : dried matter**	**Digestate**	**Units : per fresh tonne**	**Digestate**	**"Standard" farm fibre**
Total Nitrogen	% w/w	2.22	kg N	5.11	5.6
Ammonium Nitrogen	mg/kg	2443	kg NH_4-N	0.56	
Nitrate Nitrogen	mg/kg	<10	kg NO_3-N	<0.01	
Total Phosphorus (P)	% w/w	0.732	kg P_2O_5	3.86	4.7
Total Potassium (K)	% w/w	2.04	kg K_2O	5.63	6.0
Total Magnesium (Mg)	% w/w	0.428	kg MgO	1.63	1.8
Total Sulphur (S)	% w/w	0.275	kg SO_3	1.58	1.2
Total Copper (Cu)	mg/kg	12.8	kg Cu	<0.01	*
Total Zinc (Zn)	mg/kg	81.1	kg Zn	0.02	*
Total Sodium (Na)	% w/w	0.022	kg Na_2O	0.07	
Total Calcium (Ca)	mg/kg	7061	kg Ca	1.62	

* Upper limits for PAS 110 certification 96 mg Copper/kg and 192 mg Zinc/kg fresh weight

4.3.3 Plant assessments

4.3.3.1 Phytotoxicity, plant establishment and wilting

When the plants were examined on 16 June 2017 for any phytotoxicity 10 days after planting, just before application of the Serenade ASO, the vast majority of plants were healthy. A slightly wilted plant was seen in plots 18 and 24 and one scorched leaf margin plant in plot 12 (all T2 digestate). Two further slightly wilting plants were seen in plot 6 (T3 Bio-Fence) and one scorched plant in plot 7 (T5 including Bio-Fence and Serenade ASO).

The second examination for phytotoxicity on 29 June 2017 in order to assess any adverse effect of the Serenade ASO drench was after a period of very hot weather. More plants were seen to have scorched margins across all the treatments (Figure 4.7) and in the commercial crop. Scorched margins were therefore not a sign of phytotoxicity, but were caused by desiccation in the hot sun. Some plants also appeared to be dead as all the leaves were

desiccated, however a number subsequently re-grew. The grower later removed those that did not survive. The Serenade ASO alone (T4) treated plots did not differ from those left untreated (T1), with no plants looking dead and only some traces of scorched leaves (**Table 4.8**). The other treatments (T2, T3 and T5) had a mean 19% of plants with leaf margin scorch, highly significantly more than T1 and T4 (P<0.001). This high proportion and high standard error of the mean in T3 and T5 (both treated with Bio-Fence and planted 11 days later) resulted from unusually high numbers of near-dead plants in the front two plots of one bed (eight plants in plot 6 of T3 and six plants in plot 7 of T7), whereas no plants were affected after the same treatments in the adjacent bed. Elsewhere these Bio-Fence treatments had one to four near-dead plants. Two plots with digestate (T2) had a near-dead plant, otherwise all plants had survived. The necrotic symptoms had developed too suddenly for Verticillium wilt infection from the soil and were too soon after planting.

Table 4.8 Assessment of the proportion of plants with scorched leaves or appearing to be dead and the proportion remaining healthy out of around 27 plants within each 6 m long treated area on 29 June 2017 at the Oxfordshire strawberry field planted on 6 June. Two weeks after Serenade ASO application, 5 weeks after digestate incorporation and Bio-Fence activation.

Treat-ment	Product	Mean % scorched plants	S.E.	Mean % dying plants	S.E.	Mean % healthy plants	S.E.
1	Untreated	2.66	1.34	0.00	0.00	96.83	1.56
2	Anaerobic digestate solids	16.40	3.05	3.40	50.57	82.79	3.13
3	Bio-Fence	17.50	3.47	33.18	99.22	68.32	3.80
4	Serenade ASO	0.59	0.59	0.00	0.00	99.42	0.58
5	Bio-Fence + Serenade ASO	23.09	3.75	17.31	63.62	71.62	3.80
Chi Probability		<0.001		<0.001		<0.001	

Note: Regression analysis provides only approximate probabilities (Chi Pr.) and Standard Errors (S.E) since the model is not linear. The means are predictions from the regression.

When a further assessment was carried out on 6 September 2017 (two months after planting) some weakly growing or dead-looking plants were still present, and some symptoms which could have been the start of Verticillium wilt were seen, but at this stage it was considered too early to separate out disruption to water uptake from Verticillium as opposed to that resulting from poor root establishment. The untreated plots had significantly fewer (P<0.001) poorly established plants than in all four of the treatments (**Table 4.9**). The Bio-Fence

treatment (T3) had significantly more weakly established or dying plants (26.5% affected) than either the digestate (T2) or Serenade ASO (T4) treated plots (mean 15% weak or dying).

Table 4.9 Proportion of unhealthy (weak, wilting or dead) strawberry plants in September 2017 at the Oxfordshire field site. Proportion of plants starting to show Verticillium wilt in October 2017 following removal of dead (un-established) plants and the total number of live plants

Treat-ment	Product	6 September 2017		11 October 2017		
		Mean % weak, wilting or dead plants	S.E.	Mean % plants wilting	S.E.	Mean number of live plants
1	Untreated	5.61	2.01	11.97	2.99	25.8
2	Anaerobic digestate solids	14.69	3.18	8.61	2.44	24.6
3	Bio-Fence	26.51	3.91	5.90	2.33	21.6
4	Serenade ASO	15.38	3.23	11.54	2.99	25.6
5	Bio-Fence + Serenade ASO	18.75	3.47	5.15	1.98	22.6
Chi probability		<0.001		0.247		

On 11 October 2017 the total of number of plants alive in the treated 6 m length of each plot was counted (**Table 4.9**). Any dry, dead plants had been removed across the field by the farm staff during weeding operations. Most plots had only one or two less plants than the 28 that had been planted in the treatment length, but a number of the bare-root plants in plots 6 (T3, Bio-Fence alone) and 7 (T5, Bio-Fence then Serenade ASO), mainly one after the other in a row, never established following their original observation of weak growth (**Figure 4.11**). However, whereas plots 4, 20 and 22 (T3) had lost four to five plants, plots 3, 19 and 20 (T5, the two products) had lost two or three.

In October, once only wilting plants remained in the field (with symptoms now attributed to Verticillium wilt rather than failure to establish) there was no significant difference between any of the treatments (**Table 4.9**). Affected plants were diminished in size and there was complete death of the first expanded leaves and very small and weak recently emerged foliage around the centre of the plant. The similarity between treatments in October was in contrast to the records of significantly fewer healthy plants in the treated plots in September compared with untreated. By October, the Bio-Fence treated plots (T3 and T5) ranked as having the smaller proportion of wilted plants (mean 5.5% i.e. on average one plant in a plot), with the untreated and Serenade ASO plots having a mean 11.7% wilted. The severity of symptoms of individual plants ranged principally between index 4 (showing symptoms but not completely wilted) to index 7 (showing the beginning signs of Verticillium wilt, but mostly healthy), but numbers affected were too low at this date to make valid comparisons of severity between treatments (and data for these indices is not presented).

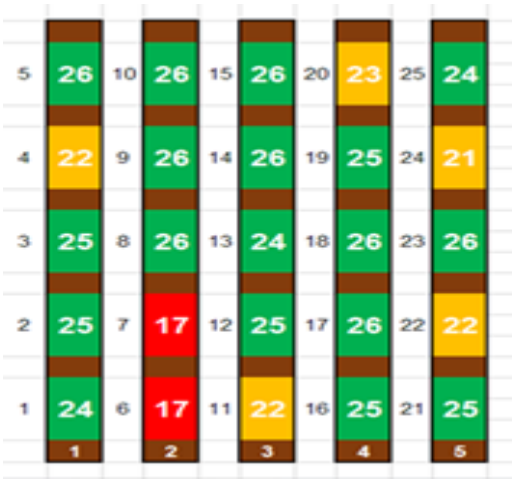


Figure 4.11 The number of live plants in each treated length of plot by 11 October 2017 following final plant losses due to poor establishment, with no mortality to Verticillium yet.

In mid-January 2018 plants with Verticillium wilt were clearly seen to have stunted growth with small and red tinged leaves in the centre of the crown and surrounding, more mature, leaves dying (**Figure 4.12**) .

Figure 4.12. Example of Verticillium wilt on a plant - small reddening young central leaves, and outer necrotic leaves, compared with a vigorous plant. Oxfordshire, 15 January 2018

On 18 July 2017, the first ripe fruit were present and there was no obvious difference in fruit production between plots, however after the plots were walked along and a record made of the average (commonest i.e. modal) number of full sized fruit on plants in each plot and the

highest number of fruit seen on a plant per plot. Analysis (Anova of a Latin Square) showed that fewer (P<0.009) fruit per plant (mean 1.4 fruit) were commonly being produced by plants in T2 plots in which anaerobic digestate had been incorporated (**Table 4.10**). The untreated plants usually had a mean 5.6 fruit and this did not differ significantly from the modal number produced in T3, T4 or T5. Other plants in T2 were capable of producing a greater number of fruit (a mean 6.4) and this did not differ significantly from the highest number of fruit/plant seen in any of the other treatments including the untreated (ranging between a maximum mean 7.6 and 9.8 fruit per plant) (**Table 4.10**). No problems, such as deformity or small size, of the fruit were reported, the only issue was some bird pecking.

Table 4.10 Observation on 18 July 2017 of the most common, and the highest number, of fruit produced by the strawberry plants in each of the five plots of the five treatments at the Oxfordshire site.

Treat-ment	Product	Mean commonest number of fruit on a plant	Mean highest number of fruit on a plant
1	Untreated	5.60	9.80
2	Anaerobic digestate solids	1.40	6.40
3	Bio-Fence	4.60	7.60
4	Serenade ASO	5.40	8.20
5	Bio-Fence + Serenade ASO	6.60	9.60
	F Pr.	<0.009	0.144
	L.S.D	2.576	3.107

Farm staff started the first of only two picks from the field on 18 July 2017. The fruit yield, as had been expected, was low. After the second pick the fruit left was very small and so the remaining fruit trusses in the crop and experiment area were removed by the farm staff to help the plants to grow on.

4.3.4 Weather

The air temperature and relative humidity in a screened enclosure on the polythene surface of the bed amongst the plants is shown in **Figures 4.13** and **4.14**. The weather during the scattering of the digestate between 14.00 and 15.00 h on 23 May 2017 was 18°C and overcast, it then became sunny and temperatures rose to 23°C in the afternoon. When the Bio-Fence was scattered on the 24 May 2017 and all plots rotavated at 11:00 h the temperature was 23°C soil was dry at the surface, but moist (not wet) beneath and produced a good tilth to incorporate the treatments. The weather remained warm and dry prior to the

irrigation and polythene covering of the Bio-Fence on the 25 May 2017. After covering, the afternoon air temperature (in the ventilated screen) remained around 27°C from midday to after 18:00 h. On the 26 May temperatures of 30°C were recorded, but moderated over the next days with daytime temperatures around 23°C on the day the polythene was slit to ventilate in the experiment and across the field destined for the commercial crop across which chloropicrin had been applied. An unusually hot period occurred from 17 June when temperatures of over 30°C were recorded. Another very hot period occurred during flowering.

On 12 June 2107 when the Serenade ASO was applied at the start of drenching at 11:00 h there was an average wind speed of 2.6 mph, with air temperature of 18.2°C and 61% relative humidity. By completion of application at 12:30 h the wind speed was 2 mph, the temperature 21°C and the air at 60.3 % relative humidity.

Records are shown for air temperature and humidity. This probe was readily removed and replaced from the screen stood on the soil surface for downloading, however the soil loggers were not disturbed as they were buried under the polythene mulch and are constructed for longer-term recording. They will be taken out and replaced in January 2018, prior to soil warming in spring.

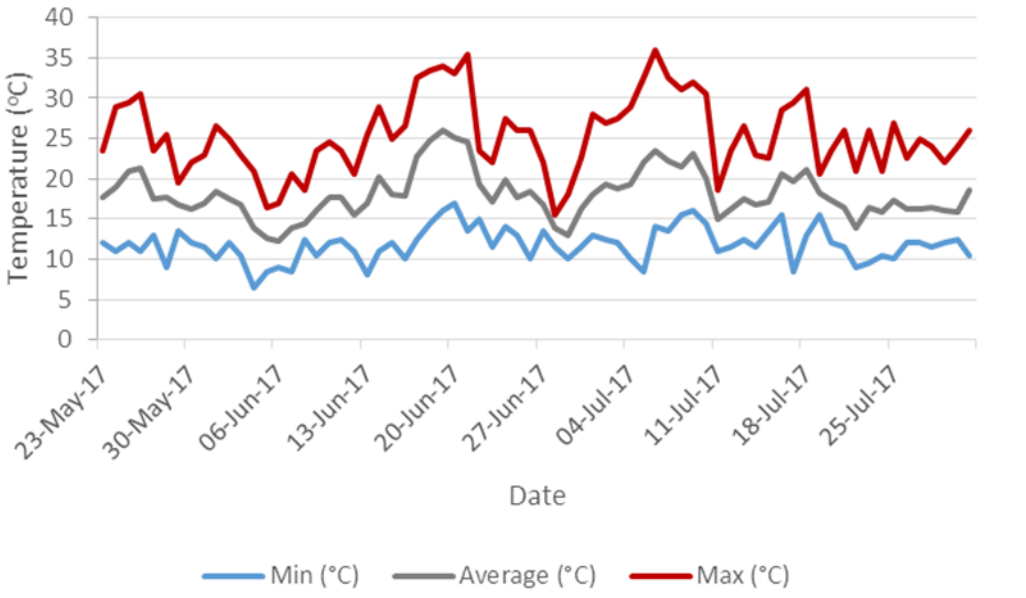


Figure 4.13 Weekly air temperatures at crop height from 23 May 2017 at the Oxfordshire site when digestate was spread, then showing a period of very hot weather during plant establishment following planting on 6 June and again leading up to fruiting in July 2017.

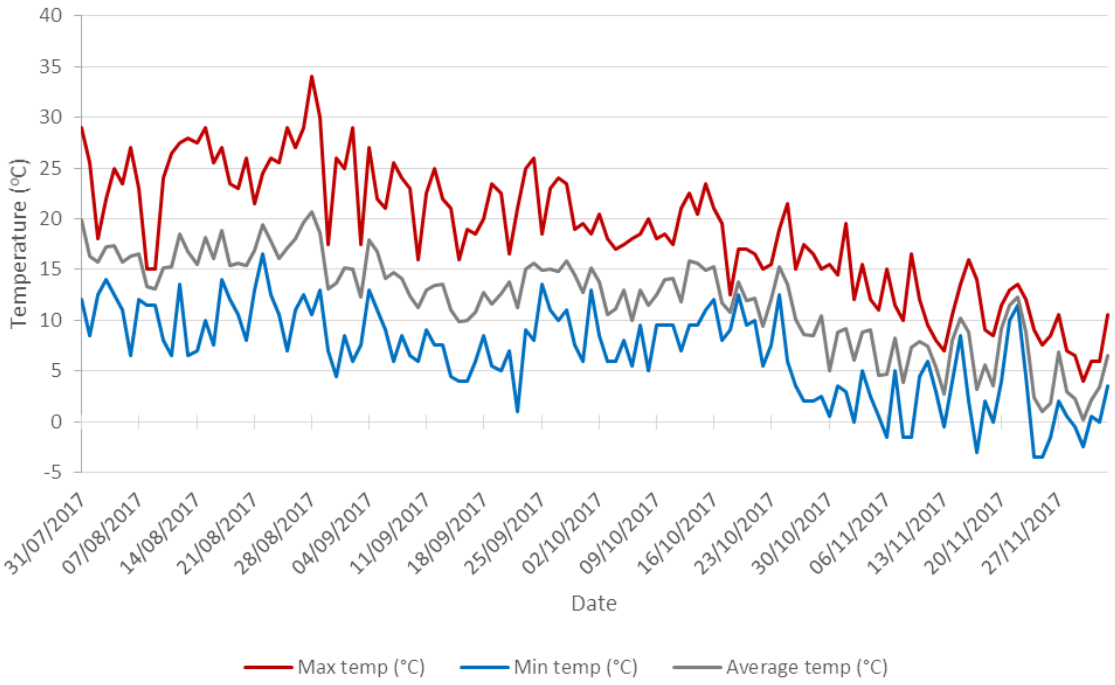


Figure 4.14 Weekly air temperatures at crop height continued from 31 July 2017 at the Oxfordshire site outdoor strawberry experiment after the completion of fruiting

The soil loggers buried at 100 mm after product incorporation on 23 May 2017 showed two of the highest temperature peaks of the year within the month of plant establishment (**Figures 4.15, 4.16 and 4.17**). These readings from the Bio-Fence, digestate and untreated plots were similar, recording unusually high maxima of 30.9°C, 30.3°C and 31.1°C, respectively on the 24 May and maxima of 32.0°C, 29.6°C and 30.3°C, respectively on the second peak on 20 June. There was a fall to a maximum of 16°C on 6 June between the two temperature peaks.

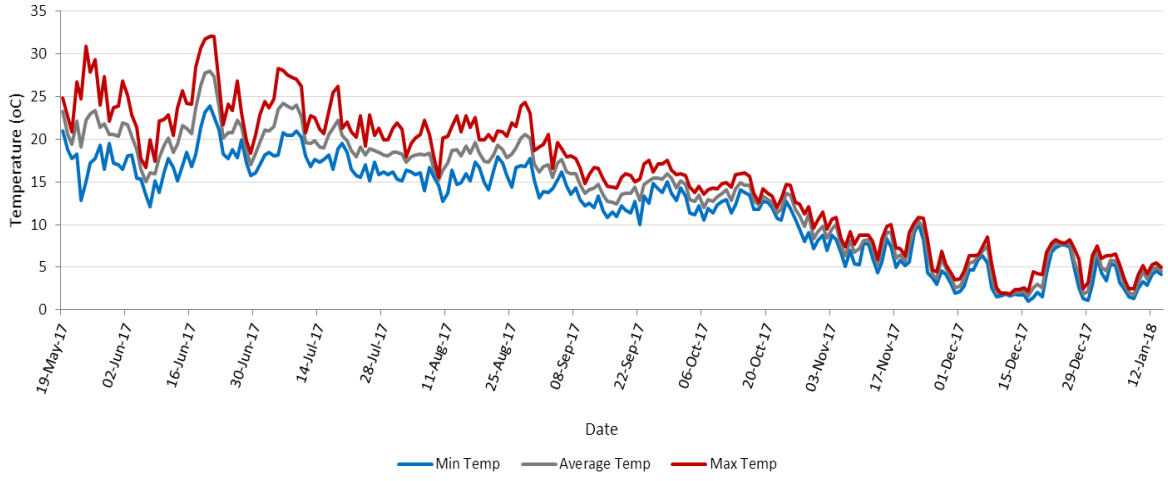


Figure 4.15. Weekly soil temperatures at 100 mm depth from 24 May 2017 date of Bio-Fence incorporation to January 2018 at the Oxfordshire site outdoor strawberry experiment.

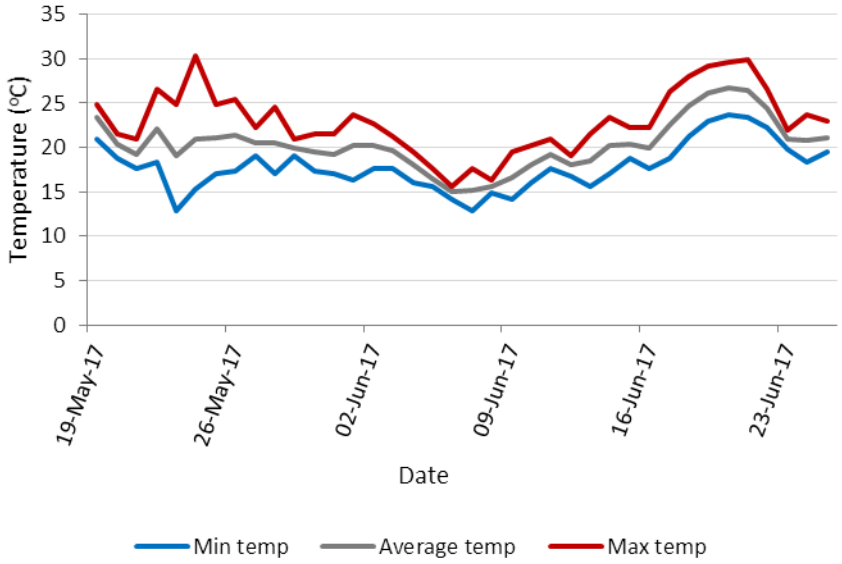


Figure 4.16. Weekly soil temperatures at 100 mm depth from 24 May 2017 date of anaerobic digestate solids incorporation to January 2018 at the Oxfordshire site outdoor strawberries.

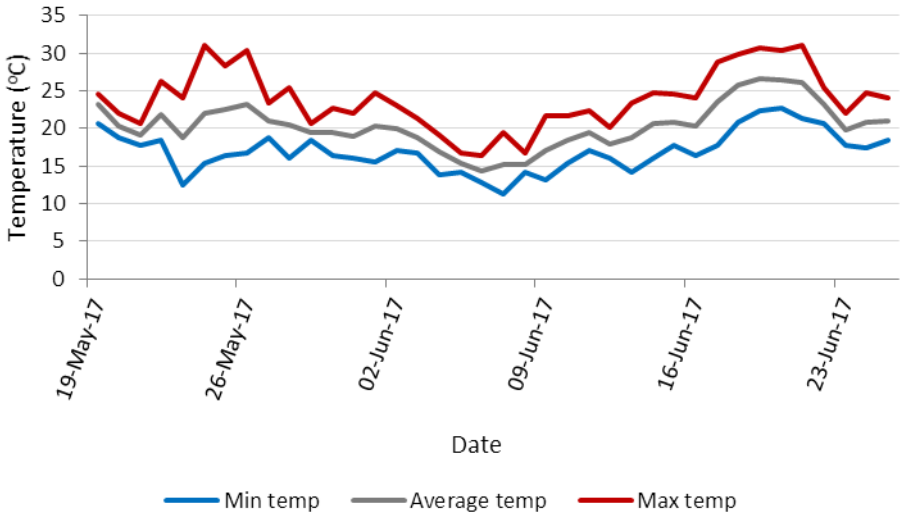


Figure 4.17. Weekly soil temperatures in an untreated plot at 100 mm depth following soil rotavation on 24 May 2017 until January 2018 at the Oxfordshire site outdoor strawberries.

4.4 Discussion

The cause of the very poor plant establishment in two adjacent plots in a bed was not determined, but it is possible that these plants were not pushed firmly enough into the bed either because the rotavation for incorporations had loosened the soil more in that location or that the planting holes were above some of the large stones on the field. Plants with brown

dry leaf margins appeared soon after planting and it is probable that although the grower increased the irrigation that some plants suffered from the very hot weather in the weeks not long after planting when both air and soil temperatures reached around 30°C. Scorched plants were present across the field, but not at the density seen where there had been incorporation of either the digestate solids or the Bio-Fence. It was possible that as both these treatments (but in particular the digestate) added organic matter to the soil that the more-open structure did not pack as well around the roots and so allowed them to desiccate and cause the plants to dry back from the leaf margins. Both materials could also have left the soil drier after incorporation. Recommendations could be made to growers to in future roll after incorporations, or if used pre-bed forming this process itself could have the required compacting effect. It was possible that there was also, or alternatively, in the hot conditions a chemical release from under the plastic mulch which scorched the plants where it exited the planting holes. Bio-Fence soil disinfestation works by producing isothiocyanates and tests can be done which involve growing cress in a sample of soil to see if it is safe to plant. However, in the current experiment the planting interval was kept to the same timing as that used for the commercial crop post-ventilation of chloropicrin as extending the interval would have put the experiment out of synchrony with farming operations on the same field and knowledge about the use of one week interval was gained.

Verticillium often starts to manifest in plants following the stress of fruiting and this was shown with wilt starting in September in plants with roots that were in contact with microsclerotia. The typical symptoms of wilt on one side of a plant and leaf collapse was more apparent by October, but at this time there were no significant treatment differences, but there were trends in that both the Bio-Fence treatments had half the proportion with symptoms in the untreated. Further assessments will be carried out in spring 2018 as overwinter stresses usually result in an increased incidence of wilting. The treatments were not expected to eliminate the Verticillium in the soil (unlike chloropicrin), but to either, (in particular with the Bio-Fence), reduce the level and so reduce infection severity, or to increase the resilience of the plants. The latter is particularly the case for the Serenade ASO and digestate treatments which may increase the beneficial microflora in the soil around the plant roots. Serenade ASO was used with the Bio-Fence to prevent a microbial ‘vacuum’ that would be open to pathogen colonisation. Potentially Serenade ASO could induce systemic acquired resistance to pathogens. The variety Symphony was selected because it can become infested by Verticillium and show symptoms, but to still produce fruit, whereas a more susceptible variety would die. If biological soil treatment is adopted by growers in future, it would be recommended to be integrated with the use of the least susceptible varieties producing fruit still acceptable to the market.

Although plants in digestate treated plots commonly had fewer fruit when observed at the first pick it is possible that their formation was delayed and a greater number than in the other treatments could have arisen later. It is alternatively possible that the higher potassium in these plots could have encouraged vegetative growth rather than fruit production and if so this might result in stronger plants and more fruit in 2018.

4.5 Conclusions

There was potentially some initial phytotoxicity in the form of leaf margin scorch in over 16% of plants in which either digestate or Bio-Fence had been incorporated. There was also more severe damage so that in addition over 17% of plants looked like they were dying in the two treatments in which Bio-Fence was used, however this result was probably adversely influenced by what was probably unrelated poor establishment in two plots. Some dying plants subsequently revived after the period of very hot weather. Dying plants were absent from the untreated plots and the Serenade ASO treated plots without any Bio-Fence incorporation.

When Verticillium wilt symptoms developed post-harvest there was no significant difference in incidence between any of the treatments and the untreated. Numbers affected were low. A greater incidence and severity of Verticillium wilt would normally be expected after the pathogen has had longer for microsclerotia to grow and mycelium to invade the crowns of the strawberry plants. Assessments in 2018 will determine if the higher ranking incidence in the untreated and Serenade ASO alone treatments is a trend that continues

The first year of fruit production was low and variable between plants of the same treatment, as expected. Plants in the digestate treated plots had commonly produced fewer fruit by the date of the single assessment made in the short fruiting period. More fruit should be produced across the experiment in the second year, over a longer period and so aid comparisons.

Knowledge and Technology Transfer

May 2017, the project consortium visited New Forest Farm

13 September 2017 – Presentation of mildew trial results at AHDB Agronomists Day at NIAB EMR

21 November 2017 – Presentation of mildew trial results at AHDB/EMRA Soft Fruit day at NIAB EMR

7th March 2018 – Presentation to fruit researchers at University of Aarhus, Denmark

References

Anonymous (1976). Strawberry powdery mildew ADAS Key No 8.1.1. MAFF, Plant Pathology Laboratory, Harpenden, Herts.

Chamorro, M., Aguado, A., and De los Santos, B. (2016). First report of root and crown rot caused by Pestalotiopsis clavispora (Neopestalotiopsis clavispora) on strawberry in spain. Plant Disease 100, 1495-1495. doi: 10.1094/pdis-11-15-1308-pdn.

Embaby, E. (2007). Pestalotia Fruit Rot on Strawberry Plants in Egypt. Egypt. J. Phytopathol. 35, 99-110.

Jeewon, R., Liew, E.C.Y., and Hyde, K.D. (2004). Phylogenetic evaluation of species nomenclature of Pestalotiopsis in relation to host association. Fungal Diversity 17, 39-55.

Mertely, J.C., Chamorro, M., Tompkins, D., Mertely, J.A., and Peres, N.A. Fungi associated with diseased roots of strawberry runner plants after transplanting. APS and MSA Joint Meting, August 10–14, Austin, Texas.

Mouden, N., Benkirane, R., Ouazzani Touhami, A., and Douira, A. (2014). Pathogenic capacity of Pestalotia longisetula Guba reported for the first time on strawberry (Fragaria ananassa Duch.) in Morocco. International Journal of Pure & Applied Bioscience 2, 132-141.

Pettitt, T.R., and Pegg, G.F. (1994). Sources of crown rot (Phytophthora cactorum) infection in strawberry and the effect of cold storage on susceptibility to the disease. Annals of Applied Biology 125, 279-292.

Rodrigues, F.A., Silva, I.T., Antunes Cruz, M.F., and Carré-Missio, V. (2014). The infection process of Pestalotiopsis Longisetula leaf spot on strawberry leaves. Journal of Phytopathology 162, 690-692. doi: 10.1111/jph.12226.

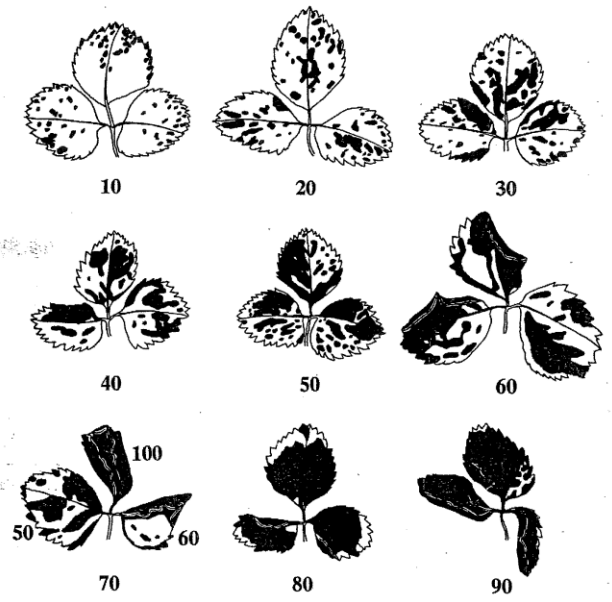
Appendix

Appendix F1: scanned copy of the mildew assessment key used in the mildew trial at NIAB EMR

AGRICULTURAL DEVELOPMENT AND ADVISORY SERVICE

Strawberry Powdery Mildew **Key No. 8·1·1**

Sphaerotheca alchemillae Grev.



Percentage of leaf area affected

Black shaded areas represent red/yellow blotches and brown dead tissue

Examine 10 *fully expanded* leaves on one typical plant. Grade these using the diagrams and key. Repeat on 9 further typical plants, giving assessments from 100 leaves. Calculate the mean percentage mildew.

Powdery mildew (%)

0 Leaves fully extended, flat and green.

5 Slight curling noticeable; mildew found with difficulty.

10 Leaves with small red-purple spots. Curling slight. Mildew visible on lower surface.

20 Red blotches tending to be confluent. Some browning. Curling obvious from a distance.

30 More blotches confluent, with browning becoming more severe. Splitting in centre of larger lesions and curling severe.

40 Confluent red and brown blotches. Splitting in centre of larger lesions. Curling now approaching rolling. Leaf becoming brittle.

50 Half of leaf area affected and apparently dead.

60 Some yellowing in addition to reddening and browning may be present.

70 Severe distortion of at least one leaflet.

80 Much of leaf affected. Distortion of all leaflets.

90 Small marginal areas only remain green.

100 Whole leaf red or brown. Severe distortion and very brittle.

Notes:

This key is based on measurements of the reddening and browning symptoms which may be seen on Royal Sovereign at picking time. It is not possible to use the extent of white sporing mycelium as a guide to severity, as this is very difficult to see even when 100% of the lower surface is infected.

PLANT PATHOLOGY LABORATORY
HARPENDEN, HERTS

Ministry of Agriculture, Fisheries and Food
(Publications), Tolcarne Drive, Pinner, Middlesex HA5 2DT

Table A1. Summary of efficacy tests of new fungicides against SPM when applied after inoculation of strawberry leaves of cv. “Malling Centenary”

Exposure period before treatment	Fungicide	Number of infected leaflets	Total number of inoculated leaf lets	incidence	Number of lesions (standard error)
24 h	Control	40	78	51.28	46.1 (10.45)
	Luna Sensation	0	87	0	0 (0)
	Takumi	4	84	4.76	1 (0.73)
	Talius	4	90	4.44	5.3 (4.98)
48 h	Control	28	90	31.11	33 (9.94)
	Luna Sensation	0	87	0	0 (0)
	Takumi	0	87	0	0 (0)
	Talius	0	87	0	0 (0)
72	Control	22	89	24.72	10 (4.12)
	Luna Sensation	0	90	0	0 (0)
	Takumi	0	87	0	0 (0)
	Talius	1	90	1.11	0.1 (0.1)

Table A2. Summary of efficacy tests of new fungicides against SPM when applied before inoculation of strawberry leaves of cv. “Malling Centenary”

Exposure period after treatment	Fungicide	Number of infected leaflets	Total number of inoculated leaf lets	incidence	Number of lesions (standard error)
24-96 h	AQ10	12	60	20.00	4.6 (2.68)
	Control	17	60	28.33	6.8 (2.95)
	Luna Sensation	10	60	16.67	4.9 (2.58)
	HDC F208	4	58	6.90	0.8 (0.47)
	Takumi	10	60	16.67	2.6 (1.64)
	Talius	6	60	10.00	1.6 (0.83)
48-120 h	AQ10	14	59	23.73	3.7 (1.3)
	Control	15	54	27.78	11.33 (6.88)
	Luna Sensation	5	60	8.33	1.5 (1.20)
	HDC F208	13	60	21.67	16.8 (12.21)
	Takumi	4	60	6.67	1.3 (0.82)
	Talius	2	60	3.33	0.4 (0.27)
96-168 h	AQ10	17	60	28.33	15.3 (7.28)
	Control	22	60	36.67	30.7 (15.64)
	Luna Sensation	11	60	18.33	21.1 (12.06)
	HDC F208	22	60	36.67	23.5 (9.22)
	Takumi	15	60	25.00	19 (9.10)
	Talius	4	60	6.67	5.4 (4.77)
168-240 h	AQ10	11	60	18.33	8.6 (4.53)
	Control	11	60	18.33	3.8 (1.67)
	Luna Sensation	10	60	16.67	4.7 (3.59)
	HDC F208	18	60	30.00	19.6 (9.74)
	Takumi	8	60	13.33	3.2 (1.63)
7	Talius	6	60	10.00	1.5 (0.78)

Table A3. Air temperature and humidity conditions at the time of spray applications

Date	At start of spray applications					At end of spray applications					Weather conditions
		Temp (°C)			Wind speed (km/h) Direction		Temp (°C)			Wind speed (km/h) Direction	
	Time	Dry bulb	Wet bulb	RH%		Time	Dry bulb	Wet bulb	RH %		
10 July	9.17	21	19	82.8	0	10.20	24	21	76.4	0	Sunny spells
17 July	9.0	20	18	82.4	0	9.51	21	18	74.7	1.6 NE	Sunny
24 July	9.37	18	17	90.6	0	10.32	18	17	90.6	0	Overcast
31 July	8.24	16.5	15.5	90.2	0	9.35	21	19	82.8	0	Sunny spells
7 Aug	16.00	21.5	18	71.1	0	17.0	20	18	82.4	0	Heavy cloud
14 Aug	8.18	16	15.5	95	0	9.09	22	19	75.3	0	Sunny spells
21 Aug	11.00	17	16.5	95.1	0	12.00	18	18	100	0	Drizzle, muggy
29 Aug	7.40	18	18	100	0	8.59	21	19	82.8	0	Hazy sunshine
5 Sep	10.45	19.5	18	86.5	0	11.43	21	19.5	87	0	Overcast 80%
11 Sep	7.40	12	11	88.8	0	8.32	12	12	100	0	Sunny
18 Sep	7.55	12.5	11	83.6	0	8.50	11.5	11	94.2	0	Sunny spells

Table A4. % accuracy of spray applications (volume applied / volume required expressed as a percentage)

Spray date	Treatment number			
	2	3	4	5
10 July	125	106	114	104
17 July	116	115	94	107
24 July	112	110	107	112
31 July	107	109	111	105
7 Aug	92	115	86	115
14 Aug	110	101	102	99
21 Aug	101	101	96	99
29 Aug	95	95	100	99
5 Sep	90	95	95	76
11 Sep	100	102	105	93
18 Sep	95	105	100	104

Table A5. Strawberry Verticillium wilt - grower management programme

Date applied	Product	Active ingredient	Rate used/ha	Target pest or disease
22 June 2017	Fortress (EAMU) + Fenomenal	Quinoxyfen + Fenamidone + fosetyl aluminium	250ml + 4.5kg	Powdery mildew Crown rot/red core
10 July 2017	Teldor + Systhane 20EW	Fenhexamid + myclobutanil	1.5kg + 330ml	Fruit botrytis, powdery mildew
17 July 2017	Tracer (120 day EAMU) + Teldor + Nimrod	Spinosad + Fenhexamid + bupirimate	150ml + 1.5kg + 1.4L	SWD + Fruit botrytis, powdery mildew
25 July 2017	Systhane 20EW	myclobutanil	330ml	Powdery mildew
16 August 2017	Systhane 20EW	myclobutanil	330ml	Powdery mildew
18 September 2017	Plenum WG (EAMU) + Masai	Pymetrozine + Tebufenpyrad	400g + 750g	Aphids TSSM & Tarsonemid mite
19 September 2017	Goltix 70SC (EAMU) + Flexidor + Retro + Shark (EAMU)	Metamitron + Isoxaben + Diquat + Carfentrazone ethyl	2L + 0.5L + 3L + 330ml Shielded/directed application down alleys between raised beds	For pre & post emergence weed & unwanted runner control in alleys between raised beds
15 November 2017	Kerb Flo	propyzamide	2.75L applied overall beds & alleys	Primarily for pre & post emergence grass weed control

- No predatory mites introduced for TSSM or Tarsonemid mite control as plants very small when planted & until post-harvest i.e. mid-August when weather cooler & plants started to grow away. TSSM levels on plants low until early September.
- Hand weeding took place prior to harvest in 2017.
- Straw was applied to field 26.06.17 prior to harvest. Then straw applied 23.03.18 to whole field to supress disease. Straw was removed from trial area, as to not interfere with disease pressure.