Project title:	Improving integrated disease management in strawberry
Project number:	SF 157
Project leader:	Prof Xiangming Xu NIAB EMR
Report:	Annual, 2016/2017
Previous report:	Year 1
Key staff:	Erika Wedgwood (ADAS) Angela Berrie (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 2017.....

Erika Wedge

Senior Plant Pathologist

ADAS

Signature ...  ... Date ...28 March 2017...

Report authorised by:

Xiangming Xu

Head of Pest & Pathogen Ecology Department

NIAB EMR

Signature .....  .. Date .....29 March 2017.....

[Name]

[Position]

[Organisation]

Signature ..... Date .....

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

- Contamination of *P. cactorum* in asymptomatic runners could cause considerable losses to growers.

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 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. 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 2

Year 1 results suggested that the incidence of contamination by *P. fragariae* (causal agent of red core) in runners is very low; however, the level of contamination of *P. cactorum* (causal agent of crown rot) could reach 25-30%. Year 2 sampling and screening therefore focussed on *P. cactorum*. The survey results in Year 2 agreed with those from Year 1. The level of *P.*

cactorum in runners varied greatly from sample to sample, and could be as high as 20%, detected mostly in asymptomatic crowns. In addition, it appears that the level of *P. cactorum* is not associated with particular cultivars.

Small-scale experiments were conducted at NIAB EMR to determine whether separate or joint use of AMF (arbuscular mycorrhizal fungi) and PGPR (Plant growth promoting bacteria) could reduce *Phytophthora* development. Results suggested that amendment of compost with both AMF and PGPR together could reduce severity of red-core development. However, these treatments failed to achieve significant reduction in the development of *P. cactorum* in inoculated crowns; further experiments are needed to confirm this result.

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 therefor 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)

Headline

- The two biocontrol substances, Ampelomyces quisqualis (approved on protected strawberry) and Bacillus pumilus (not currently an approved plant protection product), when applied with Silwet, achieved a similar level of control of powdery mildew to that achieved by a standard 7-day fungicide programme.

Background and expected deliverables

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 currently used against strawberry powdery mildew (SPM).

Projects SF 62, SF 62a and SF 94 (Defra Horticulture LINK HL0191) focussed on development, implementation and use of an SPM prediction system 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 the UK and Norway showed the importance of chasmothecia as a source of inoculum, particularly for perennial cropping systems, and indicated the importance of removing debris from previous crops. Research in Norway also suggested young leaves and fruit are most susceptible to SPM infection.

An EU-interreg funded project at NIAB EMR demonstrated 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.

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

Summary of the project and main conclusions in Year 2

In a replicated small plot field trial on strawberries (cv. Elsanta), grown under Spanish tunnels at NIAB EMR and in coir bags under a tunnel at ADAS Boxworth, the efficacy of programmes combining biocontrol agents (BCA) and elicitors, with and without fungicides at 14 day intervals, was compared to a 7 or 14 day interval standard fungicide programme and untreated plots for control of SPM on leaves and fruits. Conditions at NIAB EMR were exceptionally favourable for SPM (average 60% leaf area with SPM in untreated control), indicating that the trial gave a good test of efficacy of management programmes. Significant treatment effects on SPM were only detected at the NIAB EMR site.

At NIAB EMR, all spray treatments significantly reduced SPM incidence on both leaves and flowers/fruit. The lowest SPM incidence was on plots treated with the standard 7-day fungicide programme. However the efficacy of two BCA only programmes (in admixture with Silwet) were as effective as the standard 7-day fungicide programme. These BCA programmes included *Ampelomyces quisqualis* (AQ10 – currently approved on protected strawberries) and *Bacillus pumilus* QRD2808 (Sonata – not currently approved as a plant protection product). Surprisingly, when applied alone, the two BCAs provided better control than when used with other products. A crop ‘strengthener only’ programme offered the least effective control. Because conditions were so conducive to SPM, around 25% of leaf area was infected with SPM on the best treatment (standard 7-day fungicide programme).

There were obvious visual differences in plant vigour in the plots receiving the different programmes. All treated plots had a higher vigour score than the untreated control which was obviously stunted, but only the plants in the plots receiving 7 day fungicide programme were

significantly better, despite the similar incidence of foliar SPM in plots receiving biocontrol products. There was a negative relationship between yield (fruit number) and the level of SPM.

The incidence of fungal rots (*Botrytis* and *Mucor*) at harvest was low and sporadic. However, there was a much higher post-harvest rot incidence, particularly *Botrytis*. Although there were significant differences in the *Botrytis* incidence between treatments, none of the programmes were effective in reducing rots to acceptable levels. The incidence of other rots (e.g. *Mucor*, *Penicillium*) was very low and none of the programmes were effective in controlling the other rots.

Results from independent trials at a commercial farm over the last few years (conducted by University of Hertfordshire) showed that weekly application of silicon through drip fertigation can lead to reduced SPM development.

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.

Action points for growers

- At low/moderate SPM levels (< 15% leaf area with SPM), programmes using biofungicides alone (with Silwet) are as effective as weekly standard fungicide applications. However, it is important to ensure early control of SPM.
- This work identified *Ampelomyces quisqualis* (AQ10) as one biofungicide which gave comparable control to standard fungicide programmes and this is currently approved for use on protected strawberry.
- Weekly application of silicon through drip fertigation can also lead to reduced SPM development.

Fruit rot complex

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

Botrytis cinerea, causing grey mould, is the most-studied disease in strawberry worldwide. Infection at flowering stages leads to the establishment of latent infection, which becomes active during fruit ripening. Direct infection of fruit by conidia during ripening is also possible, which may account for a high proportion of post-harvest rots. Previous work (Project SF 94, Defra Horticulture LINK HL0191) has shown that it is possible to avoid using fungicides against *Botrytis* for early-covered June-bearers. Controlling *Botrytis* in late season strawberry, particularly ever-bearers, is problematic. Use of bees to deliver biocontrol agents to flowers gave the same level of *Botrytis* control as a fungicide programme on one strawberry farm. There is an on-going European core organic project which is assessing the use of bees to deliver biocontrol agents to strawberry flowers. However, it may face registration hurdles or even negative public responses. To manage spotted wing drosophila (SWD) risk, growers are now implementing strict hygiene measures by removing all old, damaged or diseased fruit from the plantation during and after harvest. This will also help to reduce *Botrytis* risk in late season crops.

Projects SF 74 (Defra Horticulture LINK HL0175) and SF 94 (Defra Horticulture LINK HL0191) demonstrated that rapid post-harvest cooling to 2°C is effective in delaying *Botrytis* development in raspberry and strawberry. However, such cooling treatment is not effective against *Mucor* which can develop in cold conditions. In Project SF 98, NIAB EMR identified a few fungicides that can give 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.

For fruit rot complex in this project, the integration of biocontrol products with reduced fungicides will be investigated, along with post-harvest handling to reduce fruit rot and/or delay rot development. Work to understand the epidemiology of fruit rot complex and to develop management strategies will start in Year 4 of the project.

Verticillium wilt

Background and expected deliverables

Recent withdrawal of methyl bromide and other soil fumigants has instigated new research to find alternative soil treatments for *Verticillium*. Disappointingly, a new microencapsulated product did not have sufficient efficacy to offer any commercial future (a TSB funded project which ended in December 2014). AHDB Horticulture funded a PhD studentship project to assess the use of pre-colonisation of strawberry runners with arbuscular mycorrhizal fungi (AMF) to manage wilt.

With AHDB funding, Fera developed a molecular diagnostic tool to quantify soil inoculum and ADAS is currently using this tool to investigate wilt development in relation to nematodes. Separately, NIAB EMR (in collaboration with Chinese researchers) has developed a more sensitive qPCR tool for quantifying *Verticillium* inoculum in soils. In an on-going TSB project, significant yield reduction associated with stunted strawberry growth has been observed that is apparently not associated with *Verticillium*. Further metagenomics research suggested that several candidate organisms are responsible for this stunted growth (though further research is needed to confirm this), including two fungal pathogens *Ilyonectria robusta* and *I. coprosmae* (former *Cylindrocarpon* spp.) and the suppressive effects of *Bacillus* and *Pseudomonas* species.

Summary of the project and main conclusions in Year 2

Originally, we planned to conduct an experiment to study the use of anaerobic soil disinfestation (ASD) on wilt development in Year 2. Unfortunately, CRD informed us that ASD requires registration as a plant protection product (PPP). As the Dutch manufacturer is was unwilling to do this, we had to cancel this experiment.

Financial benefits

No financial benefits are relevant to this part of the project so far.

Action points for growers

- No action points have been developed from the work on Verticillium wilt to date.

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. Integrated Pest Management (IPM) best practice involves using biopesticides in combination with the remaining synthetic pesticides and other cultural and manipulative measures including the use of clean (certified) planting materials, resistant cultivars, semiochemicals, biocontrol agents, disease forecasting and other IPM tools to achieve commercially acceptable control of pests, diseases and weeds.

Crown rot and red-core caused by *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 (CP 077). Recent research on *Phytophthora* spp. has concentrated on detecting the pathogens and seeking products to reduce root rotting. Two HDC projects have just been completed; SF 130 focussed on fungal molecular quantification and an assay was developed that detected *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 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 conduct a five-year project to identify *Phytophthora* virulence factors against strawberry. More research is required to assist growers to be able to plant disease-free propagation material in order to reduce pesticide use and crop losses.

Strawberry powdery mildew

Hort-LINK project SF 62, 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 SPMs 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 a more sensitive qPCR tool for quantifying *Verticillium* inoculum in soils. In an on-going TSB project, we have observed significant yield reduction associated with stunted strawberry growth that is apparently not associated with *Verticillium*. Further metagenomics research suggested several candidate organisms responsible for this stunted growth (though further research is needed to confirm this), including two fungal pathogens *Ilyonectria robusta* and *I. coprosmae* (former *Cylindrocarpon* spp.) and the suppressive effects by *Bacillus* and *Pseudomonas* species.

Objective 1: *Phytophthora*

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

Year 1 results showed that the incidence of contamination by *P. fragariae* in runners is extremely low (only one out of 600 plants). However, the level of contamination of *P. cactorum* could reach 25-30%. Thus, the survey and screening in Year 2 has focussed on *P. cactorum*.

1.1 Survey

1.1.1 Materials and methods

A total of ten consignments of bare-root runners (six different cultivars) were sampled in 2016 from six different growers prior to planting (Table 1.1). Five runners were taken from 4-10 boxes from each of 2-5 pallets (depending on availability); 100 runners in total were taken from each consignment except from grower G2 where runners of the same cultivar had come from two separate countries therefore 50 were sampled from each and treated as separate consignments [Consignment numbers R33/16(i) and R33/16(ii), Table 1.1].

Table 1.1 Sites visited for screening of runners prior to planting for *Phytophthora* spp.; these runners were cold-stored at the growers holding, and sampled just prior to planting.

Consignment number	Grower Number	Location	Cultivar (susceptibility to crown rot)	Country of origin	Date collected
R28/16	G1	Kent	V1 (S)*	C1	07/03/2016
R34/16	G1	Kent	V2 (U)	C1	15/03/2016
R33/16(i)	G2	Northamptonshire	V2 (U)	C1	14/03/2016
R33/16(ii)	G2	Northamptonshire	V2 (U)	C2	14/03/2016
R52/16	G3	Surrey	V3 (S)	C1	21/03/2016
R53/16	G3	Surrey	V4 (M)	C1	30/03/2016
R54/16	G4	Kent	V5 (U)	C1 – TBC	05/04/2016
R55/16	G4	Kent	V1 (S)	C1 – TBC	05/04/2016
R56/16	G5	Yorkshire	V6 (M)	C3	07/04/2016
R57/16	G6	Kent	V1 (S)	C4	09/05/2016
*: S – Susceptible, M – Moderately susceptible, and U – not known.					

Runners were kept separately in bags at 4°C at NIAB EMR until processed within 1-2 days. The roots and shoots were removed from the runners and soil washed from the remaining crown. Crowns were cut in half to look for symptoms of *P. cactorum*. Samples with symptoms of crown rot were tested with a Pocket diagnostic lateral flow device (LFD) for *Phytophthora spp*. (Forsite Diagnostics, York). For all samples, approximately 100-250 mg of internal crown material (the edge of the crown was cut away to avoid soil contamination) was cut into small pieces with a scalpel and placed into a 2 mL microtube with two 4 mm ball bearings. Samples were placed into a -80 °C freezer until DNA extraction.

DNA was extracted using DNeasy Plant Mini Kit (Qiagen) following the manufacturer's instructions with all optional steps. DNA was quantified and quality-checked using a Nanodrop 1000 spectrophotometer and stored at -20 °C. Extractions from crown material were more successful than the 2015 screening (100% in 2016 vs. 65% success in 2015), most likely due to the use of a new tissue disruptor - a 2010 Geno/Grinder (SPEX SamplePrep). However, this difference should not affect the results since the incidence of *Phytophthora* detection is expressed in terms of those samples with successful DNA extractions (of course assuming that the presence of *Phytophthora* does not affect DNA extraction)

DNA from crown was run in a PCR with FaEF primers (Table 1.2) as a control for strawberry DNA to indicate whether DNA extraction was successful. *Phytophthora cactorum* was tested for in a nested PCR using Yph (*Phytophthora* Spp., Table 1.2) primer set in the first PCR and then Ycac (*P. cactorum* specific) primers (Schena et al., 2008) in the second PCR with 1/10 dilutions of the amplicons from the 1st (YPh) PCR. Table 1.2 shows sequences of all primers used. All PCRs were performed with 2 µl of DNA (Ca. 1-4 ng/µl in PCRs with FaEF and Yph primer sets), 1x buffer, 2 mM $MgCl_2$, 0.2mM dNTPs, 0.25 U Taq and 0.2µM of each primer in a total volume of 12.5 µl. All PCRs were performed on a thermal cycler using the following touchdown cycle: an initial 95°C for 3 min, followed by 35 cycles of 95°C for 30 s, 60°C for 60 s (decreasing 0.5°C per cycle until 58°C) and 72°C for 60 s, followed by a final extension at 72°C for 5 min. PCR amplicons were run by gel electrophoresis on a 1.5% agarose gel with Gel Red at 100V for 60 mins and viewed under UV light on a GelDoc XR+ (Bio-Rad, California, USA). For each assignment of materials, at least 50 samples were extracted for DNA and subjected to the nested-PCR screening for *P. cactorum*.

Simple pairwise proportional tests (based on the z-score) were used to compare the incidence of samples with symptomatic and asymptomatic *P. cactorum* based on the nested PCR results.

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

Primer set	Target	Forward primer	Reverse Primer
Yph[a]	*Phytophthora* spp.	CGACCATKGGTGTGGACTTT	ACGTTCTCMCAGGCGTATCT
Ycac[a]	*P. cactorum*	CCATACAAAATTCTGCGCTAGG	AGACACACAAGTGGACCGTTAG
FaEF	*Fragaria*	TGGATTTGAGGGTGACAACATGA	GTATACATCCTGAAGTGGTAGACGGAGG
[a](Schena et al., 2008)			

1.1.2 Results

There were a total of 25 runners from the 900 sampled with dark red/brownish discolouration of the crown, resembling *P. cactorum* symptoms. For three of these 25 samples, the tissues with symptoms were too small to permit the LFD test. Of the 22 symptomatic samples tested with LFD, 11 were from one consignment and all gave negative LFD test results (Appendix Photo 1), which were further confirmed by the subsequent PCR. Of the remaining 11 samples tested with the LFD, seven gave a positive result for *Phytophthora*, six of which were confirmed as *P. cactorum* by PCR; but the seventh failed to amplify both the FaEF and the Ycac primer sets, indicating failures in DNA extraction. Two of the three symptomatic samples that were not tested with the LFD were also confirmed as positive for *P. cactorum* by PCR. In total eight symptomatic samples (from the 900 total) were confirmed as having crown rot (Table 1.3)

Table 1.3 Number of symptomatic samples in each consignment of strawberry runners, and the results of the LFD and nested PCR of these symptomatic samples for *Phytophthora cactorum*

	Number of samples			
Consignment	Total	Symptoms	LFD positives	PCR positives
R28/16	100	3	1[a]	2
R33/16(i)	50	2	1	1
R33/16(ii)	50	0	NA[b]	NA
R34/16	100	0	NA	NA
R52/16	100	2	0[a]	0
R53/16	100	1	1	1
R54/16	100	3	1[a]	2
R55/16	100	3	3	2
R56/16	100	0	NA	NA
R57/16	100	11	0	0

[a]: For these consignments, there was one symptomatic sample that cannot be subjected to the LFD test because of small amount of symptomatic tissues;

[b]: Not applicable.

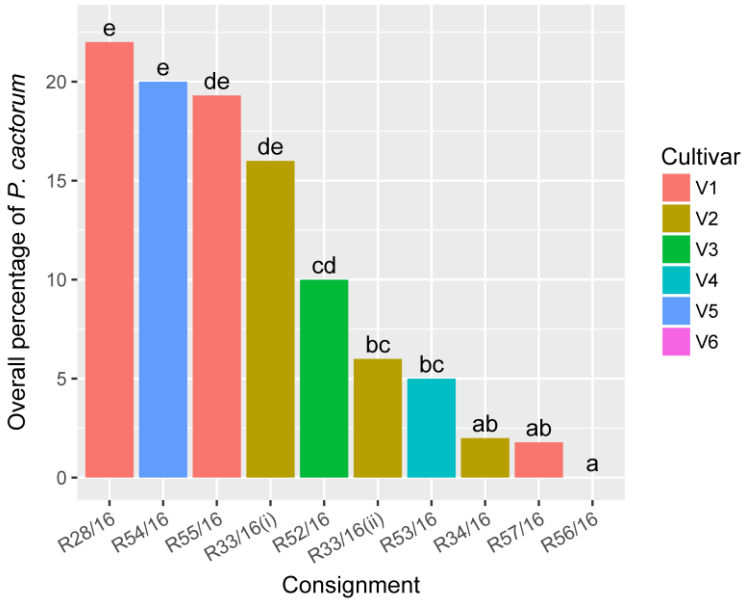
We extracted DNA from 505 asymptomatic samples, out of which, 495 samples amplified with FaEF primers (indicating successful DNA extraction from crown material). Presence of *P. cactorum* was detected in 47 out of these 495 samples (Table 1.4), giving the overall incidence of 9.5%. The incidence of positive detection results of *P. cactorum* ranging from 0% to 20% for all the consignments sampled.

Table 1.4 Number of asymptomatic samples in each consignment of strawberry runners with positive PCR test results for presence of *Phytophthora cactorum*

		Number of samples		
Consignment	Cultivar	DNA extracted	Successful extractions	PCR positive
R28/16	V1	59	50	10
R55/16	V1	50	50	9
R57/16	V1	51	50	1
R33/16(i)	V2	48	48	7
R33/16(ii)	V2	50	50	3
R34/16	V2	50	50	1
R52/16	V3	48	50	5
R53/16	V4	49	50	2
R54/16	V5	50	50	9
R56/16	V6 - TBC	50	50	0

There are significant differences in the overall incidence of *P. cactorum* (symptomatic and latent) between consignments (Figure 1.1). The overall level of *P. cactorum* ranged from 0 (consignment R56/16) to 22% (consignment R28/16). Consignments may be divided in to three groups: low (< 2.5%), moderate (2.5 ≤ and < 10%) and high (≥ 10%) (Figure 1.1). For two cultivars (V1 and V2) with multiple consignments, there were significant differences between three consignments for both V1 and V2.

Figure 1.1 The overall percentage of *P. cactorum* (symptomatic and latent) of strawberry runners sampled in 2016. The letters above each bar indicated the significance test results at *P* = 0.05: consignments with at least one common letter do not differ in the overall incidence of *P. cactorum.* For V6, the incidence of Phytophthora is zero (i.e. consignment R56/16)



1.1.3 Discussion

Results from 2016 agreed with those of 2015: the level of *P. cactorum* varied greatly among samples, and could reach a very high level (> 20%). In most cases *P. cactorum* stays latent at the time of planting. In one consignment of runners, we observed a high incidence of symptoms, resembling those caused by *P. cactorum*. However, both LFD and PCR tests did not support the presence of *P. cactorum.* We speculate that these symptoms (Photo 1 in Appendix) may have resulted from frost damage or be caused by another fungal pathogen, possibly *Pestalotiopsis* spp. (Sati and Belwal, 2005; Sowndhararajan et al., 2013; Rodrigues et al., 2014; Chamorro et al., 2016). This pathogen has recently been shown to infect crowns, leading to wilting and death; the symptom is similar to that of *P. cactorum*. Indeed, in some cases both *Pestalotiopsis* spp. and *P. cactorum* can be detected from the same crown sample. Further research is needed to assess the importance of *Pestalotiopsis* spp. in the UK.

1.2 Use of PGPR and AMF to manage *Phytophthora* spp.

Year 1 results showed that the incidence of *P. fragariae* in runners is extremely low (only one out of 600 plants) whereas the incidence of *P. cactorum* could reach 25-30%. Thus, we focussed on the effects of AMF on *P. cactorum* in Year 2, whilst completing the repeat experiment on the effect of AMF and PGPR on *P. fragariae* (initiated in Year 1)

1.2.1 Materials and methods

We completed the assessment of *P. fragariae* development in the second repeat on the effect of AMF and PGPR against *P. fragariae*, which was initiated in Year 1. The experimental details were given in the Year 1 report and hence are not included in this report. Both experiments were conducted in a glasshouse compartment (Photo 1.1)

In Year 2, two experiments were carried out; the first one on the protective effect of AMF against *P. cactorum* and the second on the effect of AMF and PGPR against *P. cactorum* on wounded plants. For Experiment 1, there were only two treatments: (1) AMF inoculated at planting and (2) control without AMF inoculation. For the second, there were eight treatments: (wound, healthy) x (AMF, control) x (PGPR, control). In Experiment 1, there were 20 replicate plants for each treatment; a completed randomised block (four blocks, each with 5 plants) design was used. In Experiment 2, there were 10 replicate plants for each treatment; a completed randomised block (two blocks, each with 5 plants) design was used. Experiment 1 was repeated once over time. The experiments were conducted in a glasshouse compartment. Cold stored (-2°C) strawberry plugs of cv. 'Malling Centenary' used were obtained from Hargreaves Plants, Norfolk, UK; plants derived in this way have shown in previous work to be free from AMF colonisation {Robinson Boyer, 2016 #21219} and a number of plants were tested prior to the main experiments to confirm this using the root staining method {Robinson-Boyer, 2015 #21006}.

Photo 1.1 Picture of experimental plants in assessing the effects of AMF and PGPR on *Phytophthora cactorum* development in a glasshouse compartment.

1.2.1.1 AMF and PGPR inoculations

Inoculum of AMF was provided by PlantWorks Ltd, Kent, UK. The AMF granular formulation was applied as commercially available Rootgrow™, a clay/pumice/zeolite mix containing spores, mycelium, and host plants root fragments colonised by five different AMF species (*Funneliformis mosseae*, *F. geosporum, Rhizophagus irregularis*, *Claroideoglomus claroideum*, *Glomus microagregatum*). Rootgrow™ contained ca. 350 propagules ml^{-1} as determined by MPN analysis (Cochran, 1950). In each experiment, the negative control (Cb^-) was not inoculated at planting. At the time of transplanting the granular AMF inoculum was placed into each planting hole before transplantation of the strawberry plug. Each pot received 25 ml of granular AMF inoculum (recommended by the manufacturer). The formulated PGPR

experimental product contained 10^8 CFU ml^{-1} (specified by the manufacturer) and was supplied as fine grade (0.5-1.0 mm) pumice containing four different rhizobacterial species (*Rhizobium strain* IRBG74, *Derxia lacustris* HL-12, *Bacillus megaterium* and *B. amyloliquefaciens*); each plant received 7.6 ml of PGPR (recommended by the manufacturer.

1.2.1.2 Pathogen inoculation

One *P. cactorum* isolate (P414; known to be pathogenic against 'Malling Centenary' from the host-pathogen interaction study in the on-going BBSRC LINK project on the resistance of strawberry to Phytophthora diseases at NIAB EMR) from the pathogen collection of NIAB EMR was used for inoculation. The stock culture was cultured on V8 agar for seven days in the dark at 18 ± 1 °C. Discs (10 mm diameter) were cut from the margins of actively growing cultures with a sterilised cork borer, immersed in a non-sterile compost extract (2 L distilled water drained through 50 g compost and diluted two fold before use) and incubated for 2 days at 20 °C in an illuminated incubator. A suspension of 10^3 (experiments 1) or 10^2 (experiment 2) zoospores ml^{-1} was then produced following the method described by Harris *et al.* (Harris et al., 1997). A vertical slit (ca. 10 mm long) was made using a scalpel blade at the base of an internal leaf (close to the crown). The inoculum was then directly pipetted onto the wounded or healthy (experiment 2 only) area, 5 mL per plant. Inoculated plants were placed into a glasshouse compartment (ca. 20 °C). A lower dose of inoculum was used in Experiment 2 because of very high mortality of plants observed in Experiment 1.

1.2.1.3 Transplantation

Before pathogen inoculation, cold stored (-2 °C) plugs of cv. 'Malling Centenary' were transplanted in 500 mL (9 × 9 × 10 cm, experiment 1) or 1 L (11 x 11 x12 cm, experiment 2) plastic pots (Desch Plantpak Ltd, Essex, UK) filled up with ca. 450 mL of Irish dark peat (Clover Peat Products Ltd, Dungannon, Ireland) and 2-5 mm perlite (Sinclair Horticulture Ltd, UK) mix (7:3) inoculated with AMF or PGPR as appropriate. Large pots were used in Experiment 2 in order to better support plant growth as many plants survived at least six weeks after inoculation. Plants were then transferred to a polytunnel under natural temperature and light conditions for ca. five (Experiment 1, in May 2016) or three (experiment 2, in November 2016) weeks to induce plant growth and AMF colonisation. All plants were then inoculated with *P. cactorum* as described above; for Experiment 2, each plant was re-inoculated again the next day to increase disease pressure. Finally, each plant was randomised placed into a glasshouse compartment (temperature set at 20 °C during the day and 15 °C during the night, with natural day/night cycle). Plants were manually watered individually 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. No additional fertiliser was added.

To speed up disease development, plants in Experiment 2 were not watered from early February 2017 for 10 days before final assessment and sampling crown tissues.

1.2.1.4 Disease assessment, plant productivity and root sample analysis

Plants were assessed once a week for aboveground disease symptoms on a rating scale: 1 – no symptoms, 2 – floppy foliage, and 3 – totally collapsed and dead (Photo 1.2). After the final aboveground disease assessment (just prior to the time when first batch of ripen fruits were ready to pick), the crowns were cut longitudinally and the extent of internal necrosis was recorded: 1 – no necrosis, 2 – up to 25 % necrosis, 3 – 25 to 50 % necrosis, 4 – 50 to 75 % necrosis, and 5 – 75 to 100 % necrosis. At the end of Experiment 1, a composite sample of root tips (ca. 2-4 g) was taken for each pot for assessment of AMF root colonisation preferentially sampling younger roots. The roots were then cleared with KOH before being stained with Trypan blue and microscopically assessed for root length colonisation (RLC). For experiment 2, crown samples were taken in mid-February 2017 from each plant for quantification of *P. Cactorum* DNA by nested qPCR.

Healthy **Floppy** **Dead**

Photo 1.2 Disease assessment keys on strawberry plants inoculated with Phytophthora spp.: 1 – healthy, 2 – floppy foliage and 3 – dead.

1.2.1.5 Data analysis

All data were analysed using R (version 3.2). Only significant differences are reported in the text. The disease data were all analysed using generalised linear models (GLM) with residual errors assumed to follow a binomial distribution; the log link function was used. Individual experiments conducted at different times were treated as a blocking factor.

1.2.2 Results

1.2.2.1 AMF & PGPR on *P. fragariae*

The first wilting symptoms appeared in less than a week after inoculation. No more symptoms appeared after 20 days from red core inoculation and the overall number of wilted plants remained lower than in the first repeat (75% diseased in 2015); 40 out 128 plants showed

disease symptoms. Diseased plants were evenly distributed in the four blocks of the experiment. However, there were no significant treatment effects on the number of diseased plants or the rate of disease development, in contrast to the first repeat experiment.

1.2.2.2 AMF on *P. cactorum*

The level of AMF colonisation of plant roots was very low, often less than 3 % RLC. In both replicate experiments, the first wilting symptoms appeared about two weeks after inoculation (Photo 1.3A) and the number of plants with visible symptoms remained stable after five weeks from inoculation. In total, 31 and 33 out of 40 plants showed crown necrosis for the first and second replicate experiment, respectively. However, there were no significant effects of AMF inoculation on the number of diseased plants and crown necrosis level.

Photo 1.3 Example of plant inoculated with *Phytophthora cactorum* in experiment 1 (the first repeat experiment) seven weeks after pathogen inoculation: (A) severely wilted plant and (B) observation of crown necrosis.

1.2.2.3 AMF & PGPR on *P. cactorum*

Visual assessment was completed in mid-February, 2017. Of the 160 plants, 29 plants and 106 plants showed wilt symptoms immediately before and after 10 days of imposed drought, respectively. Only 24 had healthy crown tissues.

Currently, we are discussing with an American company about the possibility of using this set of crown material to cross validate their quantification/detection tool for *Phytophthora* spp. If no agreement is reached before June 2017, we shall proceed with a nested qPCR quantification of Phytophthora DNA in these crown samples. Once DNA has been quantified, we shall conduct full statistical analysis to determine the effect of AMF, PGPR and wounding on visual wilt, crown symptoms and accumulation of pathogen biomass.

1.2.3 Discussion

In Experiment 1, disease reduction was not achieved by AMF. Several reasons are possible to explain this. First, we wounded the plant and inoculated with a high *P. cactorum* inoculum dose, probably giving too much advantage to the pathogen; this protocol was used as it is the standard method used to assess plant resistance to this pathogen. Thus, Experiment 2 was designed to clarify whether the effect of AMF & PGPR depends on how the pathogen was inoculated and on the inoculum dose. Second, the lack of effect of AMF could be due to the low level of root colonisation by AMF; however, a low level of AMF colonisation could lead to significant benefit to plants in coir substrate (Robinson Boyer et al., 2016). Finally, maybe AMF & PGPR do not have the ability to reduce *P. cactorum* in strawberry crowns.

Objective 2: Strawberry Powdery Mildew (SPM)

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

In 2015, two trials were conducted (one at NIAB EMR and one at ADAS, Boxworth) to study the effects of combining alternative products with reduced fungicide input on SPM development. Results suggested that combining certain alternative products with reduced fungicide input could be effective in controlling SPM, particularly when the level of inoculum was low.

In 2016, further trials were conducted at the two sites in which programmes were evaluated for control of SPM where biocontrols (Sonata or AQ10) were combined in programmes with a plant strengthener (CropBiolife) with and without a reduced fungicide programme. Table 2.1 gives the details of individual products, which were combined over time to form different SPM management programmes for testing. This experiment was conducted at both NIAB EMR and ADAS Boxworth. The ADAS site had fewer treatments than the NIAB EMR site because of the constraint of tunnel size.

In addition to the trials at NIAB EMR and ADAS, UoH focused on the use of a silicon nutrient product in the fertigation system to reduce the severity of SPM epidemics in commercial strawberry tunnels. It should be noted that UoH was not funded by AHDB to carry out this work – it was funded by a commercial company, who agreed to share the results with SF157 under an AHDB consultancy with Dr Avice Hall of UoH.

Table 2.1 Details of products used in SPM control in strawberry 2016

Product	Active ingredient	Rate of product ha^{-1}	Other information	Product type	Target
AQ10 + Silwet	*Ampelomyces quisqualis*	70 g + 0.05%		BCA	SPM
CropBiolife	flavonoids	250 ml	21 d harvest interval	Strengthener	Plants
Fortress	quinoxyfen	0.25 L		Fungicide	SPM
Nimrod	bupirimate	1.4 L		Fungicide	SPM
Rovral	iprodione	1.0 kg	4 sprays max	Fungicide	*Botrytis*
Signum	pyroxystrobin + boscalid	1.8 kg	2 sprays max	Fungicide	SPM/*Botrytis*
Sonata + Silwet	*Bacillus pumilus* QRD2808	5 L + 0.05%		BCA	SPM
Switch	cyprodinil + fludioxonil	1.0 kg	2 sprays max	Fungicide	SPM/*Botrytis*
Systhane	myclobutanil	450 ml		Fungicide	SPM

2.1 NIAB EMR

2.1.1 Materials and methods

2.1.1.1 Site and plot details

A new plantation was constructed, similar in design to the established planting used in 2015. The plantation consisted of two Spanish tunnels with three raised beds per tunnel with polythene mulch and was planted on August 5 2016 with cv. Elsanta. Each bed had trickle irrigation. The ground was sterilised prior to planting. The outer two beds were used as plots, with the middle bed as a guard row. Each plot consisted of a double row of 15 plants, 30 plants in total (approximately 4 m in length). Plots were separated in the row by 2.0 metres. The tunnel was not covered with polythene until 18 August to allow the plants time to establish at lower temperatures and with natural rainfall.

We used this newly-planted crop from cold-stored runners in late summer to ensure the plants developed in conditions of high risk for SPM infection and development. SPM was present at low incidence in adjacent established strawberry crops which acted as a source of inoculum.

In addition plants with SPM from adjacent crops were also planted throughout the trial on 25 August to ensure the disease developed rapidly in the trial.

2.1.1.2 Treatments and experimental design

All products received for inclusion in the trial were stored, handled and applied according to the manufacturer's instructions on the product label. In total there were 11 experimental treatments (programmes); each programme detail is given in Table 2.2. Treatments were replicated four times in a randomised block design. Treatments at the start of the programme were targeted at SPM only. Products for *Botrytis* control were included from flowering onwards. Each treatment was applied at 7-14 day intervals as specified until the beginning of October, including through the harvest period. All treatments apart from the untreated control received a spray of Fortress at the start of the trial as preventing SPM development in early stage is one key ingredient of successful SPM management programme.

Treatments were started once the plants had established and were producing new leaves. Treatments were applied by hand using a CP15 knapsack sprayer at 1000 L ha^{-1} according to NIAB EMR SOP724. Care was taken to ensure the spray was directed to the developing leaves and flowers in the plant centre and to the underside of the leaves. The dates treatments were applied are shown in Table 2.2. Application is either applied early in the morning or late in the afternoon.

2.1.1.3 Other treatments and information

All plots received a standard programme for pest and disease as required up to the start of the trial (Appendix Table 1). Treatments for insects and soil-borne diseases were applied as routine to all plots, including the guard rows as needed. Monitoring of plots indicated an infestation of capsid bugs at the end of August. Calypso (thiacloprid) was applied on 9 and 23 September for control. Any nutrients were applied to all plots via the trickle irrigation. Other treatments for pests or disease applied to all plots are given in Table 1 in the Appendix.

Records of temperature and humidity were taken from a USB 502 logger placed in the central guard row in the tunnel at crop height. The records were used to run a SPM risk prediction model. Records of rainfall were also taken from a weather station located at NIAB EMR, approximately 500 m away.

2.1.1.4 Assessment

Table 2.3 provides dates of activities related to the trial.

Phytotoxicity: Plots were inspected for damage after each spray and any effects recorded as detailed in EPPO Guideline PP1/135(4).

SPM: The plots were regularly inspected for SPM and the incidence on foliage assessed on five expanded leaves on each of five plants per plot on three occasions 20 September, 25 October and 1 November. On the first and third dates, assessments started from the youngest expanded leaf; at the second assessment the assessments started from the third youngest leaf (equivalent to youngest leaf two weeks earlier). A modified standard key (Anon, 1976) was used. The incidence of SPM on flowers and fruitlets was assessed as presence or absence once on 27 September on 10 flowers / fruitlets on each of five plants per plot.

Plant vigour: The height and spread of five plants per plot was measured and the figures multiplied together to give a vigour score for each plot. The five plants were selected from the same position in each plot but excluding any atypical plants

Harvest: Fruit was first picked on 21 September and then weekly until the final pick on 12 October; the picking dates were dependent on the development of specific crops. The crop at NIAB EMR was planted a month later and harvested from late September in lower temperatures. Hence the fruit was slower to ripen than the ADAS crop. Fruit weight and number were recorded. Rots were identified, including SPM and recorded. A sample of 50 fruit was taken from each plot at each harvest and incubated at ambient temperature at high humidity in plastic module trays where each fruit occupied an individual module separating it from adjacent fruit. After seven days rots were identified and incidence recorded.

Table 2.2 Treatment details of SPM programmes evaluated at NIAB EMR in 2016; for all programmes, Fortress was applied at the beginning on its own or with other products (depending on individual treatments) to reduce level of SPM inoculum (particularly that came with planting materials)

Programme	Date treatment applied							
	22 August	**30 August**	**6 September**	**13 September**	**20 September**	**27 September**	**4 October**	**11 October**
	BBCH 12/13	**BBCH 60**	**BBCH 61**	**BBCH 61-65**	**BBCH 61-81**	**BBCH 85**	**Harvest**	**Harvest**
P1 Untreated								
P2 Standard fungicide 7 days	Fortress	Rovral + Systhane	Signum	Switch + Nimrod	Signum	Switch + Systhane	Nimrod	Systhane
P3 Fungicide 14 days	Fortress		Rovral + Systhane		Switch + Nimrod		Nimrod	
P4 CropBiolife	Fortress + CropBiolife			CropBiolife			CropBiolife	
P5 CropBiolife + Sonata + Silwet	Fortress + CropBiolife	Sonata + Silwet	Sonata + Silwet	CropBiolife	Sonata + Silwet	Sonata + Silwet	CropBiolife	Sonata + Silwet
P6 Sonata + Silwet	Fortress	Sonata + Silwet	Sonata + Silwet	Sonata + Silwet	Sonata + Silwet	Sonata + Silwet	Sonata + Silwet	Sonata + Silwet
P7 CropBiolife + Sonata + Silwet + 14 day fungicide (P3)	Fortress + CropBiolife	Rovral + Systhane	Sonata + Silwet	Switch + CropBiolife	Sonata + Silwet	Switch + Systhane	Nimrod + CropBiolife	Sonata + Silwet

P8 CropBiolife + AQ10 + Silwet	Fortress + CropBiolife	AQ10 + Silwet	AQ10 + Silwet	CropBiolife	AQ10 + Silwet	AQ10 + Silwet	CropBiolife	AQ10 + Silwet
P9 AQ10 + Silwet	Fortress	AQ10 + Silwet	AQ10 + Silwet	AQ10 + Silwet	AQ10 + Silwet	AQ10 + Silwet	AQ10 + Silwet	AQ10 + Silwet
P10 CropBiolife + AQ10 + Silwet + 14 day fungicide P3	Fortress + CropBiolife	Rovral + Systhane	AQ10 + Silwet	Switch + CropBiolife	AQ10 + Silwet	Switch + Systhane	Nimrod + CropBiolife	AQ10 + Silwet
P11 CropBiolife, AQ10 alone and in tank mix Rovral	Fortress + CropBiolife	AQ10 + Rovral	AQ10 + Rovral	CropBiolife	AQ10 + Silwet	AQ10 + Rovral	CropBiolife	AQ10 + Silwet

Table 2.3 Summary of treatment and assessment timings for the SPM trial at NIAB EMR in 2016

Activity	Date
Trial planted	5 August
Trial marked out	16 August
Plots covered	18-19 August
First spray	22 August
Logger placed in tunnel	23 August
First SPM visible	24 August
Second spray applied, flowers tagged	30 August
Spray for capsid applied	1 September
Third spray applied	6 September
Fourth spray applied	13 September
Fifth spray	20 September
SPM assessed	20 September
First harvest	21 September
Second Capsid spray	22 September
Sixth spray	27 September
Assessed SPM on flowers/fruitlets	27 September
Second harvest	28 September
Post-harvest rots assessed from first harvest	28 September
Seventh spray	4 October
Third harvest	5 October
Post-harvest rots assessed from second harvest	5 October
Eighth spray	11 October
Fourth harvest	12 October
Post-harvest rots assessed from third harvest	12 October
Post-harvest rots assessed from fourth harvest	19 October
Second SPM assessment	25 October
Plant vigour assessed	26 October
Third SPM assessment	1 November

2.1.1.4 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 four harvests is also included. Fruit number was square root transformed and fruit size log transformed prior to analysis.

2.1.2 Results

The weather conditions were relatively warm to hot up to the start and during the trial up until October. Warm temperatures coupled with high humidity were very conducive to SPM development throughout the trial period except the last few days (Figure 2.1).

There were no obvious phytotoxic symptoms observed on foliage or fruit.

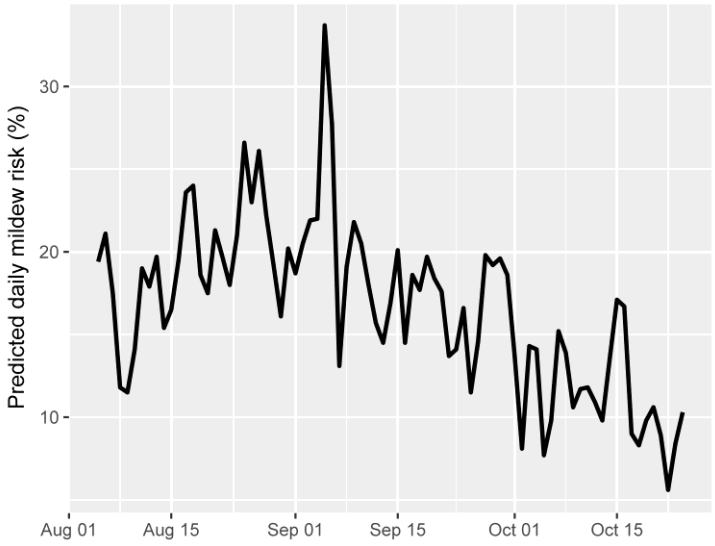


Figure 2.1 Predicted daily risks of SPM on susceptible cultivars for the NIAB EMR site in 2016; the predictions were given by the EMR model where a period of four consecutive days with risks > 10% is considered to need growers' intervention with a moderate to high level of inoculum (usually when the incidence of leaves with SPM is above 5%).

2.1.2.1 SPM on leaves

The mean SPM incidence on leaves over three assessments is given in Table 2.4 and Figure 2.2 with SPM incidence at each assessment date given in the Appendix Table 2. The conditions in August and September were exceptionally favourable for SPM with a high incidence of more than 90% leaf area with SPM in untreated plots at the first two assessments. By the third assessment in November the SPM epidemic had slowed with the lower temperatures, with a 50% reduction in SPM incidence on untreated plots.

All spray treatments significantly reduced SPM incidence ($P < 0.001$, Figure 2.2). The lowest incidence was on plots treated with the standard 7 day fungicide programme (P2) and those treated with Sonata + Silwet (P6), which had significantly less SPM than all other treatments.

P9 (AQ10 + Silwet) was almost as good as P6. The least effective programme was P4 (CropBiolife only). Plots treated with P11 (CropBiolife + AQ10 and AQ10 in tank mix with Rovral) had a significantly higher incidence than P8 where Rovral was not included. In general Sonata and AQ10 had significantly less SPM in plots where they were applied alone compared to those where either CropBiolife or fungicides had been included in the programme (P6 and P9 compared to P5, P7, P8 and P10). Treatments accounts for most variability in SPM incidence (Table 2.5).

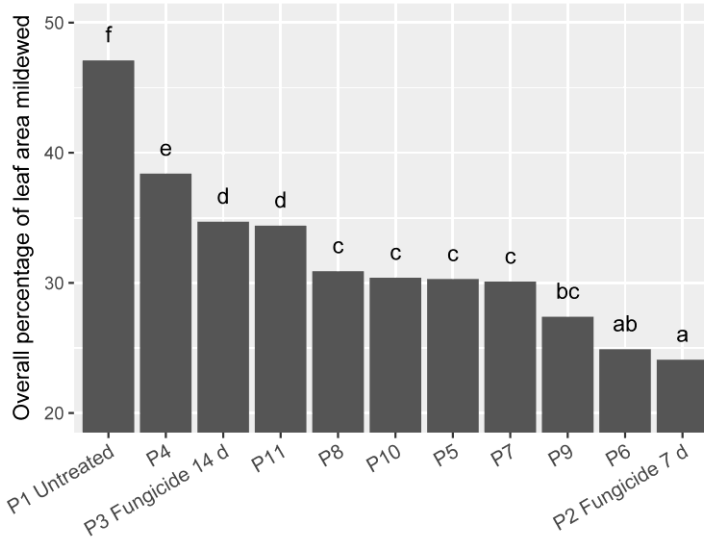


Figure 2.2 Mean % leaf area with SPM over three assessments on cv. Elsanta following treatment with various programmes of fungicides, biocontrol agents and plant strengtheners at NIAB EMR in 2016 (Table 2.2). The SPM level for the best treatment (P2) was still 25% due to conducive conditions for SPM. The SPM levels for programmes with at least one common letter (above the bar) did not differ significantly from each other at the level of P = 0.05 based on the LSD test with residual errors from a repeated measure ANOVA.

Table 2.4 Overall mean % leaf area with SPM, % flowers with SPM, % fruit with SPM, plant vigour, total yield and number of fruit for strawberry cv. Elsanta following treatments with various programmes of fungicides, elicitors and biocontrol products applied pre-harvest at NIAB EMR 2016 (Table 2.2). Figures in brackets are back transformed data; programmes with at least one common letter did not differ from each other at the level of P = 0.05.

Programme	% leaf area with SPM	% flowers/fruitlets with SPM: 27 September	% fruit with SPM at harvest	Plant vigour 26 October	Total yield (Kg)	Total fruit number
P1 Untreated	47.1f	88.0 (99.9)d	33.4c	839.5b	3.850c	16.4 (270.0)c
P2 Standard fungicide 7 days	24.1a	35.0 (32.8)a	11.1ab	1266.6a	4.575ab	18.7 (348.2)ab
P3 Fungicide 14 days	34.7d	66.4 (83.9)c	22.1b	931.5b	4.050bc	18.2 (332.8)ab
P4 CropBiolife	38.4e	75.4 (93.6)c	24.6bc	902.4b	4.025bc	17.0 (288.3)bc
P5 CropBiolife + Sonata + Silwet	30.3c	51.2 (60.8)b	13.7ab	991.2b	4.450abc	18.0 (323.9)bc
P6 Sonata + Silwet	24.9ab	32.8 (29.4)a	7.5a	972.7b	4.425bc	18.5 (342.6)ab
P7 CropBiolife + Sonata + Silwet + P3	30.1c	47.6 (54.5)b	14.3ab	942.5b	3.750c	17.8 (315.5)bc
P8 CropBiolife + AQ10 + Silwet	30.9c	57.0 (70.3)bc	15.8ab	935.0b	5.138a	20.0 (398.1)a
P9 AQ10 + Silwet	27.4bc	43.3 (47.1)ab	7.4a	914.0b	4.750ab	19.0 (362.6)ab
P10 CropBiolife + AQ10 + Silwet + P3	30.4c	50.6 (59.6)b	10.4a	965.6b	4.600ab	18.3 (336.5)ab
P11 CropBiolife, AQ10 alone and in tank mix with Rovral	34.4d	72.5 (90.9)c	19.8b	948.8b	4.075bc	17.9 (320.8)bc
P. value	<0.001	<0.001	<0.001	<0.001	0.011	0.036
LSD (P=0.05)	3.249	12.081	9.486	152.870	0.714	1.799

Table 2.5 % variation accounted for by treatment, time and their interactions for those variables present in Table 2.3

Terms	% leaf area with SPM	% flowers & fruits with SPM	% fruit with SPM at harvest	Plant vigour	Total yield	Total fruit number
Treatment	68.8	82.4	30.5	55.7	6.4	4.8
Time	10.1	-	15.5	-	48.2	54.0
Treatment x Time	7.2	-	13.7	-	5.7	7.4

2.1.2.2 SPM on fruit

The favourable weather conditions also resulted in a high incidence of SPM on the developing flowers and fruits assessed after the first harvest. Almost all flowers and developing fruitlets were infected with SPM (Table 2.4, Figure 2.3, Appendix Table 2). All treatments reduced SPM incidence significantly ($P < 0.001$) with the pattern of efficacy similar to that for foliar SPM although the SPM level was too high to be commercially acceptable for all treatments.

No SPM was recorded on the fruit at the first harvest on 21 September. Thereafter SPM incidence steadily increased. The highest incidence of fruit with SPM was in untreated plots (Table 2.4). Over all four harvests, all treatments, apart from programme 4 (CropBiolife) significantly reduced the incidence of fruits with SPM ($P < 0.001$) with the pattern of efficacy similar to that for foliar SPM. Treatments accounts for most variability in the incidence of fruit SPM (Table 2.5).

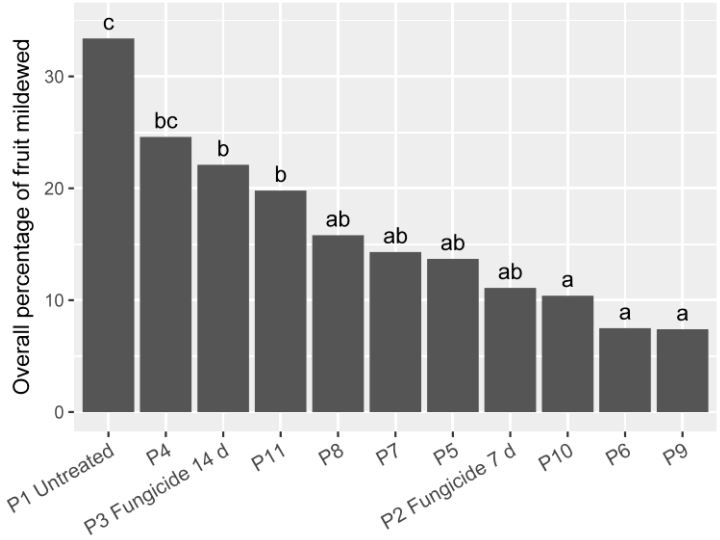


Figure 2.3 Mean % fruit with SPM at harvest, over four harvests on cv. Elsanta following treatment with various programmes of fungicides, biocontrol agents and plant strengtheners at NIAB EMR in 2016 (Table 2.2). SPM levels for treatments (programmes) with at least one common letter (above the bar) did not differ significantly from each other at the level of P = 0.05 based on the LSD test with residual errors from a repeated measure ANOVA.

2.1.2.3 Plant vigour and yield

There were obvious visual differences in plant vigour in the plots receiving the different programmes. All treated plots had a higher vigour score than the untreated control which was obviously stunted, but only the plants in the plots receiving P2 (7 day fungicide) were significantly better, despite the similar incidence of foliar SPM in plots receiving P6 and P9 (Table 2.4). Photo 2.1 shows examples of crop growth for a few treatments with photos for all treatments given in Appendix Photo 2.

The high incidence of SPM, as expected from the damage to developing flowers, had a significant effect on yield and fruit number. Plots receiving P2 (7 day fungicide) and plots receiving AQ10 (P8, P9, P10 except P11 where Rovral included) had significantly higher yield ($P = 0.011$). Similarly a significantly higher number of fruits were also recorded in these plots

(P = 0.036). Higher fruit numbers were also recorded in plots treated with P3 (14 day fungicide) and P6 (Sonata + Silwet). Yield data for individual treatments at each assessment are given in the Appendix Tables 3-5.

A: Untreated

B: P2 7 day fungicide

C: P6 Sonata + Silwet

D: P9 AQ10 + Silwet

Photo 2.1 Examples of plant growth for four selected SPM programmes at NIAB EMR in 2016; the photos were taken after the last SPM assessment in early November

2.1.2.4 Fungal rots at harvest and in post-harvest tests

The incidence of fungal rots (*Botrytis* and *Mucor*) at harvest was low and sporadic (Table 2.6 and Appendix Tables 6-7), although some significant effects were recorded, the rot incidence was too low for these to be meaningful.

Post-harvest tests were done for all harvests with the summary given in Table 2.6. The conditions in September and October were humid and very favourable for *Botrytis* development; *Botrytis* was the main rot present with a mean of over 60% rot in untreated plots over the four harvests (Appendix Table 8). *Penicillium* (Appendix Table 9), *Mucor* (Appendix Table 10) and *Cladosporium* (Appendix Table 11) were important on some harvest dates. Only programmes that included fungicides (P2, P3, P7, P10) significantly reduced rot incidence,

but none of the programmes were effective in reducing rots to acceptable levels. Similarly, the programmes were not very effective in controlling the other rots, although for *Mucor* and *Penicillium* rots P2 (7 day fungicide) significantly reduced these rots. For *Cladosporium* rot, none of the programmes were effective with the lowest incidence recorded in untreated plots and significantly more *Cladosporium* recorded in fungicide-treated plots. The incidence of total post-harvest fruit rot is given in Appendix Table 12.

Table 2.6 Overall % fruit rots at harvest and after post-harvest test on Strawberry cv. Elsanta following treatment with various programmes of fungicides, elicitors and biocontrol products applied pre-harvest at NIAB EMR 2016. Figures in brackets are back transformed data; programmes with at least one common letter did not differ from each other at the level of P = 0.05.

Programme	**% *Botrytis* rot at harvest**	**% *Botrytis* rot in post-harvest**	**% *Mucor* rot at harvest**	**% *Mucor* rot in post-harvest**	**% *Penicillium* rot in post-harvest**	**% *Cladosporium* rot in post-harvest**	**% total rot in post-harvest**
P1 Untreated	2.7bc	61.3d	1.0 (0.03)	24.6bc	36.4c	23.2a	76.7bc
P2 Standard fungicide 7 days	1.0ab	50.2abc	2.3 (0.16)	16.3a	30.1ab	36.9cd	62.2a
P3 Fungicide 14 days	2.6abc	49.3ab	1.2 (0.05)	23.0ab	34.9bc	28.0ab	62.1a
P4 CropBiolife	3.3bc	56.5bcd	1.4 (0.06)	24.5bc	34.2bc	26.6ab	73.0bc
P5 CropBiolife + Sonata + Silwet	5.2c	61.3d	0.9 (0.03)	24.9bc	33.7bc	30.4bc	78.7c
P6 Sonata + Silwet	5.2c	56.3bcd	2.5 (0.19)	31.5c	29.1ab	28.0ab	78.4c
P7 CropBiolife + Sonata + Silwet + P3	0a	48.4ab	0.3 (0)	21.2ab	29.7ab	41.2d	59.9a
P8 CropBiolife + AQ10 + Silwet	3.3bc	54.9bcd	1.1 (0.04)	29.7c	24.7a	28.0ab	75.0bc
P9 AQ10 + Silwet	3.6bc	59.5d	1.3 (0.05)	26.3bc	31.6bc	27.8ab	74.5bc
P10 CropBiolife + AQ10 + Silwet + P3	0.7ab	43.8a	1.0 (0.03)	24.3bc	31.4bc	36.0cd	59.2a

P11 CropBiolife, AQ10 alone and in tank mix with Rovral	3.1bc	57.3cd	0.3 (0)	26.6bc	31.7bc	28.3ab	71.0b
P. value	0.006	<0.001	0.429	0.041	0.025	<0.001	<0.001
LSD (P = 0.05)	2.661	6.972	1.937	7.749	5.924	6.955	6.428

2.1.3 Discussion

Conditions were exceptionally favourable for SPM compared to the previous trial in 2015 where the average leaf area with SPM on untreated plots was around 30% compared to around 60% for 2016. However, the conditions did provide a good test of SPM efficacy. All spray treatments significantly reduced the incidence of SPM compared to the untreated control. Programmes P2 (7 day fungicide), P6 (Sonata + Silwet) and P9 (AQ10 + Silwet) were consistently significantly better than the other treatments. AQ10 and Sonata also consistently performed better when in full programmes rather than those that included fungicides or CropBiolife. Reasons for this are not clear. The incidence of SPM was always significantly higher in P11 (AQ10 + Rovral) compared to the same programme without Rovral (P8). This suggests that tank mixing Rovral with AQ10 may reduce efficacy. The field plots receiving the 7 day fungicide programme (P2) stood out in appearance as larger more vigorous plants compared to the other plots, especially the untreated which was white and stunted (Figs 4-6, Photo 2 in appendix). This difference was borne out in the vigour score (Table 2.4). However, vigour scores for the other treatments was not significant, even those where SPM control was similar. No obvious phytotoxicity was observed on any of the plots which could account for this discrepancy. It is possible that the fungicide was controlling other fungal problems which were not visible such as endophytes. SPM incidence on flowers and fruits generally followed a similar pattern to that on the leaves in terms of efficacy.

The high incidence of SPM on developing flowers and fruits had significant effects on yield and fruit number. However, reductions in yield and fruit number were not as great as expected from the high incidence of SPM present.

The BCAs Sonata and AQ10 are SPM specific products and not expected to reduce fungal rots. So it was expected that only the programmes including fungicides would reduce rotting. In practice none of the programmes were effective with high levels of rotting recorded in all. A higher incidence of *Cladosporium* was recorded in treated plots than in control plots. This was not unexpected. *Cladosporium* is usually regarded as a saprophyte or weak pathogen and often colonises damaged or over ripe tissue. Such rots are not usually well controlled by fungicides and can be increased due to the elimination of antagonistic fungi.

2.1.4 Conclusions

The conditions during the trial were exceptionally favourable for SPM. All spray treatments significantly reduced SPM incidence on both leaves and flowers/fruit. The lowest SPM incidence was on plots treated with the standard 7 day fungicide programme (P2), but still a mean of 25% leaf area with SPM; the efficacy of two BCA only programmes (applied together with Silwet) did not differ from the 7-day fungicide programme. A crop strengthener only

programme led to the least SPM control efficacy. In general the two BCAs applied alone led to better control than with other products.

There were obvious visual differences in plant vigour in the plots receiving the different programmes. All treated plots had a higher vigour score than the untreated control which was obviously stunted, but only the plants in the plots receiving 7 day fungicide were significantly better, despite the similar incidence of foliar SPM in plots receiving biocontrol products. There was a negative relationship between yield (fruit number) and the level of SPM.

The incidence of fungal rots (*Botrytis* and *Mucor*) at harvest was low and sporadic. However, there was a much higher post-harvest rot incidence, particularly *Botrytis*. Although there were significant differences in the *Botrytis* incidence between treatments, none of the programmes were effective in reducing rots to acceptable levels. The incidence for non-*Botrytis* rot is very low and all programmes were not very effective in controlling the other rots.

2.2 ADAS Boxworth

2.2.1 Materials and methods

2.2.1.1 Site and plot details

Strawberries cv. Elsanta, mini tray plants, were planted in coir grow bags in a Spanish tunnel with netted sides at ADAS Boxworth on 21 July 2016. The plants were misted for five days after planting and trickle fertigated throughout the trial period. Each plot consisted of two grow-bags of eight plants (Appendix Table 13 and Photo 2.2).

Photo 2.2 Trial layout at ADAS Boxworth in 2016 for evaluating SPM management programmes combining fungicides with alternative products

2.2.1.2 Treatments and experimental design

The trial included eight treatment programmes (Table 2.7), including an untreated control, and each of the treatments was replicated four times. Two packets of AQ10 were received from the supplier and placed immediately unopened in a cold store at 4°C. One packet of AQ10 was used for the applications between 2 August and 15 August and the other pack was used for the applications between 22 August and 6 September in accordance with the shelf life recommendations. Treatments were applied according to the treatment schedule in Table 2.7 at the rates detailed in Table 2.1. AQ10 was left to soak for 30 minutes prior to use in line with technical recommendations and agitated in the spray tank during use. Spray treatments were applied to each plot at 1000 L water ha^{-1} using an air assisted knapsack (Oxford Precision Sprayer) and hand lance with a 02F110 nozzle at a medium pressure (3 bar) and fine spray. Spray guard boards were used between plots to avoid any drift. Each spray was applied at a time in the day when temperatures were predicted to be at their lowest. Application start times varied from 8.51 am to 2.50 pm with temperatures varying from 21.1°C to 31.7°C Met data is recorded from the ADAS Boxworth weather station, as recorded on a handheld digital thermometer device, with temperature and humidity recorded throughout the trial at crop level by a logger in a ventilated screen.

The first spray treatments were applied on 26 July 2016 and the final treatment applications were made on 6 September 2016. Once flowers were present, the youngest three per plot were marked at intervals with wool, changing the wool colour between dates; this was done to indicate when fruit was picked from that flower so the treatment that the flower received could be determined.

Table 2.7 Treatment details of SPM programmes evaluated at ADAS Boxworth 2016

Programme	Date treatment applied						
	26 July	**02 August**	**09 August**	**15 August**	**22 August**	**30 August**	**06 September**
P1 Untreated control	-	-	-	-	-	-	-
P2 Standard fungicides 7 days	Fortress	Nimrod	Rovral WG + Systhane	Signum	Switch	Signum	Switch + Systhane
P3 Standard fungicides 14 days	Fortress	-	Rovral WG + Systhane	-	Switch	-	Switch + Systhane
P4 CropBiolife	CropBiolife + Fortress	-	-	CropBiolife	-	-	CropBiolife
P8 CropBiolife + AQ10	CropBiolife + Fortress	AQ10 + Silwet	AQ10 + Silwet	CropBiolife	AQ10 + Silwet	AQ10 + Silwet	CropBiolife
P9 AQ10	Fortress	AQ10 + Silwet	AQ10 + Silwet	AQ10 + Silwet	AQ10 + Silwet	AQ10 + Silwet	AQ10 + Silwet
P10 CropBiolife + AQ10 + fungicides at 14 days	CropBiolife + Fortress	AQ10 + Silwet	Rovral WG + Systhane	CropBiolife + AQ10 + Silwet	Switch	AQ10 + Silwet	CropBiolife + Switch + Systhane
P11 CropBiolife + AQ10 alone and in tank mix with Rovral	CropBiolife + Fortress	AQ10 + Silwet	AQ10 + Rovral WG	CropBiolife	AQ10 + Rovral WG	AQ10 + Silwet	CropBiolife

SPM infection was anticipated to arrive naturally in the crop. However, since symptoms were slow to develop in the previous year of the project it was decided that 36 pots of naturally infected plants of cv. Sonata (sourced from last year's trial) would be placed evenly around the trial. In addition, infected runners of cv. Elsanta were collected from a commercial farm in Oxfordshire on 8 August and planted in the ends of each grow bag; infected leaves were also collected and scattered across the trial, several per grow bag. On 19 August a coir grow bag containing eight plants, cv. Prize, infected with SPM (sourced from a commercial farm in Cambridgeshire) was placed at the top end of the trial on raised crates to allow air movement through the tunnel to disperse SPM spores. On the same date bags of fruit and flower stalks with SPM were collected from the same farm and placed around the trial to encourage SPM infection. The floor of the tunnel was hosed and the doors of the tunnel were closed to increase humidity to encourage the germination of SPM spores on the plants. On 26 August further fruit and flower stalks with SPM were collected from the same commercial farm in Cambridgeshire, cv. Prize, and placed throughout the trial area.

2.2.1.3 Assessment

Plants were monitored weekly before each spray application for any evidence of phytotoxicity or SPM infection (Table 2.8).

SPM symptoms were assessed on the youngest five fully expanded leaves of five plants per plot. SPM was recorded as the percentage leaf area that was covered by mycelium and any purpling, using a standard key as used at NIAB EMR. From 5 September onwards SPM developing on the calyxes of the strawberries was assessed. SPM developing on the calyxes was recorded as a percentage of infected calyxes per plot. From 19 September onwards SPM was assessed on harvested fruit as both a count and a percentage of fruit with SPM.

Fruit harvesting commenced on 5 September 2016 and finished on 12 October 2016. Fruit was harvested twice weekly; all the ripe fruit was picked and graded as either marketable (Class I) or waste fruit. The number and mass of marketable and waste fruit per plot were recorded. The waste fruit was also assessed for SPM, *Botrytis*, *Mucor* spp., misshapen fruit, split fruit and pest damage. The number of fruit per plot in each of these categories was recorded. Small healthy fruit were included in the marketable group.

On three occasions, at the beginning (when there was enough fruit per plot), middle and end of harvest 45 healthy fruit per plot were sampled and incubated individually at a high humidity in plastic module trays so that each fruit occupied an individual module cell separating it from adjacent fruit. The fruit was incubated at ambient temperature for seven days, after which the rot types were identified and the number of marketable and rotten fruit were recorded.

SPAD (Single Photon Avalanche Diode) measurements were taken on 6 October. This machine records the chlorophyll content of leaves. Eight plants in a plot were assessed taking readings from the three youngest fully expanded leaves per plant.

Table 2.8 Summary of strawberry treatment and assessment timings – ADAS Boxworth 2016

Activity	**Date**
Strawberry plants planted	21/07/2016
Pre-spray assessment	25/07/2016
Applied spray 1 (all treatment programmes)	26/07/2016
Phytotoxicity and SPM assessment after spray 1	01/08/2016
Applied spray 2 (treatments 2,5,6,7 and 8)	02/08/2016
Phytotoxicity and SPM assessment after spray 2	08/08/2016
Applied spray 3 (treatments 2,3,5,6,7 and 8)	09/08/2016
Phytotoxicity and SPM assessment after spray 3	15/08/2016
Applied spray 4 (treatments 2,4,5,6,7 and 8)	16/08/2016
Phytotoxicity and SPM assessment after spray 4	22/08/2016
Applied spray 5 (treatments 2,3,5,6,7 and 8)	23/08/2016
Phytotoxicity and SPM assessment	30/08/2016
Applied spray 6 (treatments 2,5,6,7 and 8)	30/08/2016
First occurrence of SPM on calyxes	05/09/2016
Harvest 1 assessment	05/09/2016
Phytotoxicity and SPM assessment after spray 6	05/09/2016
Applied spray 7 (treatments 2,3,4,5,6,7 and 8)	06/09/2016
Harvest 2 assessment	07/09/2016
Post-storage *Botrytis* assessment 1 of fruit from harvest 1	12/09/2016
Harvest 3 assessment	12/09/2016
SPM assessment	12/09/2016
Harvest 4 assessment	15/09/2016
SPM first noticed on harvested fruit	19/09/2016
Phytotoxicity and SPM assessment after spray 7	19/09/2016
Post-storage *Botrytis* assessment 2 of fruit from harvest 3	19/09/2016
Harvest 5 assessment	19/09/2016
Harvest 6 assessment	22/09/2016
SPM first noticed on leaf clusters	26/09/2016
SPM assessment	26/09/2016
Harvest 7 assessment	26/09/2016
Post-storage *Botrytis* assessment 3 of fruit from harvest 5	27/09/2016

Harvest 8 assessment	29/09/2016
SPM assessment	04/10/2016
Harvest 9 assessment	04/10/2016
SPAD assessment	06/10/2016
Harvest 10 assessment	06/10/2016
Harvest 11 assessment	12/10/2016
SPM assessment	12/10/2016
SPM assessment	19/10/2016
SPM assessment	26/10/2016

2.2.1.4 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. Preliminary analysis suggested that there was no significant relationship of the SPM severity with the distance of individual bags to the spreader plants.

2.2.2 Results

The weather conditions varied from being relatively warm to hot up to the start and during the trial up until October. Warm temperatures coupled with high humidity were very conducive to SPM development throughout the trial period, except a few days in mid-August (Figure 2.4) and the last couple of weeks.

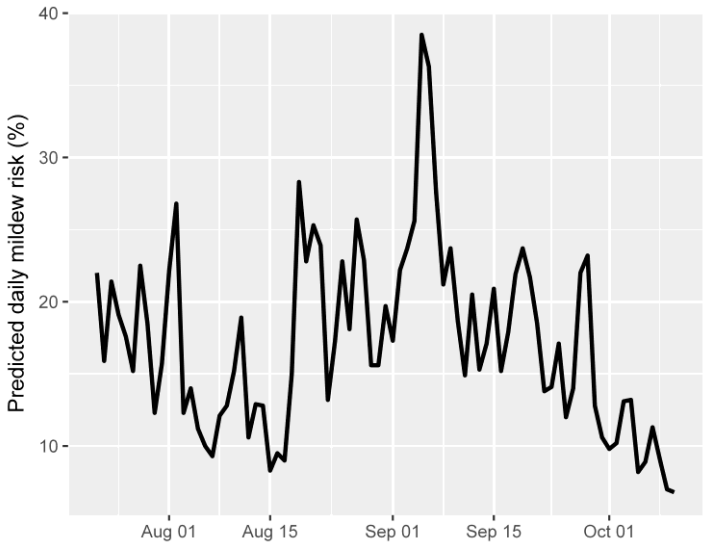


Figure 2.4 Predicted daily risks of SPM on susceptible cultivars for the ADAS Boxworth site in 2016; the predictions were given by the EMR model where a period of consecutive four days with risks > 10% is considered to need growers' intervention with a moderate to high level of inoculum (when the incidence of leaves with SPM is above 5%.

The SPM was slow to develop during the trial period. The first signs of SPM were observed on the strawberry calyxes on 5 September at the assessment carried out after spray six had been applied. SPM incidence at each assessment date is given in the Appendix Tables 15 (calyxes), 16 (fruit) and 18 (leaves). The symptoms involved purple patches on the calyxes. Overall, there were no significant differences between treatments, which accounted for only 2.8% of the total variability (Table 2.9). Most of variability in % calyx with SPM was due to time, accounting for ca. 56% of the total variability (Table 2.9). Only on one assessment occasion (19 September) did the treatments differ in the % calyx with SPM (Figure 2.5, $P < 0.05$). P2 (the standard fungicide programme at 7 day intervals) had significantly higher percentages of calyxes with purpling than the plants in the untreated programme (P1) and also than plants in P4 (CropBiolife), P8 (CropBiolife + AQ10) and P11 (CropBiolife + AQ10 alone and in tank mix with Rovral).

Table 2.9 % variation accounted for by treatment, time and their interactions for percentage of host tissues with SPM (based on angular transformation) for the 2016 ADAS trial at Boxworth

Terms	% leaves with SPM	% calyx with SPM	% fruit with SPM	% *Botrytis* rot
Treatment	7.1	2.8	5.4	3.3
Time	2.9	56.4	32.1	33.4
Treatment x Time	16.2	8.8	10.3	10.0

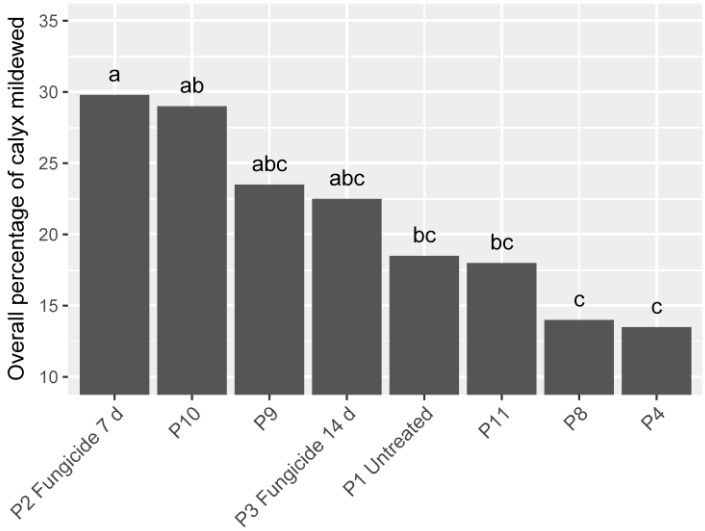


Figure 2.5 % calyx with SPM (based on a single assessment on 19 September) on cv. Elsanta following treatment with various programmes of fungicides, biocontrol agents and plant strengtheners at ADAS in 2016 (Table 2.7). SPM levels for treatments (programmes) with at least one common letter (above the bar) did not differ significantly from each other at the level of P = 0.05. Even the best treatment had a SPM level of 14%, indicating the favourability of conditions for SPM.

Similar results were obtained on fruit SPM. SPM was first seen on the fruit in the trial on the 19 September 2016. Overall, there were no significant differences in the amount of SPM on fruit between management programmes. On 12 October, SPM on fruit was significantly higher in the untreated programme (P1) than in any of the other treatment programmes ($P < 0.05$). On this date 25 % of the berries had SPM in P1, whereas none of the berries in the other treatments had SPM.

SPM was first seen on leaves on 26 September 2016. Overall, there were no significant differences between treatment programmes regarding levels of SPM on leaf clusters. However, there was a significant interaction between assessment time and treatment programme ($P < 0.05$), though it is difficult to interpret the interactions.

There were no phytotoxicity effects observed after any of the treatments were applied over the trial period. There were no significant differences in SPAD measurements between the different treatment programmes at the assessment carried out on 6 October 2016 (Appendix Table 17).

No significant differences between treatments were observed in levels of *Botrytis* or *Mucor* at assessments carried out on 12 September, 19 September or 27 September 2016 (Appendix Tables 19-20). There were no significant differences between marketable yields or waste yields between the different treatments over the entire harvest period (Appendix Table 14).

2.2.3 Discussion

No clear benefits were seen from any of the treatment programmes compared to the untreated control. There is no apparent reason why strawberries that had received applications of standard fungicides at seven day intervals should have more SPM (purple lesions on the calyxes) than the untreated control which was seen on one occasion in the trial period. During the trial there was one occasion when fruit in the untreated control programme had higher levels of SPM compared to fruit in all of the other treatment programmes. This result suggests that all of the treatment programmes improved protection on this date; however this result was not repeated at any of the other assessments where fruit were assessed for SPM.

No benefits in terms of higher marketable yields or lower waste yields were seen from any of the treatments which might be because there was not enough SPM present in the trial to have an effect on the yields.

2.2.4 Conclusion

Overall, there were no significant differences in the percentage of calyxes with SPM between the treatment programmes that were tested in the trial. On one occasion, 19 September 2016, plants in the 7 day fungicide programme had a significantly higher percentage of calyxes with SPM compared to strawberries in the untreated programme and the strawberries in P4

(CropBiolife), P8 (CropBiolife + AQ10) and P11 (CropBiolife + AQ10 alone and in tank mix with Rovral). Overall, no significant differences were observed in the percentages of fruit with SPM between the different treatment programmes. However, on one occasion, 12 October 2016, all of the treatment programmes had significantly lower percentages of fruit with SPM compared to the untreated control meaning that on this occasion all of the treatment programmes provided better control of SPM than the untreated programme.

None of the treatments tested during the trial caused any damage to the plants after each of their applications in terms of phytotoxicity. There were no significant differences in SPAD measurements between the different treatments, meaning there were no differences in chlorophyll levels of the leaves. Marketable yield was unaffected by the different treatment programmes as no significant differences were seen between the different treatments. There were also no significant differences between the numbers of waste fruit that were harvested from the different treatment programmes. Finally, the different treatment programmes had no effect on the number of fugal rots recorded at the harvest assessments during the trial.

2.3 Summary of silicon nutrient trials 2014 and 2015 (Shared under an AHDB consultancy with Dr Avice Hall, UoH and SF157)

The hypothesis tested in these trials was that a weekly application of a Silicon (Si) nutrient in the fertigation tubes would reduce susceptibility of strawberry plants to SPM. The trials were set up on a commercial farm in commercially managed polythene tunnels. In each year, there were four treatments each with five replicates. The treatments were (a) Untreated Control (no commercial fungicides and no Si nutrient), (b) Si nutrient and no commercial fungicides, (c) Commercial fungicides only and no Si nutrient, and (d) Si nutrient and commercial fungicides. In both years 15 leaves were sampled and assessed for SPM every two weeks from April to September. SPM data were first summarised as the Area Under the Disease Progress Curve (AUDPC) and AUDPC values were then subjected to ANOVA for comparing the four treatments.

Figures 2.6 and 2.7 present the summary of results obtained in 2014 and 2015, respectively. There was a difference in disease levels between 2014 and 2015, a reflection of differences in the weather between the years. The 2014 trial had much higher average disease level than the following year. In 2014, ANOVA showed that there was a significant difference ($P = 0.03$) in the AUDPC values between Si (b & d) and no-Si treatments (a & c). From 17/06/14 onwards, there was a significant difference ($P < 0.001$) in the disease level between untreated control (a) and Si only (b), as well as between untreated control (a) and Si + fungicides (d). This suggests that the use of Si alone could significantly reduce SPM development compared to

untreated control. The use of Si and fungicides together led to the best SPM reduction compared with the untreated control.

In 2015, there was an overall significant difference (P = 0.012) in the AUDPC values between Si and no-Si treatments, i.e. treatment (a & b) versus treatment (c & d). In addition, there was a significant difference in the SPM level (P < 0.001) between untreated control (a) and fungicides only (c). Moreover, there was a significant difference (P < 0.001) between untreated control (a) and Si + fungicides (d), indicating that adding Si into fungicides may improve the efficacy of fungicides. There was a significant difference (P < 0.01) between Si (b) and Si + fungicides (d). It should be pointed out that from May 2015 onwards growers had been adding SW7, which is a Si based wetter into fungicides treatment. Thus, there could be a small amount of Si even in the fungicide alone treatment. Therefore, the SPM level from the fungicide alone treatment may have been higher than observed.

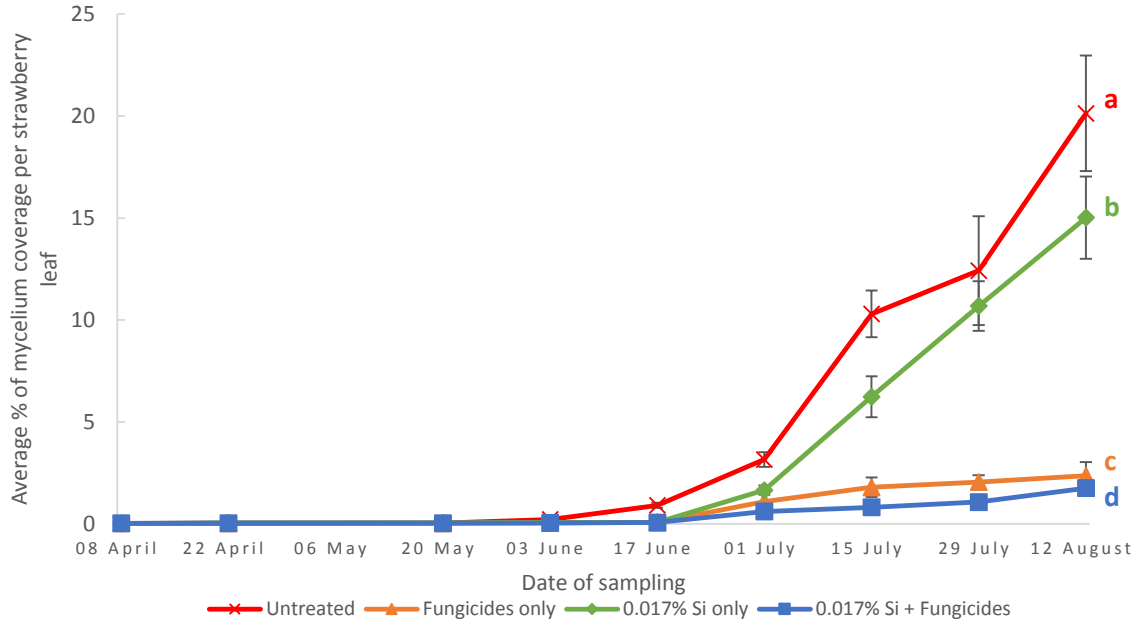


Figure 2.6 Average % leaf area with SPM in the 2014 Silicon trial for no Si and no fungicide treatments (a), Si only (b), fungicides only (c), and Si plus fungicides (d). The final AUDPC values of the four treatments are shown in the graph as well, with the font colour matching the colour of the disease progress curve that represents each treatment.

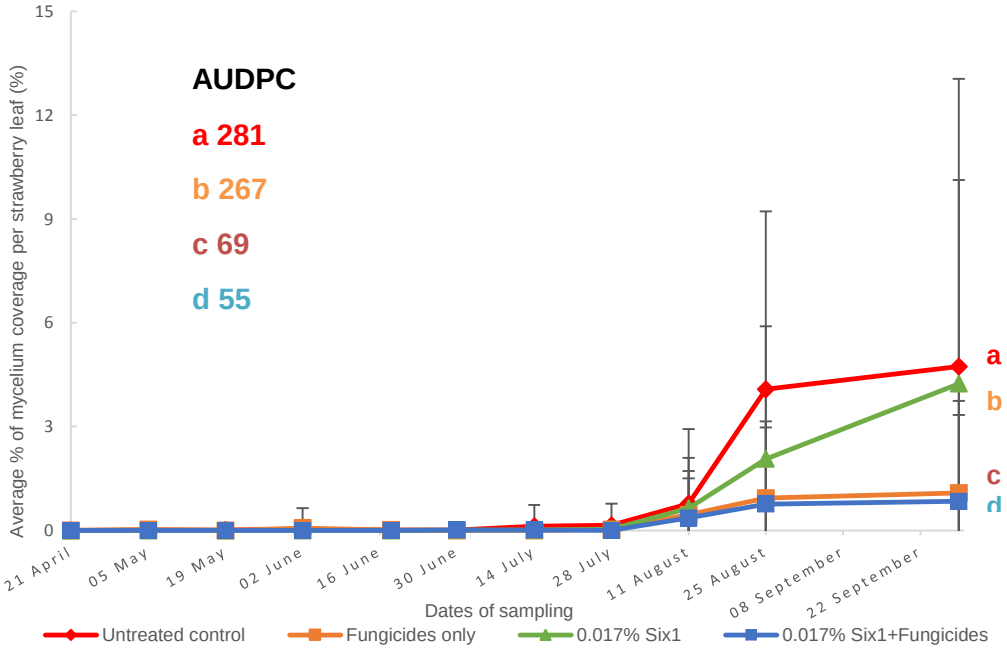


Figure 2.7 Average % leaf area with SPM in the 2015 Pheasant Field Silicon trial for no Si and no fungicide treatments (a), Si only (b), fungicides only (c), and Si plus fungicides (d). The final AUDPC values of the four treatments are shown in the graph as well, with the font colour matching the colour of the disease progress curve that represents each treatment.

In summary, over the two years, using Si delayed SPM development irrespective of the use of fungicides. The 2016 silicon results are consistent with 2014 and 2015, but have not yet been fully analysed.

Objective 3: Fruit rot complex

To understand epidemiology of fruit rot complex and to develop management strategies (to start in Year 4)

Objective 4: Wilt

To develop alternative methods to reduce wilt in soils

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

Unfortunately, CRD informed us that ASD is treated as a normal pesticide and requires registration, which the Dutch manufacturer is not willing to do. Hence, we had to cancel this experiment.

We plan to conduct a wilt field trial in 2017 evaluating efficacy of combining alternative treatments. For this purpose, we selected a site and sampled soil for estimating wilt inoculum to decide whether the site could be used for the trial. This site is of the type of sandy loam soil with a history of *Verticillium* wilt in strawberries. Soil was sampled on 15 September 2016 after cultivations following a barley crop to estimate the level of wilt inoculum using a standard procedure (Harris et al., 1993), taking a series of cores to 200 mm deep across the area. The area 7.5 m wide (equivalent to five bed widths) that would be kept from the growers' chloropicrin sterilisation in spring 2017 was divided into three bands to 20 m up the field and 1 kg of soil collected from each, for Harris testing of viable propagules by ADAS. Further soil sample cores were taken for standard soil analysis of P, K and Mg and available Nitrogen at three depths down to 900 mm.

The results showed that there were 5.6, 3.6 and 2.6 Verticillium propagules per g of soil in the bands progressing into the field. A strawberry plant with a moderate resistance to Verticillium wilt would have a medium chance of wilt; a susceptible variety would have a high chance if planted in an area that falls within the 2.1 to 5.0 propagule range. This showed that the field would be a suitable site for testing material for the reduction of Verticillium wilt in strawberry. Soil analysis gave a pH of 7.8 and information for use in determining fertiliser inputs for the next crop.

Knowledge and Technology Transfer

1. Dr Berrie presented the summary of strawberry management work at the AHDB Soft Fruit day at NIAB EMR, November 2016.
2. Dr Berrie presented the strawberry management work at the IOBC Soft Fruit conference, Greece, September 2016.
3. Prof Xu presented a poster on the effects of AMF on *P. fragariae* at the International Society of Microbial Ecology Conference in Montreal August 2016
4. Dr Hall presented an oral paper on 'Integrated Control of SPM' at the International Strawberry Symposium, August 2016 in Quebec, and two posters, one on localisation of Silicon in treated plants, the other on integrated control
5. Ms Liu presented a poster on 'What is the origin of the initial inoculum for SPM epidemics' at the BSPP Meeting, September 2016 Oxford
6. Dr Hall presented a lecture on 'Integrated control of SPM control in Strawberry' in LMHIA (Lower mainland Horticulture Improvement Association) Horticultural Growers Short Course Abbotsford, British Columbia, Canada Thursday 26 January 2017
7. The updated AHDB factsheet on 'SPM' should be out in February/ March 2017 by Jolyon Dodgson, Avice Hall, Xiaolei Jin

References

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.

Cochran, W.G. (1950). Estimation of bacterial densities by means of the most probable number. *Biometrics* 6**,** 105-116.

Harris, D., Simpson, D., and Bell, J. (1997). Studies on the possible role of micropropagation in the dissemination of the strawberry crown rot pathogen *Phytophthora cactorum*. *Journal of Horticultural Science & Biotechnology* 72**,** 125-133.

Harris, D.C., Yang, J.R., and Ridout, M.S. (1993). The detection and estimation of *Verticillium dahliae* in naturally infested soil. *Plant Pathology* 42**,** 238-250. doi: 10.1111/j.1365-3059.1993.tb01496.x.

Robinson Boyer, L., Feng, W., Gulbis, N., Hajdu, K., Harrison, R.J., Jeffries, P., and Xu, X. (2016). The use of arbuscular mycorrhizal fungi to improve strawberry production in coir substrate. *Frontiers in Plant Science* 7**,** 1237. doi: 10.3389/fpls.2016.01237.

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.

Sati, S.C., and Belwal, M. (2005). Aquatic hyphomycetes as endophytes of riparian plant roots. *Mycologia* 97**,** 45-49. doi: 10.3852/mycologia.97.1.45.

Schena, L., Duncan, J.M., and Cooke, D.E.L. (2008). Development and application of a PCR-based 'molecular tool box' for the identification of *Phytophthora* species damaging forests and natural ecosystems. *Plant Pathology* 57**,** 64-75. doi: 10.1111/j.1365-3059.2007.01689.x.

Sowndhararajan, K., Marimuthu, S., and Manian, S. (2013). Biocontrol potential of phylloplane bacterium *Ochrobactrum anthropi* BMO-111 against blister blight disease of tea. *Journal of Applied Microbiology* 114**,** 209-218. doi: 10.1111/jam.12026.

Appendix

Photo 1 Samples with '*P.* cactorum' like symptoms but with negative test results for presence of *P. cactorum* using LFD and nested PCR methods.

Table 1 Treatments applied to all plots during the NIAB EMR SPM trial 2016

Date applied	Product	Type / target	Rate (ha^{-1})
22 August	Aliette	Fungicide / Phytophthora	3.75 kg
9 September	Calypso	Insecticide / capsids	250 mL
23 September	Calypso	Insecticide / capsids	250 mL

Table 2 % leaf area with SPM on strawberry cv. Elsanta assessed on 20 September, 25 October and 1 November and % flowers / fruitlets with SPM following treatment with various programmes of fungicides, elicitors and biofungicides applied pre-harvest at NIAB EMR 2016. Figures in brackets are back transformed data.

Programme	% leaf area with SPM			% flowers/fruitlets with SPM				Plant vigour 26 October
	20 September	25 October	1 November	27 September	28 September	5 October	12 October	
P1 Untreated	52.0 (62.0)	51.3 (60.9)	38.1 (38.0)	88.0 (99.9)	9.0 (2.5)	49.5 (57.8)	41.8 (44.4)	839.5
P2 Standard fungicide 7 days	25.6 (18.7)	24.8 (17.6)	22.0 (14.0)	35.0 (32.8)	3.9 (0.5)	18.8 (10.3)	10.8 (3.5)	1266.6
P3 Fungicide 14 days	39.5 (40.5)	36.5 (35.3)	28.2 (22.4)	66.4 (83.9)	12.5 (4.7)	29.8 (24.8)	23.9 (16.5)	931.5
P4 CropBiolife	42.9 (46.4)	38.0 (38.0)	34.3 (31.8)	75.4 (93.6)	18.1 (9.7)	23.4 (15.8)	32.3 (28.6)	902.4
P5 CropBiolife + Sonata + Silwet	31.3 (26.9)	30.7 (26.1)	28.8 (23.3)	51.2 (60.8)	8.4 (2.1)	11.8 (4.2)	20.8 (12.7)	991.2
P6 Sonata + Silwet	24.3 (17.0)	27.4 (21.2)	23.0 (15.3)	32.8 (29.4)	5.9 (1.1)	10.1 (3.0)	6.5 (1.3)	972.7
P7 CropBiolife + Sonata + Silwet + P3	34.0 (31.3)	29.4 (24.2)	26.8 (20.3)	47.6 (54.5)	9.3 (2.6)	15.8 (7.4)	17.6 (9.1)	942.5
P8 CropBiolife + AQ10 + Silwet	29.6 (24.3)	32.5 (28.8)	30.7 (26.0)	57.0 (70.3)	10.4 (3.3)	13.9 (5.8)	23.1 (15.4)	935.0
P9 AQ10 + Silwet	27.3 (21.1)	29.5 (24.3)	25.3 (18.3)	43.3 (47.1)	2.8 (0.2)	6.1 (1.1)	13.4 (5.3)	914.0
P10 CropBiolife + AQ10 + Silwet + P3	32.1 (28.3)	31.5 (27.3)	27.5 (21.3)	50.6 (59.6)	3.8 (0.4)	6.0 (1.1)	21.5 (13.4)	965.6

P11 CropBiolife, AQ10 alone and in tank mix with Rovral	34.9 (32.8)	37.3 (36.7)	31.0 (26.5)		72.5 (90.9)	12.9 (5.0)	24.6 (17.3)	21.9 (13.9)	948.8
P. value	<0.0001	<0.0001	<0.0001		<0.0001	0.132	<0.0001	0.008	0.001
LSD (p=0.05)	3.516	3.884	3.249		12.081	10.313	14.739	16.079	152.870

Table 3 Yield kg per plot following treatment with various programmes of fungicides, elicitors and biofungicides applied pre-harvest at NIAB EMR 2016

Programme	21 September	28 September	5 October	12 October	Total yield
P1 Untreated	0.975	1.475	0.725	0.675	3.850
P2 Standard fungicide 7 days	1.375	1.375	0.925	0.900	4.575
P3 Fungicide 14 days	1.025	1.425	0.900	0.700	4.050
P4 CropBiolife	1.025	1.525	0.850	0.625	4.025
P5 CropBiolife + Sonata + Silwet	1.450	1.475	0.875	0.650	4.450
P6 Sonata + Silwet	1.350	1.350	0.850	0.875	4.425
P7 CropBiolife + Sonata + Silwet + P3	0.950	1.275	0.825	0.700	3.750
P8 CropBiolife + AQ10 + Silwet	1.550	1.713	1.125	0.750	5.138
P9 AQ10 + Silwet	1.450	1.550	0.975	0.775	4.750
P10 CropBiolife + AQ10 + Silwet + P3	1.275	1.525	1.025	0.775	4.600
P11 CropBiolife, AQ10 alone and in tank mix with Rovral	1.000	1.400	0.900	0.775	4.075
P. value	0.002	0.253	0.017	0.006	0.011
LSD (P = 0.05)	0.333	0.294	0.188	0.140	0.714

Table 4 Fruit number per plot (square root transformed), following treatment with various programmes of fungicides, elicitors and biofungicides applied pre-harvest at NIAB EMR 2016. Figures in brackets are back transformed data

Programme	21 September	28 September	5 October	12 October	Total fruit number
P1 Untreated	6.3 (39.9)	11.7 (137.0)	6.9 (48.1)	6.4 (41.2)	16.4 (270.0)
P2 Standard fungicide 7 days	8.6 (73.3)	11.5 (131.8)	8.4 (70.6)	8.1 (64.9)	18.7 (348.2)
P3 Fungicide 14 days	6.5 (42.6)	11.8 (138.5)	9.8 (96.0)	7.2 (52.5)	18.2 (332.8)
P4 CropBiolife	6.7 (45.0)	10.5 (109.9)	9.6 (91.2)	6.1 (37.8)	17.0 (288.3)
P5 CropBiolife + Sonata + Silwet	8.7 (75.6)	11.4 (130.3)	8.7 (75.2)	6.4 (40.5)	18.0 (323.9)
P6 Sonata + Silwet	8.5 (71.5)	11.0 (121.3)	8.9 (79.9)	8.3 (68.3)	18.5 (342.6)
P7 CropBiolife + Sonata + Silwet + P3	6.8 (46.4)	11.3 (126.8)	9.5 (89.8)	7.0 (48.4)	17.8 (315.5)
P8 CropBiolife + AQ10 + Silwet	9.0 (80.1)	12.3 (150.9)	10.6 (113.1)	7.2 (52.0)	20.0 (398.1)
P9 AQ10 + Silwet	9.0 (81.3)	12.0 (144.6)	9.3 (86.4)	6.9 (47.2)	19.0 (362.6)
P10 CropBiolife + AQ10 + Silwet + P3	7.8 (60.7)	11.1 (123.9)	9.7 (94.5)	7.4 (54.3)	18.3 (336.5)
P11 CropBiolife, AQ10 alone and in tank mix with Rovral	6.8 (46.0)	11.3 (127.7)	9.1 (83.2)	7.7 (59.3)	17.9 (320.8)
P. value	0.003	0.316	0.001	0.095	0.036
LSD (P = 0.05)	1.613	1.342	1.361	1.457	1.799

Table 5 Mean fruit weight in grams (Log transformed), following treatment with various programmes of fungicides, elicitors and biofungicides applied pre-harvest at NIAB EMR 2016. Figures in brackets are back transformed data

Programme	21 September	28 September	5 October	12 October	Mean fruit size
P1 Untreated	3.2 (25)	2.3 (11)	2.8 (16)	2.8 (17)	2.8 (16)
P2 Standard fungicide 7 days	2.9 (19)	2.3 (10)	2.6 (14)	2.7 (14)	2.6 (14)
P3 Fungicide 14 days	3.2 (24)	2.3 (10)	2.2 (9)	2.6 (14)	2.6 (13)
P4 CropBiolife	3.1 (23)	2.6 (14)	2.2 (9)	2.8 (17)	2.7 (15)
P5 CropBiolife + Sonata + Silwet	2.9 (19)	2.4 (11)	2.5 (12)	2.8 (16)	2.6 (14)
P6 Sonata + Silwet	2.9 (19)	2.4 (11)	2.4 (11)	2.6 (13)	2.6 (13)
P7 CropBiolife + Sonata + Silwet + P3	3.0 (21)	2.3 (10)	2.2 (9)	2.7 (15)	2.5 (13)
P8 CropBiolife + AQ10 + Silwet	2.9 (19)	2.4 (11)	2.3 (10)	2.7 (15)	2.6 (13)
P9 AQ10 + Silwet	2.9 (18)	2.4 (11)	2.4 (11)	2.8 (17)	2.6 (14)
P10 CropBiolife + AQ10 + Silwet + P3	3.0 (21)	2.5 (12)	2.4 (11)	2.7 (15)	2.6 (14)
P11 CropBiolife, AQ10 alone and in tank mix with Rovral	3.1 (22)	2.4 (11)	2.4 (11)	2.6 (14)	2.6 (14)
P. value	0.095	0.150	0.031	0.572	0.078
LSD (P = 0.05)	0.247	0.208	0.332	0.295	0.1422

Table 6 Mean % *Botrytis* fruit rot at harvest (angular transformed), following treatment with various programmes of fungicides, elicitors and biofungicides applied pre-harvest at NIAB EMR 2016. Figures in brackets are back transformed data

Programme	**21 September**	**28 September**	**5 October**	**12 October**	**Overall *Botrytis* rot**
P1 Untreated	3.9 (0.5)	2.7 (0.2)	2.5 (0.19)	1.7 (0.09)	2.7
P2 Standard fungicide 7 days	0	1.2 (0.04)	0	2.7 (0.23)	1.0
P3 Fungicide 14 days	3.5 (0.4)	1.4 (0.06)	1.6 (0.08)	4.0 (0.50)	2.6
P4 CropBiolife	3.0 (0.3)	4.8 (0.70)	3.4 (0.36)	1.9 (0.11)	3.3
P5 CropBiolife + Sonata + Silwet	0	5.5 (0.9)	11.1 (3.68)	4.1 (0.52)	5.2
P6 Sonata + Silwet	1.6 (0.1)	8.4 (2.2)	5.6 (0.94)	4.9 (0.74)	5.2
P7 CropBiolife + Sonata + Silwet + P3	0	0	0	0	0
P8 CropBiolife + AQ10 + Silwet	3.4 (0.3)	5.3 (0.8)	4.7 0.68)	0	3.3
P9 AQ10 + Silwet	1.3 (0.05)	6.9 (1.4)	4.8 (0.70)	1.6 (0.08)	3.6
P10 CropBiolife + AQ10 + Silwet + P3	0	2.8 (0.24)	0	0	0.7
P11 CropBiolife, AQ10 alone and in tank mix with Rovral	0	3.2 (0.31)	1.7 (0.09)	7.3 (1.62)	3.1
CropBiolife	0.497	0.021	0.003	0.110	0.006
LSD (P = 0.05)	4.847	4.619	4.952	5.072	2.661

Table 7 Mean % Mucor fruit rot at harvest (angular transformed), following treatment with various programmes of fungicides, elicitors and biofungicides applied pre-harvest at NIAB EMR 2016. Figures in brackets are back transformed data

Programme	21 September	28 September	5 October	12 October	Overall *Mucor* rot
P1 Untreated	2.5 (0.20)	1.6 (0.08)	0	0	1.0 (0.03)
P2 Standard fungicide 7 days	1.5 (0.07)	3.1 (0.29)	2.6 (0.21)	1.9 (0.11)	2.3 (0.16)
P3 Fungicide 14 days	2.2 (0.15)	1.1 (0.04)	1.6 (0.08)	0	1.2 (0.05)
P4 CropBiolife	2.2 (0.15)	1.4 (0.06)	1.9 (0.11)	0	1.4 (0.06)
P5 CropBiolife + Sonata + Silwet	0	1.1 (0.04)	2.5 (0.20)	0	0.9 (0.03)
P6 Sonata + Silwet	0	3.4 (0.35)	5.2 (0.81)	1.5 (0.07)	2.5 (0.19)
P7 CropBiolife + Sonata + Silwet + P3	0	1.1 (0.04)	0	0	0.3 (0)
P8 CropBiolife + AQ10 + Silwet	0	3.3 (0.33)	1.2 (0.04)	0	1.1 (0.04)
P9 AQ10 + Silwet	1.8 (0.10)	1.9 (0.11)	1.6 (0.08)	0	1.3 (0.05)
P10 CropBiolife + AQ10 + Silwet + P3	0	2.4 (0.17)	1.6 (0.08)	0	1.0 (0.03)
P11 CropBiolife, AQ10 alone and in tank mix with Rovral	0	0	1.3 (0.05)	0	0.3 (0)
P. value	0.751	0.487	0.795	0.465	0.429
LSD (P = 0.05)	3.915	3.134	5.212	2.024	1.937

Table 8 Mean % Botrytis fruit rot in post-harvest (angular transformed), following treatment with various programmes of fungicides, elicitors and biofungicides applied pre-harvest at NIAB EMR 2016. Figures in brackets are back transformed data

Programme	**21 September**	**28 September**	**5 October**	**12 October**	**Overall *Botrytis* rot**
P1 Untreated	69.7 (87.9)	52.7 (63.3)	65.8 (83.1)	56.9 (70.1)	61.3
P2 Standard fungicide 7 days	61.0 (76.5)	47.3 (54.0)	47.9 (55.0)	44.5 (49.1)	50.2
P3 Fungicide 14 days	50.5 (59.5)	48.4 (55.9)	53.0 (63.8)	45.5 (50.9)	49.3
P4 CropBiolife	62.1 (78.0)	55.0 (67.1)	62.5 (78.7)	46.2 (52.2)	56.5
P5 CropBiolife + Sonata + Silwet	69.3 (87.5)	56.6 (69.7)	63.4 (79.9)	55.9 (68.5)	61.3
P6 Sonata + Silwet	60.5 (75.7)	51.8 (61.7)	58.1 (72.1)	54.9 (66.9)	56.3
P7 CropBiolife + Sonata + Silwet + P3	49.0 (56.9)	43.3 (47.0)	52.8 (63.4)	47.0 (53.6)	48.4
P8 CropBiolife + AQ10 + Silwet	56.5 (69.6)	51.0 (60.3)	58.2 (72.2)	54.0 (65.4)	54.9
P9 AQ10 + Silwet	68.6 (86.7)	54.1 (65.7)	62.1 (78.1)	53.3 (64.2)	59.5
P10 CropBiolife + AQ10 + Silwet + P3	47.8 (54.9)	35.9 (34.3)	49.7 (58.1)	42.0 (44.7)	43.8
P11 CropBiolife, AQ10 alone and in tank mix with Rovral	58.1 (72.0)	47.4 (54.2)	58.9 (73.3)	64.8 (81.9)	57.3
P. value	0.003	0.049	0.059	0.031	<0.001
LSD (P = 0.05)	12.054	11.663	11.789	12.738	6.972

Table 9 Mean % *Penicillium* fruit rot in post-harvest (angular transformed), following treatment with various programmes of fungicides, elicitors and biofungicides applied pre-harvest at NIAB EMR 2016. Figures in brackets are back transformed data

Programme	**21 September**	**28 September**	**5 October**	**12 October**	**Overall *Penicillium* rot**
P1 Untreated	9.8 (2.9)	44.3 (48.8)	52.1 (62.3)	39.4 (40.2)	36.4
P2 Standard fungicide 7 days	9.6 (2.8)	43.9 (48.0)	38.9 (39.4)	28.1 (22.2)	30.1
P3 Fungicide 14 days	16.4 (7.9)	44.4 (49.0)	44.4 (48.9)	34.4 (31.9)	34.9
P4 CropBiolife	11.1 (3.7)	46.2 (52.0)	48.5 (56.2)	31.1 (26.7)	34.2
P5 CropBiolife + Sonata + Silwet	16.4 (8.0)	45.4 (50.7)	43.0 (46.6)	30.1 (25.1)	33.7
P6 Sonata + Silwet	15.3 (7.0)	36.2 (34.9)	40.7 (42.4)	24.0 (16.6)	29.1
P7 CropBiolife + Sonata + Silwet + P3	4.1 (0.5)	42.8 (46.1)	38.4 (38.5)	30.7 (26.1)	29.7
P8 CropBiolife + AQ10 + Silwet	10.5 (3.3)	35.5 (33.7)	32.7 (29.2)	19.8 (11.5)	24.7
P9 AQ10 + Silwet	16.3 (7.8)	38.2 (38.3)	41.5 (44.0)	30.3 (25.4)	31.6
P10 CropBiolife + AQ10 + Silwet + P3	14.1 (5.9)	43.4 (47.2)	37.5 (37.1)	30.6 (25.9)	31.4
P11 CropBiolife, AQ10 alone and in tank mix with Rovral	10.6 (3.4)	42.2 (45.2)	45.0 (50.0)	28.9 (23.3)	31.7
P. value	0.520	0.495	0.008	0.335	0.025
LSD (P = 0.05)	11.578	10.869	8.870	13.277	5.924

Table 10 Mean % *Mucor* fruit rot in post- harvest angular transformed, following treatment with various programmes of fungicides, elicitors and biofungicides applied pre-harvest at NIAB EMR 2016. Figures in brackets are back transformed data

Programme	**21 September**	**28 September**	**5 October**	**12 October**	**Overall *Mucor* rot**
P1 Untreated	13.5 (5.5)	38.2 (38.3)	16.8 (8.4)	29.8 (24.7)	24.6
P2 Standard fungicide 7 days	10.3 (3.2)	24.4 (17.1	15.2 (6.9)	15.3 (7.0)	16.3
P3 Fungicide 14 days	16.3 (7.9)	30.2 (25.2)	22.5 (14.6)	22.8 (15.0)	23.0
P4 CropBiolife	9.8 (2.9)	35.5 (33.8)	14.7 (6.4)	38.1 (38.1)	24.5
P5 CropBiolife + Sonata + Silwet	14.7 (6.5)	34.1 (31.4)	21.0 (12.8)	29.7 (24.5)	24.9
P6 Sonata + Silwet	26.1 (19.4)	38.7 (39.1)	30.4 (25.6)	30.6 (25.9)	31.5
P7 CropBiolife + Sonata + Silwet + P3	15.4 (7.0)	33.5 (30.5)	8.8 (2.3)	25.8 (19.1)	21.2
P8 CropBiolife + AQ10 + Silwet	27.0 (20.7)	37.2 (36.5)	27.2 (21.0)	27.3 (21.1)	29.7
P9 AQ10 + Silwet	18.2 (9.8)	36.5 (35.4)	27.1 (20.7)	23.5 (15.9)	26.3
P10 CropBiolife + AQ10 + Silwet + P3	18.0 (9.5)	40.1 (41.5)	18.3 (9.8)	20.9 (12.7)	24.3
P11 CropBiolife, AQ10 alone and in tank mix with Rovral	20.5 (12.3)	38.1 (38.1)	25.3 (18.3)	22.3 (14.5)	26.6
P. value	0.009	0.535	0.222	0.062	0.041
LSD (P = 0.05)	9.269	13.707	15.958	12.210	7.749

Table 11 Mean % *Cladosporium* fruit rot in post-harvest (angular transformed), following treatment with various programmes of fungicides, elicitors and biofungicides applied pre-harvest at NIAB EMR 2016. Figures in brackets are back transformed data

Programme	**28 September**	**5 October**	**Overall *Cladosporium* rot**
P1 Untreated	6.9 (1.5)	39.4 (40.3)	23.2
P2 Standard fungicide 7 days	34.1 (31.4)	39.7 (40.8)	36.9
P3 Fungicide 14 days	21.0 (12.9)	35.0 (32.9)	28.0
P4 CropBiolife	17.6 (9.1)	35.5 (33.8)	26.6
P5 CropBiolife + Sonata + Silwet	20.2 (11.9)	40.6 (42.3)	30.4
P6 Sonata + Silwet	20.5 (12.3)	35.5 (33.8)	28.0
P7 CropBiolife + Sonata + Silwet + P3	36.3 (35.1)	43.9 (48.0)	41.2
P8 CropBiolife + AQ10 + Silwet	18.8 (10.4)	37.3 (36.7)	28.0
P9 AQ10 + Silwet	17.9 (9.4)	37.7 (37.3)	27.8
P10 CropBiolife + AQ10 + Silwet + P3	29.5 (24.3)	42.4 (45.5)	36.0
P11 CropBiolife, AQ10 alone and in tank mix with Rovral	16.1 (7.7)	40.5 (42.2)	28.3
P. value	0.001	0.384	<0.001
LSD (P = 0.05)	12.044	7.985	6.955

Table 12 Mean % total fruit rot in post-harvest (angular transformed), following treatment with various programmes of fungicides, elicitors and biofungicides applied pre-harvest at NIAB EMR 2016. Figures in brackets are back transformed data

Programme	21 September	28 September	5 October	12 October	Overall total rot
P1 Untreated	76.0 (94.2)	81.8 (97.9)	82.8 (98.4)	66.2 (83.7)	76.7
P2 Standard fungicide 7 days	63.9 (80.7)	67.1 (84.8)	65.7 (83.1)	52.2 (62.4)	62.2
P3 Fungicide 14 days	54.4 (66.1)	68.5 (86.6)	68.9 (87.1)	56.6 (69.8)	62.1
P4 CropBiolife	75.8 (94.0)	78.9 (96.3)	72.7 (91.1)	64.4 (81.3)	73.0
P5 CropBiolife + Sonata + Silwet	78.2 (95.9)	85.9 (99.5)	82.5 (98.3)	68.0 (86.0)	78.7
P6 Sonata + Silwet	81.3 (97.7)	83.6 (98.7)	81.3 (97.7)	67.4 (85.2)	78.4
P7 CropBiolife + Sonata + Silwet + P3	52.2 (62.5)	68.0 (86.0)	60.9 (76.4)	58.0 (71.9)	59.9
P8 CropBiolife + AQ10 + Silwet	71.5 (89.9)	80.4 (97.2)	82.1 (98.1)	66.0 (83.4)	75.0
P9 AQ10 + Silwet	78.8 (96.2)	80.3 (97.2)	79.5 (96.7)	59.6 (74.4)	74.5
P10 CropBiolife + AQ10 + Silwet + P3	56.9 (70.2)	69.6(87.9)	60.3 (75.4)	50.0 (58.7)	59.2
P11 CropBiolife, AQ10 alone and in tank mix with Rovral	66.9 (84.6)	71.0 (89.4)	75.7 (93.9)	70.3 (88.6)	71.0
P. value	<0.0001	0.001	<0.0001	0.008	<0.001
LSD (P = 0.05)	13.011	9.894	8.333	11.162	6.428

A: P1 Untreated

B: P2 7 day fungicide

C: P3 14 day fungicide

D: P4 CropBiolife

E: P5 CropBiolife + Sonata/Silwet

F: P6 Sonata/Silwet

G: P7 CropBiolife + Sonata/Silwet + P3

H: P8 CropBiolife + AQ10/Silwet

I: P9 AQ10/Silwet mix with Rovral

J: P10 CropBiolife + AQ10/Silwet + P3

K: P11 CropBiolife + AQ10 in tank

Photo 2 Photos of plant growth at the end of the strawberry SPM trial at NIAB EMR (taken in early November 2016).

Table 13 Trial Plan for coir grow-bags in polythene tunnel at ADAS Boxworth July to October 2016; actual SPM management programme details are given in Table 2.2

Plot	Block	Prog-ramme	Plot	Block	Prog-ramme	Plot	Block	Prog-ramme	Plot	Block	Prog-ramme
1	**1**	**2**	**9**	**2**	**1**	**17**	**3**	**4**	**25**	**4**	**6**
2	**1**	**3**	**10**	**2**	**6**	**18**	**3**	**5**	**26**	**4**	**2**
3	**1**	**6**	**11**	**2**	**7**	**19**	**3**	**2**	**27**	**4**	**5**
4	**1**	**8**	**12**	**2**	**4**	**20**	**3**	**7**	**28**	**4**	**3**
5	**1**	**5**	**13**	**2**	**8**	**21**	**3**	**3**	**29**	**4**	**7**
6	**1**	**1**	**14**	**2**	**5**	**22**	**3**	**6**	**30**	**4**	**4**
7	**1**	**7**	**15**	**2**	**3**	**23**	**3**	**1**	**31**	**4**	**8**
8	**1**	**4**	**16**	**2**	**2**	**24**	**3**	**8**	**32**	**4**	**1**

Table 14 Combined plot yields (marketable and waste) and number of fruit (marketable and waste) over all harvests 9 July to 14 August at ADAS Boxworth 2016

Programme	Marketable weight (g)	Number of marketable fruit	Waste weight (g)	Number of waste fruit
P1 Untreated control	1925	190	620	54
P2 Standard fungicides 7 days	1845	190	534	52
P3 Standard fungicides 14 days	1901	190	649	63
P4 CropBiolife	1592	166	695	66
P8 CropBiolife + AQ10	1836	185	786	74
P9 AQ10	1877	206	502	49
P10 CropBiolife + AQ10 + fungicides at 14 days	1767	183	652	67
P11 CropBiolife + AQ10 alone and in tank mix with Rovral	1714	170	777	75.5
P. value	0.653	0.352	0.070	0.108

Table 15 Percentage of strawberry calyxes with purpling (likely to be SPM) in the different treatment programmes over the trial period at ADAS Boxworth 2016

Programme	5 Sept	12 Sept	19 Sept	26 Sept	4 Oct	12 Oct	19 Oct
P1 Untreated control	13.2	13.8	18.5	15.8	3.3	8.0	3.0
P2 Standard fungicides 7 days	12.2	12.5	29.8	9.0	0.5	1.0	0.8
P3 Standard fungicides 14 days	15.2	12.0	22.5	12.5	4.8	5.0	2.3
P4 CropBiolife	18.2	14.2	13.5	9.3	0.3	3.0	2.8
P8 CropBiolife + AQ10	9.5	11.8	14.0	12.0	3.8	3.8	3.0
P9 AQ10	32.0	21.0	23.5	14.3	3.0	2.3	0.3
P10 CropBiolife + AQ10 + fungicides at 14 days	20.5	22.2	29.0	18.0	2.3	2.0	0.0
P11 CropBiolife + AQ10 alone and in tank mix with Rovral	13.0	10.8	18.0	9.3	2.5	3.5	1.0
P. value	0.059	0.330	0.041	0.142	0.314	0.071	0.186

Table 16 Percentage of strawberry fruit with SPM in the different treatment programmes over the trial period at ADAS Boxworth 2016

Programme	19 Sept	26 Sept	4 Oct	12 Oct
P1 Untreated control	7.3	10.8	35.5	25.0
P2 Standard fungicides 7 days	0.0	0.0	18.0	0.0
P3 Standard fungicides 14 days	1.0	4.0	36.8	0.0
P4 CropBiolife	0.0	9.0	10.0	0.0
P8 CropBiolife + AQ10	1.0	0.8	30.0	0.0
P9 AQ10	0.8	2.5	23.0	0.0
P10 CropBiolife + AQ10 + fungicides at 14 days	0.0	9.8	12.5	0.0
P11 CropBiolife + AQ10 alone and in tank mix with Rovral	1.3	8.2	22.5	0.0
P. value	0.184	0.190	0.720	0.024

Table 17 SPAD measurements of leaves from the different treatment programmes at ADAS Boxworth 2016

Programme	6 October
P1 Untreated control	35.55
P2 Standard fungicides 7 days	37.67
P3 Standard fungicides 14 days	37.11
P4 CropBiolife	37.61
P8 CropBiolife + AQ10	34.86
P9 AQ10	37.34
P10 CropBiolife + AQ10 + fungicides at 14 days	36.15
P11 CropBiolife + AQ10 alone and in tank mix with Rovral	36.38
P. value	0.233

Table 18 Percentage of leaf area with SPM in the different treatment programmes over the trial period at ADAS Boxworth 2016

Programme	26 Sept	4 Oct	12 Oct	19 Oct	26 Oct
P1 Untreated control	0.00	0.00	0.00	0.00	0.16
P2 Standard fungicides 7 days	0.06	0.11	0.14	0.14	0.16
P3 Standard fungicides 14 days	0.01	0.09	0.11	0.13	0.08
P4 CropBiolife	0.12	0.24	0.28	0.31	0.02
P8 CropBiolife + AQ10	0.00	0.09	0.10	0.14	0.00
P9 AQ10	0.00	0.00	0.00	0.00	0.47
P10 CropBiolife + AQ10 + fungicides at 14 days	0.00	0.01	0.01	0.02	0.07
P11 CropBiolife + AQ10 alone and in tank mix with Rovral	0.17	0.18	0.23	0.25	0.00
P. value	0.668	0.725	0.699	0.687	0.297

Table 19 Percentage of strawberry fruit with *Botrytis* in the different treatment programmes over the trial period at ADAS Boxworth 2016

Programme	12 Sept	19 Sept	26 Sept
P1 Untreated control	21.7	13.3	12.2
P2 Standard fungicides 7 days	30.6	19.4	13.9
P3 Standard fungicides 14 days	45.6	6.9	11.1
P4 CropBiolife	36.1	23.1	15.6
P8 CropBiolife + AQ10	36.1	9.4	17.2
P9 AQ10	42.8	12.5	19.4
P10 CropBiolife + AQ10 + fungicides at 14 days	34.4	16.1	13.3
P11 CropBiolife + AQ10 alone and in tank mix with Rovral	26.7	21.9	16.1
P. value	0.508	0.472	0.958

Table 20 Percentage of strawberry fruit with *Mucor* in the different treatment programmes over the trial period at ADAS Boxworth 2016

Programme	12 Sept	19 Sept	26 Sept
P1 Untreated control	41.7	85.6	35.6
P2 Standard fungicides 7 days	33.9	61.9	35.6
P3 Standard fungicides 14 days	32.2	81.1	37.8
P4 CropBiolife	45.6	66.4	27.2
P8 CropBiolife + AQ10	19.4	72.8	43.3
P9 AQ10	33.9	78.3	40.0
P10 CropBiolife + AQ10 + fungicides at 14 days	34.4	76.7	32.2
P11 CropBiolife + AQ10 alone and in tank mix with Rovral	31.7	72.2	40.0
P. value	0.653	0.148	0.847