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| <b>Project leader:</b>   | Prof Xiangming Xu<br>NIAB EMR  |
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| <b>Key staff:</b>  | Erika Wedgwood (ADAS)<br>Angela Berrie (NIAB EMR)<br>Tom Passey (NIAB EMR)<br>Avice Hall (University of Hertfordshire) |
| <b>Location of project:</b>                                      | East Malling; Cambridge; Wisbech   |
| <b>Industry Representative:</b>                                  | Louise Sutherland  |
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## AUTHENTICATION

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

Xiangming Xu

Head of Pest & Pathogen Ecology Department

NIAB EMR

Signature ..... Date ...28 March 2019.....

Erika Wedgwood

Senior Plant Pathologist

ADAS

Signature ... Date ...28 March 2019...

### **Report authorised by:**

Xiangming Xu

Head of Pest & Pathogen Ecology Department

NIAB EMR

Signature ..... Date ...29 March 2019.....

## TABLE OF CONTENTS

|   |           |
|---|-----------|
| <b>GROWER SUMMARY</b>   | <b>1</b>  |
| Crown rot and red-core caused by <i>Phytophthora</i> species  | 1         |
| Strawberry powdery mildew (SPM)   | 3         |
| Fruit rot complex   | 9         |
| Verticillium wilt   | 10        |
| <b>SCIENCE SECTION</b>  | <b>14</b> |
| Introduction  | 14        |
| Crown rot and red-core caused by <i>Phytophthora</i> spp.   | 14        |
| Strawberry powdery mildew (SPM)   | 14        |
| Fruit rot complex: <i>Botrytis cinerea</i> , <i>Mucor</i> and <i>Rhizopus</i>   | 15        |
| Verticillium wilt   | 16        |
| <b>Objective 1: Phytophthora</b>  | <b>17</b> |
| Materials and methods   | 17        |
| Results   | 22        |
| Discussion  | 26        |
| <b>Objective 2: Epidemiological mode of action of new products against strawberry powdery mildew (SPM)</b>  | <b>28</b> |
| Background  | 28        |
| Materials and method  | 29        |
| Results   | 32        |
| Discussion  | 35        |
| <b>Objective 2: Integration of managed programmes for control of powdery mildew and fruit rots in protected strawberries (ORETO Trial 18/004)</b> | <b>37</b> |
| Introduction and objectives   | 37        |
| Materials and methods   | 37        |
| Results   | 45        |
| Discussion  | 55        |
| Summary and conclusions   | 57        |
| Reference   | 58        |
| <b>Objective 2: Demonstration of a SPM management strategy on strawberry on a commercial farm</b>   | <b>59</b> |
| Materials and methods   | 59        |
| Results   | 62        |
| Discussion  | 69        |
| Summary and conclusions   | 70        |
| <b>Objective 2: Strawberry powdery mildew research at University of Hertfordshire (UoH)</b>   | <b>72</b> |
| <b>Objective 3: Fruit rot complex</b>   | <b>77</b> |
| Background  | 77        |
| Materials and Methods   | 77        |
| Results   | 78        |
| Discussion  | 79        |
| Conclusions   | 79        |
| References  | 79        |

|  |           |
|--|-----------|
| <b>Objective 4: To evaluate the effects of individual and combined use of alternative products against Verticillium wilt of strawberry .....</b> | <b>81</b> |
| Introduction .....   | 81        |
| Methods and Materials .....  | 81        |
| Results.....   | 84        |
| Discussion .....   | 93        |
| Conclusions .....  | 95        |
| <b>Knowledge and Technology Transfer .....</b>   | <b>97</b> |
| <b>Appendix .....</b>  | <b>98</b> |

## GROWER SUMMARY

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

### Crown rot and red-core caused by *Phytophthora* species

#### Headline

- Several fungicide and bio-fungicide products significantly reduced the losses due to latent infection by *Phytophthora cactorum* when applied as a dipping treatment at planting

#### Background and expected deliverables

Adopting a clean propagation system is the first line of defence against crown rot and red-core diseases. This strategy has been working for many years until recent times. Currently, crown rot and red-core can cause significant damage in strawberry even in substrate production. The most likely cause is asymptomatic infection in planting material. Fenomenal (fenamidone + fosetyl-aluminium), an effective product against *Phytophthora*, is not approved for use beyond November 14 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. Two AHDB Horticulture projects have just been completed; SF 130 focussed on fungal molecular quantification and an assay was developed that detected *Phytophthora rubi*, although it was not as sensitive as the *Phytophthora fragariae* assay (which however detects both pathogens); SF 123 investigated alternative products against *P. rubi* on raspberry where one novel chemical product gave disease 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.

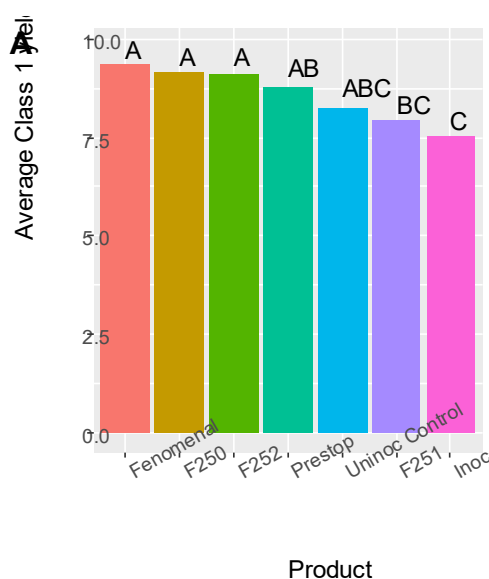


Figure A: Average Class I yield of “Malling Centenary” plants for each product treatment. Each product was applied as dipping or drenching two weeks after dipping. Additional drenching did not affect fruit yield. Treatments sharing at least one common letter (above the bar) are not statistically different from each other at  $P = 0.05$ .

In the first three years of this project, we showed that (1) *P. fragariae* (red core) was rarely detected in planting material, (2) in contrast, incidence of *P. cactorum* could be up to 30% in planting material, though varying greatly among batches, and (3) neither arbuscular mycorrhizal fungi (AMF) nor plant growth promoting rhizobacteria (PGPR) managed to reduce the losses caused by *P. cactorum*. The aim of this project in year 4 on Phytophthora is to assess whether treating plants at planting time can reduce the losses due to *P. cactorum*.

## **Summary of the project and main conclusions**

In Year 4, we conducted a large study to test existing and new products as dipping/drenching treatments at 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 only is sufficient to reduce the level of *P. cactorum* to the level comparable to the un-inoculated control; thus additional drenching is not necessary. Of the five products tested, four significantly reduced *P. cactorum* development and resulted in similar yield as the un-inoculated control as shown in Figure A. Of the four products, two are registered products; Fenomenal (use-up date 14 November 2019) and Prestop (*Gliocladium catenulatum*); the other two are experimental products: one chemical (F250) and one biological (F252). In contrast, the other experimental biological product (F251) led to increased plant mortality.

## **Financial benefits**

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

## **Action points**

- Results from Year 1 and 2 suggested that growers should consider treating runners for *P. cactorum* at the time of planting
- Year 3-4 results suggested that dipping plants with chemical and biological products should be considered at planting when the level of crown rot in planting material is expected to be high.

## Strawberry powdery mildew (SPM)

### Headline

- A managed approach to strawberry powdery mildew control using a risk prediction model can reduce fungicide use by half.

### Background and expected deliverables

Powdery mildew, 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 in 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.

Mildew is favoured by warm temperatures and high humidity such that conditions are most favourable for mildew from late June to October. Hence mildew 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 mildew is relatively straightforward. However, management of mildew in everbearer crops is much more challenging. The long growing period from March to November with flowering, fruiting and harvest continuous from June-November, a range of crop protection products is usually required with a continuous series of spray rounds needed to cover the whole period. Disease control is currently based on use of fungicides. Given the pressure to reduce use of conventional plant protection products and continuing loss of approved actives, this approach is not sustainable.

The SCEPTRE project (2010-2014) identified alternative products, including Cultigrow (a biostimulant / elicitor) and two biofungicides (biological control agents - BCAs) – AQ10 (*Ampelomyces quisqualis*) and a bacterial based biofungicide (F208). The purpose of the work in this project was to confirm the efficacy of these products, evaluate them in programmes with fungicides and develop a simple decision-based management system for mildew control.

The trial in 2015 confirmed the efficacy of the BCAs AQ10 and F208 and the biostimulant Cultigrow alone or in combination with fungicides, in controlling mildew. In 2016 further trials were conducted in which programmes were evaluated for control of powdery mildew where the biofungicides (F208 or AQ10) were combined in programmes with Cultigrow with and



without a reduced fungicide programme 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 BCAs were as effective in controlling mildew as the standard 7-day fungicide programme, particularly when applied alone in a programme and especially in reducing mildew on fruit.

Having identified alternative products that were effective on June-bearer crops, the next step was to combine their use in programmes and incorporate other factors such as disease risk (determined from model predictions based on tunnel humidity and temperature and also the forward weather forecast), 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 an everbearer cultivar. The mildew control achieved by managed programmes of fungicides and BCAs 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), 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 mildew, there was little opportunity to omit sprays. However, in the managed treatment, intervention with a fungicide in place of the BCA (F208) occurred only twice. The mildew risk throughout the trial was high. Mildew incidence on the leaves was very low. However, on fruit the mildew 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. Mildew incidence on the fruit in all treated plots was negligible throughout the harvest period. This trial demonstrated that use of BCAs, with or without Sirius or Cultigrow, gave good control of mildew in strawberry comparable to a fungicide-based programme.

The objective in 2018 was to explore how the approach for managing mildew could be integrated with control of botrytis and other fruit rots on everbearer crops in a replicated trial at NIAB EMR. In addition, a trial was conducted on a commercial farm as a demonstration to encourage growers to take up a more managed approach to disease control.

## **Summary of the project and main conclusions**

### ***Management trials***

At NIAB-EMR, 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 mildew and botrytis risks were lower. Three managed treatments were compared to a routine 7 day

fungicide programme and an untreated control. Simple 'Look up' tables were produced from SPM and Botrytis computer models (previously developed at NIAB-EMR) for use in conjunction with the forward weather forecast (from BBC Weather website) to determine disease risk for spray decisions.

The weather conditions (warm temperatures coupled with high humidity) were very conducive to powdery mildew 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 mildew at planting time and this combined with the hot dry weather in June and July meant that mildew failed to establish in the crop, despite the higher risk identified in August and September. Therefore, only four fungicide sprays (and seven BCAs) for mildew were applied in the managed plots compared to 14 (and two BCAs) in the routine treated plots, a saving of £356 /ha. 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. There was a saving of only two fungicides compared to the routine treatment with a cost saving of £485 /ha (see table below). However, the incidence of Botrytis in post-harvest tests showed that for most of the 20 harvests, differences in Botrytis between the untreated control and treated plots was very small, questioning the need for the fungicide inputs with potential savings in cost. There were also no treatment effects on yield and fruit quality.

## Summary of fungicides, BCAs and biostimulants applied to strawberry plots at NIAB EMR in 2018 and the programme costs

| Treatment period | Treatment           | Management treatment |             |                                   |                                   |                              |
|------------------|---------------------|----------------------|-------------|-----------------------------------|-----------------------------------|------------------------------|
|                  |                     | T1: Untreated        | T2: Routine | T3: SPM managed, routine Botrytis | T4: Routine SPM. Managed Botrytis | T5: Managed SPM and Botrytis |
| 5 June- 2 July   | Botrytis Fungicide  | 0                    | 4           | 4                                 | 1                                 | 1                            |
|                  | Mildew Fungicide    | 0                    | 5           | 2                                 | 5                                 | 2                            |
|                  | BCA                 | 0                    | 0           | 0                                 | 0                                 | 0                            |
|                  | biostimulant        | 0                    | 0           | 1                                 | 0                                 | 1                            |
| 9 July-30 July   | Botrytis Fungicide  | 0                    | 4           | 5                                 | 4                                 | 3                            |
|                  | Mildew Fungicide    | 0                    | 4           | 1                                 | 5                                 | 1                            |
|                  | BCA                 | 0                    | 0           | 2                                 | 0                                 | 2                            |
|                  | Biostimulant        | 0                    | 0           | 1                                 | 0                                 | 1                            |
| 6 Aug-17 Sep     | Botrytis Fungicide  | 0                    | 5           | 5                                 | 7                                 | 7                            |
|                  | Mildew Fungicide    | 0                    | 5           | 1                                 | 5                                 | 1                            |
|                  | BCA                 | 0                    | 2           | 5                                 | 0                                 | 3                            |
|                  | Biostimulant        | 0                    | 0           | 2                                 | 0                                 | 2                            |
| Total            | Botrytis fungicides | 0                    | 13          | 14                                | 12                                | 11                           |
|                  | Mildew fungicides   | 0                    | 14          | 4                                 | 15                                | 4                            |
|                  | Total fungicides    | 0                    | 27          | 18                                | 27                                | 15                           |
|                  | Biofungicides       | 0                    | 2           | 7                                 | 0                                 | 5                            |
|                  | Biostimulant        | 0                    | 0           | 4                                 | 0                                 | 4                            |
| Cost £/ha        | Total programme     | 0                    | 2,278       | 2,169                             | 1,905                             | 1,579                        |
|                  | Mildew only         | 0                    | 1,033       | 677                               | 890                               | 677                          |
|                  | Botrytis s only     | 0                    | 1,596       | 1,700                             | 1,223                             | 1,111                        |

### Commercial Demonstration

A demonstration trial was established on a commercial farm on an everbearer variety. In this trial, two tunnel treatments were compared. One tunnel followed the same mildew and Botrytis control programme as the rest of the farm. In the other, the control criteria used for powdery mildew and rots in the NIAB EMR trial were adopted. As in the trial at NIAB EMR, strawberry powdery mildew 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 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 eight in the trial tunnel. There was 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.

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

| Item                    | Control tunnels | Trial tunnel |
|-------------------------|-----------------|--------------|
| <b>Total Fungicides</b> |                 |              |
| for <i>Botrytis</i>     | 13              | 8            |
| for mildew              | 19              | 10           |
| Total                   | 26              | 15           |
| <b>Other products</b>   |                 |              |
| BCAs                    | 2               | 1            |
| Cultigrows              | 0               | 5            |
| Other biostimulants     | 13              | 11           |
| <b>Cost £/ha</b>        |                 |              |
| Total                   | 1715.08         | 1272.22      |
| Mildew only             | 1110.10         | 848.23       |
| Botrytis only           | 934.44          | 623.99       |

**Mode of action**

Three new fungicides, Luna Sensation (fluopyram & trifloxystrobin), Takumi (cyflufenamid) and Talius (proquinazid), have good anti-sporulant ability, especially Luna Sensation. They could reduce sporulation by up to 50% within 4 days of their application. Silwet on its own also achieved a comparable level of anti-sporulant effect to the three fungicides especially for the periods immediately following its application. AQ10 and F208 were each applied together with Silwet, giving a similar level of control to Silwet. It is therefore open to question as to how much additional effect each biocontrol agent contributed to the observed effect. Nevertheless, over the four sampling occasions, AQ10 (with Silwet) gave better control than Silwet alone and F208 (+ Silwet), although the actual difference was small. The overall test results from two-year testing are summarised in the table below:

**Effectiveness of several products applied as a curative, protectant or anti-sporulant treatment against strawberry powdery mildew**

|                | Curative:<br>number of days<br>applied after<br>infection | Protectant:<br>number of days<br>applied before<br>infection | Anti-sporulant:<br>number of days<br>with good<br>suppression of<br>sporulation |
|----------------|---|--|---|
| Talius         | 2-3   | 7-8  | 2-3   |
| Takumi         | 2-3   | 4-5  | 2-3   |
| Luna Sensation | 2-3   | 4-5  | 4   |
| Charm          | Not tested  | To be tested   | 4   |
| Silwet         |   | Not tested   | 2-3   |
| Silwet + AQ10  | Not tested (not expect<br>to have an effect)              | 2 (AQ10 only)  | 4   |
| Silwet + F208  |   | 2-3 (F208 only)  | 2-3   |

### ***Main conclusions***

A simple decision-based system for determining treatments for powdery mildew 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. This system incurred no adverse effects in yield, fruit quality or disease control.

In addition, while the routine programme employed all permitted applications of approved fungicide products through the season, some permitted fungicide applications were held in reserve for use at the end of the season where the managed programme was adopted. This could be helpful should a late outbreak of infection occur.

### **Financial benefits**

Both the replicated trial at NIAB EMR and the demonstration trial on the commercial farm 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. In both cases cost savings were made (£699 /ha and £443 /ha respectively) with no adverse effects on yield, fruit quality or rot incidence. 2018 was a low mildew year for both trial sites and this will need to be taken into account. There were also advantages in reduced residues in the fruit, particularly for sprays targeted at SPM.

### **Action points for growers**

- Three new products including Luna Sensation and Takumi (both curative and anti-sporulant activity) along with Talius (curative activity) offer growers with additional protection against powdery mildew.
- All three can be integrated within spray programmes.
- 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 growth stage.
- Use of such a system can reduce both the number of fungicides applied and the subsequent total cost of the spray programme.

- The model is being used and demonstrated at the NIAB EMR WET Centre and those growers who employ the Precision Irrigation Package are supplied with the model and are trained in its use.

## **Fruit rot complex**

### **Headline**

- *Pestalotiopsis* species are unimportant in fruit rots and plant death in UK strawberry.

### **Background and expected deliverables**

Recent evidence in the UK and New Zealand has shown that *Botrytis cinerea* is not the only pathogen causing fruit rot in strawberry. The importance of *B. cinerea* may have been overstated because of similar morphological characteristics of *Botrytis* fungal morphology with two other rotting fungi – *Mucor* and *Rhizopus* species. 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*. 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.

Towards the end of the second year of this project, there were increasing reports on the occurrence of a new pathogen isolated from the crowns of wilting plants. In addition, this pathogen was shown to cause fruit rot on strawberry in Egypt. In year three, 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

We used the molecular primers developed in Year three to screen for the presence of *Pestalotiopsis* species. in a number of selected samples taken for testing *P. cactorum* in Year 1 and 2. Of the 136 samples tested, only one sample showed positive for presence of *Pestalotiopsis*.

In addition, we carried out a preliminary study to investigate the survival of two commercial biocontrol agents in strawberry flowers; this work will be completed by May 2019 and reported in 2020.

## Financial benefits

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

## Action points for growers

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

## Verticillium wilt

### Headline

- A drench of Serenade ASO at plant establishment appears to reduce crown wilting over a year later.

## 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 is used by growers, but methyl bromide is no longer authorised and chloropicrin use now requires annual Emergency Authorisation.

Some varieties have greater resistance to Verticillium wilt, but other measures are also required to reduce the impact of the disease. There is the potential for soil amendment 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.

## Summary of the project and main conclusions in Year 4

In May 2017 part of a field with a *Verticillium dahliae* count of four propagules per gram of soil was withheld from chloropicrin fumigation. Replicated 7 m lengths of bed were instead left untreated or given one of two different pre-planting treatments;

1. The incorporation of pasteurised anaerobic maize and vegetable digestate solids.
2. The incorporation of *Brassica carinata* pellets (Bio-Fence) which released isothiocyanates under the polythene.

Cold-stored strawberry runners (cv. Symphony) were planted in the trial area on 6 June 2017. Symphony plants were established on the same date in the adjacent chemically fumigated commercial beds. A week after planting, Serenade ASO (*Bacillus subtilis*) was applied to half of the Bio-Fence treated plots and to half of the untreated plots. This resulted in four treated plots and one untreated plot, randomised within each of five replicate beds.

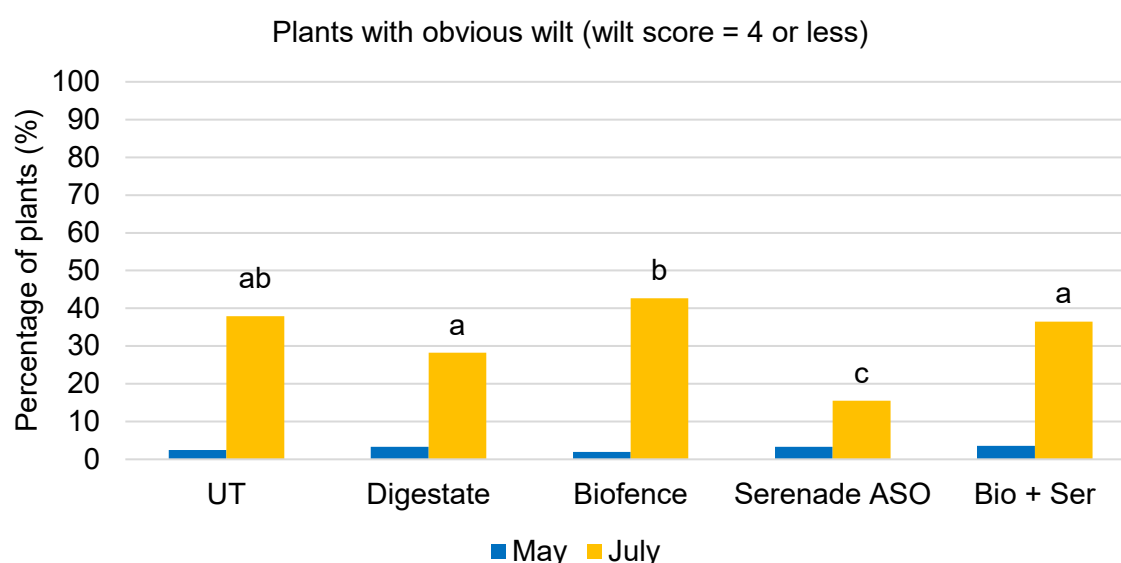
The week of planting was exceptionally hot and some plants struggled to establish, especially in the Bio-Fence plots. It is possible that the seven-day ventilation period used for the chloropicrin treated area should have been extended. By May 2018, only occasional plants were starting to wilt. Fruit harvesting was carried out between 11 and 27 June 2018 during a period of exceptionally hot weather. The total weight of fruit and the weights that were marketable or unmarketable did not differ between the untreated and any of the four treatments, with a mean 555 g total weight per plant and 89% marketable. On the one date (27 June) that fruit yield and berry weights were also recorded from the commercial crop, the total weight of marketable fruit was 104 g per plant with 91% marketable, compared with 43 g per plant in the trial area. Average fruit weight of Class 1 fruit harvested on 27 June was 21 g, whereas from the trial plots, the mean was 12 g.

In July 2018, after plants had experienced both the stress of fruiting and enough heat to scorch the fruit in the field, wilting was seen across the trial area (Figure B). A significantly ( $P < 0.001$ ) greater proportion of the plants (42.6%) had severe wilt after receiving Bio-Fence than after all other treatments except the untreated. Of plants which received Serenade ASO, only 15.5% had severe wilt, significantly ( $P < 0.001$ ) fewer than any of the other treatments.

Harris testing of the soil for *V. dahliae* before treatment with Serenade ASO determined that this had not followed a lower starting population of micro-sclerotia than in the untreated plots. The Bio-Fence plots had only 16.2% of plants with very slight or zero visible wilt, significantly



( $P < 0.05$ ) fewer than in all the other four treatments (with a mean 31.5%). That the plots which received both Bio-Fence plus Serenade ASO had significantly more plants that were healthy compared with plots with Bio-Fence alone suggests that Serenade ASO helped to prevent wilt that would otherwise have occurred. Serenade ASO may have triggered plant defences and/or the *B. subtilis* competed with the *V. dahliae*. *Verticillium* presence was not able to be confirmed by isolation in wilted plants sent for laboratory examination in 2018, even though soil infestation by *V. dahliae* of 4 propagules / gram of soil in 2018 was confirmed from three untreated plots.



**Figure B.** Percentage of plants with obvious wilt (vigour/wilt index 4 or less), on 17 May and 19 July 2018. Significant differences ( $P < 0.001$ ) from regression analysis indicated by letters in July. No significant difference ( $P = 0.945$ ) in May. 'UT' refers to untreated plots; 'Bio + Ser' refers to the treatment with both Biofence and Serenade ASO.

## Financial benefits

Up to and including harvest in 2018 no financial benefits were shown from the use of the products at planting. However, post-harvest by July 2018, 38% of the plants in the untreated plots had severe wilt compared with those given a single drench of Serenade ASO at planting (where 16% of plants had obvious wilt). If over a third of plants are weakened or die in a commercial crop then this will result in a substantial yield reduction, potentially leading to early termination of the crop. Serenade ASO could therefore save the crop from destruction, making a third year of production financially viable.

## Action points for growers

- In soil grown strawberry production, carry out a soil (Harris) test for the presence of *Verticillium dahliae* before establishing a new crop.
- The result will determine the need to fumigate the soil before planting.
- If infected soil is not fumigated, most commercially grown varieties are likely to be affected, leading to reduced yield and fruit size.
- Be aware that if a biofumigant is used, an adequate ventilation period before planting should be allowed, potentially longer than that used for chloropicrin.
- Consider a drench application of Serenade ASO at plant establishment, as this can reduce crown wilting over a year later.

## SCIENCE SECTION

### Introduction

Strawberry is attacked by several pathogens, including *Botrytis cinerea*, strawberry powdery mildew (SPM) and *Phytophthora* spp. In recent years, *Phytophthora* species have gradually increased in their prevalence. Other fungal fruit rot pathogens have also become more prevalent but have received less 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 has been working for many years until recent times. Currently, crown rot and red-core can cause significant damage in strawberry even in substrate production. The most likely cause is asymptomatic infection in planting materials. Frequent application of fungicides, alleged to have occurred in overseas nurseries, may delay the onset of symptom development until post-transplanting. Subsequent disease spread is likely to occur because of over-irrigation or rain-splash. Alternative products for control of crown rot (both conventional and biological fungicides) were identified in trials conducted by NIAB EMR as part of the SCEPTRE project. Recent research on *Phytophthora* spp. has concentrated on detecting the pathogens and seeking products to reduce root rotting. AHDB 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 however 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. NIAB EMR has just completed a BBSRC project, in which we have identified a number of quantitative resistance factors against *P. cactorum*. These resistance factors will be exploited in breeding programmes at NIBA EMR. More research is required to assist growers to be able to plant disease-free propagation material and to reduce impact of the disease during cropping.

### **Strawberry powdery mildew (SPM)**

A Hort-LINK project (HL0191) focussed on development, implementation and use of a SPM prediction system. The prediction system was based on the one developed at the University

of Hertfordshire. The project clearly demonstrated the benefit of using the system for early crops where initial SPM inoculum is low. Recent research in UK (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***

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 overstated 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**

Withdrawal of methyl bromide and recent withdrawal of chloropicrin (followed by emergency approvals) as soil fumigants have focussed the industry on searching for alternative soil treatments against this pathogen. (Update May 2019: An emergency authorisation for use of chloropicrin in soft fruit, tree fruit and ornamentals was submitted in 2018. If successful, this authorisation will replace the current emergency authorisation (1432/18) which expires at the end of June 2019).

Disappointingly, a new microencapsulated product did not have sufficient efficacy to have any commercial future (TSB project ended December 2014). AHDB Horticulture recently funded a project (CP 103) at NIAB EMR on pre-colonising strawberry runners or tipping plants to manage wilt and results showed that pre-colonising strawberry plants did not help plants to reduce wilt development. With AHDB funding, Fera developed a molecular diagnostic tool to quantify soil inoculum and currently ADAS is using this tool to investigate the relationship of wilt development in relation to nematodes. Separately, 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* spp.) and the suppressive effects by *Bacillus* and *Pseudomonas* species.

## **Objective 1: Phytophthora**

Field survey work in year 1 suggested that *P. cactorum* is more prevalent than *P. fragariae* in strawberry planting material received by UK growers. Most *P. cactorum* detected in planting material in years 1 & 2 was latent. For only one of the 12 batches with at least 5% plants showing positive *P. cactorum* PCR results was there noticeable disease development post-planting. Thus, plants may grow out of the latent infection. In year 2, we demonstrated that neither individual nor joint use of arbuscular mycorrhizal fungi (AMF) and plant growth promoting rhizobacteria (PGPR) significantly reduced *P. cactorum* development when plants were inoculated with *P. cactorum* at the time of planting (post-cold storage). This may not be surprising because under high disease pressures the curative effect (killing young developing infection) of AMF and PGPR is unlikely to be observed. The lack of effect of AMF and PGPR on *P. cactorum* was confirmed in year 3.

In year 4, we initiated a new experiment to evaluate the effects of post-cold storage (prior to planting) dipping/drenching treatment with selected products on the development of latent infection of *P. cactorum*. In addition to disease development, we also assessed plant vigour and fruit yield.

## **Materials and methods**

The aim of the experiment was to assess the effects of fungicide and biocontrol product treatments at planting on strawberry plants (a June bearer cultivar) inoculated with *P. cactorum* prior to cold storage.

### **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 were obtained from a commercial nursery and delivered to NIAB EMR (Photo 1.1) in early October 2017. Tray plants (instead of runners) were used as we wanted to minimise the extent of natural infection from nursery (particularly soil). Because of the expected high mortality of inoculated plants (ca. 30-50%) in cold store, we ordered 3000 plants for this experiment.

Two *P. cactorum* isolates (P 404 and P414), known to be pathogenic against the relevant cultivar were used. A suspension of  $10^5$  zoospores  $\text{ml}^{-1}$  was produced following a previously published method (Harris, Simpson, and Bell 1997). Each crown was inoculated without wounding by directly pipetting 3 ml inoculum onto the crown. Inoculated plants were placed into a polytunnel for 3-5 weeks to allow infection to take place and to harden before cold storage. Because of the large variability in the incidence of latent infection following inoculation, we divided the plants into three groups, each with 850 plants for inoculation; the remaining 350 plants as un-inoculated control. The first group of plants were inoculated once, the second twice, and the third three times. There was an interval of a week between consecutive inoculations. This inoculation schedule was used to increase the probability of more plants with latent infection and at the same time to ensure we had a sufficient number of inoculated plants surviving the cold storage for treatment application at planting. These plants were placed into a cold store ( $-2^{\circ}\text{C}$ ) on 18 December 2017.



**Photo 1.1.** Picture of the tray plants in late September before inoculation with *P. cactorum* for post cold storage treatment

**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  |
|---------------|--|
| 04/10/2017    | 3000 fresh tray plants delivered and maintained in trays in a polytunnel   |
| 08-09/11/2017 | Inoculating healthy crown tissues of all plants (except those allocated to the control) with <i>P. cactorum</i> spore suspensions                                |
| 15-16/11/2017 | Inoculating plants (allocated to receive 2 <sup>nd</sup> and 3 <sup>rd</sup> inoculations) with <i>P. cactorum</i> spore suspensions                             |
| 22-23/11/2017 | Inoculating plants (allocated to receive 3 <sup>rd</sup> inoculation) with <i>P. cactorum</i> spore suspensions  |
| 18/12/2017    | Plants moved to $-2^{\circ}\text{C}$ cold store  |
| 02/05/2018    | Health check on sub-sample of plants removed from cold store on 30/04/18   |
| 17/05/2018    | Plants taken out from the cold-store moved to the Middle Park at NIAB EMR and left in shade to allow plants to defrost for 24 hours before treating and planting |
| 18/05/2018    | Plants treated (dipped) and planted  |

|            |   |
|------------|---|
| 01/06/2018 | Drench treatments applied to appropriate plots  |
| 15/06/2018 | Conducted first disease assessment.   |
| 29/06/2018 | Added <i>Amblyseius cucumeris</i> (1 sachet of slow release ripped open and spread over four bags); conducted second disease assessment |
| 05/07/2018 | 14000 <i>Phytoseiulus persimilis</i> added to the plot  |
| 06/07/2018 | First fruit pick  |
| 16/07/2018 | Conducted the third disease assessment  |
| 03/08/2018 | Last (the eighth) fruit pick  |
| 06/08/2018 | Symptoms assessed first two blocks  |
| 07/08/2018 | Symptoms assessed in the other two blocks   |
| 17/08/2018 | Decreased irrigation from 8 to 4 minutes every 8 hours to stress plants   |
| 24/08/2018 | Decreased irrigation from 4 to 2 minutes every 8 hours to stress plants   |
| 03/09/2018 | Final assessment of plant wilting/death; sampled crowns for molecular test  |

## Treatments and experimental design

The single main experimental treatment factor was the selected products:

1. Fenomenal (fenamidone + fosetyl aluminium) (control product); approval for use of fenomenal is to be withdrawn (use up date 14<sup>th</sup> November 2019) but was included in the trial because of its known efficacy against *P. cactorum* as the available industry standard.
2. Prestop (a product based on formulated *Gliocladium catenulatum* strain J1446)
3. A commercial microbial product but not registered for this specific use (AHDB code – F252)
4. A commercial microbial product but not registered for this specific use (AHDB code – F251)
5. A new fungicide product (AHDB code – F250).

For each product, there were two treatments: dipping only at planting time, and dipping plus additional drenching 2 weeks after planting. Table 1.2 gives the rates from labels or from unpublished information from relevant manufacturers. In addition, there were two control treatments: (1) untreated but inoculated control (positive control) and (2) un-inoculated and untreated control (negative control). Thus, there were 12 treatments.

A randomised block design (with four blocks) was used (Appendix 1). Within each block, there were six coir bags (CoCo Green) for each treatment; two bags were allocated to plants that were inoculated once, twice or three times with *P. cactorum* the previous autumn. There were eight plants per bag, giving 48 plants per replicate, i.e. total 192 plants in the entire trial for each treatment. For the negative control, all plants were neither inoculated nor treated with products.



## Applying treatments

Generally speaking, symptoms of crown rot in infected planting materials are likely to be induced by post-planting stresses. Thus, the planting date was postponed to mid May 2018 when the temperature was high.

Plants were moved out the cold store to the shade area near the tunnel the day before planting for defrosting. All dipping treatments (15 minute treatment) were applied inside a glasshouse compartment on 18<sup>th</sup> May 2018 and then immediately planted. The bags were laid on the top of plastic grey boxes (with holes to allow water through). Plants were fertigated with a 6 L per hour dripper per bag [with four sub-drippers per bag]. Fertigation was developed specifically 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 were poured slowly over the top of the crown of each plant in specific plots as an additional drenching application.

**Table 1.2.** Products for crown rot control in strawberry

| Product          | Active ingredient                                | Rate (g/L) | Application method       |
|------------------|--|------------|--------------------------|
| Fenomenal*       | fosetyl-Al + fenamidone                          | 1.5        | Pre-plant dip 15 mins    |
|                  |  | 0.75       | Drench 100 ml/plant      |
| F250 (AHDB code) | Experimental fungicide from a commercial company | 5 (mL/L)   | Pre-plant dip 15 mins    |
|                  |  | 5 (mL/L)   | Drench 100 ml/plant      |
| Prestop          | <i>Gladiolus catenlanum</i>                      | 5          | Pre-plant dip 15 mins    |
|                  |  | 5          | Drench 100 ml/plant      |
| F252 (AHDB code) | Microbial biofungicide                           | 0.1        | Pre-plant dip 15 mins    |
|                  |  | 0.25       | Drench 100 ml/plant      |
| F251 (AHDB code) | Microbial biofungicide                           | 91         | Pre-plant dip 15 seconds |
|                  |  | 0.07-1.0   | Drench 100 ml/plant      |

\*: Fenomenal is being withdrawn but used as a standard treatment for comparison.

## Assessment

One crate of each of the un-inoculated control, those plants inoculated with *P. cactorum*, twice and three times were taken out of the -2°C cold store on 30<sup>th</sup> April 2018, left at ambient conditions, and then assessed on 2 May 2018. There were 36, 40, 44 and 44 plants for the un-inoculated control, those plants inoculated with *P. cactorum*, twice and three times, respectively. Plants were assessed on general health and any sign of mycelia on healthy tissue only; crowns were then cut in half with secateurs to check for any browning of the tissue, which indicates potential *P. cactorum* infection (SF 157 year 3 Annual Report). Where

browning was seen in un-inoculated control plants, the material was tested with a LFD to check for *Phytophthora*. However, it was cost prohibitive to do this test for inoculated plants too. Four plants from each group (inoculated once, twice and three times, and the un-inoculated control) were randomly taken and send for quantification of total water-soluble carbohydrate on the day of planting (18<sup>th</sup> May 2018).

There were eight fruit picks: starting from 06 July to 03 August 2018; for every pick, total weight of class I and II fruit was obtained for each replicate (six bags). Residues for multiple pesticides were tested for the third pick for those fruit from fungicide-treated plots).

Before the last fruit pick (6<sup>th</sup> August), plants were irrigated as in commercial production; water was not withdrawn as initially planned because of the exceptional hot conditions in the early summer. Irrigation was not reduced until 17<sup>th</sup> August because of hot conditions immediately post-harvest. Irrigation was reduced by 50% on 17<sup>th</sup> August and further by 50% on 24<sup>th</sup> August to induce disease development before final disease assessment on 3 September.

Visual plant symptoms were assessed five times: 15<sup>th</sup> June, 29<sup>th</sup> June, 16<sup>th</sup> July, 6<sup>th</sup> August (last pick) and 3<sup>rd</sup> September (Photo 1.2). Because of the cost constraint, we could not sample all plants for molecular screening of *P. cactorum*. Year 1-3 results showed that most plants with positive PCR results for presence of *P. cactorum* are from those plants that had crowns with internal browning. [Please note: **not all plants with discoloured crown tissues had *P. cactorum***]. The chance of *P. cactorum* present in the healthy crown tissues was very low. Thus, to increase the efficiency of detecting *P. cactorum*, we focused on molecular screening of discoloured crown tissues. We first examined crowns of all surviving plants on 3<sup>rd</sup> September for internal browning. Then, for each combination of product, application method and number of times plants inoculated with *P. cactorum* we randomly sampled one plants with browning crown tissue for molecular detection of *P. cactorum* DNA. A total of 144 plants were sampled. From the incidence of discoloured crowns, and proportion of discoloured crown tissues with positive detection of *P. cactorum*, we estimated the incidence of *P. cactorum*.



**Healthy**



**Floppy**



**Dead**

**Photo 1.2.** Visual plant assessment keys on strawberry plants inoculated with *Phytophthora* spp. from left to right: healthy, wilting, and severe wilting (dead).

## Data analysis

Three disease-related and two yield variables were statistically analysed: number of dead plants and plants with wilting symptoms (including those dead ones) at the final pick, number of plants with browning crown tissues four weeks after the final pick, and class I and total yield. These data were analysed using R (version 3.5.1). Only significant ( $P < 0.05$ ) or close-to-significant ( $P < 0.1$ ) [this is now recommended as a good practice in data presentation] differences are reported in the text. The disease-related data were analysed using generalised linear models (GLM) with residual errors assumed to follow a binomial distribution. Because of the nature of GLM, significance of treatment differences is not directly based on the standard errors on the original measurement scale; thus we did not present error bars on the original scale in graphs. Pairwise treatment comparisons were based on deviance testing following the nest-model analysis in GLM. For yield data, standard analysis of variance (ANOVA) was applied (no data transformation was necessary). In addition, ANOVA was applied to the carbohydrate data without data transformation.

## Results

### Pre-planting assessment of latent infection

A sub-sample of plants taken from cold storage on 30 April 2018 was assessed for disease on 2<sup>nd</sup> May 2018. *Botrytis* mycelia were present on dead tissues of many plants. Table 1.2 shows the summary of the results. Only one out of 36 un-inoculated plants had *P. cactorum* based on both crown tissue browning assessment and LFD test. The results suggested that inoculation was partially successful in establishing latent infection, with approximately 50% inoculated plants showing slight or severe crown browning, indicative of potential infection by *P. cactorum*.

Total water-soluble carbohydrate varied greatly among individual plants, ranging from 7.5 to 38.9 g kg<sup>-1</sup>, but was not dependent on the number of times the plants were inoculated.

**Table 1.3.** Number of cold-stored June bearer plants assessed for crown tissue symptoms 2 weeks before planting; most of these plants were inoculated with *P. cactorum* the previous autumn

| # of times inoculated with <i>P. cactorum</i> | Total number | Healthy foliar | Healthy looking crown | Very symptomatic | Slight browning of crown |
|---|--------------|----------------|-----------------------|------------------|--------------------------|
| 0 (Control)                                   | 36           | 35             | 33                    | 1 <sup>a</sup>   | 2 <sup>b</sup>           |
| 1x  | 40           | 40             | 22                    | 3                | 15                       |
| 2x  | 44           | 41             | 20                    | 6                | 18                       |
| 3x  | 40           | 40             | 17                    | 6                | 17                       |

<sup>a</sup> Tested positive for *Phytophthora* with LFD; <sup>b</sup> tested negative for *Phytophthora* with LFD.

## Post-planting disease development

For all studied variables, GLM deviance analysis of nest models showed:

1. there were no significant differences between the dipping only and both dipping and drenching treatments; and
2. there were no significant interactions between products and application methods (dipping or, or both dipping and drenching) in affecting yield and symptom development.

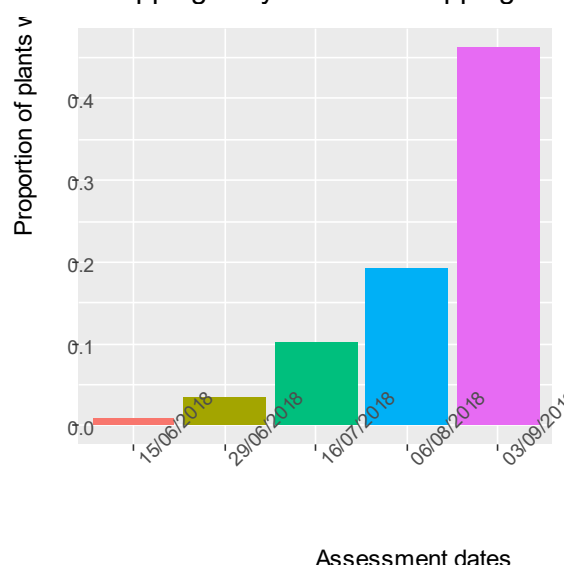
Thus, all subsequent analyses focused on comparisons among products and the two control treatments with data pooled over the application timings and the number of times the plants were inoculated.

The proportion of plants with wilting symptoms (including dead ones) increased steadily over time, from 1% on 16<sup>th</sup> June to 47% on 3<sup>rd</sup> September (Fig. 1.1). At the final pick (6<sup>th</sup> August), 19% plants had visual symptoms [wilting]. However, only 2.7% plants were dead at the final pick; even at the final assessment (3<sup>rd</sup> September) when all plants were destructively assessed for crown health status, only 3.1% were dead.

Four (F250, F252, Fenomenal and Prestop) of the five products tested significantly ( $P < 0.05$ ) reduced the proportion of plants with wilting symptoms at the final pick to the level (13% to 16%), similar to the un-inoculated control (Fig. 1.2A). However, F251 significantly ( $P < 0.05$ ) increased wilting development when compared with the inoculated control: 35% versus 27% (Fig. 1.2A). The number of dead plants at the final pick was very low for all treatments except those treated with F251, which led to a significant ( $P < 0.05$ ) increase in the plant mortality when compared to all other treatments: 10% versus 1-2% (Fig. 1.2B).

Four weeks after the final pick, the proportion of plants with crown tissue browning was very high, reaching nearly 68%. The treatment effect on crown tissue browning followed the same pattern as for the number of plants with wilting at the final pick (Fig. 1.2AC). F251 led to increased crown browning whilst the other four products lessened crown browning.

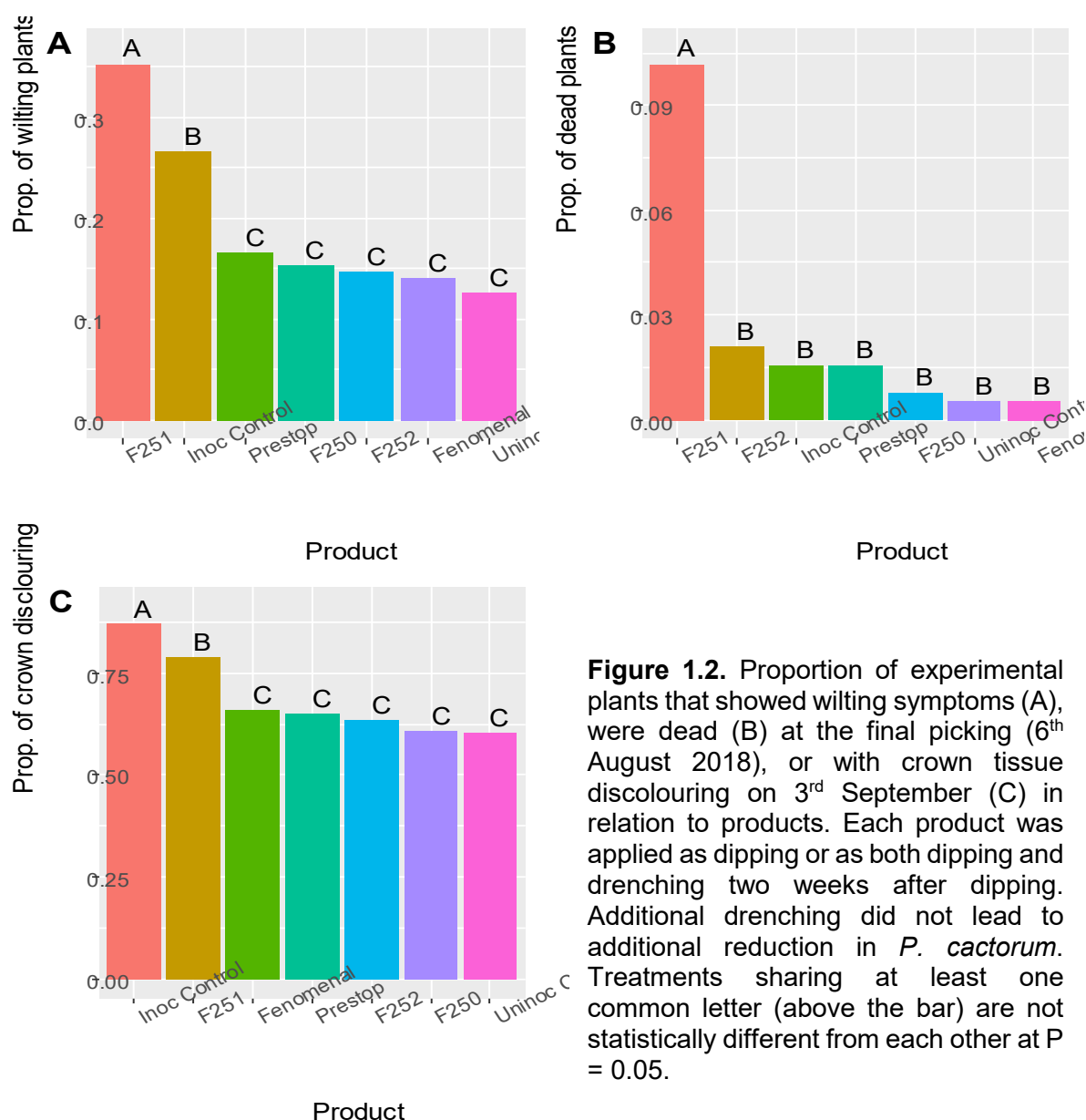
Irrigation was reduced on 17<sup>th</sup> and 24<sup>th</sup> August to stress plants to encourage *P. cactorum* development prior to the final destructive observation of crown tissues (Table 1.1). We did not present the September crown discolouring data as the DNA testing was used to detect the



**Figure 1.1.** Proportion of plants with wilting symptoms (including dead ones) over time on June bearer plants; planting date was 18<sup>th</sup> May 2018.

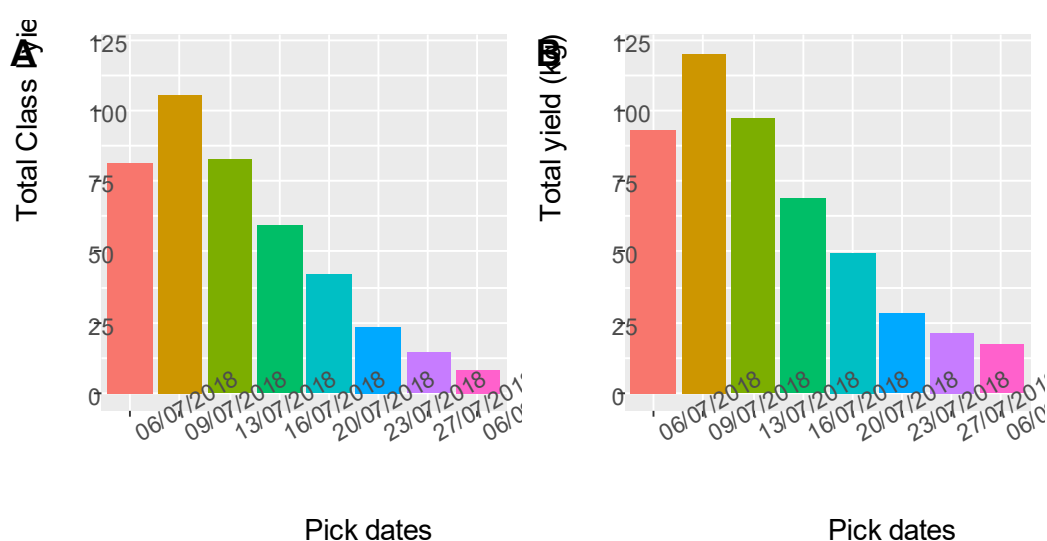
presence of *P. cactorum* in discolouring crown samples after the September visual assessment. We sampled a total 144 discoloured crown tissues for molecular detection of *P. cactorum* since Year 1-3 results showed that *Phytophthora* DNA was usually only detected in discoloured crown tissues [not in healthy tissues].

The number of samples was only 12 for the two control treatments and 24 for other product treatments, giving a total 144 plants. In 48% of the 144 samples, molecular testing showed positive results for presence of *P. cactorum*. Of the seven treatments (five products and two controls), the incidence (8%) of positive detection of *P. cactorum* following Fenomenal treatment was significantly ( $P < 0.01$ ) lower than all other six treatments based on simple pairwise comparisons of proportions. The incidence of positive detection of *P. cactorum* did not differ significantly among the other six treatments: inoculated control (50%), un-inoculated control (75%), F251 (50%), Prestop (67%), F250 (53%) and F252 (60%).

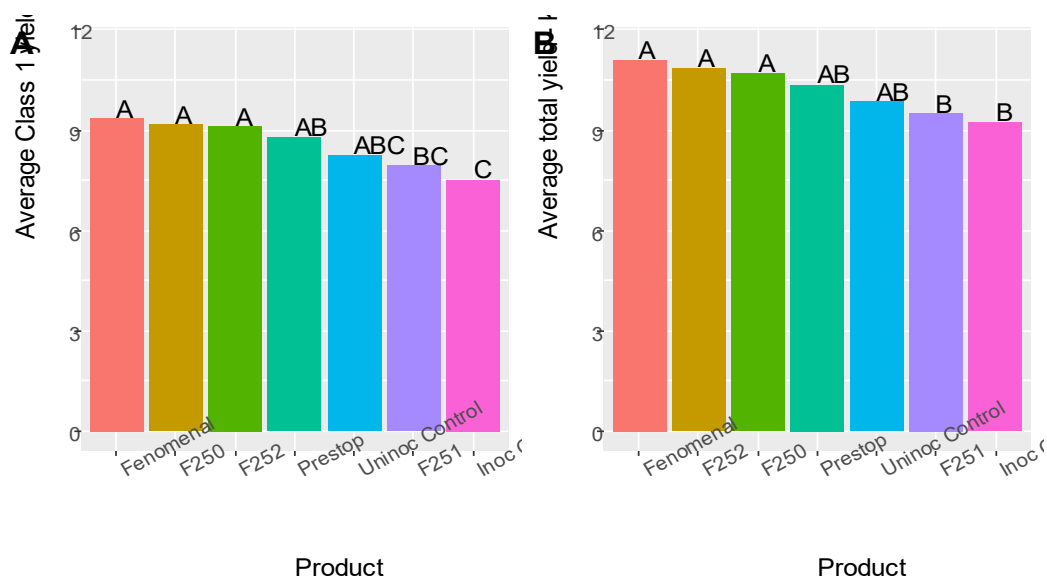


## Fruit yield

Fig. 1.3 shows Class 1 and total yield for each pick; the first five picks accounted for 89% and 87% of the total Class 1 and total yield, respectively. ANOVA indicated that additional drenching did not significantly affect fruit yield. Both Class 1 and total yield differed ( $P < 0.05$ ) among treatments. Of the five products tested, all the products except F251 significantly increased Class 1 yield over the inoculated control (Fig. 1.4A); there were no significant differences in the total class 1 yield among F250, F252, Prestop and Fenomenal. In addition to Fenomenal, both F252 and F250 significantly ( $P < 0.05$ ) increased Class 1 and total yield (Fig. 1.4) over the inoculated control but did not differ with the un-inoculated control.



**Figure 1.3.** Class I (A) and total (B) fruit yield on each picking date for the *P. cactorum* trial with June bearer plants.



**Figure 1.4.** Average Class I (A) and total (B) fruit yield of the June bearer plants per replicate for each product treatment. Each product was applied as dipping or drenching two weeks after dipping. Additional drenching did not affect fruit yield. Treatments sharing at least one common letter (above the bar) are not statistically different from each other at  $P = 0.05$ .

## Discussion

The present results indicated that treating plants with synthetic chemical or biological products at planting can significantly reduce the losses due to latent infection from artificial inoculation of *P. cactorum* in cold-stored plants.

However, additional drench treatment did not result in additional benefit. In some situations, additional post-planting drenching might offer some benefit in terms of protecting plants from new infections when new oospores (pathogen inoculum) are produced from the diseased plants in the growing media. This protection effect was demonstrated in previous AHDB funded work where plants in coir were drenched after diseased plants were first introduced as inoculum. In the present study, we did not observe any benefit associated with additional post-planting drenching; this may be explained by the following reasons. Firstly, in the present study, every plant was dip-treated at planting, which may have reduced inoculum production. Secondly, the disease development was not as severe as it might be: only ca 20% plants had visual wilting symptoms and 3% mortality at the final pick. Thus inoculum production from infected plants at the time of drenching, if any, would most likely have been minimal.

Of the five products tested, four performed similarly, achieving outcomes as good as the uninoculated control but better than the inoculated control, in terms of both disease development and fruit yield. Of the four products, Fenomenal is going to be withdrawn very soon with a final use date of November 2019 but was used as a standard for the purpose of treatment comparison. F250 is a new conventional fungicide under development and thus it is unlikely

to be available to commercial growers immediately. The other two are commercially formulated biocontrol products. F252 is approved for strawberries (under protection) for peat incorporation, drench, or via irrigation and so it should be able to be used in commercial strawberry production for managing *P. cactorum*. Prestop is also approved for use on strawberries (under protection) and is therefore available to growers.

One product (F251) led to significant an increase in disease development, mostly in the category of dead plants, and did not result in any improvement in fruit yield when compared with the inoculated control. Interestingly, this is a formulated microbial strain, which claims to induce plant resistance. Coincidentally, in one experiment we conducted in Year 2/3, the results also suggested that the use of mixed PGPR strains may also lead to increased *P. cactorum* development. This suggests that care may be needed when using ‘beneficial’ bacteria to manage plant diseases through induced plant defence responses.

There were high levels of discoloured crown tissues in all treatments – with the inoculated control being highest (nearly 90%), ca. 80% for F251, and ca. 60% for all the other treatments (including the un-inoculated control). Crown tissue discolouring can result from several factors, including infection by *P. cactorum*; results from years 3 showed that positive detection of *P. cactorum* DNA was nearly all in discoloured crown tissues. For the Fenomenal-treated plants, only 8% of discoloured tissues tested positive for *P. cactorum*. In contrast, a high proportion of the discoloured tissues (ranging from 50% to 75%) for all the other six treatments (including the un-inoculated) showed positive results for presence of *P. cactorum* via molecular testing, significantly higher than the Fenomenal-treated plants. This suggests that Fenomenal is able to kill latent infection whereas other products are probably only able to restrict pathogen development, avoiding crop losses.

The present results also suggested that the level of latent infection in the initial planting material could be as high as 30%; our inoculation managed to increase this significantly to ca. 45%. This level of latent infection in the initial material is high but not unexpectedly high given the survey results in Year 1-2. In 19 batches of plants we sampled at the planting time, six had incidence of *P. cactorum* higher than 15% with highest of 37.5%. Indeed, for a different batch of plants used in another study at NIAB EMR in 2018, we lost nearly 75% of plants due to *P. cactorum* from the start of flowering to the first pick. These results do suggest the magnitude of potential risks associated with latent infection of *P. cactorum* in initial planting material.



## **Objective 2: Epidemiological mode of action of new products against strawberry powdery mildew (SPM)**

### **Background**

Fungicides are often sprayed at regular intervals throughout a growing season to manage SPM. Usually, field trials are conducted to evaluate the effect of fungicide doses and application intervals on their mildew control efficacy. This approach of using fungicides based on the application dose and interval does not fully exploit the different characteristics conferred by modern fungicides, targeting different aspects of pathogen life cycles. This epidemiological mode of action against mildew 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 mildew fungicides, there is no information on their modes of actions, preventing their effective use in management programmes within the framework of disease predictions.

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

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

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

## Materials and method

The main objective was to determine the protective and anti-sporulant 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 F208 (coded biofungicide).

## General procedure

Table 2.1 gives the products tested and their rate of use. A wetter (Silwet) was applied together with AQ10 and F208; 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<br>(300 ppm) | 30000 ppm           | <ul style="list-style-type: none"> <li>• 1 ml product into 32.3 ml water (stock solution)</li> <li>• 2 ml stock solution to 198 ml water</li> </ul>    |
| Luna Sensation | 0.8 L<br>(1600 ppm) | 160000 ppm          | <ul style="list-style-type: none"> <li>• 4 ml product into 21 ml water (stock solution)</li> <li>• 2 ml stock solution to 198 ml water</li> </ul>      |
| Talius         | 0.25 L<br>(500 ppm) | 50000 ppm           | <ul style="list-style-type: none"> <li>• 1 ml product into 19 ml water (stock solution)</li> <li>• 2 ml stock solution to 198 ml water</li> </ul>      |
| AQ10 + Silwet  | 75 g<br>(150 ppm)   | 15000 ppm           | <ul style="list-style-type: none"> <li>• 0.5 g product into 33.3 ml water (stock solution)</li> <li>• 2 ml stock solution to 198 ml water</li> </ul>   |
| F208 + Silwet  | 5 L<br>(10000 ppm)  | 100000 ppm          | <ul style="list-style-type: none"> <li>• 2 ml product in 18 ml water (stock solution)</li> <li>• 20 ml stock solution to 180 ml water</li> </ul>       |
| Silwet         | 0.25 L<br>(500 ppm) | 50000 ppm           | <ul style="list-style-type: none"> <li>• 1 ml product into 19 ml water (stock solution)</li> <li>• 2 ml stock solution to 198 ml water</li> </ul>      |
| Charm          | 0.6 L<br>(1200 ppm) | 96000 ppm           | <ul style="list-style-type: none"> <li>• 0.96 ml product into 9.04 ml water (stock solution)</li> <li>• 2 ml stock solution to 158 ml water</li> </ul> |

## Location and plants

Tray plants of cv. “Malling Centenary” were used. This work was done in a glasshouse. A key requirement for this experiment was to keep batches of plants free from external SPM before

the exposure of treated plants to SPM inoculum. A glasshouse compartment was used as a 'clean' area with 'restricted' entry and plants in this area were checked at least twice weekly for SPM. If SPM was found, the infected leaves were removed and all plants sprayed with a standard 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.

## **Inoculation**

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

## **Environmental conditions**

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

## **Experimental design and assessment**

In all experiments, a completely randomised design was used; each treatment had five replicate plants. Each type of experiment was repeated once. 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), we estimated the % of leaf areas with SPM. Statistical comparisons were between treated and the controls in the same period [hence subjected to the same climatic conditions].

## **Protectant test**

The seven products (Charm, Takumi, Talius and Luna Sensation, AQ10, F208 and Silwet) were included for this test. 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 (or control)], each with five replicate plants. Only one study was carried out in June

as one replicate study had already been done in 2017. % leaf area with SPM on each treated/inoculated leaf was recorded 10 days after inoculation. Each plant had two leaves assessed; these two youngest leaves [one still curled, and the other one just fully/nearly unrolled] were susceptible to infection by SPM at the time of inoculation.

### **Anti-sporulant test**

Plants with fresh sporulating SPM lesions were selected for the antisporeulant test. Each of the seven products (Charm, Takumi, Talius and Luna Sensation, AQ10, F208 and Silwet) was applied directly to all actively sporulating SPM lesions on two plants with a hand-held sprayer until run-off. Water was used as the control treatment. To avoid cross contamination, plants allocated to one specific product were taken to another compartment for treatment and then moved back to the same compartment.

The cello-tape imprint technique was used to sample spores from three lesions for each treatment on each of the four sampling occasions: 1, 2, 4 and 7 days after treatment application. Plants were shaken gently one day before sampling to remove previously mature spores. A piece of the cello-tape was firmly pressed against a treated lesion, peeled off and placed onto a glass slide. On each glass slide, the number of deformed spores out of 200 was estimated under a microscope.

Two replicate experiments were conducted during the period of late September to mid-October.

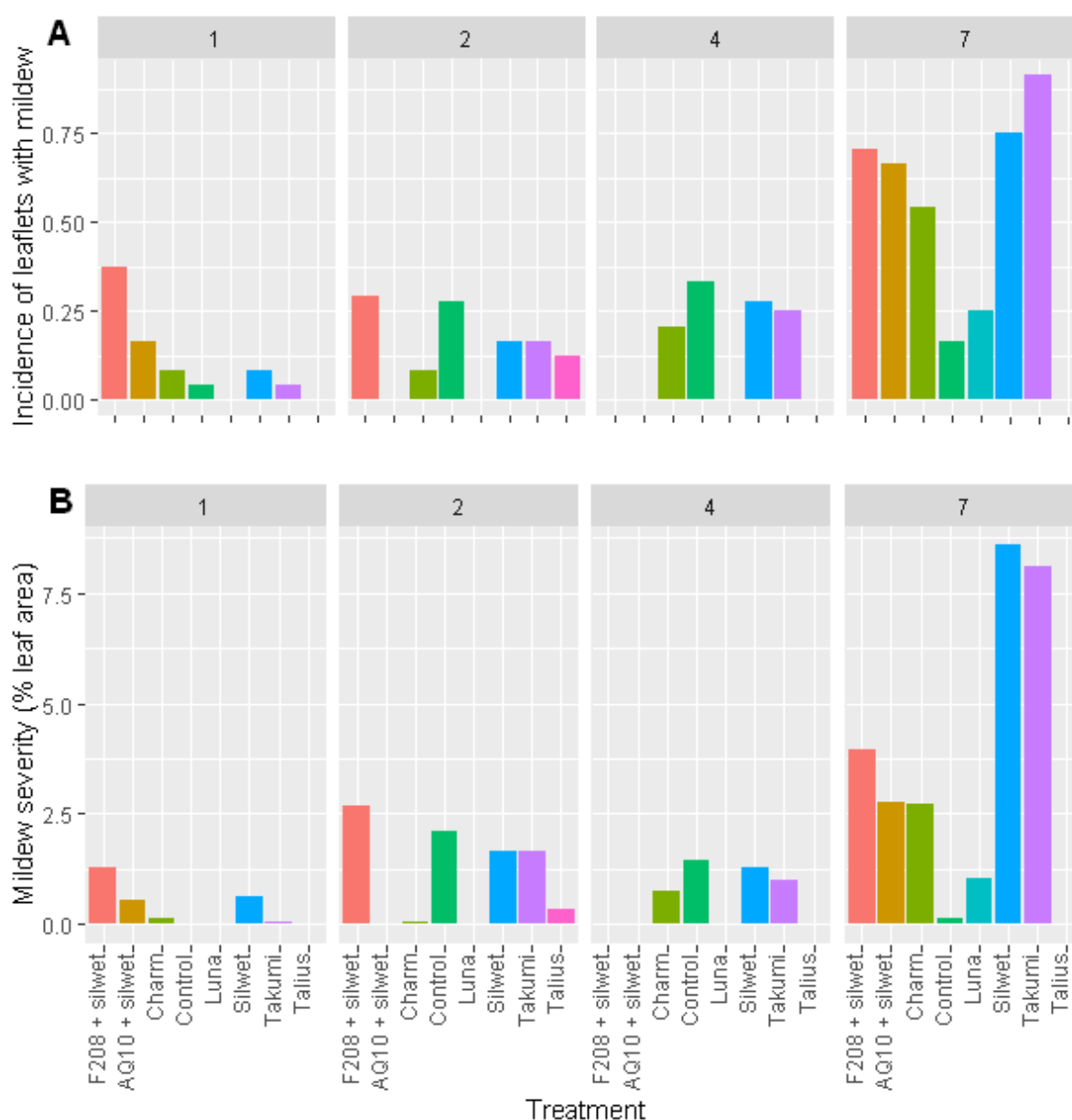
### **Data analysis**

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. As for the incidence of leaflets with SPM, GLM (with a binomial distribution assumed for errors) was used to analyse the antisporeulant test data. 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.

## Results

### Protectant tests

The level of SPM was low, except for the exposure 7 days after treatment (Fig. 2.1): overall, only 15% of inoculated leaflets developed visible lesions. SPM severity also varied greatly from plant to plant. There was a higher level of SPM for the last inoculation date (Fig. 2.1). However, the control had the lowest level of SPM, which meant that conclusions on fungicide protectant activity cannot be made from this experiment.



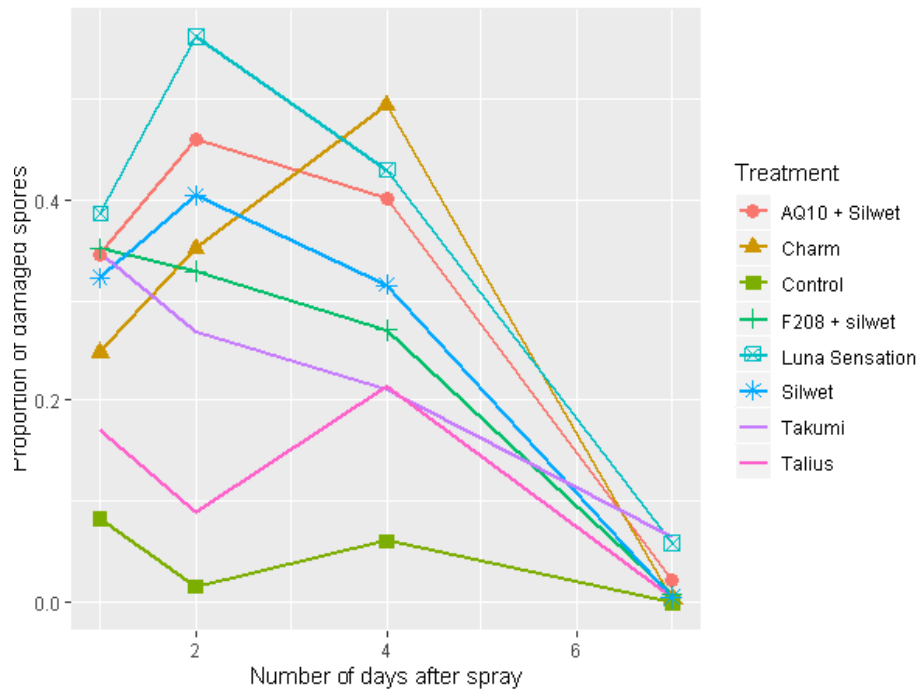
**Figure 2.1.** Protectant activity: proportion of strawberry leaflets with visible mildew lesions (A) and percentage of leaf area with mildew lesions (B) when inoculated one, two, four and seven (as indicated on the top frame of each graph) days after treatment application.

## Anti-sporulation tests

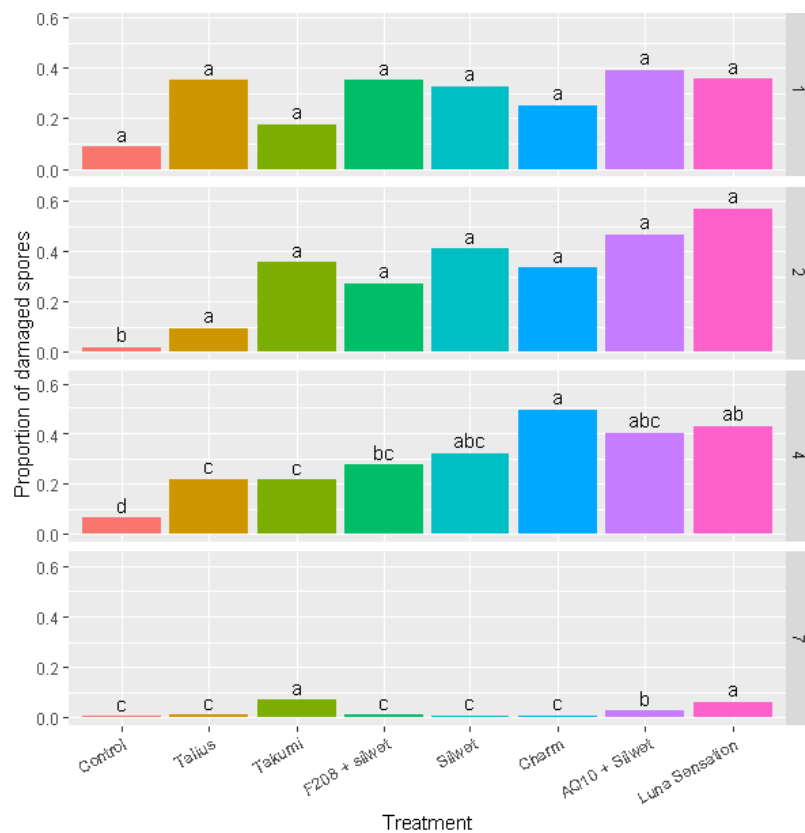
The proportion of deformed spores generally remained at a similar level for the first three assessments and decreased sharply at the last assessment (Fig. 2.2). The average proportion of deformed spores was 0.28, 0.31, 0.30 and 0.02 when assessed 1, 2, 4 and 7 days after treatment. Applying water alone (control treatment) led to 10% suppression of sporulation within 24 h; however, SPM colonies recovered sporulation ability within 48 h (Fig. 2.2). The negative impact of water (rainfall) on sporulation of powdery mildew fungi in general is a well-known phenomenon

Differences among the four assessment occasions accounted for 41% of the total variability in the number of deformed spores (on the logarithm scale) whereas treatment differences accounted for 15% of the total variability; both these differences were highly significant ( $P < 0.001$ ). The interaction between assessment time and treatment was close to statistical significance, mainly due to the much higher than expected effects for Charm on day 4 (Fig. 2.2). The overall percentage of deformed spores over the four assessments were 36%, 31%, 27%, 26%, 24%, 22%, 12% and 4% for Luna Sensation, AQ10, Charm, Silwet, F208, Takumi, Talius and water control, respectively.

One day after product application, all treatments except Talius had inhibited sporulation more ( $P < 0.05$ ) than the water control; nevertheless, there were no significant differences among the seven treatments (Fig. 2.3). On day 2 and 4, all treatments had inhibited ( $P < 0.05$ ) sporulation when compared with the water control (Fig. 2.3). Seven days after treatment, Luna Sensation, Takumi and AQ10 still had some antispore effects: with 6%, 7% and 2% deformed spores respectively, compared with 0% deformed spores for the water control (Fig. 2.3). AQ10 appeared to have consistently better antispore effects than Silwet used alone and F208+Silwet, albeit small, and only statistically significant on day 7.



**Figure 2.2.** Anti-sporulant activity: proportion of deformed spores (conidia) when assessed over time following application of treatments directly to sporulating lesions.



**Figure 2.3.** Proportion of deformed spores (conidia) when assessed over time following application of treatments directly to sporulating lesions. Within each assessment, treatments sharing at least one common letter did not differ significantly.

Based on the results from the last two years, we may summarise key findings of the fungicide work in the following table:

**Table 2.1a.** Protectant, curative and anti-sporulant properties of products effective for the control of powdery mildew on strawberry

| <b>Product</b> (approval status on strawberry)                       | <b>Protectant</b> (number of days applied before infection occurred) | <b>Curative</b> (number of days applied after infection where disease was controlled) | <b>Anti-sporulant</b> (number of days with good suppression of sporulation) |
|--|--|---|---|
| <b>Talius:</b> proquinazid (protected)                               | 7-8  | 2-3   | 2-3   |
| <b>Takumi:</b> cyflufenamid (outdoor & protected)                    | 4-5  | 2-3   | 2-3   |
| <b>Luna Sensation:</b> fluopyram/trifloxystrobin (protected)         | 4-5  | 2-3   | 4   |
| <b>Charm:</b> difenoconazole/fluxapyroxad (outdoor & protected)      | Yet to be tested   | Not tested  | 4   |
| <b>Silwet:</b> wetting agent (outdoor & protected)                   | Not tested   | Not tested (not expected to have an effect)   | 2-3   |
| <b>Silwet &amp; AQ 10:</b> <i>Ampelomyces quisqualis</i> (protected) | 2 (without Silwet)   | Not tested (not expected to have an effect)   | 4   |
| <b>Silwet &amp; F208:</b> coded (not approved)                       | 2-3 (without Silwet)   | Not tested (not expected to have an effect)   | 2-3   |

## Discussion

### Protectant test

The SPM level was very low in the repeat protectant trial in 2018, particularly for the first exposure periods (1, 2 and 4 days after treatment). This low level of SPM was most likely due to the extreme hot weather during the early summer of 2018. Another possible reason is the high level of latent infection by *P. cactorum* in this batch of planting material used in this study. In another experiment with the same batch of plants, we experienced a loss of 70% of plants due to *P. cactorum* from the start of flowering to the first pick, leading us to abandon the experiment. A high level of *P. cactorum* infection can affect SPM development: SPM generally develops better on young and actively growing leaves.

For the final exposure period (7 days after treatment), the level of SPM was higher than the



previous three exposure periods. However, because the control treatment had the least SPM development for the fourth exposure period, this repeat did not produce any useful data. Therefore, we need to re-do this repeat in 2019. We do not have biological explanations for this high level of SPM in the control treatment. The only explanation is that control plants allocated to the fourth exposure period by chance had more severe latent infection of *P. cactorum* than other treatments.

### **Antisporulant test**

When fungicides were applied to SPM lesions, the results demonstrated that all products except Talus can considerably suppress SPM sporulation up to 4 days after application. Although the antisporulant effects of Takumi and Luna Sensation were still significant 7 days after treatment, the percentages of deformed spores were very small and hence can be ignored in practical disease management. Although suppression of spore production by these products did not exceed 50%, the impact of this level of suppression on powdery mildew epidemics can be very important given the nature of exponential increase in inoculum levels (i.e. polycyclic disease development).

Although AQ10 and F208 demonstrated a good effect in suppressing SPM sporulation, we cannot be certain whether the biocontrol products AQ10 and F208 contributed significantly to the observed suppression as they were applied in combination with Silwet. Silwet on its own also led to comparable effects of suppressing sporulation (Fig. 2.3). It appears that AQ10 had consistently better antisporulant effects (albeit very small) than Silwet used alone and F208 + Silwet. This could be due to the specific biocontrol mechanism of AQ10 against powdery mildew: it parasitizes powdery mildew mycelia and spores and so its effect can be slow but persistent.

## **Objective 2: Integration of managed programmes for control of powdery mildew and fruit rots in protected strawberries (ORETO Trial 18/004)**

### **Introduction and objectives**

Trials in 2015 - 2016 identified effective products for control of powdery mildew 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 mildew 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 mildew, giving few opportunities to omit sprays. If the trial had been started in March, then there would have been more opportunities to manage the mildew during the period up to June when mildew risks are generally much lower.

The objective in 2018 was to explore how the approach for managing mildew could be integrated with control of botrytis and other fruit rots on everbearer crops.

### **Materials and methods**

#### **Study design**

##### ***Strawberry planting***

Ever bearer strawberry module plants were delivered in late March and held in a cold glasshouse until the start of the trial. The plants were planted on 20 April. A plantation at NIAB EMR, East Malling, Kent was used; it consisted of two Spanish tunnels with three mypex covered raised beds in each. To minimise the risk of waterlogging, plastic boxes (with holes to allow water through) were laid directly onto the mypex. The plants were planted into peat/coir bags (Botanicoir) on 20 April. Each bag contained eight plants, staggered in the bag, irrigated with two sub-drippers with trickle irrigation, located in the mid end section of each bag. Plants were fertigated with a 6 L per hour dripper (with four sub-drippers) shared between two bags, i.e. 3 L per hour per bag. There were 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 were slow to establish due to cool weather conditions but by the end of May had established well and were growing away. During this period the plants were treated for aphids with Caylpsa (thiacloprid) and for Phytophthora diseases with Fenomenal (fenamidone + fosetyl-AI). No other fungicides were applied.

## ***Treatments***

The programmes evaluated are given in Table 2.2. Details of the fungicides, BCAs, plant strengtheners and nutrients used in the programmes are given in Tables 2.3 - 2.5. All products received for inclusion in the trial were stored, handled and applied according to the manufacturer's instructions on the product label. All were applied as foliar sprays.

All treated plots were sprayed with Amistar (azoxystrobin) on 5 June. In addition, Cultigrow was applied to Treatments 3 and 5. The trial decision-based treatments were then started on 11 June. Decisions on spray applications to treatments 3 and 5 (SPM-managed) were based on the criteria given below in Tables 2.5 and 2.6 and 2.7. Decisions on spray applications to treatments 4 and 5 (Botrytis-managed) were based on criteria in Tables 2.8 and 2.9. 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 2.10.

## ***Spray application***

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

## ***Experimental Design***

The experiment was conducted with a randomised block design with four blocks (i.e. rows). Within each block there were five plots, each randomly assigned to one of the five treatments.

Within each plot, there were 10 bags (i.e. 80 plants). Plots were separated in the row by 2 metres.

**Table 2.2.** Treatment programmes evaluated at NIAB EMR in 2018

| <b>Treatment</b> | <b>Type</b>   | <b>Products</b>              | <b>Other</b>                                   |
|------------------|---|------------------------------|--|
| 1                | Untreated   | -                            | -  |
| 2                | Routine   | Fungicides                   | None   |
| 3                | Managed SPM<br>(Sprays for Botrytis as for T2)                | Fungicides and biofungicides | Cultigrow applied monthly from start of growth |
| 4                | Managed Botrytis and other rots.<br>(Sprays for SPM as in T2) | Fungicides and biofungicides | None   |
| 5                | Managed SPM and Botrytis / other rots                         | Fungicides and biofungicides | Cultigrow applied monthly from start of growth |

## Assessments

### *SPM and other diseases*

Plots were inspected for the presence of SPM twice 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 were assessed at an interval of three weeks using a standard key (Anonymous, 1976). A copy of the key is included in Appendix 2. This interval of assessment was used initially to allow for six assessments by late September. However, only one mildewed leaf was found in the trial plots during the whole of the trial period. So no full mildew assessments were carried out. Assessments on fruit were conducted at harvest as presence or absence of SPM.

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

**Table 2.3.** 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                           |
| Rovral         | iprodione                     | 1 kg                 | No          | 4                          | 2                     | dicarboximide                       | Botrytis, not compatible with AQ10 |
| 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       | quinoxifen                    | 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                      |
| 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 2.4.** Biofungicides for disease control on strawberry applied as foliar sprays

| Product       | Active ingredient              | Rate of product / ha | Maximum number of sprays | Product type        |
|---------------|--------------------------------|----------------------|--------------------------|---------------------|
| F208 + Silwet | Bacterial-based BCA            | 5 L + 0.05%          | Not specified            | BCA: SPM            |
| AQ10 + Silwet | <i>Ampelomyces quisqualis</i>  | 70 g + 0.05%         | 12                       | BCA: SPM            |
| Prestop       | <i>Gliocladium catenulatum</i> | 3 kg                 | 3                        | BCA: Botrytis       |
| Serenade      | <i>Bacillus subtilis</i>       | 10 L                 | 6                        | BCA: SPM / Botrytis |

**Table 2.5.** 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 |
| Sirius                      | silicon           | 0.05-0.1%            | 2-6 at 10-14 day intervals | Nutrient     |

**Table 2.6.** Criteria for SPM management decisions

| Item   | How determined  | Risk  | Management options  |
|--|---|---|---|
| Disease risk<br><b>Less important</b>  | 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  | <b>Product choice</b> – Fungicide or BCA                          |
| Growth stage and rate of growth  | Inspections 1-2 times per week  | Rapid leaf production, start of flowering/ fruiting indicates increased risk and possible change of product   | <b>Spray interval</b> – 7 or 14 days<br><b>Tunnel ventilation</b> |
| Mildew monitoring<br><b>Most important as short time between infection and visible mildew; need to spot new mildew on leaves</b> | Inspections 1-2 times per week on youngest leaves on 5 plants per plot. Plants selected at random for each inspection   | Scored 0-5,<br>0 = no SPM on leaves,<br>1 = <1% (new SPM lesion), 2 = 1-5 %, 3 = 5-10%, 4 = up to 20%, and 5 = > 20%<br><br>Flowers and fruit scored as presence or absence |   |

**Table 2.7.** Decision making criteria for selecting SPM treatments

| Predicted risk from model and forecast |                | Plant growth rate | Current disease level | Decisions (& product type)* |                |            |             |
|--|----------------|-------------------|-----------------------|-----------------------------|----------------|------------|-------------|
| Last 2 days                            | Last 7-10 days |                   |                       | Curative                    | Anti-sporulant | Protectant | Biocontrol* |
| Low                                    | Low            | Low               | Low                   |                             |                | X          | X           |
| Low                                    | Low            | Low               | High                  |                             | X              | X          | X (F208)    |
| Low                                    | Low            | High              | Low                   |                             |                | X          | X           |
| Low                                    | Low            | High              | High                  | X                           | X              | X          | X (F208)    |
| Low                                    | High           | Low               | Low                   | X                           |                |            | X           |
| Low                                    | High           | Low               | High                  | X                           | X              | X          | X (F208)    |
| Low                                    | High           | High              | Low                   | X                           |                |            | X           |
| Low                                    | High           | High              | High                  | X                           | X              | X          | X (F208)    |
| High                                   | Low            | Low               | Low                   |                             |                |            | X           |
| High                                   | Low            | Low               | High                  |                             | X              | X          | X (F208)    |
| High                                   | Low            | High              | Low                   |                             |                | X          | X           |
| High                                   | Low            | High              | High                  |                             | X              | X          | X (F208)    |
| High                                   | High           | Low               | Low                   | X                           | X              |            | X           |
| High                                   | High           | Low               | High                  | X                           | X              | X          | X (F208)    |
| High                                   | High           | High              | Low                   | X                           | X              | X          | X           |
| High                                   | High           | High              | High                  | X                           | X              | X          | X (F208)    |

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

**Table 2.8.** 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 2.9.** Criteria for Botrytis management decisions

| Item  | How determined   | Risk  | Management options  |
|---|--|---|---|
| Disease risk<br><b>Most important</b>   | 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 %   | <b>Product choice</b> – Fungicide BCA<br><br><b>Spray interval</b> – 7 or 14 days |
| Growth stage  | Weekly inspections   | Start of flowering  |   |
| Disease monitoring<br><b>Less important as long time between infection and visible Botrytis</b> | 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, 5=Sporing botrytis abundant throughout crop |   |

**Table 2.10.** 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          |

### **Harvest**

All fruit was picked and assessed for the presence of powdery mildew and other defects. For each plot at each pick, total yield, total number of fruit, total number of Class 1 fruit, and number of mildewed fruit and number of fruit with rots were recorded. At each harvest a random sample of 50 sound fruit was taken from each plot and placed in plastic trays or module trays in polythene bags and incubated at ambient temperature (20-25°C) for 7 days after which the rots present were recorded. This gave the total rot potential for the plot. The first pick was on 6 July and the last pick was on 17<sup>th</sup> September; a total of 20 picks.

### **Plant vigour**

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



### ***Phytotoxicity***

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

### ***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.

### ***Meteorological records***

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

The forward weather forecast used in the 'look up' tables was obtained from the BBC Weather website.

### ***Simple 'look-up' tables and models***

The 'look-up' tables developed for this experiment were devised as follows:

- Weather data for the last three years (2015-2017) was used to generate daily forecasts (using the original models implemented as computer software – see below).
- Daily average temperature and relative humidity values were derived.
- Based on researcher experience, powdery mildew or Botrytis daily risks were divided into three categories.
- Finally, AI algorithms (random forest tree) were used to derive the criteria (daily temperature and RH) for classifying daily risks into the three categories.

In this experiment, Botrytis and SPM models were run alongside the look-up tables 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 in 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  $\leq 1$  h. The model is driven by ambient relative humidity and shade temperature ( $^{\circ}\text{C}$ ).

### **Statistical analysis**

The data were analysed using a repeated measures ANOVA, combining data recorded over time for each type of variable. This takes account of the correlations between successive measurements from the same plot. All percentage figures were transformed to the angular scale before analysis. In addition, mean yield per plot for the fourteen harvests was also included. Fruit number was square root transformed and fruit size log transformed prior to analysis.

## **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**

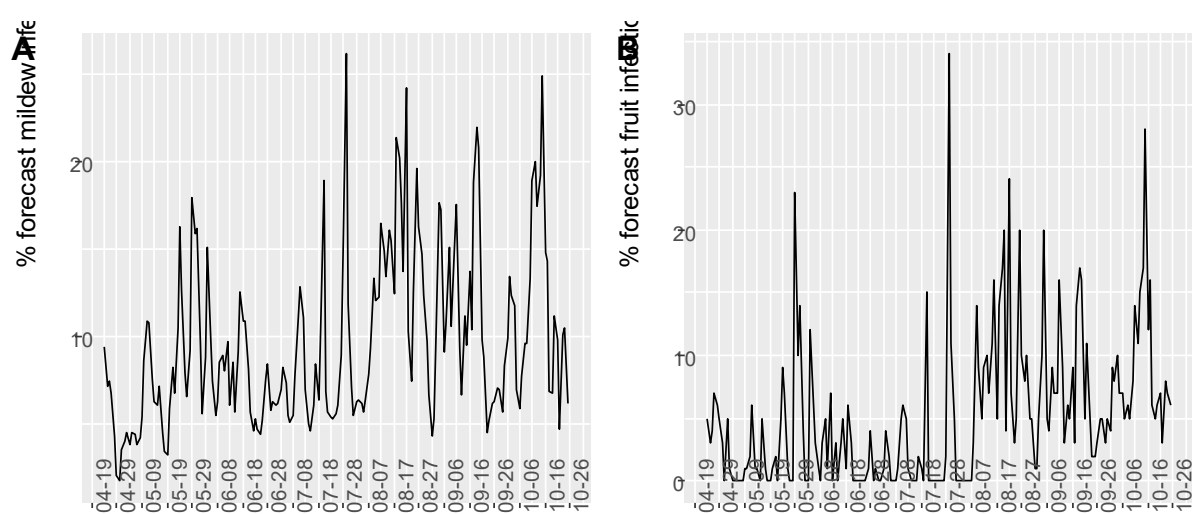
The weather conditions (warm temperatures coupled with high humidity) were very conducive to SPM development in late May / early June and from end of July onwards (Fig. 2.4A). The programmes applied to all treatments are given in Table 2.11 and summarised in Table 2.12. The trial activities, disease monitoring and assessments together with the decisions in response to the predicted risks, based on SPM monitoring in the crop and the model, are shown in Table 2.13. For treatments 3 and 5 the principle was to apply the BCA F208 as the basic treatment. Cultigrow was applied routinely at monthly intervals with Sirius as an option for an additional treatment. If the incidence of SPM increased or was predicted to increase, then the option was to switch to a fungicide or to change to AQ10 as an alternative BCA. However, SPM was found on only one leaf (23 July) and around two fruit throughout the whole

trial period which gave opportunities for 'saving' on treatments. The BCA F208 remained as the basic BCA treatment in programmes 3 and 5 and was applied on 5 occasions. Conventional fungicides for SPM were only applied twice. In comparison fungicides for SPM were applied at 7 day intervals to treatments 2 and 4 and a total of 13-14 fungicides were applied to these plots.

The simplified look-up prediction scheme did not perform well against the original model (Fig.2.5A). The look-up table appears to over-forecast days with 'moderate risks', namely there are many days with low levels of risks but classified as 'moderate' risk days. However, in 2018 this did not actually affect the decisions made as the absence of mildew in the crop inspections was an overriding factor.

### **SPM incidence**

Despite the high risk of SPM development in late May / early June and from late July onwards SPM failed to develop in the trial with the disease seen on only one leaf and two fruits throughout the trial period. No SPM was seen on the plants at planting time and no SPM appeared during the plant establishment in May. The incidence of SPM on other strawberry crops on the farm nearby was high but the disease failed to establish in this trial.



**Figure 2.4.** Predicted daily risk of (A) SPM and (B) Botrytis on susceptible cultivars for the NIAB EMR site in 2018. The predictions were given by the NIAB EMR model where a period of four (or more) consecutive days with risks > 10% is considered to need growers' intervention with a moderate to high level of inoculum (usually when the incidence of leaves with SPM is above 5%). Botrytis risk threshold risk is 10%.

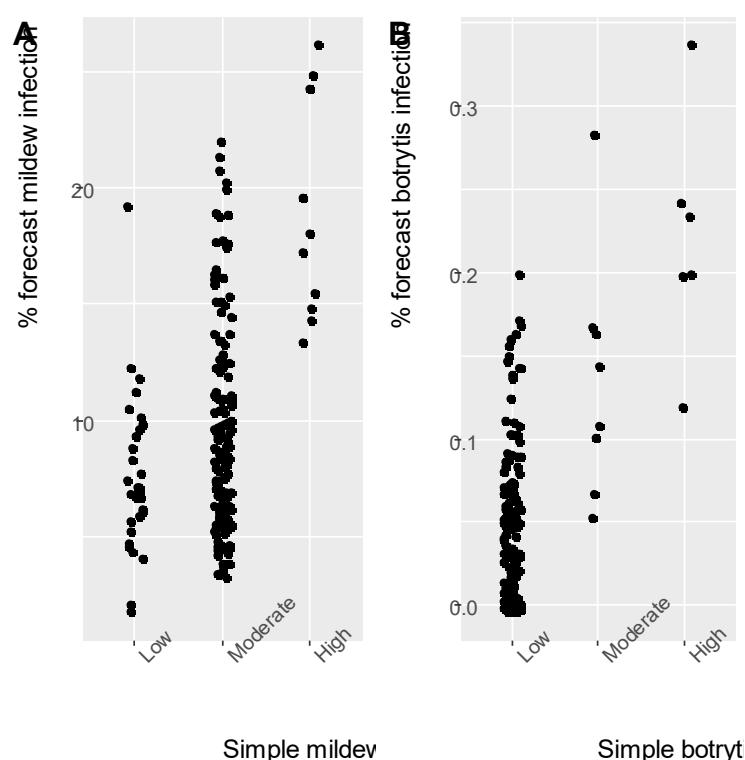
## Botrytis fruit rot

### **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 late May / early June and from end of July onwards (Fig. 2.4B). 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 Botrytis monitoring in the crop and the model, are shown in Table 2.13. Fungicides for

Botrytis control in the routine sprayed plots (T2 and T3) were applied at 7 days intervals from early June amounting to 13-14 fungicides in total over the trial period. Fewer conventional fungicides were used on these treatments at the end of the season as all available products had been used earlier and the BCA Serenade was used instead. In the managed treatments T4 and T5, only one fungicide for Botrytis was applied in June with 3-4 applied in July and 7 applied in the high risk period in August and September. As fewer fungicides had been applied earlier in the season there was no limit of fungicide choice in these treatments. Overall 11-12 fungicides for Botrytis were applied to T4 and T5. This was a saving of only 2 fungicides compared to the routine treatments. This was due to the high risk for Botrytis in August and September and the lack of confidence in using BCAs for Botrytis in high risk periods.

The simplified lookup disease prediction scheme did not perform well against the original model (Fig. 2.5B). The lookup table appears to under-forecast days with 'moderate risks', namely, there are many days with moderate levels of risks but classified as 'low' risk days. The lookup table was used to assist in the decisions on Botrytis control but as the sprays applied covered a seven day period, the disease risk was based on the forecast for seven



**Figure 2.5.** Disease forecasts from the simplified mildew (A) and botrytis (B) lookup tables compared against the disease forecasts from the original models.

days ahead, and decisions based on the average humidity and temperatures for that period, particularly during the night.

### ***Botrytis incidence***

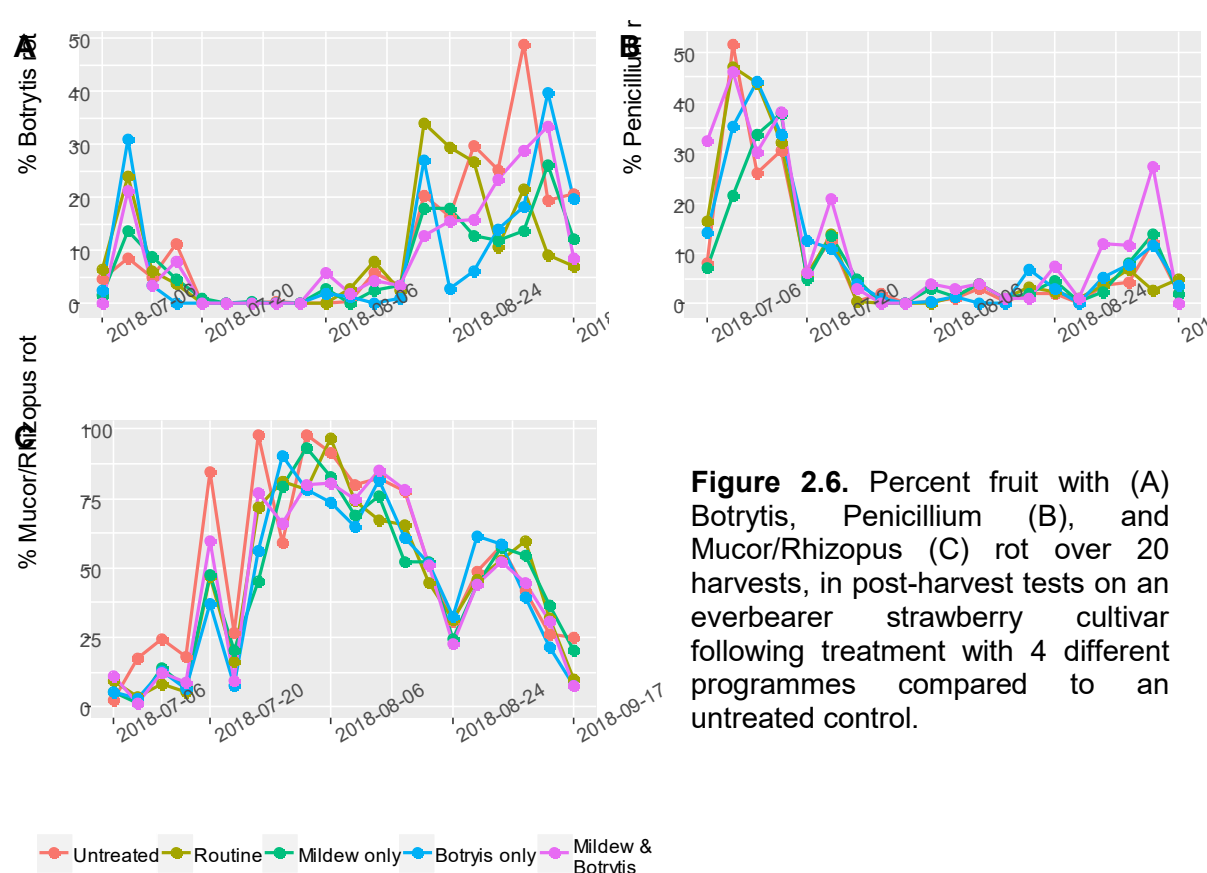
The incidence of post-harvest *Botrytis* rot for each individual pick is given in Appendix 3, whereas the incidence of all fruit rot is given in Appendix 6 for individual picks. The incidence of rots recorded at harvest was low with a mean of around 4% rots in untreated plots at the first harvest on 6 July, corresponding to the moderate *Botrytis* risk at the end of May / early June. Thereafter rot incidence at harvest was negligible until the harvest on 13 August when rot incidence increased (corresponding to the increased *Botrytis* risk from end of July to September Fig. 2.4B) but only to around a mean of 2.6% on untreated plots. Rots were recorded at harvest from 13 July to the final pick on 17 September but never at high incidence. *Botrytis* was the main rot present. The overall rot incidence at harvest over 20 picks is given in Table 2.14. There was no overall significant effect of the treatments on the incidence of rots. None of the treatments had any significant effect on rot incidence compared to the untreated control at any of the 20 harvests.

The incidence of *Botrytis* 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 (Fig. 2.4B). The actual incidence of *Botrytis* in the post-harvest tests was much higher than at harvest, particularly from mid-August onwards (Fig. 2.6A). The overall incidence of *Botrytis* in post-harvest tests is given in Table 2.15. There was no significant effect of treatments on the overall incidence of *Botrytis* in post-harvest tests. Over the 20 harvests the incidence of *Botrytis* in untreated plots in post-harvest tests ranged from 0 to 47.4%. There were no significant effects of treatments on *Botrytis* rot incidence compared to the untreated control in any of the 20 harvests.

### **Other fruit rots**

Rots due to *Penicillium* spp (Appendix 4), and soft rots, mainly *Rhizopus* spp (Appendix 5), were the other main rots recorded in post-harvest tests. *Penicillium* rot was recorded from 6 July to 23 July and then from 31 August to 17 September, at similar incidence to *Botrytis* (Fig. 2.6B). The overall incidence of *Penicillium* in post-harvest tests is given in Table 2.15. There was no significant effect of treatments on the incidence of *Penicillium* in fruit from the different programmes. Over the 20 harvests the incidence of *Penicillium* rot in untreated plots in post-harvest tests ranged from 0 to 51.9%. There were no significant effects of treatments on *Penicillium* rot incidence compared to the untreated control in any of the 20 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

during the hot period of July to late August where incidence was between 60-100% (Fig. 2.6C). 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 40%. Over the 20 harvests the incidence of *Mucor* / *Rhizopus* in untreated plots in post-harvest tests ranged from 1.2 to 99%. 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 40% soft rots. Fungicides in general have limited efficacy against *Penicillium* and *Mucor* / *Rhizopus* species.



**Figure 2.6.** Percent fruit with (A) Botrytis, Penicillium (B), and Mucor/Rhizopus (C) rot over 20 harvests, in post-harvest tests on an everbearer strawberry cultivar following treatment with 4 different programmes compared to an untreated control.

## **Other diseases**

The unknown fungus found colonising the stigmas on flowers in 2017 was again recorded in plots but later than in 2017 (29 August). The incidence was much lower. The fungus is still to be identified.

## **Harvest**

Fruit was harvested weekly or twice weekly from 6 July to 17 September, a total of 20 harvests. There were no overall significant effects of treatments on yield, fruit number, % Class 1 fruit, % unmarketable fruit or % rots at harvest (Table 2.14).

**Table 2.11.** Summary of fungicides, BCAs, biostimulants applied to strawberry plots at NIAB EMR 2018

| Growth stage / Date                | June                  |                 |                 |                 | July            |                 |                |                | August              |                 |                |                     | September      |                                   |          |                |                |
|------------------------------------|-----------------------|-----------------|-----------------|-----------------|-----------------|-----------------|----------------|----------------|---------------------|-----------------|----------------|---------------------|----------------|-----------------------------------|----------|----------------|----------------|
|                                    | 5                     | 11              | 18              | 25              | 2               | 9               | 16             | 23             | 25                  | 30              | 6              | 13                  | 20             | 28                                | 3        | 10             | 17             |
| T1 - Untreated                     | Nil                   | Nil             | Nil             | Nil             | Nil             | Nil             | Nil            | Nil            | Nil                 | Nil             | Nil            | Nil                 | Nil            | Nil                               | Nil      | Nil            | Nil            |
| T2 – Routine fungicide             | Amistar               | Topas + Frupica | Nimrod + Switch | Stroby + Signum | Topas + Frupica | Takumi + Teldor | Charm + Switch | Topas + Signum | Nil                 | Nimrod + Teldor | Takumi + Scala | Topas + Teldor      | Nimrod + Scala | Charm + Teldor                    | Serenade | Serenade       | Luna Sensation |
| T3 – SPM managed Routine Botrytis  | CBL + Amistar         | Frupica         | Switch          | Stroby + Signum | Frupica         | CBL + Teldor    | F208 + Switch  | Signum         | Luna Sensation      | F208 + Teldor   | F208 + Scala   | CBL + F208 + Teldor | Scala          | F208 + Teldor                     | Serenade | CBL + Serenade | Luna Sensation |
| T4 – Botrytis managed, routine SPM | Amistar               | Topas           | Nimrod          | Stroby + Signum | Topas           | Takumi + Teldor | Charm + Switch | Topas          | Luna Sensation      | Nimrod + Teldor | Takumi + Scala | Topas + Teldor      | Nimrod + Scala | Charm + Teldor                    | Signum   | Switch         | Luna Sensation |
| T5 – Managed SPM and Botrytis      | CBL + Amistar         | Nil             | Nil             | Stroby + Signum | Nil             | CBL             | F208 + Switch  | Nil            | Luna Sensation      | F208 + Teldor   | F208 + Scala   | CBL + F208+ Teldor  | Scala          | F208 + Teldor                     | Signum   | CBL + Switch   | Luna Sensation |
| Comments                           | 1 <sup>st</sup> spray | Low risk        | Low risk        | Mod risk        | Low risk        | Low risk        | Mod risk       | Low risk       | Mildew seen 23 July | Mod risk        | High risk      | High risk           | Low risk       | High Bot risk. Trace mildew found | Bot risk | Bot risk       | Bot risk       |



**Table 2.12.** Summary of number of fungicides, BCAs, biostimulants applied to strawberry plots at NIAB EMR 2018 and the programme costs

| Treatment period | Treatment                  | Management treatment |             |                                   |                                   |                              |
|------------------|----------------------------|----------------------|-------------|-----------------------------------|-----------------------------------|------------------------------|
|                  |                            | T1: Untreated        | T2: Routine | T3: SPM managed, routine Botrytis | T4: Routine SPM. Managed Botrytis | T5: Managed SPM and Botrytis |
| 5 June- 2 July   | Botrytis Fungicide         | 0                    | 4           | 4                                 | 1                                 | 1                            |
|                  | Mildew Fungicide           | 0                    | 5           | 2                                 | 5                                 | 2                            |
|                  | Biofungicide               | 0                    | 0           | 0                                 | 0                                 | 0                            |
|                  | Biostimulant               | 0                    | 0           | 1                                 | 0                                 | 1                            |
|                  |                            |                      |             |                                   |                                   |                              |
| 9 July-30 July   | Botrytis Fungicide         | 0                    | 4           | 5                                 | 4                                 | 3                            |
|                  | Mildew Fungicide           | 0                    | 4           | 1                                 | 5                                 | 1                            |
|                  | Biofungicide               | 0                    | 0           | 2                                 | 0                                 | 2                            |
|                  | Biostimulant               | 0                    | 0           | 1                                 | 0                                 | 1                            |
|                  |                            |                      |             |                                   |                                   |                              |
| 6 Aug-17 Sep     | Botrytis Fungicide         | 0                    | 5           | 5                                 | 7                                 | 7                            |
|                  | Mildew Fungicide           | 0                    | 5           | 1                                 | 5                                 | 1                            |
|                  | Biofungicide               | 0                    | 2           | 5                                 | 0                                 | 3                            |
|                  | Biostimulant               | 0                    | 0           | 2                                 | 0                                 | 2                            |
|                  |                            |                      |             |                                   |                                   |                              |
| Total            | Botrytis fungicides        | 0                    | 13          | 14                                | 12                                | 11                           |
|                  | Mildew fungicides          | 0                    | 14          | 4                                 | 15                                | 4                            |
|                  | Total fungicides           | 0                    | 27          | 18                                | 27                                | 15                           |
|                  | Biofungicides              | 0                    | 2           | 7                                 | 0                                 | 5                            |
|                  | Biostimulant               | 0                    | 0           | 4                                 | 0                                 | 4                            |
|                  | Fungicides + biofungicides | 0                    | 29          | 25                                | 27                                | 20                           |
|                  | Total products             | 0                    | 29          | 29                                | 27                                | 24                           |
| Cost £/ha        | Total programme            | 0                    | 2,278       | 2,169                             | 1,905                             | 1,579                        |
|                  | Powdery mildew only        | 0                    | 1,033       | 677                               | 890                               | 677                          |
|                  | Botrytis only              | 0                    | 1,596       | 1,700                             | 1,223                             | 1,111                        |

**Table 2.13.** Summary of strawberry treatments, assessments and management decisions in SPM and Botrytis management trial – NIAB EMR 2018

| Date   | Activity  |
|--------|---|
| 22 Mar | Plants delivered in modules and put in unheated glasshouse L until needed             |
| 14 Apr | Polytunnels, trays and bags set up. Plants de-blossomed in glasshouse                 |
| 20 Apr | Plants planted out in bags, 8 plants per bag, 10 bags per plot. No SPM seen on plants |
| 29 Apr | Cold weather polytunnel sealed up to protect plants from frost                        |

|         |   |
|---------|---|
| 4 May   | Calypso applied for aphids  |
| 9 May   | Checked tunnel with agronomist. No SPM seen. Some yellow leaves, possibly cold. Plants de-blossomed   |
| 5 Jun   | Amistar applied to treated plots for Botrytis and Cultigrow to T3 and 5   |
| 6 Jun   | No SPM seen. Botrytis low risk. No sprays on managed plots. Aphids still alive  |
| 8 Jun   | Chess applied for aphids  |
| 13 Jun  | No SPM seen. Botrytis low risk. No sprays on managed plots  |
| 20 Jun  | Forward forecast indicates moderate risk for Botrytis and SPM. Fungicide applied to managed plots   |
| 27 Jun  | No SPM seen. Botrytis low risk. No sprays on managed plots  |
| 4 Jul   | No SPM seen. Forecast indicates Botrytis risk. Botrytis fungicide to managed plots  |
| 6 Jul   | First harvest. Botrytis present at low incidence  |
| 9 Jul   | Second harvest. Botrytis present at low incidence   |
| 11 Jul  | Forecast indicates moderate risk for Botrytis and SPM. No SPM seen. F208 applied for SPM in managed plots and Botrytis fungicide  |
| 13 Jul  | Third harvest. Botrytis present at low incidence  |
| 16 Jul  | Fourth harvest. Low incidence of Botrytis   |
| 18 Jul  | No SPM seen. Low risk Botrytis and SPM forecast. No sprays managed plots  |
| 20 Jul  | Fifth harvest. Very low incidence of Botrytis   |
| 23 Jul  | Sixth harvest. SPM reported on one leaf by agronomist. No Botrytis  |
| 25 Jul  | Luna Sensation applied to T3-T5. No further SPM seen. Forecast indicates SPM and Botrytis risk moderate. Fungicide + F208 for managed plots                                     |
| 27 Jul  | Seventh harvest. No Botrytis  |
| 30 Jul  | Eighth harvest. No Botrytis   |
| 2 Aug   | No SPM seen. Forecast indicates high disease risk. Fungicide + F208 for managed plots   |
| 3 Aug   | Ninth harvest. No Botrytis  |
| 6 Aug   | Tenth harvest. Very low incidence of Botrytis   |
| 8 Aug   | No SPM seen. Forecast indicates high disease risk. Fungicide + F208 for managed plots. Capsid present and some yellowed plants  |
| 9 Aug   | Eleventh harvest. Very low incidence of Botrytis  |
| 13 Aug  | Twelfth harvest. Low incidence of Botrytis  |
| 15 Aug  | No SPM seen. Botrytis noted on fruit at harvest. Growth slowing. Forecast indicates low risk for both diseases. No sprays for managed plots                                     |
| 17 Aug  | Thirteenth harvest. SPM seen on 2 fruits at harvest. Low incidence of Botrytis  |
| 20 Aug  | Fourteenth harvest. Low incidence of Botrytis   |
| 22 Aug  | Trace SPM seen. Forecast indicates high disease risk. Fungicide + F208 for managed plots  |
| 24 Aug  | Fifteenth harvest. Low incidence of Botrytis  |
| 28 Aug  | Sixteenth harvest. Low incidence of Botrytis  |
| 29 Aug  | No SPM seen. Very few new flowers appearing. Flower fungus now present. Forecast high risk for Botrytis. Botrytis sprays for managed plots                                      |
| 31 Aug  | Seventeenth harvest. Low incidence of Botrytis  |
| 2 Sept  | No SPM seen. Low SPM risk but forecast indicates high Botrytis risk. Routine sprayed run out of fungicides so Serenade applied. Fungicide applied for Botrytis in managed plots |
| 4 Sept  | Eighteenth harvest. Weekly harvests as ripening slowing. Low incidence of Botrytis  |
| 6 Sept  | No SPM seen. Low SPM risk. Botrytis visible on some rotted fruit in plots. High risk Botrytis. Fungicide applied to managed plots   |
| 10 Sept | Last sprays applied. Nineteenth harvest. Low incidence of Botrytis  |
| 17 Sept | Final harvest (20 <sup>th</sup> ). Low incidence of Botrytis  |

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**Table 2.14.** Mean yield, fruit number (Square root transformed), % Class 1 fruit, % unmarketable and mean % total rots at harvest. (Both angular transformed) Mean of 20 harvests. (Figures in brackets are back transformed data)

| Treatment                     | Mean Total yield kg | Mean Total fruit number | Mean % Class 1 fruit | Mean % unmarketable fruit | Mean % Total rot |
|-------------------------------|---------------------|-------------------------|----------------------|---------------------------|------------------|
| T1: Untreated                 | 29.9                | 48.9 (2387.5)           | 56.5 (69.5)          | 31.7 (27.6)               | 8.5 (2.2)        |
| T2: Routine fungicide         | 26.8                | 47.2 (2227.9)           | 55.2 (67.4)          | 33.3 (30.2)               | 5.8 (1.0)        |
| T3: Mildew managed            | 28.4                | 47.9 (2293.2)           | 57.2 (70.6)          | 31.2 (26.8)               | 6.9 (1.4)        |
| T4: Botrytis managed          | 29.5                | 47.9 (2292.5)           | 57.3 (70.8)          | 30.9 (26.4)               | 7.3 (1.6)        |
| T5: Mildew + Botrytis managed | 27.8                | 47.3 (2236.6)           | 58.0 (71.9)          | 30.2 (25.3)               | 6.9 (1.4)        |
| F Prob                        | 0.28                | 0.62                    | 0.27                 | 0.22                      | 0.14             |
| SED (12)                      | 1.49                | 1.13                    | 1.23                 | 1.27                      | 0.92             |
| LSD (p=0.05)                  | 3.25                | 2.47                    | 2.67                 | 2.76                      | 2.00             |

**Table 2.15.** Mean % incidence of fruit rots (angular transformed) in post-harvest tests following incubation for 7 days at ambient temperature. Mean of 20 harvests. (figures in brackets are back transformed data)

| Treatment                     | Mean % Botrytis | Mean % Penicillium | Mean % Mucor / Rhizopus |
|-------------------------------|-----------------|--------------------|-------------------------|
| T1: Untreated                 | 19.3 (11.0)     | 16.8 (8.4)         | 46.2 b (52.1)           |
| T2: Routine fungicide         | 17.8 (9.3)      | 17.9 (9.4)         | 42.1 a (45.0)           |
| T3: Mildew managed            | 15.9 (7.5)      | 16.4 (8.0)         | 41.9 a (44.6)           |
| T4: Botrytis managed          | 16.9 (8.4)      | 18.1 (9.7)         | 40.7 a (42.6)           |
| T5: Mildew + Botrytis managed | 17.4 (8.9)      | 20.6 (12.4)        | 42.1 a (44.9)           |
| F Prob                        | 0.67            | 0.11               | 0.023                   |
| SED (12)                      | 2.27            | 1.49               | 1.44                    |
| LSD (p=0.05)                  | 4.94            | 3.24               | 3.13                    |

## Residue analysis

The results from residue analysis are shown in Table 2.16. The residues detected were all below the MRL with fewer residues detected in the first sampling on 22 August, when half the products were directed at SPM control. Least residues were detected in programmes 3 and 5 where SPM control was managed. By contrast at the second sampling, most residues were from products directed at Botrytis control, as expected given the increased use of products for Botrytis control in August and September. Most residues at this time were detected in programmes 4 and 5, which were managed for Botrytis control. Fewer were detected in programmes 2 and 3 as these were using biofungicides for control at this time, having run out of fungicides to apply. Up to eight residues were detected in fruit samples. At each of the

sampling times residues were also detected in the fruit from untreated plots which had received no fungicide sprays. This must have resulted from spray drift.

**Table 2.16.** Residues present in strawberry samples taken from Treatments T1-T5 on 22 August and 19 September

| Sample date  | Active ingredient | Treatment / Residue detected (mg/kg) |             |                                   |                                   |                              | EU MRL Mg/kg |
|--------------|-------------------|--------------------------------------|-------------|-----------------------------------|-----------------------------------|------------------------------|--------------|
|              |                   | T1: Untreated                        | T2: Routine | T3: SPM managed, routine Botrytis | T4: Routine SPM. Managed Botrytis | T5: Managed SPM and Botrytis |              |
| 22 August    | pyrimethanil      | 0.036                                |             |                                   | 4.0                               |                              | 5.0          |
|              | boscalid          |                                      | 0.039       | 0.04                              | 0.03                              |                              | 6.0          |
|              | bupirimate        |                                      | 1.0         |                                   | 1.1                               |                              | 2.0          |
|              | cyflufenamid      |                                      | 0.015       |                                   | 0.019                             |                              | 0.04         |
|              | fenhexamid        |                                      | 1.2         | 1.3                               | 2.6                               | 4.4                          | 10.0         |
|              | fludioxonil       |                                      | 0.022       | 0.018                             |                                   |                              | 4.0          |
|              | penconazole       |                                      | 0.021       |                                   | 0.03                              |                              | 0.5          |
|              | fluopyram         |                                      |             | 0.011                             | 0.06                              |                              | 2.0          |
| 19 September | cyprodonil        | 0.23                                 | 0.28        | 0.22                              | 0.63                              | 0.67                         | 5.0          |
|              | fludioxonil       | 0.22                                 | 0.27        | 0.21                              | 0.54                              | 0.63                         | 4.0          |
|              | pyrimethanil      | 0.23                                 | 0.65        | 0.38                              | 0.51                              | 0.35                         | 5.0          |
|              | fenhexamid        |                                      | 0.55        | 0.52                              | 0.23                              | 0.43                         | 10.0         |
|              | fluopyram         |                                      | 0.36        | 0.45                              | 0.22                              | 0.35                         | 2.0          |
|              | trifloxystrobin   |                                      | 0.43        | 0.54                              | 0.24                              | 0.44                         | 1.0          |
|              | boscalid          |                                      |             |                                   | 0.31                              | 0.36                         | 6.0          |
|              | pyraclostrobin    |                                      |             |                                   | 0.056                             | 0.043                        | 1.5          |

## Economic appraisal

The relative costs of the programmes are given in Table 2.12. The total cost of the programme applied to the routine plots was £2,278 /ha. A saving of £699 /ha was achieved by managing the SPM and Botrytis spray inputs. There was a saving of £356 /ha on sprays targeted at SPM and £485 /ha on sprays targeted at Botrytis. There were no penalties in yield or fruit quality as a result of the managed programmes.

## Discussion

The results from the managed trial have provided some interesting points for discussion. Of the two important disease problems in protected strawberry production, it is becoming clear that SPM is the more significant, as 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 (SWD), have restricted the build-up of rot inoculum in the crop, which previously was a significant factor, meaning that any impact on yield is now minimal. Throughout this trial the incidence of SPM was negligible both on leaves and fruit. This was most likely due to SPM-free plants at the start and the hot / dry weather in June and July was not conducive to mildew infection and establishment. So, despite favourable weather in August and September the absence of SPM inoculum in the plots meant the predicted mildew epidemic did not occur. This permitted large savings in fungicide sprays (by 10 sprays) and hence costs in the SPM-managed plots (£356 / ha). 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. So, there was little opportunity for reducing fungicide inputs in August and September if treatments were applied in the managed plots according to the risk. Overall, fewer fungicides were applied in the managed plots compared to the routine sprayed plots with a small saving in costs. However, in none of the 20 harvests was there a significant effect of treatment on Botrytis incidence compared to the untreated control. 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 40%). Therefore do the fungicide treatments applied for rot control have any benefit? In this trial, omitting the sprays for rots would have saved up to £1,600 / ha with little effect on yield and fruit quality, but also with a significant reduction in residues in the fruit. The post-harvest tests used in this trial give the maximum rot potential. In commercial practice the use of Cool chain management would delay the development of any rots.

As expected multiple residues were detected in the fruit, particularly in the samples taken in September, most of which related to products used for Botrytis control. Fruit sampled in August had fewer residues especially in the mildew-managed treatments (T3 and T5). All residues were below the MRL.

Overall using the simple decision-based system for determining treatments for powdery mildew 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, in the managed programme there were fungicide products in reserve for use towards the end of the season, whereas all products had been used up a month earlier in the routine programme

The trial planned for 2019 will continue to develop the managed approach to SPM and rot control but will also look at the use of sprays for rot control. It is important to establish whether the results obtained in this trial were related to the exceptional weather experienced in June and July.

## Summary and conclusions

- Weather conditions were very favourable for development of SPM in late May / early June and from late July onwards which was confirmed by the high risk (consecutive days with risk > 10%) shown by the mildew risk model. However, weather in June and July was hot and dry and not favourable for SPM
- The strawberry plants were mildew-free at planting and the disease failed to develop in the crop despite the favourable conditions in August and September. Only negligible levels were found on leaves and fruit
- This allowed savings in fungicide inputs in SPM managed plots with only four fungicides and five biofungicides applied compared to 14 fungicides and two biofungicides in the routine treated plots and with a cost saving of £356 /ha
- 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
- The incidence of rots recorded at harvest followed the Botrytis risk but was very low, ranging from 0 to 6% in untreated plots. None of the treatments had any significant effect on rot incidence compared to the untreated control at any of the 20 harvests.
- The incidence of Botrytis 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 20 harvests the incidence of Botrytis in untreated plots in post-harvest tests ranged from 0 to 47.4%. There were no significant effects of treatments on Botrytis rot incidence compared to the untreated control in any of the 20 harvests.
- A total of 13-14 fungicides over the trial period were applied for Botrytis control in routine sprayed plots (T2). Fewer fungicides were used on these treatments at the end of the season as all available products had been used earlier and the BCA Serenade was used instead. In the managed treatments T4 and T5, 11-12 fungicides for Botrytis were applied overall. This was a saving of only two fungicides compared to the routine treatments due to the high risk for Botrytis in August and September. This was a cost saving of £485 /ha
- Rots due to *Penicillium* spp. and soft rots, mainly *Rhizopus* spp. were the other main rots recorded in post-harvest tests. *Penicillium* rot had a similar incidence pattern to Botrytis. Over the 20 harvests the incidence of *Penicillium* rot in untreated plots in post-harvest tests

ranged from 0 to 51.9%. There were no significant effects of treatments on *Penicillium* rot incidence compared to the untreated control in any of the 20 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 during the hot period of July to late August where incidence was between 60-100%. Over the 20 harvests the incidence of *Mucor* / *Rhizopus* in untreated plots in post-harvest tests ranged from 1.2 to 99%. 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 40% soft rots
- The residues detected were all below the MRL with fewer residues detected in the first sampling on 22 August, when half the products were directed at SPM control. Least residues were detected in programmes 3 and 5 where SPM control was managed. By contrast at the second sampling, most residues were from products directed at *Botrytis* control, as expected given the increased use of products for *Botrytis* control in August and September. Most residues at this time were detected in programmes 4 and 5, which were managed for *Botrytis* control. Fewer were detected in programmes 2 and 3 as these were using BCAs for control at this time, having run out of fungicides to apply. Up to eight residues were detected in fruit samples.
- 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
- Overall a simple decision-based system for determining treatments for powdery mildew 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, in the managed programme there were fungicide products in reserve for use at the end of the season whereas all products had been used up a month earlier in the routine programme

## Reference

X.-M. Xu, D.C. Harris and A.M. Berrie, 2000. Modelling infection of strawberry flowers by *Botrytis cinerea* using field data. *Phytopathology*, **90**: 1367-1374

## **Objective 2: Demonstration of a SPM management strategy on strawberry on a commercial farm**

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, antisporeulant) 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. If growers were to take up the managed SPM strategy then the system needed to be evaluated and demonstrated on a commercial farm. In 2018 such a trial was established at a commercial site.

### **Materials and methods**

#### **Site**

Clock House Farms, Hatchgate Site (51.216764 N, 0.439398 E) using a commercial everbearer cultivar. The trial tunnel was 0.03 ha in size and consisted of five table tops with the everbearer planted in cocogreen coir bags (Fig. 2.7 yellow plot). This was compared to a similar sized end tunnel (Fig. 2.7 red plot) which received the standard Farm programme.





**Figure 2.7.** Site at Hatchgate Farm, Mill Lane Yalding, Kent

## Treatments

The treatment programmes are given in Table 2.17. The trial tunnel was used for the managed SPM programme and compared to SPM control in the control tunnel. Visits were made twice weekly from April to September to check the trial tunnel for SPM and other diseases. Spray decisions were based on visual assessments and the SPM risk obtained from the forward forecast obtained from the internet and using the criteria (look-up tables) given in Tables 2.6 to 2.8. Alternative products used in the trial tunnel are given in Table 2.18. It was not possible to include the BCA F208 in this trial as the product only has an experimental approval for trials use with crop destruct. Decisions on treatments for the trial tunnel were sent to the spray man at Clockhouse responsible for the trial by email. All treatments to the trial tunnel were applied by the farm using commercial sprayers

Pest monitoring and control was carried out by the farm agronomist and treatments applied as needed. Irrigation and nutrition for the trial tunnel followed the standard farm practice.

Initially it was planned for management decisions to be made on SPM control only with treatments for control of Botrytis and other diseases applied routinely as needed for the management programme. However, it soon became clear that separating management of the

two diseases in the commercial situation was not practical so spray decisions regarding treatment for the trial tunnel applied to both SPM and Botrytis using the look-up table criteria in Tables 2.9 and 2.10 for decisions on Botrytis control.

## **Assessments**

### ***SPM and other diseases***

A full assessment for SPM on leaves as percentage leaf area infected on the youngest five expanded leaves on each of ten plants per row (5 rows per tunnel = 50 plants in total) was done monthly using a standard key (Anon, 1976). SPM on flowers or fruitlets was assessed separately if needed. BBCH crop growth stage was recorded at each assessment time.

Assessments for other diseases (eg leaf spots) were made as needed.

### ***Plant vigour***

If during the trial differences in plant vigour become apparent between the treatments then formal assessments were made by measuring the height and spread of 50 plants per plot.

### ***Harvest***

A sample of 150 fruit was picked from the trial and control tunnel at monthly intervals from June until September. The weight was recorded and fruit assessed for size, quality and rots. The fruit was then incubated at ambient temperature at high humidity and the rots recorded after 7 days. For the first month of harvest (June) records of yield were taken by the farm for the trial and control tunnels.

### ***Residues***

Fruit from the trial and control tunnels was sampled on three occasions and sent for multi-residue analysis.

### ***Meteorological records***

A data logger (USB-502) was placed at crop height in the tunnel to monitor temperature and humidity. This was downloaded weekly and used to estimate daily average maximum and minimum temperature / humidity. This data was used to run the SPM and botrytis models.

The forward forecast was obtained from the BBC Weather website.

### ***Simple 'look-up' tables and models***

The same 'look-up' tables derived to determine SPM and Botrytis risk in the previous experiment were used. Again, the NIAB-EMR SPM and Botrytis models were run alongside the look-up tables to allow a comparison of the two approaches.

### ***Economic appraisal***

At the end of the season, the cost associated with the two treatments as well as fruit yield were 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 powdery mildew development in late May / early June and from end of July onwards (Fig.2.8A). The programmes applied to all treatments are given in Table 2.20. 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 2.19. Although conditions were favourable for SPM in early June and from end of July onwards no mildew was observed in the tunnel until 13 September when fresh colonies were found on runners hanging down in the crop. A spray of Luna Sensation was advised to ensure eradication of the mildew combined with removal of the runners. The absence of SPM in the trial tunnel gave the opportunity to omit fungicide sprays (Table 2.20) and over the trial period 10 sprays were applied for mildew in the trial tunnel compared to 19 sprays on the control tunnels. Similarly no mildew was seen in the control tunnel either.

**Table 2.17.** Treatment programmes evaluated at Hatchgate Farm in 2018

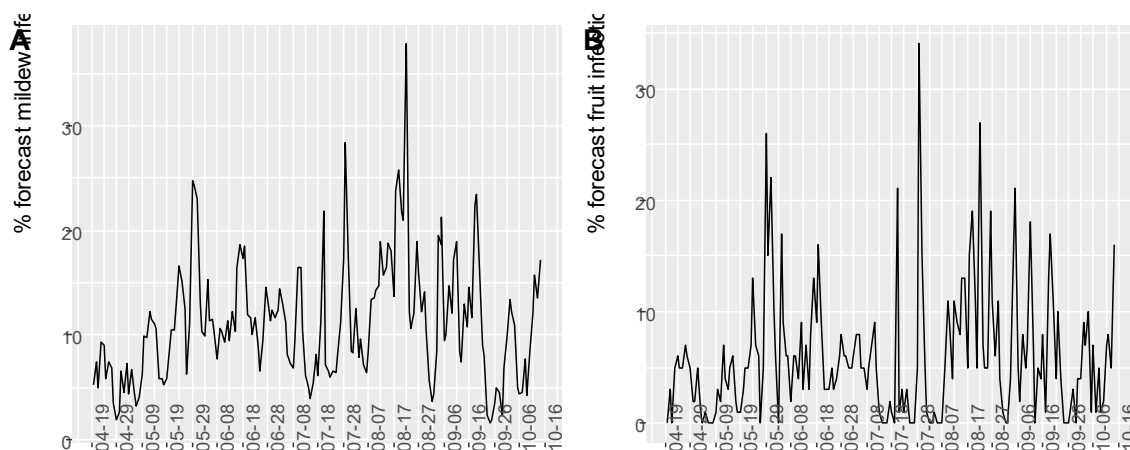
| <b>Treatment</b> | <b>Type</b>              | <b>Products</b>                               | <b>Other</b>   |
|------------------|--------------------------|---|--|
| 1 (Red)          | Routine Farm programme   | Fungicides +<br>(depending on Farm programme) | None (but depending on Farm programme)                       |
| 2 (Yellow)       | Managed SPM and Botrytis | Fungicides, AQ10, Amylo X WG                  | Cultigrow (Cropbiolife) applied monthly from start of growth |

**Table 2.18.** BCAs and plant extracts products for disease control on strawberry to be applied as foliar sprays

| Product                     | Active ingredient                      | Rate of product / ha | Maximum number of sprays                             | Product type          |
|-----------------------------|--|----------------------|--|-----------------------|
| AQ10 + Silwet               | <i>Ampelomyces quisqualis</i>          | 70 g + 0.05%         | 12   | BCA, SPM              |
| Amylo X WG                  | <i>Bacillus amyloliquefaciens</i> D747 | 2.5 kg               | 6 at 7 day intervals up to BBCH 89 (fruits coloured) | BCA, SPM and Botrytis |
| Serenade                    | <i>Bacillus subtilis</i> QST 713       | 10 L                 | 20 per crop  | BCA, SPM and Botrytis |
| Cultigrow CBL (Cropbiolife) | flavonoids                             | 250 ml               | 5 at 28 day intervals                                | Plant strengthener    |
| Sirius                      | silicon                                | 0.05-0.1%            | 2-6 at 10-14 day intervals                           | Nutrient              |

### Botrytis risk

The weather conditions (warm temperatures coupled with high humidity) were very conducive to Botrytis infection and development in late May / early June and from end of July onwards (Fig.2.8B). The programmes applied to all treatments are given in Table 2.20. 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 2.19. A low incidence of Botrytis was observed in the trial tunnel in early April on old flowers which were being removed. Sporulating Botrytis was also noted on old fruits from 2017 crop present as debris in the bags (bags in use for second year). The hot dry weather in June and July was not conducive to Botrytis with opportunities for reducing sprays. The incidence of Botrytis inoculum in the Trial tunnel increased particularly in August and September. Most of this was on shrivelled fruit. A total of 13 fungicides were applied for Botrytis control to the control tunnels compared to 8 in the trial tunnel.



**Figure 2.8.** Predicted daily risk of (A) SPM and (B) Botrytis on susceptible cultivars for the Clock House Farm site in 2018. The predictions were given by the NIAB EMR model where a period of four (or more) consecutive days with risks > 10% is considered to need growers' intervention with a moderate to high level of inoculum. Botrytis risk threshold risk is 10%.

**Table 2.19.** Record of visits, assessments and risks for spray decisions on the trial tunnel at Clock House Farm at Hatchgate, Yalding in 2018

| Date     | Record of work done, observations made or reference to lab or field book entry   |
|----------|--|
| 10 April | Recently planted. No new growth, cool / humid. Botrytis present on old flowers. Requested Amistar spray same as other tunnels                  |
| 12 April | Some new leaf showing, cool / humid plants de-blossomed  |
| 17 April | Some new leaves, warmer, breezy. Low RH No mildew seen. No spray   |
| 19 April | More leaf growth, Warm / breezy. No mildew seen. No spray  |
| 24 April | 2-3 new leaves. 13-15°C, breezy. Moderate RH. No mildew seen. Requested Mildew spray (Fortress) + CBL as new leaves                            |
| 26 April | No mildew seen. Leaf growth slowed. Cold wind low RH   |
| 1 May    | Cool, slight breeze. Very wet and windy 30 April. No mildew seen. No spray.  |
| 8 May    | New leaf growth and flowers. Warmer 15°C+25°C expected. No mildew seen. Full assessment trial and Control tunnels. No mildew. Requested spray. |
| 10 May   | Moderate leaf growth. Lower temperature, Breezy low RH. No mildew seen   |
| 15 May   | Moderate leaf growth + flowers Temperatures 15-16°C. Breezy, cold nights. No mildew seen. No spray.  |
| 17 May   | Well-developed plants + new flowers. Low/moderate temperature cool nights. No spray.   |
| 22 May   | Moderate leaf and flower development. Cool temperatures, cool nights. Low risk both. No mildew seen. No spray                                  |
| 24 May   | Moderate leaf and flower development. Cool temperatures 14-16°C, cool nights. Low risk both. No mildew seen. CBL spray requested.              |
| 28 May   | Moderate-high growth leaves and flowers. High temperatures and RH over last few days. No mildew seen. Request Botrytis and mildew spray.       |
| 5 June   | Some ripe fruit and runners present. Moderate – high leaf and flower growth. Weather cooler and breezy. Lower risk for both. No mildew seen.   |
| 7 June   | First harvest. Moderate-high leaf and flower growth. Cool / breezy. No mildew seen. Low risk. No sprays.                                       |
| 12 June  | Moderate-high leaf and flower growth. Cool / breezy. No mildew seen. Low risk. No sprays.  |
| 14 June  | Moderate-high leaf and flower growth. Cool 15°C/ breezy. No mildew seen. Low risk. No sprays.  |

|              |   |
|--------------|---|
| 19 June      | Moderate-high leaf and flower growth. Cloudy / warm 20°C. No mildew seen. Moderate risk. Sprays for both requested. First fruit samples taken from Trial and Control tunnels. Residue samples taken.  |
| 21 June      | Moderate-high leaf and flower growth. Cloudy / cooler 15-20°C. No mildew seen. Moderate risk. Sprayed 20 June.  |
| 26 June      | Moderate-high leaf and flower growth. Hot 20-25°C. No mildew seen. Low risk No spray.   |
| 28 June      | Moderate-high leaf and flower growth. Warm 19-21°C. No mildew seen. Low risk No spray.  |
| 3 July       | Moderate-high leaf and flower growth. Warm 22°C. Humid No mildew seen. Moderate risk CBL + fungicide requested.   |
| 5 July       | Moderate-high leaf and flower growth. Warm 22°C. Humid No mildew seen. Moderate risk.   |
| 10 July      | Moderate-high leaf and flower growth. Much cooler than last week. 17°C. Breezy No mildew seen. Second fruit sample from trial and control tunnel.   |
| 12 July      | Moderate-high leaf and flower growth. Much cooler than last week. 16°C. Breezy No mildew seen. No spray.  |
| 17 July      | Moderate-high leaf and flower growth. Hotter over last few days. 21°C. Breezy No mildew seen. No spray  |
| 19 July      | Moderate-high leaf and flower growth. Cooler over last few days. Breezy No mildew seen. No spray.   |
| 25 July      | Moderate-high leaf and flower growth. Hotter over last few days. 21°C + High RH. No mildew seen. CBL + fungicide spray requested.   |
| 31 July      | Moderate-high leaf and flower growth. Fruit size decreased. Some rain in last few days > RH No mildew seen. 200 fruit sample taken from both tunnels. Residue sample taken  |
| 2 August     | Moderate-high leaf and flower growth. Hotter temperature. No mildew seen. Flower fungus present. Spray requested  |
| 7 August     | Moderate-high leaf and flower growth. Hot / humid. Rain forecast. No mildew seen. Flower fungus present also in Control tunnel. Spray requested.  |
| 9 August     | Moderate-high leaf and flower growth. Temperature cooler 15-18°C High RH. No mildew seen. Flower fungus very obvious.   |
| 14 August    | Moderate-high leaf and flower growth. Smaller fruit size. Cooler temperature 18-19°C. Breezy. No mildew seen. Sporing Botrytis seen on shrivelled fruits. Flower fungus present. High Botrytis risk Spray requested.  |
| 16 August    | Growth slowing Cooler temperature 18-19°C. Breezy. No mildew seen.  |
| 21 August    | Growth slowing. Cooler temperature 18-19°C. Breezy. Humid night No mildew seen. Sporing Botrytis seen on shrivelled fruits. Flower fungus incidence assessed (50% flowers infected). High Botrytis risk Spray requested. Fruit samples taken from both tunnels. |
| 23 August    | Growth. Smaller fruit size. Cool. Breezy. Lower RH. No mildew seen. Sporing Botrytis seen on shrivelled fruits. Flower fungus present.  |
| 28 August    | Growth. Smaller fruit size. Cool. RH increasing. No mildew seen. Sporing Botrytis seen on shrivelled fruits. Flower fungus present. High Botrytis risk. Spray requested.  |
| 4 September  | Growth. Smaller fruit size. Cool. Breezy. Low RH No mildew seen. Sporing Botrytis seen on shrivelled fruits. Botrytis is main risk.   |
| 6 September  | Growth. Smaller fruit size. Cool. Breezy. Low RH No mildew seen. Sporing Botrytis seen on shrivelled fruits. Botrytis is main risk. Spray requested.  |
| 11 September | Growth. Smaller fruit size. Warm, Breezy. Low RH No mildew seen. Sporing Botrytis seen on shrivelled fruits. Botrytis is main risk. 150 fruit sample from Trial and Control tunnel.   |
| 13 September | Growth. Smaller fruit size. Warm, high RH 1 <sup>st</sup> mildew seen on runner. Sporing Botrytis seen on shrivelled fruits. Requested mildew spray – Luna Sensation and to remove runners ASAP.  |
| 18 September | Growth slowing down. Cool, windy, Low RH. No new mildew seen. None seen in Control tunnel. Residue samples taken  |
| 20 September | Growth slowing down. Cool, windy, Low RH. No new mildew seen. Plenty of sporing Botrytis. Spray requested.  |
| 25 September | Growth slowing down. Cool, cold nights, High RH. No new mildew seen. Plenty of sporing Botrytis.  |
| 16 October   | Final visit. Very few new flowers. Warm days. Cool nights. No new mildew seen. Flower fungus present at high incidence.   |

**Table 2.20.** Fungicide sprays applied to the trial tunnel versus rest of site at Hatchgate Farm, Yalding in 2018

| <b>Spray date</b>         | <b>Control tunnels</b>                        | <b>Trial tunnel</b>                           |
|---------------------------|---|---|
| 12 April                  | Amistar; Hortiphyte                           | Amistar; Hortiphyte                           |
| 13 April                  | Paraat  |   |
| 24 April                  |   | Cultigrow; Fortress                           |
| 7 May                     | Stroby; Fortress; Hortiphyte                  |   |
| 14 May                    | Topas   | Topas   |
| 21 May                    | Charm   | Hortiphyte                                    |
| 25 May                    | Takumi; Teldor                                |   |
| 30 May                    |   | Takumi; Teldor                                |
| 31 May                    | Talius; Prolectus; Hortiphyte                 | Cultigrow; Hortiphyte                         |
| 7 June                    | Luna Sensation; Maxicrop Triple               | Maxicrop Triple                               |
| 14 June                   | Frupica; Nimrod; Calmax Ultra                 | Calmax Ultra                                  |
| 20 June                   | Luna Sensation; Maxicrop Triple; Calmax Ultra | Luna Sensation; Maxicrop Triple; Calmax Ultra |
| 2 July                    | Nimrod  | Cultigrow; Nimrod                             |
| 7 July                    | AQ10  |   |
| 11 July                   | Amistar; Maxicrop Triple; Calmax Ultra        | Maxicrop Triple; Calmax Ultra                 |
| 20 July                   | Maxicrop Triple                               | Maxicrop Triple                               |
| 28 July                   | Takumi; Calmax Ultra                          | Takumi; Calmax Ultra; Ametros                 |
| 3-7 August                | Amistar; Maxicrop Triple                      | Charm; Maxicrop Triple                        |
| 14 August                 | Frupica; Hortiphyte                           | Frupica; Hortiphyte                           |
| 21 August                 | AQ10  | AQ10  |
| 27 August                 | Topas; Teldor; Maxicrop Triple                | Teldor; Maxicrop Triple                       |
| 30 August                 | Amistar Top; Maxicrop Triple                  | Charm; Cultigrow                              |
| 7 September               | Scala; Kindred; Nimrod; Maxicrop Triple       | Scala; Maxicrop Triple                        |
| 15 September              | Amistar Top; Maxicrop Triple                  | Luna Sensation                                |
| 22 September              | Teldor  | Teldor  |
| <b>Total Fungicides</b>   |   |   |
| for <i>Botrytis</i>       | 13  | 8   |
| for SPM                   | 19  | 10  |
| <b>Total</b>              | <b>26</b>                                     | <b>15</b>                                     |
| <b>Other products</b>     |   |   |
| Biofungicides             | 2   | 1   |
| Cultigrow                 | 0   | 5   |
| Other biostimulants       | 13  | 11  |
| <b>Total all products</b> | <b>41</b>                                     | <b>32</b>                                     |
| <b>Cost £/ha</b>          |   |   |
| Total                     | 1715.08                                       | 1272.22                                       |
| Mildew only               | 1110.10                                       | 848.23  |
| Botrytis only             | 934.44  | 623.99  |

## Residues

The result of residue analysis on three samples of fruit is shown in Table 2.21. Most residues were recorded in the June and September samples. For the first sample taken in June nine

different fungicide residues were recorded in fruit from the control tunnel compared to four in the trial fruit. Actual residues for the chemicals found were also lower in the trial fruit sample. In the September sample eight residues were recorded in the fruit from the trial tunnel compared to six in the control fruit. The additional two residues in the trial fruit – fluopyram + trifloxystrobin resulted from the Luna Sensation applied on 15 September to control the first SPM seen. All residues found were below the MRL for the chemical.

**Table 2.21.** Residues (mg/kg) present in strawberry samples taken from the control and trial tunnels on 19 June, 31 July and 18 September

| Sample date  | Active ingredient | Treatment |       | EU MRL |
|--------------|-------------------|-----------|-------|--------|
|              |                   | Control   | Trial |        |
| 19 June      | bupirimate        | 0.16      | 0.046 | 2.0    |
|              | cyflufenamid      | 0.029     | 0.016 | 0.04   |
|              | fenhexamid        | 0.35      | 0.19  | 10.0   |
|              | fluopyram         | 0.17      |       | 2.0    |
|              | difenoconazole    | 0.027     |       | 0.5    |
|              | mepanipyrim       | 0.42      | 0.11  | 3.0    |
|              | proquinazid       | 0.047     |       | 1.5    |
|              | trifloxystrobin   | 0.18      |       | 1.0    |
|              | azoxystrobin      | 0.016     |       | 10.0   |
|              |                   |           |       |        |
| 31 July      | cyflufenamid      | 0.023     | 0.02  | 0.04   |
|              | difenoconazole    | 0.011     |       | 0.5    |
| 18 September | azoxystrobin      | 0.34      | 0.11  | 10.0   |
|              | bupirimate        | 0.064     | 0.054 | 2.0    |
|              | difenoconazole    | 0.26      | 0.07  | 0.5    |
|              | fenhexamid        | 0.2       | 0.21  | 10.0   |
|              | mepanipyrim       | 0.013     | 0.011 | 3.0    |
|              | pyrimethanil      | 0.46      | 0.39  | 5.0    |
|              | fluopyram         |           | 0.19  | 2.0    |
|              | trifloxystrobin   |           | 0.22  | 1.0    |

## Yield and Fruit quality

The weight and size of the 150 fruit samples is shown in Table 2.22. Fruit size was consistently slightly larger in fruit from the trial plot. Waste was also lower. The yield for the two tunnels recorded by the Farm in June (Table 2.23) was lower for the trial tunnel by around 13 kg.

## Incidence of Botrytis and other diseases

The incidence of Botrytis in post-harvest tests on fruit sampled from control and trial tunnels is shown in Table 2.24. Botrytis incidence was similar in the control and trial fruit with the highest incidence in both around 85% recorded in the first sample and corresponding to the



moderate Botrytis risk at the end of May. Other rots recorded were *Penicillium*, which was at similar incidence in samples and *Mucor* / *Rhizopus* which tended to be at higher incidence in the trial tunnel (Table 2.24).

The flower fungus noted at NIAB EMR in 2017 was first observed in the Trial tunnel on 2 August and steadily increased in incidence from then onwards. An assessment of infected flowers on 25 August showed a mean of 50% of the flowers in the Trial tunnel were infected with a similar incidence in the Control tunnel. Shrivelled fruit present appeared to have obvious flower fungus present on the stigmas. Infected flowers were tagged in an attempt to see whether they resulted in shrivelled fruit but the results were inconclusive. Comments from growers suggested the shrivelled fruit related to the hot dry conditions, rather than any fungal cause.

## Economic appraisal

The relative costs of the programmes are given in Table 2.20. The total cost of the programme applied to the routine plots was £1715.08/ha. A saving of £442.86 /ha was achieved by managing the SPM and Botrytis spray inputs. There was a saving of £261.87 /ha on sprays targeted at SPM and £310.45 /ha on sprays targeted at Botrytis. There were no penalties in yield, fruit quality or rots as a result of the managed programmes.

**Table 2.22.** Fruit quality data for everbearer strawberry for Trial tunnel versus Control tunnel at Clock House Farm site at Yalding, Kent in 2018

| Sample date         | Treatment      | Weight kg<br>150 fruit | Fruit quality data / % fruit in size categories |             |             |            |             |
|---------------------|----------------|------------------------|---|-------------|-------------|------------|-------------|
|                     |                |                        | > 45 mm   | 35-45 mm    | < 35 mm     | < 25 mm    | Waste       |
| 19 June             | Control        | 5.53                   | 50.0  | 50.0        | 0           | 0          | 11.5        |
|                     | Trial          | 6.03                   | 63.6  | 35.8        | 0.7         | 0          | 7.9         |
| 10 July             | Control        | 4.09                   | 81.3  | 18.7        | 0           | 0          | 10.7        |
|                     | Trial          | 4.05                   | 71.3  | 28.7        | 0           | 0          | 4.7         |
| 31 July             | Control        | 4.12                   | 5.0   | 49.5        | 45.5        | 0          | 8.5         |
|                     | Trial          | 4.15                   | 4.8   | 57.6        | 37.6        | 0          | 8.6         |
| 21 August           | Control        | 1.66                   | 0.7   | 7.3         | 86.8        | 5.3        | 23.2        |
|                     | Trial          | 1.85                   | 1.3   | 13.9        | 74.8        | 9.9        | 17.9        |
| 11 September        | Control        | 2.07                   | 0.7   | 20.7        | 73.3        | 5.3        | 8.0         |
|                     | Trial          | 2.2                    | 5.3   | 23.3        | 66.6        | 4.7        | 7.3         |
| <b>Total / Mean</b> | <b>Control</b> | <b>17.47</b>           | <b>27.5</b>                                     | <b>29.2</b> | <b>41.1</b> | <b>2.1</b> | <b>12.4</b> |
|                     | <b>Trial</b>   | <b>18.28</b>           | <b>29.3</b>                                     | <b>31.9</b> | <b>35.9</b> | <b>2.9</b> | <b>9.3</b>  |

**Table 2.23.** Yield data for everbearer strawberry for Trial tunnel versus Control tunnel at Clock House Farm site at Yalding, Kent in June 2018

| Harvest date                  | Control            |                   | Trial              |                   |
|-------------------------------|--------------------|-------------------|--------------------|-------------------|
|                               | Number of trays    | Fruit weight (kg) | Number of trays    | Fruit weight (kg) |
| 6 June (1 <sup>st</sup> pick) | 0                  | 2.50              | 0                  | 2.50              |
| 16 June                       | 9.9                | 39.60             | 8.4                | 33.60             |
| 19 June                       | 11.6               | 46.40             | 10.4               | 41.60             |
| 22 June                       | 15.4               | 61.60             | 13.7               | 54.80             |
| 26 June                       | 11.1+1 market tray | 30.64             | 13.1+1 market tray | 35.44             |
| <b>Total</b>                  | <b>49</b>          | <b>180.74</b>     | <b>46.6</b>        | <b>167.94</b>     |

**Table 2.24.** Incidence of Botrytis (%) fruit rot in everbearer strawberries from Control and trial tunnels at Clock House Farm Yalding Site following incubation for 7 days post-harvest

| Sample date  | Treatment | % Rot    |             |                  |
|--------------|-----------|----------|-------------|------------------|
|              |           | Botrytis | Penicillium | Mucor / Rhizopus |
| 19 June      | Control   | 85.7     | 10.9        | 0                |
|              | Trial     | 86.4     | 4.0         | 8.8              |
| 10 July      | Control   | 22.0     | 1.3         | 0                |
|              | Trial     | 20.3     | 3.3         | 3.3              |
| 31 July      | Control   | 6.8      | 3.7         | 42.0             |
|              | Trial     | 12.3     | 6.8         | 60.5             |
| 21 August    | Control   | 36.7     | 4.7         | 39.3             |
|              | Trial     | 20.0     | 0.7         | 64.0             |
| 11 September | Control   | 4.3      | 0           | 17.9             |
|              | Trial     | 4.9      | 0           | 9.7              |

## Discussion

As with the trial at NIAB EMR, in this trial the incidence of SPM was negligible both on leaves and fruit. The same everbearer was used in both trials and probably supplied from the same source. The plants were free from SPM at the start and the hot / dry weather in June and July contributed to the negligible incidence of SPM at the site, which was also quite isolated from other strawberry plantings. This permitted large savings in fungicide sprays and hence costs in the SPM-managed plots (£261.87 / ha). For Botrytis the risks shown by the models were at the beginning of the crop and again from late July onwards. There were opportunities for reducing sprays in the trial tunnel in the first part of the season but less in August and September. The incidence of Botrytis in the fruit at the first harvest in post-harvest tests was high and similar in both plots. Botrytis incidence was also similar at each of the other harvest dates. Penicillium and soft rots were also recorded. The incidence of soft rots was again high in July and August and generally at higher incidence in fruit from the trial tunnel. The reason

for this is not clear. A total of 13 fungicides were applied for Botrytis control to the control tunnels compared to 8 in the trial tunnel, a saving in cost of £310.45 /ha. As in the trial at NIAB EMR reducing the fungicide inputs appeared to have little impact on rot control, although without an untreated control it is not possible to say what the rot incidence would have been in the absence of fungicides. Residues in fruit in the trial tunnel were less than in the control tunnel in the first two samples but similar in the last sample when more fungicides were being used for Botrytis control.

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.

Both this trial and the trial at NIAB EMR have demonstrated the potential in reducing spray inputs, reducing costs and residues by following a decision-based management system.

## **Summary and conclusions**

- Weather conditions were very favourable for development of SPM in late May / early June and from late July onwards which was confirmed by the high risk (consecutive days with risk > 10%) shown by the mildew risk model. However, weather in June and July was hot and dry and not favourable for SPM
- The strawberry plants were mildew-free at planting and the disease failed to develop in the crop despite the favourable conditions in August and September. Mildew was only found in the crop on runners in September
- This allowed savings in fungicide inputs in SPM managed tunnel with only 10 fungicides applied compared to 19 fungicides in the control and with a cost saving of £261.87 /ha
- 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 control to the control tunnels compared to 8 in the trial tunnel. 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.
- Penicillium and soft rots were also recorded. The incidence of soft rots was again high in July and August and generally at higher incidence in fruit from the trial tunnel. The reason for this is not clear
- Most residues were recorded in the June and September samples. For the first sample taken in June nine different fungicide residues were recorded in fruit from the control tunnel

compared to four in the trial fruit, reflecting the lower fungicide use in the trial tunnel. Actual residues for the chemicals found were also lower in the trial fruit sample. In the September sample eight residues were recorded in the fruit from the trial tunnel compared to six in the control fruit. The additional two residues in the trial fruit – fluopyram + trifloxystrobin resulted from the Luna Sensation applied on 15 September to control the first SPM seen. All residues found were below the MRL for the chemical.

- 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.

## **References**

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

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

The following posters are presented below:

1. Wileman, H., Liu, B. & Hall, A. 2018. A rule-based prediction system improves spray precision for the control of strawberry powdery mildew. ICPP Conference, Boston, USA, 2018.
2. Wileman, H. & Hall, A. 2018. Use of a real-time decision support system to give accurate timings for fungicide applications. BSPP Annual Presidential Meeting, University of Warwick, 2018.
3. Asiana, I., Hall, A.M. & Davies, K. 2018. The deposition of silicon linked to the reduction in susceptibility to strawberry powdery mildew. ICPP Conference, Boston, USA, 2018.
4. Asiana, I., Hall, A.M. & Davies, K. 2018. Are strawberries ever deficient in silicon? BSPP Annual Presidential Meeting, University of Warwick, 2018.



# A rule-based prediction system improves spray precision for the control of strawberry powdery mildew

Hannah Wileman, Bo Liu & Avice Hall

University of Hertfordshire, Hatfield, Hertfordshire, AL109AB, UK.

## INTRODUCTION

Strawberry powdery mildew (SPM) is a serious disease of the strawberry crop, caused by the fungus *Podosphaera aphanis*. An infection can result in yield losses of 20-70%, resulting in £56.8-196 million losses. Symptoms include leaf cupping (Fig.1), red blotches and white mycelium on the leaves, petioles, flowers and/or fruit.



**Fig. 1:** Leaf cupping is an early symptom of a SPM infection. (Jin, 2015)

Environmental conditions such as temperature and relative humidity (RH) were found to affect SPM disease development. A rule-based prediction system was devised recording the accumulated numbers of hours (144) of conditions necessary for the fungus to germinate, grow and produce spores. For germination: RH >60%, temperature 15.5-30°C; with optimum temperature for growth 18-30°C. These parameters identify the high risk days when sporulation may occur, thus allows growers to spray fungicide at the optimal time of the primary infection<sup>1</sup>.

After the primary research<sup>2</sup> based on the above parameters, prediction software was delivered as a CD-format. It predicted when to spray fungicides accurately, controlled disease with reduced sprays but the usability was challenging. Using the same parameters a web-based real-time system was developed, to be easier to use and act as a decision support system.

In order for the prediction system to be more widely adopted, it needs to effectively control the disease, be trusted by growers and easy to use. It should be validated to ensure that it meets these criteria.

## MATERIALS & METHODS

Validation in 2017 of the web-based real-time prediction system was conducted at a farm in the West Midlands, England. In several tunnels fungicide applications were performed according to the system. This was compared to a control field of equal size, containing the same strawberry variety (Prize), however, fungicide was sprayed according to a normal commercial spray programme.

Monthly, 100 leaves were assessed for mycelium, giving the total disease level across the sample and percentage disease cover using an assessment key.

The prediction system was used by the growers, who checked the system daily to decide when a fungicide spray was needed. When using the system, green suggests low disease level, up to 115 hours; amber then indicates to the grower that they should prepare to spray; at 125 hours, red represents when sporulation is likely to occur. Fungicide was often applied when the system gave an amber warning; once complete the system was reset (Fig. 2).

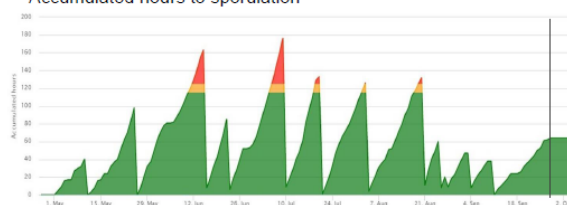
Parameters analysed:

- Disease control
- Number of fungicide applications and modes of action used
- Cost-benefit analysis
- Usability of system

## RESULTS

- Use of the prediction system provided satisfactory control of SPM development
- Fewer fungicide applications were used in prediction system (15) than control field (20), a reduction by five
- Prediction system gave better use of fungicide modes of action. Each mode of action was used one less time than in control field, due to fewer applications
- Over £300 per hectare was saved using the prediction system, due to reduced fungicide usage and labour costs
- The grower on the farm in the West Midlands, England was able to successfully utilise the prediction system to support the management of fungicide applications (Fig. 2)

Accumulated hours to sporulation



**Fig. 2:** Web-based prediction system graph used by farm in the West Midlands (2017). Peaks on the graph show where hours have accumulated under disease conducive conditions. When fungicide spray is applied, accumulated hours is reset to 0.

## DISCUSSION

By using the web-based real-time prediction system as a decision support system the grower was able to use fewer fungicide applications whilst maintaining control of the disease. Therefore, reducing the potential for fungicide residues on the fruit, which is in the consumers interest. By using less fungicide applications and fewer modes of action of those applied; the selection pressure on the fungus is reduced, consequently, decreasing the risk of fungicide resistance.

The use of the prediction system enables the grower to spray with precision, therefore, maximising fungicide effectivity on disease control, whilst making monetary savings.

## FUTURE WORK

In the 2018 season the prediction system is continuing to be validated and used on six farm sites across Great Britain, two in England and four in Scotland. Effect on disease control, number of fungicide applications and mode of action used will again be assessed, as well as additional cost benefit analyses.

## REFERENCES

- [1] Dodgson J, Hall A.M. & Parker S. (2007) System to predict high risk periods for *Podosphaera aphanis* infection of strawberries grown in polythene tunnels. *Aspects of Applied Biology*, No. 83.
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## Acknowledgements

Many thanks to Henry and Harriet Duncalfe of Maltmas farm, who have used and continue to use the prediction system for the duration of this research, additionally providing information that has aided in the analysis of this data. Also many thanks to Richard Hibbard of E.C. Drummond Ltd for using the prediction system through the 2017 period. Finally thanks to Carmilla Asiana and project students involved in this research.



# Use of a real-time decision support system to give accurate timings for fungicide applications

Hannah Wileman & Avice Hall

University of Hertfordshire, Hatfield, Hertfordshire, AL109AB, UK.

## INTRODUCTION

Strawberry powdery mildew is a serious disease of the strawberry crop, caused by the fungus *Podosphaera aphanis*. An infection can result in yield losses up to 70%, at 20% this results in £56.8 million losses<sup>1</sup>. Symptoms include leaf cupping (Figure 1), red blotches and white mycelium on the leaves, petioles, flowers and/or fruit.

Environmental conditions such as temperature and relative humidity (RH) were found to affect strawberry powdery mildew development. A rule-based prediction system was devised recording the accumulated numbers of hours (144) of conditions necessary for the fungus to germinate, grow and produce spores. For germination: RH >60%, temperature 15.5-30°C; with optimum temperature for growth 18-30°C. These parameters identify the high risk days when sporulation may occur, thus allows growers to spray fungicide at the optimal time of the primary infection<sup>2</sup>.

After the primary research<sup>3</sup> based on the above parameters, prediction software was delivered as a CD-format. It predicted when to spray fungicides accurately, controlling disease with reduced sprays but the usability was challenging. Using the same parameters a web-based real-time system was developed, to be easier to use and act as a decision support system.

In 2017, the real-time web-based prediction system was successfully used by a grower in Ross on Wye. By being used as a decision support system, the grower was able to spray with precision, therefore, maximising fungicide efficacy on disease control, whilst making monetary savings. Growers now want to know how many temperature and relative humidity monitors would be needed to successfully implement the prediction system on a whole farm.

**Aim:** To determine how temperature and relative humidity may differ spatially across a farm site, recorded by in tunnel weather monitors.

## MATERIALS & METHODS

In 2018, validation of the prediction system was continued. Temperature and relative humidity monitors (TinyTags<sup>®</sup>) were placed in several fields and tunnels at a farm near Wisbech, Cambridgeshire, as shown in Figure 2. This data was recorded from 7<sup>th</sup> August to 11<sup>th</sup> September 2018.

The grower carried out fungicide sprays according to the real-time web-based prediction system. Disease assessment for mycelium, which may be present on the leaves was carried out, gaining samples in Amelia Field, tunnels 1, 3, 5, 7, 9, 11 & 13, with 30 leaves assessed monthly from each.

## RESULTS

- Estimated spray dates were generated inputting the TinyTag<sup>®</sup> data into the CD prediction software, producing prediction graphs. Suggested spray dates are shown by the beginning of the yellow lines (Figures 3 & 4)
- Table 1 shows that several estimated dates for fungicide application were the same; with some estimating two dates in the time period studied to spray a fungicide
- No disease was found on the leaf samples collected

Table 1: Comparison of estimated spray dates, produced at the end of the season for different fields/ tunnels (Figure 2)

| Field-Tunnel           | Estimated Spray Date |
|------------------------|----------------------|
| Amelia Field- Tunnel 1 | 21/08/18             |
| Amelia Field- Tunnel 3 | 18/08/18, 03/09/18   |
| Biketrack Field        | 18/08/18, 02/09/18   |
| Ladybird Field         | 18/08/18             |
| Dome Field             | 18/08/18, 30/08/18   |

Figure 3: Prediction graph of accumulated hours to sporulation for Dome Field.

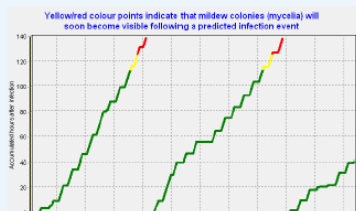
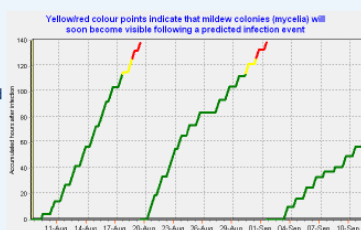


Figure 4: Prediction graph of accumulated hours to sporulation for Biketrack Field.



Figure 2: Map of farm located near Wisbech. Each coloured box represents field where TinyTags<sup>®</sup> were located, red dot indicates approximate location of TinyTags<sup>®</sup>. T1 indicates Tunnel 1 of Amelia Field.

## DISCUSSION

In Table 1, there is a slight variation in estimated spray dates amongst the different fields and tunnels. However, if the grower sprayed all fields at the earlier date, this would stop the development of the disease. The later date estimated in Amelia Field Tunnel 1 is within the red period, therefore, spraying a fungicide at this time would still control the disease.

This research shows that weather monitors are not required in every field, as the prediction is similar in most fields studied. However, environmental features and their effect on temperature and relative humidity need to be understood. Features which influence temperature and humidity include location of wind breaks, areas that are prone to flooding and location of dykes. The weather monitor needs to be located where the impact of these environmental features has been considered.

As shown in Table 1, there is approximately two weeks between the first and second estimated spray date. When compared to a commercial spray programme, which typically applies fungicides every 7 or 8 days; the use of the prediction system has the potential to save fungicide sprays.

The use of the prediction system enables the grower to spray with precision, therefore, maximising fungicide efficacy.

In 2019, this research will be repeated at several farm sites. These results here will ensure the weather monitor is best placed at the growers' sites, who are participating in the continued validation of the web-based real-time prediction system.

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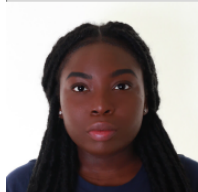
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- [3] Liu B. and Hall A.M. (2013) Practical experience of using a prediction system to control strawberry powdery mildew. Aspects of Applied Biology. 119, 227-232.

## ACKNOWLEDGEMENTS

Many thanks to Henry and Harriet Duncliffe of Maltmas farm, Richard Hibbard of E.C. Drummond Ltd and Stuart Arbuckle of Star Inn Farm, who have used and continue to use the prediction system for the duration of this research. Finally thanks to Camilla Asiana and Bo Liu.



# THE DEPOSITION OF SILICON LINKED TO THE REDUCTION IN SUSCEPTIBILITY TO STRAWBERRY POWDERY MILDEW



Authors: Asiana, I., Hall A.M., Davies K.  
University of Hertfordshire, College Lane, Hatfield AL10 9AB, UK

University of  
Hertfordshire



## Introduction

The most important disease of protected strawberries in the UK is strawberry powdery mildew caused by *Podosphaera aphanis*, which has to be controlled by the frequent use of fungicides (Dodgson, Hall & Jin 2016).

Work carried out at the University of Hertfordshire has shown that the weekly use of silicon nutrient in the fertigation tubes at a commercial strawberry farm results in reduced susceptibility to this disease.

Previous work at the university has also shown that the use of a silicon nutrient through a fertigation system enhances the constitutive defence mechanism of the plant.

## Aim

To examine the effect of silicon nutrient applied in a silicon fertigation field trial through fortnightly disease assessment and the deposition of silicon in strawberry plants in a glasshouse in reducing strawberry powdery mildew.

## Materials and methods

The silicon fertigation field trial had 6 treatments (table 1) and silicon was added twice weekly. Samples were collected fortnightly for disease assessments.

❖The silicon deposition experiment in the glasshouse had 12 treated and 12 untreated strawberry plants in a glasshouse. 0.017% silicon nutrient (as in the fertigation field trial) was delivered for 8 weeks into two ways; a). Through the root application. b). Hydroponically

❖Cross-sections of leaves, petioles and roots were stained with a fluorescence dye (Basic amine Lyso tracker yellow HCK -123), final concentration 1µM (Shetty et al., 2012).

❖Examination was conducted using a confocal microscope at x400 magnification and wavelength 450nm.

## Results

Figure 1 and Table 1 shows the largest epidemic took place in the untreated and the silicon nutrient reduced the epidemic even in the absence of the fungicide. The results in figures 2-7 showed that in the leaf, silicon was found in the cuticle, epidermis, palisade layer, stomata and vascular tissue. In the petiole, the silicon was found in the epidermis and xylem and in the roots, the silicon was found in the xylem only.

The fluorescence intensity (Table 2) of the cross sections was quantified and this shows that the silicon was 5 times higher in the treated plants than the untreated. In addition, the silicon fertigation field trial has shown that plants with higher levels of silicon are less susceptible to the disease (Figure 1 and Table 1).

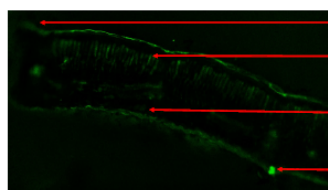


Figure 2: Strawberry leaf section with a silicon nutrient under a confocal microscope. Magnification x400.

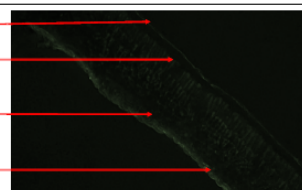


Figure 3: Strawberry leaf section without a silicon nutrient under a confocal microscope. Magnification x400.

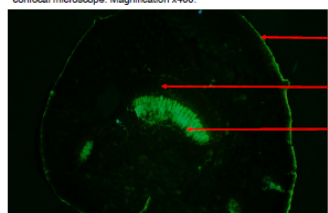


Figure 4: Strawberry petiole cross section with a silicon nutrient under a confocal microscope. Magnification x400.

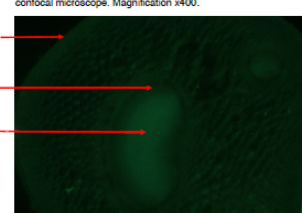


Figure 5: Strawberry petiole cross section without a silicon nutrient under a confocal microscope. Magnification x400.

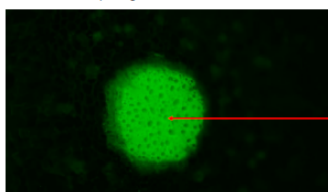


Figure 6: Strawberry root cross section with a silicon nutrient under a confocal microscope. Magnification x400.

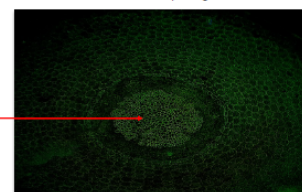


Figure 7: Strawberry root cross section without a silicon nutrient under a confocal microscope. Magnification x400.

Figure 1: Silicon fertigation field trial epidemic curve

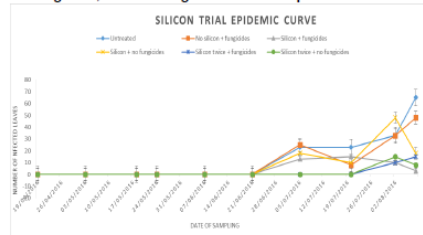


Table 1: Area under the disease progress curve (AUDPC)

| Treatments                                     | AUDPC |
|--|-------|
| Untreated - no fungicides, no silicon nutrient | 3,423 |
| No silicon nutrient + fungicides               | 2,825 |
| Silicon nutrient + fungicides                  | 732   |
| Silicon nutrient + no fungicides               | 1,610 |
| Silicon nutrient twice + fungicides            | 410   |
| Silicon nutrient twice without fungicides      | 375   |

Table 2: Fluorescence Integrated density

| Cross-section | Untreated Fluorescence intensity | Treated Fluorescence intensity |
|---------------|----------------------------------|--------------------------------|
| Leaf          | 2.209cps                         | 7.923cps                       |
| Petiole       | 1.913cps                         | 7.770cps                       |
| Root          | 1.266cps                         | 11.594cps                      |

## Acknowledgement

Special thanks to Henry & Harriet Duncalfe, Maltmas farm for the provision of the field trial, Gidon Bahiri (OrionFT) for providing Sirius for the silicon field trial, also my supervisors Dr Avice M. Hall and Dr Keith Davies for their contributions towards my research journey.

## References

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## Discussion and conclusion

In the glasshouse experiment, treated plants showed that the silicon was found in the vascular tissue throughout the plant, in the leaf deposited in the epidermis, palisade layer and stomata, and in the xylem of the petiole and roots compared to untreated plants.

Additionally, in the silicon fertigation field trial there was more silicon in the treated than the untreated plants and level of silicon correlates with reduced disease susceptibility. This suggests that the addition of silicon nutrient through the fertigation fields enhances the constitutive defence mechanism of the plant.





# ARE STRAWBERRIES EVER DEFICIENT IN SILICON ?

Authors: Asiana ,(i.asiana@herts.ac.uk), Hall A.M., Davies K.

School of Life and Medical Sciences  
University of Hertfordshire, College Lane, Hatfield AL10 9AB, UK

## Introduction

The most important disease of protected strawberries in the UK is strawberry powdery mildew caused by *Podosphaera aphanis*, which has to be controlled by the frequent use of fungicides (Dodgson, Hall & Jin 2016).

Work carried out at the University of Hertfordshire has shown that the weekly use of silicon nutrient in the fertigation tubes at a commercial strawberry farm results in reduced susceptibility to this disease. Previous work done at the university has also shown that the use of silicon nutrient enhances the constitutive defence mechanism of the plant to infection and additional crop benefits (Jin, 2015 & Liu, 2017). Different soils have different levels of silicon and most strawberries are grown in coir which has little bioavailable silicon. This could lead to silicon deficiency in most strawberry plants.

## Aim

To characterise the symptoms of silicon deficiency in strawberry plants and to evaluate what occurred when grown without silicon.

## Material and methods

A hydroponic experiment was set up for 23 weeks (24 January – June 2018) in plastic tubs containing Hoagland's solution (Jones, 2016). All work was carried out in plastic (to exclude glass) See figure 1. Tubs were wrapped with black polythene bags to reduce light penetration. Bare roots Malling Centenary strawberry plants were planted in tubs with black lids. Plants were spaced apart to minimize crowding in tubs. Air supply was provided to the roots of the plants by means of aeration pumps.

Treatment was a weekly application of silicon nutrient 50mls per tub. The nutrient used was "Sirius", a bioavailable form of silicon "Si(OH)<sub>4</sub>" (Polyether modified Polysioxane, Ethanol, tetraethyl silicate and alkoxypoly Ethyleneoxy ethanol) v/v 0.25% for the treated. The untreated (control) used de-ionized water only. Additionally, they were topped up with Hoagland's solution twice weekly.

## Results

Results in table 1 found that there were significantly more leaves ( $P < 0.05$ ), significantly more runners ( $P < 0.05$ ), significantly more fruits ( $P < 0.05$ ), significantly higher °Brix levels ( $P < 0.05$ ) and significantly more chlorophyll ( $P < 0.05$ ) in 'Sirius' treated strawberries compared to the untreated. See table 1.

Additionally, there was an increase in weight, size and biomass in the treated than the untreated plants (see table 1, figure 2 and 3). Flowering was a week earlier in the treated strawberries compared to the untreated. See table 1. (Data was analysed using ANOVA, Regression statistics and the dependent "paired" t test respectively).

Results from statistics and table 1 show a summary of additional benefits of using silicon nutrient in growing strawberries.

Table 1: Cumulative averages from the hydroponic deficiency experiment January – June 2018

| Strawberry plants                  | Untreated (no silicon nutrient) de-ionized water         | Treated (silicon nutrient) v/v 0.25%             |
|------------------------------------|--|--|
| Leaves                             | 115 (Counted weekly for 26 weeks)                        | 124 (Counted weekly for 26 weeks)                |
| Runners                            | 24 (Counted weekly for 26 weeks)                         | 37 (Counted weekly for 26 weeks)                 |
| Flowering dates                    | 22 May 2018 (First sight of flowering)                   | 15 May 2018 (First sight of flowering)           |
| Number of fruits                   | 8 (Counted during fruiting period)                       | 16 (Counted during fruiting period)              |
| °Brix                              | 9 (Measurements taken after each sample)                 | 17 (Measurements taken after each sample)        |
| Weight of fruit (g)                | 12.9 g (Fruits weighed after each sample)                | 20.6 g (Fruits weighed after each sample)        |
| Size (centimetres)                 | 1.18cm (Fruit size was measured after sample)            | 1.38cm (Fruit size was measured after sample)    |
| Chlorophyll (µmol/m <sup>2</sup> ) | 665.1 µmol/m <sup>2</sup> (Measured by SPAD once weekly) | 813.5 µmol/m <sup>2</sup> (Measured once weekly) |
| Biomass (g)                        | 140.28 g (At the end of experiment)                      | 184.40 g (At the end of experiment)              |



Figure 1: Hydroponic tubs on glasshouse bench after three weeks of planting



Figure 2: First week of planting. Strawberry roots forming new roots. Old mature roots are brown and new roots are white in colour.

Figure 3: After three weeks of planting. Strawberry roots forming new roots. Old mature roots are brown and new roots are white in colour.

## Acknowledgement

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## References

- Dodgson J, Hall A, Jin S. (2016). Control of strawberry powdery mildew under protection (project SF 62 & SF 62a). Factsheet 17/02. spp. Stoneleigh, Warwickshire: AHDB horticulture.  
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Liu B (2017) Sustainable strawberry production and management including control of strawberry powdery mildew. Unpublished doctoral thesis. Hatfield: University of Hertfordshire

## Discussion and conclusion

Whilst the results from the hydroponic deficiency experiment showed no deficiency symptoms, the leaves, runners, fruits and chlorophyll of silicon treated strawberries were significantly higher than the untreated. The concentration of silicon nutrient used significantly increased productivity.

The results suggests that though silicon is not an essential element, it is probably a limiting factor in strawberry productivity. It is therefore recommended that growers use silicon nutrient throughout the growing season, particularly when growing in coir.

## Objective 3: Fruit rot complex

### Background

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

In Year 4, we did a small piece of work in relation to fruit rot: testing for presence of *Pestalotiopsis* spp. in those samples used for testing *P. cactorum* in the Years 1-2.

### Materials and Methods

#### Molecular screen of *Pestalotiopsis* spp.

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
- (2) 10 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. *Pestalotiopsis* 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, *Pestalotiopsis* 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 1<sup>st</sup> 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<sub>2</sub>, 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

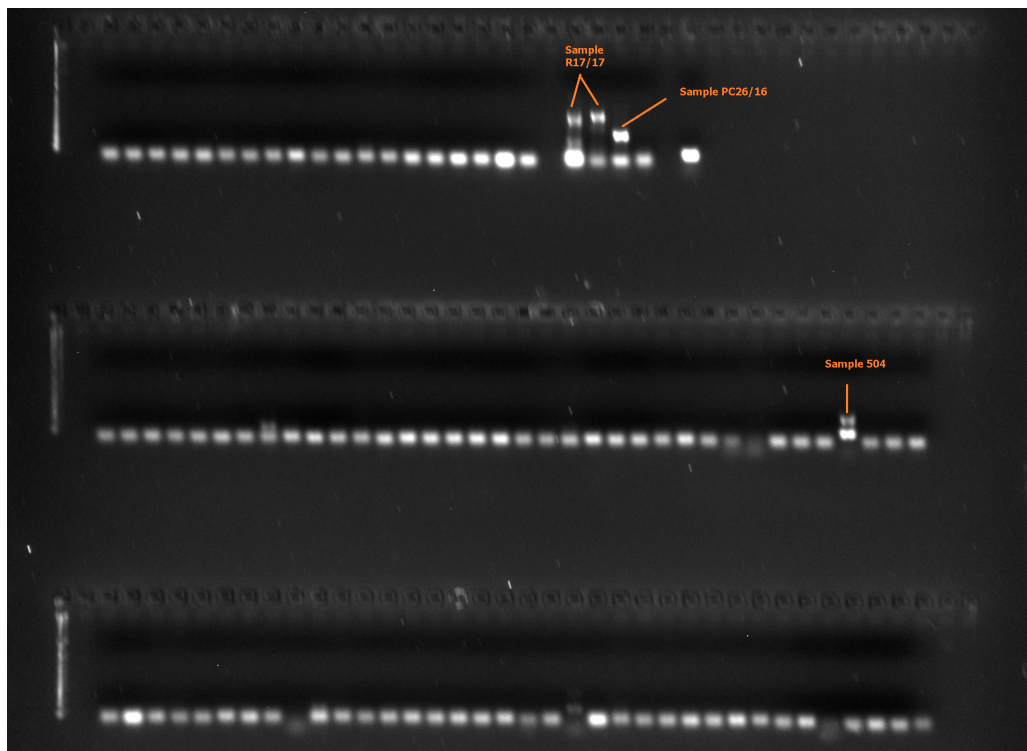
| Primer set | Target                | Forward primer          | Reverse primer                   |
|------------|-----------------------|-------------------------|----------------------------------|
| Pesta      | <i>Pestalotiopsis</i> | CTTACCTTTTGTTCCTCGG     | TCTTGTTCAAGAACGCAGC              |
| FaEF       | <i>Fragaria</i>       | TGGATTTGAGGGTGACAACATGA | GTATACATCCTGAAGTGGTAGACGGA<br>GG |

## Results

### Molecular screen of *Pestalotiopsis* spp

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.



**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 *Pestalotiopsis* spp. There were two positive controls: R17/17 from pear and PC26/16 from strawberry. The image is composed of results from three separate gels.

## Discussion

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.

## Conclusions

- *Pestalotiopsis* species appear not to be important on strawberry in the UK

## References

- 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.

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

### **Introduction**

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.

### **Methods and Materials**

The experiment was set up in Oxfordshire in spring 2017 in a sandy-loam field containing a relatively high number of micro-sclerotia (four propagules per gramme of soil) where the grower was to treat the rest of the field with chloropicrin. The cold-stored bare-root plants of a moderately susceptible strawberry variety, Symphony, were expected to allow wilt symptoms to be seen in the second year of the trial. The beds were outdoors, covered tightly with standard blue weed-suppression polythene mulch with irrigation drip-lines underneath. There were 27 plants within the assessed central 6 m length (with 7 m treated) and two rows.

## Experimental design and treatment

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

Two alternatives to chemical soil disinfestation were rotavated into the soil to 150 mm deep on 23 May 2017. For uniformity, the soil on top of every bed in the trial was also rotavated. One product was a bio-fumigant, Bio-Fence, a granular product made from *Brassica carinata* meal, applied at 2000 kg/ha a week before planting followed by 150 mm depth of irrigation water before immediately covering the beds with polythene mulch. Isothiocyanates were anticipated to be released, which have been known to reduce *V.*

*dahliae* inoculum in soil. As in the grower's adjacent chloropicrin treated beds, planting-hole slits were made in the polythene mulch to allow fumes out before both areas were planted on 6 June 2017 with cold-stored strawberry runners cv. Symphony.

Anaerobic digestate solids were the second organic material incorporated on 23 May. They have potential to suppress plant pathogens by encouraging the build-up of beneficial microbial populations. The material was applied at 50 t/ha, was PAS 110 certified, and composed of pasteurised shredded maize crop plus pack-house produced vegetable waste. The third treatment was applied on 12 June 2017, a week after planting on the 6 June. Serenade ASO (*Bacillus subtilis* strain QST 713) was used at 10 L/ha in 1000 L of water / ha. It has an EAMU for other crops as a soil drench at planting (via sprayer or irrigation lines) against soil-borne pathogens such as *Pythium*, *Phytophthora* and *Rhizoctonia*. In outdoor strawberry, the new EAMU (2018 1855) permits six overhead applications at 10 L/ha dose at 1000 L/ha water volume (EAMU 2016 2638 permitting 20 applications will still be possible with old stock to February 2020).

A fourth treatment was a combination of the Bio-Fence and Serenade ASO treatments.

The commercial crop and trial area were managed in the same way by the grower, except that the grower covered the chloropicrin treated crop with straw at the end of February 2018

|   |    |    |    |    |
|---|----|----|----|----|
| 5 | 10 | 15 | 20 | 25 |
| 4 | 9  | 14 | 19 | 24 |
| 3 | 8  | 13 | 18 | 23 |
| 2 | 7  | 12 | 17 | 22 |
| 1 | 6  | 11 | 16 | 21 |

|    |                              |
|----|------------------------------|
| T1 | Untreated                    |
| T2 | Maize & vegetable crop waste |
| T3 | Bio-Fence                    |
| T4 | Serenade ASO                 |
| T5 | Bio-Fence + Serenade ASO     |

**Figure 4.1.** Oxfordshire, 2018. Layout of 6 m long plots, with beds running plot 1 to 5 etc., each with two rows of strawberries.



to keep it cool and so delay harvest so that picking would start a fortnight later than other fields.

**Table 4.1.** Materials applied to plots before and after planting cv. Symphony cold-stored strawberry runners on 6 June 2017 in a *Verticillium* infested field in Oxfordshire

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

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

## Assessments

### *Verticillium* wilt pre- and post-harvest score

Plants were assessed before and after harvest (May and July 2018 respectively), using an index that recorded both the condition of the foliage vigour (canopy density and greenness) and leaf wilting and discolouration. Dead / near dead plants scored index 1 (no vigour) with obviously wilting plants of decreasing wilt severity having indices of 2 (poor vigour) through to an index of 4. Plants of index 5 had developing wilt and index 6 had symptoms potentially starting. Plants that appeared healthy, were assigned indices of increasing vigour between 7 (good vigour) and 9 (excellent vigour) (Figure 4.2).

### Harvest

Fruit harvesting in the field started on the 11 June 2018, as the country entered an extremely hot few weeks, and was stopped after a further four harvests following the 27 June. The plots were harvested and recorded by the farm staff according to the grading used on the commercial crop. Under or oversized or miss-shaped fruit were put into Class 2 and weighed separately from Class 1. Fruit damaged in any way were classed as Waste. Ten Class 1 berries were weighed at random from each plot on 27 June and from equivalent row lengths (6 m) in the chloropicrin treated commercial crop to record mean berry weight. On the same date, yield was also measured in these commercial plots.



### ***Fera plant examination for Verticillium***

Two samples of six plants removed from the crop on 18 July, showing either mild (petiole necrosis) or severe (dead crowns with some wilted leaves) *Verticillium* wilt symptoms and were sent to the Fera Plant Clinic for standard diagnosis. On receipt at Fera the plants were inspected and crowns cut open to look for vascular staining. Where staining was observed, isolations were made from that tissue, and the rest of the crown was incubated for 10 days with the vascular tissue exposed. Verticils (spore-bearing structures) and microsclerotia were also looked for and if found, isolated onto agar to confirm *Verticillium*. Molecular testing of plants for *Verticillium* is not available at Fera (only Taqman on strawberries for *Phytophthora fragariae*).

### ***ADAS Soil Harris testing***

After the final wilt assessments in the crop in July 2019 soil samples were taken in August 2019 from the treatment with fewest wilted plants and also the untreated plots for comparison. Fewest obviously wilted plants (scoring indices 1 to 4) were present in the plots treated only with Serenade ASO. This was to determine whether the fewer wilted plants might have resulted from by chance a lower density of *V. dahliae* micro-sclerotia initially in the Serenade ASO allocated plots rather than any subsequent reduction of symptom severity by the Serenade ASO.

In August 2019, the soil from down to 150 mm was sampled with a corer around the plants inside the planting holes cut in the plastic mulch of untreated plots 5, 8 and 21 and Serenade ASO treated plots 15, 16 and 23. To be able to provide sufficient soil from around the plants for the test, the soil from the plots of each treatment were combined. Soil that had been collected before the beds were treated and plastic mulch covered in May 2017 were retrieved from cold storage and individual samples from each of the same six plots as sampled in August 2019 were sent for Harris testing together with the August 2019 samples.

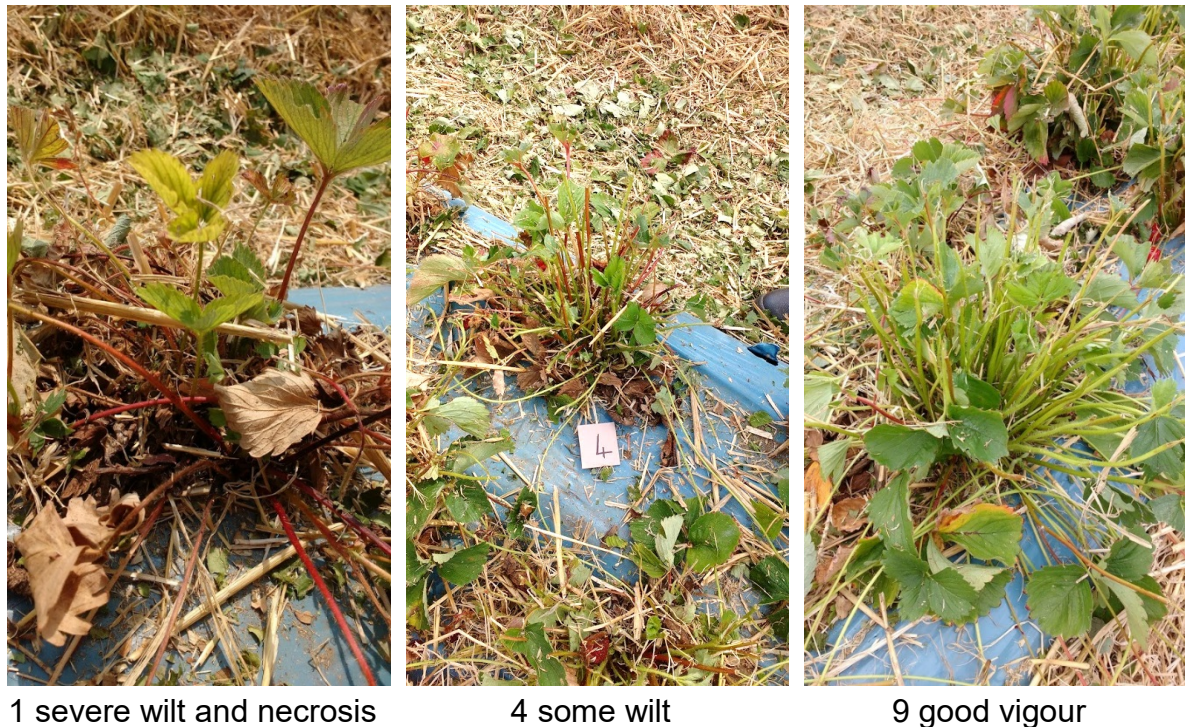
## **Results**

### ***Verticillium wilt assessment (pre- and post-harvest) 2018***

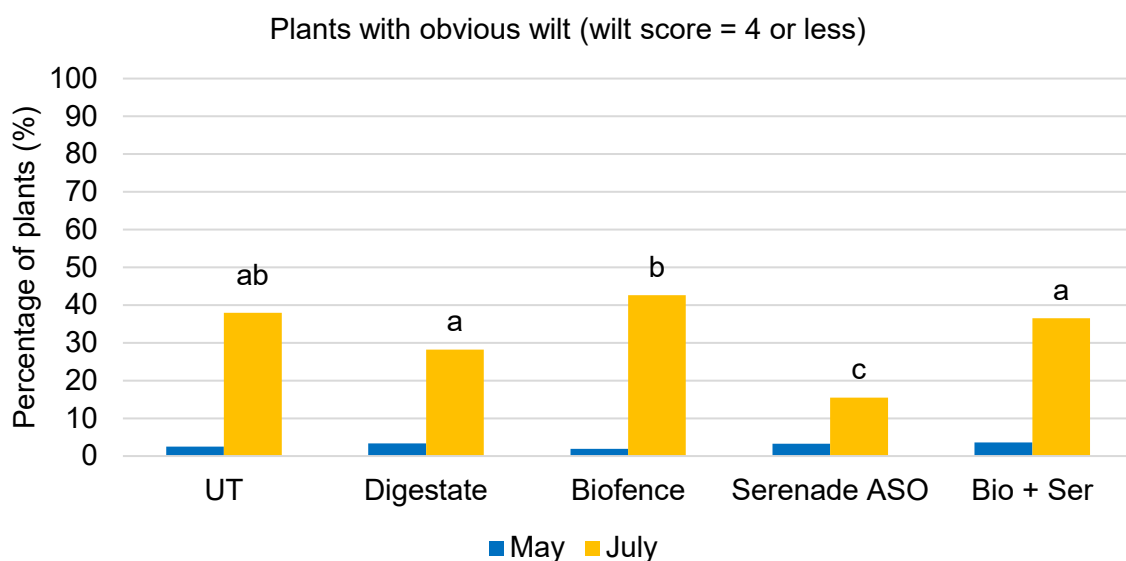
More plants were wilting (scored 4 or less on the wilted to good vigour scale) in July after harvest than before in May (Figure 4.3). Regression analysis of the proportion scoring 4 or less showed highly significant treatment differences in the July *Verticillium* wilt scores ( $P < 0.001$ ), but not in the May data where few plants scored 4 or less. By July, there were significantly more plants wilted (vigour score  $\leq 4$ ) in the Bio-Fence treated plots (with 42.6% wilted), compared to all other treatments, except the untreated control. The 15.5% of

Serenade ASO treated plants wilted (vigour score  $\leq 4$ ) was significantly less than the untreated control plants (with 37.9% of plants wilting).

Bio-Fence treated plots had significantly fewer ( $P < 0.05$ ) plants of good vigour and no wilt (16.2%), than the untreated (31.6%) in the July post-harvest assessment (a vigour score of 7 or higher) (Figure 4.4). Pre-harvest in May, there had been no significant differences between treatments in the proportion of plants without visible *Verticillium* (Figure 4.4).



**Figure 4.2.** Examples of wilt severity and foliar vigour index used on strawberry plants post-harvest on 19 Jul 2018. Index 1 = severe wilt, 4 = some wilt, 9 = no wilt excellent vigour.



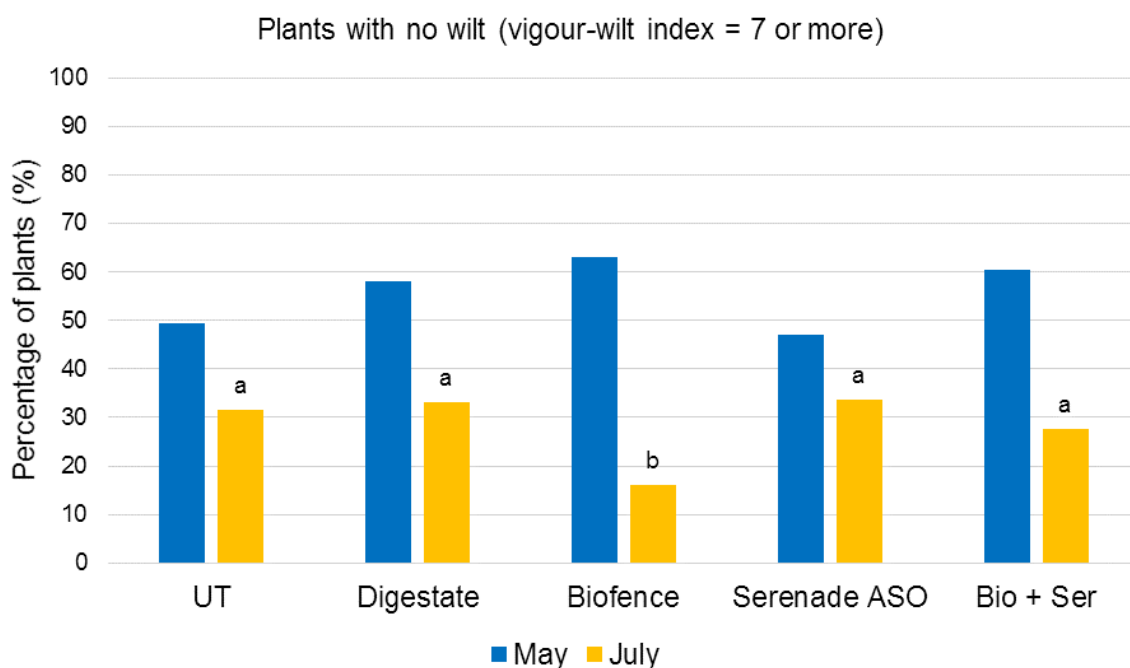
**Figure 4.3.** Percentage of plants with obvious wilt (vigour/wilt index 4 or less), on 17 May and 19 July 2018. Significant differences ( $P < 0.001$ ) from regression analysis indicated by letters in July. No significant difference ( $P = 0.945$ ) in May. 'UT' refers to untreated plots; 'Bio + Ser' refers to the treatment with both Biofence and Serenade ASO.

## Harvest

There were problems throughout the industry in 2018 with fruit ripening faster than they could be picked and berries desiccating on the stalks.

All analysis was carried out by Analysis of Variance (ANOVA) with 16 degrees of freedom (d.f.) and least significant differences (LSD) are shown in the following tables along with the probability ( $P$ ) value. Significant differences were taken as being where  $P < 0.05$ . Duncan's Test was used to show relative differences between means where  $P < 0.05$ .

Fruit picking commenced on the 11 June 2018 (with a mean total of marketable plus waste of 548 g per plot) with the volume then increasing at each of the subsequent two harvests (15 and 18 June) and peaking on 25 June (at a mean total of 6.7 kg per plot). A final pick was made on the 27 June (Table 4.2). Over this period extremely hot weather was speeding the ripening of the fruit and fruit was becoming scorched on the plants and comprised the bulk of the waste fruit picked. The host grower and others nationally were having difficulties in being able to keep up with harvesting the commercial crops and in supplying a quality product to the market because of the weather conditions.



**Figure 4.4.** Percentage of plants with no wilt symptoms (a vigour/wilt index 7 or more), on 17 May and 19 July 2018. Significant differences ( $P = 0.012$ ) from regression analysis indicated by letters in July. No significant difference ( $P = 0.055$ ) in May. UT refers to untreated plots; 'Bio + Ser' refers to the treatment with both Biofence and Serenade ASO.

**Table 4.2.** Mean Weight of Fruit (g) classed as Marketable (Class 1 + 2) or Waste at each of five harvest dates

| Harvest 2018 | date | Class 1 + Class 2 | Waste | Total  | Waste as % of total |
|--------------|------|-------------------|-------|--------|---------------------|
| 11 Jun       |      | 479.1             | 68.6  | 547.7  | 12.52               |
| 15 Jun       |      | 1748.6            | 175.7 | 1924.5 | 9.13                |
| 18 Jun       |      | 2527.5            | 177.6 | 2705.1 | 6.56                |
| 25 Jun       |      | 5739.6            | 922.5 | 6662.1 | 13.85               |
| 27 Jun       |      | 1000.2            | 147.0 | 1147.2 | 12.81               |

There was no significant difference between treatments in the total of marketable plus waste fruit picked per plot by the end of harvest (Table 4.3), with a mean 12.99 kg fruit per plot. Of the fruit that was marketable there was again no significant difference between treatments with a mean 11.50 kg produced per plot (Table 4.3).

**Table 4.3.** Mean Total Marketable Fruit (g) and Total including Waste over all harvests

|                    | Untreated | Digestate | Bio-Fence | Serenade ASO | Bio-Fence + Serenade ASO | P     | LSD     |
|--------------------|-----------|-----------|-----------|--------------|--------------------------|-------|---------|
| Marketable + waste | 13086.6   | 12684.2   | 12723.2   | 13326.6      | 13110.8                  | 0.982 | 2654.32 |
| Marketable         | 11687.8   | 11060.4   | 11177.4   | 11843.2      | 11706.0                  | 0.913 | 2160.85 |

Following some failure of plant establishment in May 2017 in the Bio-Fence treated plots in particular (influenced by the exceptionally hot weather at planting, as discussed in the previous annual report) these plots had on average three fewer plants, but the yield was shown to have been made up by those remaining so that there were no treatment differences in total fruit yield per plant (a mean 555 g) (Table 4.4).

**Table 4.4.** Mean Number of Plants present at harvest (as recorded on 19 July 2018) and the Mean Total Weight (g) of Marketable + Waste fruit per plant over all harvests

|                     | Treatments applied in 2017 |           |           |              |                          | Analysis |       |
|---------------------|----------------------------|-----------|-----------|--------------|--------------------------|----------|-------|
|                     | Untreated                  | Digestate | Bio-Fence | Serenade ASO | Bio-Fence + Serenade ASO | P        | LSD   |
| Mean plants/plot    | 25                         | 24        | 21        | 25           | 23                       | -        | -     |
| Fruit weight /plant | 523.5                      | 519.9     | 619.7     | 536.4        | 575.1                    | 0.204    | 97.92 |

The weight of ten Class 1 berries taken at random was recorded per plot on the 27 June 2018 and also in three lengths of a bed nearby in the commercial (chloropicrin treated) crop. The commercial crop had three, not two, rows of plants in the bed because the grower had found he had surplus material at planting. Berry weight was measured as it was possible that this could have been reduced owing to a reduced ability of plants affected by *Verticillium* to take up water, however there was no significant difference in berry weight per plot (each fruit weighed a mean 12.0 g) between the treatments (Table 4.5). The berry weight recorded by the farm staff from the commercial crop at the same time was greater, with ten berry weights of 218 g, 195 g and 205 g recorded from the three plots, giving a mean single berry weight of 20.6 g. However, the commercial crop was towards the start of harvest at this time (because fruiting had been delayed by strawing-over in cold weather in late February 2018 to keep the plants chilled) whereas this was the third week of harvest in the trial plots and the weight of fruit harvested on the 27 June had declined after peaking two days earlier.

**Table 4.5.** Mean Weight (g) of ten Class 1 Berries on 27 June 2018

|                   | Treatments applied in 2017 |           |           |              |                          | Analysis |       |
|-------------------|----------------------------|-----------|-----------|--------------|--------------------------|----------|-------|
| Harvest date 2018 | Untreated                  | Digestate | Bio-Fence | Serenade ASO | Bio-Fence + Serenade ASO | P        | LSD   |
| 27 June           | 125.8                      | 124.2     | 117.2     | 109.2        | 121.2                    | 0.83     | 32.95 |

There were no significant differences between treatments at any of the five harvest dates in the weight per plot of Class 1 fruit, Class 2 fruit, or Class 1 + 2 combined (Tables 4.6, 4.7 and 4.8). Total yields (Table 4.9) of all treatments fell between 14 kg and 16 kg per 6 m bed length. At the final harvest date, ANOVAs of Class 2 and Class 1+Class 2 fruit weights gave P values of 0.077 and 0.060, respectively (Tables 4.7 and 4.8) and these approach significance at  $P < 0.05$ . The highest weights (for Serenade ASO plots) and lowest weights (for digestate) can be separated by least significant difference, but the results for neither differ from the untreated or other treatments.

On 11 June alone there was a significantly higher ( $P < 0.01$ ) weight of waste fruit in plots which had received digestate at planting with a mean 142 g (individual plots ranged from 90 g to 179 g) compared with a mean for the other treatments of 50 g waste per plot which would equate to approximately the weight of five fruit (Table 4.9).

**Table 4.6.** Mean Weight (g) of Class 1 Fruit

| Harvest date 2018 | Treatments applied in 2017 |           |           |              |                          | Analysis |         |
|-------------------|----------------------------|-----------|-----------|--------------|--------------------------|----------|---------|
|                   | Untreated                  | Digestate | Bio-Fence | Serenade ASO | Bio-Fence + Serenade ASO | P        | LSD     |
| 11 June           | 260.8                      | 516.8     | 598.4     | 379          | 439.8                    | 0.119    | 263.25  |
| 15 June           | 1396.4                     | 1654.6    | 1620      | 1489         | 1490.4                   | 0.886    | 598.60  |
| 18 June           | 2463.8                     | 2261.8    | 2073.2    | 2436.2       | 2154.4                   | 0.49     | 542.50  |
| 25 June           | 4665.2                     | 4376.4    | 4211.8    | 4895.4       | 4737                     | 0.64     | 1038.91 |
| 27 June           | 670.6                      | 536.8     | 607.4     | 804.4        | 711.6                    | 0.18     | 227.89  |

**Table 4.7.** Mean weight (g) of Class 2 Fruit

| Harvest date 2018 | Treatments applied in 2017 |           |           |              |                          | Analysis |        |
|-------------------|----------------------------|-----------|-----------|--------------|--------------------------|----------|--------|
|                   | Untreated                  | Digestate | Bio-Fence | Serenade ASO | Bio-Fence + Serenade ASO | P        | LSD    |
| 11 June           | 56.0                       | 34.4      | 35.8      | 39.2         | 35.4                     | 0.95     | 65.61  |
| 15 June           | 208.2                      | 257       | 204.2     | 160.6        | 262.6                    | 0.667    | 162.75 |
| 18 June           | 260.6                      | 238.4     | 236.6     | 238.4        | 274                      | 0.988    | 181.41 |
| 25 June           | 1334.6                     | 956.0     | 1258.0    | 982.8        | 1280.8                   | 0.381    | 505.63 |
| 27 June           | 372.0                      | 228.0     | 332.0     | 418.0        | 320.0                    | 0.077    | 131.70 |

**Table 4.8.** Mean Marketable Fruit Yield (g) Class 1 + Class 2

| Harvest date 2018 | Treatments applied in 2017 |           |           |              |                          | Analysis |         |
|-------------------|----------------------------|-----------|-----------|--------------|--------------------------|----------|---------|
|                   | Untreated                  | Digestate | Bio-Fence | Serenade ASO | Bio-Fence + Serenade ASO | P        | LSD     |
| 11 June           | 316.8                      | 551.2     | 634.2     | 418.2        | 475.2                    | 0.214    | 285.55  |
| 15 June           | 1604.6                     | 1911.6    | 1824.2    | 1649.6       | 1753                     | 0.794    | 582.36  |
| 18 June           | 2724.4                     | 2500.2    | 2309.8    | 2674.6       | 2428.4                   | 0.552    | 582.63  |
| 25 June           | 5999.8                     | 5332.4    | 5469.8    | 5878.2       | 6017.8                   | 0.511    | 1028.44 |
| 27 June           | 1042.2                     | 765.0     | 939.4     | 1222.6       | 1031.6                   | 0.060    | 297.43  |

**Table 4.9.** Mean Total Fruit Waste (g) at each harvest in June 2018. Duncan's test given on 11 June with a & b significantly different

| Harvest date 2018 | Treatments applied in 2017 |           |           |              |                          | Analysis |       |
|-------------------|----------------------------|-----------|-----------|--------------|--------------------------|----------|-------|
|                   | Untreated                  | Digestate | Bio-Fence | Serenade ASO | Bio-Fence + Serenade ASO | P        | LSD   |
| 11 June           | a 26.4                     | b 142.0   | a 64.0    | a 30.2       | a 80.2                   | 0.004    | 58.06 |
| 15 June           | 130.2                      | 135.4     | 207.6     | 191          | 214.4                    | 0.399    | 115.8 |
| 18 June           | 252.8                      | 188.6     | 122.6     | 190.8        | 133.2                    | 0.428    | 155.6 |
| 25 June           | 848.6                      | 983.8     | 1001.6    | 938.8        | 839.6                    | 0.979    | 693.2 |
| 27 June           | 140.8                      | 174.0     | 150.0     | 132.6        | 137.4                    | 0.755    | 71.55 |
| Total             | 1398.8                     | 1623.8    | 1545.8    | 1483.4       | 1404.8                   | 0.961    | 744.2 |

When calculations were performed to estimate the yield per plant rather than per plot (to account for the fewer plants present in the Bio-Fence treated plots) then significant differences were not shown between treatments, except for at the 11 June harvest date (Tables 4.10, 4.11, 4.12 and 4.13). On 11 June, a significantly ( $P < 0.05$ ) greater weight of Class 1 fruit was collected from the Bio-Fence only plants (28.2 g) than produced by either the untreated or Serenade ASO alone treated plants (mean 12.8 g). There were no differences on 11 June in the weight of Class 2 fruit (Table 4.11), but the differences seen in Class 1 fruit weight per plant on 11 June were reflected in the marketable yield for this date (Table 4.12). The overall total marketable yield per plant was on average 491.6 g and the waste fruit was 63.3 g.

**Table 4.10.** Mean Weight of Class 1 fruit per Plant (g). Duncan's test given on 11 June with a & b significantly different.

| Harvest date 2018 | Treatments applied in 2017 |           |           |              |                          | Analysis |       |
|-------------------|----------------------------|-----------|-----------|--------------|--------------------------|----------|-------|
|                   | Untreated                  | Digestate | Bio-Fence | Serenade ASO | Bio-Fence + Serenade ASO | P        | LSD   |
| 11 June           | a 10.4                     | ab 21.4   | b 28.2    | a 15.2       | ab 18.8                  | 0.026    | 10.42 |
| 15 June           | 55.9                       | 68.1      | 78.5      | 59.9         | 65.1                     | 0.336    | 23.42 |
| 18 June           | 98.6                       | 92.8      | 100.0     | 98.1         | 93.6                     | 0.928    | 20.79 |
| 25 June           | 186.6                      | 178.6     | 206.9     | 196.9        | 209.3                    | 0.436    | 39.27 |
| 27 June           | 26.8                       | 22.0      | 31.0      | 32.4         | 31.6                     | 0.397    | 12.52 |

**Table 4.11.** Mean Weight of Class 2 Fruit (g) per Plant at each harvest date in June 2018.

| Harvest date 2018 | Treatments applied in 2017 |           |           |              |                          | Analysis |       |
|-------------------|----------------------------|-----------|-----------|--------------|--------------------------|----------|-------|
|                   | Untreated                  | Digestate | Bio-Fence | Serenade ASO | Bio-Fence + Serenade ASO | P        | LSD   |
| 11 June           | 2.24                       | 1.35      | 1.69      | 1.57         | 1.56                     | 0.966    | 2.704 |
| 15 June           | 8.30                       | 10.50     | 9.90      | 6.50         | 11.30                    | 0.613    | 6.96  |
| 18 June           | 10.40                      | 9.90      | 11.00     | 9.60         | 12.00                    | 0.965    | 7.60  |
| 25 June           | 53.40                      | 39.10     | 62.30     | 39.50        | 56.80                    | 0.165    | 22.94 |
| 27 June           | 14.90                      | 9.20      | 16.90     | 16.80        | 14.40                    | 0.163    | 6.82  |

**Table 4.12.** Mean Marketable Yield per Plant (g). Duncan's test given on 11 June with a & b significantly different.

| Harvest date 2018 | Treatments applied in 2017 |           |           |              |                         | Analysis |       |
|-------------------|----------------------------|-----------|-----------|--------------|-------------------------|----------|-------|
|                   | Untreated                  | Digestate | Bio-Fence | Serenade ASO | Bio-Fence +Serenade ASO | P        | LSD   |
| 11 June           | a 12.7                     | ab 22.8   | b 29.9    | a 16.8       | ab 20.4                 | 0.050    | 11.21 |
| 15 June           | 64.2                       | 78.6      | 88.4      | 66.4         | 76.4                    | 0.180    | 22.03 |
| 18 June           | 109                        | 102.7     | 111       | 107.7        | 105.6                   | 0.942    | 22.22 |
| 25 June           | 240                        | 217.8     | 269.3     | 236.4        | 266.1                   | 0.090    | 41.57 |
| 27 June           | 41.7                       | 31.2      | 47.9      | 49.2         | 46                      | 0.231    | 17.42 |
| Total             | 467.5                      | 453.1     | 546.5     | 476.5        | 514.5                   | 0.163    | 83.37 |

There was significantly ( $P < 0.01$ ) less waste per plant on 11 June in the Untreated, Serenade ASO alone and Bio-Fence alone treated plots than in those which received digestate (Table 4.13) (as also shown in the results per plot). However, the mean weight of all waste fruit for the digestate was 5.8 g whereas the mean weight of a single fruit in this experiment was approximately 12 g. Although there were differences per plot on 11 June, there was no difference between treatments per plant in the total weight of fruit produced (Table 4.14).

Fruit was collected at the final harvest on 27 June (preceded by a month of plant stress with potential to reduce fruit fill) from four lengths of bed within the crop planted in chloropicrin treated soil. There were 44 plants in each 6 m length, more than in the equivalent bed length in the trial because three, not two, rows were planted. Therefore, direct comparison can only be made per plant (although differing proximities of plants could affect their growth and yield).

**Table 4.13.** Mean Waste Fruit per Plant (g). Duncan's test given on 11 June with a & b significantly different

| Harvest date 2018 | Treatments applied in 2017 |           |           |              |                          | Analysis |        |
|-------------------|----------------------------|-----------|-----------|--------------|--------------------------|----------|--------|
|                   | Untreated                  | Digestate | Bio-Fence | Serenade ASO | Bio-Fence + Serenade ASO | P        | L.s.d. |
| 11 June           | a 1.06                     | b 5.82    | a 2.91    | a 1.21       | ab 3.48                  | 0.008    | 2.585  |
| 15 June           | 5.21                       | 5.55      | 9.63      | 7.69         | 9.42                     | 0.185    | 4.691  |
| 18 June           | 10.11                      | 7.66      | 6.29      | 7.66         | 5.62                     | 0.619    | 6.29   |
| 25 June           | 33.9                       | 40.7      | 46.7      | 37.9         | 35.9                     | 0.892    | 28.55  |
| 27 June           | 5.63                       | 7.07      | 7.65      | 5.34         | 6.17                     | 0.592    | 3.435  |
| Total             | 56                         | 66.8      | 73.2      | 59.9         | 60.6                     | 0.763    | 29.87  |

**Table 4.14.** Mean Weight of Marketable + Waste fruit (g) per plant over all harvests

| Treatments applied before planting & fruit weights (g) |           |           |           |              |                          | Analysis |       |
|--|-----------|-----------|-----------|--------------|--------------------------|----------|-------|
|  | Untreated | Digestate | Bio-Fence | Serenade ASO | Bio-Fence + Serenade ASO | P        | LSD   |
| Total  | 523.5     | 519.9     | 619.7     | 536.4        | 575.1                    | 0.204    | 97.92 |



Class 1 yield per plant on 27 June in the commercial crop was a mean 97.5 g (with a range from 72 g to 131 g / plant). There was a mean 6.75 g Class 2 fruit per plant (with a range from 6 to 14 g / plant). The mean 104.2 g of marketable fruit per commercial plant was more than double that collected from the plants in the trial. One commercial plot had no waste recorded, but plants in the other plots had between 11 and 14 g / plant resulting in a mean of 6.37 g of waste fruit per plant in the trial area, meaning that 12.8% of the fruit picked was waste, whereas in the commercial crop 9.2% of the fruit picked was classed as waste (Table 4.15).

**Table 4.15.** Mean fruit weights (g) per plant on 27 June 2018 in the commercial crop growing in chloropicrin treated soil, and all trial plots, with proportion Waste

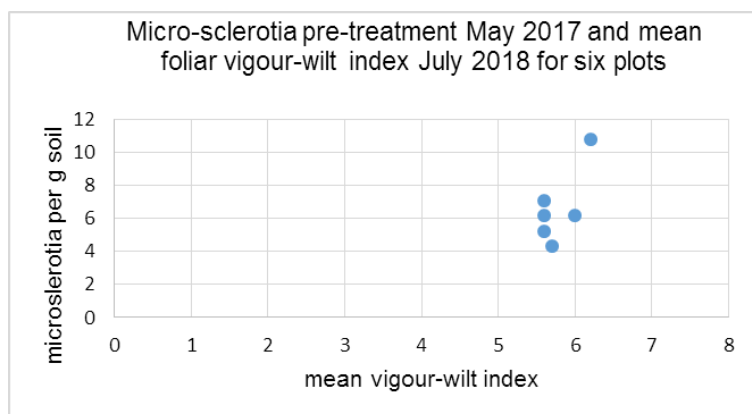
| <b>Crop harvested</b>                  | <b>Class 1 +<br/>Class 2</b> | <b>Waste</b> | <b>Total</b> | <b>Waste as % of<br/>Total</b> |
|--|------------------------------|--------------|--------------|--------------------------------|
| Chloropicrin treated bed               | 104.25                       | 10.50        | 114.75       | 9.15                           |
| Trial plots (mean untreated & treated) | 43.2                         | 6.37         | 49.57        | 12.85                          |

### **Fera wilted plant examination**

Initial observations showed roots of both samples (mild and severe wilt symptoms) to be blackened and necrotic and some minimal vascular staining in the crowns. Microscopic analysis and root preparations revealed a small amount of *Rhizoctonia* in both samples but no other root pathogens, including no *Verticillium*.

### **Soil Harris testing**

In May 2017, before any treatments had been carried out, soil from five of the six plots sent for Harris testing had between four and seven micro-sclerotia per gram of soil (Figure 4.1 for plots 5, 8, 15, 12, 21 and 23). However, Plot 5 (at the end of replicate block 1) had 10.8 micro-sclerotia and this remained an untreated plot. When plants were assessed in July 2018 for vigour (encompassing a lack of vigour due to wilting) the mean index of five of the plots was within the range of index 5 to 6 (indicating slight wilt), with plot 5 conversely showing the highest mean vigour index of 6.2 and thus indicating less severe foliar wilt on average.

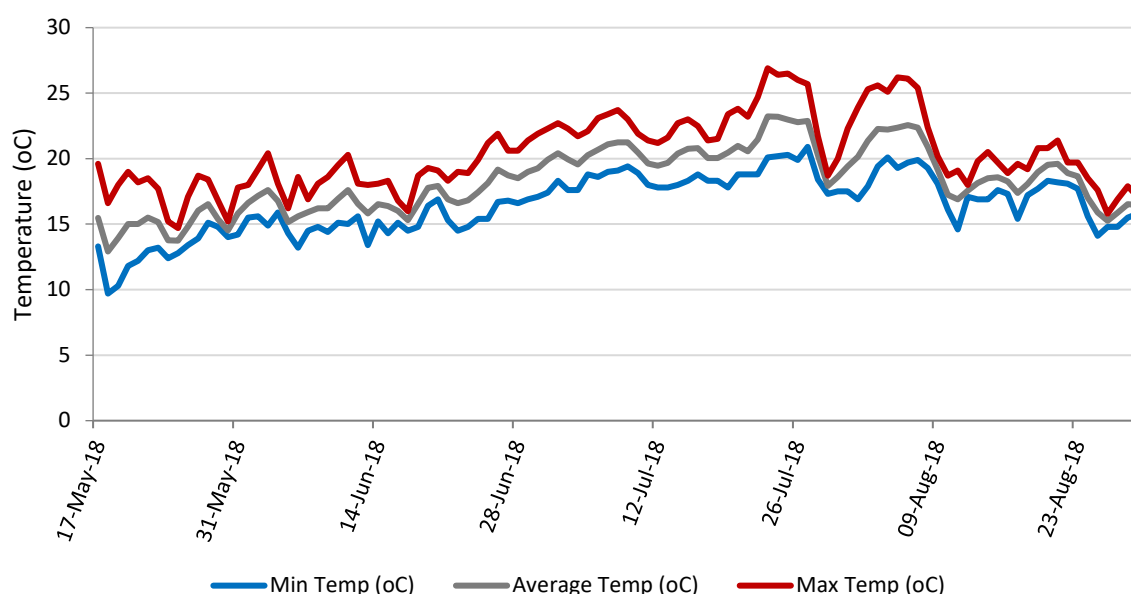


**Figure 4.5.** Harris test results per plot of micro-sclerotia per g /soil sampled pre-treatment in May 2017 plotted against mean foliar vigour-wilt index in July 2018 for each of the plots.

Indices 5 & 6 = first signs of wilt and 7 = appeared healthy).

## Temperature

Maximum soil temperatures at 100 mm depth (under the pale blue poly-mulch) built up during June to a maximum during the harvest period of 21.2°C on 25 June, and continued to rise to the day of post-harvest plant wilt assessment on 19 July (maximum 23.4°C) (Figure 4.6). The grower adjusted the flow of the irrigation by trickle tape to account for the unusually hot and dry weather experienced by this outdoor crop.



**Figure 4.6.** Soil temperature at 100 mm depth in a central (untreated) blue plastic-mulched strawberry bed, May to August 2018, Oxfordshire.

## Discussion

By July 2018, plants had been put under stress by fruit production, and infected plants were less able to keep up with water demand in the heat so causing them to succumb to *Verticillium* blocking of the vascular tissue, resulting in more plants wilting post-harvest than prior to it.

Of the plants in plots that received both Bio-Fence and Serenade ASO, by July 2019 36.5% were obviously wilted. This was significantly ( $P < 0.001$ ) many more than when Serenade ASO alone was used (15.5%), but significantly less than when Bio-Fence alone was used (42.6%). It is possible that the Bio-Fence could have weakened the plants, making them more susceptible to the *Verticillium*. Compared with plots which received only Serenade ASO, significantly over twice the number of plants was severely affected when left untreated (38.0%), but untreated plots were not significantly different from the two Bio-Fence treatments, and the digestate (28.2%). Serenade ASO was drenched over the plants once only (after plant establishment in spring 2017) and this treatment appears to have had a benefit visible in July 2018. There may have been either an indirect effect on the ability of the *Verticillium* mycelium to colonise the plants (perhaps by plant strengthening as a result of systemic acquired resistance) or the *Bacillus subtilis* bacteria in the product which entered the rhizosphere might have directly competed with the *Verticillium* for space and nutrients on the roots. As the micro-sclerotia density in the soil was no different in August 2018 between the Serenade ASO treated plots and the untreated it is unlikely that the biofungicide had a direct effect on the *Verticillium* micro-sclerotia. However, micro-sclerotia production per plot on dying plants would be expected to be lower where a smaller proportion of plants are infected by *Verticillium*.

There was neither significant benefit nor detriment to fruit yield from any of the treatments, compared with the untreated plots. The soil had been confirmed to contain *Verticillium* and wilting was starting to be seen in all the treatments pre-harvest. It is probable that the yield was reduced across the trial area resulting in the treated plots responding as if untreated. That the crop had become infected and yield affected is supported both by the marketable yield being only 41% of the commercial crop. There was also a contrast in berry weight at the final pick between the trial area (mean 12.0 g) and the chloropicrin treated farm crop (mean 20.6 g). However the commercial crop was a fortnight behind the trial area because the former had been strawed when cold in March to hold back fruiting and it is likely that towards the end of harvest the fruit size would be smaller than towards the start. *Verticillium* affects water uptake by blocking the vascular system and so, even when adequate irrigation is provided, the pathogen reduces the ability of the plants to fill fruit resulting in smaller fruit and thereby reducing yield. Both crops used the variety Symphony (from the same delivery batch). This variety is moderately susceptible to *Verticillium* wilt and was selected for the trial to be able to observe some wilting but still allow plants to produce fruit, however if a more resistant variety were to be tested then integrated measures could improve plant tolerance further.

The Serenade ASO drench application (over the plant and into the soil) had been applied alone to utilise its reported plant defence activation mode of action and potentially to protect

against infection by *Verticillium* using competition and enzymatic activity. However, the EAMU for the product states a single drench application at planting and this may have been insufficient to elicit a plant response or provide sufficient barrier. Foliar application is permitted more frequently (previously 20 applications, but now reduced to six) and it is possible that this could help plant defence stimulation. Serenade ASO was applied to the Bio-Fence plots with the aim of replacing with *Bacillus subtilis* the beneficial and pathogenic micro-organisms killed by the Bio-Fence biofumigation. These beneficial bacteria could have potentially colonised around the roots and been present ready to resist any *Verticillium* that grew from microsclerotia unaffected by the bio-fumigant.

The anaerobic digestate was applied to increase the soil organic matter and potentially encourage the development of beneficial micro-organisms in the soil. However, the amount permitted to be incorporated was not that great and having been pasteurised it did not have a well-developed microbial flora of its own at the time of incorporation.

Where crops are sufficiently vigorous at the end of their second year of fruiting (with fruit harvest expected to be minimal in the first year) then they would be left to a third year of fruiting. However, plant crowns dying in trial plots after harvest owing to *Verticillium* wilt and further plants lacking vigour would mean that if this were a commercial crop then the grower would need to assess if the crop should be left to fruit again. Already in 2018, the Class 1 fruit had been lighter and overall the trial area yielded less than the chloropicrin treated crop.

## Conclusions

- Serenade ASO treated plants had significantly fewer plants showing *Verticillium* wilt symptoms at the end of the growing season than untreated control plants. This was shown not to be a consequence of lower soil infestation in these plots initially.
- Bio-Fence treated soil plots had significantly fewer healthy plants at the end of the growing season in July 2019 than present in untreated control plots.
- By the end of the harvest in June 2018 there was no difference in the total weight of fruit produced from plots that had been left untreated compared with those treated to reduce the effect of *Verticillium* in the soil. Plots that had either received anaerobic digestate or Bio-Fence incorporation prior to planting at the end of May 2017 had similar yields to those drenched with Serenade ASO in early June 2017 and these yields were all similar to plots receiving both Bio-Fence and Serenade ASO.
- There were no significant differences in either the total marketable fruit yield or the total weight of waste fruit harvested from either the untreated or the four treatments.

- There was a significant difference at the first pick only, when yields were low, when a few more fruit were classed as waste in the treatment in which digestate had been incorporated than recorded in the other treatments.
- When allowance was made for lower plant establishment in the Bio-Fence alone treated plots there was a significant difference at the first pick only whereby these produced a greater weight of marketable fruit per plant than the untreated and Serenade ASO alone treated plants. The digestate or Bio-Fence plus Serenade ASO plots had similar, in between, yields per plant to all the other plots.
- At the final harvest of the trial, plants in this area of the field produced only 41% of the weight of marketable fruit harvested in the commercial crop, and of the total amount of fruit produced per plant a greater proportion was waste. This indicates that the plants in the trial area were not producing fruit to their full potential and (as husbandry was similar in both areas of the field, exceptions being chloropicrin use, planting three rows per bed and strawing-over in the commercial crop) this may demonstrate the need for effective soil sterilisation to ensure good economic return from the crop.

## Knowledge and Technology Transfer

- 21<sup>st</sup> November 2018, 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
- An article on strawberry *Phytophthora* management submitted (November 2018) for AHDB Grower magazine
- An article on strawberry powdery mildew management submitted (February 2019) for AHDB Grower magazine

## Appendix

### Appendix 1: Plot layout for the *Phytophthora cactorum* study

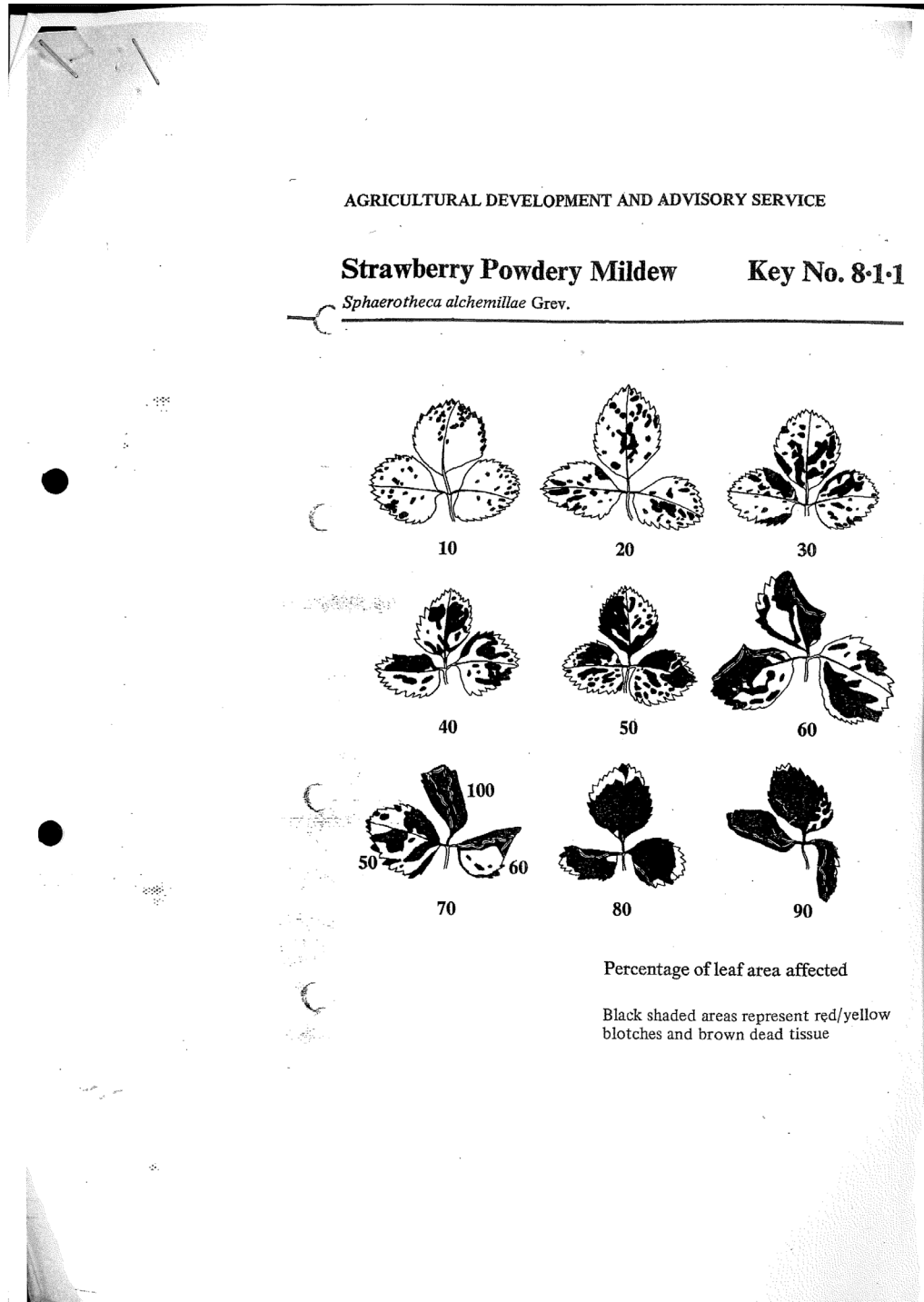
Plot plan for the trial "Managing nursery-originated infection of *P. cactorum*"

| Block 4      |    | Block 3      |   | Block 2      |   | Block 1      |   |
|--------------|----|--------------|---|--------------|---|--------------|---|
| F252         | 3* | Prestop      | 2 | Uninoculated |   | F251         | 2 |
| Dipping only | 2  | Dipping only | 3 | Control      |   | Dipping only | 1 |
|              | 1  |              | 1 |              |   |              | 3 |
| F250         | 1  | F252         | 1 | Prestop      | 1 | Uninoculated |   |
| Dipping +    | 2  | Dipping only | 3 | Dipping only | 3 | Control      |   |
| Drenching    | 3  |              | 2 |              | 2 |              |   |
| F251         | 2  | Fenomenal    | 2 | F251         | 3 | Fenomenal    | 1 |
| Dipping +    | 1  | Dipping +    | 3 | Dipping +    | 1 | Dipping only | 3 |
| Drenching    | 3  | Drenching    | 1 | Drenching    | 2 |              | 2 |
| F251         | 3  | F251         | 3 | Prestop      | 2 | Prestop      | 3 |
| Dipping only | 2  | Dipping only | 1 | Dipping +    | 3 | Dipping only | 1 |
|              | 1  |              | 2 | Drenching    | 1 |              | 2 |
| Fenomenal    | 1  | Prestop      | 1 | F250         | 3 | F252         | 1 |
| Dipping +    | 3  | Dipping +    | 2 | Dipping +    | 1 | Dipping +    | 3 |
| Drenching    | 2  | Drenching    | 3 | Drenching    | 2 | Drenching    | 2 |
| F250         | 1  | Inoculated   | 2 | Inoculated   | 1 | Prestop      | 1 |
| Dipping only | 2  | Control      | 3 | Control      | 2 | Dipping +    | 2 |
|              | 3  |              | 1 |              | 3 | Drenching    | 3 |
| F252         | 2  | F252         | 1 | Fenomenal    | 3 | F251         | 1 |
| Dipping +    | 3  | Dipping +    | 3 | Dipping +    | 2 | Dipping +    | 2 |
| Drenching    | 1  | Drenching    | 2 | Drenching    | 1 | Drenching    | 3 |
| Uninoculated |    | F250         | 3 | F251         | 3 | Fenomenal    | 2 |
| Control      |    | Dipping +    | 1 | Dipping only | 1 | Dipping +    | 3 |
|              |    | Drenching    | 2 |              | 2 | Drenching    | 1 |
| Prestop      | 2  | F251         | 3 | F252         | 3 | F252         | 2 |
| Dipping +    | 1  | Dipping +    | 1 | Dipping only | 2 | Dipping only | 3 |
| Drenching    | 3  | Drenching    | 2 |              | 1 |              | 1 |
| Fenomenal    | 3  | Uninoculated |   | Fenomenal    | 2 | Inoculated   | 2 |
| Dipping only | 2  | Control      |   | Dipping only | 3 | Control      | 1 |
|              | 1  |              |   |              | 1 |              | 3 |
| Prestop      | 1  | Fenomenal    | 3 | F252         | 3 | F250         | 3 |
| Dipping only | 3  | Dipping only | 2 | Dipping +    | 1 | Dipping +    | 1 |
|              | 2  |              | 1 | Drenching    | 2 | Drenching    | 2 |
| Inoculated   | 1  | F250         | 2 | F250         | 1 | F250         | 2 |
| Control      | 3  | Dipping only | 3 | Dipping only | 2 | Dipping only | 1 |
|              | 2  |              | 1 |              | 3 |              | 3 |



\*: number of times the plants were inoculated with *P. cactorum* in the autumn 2017.  
There were two for each inoculation time, i.e. six bags in each product treatment

Appendix 2: scanned copy of the mildew assessment key used in the mildew trial at NIAB EMR





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

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**Appendix 3.** % Botrytis rot (angular transformed) in post-harvest tests in 20 picks from everbearer strawberries following treatment with 4 different programmes compared to an untreated control at NIAB EMR in 2018. (figures in brackets are back transformed data)

| Treatment                     | Pick date / % botrytis rot |                |               |               |              |         |              |         |       |              |              |               |              |                |                      |                |                |                |                |                | Over all rot   |
|-------------------------------|----------------------------|----------------|---------------|---------------|--------------|---------|--------------|---------|-------|--------------|--------------|---------------|--------------|----------------|----------------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                               | 6 July                     | 9 July         | 13 July       | 16 July       | 20 July      | 23 July | 27 July      | 30 July | 3 Aug | 6 Aug        | 9 Aug        | 13 Aug        | 17 Aug       | 20 Aug         | 24 Aug               | 28 Aug         | 31 Aug         | 4 Sep          | 10 Sep         | 17 Sep         |                |
| T1: Untreated                 | 8.6<br>(2.2)               | 12.2<br>(4.5)  | 9.1<br>(2.5)  | 16.4<br>(8.0) | 0            | 0       | 0            | 2.0 (1) | 0     | 0            | 2.0<br>(0.1) | 9.8<br>(2.9)  | 7.1<br>(1.5) | 25.3<br>(18.3) | 23.8b<br>(16.2)      | 32.6<br>(29.0) | 29.6<br>(24.4) | 43.5<br>(47.4) | 23.9<br>(16.4) | 22.9<br>(15.2) | 19.3<br>(10.9) |
| T2: Routine fungicide         | 10.0<br>(3.0)              | 25.3<br>(18.2) | 7.5<br>(1.7)  | 8.0<br>(1.9)  | 0            | 0       | 0            | 0       | 0     | 0            | 5.1<br>(0.8) | 14.3<br>(6.1) | 6.4<br>(1.3) | 33.8<br>(30.9) | 32.0b<br>(28.0)      | 30.0<br>(25.0) | 18.5<br>(10.0) | 26.2<br>(19.5) | 15.1<br>(6.8)  | 10.3<br>(3.2)  | 17.8<br>(9.3)  |
| T3: Mildew managed            | 3.7<br>(0.4)               | 15.8<br>(7.4)  | 10.7<br>(3.4) | 8.7<br>(2.3)  | 3.0<br>(0.3) | 0       | 2.0<br>(0.1) | 0       | 0     | 6.6<br>(1.3) | 0            | 6.4<br>(1.3)  | 7.5<br>(1.7) | 22.8<br>(15.0) | 23.0a<br>b<br>(15.3) | 19.0<br>(10.6) | 19.9<br>(11.6) | 18.7<br>(10.3) | 30.6<br>(26.0) | 19.8<br>(11.4) | 15.9<br>(7.5)  |
| T4 :Botrytis managed          | 6.3<br>(1.2)               | 32.5<br>(28.9) | 9.1<br>(2.5)  | 0             | 0            | 0       | 0            | 0       | 0     | 5.6<br>(0.9) | 4.9<br>(0.7) | 0             | 4.1<br>(0.5) | 23.7<br>(16.2) | 9.8a<br>(2.9)        | 13.2<br>(5.2)  | 18.6<br>(10.2) | 22.3<br>(14.3) | 37.2<br>(36.5) | 22.5<br>(14.6) | 16.9<br>(8.4)  |
| T5: Mildew + Botrytis managed | 0                          | 25.9<br>(19.1) | 5.6<br>(0.9)  | 13.6<br>(5.6) | 0            | 0       | 0            | 0       | 0     | 9.0<br>(2.5) | 6.9<br>(1.5) | 8.6<br>(2.2)  | 5.5<br>(0.9) | 16.6<br>(8.2)  | 22.7a<br>b<br>(14.9) | 21.2<br>(13.1) | 28.0<br>(22.1) | 30.5<br>(25.7) | 35.1<br>(33.0) | 14.7<br>(6.4)  | 17.4<br>(8.9)  |
| F Prob                        | 0.50                       | 0.49           | 0.99          | 0.26          | 0.45         |         | 0.44         | 0.44    |       | 0.38         | 0.37         | 0.11          | 0.97         | 0.84           | 0.05                 | 0.15           | 0.19           | 0.26           | 0.15           | 0.70           | 0.67           |
| SED (12)                      | 6.0                        | 12.22          | 9.32          | 7.20          | 1.91         |         | 1.29         | 1.29    |       | 5.40         | 3.61         | 4.80          | 5.26         | 14.64          | 6.17                 | 7.93           | 5.71           | 11.08          | 8.86           | 10.36          | 2.27           |
| LSD (p=0.05)                  | 13.1                       | 26.62          | 20.52         | 15.69         | 4.17         |         | 2.80         | 2.80    |       | 11.76        | 7.86         | 10.46         | 11.45        | 31.89          | 13.45                | 17.29          | 12.45          | 24.14          | 19.30          | 22.58          | 4.95           |

**Appendix 4.** % *Penicillium* rot (angular transformed) in post-harvest tests in 20 picks from everbearer strawberries following treatment with 4 different programmes compared to an untreated control at NIAB EMR in 2018. (figures in brackets are back transformed data)

| Treatment                     | Pick date / % <i>Penicillium</i> rot |                |                |                |               |                |               |              |          |              |              |              |              |               |               |              |                |               |                      |              | Overall<br>I rot |
|-------------------------------|--------------------------------------|----------------|----------------|----------------|---------------|----------------|---------------|--------------|----------|--------------|--------------|--------------|--------------|---------------|---------------|--------------|----------------|---------------|----------------------|--------------|------------------|
|                               | 6<br>July                            | 9<br>July      | 13<br>July     | 16<br>July     | 20<br>July    | 23<br>July     | 27<br>July    | 30<br>July   | 3<br>Aug | 6<br>Aug     | 9<br>Aug     | 13<br>Aug    | 17<br>Aug    | 20<br>Aug     | 24<br>Aug     | 28<br>Aug    | 31<br>Aug      | 4<br>Sep      | 10<br>Sep            | 17<br>Sep    |                  |
| T1: Untreated                 | 11.9<br>(4.3)                        | 46.1<br>(51.9) | 30.7<br>(26.0) | 33.5<br>(30.5) | 12.4<br>(4.6) | 20.1<br>(11.8) | 2.0<br>(0.1)  | 5.8<br>(1.0) | 0        | 2.0<br>(0.1) | 2.9<br>(0.3) | 8.2<br>(2.0) | 2.0<br>(0.1) | 4.1<br>(0.5)  | 5.6<br>(0.9)  | 0            | 7.7<br>(1.8)   | 8.3<br>(2.1)  | 19.6a<br>b<br>(11.2) | 4.0<br>(0.5) | 16.8<br>(8.4)    |
| T2: Routine fungicide         | 19.9<br>(11.5)                       | 43.2<br>(46.8) | 41.1<br>(43.2) | 33.8<br>(30.9) | 11.4<br>(3.9) | 20.3<br>(12.0) | 2.0<br>(0.1)  | 0            | 0        | 0            | 6.1<br>(1.1) | 8.0<br>(1.9) | 2.9<br>(0.3) | 7.5<br>(1.7)  | 7.6<br>(1.8)  | 4.5<br>(0.6) | 7.8<br>(1.8)   | 13.0<br>(5.0) | 6.6a<br>(1.3)        | 6.6<br>(1.3) | 17.9<br>(9.4)    |
| T3: Mildew managed            | 8.1<br>(2.0)                         | 23.6<br>(16.1) | 36.1<br>(34.7) | 37.7<br>(37.4) | 11.0<br>(3.6) | 21.3<br>(13.2) | 11.0<br>(3.7) | 0            | 0        | 8.5<br>(2.2) | 3.5<br>(0.4) | 8.2<br>(2.0) | 2.9<br>(0.3) | 5.6<br>(0.9)  | 7.9<br>(1.9)  | 2.0<br>(0.1) | 6.4<br>(1.2)   | 13.7<br>(5.6) | 18.9a<br>b<br>(10.5) | 5.7<br>(1.0) | 16.4<br>(8.0)    |
| T4 :Botrytis managed          | 15.4<br>(7.1)                        | 36.3<br>(35.0) | 41.7<br>(44.3) | 35.4<br>(33.5) | 16.7<br>(8.3) | 16.5<br>(8.1)  | 7.5<br>(1.7)  | 3.0<br>(0.3) | 0        | 2.0<br>(0.1) | 4.9<br>(0.7) | 0            | 0            | 12.2<br>(4.5) | 5.1<br>(0.8)  | 0            | 9.4<br>(2.7)   | 11.5<br>(4.0) | 17.2a<br>b (8.7)     | 5.7<br>(1.0) | 18.1<br>(9.7)    |
| T5: Mildew + Botrytis managed | 32.7<br>(29.2)                       | 42.6<br>(45.9) | 32.9<br>(29.5) | 37.2<br>(36.6) | 14.3<br>(6.1) | 25.9<br>(19.1) | 5.1<br>(0.8)  | 0            | 0        | 8.0<br>(1.9) | 8.7<br>(2.3) | 8.2<br>(2.0) | 2.9<br>(0.3) | 2.9<br>(0.3)  | 13.4<br>(5.4) | 4.4<br>(0.6) | 20.1<br>(11.8) | 17.4<br>(8.9) | 31.2b<br>(26.8)      | 0            | 20.6<br>(12.4)   |
| F Prob                        | 0.45                                 | 0.25           | 0.36           | 0.96           | 0.87          | 0.69           | 0.42          | 0.17         |          | 0.17         | 0.71         | 0.45         | 0.90         | 0.65          | 0.73          | 0.23         | 0.21           | 0.85          | 0.06                 | 0.88         | 0.11             |
| SED (12)                      | 13.54                                | 10.21          | 6.29           | 6.84           | 6.16          | 6.26           | 5.31          | 2.64         |          | 3.93         | 4.41         | 5.18         | 3.50         | 6.51          | 6.49          | 2.45         | 6.00           | 8.16          | 7.07                 | 6.96         | 1.49             |
| LSD (p=0.05)                  | 29.50                                | 22.25          | 13.84          | 14.92          | 13.42         | 13.64          | 11.58         | 5.76         |          | 8.56         | 9.63         | 11.29        | 7.63         | 14.18         | 14.15         | 5.34         | 13.05          | 17.77         | 15.40                | 15.17        | 3.24             |

**Appendix 5.** % Mucor/ Rhizopus rot (angular transformed) in post-harvest tests in 20 picks from everbearer strawberries following treatment with 4 different programmes compared to an untreated control at NIAB EMR in 2018. (figures in brackets are back transformed data)

| Treatment                     | Pick date / % Mucor / Rhizopus rot |                |                |                |                  |                |                  |                |                |                  |                |                |                |                |                |                |                |                |                |                | Overall rot    |
|-------------------------------|------------------------------------|----------------|----------------|----------------|------------------|----------------|------------------|----------------|----------------|------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                               | 6 July                             | 9 July         | 13 July        | 16 July        | 20 July          | 23 July        | 27 July          | 30 July        | 3 Aug          | 6 Aug            | 9 Aug          | 13 Aug         | 17 Aug         | 20 Aug         | 24 Aug         | 28 Aug         | 31 Aug         | 4 Sep          | 10 Sep         | 17 Sep         |                |
| T1: Untreated                 | 6.3<br>(1.2)                       | 20.1<br>(11.8) | 29.4<br>(24.1) | 21.5<br>(13.4) | 68.0b<br>(86.0)  | 29.9<br>(24.9) | 84.2b<br>(99.0)  | 49.2<br>(57.3) | 84.4<br>(99.1) | 78.2bc<br>(95.8) | 64.2<br>(95.8) | 66.4<br>(84.0) | 63.1<br>(79.5) | 45.2<br>(50.3) | 33.0<br>(29.6) | 44.1<br>(48.4) | 50.1<br>(58.9) | 40.0<br>(41.4) | 30.6<br>(25.9) | 28.8<br>(23.2) | 46.2<br>(52.1) |
| T2: Routine fungicide         | 12.4<br>(4.6)                      | 7.9<br>(1.9)   | 14.4<br>(6.2)  | 11.5<br>(4.0)  | 43.2a<br>(46.8)  | 22.0<br>(14.0) | 59.1ab<br>(73.6) | 64.7<br>(81.7) | 65.5<br>(82.8) | 82.3c<br>(98.2)  | 59.9<br>(98.2) | 55.0<br>(67.2) | 55.7<br>(68.2) | 41.7<br>(44.3) | 33.5<br>(30.4) | 42.7<br>(46.0) | 46.7<br>(53.0) | 50.8<br>(60.1) | 33.4<br>(30.3) | 14.9<br>(6.7)  | 42.1<br>(45.0) |
| T3: Mildew managed            | 9.5<br>(2.7)                       | 3.2<br>(0.3)   | 17.1<br>(8.6)  | 15.2<br>(6.9)  | 43.3a<br>(47.1)  | 25.3<br>(18.3) | 42.2a<br>(45.1)  | 66.6<br>(84.3) | 77.5<br>(95.3) | 65.9ab<br>(83.4) | 57.0<br>(83.4) | 63.6<br>(80.2) | 46.2<br>(52.1) | 46.4<br>(52.5) | 27.8<br>(21.8) | 41.5<br>(43.9) | 49.6<br>(58.1) | 48.0<br>(55.3) | 37.2<br>(36.5) | 26.2<br>(19.4) | 41.9<br>(44.6) |
| T4 :Botrytis managed          | 9.6<br>(2.8)                       | 5.2<br>(0.8)   | 20.5<br>(12.3) | 14.3<br>(6.1)  | 36.9a<br>(36.0)  | 15.6<br>(7.2)  | 49.2a<br>(57.4)  | 74.3<br>(92.7) | 62.5<br>(78.7) | 59.4a<br>(74.1)  | 53.8<br>(74.1) | 66.6<br>(84.3) | 52.1<br>(62.2) | 46.1<br>(51.8) | 34.5<br>(32.1) | 51.7<br>(61.6) | 50.7<br>(59.8) | 38.8<br>(39.3) | 26.5<br>(19.9) | 11.3<br>(3.8)  | 40.7<br>(42.6) |
| T5: Mildew + Botrytis managed | 16.3<br>(7.9)                      | 3.0<br>(0.3)   | 20.5<br>(12.3) | 16.8<br>(8.4)  | 51.3ab<br>(60.9) | 16.6<br>(8.2)  | 66.4ab<br>(84.0) | 54.9<br>(67.0) | 66.1<br>(83.6) | 66.3ab<br>(83.8) | 60.9<br>(83.8) | 71.3<br>(89.7) | 64.5<br>(81.5) | 45.6<br>(51.0) | 28.5<br>(22.8) | 41.4<br>(43.8) | 46.3<br>(52.2) | 41.9<br>(44.6) | 33.4<br>(30.3) | 10.7<br>(3.4)  | 42.1<br>(44.9) |
| F Prob                        | 0.86                               | 0.31           | 0.32           | 0.61           | 0.02             | 0.32           | 0.03             | 0.27           | 0.10           | 0.04             | 0.60           | 0.44           | 0.35           | 0.91           | 0.85           | 0.19           | 0.96           | 0.25           | 0.61           | 0.15           | 0.02           |
| SED (12)                      | 9.38                               | 8.64           | 7.00           | 6.27           | 8.06             | 7.39           | 11.78            | 11.49          | 8.41           | 7.24             | 6.59           | 8.43           | 9.72           | 5.50           | 7.41           | 4.47           | 7.55           | 5.94           | 6.74           | 8.40           | 1.44           |
| LSD (p=0.05)                  | 20.44                              | 18.83          | 15.37          | 13.66          | 17.57            | 16.09          | 25.66            | 25.04          | 18.35          | 15.78            | 14.35          | 18.37          | 21.17          | 12.0           | 16.14          | 9.75           | 16.44          | 12.95          | 14.68          | 18.30          | 3.14           |

**Appendix 6.** Table ?? % Total rot (angular transformed) at harvest in 20 picks from everbearer strawberries following treatment with 4 different programmes compared to an untreated control at NIAB EMR in 2018. (Figures in brackets are back transformed data)

| Treatment                     | Pick date / % rot |              |              |              |         |              |              |              |              |              |              |               |              |               |              |              |              |               |               |               | Overall rot |
|-------------------------------|-------------------|--------------|--------------|--------------|---------|--------------|--------------|--------------|--------------|--------------|--------------|---------------|--------------|---------------|--------------|--------------|--------------|---------------|---------------|---------------|-------------|
|                               | 6 July            | 9 July       | 13 July      | 16 July      | 20 July | 23 July      | 27 July      | 30 July      | 3 Aug        | 6 Aug        | 9 Aug        | 13 Aug        | 17 Aug       | 20 Aug        | 24 Aug       | 28 Aug       | 31 Aug       | 4 Sep         | 10 Sep        | 17 Sep        |             |
| T1: Untreated                 | 9.1<br>(2.5)      | 3.4<br>(0.4) | 4.0<br>(0.5) | 3.2<br>(0.3) | 0       | 2.6<br>(0.2) | 0            | 2.3<br>(0.2) | 5.9<br>(1.1) | 1.8<br>(0.1) | 0            | 9.3<br>(2.6)  | 9.5<br>(2.7) | 6.5<br>(1.3)  | 8.1<br>(2.0) | 3.6<br>(0.4) | 4.2<br>(0.5) | 12.1<br>(4.4) | 14.3<br>(6.1) | 6.0<br>(1.1)  | 8.5 (2.2)   |
| T2: Routine fungicide         | 9.8<br>(2.9)      | 0            | 0            | 0            | 0       | 0            | 4.3<br>(0.6) | 0            | 1.7<br>(0.1) | 0            | 0            | 10.0<br>(3.0) | 7.0<br>(1.5) | 2.2<br>(0.2)  | 0            | 7.0<br>(1.5) | 0            | 0             | 7.9<br>(1.9)  | 5.2<br>(0.8)  | 5.8 (1.0)   |
| T3: Mildew managed            | 7.2<br>(1.6)      | 0            | 0            | 4.2<br>(0.5) | 0       | 2.6<br>(0.2) | 2.0<br>(0.1) | 2.6<br>(0.2) | 5.8<br>(1.0) | 1.8<br>(0.1) | 2.0<br>(0.1) | 7.0<br>(1.5)  | 7.7<br>(1.8) | 7.0<br>(1.5)  | 5.3<br>(0.8) | 6.6<br>(1.3) | 4.2<br>(0.5) | 3.9<br>(0.5)  | 0             | 10.9<br>(3.6) | 6.9 (1.4)   |
| T4 :Botrytis managed          | 2.9<br>(0.3)      | 0            | 0            | 0            | 0       | 0            | 0            | 2.3<br>(0.2) | 0            | 1.6<br>(0.1) | 0            | 11.1<br>(3.7) | 7.0<br>(1.5) | 10.0<br>(3.0) | 6.6<br>(1.3) | 3.3<br>(0.3) | 7.1<br>(1.5) | 4.2<br>(0.5)  | 10.4<br>(3.3) | 9.6<br>(2.8)  | 7.3 (1.6)   |
| T5: Mildew + Botrytis managed | 2.5<br>(0.2)      | 0            | 0            | 5.2<br>(0.8) | 0       | 0            | 0            | 0            | 2.1<br>(0.1) | 0            | 0            | 4.7<br>(0.7)  | 7.9<br>(1.9) | 4.8<br>(0.7)  | 7.6<br>(1.8) | 3.5<br>(0.4) | 0            | 13.1<br>(5.1) | 4.6<br>(0.6)  | 12.7<br>(4.8) | 6.9 (1.4)   |
| F Prob                        | 0.41              | 0.45         | 0.45         | 0.77         |         | 0.61         | 0.25         | 0.78         | 0.14         | 0.71         | 0.45         | 0.35          | 0.87         | 0.07          | 0.28         | 0.85         | 0.56         | 0.16          | 0.29          | 0.70          | 0.14        |
| SED (12)                      | 4.64              | 2.16         | 2.55         | 5.04         |         | 2.4          | 2.17         | 2.86         | 2.53         | 1.85         | 1.27         | 3.27          | 2.59         | 2.39          | 3.82         | 4.49         | 4.91         | 5.67          | 6.50          | 6.08          | 0.92        |
| LSD (p=0.05)                  | 10.11             | 4.70         | 5.55         | 10.98        |         | 5.2          | 4.72         | 6.24         | 5.52         | 4.04         | 2.77         | 7.13          | 5.69         | 5.20          | 8.42         | 9.78         | 10.69        | 12.35         | 14.16         | 13.24         | 2.00        |