# Final Trial Report

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|---|---|
| **Trial code:** | SP70 |
| **Title:** | **Managing nursery-originated infection of *Phytophthora cactorum* in planting material via flush irrigation treatments with selected chemical and biocontrol products** |
| **Crop** | **Protected strawberry grown in coir bags** |
| **Target** | Strawberry crown rot |
| **Lead researcher:** | Xiangming Xu |
| **Organisation:** | NIAB EMR |
| **Period:** | 01/10/2019 to 31/01/2021 |
| **Report date:** | 04/03/2021 |
| **Report author:** | Xiangming |
| **ORETO Number: (certificate should be attached)** | |

I the undersigned, hereby declare that the work was performed according to the procedures herein described and that this report is an accurate and faithful record of the results obtained

24/03/2021……
Date

…… 
Authors signature

# Trial Summary

## Introduction

In SF157 (04/2015 – 03/2020), we conducted studies on strawberry crown rot and showed that: (1) the level of *Phytophthora cactorum* DNA detected in runners at planting can reach 30%; (2) a few products when applied as a dipping treatment at planting time, significantly reduced the losses due to *P. cactorum*; and (3) applying products post-planting through fertigation can be as effective at controlling crown rot as dipping.

## Methods

In late autumn 2019, 14 crop protection products were tested via fertigation for their efficacy against crown rot using the protocol developed in SF157: (1) tray plants were inoculated with *P. cactorum* in autumn; then placed into cold store several weeks after inoculation; (2) planted out on 4th June 2021 and treatments applied as flush fertigation one week after; (3) plants were monitored for visual disease symptoms; (4) crown tissue health was assessed post-harvest; and (4) crowns were screened molecularly for *P. cactorum DNA*.

## Results

There were no significant differences among treatments for wilting and incidence of the pathogen DNA; but a few treatments led to reduced crown tissue browning (Table S1).

**Table S1. Summary of visual assessment (incidence of plant wilting and crown browning) and molecular screening (incidence of pathogen DNA detected)**

| Treatment | Incidence of plant wilting (including dead plants) | | | Crown tissue browning | Positive PCR |
|---|---|---|---|---|---|
| | 24/07 | 06/08 | 24/08 | | |
| Control | 0.033 | 0.167 | 0.225 | 0.158 | 0.472 (0.359)[$] |
| AHDB 9958 | 0.017 | 0.042 | 0.083 | 0.067 | 0.280 (0.339) |
| AHDB 9809 | 0.050 | 0.083 | 0.142 | 0.033 | 0.125 (0.332) |
| AHDB 9783 | 0.017 | 0.033 | 0.117 | 0.058 | 0.450 (0.337) |
| AHDB 9777 | 0.083 | 0.192 | 0.258 | 0.042 | 0.263 (0.334) |
| AHDB 9808 | 0.017 | 0.058 | 0.158 | 0.083 | 0.440 (0.343) |
| AHDB 9882 | 0.075 | 0.117 | 0.142 | 0.017* | 0.318 (0.328) |
| AHDB 9814 | 0.075 | 0.100 | 0.100 | 0.017* | 0.412 (0.328) |
| AHDB 9942 | 0.017 | 0.050 | 0.167 | 0.133 | 0.550 (0.354) |
| AHDB 9937 | 0.042 | 0.108 | 0.242 | 0.133 | 0.286 (0.354) |
| AHDB 9941 | 0.008 | 0.100 | 0.108 | 0.092 | 0.600 (0.345) |
| AHDB 9883 | 0.025 | 0.075 | 0.183 | 0.025+ | 0.368 (0.330) |
| AHDB 9939 | 0.008 | 0.200 | 0.258 | 0.108 | 0.368 (0.348) |
| AHDB 9955 | 0.025 | 0.117 | 0.158 | 0.050 | 0.381 (0.336) |
| AHDB 9730 | 0.017 | 0.158 | 0.242 | 0.133 | 0.450 (0.354) |

[$]: Figures in the brackets are the estimated incidence of positive PCR results adjusted for crown health

| | |
|---|---|
| | Not significantly different from untreated control (p>0.10) |
| | Difference with the control close to significance (0.05<p<0.10) |
| | Significantly different from untreated control (p<0.05) |

## Conclusions

Results from the group testing and comparisons of individual treatments with the control indicated that AHDB 9814, AHDB 9883, AHDB 9955, AHDB 9809, AHDB 9958 and AHDB 9783 treatments resulted in a greater (P < 0.10) reduction in crown tissue browning than the other products and could be further tested in a larger trial under (close to) commercial conditions. AHDB 9955, AHDB 9809, AHDB 9958 and AHDB 9783 was also very close to AHDB 9814. AHDB 9882 was able to reduce crown tissue browning but its application led to residues on fruit, exceeding the permitted MRL.

### Take home message:

AHDB 9955 may be used via fertigation to manage crown rot (as also showed activity in SF157). Further testing of three commercial products (AHDB 9955, AHDB 9814 and AHDB 9883) is needed under commercial conditions.

## Objectives

Following evaluation of some treatments for disease control in SF157 (04/2015 – 03/2020), 14 crop protection products (synthetic fungicides, biopesticides) were tested for their efficacy against crown rot in strawberry; these products were applied via bursts of fertigation events following the protocols developed in SF157.

### Trial conduct

This trial was not designated an official SCEPTRE PLUS trial, rather an extension of SF157 with funding from the soft fruit panel but results channeled through SCEPTRE PLUS.

### Test site

| Item | Details |
|---|---|
| Location address | NIAB EMR, East Malling, ME19 6BJ |
| Crop | Strawberry (protected) |
| Cultivar | Malling Centenary |
| Soil or substrate type | Botanicoir |
| Agronomic practice | Standard commercial practice |
| Prior history of site | Used for crown rot trial in SF157 |

### Trial design

| Item | Details |
|---|---|
| Trial design: | Randomized block design |
| Number of replicates: | 4 blocks; in each block one plot has 30 plants |
| Row spacing: | 50 cm |
| Plot size: (w x l) | 3 coir bags [bag- ca 1 m x 0.35 m] |
| Plot size: ($m^2$) | 1 $m^2$ |
| Number of plants per plot: | 30 |
| *Leaf Wall Area calculations* | |

## Treatment details (no information on adjuvant)

| AHDB Code | Active substance | Product name/ manufacturers code | Formulation batch number | Content of active substance in product | Formulation type | Rate (g, ml/L) (100 ml / plant = 10 plants (1 bag) |
|---|---|---|---|---|---|---|
| | | **Untreated - inoculated** | | | | |
| | | **Untreated - uninoculated** | | | | |
| AHDB9777 | N/D | N/D | N/D | N/D | N/D | N/D |
| AHDB 9937 | N/D | N/D | N/D | N/D | N/D | N/D |
| AHDB 9955 | *Trichoderma asperellum* strain T34 | **T34, Fargo** | 07191710 | Minimum $1x10^{12}$ *Trichoderma asperellum* cfu/Kg (12%ww) | Wettable powder | 0.25 g |
| AHDB 9942 | N/D | N/D | N/D | N/D | N/D | N/D |
| AHDB 9783 | N/D | N/D | N/D | N/D | N/D | N/D |
| AHDB 9809 | N/D | N/D | N/D | N/D | N/D | N/D |
| AHDB 9958 | N/D | N/D | N/D | N/D | N/D | N/D |
| AHDB 9808 | N/D | N/D | N/D | N/D | N/D | N/D |
| AHDB 9939 | N/D | N/D | N/D | N/D | N/D | N/D |
| AHDB 9730 | N/D | N/D | N/D | N/D | N/D | N/D |
| AHDB 9814 | N/D | N/D | N/D | N/D | N/D | N/D |
| AHDB 9882 | N/D | N/D | N/D | N/D | N/D | N/D |
| AHDB 9941 | N/D | N/D | N/D | N/D | N/D | N/D |
| AHDB 9883 | N/D | N/D | N/D | N/D | N/D | N/D |

## Application schedule:

Applied via flush fertigation about one week after planting, with application details presented in the report section.

## Untreated levels of pathogens at application and through the assessment period

This is not applicable since we are dealing with latent *P. cactorum* infections pre-existing in planting materials prior to production application. Because these infections can remain latent for a varying period of time, it is not possible to assess the background infection level until the final molecular screening using PCR of crown tissues. The PCR test indicated the presence / absence of pathogen DNA and results are presented below.

### Assessment details

Plants were assessed in early fruiting, mid-fruiting, and immediately post-harvest for wilting symptoms. Crown health was assessed in late September 2020 after a period of mild drought stress; then a number of crowns from each treatment were sampled and stored and later processed for detection of *P. cactorum* using PCR during the winter in the laboratory.

## Introduction

Adopting a clean propagation system is the first line of defence against crown rot and red-core diseases. This strategy had worked for many years until recent times. Currently, crown rot (*P. cactorum*) and red-core (*P. fragariae*) can cause significant damage in strawberry even in substrate production. The most likely cause is asymptomatic infection in planting materials. Frequent application of conventional chemical fungicides, alleged to have occurred in overseas nurseries, may delay the onset of symptom development until post-transplanting. Subsequent disease spread is more 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 (SF121). Recent research on *Phytophthora* spp. has concentrated on detecting the pathogens and seeking products to reduce root rotting. HDC project, SF130 focussed on fungal molecular quantification; a PCR assay was developed that detected *P. rubi*, although it was not as sensitive as the *P. fragariae* assay (which detects both pathogens). SF123 looked at alternative products against *P. rubi* on raspberry where one novel chemical product gave disease reduction. Red-core is more difficult to control and currently there is no work on controlling this disease; survey results from AHDB SF157 showed that *P. fragariae* was rarely detected in planting materials.

NIAB EMR has just completed a BBSRC project (BB/K017071/2), in which a number of quantitative resistance factors against *P. cactorum* were identified. These resistance factors will be exploited in breeding programmes at NIAB EMR. More research is required to assist growers in applying appropriate treatments as AHDB recognised that it is not feasible to change the behaviour of continental propagators.

In SF157 (04/2015 – 03/2020), a number of studies were conducted on strawberry crown rot and arrived at a several conclusions:

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

## Methods and Materials

### General considerations

The survey of strawberry planting material from multiple batches in year 1 and 2 of AHDB SF157, coupled with detection of pathogen DNA in bare-rooted runners, indicated that the level of contamination of *P. cactorum* could reach 25-30% of the plants although more commonly the level is less than 5%. Thus, un-inoculated plants were not expected to be ‘disease-free’. On the other hand, the background level of latent infection is variable and usually not high enough to assess treatment effects reliably under the usual size of experiments. Thus, plants were inoculated to ensure consistent

"high" levels of infection across the treatments. Thus, crown rot symptoms in assessed plants could result from two sources: (1) background infections that were present in all plants; and (2) artificial inoculation as carried out at NIAB EMR prior to cold storage. The differences between the inoculated and un-inoculated controls indicate the extent of successfulness of the artificial inoculation; however, such differences do not affect the conclusions drawn from the experiment. From the same reasoning, some treatments may have lower levels of diseases than the **un-inoculated** plants if (1) the level of the background infection is high; and/or (2) these treatments could delay/reduce crown rot development resulting from the background infections as well as from the artificial inoculations.

## Plants, pathogen and inoculation

The timeline for all key tasks is given in Appendix 1. Fresh tray plants (super elite) of a June-bearer strawberry cultivar (Malling Centenary) were obtained from a commercial nursery in October 2019.

Based on previous studies in SF 157, three pathogenic *P. cactorum* isolates were used; plants were inoculated twice (about 2 weeks apart) to increase latent infection. A suspension of $10^4$ - $10^5$ zoospores $ml^{-1}$ was produced following a previously published method (Harris, Simpson and Bell, 1997). Each crown was inoculated without wounding by directly pipetting 3 ml inoculum onto the crown. Inoculated plants were kept in a polytunnel for several weeks to allow infection to take place and to harden before cold storage. Several days before being moved to the cold store, these plants were sprayed with Teldor (fenhexamid) to control Botrytis. Un-inoculated plants (around 400) were placed in separate clean crates in the cold store to avoid cross-contamination.

## Treatments and experimental design

There was only one treatment application method: products applied via irrigation line. There were 14 products (Table 1), including Fenomenal used for comparative purposes. In addition, there were two control treatments: (1) untreated inoculated control (positive control) and (2) un-inoculated untreated control (negative control).

Each treatment had 120 plants planted in 12 bags. Ten plants in each bag (Botanicoir) were planted in a zig-zag pattern. A randomised block design was used with four blocks. Within each block, each treatment had 30 plants (3 coir bags). The experiment was conducted in a polytunnel with the bags laid on the top of plastic boxes (with holes allowing water through).

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### Applying treatments

Due to COVID 19, plants were planted out on 4th June instead of late May as originally planned. However, this should not matter as symptoms of crown rot in infected planting materials are likely to be induced by post-planting stresses – hence late planting may have increase crown rot development. Plants were fertigated with a total of 6 L per hour per bag (using four sub-drippers per bag).

To calculate the length of time for the product to reach the drippers from the irrigation rig a dye (used for spray deposition trials) visible to the eye was added to the irrigation rig to calculate the time taken from the rig through to the drippers with the end of the irrigation pipe open. As a result, treatments were applied to the irrigation rig through a Dosatron with the irrigation pipe open and drippers out of the bags for 12 seconds before closing the end of the pipe, inserting drippers into bags to be treated and the timer then started for the product application time of 3 min 10 s for each application event. For fertigation treatments, each product was applied through the irrigation pipe twice (i.e., 6min 20s total treatment application time, Photo 2), with a minimum two hours between two consecutive irrigation events to avoid overflowing (determined from previous SF157 experiments). The pipes were cleaned between treatments by opening up the end of the pipe for 45-60 s to allow the flow of clean water through the pipes and the drippers. To avoid potential harmful effects of conventional chemical pesticides on biofungicides, a separate irrigation pipe was used for application of the biofungicides. The control treatment bags were irrigated with water for the same duration through the regular irrigation lines whilst drippers were not inserted for the treated bags. For each product, the stock solution was made at a specific concentration so that the total amount of product delivered to each plant after the two irrigation events was at the selected rate (Table 2).

Thereafter, the exact fertigation frequency/time was determined by regular measurement of coir substrate moisture.

## Assessment

Overall, strawberry fruit production was not as good as in a commercial production. All fruit were picked and classified into marketable and unmarketable categories. As there were no statistically significant differences between treatments, yield data are not presented.

**Residue testing**

In late July, one composite sample of fruit from across the four blocks was taken for each of the chemical treatments and sent to QTS Analytical Ltd for residue testing.

**Plant symptoms**

Assessments were made on plant symptoms (Photo 2, potentially caused by *P. cactorum*) in the early (24th July) and mid fruiting (6th August) period as well as post-harvesting (24th August) and in late September before destructive sampling of crown tissue. Each plant was recorded in three categories: healthy, witling, and dead. During harvest onwards, periods of drought stress were applied by reducing irrigation to induce disease development, before a final visual assessment for crown tissue discolouring of all surviving plants in late September.

## Molecular screening to determine presence of *P. cactorum* using PCR

Two, or occasionally three, crown tissues (i.e., 2-3 plants) were sampled from each coir bag for molecular screening, aiming to sample as many crown tissues as possible with the "browning" symptoms since SF157 results indicated that the pathogen is most likely to be present in the discoloured crown tissues. In total, we sampled 430 crowns for molecular screening for the presence of *P. cactorum* DNA and the sampled crowns were stored in -20°C fridge for subsequent molecular detection of *P. cactorum* DNA.

DNA was extracted from strawberry crown samples using DNeasy Plant Mini Kit (Qiagen) following the manufacturer's instructions with all optional steps. DNA was quantified and quality-checked using a Nanodrop 1000 spectrophotometer and stored at -20 °C.

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

**Table 2. Sequences (5'-3') for primer pairs used to screen strawberry runners**

| Primer set | Target | Forward primer | Reverse Primer |
|---|---|---|---|
| Yph[a] | *Phytophthora* spp. | CGACCATKGGTGTGGACTTT | ACGTTCTCMCAGGCGTATCT |
| Ycac[a] | *P. cactorum* | CCATACAAAATTCTGCGCTAGG | AGACACACAAGTGGACCGTTAG |
| FaEF | *Fragaria* | TGGATTTGAGGGTGACAACATGA | GTATACATCCTGAAGTGGTAGACGGAGG |

[a] Schena, Duncan, and Cooke (2008)

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

### Statistical analysis

There were two disease-related variables: number of plants with wilting symptoms (including dead plants), and the number of plants with the presence of *P. cactorum* DNA. These data were analysed using R (version 3.6.1). The 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 association between plant wilting, crown tissue browning and molecular screening test was assessed with the Fisher's exact test.

The symptom data were analysed using generalised linear models (GLM) with residual errors assumed to follow a binomial or quasi-binomial (to account for over-dispersion) distribution. Because of the nature of GLM, significance of treatment differences is not directly based on the standard errors on the original measurement scale; thus error bars were not presented on the original scale in graphs. Comparison of individual treatments with the untreated control treatments was based on deviance testing following the nest-model analysis in GLM.

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

## Results

### Efficacy summary

Table 3 show the overall summary of product efficacy for plant wilting, crown tissue browning and pathogen DNA presence.

**Table 3. Summary of visual assessment (incidence of plant wilting and crown discolouring) and molecular screening (incidence of pathogen DNA detected)**

| Treatment | Incidence of plant wilting (including dead plants) | | | | Crown tissue browning | Positive PCR |
|---|---|---|---|---|---|---|
| | 24/07 | 06/08 | 24/08 | 28/09 | | |
| Control | 0.033 | 0.167 | 0.225 | 0.317 | 0.158 | 0.472 (0.359)[$] |
| AHDB 9958 | 0.017 | 0.042 | 0.083 | 0.275 | 0.067 | 0.280 (0.339) |
| AHDB 9809 | 0.050 | 0.083 | 0.142 | 0.175 | 0.033 | 0.125 (0.332) |
| AHDB 9783 | 0.017 | 0.033 | 0.117 | 0.325 | 0.058 | 0.450 (0.337) |
| AHDB 9777 | 0.083 | 0.192 | 0.258 | 0.325 | 0.042 | 0.263 (0.334) |
| AHDB 9808 | 0.017 | 0.058 | 0.158 | 0.250 | 0.083 | 0.440 (0.343) |
| AHDB 9882 | 0.075 | 0.117 | 0.142 | 0.200 | 0.017* | 0.318 (0.328) |
| AHDB 9814 | 0.075 | 0.100 | 0.100 | 0.233 | 0.017* | 0.412 (0.328) |
| AHDB 9942 | 0.017 | 0.050 | 0.167 | 0.400 | 0.133 | 0.550 (0.354) |
| AHDB 9937 | 0.042 | 0.108 | 0.242 | 0.400 | 0.133 | 0.286 (0.354) |
| AHDB 9941 | 0.008 | 0.100 | 0.108 | 0.225 | 0.092 | 0.600 (0.345) |
| AHDB 9883 | 0.025 | 0.075 | 0.183 | 0.225 | 0.025+ | 0.368 (0.330) |
| AHDB 9939 | 0.008 | 0.200 | 0.258 | 0.350 | 0.108 | 0.368 (0.348) |
| AHDB 9955 | 0.025 | 0.117 | 0.158 | 0.283 | 0.050 | 0.381 (0.336) |
| AHDB 9730 | 0.017 | 0.158 | 0.242 | 0.392 | 0.133 | 0.450 (0.354) |

[$]: Figures in the brackets are the estimated incidence of positive PCR results adjusted for crown health

| | |
|---|---|
| | Not significantly different from untreated control ($p>0.10$) |
| | Difference with the control close to significance ($0.05<p<0.10$) |
| | Significantly different from untreated control ($p<0.05$) |

## Plant wilting

Out of all 1920 plants, only 1, 4 and 10 plants were dead when assessed on 24th July, and 6th and 26th August, respectively. The corresponding values for wilting were 61, 189 and 310 on the three assessment dates. Only 138 plants had clear "browning" crown tissues when assessed in late September. For the assessment in late August and September, there were a number of bags near one end of the tunnel in which nearly all plants showed wilting symptoms, which is likely due to increased droughting. Furthermore, the differences between the inoculated and un-inoculated controls were small and not statistically significant; thus in all subsequent statistical analysis, the two control treatments were aggregated into one.

The overall incidence of plant wilting was 0.034, 0.107, 0.172 and 0.292 for assessments made on 24/07, 06/08, 26/08 and 28/09, respectively; the corresponding range was 0.008 to 0.083, 0.033 to 0.200, 0.083 to 0.258, and 0.175 to 0.400. For all wilting incidence data, the GLM analysis indicated no significant differences among treatments. For the late September assessment, the differences of AHDB 9942, AHDB 9937 and AHDB 9730 with the control were close to the 5% significance level but these three treatments had a higher incidence of wilting than the control (Table 3).

## Crown tissue browning

In late September, the overall incidence of crown tissue browning was only 0.077, with respective minimum and maximum values of 0.017 (AHDB 9882 and AHDB 9814) and 0.158 (the control). When a plant had crown tissue browning, it was more likely to show the wilting symptoms; such an association was highly significant for all four witling assessments ($P \leq 0.001$). The odds ratio was 3.37, 4.37, 4.10 and 4.73 for the wilting assessments made on 24/07, 06/08, 26/08 and 28/09, respectively.

There were significant differences among treatments in the incidence of crown browning. AHDB 9882 and AHDB 9814 had a lower ($P < 0.05$) incidence of crown tissue browning; in addition, AHDB 9883 also had a lower incidence than the control, which was close to statistical significance ($P = 0.088$).

Five other treatments (AHDB 9777, AHDB 9955, AHDB 9809, AHDB 9958 and AHDB 9783) did not differ significantly from AHDB 9882 and AHDB 9814 (Table 4). The best fit model aggregated treatments into two groups: (1) AHDB 9882, AHDB 9814, AHDB 9883, AHDB 9777, AHDB 9955, AHDB 9809, AHDB 9958 and AHDB 9783, and (2) the other eight treatments (two controls and six products). The former had an overall reduced incidence of crown tissue browning when compared with the latter group.

## PCR test to determine presence of *P. cactorum*

In total, 430 crown tissues were screened for the presence of *P. cactorum* DNA. Of the 430 samples, 115 samples failed to produce definitive results. Of the remaining 315 samples, 193 generated negative results and 122 samples with positive results. There was some evidence of a positive association of a positive PCR result with plant wilting assessed on 24th July: $P = 0.10$ with the odds ratio of 2.29. There was no indication of association of positive PCR results with late wilting assessments.

There was a significant positive association of positive PCR results with crown tissue browning: $P < 0.001$ with the odds ratio of 2.47. Thus, about 32.5% and 54.4% of healthy and browning crown tissues had positive PCR results, respectively. The overall level of positive PCR tests ranged from 0.125 to 0.600 with an average of 0.378 (Table 4); treatments did not differ significantly from each other in the incidence of positive PCR tests. The incidence of positive PCR results adjusted for crown health status in each treatment was very close, ranging from 0.328 (AHDB 9882 & AHDB 9814dis) to 0.359 (control).

## Residue analysis

Residues were analysed by a local accredited company. Residues were detected in three treatments: AHDB 9777, AHDB 9941, and AHDB 9882.

# Discussion

Both the number of dead/wilting strawberry plants, discoloured crown tissues and molecular screening results showed that there were virtually no differences between the inoculated and un-inoculated control treatments. This suggests that the artificial inoculation did not appreciably increase the level of *P.*

*cactorum* over the background infection level. The high proportion of plants with *P. cactorum* DNA is close to the higher end of the range of latent infection in planting materials found in SF157 where the level of background infection was in the range of 0 to 40%.

Surprisingly, such a high background level of background infection did not translate into a similar level of visible plant symptoms (mortality or wilting) even under imposed mild water stress. At the beginning of fruit picking (late July), the incidence of plant witling was associated with the presence of pathogen DNA; thus, the odds of plant wilting were increased more than 5 times if the pathogen DNA was present. Unfortunately, the incidence of plant wilting in late July was too low to be able to compare treatment effects. The incidence of wilting assessed at later dates was no longer associated with the presence of pathogen DNA. This is most likely due to the fact that water stress may have accounted for a considerable proportion of the plant wilting. Indeed, there were a number of plots in both ends of the tunnel with many plants with wilting, which appeared to result from water stress. However, this still does not explain why such mild stress did not result in more symptom expression of crown rot given such a high level of pathogen DNA present in the crowns. Crown rot symptom development is usually associated with stresses during the fruiting period; maybe such a 'fruit production' stress can only be found in a commercial setting rather than in an experimental plot. Alternatively, maybe a considerable proportion of pathogen inoculum or latent infection was no longer viable or too weak to induce crown rot symptoms. This needs further investigation as P. cactorum has recently reclaimed its prominence in commercial strawberry production in the UK.

Previous work in SF157 suggested that the pathogen was most likely to be present in crowns with "discolouring" or "browning", which was further supported by the present data. However, unlike in SF157 despite the high level of pathogen DNA detection in crown tissues the incidence of plants with crown tissue browning is still very low. This might provide some evidence that the pathogen may have lost much of its virulence/ability to induce plant symptoms.

None of the treatments reduced the incidence of pathogen DNA in crown tissues. Similarly, they did not affect the incidence of wilting. Given that wilting is likely to be largely caused by imposed mild water stress, it is not unexpected that treatments would not have effects in reducing the drought-induced wilting. It is unfortunate that the wilting in late July was associated with the pathogen presence, but its incidence was too low to permit statistical comparisons.

It is reasonable to use crown tissue browning metric to represent pathogen development in the present study because (as we discussed above) (1) the high level of pathogen DNA detection in all treatments, (2) the wilting (further development of plant symptoms) was confounded with drought in few specific locations, and (3) crown tissue browning was positively associated with pathogen DNA detection. The untreated control treatments had the highest incidence of tissue browning (ca. 16%), very similar to the AHDB 9942 and AHDB 9937 treatments. Of all the treatments, only AHDB 9882 and AHDB 9814 significantly reduced the incidence of crown tissue browning, whilst the reduction achieved by AHDB 9883 was close to statistical significance. The incidence of crown tissue browning for AHDB 9955, AHDB 9809, AHDB 9958 and AHDB 9783 was also very close to AHDB 9882 and AHDB 9814. Unfortunately, residues above MRL were detected in the fruit from plants treated with AHDB 9882.

# Conclusions

Despite the relatively high incidence (close 0.40) of pathogen DNA presence in crown tissues the level of visual crown rot symptoms is very low. Thus, more research may be needed to understand conditions that induce crown rot symptom development under commercial production conditions. There are a number of products with similar performance as the best treatment (AHDB 9882); of these products, three are commercial products: AHDB 9814, AHDB 9883 and AHDB 9955, and could be further tested in a large trial under conditions close to the commercial production systems. Unfortunately, residues from the AHDB 9882 treatment greatly exceeded the MRL.

# Acknowledgements

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# Appendix

a. Crop diary and trial events

| **Appendix 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** |
| 17/10/19 | Plants arrived from a commercial supplier in 9-hole trays – placed straight into Polytunnel P13 |
| 06/11/19 | 1st inoculation of plants, 3 ml of spore suspension straight onto crown of plant |
| 20/11/19 | 2nd inoculation of plants (as in 1st) |
| 06/12/19 | Plants sprayed to control *Botrytis* |
| 09/12/19 | Plants moved to Glasshouse cold store #2 at -2◦C |
| 01/06/20 | Trays and coir bags laid out in the experimental tunnel |
| 02/06/20 | Irrigation set up and bags irrigated |
| 03/06/20 | Plants moved out of the cold store to South Park Crown rot tunnel to defrost prior to planting |
| 04/06/20 | 10 plants of the same treatment per bag planted. Irrigation set at 4 x 1 min per day |
| 12/06/20 | Irrigation turned off to allow bags to dry so treatments would not run straight out of bags (bags currently well irrigated). |
| 16/06/20 | Treatments applied through an extra irrigation line, moving labelled bags to the line for treatment. Irrigation was open for 3 min 10 s irrigation; one hour later, irrigation was open for further 3 min 10 s. Inoculated and uninoculated controls received equivalent water through irrigation lines |
| 17/06/20 | Drippers put back in all bags and labels placed in bags. Irrigation put back on for 4 x 2 mins per day |
| 23/06/20 | Irrigation changed to 4 x 5 mins per day |
| 24/06/20 | Irrigation changed to 4 x 8 mins per day |
| 13/07/20 | Reduced to 4 x 2 min to mild stress plants to encourage crown rot development |
| 17/07/20 | Increased irrigation to 4 x 6 mins per day |
| 23/07/20 | Picked 1 punnet of class 1 fruit from each plot, weighed plots and combined fruit for treatments from each block for residue analysis. |
| 24/07/20 | Carried out first plant health assessment. Reduced irrigation to 4 x 2 mins to impose mild stress |
| 28/07/20 | Increased irrigation to 4 x 8 mins |
| 31/07/20 | Decreased irrigation to 4 x 3 mins to impose mild stress |
| 03/08/20 | Increased irrigation on both trials to 4 x 10 mins |
| 05/08/20 | Increased irrigation to 4 x 15 mins before assessment next day to reduce wilting of plants due to under irrigation |
| 06/08/20 | Carried out second plant health assessment |
| 10/08/20 | Dropped irrigation on to 4 x 3 mins to induce stress |
| 12/08/20 | Increased irrigation to 4 x 4 mins |
| 17/08/20 | Decreased irrigation to 4 x 2 mins |
| 21/08/20 | Increased irrigation to 4 x 10 mins before assessment start of next week |
| 26/08/20 | Carried out third plant health assessment; decreased irrigation to 3 x 2 mins a day |
| 04/09/20 | Irrigation increased to 3 x 4 mins a day |
| 11/09/20 | Increased irrigation to 3 x 10 mins over weekend as some bags very dry |
| 14/09/20 | Decreased irrigation to 3 x 2 mins for final stress |
| 18/09/20 | Increased irrigation to 3 x 5 mins and gave very dry bags with wilting plants extra water |
| Late Sept. | Carried out crown assessment; sampling crowns for molecular screen |

| | |
|---|---|
| October | Crown material taken from each plant, chopped up, placed into microtubes and frozen prior to DNA extraction |
| Nov-Jan | Extracted DNA and carried out molecular screening |

**Photo 1**. A product is being applied to the centre row of plants via an irrigation line.

**Photo 2**. Example of one plant developing *Phytophthora* crown rot symptoms.

**Photo 3**. Trial plants in early July (about 4 weeks after planting.

b. Average daily temperature and humidity inside a tunnel about 500 m away from the trial tunnel

| Date | Temp | RH | Date | Temp | RH | Date | Temp | RH |
|---|---|---|---|---|---|---|---|---|
| 25/05/20 | 19.8 | 69.2 | 07/07/20 | 17.9 | 67.8 | 19/08/20 | 18.3 | 97.2 |
| 26/05/20 | 20.2 | 64.3 | 08/07/20 | 17.8 | 94.6 | 20/08/20 | 23.0 | 76.8 |
| 27/05/20 | 19.6 | 66.8 | 09/07/20 | 18.5 | 91.1 | 21/08/20 | 19.7 | 78.1 |
| 28/05/20 | 17.9 | 66.0 | 10/07/20 | 17.9 | 75.7 | 22/08/20 | 18.8 | 78.2 |
| 29/05/20 | 17.5 | 65.8 | 11/07/20 | 17.0 | 69.7 | 23/08/20 | 17.8 | 77.7 |
| 30/05/20 | 18.7 | 66.9 | 12/07/20 | 19.4 | 68.1 | 24/08/20 | 17.4 | 81.7 |
| 31/05/20 | 19.3 | 63.8 | 13/07/20 | 20.2 | 64.6 | 25/08/20 | 17.2 | 91.0 |
| 01/06/20 | 19.5 | 65.7 | 14/07/20 | 19.3 | 77.5 | 26/08/20 | 18.8 | 69.9 |
| 02/06/20 | 21.1 | 60.5 | 15/07/20 | 17.9 | 74.6 | 27/08/20 | 16.3 | 88.6 |
| 03/06/20 | 16.8 | 76.0 | 16/07/20 | 18.8 | 81.4 | 28/08/20 | 16.6 | 87.3 |
| 04/06/20 | 14.3 | 72.0 | 17/07/20 | 23.8 | 73.1 | 29/08/20 | 13.6 | 89.3 |
| 05/06/20 | 13.6 | 70.2 | 18/07/20 | 22.5 | 70.8 | 30/08/20 | 14.3 | 82.0 |
| 06/06/20 | 11.6 | 73.8 | 19/07/20 | 17.9 | 87.1 | 31/08/20 | 15.3 | 77.7 |
| 07/06/20 | 14.0 | 82.0 | 20/07/20 | 19.7 | 70.9 | 01/09/20 | 15.3 | 78.1 |
| 08/06/20 | 13.2 | 76.5 | 21/07/20 | 18.7 | 67.8 | 02/09/20 | 15.5 | 84.1 |
| 09/06/20 | 15.6 | 69.0 | 22/07/20 | 20.4 | 68.5 | 03/09/20 | 18.4 | 93.4 |
| 10/06/20 | 13.6 | 86.1 | 23/07/20 | 20.1 | 67.8 | 04/09/20 | 16.7 | 80.6 |
| 11/06/20 | 15.0 | 88.7 | 24/07/20 | 20.4 | 80.3 | 05/09/20 | 15.8 | 74.9 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 12/06/20 | 17.5 | 86.0 | 25/07/20 | 18.3 | 93.6 | 06/09/20 | 14.5 | 80.2 |
| 13/06/20 | 20.8 | 73.2 | 26/07/20 | 19.1 | 81.9 | 07/09/20 | 14.9 | 86.8 |
| 14/06/20 | 20.5 | 74.7 | 27/07/20 | 18.3 | 90.0 | 08/09/20 | 20.3 | 85.7 |
| 15/06/20 | 20.6 | 73.9 | 28/07/20 | 18.8 | 68.2 | 09/09/20 | 20.2 | 83.4 |
| 16/06/20 | 21.0 | 76.0 | 29/07/20 | 17.9 | 69.3 | 10/09/20 | 16.0 | 75.9 |
| 17/06/20 | 19.2 | 81.0 | 30/07/20 | 20.8 | 62.3 | 11/09/20 | 13.6 | 81.9 |
| 18/06/20 | 19.8 | 82.2 | 31/07/20 | 24.0 | 64.3 | 12/09/20 | 16.1 | 80.7 |
| 19/06/20 | 17.1 | 82.3 | 01/08/20 | 21.6 | 75.6 | 13/09/20 | 19.1 | 79.8 |
| 20/06/20 | 19.6 | 76.4 | 02/08/20 | 19.2 | 68.8 | 14/09/20 | 19.5 | 78.1 |
| 21/06/20 | 19.5 | 77.5 | 03/08/20 | 18.8 | 67.9 | 15/09/20 | 22.1 | 80.9 |
| 22/06/20 | 19.9 | 67.6 | 04/08/20 | 17.8 | 73.2 | 16/09/20 | 20.0 | 82.4 |
| 23/06/20 | 22.8 | 67.1 | 05/08/20 | 20.2 | 70.8 | 17/09/20 | 16.9 | 77.4 |
| 24/06/20 | 24.1 | 65.8 | 06/08/20 | 23.1 | 72.7 | 18/09/20 | 15.4 | 82.5 |
| 25/06/20 | 24.7 | 65.7 | 07/08/20 | 24.4 | 64.8 | 19/09/20 | 18.1 | 82.9 |
| 26/06/20 | 25.3 | 74.9 | 08/08/20 | 25.4 | 66.8 | 20/09/20 | 18.3 | 81.7 |
| 27/06/20 | 18.0 | 85.4 | 09/08/20 | 23.8 | 77.4 | 21/09/20 | 17.8 | 82.1 |
| 28/06/20 | 18.0 | 69.9 | 10/08/20 | 26.2 | 74.2 | 22/09/20 | 17.6 | 81.9 |
| 29/06/20 | 17.1 | 68.1 | 11/08/20 | 26.6 | 75.5 | 23/09/20 | 16.6 | 90.3 |
| 30/06/20 | 16.5 | 89.3 | 12/08/20 | 25.3 | 76.3 | 24/09/20 | 11.5 | 87.0 |
| 01/07/20 | 19.0 | 80.9 | 13/08/20 | 24.0 | 84.3 | 25/09/20 | 10.2 | 79.9 |
| 02/07/20 | 18.4 | 78.9 | 14/08/20 | 21.1 | 89.5 | 26/09/20 | 10.1 | 75.3 |
| 03/07/20 | 17.9 | 79.4 | 15/08/20 | 20.8 | 95.1 | 27/09/20 | 11.1 | 86.6 |
| 04/07/20 | 17.8 | 90.9 | 16/08/20 | 21.1 | 88.8 | 28/09/20 | 14.3 | 76.7 |
| 05/07/20 | 20.4 | 69.4 | 17/08/20 | 20.1 | 84.9 | 29/09/20 | 15.9 | 87.6 |
| 06/07/20 | 17.5 | 65.8 | 18/08/20 | 20.2 | 83.0 | 30/09/20 | 13.7 | 94.1 |