Project title:	Improving integrated disease management in strawberry
Project number:	SF 157
Project leader:	Prof Xiangming Xu NIAB EMR
Report:	Final 2019/2020
Previous reports:	Annual, Years 1, 2, 3 and 4
Key staff:	Erika Wedgwood (ADAS) Angela Berrie (NIAB EMR) Tom Passey (NIAB EMR) Avice Hall (University of Hertfordshire)
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 ...25 March 2020.....

Erika Wedgwood
Senior Plant Pathologist
ADAS
Signature ... Date ...28 March 2020...

Report authorised by:
Xiangming Xu
Head of Pest & Pathogen Ecology Department
NIAB EMR
Signature ..... Date ...29 March 2020.....

CONTENTS

GROWER SUMMARY

For ease of reading, this Grower Summary report is divided into sections for each of the diseases being worked upon in the project. **This summary covers all the main findings from the five-year project, not just the final year.**

Crown rot and red-core caused by Phytophthora spp.

Headlines

- Prestop when applied through drip irrigation lines led to significant reductions in plant wilting/death (following inoculation with *P. cactorum*) whilst T34 Biocontrol also showed some promising results in improving plant health.
- A new experimental chemical compound (AHDB code F250) when applied through drip irrigation lines led to significant reductions in plant wilting/death (following inoculation with *P. cactorum*). This compound will be further evaluated in a SCEPTREplus trial in 2020.

Background and expected deliverables

Adopting a clean propagation system is the first line of defence against crown rot and red-core diseases. This strategy worked for many years but prior to project commencement, crown rot and red-core caused significant damage in strawberry even in substrate production. Fenomenal (fenamidone + fosetyl-aluminium), an effective product against Phytophthora, has not been approved for use in strawberry since November 2019. Alternative products for control of crown rot (both fungicides and biocontrol products) were identified in trials conducted by NIAB EMR as part of the SCEPTRE project. In previous AHDB-funded research, SF 130 focussed on fungal molecular quantification; 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. More research is required to provide growers with disease-free propagation material in order to reduce crop protection product use and crop losses.

Summary of the project and main conclusions

A survey was conducted along with a molecular screening of bare-rooted runners for the presence of *Phytophthora* spp. The percentage of runners with contamination of *P. fragariae* (causal agent of red core) was so low that subsequent project work did not focus on this

pathogen. However, the level of contamination of *P. cactorum* (causal agent of crown rot) could reach 25-30% in some batches of plants although more usually, it was less than 5%; nevertheless there may only be 5% of runners with visible symptoms of crown rot at the time of planting. Further studies assessed the effect of pre-inoculating plants either with arbuscular mycorrhizal fungi (AMF) or plant growth promoting rhizobacteria (PGPR). Neither managed to reduce the losses caused by *P. cactorum*. In addition, we found that latent infection of plants with *P. cactorum* led to reduced tolerance of plants to drought stress.

Two large studies were done to test existing and new products as dipping (at planting) or drenching/irrigation treatments post-planting, to minimise the losses due to latent infection by *P. cactorum*. To ensure a certain level of latent infection by *P. cactorum,* tray plants were inoculated several times (without wounding) before cold storage. Results showed that dipping alone is sufficient to reduce the level of *P. cactorum* to the level comparable to the un-inoculated control; thus additional drenching is not necessary. Further work demonstrated that applying products through drip irrigation lines (more practical for growers) can be as effective as dipping treatments and better than the drenching only treatments. Treatments appeared to delay disease symptom development and/or reduce the disease severity but did not eliminate latent infection. Although no longer approved, Fenomenal was found to offer the best control in managing crown rot on strawberry. Prestop showed promising results, particularly when applied through irrigation lines (giving ca. 45% reduction in plant mortality). T34 Biocontrol showed some reduction in plant mortality by ca. 30%, close to being statistically significant, and thus should be evaluated further. A new experimental compound, when applied through irrigation lines, led to nearly 50% reduction in plant mortality.

Main conclusions (years 1-5)

- The level of bare-root runners with *Phytophthora fragariae* (red-core) DNA detected in commercial planting material is currently very low and can be ignored.
- The level of *P. cactorum* DNA detected in samples of runners can reach 30% although more usually it is less than 5%. The material is mostly in an asymptomatic state; the level of *P. cactorum* detection in runners is not associated with specific cultivars.
- Latent infection by *P. cactorum* reduced plant tolerance to drought stress.
- Pre-inoculation of plants with AMF and PGPR did not reduce the infection of strawberry crowns by *P. cactorum* but may have positive effects against *P. fragariae*.
- Several products when applied as a dipping treatment at planting time, significantly reduced the losses due to plant wilting/death, mostly due to infection by *P. cactorum*.
- Applying products post-planting through irrigation lines can be as effective at controlling crown rot as dipping and better than post-planting drenches alone.

Financial benefits

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

The project results suggested that growers should consider treating runners for *P. cactorum* at the time of planting. Effective control as a result of this research could reduce crown rot development by 40-50%, amounting to savings of £48 million across the industry.

Action points for growers

- Growers should consider treating runners with a post-planting application of Prestop via irrigation lines to improve plant health.

Strawberry powdery mildew (SPM)

Headlines

- Employing a managed approach to strawberry powdery mildew control can reduce fungicide use by up to 50% whilst maintaining the same level of control as a routine 7-day fungicide programme.
- The use of fungicides for Botrytis control in protected table-top strawberries offers no advantages over an unsprayed control.

Background and expected deliverables

Strawberry powdery mildew (SPM), caused by the fungus *Podosphaera aphanis*, is one of the most important diseases affecting strawberry production in the UK. All above ground parts of the plant are attacked and severe infection can have a significant effect on yield and fruit quality. The disease is more prevalent on protected crops and hence a particular problem in the UK where the majority of commercial crops are grown under polytunnels or in glasshouses. Strawberry cultivars do vary in susceptibility but most of the cultivars preferred by the market are susceptible. SPM is favoured by warm temperatures and high humidity such that conditions are most favourable from late June to October. Hence SPM problems are mainly seen in late cropping June-bearers (planted in May and cropping in August and September) or in the later production of the everbearer crops. In June-bearer type crops with the short harvesting period, control of SPM is relatively straightforward. However, SPM management in everbearer crops is much more challenging. Due to the long growing period from March to

November coupled with flowering, fruiting and harvest continuous from June-November, a range of crop protection products is usually required to control SPM with around 15 or more spray rounds needed to cover the whole period. Control is currently based on fungicides, an approach which, given the concerns about residues in the fruit and the likely reduction in fungicide availability in the future, is not sustainable. The SCEPTRE project (2010-2014) identified alternative products, including Cultigrow (a biostimulant / elicitor) and two biofungicides (BCAs) – AQ10 (*Ampelomyces quisqualis*) and Sonata (*Bacillus pumilis*) a bacterial based biofungicide from Bayer. The purpose of the work in SF157 was to confirm the efficacy of these products, evaluate them in programmes with fungicides and develop a simple decision-based management system for SPM control.

Evaluating biofungicide and biostimulant products (Year 1 and 2)

This work was conducted at NIAB EMR under polytunnels. Efficacy trials in 2015 and 2016 were done in small plots (30 plants per plot) on cv. Elsanta planted in July / August to ensure the crop was growing in the high SPM risk part of the season. They were planted in soil on plastic-covered raised beds with trickle irrigation. The trial in 2015 confirmed the efficacy of the biofungicides AQ10 and Sonata (both applied with a wetter if used alone) and the biostimulant Cultigrow in controlling SPM either alone or in combination with fungicides. In 2016, further trials were conducted in which programmes were evaluated for control of SPM where the biofungicides (Sonata or AQ10) were combined in programmes with Cultigrow (CBL) with and without a reduced fungicide programme and compared to a 7- or 14-day fungicide programme and an untreated control. The mildew risk was high in 2016 but the results showed that the biofungicides were as effective in controlling SPM as the standard 7-day fungicide programme, particularly when applied alone in a programme and especially in reducing SPM on fruit.

Developing simple decision-based programmes to control SPM (Years 3-5)

Having identified alternative products that were effective on June-bearer crops, we then turned our attention to everbearer crops. We combined alternative products in programmes and incorporated other factors such as disease risk, growth stage, type of fungicide (curative, protectant, anti-sporulant) in order to develop a simple decision-based management programme for use on everbearer crops.

In 2017, programmes were tested in larger plot trials on a commercial everbearer. The SPM control achieved by managed programmes of fungicides and the biofungicide Sonata (used with a wetter if applied alone) was compared with that achieved by a routine 7-day fungicide programme and an untreated control. The managed programmes included routine applications of either a silicon-based product Sirius (applied every two weeks), based on the research of Dr Avice Hall (University of Hertfordshire) or Cultigrow (applied monthly) or no

additional treatment. A total of 11 spray rounds were applied from 10 July to 18 September. As the trial was conducted from July to September in the high-risk part of the year for SPM, there was little opportunity to omit sprays. However, in the managed treatment, intervention with a fungicide in place of the biofungicide Sonata occurred only twice. The mildew risk throughout the trial was high. SPM incidence on the leaves was very low. However, on fruit the SPM incidence on untreated plots rose rapidly to more than 90% after four harvests and remained at that level for the remaining ten harvests with consequent reductions in yield and fruit quality. SPM incidence on the fruit in all treated plots was negligible throughout the harvest period (Fig. 1).

This trial demonstrated that use of biofungicides, with or without Sirius or Cultigrow, gave good control of SPM in strawberry, which was comparable to a traditional 7-day fungicide-based programme.

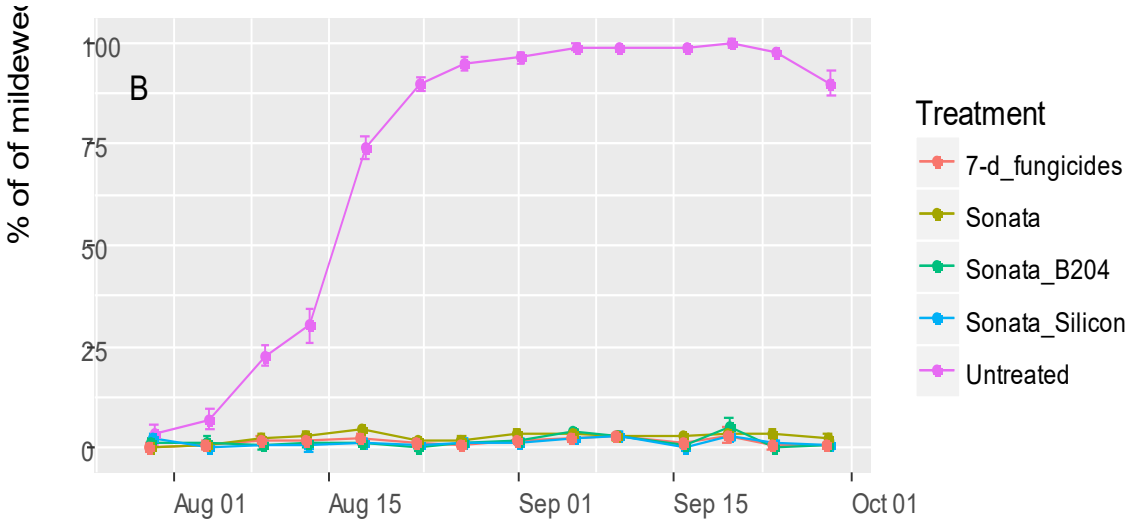


Figure 1. Percentage mildewed fruit at each harvest for a commercial everbearer following treatment with five management programmes against powdery mildew at NIAB EMR in 2017.

In 2018, trials were conducted on the same everbearer cultivar to further develop the managed approach and explore how the system could be integrated with control of Botrytis and other fruit rots. The crop was planted in April and cropped from early July to mid- September, giving the opportunity for saving sprays in the early part of the season, when the SPM and Botrytis risks are usually lower. Three managed treatments were compared to a routine 7-day fungicide programme and an untreated control (Table 1). The managed treatments were derived from the SPM risk prediction model developed by NIAB EMR and employed in previous AHDB funded SPM research projects managed by the University of Hertfordshire (SF 62 and SF 62a). From the model, simplified 'look up' tables were produced for use in conjunction with the forward weather forecast, obtained from the internet, to determine disease

risk. Decisions on when to start the programme for SPM control along with choice of product were based on this (Table 2).

The weather conditions (warm temperatures coupled with high humidity) were very conducive to SPM and Botrytis development in late May / early June and from the end of July onwards. The high temperatures with very low rain in June and July gave a low risk for both diseases. There was a very low incidence of SPM at planting time and this combined with the hot dry weather in June and July meant that SPM failed to establish in the crop, despite the higher risk identified in August and September. Therefore, only four fungicide sprays (and 7 biofungicides - Sonata) for SPM were applied in the managed plots compared to 14 (and 2 biofungicides - Serenade) in the routine treated plots (Table 3). By contrast, the high risk of Botrytis rot identified in August and September required frequent applications of fungicides with little opportunity for saving sprays in the managed plots (Table 3). However, the incidence of Botrytis in post-harvest tests (Fig. 2) showed for most of the 20 harvests, differences in Botrytis between the untreated control and treated plots were very small. This questioned whether fungicides are needed at all for Botrytis control, which could lead to potential savings in cost and a reduction in fruit residues (Table 3).

Table 1. Treatment programmes evaluated in 2018

Treatment	Type	Products	Other
1	Untreated	-	-
2	Routine	Fungicides	None
3	Managed for SPM Sprays for Botrytis as for T2	Fungicides, Biofungicides,	Cultigrow applied monthly from start of growth
4	Managed for Botrytis and rots; sprays for SPM as in T2	Fungicides, Biofungicides	None
5	Managed SPM, Botrytis, and rots	Fungicides, Biofungicides	Cultigrow applied monthly from start of growth

Table 2. Simplified strawberry SPM and Botrytis risk in relation to daily average temperature and relative humidity

Condition		SPM risk
Temperature	**Humidity**	
< 14	Not relevant	Low
≥ 14	< 82%	Moderate
≥ 14	≥ 82%	High
		Botrytis risk
Not relevant	< 82%	Low
< 16	82% - 87%	
< 16	≥ 87%	Moderate
≥ 16	≥ 82%	High

In 2019, trials were conducted on the same everbearer cultivar to further evaluate the SPM management system and to reassess the value of fungicides for rot control. Cool chain management of the fruit post-harvest was also included as part of the fruit rot management programme. The crop was planted on 1 May and cropped from 9 July to 17 September. This provided the opportunity for saving sprays in the early part of the season, when the risks of SPM and Botrytis infection are usually lower. Two managed treatment programmes were compared to a routine 7-day fungicide programme and an untreated control. Both managed programmes were based on the biofungicide Sonata (used with a wetter if applied alone) applied as a protectant programme once a mildew risk had been determined, with the option to intervene with a traditional fungicide when the risk was high. One managed treatment included fungicides for Botrytis applied according to risk (Treatment 3). No fungicides for Botrytis control were included in the second managed treatment (Treatment 4). As in 2018, the simple 'look up' table (Table 2) derived from the SPM risk model, was used in conjunction with the forward weather forecast, obtained from the internet, to determine disease risk, dictating decisions on the when to start sprays for SPM and choice of product.

Table 3. Summary of fungicides, Biofungicides, biostimulants applied to strawberry plots at NIAB EMR 2018 and programme costs

Treatment	Management treatment				
	Untreated	Routine fungicide	SPM managed / Routine, Botrytis	Routine SPM / Managed Botrytis	Managed for SPM and Botrytis
Botrytis fungicides	0	13	14	12	11
SPM fungicides	0	14	4	15	4
Total fungicides	0	27	18	27	15
Biofungicide	0	2	7	0	5
Biostimulant	0	0	4	0	4
Cost £/ha					
Total cost	0	2,278	2,169	1,905	1,579
SPM only	0	1,033	677	890	677
Botrytis only	0	1,596	1,700	1,223	1,111

In 2019, the weather conditions (warm temperatures coupled with high humidity) were very conducive to SPM and Botrytis development in late May / early June and continued for much of the trial period from the end of June onwards. Despite the favourable conditions for most of the trial period, only a low incidence of SPM was present on leaves in untreated plots with negligible incidence on treated plots. SPM eventually established on fruit in early August reaching a mean of around 15% of fruit by the final harvest (Fig. 3). The incidence on treated plots was similar and remained very low. On SPM managed plots the first spray was delayed

until 20 June. Fungicide intervention in response to increased mildew risk was made on two occasions. A total of 10 biofungicides, 2 fungicides and 4 biostimulants were applied to the two managed treatments compared to the routine treated plots where sprays started on 15 May and a total of 18 fungicides were applied (Table 4).

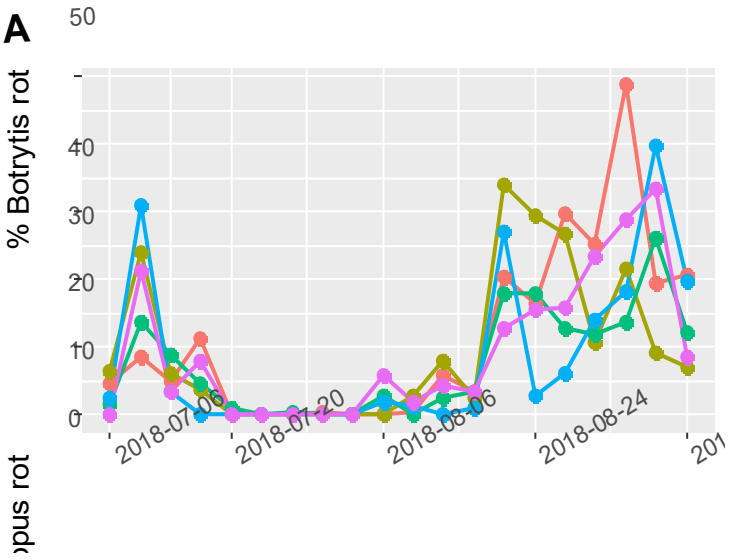


Figure 2. Percentage fruit with Botrytis rot over 20 harvests, in post-harvest tests on a commercial everbearer strawberry following treatment with 4 different programmes compared to an untreated control at NIAB EMR in 2018:

- Red: untreated;
- Pale green: routine;
- Green: mildew only;
- Blue: Botrytis only;
- Purple: both managed.

Table 4. Summary of fungicides, Biofungicides, biostimulants applied to strawberry plots at NIAB EMR 2019 and programme costs

Item	Management treatment / Number of sprays			
	Untreated	Routine fungicide	Routine SPM and Botrytis managed	SPM managed No Botrytis fungicides
Botrytis fungicides	0	15	12	2
SPM fungicides	0	18	2	2
Total fungicides	0	29	12	2
Biofungicide	0	0	10	10
Biostimulant	0	0	4	4
Total products	0	29	26	16
Cost £/ha				
Total cost	0	2006.09	1933.76	1081.99
SPM only	0	888.97	933.94	1081.99
Botrytis only	0	1360.66	1184.16	184.34

A total of 15 fungicide sprays were applied for Botrytis control in routine-treated plots compared to 12 fungicide sprays in the managed plot (Treatment 3). No fungicide treatments for Botrytis were applied to Treatment 4 managed plots. The incidence of rots recorded at harvest was very low ranging from 0 to 1.3% in untreated plots. The incidence of Botrytis in post-harvest tests at ambient temperature (maximum rot potential) in untreated plots ranged from 0 to 27.5% (Fig. 4). There were no consistent effects of treatments on Botrytis rot incidence in any of the 19 harvests, indicating that the 12-15 fungicides applied had little

benefit. There were obvious reductions in the incidence of soft rots (*Mucor* and *Rhizopus*) in treated plots compared to the untreated control, however, the reduction in rot incidence in the treated plots was small and still resulted in more than 55% soft rots and therefore of little value. The incidence of rots in the cool chain fruit management was very low compared to the fruit held at ambient temperature (maximum rot potential) for the same period.

Between 3 and 7 fungicide (mainly Botrytis fungicides) residues were detected in the routine and SPM / Botrytis managed treatments in fruit sampled in August and September compared to no residues detected in the August sampling and 2 fungicides in the September sampling of fruit from Treatment 4. All residues were below the MRL.

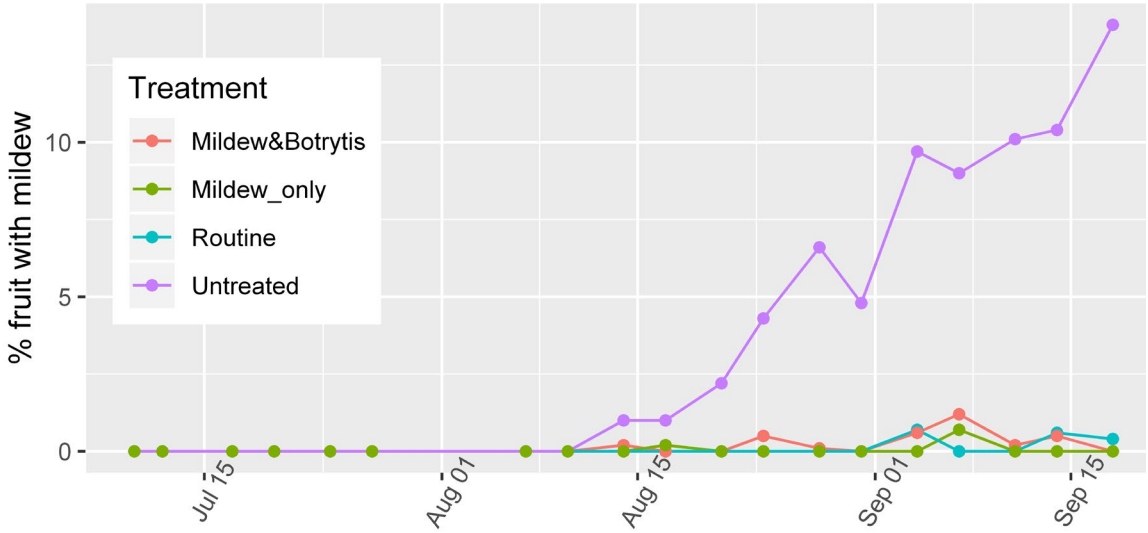


Figure 3. Percentage fruit with SPM at harvest on strawberry cv. everbearer in 2019 at NIAB EMR following treatment with three different programmes compared to an untreated control.

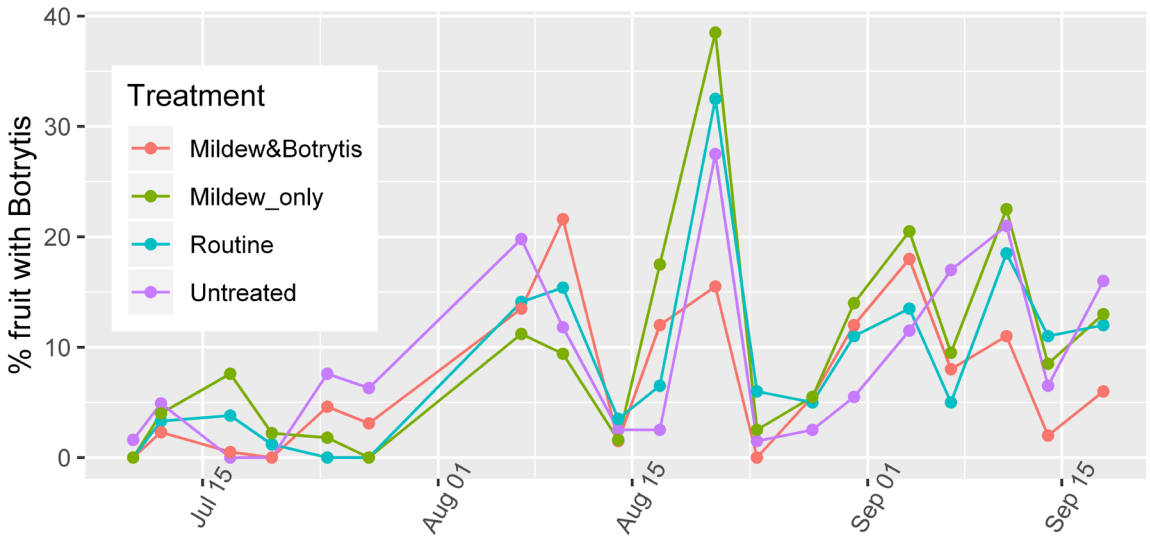


Figure 4. Percentage fruit with Botrytis rot over 19 harvests, in post-harvest tests (7days at ambient temperature) on an everbearer strawberry cultivar following treatment with four different programmes compared to an untreated control.

Overall, a simple decision based system for determining treatments for SPM and rots in protected everbearer strawberries based on biofungicides as protectants (with fungicides included when the risk determines it) for mildew control and omitting fungicides for Botrytis control resulted in a 90 % reduction in fungicide use and a cost saving of around £900 /ha compared to a routine programme with no penalties in yield, fruit quality or disease control.

Commercial Demonstration (Years 4 and 5)

Commercial site 2018

A separate demonstration trial was established on a commercial farm using an everbearer cultivar. The treatments applied were based on the same criteria for SPM and rots as used in the trials at NIAB EMR. They were compared to that in a similar sized tunnel following the standard farm programme. Similar to the trial at NIAB EMR, SPM failed to establish in the trial allowing savings in fungicide inputs in the SPM managed tunnel with only 10 fungicides applied compared to 19 fungicides in the control and with a cost saving of £261.87 /ha (See Table 5 below).

The Botrytis risk was similar to that for SPM with the main risk period shown by the model in late May / early June and from late July onwards and very low risks in June and July. Savings in fungicide use were made in the early part of the season but there was little opportunity in August and September. However, a total of 13 fungicides were applied for Botrytis in the control tunnel compared to 8 in the trial tunnel. There is a saving in cost of £310.45 /ha but

with little effect on Botrytis incidence in fruit from the two tunnels which was similar in both plots at each of the harvest dates. There were also no clear differences in fruit quality.

WET Centre site 2019

In 2019, the system was further evaluated in the WET Centre demonstration area at NIAB EMR on the everbearer cultivar Malling Champion. In two tunnels, the treatments applied were based on the same criteria for SPM and rots as used in the trials at NIAB EMR. These were compared to the rest of the WET Centre, which followed the standard farm programme. Weather conditions from June onwards were favourable for SPM and Botrytis. The cultivar used in the planting – Malling Champion - was newly introduced and classified as moderately susceptible to SPM but with no experience in large commercial plantings. Hence caution was needed as the development of SPM on leaves and fruit in response to favourable conditions was not known. At the start of the trial, the biofungicide Sonata was not approved for use and therefore could not be used in this trial and hence control was based on fungicides only. A very low incidence of SPM developed in the trial from mid-June, and therefore there was little opportunity to reduce fungicide inputs. However, delaying the start of the 7-day programme in the managed area resulted in a small saving of 3 mildew fungicides compared to the routine programme. Although the incidence of SPM was always higher in the managed plots, SPM on leaves and fruit generally remained very low. Similarly sprays for Botrytis were delayed in the managed area until the weather risk increased. A total of 13 fungicides were applied for Botrytis control to the routine tunnels compared to 11 in the managed tunnel.

Table 5. Summary of fungicides, BCAs, biostimulants applied in demonstration strawberry trial on a commercial farm in Kent in 2018 and the programme costs

Item	Control tunnels	Trial tunnel
Total Fungicides		
for Botrytis	13	8
for SPM	19	10
Total	**26**	**15**
Other products		
BCAs	2	1
Cultigrows	0	5
Other biostimulants	13	11
Cost £/ha		
Total	**1715.08**	**1272.22**
SPM only	1110.10	848.23
Botrytis only	934.44	623.99

Overall, the experience with the simple decision-based management system for SPM in the commercial trials, especially in 2018, supported the results in the trial at NIAB EMR. The commercial trials also showed little benefit in controlling Botrytis using fungicides.

Mode of action

The results from this work have indicated that the three new fungicides (Luna Sensation, Takumi and Talius) all have good effects against SPM. Charm is not very effective against SPM when applied as a protectant. The two biocontrol products have some effects against SPM but are not expected to be useful on their own where more than a trace level of fresh SPM lesions are already present in the crop. The overall test results from three-year testing are summarised in Table 6 below:

Table 6. Protectant, curative and anti-sporulant properties of **new** products effective for the control of SPM

Product	Curative: number of days applied after infection	Protectant: number of days applied before infection	Anti-sporulant: number of days with good suppression of sporulation
Talius	7-8	2-3	2-3
Takumi	4-5	2-3	2-3
Luna Sensation	4-5	2-3	4
Charm	2-3	Not tested	4
Silwet	1-2	Not tested (but not	2-3
Silwet & **AQ 10**	1-2	expected to have an	4
Silwet & **Sonata**	1-2	effect)	2-3

Overall conclusions

- Strawberry powdery mildew (SPM) is one of most important diseases in protected strawberry production. Once the disease is established in the crop, control is difficult to achieve and losses in yield and quality are likely with crop abandonment a possibility.
- Managing SPM with a simple decision-based system to determine treatment enables savings in fungicide use when the risk of infection is low in the early part of the season, ensuring products are available for the higher risk period in late summer, reducing fungicide input by at least 40%.
- Biofungicides such as Sonata and biostimulants were shown to be effective against SPM in the trials and can form the basis of a protectant programme for control of SPM, using fungicides only in high risk periods.
- Botrytis still remains a potential problem in protected strawberries. However, the importance has declined compared to SPM.
- Growing under protection and use of cool chain management of the fruit has considerably reduced the development of Botrytis fruit rot. In addition, management of fruit waste at harvest to control SWD has reduced the Botrytis inoculum and hence the build-up of the disease in the everbearer crop.

- The results from 2018 and 2019 consistently show little benefit in Botrytis control from the use of fungicides.
- There was a consistent effect of fungicides in reducing soft rots from around 80-90% in untreated to 50-70% in sprayed plots; but this was observed when fruit were stored under ambient conditions post-harvest, hence representing the maximum potential of pot-harvest rot development.
- Development of any Botrytis or soft rots can be delayed by cool chain management of the harvested crop.
- Basing the disease control programme for protected everbearer strawberries on biofungicides for SPM control with intervention with fungicides during high-risk infection periods and minimising the use of fungicides for rot control, offers large potential savings in residues in the fruit.
- Several new fungicides were shown to have good effects against SPM (Table 6); when these products are used, special attention should be paid to their efficacies when applied as protectant, anti-sporulant or curative treatments.

Financial benefits

The replicated trial at NIAB EMR and the demonstration trial on the commercial farm in 2018 have demonstrated the ability to reduce fungicide inputs where treatments used for SPM and fungal rots are based on a simple decision based system compared to a routine or standard farm programme. The results were confirmed in the replicated trial at NIAB EMR in 2019. In both cases in 2018, cost savings were made (£699 /ha and £443 /ha respectively) with no adverse effects on yield, fruit quality or rot incidence. There were also advantages in reduced residues in the fruit, particularly for sprays targeted at SPM. The results from 2018 and 2019 also consistently show little benefit in Botrytis control from the use of fungicides hence offering further savings in fungicide costs and residues in the fruit.

Action points for growers

- Integrate the new fungicide products Luna Sensation and Takumi (both curative and anti-sporulant activity) and Talius (curative activity) with other control measures
- These products should be saved for use in the programme when the SPM risk is high.
- The adjuvant Silwet on its own also offers good anti-sporulant activity and can complement traditional spray programmes.

- Growers should consider adopting a decision-based managed approach to powdery mildew control using the mildew risk model along with forward weather forecasts and crop stage.
- Basing the disease control programme for protected everbearer strawberries on biofungicides (used with adjuvants if applied alone) for SPM control with intervention with fungicides during high risk periods and minimising the use of fungicides for rot control, offers large potential savings in costs and residues in the fruit.
- Growers should consider trying the approach on part of their farm to gain experience and confidence in the system.

Fruit rot complex

Headline

- *Pestalotiopsis* spp. do not appear to be important as pathogens of strawberry in the UK.

Background and expected deliverables

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

Projects SF 74 (Defra Horticulture LINK HL0175) and SF 94 (Defra Horticulture LINK HL0191) suggested that in raspberry and strawberry, rapid post-harvest cooling to storage at 2°C is effective in delaying Botrytis development. However, such cooling treatment is not effective against *Mucor,* 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.

Towards the end of Year 2 of this project, there were increasing reports on the occurrence of *Pestalotiopsis*, a new pathogen being isolated from the crowns of wilting plants. In addition, this pathogen was shown to cause fruit rot on strawberry in Egypt. In year 3, we carried out

preliminary work on this new pathogen of strawberry to determine the importance of this disease to the UK industry. Although *Pestalotiopsis* strains can produce disease lesions on detached leaves and fruit, they failed to infect crowns of intact plants in artificial inoculation even under disease conducive conditions.

Summary of the project and main conclusions

Using a detached fruit and leaf pathogenicity test, we demonstrated that all the *Pestalotiopsis* isolates tested can establish infection and colonise the host tissue. The pathogen was also able to cause a post-harvest rot following inoculation during fruit development. However, we failed to show that the isolates tested were able to cause a disease in the crown. Plant leaves and crown were inoculated with the *Pestalotiopsis* spore and mycelium inoculum and despite providing highly favourable conditions, only a background level of disease was recorded. Based on our findings and the literature, we conclude that *Pestalotiopsis* is a weak pathogen, which is able to infect the plant when it is under other stresses. Furthermore, only one sample from more than 100 plant samples of year 1-2 Phytophthora survey had positive DNA result for *Pestalotiopsis.*

Work on developing strategies for managing Botrytis fruit rot is presented in the previous section (SPM). The key message is that post-harvest cool-chain management is essential for managing *B. cinerea* without fungicide input.

Preliminary data also showed that *B. subtilis* (Serenade ASO, Solani) can maintain sufficient densities of viable propagules for 10 days after application to control *B. cinerea* under protected conditions in the autumn; whereas the corresponding period is 4 days for *Gliocladium catenulatum* (Prestop). However, previous results at NIAB EMR showed the limited movement of *B. subtilis* among flowers under protection, which meant that frequent application is necessary to protect flowers from infection because of the nature of continuous flower development in everbearers. Further work is needed to study the extent of spread/movement of *G. catenulatum* among plants following its application.

Financial benefits

Based on the results so far, we conclude that *Pestalotiopsis* spp. are not important on strawberry under the UK conditions. Indeed, there were no reports of this pathogen in the UK in 2018.

Action points for growers

- Current results are insufficient for making any recommendations. Be vigilant for this disease in plantations, manifesting itself either as a crown rot or a fruit rot.

- When biocontrol products are used, special attentions need to be paid to survival/movement of biocontrol organisms and the rate of crop growth.

Verticillium wilt

Headline

- *Bacillus subtilis* (Serenade ASO, Solani) showed some promise in reducing the level of *Verticillium dahliae* inoculum in the field.

Background and expected deliverables

Verticillium wilt of strawberry develops from micro-sclerotia of *Verticillium dahliae* in the soil and can reduce yields by 75% through death of plant crowns and reduced water movement into the fruit. Chemical soil fumigation was traditionally used by growers to reduce the pathogen in the soil to levels safe for strawberry production, but the most successful fumigant methyl bromide, is no longer authorised and the best alternative chloropicrin, now requires annual Emergency Authorisation.

Some cultivars have greater resistance to *Verticillium* wilt, but other measures are also required to reduce the impact of the disease. There is the potential to use soil amendments with either organic matter or a biofungicide drench to change the microbial population and so compete for resources with *Verticillium*. Biofumigation may result in reduced viability of *Verticillium* microsclerotia. This work set out to investigate this approach.

Summary of the project and main conclusions

In 2015, we investigated an alternative to the use of pre-planting chloropicrin applied in a soil grown crop using plastic mulched raised beds. Anaerobic soil disinfestation (“soil-setting”) was carried out on a sandy silt soil collected from a soft fruit farm that had a natural infestation of 2.3 microsclerotia of *Verticillium dahliae* per gram of soil. The soil was collected into replicated 10 L pots treated with either just Herbie 82 or this plus a “starter” of 670 ml of soil pre-incubated anaerobically with Herbie 67P. Both products from Thatchtec BV were organic by-products from the food industry and purported to provide nutrition to encourage the activity of anaerobic bacteria present in the soil and allow metabolites anticipated to be produced by these bacteria to reduce the viability of microsclerotia. Pots were irrigated with either 5 mm or 10 mm of water (resulting in 10% or 14% moisture content) and then sealed for eight weeks with a mean soil temperature of 16°C. Significant reduction in propagule viability occurred at either moisture level after the incorporation of Herbie 82 with or without the “starter soil”, to give a mean 0.28

microsclerotia/g of soil, with four out of the sixteen pots having zero. No further work was done with Herbie.

With increasing restrictions on the use of the soil fumigant chloropicrin, a randomised block experiment was set up in 2017 with the pre-planting incorporation of products into *V. dahliae* infested sandy-loam soil (4 propagules/gram of soil) to investigate their potential to reduce losses in a strawberry crop. Either a bio-fumigant, Bio-Fence, (a granular product made from *Brassica carinata* meal) applied at 2,000 kg/ha, or anaerobic digestate solids (composed of maize plants plus vegetable waste and PAS 110 certified) at 50 t/ha, were incorporated into the top 150 mm of formed soil beds before sheeting and then irrigated using trickle tape under the plastic. A week later, on 6 June 2017, cold-stored bare-root plants of a variety moderately susceptible to *Verticillium*, cv. Symphony, were planted into slits in the plastic mulch. A week later, Serenade ASO (*Bacillus subtilis* strain QST 713) was sprayed at 10 L/ha in 1,000 L of water / ha on both half of the untreated plots and half of the BioFence treated plots. Fruit were too few to pick, and only a few indications of *Verticillium* wilt had appeared by the end of 2017, but in June 2018 total fruit yield was similar across treated and untreated plots. Clear *Verticillium* wilt symptoms did not show until July 2018, after fruiting, when there were significantly more plants obviously wilted in the Bio-Fence alone treated plots (43% wilted), compared with the Serenade ASO alone (15% wilted) or in combination with Bio-Fence (37%). Some BioFence treated plots had been poor to establish in 2017, possibly because of too short a ventilation period after isothiocyanate release, and this may have led to their greater wilt susceptibility. The small proportion of Serenade ASO treated plants that wilted was significantly less than the untreated control plants (38% wilted) and it was confirmed by Harris testing that this was not because of initially lower soil microsclerotia infestation in the Serenade ASO plots.

Financial benefits

Potential loss of plants due to *V. dahliae* in soil grown crops can vary between 5-90%. In 2016, 90,000 tonnes of strawberries were sold in the UK season with the market valued at £386 million (Data from Kantar). At present, it is estimated that around 10% of the UK strawberry crop is grown in field soils, equating to £38.6 million. Should 25% of plant losses occur in the UK as a result of Verticillium wilt, this would represent lost revenue of £9.6 million. Techniques and measures to control Verticillium wilt could therefore save such potential losses.

Action points for growers

- If growing strawberries in field soils, ensure soil samples are sent for enumeration of *Verticillium* microsclerotia several months before preparing for planting, so that results can

be returned in time to make decisions about the need for soil fumigation and cultivar selection.

- Leaving *Verticillium* affected soils untreated is likely to result in reduced berry weight through reduced water-fill ability and thus lower total fruit yield
- Be aware that if a biofumigant is used, an adequate ventilation period before planting should be allowed, potentially longer than that used for chloropicrin or traditional fumigants. Use a cress test to ensure crop safety.
- Consider a drench application of Serenade ASO at plant establishment as it can reduce crown wilting over a year later.

.

SCIENCE SECTION

Introduction

Strawberry is attacked by several pathogens, including Botrytis *cinerea,* strawberry powdery mildew (*Podosphaera aphanis*) and *Phytophthora* species. In recent years, industry empirical evidence suggested that *Phytophthora* species may have gradually increased in their prevalence. In addition to *Botrytis* fruit rot, recent research at NIAB EMR suggested that *Mucor and Rhizopus* fruit rot pathogens have also become more prevalent but received insufficient research attention. IPM best practice involves using biopesticides in combination with the remaining synthetic pesticides and other cultural and manipulative measures, including the use of clean (certified) planting materials, resistant cultivars, disease forecasting and other IPM tools to achieve commercially acceptable control of pests, diseases and weeds.

Crown rot and red-core caused by *Phytophthora* spp.

Adopting a clean propagation system is the first line of defence against crown rot and red-core diseases. This strategy had worked for many years until recent times. Prior to this project, however, crown rot (*P. cactorum*) and red-core (*P. fragariae*) were thought to be causing significant damage in strawberry even in substrate production, with asymptomatic infection in planting materials considered the most likely cause. 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 conventional and biological fungicides) were identified in trials conducted by NIAB EMR as part of the SCEPTRE project (SF 121). Recent research on *Phytophthora* spp. has concentrated on detecting the pathogens and seeking products to reduce root rotting. HDC project SF 130 focussed on fungal molecular quantification; an assay was developed that detected *P. rubi*, although it was not as sensitive as the *P. fragariae* assay (which detects both pathogens). SF 123 looked at alternative products against *P. rubi* on raspberry where one novel chemical product gave disease reduction. Red-core is more difficult to control and currently there is no work on controlling this disease; survey results from year 1 and 2 of this project (SF 157) showed that *P. fragariae* was rarely detected in planting materials. NIAB EMR has just completed a BBSRC project (BB/K017071/2), in which we have identified a number of quantitative resistance factors against *P. cactorum*. These resistance factors will be exploited in breeding programmes at NIAB EMR. More research is required to assist growers in applying appropriate treatments as AHDB recognised that it is not possible to change the behaviour of continental propagators.

Strawberry powdery mildew (SPM)

A Hort-LINK project (HL0191) focussed on development, implementation and use of an 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 (e.g. HH3288SSF, SF 062, SF 062a) and Norway showed the importance of chasmothecia as a source of 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. In another Horticulture LINK project (HL01107), we also showed 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 (TSB 100875).

Work in a recent AHDB project (CP 77) on edible crops highlighted the efficacy of at least three biological plant protection products against powdery mildews on crops other than strawberries. These biofungicides could gain approval for use on strawberry; however work was required to determine how these might be integrated into crop protection programmes used against SPM.

Fruit rot complex: *Botrytis cinerea*, *Mucor* and *Rhizopus* species

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 previously funded a project (CP 103) at NIAB EMR on pre-colonising strawberry runners or tipping plants to manage wilt; results showed that pre-colonising strawberry plants did not help plants to reduce wilt development. With AHDB funding, Fera developed a molecular diagnostic tool to quantify soil inoculum and ADAS used this tool to investigate the relationship of wilt development in relation to nematodes (AHDB Project 21140029). Separately, NIAB EMR (in collaboration with Chinese researchers) has developed another qPCR tool for quantifying *Verticillium* inoculum in soils. However, neither of these two methods is sensitive enough to quantify inoculum below 0.5 CFU per gram of soils, at which level wilt can still be caused on susceptible strawberry cultivars. In a recently completed project funded by Innovate UK (1001-CRD-SAF-NACP), we 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* sp.) and the suppressive effects by *Bacillus* and *Pseudomonas* species.

Objective 1: *Phytophthora*

In year 1, it was demonstrated that the combined use of arbuscular mycorrhizal fungi (AMF) and plant growth promoting bacteria (PGPR) can reduce the development of red core (*P. fragariae*) on roots that were dipped into spore suspensions; however, a survey of planting material in year 1 suggested that *P. cactorum* is more important than *P. fragariae* therefore work for the remainder of the project focussed on *P. cactorum*. Most *P. cactorum* detected in plant materials in years 1 and 2 was latent. Indeed, most of these latent infections failed to develop into visual symptoms after planting in the field. Thus, plants may grow out of the latent infection and/or some of these positive detections based on the nested PCR technique used could be due to non-viable microbial DNA present in the crown material. In year 4, it was shown that several products can be partially effective in reducing post-planting development of *Phytophthora* when applied as dipping or dipping-drenching treatments. See previous SF 157 Annual Reports for details.

The objective was to evaluate the effects of selected products from Year 4 on the development of latent infection by *P. cactorum* in terms of symptom development, plant vigour, and fruit production when applied as post-planting drenching or via the irrigation system.

Materials and methods

General considerations

The survey of planting material from multiple batches in project years 1 and 2, coupled with molecular screening of bare-rooted runners, indicated that the level of contamination of *P. cactorum* could reach 25-30% of the plants although more usually, it is less than 5%. Thus, un-inoculated plants were not expected to be ‘disease-free’. On the other hand, the background level of latent infection is variable and usually not high enough to assess treatment effects reliably under the usual size of experiments. Therefore in this experiment, plants were inoculated to ensure consistent “high” levels of infection across the treatments. Thus, crown rot symptoms in assessed plants could result from two sources: (1) background infections that were present in all plants; and (2) artificial inoculation as carried out at NIAB EMR prior to cold storage. The differences between the inoculated and un-inoculated controls indicate the extent of success of the artificial inoculation; however, such differences do not affect the conclusions drawn from the experiment. From the same reasoning, some treatments may have lower levels of diseases than the **un-inoculated** plants if (1) the level of the background infection is high; and (2) these treatments could delay/reduce crown rot development resulting from the background infections as well as from the artificial inoculations.

Plants, pathogen and inoculation

The timeline for all key tasks is given in Table 1.1. Fresh tray plants (super elite) of a June-bearer cultivar (Malling Centenary) were obtained from a commercial nursery in January 2019. Because of the expected high mortality of inoculated plants (ca. 30-50%) in cold store, 3000 plants were ordered for this experiment.

Based on previous studies in SF 157, three pathogenic *P. cactorum* isolates were used; plants were inoculated twice (about 2 weeks apart) to increase the incidence of latent infection. A suspension of 10^4 - 10^5 zoospores ml^{-1} was produced following a previously published method (Harris, Simpson and Bell, 1997). Each crown was inoculated without wounding by directly pipetting 3 or 4 ml inoculum onto the crown (Table 1.1). Inoculated plants were kept in glasshouse compartments for 4-6 weeks to allow infection to take place and to harden before cold storage. About 3 weeks before being moved to the cold store, these plants were sprayed with Teldor (fenhexamid) to control Botrytis (because of warm and moist conditions in the compartment). These plants were first placed into a cold store of 4-5°C, then to a cold store of 2-4°C and finally to a cold store of -2°C in late March 2019 (packed in grey plastic boxes lined with plastics). Un-inoculated plants (around 400) were kept in a separate glasshouse compartment to the inoculated plants and were then placed in separate clean crates in the cold store to avoid cross-contamination.

Treatments and experimental design

There were two treatment factors: application methods and products. Each product was applied in two ways: (1) post-planting drenching, and (2) post-planting flush application via the irrigation line.

There were five products, four of which were tested in the 2017-18 season with positive results; these were

(1) Fenomenal (product control); this product is no longer approved for use but was included as a standard treatment for comparison
(2) Prestop (*Gliocladium catenulatum* strain J1446)
(3) T34 Biocontrol (*Trichoderma asperellum* strain T34) (previously AHDB coded as F252)
(4) A chemical fungicide (AHDB code: F250)
(5) A chemical product based on co-formulation of F250 (AHDB code: F276)

In addition, there were two control treatments: (1) untreated but inoculated control (positive control) and (2) un-inoculated and untreated control (negative control). Furthermore, a dipping treatment with Fenomenal at planting time was also included for comparison with the 2017-18

results. Table 1.2 gives the rates from labels or from unpublished information from relevant manufacturers.

Each treatment had 200 plants planted in 20 bags. Ten plants in each bag (Botanicoir) were planted in a zig-zag pattern. The fertigation regime used was developed specifically for this cultivar. A randomised block design was used with five blocks. Within each block, each treatment had 40 plants (four coir bags). The experiment was conducted in a polytunnel with the bags laid on the top of plastic grey boxes (with holes allowing water through).

Table 1.1. Dates of key tasks in an experiment to assess effects of treatments at planting on strawberry plants inoculated with *P. cactorum* prior to cold storage (a June-bearer cultivar was used)

Date	Tasks
22/01/19	Trials team collected pallet of 3000 tray plants from propagator. On return to EMR plants kept in Egham shed over night to protect from frost
23/01/19	Plants placed in 9-hole trays in compartments C18, C19 and C20. Plants in C20 were designated as un-inoculated control plants to avoid cross-contamination during inoculations.
28/01/19	Plugs from three *P. cactorum* isolates placed in plates of compost water, left in two different incubators with lights
30/01/19	Zoospores harvested and concentration calculated at $4.8x10^4$; 3 ml inoculum applied to each of the plants in C18 and C19
13/02/19	Zoospores harvested and concentration calculated at $1.3x10^5$; 4 ml inoculum applied to each of the plants in C18 and C19; the exact inoculum dose was not important as long as it was the same for all plants. Glasshouse conditions changed to frost protect and vents turned on for opening during the day. Outside conditions and forecast for mild weather (early-mid teens) for next couple of weeks
22/02/19	Teldor spray applied to plants for Botrytis
26/02/19	Last water of plants
27/02/19 – 01/03/19	Plants had vast majority of leaves taken off and then packed into boxes lined with plastics, control plants in grey crates and inoculated plants into the boxes the plants arrived in. Control plants were done first to avoid cross contamination. It was noted that a number of the inoculated plants were showing symptoms of crown rot. Plants were moved to a cold store at 4-5◦C of NIAB EMR
15/03/19	Plants moved from cold store 1 in EMB to glasshouse cold store 3 (ca. 2-4 ◦C) and a week later moved cold store 2 (-2◦C)
30/05/19	Botanicoir bags put on irrigation and plants out of cold store. Measured the volume of irrigation through 4 drippers in 4 mins; 360 ml, 365 ml; 355ml, 250 ml (one dripper fell out at some point), 365 ml, 350 ml; so approx. 90 ml a min to each bag
31/05/19	Plants planted. Fenomenal root dip applied to 20 bags worth of plants (15 g in 10 l) as per product label
07/06/19	Visited plot and many plants showing no signs of new growth and appear to be dead. Reduced irrigation to 1 min every 8 hours
12/06/19	Due to the high number of dead plants (with symptoms consistent with Botrytis), consolidated the healthier plants – 8 bags of 10 plants per treatment (i.e. 2 bags per plot rather than 5)
17/06/19	Applied drench treatments, 100 ml per plant directly on to base of crown. Applied treatments through irrigation 3 x 4 mins. Washed out irrigation pipes (one for the 2 BCAS, one for the other 3 treatments) between treatments by opening up the end of the pipe to allow flow through of water and then through drippers. The three doses of each treatment were applied with a minimum 1 hour between doses to avoid overflowing
28/06/19	Hot weather forecast so increased irrigation to 6 mins every 8 hours

04/07/19	Weather quite warm and plants getting bigger so increased irrigation to 8 mins
05/07/19	Increased irrigation to 10 mins every 8 hours (fruiting period). Carried out assessment of plants in first 3 blocks. Collected dead/dying plants for DNA extraction at a later date (kept in cold store). Carried out assessment of block 4 plants on 08/07/19
15/07/19	Some bags a little dry in places. Put on Pekacid (1kg in 10L) on at 1.5% on dosatron to clear lines. Gave 10 mins extra manual irrigation to check drippers in drier looking bags
30/07/19	Sufficient fruit were harvest and sent for residue analysis
12-13/09/19	Post-harvest plant assessment: block 1&3 12/09/19, blocks 2&4 13/09/19. In addition to those dead or wilting plants, all crown tissues of "healthy" were sampled for DNA analysis.

Table 1.2. Products for crown rot control in strawberry

Product	Rate (g/l)	Active ingredient	Application method
Fenomenal*	0.75	fosetyl-Al + fenamidone	Dipping (15 mins) Drench (100 ml/plant) Fertigation (3 x 4 mins, 1 h apart)
F250	0.63 (ml/l)	-	Drench (100 ml/plant) Fertigation (3 x 4 mins, 1 h apart)
Prestop	5	*Gliocladium catenulatum*	Drench (100 ml/plant) Fertigation (3 x 4 mins, 1 h apart)
T34 Biocontrol	0.25	*Trichoderma asperellum*	Drench (100 ml/plant) Fertigation (3 x 4 mins, 1 h apart)
Co-formulated F250[$]	0.67 (ml/l)	-	Drench (100 ml/plant) Fertigation (3 x 4 mins, 1 h apart)

*: Fenomenal is no longer approved for use but was included as a standard treatment for comparison.

[$]; Due to misunderstanding through emails, a lower 0.67 (ml/l) rate was used (instead of the recommended rate 1.06 ml/l).

Applying treatments

Symptoms of crown rot in infected planting materials are usually likely to be induced by post-planting stresses. Thus, the planting date was postponed to late May 2019 when the temperature was high. Plants were moved out of the cold store to the shade area near the tunnel the day before planting for defrosting (10-25°C). The bags were laid on the top of plastic grey boxes (with holes to allow water through). Plants were fertigated with a total of 6 L per hour per bag (using four sub-drippers per bag).

For Fenomenal, the dipping treatment (15 minutes) was applied to plants inside a glasshouse compartment on 31st May 2019 and then they were immediately planted when the excessive chemicals were drained off from the roots. For drench treatments, 100 ml of each product was directly poured slowly on to the base of the crown of each plant. For fertigation treatments, each product was applied through the irrigation pipe three times, each lasting for 4 minutes, with a minimum of 1 hour between two consecutive irrigation events to avoid overflowing (determined from preliminary experimentation). To avoid potential harmful effects of

conventional pesticides on biofungicides, separate irrigation pipes were used for the two biofungicides and the other three fungicides. The pipes were cleaned between treatments by opening up the end of the pipe to allow the flow of clean water through the pipes and the drippers.

We used the fertigation regime specifically developed for this cultivar by the industry; the exact fertigation frequency/time was determined by regular measurement of coir substrate moisture. Two weeks after planting, 100 ml of each product was poured slowly over the top of the crown of each plant in specific plots as an additional drenching application.

Unfortunately, many plants (ca. 40%) failed to establish or were not growing well following planting. Preliminary assessment suggested that these plants were not associated with any particular product treatments but were probably suffering from Botrytis. The issue was discussed with AHDB representatives about how to deal with this experiment. In the end, it was decided to reduce the size of this experiment but continue with the trial. After removal of those dead or dying plants, there were still ca. 80 plants per treatment. Instead of five blocks, there were four blocks each with 20 plants per treatment, which was still considered statistically robust.

Assessment

Overall, fruit production was poor for plants in all treatments and hence no assessments were made of fruit yield or quality. There was, however, a sufficient number of fruit for residue testing following one harvest in late July following the requirement from QTS Analytical Ltd. For each chemical and control treatment, 1 kg fruit was picked and gloves were changed between treatments. Assessments were made on *Phytophthora* in the early fruiting periods (early July, Table 1.1) – a considerable number of plants were showing *Phytophthora* crown rot symptoms (Photo 1.1). Each plant was recorded in three categories: healthy, witling, and dead. After the last fruit harvest (late August), irrigation was reduced by 50%, and further by 50% a week later to induce disease development before a final disease assessment in early September (Table 1.1). The crowns of all visually healthy surviving plants were examined in the early September assessment.

Photo 1.1. Example of one plant developing *Phytophthora* crown rot symptoms

Crown tissue was collected from every plant ('healthy', wilting and dead). At each assessment date, crown tissues of those dead and wilting plants were sampled and stored in -20°C fridge. The crowns of all surviving plants were sampled for molecular detection of *P. cactorum* DNA after visual assessment of crown tissues in early September and stored in -20°C fridge for subsequent molecular detection of *Phytophthora* DNA. Because of the budget constraint, DNA was not extracted for all samples but nearly 75% of all plants ('healthy', wilting and dead). Samples for DNA extraction were focussed more on the wilting and dead plants because this is where commercial loss would occur.

Data analysis

There were two disease-related variables: number of plants with wilting symptoms (including those dead ones), and number of plants with the presence of *P. cactorum* DNA. These data were analysed using R (version 3.6.1). Only significant ($P < 0.05$) or close-to-significant ($P < 0.1$) differences are reported in the text (recommended as good practice in data presentation).

- The symptom data were analysed using generalised linear models (GLM) with residual errors assumed to follow a binomial or quasi-binomial (to account for over-dispersion) distribution. Because of the nature of GLM, significance of treatment differences is not directly based on the standard errors on the original measurement scale; thus error bars were not presented on the original scale in graphs. Pairwise treatment comparisons were based on deviance testing following the nest-model analysis in GLM.
- As not all crown tissues were subjected to DNA screening (because of the budget constraints), there was an insufficient number of plants for each block to conduct a robust GLM analysis as for the symptom data. We had to estimate the overall proportion of plants with *Phytophthora* DNA present from those plants subjected to DNA screening for each treatment. Thus, it was not possible to conduct a formal statistical analysis of the DNA data.

It should be noted that (1) dead and wilting plants were not necessarily all due to *Phytophthora*, and (2) not all plants with *Phytophthora* DNA would have shown typical wilting symptoms (dead or wilting). For commercial production, the important variable is the 'symptom' data as commercial crop losses would have resulted from these plants with symptoms.

Results

Figure 1.1 shows the overall incidence of wilting and dead plants when assessed in early July and September 2019. About 16% and 27% of plants were dead in early July and September, respectively; the corresponding values for the wilting plants were 25% and 52%.

GLM was applied to assess whether products and application methods affected *P. cactorum* development (note that the Fenomenal dipping treatment and the two control treatments were not included in this analysis). The analysis showed that:

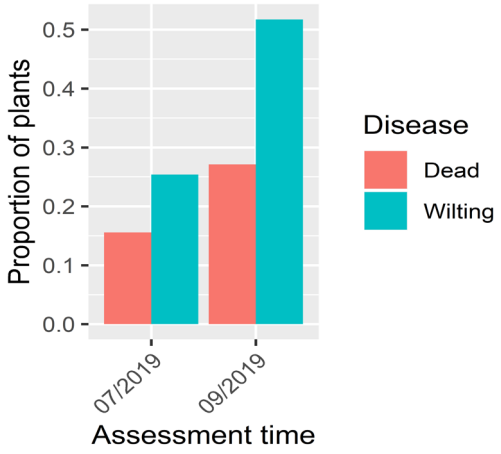


Figure 1.1. Proportion of wilting and dead plants when assessed in early July and September 2019.

1. Neither products nor application methods differed in their effects on the incidence of wilting plants. Nevertheless, the product effect was nearly statistically significant (P = 0.073), primarily due to lower incidence associated with Fenomenal compared with the other products.

2. The products tested differed significantly (P < 0.01) in their effects on the number of dead plants. For the July assessment, about 8% of plants were dead for Fenomenal, lower (P < 0.05) than Prestop and T34 Biocontrol, which, in turn, were less than for F250. Similar results were obtained for the September assessment as well (Figure 1.2) except that the relative performance of Prestop was better.

3. Overall, the irrigation method led to a lower (P = 0.05) incidence of dead plants than the drench method for the September assessment only: 23.5% vs. 29.5%.

4. There were no significant interactions between products and application methods in affecting the incidence of dead and wilting plants.

When GLM analysis was applied to all the data from 13 treatments, there were highly significant (P < 0.001) differences in the incidence of wilting plants among the 13 treatments. Of all the 78 pairwise comparisons, 14 were significant for incidence of wilting in early July. All the 14 comparisons involved Fenomenal dipping or Fenomenal irrigation (Figure 1.3A and Table 1.3): both dipping and irrigation of Fenomenal led to reduced incidences of wilting plants. In early September, the differences in the wilting incidence was smaller among treatments: only five pairwise comparisons were significant – four involving Fenomenal and one Prestop (Figure 1.3B and Table 1.3).

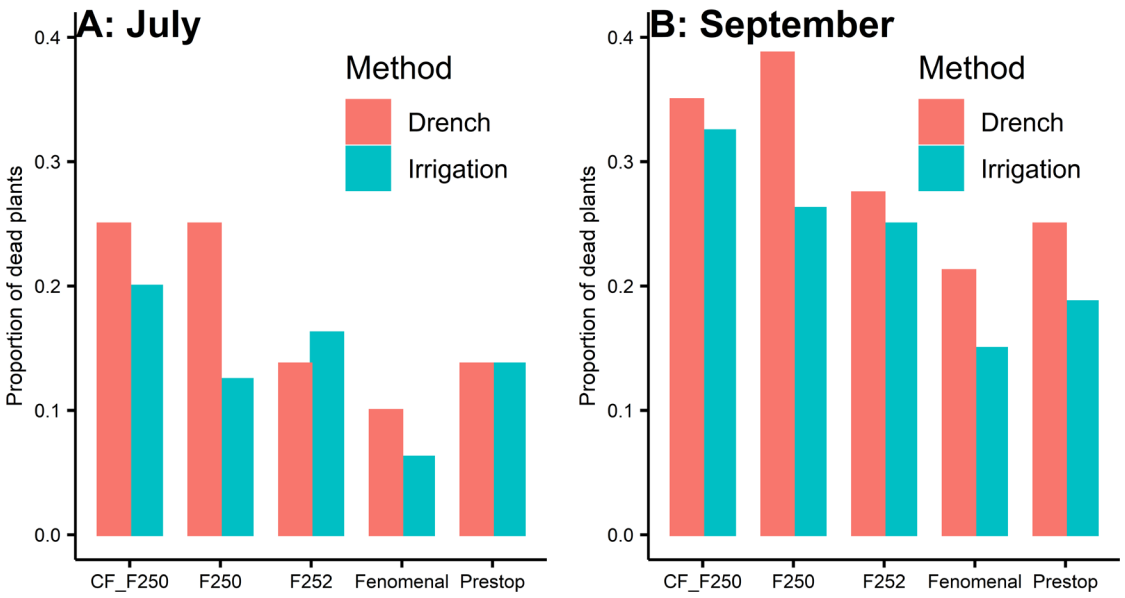


Figure 1.2. Proportion of dead plants due to *P. cactorum* in early July and September for each combination of product and application method (irrigation or drenching). F252 is T34 Biocontrol.

GLM analysis showed that the 13 treatments differed ($P < 0.001$) in their effects on the number of dead plants. Of all the 78 pairwise comparisons, 24 were significant for the incidence of dead plants in the early July. Twenty of the 24 comparisons involved Fenomenal and in addition both the Prestop and F250 irrigation treatments led to fewer ($P < 0.05$) dead plants than the control (Figure 1.4A and Table 1.3). Fenomenal, irrespective of the application method, generally led to reduced number of dead plants. In early September 15 pairwise comparisons were significant – 12 involving Fenomenal and three involving Prestop (via irrigation) (Figure 1.4B and Table 1.3): Fenomenal and Prestop led to a reduced number of dead plants. It should be noted that T34 Biocontrol also led to a reduced number of dead plants when compared with both control treatments although not statistically significant.

The high number of dead plants in both assessments was highest for the un-inoculated control but did not statistically differ significant from the inoculated control. This may suggest that (1) the artificial inoculation failed to increase the level of *Phytophthora* over the background infection appreciably, and (2) the background level of infection was relatively high.

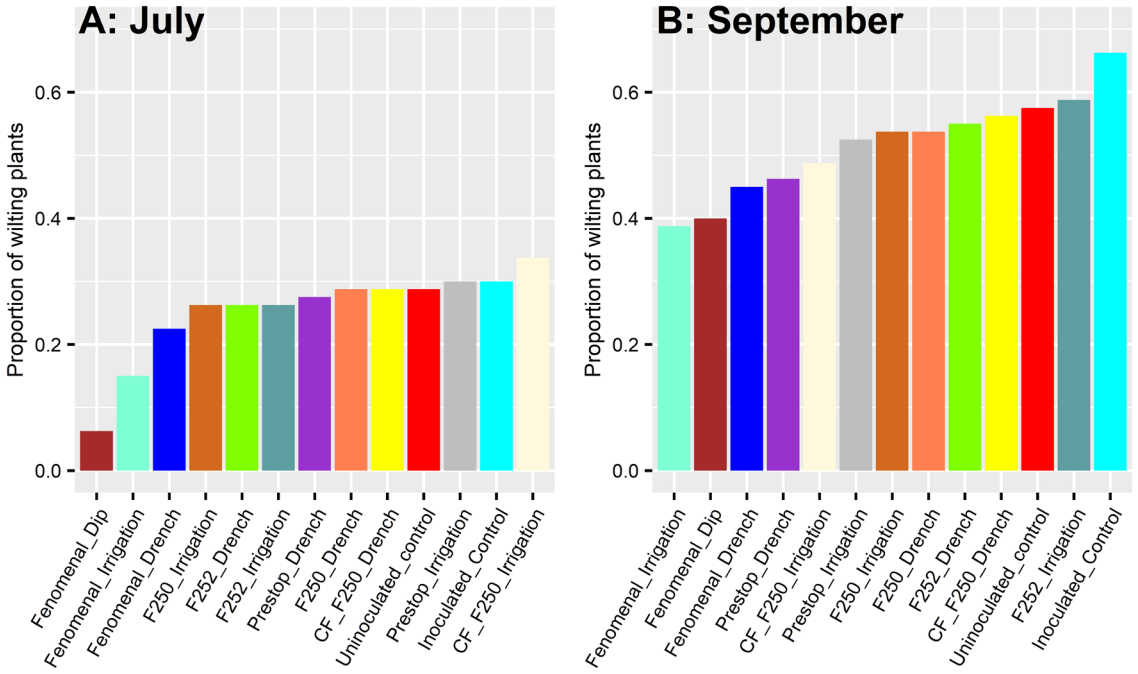


Figure 1.3. Proportion of wilting plants due to *P. cactorum* when assessed in early July (A) and September (B) for all treatments. Results of pairwise treatment comparisons are given in Table 1.3. F252 is T34 Biocontrol.

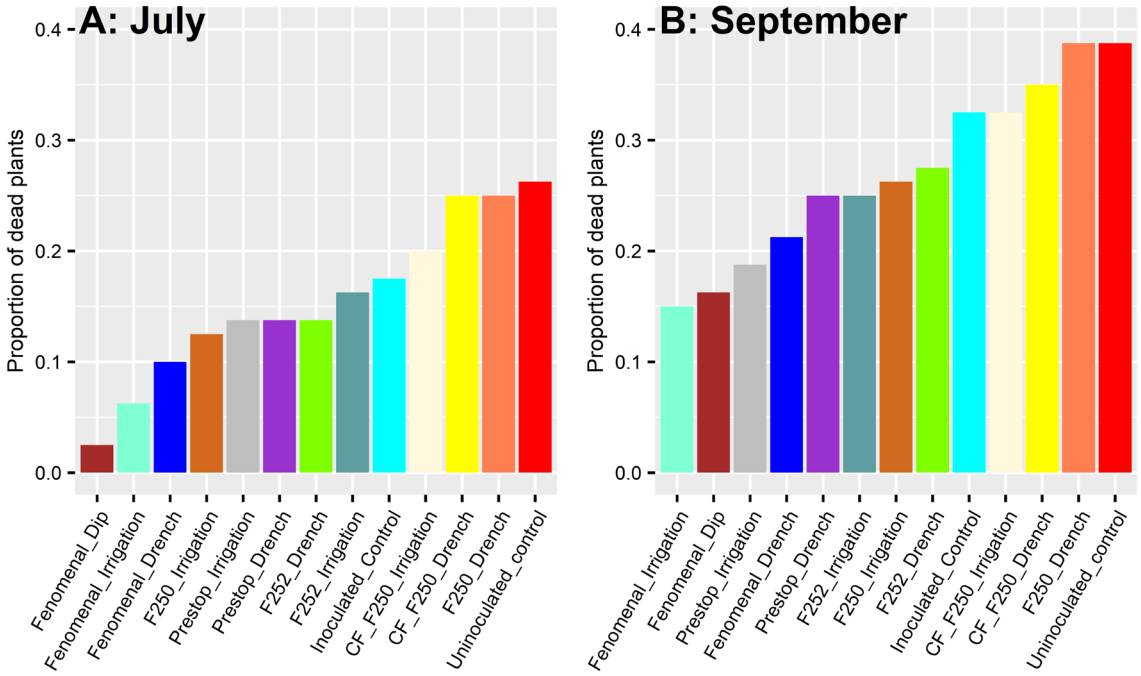


Figure 1.4. Proportion of dead plants (due to *P. cactorum*) when assessed in early July (A) and September (B) for all treatments. Results of pairwise treatment comparisons are given in Table 1.3. F252 is T34 Biocontrol.

Table 1.3. The list of pairwise treatment comparisons where there is significant difference for at least one of the four disease variables: incidence of wilting and dead plants in early July and September. Pairwise testing is based on the analysis of nested models under the GLM framework.

Treatment higher incidence	Treatment lower incidence	July		September	
		Wilting	Dead	Wilting	Dead
CF_F250; drench	F250; irrigation		0.046		
CF_F250; drench	Fenomenal; dip	0.001	0.000		0.008
CF_F250; drench	Fenomenal; drench		0.013		
CF_F250; drench	Fenomenal; irrigation		0.001		0.005
CF_F250; drench	Prestop; irrigation				0.025
CF_F250; irrigation	Fenomenal; dip	0.000	0.000		0.021
CF_F250; irrigation	Fenomenal; irrigation	0.014	0.010		0.012
F250; drench	F250; irrigation		0.046		
F250; drench	Fenomenal; dip	0.001	0.000		0.002
F250; drench	Fenomenal; drench		0.013		0.020
F250; drench	Fenomenal; irrigation		0.001		0.001
F250; drench	Prestop; irrigation				0.007
F250; irrigation	Fenomenal; dip	0.002	0.015		
T34; drench	Fenomenal; dip	0.002	0.008		
T34; irrigation	Fenomenal; dip	0.002	0.002		
T34; irrigation	Fenomenal; irrigation		0.048	0.041	
Fenomenal; drench	Fenomenal; dip	0.008	0.050		
Inoculated control	Fenomenal; dip	0.000	0.001	0.007	0.021
Inoculated control	Fenomenal; drench			0.029	
Inoculated control	Fenomenal; irrigation	0.043	0.029	0.005	0.012
Inoculated control	Prestop; drench			0.040	
Prestop; drench	Fenomenal; dip	0.001	0.008		
Prestop; irrigation	Fenomenal; dip	0.000	0.008		
Prestop; irrigation	Fenomenal; irrigation	0.043			
Un-inoculated	F250; irrigation		0.030		
Un-inoculated	Fenomenal; dip	0.001	0.000		0.002
Un-inoculated	Fenomenal; drench		0.008		0.020
Un-inoculated	Fenomenal; irrigation		0.001		0.001
Un-inoculated	Prestop; irrigation		0.050		0.007

Molecular screening

After the last disease assessment in early September, there were 152 and 350 plants with healthy and discoloured crown tissues, respectively from the 502 plants that looked visibly 'healthy'. DNA was extracted from 775 crown tissue samples; of these extractions, 559 were successful. Table 1.4 shows the number of plants with *Phytophthora* DNA in relation to plant health status. The results indicated that the proportion of plants with *Phytophthora* DNA detected increased with increasing disease symptom 'severity': 37%, 68% and 73% for plants with healthy crown and discoloured crown tissue), and dead/wilting plants, respectively.

Table 1.4. Of the total 559 samples with successful DNA extractions, the number of plants with *Phytophthora* DNA for each category of plant health status: healthy (without discoloured tissue), healthy (with discoloured crown tissue), and dead/wilting.

Plant health status	*Phytophthora* DNA detected		
	No	Yes	Total
healthy (without discoloured tissue)	45	26	71
healthy (with discoloured crown tissue)	55	117	172
dead/wilting	84	232	316

Subsequent molecular data analysis was focused on the dead/wilting plants for two reasons. Firstly, there were many more dead/wilting plants than 'apparently' healthy plants, making comparisons more reliable. Secondly, those 'apparently' healthy plants (assessed post-harvest) should not have affected cropping potentials. The percentage of dead/wilting plants with positive *P. cactorum* DNA detection ranged from 59% to 96%. Most of the pairwise differences between treatments were not statistically significant except several comparisons involving T34 Biocontrol when applied as drench, with higher percentages of positive *P. cactorum* DNA. Fig. 1.5 shows the estimated incidence of wilting plants with positive detection of *P. cactorum* DNA, which was calculated as the product of the overall incidence of dead/wilting plants (Fig 1.3B) and the proportion of tested dead/wilting plants with *P. cactorum* DNA. As Fig. 1.5 shows the estimated incidence from the overall treatment data, it is not possible to conduct formal statistical analysis. However, by comparing Fig. 1.3B with Fig. 1.5, we could see the following trends:

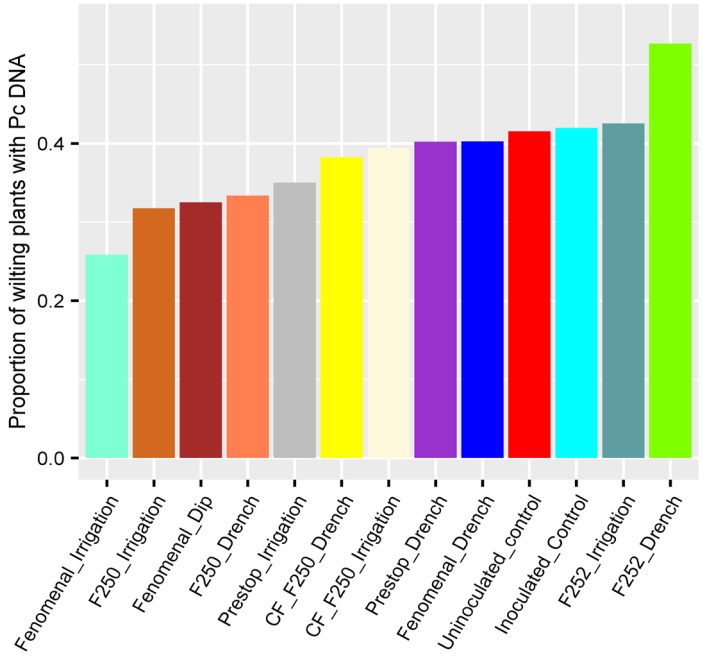


Figure 1.5. Estimated proportion of "Phytophthora infected" wilting plants when assessed in September for all treatments. F252 is T34 Biocontrol.

1. Fenomenal remained the best treatment for reducing *P. cactorum*, particularly when applied through irrigation
2. Application through irrigation was more effective than drenching in reducing *P. cactorum*

3. The two biocontrol treatments appeared to reduce the incidence of dead/wilting plants but did not reduce so much the incidence of plants with *P. cactorum* DNA

4. F250, when applied through the irrigation line, led to reduced incidences of dead/wilting plants, particularly in the early assessment (July), and plants with *P. cactorum* DNA. This product will be further evaluated in a SCEPTREplus trial in 2020.

Residue analysis

A very low level of active ingredient compounds (much less than MRL) were detected for all F250, co-formulated F250 and Fenomenal treatments irrespective of application methods. The difference in the residue concentration was small among different application methods

Discussion

Both the number of dead/wilting plants and molecular screening results showed that there were virtually no differences between the two control treatments (inoculated and un-inoculated). This lack of differences suggest that the artificial inoculation did not appreciably increase the level of *Phytophthora* over the background infection level. Furthermore, the level of plants with *P. cactorum* DNA detected also indicated a high level of background infection. This was not totally unexpected, given the level of background infection observed here was comparable to the highest level observed in the year 1-2 survey. This high level could be partially because we requested the plants very late and hence they were not managed as normal tray plants. However, it should be stressed that the failure of artificial inoculation in increasing the level of latent infection does not affect the data analysis and interpretation as there is was a sufficient level of background infection.

Overall, the irrigation application appeared to result in better control of crown rot in terms of plant mortality. This is likely due to possible leaching/overflow of products following the drenching treatment – this is difficult to eliminate in spite of our care in applying the drenching treatment. It is also speculated that applying a lower dose of products but over a prolonged period of time (say first two weeks post-planting) through irrigation lines may provide better management of crown rot development by ensuring more contact between the expanding root system and crown tissues.

The results indicated that treating plants with Fenomenal at planting time whether through dipping, drench or irrigation can lead to significant reduction of crown rot symptom development, particularly plant death. Thus, we may conclude from both the trials (2018 and 2019) that Fenomenal was the best performing product. Unfortunately, this product is no longer approved for use and was included in order to represent the previous industry standard

and to allow comparison with the previous year's results. One coded compound (F250), when applied through the irrigation line, reduced the incidence of dead/witling plants in the July assessment and also the incidence of plants with *P. cactorum* DNA. The F250 co-formulated product failed to reduce wilting development in the present study. However, it should be noted that F250 + co-formulation was mistakenly applied at a rate that was lower than recommended by the manufacturer.

The differences between products are primarily expressed as effects on plant mortality rather than wilting symptoms. Furthermore, the differences between treatments or products were much reduced in the September relative to the July assessment. This is particularly true for the Fenomenal dipping treatment. All these suggest that these products did not cure/eliminate latent infection by *P. cactorum* but delayed the onset of wilting and/or reduced the severity of wilting symptoms, leading to much reduced wilting and mortality early in the season. This is further supported by the molecular results, which showed that the differences in the incidence of dead/wilting plants with the pathogen DNA were smaller than differences in the wilting incidence.

There are also promising results for two biocontrol products, particularly Prestop. When applied through the irrigation line, Prestop significantly reduced plant death by ca. 45% when compared to the untreated inoculated control. In addition, T34 showed some reduction in plant mortality by ca. 30%, close to being statistically significantly. However, molecular data suggested that the two biocontrol products did not have effects directly on *P. cactorum*. Therefore, their apparent effects in reducing plant symptom development may be due to growth promotion leading to positive effects against other biotic (e.g. *Botrytis*) and abiotic (e.g. hot weather) factors. T34 Biocontrol is currently approved for strawberries (under permanent protection) for peat incorporation, drench, or via irrigation and so it should be able to be used in commercial strawberry production for improving strawberry plant health (and growth). As discussed above, the control efficacy may be expected to increase if the biocontrol products were to be applied soon after planting for a longer period. Furthermore, as they are biocontrol products, application over a prolonged period will not lead to any residue problem as long as it can be justified financially.

In summary, the present results suggest that application of products through irrigation is as effective as drenching. Further research should be conducted to study the control and growth-promotion effects when the two biocontrol products are applied soon after planting for varying durations.

Epidemiological mode of action of new products against strawberry powdery (SPM) (Objective 2)

Introduction

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

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

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

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

- If there are high risks of infection over the last few days based on weather conditions and/or inoculum, you would need to choose a fungicide with good curative efficacy to kill these young developing colonies
- If high risks of infection are anticipated based on weather forecasts (particularly over a long bank holiday weekend), you would choose a fungicide with good protectant ability to protect tissues from infection

- If the level of [fresh, i.e., sporulating] visual SPM is moderate to high [indicating failure of SPM control in the recent past], you would choose a fungicide with good anti-sporulant efficacy.

Experiments to look at the epidemiological mode of action of SPM fungicides commenced in 2017 looking at protectant, curative and anti-sporulant effects. Interim results have been reported in previous SF 157 Annual Reports. In 2019, the aim was to gain further information on the protectant efficacy of a range of fungicides and to enable analyses of data obtained in earlier seasons.

Materials and methods

The main objective was to determine the protective effects of new products against SPM: Takumi (a.i. cyflufenamid), Talius (a.i. proquinazid), Luna Sensation (a.i. fluopyram and trifloxystrobin), Charm (a.i. fluxapyroxad [SDHI] + difenoconazole [triazole]), AQ10 (*Ampelomyces quisqualis* strain AQ 10) and Sonata (*Bacillus pumilus* strain QST 2808).

Products tested

Table 2.1 gives the products tested and their rate of use. A wetter (Silwet) was applied together with AQ10 and Sonata; for comparison, Silwet was also applied on its own. All products were applied at the recommended dose to run-off (unless otherwise specified by the manufacturers) – spray to run-off is necessary to avoid potential differences in spray coverages between leaves and between treatments over time.

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

Product	Rate (/ha)	Stock concentration	How to make
Takumi	0.15 L (300 ppm)	30000 ppm	• 1 ml product into 32.3 ml water (stock solution) • 2 ml stock solution to 198 ml water
Luna Sensation	0.8 L (1600 ppm)	160000 ppm	• 4 ml product into 21 ml water (stock solution) • 2 ml stock solution to 198 ml water
Talius	0.25 L (500 ppm)	50000 ppm	• 1 ml product into 19 ml water (stock solution) • 2 ml stock solution to 198 ml water
AQ10 + Silwet	75 g (150 ppm)	15000 ppm	• 0.5 g product into 33.3 ml water (stock solution) • 2 ml stock solution to 198 ml water
Sonata + Silwet	5 L (10000 ppm)	100000 ppm	• 2 ml product in 18 ml water (stock solution) • 20 ml stock solution to 180 ml water
Silwet	0.25 L (500 ppm)	50000 ppm	• 1 ml product into 19 ml water (stock solution) • 2 ml stock solution to 198 ml water
Charm	0.6 L (1200 ppm)	96000 ppm	• 0.96 ml product into 9.04 ml water (stock solution) • 2 ml stock solution to 158 ml water

Location and plants

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

The temperature / humidity was not controlled or recorded, as climatic conditions are in general suitable for SPM infection from spring to autumn in the UK. For every single study an appropriate untreated (but inoculated) control was included – treatments were only compared against the control for the same exposure (inoculation) period (hence not over time).

Inoculation

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

Experimental design, procedure and assessment

A completely randomised design was used; each treatment had five replicate plants. All products were applied to the plants with a hand-held sprayed on the 13th August 2019. For each of the seven products (Charm, Takumi, Talius and Luna Sensation, AQ10, Sonata and Silwet), there were four inoculation (exposure) times: 1, 2, 4 and 7 days after chemical treatment. For each inoculation, plants were inoculated and exposed to SPM inoculum for 3 days. In total there were 32 treatment combinations [4 inoculation times x 8 products (including the control)], each with five replicate plants.

The number of lesions on each inoculated leaflet was recorded 8-10 days after inoculation. In a few cases, where counting lesions was not possible (due to high numbers), percentage (%) leaf area with SPM was estimated. Statistical comparisons were between treated and the controls in the same period [hence subjected to the same climatic conditions]. Each plant had three leaves assessed; these were the youngest leaves, which were susceptible to infection

by SPM at the time of inoculation.

Data analysis

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

Three protectant tests (two conducted in 2017 although the level of SPM in one of the two tests was low; one done in 2019) were analysed. Different experimental studies were treated as a blocking factor in GLM.

Results

Figure 2.1 shows the overall results of the protectant tests summarised over all three experiments. Table 2.1 gives those pairwise comparisons where there were significant ($P \leq 0.05$) differences for at least one of the eight SPM measurements: incidence of leaflets with SPM and number of lesions per leaflet when inoculated 1, 2, 4 or 7 days after product application. Of the 224 pairwise comparisons, 70 were significant. For the ease of presentation, the products were divided into three categories: control, biocontrol (three products, including Silwet), and chemical (four products).

Despite the large variability among three experiments, and among individual plants within the same treatment, the following trends on the protectant testing can be observed:

- In total, 56 of the 70 significant comparisons were between chemicals and biocontrol/untreated control treatments. In all cases, chemical products led to reduced SPM development when compared to the control or biocontrol products.
- The effects of biocontrol products were much smaller than the chemicals. Only 25% of the pairwise comparisons of the control with biocontrol products were significant; the corresponding value for the chemical products was 53%. In 37 out of the total 96 pairwise comparisons, fungicides performed better than the biocontrols (Table 2.1)
- Only five out of the total 48 pairwise fungicides were significant, all involving Talius (better than Takumi or Charm) for later inoculation periods (Table 2.1).

- Talius was the most effective product and may protect tissues from infection for a minimum of 7-8 days, whereas Charm was the least effective conventional product and may only protect tissues from infection for 1-2 days.

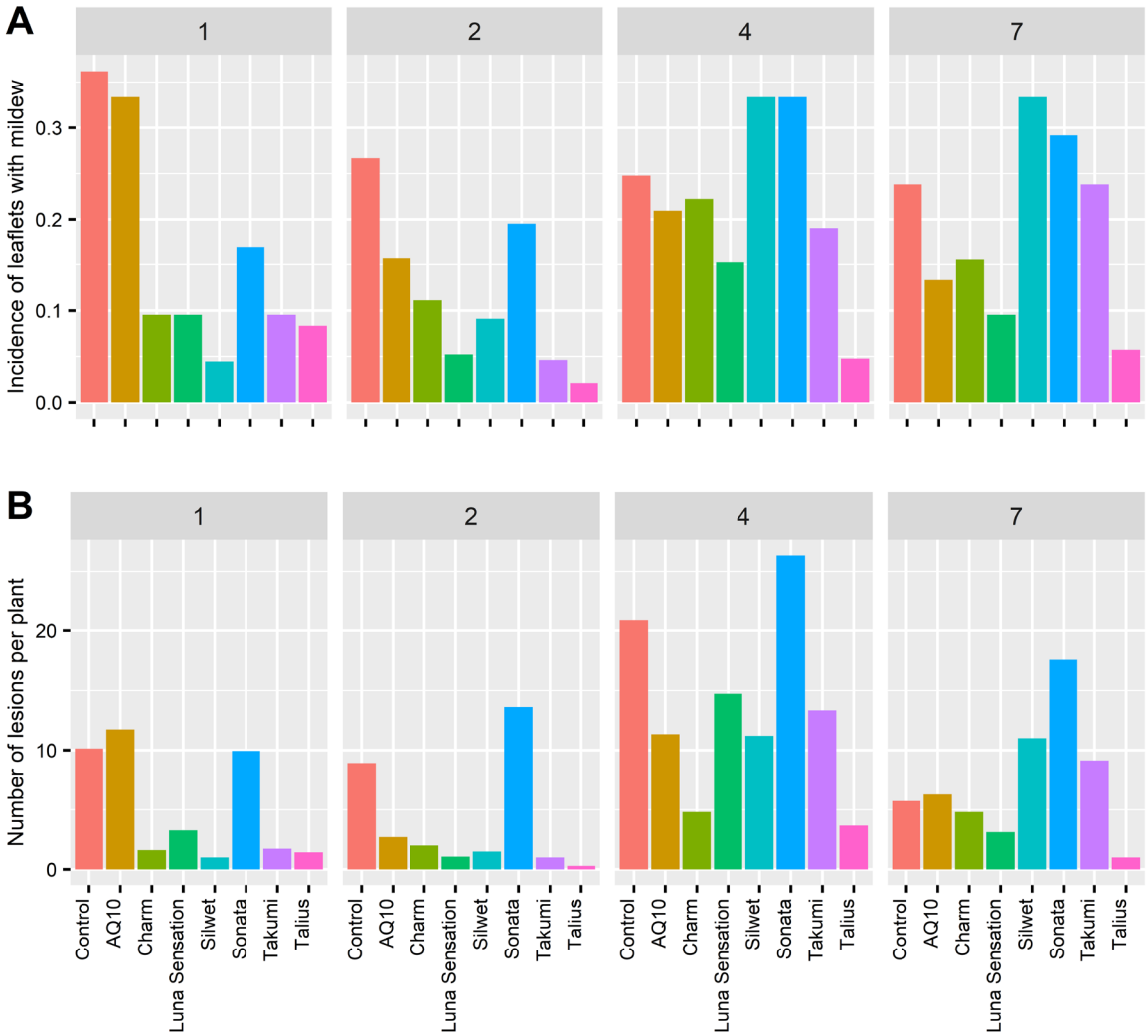


Figure 2.1. Protectant activity: proportion of strawberry leaflets with visible mildew lesions (A) and number of lesions per plant (B) when inoculated one, two, four and seven (as indicated on the top frame of each graph) days after treatment application.

Table 2.1. The list of pairwise treatment comparisons whether there is significant (P < 0.05) or close-to-significant (0.05 < P ≤ 0.1) difference for at least one of the eight SPM measurements: incidence of leaflets with SPM and number of lesions per leaflet when inoculated 1, 2, 4 or 7 days after product application. Pairwise testing is based on the analysis of nested models under the GLM framework.

Treatment		1 day		2 days		4 days		7 days	
1	2	Incidence	Lesion	Incidence	Lesion	Incidence	Lesion	Incidence	Lesion
Control	AQ10			0.041	0.005				
Control	Sonata	0.050							0.019
Control	Silwet	0.003	0.004						
Control	Charm	0.017	0.007						
Control	Luna S.	0.003	0.042	0	0				
Control	Takumi	0.003	0.007	0	0				
Control	Talius	0.003	0.007	0	0	0.003	0.017	0.013	
AQ10	Silwet	0.006	0.002						
AQ10	Sonata				0				0.027
AQ10	Charm	0.029	0.003						
AQ10	Luna S.	0.007	0.017	0.023					
AQ10	Takumi	0.007	0.002	0.013					
AQ10	Talius	0.006	0.002	0.001	0.038	0.013			0.050
Silwet	Sonata		0.004						
Silwet	Talius			0.03		0		0.007	0.029
Silwet	Luna S.					0.017		0.029	
Sonata	Takumi		0.008	0.003	0				
Sonata	Talius		0.008	0	0	0	0.004	0.003	0
Sonata	Luna S.		0.047	0.006	0	0.028		0.018	0.002
Sonata	Charm		0.008						
Charm	Talius			0.01		0.006			
Takumi	Talius					0.024		0.013	0.01

*: Luna Sensation

Discussion and conclusions

In total, four studies have been conducted on the protectant effects of the selected products. Of these four studies, there was virtually no SPM development for the 2018 test, particularly for the exposure periods of 1, 2 and 4 days after treatment. On the other hand, for the final exposure period (7 days after treatment), the level of SPM was reasonably high but the control treatment had the least SPM development. Thus, the 2018 study was excluded from combined analysis.

Based on the results from the last three years, key findings of the fungicide mode-of-action work are summarised in the following table:

Table 2.2. Protectant, curative and anti-sporulant properties of **new** products effective for the control of SPM

Product (approval status on strawberry)	**Protectant** (number of days applied before infection occurred)	**Curative** (number of days applied after infection where disease was controlled)	**Anti-sporulant** (number of days with good suppression of sporulation)
Talius: proquinazid (protected)	7-8	2-3	2-3
Takumi: cyflufenamid (outdoor & protected)	4-5	2-3	2-3
Luna Sensation: fluopyram/trifloxystrobin (protected)	4-5	2-3	4
Charm: difenoconazole/fluxapyroxad (outdoor & protected)	2-3	Not tested	4
Silwet: wetting agent (outdoor & protected)	1-2	Not tested (but not expected to have an effect)	2-3
Silwet & **AQ 10:** *Ampelomcyes quisqualis* (protected)	1-2 (with or without Silwet)	Not tested (but not expected to have an effect)	4
Silwet & **Sonata** *Bacillus pumilus* strain QST 2808 (protected)	1-2 (with or without Silwet)	Not tested (but not expected to have an effect)	2-3

Integration of managed programmes for control of SPM and fruit rots in protected strawberries (ORETO Trial 19/014) (Objective 2)

Introduction and objectives

Trials in 2015 - 2016 identified effective products for control of SPM in strawberries. The trial in 2017 combined their use in programmes and incorporated other factors such as disease risk, growth stage, type of fungicide (curative, protectant, anti-sporulant) to develop a decision-based management programme for growers. This trial demonstrated that use of biofungicides gave good control of SPM in strawberry comparable to a fungicide-based programme. The trial was conducted from late June to September, a time of year when weather conditions are usually very favourable to SPM, giving few opportunities to omit sprays. If the trial had been started in March, then there would have been more opportunities to manage the SPM during the period up to June when mildew risks are generally much lower. In 2018 the approach for managing SPM was integrated with control of Botrytis and other fruit rots on an everbearer crop. The results showed that, overall a simple decision based system for determining treatments for SPM and rots in protected everbearer strawberries resulted in a 50 % reduction in fungicide use and a cost saving of £699 /ha compared to a routine programme with no penalties in yield, fruit quality or disease control. In addition, over the 20 harvests there were no significant effects of treatments on Botrytis rot incidence compared to the untreated control, suggesting that the fungicides applied for Botrytis control gave no benefit. Obviously, such a result has potential for large reductions in fungicide costs and in reducing residues in fruit. The purpose of the trial in 2019 was therefore to further evaluate the SPM management system and to reassess the value of fungicides for rot control. Cool chain management of the fruit post-harvest was also included as part of the fruit rot management programme.

Materials and methods

Study design

Strawberry planting

Ever-bearer strawberry module plants were delivered in late March and held in a cold store at 2^{o}C until the start of the trial. The plants were planted on 1-3 May. There was evidence of *Botrytis* on the old leaves in the cold store and this was removed at the time of planting with a post-planting spray of Teldor applied to all plots on 3rd May. A plantation at NIAB EMR, East

Malling, Kent was used; it consisted of two Spanish tunnels with three low table tops in each tunnel (Fig. 2.1).

The plants were planted into peat/coir bags (Botanicoir) on 1-3 May. Each bag contained eight plants, staggered in the bag, irrigated with four 2 L/h drippers with trickle irrigation. There were 10 bags per plot giving a total of 80 plants per plot. Each plot was 10 m in length and separated in the row by 2 m. The plants established well and by 7 May most were producing new leaves. During this period the plants were treated for aphids with Calypso (thiacloprid) and for Phytophthora diseases with Fenomenal (fenamidone + fosetyl-Al) on 24 May.

Figure 2.1. Trial layout in polytunnels at NIAB EMR in 2019 showing low table tops.

Treatments

The programmes evaluated are given in Table 2.1. Details of the fungicides, BCAs, biostimulants and nutrients used in the programmes are given in Tables 2.2-2.4. All products received for inclusion in the trial were stored, handled and applied according to the manufacturer's instructions on the product label. All were applied as foliar sprays.

The trial decision-based treatments were then started on 15 May. Decisions on spray applications to treatments 3 and 4 (SPM-managed) were based on the criteria given below in Tables 2.5 and 2.6. Decisions on spray applications to treatment 3 (Botrytis-managed) were based on criteria in Tables 2.7 and 2.8, which were simplified rules derived from more complex models as implemented in a computer package. All management decisions were recorded (Table 2.13). These treatments were compared to a routine fungicide programme applied every 7 days (Treatment 2) and to an untreated control (Treatment 1). Details of the programmes applied are given in Table 2.11.

Table 2.1. Treatment programmes evaluated at NIAB EMR in 2019

Treatment	Type	Products	Other
1	Untreated	-	-
2	Routine	Fungicides	None
3	Managed SPM and Botrytis and other rots	Fungicides and biofungicides	Cultigrow applied monthly from start of growth
4	Managed SPM	Fungicides and biofungicides. No fungicides for Botrytis	Cultigrow applied monthly from start of growth

Spray application

Treatments were applied using a Birchmeier electric knapsack sprayer (without air assistance) with Albuz hollow cone red nozzle at 1000 L/ha following NIAB EMR SOP 724. The sprayer lance was used to ruffle the strawberry plants to ensure spray penetration to the centre of the plant, the youngest leaves and to the leaf undersides. All treatments were applied using the same sprayer for the fungicides and for the biofungicide Sonata as it is compatible with all fungicides.

Other treatments

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

All plots received a standard nutrient programme via the irrigation suitable for the everbearer cultivar (pre and post-flowering). The amount of irrigation provided varied from time to time, depending on the substrate moisture level and advice from Alex Cooke of BGG and Scott Raffle.

Experimental Design

The experiment was conducted with a randomised block design with four blocks (i.e. rows). Within each block there were four plots, each randomly assigned to one of the four treatments. Within each plot, there were 10 bags (i.e. 80 plants). Plots were separated in the row by 2 metres.

Assessment

SPM and other diseases

Plots were inspected for the presence of SPM weekly for management decisions. A full assessment for SPM on leaves as percentage leaf area infected on the youngest five expanded leaves on each of ten plants per plot was done when the SPM incidence was sufficient to assess using a standard key (Anonoymous, 1976). A copy of the key is included in the Appendix 11. However, the incidence of SPM on leaves in the trial plots during the whole of the trial period was very low. So only one full SPM assessment on leaves was conducted on 19 September on a random sample of 40 leaves per plot. Assessments on fruit were conducted at harvest as presence or absence of SPM.

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

Harvest

All fruit was picked and assessed for the presence of SPM and other defects. For each plot at each pick, total yield, total number of fruit, total number of Class 1 fruit, and number of mildewed fruit and number of fruit with rots were recorded. A sample of 2 x 50 healthy Class 1 fruit was taken from each plot at each harvest and treated as follows. Half was incubated at high humidity in plastic module trays where each fruit occupied an individual module separating it from adjacent fruit. The fruit was incubated at ambient temperature for 6 days after which the rots were identified and incidence recorded. The other half (Sample B) was put into cool chain management to simulate commercial management of the fruit from harvest to consumer as detailed in Tables 2.9a and 2.9b, based on information from technical support at a commercial soft fruit pack house. Sample A represented the maximum rot potential for the sample compared to sample B where rot development was suppressed due to the cool chain regime.

The first pick was on 9 July and the last pick was on 17 September; a total of 19 picks. However, there was insufficient fruit in each plot to provide fruit for all the post-harvest tests until 13 August. Therefore, the sample A fruit post-harvest tests were conducted on all harvests and the Sample B (cool-chain-managed fruits) only on harvests from 13 August.

Phytotoxicity

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

Residue samples

Samples for residue analysis were taken on two occasions – at the mid and end of the harvest period. At least one kilo of fruit was sampled from each treatment, sampling a similar number of fruit from each plot and from similar positions within the fruit canopy. Fruit was stored at 3-4°C until collected by the residue analysis company, usually within one day of sampling.

Basis for spray-decision criteria and models

In this experiment, Botrytis and SPM models were run alongside the look-up tables (simplified versions of the models – hence less accurate but easier to use) to allow a comparison of the two approaches. The two models were previously developed at NIAB-EMR to forecast the development of Botrytis and strawberry powdery mildew, respectively. Both models were written in Delphi (version XE13) as a Windows programme.

The Botrytis warning system (BOTEM) was based on one of the models described previously (Xu et al., 2000). The model first predicts the incidence of daily flower infection, and then the incidence of daily fruit infection resulting from the flower infections. The SPM model (unpublished) simulates the epidemics of secondary mildew at daily intervals but estimates percentage infection and accumulated development for the incubation (latent) period on each day using weather data recorded at an interval ≤ 1 h. The model is driven by ambient relative humidity and shade temperature (°C).

Table 2.2. Available fungicide products for disease control on strawberry

Product	Active ingredient	Rate of product / ha	Against SPM	Max number of sprays	Harvest interval days	Chemical group	Disease controlled
Switch	cyprodonil + fludioxonil	1 kg	No	2	3	Anilino-pyrimidine + phenylpyrroles	Botrytis
Frupica	mepanipyrim	0.9 L	No	2	3	Anilino-pyrimidine	Botrytis
Prolectus	fenpyrazamine	1.2 kg	No	3	1	Amino-pyrazolinone (KRI fungicide)	Botrytis
Scala	pyrimethanil	2 L	No	2	3	Anilino-pyrimidine	Botrytis
Signum	pyraclostrobin + boscalid	1.5	P	2	3	QoI + SDHI	Botrytis
Teldor	fenhexamid	1.5 kg	No	4	1	Hydroxyanilides (KRI fungicide)	Botrytis
Kindred	meptyldinocap	0.6 L	P	3	3	Dinitrophenyl-crotonates	SPM
Charm	difenoconazole + fluxapyroxad	0.6 L	P	3	1	Triazole + SDHI	SPM
Fortress	quinoxyfen	0.25 L	P	2	14	Aza naphthalenes	SPM
Nimrod	bupirimate	1.4 L	AS*/C/P	3	1	Hydroxyl-pyrimidine	SPM
Amistar	azoxystrobin	1.0 L	P	4	7	QoI	SPM, Botrytis
Amistar Top	Azoxystrobin + difenoconazole	1.0 L	P	2	3	QoI + triazole	SPM + Botrytis
Karma	Potassium bicarbonate	3 kg	AS	8	1	Inorganic	SPM
Luna Sensation	trifloxystrobin + fluopyram	0.8 L	AS/C/P	2	1	SDHI + QoI	SPM, Botrytis
	potassium bicarbonate	20 kg	AS	Max total dose of 60 kg/ha	0?	Inorganic	SPM
Stroby	kresoxim-methyl	0.3 kg	P	3	14	QoI	SPM
Takumi	cyflufenamid	150 ml	AS/C/P	2	3	Phenyl-acetamide	SPM
Kumulus	sulphur	200g/100 L	P	No limit	0	inorganic	SPM
Topas	penconazole	0.5 L	AS/C/P	4	3	DMI	SPM
Talius	proquinazid	190 ml	AS/C/P	1	3	Aza-naphthalenes	SPM

AS = Antisporulant, P = protectant, C=curative

Table 2.3. Biofungicides for disease control on strawberry applied as foliar sprays

Product	Active ingredient	Rate of product / ha	Maximum number of sprays	Product type
Sonata* + Slither	Bacillus pumilis	5 L + 0.05%	6	BCA: SPM
AQ10 + Slither	*Ampelomyces quisqualis*	70 g + 0.05%	12	BCA: SPM
Prestop	*Gliocladium catenulatum*	3 kg	3	BCA: Botrytis
Serenade	*Bacillus subtilis*	10 L	6	BCA: SPM / Botrytis

*Slither (a wetter) was included with Sonata when Sonata was applied alone. If Sonata was applied with a fungicide then Slither was not included as the fungicide was already formulated with a wetter. Sonata was approved for use on strawberries in polytunnels with a maximum of 6 sprays per season in 2019 after the start of the trial.

Table 2.4. Other products used on strawberry applied as foliar sprays

Product	Active ingredient	Rate of product / ha	Maximum number of sprays	Product type
Cultigrow CBL (Cropbiolife)	flavonoids	250 ml	5 at 28 day intervals	Biostimulant

Table 2.5. Criteria for SPM management decisions

Item	How determined	Risk	Management options
Disease risk Less important	Determined from input of humidity and temperature from logger in tunnel to NIAB EMR disease risk model (see below) and forward weather forecast from internet	More than 4 days with risk above 10% requires action	**Product choice** – Fungicide or Biofungicide **Spray interval** – 7 or 14 days
Growth stage and rate of growth	Inspections 1-2 times per week	Rapid leaf production, start of flowering/ fruiting indicates increased risk and possible change of product	**Tunnel ventilation**
SPM monitoring Most important as short time between infection and visible SPM; need to spot new SPM on leaves	Inspections 1-2 times per week on youngest leaves on 5 plants per plot. Plants will be selected at random for each inspection	Scored 0-5, 0 = no SPM on leaves, 1 = <1% (new SPM lesion), 2 = 1-5 %, 3 = 5-10%, 4 = up to 20%, and 5 = > 20% Flowers and fruit scored as presence or absence	

Table 2.6 Simplified SPM risk in relation to daily average temperature and relative humidity

Condition		SPM risk
Temperature	**Humidity**	
< 14	Not relevant	Low
≥ 14	< 82%	Moderate
≥ 14	≥ 82%	High

Table 2.7. Criteria for Botrytis rot management decisions

Item	How determined	Risk	Management options
Disease risk Most important	Determined from input of humidity and temperature from logger in tunnel to disease risk model (see below) and forward weather forecast from internet	Important factors- Day time humidity and night temperature. Predicted risk above 10 %	**Product choice:** Fungicide, Biofungicide **Spray interval:** 7 or 14 days
Growth stage	Weekly inspections	Start of flowering	
Disease monitoring Less important as long time between infection and visible *Botrytis*	Inspections 1-2 times per week for visible sporing *Botrytis*	Scored 0-5, where 0 = no *Botrytis*, 1 = trace of inoculum, 2 = sporing *Botrytis* found with difficulty, 3 = sporing *Botrytis* easily found, 4 = sporing *Botrytis* visible in 30% crop, and 5 = sporing *Botrytis* abundant throughout crop	

Table 2.8. Simplified strawberry Botrytis risk in relation to daily average temperature and relative humidity

Condition		Botrytis risk
Temperature	**Humidity**	
Not relevant	< 82%	Low
< 16	82% - 87%	
< 16	≥ 87%	Moderate
≥ 16	≥ 82%	High

Table 2.9a. Cool chain regime for strawberry trials 2019 - Pick on Tuesday

Item		Day 1 Tuesday		Day 2 Wednesday			Day 3 Thursday	Day 4 Friday	Day 5 Saturday	Day 6 Sunday	Day 7 Monday
Site/Task	Pick	Fast cool Plant Pathology cold store	Fridge: Jim Mount (JM)	Fridge JM	JM corridor	Fridge JM	Fridge JM	Fridge JM	Fridge JM	Fridge JM	Assess
Time		11.00-14.00	14.00-	11.00	11.00-13.00	13.00	All day	All day	All day	All day	
Temperature		8°C	3°C	3°C	12°C	3°C	3°C	3°C (to 5°C at end of day)	5°C	5°C	

Table 2.9b. Cool chain regime for strawberry trials 2019 - Pick on Friday

Item		Day 1 Friday			Day 2 Saturday	Day 3 Sunday	Day 4 Monday	Day 5 Tuesday	Day 6 Wednesday	Day 7 Thursday
Site/Task	Pick	Fast cool Plant Pathology cold store	JM corridor	Fridge JM	Fridge JM	Fridge JM	Fridge JM	Fridge JM	Fridge JM	Assess
Time		10.00-13.00	13.00-15.00	15.00	All day	All day	All day	All day	All day	
Temperature		8°C	12°C	3°C	3°C	3°C	3°C (to 5°C at end of day)	5°C	5°C	

Meteorological records

A data logger (USB-502) was placed at crop height in each tunnel to monitor temperature and humidity. This was down-loaded weekly and the data input to the NIAB EMR SPM and *Botrytis* models for disease risk determination. In addition, information on temperature and humidity forecast for the week ahead were obtained from the BBC weather on the internet. This gives hourly forecasts for the week ahead and can be used in conjunction with the criteria in Tables 2.6 and 2.8 to make decisions on risk to decide on sprays in Treatments 3 and 4. Records of daily maximum and minimum temperature and rainfall were also taken from a weather station located at East Malling main site, approximately 500 m east of the trial.

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.

Results

General

After plot establishment on 20 April, plants resumed growth and started flower production. Flowers were removed until end of May. Plant growth was good and at the level commercially acceptable for most of the trial period. There were no obvious phytotoxic symptoms observed on foliage or fruit in any of the plots following the spray treatments. There were also no obvious differences in plant vigour (height and spread) between the plots.

SPM

SPM risk

The weather conditions (warm temperatures coupled with high humidity) were very conducive to SPM development in late May / early June which continued for much of the trial period (Fig. 2.2). The programmes applied to all treatments are given in Table 2.11 and summarised in Table 2.12. The trial activities, disease monitoring and assessments together with the decisions in response to the predicted risks, based on SPM monitoring in the crop, the model and the forward weather forecast from the internet are shown in Table 2.13. The routine fungicide programme (T2) for SPM control started on 15 May and continued at 7 day intervals until 12 September, a total

of 18 spray rounds. For the managed programmes T3 and T4 the principle was to apply the biofungicide Sonata as the basic treatment. Cultigrow was applied routinely at monthly intervals from 24 May. Old mildew mycelium was detected on the old leaves on the strawberry plants at the time of planting. There was no weather mildew risk until 20 June so the first applications of Sonata were delayed until 20 June and then at 7 day intervals from 18 July as the weather risk remained moderate to high. New mildew lesions were detected on 11 July and the managed plots received a fungicide spray of Charm to eradicate the mildew in place of Sonata. Sonata was continued as the main treatment on T3 and T4 until 15 August when the weather risk was high and new mildew lesions were found on leaves and flowers. Luna Sensation was then applied in place of Sonata. A total of 10 treatments of Sonata were applied to T3 and T4 plots, with only two interventions with fungicide.

SPM incidence

Despite the moderate to high risk of SPM development in late May / early June and then onwards for most of the trial period the incidence of SPM on leaves and flowers and fruit in treated plots remained very low (Table 2.10, Appendix 2 and Fig. 2.3). In untreated plots the incidence on leaves was also low but was found at a moderate to high incidence on flowers and fruits from early August and increased to a mean of nearly 15% of fruit infected by the final harvest. All treated plots had significantly less mildew over the 19 harvests than fruit in the untreated plots. The incidence in replicate 3 of the untreated plots was considerably higher than in the other replicates with 31% of fruit with SPM recorded on 10 September. Reasons for the higher SPM incidence in this plot are not clear.

Table 2.10. Table 2.10. Mean % leaf area with SPM on 19 September and arcsine-transformed % fruit with SPM at harvest (mean of 19 harvests) at NIAB EMR in 2019 (the number in the bracket is the untransformed % fruit with SPM)

Treatment	Mean % leaf area mildewed	Mean % fruit with SPM at harvest
T1: Untreated	0.33	1.120 (3.80) a
T2: Routine fungicide	0	0.060 (0.09) b
T3: SPM + Botrytis managed	0.01	0.017 (0.17) b
T4: SPM managed No Botrytis sprays	0	0.001 (0.05) b
F Prob		0.055
SED (9)		0.014
LSD (p=0.05)		0.054

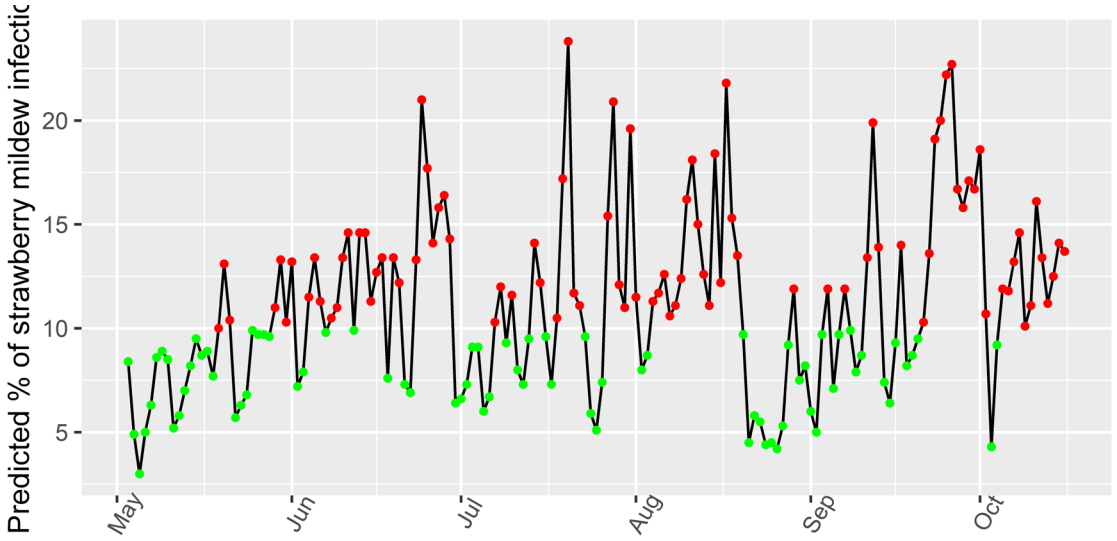


Figure 2.2. Predicted daily risk of SPM for the NIAB EMR site in 2019. The predictions were given by the NIAB EMR model where a period of 4 (or more) consecutive days with risks > 10% on susceptible cultivars (the red points) is considered to need growers' intervention with a moderate to high level of inoculum (usually when the incidence of leaves with SPM is above 5%).

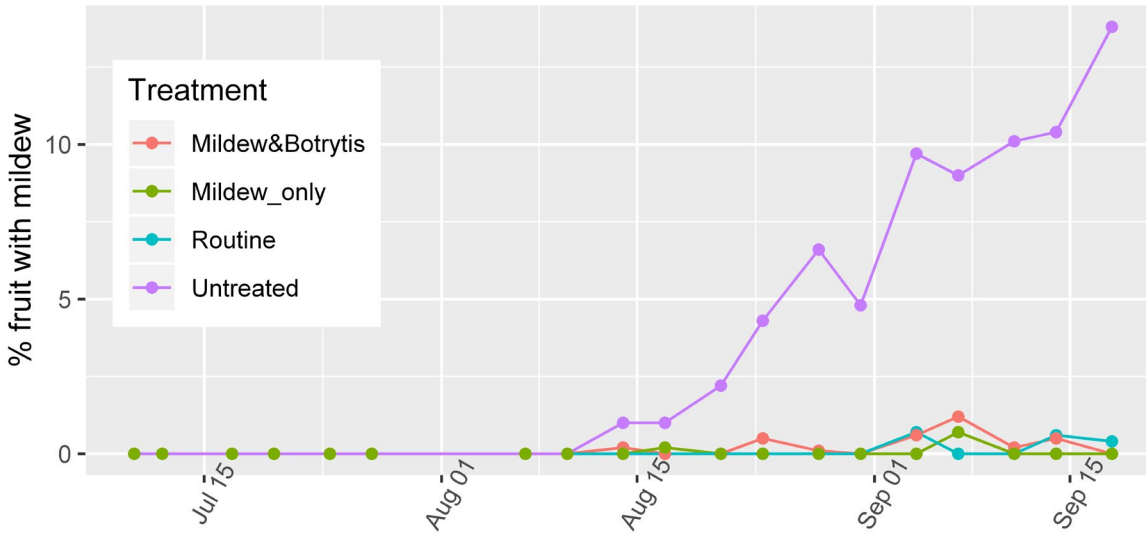


Figure 2.3. % fruit with SPM at harvest on strawberry cv. Everbearer in 2019 at NIAB EMR following treatment with three different programmes compared to an untreated control.

Botrytis risk

Botrytis risk

As for SPM, the weather conditions (warm temperatures coupled with high humidity) were very conducive to *Botrytis* infection of flowers and development in early June and from end of July onwards with a reduced risk in early September due to cooler nights (Fig. 2.4). The

programmes applied to all treatments are given in Table 2.11 and summarised in Table 2.12. The trial activities, disease monitoring and assessments together with the decisions in response to the predicted risks, based on monitoring in the crop, the model and the weather forecast from the internet are shown in Table 2.13. Fungicides for Botrytis control in the routine sprayed plots (T2) were applied at 7 day intervals from 20 June when flowering started, amounting to 15 fungicides in total over the trial period. In the managed treatment T3 only one fungicide for Botrytis was applied up to 20 June with five applied in July and six applied in the high risk period in August and September. Overall 12 fungicides for Botrytis were applied to T3. This was a saving of only 2 fungicides compared to the routine treatment and was due to the high risk for Botrytis in August and September. No fungicides specifically for Botrytis were applied to T4. The two fungicides applied for SPM control were also active against Botrytis but were not applied for this purpose.

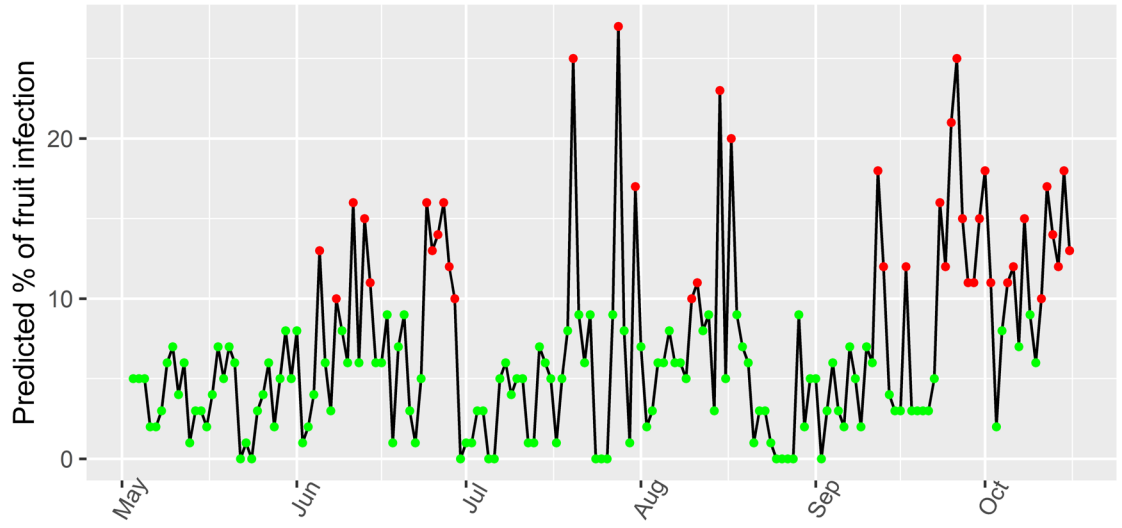


Figure 2.4. Predicted daily infection of flowers by *Botrytis* in 2019. The predictions were given by the NIAB EMR model where risks > 10% are considered to need growers' attention.

Botrytis incidence

The incidence of Botrytis at harvest for each pick is given in Appendix 1 and summarised in Table 2.14 and was very low ranging from 1.5% to 0 with no Botrytis recorded for most treatments and harvests. The highest incidence was in August, corresponding with the higher risk at this time. The incidence of post-harvest Botrytis rot for each individual pick is given in Appendix 4 and Fig.2.5. Botrytis rot incidence was much higher following 7-days incubation at ambient temperature ranging from 0-38.5% in untreated plots with the highest incidence in August and late September, corresponding to the higher risk during this period. For the individual harvests there was no clear pattern in Botrytis rot incidence to differentiate T2 and T3 (treated for Botrytis) from T1 and T4 (no Botrytis treatments applied). Of the 19 harvests

there were significant differences in Botrytis rot incidence on two occasions only (20 August and 13 September see Appendix 4) with significantly higher Botrytis rot in treated plots. Over the 19 harvests there was no significant effect of treatment on Botrytis rot (Table 2.15). This suggests there was not much benefit from the fungicide treatments applied.

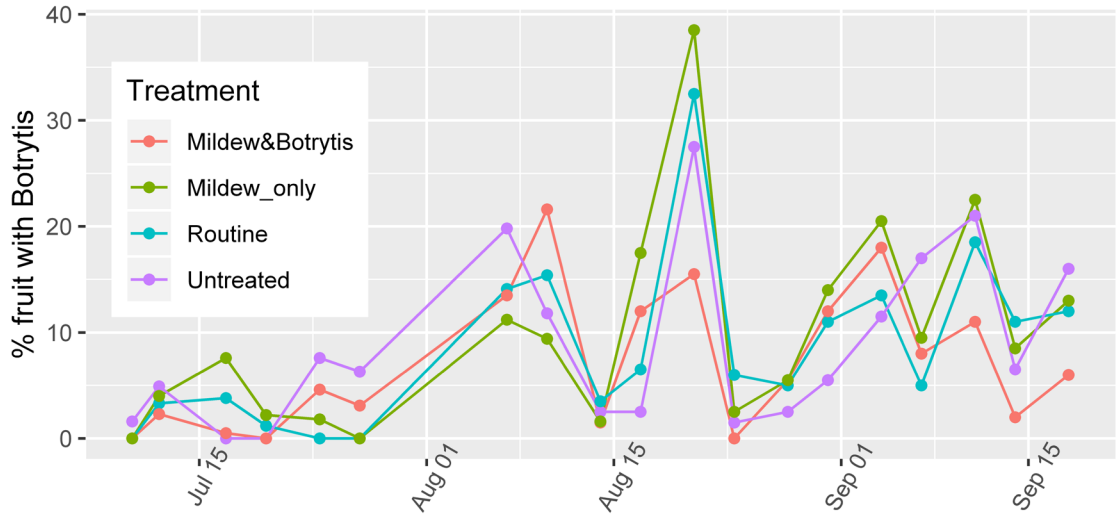


Figure 2.5. Percent fruit with *Botrytis* rot over 19 harvests, in post-harvest tests (7days at ambient temperature) on an everbearer strawberry cultivar following treatment with four different programmes compared to an untreated control.

Other fruit rots

Rots due to *Penicillium* spp (Fig. 2.6 and Appendix 5), and soft rots, mainly *Rhizopus* spp (Fig. 2.7 and Appendix 6). were the other main rots recorded in post-harvest tests. Penicillium rot incidence started high, up to 40% and then decreased with a slight increase in August. The overall incidence of Penicillium in post-harvest tests is given in Table 2.15. There was no consistent effect of treatments on the incidence of Penicillium in fruit from the different programmes. Over the 19 harvests the incidence of Penicillium rot in untreated plots in post-harvest tests ranged from 1 to 33%. There was only one occasion on 30 August when there was a significant effect of treatment on Penicillium rot incidence and significantly more Penicillium was recorded in Treatments 2 and 3 which received fungicide sprays, than in untreated plots. Overall there were no significant effects of treatments on Penicillium rot incidence compared to the untreated control.

In contrast to Penicillium and Botrytis rots the incidence of soft rots started off low in the early harvests (Fig. 2.7 and Appendix 6) and rapidly increased; soft rots were the predominant rot recorded for most of the harvests ranging from 30-90% in untreated plots. The overall incidence of soft rots in post-harvest tests is given in Table 2.15. Overall, fruit from treated plots had significantly less soft rot than the untreated control. However, the incidence of soft

rots in the treated plots was still high at more than 55%. There were significant effects of treatments on rot incidence compared to the untreated control on three occasions (Appendix 6). However, the reduction in rot incidence in the treated plots was small and still resulted in more than 55% soft rots. Fungicides in general have limited efficacy against Penicillium and Mucor / Rhizopus species.

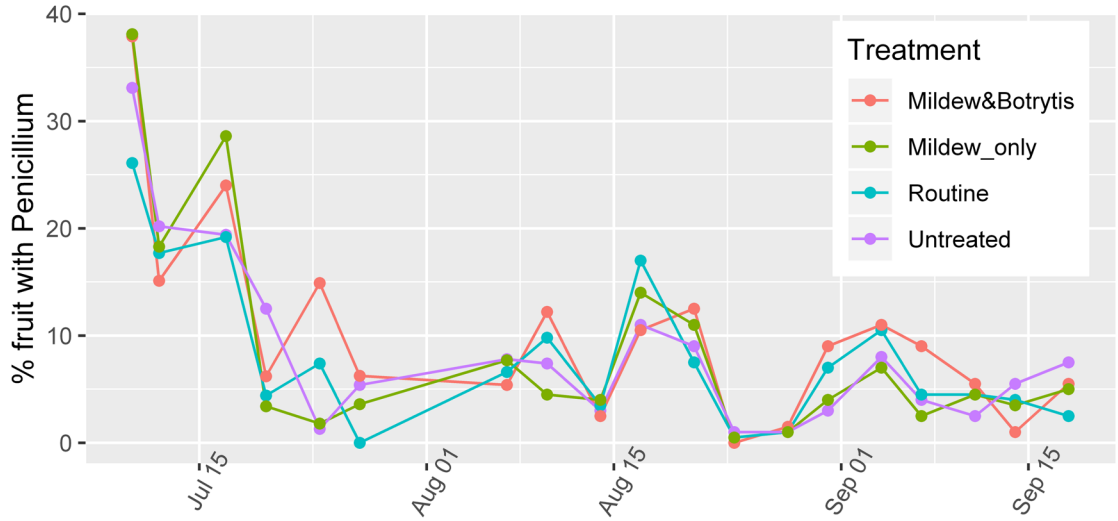


Figure 2.6. Percent fruit with Penicillium rot over 19 harvests, in post-harvest tests (7days at ambient temperature) on an everbearer strawberry cultivar following treatment with four different programmes compared to an untreated control.

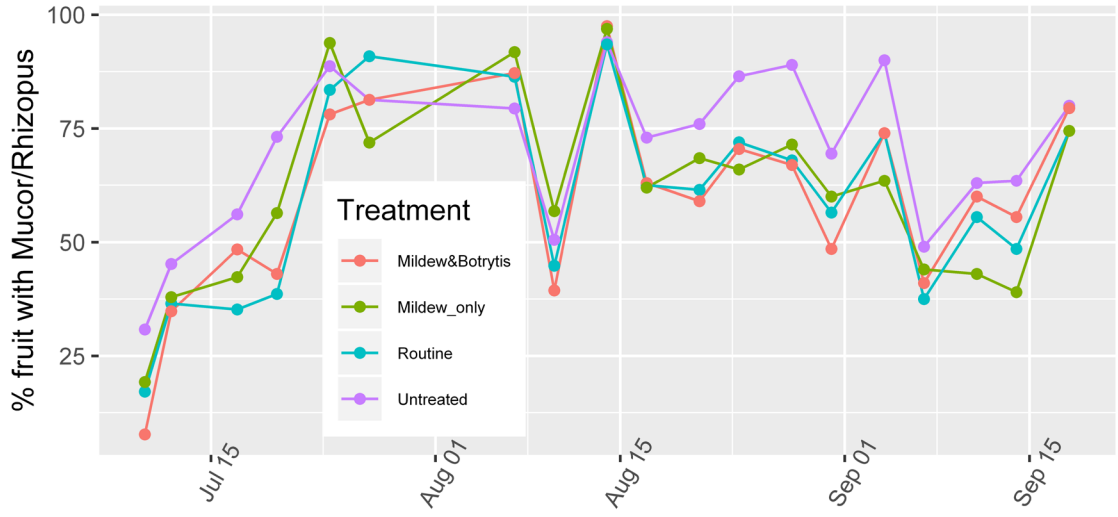


Figure 2.7. Percent fruit with Mucor / Rhizopus soft rots over 19 harvests, in post-harvest tests (7days at ambient temperature) on an everbearer strawberry cultivar following treatment with four different programmes compared to an untreated control.

Total rots in post-harvest tests is also given in Table 2.15 and in Appendix 7. There were significant effects of the treatments on total rots, mainly accounted for by the effects on soft rots.

Cool chain management

A comparison of rot incidence in fruit following 7-days incubation at ambient temperature with that following cool chain management for the same period was conducted on harvests from 13 August to 17 September only (as there was insufficient fruit available from each plot prior to this). The incidence of rots in the cool chain fruit was very low and predominantly soft rots (*Mucor / Rhizopus* spp.) with only a negligible incidence of Botrytis and Penicillium rots. Data presented in Figs.2.8 and 2.9 and Table 2.16 (Appendix 7 and 8) are for total rots. Mean total rot incidence in cool chain fruit varied from 0.65-1.74% for the 11 harvests compared to 73.6-84.2% for the fruit held at ambient temperature (maximum rot potential) for the same period. There were no significant effects of treatments on rots following cool chain management.

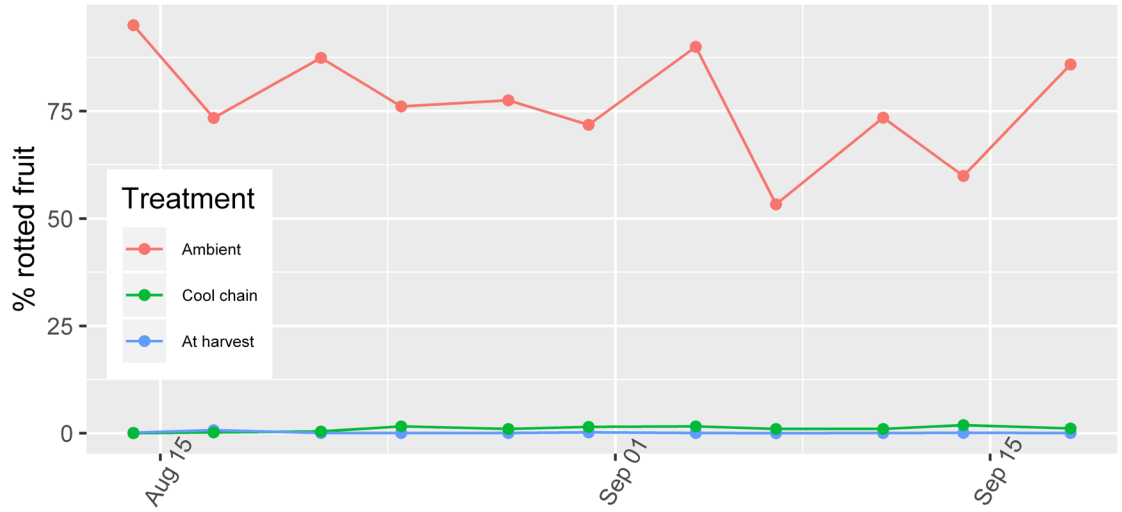


Figure 2.8. Percent fruit with rots over 11 harvests, at harvest, in post-harvest tests (7 days at ambient temperature) and after 7-days cool chain management on an everbearer strawberry cultivar following treatment with four different programmes compared to an untreated control.

Other diseases

The fungus, found colonising the stigmas of strawberry flowers in 2017 was again recorded in plots in 2019 and appeared earlier (2 July) than in previous years. Most plots were affected. The fungus is still to be fully identified but is closely related to Smut fungi.

Yield and fruit quality

Fruit was harvested weekly or twice weekly from 9 July to 17 September, a total of 19 harvests. Yield and % Class 1 fruit are shown in Figs. 2.10 and 2.11, Appendix 9 and 10 and Table 2.14. There was a drop in yield around late July / early August corresponding to the stop in flower production in early July. Thereafter yield steadily increased, peaking at the end of August. The percentage of Class 1 fruit was mainly high at over 90% (Fig. 2.11). There was a decline in % Class 1 fruit at the end of July mainly due to the fall in yield. Per cent Class 1 fruit decreased

in the untreated plots from mid-August due to the increase in fruit infected with SPM. There was significantly less Class 1 fruit in untreated plots on two occasions (13 August and 17 September) but overall there were no significant effects of treatments on yield, % Class 1 fruit, or % rots at harvest (Table 2.14).

Figure 2.9. Rots in strawberry fruit following 7-days incubation at ambient temperature (LHS) and 7 days in cool chain management (RHS)

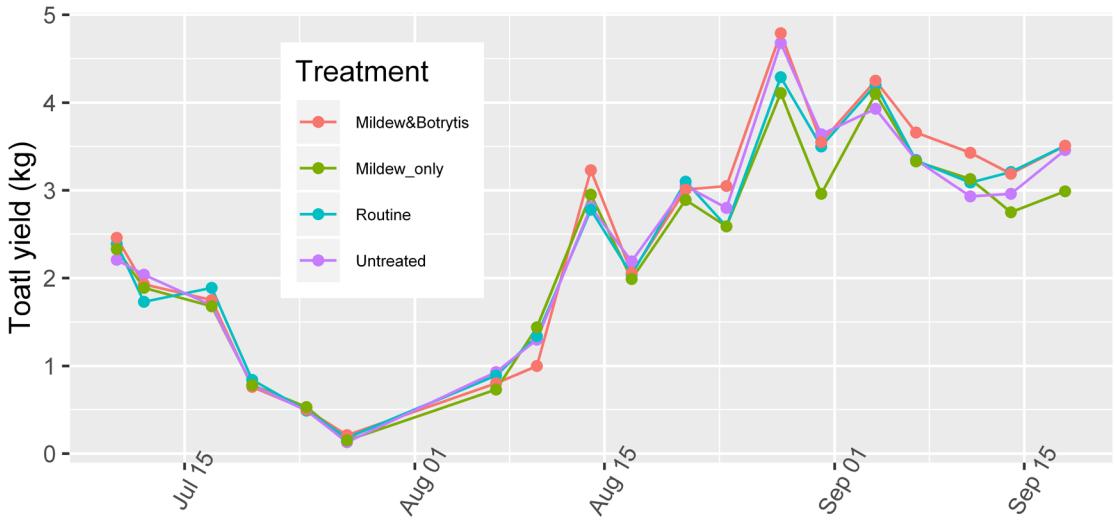


Figure 2.10. Total fruit yield (kg) of 19 harvests following treatment with 4 different programmes compared to an untreated control.

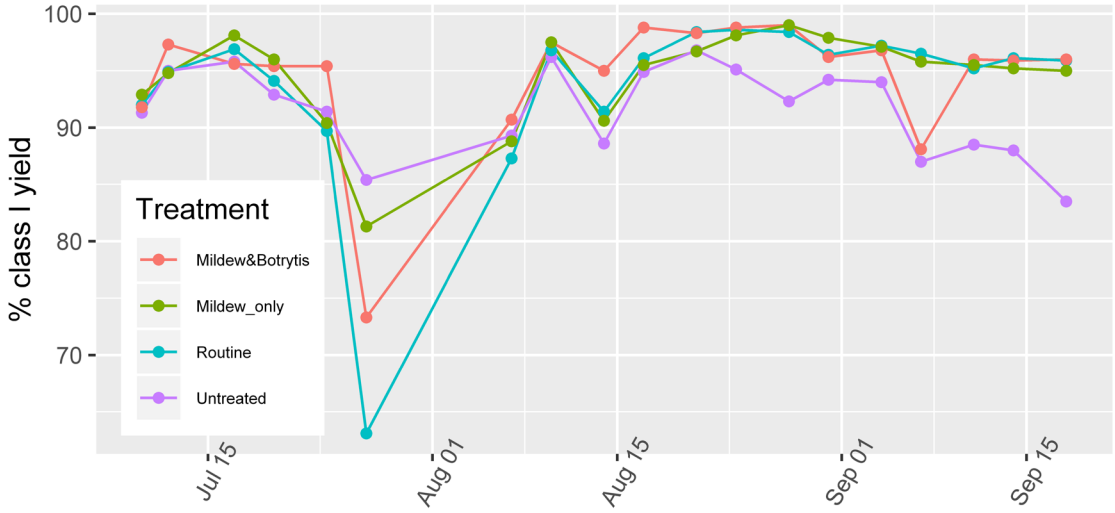


Figure 2.11. Mean % Class 1 strawberry fruit from 19 harvests following treatment with 4 different programmes compared to an untreated control.

Table 2.11. Fungicides, BCAs and biostimulants applied to strawberry plots at NIAB EMR 2019

Treatment	May		June					July				August					September	
	15	24	31	6	14	20	28	4	11	18	26	1	8	15	21	29	5	12
T1 - Untreated	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
T2 – Routine fungicide	Amistar	Fortress	Amistar	Kindred	Stroby	Nimrod + Signum	Stroby + Switch	Topas + Frupica	Charm	Nimrod + Signum	Topas + Frupica	Takumi + Teldor	Nimrod + Scala	Luna Sensation	Topas + Switch	Takumi + Teldor	Talius + Scala	Topas + Prolectus
T3 – SPM and Botrytis managed		CBL				CBL + Sonata + Signum	Sonata + Switch		Charm	CBL + Sonata + Signum	Sonata + Frupica	Sonata + Teldor	Sonata + Scala	Luna Sensation + CBL	Sonata + Switch	Sonata + Teldor	Sonata + Scala	Sonata + Prolectus
T4 – SPM managed. No Botrytis sprays		CBL				CBL + Sonata	Sonata + Slither		Charm	CBL + Sonata + Slither	Sonata + Slither	Sonata + Slither	Sonata + Slither	Luna Sensation + CBL	Sonata + Slither	Sonata + Slither	Sonata + Slither	Sonata + Slither
Comments	No mildew Low risk	No mildew Low risk	No risk	No risk	Low Risk	No mildew High risk		No mildew risk	Trace mildew on T3	Mod risk	High risk	Mildew on T1 Mod risk	High mildew + Botrytis risk	High risk New mildew in T1 and sprayed plots		Mildew in T1 Mod risk	Mod risk	Mildew present Mod risk

Table 2.12. Summary of fungicides, biofungicides, biostimulants applied to strawberry plots at NIAB EMR 2019 and the programme costs

Treatment period	Treatment	Management treatment			
		T1: Untreated	T2: Routine	T3: SPM + Botrytis managed	T4: SPM. Managed No Botrytis sprays
15 May-20 June	Botrytis Fungicide	0	3	1	0
	Mildew Fungicide	0	6	0	0
	BCA	0	0	1	1
	biostimulant	0	0	2	2
28 June-1 August	Botrytis Fungicide	0	6	5	0
	Mildew Fungicide	0	6	1	1
	BCA	0	0	4	4
	Biostimulant	0	0	1	1
8 August-12 September	Botrytis Fungicide	0	6	6	(1)
	Mildew Fungicide	0	6	1	1
	BCA	0	0	5	5
	Biostimulant	0	0	1	1
Total	Botrytis fungicides	0	15	12	1
	Mildew fungicides	0	18	2	2
	Total fungicides	0	29	12	2
	Biofungicides	0	0	10	10
	Biostimulant	0	0	4	4
	Total products	0	29	26	16
Cost £/ha	Total programme	0	2006.09	1933.76	1081.99
	Mildew only	0	888.97	933.94	1081.99
	Botrytis only	0	1360.66	1184.16	184.34

Table 2.13. Summary of strawberry treatments, assessments and management decisions in SPM and Botrytis management trial – NIAB EMR 2019

Date	Activity
1-3 May	Trial planted. Evidence of Botrytis in plants in cold store. Any old
	Leaves with Botrytis removed at planting. Botrytis spray requested
7 May	Checked plants. Most growing away with signs of new leaves. Trace of
	Mildew mycelium seen on old leaves. Not sporing. Forecast weather
	Up to 18°C in day and cool at night. RH less than 80% as windy. Low risk mildew. Mildew spray to routine plots WB 13 May
15 May	Spray applied to T2
21 May	Checked plants. No mildew seen. Growing well. Some wilting plants probably crown rot. Tom to apply Fenomenal
22 May	Fenomenal applied
24 May	Spray applied T2-T4
29 May	De-blossomed plants. No mildew seen. Some wilting plants
31 May	Spray to T2
4 June	No mildew seen, Green aphids severe in patches. Calypso applied later. Forecast mildew risk low

6 June	Spray to T2
11 June	No mildew seen. Aphids mostly gone. Low / moderate mildew risk
18 June	No mildew seen. Some black aphids. Temperature >18^{o}C. High humidity forecast. Flowering. Spray needed on managed plots
20 June	Sprays to T2-T4
25 June	No mildew seen. Still aphids. Warm and humid = High mildew and Botrytis risk
28 June	Sprays to T2-T4
2 July	No mildew seen. One flower seen with flower fungus. More wilting plants. Some ripe fruit. Very few new flowers. Mildew risk low/moderate. Omit sprays T3, T4
4 July	Sprays T2
9 July	Traces of mildew seen on young leaf in one plot. Fungicide treatment to all. 1st harvest
11 July	Fungicide applied to T2-T4
12 July	2nd harvest
16 July	No mildew seen. Moderate risk. Flower fungus easily found, 3rd harvest
18 July	Sprays to T2-T4
19 July	4th harvest
23 July	No mildew found. Flower fungus present in all plots at low-moderate incidence. High mildew risk. 5th harvest
26 July	6th harvest. Sprays to T2-T4 after picking
31 July	Mildew found at low incidence in one untreated plot. New lesion on leaves. None seen on flowers and fruit. Aphid still present. Moderate risk
1 August	Sprays to T2-T4
6 August	Mildew found low incidence in T1. None seen in sprayed plots. Flower fungus prevalent. High mildew and Botrytis risk. 7th harvest
8 August	Sprays to T2-T4
9 August	8th harvest
13 August	Mildew found on fruit stalk in one T1 plot. Trace on leaf in T3 plot. High mildew and Botrytis risk. Fungicide spray to all. 9th harvest
14 August	Sample of fruit for residue analysis taken from all plots
15 August	Fungicide spray to T2-T4
16 August	10th harvest
20 August	No new mildew seen on treated plots. New mildew in T1 plots. High mildew and Botrytis risk. 11th harvest
21 August	Sprays to T2-T4
23 August	12th harvest
27 August	Trace mildew seen in T2 plots on flowers and fruit. Increasing in T1 plots. 13th harvest
29 August	Sprays to T2-T4 plots
30 August	14th harvest
3 September	Mildew in T1 plots. Trace mildew in T2 plots. Moderate risk.15th harvest
5 September	Sprays to T2-T4 plots
6 September	16th harvest
10 September	Mildew in T1 plots. Trace mildew in T2 and some managed plots. Moderate risk. 17th harvest
12 September	Sprays to T2-T4 plots
13 September	18th harvest
17 September	19th harvest. Last harvest
18 September	Sample of fruit for residue analysis taken from all plots
19 September	Assessment of leaf area mildewed on random sample of 40 leaves per plot

Table 2.14. Mean yield, % Class 1 fruit, and mean % Botrytis and total rots at harvest in 2019. Mean of 19 harvests.

Treatment	Mean Total yield kg	Mean % Class 1 fruit	Mean % fruit with Botrytis at harvest	Mean % Total rot
T1: Untreated	45.35	91.6	0.15	0.23
T2: Routine fungicide	45.36	93.3	0.2	0.31
T3: SPM + Botrytis managed	47.14	94.5	0.3	0.37
T4: SPM managed No Botrytis sprays	43.28	94.5	0.04	0.20
F Prob	0.416	0.142	0.276	0.712
LSD (p=0.05)	NS	NS	NS	NS

Table 2.15. Mean % incidence of fruit rots (angular transformed) in post-harvest tests in 2019 following incubation for 7 days at ambient temperature. Mean of 19 harvests. (figures in brackets are original data)

Treatment	Mean % Botrytis	Mean % Penicillium	Mean % Mucor / Rhizopus	Mean % total rotted fruit
T1: Untreated	8.7	8.6	74.7 (71.3) a	87.7 (83.4) a
T2: Routine fungicide	8.5	8.1	62.3 (59.5) b	78.0 (73.8) b
T3: SPM + Botrytis managed	7.2	10.1	62.2 (59.8) b	78.5 (74.2) b
T4: SPM managed No Botrytis sprays	10.0	8.6	63.6 (61.0) b	77.5 (73.8) b
F Prob	0.637	0.757	<0.001	0.009
SED (12)			0.133	0.111
LSD (p=0.05)	NS	NS	0.514	0.431

Table 2.16. Mean % incidence of Total fruit rots at harvest, in post-harvest tests following incubation for 7 days at ambient temperature and after 7 days in cool chain management in 2019. Mean of 11 harvests.

Treatment	Mean % Total rot at harvest	Mean % Total rot after 7 days at ambient	Mean % Total rot after 7 days cool chain
T1: Untreated	0.09	84.2 a	0.73
T2: Routine fungicide	0.15	74.7 b	1.74
T3: SPM + Botrytis managed	0.12	74.2 b	0.98
T4: SPM managed No Botrytis sprays	0.12	73.6 b	0.65
F Prob		0.009	0.605
LSD (p=0.05)	NS		NS

Fungicide residues

The fungicide residues obtained in the samples taken in August and September are shown in Table 2.17. None of the residues obtained exceeded the Maximum Residue Level (MRL). The only residue detected in the fruit from untreated plots was of the insecticide thiacloprid, which was applied to all plots for aphid control. At both sampling times, 6-7 fungicide residues were detected in fruit from the plots receiving the standard fungicide programme. Only three fungicide residues were detected in fruit from Treatment 3 at the first sampling, but six in the second sampling. By contrast no residues were detected in fruit from Treatment 4 at the first sampling and only two fungicide residues at the second sampling. Most of the fungicide residues detected in the fruit were from fungicides relating to Botrytis control.

Table 2.17. Residues present in strawberry samples taken from Treatments T1-T4 on 14 August and 18 September 2019

Sample date	Active ingredient	Treatment / residue mg/kg				
		T1: Untreated	T2: Routine	T3: SPM and *Botrytis* managed	T4: SPM managed No *Botrytis* sprays	EU MRL mg/kg
14 August	bupirimate		0.1			2.0
	cyflufenamid		0.01			0.04
	fenhexamid		0.31	0.51		10.0
	mepanipyrim		0.074	0.059		3.0
	ethirimol*		0.05			0.2
	pyrimethanil		0.82	0.67		5.0
	thiacloprid	0.017				1.0
18 September	cyprodonil		0.052	0.051		5.0
	fludioxonil		0.15	0.15		4.0
	penconazole		0.026			0.5
	fenhexamid		0.99	0.81		10.0
	pyrimethanil		0.38	0.46		5.0
	fluopyram		0.053	0.052	0.055	2.0
	trifloxystrobin		0.044	0.044	0.032	1.0
	thiacloprid	0.34	0.35	0.29	0.34	1.0

*Ethirimol is a break down product of bupirimate

Economic appraisal

The cost of the programmes applied is shown in Table 2.12. Total programme costs were highest for the standard fungicide programme and cheapest for Treatment 4, where no fungicides for Botrytis control were applied. The fungicide only programme for mildew control in the standard programme was slightly cheaper than in Treatments 3 and 4, mainly due to the fungicides for mildew control being cheaper than the combined costs of biofungicides and wetters. The biggest savings were in omitting Botrytis fungicides in Treatment 4 which resulted

in a cost saving of over £1,000 per hectare and the added advantage of considerably reduced residues in the fruit.

Discussion

The trials in 2019 further demonstrated the value of managing SPM with integration of biofungicides and conventional fungicide products with the timing depending on model predictions, crop growth, forecast weather and current level of SPM. Furthermore, the results questioned the value of using conventional fungicides to control Botrytis fruit rotting under production, provided that a post-harvest cool-chain management is adopted.

While SPM and Botrytis remain two important disease problems in protected strawberry production, it is becoming clear that SPM has now become the more significant, particularly in everbearers. If SPM becomes established in the crop, control is difficult and epidemics result in significant losses in yield and fruit quality and even crop abandonment. Botrytis however, is more of a post-harvest problem and the fruit management procedures adopted to cope with the arrival of Spotted Wing Drosophila, have restricted the build-up of rot inoculum in the crop, which previously was a significant factor, meaning that any impact on yield is minimal.

In this trial, SPM was present at very low incidence on the old leaves at the time of planting. Weather conditions were not conducive to SPM until mid-June but thereafter, in contrast to 2018, the weather risk continued at moderate to high for most of the trial period. Despite this, SPM was slow to develop on leaves in untreated plots and never reached high incidence but was higher than in the treated plots where on leaves it remained at negligible incidence. On flowers and fruit SPM did not appear in untreated plots until early August but thereafter steadily increased to a mean of nearly 15 % mildewed fruit by the final harvest. The incidence in treated plots remained very low. The strategy in the SPM managed plots (where control was based on the biofungicide Sonata protectant programme with fungicide intervention when the SPM risk, based on disease monitoring and weather risks, increased), performed as well as the routine 7 day fungicide programme. The start of the Sonata programme was delayed until 20 June and only two fungicide sprays were applied. So there were large savings in fungicide sprays.

For Botrytis, the risks shown by the models were at the beginning of the crop and again from late July onwards. The incidence of Botrytis in the fruit at harvest and in post-harvest tests followed this pattern but with low incidence at harvest and higher rot incidence in the post-harvest tests. Thus, there was little opportunity for reducing fungicide inputs from July onwards in Treatment 3. Overall, fewer fungicides for Botrytis were applied in the managed plots compared to the routine sprayed plots with a small saving in costs. No fungicides targeted at Botrytis were applied in Treatment 4. However, in only two of the 19 harvests was there a

significant effect of treatment on Botrytis incidence and in both cases Botrytis was higher in treated than in untreated plots. Similarly, with the other rots – Penicillium and soft rots – there were no or few significant reductions in rots in treated plots. Significant reductions recorded in soft rots were of little importance as the rot incidence in the treated plots was still high (more than 55%).

In the post-harvest tests rot incidence in the cool chain managed fruit was very low after 7 days compared to the fruit maintained at ambient temperature and demonstrated the importance of including this system in strawberry production.

Therefore, as in 2018, the fungicide treatments applied for rot control had little benefit. Omitting the sprays for rots has the potential for large savings in costs (around £1,000 per hectare) and perhaps of greater importance, reduced fungicide residues, with little effect on yield and fruit quality.

As expected multiple residues were detected in the fruit (although none above MRL), most of which related to products used for Botrytis control. Very few residues were detected in fruit from Treatment 4 where no fungicides for Botrytis control were applied.

Overall using the simple decision-based system for determining treatments for SPM and rots and omitting fungicides for Botrytis control (Treatment 4) in protected everbearer strawberries resulted in a 90 % reduction in fungicide use and a cost saving of £900 /ha compared to a routine programme, with no penalties in yield, fruit quality or disease control. The results from this year's trial have confirmed, that provided SPM is monitored in the crop and the weather risk identified (either from using a model which includes a forward forecast or sourcing the forecast from the internet) the SPM control programme can be based on biofungicides with intervention from fungicides when a higher risk is identified.

Summary and conclusions

- Weather conditions were very favourable for development of SPM in late May / early June and continued for much of the trial period from late July onwards which was confirmed by the high risk (consecutive days with risk > 10%) shown by the mildew risk model
- A low incidence of old SPM was present on the old leaves of the strawberry plants at planting. Despite the favourable conditions for most of the trial period only a low incidence of SPM was present on leaves in untreated plots with negligible incidence on treated plots
- SPM eventually established on fruit in early August and steadily increased from then until the final harvest on 17 September reaching a mean of around 15% of fruit with SPM in control plots. The incidence on treated plots was similar and remained very low

- On plots managed for SPM control the SPM programme was based on the biofungicide Sonata and did not start until 20 June. Fungicide intervention in response to increased SPM risk was made on two occasions. A total of 10 biofungicides, two fungicides and four biostimulants were applied to Treatments 3 and 4 for SPM
- On routinely treated plots (T2) the programme for SPM control started on 15 May and continued at 7 day intervals until 12 September resulting in a total of 18 fungicides applied
- The Botrytis model showed a risk in early June and from late July onwards, August with a decreased risk in early September.
- In routinely treated plots Botrytis sprays started on 15 and 31 May and then every 7 days from 20 June. A total of 15 fungicide sprays were applied. In the managed treatment (T3, both SPM and Botrytis) fungicide sprays started on 20 June and 28 June and then continued every 7 days from 11 July. A total of 12 fungicide sprays were applied. No fungicide treatments for *Botrytis* were applied to T4 plots
- The incidence of rots recorded at harvest followed the Botrytis risk but was very low ranging from 0 to 1.3% in untreated plots. None of the treatments had any significant effect on rot incidence compared to the untreated control at any of the 19 harvests.
- The incidence of Botrytis rots in post-harvest tests followed a similar pattern with Botrytis present at the early harvests in July and from early August onwards relating to the identified Botrytis risk. Over the 19 harvests the incidence of Botrytis rots in untreated plots in post-harvest tests ranged from 0 to 27.5%. There were significant effects of treatments on Botrytis rot incidence in only two of the 19 harvests, but in each case there was significantly more Botrytis rot in fungicide-treated plots than in the untreated control indicating that the 12-15 fungicides applied had little benefit
- Rots due to *Penicillium* spp. and soft rots, mainly *Rhizopus* spp. were the other main rots recorded in post-harvest tests. Over the 19 harvests the incidence of Penicillium rot in untreated plots in post-harvest tests ranged from 1 to 33.1%. There were no significant effects of treatments on Penicillium rot incidence compared to the untreated control in any of the 19 harvests
- In contrast to Penicillium and Botrytis rots, the incidence of soft rots started off in the early harvests at low incidence and gradually increased and was the predominant rot recorded in most harvests where incidence was between 30.8-94% in untreated plots. There were significant effects of treatments on rot incidence compared to the untreated control on three occasions. However, the reduction in rot incidence in the treated plots was small and still resulted in more than 55% soft rots

- The incidence of rots in the cool chain fruit management over 11 harvests from 11 August to 17 September was very low and predominantly soft rots with only a negligible incidence of Botrytis and Penicillium rots. Mean total rot incidence in cool chain fruit varied from 0.65-1.74% for the 11 harvests compared to 73.6-84.2% for the fruit held at ambient temperature (maximum rot potential) for the same period
- The residues detected were all below the MRL. Fewer residues were detected in the first sampling on 14 August and at both sample dates most of the residues were from Botrytis fungicides. Most residues were detected in T2, which received the routine 7-day programme. Fewer residues were detected in programmes T3 and were mainly from Botrytis fungicides. No residues were detected in T4 at the first sampling and only two fungicides in the second sample
- Fruit was harvested twice weekly from 9 July to 17 September, a total of 19 harvests. There was a drop in yield around late July / early August corresponding to the stop in flower production in early July. Thereafter yield steadily increased, peaking at the end of August. The percentage of Class 1 fruit was mainly high at over 90%. There was a decline in % Class 1 fruit at the end of July mainly due to the fall in yield. Per cent Class 1 fruit decreased in the untreated plots from mid- August due to the increase in fruit infected with SPM. There were no significant differences in yield, % Class 1 fruit and % unmarketable fruit between the managed programmes and the routine fungicide programme
- There were no obvious phytotoxic symptoms observed on foliage or fruit in any of the plots following the spray treatments
- There were no obvious differences in plant vigour (height and spread) between the plots
- Total programme costs were highest for the standard fungicide programme and cheapest for Treatment 4, where no fungicides for Botrytis control were applied. The biggest savings were in omitting Botrytis fungicides in Treatment 4 which resulted in a cost saving of over £1,000 per hectare
- Overall a simple decision based system for determining treatments for SPM and rots in protected everbearer strawberries based on biofungicides for mildew control and omitting fungicides for Botrytis control resulted in a 90 % reduction in fungicide use and a cost saving of around £900 /ha compared to a routine programme with no penalties in yield, fruit quality or disease control
- SPM has now become one of the most important diseases in protected strawberry production. Once the disease is established in the crop control is difficult with losses in yield and quality and potential crop abandonment

- Managing SPM enables savings in fungicide use when the risk is low in the early part of the season, ensuring products are available for the higher risk period in late summer
- There are now effective biofungicides such as Sonata that can form the basis of a protectant programme for control of SPM, using fungicides only in high risk periods
- Botrytis remains a potential problem in protected strawberries. However, the importance has declined compared to SPM
- Growing under protection together with cool chain management of the fruit, has considerably reduced the development of Botrytis fruit rot. In addition, management of fruit waste at harvest to control SWD has reduced the Botrytis inoculum and hence the build-up of the disease in the ever bearer crop
- The results from 2018 and this year consistently show little benefit in Botrytis control from the use of fungicides
- There was a consistent effect of fungicides in reducing soft rots from around 80-90% in untreated to 50-70% in sprayed plots, but this small reduction in rot does little to justify the fungicide inputs
- Both Botrytis and soft rots can be delayed by cool chain management of the harvested crop

Demonstration of a SPM management strategy on strawberry in Wet Centre at NIAB EMR (Objective 2)

Trials in 2015 - 2016 identified effective products for control of SPM in strawberries. The trial in 2017 combined their use in programmes and incorporated other factors such as disease risk, growth stage, type of fungicide (curative, protectant, anti-sporulant) to develop a decision-based management programme for growers. This trial demonstrated that use of biofungicides in a managed programme, gave good control of mildew in strawberry comparable to a fungicide-based programme. In 2018 the managed SPM strategy was evaluated and demonstrated on a commercial farm site (Hatchgate site by kind permission of Clock House Farms). Overall using the simple decision based system for determining treatments for powdery mildew and rots in the trial tunnel resulted in around a 50% reduction in fungicide use and a cost saving of £442.86 /ha compared to the control tunnel receiving the farm programme with no obvious penalties in yield, fruit quality or disease control. In 2019 the system was further evaluated in the Wet Centre demonstration area at NIAB EMR

The objective was to compare the disease control achieved and the residues in the fruit by managed programmes of fungicides and biofungicides with that achieved by a routine fungicide programme.

Materials and methods

Site and planting

The demonstration was conducted at the Wet Centre NIAB EMR East Malling, Kent. The site consisted of established table tops in a multi span polytunnel. Coir substrate bags (Cocogreen) were used and planted with everbearer strawberry cv. Malling Champion in mid- April. Nutrition and irrigation of the bags were managed by sensors linked to dashboard display in tunnels (by Wet Centre management staff).

Treatments

The Wet Centre area was divided into two areas, one to be treated routinely with fungicides (Treatment 1, Table 2.18) and the other (2 blocks in South west end, labelled red) were managed (Treatment 2, Table 2.18). Fungicides and BCAs used in the programmes are given in Tables 2.2 and 2.19. All were applied as foliar sprays.

Treatment 1 was managed as a routine fungicide programme, initially at 10-14 day intervals and then at 7 day intervals once plant growth increased. Decisions on spray applications to Treatment 2 were based on the criteria given above in Tables 2.5 and 2.6 for mildew and Tables 2.7 and 2.8 for Botrytis and other rots. Leaves, flowers and fruits were checked for

mildew and other diseases twice weekly from 30 April. Sensors in the tunnels provided temperature and humidity data for input directly into the powdery mildew model which was then available on the dashboard display in the tunnel. Treatments in the managed area were based along with the disease monitoring of the crop on the criteria in Tables 2.5 - 2.8 above and from the forward forecast on the BBC weather on the internet (Table 2.20). Decisions on the programme applied to the routine area were made by Alex Cook of BGG. Decisions on treatments in the managed area were also made by Alex with assistance from A Berrie to ensure understanding of the management system used.

Table 2.18. Treatment programmes evaluated in Wet Centre at NIAB EMR in 2019

Treatment	Type	Products
1 unlabelled	Routine	Fungicides, biofungicides, biostimulants
2: red tape	Managed SPM and fruit rots	Fungicides, biofungicides, biostimulants

Table 2.19. Biofungicides for disease control on strawberry used in the trial in the Wet Centre at NIAB EMR in 2019

Product	Active ingredient	Rate of product / ha	Maximum number of sprays	Product type and disease targeted
Amylo X WG	*Bacillus amyloliquefaciens* D747	2.5 kg	6 at 7 day intervals up to BBCH 89 (fruits coloured)	Biofungicide:SPM and Botrytis
AQ 10 + Silwet	*Ampelomyces quisqualis* strain AQ 10	70 g + 0.05%	12	Biofungicide: SPM
Prestop	*Gliocladium catenulatum*	3 kg	3	Biofungicide: Botrytis
Serenade	*Bacillus subtilis* strain QST 713	10 L	6	Biofungicide: SPM/ Botrytis

Assessments

SPM and other diseases

A full assessment for SPM on leaves as percentage leaf area infected on a random sample of 100 leaves was done once on 21 August using a standard key (Anon, 1976) (Appendix 11). Similarly, a full assessment of powdery mildew on a random sample of 100 flower trusses (% incidence) was conducted once on 21 August.

A random sample of 100 fruit was picked from the trial and control tunnels on 13 August. The weight was recorded and fruit assessed for mildew and rots. The fruit was then incubated at ambient temperature at high humidity as described earlier and the rots recorded after 7 days.

Economic appraisal

At the end of the season, the cost associated with the two treatments was assessed to conduct a simple economic appraisal of the management programme.

Results

SPM risk and incidence

The weather conditions (warm temperatures coupled with high humidity) were very conducive to SPM development in late May / early June and continued for much of the trial period from end of July onwards (Fig.2.2). The programmes applied to the two tunnel areas are shown in Table 2.21. The trial activities, disease monitoring and assessments together with the decisions in response to the predicted risks, based on SPM monitoring in the crop and the model, are shown in Table 2.20. Traces of mildew mycelium on the old leaves were noted on the plants in all tunnels on 30 April. All tunnels were sprayed with Fortress on 26 April. The routine treated areas continued to be sprayed at 7 day intervals. Further treatment in the managed area, apart from a spray on 29 May, was then delayed until the appearance of new SPM. New mildew lesions were first observed in the managed area on 11 June and continued to be noted in most crop inspections from then onwards. Sprays for SPM and Botrytis in the managed area were then applied from 14 June at 7 day intervals, following the same programme as the routine treated areas. SPM was first noted on fruit stalks on 9 July. SPM was also observed in the routine treated areas but at lower incidence. Although new lesions of SPM continued to be found on leaves and fruit in the managed area throughout the trial period, the disease remained at a low incidence, but there were few opportunities to reduce fungicide inputs. The incidence of SPM on leaves on 21 August was less than 1% mildewed leaf area (Table 2.22) and on fruit trusses 6-10%. The incidence was low but higher in the managed areas. SPM on a sample of 100 harvested fruit assessed on 13 August was 1% on fruit from the managed area and 0% on routine sprayed fruit. Delaying the start of the 7-day programme in the managed area resulted in a small saving of three fungicides (Table 2.21).

Table 2.20. Record of visits, assessments and risks for spray decisions on the trial tunnel at in Wet Centre at NIAB EMR in 2019

Date	Record of work done, observations made or reference to lab or field book entry
30 April	Inspected plants. Found trace mildew mycelium on old leaf. Non sporing. Weather indicates low risk
2 May	Inspected plants. Found trace mildew mycelium on old leaf. Non sporing. Weather indicates low risk. Sprayed with Amistar Top last week. Fortress due on 3/5. Omit mildew spray for 10/5
3 May	Crop sprayed
7 May	Checked trial area for mildew. No new mildew seen
21 May	Checked trial area. No new mildew found. Aphid + low level of spider present. Predators needed. Forward forecast = low risk mildew and Botrytis. No sprays
23 May	No new mildew found
28 May	No new mildew found. Forecast low risk. No sprays
30 May	No new mildew found. Model no risk. No sprays
4 June	No new mildew found. Forecast low risk
11 June	Two very new mildew lesions found on young leaves. Forecast low / moderate mildew risk. Spray needed
18 June	Two very new mildew lesions found on young leaves. Forecast High RH and temperature > 18^{o}C. Spray needed
20 June	One new mildew lesion seen
25 June	Several young leaves with new mildew. Forecast weather = high risk, Spray
2 July	New mildew lesions on young leaves. Similar number of leaves affected to last week, but larger area affected. Risk moderate. Spray Mildew and Botrytis
9 July	New mildew on young leaves and also on fruit stalk and young fruit. Low incidence. Mildew also seen on young leaves and fruit in routine sprayed but lower incidence. Southern tunnel less humid than North tunnels (different poly cover). Forecast moderate risk spray needed. Also noted petiole and fruit stalk base rot. Sample taken to check cause
16 July	Low incidence of new mildew on young leaves and occasional fruit. One fruit with Botrytis. Forecast risk mod/high. Spray. Petiole base rot worse and some collapsed plants with rot in crown. LFD test negative so not *Phytophthora*. Isolations onto agar show base rot is Botrytis
23 July	Low incidence of mildew on young leaves and on young fruit. Incidence still very low so treatments are containing the disease Forecast risk mod/high. Spray
31 July	Leaf production slowing so fewer new mildew lesions. Mildew more obvious on older leaves and fruit but still at very low incidence
6 August	As above. More mildew on fruit but still at low incidence
13 August	As above. Low incidence of flower fungus. 100 fruit sampled from routine and managed tunnels. Assessed for rots, mildew and weight
21 August	Mildew assessed on a random sample of 100 leaves from each of routine and managed tunnels and on 100 flower / fruit trusses from each

Table 2.21. Products applied to the trial tunnel versus routine tunnels of site in Wet Centre at NIAB EMR in 2019

Spray date	Control tunnels		Trial tunnel	
	Product / Rate/ha	Target	Product /. Rate/ha	Target
26 April	Fortress 0.25 L	SPM	Fortress 0.25 L	SPM
	Hortiphyte 3 L	Plant health	Hortiphyte 3 L	Plant health
28 April	Paraat 3 kg	Phytophthora	Paraat 3 kg	Phytophthora
3 May	Amistar Top 1 L	Mildew, Botrytis		
	Maxicrop 3 L	Plant health	Maxicrop 3 L	Plant health
	Hortiphyte 3 L	Plant Health	Hortiphyte 3 L	Plant Health
10 May	Stroby 0.3 kg	SPM		
	Maxicrop 3 L	Plant health		
17 May	Fortress 0.25 L	SPM		
	Hortiphyte 3 L	Plant health		
29 May	Amistar Top 1 L	SPM / Botrytis	Amistar Top 1 L	SPM / Botrytis
	Maxicrop 3 L	Plant health	Maxicrop 3 L	Plant health
	Calypso 0.25 L	Aphids	Calypso 0.25 L	Aphids
31 May	Takumi 0.15 L	SPM		
	Maxicrop 3 L	Plant health	Maxicrop 3 L	Plant health
7 June	Frupica 0.9 L	Botrytis		
	Maxicrop 3 L	Plant health	Maxicrop 3 L	Plant health
14 June	Takumi 0.15 L	SPM	Takumi 0.15 L	SPM
	Scala 2 L	Botrytis	Scala 2 L	Botrytis
	Maxicrop 3 L	Plant health	Maxicrop 3 L	Plant health
21 June	Topas 0.5 L	SPM	Topas 0.5 L	SPM
	Switch 1 kg	Botrytis	Switch 1 kg	Botrytis
	Maxicrop 3 L	Plant health	Maxicrop 3 L	Plant health
28 June	Charm 0.6 L	SPM	Charm 0.6 L	SPM
	Calypso 0.25 L	Aphids	Calypso 0.25 L	Aphids
	Maxicrop 3 L	Plant health	Maxicrop 3 L	Plant health
5 July	Scala 2 L	Botrytis	Scala 2 L	Botrytis
	Maxicrop 3 L	Plant health	Maxicrop 3 L	Plant health
	Topas 0.5 L	SPM	Topas 0.5 L	SPM
12 July			Potassium bicarb 7.5 kg	SPM
12 July	Luna Sensation 0.8 L	SPM		
15 July			Luna Sensation 0.8 L	SPM
19 July	Frupica 0.9 L	Botrytis	Frupica 0.9 L	Botrytis
	Maxicrop 3 L	Plant health	Maxicrop 3 L	Plant health
26 July	Topas 0.5 L	SPM	Topas 0.5 L	SPM
2 August	Amylo X 2.5 kg	SPM, Botrytis	Amylo X 2.5 kg	SPM, Botrytis
	Maxicrop 3 L	Plant health	Maxicrop 3 L	Plant health
16 August	Signum 1.8 kg	Botrytis	Signum 1.8 kg	Botrytis
	Topas 0.5 L	SPM	Topas 0.5 L	SPM
	Maxicrop 3 L	Plant health	Maxicrop 3 L	Plant health
	Tracer 0.15 L	SWD	Tracer 0.15 L	SWD
	Calypso	Aphids, Capsid	Calypso	Aphids, Capsid
23 August	Maxicrop 3 L	Plant health	Maxicrop 3 L	Plant health
	Talius 0.19 L	SPM	Talius 0.19 L	SPM
	Switch 1 kg	Botrytis	Switch 1 kg	Botrytis
	SP058	Wetter	SP058	Wetter
25 August	Pot bicarb 8 kg	SPM	Pot bicarb 8 kg	SPM

30 August	Topas 0.5 L	SPM	Topas 0.5 L	SPM
	Calypso 0.25 L	Aphids	Calypso 0.25 L	Aphids
	Maxicrop 3 L	Plant health	Maxicrop 3 L	Plant health
6 September	Signum 1.8 kg	Botrytis	Signum 1.8 kg	Botrytis
	Systhane 20 0.3 L	SPM	Systhane 20 0.3 L	SPM
	Maxicrop 3 L	Plant health	Maxicrop 3 L	Plant health
13 September	Serenade 10 L	SPM, Botrytis	Serenade 10 L	SPM, Botrytis
	Maxicrop 3 L	Plant health	Maxicrop 3 L	Plant health
19 September	Teldor 1.5 kg	Botrytis	Teldor 1.5 kg	Botrytis
	Maxicrop 3 L	Plant health	Maxicrop 3 L	Plant health
	Tracer 0.15 L	SWD	Tracer 0.15 L	SWD
26 September	Pot bicarb 8 kg	SPM	Pot bicarb 8 kg	SPM
	Batavia 1 L	SWD	Batavia 1 L	SWD

Total SPM fungicides	20	17
Total Botrytis fungicides	13	11
Total fungicides	29	24
BCAs	2	2
Bisostimulants	20	18
Total products	51	44
Cost £/ha		
Total programme	**2197.86**	**1966.32**
Mildew only	721.41	643.62
Botrytis only	1214.51	1067.61
Total fungicide cost	1712.38	1497.29
Biofungicides	261.67	261.67
Biostimulants	207.36	207.36

Table 2.22. Incidence of SPM on leaves and fruit in routine treated and managed areas of strawberry cv. Malling Champion in Wet Centre at NIAB EMR in 2019

Treatment	Mean % leaf area mildewed 21/081/19	Mean % fruit trusses with SPM on 21 /08/19	% fruit with SPM at harvest 13/08/19 August
Routine	0.28	6.0	0
Managed	0.57	10.0	1.0

Botrytis risk

The weather conditions (warm temperatures coupled with high humidity) were very conducive to Botrytis infection and development in early June and from end of July onwards (Fig.2.4). The programmes applied to the routine and managed areas are given in Table 2.21. The trial activities, disease monitoring and assessments together with the decisions in response to the predicted risks, based on monitoring in the crop and the model, are shown in Table 2.22. Botrytis inoculum was not seen in the managed plots until the appearance of the stem base

rot on 9 July. Sprays for Botrytis were delayed in the managed area until the weather risk increased and started on14 June with one earlier treatment on 29 May. A total of 13 fungicides were applied for Botrytis control to the routine tunnels compared to 11 in the managed tunnel. Fruit was sampled once for Botrytis on 13 August (Table 2.23). No Botrytis was seen on the harvested fruit, but after 7 days' incubation at ambient temperature 46% of fruit from the managed area developed Botrytis compared to 33% in fruit from the routine area.

Table 2.23. Weight of 100 fruit and Incidence of Botrytis on 13 August and after 7 days' incubation at Ambient temperature on harvested fruit from routine treated and managed areas of strawberry cv. Malling Champion in Wet Centre at NIAB EMR in 2019

Treatment	Weight of 100 fruit (g)	% fruit with Botrytis at harvest	% Rot after 7 days at ambient temperature			
			Botrytis	Penicillium	Mucor & Rhizopus	Total rot
Routine	815	0	33.0	6.0	38.0	71.0
Managed	950	0	46.0	9.0	25.0	76.0

Other diseases

A low incidence of the flower fungus was noted on 13 August in the tunnels

Economic appraisal

The cost of the programmes applied is shown in Table 2.21. Total programme costs were highest for the standard farm programme with a saving of £231.54 /ha in the managed trial programme. Costs for biofungicides and biostimulants were the same for both programmes. Managing the fungicides for SPM and Botrytis control in the trial tunnel resulted in a reduction of five fungicide applications, all at the start of the season, with a reduction of £215.09/ha in costs.

Discussion

Weather conditions from June onwards were favourable for SPM and Botrytis. The cultivar used in the planting – Malling Champion - was newly introduced and classified as moderately susceptible to SPM but with no experience in large commercial plantings. Hence caution was needed as the development of SPM on leaves and fruit in response to favourable conditions was not known. In addition, as the Wet Centre is frequently visited by growers and other visitors any epidemics of SPM would have been undesirable. Therefore, as SPM at low incidence developed in the trial from mid-June, although at very low incidence, there was little opportunity to reduce fungicide inputs. In the other trial reported earlier, the SPM managed plots were treated with the biofungicide Sonata, which was not possible in this trial as the fruit was marketed commercially and at the outset of the trial Sonata was not approved for use.

Experience with other biofungicides such as Amylo X for SPM control was limited. Some reduction in fungicide use was possible by delaying the start of the programme and although the incidence of SPM was always higher in the managed plots, the incidence generally remained low.

Summary and conclusions

- Weather conditions were very favourable for development of SPM in late May / early June and remained favourable for most of the trial period
- A low incidence of old mildew was present on the old leaves of the strawberry plants at the time of planting
- The routine treated areas were sprayed at 7 day intervals from 26 April
- The managed area received sprays for SPM on 26 April and 29 May, but the 7-day programme was delayed until 14 June following detection of new SPM lesions in the crop and favourable weather
- New mildew lesions continued to appear in most crop inspections from then onwards with little opportunity for saving sprays
- SPM was also observed in the routine treated areas but at lower incidence
- Although new lesions of SPM continued to be found on leaves and fruit in the managed area throughout the trial period, the disease remained at a low incidence. The incidence of SPM on leaves on 21 August was less than 1% mildewed leaf area and on fruit trusses 6-10%. The incidence was low but higher in the managed areas
- SPM on a sample of 100 harvested fruit assessed on 13 August was 1% on fruit from the managed area and 0% on routine sprayed fruit
- Delaying the start of the 7-day programme in the managed area resulted in a small saving of 3 fungicides
- The weather conditions (warm temperatures coupled with high humidity) were very conducive to Botrytis infection and development in early June and from end of July onwards
- Botrytis inoculum was not seen in the managed plots until the appearance of the stem base rot on 9 July
- Sprays for Botrytis were delayed in the managed area until the weather risk increased and started on14 June with one earlier treatment on 29 May. A total of 13 fungicides were applied for Botrytis control to the routine tunnels compared to 11 in the managed tunnel.

- Fruit was sampled once for Botrytis on 13 August. No Botrytis was seen on the harvested fruit, but after 7 days' incubation at ambient temperature 46% of fruit from the managed area developed Botrytis compared to 33% in fruit from the routine area.
- Total programme costs were highest for the standard farm programme with a saving of £231.54 /ha in the managed trial programme. Costs for biofungicides and biostimulants were the same for both programmes. Managing the fungicides for SPM and Botrytis control in the trial tunnel resulted in a reduction of five fungicide applications, all at the start of the season, with a reduction of £215.09/ha in costs.
- Overall using the simple decision based system for determining treatments for powdery mildew and rots in the trial did result in reduced fungicide inputs but the opportunities for saving fungicide inputs in this trial were limited by the moderate –high risk conditions for SPM and Botrytis and the lack of experience with the new cultivar Malling Champion

References

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

Strawberry powdery mildew research at University of Hertfordshire (UoH) (Objective 2)

The following posters from the University of Hertfordshire presented at the 5th Biostimulants World Congress, Barcelona, November 2019 are included below:

1. Hall, A.M., Liu, B., Asiana, C. & Wileman, H. 2019. An overview of the use of a bioavailable silicon nutrient in sustainable strawberry production.
2. Liu, B., Hall, A.M., Asiana, C. & Jiu, X. 2019. Silicon enhances the constitutive defence pathway in strawberries against strawberry powdery mildew and two-spotted spider mites.
3. Asiana, C., Wileman, H. & Hall, A.M. 2019. What are the benefits of using silicon as a nutrient for strawberry growth.

An Overview of the use of a Bioavailable Silicon Nutrient in Sustainable Strawberry Production

Dr Avice M Hall, Dr Bo Liu, Carmilla Asiana & Hannah Wileman

Introduction

The UK now produces 80% of the strawberries bought in the UK. In the last 25 years, yield has gone from 41,600 tonnes to 115,500 tonnes, but the area utilised has remained around 4,500ha. This has been achieved by intensification through use of polythene tunnels, new cultivars, the optimisation of nutrients in fertigation and managed use of pesticides. However, Strawberry Powdery Mildew (caused by *Podpspheara aphanis*) is the biggest threat to strawberry production under protection in the UK and wherever strawberries are produced under protection worldwide.

Silicon is not considered an essential plant nutrient. Along with most dicotyledons, strawberries are not considered to be silicon accumulators, however like all plants silicon is an integral part of the trichomes (leaf hairs).

Work at the University of Hertfordshire over 10 years, both in the glasshouse and on commercial farms shows that the use of a bioavailable silicon nutrient has a multifaceted beneficial effect on strawberry plants.

Results

The results from the last ten years have shown:

- An increase in the length and density of leaf hairs (Fatema, 2014)
- Increase in leaf thickness and cuticle thickness (Jin, 2016) (Figures 1 & 2)
- Changes in the wax morphology and the presence of phytoliths on the leaf surface (Jin 2016) (Figure 3 & 4)
- Deposition of silicon in the cuticle, epidermis and palisade cells of the leaf and in xylem vessels (Asiana) (Figures 5 & 6)
- A delay in the start of *P. aphanis* epidemics by 8 to 12 days (Jin, 2015; Liu, 2017) (Figure 7)
- A reduction in the number of two spotted spider mites
  (See: 'Silicon enhances the constitutive defence pathway in strawberries against strawberry powdery mildew and two spotted spider mites' Liu, Biostimulants World Congress, 2019)

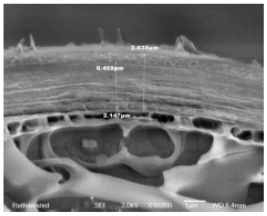


Figure 1: Untreated, SEM image of adaxial surface of leaf, measuring cuticle thickness (at x10,000) (Jin, 2016)

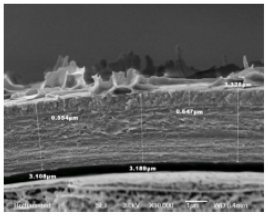


Figure 2: 0.1% Silicon (v/v) treated through roots, SEM image of adaxial surface of leaf, measuring cuticle thickness (at x10,000) (Jin, 2016)

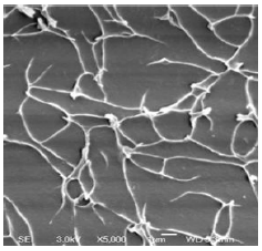


Figure 3: Untreated, SEM image of adaxial surface of leaf (at x5000) (Jin, 2016)

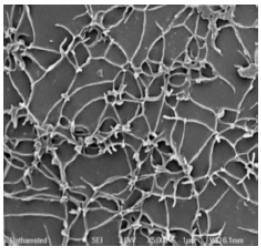


Figure 4: 0.25% Silicon treated (v/v), SEM image of adaxial surface of leaf (at x5000) (Jin, 2016)

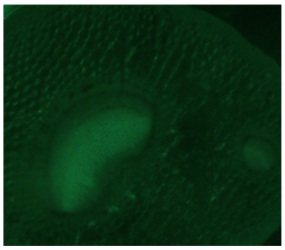


Figure 5: Silicon deposition in untreated strawberry petiole cross section, stained with lyso-tracker HCK-123 yellow dye, visualised using a LED fluorescent microscope (at x400).

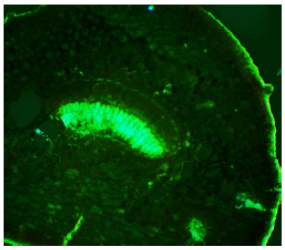


Figure 6: Silicon deposition in plant treated with 0.017% silicon (v/v) through roots. Strawberry petiole cross section, stained with lyso-tracker HCK-123 yellow dye, visualised using a LED fluorescent microscope (at x400).

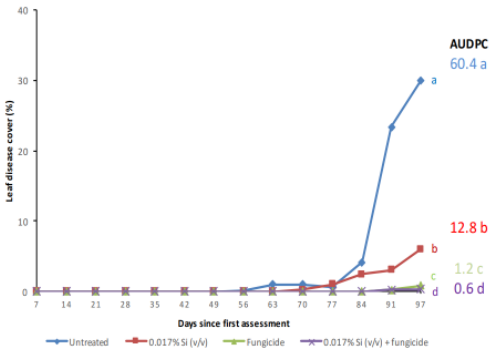


Figure 7: The progression of disease severity (AUDPC) (as measured by % of disease) for the strawberry powdery mildew (root treatments, polythene tunnel). There are four test groups, two treated with Si nutrient (root treatments) and two without. The disease severity was assessed each week. Si nutrient applied through fertigation tubes twice a week from 24 April 2013 to 31 July 2013. The first assessment was on 24 April 2013 (Jin, 2016).

Acknowledgements

Many thanks to our grower partners Henry and Harriet Duncalfe for their provision of field trials from 2004 to 2017; Gidon Bahari and Martyn Charik of Orion FT for supplying the silicon solution used and present funding and PhD students who have carried out this work Dr Kaneez Fatema and Dr Xiaolei Jin.

These results all support the hypothesis that the use of a bioavailable silicon nutrient enhances the passive (constitutive) defence pathway of strawberry.

Additional benefits have also been demonstrated, including larger plants with more leaves, enhanced chlorophyll, earlier flowering, and higher Brix° levels in fruit.
(See: 'What are the benefits of using silicon as a nutrient for strawberry growth?', Asiana and Wileman, Biostimulants World Congress, 2019)

Discussion

Overall the work reported here shows that bioavailable silicon nutrient can make a valuable contribution to sustainable strawberry production by enhancing the passive defence pathway, giving measurable protection against pests and diseases, thus reducing pesticide usage. Furthermore, the stimulatory effect on plant growth, and Brix° is of great benefit to growers in a tight market. Finally, though silicon is not considered an essential element for plant growth these results suggest that it is indeed 'quasi essential'.

References

Asiana, C. & Wileman, H. (2019) What are the benefits of using silicon as a nutrient for strawberry growth?'. Poster. Biostimulants World Congress.

Asiana, C. Thesis: Unpublished

Fatema, K. (2014). The Effect of Silicon on Strawberry Plants and its Role in Reducing Infection by *Podosphaera aphanis*. Thesis. Hatfield: University of Hertfordshire.

Jin, X. (2016). Epidemiology and control of powdery mildew (*Podosphaera aphanis*) on strawberry. Thesis. Hatfield: University of Hertfordshire.

Liu B (2017) Thesis. Hatfield: University of Hertfordshire Sustainable strawberry production and management including control of strawberry powdery mildew.

Liu B. (2019) Silicon enhances the constitutive defence pathway in strawberries against strawberry powdery mildew and two-spotted spider mites. Poster: Biostimulants World Congress.

Silicon enhances the constitutive defence pathway in strawberries against strawberry powdery mildew and two-spotted spider mites

Bo Liu[a], Avice M Hall[a], Carmilia Asiana[a], Xiaolei Jin[b]

a. School of Life and Medical Sciences, University of Hertfordshire, College Lane, Hatfield, UK AL10 9AB. Tel: +44 (0)1707 284339; email: b.liu7@herts.ac.uk

b. Department of Biological Sciences, National Sun Yat-Sen University, Kaohsiung, 80424, Taiwan

Introduction

Strawberry powdery mildew (*Podosphaera aphanis*) is a major fungal disease affecting strawberry production worldwide and can result in great yield losses. Work at University of Hertfordshire has shown that the use of a silicon (Si) nutrient 'Sirius®' (OrionFT) contributed to enhanced constitutive (passive) defence pathway (i.e. morphological changes in the leaf structure) in strawberry plants. The work reported here assessed the use of this silicon nutrient applied via the fertigation tubes in contributing to delayed *P. aphanis* epidemic build-up and reduced number of two-spotted spider mites (*Tetranychus urticae*) in commercial strawberry tunnels, as a result of such enhanced passive defence pathway.

Aims

To investigate the effects of the silicon nutrient in enhancing the constitutive defence pathway in strawberries, and how did these changes contributed to reduced severity of *P. aphanis* and *T. urticae* on strawberry leaves.

Materials and Methods

The experiments were carried out in polythene tunnels at a commercial strawberry farm in Wisbech, UK in 2014 and 2015. In the 2014 experiment, the silicon nutrient was applied once a week at a concentration of 0.017% (volume/volume) in the fertigation system. Four treatments were undertaken, which were: untreated control, commercial fungicide only, 0.017% Si only and 0.017% Si plus commercial fungicide. 75 leaf samples (15 leaves x 5 replicates) were taken per treatment fortnightly for leaf mycelium and spider mite assessment. In the 2015 experiment, two more treatments (0.017% Si double dosage only and 0.017% Si double dosage plus commercial fungicide) were added based on the 2014 treatments. Sampling and assessment methods were consistent with the 2014 experiment. Strawberry crops received commercial fungicide applications according to the farm normal spray programme. Area Under the Disease Progress Curve (AUDPC) was calculated for each treatment.

Fig. Strawberry powdery mildew infected leaf (a) peduncles (b) and fruit (c)

Results

The strawberry plants that received silicon were significantly less infected by *P. aphanis* ($P<0.05$) for the period of 2014-2015 (Fig.1A(2014) & Fig.1B(2015)). 0.017% Silicon nutrient plus commercial fungicide treatment had the smallest disease score (AUDPC=63 in 2014, and 53 in 2015) among all treatments in both years. Crops from the 0.017% Silicon nutrient alone treatment also showed a smaller disease severity (AUDPC=475 in 2014 and 267 in 2015) than untreated control (AUDPC=662 in 2014 and 281 in 2015). Plants showed a delayed epidemic build-up for approximately two weeks compared to untreated control in 2014 (Fig.1A).

Plants from silicon treatments were also less infested by *T. urticae* ($P<0.001$) for the period of 2014-2015 (Fig.2A(2014) & Fig.2B(2015)). In 2014, two silicon treatments had smaller numbers of *T. urticae* per strawberry leaf than untreated control. Similarly, in the 2015 experiment, 0.017% silicon nutrient only and 0.017% silicon nutrient plus commercial fungicide treatments had an average of 2 *T. urticae* per leaf compared to 9 in the untreated control.

Silicon treated plants had thicker leaf cuticle (Fig 3A&B) and denser layer of leaf wax (Fig 3C&D), and silicon was mainly deposited in leaf epidermis and palisade layers (Fig. 4B).

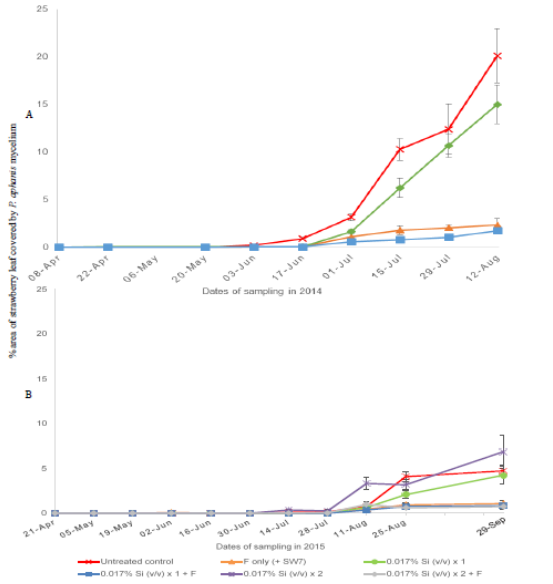


Fig. 1 % area of strawberry leaf covered by *P. aphanis* mycelium from 2014 (A) and 2015 (B) Si fertigation trials. Treatments were: untreated control (2014 & 2015), commercial fungicide only (2014 & 2015), 0.017% Silicon only (2014 & 2015), 0.017% Silicon plus commercial fungicide (2014 & 2015), 0.017% Silicon double dosage only (2015), 0.017% Silicon double dosage plus commercial fungicide (2015).

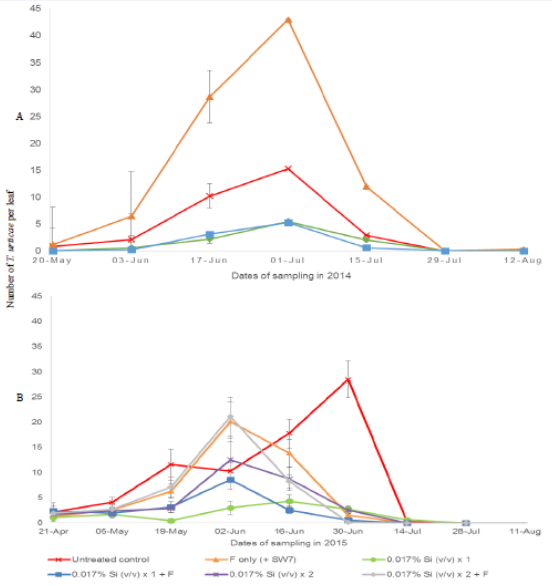


Fig. 2 Number of *T. urticae* per strawberry leaf from 2014 (A) and 2015 (B) Si fertigation trials. Treatments were: untreated control (2014 & 2015), commercial fungicide only (2014 & 2015), 0.017% Silicon only (2014 & 2015), 0.017% Silicon plus commercial fungicide (2014 & 2015), 0.017% Silicon double dosage only (2015), 0.017% Silicon double dosage plus commercial fungicide (2015).

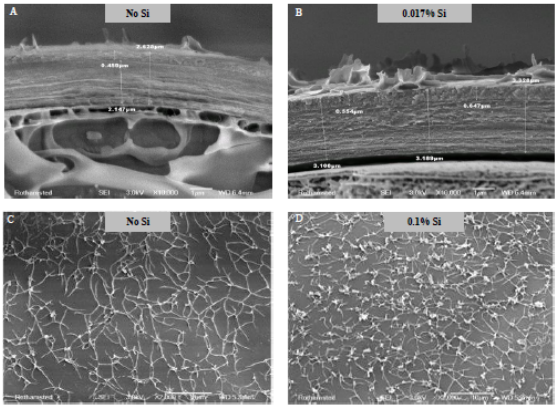


Fig. 3 Scanning Electron Microscope images taken at Rothamsted Research (UK) of strawberry leaf cuticle without silicon application (A) and with 0.017% silicon root application (B); Wax formation on strawberry leaves without silicon (C) and with 0.1% silicon root application (D). (Jin, 2015)

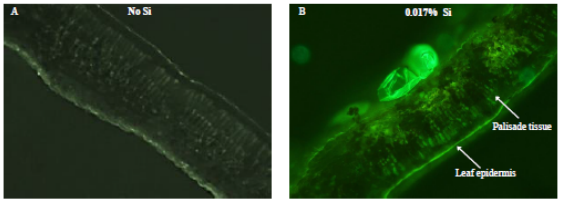


Fig. 4 Cross section of strawberry leaves from control (A) and 0.017% silicon root application (B) treatments stained with molecular tracker dye observed under the Confocal microscope. Silicon was mainly deposited (areas showing fluorescence) in leaf epidermis and palisade layers (B) (Asiana, unpublished)

Conclusion & Discussion

The results indicated that silicon nutrient enhanced the constitutive defence pathway of strawberry plants, resulted in increased leaf cuticle thickness and wax density, which contributed to the reduction of strawberry powdery mildew and two-spotted spider mites. Silicon was found mainly deposited in leaf epidermis and palisade layers, and have other beneficial effects on strawberries, e.g. increased pollen viability, increased °Brix level in the fruits etc., which all worth to be further investigated. The questions lay ahead will be find out how much silicon a plant can take in order to benefit its growth therefore to work out the most suitable application rate for commercial growing.

Acknowledgement

Thanks to Harriet & Henry Duncalfe at Maltmas Farm for providing the field trial. Thanks to Gidon Bahiri and OrionFT Ltd for providing Sirius® and for their support to this work.

What are the benefits of using silicon as a nutrient for strawberry growth?

University of Hertfordshire UH

Carmilla Asiana, Hannah Wileman & Dr Avice M Hall

University of Hertfordshire
College Lane, Hatfield,
Hertfordshire, AL109AB, UK.
(+44)1707 284539
h.wileman3@herts.ac.uk

Introduction

- Strawberries produced in substrate (e.g. coir) are grown in the presence of very low levels or no bioavailable silicon (Liu, 2017)
- Silicon has been shown to reduce strawberry powdery mildew epidemics, caused by *P. aphanis,* in consecutive field trials (2013-2016) (Liu, 2019)
- Reduction in disease levels may be a result of deposition of silicon within leaves and petioles, which enhances the passive defence pathway
- Deposition of silicon occurs in the cuticle, epidermis, palisade layers and stomata of the leaves (Figure 1); xylem and epidermis of the petioles and xylem of the roots
- Silicon enhances the passive defence pathway by modifying the wax and cuticle thickness of leaves (Jin 2016)

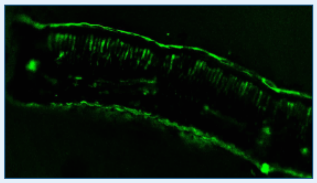


Figure 1: Silicon deposition in a strawberry leaf cross section, stained with lyso-tracker HCK-123 yellow dye, visualised using a LED fluorescent microscope.

Aim: To evaluate additional benefits of the use of silicon and to determine any deficiency and toxicity effects of silicon in strawberry growth.

Deficiency Experiment

There were no classic deficiency symptoms observed in untreated plants, however the plants were smaller compared to silicon treated plants. The wet biomass of the untreated plants was significantly lower than the silicon treated plants ($p<0.05$).

Results in Table 1 found that there were significantly fewer leaves and chlorophyll content ($P<0.05$) and significantly less fruits and higher Brix° levels in the fruit ($P<0.05$), in untreated plants, compared to silicon treated plants. Flowering was a week later in untreated plants compared to silicon treated plants. Data was analysed using ANOVA, regression statistics and the dependant "paired" t test.

Table 1: Results from hydroponic deficiency experiment (January-June 2018)

	Untreated Control	Silicon rate: 0.017%
Average number of leaves at end of treatment	20	29
Average chlorophyll content of leaves (throughout experimental period)	665.1µmol/m²	813.5µmol/m²
Initial flowering date	22nd May 2018	15th May 2018
Total number of fruits counted during fruiting period	8	16
Average Brix° content of fruit	9	17
Total number of runners at end of treatment	24	37

Discussion

Whilst the results from the hydroponic deficiency experiment showed no classic deficiency symptoms, the number of leaves, runners, fruits and chlorophyll content of untreated plants were significantly lower than the silicon treated plants. Therefore, using a silicon treatment "Sirius" at a rate of 0.017% (normal field rate), significantly increases productivity.

However, in the toxicity experiment, the silicon treatment at the rate of 1.7% greatly reduced productivity; this may be due to the increased concentration of silicon within the growing system. The level of silicon measured in the 1.7% silicon treatment is above maximum level (22mg/L) recommended for water sources in strawberry irrigation (AHDB, 2011). The silicon content in 0.017% and 0.17% silicon treatments are both below 22mg/L. The pH measured (7.5) was also higher than the recommended pH of 6-6.5. The rate of 0.17% was not toxic to the plants, instead increased productivity, however this was not significantly different to the untreated plants.

The results suggest that though silicon is not an essential element it is probably a limiting factor in strawberry productivity, but at very high levels can be toxic. It is therefore recommended that growers use silicon at a rate of 0.017% throughout the growing season, particularly when growing in coir.

Methods

Two glasshouse hydroponic experiments were set up in 2018 & 2019, in 5L plastic containers with aeration pumps providing an air supply (Figure 2). These contained Hoagland's solution, comprised of deionised water, macronutrients and micronutrients essential for plant growth (no silicon) (Jones, 2016). Plastic was used throughout the experiments to eliminate silicon. Bare root Malling Centenary™ strawberry crops were planted for both experiments.

Deficiency experiment: this contained two treatments,

1. Silicon treatment: 50 ml treatment of "Sirius" (a commercial bioavailable form of silicon) at 0.017% (v/v) - applied weekly
2. Untreated control: 50ml treatment of deionised water - applied weekly

Toxicity experiment: this contained four treatments,

1. Silicon treatment: 50ml treatment of "Sirius" at 0.017% (v/v) (normal field rate) - applied weekly
2. Silicon treatment: 50ml treatment of "Sirius" at 0.17% (v/v) - applied weekly
3. Silicon treatment: 50ml treatment of "Sirius" at 1.7% (v/v) - applied weekly
4. Untreated control: 50ml treatment of deionised water - applied weekly

Figure 2: Hydroponic tubs containing strawberry plants in Hoagland's solution on glasshouse bench four weeks after planting (2018).

Results

Toxicity Experiment

The silicon treatments of 0.017% and 0.17% showed no detrimental effects to the plants treated, however, the silicon treatment of 1.7% gave toxicity symptoms and caused plant death. As shown in Table 2, the 1.7% silicon treatment reduced leaf number and plant biomass. The silicon treatment of 1.7% caused a reduction in the total number of fruit, average weight and size of fruit (Table 2).

The number of leaves in silicon treatment of 1.7% is significantly reduced compared to untreated, 0.017% and 0.17% silicon treated ($p<0.05$), by two-way ANOVA test.

Table 2: Results from hydroponic toxicity experiment (January-June 2019)

	Untreated Control	Silicon rate: 0.017%	Silicon rate: 0.17%	Silicon rate: 1.7%
Average number of leaves at end of treatment	15	15	18	7
Average Total dry biomass (g)	28.42	23.90	26.67	9.06
Average Total number of fruits	55	75	73	25
Average weight of fruit (g)	13.97	13.46	13.71	7.98
Average size of fruit (LxW) (mm)	27.42 x 28.6	27.37 x 29.5	29.3 x 30.0	22.7 x 23.2

The pH and EC of all hydroponic tubs was measured each week, the optimal pH for strawberry growth is 6-6.5 and the EC is 1.8-2.0. The pH gradually increased in the 1.7% silicon treatment through the duration of the experiment, from 6.42 to 8.59. In week 10 (4th April 2019) some of the 1.7% silicon treated plants started to show deterioration, the pH was 7.5 and the concentration of silicon in the tubs was 33.28mg/L.

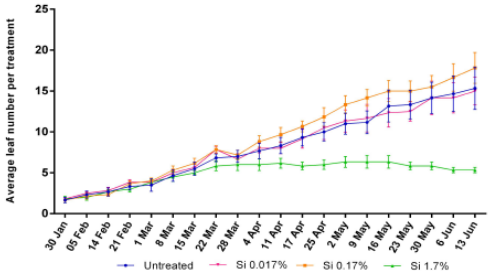


Figure 2: Average leaf number each week for duration of experimental period (January- June 2019)

References

AHDB. (2011). *Strawberry analysis chart- optimum ranges*. Poster. Available: https://horticulture.ahdb.org.uk/publication/strawberry-analysis-chart-optimum-ranges

Jin, X. (2016). *Epidemiology and control of powdery mildew (Podosphaera aphanis) on strawberry.* Thesis. Hatfield: University of Hertfordshire.

Jones Jr, J. B. (2016). *Hydroponics: a practical guide for the soilless grower*. CRC press.

Liu B (2017) Thesis. Hatfield: University of Hertfordshire *Sustainable strawberry production and management including control of strawberry powdery mildew.*

Liu B. (2019) *Silicon enhances the constitutive defence pathway in strawberries against strawberry powdery mildew and two-spotted spider mites*. Poster. Biostimulants World Congress.

Acknowledgments

Many thanks to Gidon Bahiri and Martyn Charik of Orion FT for providing "Sirius" for silicon experiments in both 2018 and 2019. Also, thanks to Dr Avice Hall and Dr Bo Liu for their contributions to this work. Thanks to both the chemistry and microbiological technical staff of University of Hertfordshire Science Building and technical staff based at Bayfordbury field station.

Objective 3: Fruit rot complex

Molecular screen of *Pestalotiopsis* spp.

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

Before we embarked on developing diagnostic tools for the new pathogens, we needed to prove that they are pathogenic against popular commercial strawberry cultivars and hence can be a primary pathogen. We reported that several *Pestalotiopsis* isolates can cause disease symptoms on detached leaves and fruit inoculated with either spore suspension or mycelial plugs, but failed to produce symptoms *in vivo* tests on whole strawberry plants and attached fruit.

In the survey for *Phytophthora* spp. in year 1 and 2 (SF 157), we observed typical crown rot symptoms in a number of samples but molecular testing failed to detect *P. cactorum*. These symptoms could be due to frost damage or infection by other pathogens, as such further work is needed to assess the importance of *Pestalotiopsis* spp. in the UK, and DNA extracted from crown tissues sampled in the Years 1-2 provided a great opportunity to maximise the value for AHDB funding. Thus, we tested for presence of *Pestalotiopsis* spp. in those samples used for testing *P. cactorum* in the Years 1-2,

Materials and Methods

DNA extracted from the following Year 1-2 crown samples was included for molecular screening of *Pestalotiopsis* spp.: (1) all samples with crown browning (discolouring), including those samples tested positive for *P. cactorum* as reported in previous years; and (2) ten random samples with apparently healthy crowns. As reported previously (Annual Report year 3), DNA extracted from crown tissues was run in a PCR with FaEF primers (Table 3.1) as a control for strawberry DNA to indicate whether DNA extraction was successful. *Pestalotiopisis*

primers (Table 3.1) were designed in house at NIAB EMR (as reported in Year 3). In an attempt to increase the detection of the pathogen within strawberry material, *Pestalotiopisis* was tested for in a nested PCR using the in-house designed Pesta primer set in the first PCR and then again in the second PCR with 1/10 dilutions of the amplicons from the 1st Pesta PCR.

All PCRs were performed with 2 µl of DNA (Ca. 1-4 ng/µl in PCRs with FaEF and Pesta 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. FaEF 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. Pesta 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, 49°C for 30 s and 72°C for 60 s, followed by a final extension at 72°C for 5 min. Two isolates were used as a positive control: R17/17 isolated from pear in 2017 and PC26/16 isolated from strawberry in 2016. PCR products were run by gel electrophoresis on a 1.5% agarose gel with Gel Red at 100V for 60 mins alongside a 1KB+ ladder and viewed under UV light on a GelDoc XR+ (Bio-Rad, California, USA).

Table 3.1. Sequences (5'-3') for primer pairs used to screen strawberry runners

Primer set	Target	Forward primer	Reverse primer
Pesta	*Pestalotiopsis*	CTTACCTTTTGTTGCCTCGG	TCTTGGTTCAAGAACGCAGC
FaEF	*Fragaria*	TGGATTTGAGGGTGACAACATGA	GTATACATCCTGAAGTGGTAGACGGAGG

Results

A total of 182 samples were screened for presence of *Pestalotiopsis* from the 1500 samples collected from growers in Years 1 and 2. Out of the 182 samples, 136 DNA samples were amplified with FaEF primers, indicating successful DNA extraction from crown material. Only one sample (504 – sampled in 2016) showed a positive band for presence of *Pestalotiopsis* (Fig. 3.1). It should be noted that the amplicon size for the positive controls was different for the two strains from pear and apple, indicating that the isolates are likely to be different *Pestalotiopsis* species.

Conclusions

Molecular screening of 136 strawberry crown samples only showed one positive result for *Pestalotiopsis* presence. Combined with the results from year 3, we may conclude that *Pestalotiopsis* spp. are not important on strawberry in the UK at the current time.

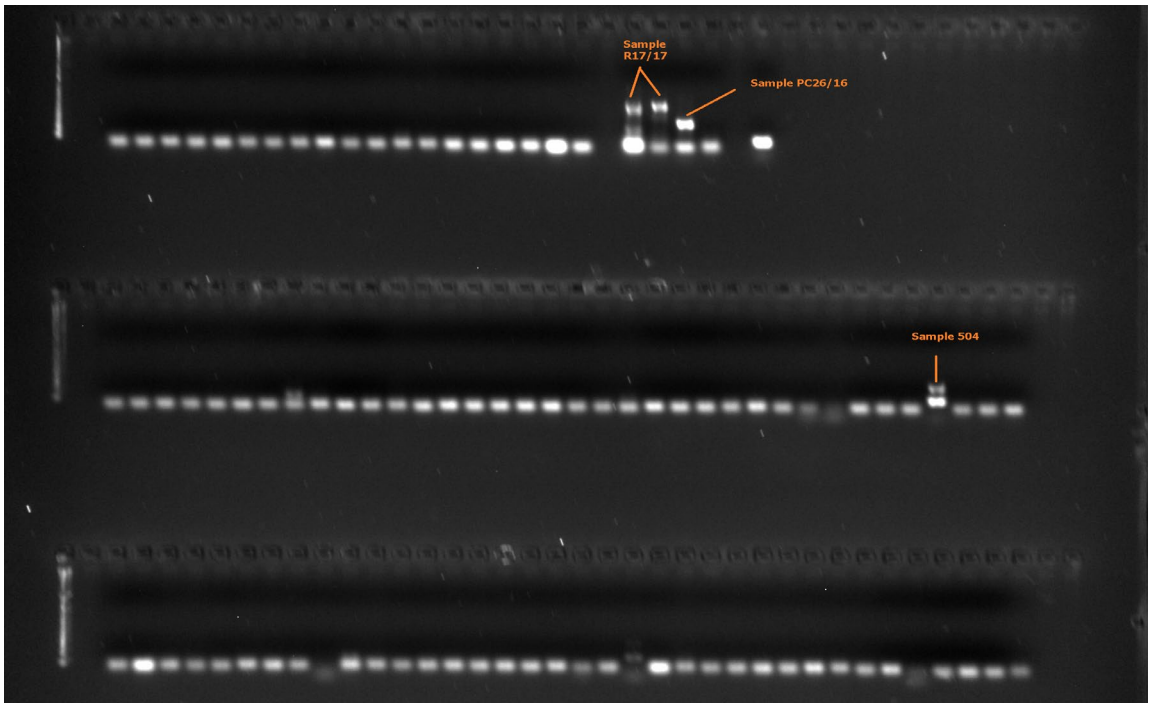


Figure 3.1. The image of gel electrophoresis of PCR products of DNA extracted from strawberry crown tissues with the primers specifically designed for detecting *Pestalotiopisis* spp. There were two positive controls: R17/17 from pear and PC26/16 from strawberry. The image is composes of results from three separate gels.

Survival of biocontrol microbes on strawberry flowers

Currently, bio-pesticides are usually applied as if they were conventional pesticides without considering their survival and dispersal under natural conditions. Understanding their survival and potential spread in commercial crops is critically important for timing bio-pesticide applications. In Year 4, we studied the survival of two commercial biocontrol agents in strawberry flowers.

Materials and Methods

The same strawberry planting used for evaluating SPM/ Botrytis management programmes at NIAB EMR (section 2.2) was used for studying the survival of two biocontrol agents after the system evaluation trial terminated in late September.

Pure cultures of the *B. subtilis* strain QST 713 (isolated from the commercial Serenade product) and the commercial product Prestop (*G. catenulatum*) were applied with a hand-held sprayer to individual flowers on three occasions (26 and 28 September, and 1 October). The commercial product Serenade was not used directly because the commercial formulation somehow interferes with the PMQ-qPCR process, resulting in inaccurate estimates of viable population sizes. This PMA-qPCR technique for both biocontrol organisms was developed by

Gurkan Tut, a PhD student (supervised by Xiangming Xu at NIAB EMR) working on an AHDB funded PhD project (CP 140: Optimising the use of biocontrol agents to improve the control of *Botrytis cinerea* in key vegetable and fruit crops).

For each application, each biofungicide was applied to flowers attached to 60 plants; these 60 plants were divided into 10 blocks (each with six plants) based on their spatial location inside the tunnel. To quantify the viable population size of each biocontrol strain following its application, ten flowers (one from each block) were randomly taken on six occasions: 0, 2, 4, 6, 8 and 10 days after applications. For the "0" day sample, flowers were taken immediately after application. The ten flowers were then pooled together as one composite sample for use in the PMA-based qPCR method to estimate viable population sizes.

Results

Gurkan Tut, a PhD student funded by AHDB (who finished study in Oct 2019), quantified the number of viable BCA propagules following their application. The quantification was done with the PMA-based qPCR method developed by Gurkan. Figure 3.2 show the overall results on the number of viable propagules for both BCAs on the surface of strawberry flowers over time. *Bacillus subtilis* (Serenade) appears to be able to survive much better than *Gliocladium catenulatum* (Prestop). The viable population size of *B. subtilis* did not decrease much within 8 days after application. In contrast, the viable population size of *G. catenulatum* decreased sharply four days after application ($P < 0.05$, Fig. 3.2).

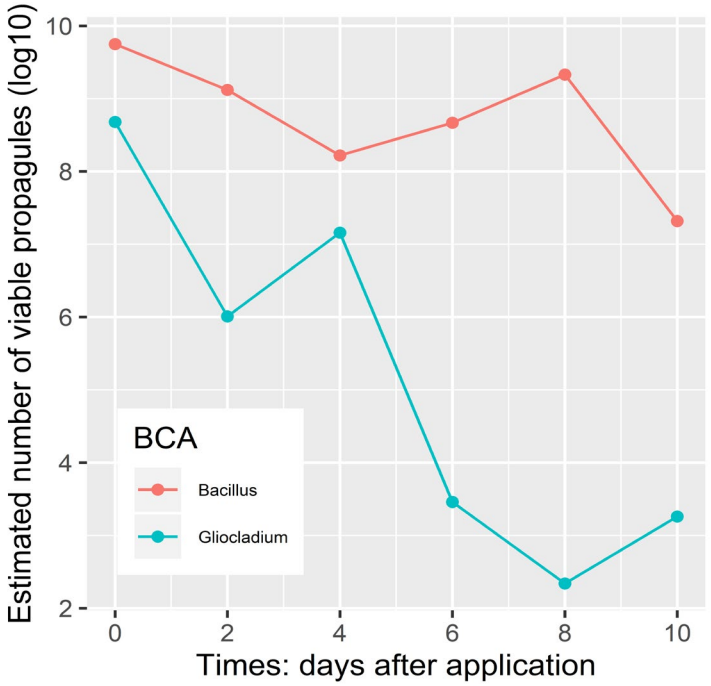


Figure 3.2. The estimated number of viable BCA propagules on the surface of 10 strawberry flowers following their application.

Discussion and conclusions

The present results suggested that a large proportion of *B. subtilis* propagules (> 10^7 propagules for flowers) can survive on the flower surface for at least 10 days in the autumn

under protection. Whereas *G. catenulatum* suffers much greater mortality after 4 days. The number of biocontrol propagules required for effective control of *B. cinerea* was shown to be 10^6 propagules for both *B. subtilis* and *G. catenulatum* (G. Tut, PhD thesis). Thus, we may conclude that the density of viable propagules (required for *B. cinerea* control) should be sufficient for 10 and 4 days after application for *B. subtilis* and *G. catenulatum*, respectively, in the autumn under UK conditions. Further research is needed to confirm these findings, particularly over the entire period when these biocontrol products are applied.

The survival of a sufficient number of microbial propagules is one of many necessary requirements for achieving satisfactory biocontrol. Another key requirement is to possess the ability to be easily dispersed to new host tissues. This is particularly important for protecting fungal infection of flowers, since new flowers are continuously produced and open. Unfortunately, recent research at NIAB EMR suggested that very little dispersal of *B. subtilis* occurred among flowers (Wei *et al.*, 2016) under protected conditions. Thus, despite the fact *B. subtilis* may survive for 10 days following application, the current 7-10 day application may be insufficient to protect newly opened flowers due to the limited microbial dispersal under protection. Thus, timing the applications at those periods with severe disease forecast risks is critically important. Currently we have no knowledge about the dispersal characteristics of *G. catenulatum* under protection. Further research is needed.

We thus conclude: (1) *Pestalotiopsis* spp. appear not to be important on strawberry in the UK; and (2) under protected conditions in the autumn, the density of viable population should be sufficient for 10 and 4 days within application for *B. subtilis* and *G. catenulatum*, respectively.

References cited

Chamorro M, Aguado A, 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.

Embaby EM, 2007. Pestalotia Fruit Rot on Strawberry Plants in Egypt. *Egypt. J. Phytopathol.* **35**, 99–110.

Rodrigues FA, Silva IT, Antunes Cruz MF, Carré-Missio V, 2014. The infection process of Pestalotiopsis Longisetula leaf spot on strawberry leaves. *Journal of Phytopathology* **162**, 690–692.

Feng Wei, Xiaoping Hu, X-M. Xu (2016) Dispersal of *Bacillus subtilis* and its effect on strawberry phyllosphere microbiota under open field and protection conditions. *Scientific Report* **6,** Article number: 22611 (2016) doi:10.1038/srep22611

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

The cause of strawberry wilt, *Verticillium dahliae*, can persist as micro-sclerotia in soil for around 10 years and can infect crowns via roots to reduce yields by 75% through the death of some or all plant crowns and reduced water movement into the fruit. Soil sampling followed by Harris tests is used to determine the severity of soil infestation and varieties can be selected that will tolerate low levels, but soil fumigation pre-planting is often necessary (AHDB Factsheet 16/06). However, methyl bromide is no longer authorised, and treatment with chloropicrin has recently only been possible using annual Emergency Authorisation. *V. dahliae* is also a pathogen of potatoes, linseed, peas and some weeds and, as long crop rotations are rarely feasible, alternative methods for reducing soil-borne pathogens are urgently needed.

A field experiment to evaluate the effects of individual and combined use of alternative products against Verticillium wilt of strawberry was completed in 2018. Details of the work are included in previous SF 157 annual reports and a summary is included in the Grower Summary of this report.

Knowledge and Technology Transfer

- 11th September AHDB Agronomists Day, training participants on powdery mildew identification
- 20th November 2019, two presentations were given on the AHDB Soft Fruit Day
    - one on the management of Phytophthora infection in planting material
    - the other on the management of strawberry powdery mildew and Botrytis
- 4 December – Discussion with David Thomson, agronomist with BGG on powdery mildew management
- On 25th Feb 2020, Avice gave a paper at Crop Protection in Northern Britain on the field trials in Scotland of the SPM prediction system
- 10 March – Talk to BGG agronomists on powdery mildew management.

Appendices

Appendix 1. % Botrytis rot at harvest in 19 picks from everbearer strawberries following treatment with 4 different programmes compared to an untreated control at NIAB EMR in 2019.

Treatment	Pick date / % *Botrytis*																		
	9 July	11 July	16 July	19 July	23 July	26 July	6 Aug	9 Aug	13 Aug	16 Aug	20 Aug	23 Aug	27 Aug	30 Aug	3 Sep	6 Sep	10 Sep	13 Sep	17 Sep	Overall Mean
T1: Untreated	1.3	0	0	0	0	0	1.0	0	0.2	0	0	0	0	0.2	0	0	0	0.2	0	0.15
T2: Routine fungicide	0	0.6	0	0	1.5	0	1.0	0	0.2	0.3	0	0	0	0	0.1	0	0	0	0.1	0.2
T3: SPM + Botrytis managed	1.3	0	0	0	0	3.1	0	0.5	0	0.3	0.2	0	0	0	0	0	0.1	0	0.1	0.3
T4: SPM managed No Botrytis sprays	0	0	0	0	0	0	0	0	0	0.5	0	0.2	0	0	0.1	0	0	0	0	0.04
F Prob	0.101	0.436	-	-	0.436	0.436	0.631	0.436	0.629	0.767	0.436	0.436	-	0.436	0.436	-	0.436	0.436	0.631	0.276
SED (12)																				
LSD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS = Not significant

Appendix 2. % Mildew on fruit at harvest in 19 picks from everbearer strawberries following treatment with 4 different programmes compared to an untreated control at NIAB EMR in 2019 (figures in brackets are original data where data has been angular transformed for statistical analysis)

Treatment	Pick date / % Mildew on fruit																		
	9 July	11 July	16 July	19 July	23 July	26 July	6 Aug	9 Aug	13 Aug	16 Aug	20 Aug	23 Aug	27 Aug	30 Aug	3 Sep	6 Sep	10 Sep	13 Sep	17 Sep	Overall Mean
T1: Untreated	0	0	0	0	0	0	0	0	1.0	1.0	2.2	4.3	6.6	4.8	9.7	9.0	7.2 (10.1) a	7.6 (10.4)	12.0 (13.8) a	1.12 (3.8) a
T2: Routine fungicide	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0 b	0.3 (0.6)	0.2 (0.4) b	0.006 (0.09) b
T3: SPM + Botrytis managed	0	0	0	0	0	0	0	0	0.2	0	0	0.5	0.1	0	0.6	1.2	0.04 (0.2) ab	0.1 (0.5)	0 b	0.017 (0.17) b
T4: SPM managed No Botrytis sprays	0	0	0	0	0	0	0	0	0	0.2	0	0	0	0	0	0.7	0 b	0	0 b	0.001 (0.05) b
F Prob	-	-	-	-	-	-	-	-	0.436	0.280	0.436	0.128	0.223	0.289	0.090	0.080	0.024	0.050	0.001	0.055
SED (9)																	0.685	0.827	0.445	0.014
LSD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	3.47	4.18	2.27	0.054

NS = Not significant

Appendix 3. % Total rot at harvest in 19 picks from everbearer strawberries following treatment with 4 different programmes compared to an untreated control at NIAB EMR in 2019.

Treatment	Pick date / % Total rot																			
	9 July	11 July	16 July	19 July	23 July	26 July	6 Aug	9 Aug	13 Aug	16 Aug	20 Aug	23 Aug	27 Aug	30 Aug	3 Sep	6 Sep	10 Sep	13 Sep	17 Sep	Overall Mean
T1: Untreated	1.3	0	0	0	0	0	1.6	0.4	0.2	0	0	0	0	0.6	0	0	0	0.2	0	0.23
T2: Routine fungicide	0.6	0.6	0.5	0	1.5	0	1.0	0	0.2	1.3	0	0	0	0	0.1	0	0	0	0.1	0.31
T3: SPM + Botrytis managed	1.3	0	0	0	0	3.1	0.8	0.5	0	0.7	0.2	0	0	0.1	0	0	0.1	0.1	0.1	0.37
T4: SPM managed No Botrytis sprays	2.8	0	0	0	0	0	0	0	0	0.8	0	0.2	0.2	0	0.1	0	0	0	0	0.20
F Prob	0.647	0.436	0.436	-	0.436	0.436	0.601	0.631	0.629	0.292	0.436	0.436	0.436	0.234	0.436	-	0.436	0.630	0.631	0.712
SED (9)																				
LSD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS = Not significant

Appendix 4. % Botrytis rot in post-harvest tests in 19 picks from everbearer strawberries following treatment with four different programmes compared to an untreated control at NIAB EMR in 2019 (figures in brackets are original data where data has been angular transformed for statistical analysis).

Treatment	Pick date / % *Botrytis*																			
	9 July	**11 July**	**16 July**	**19 July**	**23 July**	**26 July**	**6 Aug**	**9 Aug**	**13 Aug**	**16 Aug**	**20 Aug**	**23 Aug**	**27 Aug**	**30 Aug**	**3 Sep**	**6 Sep**	**10 Sep**	**13 Sep**	**17 Sep**	**Overall Mean**
T1: Untreated	1.6	4.9	0	0	7.6	6.3	19.8	11.8	2.5	2.5	27 2 (27.5) ab	1.5	2.5	5.5	11.5	17.0	21.0	4.8 (6.5) ab	16.0	8.7
T2: Routine fungicide	0	3.3	3.8	1.2	0	0	14.1	15.4	3.5	6.5	32.1 (32.5) a	6.0	5.0	11.0	13.5	5.0	18.5	10.9 (11.0) a	12.0	8.5
T3: SPM + Botrytis managed	0	2.3	0.5	0	4.6	3.1	13.5	21.6	1.5	12.0	15.1 (15.5) b	0	5.5	12.0	18.0	8.0	11.0	1.5 (2.0) b	6.0	7.2
T4: SPM managed No Botrytis sprays	0	4.0	7.6	2.2	1.8	0	11.2	9.4	1.6	17.5	37.7 (38.5) a	2.5	5.5	14.0	20.5	9.5	22.5	6.3 (8.5) ab	13.0	10.0
F Prob	0.436	0.907	0.436	0.622	0.164	0.436	0.576	0.537	0.842	0.268	0.051	0.625	0.574	0.276	0.664	0.131	0.460	0.084	0.427	0.637
SED (12)											0.661							0.515		
LSD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	3.35	NS	NS	NS	NS	NS	NS	2.62	NS	NS

NS = Not significant

Appendix 5. % Penicillium rot in post-harvest tests in 19 picks from everbearer strawberries following treatment with four different programmes compared to an untreated control at NIAB EMR in 2019 (figures in brackets are original data where data has been angular transformed for statistical analysis).

Treatment	Pick date / % Penicillium																		
	9 July	11 July	16 July	19 July	23 July	26 July	6 Aug	9 Aug	13 Aug	16 Aug	20 Aug	23 Aug	27 Aug	30 Aug	3 Sep	6 Sep	10 Sep	13 Sep	17 Sep	Overall Mean
T1: Untreated	33.1	20.2	19.4	12.5	1.3	5.4	7.8	7.4	3.0	11.0	9.0	1.0	1.0	2.2 (3.0) c	8.0	4.0	2.5	5.5	7.5	8.6
T2: Routine fungicide	26.1	17.7	19.2	4.4	7.4	0	6.6	9.8	3.5	17.0	7.5	0.5	1.0	6.4 (7.0) ab	10.5	4.5	4.5	4.0	2.5	8.1
T3: SPM + Botrytis managed	37.9	15.1	24.0	6.2	14.9	6.3	5.4	12.2	2.5	10.5	12.5	0	1.5	8.3 (9.0) a	11.0	9.0	5.5	1.0	5.5	10.0
T4: SPM managed No Botrytis sprays	38.1	18.3	28.6	3.4	1.8	3.6	7.7	4.5	4.0	14.0	11.0	0.5	1.0	3.7 (4.0) bc	7.0	2.5	4.5	3.5	5.0	8.6
F Prob	0.774	0.800	0.441	0.157	0.397	0.345	0.754	0.165	0.842	0.512	0.712	0.825	0.994	0.007	0.662	0.116	0.708	0.215	0.347	0.757
SED (12)														0.107						
LSD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.544	NS	NS	NS	NS	NS	NS

NS = Not significant

Appendix 6. % Mucor / Rhizopus rot (angular transformed) in post-harvest tests in 19 picks from everbearer strawberries following treatment with four different programmes compared to an untreated control at NIAB EMR in 2019 (figures in brackets are original data where data has been angular transformed for statistical analysis).

Treatment	Pick date / % Mucor / Rhizopus																		
	9 July	11 July	16 July	19 July	23 July	26 July	6 Aug	9 Aug	13 Aug	16 Aug	20 Aug	23 Aug	27 Aug	30 Aug	3 Sep	6 Sep	10 Sep	13 Sep	17 Sep	Overall Mean
T1: Untreated	30.8	45.2	56.1	73.2	88.7	81.3	50.5	79.4	94.0	73.0	76.0	86.5	89 8 (89.0) a	70.3 (69.5) a	90.6 (90.0) a	49.0	63.0	63.5	80.0	74.7 (71.3) a
T2: Routine fungicide	17.1	36.5	35.2	38.6	83.5	90.9	44.8	86.4	93.5	62.5	61.5	72.0	68.7 (68.0) b	56.6 (56.5) ab	76.1 (74.0) ab	37.5	55.5	48.5	74.5	62.3 (59.5) b
T3: SPM + Botrytis managed	7.7	34.8	48.4	43.0	78.1	81.3	39.4	87.2	97.5	63.0	59.0	70.5	68.0 (67.0) b	48.5 (48.5) b	75.1 (74.0) ab	41.0	60.0	55.5	79.5	62.2 (59.8) b
T4: SPM managed No Botrytis sprays	19.2	37.9	42.3	56.4	93.8	71.9	56.8	91.8	96.9	62.0	68.5	66.0	71.7 (71.5) b	60.0 (60.0) ab	64.6 (63.5) b	44.0	43.0	39.0	74.5	63.6 (61.0) b
F Prob	0.116	0.822	0.312	0.309	0.886	0.901	0.276	0.726	0.921	0.679	0.316	0.268	0.057	0.074	0.092	0.532	0.352	0.117	0.930	<0.001
SED (12)													0.940	0.530	1.247					0.133
LSD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	4.747	2.695	6.272	NS	NS	NS	NS	0.514

NS = Not significant

Appendix 7. % Total rot (angular transformed) in post-harvest tests in 19 picks from everbearer strawberries following treatment with four different programmes compared to an untreated control at NIAB EMR in 2019 (figures in brackets are original data where data has been angular transformed for statistical analysis).

Treatment	Pick date / % Total rot																		
	9 July	11 July	16 July	19 July	23 July	26 July	6 Aug	9 Aug	13 Aug	16 Aug	20 Aug	23 Aug	27 Aug	30 Aug	3 Sep	6 Sep	10 Sep	13 Sep	17 Sep	Overall Mean
T1: Untreated	76.4	80.4	72.4	79.7	97.6	93.8	94.4	62.6	94.5	74.5	95.5	88.0	91.5 a	77.0	97.5	62.5	83.5	71.5	90.5	87.7 (83.4) a
T2: Routine fungicide	57.7	84.5	68.4	40.8	85.3	90.9	94.1	58.2	94.5	72.5	85.5	78.0	72.0 b	72.5	87.0	43.5	71.5	60.0	85.0	78.0 (73.8) b
T3: SPM + Botrytis managed	57.2	74.9	69.4	47.3	94.7	92.7	97.8	60.3	98.5	73.0	76.5	70.5	71.5 b	64.0	93.5	52.0	74.0	58.5	84.0	78.5 (74.2) b
T4: SPM managed No Botrytis sprays	62.2	71.1	65.8	61.7	96.9	71.9	96.6	65.6	92.3	73.5	92.0	68.0	75.0 b	73.5	82.0	55.0	65.0	49.5	84.0	77.5 (73.8) b
F Prob	0.785	0.420	0.549	0.140	0.684	0.700	0.370	0.957	0.866	0.987	0.002	0.228	0.051	0.246	0.286	0.159	0.187	0.171	0.639	0.009
SED (12)																				0.111
LSD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		NS		NS	NS	NS	NS	NS	NS	0.431

NS = Not significant

Appendix 8. % Total rot in Cool chain management tests in 11 picks from everbearer strawberries following treatment with four different programmes compared to an untreated control at NIAB EMR in 2019.

Treatment	13 Aug	16 Aug	20 Aug	23 Aug	27 Aug	30 Aug	3 Sep	6 Sep	10 Sep	13 Sep	17 Sep	Overall Mean
T1: Untreated	0	0	0	2.0	1.0	1.5	1.5	0	0	0.5	1.5	0.73
T1: Untreated	0	0	1.0	2.0	1.0	3.1	2.0	2.5	2.5	3.5	1.5	1.74
T2: Routine fungicide	0	0	0.5	2.0	1.5	0.5	1.5	1.0	0.5	2.5	1.0	0.98
T3: SPM + Botrytis managed	0	0.7	0	0.5	0.5	1.0	1.5	0.5	1.0	1.0	0.5	0.65
F Prob	-	0.436	0.194	0.817	0.411	0.796	0.935	0.392	0.321	0.539	0.670	0.605
SED (12)												
LSD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS = Not significant

Appendix 9. Total Yield from 19 picks from everbearer strawberries following treatment with four different programmes compared to an untreated control at NIAB EMR in 2019.

Treatment	Pick date / Yield kg																		
	9 July	11 July	16 July	19 July	23 July	26 July	6 Aug	9 Aug	13 Aug	16 Aug	20 Aug	23 Aug	27 Aug	30 Aug	3 Sep	6 Sep	10 Sep	13 Sep	17 Sep	Total yield
T1: Untreated	2.21	2.04	1.69	0.78	0.49	0.13	0.93	1.3	2.81	2.19	3.05	2.8 ab	4.68	3.64	3.93	3.35	2.93	2.96	3.46	45.35
T2: Routine fungicide	2.39	1.73	1.89	0.84	0.49	0.18	0.89	1.34	2.78	2.04	3.1	2.59 b	4.29	3.5	4.2	3.34	3.09	3.21	3.51	45.36
T3: SPM + Botrytis managed	2.46	1.93	1.75	0.76	0.5	0.21	0.8	1.0	3.23	2.06	3.01	3.05 a	4.79	3.55	4.25	3.66	3.43	3.19	3.51	47.14
T4: SPM managed No Botrytis sprays	2.33	1.89	1.68	0.78	0.53	0.15	0.73	1.44	2.95	1.99	2.89	2.59 b	4.11	2.96	4.1	3.33	3.13	2.75	2.99	43.28
F Prob	0.881	0.406	0.893	0.980	0.969	0.465	0.829	0.532	0.711	0.837	0.820	0.006	0.098	0.127	0.691	0.671	0.699	0.537	0.535	0.416
SED (9)												0.109								
LSD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.248	NS	NS	NS	NS	NS	NS	NS	NS

NS = Not significant

Appendix 10 % Class 1 fruit from 19 picks from everbearer strawberries following treatment with four different programmes compared to an untreated control at NIAB EMR in 2019 (figures in brackets are original data where data has been angular transformed for statistical analysis).

Treatment	Pick date / % Class 1																		
	9 July	11 July	16 July	19 July	23 July	26 July	6 Aug	9 Aug	13 Aug	16 Aug	20 Aug	23 Aug	27 Aug	30 Aug	3 Sep	6 Sep	10 Sep	13 Sep	17 Sep	Overall % Class 1
T1: Untreated	91.3	95.0	95.8	92.9	91.4	85.4	89.3	96.2	88.6 b	94.9	96.8	95.1	92.3	94.2	94.0	87.0	88.5	88.0	84.4 (83.5) b	91.6
T2: Routine fungicide	92.0	94.9	96.9	94.1	89.7	63.1	87.3	96.8	91.4 b	96.1	98.4	98.6	98.4	96.4	97.2	96.5	95.2	96.1	96.1 (95.9) a	93.3
T3: SPM + Botrytis managed	91.8	97.3	95.6	95.4	95.4	73.3	90.7	97.5	95.0 a	98.8	98.3	98.8	99.0	96.2	96.8	88.1	96.0	95.9	96.3 (96.0) a	94.5
T4: SPM managed No Botrytis sprays	92.9	94.8	98.1	96.0	90.4	81.3	88.8	97.5	90.6 b	95.5	96.7	98.1	99.0	97.9	97.1	95.8	95.5	95.2	95.3 (95.0) ab	94.5
F Prob	0.889	0.577	0.464	0.647	0.796	0.410	0.951	0.879	0.003	0.158	0.389	0.079	0.254	0.443	0.328	0.268	0.374	0.215	0.028	0.142
SED (9)									0.050											
LSD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	0.257	NS	NS	NS	NS	NS	NS	NS	NS	NS		NS

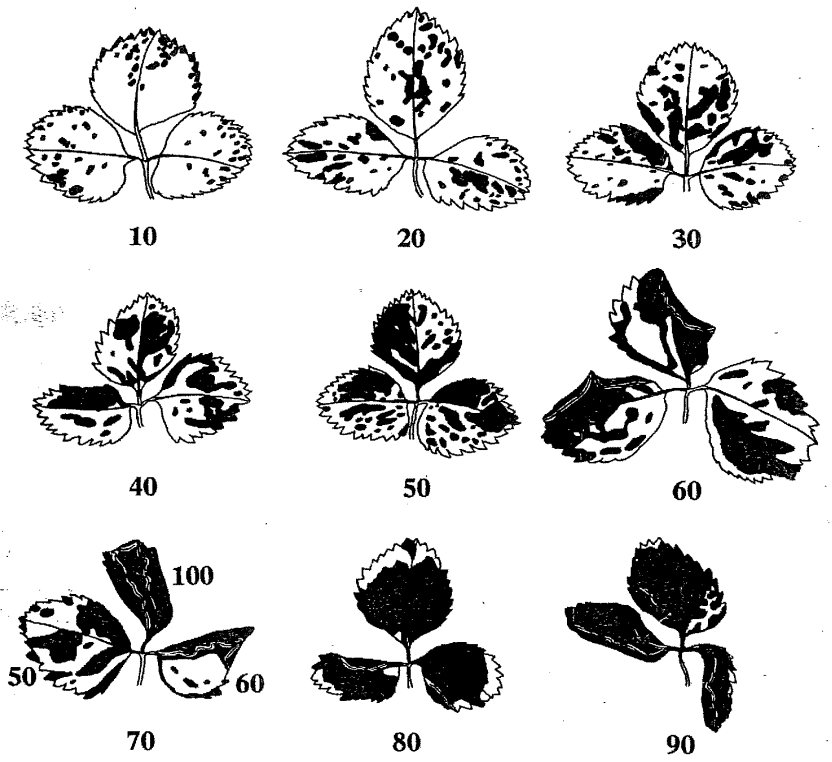
NS = Not significant

Appendix 11: Assessment key for strawberry powdery mildew

AGRICULTURAL DEVELOPMENT AND ADVISORY SERVICE

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

Sphaerotheca alchemillae Grev.



Percentage of leaf area affected

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

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

Powdery mildew (%)

0 Leaves fully extended, flat and green.

5 Slight curling noticeable; mildew found with difficulty.

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

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

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

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

50 Half of leaf area affected and apparently dead.

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

70 Severe distortion of at least one leaflet.

80 Much of leaf affected. Distortion of all leaflets.

90 Small marginal areas only remain green.

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

Notes:

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

PLANT PATHOLOGY LABORATORY
HARPENDEN, HERTS

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