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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

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

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GROWER SUMMARY

Headline

Overall microorganism species richness increased in irrigation water as crops aged, and the abundance of common tomato pathogens increased

Low levels of root disease were observed in the 2015 crops monitored, but associations between observable root issues and low species richness and diversity were observed

Background

Root diseases of tomato are numerous, widespread and potentially devastating. Plant losses due to root disease have cost over £50,000 on each of at least two nurseries in recent years. There may be potential yield loss occurring on many nurseries due to root death from low levels of disease. In the UK, the most common root diseases in hydroponic crops are Pythium root rot, Phytophthora root and stem base rot, black dot (*Colletotrichum coccodes*), root mat disease (vectored by *Rhizobium radibacter*) and Verticillium wilt; a range of other diseases occur from time to time. A new race (race 3) of *Fusarium oxysporum* f. sp*. lycopersici*, cause of Fusarium wilt in tomato, has now been reported in several countries and is a potential threat to UK crops. The fungus *Plectosphaerella cucumerina* was recently reported to be causing severe root disease of tomato in China; this fungus was common and abundant on UK tomato crops monitored in 2013 (Final report, PC 281a). Root diseases are generally difficult to diagnose as the range of symptoms is limited, symptoms can overlap, plants can be infected by several pathogens simultaneously, and some causal microorganisms are difficult to isolate.

Increasingly tomato crops are grown, from planting, in closed systems with the nutrient solution recycled in order to reduce water and nutrient use and protect the environment; this may pose a threat to production in terms of pathogenic microorganisms establishing in solution. Fungal and Oomycete spores and virus particles released from roots of a few infected plants can potentially be spread, very rapidly, to all plants in a glasshouse when the waste solution is recycled. Most growers treat recycled water (e.g. UV, heat, slow sand filter (SSF)) with the aim of reducing pathogen inoculum and disease risk, but the efficacy of systems in practice is not monitored and their impact on root disease is unclear. Breakdowns in the water disinfection treatment are not uncommon, and efficiency of systems may be reduced over time. There is increasing evidence that microbial diversity on roots can benefit plant health through reducing root disease and inducing systemic resistance to some foliar pathogens. Molecular methods now provide an

excellent tool for studying the largely unexplored world of root zone and irrigation water microbial ecosystems.

Work in AHDB Horticulture funded project PC 281a detected consistent occurrence of several root-infecting plant pathogens including *Colletotrichum coccodes, Fusarium oxysporum, Pythium* spp. and *Verticillium albo-atrum* in three hydroponic crops monitored throughout 2013. All three crops were grown on Maxifort rootstock and none developed serious root disease. PC 281a found that a relatively stable microbial population was present on roots from planting, and that the microbial diversity on roots was greater in closed systems than run-to-waste crops. Work was restricted to three crops, one rootstock and one season, so it is unclear if lack of root disease was due to rootstock vigour, microbial diversity or a hitherto unknown reason.

The overall objective of this project was to utilise the tomato root microarray developed in PC 281a, in combination with specific quantitative diagnostic techniques (multiplex qPCR) for four key tomato root pathogens, to characterise and quantify microorganism populations in irrigation water and assess the risk of root disease when tomato crops are grown in closed irrigation systems with recirculation of the nutrient solution.

Specifically, the project objectives were:

1. To further validate the microarray previously used for monitoring tomato rhizosphere microorganisms, and to integrate this with specific quantitative PCR based diagnostics for four specified tomato root pathogens.
2. To determine the effect of water disinfection treatment, sampling location and crop age on the occurrence of microorganisms in water in tomato crops grown in closed systems with recycled nutrient solution.
3. To compare the pathogenicity of four fungal and oomycete root pathogens commonly found in hydroponic crops to own-root and grafted plants of tomato.
4. To monitor 10 commercial crops grown in closed systems with recycled irrigation solution for root pathogens and root disease.
5. To communicate results to tomato growers and the wider horticulture industry.

Summary

Objective 1 – Optimise microarray and qPCR diagnostics

Whilst the array technology developed in PC 281a has the capacity to rapidly screen for the presence of multiple organisms in each sample, real-time PCR is more sensitive for specific organisms and can give much better quantitative data. There is therefore a balance to strike between using the array to screen a sample for multiple pathogens at the same time but with lower sensitivity, versus real-time PCR assays that have greater sensitivity for individual species but have to be run essentially as separate tests for each organism in turn. Therefore it was decided to develop more sensitive real-time PCR assays for four species that are commonly detected on the array to be able to further validate the array as a diagnostic tool. For the organisms *Colletotrichum coccodes, Plectospharella cucumerina, Pythium aphanidermatum* and *Pythium myriotylum* a TaqMan assay was developed where all four probes had different fluorophores so it was possible to measure them on the same plate under different wavelengths. However, some filters had slight overlaps in recording that may lead to difficulty in using them in the same sample well.

Two of four of the primer assays seemed to have good specificity. Both *C. coccodes* and *P. cucumerina* were validated using three separate strains of pure culture and there were no cross-reactions with the broad range of other fungi examined. However the *P. aphanidermatum* and *P. myriotylum* assays were tested with only one isolate each, so, despite initial positive results, they need more tests to confirm their reliability. *P. myriotylum* did not amplify in samples that had been positively detected using the microarray, so needs further assessment using more pure culture strains to identify the reason for this result. It appears that the use of the ITS regions (internal transcribed spacer region, commonly used for identification of fungal species) for each of the primers was sufficient for specific and accurate identification. From the serial dilutions, it was shown that the assays had sufficient efficiency falling within the guideline range, with the exception of *C. coccodes*. Further refining of the protocol and mastermixes is required for *C. coccodes.*

The real-time PCR results for *C. coccodes* and *P. cucumerina* generally confirmed the microarray results with the majority of microarray-positive results also showing positive results with the real-time PCR assays. However, the real-time PCR also amplified from some samples that were

negative with the array, confirming that the sensitivity of the PCR assays was greater than the array. For the *Pythium* tests, the PCR primers used were ones that had been previously validated and published by others; however, it should be noted that because of the lack of positive control cultures for validating the specificity of these primers in our tests, the results with these primers were inconclusive, especially for *P. myriotylum*, the presence of which had occasionally been indicated in the microarrays, but could not be confirmed by PCR.

Objective 2 – Effect of water treatment system, sampling location and crop age on microorganisms in recycled water

In 2015, five commercial tomato crops grown in rockwool substrate (plus an NFT crop) and with a closed, re-circulating irrigation system were monitored. Crops were visited in January/February, April, July and October. At each visit three replicate root samples were taken, and an assessment carried out of root disease. Samples of irrigation water were also taken at five locations around the loop. Each site differed in the set-up and layout of the irrigation system, but generally the sample locations included the source water, the mixing tank, slab water, drain water (pre-disinfection treatment) and water immediately post-disinfection treatment. Each site had a different method of water disinfection, and site details are summarised in Table 1.

Table 1. Details of monitored crops and their water disinfection treatment – 2015

Crop	Scion	Rootstock	Water treatment	Additional treatment
1.	Garincha	Maxifort	fSSF	-
2.	Piccolo	Maxifort	pSSF	Reciclean
3.	Piccolo	Emperador and own-roots	Nil	Proplant (early in season)
4.	Dometica	Optifort	UV	Proparva
5.	Dometica	Optifort	Heat	Proparva

Throughout cropping, no wilting, yellowing or plant death that could be attributed to root disease occurred in the crops monitored. There was noticeable root browning in some of the crops, especially by the end of the season. At the final visit in October, vascular staining was present in all crops at varying levels (0-37% of stems), though it was not observed severely in any crop.

Root mat symptoms were also observed in three of the five crops (pSSF, UV and Heat).

Root and water samples were sent to Nottingham University and tested using the microarray developed in PC 281a. Sampling location around the irrigation loop influenced the microorganisms detected, with a greater number of pathogenic species generally detected in the slab and drain water. Pathogens including *Fusarium oxysporum*, a number of *Pythium* species, a weakly pathogenic *Verticillium* species and two *Phytophthora* species were detected on commercial sites over the season, without associated symptoms of disease in the crops.

Of the five crops sampled, the NFT crop had notably lower total species richness in both the rhizosphere and irrigation water when compared to the other sites, despite water disinfection treatments being present at the latter sites. Site 1, fSSF, had the highest total species richness in both roots and water, potentially due to all water being treated with a biological filter.

Disinfection treatment was observed to lower species richness and remove a variety of common pathogens from irrigation water. The most effective treatments appeared to be the full slow sand filter, and UV treatment, though effects were variable. As the pasteuriser at Site 5 was only working at the final visit, its efficacy was difficult to quantify in relation to the other treatments. Water disinfection treatments also reduced pathogen loads in irrigation water, most noticeably the UV and Slow Sand Filter treatments. Sampling location has a strong effect on the microbial life detected in irrigation water, dependent on the water's source and the specific irrigation system present at each site (Table 2). See glossary for explanation of species richness and species diversity.

Relatively little root disease was seen over the season in the five main sites monitored, and so linking visible symptoms to the pathogens detected by the microarray proved difficult. Pathogen species richness in the rhizosphere over the season was highest at Sites 2 and 4, the same sites which exhibited highest root browning scores and most visible vascular staining at the end of the season. Average total species diversity over the season was also lowest at Site 2, and highest at Site 1 where no severe symptoms were observed. By the end of the season in October, total species diversity from irrigation water sampled from the slab was lowest at Site 2, and this may be associated with the root browning observed.

Table 2. Summary of the effect of water treatment systems, and sample location on microorganisms detected once or more in recycled irrigation water by microarray (Apr-Oct samples) – 2015

Factor			Potential pathogens detected [a]						Fungal pathogen species richness in October (no. species)	Pathogen species diversity in October	Total species richness in October	Total species diversity in October
Water treatment	Sample location	Cc	Fo	Pn	Pyt	Ple	Tb*	Vn				
1. f SSF	1 Mains	-	✓	-	-	✓	-	-	2	0.87	9	2.11
	2 Mix/Coll	-	✓	✓	-	✓	-	-	0	0.62	5	1.50
	3 Slab	-	-	-	✓	✓	-	✓	4	0.79	10	2.33
	4 Pre-t	✓	✓	-	✓	✓	-	-	6	0.95	12	2.36
	5 Post-t	-	-	-	✓	-	-	-	1	1.03	9	2.00
2. p SSF	1 Res	-	✓	✓	✓	-	-	-	4	0.62	6	2.09
	2 Mix/Coll	-	-	-	✓	-	-	-	3	0.73	5	1.84
	3 Slab	✓	-	-	✓	✓	-	✓	4	0.55	5	1.96
	4 Pre-t	-	-	-	✓	-	-	-	2	0.50	6	2.09
	5 Post-t	-	-	-	✓	-	-	-	0	0.00	3	1.68
3. Nil	1 Res	-	✓	-	✓	-	-	-	5	0.86	6	1.89
(NFT)	2 Res/Mains	-	-	-	-	-	-	-	0	0.00	0	0.62
	3 Tank	-	✓	-	✓	✓	-	✓	7	1.17	11	2.61
	4 Slab (graft)	-	✓	-	✓	✓	-	✓	7	1.14	14	2.72
	5 Slab (OR)	✓	-	✓	✓	✓	-	-	4	0.20	4	1.86
4. UV	1 Res	-	-	-	✓	-	-	-	6	0.75	7	2.31
	2 Mix/Coll	-	-	-	✓	-	-	-	3	0.60	8	2.36
	3 Slab	✓	-	-	✓	✓	-	✓	10	0.94	15	2.77
	4 Pre-t	-	✓	-	✓	✓	-	✓	6	0.78	14	2.72
	5 Post-t	-	-	-	✓	-	-	-	4	0.38	11	2.50
5. Heat	1 Res	-	✓	-	✓	-	-	-	4	0.39	5	1.97
	2 Mix/Coll	-	-	-	✓	-	-	-	3	0.53	5	1.98
	3 Slab	✓	✓	-	✓	✓	-	✓	5	0.83	7	2.26
	4 Pre-t	✓	✓	-	✓	✓	-	✓	8	0.66	17	2.91
	5 Post-t**	-	✓	-	✓	-	-	-	2	0.20	3	1.36

[a]Cc– *Colletotrichum coccodes,* Fo – *Fusarium oxysporum*, Pn – *Phytophthora nicotianae*, Pyt – *Pythium* species, Ple – *Plectosphaerella cucumerina*, Tb – *Thielaviopsis basicola*, Vn – *Verticillium nigrescens,* OR – own roots

*Note that Tb did occur at Sites 2 and 3, but was only detected on roots

**October sample only

Objective 3 – Pathogenicity of root pathogens on own-root tomato and grafted plants

Preliminary trial

A preliminary experiment was set up in February 2015 to establish pathogenicity of isolates of four common pathogens collected from end of season tomato crops in 2014. Tomato plants (cv. Elegance, ungrafted) were inoculated with two isolates of each pathogen in a randomised, split-plot design. Plants were periodically assessed for root health indicators over a six week period. Plants were inoculated with a drench of 1 x 10^5 spores per ml spore suspension at the stem base (the *Pythium* spp. were applied as an inoculum of 1 x 10^6 zoospores per ml). Results are summarised in Table 3.

Table 3. Summary of mean effect of root inoculation treatments at 4 weeks after inoculation – March 2015

Treatment	Incidence of yellowing	Severity of yellowing	% roots discoloured & rotten	% roots white
1. Untreated	1.0	9.5	36.2	63.8
2. Pythium	**0.1**	**0.1**	23.6	76.4
3. Fusarium	0.9	**14.0**	51.3	48.7
4. Plectosphaerella	0.9	6.7	46.2	53.8
5. Colletotrichum	**0.8**	2.8	51.3	**48.7**
Significance	<0.001	<0.001	<0.001	<0.001
LSD	0.19	4.14	14.24	14.24

Bold – significantly different from untreated.

Six weeks after inoculation leaf yellowing was significantly more severe in plots inoculated with *Fusarium* (p=<0.001). Rotten roots in the inoculated treatments were not significantly different from the untreated, but % white roots were significantly reduced by inoculation with *Colletotrichum coccodes*.

Following this pathogenicity trial, main trials looking at the four pathogens individually, on both own root Elegance and on grafted plants, were established in summer 2015.

Inoculated trials

Pythium sp. & *Plectosphaerella cucumerina* inoculated trials were conducted at ADAS Boxworth. Half of the plots contained plants grown on the rootstock Maxifort, and the other half ungrafted plants, cv. Elegance, in order to determine the effect of rootstock on disease susceptibility. At the date of inoculation (8th July), a week after plant arrival, *Pythium* cultures had failed to produce zoospores. The *Pythium* trial was therefore inoculated with plugs of mycelium, placed into the rockwool cubes. Both ungrafted and Maxifort grafted plants were inoculated at low (2 x 0.8 mm plugs of mycelium), medium (4 x 0.8 mm plugs of mycelium) and high (6 x 0.8 mm plugs of mycelium) levels. The two isolates of *Pythium* used in the preliminary work were used, with half of the plugs from each isolate. The *Plectosphaerella* trial was inoculated a week after plant arrival on July 8th with low (1×10^2 spores per ml), medium (1×10^4 spores per ml) and high (1×10^6 spores per ml) concentrations. Spore suspensions contained two isolates of *Plectosphaerella cucumerina* in equal amounts.

Plants were grown on for nine weeks, and assessed regularly for signs of root disease. At the final assessment, plants were destructively assessed, with the rockwool cube cut open and the roots inside scored. Stem bases were scraped to reveal any staining present in the vascular tissue.

Inoculated trials using *Colletotrichum coccodes* and *Plectosphaerella cucumerina* inoculated trials were established at the University of Nottingham. Pathogens consisted of a mix of three isolates of each species and were inoculated at three levels. However, rather than being grown in cubes held in trays, the rockwool cubes were planted onto slabs in trays. Additionally, the *F. oxysproum* trial included two different rootstocks, Arnold as well as Maxifort. The rootstock Arnold claims resistance to races 1 and 2 of *F. oysporum* f. sp. *lycopersici* (Fol), whereas Maxifort claims resistance to race 1 only. This trial aimed to examine the effect these differing resistances had on infection and symptom expression.

Inoculation with *Pythium* sp. or *Plectosphaerella*, even at the high level, did not adversely affect root growth or cause increased browning compared with the uninoculated control plants. It was therefore not possible to determine if grafted plants were more resistant to *Pythium* root rot or *P. cucumerina* than ungrafted plants. A small amount of vascular staining (5% of plants) was observed in the *P. cucumerina* inoculated trial. This was not in sufficient amounts to be statistically significant, however it did occur in own-root plants only, at the higher rates of inoculation.

Inoculation with *C. coccodes* and *P. cucumerina*, even at the high level, caused no foliar symptoms and little obvious root disease were observed when the *C. coccodes* and *P. cucumerina* trials were concluded at 8 weeks after planting.

Monitoring of grafted and ungrafted plants on a commercial site

On a commercial site, scion variety Piccolo was grown both grafted and ungrafted in the same crop, providing an opportunity for comparison. However, it should be noted that this crop was grown on an NFT system and results may differ from rockwool crops.

In October, Piccolo grafted to a rootstock Emperador exhibited less vascular staining than Piccolo grown on its own roots, but crops did not appear to differ greatly in terms of crop health over the season. Roots sampled from both grafted and ungrafted plants showed a greater abundance of pathogens in January and April than later in the year. Grafted roots of variety Emperador typically had greater abundance of *Pythium* species than ungrafted roots, on which detection of true fungi such as *V. nigrescens* was more common than on grafted roots. Oomycetes and true fungi were detected in both irrigation water and roots, but the sampling times at which common pathogens were prevalent differed. Irrigation water differed from roots, with few microorganisms detected in water taken from either rootzone in January, a higher abundance of fungal and Oomycete pathogens on ungrafted plants in April, but a higher abundance on Emperador (i.e. grafted) roots by October.

Objective 4 – Monitor additional crops grown with re-cycled irrigation for root pathogens and root disease

In order to gain a more reliable estimate of the occurrence of root disease in crops grown with recycled irrigation water, an additional five sites were identified (Table 4). Crops on these sites were assessed for root disease in July and October 2015, and three replicate root samples were examined by the microarray.

Table 4. Details of additional commercial sites monitored in July and October 2015

Site	Scion	Rootstock	Substrate	Water treatment	Additional treatment
6.	Olinta	Beaufort	Coir	Heat	Natugro programme
7.	Sunstream	Maxifort	Rockwool	Heat	-
8.	Roterno	Optifort	Coir	Heat	Compete Plus
9.	Conchita	Ungrafted	NFT	Nil	-
10.	Conchita	Ungrafted	NFT	Nil	-

A similar spectrum of microorganisms was found as on the roots of sites 1-5. Interestingly, one of the additional NFT sites monitored (Site 10) had a much higher species richness across the year, more similar to the crops grown in substrates. This shows that many more factors other than water disinfection treatment and growing system are working to affect the microbial community in irrigation water and in the rhizosphere. The species richness for the full set of 10 sites monitored in July and October is given in Table 5.

Table 5. Species richness in the rhizosphere of 10 commercial sites monitored in July and October 2015 (1-5 from Objective 2, 6-10 as above)

			No. species detected (pathogens)		No. species detected (saprophytes & bacteria)	
Date			Jul	Oct	Jul	Oct
Site	Substrate	Disinfection				
1.	Rockwool	SSF	8	5	7	5
2.	Rockwool	pSSF	4	0	2	0
3a. (grafted)	NFT	Nil	0	1	4	6
3b. (ungrafted)	NFT	Nil	0	0	5	0
4.	Rockwool	UV	9	10	4	8
5.	Rockwool	Heat	8	2	6	6
6.	Coir	Heat	4	8	4	8
7.	Rockwool	Heat	4	6	4	5
8.	Coir	Heat	3	8	4	5
9.	NFT	Nil	5	1	8	0
10.	NFT	Nil	8	9	10	8

Financial Benefits

The project outputs can be seen to have the following benefits to growers:

- Targeted molecular tests for use by researchers to study tomato root microorganisms could reduce workload and expenses associated with pathogen identification.
- Increased knowledge of the distribution of potential pathogenic and beneficial microorganisms in the water 'closed loop' when crops are grown with re-circulation.
- Insight into the effect of different water disinfection treatment systems on key plant pathogens and beneficial microorganisms in irrigation water.
- Increased confidence for growers to grow crops in closed systems with re-circulation has the potential to save water, as enforced by the Sustainable Use Directive and restrictions on water abstraction, and has been estimated to save approximately 40% in water and nutrients.

Action Points

There are no immediate action points. However, there are several points of interest arising from this project which growers should note with regard to detection and control of root diseases.

1. Tomato plants can have a diverse microbial population on roots, including potential pathogens, with no associated disease symptoms.
2. It is an ecological principle that a diverse community is likely to be more resistant to change than a simple community. Of the three NFT crops sampled, two of them had noticeably fewer microorganisms detected than the rockwool crops sampled. The crop with the highest average diversity across the season (both of pathogens and total microorganisms) was Site 1, where no severe root disease was observed. In general, lowest total species diversity was observed at Site 2 (pSSF), and diversity in the slab water was lowest by the end of the season when visible root browning was observed.
3. Although many microorganism species are present on tomato roots early in the season and persist throughout cropping, additional species, including pathogens (e.g. *Colletotrichum coccodes*; *Fusarium oxysporum*) may occur during crop production. Two samples were taken from crops exhibiting severe root disease symptoms in late 2015, and there were additional pathogenic species (*Pythium diclinum* and a greater abundance of *P. myriotylum* and *P. cucumerina* at Site 6; *F. oxysporum, Phytophthora areacae* and *nicotianae, V. nigrescens* and

P. cucumerina at Site 8) detected by the microarray compared to healthy crops on those sites. This indicates a potential benefit from maintaining disease precautions during crop production.

4. *Plectosphaeralla cucumerina* (*Fusarium tabacinum*) was, as in previous work, commonly and consistently detected on tomato roots in this project. Previous work indicates this fungus is common in hydroponic crop production. Although generally regarded as a weak pathogen, inoculation of plants did result in visible root browning and severe root rot associated with this pathogen has been reported in China. Growers should be alert to any reports of *Plectosphaerella* associated with a tomato disease in Europe.

5. Nurseries can differ in the range of pathogens commonly found on tomato roots, and it is important to note that disinfection systems may not always remove 100% of potential pathogens. Water treatment systems that resulted in large reductions in potential pathogens detected in recycled irrigation water were fSSF, pSSF and UV. On the one occasion where the heat treatment was working it also appeared highly effective.

6. The detection of several potential pathogens on roots through cropping, and the lack of any visible deleterious effect on crop growth observed in 2013 (PC 281a) was found again in 2015. The conditions necessary for root-infecting pathogens to cause severe root disease remain unknown. Until there is experience over several seasons of growing grafted plants on rockwool slabs, it is recommended that between-crop hygiene and water treatment are maintained as precautionary measures against damaging root disease.

7. A microarray for detection of tomato rhizosphere microorganisms has been validated and can be used for investigation of root diseases. Eighteen of the probes on the array are species-specific, were self-validated and showed no or low-level cross-hybridisation with other species. These comprise 12 fungi and oomycetes (including *Plectosphaerella cucumerina*, *Fusarium oxysporum, Fusarium solani*, *Rhizoctonia solani*, *Thielaviopsis basicola*, *Trichoderma harzianum* and *Verticillium dahliae*) and six bacteria (including *Bacillus amyloliquefaciens*, *Bacillus subtilis* and *Ralstonia solanacearum*). The usefulness of the microarray would be enhanced by increasing the number of probes with nil or low level cross-reaction. Additional molecular methods with a greater degree of sensitivity and quantification have been developed in this project for *Colletotrichum coccodes* and *Plectosphaerella cucumerina.*

SCIENCE SECTION

Introduction

Traditionally, studies of root disease in recirculating systems have focussed on Oomycetes, known to spread easily in aquatic environments, but more recently PepMV has been shown to be effectively spread in nutrient solution. A French study has also focused on microbial diversity in the root zone, as well as the presence of beneficial or antagonistic organisms that may exert an effect on pathogens. Similarly, it has been shown that severity of Fusarium crown and root rot is reduced on substrate that has been used previously. The mechanism of suppression seems to be mediated by substances held by the substrate, the previous growing history and the presence of resident microflora. In terms of rootstocks and their effect, Italian work has shown rootstocks to differ in the resistance to *Colletotrichum coccodes* provided in a commercial crop, though all provided adequate control.

Project PC 281 using the molecular method T-RFLP revealed a tremendous diversity of microorganisms on tomato roots and variations between crops. Attempts were made to manipulate root microbial populations of a soil grown crop by addition of microbial products (e.g. Compete Plus, *Trichoderma*), but no effects were detected. Building on this information, PC 281a (2013) used a microarray to examine in detail (every 2 weeks) the root zone microorganisms in three hydroponic crops grown on rockwool. This work showed:

1. a wide diversity of microorganisms on Maxifort roots;
2. a relatively stable microbial population over the year;
3. largely similar populations between the three crops;
4. a gradual increase in Oomycetes with time;
5. no obvious root disease in any crop despite the presence of pathogens, some throughout the year from planting onto slabs.

A further opportunity with next generation molecular diagnostics is to identify the presence/absence of specific microorganisms in the rhizosphere that have been shown to provide biological control of root diseases, induce systemic resistance to foliar or whole plant diseases or reduce crop growth when a low level of root infection is present.

The overall aim of the current project was to increase understanding of microorganisms on roots of tomato grown in closed hydroponic systems in order to better assess the risk of severe root rot. Specifically, the project objectives were to:

1. Identify microorganisms present at different positions in the closed water system of hydroponic tomato crops;
2. Compare the microbial populations before and after different water disinfection treatments;
3. Compare the susceptibility of own-root and grafted tomato plants to four fungal and oomycete pathogens commonly found in hydroponic solution;
4. Determine occurrence of root disease and root microbial populations in 10 tomato crops grown in closed systems with re-circulation;
5. Communicate results to growers.

Materials and methods

Objective 1 – Optimise microarray and qPCR diagnostics

The microarray was further validated by development of qPCR tests for four of the microarray targets and then comparing the microarray and qPCR methods for selected samples. Compared with microarray detection, a qPCR test potentially offers greater specificity and sensitivity and the ability to accurately quantify a microorganism. Isolates of four common pathogens of tomato crops were sought from end of season crops in 2014. Growers were requested to submit samples of visibly browning tomato roots, which were processed in the ADAS Pathology Laboratory. Fungi were isolated onto PDA + S agar plates, and fungal colonies suspected to be *Fusarium oxysporum*, *Colletotrichum coccodes*, *Plectosphaerella cucumerina* and *Pythium* spp. were sent to the University of Nottingham for PCR identification, and for use in further validating the tomato pathogen microarray.

Four microorganisms were identified for assay development, based on their perceived importance and prevalence as root-infecting pathogens: *Colletotrichum coccodes, Plectospharella cucumerina, Pythium aphanidermatum* and *Pythium myriotylum.* Potential primers were then developed by a mix of looking for these in previous literature and some self-modification of the primers. Common barcoding genes were appropriate to use, targeting either the ITS 1 or ITS 2 region (Table 6). These primers were then validated using cultures from a variety of sources including ADAS's culture collection and the CBS culture collection. DNA was extracted from these

cultures, the rDNA amplified using universal primers, and the resultant DNA sequenced to confirm their identity.

For each q-PCR assay reaction mixture, Promega GoTaq® Probe qPCR systems ready-to-use 2x master mix was used and each well volume was made up to 20 µl. The primer and probe working concentrations are listed in Table 7. For each assay 2 µl of sample was added. Assays were carried out on Roche LightCycler® 480 white 384 well plates, in a Roche LightCycler® 480 II.

Assays were then validated using as many of these pure culture sample DNAs as possible for each assay. These pure culture DNA samples were in a range of concentrations: one fold, tenfold and hundredfold. The serial dilution of the samples enables the assay efficiency to be tested when using the standardised amplification programme (Table 8). Specificity was tested by the use of negative control samples, containing non-target organisms. The non-target organisms used included *Botrytis cinerea, Penicillium* spp., *Aspergillus* spp. and *Verticillium* spp. For each sample of roots used, there were three replicates and an average calculated.

Following initial validation of the primers they were then used on fertigation solution samples and root samples from the microarray analysis. This was to test the sensitivity of the microarray species DNA in the samples.

The final stage was to test the quantification abilities of both the microarray and qPCR assays in tandem. This involved the ranking of microarray scores as low, medium and high. This was done by ranking microarray scores from 0.1-0.3 as low concentration, 0.3-0.7 medium and 0.7-1.0 as high. A sample set including all three levels was tested using the qPCR assay to assess the reliability of the semi-quantitative rankings.

Table 6. List and detail of TaqMan primers used for detecting four tomato root pathogens

Primer	Sequence (5'-3')	Target DNA	Size of product (bp)	Fluorophore
C. coccodes				
CcTqF1	TCTATAACCCTTTG TGAACATACCTAAC TG	ITS 1	145	
CcTqR1	CACTCAGAAGAAAC GTCGTTAAAATAGA G			
Taqman probe	CGCAGGCGGCACC CCCT			Cy5
P. cucumerina				
PcRtF1	GTGCCCGCCGGTC TC	ITS 1	72	
PcRtR1	GACAGTTCGCTAA GAACACTCAGAAGT			
Taqman probe	TCAGAATCTCTGTT TTCGAACCCGACG A			Fam1
P. myriotylum				
PyuniTqF1	CTGTTCTTTCCTTG AGGTG	ITS 2	149	
PmTqR1	GGAGCCGAAACTC TCACAAGAC			Tex Red
Taqman probe	TCCCAAATTGGTGT TGCCTTCTTTACCC			
P. aphanidermatum		ITS 2	163	

PyuniTqF1 PaTqR1 TaqMan probe	CTGTTCTTTCCTTG AGGTG GCGCGTTGTTCAC AATAAATTGC CATTTGCCCAGACC ATTGCCTC			Hex

Table 7. qPCR Primers and probes working concentrations.

Assay		Working concentration
C. coccodes	Primer Probe	0.3 µM 0.1 µM
P. cucumerina	Primer Probe	0.3 µM 0.23 µM
P. aphanidermatum and *myriotylum*	Primer Probe	0.5 µM 0.25 µM

Table 8. Standardised PCR protocol used in this work

Stage	Temperature (ºC)	Time (s)	Cycles
Pre-incubation	50º 90º	120 120	 1
Amplification	95º 60º	15 60	 45
Cooling	40º	30	1

Objective 2 – Effect of water treatment system, sampling location and crop age on microorganisms in recycled water

Five commercial tomato sites were selected at the start of the 2015 growing season where tomatoes were grown hydroponically in rockwool, and on recirculating water systems. Each of the sites selected employed a different method of water disinfection, detailed in Table 9 below.

Table 9. Disinfection treatments in use at each of five monitored sites with recirculated irrigation; sites 1, 2, 4 & 5 were growing on rockwool - 2015

Site	Disinfection treatment	Scion	Rootstock
1.	Full SSF	Garincha	Maxifort
2.	Partial SSF (10% total volume)	Piccolo	Maxifort
3a.	None (NFT)	Piccolo	Emperador
3b.	None (NFT)	Piccolo	Own root
4.	UV	Dometica	Optifort
5.	Heat	Dometica	Optifort

Sites were visited on four occasions throughout the growing season, in January, April, July and October. At each site, a row of grafted plants was selected at the January visit, and the same row was sampled at each subsequent visit. Both water and roots were sampled at each date from each site, and root health was assessed along the chosen row, and the two rows either side of it. Fresh coveralls and overshoes were worn for each site visit. When visits were being organised the status of disinfection equipment on site was confirmed. Additionally, on arrival, any fungicide or other product application to the root zone of monitored plants was recorded. For each crop, the variety, rootstock, water treatment, growing medium, planting date and date recirculation commenced were recorded.

Root health was assessed by looking for wilting or yellowing heads along the row for 50 cubes. Missing plants, plants with fungal growth visible at the stem base, and cubes with obvious root mat (rhizogenic *Rhizobium radiobacter*) were also assessed. Additionally, the plastic was pulled back around 10 cubes to expose the roots, which were assessed for root colour on a 1-5 index, where 1 indicated totally white, healthy roots, and 5 indicated severely brown, diseased roots. At the final assessment in October, the 50 stem bases were also scraped in order to assess the presence or absence of vascular staining.

Root sampling at each date involved sampling roots from 3 slabs along a row. Fresh gloves were worn at each site, including where sites were on the same nursery, and samples were taken further along the row at each visit to avoid sampling previously damaged root tissue. If roots were thick and difficult to remove by hand, laboratory scissors that had been sterilised with 100% ethanol were used to remove them. From the NFT site without disinfection, additional root

samples were taken. Here, own root plants and grafted plants were present on the same crop row, and three replicate root samples were taken from both sets of plants for comparison. The root samples were then packaged into separate, labelled grip seal bags and posted to the University of Nottingham for processing on the same day.

Irrigation water was sampled at each site from five locations around the water loop on each occasion. At early sampling dates on some sites only 4 samples were taken as the disinfection equipment was not yet in use. 250 mls of water was sampled at each point, in labelled new plastic screw cap bottles. A generalised plan of the sampling at each sites can be seen in Figure 1 below.

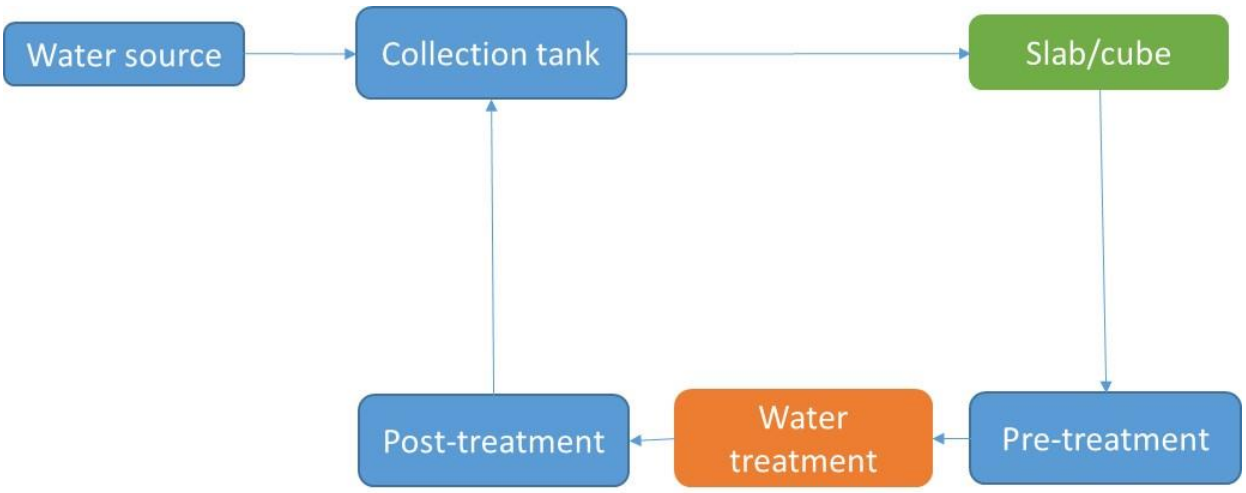


Figure 1. Generalised sampling locations points (1-5) across five commercial tomato sites – 2015

Each site differed slightly in their irrigation system, and in where samples could be taken from. The sample points at each site can be seed in Table 10. Bottles were filled at appropriate points around the loop from taps, or from collection tanks/sumps. Water from the slabs were collected using a fresh, sterile 50 ml syringe, inserted into the rockwool slab (or small cube/rhizosphere in NFT). Samples were sealed with electrical tape and posted to the University of Nottingham for processing on the same day. It should be noted that at the January visit, recirculation had not yet commenced, and so samples from the Collection tank contained only fresh water yet to travel around the loop.

Once samples had been collected, they needed to be filtered, collecting the microorganisms in the sample before the DNA was extracted.

For the root samples, extraction of their DNA followed much the same protocol as the DNA extraction from plated samples. Root samples were first examined on sterile filter paper, removing much of the excess water, where the younger looking roots were selected. Once 120 mg ±5 mg was selected they were placed inside a autoclaved screw-cap Eppendorf tube containing 8-12 2 mm glass beads. This, as before, was then homogenised. The samples were placed in liquid nitrogen for 2 min, after which they were then placed into the Fastprep FP120 (Thermo-Savant) at 6.5 ms-1 for 45 seconds. The samples were then rested before repeating the previous steps. This sequence was repeated 3 times for each sample. Once the samples were homogenized they were then extracted using the 'DNeasy plant mini kit' (QIAGEN) following the standardised protocol provided with it. Upon extraction the samples would then be stored at -20ºCFollowing homogenizing, the protocol followed that of the 'DNeasy plant mini kit' (QIAGEN).

For irrigation water samples, upon receipt the DNA was extracted as soon as possible. The sample would be separated into two replicates for each sampling location of 100 ml and these were passed through a Nalgene™ reusable 250 ml vacuum filter unit that held a sterile 47 mm, 0.2 µm Supor®-200 PES Membrane Disc Filter (Pall product). Once 100 ml of fertigation solution was passed through, the filter was washed using 100 ml of sterile distilled water (SDW) to remove any excess salts that remained on the filter. Once the water had been passed through, the filter was then removed by cutting into thin strips using a sterile scalpel and placed in a micro Bio-Spin® chromatography column. Once samples were placed in this column, 400 µl of lysis buffer (AP1 from the DNeasy plant mini kit) and 4 µl of RNase (also from DNeasy mini kit) were added to the columns before incubation at 65ºC for 10 min within a water bath with 2-3 inversions. Once the incubation was complete the columns stopper at the bottom was removed and then placed in a receiver tube where it was then centrifuged for 7 min at 6000 rpm. Following this, the column was then discarded and the DNA extracted using the 'DNeasy plant mini kit' protocol.

DNA samples for either genetic sequencing or for microarray analysis were amplified using PCR. Two sets of universal primers were used to amplify the eukaryotic ITS region, and the prokaryotic IGS sequence. These primers were produced by Sigma-Aldrich®, and were diluted to a stock solution of 100 µM and then when used diluted further to a working concentration of 10 µM, using sterile distilled water and stored at -20ºC.

Once the sample DNA was amplified, it was then possible to analyse it using the small scale microarray. Throughout the protocol, a Thermomixer Comfort ® (Eppendorf) dry heat block was used. In the first stage of the protocol the ArrayTube itself first needs conditioning. This first means that 500 µl of SDW must be added to the tube, and is kept in the thermomixer agitating at 500 rpm for 5 minutes at 50ºC. Once the 5 min was finished the contents were discarded and replaced by 500 µl of Nexterion® Hyb which is again agitated at 500 rpm for 5 minutes at 50ºC. Once finished it was too, discarded. Once the microarray had been prepared a new 80 µl of Nexterion® Hyb was added to the array and kept at 55ºC whilst the Purified PCR products were heated to 95ºC for 5 minutes in the BioRad® s1000™ thermal cycler. This heating of the DNA allows the DNA strands to dissociate from each other.

Twenty µl of both the prokaryotic and eukaryotic biotin incorporated samples were then added to each microarray and allowed to hybridise with the arrays probes whilst kept at 55ºC and agitated at 500 rpm for 1 hour and 15 minutes. Once the hybridisation steps were finished the micro array underwent 3 stages of cleaning; first 500 µl of wash buffer 1, WB1 (2X SSC [saline sodium-citrate], 0.01 % Triton X100) was added and then agitated at 500 rpm for 5 minutes at 20ºC. Following this, the WB1 was discarded and replaced by 500 µl of WB2 (2X SSC) and kept in the same conditions as WB1 for 5 min. This was then repeated one final time following the discarding and replacement of WB2 with 500 µl of WB3 (0.2X SSC).

Once the cleaning was finished the next step of preparing the microarray for analysis was to block the spots on the microarray where there is no DNA-probe binding. This was done with the addition of a freshly produced blocking solution (n+2) made up of 200 µl PBS (phosphate-buffered saline), 4 mg of skimmed milk powder, and 1 µl of 1% Triton X100. Once vortexed 100 µl of this solution was added to each microarray and kept at 20ºC for 15 min before being discarded. The next step; conjugation, also required a freshly prepared solution. The solution was made at a quantity of n+2 with 100 µl of blocking solution and 1 µl of HRP (horse radish peroxidase) linked anti-uracil antibodies (Cell Signalling Technology®). This solution was then gently mixed and 100 µl added to each of the microarrays. This was again incubated at 20ºC for 15 min. The microarray then underwent another washing stage where 500 µl aliquots of PBST (1X PBS, 0.2% Tween 20) were added to each microarray, agitated at 500 rpm at 20ºC for 5 min and discarded. This was repeated three times. For the final step the array was stained with a 100 µl aliquot of SeramunGrün® chip (Seramun®). This is an o-Dianisidine substrate and is then cleaved by the horse radish

peroxidase enzyme linked to the antibodies. This then results in a localised colour change that can then be recorded by the array-reader. The substrate was left to incubate in the ArrayTube for precisely 16 min at room temperature before being placed in the ArrayTube transmission reader-03 (Alere™) whilst utilising the software programme Iconoclust v4.1 (Alere™) to record the images of the array. These images were then subsequently analysed using a bespoke experimental template (10429_AT_myco_2_1.0, Alere™) which aligns probes with their respective organisms. The intensities of the probes were then normalised and corrected in terms of the local background intensity, using the equation:

$$i = \frac{Average\ spot\ intensity}{Local\ background\ intensity}$$

From the results gathered through the use of Iconoclust V4.1, the data was first filtered by removing any ambiguous results or results due to background noise by using intensity cut off value of 0.1. This value was selected as it is double that of any recorded results recorded from a blank array. For all of the organisms detected on the array, there were two probes. This is to prevent lack of detection as a result of anomalous results or by damage to one probe. These two probes would then be averaged.

Species richness was calculated as a count of the total number of species detected. To maintain continuity with previous projects (PC 281a), the same method of calculating species diversity was maintained, using an adapted form of the Shannon-Wiener index (H'). All the taxa on the microarray were divided into four categories for this analysis: total array, fungi and oomycetes, saprophytic fungi, and bacteria. Universal probes, such as the fungal or bacterial probe were regarded as independent species and allocated to their associated groupings. For each category the species index was calculated and recorded following the adapted formula:

$H' = -\Sigma[(a/b)\ ln\ (a/b)]$

Where a= average intensity of a single species on the array, b= sum of all average intensities on the array.

Species diversity represents a measure of the number of different species that are represented in the community and consists of two components, species richness (a simple count of species present) and species evenness (abundance of the species present).

As part of Objective 2, a trial was also established to further investigate the effects of applying a number of commonly used commercial products on microbial populations in the rhizosphere. A commercial site was selected with four adjacent glasshouse bays of identical size, in which the same scion and rootstock varieties were being grown, and which were fed and irrigated with the same rig. In September 2015 three commercial biological or ‘grey area’ products were selected and each applied at recommended rates to a single bay only using a dosatron. One bay remained untreated, but was irrigated with the same volume of water using the dosatron at the time of treatment. Roots were sampled from the central row in each bay immediately before treatment, and along the row were sampled following treatment an hour later, one day later, three days later, one week later and fourteen days later. Sampled roots and a sample of the raw products applied were posted to Nottingham by next day delivery to be tested with the microarray. Unfortunately, due to a problem with the DNA extraction kit used, no results are available for this portion of work.

Table 10. A summary of sample points for each of sites 1-5 with different water treatment systems, sampled on four occasions through the growing season in 2015

Date	Site	1	2	3	4	5
	Location	fSSF	pSSF	NFT	UV	Heat
January	1	Mains	Reservoir	Mains	Mains	Mains
	2	Mixing/Collection	Mixing/Collection	NA	Mixing/Collection	Mixing/Collection
	3	Slab	Slab	Underground tank	Slab	Slab
	4	Pre-treatment	Pre-treatment	Grafted slab	Pre-treatment	Pre-treatment
	5	NA	Post-treatment	Ungrafted slab	NA	NA
April	1	Borehole	Reservoir	Mains	Reservoir	Reservoir
	2	Mixing/Collection*	Mixing/Collection	NA	Mixing/Collection	Mixing/Collection
	3	Slab	Slab	Underground	Slab	Slab
	4	Pre-treatment	Pre-treatment	Grafted slab	Pre-treatment	Pre-treatment
	5	Post-treatment	Post-treatment	Ungrafted slab	Post-treatment	NA
July	1	Borehole	Reservoir	Reservoir	Reservoir	Reservoir
	2	Mixing/Collection	Mixing/Collection	Reservoir plus mains	Mixing/Collection	Mixing/Collection
	3	Slab	Slab	Underground tank	Slab	Slab
	4	Pre-treatment	Pre-treatment	Grafted slab	Pre-treatment	Pre-treatment
	5	Post-treatment	Post-treatment	Ungrafted slab	Post-treatment	NA
October	1	Borehole	Reservoir	Reservoir	Reservoir	Reservoir
	2	Mixing/Collection	Mixing/Collection	Reservoir plus mains	Mixing/Collection	Mixing/Collection
	3	Slab	Slab	Underground tank	Slab	Slab
	4	Pre-treatment	Pre-treatment	Grafted slab	Pre-treatment	Pre-treatment
	5	Post-treatment	Post-treatment	Ungrafted slab	Post-treatment	Post-treatment

NA – not available as not yet recirculating/mixing, or due to break down; *mostly Borehole water as pump had tripped out on day of visit

Objective 3 – Pathogenicity of root pathogens on own-root tomato and grafted plants

Experiment 1 – Preliminary inoculation study (ADAS Boxworth)

To ensure the pathogenicity of isolates collected from UK tomato crops in late 2014, a preliminary trial was established using ungrafted tomato seedlings cv. Elegance. Tomatoes were grown on rockwool cubes by a commercial propagator, and were placed in 50 x 30 cm disinfected plastic trays in a glasshouse at ADAS Boxworth. Plants arrived at approx. 50 days old. Each tray contained two tomato plants, which were fed using liquid feed added to the trays. EC and pH in the trays were measured daily, and the feed solution adjusted or dumped and restarted as appropriate. Fungal isolates collected from UK crops whose identity had been confirmed by PCR at the University of Nottingham were used to inoculate trays.

Plants were inoculated using a drench of 20 mls spore suspension over the stem base and rockwool cube, one week after their arrival at ADAS Boxworth. Two isolates of each of the key pathogens at two spore suspension concentrations were used to inoculate tomato plants. Each isolate of *F. oxysporum* (BX14/153a and BX14/153b), *C. coccodes* (BX14/148a and BX14/149) and *P. cucumerina* (GD161 and GD107, sourced from cultures stored at the University of Nottingham) were inoculated at concentrations of $1x10^5$ and $1x10^6$, and the *Pythium* spp. (BX14/153c and a known *P. aphanidermatum*, BX13/23, confirmed by PCR) were inoculated at concentrations of $1x10^5$ and $1x10^6$. Isolates were selected that grew and sporulated readily on PDA+S agar, or in the case of the *Pythium* spp., readily produced zoospores. A plot was five tomato plants (two and a half trays) and each plot was inoculated with a high or low concentration of inoculum of each of the eight fungal isolates. Due to limited glasshouse space at experiment set up, two tomato plants (the fifth of each plot, in the same tray) received a mixed isolate inoculation for each key pathogen at both inoculum concentrations. This resulted in a total of 16 treatments, plus 2 untreated control plots.

Immediately prior to inoculation, the feed solution was adjusted to optimum pH and EC, and was not dumped until 10 days afterwards to favour successful inoculation of plants. Plants were monitored for signs of root disease daily, and were assessed weekly, and when first suspected symptoms appeared.

Plants were assessed for visible wilting or yellowing of the leaves, crop vigour and any nutrient deficiencies that occurred over the course of the experiment. Additionally, root health and

extent were assessed. Colour was assessed on a 1-5 index where 1 equalled very white, healthy roots and 5 equalled severely rotten, brown roots. Extent was assessed on a 1-5 index where 1 equalled no growth from the edge of the cube and 5 equalled extensive root growth from the cube and into the tray. Some plants were awarded a score of 0 if roots had receded so that the cube base was largely bare of roots. At the final assessment, percent rotten and white roots on the cube base were also assessed, presence of stem staining and a destructive assessment involving cutting open the rockwool cube and observing the internal root structure and health was performed. Here, a 1-5 index was used where 1 equalled a good network of fine, healthy roots and where 5 equalled only a sparse root network present. At the final assessment, root samples from plots inoculated with each isolate were also taken and sent to Nottingham to test for presence of the inoculated pathogens.

At the conclusion of the experiment, tomato plants were disposed of, and trays and the glasshouse floor and structure were cleaned and disinfected.

Experiment 2 – Pythium sp. inoculation study (ADAS Boxworth)

Following the preliminary trial, a larger scale trial examining the effect of three different inoculation strengths of *Pythium* species on tomatoes cv. Elegance grown on the vigorous rootstock, Maxifort, versus ungrafted Elegance. Plants were inoculated using mycelial plugs in a 1:1 ratio of the *Pythium* spp. (BX14/153c and BX13/24) as zoospores were not synchronously released by cultures. Two (Low), four (Medium), or six (High) 0.8 mm agar plugs taken from the edges of actively growing cultures were embedded into the rockwool at water level on the 8th July (Table 11). Each plot contained two plants in rockwool cubes, grown in trays containing nutrient solution, and inoculation took place 7 days after plant arrival to give plants time to acclimatise to their new environment.

Table 11. Pythium inoculation treatments applied to tomato plants with scion cv. Elegance – ADAS Boxworth, 2015

Treatment	Rootstock	Inoculum level
1.	Elegance	Nil
2.	Elegance	Low
3.	Elegance	Medium
4.	Elegance	High
5.	Maxifort	Nil

6.	Maxifort	Low
7.	Maxifort	Medium
8.	Maxifort	High

Tomatoes were fed with commercial standard soluble fertilisers, and amounts provided were governed by pH and EC and by plant appearance. Feed was entirely changed a minimum of weekly.

Following inoculation, plants were monitored closely for any signs of root disease. The crop was also managed to encourage this. No pruning or removal of laterals was carried out in order to put maximum strain on plant root systems. For the same reason, the trial was run throughout the peak of summer and temperature and humidity loggers were placed in the trial. The trial was assessed four times, on the 15th and 29th July, the 28th August and a final, destructive assessment carried out on 10th September. Plants were assessed for vigour and foliar signs of root disease including leaf yellowing and wilting. Roots were assessed for colour and extent. At the final assessment, a destructive assessment was also carried out where the rockwool cube was opened and root colour and extent within the cube assessed. Stem bases were also scraped at the final assessment to reveal the presence or absence of any staining of the vascular tissue. A sample of root tissue from each treatment was also taken and isolations carried out onto PDA + S agar to determine if the inoculated pathogen was still present.

Experiment 3 – Plectosphaerella cucumerina inoculation study (ADAS Boxworth)

The trial was conducted as described for Experiment 2. Plants were inoculated with 20 mls of a 1:1 mix of *P. cucumerina* isolates GD161 and GD107, sourced from cultures stored at the University of Nottingham, applied to the stem base. Uninoculated controls received 20 mls of sterile distilled water, 'Low' inoculation treatments received a spore inoculum of 1×10^2 per ml, 'medium' 1×10^4 per ml and 'high' 1×10^2 per ml.

Experiment 4 – Colletrotrichum coccodes inoculation study (University of Nottingham)

Fungal isolates were collected from a combination of UK growers and CABI culture collection and stored at the University of Nottingham. These cultures were confirmed by PCR and sequencing.

A large scale trial was devised to look at the effect of three different inoculation strengths of *C. coccode*, on tomatoes cv. Elegance grown on either its own roots or on a more vigorous rootstock cv. Maxifort. The plants were delivered at approximately 50 days old, and were laid out in plots of three plants per rockwool slab. Each slab was contained within a disinfected tray containing nutrient solution. The conditions of the glasshouse over the majority of the course of the trial were as follows; 18°-30°C day temperature, ventilated at 35°C, with a night temperature of 15°-16°C. The plants were inoculated 7 days after delivery, allowing the plants to settle. The spore solutions were produced from three separate isolates of *C. coccodes*; BX14/128b, BX14/148b and BX14/149, all supplied by ADAS from UK growers. These were each used to make a spore solution with a mix ratio of each isolate of 1:1:1. 20 ml of spore solutions were delivered to the stem base and roots of the plants at three varying concentrations; 10^3/ml, 10^4/ml and 10^5/ml each representing 'low', 'medium', and 'high' treatment levels respectively. As well as this the control group had 20 ml of distilled water added to the roots instead.

Prior to the inoculation, the nutrient solution was adjusted to be optimal pH (6-6.5) and EC (3-4), before then being dumped 10 days following inoculation to favour the successful infection of the roots. As well as adjustments to the nutrient solution, the plants were also managed in a manner conducive to infection. This included discontinuing the pruning and removal of laterals to place maximal strain on the roots, whilst also conducting the trial over the peak of summer. Following inoculation, the plants were assessed every ten days from the outside, as well as root samples being taken from inside the rockwool cube and visually assessed, until the final destructive assessment was carried out.

Plants were assessed for visible wilting or yellowing of the leaves, crop vigour and any nutrient deficiencies that occurred over the course of the experiment. Additionally, root health and extent were assessed. Colour was assessed on a 1-5 index where 1 equalled very white, healthy roots and 5 equalled severely rotten, brown roots. Extent was assessed on a 1-5 index where 1 equalled no growth from the edge of the cube and 5 equalled extensive root growth from the cube and into the tray. Some plants were awarded a score of 0 if roots had receded so that the cube base was largely bare of roots. At the final assessment, percent rotten and white roots on the cube base were also assessed, presence of stem staining and a destructive assessment involving cutting open the rockwool cube and observing the internal root structure and health was performed. Here, a 1-5 index was used where 1 equalled a good network of fine, healthy roots and where 5 equalled only a sparse root network present.

Experiment 5 – Fusarium oxysporum inoculation study (the University of Nottingham)

The trial was conducted as described for Experiment 4. Fungal isolates were collected from a combination of UK growers and CABI culture collection and stored at the University of Nottingham. These cultures were confirmed by PCR and sequencing, also at the University of Nottingham.

The spore solutions were produced from three separate isolates of *F. oxysporum*; FOL 2a, BX14/153a and BX14/168a, supplied by ADAS from UK growers and CABI. These were each used to make a spore solution with a mix ratio of each isolate of 1:1:1. 20 ml of spore solutions were delivered to the stem base and roots of the plants at three varying concentrations; 10^3/ml, 10^5/ml and 10^7/ml each representing 'low', 'medium', and 'high' treatment levels respectively.

As detailed in the methods for Objective 2, roots and water from grafted and ungrafted Piccolo were also sampled from a commercial site, to contribute to the comparison of rootstocks and the effect they have on microorganisms in the rhizosphere.

Objective 4 – Monitor additional crops grown with re-cycled irrigation for root pathogens and root disease

An additional five sites, different to those used in Objective 2, were identified that grew tomatoes hydroponically and with a recirculating water system. Crops on these sites were assessed for root disease in July and October, and root samples taken. At each sampling visit, roots were sampled from:

a) plants with no visible root disease

b) plants with obvious signs of root disease (if present)

As for Objective 2, the same row at each site was sampled at all sampling occasions, but different areas of the row were sampled at each visit so as to avoid selecting roots that had been previously damaged by sampling. Fresh coveralls and overshoes were worn on visiting each site. When visits were being organised the status of disinfection equipment on site was confirmed. Additionally, on arrival, any fungicide or other product application to the root zone of monitored plants was recorded. Three replicates of roots were sampled each time, from three separate slabs along the row. Fresh sterile gloves were worn for sampling each site, including for different sites/crops on the same nursery, and sterilised scissors were used if roots were difficult to remove by hand. Samples were packaged in separate grip seal bags

and posted to the University of Nottingham on the same day. The five additional sample sites are detailed in Table 12 below.

Table 12. A summary of the disinfection treatment, rootstock and scion present on each of the additional 5 sites sampled in July and October 2015

Site	Water disinfection treatment	Substrate	Rootstock	Scion
6.	Heat	Coir	Beaufort	Olinta
7.	Heat	Rockwool	Maxifort	Sunstream
8.	Heat	Coir	Optifort	Roturno
9.	Nil	NFT	Ungrafted	Conchita
10.	Nil	NFT	Ungrafted	Conchita

Following receipt at the University of Nottingham, root samples were treated as described for Objective 2.

Results and discussion

Objective 1 – Optimise microarray and qPCR diagnostics

Validation of primers, probes and amplification protocol

For the organisms *Colletotrichum coccodes, Plectospharella cucumerina, Pythium aphanidermatum* and *Pythium myriotylum,* a TaqMan assay was developed where all four probes had different fluorophores so it was possible to measure them on the same plate under different wavelengths. However, some filters had slight overlaps in recording that may lead to difficulty in using them in the same well.

All assays successfully amplified the pure culture DNA samples for their target organism. The numbers of pure cultures used to test each assay set are listed in Table 13. The overall

efficiency of the assays were sufficient to be used. The efficiency of *C. coccodes* on its own falls just outside the sufficient efficiency range of 90-110%, so needs further refining; the rest fell within the sufficient range. Efficiencies are not certain values but estimates as a result of them being derived from serial dilutions which contain uncertainty. This is the reason for theoretically impossible efficiencies being derived >100% so a relatively wide range of 10% either side of 100% is permitted. All confirmed pure cultures were successfully detected and amplified. In the tables below, the Ct score refers to the cycle threshold, or the cycle number on which fluorescence is first recorded. This relates to when the amplification begins and enables the amount of DNA to be quantified.

Table 13. Amplification efficiency of assay protocol. Efficiencies based on the presumption that 90%-110% is acceptable and 100% is a Ct difference of 3.32 for a tenfold dilution.

Primer set	Number of pure cultures used	Average Ct difference	Amplification efficiency
C. coccodes	3	3.76	113.6%
P. cucumerina	3	3.12	94.0%
P. aphanidermatum	2	3.18	102.1%
P. myriotylum	1	3.39	95.78%
Overall average		**3.36**	101.8%

Microarray validation in conjunction with q-PCR primers

The aim of this work was to validate microarray results for these four pathogens with regard to both no detection or detection by the microarray, and relative quantity as determined by microarray colour intensity.

a) Colletotrichum coccodes

Table 14 compares microarray results to the same samples being analysed using the *C. coccodes* qPCR assay. The ranging concentrations (low, medium and high) derived from the microarrays seem to be well supported in this case by the qPCR amplification with clear definition between each of the samples.

There is also evidence for insufficient sensitivity in the microarray, as one sample that tested negative by the microarray, was amplified using qPCR. This amplification was relatively late

in terms of qPCR amplification with a Ct of 33.21; however, this still suggests it was present at a low concentration. Relative to the microarray that recorded low concentrations of

Table 14. Result of the comparison of microarray results for detection *C. coccodes* to qPCR results.

Test samples from microarray	Concentration of test fungus	qPCR amplification expected/actual	Average Ct score	Average Ct difference
Negative microarray result	n/a n/a	-/+ -/-	33.21	
Low to high microarray score	Low Medium High	+/+ +/+ +/+	27.05 24.94 22.80	 2.11 2.14
No template control	n/a	-/-	ND	

ND – not detected

b) Plectosphaerella cucumerina

Again, in the *P. cucumerina* assay, there is a suggestion of lack of microaarray sensitivity with both supposed negative samples amplifying (Table 15). The result of the validation of the semi-quantitative side of the array gave inconclusive results with no real clear defined order.

Table 15. Comparison of microarray results for detection of *P. cucumerina* to qPCR results.

Test samples from microarray	Concentration of test fungus	qPCR amplification expected/actual	Average Ct score	Average Ct difference

Negative Microarray result	n/a n/a	-/+ -/+	28.37 32.28	
Low to high microarray score	Low Medium High	+/+ +/+ +/+	22.45 25.16 24.23	 -2.71 0.93
No template control	n/a	-/-		

c) Pythium aphanidermatum

P. aphanidermatum tests were limited by a lack of positive microarray samples to retest; however, the investigation of negative microarray samples suggests there was wasn't any of this pathogen at even low concentrations throughout the season (Table 16).

Table 16. Comparison of microarray results for detection of *P. aphanidermatum* to qPCR results.

Test samples from microarray	Concentration of test fungus	qPCR amplification expected/actual	Average Ct score
Negative Microarray result	n/a n/a	-/- -/-	ND ND
No template control	n/a	-/-	ND

ND – not detected

d) Pythium myriotylum

P. myriotylum showed evidence of specific primers in term of not detecting other organisms on the negative samples, whilst confirming there was no *P. myriotylum* present in the sample.

However, the low to high microarray results showed no amplification using the qPCR assay (Table 17).

Table 17. Comparison of microarray results for detection of *P. myriotylum* to qPCR results.

Test samples from microarray	Concentration of test fungus	qPCR amplification expected/actual	Average Ct score
Negative Microarray result	n/a	-/-	ND
	n/a	-/-	ND
Low to high microarray score	Low	+/-	ND
	Medium	+/-	ND
	High	+/-	ND
No template control	n/a	-/-	ND

ND – not detected

From these preliminary validation results, two of the four primer assays seem to have good specificity. Both *C. coccodes* and *P. cucumerina* could be validated using three separate strains of pure culture to ensure the specificity of the primers were sufficient to identify different isolates. However neither of the *P. aphanidermatum* or *P. myriotylum* assays were tested with as many samples, so, despite initial positive results, more tests are needed to confirm their reliability. *P. myriotylum* in particular did not seem to amplify anything in samples that had been positively detected using the microarray, so needs further assessment using more pure culture strains to identify the reason for this result whether it is due to experimental error, or ineffective primers; or whether the microarray's probe was not specific enough and was detecting other *Pythia* species. It seems the use of the ITS regions for each of the primers was sufficient for specific and accurate identification. On the whole, from the serial dilutions, the *P. cucumerina* assays seemed to have sufficient effciency falling within the guideline range. Futher refining of the protocol and mastermixes is probably required for *C. coccodes.*

Objective 2 – Effect of water treatment system, sampling location and crop age on microorganisms in recycled water

A full record of microorganisms detected by the microarray for each sample location, site and

sampling date is given in Appendix 1. Here, visible trends in the data relevant to the project objectives will be discussed. It is important to note that the five sites monitored differ in many other factors than simply disinfection treatment in place, and that this additional variation may make trends difficult to discern.

Water treatment system and species richness

The water treatment systems in place at the five sites monitored (fSSF, pSSF, Nil-NFT, UV and Heat) differed in their ability to remove microorganisms from the water. However, it should be noted that the pasteuriser at the fifth site utilising heat disinfection was not in action for sampling in Jan/Feb, April and July. In October, the equipment was working and disinfection achieved by the pasteuriser appeared effective, with *Pythium* and *Fusarium* spp. reduced to low levels, and *Verticillium nigrescens* removed completely. For the other four treatment systems (including NFT with nil treatment), results will be discussed in more detail.

Table 18 details the microorganisms that appeared at least once in every system monitored in 2015 (Sites 1-5, including NFT Site 3), highlighting that though different water disinfection systems were installed, and numerous other factors between sites undoubtedly differed, the hydroponic environment is inherently favourable for a number of plant pathogens, saprophytes and bacteria. Conversely, Table 19 details the microorganisms which appeared uniquely in one of the systems, but not in others. Presumably, this is due to factors which differed between sites. This could be due to the variable efficacy of water disinfection systems on particular species or groups, or could be due to any number of factors such as particular water sources, tomato cultivar, fertiliser recipe etc.

Table 18. Microorganisms that appeared at least once in each system throughout 2015

Fungal pathogens	Saprophytes	Bacteria
F. oxysporum	*A. flavus*	*Erwinia* spp.
P. irregulare	*Cladophora* spp.	*Nitrospira* spp.
P. myriotylum	*Exophiala pisciphila*	*Pseudomonas* universal
P. paroecandrum	*Penicillium* spp.	*Xanthomonas* spp.
P. cucumerina		*Yersinia* spp.
S. subterranea		
V. nigrescens		

Table 19. Microorganisms detected that appeared uniquely in each system monitored over 2015

fSSF	pSSF	NFT (ungrafted)*	NFT (grafted)*	UV	Heat
R. solani	*Armillaria mellea*	*V. dahliae*	*D. microsporus*	*P. debaryanum*	*Aspergillus* spp.
Chaetomium cochliodes	*P. arrhenomanes*	*P. griseofolium*		*Alternaria spp.*	*Phoma* spp.
	Myrothecium roridum			*P. chrysogenum*	
	Trichoderma viride			*Rhizopus oryzae*	
	A. tumefasciens				
*if the other rhizosphere and rootzone irrigation water is excluded, can be taken together for general NFT					

For sites 1-5, the microorganisms detected by the microarray at each of the sites sampled (with different water disinfection treatments) are summarised below. When data across each of the sampling points around the irrigation loop are combined, distinct differences between the disinfection treatments can be observed. Notable differences between sites for individual pathogens are highlighted in bold in Table 20 (see Appendix 1 for full names of microorganisms). For example, *F. oxysporum* was detected at relatively high levels at site 2 (pSSF) and 5 (heat) compared with the other sites. Relative abundance of individual *Pythium* species also differs between sites, for example Sites 1, 4 and 5 have higher levels of *P. irregulare*, but lower levels of *P. myriotylum*, whereas for Sites 2 and 3 this trend is reversed. This suggests that the different site disinfection treatment have better efficacy against some pathogens than others, and this could be for numerous reasons.

Table 20. Summary of microorganisms detected by the microarray in irrigation water sampled from 5 sites with different water disinfection treatments - 2015

Pathogen	1 fSSF	2 pSSF	3a NFT (grafted)	3b NFT (ungrafted)	4 UV	5 Heat
Index (maximum value)	114	114	90	90	114	102
C. coccodes	1	6	0	0	6	3
F. oxysporum	6	**12**	4	4	8	**14**
P. nicotianae	0	1	0	0	0	1

Pythium diclinum	3	5	7	6	4	0
P. irregulare	**19**	1	13	11	**25**	**18**
P. myriotylum	5	**17**	**13**	**11**	7	0
P. paroencandrum	9	10	4	2	**12**	**15**
Plectosph.	**24**	**21**	14	11	**18**	**19**
R. solani	1	0	0	0	0	0
Th. basicola	0	1	0	0	0	0
Vaa	0	0	0	0	0	0
Vd	0	0	0	0	0	0
Vn	**17**	**16**	11	8	10	**16**

Large differences between the sites for individual pathogens are shown in bold

Overall in terms of cumulative species richness, it was found that over the season the NFT system (Site 3) detected the lowest species richness within its irrigation water samples (Table 21). This was true for both potential pathogens and saprophytes and bacteria. More microorganisms were detected in the irrigation water than on the roots sampled at all time points except January. Relatively few microorganisms were detected on roots throughout the season compared to the other crops (Table 21). Apart from Site 3, species richness did not notably differ between water and roots, though specific pathogens were observed to favour certain environments. For example, *P. cucumerina* was reported far more often from the roots or slab water than from anywhere else in the system. Site 3 acted as a control treatment for this aspect of the project, as no disinfection treatment was present on site.

Table 21. Number of pathogens detected in irrigation water and roots by the microarray across 5 sites – 2015

Microorganism group	No. species detected at site:					
	1	2	3a	3b	4	5
	fSSF	pSSF	NFT ungrafted	NFT grafted	UV	Heat
<u>On roots</u>						

Pathogenic fungi and oomycetes	8	10	7	8	10	8
Saprophytic fungi and oomycetes	5	3	2	3	7	4
Bacteria	8	5	4	7	4	4
Total	28	23	17	23	27	21
In water						
Pathogenic fungi and oomycetes	13	13	11		10	9
Saprophytic fungi and oomycetes	12	6	6		11	12
Bacteria	9	6	5		7	5
Total	34	25	22		28	26

In terms of the other nurseries, the fSSF system had the highest species richness, whilst the pSSF system had the second lowest (Figs 2 and 3). It is possible that in the system where all water is cycled through the filter (Site 1), a greater number of saprophytic fungi and bacteria are introduced to the system than in the part SSF system (Site 2) where only 10% of run-off is cycled through a SSF. The high species richness reported for the fSSF is largely due to a high count of bacterial species compared to the other systems, and levels of pathogenic fungi or Oomycetes are largely in line with other sites. This further supports the role of slow sand filters in supplementing the irrigation water with potentially beneficial saprophytes.

With regards to efficacy, treatment effects can be seen by comparing sample points 4 (pre-treatment) and 5 (post-treatment) (Figs 2-6). The full SSF (Site 1) was efficient at removing pathogens from irrigation water. In October at Site 1, there was not as large a drop in species richness as previously in the season, but the treatment appeared to successfully remove *C. coccodes*, multiple *Pythium* spp., and *P. cucumerina* (Table 22). Site 2's microarray results (Fig 3), also showed a reduction in pathogens and general microbial life after treatment. At the April sampling date where species richness did not fall after treatment at Site 2, however, very few common pathogens were detected pre-treatment, and a greater number of saprophytic and bacterial species were detected afterwards. This highlights why looking at species richness alone is insufficient.

Species richness on the roots grown in the heat and UV (Figs 5 and 6) systems were relatively similar, both had relatively sporadic results in terms of appearance of organisms. Microarray

results from Site 4, where water was UV treated, showed the treatment to have a good degree of efficacy against common pathogens in the water, removing the majority of pathogens (though some *Pythium* remained). Site 5, which was heat treated, had a cumulative species richness level more in line with Sites 1 and 2 where a Slow Sand Filter was functioning. This is more likely due to the fact that disinfection was not being carried out at the majority of sampling dates, meaning microbes were not being removed from the system that otherwise would be.

Sampling location

The microorganisms detected on the microarray varied between sample locations, and this also varied between sites (Tables 22-26). A reduction in the number of species (richness) and in species diversity (a measure taking species richness into account, but also the relative proportions of each species) was recorded for all sites with disinfection treatment at sample point 5, post-treatment. However, as only a single treated sample could be taken at Site 5 (Heat) it is difficult to draw conclusions for this site.

What was detected on root samples did not always correspond to what was detected in their corresponding water samples. Detailed tables comparing pathogens on roots and in irrigation water can be found in Appendix 1. If microorganisms were detected in irrigation water, they were more likely to be detected on or in the roots. One notable exception to this is in the NFT crop (Site 3), where it was uncommon to detect any microorganisms in the irrigation loop other than when water was sampled from around the roots. At this site, where locations 4 and 5 corresponded to grafted and ungrafted roots respectively, a greater number of species was present on ungrafted roots. This could imply grafted roots are more capable at withstanding colonisation by pathogens, due to some form of in-built resistance. Conversely, water samples taken from the rootzone contained a greater number of species when taken from around grafted roots.

The original water source differed between sites, and microorganisms were detected in the water sources used by all sites. Notably, in January the source water for all sites was largely sourced from the mains supply, and was not 100% free of microbial life. In general, a greater number of species and greater species diversity was detected in water taken at the slab. This is likely a more favourable environment for plant pathogens and closely associated saprophytic species. Some species, such as *P. cucumerina*, were rarely detected prior to the

slab, and were reliably removed by disinfection, which could imply that they entered the system on the plants as seedlings.

Tables 22-26 summarise the most commonly detected pathogens at each sample location for sites 1-5.

Table 22. Effect of sampling point on microorganisms present in irrigation water, based on cumulative prevalence over four sample times of 6 most common pathogens (0-24* index) – Site 1, fSSF

Top 6 pathogens	Source	Mixing tank	Slab	Pre-treat	Post-treat
1. *S. subterranean*	4	0	8	9	2
2. *P. cucumerina*	0	0	8	2	0
3. *F. oxysporum*	1	2	0	3	0
4. *V. nigrescens*	0	0	7	0	0
5. *P. irregulare*	3	0	15	12	1
6. *P. paroecandrum*	1	0	8	9	0

*0-18 for post-treatment sample, treatment was not yet running at January visits

Table 23. Effect of sampling point on microorganisms present in irrigation water, based on cumulative prevalence over four sample times of 6 most common pathogens (0-24 index)* – Site 2, pSSF

Top 6 pathogens	Source	Mixing tank	Slab	Pre-treat	Post-treat
1. *F. oxysporum*	2	0	4	2	0
2. *P. diclinum*	0	3	0	1	0
3. *P. irregulare*	2	3	2	5	2
4. *P. myriotylum*	6	8	0	7	0
5. P. paroecandrum	2	4	1	3	0
6. *S. subterranea*	1	2	0	2	0

*0-18 for post-treatment sample, treatment was not yet running at January visit.

Table 24. Effect of sampling point on microorganisms present in irrigation water, based on cumulative prevalence over four sample times of 5 most common pathogens (0-24 index)* – Site 3, NFT

Top 5 pathogens	Source	Mixing tank	Underground tank	Slab (grafted)	Slab (ungrafted)
1. *F. oxysporum*	3	0	1	1	0
2. *P. diclinum*	0	0	6	1	7
3. *P. irregulare*	3	0	2	1	6
4. *P. myriotylum*	0	0	4	6	9
5. *V. nigrescens*	0	0	8	5	6

*0-18 for post-treatment sample, treatment was not yet running at January visit.

Table 25. Effect of sampling point on microorganisms present in irrigation water, based on cumulative prevalence over four sample times of 5 most common pathogens (0-24 index)* – Site 4, UV

Top 5 pathogens	Source	Mixing tank	Slab	Pre-treat	Post-treat
1. *P. irregulare*	6	6	13	13	3
2. *P. myriotylum*	3	2	2	0	0
3. *P. paroencandrum*	4	2	6	6	0
4. *P. cucumerina*	0	0	7	3	0
5. *S. subterranea*	6	2	7	8	2

*0-18 for post-treatment sample, treatment was not yet running at January visit.

Table 26. Effect of sampling point on microorganisms present in irrigation water, based on cumulative prevalence over four sample times of 5 most common pathogens (0-24 index)* – Site 5, Heat

Top 5 pathogens	Source	Mix tank	Slab	Pre-treat	Post-treat
1. *P. irregulare*	6	6	10	18	1
2. *F. oxysporum*	2	2	3	7	1
3. *P. paroencandrum*	5	4	6	5	0
4. *P. cucumerina*	0	0	9	6	0
5. *S. subterranea*	3	3	4	6	0

*0-6 index post-treatment as sample only available in October.

For each site, species richness in water has been graphed to illustrate the effect of the water treatment in place between sample points 4 (pre-treatment) and 5 (post-treatment) (Fig 2-6). Note there was no water disinfection treatment at site 3 (NFT). Here, samples 1-5 refer to: (1) reservoir; (2) reservoir + mains; (3) underground tank; (4) around grafted roots; (5) around ungrafted roots (see Table 9).

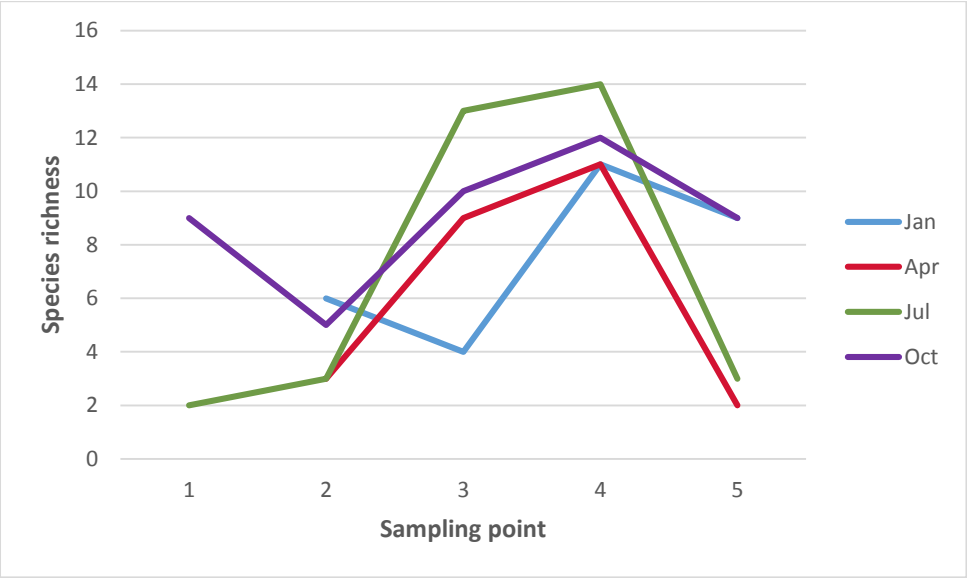


Figure 2. Species richness across sampling points for Site 1, fSSF - 2015

Site 1 (Fig 2) illustrates the high efficacy of a full slow sand filter, as species richness reliably drops after the treatment. This drop is less pronounced in October, potentially as the filter has been working all season and SSFs require regular cleaning. Species richness over sampling points for Site 1 behaves as would be expected. Sample point 1, in this case borehole water, is not free from microbial life. Any microbes present in this water, sampled from a pipe,

become less concentrated in the mixing tank. Species richness is relatively high in both the slab and pre-treatment, as might be expected.

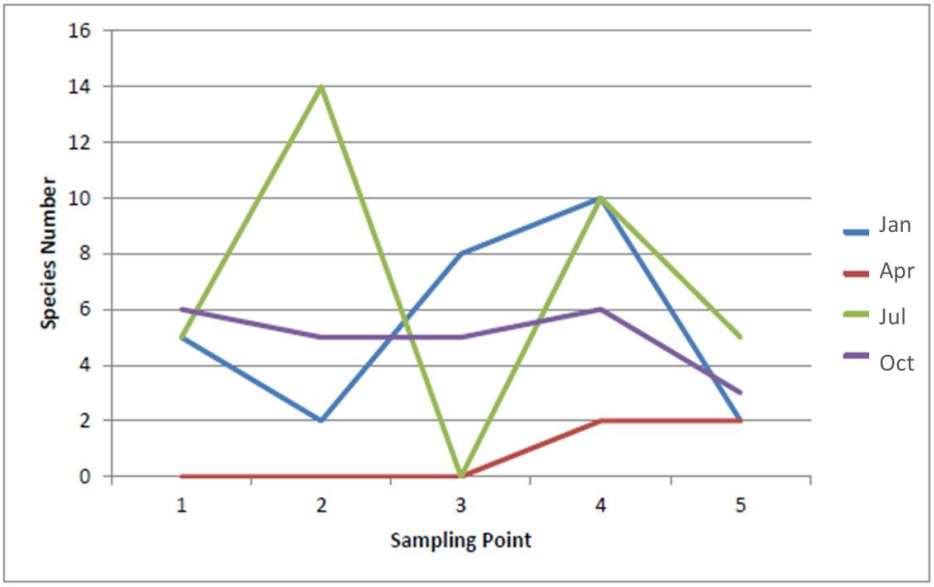


Figure 3. Species richness across sampling points for Site 2, pSSF - 2015

The impact of sampling point at Site 2 (Fig 3) was very variable across the year. The water source at this site was a clay lined reservoir, kept in a relatively natural state, which was likely subject to environmental changes. In addition, only 10% of run-off water at this site was treated with the SSF, and the fact that different factions of the water were treated differently could also have introduced additional variation. There is a clear spike in species richness at Point 2 (mixing/collection tank), which may be due to the recirculation of untreated water. Why this occurs in July and at no other time point is unclear, but may be due to the return water.

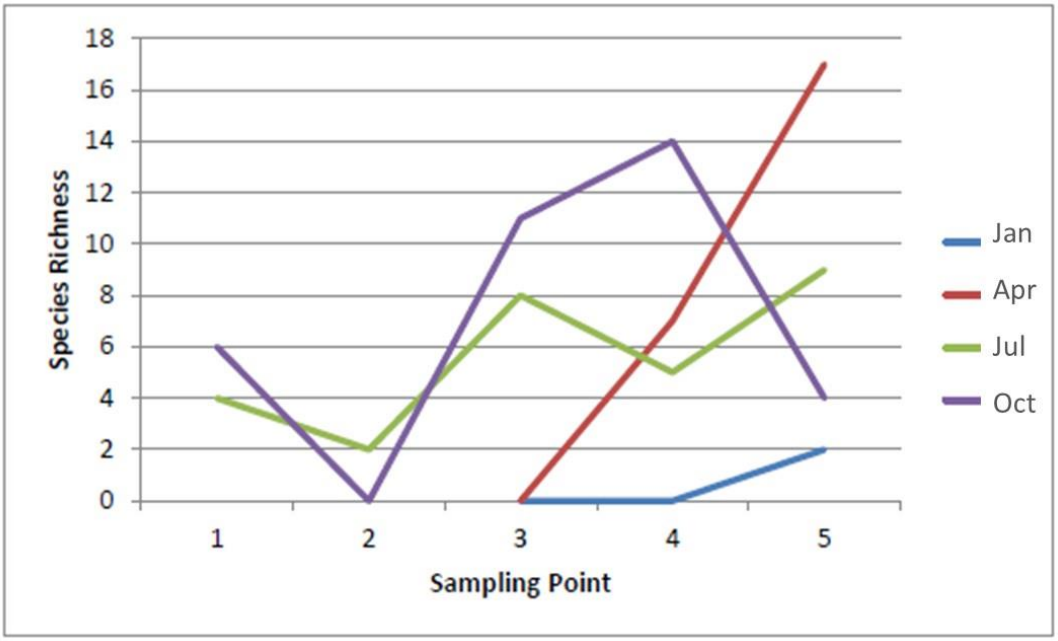


Figure 4. Species richness across sampling points for Site 3, NFT - 2015

The greatest number of species detected at any point at Site 3 (Fig 4) were detected on ungrafted roots in April (sample point 5). The only sampling point where ungrafted roots (sample point 4) had fewer species than grafted was in October, when crops were at their oldest. Sampling point 3 (underground water tank) began with no detectable species in January or April, and the levels increased over the season, from July to October. Point 2 (reservoir + mains), when sampled, reliably shows a drop in richness compared with point 1 (reservoir only). This is most likely because source water from the reservoir was diluted 50% with mains at this point, reducing the concentration of pathogens. Water also went through a large mesh size filter between Points 1 and 2. Point 3 was the large underground tank present in the NFT system, and it is likely that species were able to slowly build up in this very large body of water over the season as no disinfectant treatment was in use.

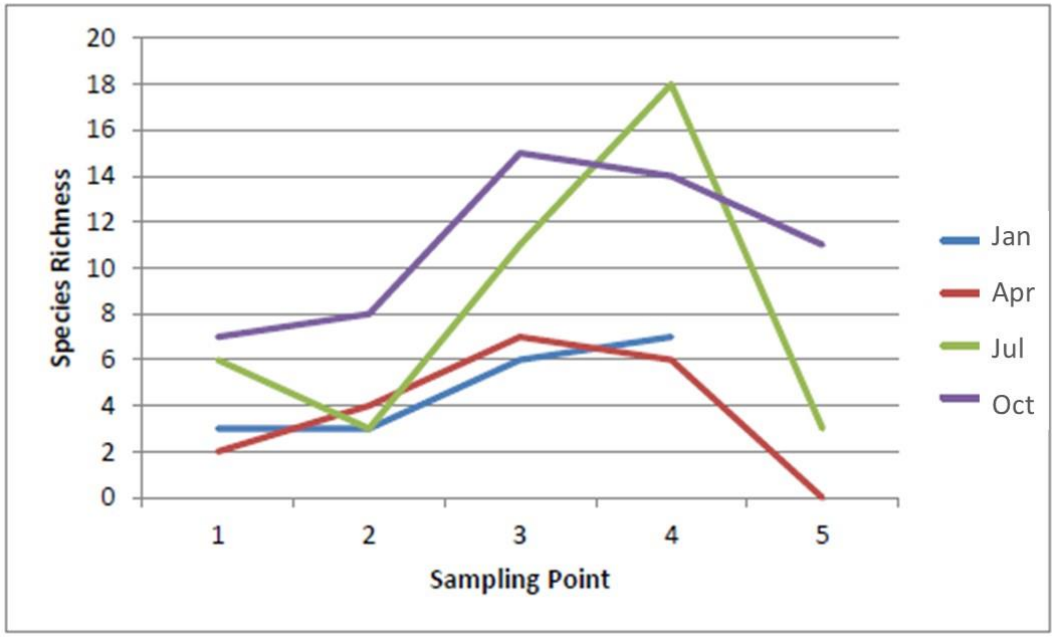


Figure 5. Species richness across sampling points for Site 4, UV – 2015

Figure 5 illustrates the good efficacy of UV treatment of water at Site 4. Species richness does not experience such a stark drop post-treatment (sample point 5) in October as earlier in the season. One potential reason for this could be an increase in water turbidity over the season, an issue known to reduce the efficacy of UV water treatments. Species richness levels at Point 1 (borehole water) for both Sites 4 and 5 are based on the same water source, but levels were not as similar as might be expected. This could reflect the variability within each body of water sampled, and though efforts were made to capture as realistic a picture as possible, sample sizes taken from each sample point were restricted by practical concerns.

Figure 6 shows that on the one occasion (October) where the heat treatment was working effectively and a sample could be taken, that it was very effective in reducing species richness (compare sample points 4 and 5). There was a peak in species richness at Point 2 (mixing/collection tank) in July, and as Point 2 includes both fresh and recirculated water this could be a result of the pasteuriser being out of action for a long period.

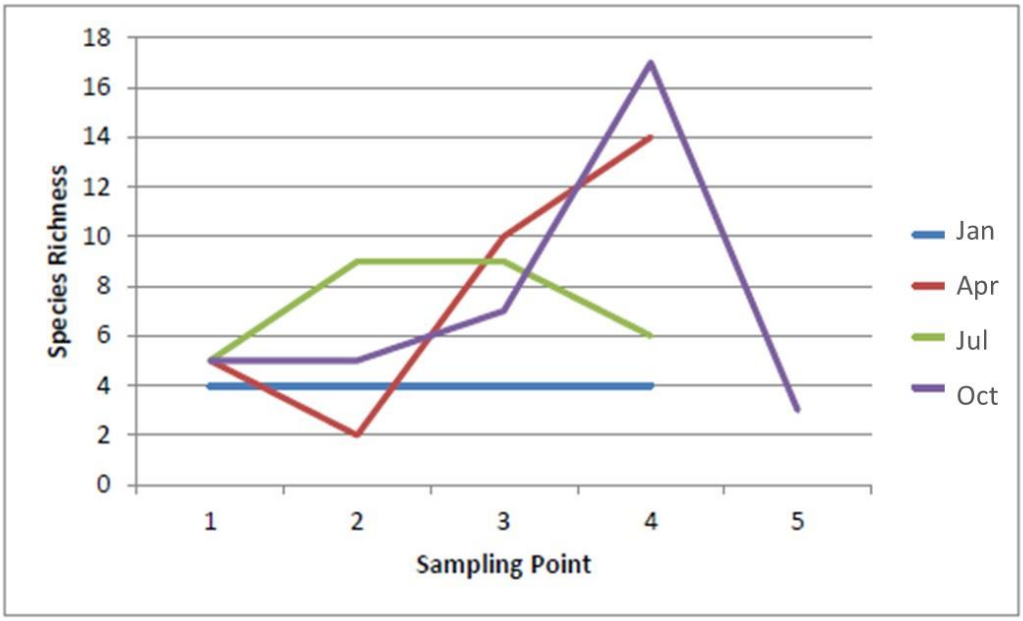


Figure 6. Species richness across sampling points for Site 5, Heat – 2015

Water treatment system and species diversity

Average species diversity has been graphed for each site (Figures 7-11).

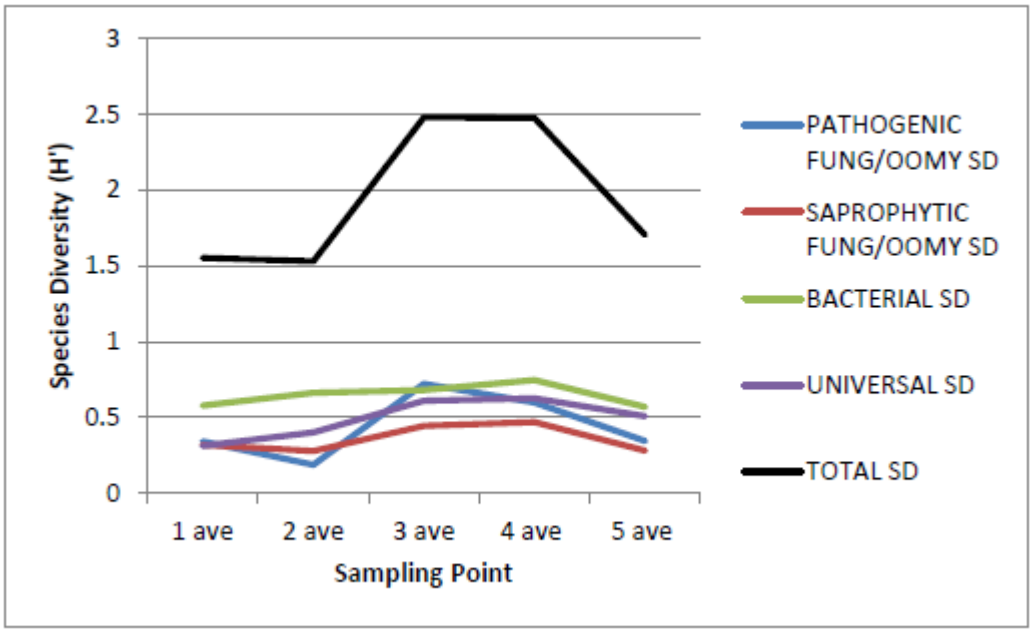


Figure 7. Seasonal average species diversity in water across 5 sampling points for Site 1, fSSF - 2015

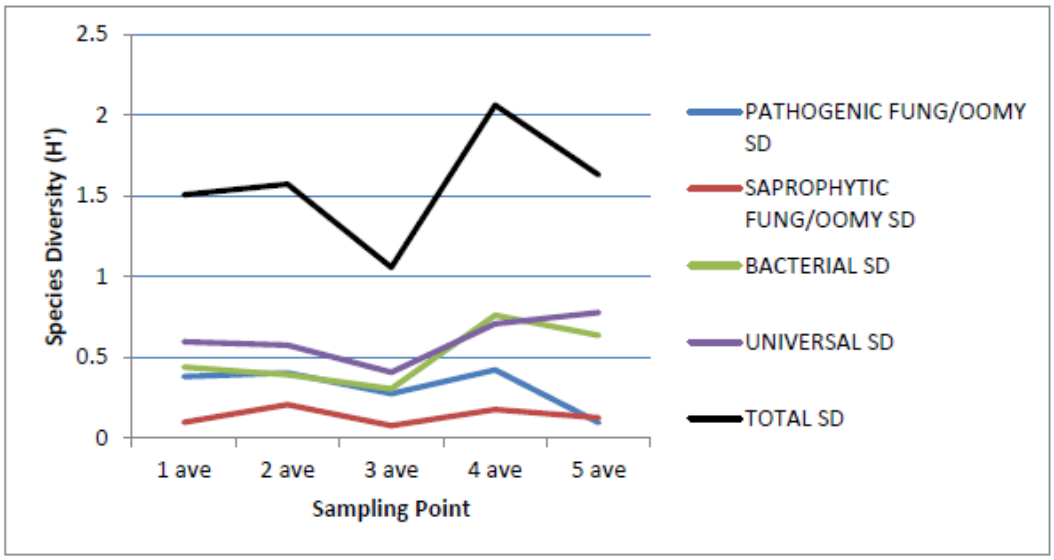


Figure 8. Seasonal average species diversity in water across 5 sampling points for Site 2, pSSF – 2015

Figures 7 and 8 show that despite slow sand filter treatments reducing diversity, this reduction is not quite as extreme as Sites 4 and 5, where a potentially harsher disinfection treatment is being used. Site 2 is the only site where diversity of all groups, including pathogenic fungi/Oomycetes is lowest at the slab.

Notably at Site 3, where no treatment was employed, total species diversity was lowest at Point 2, where source water was diluted with the mains (Figure 9).

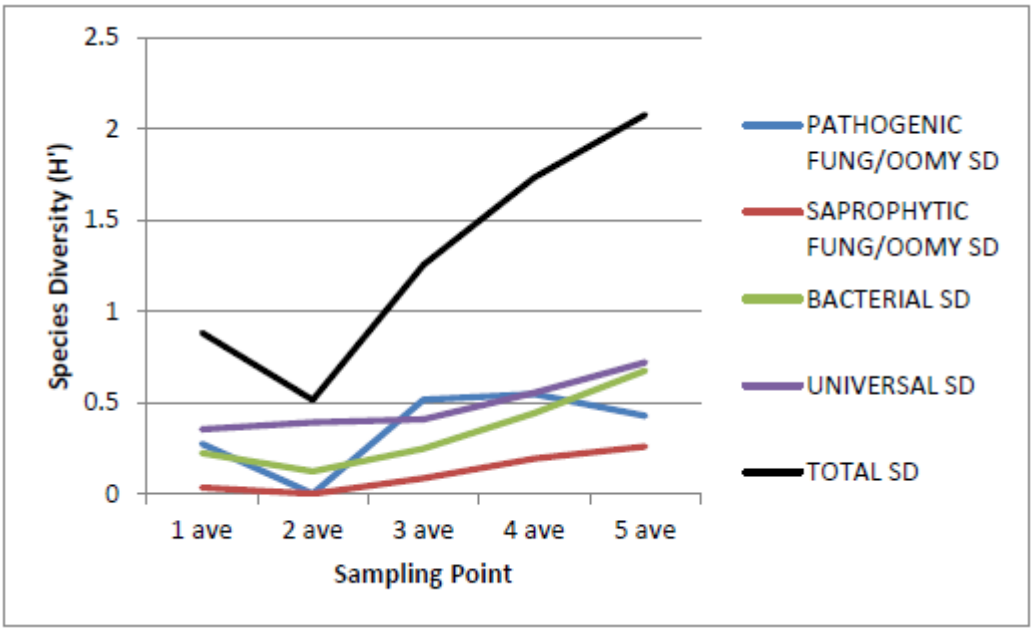


Figure 9. Season average species diversity in water across 5 sampling points for Site 3, NFT (note that here point 4 is from around grafted roots; 5 is from around ungrafted roots) - 2015

Figure 9 also illustrates that though total species diversity is higher on ungrafted roots (sampling point 5), diversity of pathogenic species is actually slightly higher on grafted roots (sampling point 4), though this difference is negligible. Water sampled from the rootzone of plants (sample points 4 and 5) has the highest diversity of all groups. Bacterial and saprophytic diversity appear more constant across sample points than pathogenic diversity.

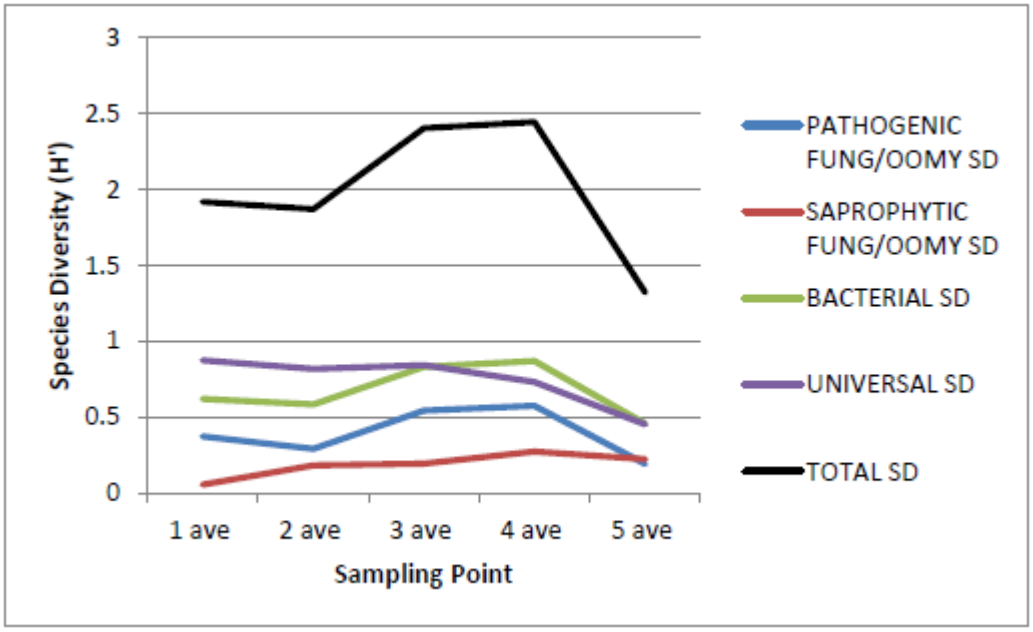


Figure 10. Seasonal average species diversity in water across 5 sampling points for Site 4, UV – 2015

For the UV treatment system (Site 4), species diversity is largely constant across sampling points (Fig 10), though it does drop at Point 5, post UV treatment, for all groups.

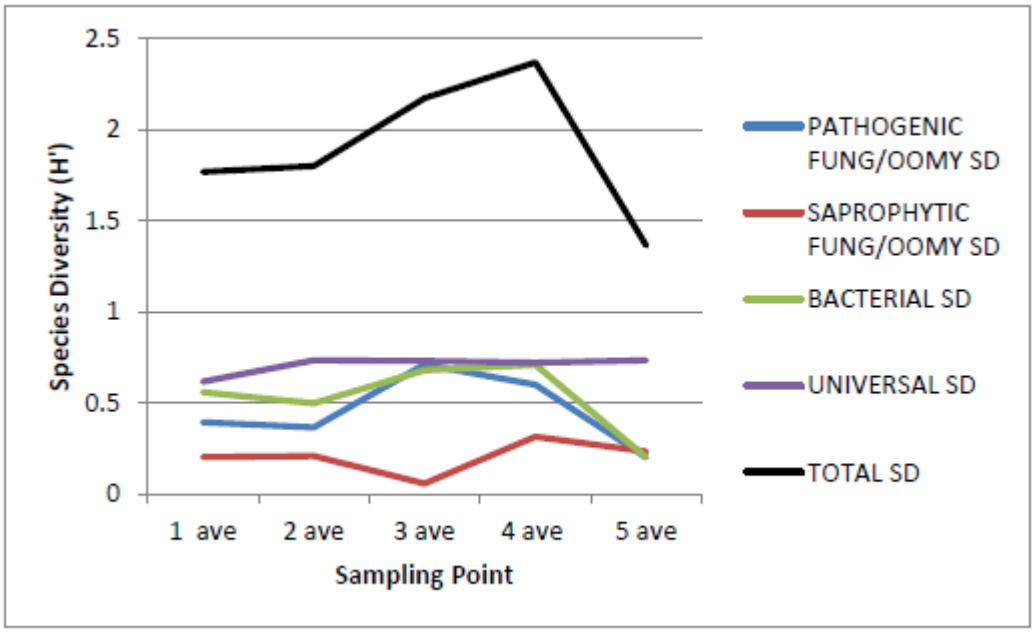


Figure 11. Average species diversity across sampling points for Site 5, Heat – 2015

The heat treatment system (Site 5) was only working sporadically during the year, so results need to be treated with caution. This site had decreased diversity of saprophytes at Point 3, the slab (Fig 11), but this did not apply to any of the other groups as at Site 2 (Fig 7).

Overall, in terms of the efficacy of the water treatments, using average species diversities, the scores tended to show a reduction in species diversity following treatment. Upon deeper investigation (analysing the data by t-test, with a significance level of $P<0.05$), both pSSF and fSSF showed significant reductions. The UV treatment was not significant by this test ($p = 0.0753$) and, although the heat treatment results appeared to decrease diversity, there were not enough post treatment samples available to test significance.

Crop age and species richness

Whereas species richness in the irrigation solution generally increased over the season (Figures 2-6 and Table 27-28), it decreased in the rhizosphere (Tables 29-30). Additionally, the proportion of the microorganisms in the rhizosphere made up of commonly pathogenic organisms decreased as the crops aged (Tables 31-35). There is a drop in species richness of pathogens in the rhizosphere for all sites except Site 4 (UV) where there is a slight increase in richness at the end of the season. This decrease is also less pronounced for Site 1 (fSSF). In terms of species richness in irrigation water, Sites 3, 4 and 5 all showed a trend for increasing richness over the season. For Sites 1 and 2, species richness in the irrigation water

did not show an increase, and for both sites the highest levels were in January. Site 1 species richness is relatively constant throughout the season, and at Site 2 there is a slight trend for decreasing species richness. Sites 1 and 2 both use slow sand filters, and it is possible that some microbes persist in the system from the previous year due to the presence of the SSF.

In terms of the number of records of common pathogens in each system, these also appeared to decrease with crop age. This was apparent for all sites except Site 4, UV, where levels did not drop in October but remained more constant over the season (Table 35). These trends were also present for saprophytes and bacteria, with species richness decreasing over the season for Sites 1, 2 and 3 (ungrafted) and 5. At Sites 3 (grafted) and Site 4 (UV) there was also a decrease from January to October, but levels increased again slightly in October samples. This may be due to crops getting older and perhaps weaker, providing more of an opportunity for some saprophytes to colonise.

For saprophytes and bacteria present in water (Table 28), ,there was an increase in species richness over the season for three of the monitored sites, up until July. Sites 3 (grafted), Site 4 (UV) and Site 5 (heat) showed a continuedincrease until October, and species richness for Site 3 (ungrafted) increased until July, after which there was a decrease. For Sites 1 and 2 there was no clear trend.

Table 27. Number of pathogens detected in irrigation water by the microarray across 5 sites and 4 sampling occasions – 2015

Date	No. species detected (pathogens) in water			
Site	Jan	Apr	Jul	Oct
1	8	5	7	7
2	12	0	8	9
3a (grafted)	0	7	7	11
3b (ungrafted)	0	9	7	10
4	3	6	8	12
5	6	9	6	9

Table 28. Number of saprophytes and bacteria detected in irrigation water by the microarray across 5 sites and 4 sampling occasions – 2015

Date	No. species detected (saprophytes and bacteria)			
Site	Jan	Apr	Jul	Oct
1	10	11	10	8
2	9	4	11	5
3a (grafted)	2	3	7	7
3b (ungrafted)	0	10	10	5
4	9	9	13	15
5	7	12	8	12

Table 29. Number of pathogens detected on roots by the microarray across 5 sites and 4 sampling occasions – 2015

Date	No. species detected (pathogens)			
Site	Jan	Apr	Jul	Oct
1	8	8	8	5
2	13	8	4	0
3a (grafted)	13	1	0	1
3b (ungrafted)	10	6	0	0
4	7	9	9	10
5	6	8	8	2

Table 30. Number of saprophytes and bacteria detected on roots by the microarray across 5 sites and 4 sampling occasions – 2015

Date	No. species detected (saprophytes and bacteria)			
Site	Jan	Apr	Jul	Oct
1	10	9	7	5
2	18	7	2	0
3a (grafted)	10	3	4	6
3b (ungrafted)	10	9	5	0

4	10	9	4	8
5	8	6	6	6

Table 31. Effect of crop age on rhizosphere microorganisms, based on prevalence of 5 most common pathogens (0-9 index) – Site 1, fSSF

Top 5 pathogens	Jan	Apr	Jul	Oct
1. *P. cucumerina*	8	9	4	0
2. *V. nigrescens*	5	7	3	0
3. *S. subterranea*	6	0	3	3
4. *F. oxysporum*	1	3	2	0
5. *P. irregulare*	6	3	4	1
6. *P. paroencandrum*	3	1	1	0

Table 32. Effect of crop age on rhizosphere microorganisms, based on prevalence of 5 most common pathogens (0-9 index) – Site 2, pSSF

Top 5 pathogens	Jan	Apr	Jul	Oct
1. *P. cucumerina*	8	8	1	0
2. *V. nigrescens*	5	7	1	0
3. *F. oxysporum*	9	2	0	0
4. *F. redolens*	7	2	0	0
5. *P. paroencandrum*	4	3	0	0

Table 33. Effect of crop age on rhizosphere microorganisms, based on prevalence of 6 most common pathogens (0-9 index) – Site 3, NFT with grafted plants

Top 6 pathogens	Jan	Apr	Jul*	Oct
1. *P. diclinum*	5	0	0	0
2. *P. irregulare*	6	0	0	0

3. *P. myriotylum*	4	0	0	0
4. *P. paroencandrum*	5	0	0	0
5. *S. subterranean*	6	0	0	0
6. V. *nigrescens*	3	0	0	1

*Index is 0-3 for this sample

Table 34. Effect of crop age on rhizosphere microorganisms, based on prevalence of 6 most common pathogens (0-9 index) – Site 3, NFT with ungrafted plants

Top 6 pathogens	Jan	Apr	Jul*	Oct
1. *P. diclinum*	2	4	0	0
2. *P. irregulare*	2	0	0	0
3. *P. myriotylum*	2	4	0	0
4. *P. cucumerina*	1	2	0	0
5. *S. subterranean*	2	3	0	0
6. *V. nigrescens*	0	2	0	0

*Index is 0-3 for this sample

Table 35. Effect of crop age on rhizosphere microorganisms, based on prevalence of 6 most common pathogens (0-9 index) – Site 4, UV

Top 6 pathogens	Jan	Apr	Jul	Oct
1. *P. cucumerina*	2	9	3	4
2. *V. nigrescens*	2	5	1	4
3. *S. subterranea*	2	2	4	4
4. *P. irregulare*	2	2	2	5
5. *C. coccodes*	0	5	0	2
6. *F. oxysporum*	0	4	1	3

Table 36. Effect of crop age on rhizosphere microorganisms, based on prevalence of 5 most common pathogens (0-9 index) – Site 5, Heat

MO	Jan	Apr	Jul	Oct
1. *P. cucumerina*	2	7	6	0

2. *F. oxysporum*	6	2	3	0
3. *V. nigrescens*	2	4	5	0
4. *S. subterranea*	1	3	4	3
5. *P. paroencandrum*	0	3	4	0

Crop health

At each sampling visit, measures of crop health such as leaf yellowing, wilting and root browning were assessed. The degree of root browning present over the season is summarised below (Table 37), and presence of vascular staining (Table 38).

Table 37. Root browning present across 5 sites assessed on 4 occasions across the growing season, on a 1-5 index (1 = healthy and white, 5 = very brown and rotten)

Site	Root browning (0-5)			
	Jan	Apr	Jul	Oct
1. fSSF	1.4	2.0	2.6	2.3
2. pSSF	1.6	1.9	2.2	4.4
3a. NFT - RS	1.2	1.0	1.0	1.1
3b. NFT – own	1.2	1.0	1.3	1.3
4. UV	1.4	1.7	2.3	4.1
5. Heat	1.4	1.9	2.4	1.7

Table 38. Incidence and severity of vascular staining across 5 sites at the end of the growing season – October 2015

Site	Vascular stain (% plants)	Mean vascular staining index (0-5)
1. fSSF	0	0
2.pSSF	23.3	0.4
3a. NFT - RS	0	0
3b. NFT – own	13.3	0.2

4. UV	36.7	0.5
5. Heat	6.7	0.1

As in previous work (PC 281a), no leaf yellowing or wilting and little visible root disease was observed over the course of monitoring, despite known pathogens being detected in irrigation water and in the rhizosphere. As might be expected, root health had visibly declined by the final visit in October 2015, as crops reached the end of their season and were soon to be pulled out. Root browning was notably worse by the end of the crop at Sites 2, (pSSF) and 4 (UV) (Table 37). *Pythium irregulare*, commonly associated with root browning, was detected at Site 4 most often across the season, whereas the greatest levels of *P. myriotylum* were detected at Site 2. However, the other sites also had pathogens capable of causing such symptoms detected over the season, and no associated symptoms were expressed. A method with greater capability to quantify the microorganisms in roots would be informative.

Given the low incidence of clear root disease symptoms, it is difficult to relate these assessments to the results of the microarray. Over the whole season, Site 2 (pSSF) and Site 4 (UV) had the greatest pathogen species richness in the rhizosphere, and it was these sites that exhibited the greatest amount and degree of vascular staining by the end of the season, and the most visible root browning. Additionally, Site 2 had the lowest total species diversity at the slab when averaged over the season, and when calculated for the October sampling. Low diversity in a system is often associated with a system more vulnerable to pathogen attack, and this could be one explanation for the symptoms observed at Site 2, even though pathogens that could be responsible for the symptoms observed were also detected at other sites. A moderate amount of vascular staining was also present at Site 4, which could be due to a number of reasons. Species richness was not decreasing as much as previously when pre- and post-treatment samples were taken in October, which could indicate lowering efficacy of the UV disinfection treatment over the season, or for another reason.

Objective 3 – Pathogenicity of root pathogens on own-root tomato and grafted plants

Experiment 1 – Preliminary inoculation study (ADAS Boxworth)

Results are summarised in Tables 39-41. Surprisingly, inoculation with a *Pythium* sp., and to a lesser extent *C. coccodes*, resulted in plants with increased root extent compared with the

untreated control. These treatments also had a lower incidence of yellowing plants at 3 weeks after inoculation. However, whereas *Pythium* sp. inoculation resulted in plants showing an overall reduction in root discolouration, *C. coccodes* inoculated plants showed greater root discolouration. *F. oxysporum* was the only treatment that increased severe leaf yellowing.

There was a trend for increased rotted roots at 4 weeks after inoculation with *C. coccodes* (51.3%), *F. oxysporum* (51.3%) and *P. cucumerina* (46.2%), compared with the untreated (36.2%). The proportion of white roots remaining was significantly reduced by *C. coccodes*. None of the treatments affected plant vigour, recorded at 3 weeks after inoculation.

At 1 week after inoculation, there was a trend for increased root discolouration using the higher inoculum concentration of *C. coccodes* (one isolate), *F. oxysporum* (both isolates), *P. cucumerina* (both isolates) and *Pythium* sp. (one isolate) (Table 39). Differences were largely maintained at assessments 2 and 3, but were not apparent at the final assessment, when roots inside the propagation cube were also examined. There was little evidence of any other consistent effect on symptoms from the higher inocula, compared with the lower. Isolates 1 of *P. cucumerina* caused more root discolouration and rotting than isolate 2. There were no other consistent differences between isolates in the severity of symptoms on plants.

Isolation from the roots of three plants in each inoculation treatment at 4 weeks after inoculation indicated occurrence of non-target pathogens in several treatments. Three treatments (*C. coccodes*, higher; *F. oxysporum*, low; and *P. cucumerina*, high) had the intended pathogen only. The other treatments were all found to contain *Fusarium* sp., including the uninoculated control. Colony appearance suggested a mixture of *Fusarium* species.

Table 39. Summary of mean effect of root inoculation treatments at intervals (weeks) after inoculation – March 2015

Treatment	Root extent (0-5)					Root colour (0-5)			
	Wk 1	Wk 2	Wk 3	Wk 4		Wk 1	Wk 2	Wk 3	Wk 4
1. Untreated	0.8a	1.3a	1.5ab	2.2a		1.3ab	1.5	2.4b	3.0b
2. Pythium	**2.1c**	**2.8b**	**3.1c**	**3.7b**		1.2a	1.9	**1.8a**	**2.4a**
3. Fusarium	1.0a	1.4a	1.2a	2.4a		1.7bc	1.9	2.9b	3.4b
4. Plectosphaerella	1.3ab	1.4a	0.9a	2.1a		**1.8c**	1.7	2.6b	3.1b
5. Colletotrichum	**1.9bc**	**2.3b**	2.2b	2.8a		**1.9c**	2.1	2.8b	3.5b
Significance	<0.001	<0.001	<0.001	<0.001		0.002	0.154	0.002	0.003
LSD	0.59	0.65	0.74	0.70		0.42	-	0.57	0.58

Bold – significantly different from untreated

Table 40. Summary of mean effect of root inoculation treatments at intervals after inoculation – March 2015

Treatment	Incidence of yellowing			Severity of yellowing			% roots rotten		% roots white		Plant vigour (0-5)
	Wk 3	Wk 4		Wk 3	Wk 4		Wk4		Wk 4		Wk 3
1. Untreated	0.8c	1.0c		0.7a	9.5c		36.2ab		63.8bcd		4.8
2. Pythium	**0a**	**0.1a**		0a	**0.1a**		23.6a		76.4d		5.0
3. Fusarium	0.7bc	0.9bc		**1.5b**	**14.0d**		51.3bc		48.7ab		4.7
4. Plectosphaerella	0.7bc	0.9bc		0.4a	6.7bc		46.2bc		53.8abc		4.6
5. Colletotrichum	**0.5b**	**0.8b**		0.3a	2.8ab		51.3bc		**48.7a**		4.9
Significance	<0.001	<0.001		<0.001	<0.001		<0.001		<0.001		0.176
LSD	0.26	0.19		0.61	4.14		14.24		14.24		-

Bold – significantly different from untreated.

Table 41. Recovery of fungi and oomycetes from roots of plants at 4 weeks after inoculation

Inoculation treatment	Microorganisms recovered			
	Pythium	Fusarium	Plectosphaerella	Colletotrichum
1. Uninoculated	-	✓	-	-
2. Pythium (L)	-	-	-	-
3. Pythium (H)	-	✓	-	-
4. Fusarium (L)	-	✓	-	-
5. Fusarium (H)	✓	✓	-	-
6. Plectosphaerella (L)	-	✓	-	-
7. Plectosphaerella (H)	-	-	✓	-
8. Colletotrichum (L)	-	✓	-	-
9. Colletotrichum (H)	-	-	-	✓

Experiment 2 – Pythium spp. inoculation study (ADAS Boxworth)

In the full scale *Pythium* inoculated trial, no yellowing or wilting was observed throughout the course of the trial. By the end of the trial, after plants had been grown for 13 weeks following inoculation, some root browning was observed. No vascular staining was apparent at the conclusion of the trial in any of the plants and all treatments retained good plant vigour.

Inoculation had no significant effect on root extent or % brown roots (Table 42) or % rotted roots (Table 43). Own root Elegance and Maxifort roots did not differ in root extent or % brown roots. Maxifort plants inoculated with the medium concentration of *Pythium* had roots with a significantly darker root colour score than own root Elegance inoculated with the medium concentration of *Pythium* (Table 42). It is a possibility that inoculation and subsequent root loss encouraged growth of new, white root in inoculated plants, whereas an uninoculated, vigourous rootstock was happy to grow as normal.

Table 42. Effect of rootstock and *Pythium* inoculum level on tomato roots (destructive assessment) – ADAS Boxworth, 2015

Trt	Rootstock	Inoculum	Extent (0-5)			% brown roots		Root colour (0-5)
			Internal	External		Internal	External	
1.	Elegance	Nil	2.9	2.9		83.8	2.5	3.1
2.	Elegance	L	3.0	3.8		64.9	2.3	3.5
3.	Elegance	M	3.0	3.0		81.4	2.3	3.3
4.	Elegance	H	2.6	4.0		65.6	2.5	3.8
5.	Maxifort	Nil	3.3	4.3		67.9	2.5	4.0
6.	Maxifort	L	3.1	4.0		70.0	2.3	3.3
7.	Maxifort	M	2.9	4.0		74.1	2.5	3.9
8.	Maxifort	H	3.6	3.3		70.0	2.6	3.6
Significance			0.347	0.112		0.621	0.954	<0.001
LSD			0.931	1.300		26.98	0.755	0.406

Table 43. Effect of rootstock and *Pythium* inoculum level on tomato roots after 13 weeks – 2015

Treatment	Rootstock	Inoculum	Roots white (% of cube base)	Roots rotten (% of cube base)
1.	Elegance	0	77.4	2.5
2.	Elegance	L	85.4	2.3
3.	Elegance	M	70.5	2.3
4.	Elegance	H	72.2	2.5
5.	Maxifort	0	76.1	2.5
6.	Maxifort	L	73.1	2.3
7.	Maxifort	M	81.9	2.5
8.	Maxifort	H	64.0	2.6
Significance			0.471	0.954
LSD			23.07	0.755

The isolations carried out on a sample of roots from each treatment at the end of the trial showed that the tomato plants had a number of pathogenic fungi present on their roots (Table 44), including some that had not been inoculated. Presumably these potential pathogens had come in with the plants or been picked up from surroundings. The lack of detection of *Pythium* in treatments 2, 4, 7 and 8 is surprising given that these plants were inoculated with *Pythium*. However, the results are consistent with the lack of root rot symptoms. These potential pathogens were identified by colony morphology on the plate and under a light microscope.

Table 44. Recovery of fungi and oomycetes from roots of plants at 4 weeks after inoculation with *Pythium* spp.

Trt	Rootstock	Inoculum	Microorganisms recovered				
			Pyt	*Fo*	*Pc*	*Cc*	*Phyt*
1.	Elegance	0		✓	✓		
2.	Elegance	L		✓			
3.	Elegance	M	✓	✓	✓		
4.	Elegance	H		✓	✓		✓
5.	Maxifort	0		✓	✓	✓	✓
6.	Maxifort	L	✓			✓	
7.	Maxifort	M		✓	✓	✓	
8.	Maxifort	H		✓	✓		

Experiment 3 – Plectosphaerella cucumerina inoculation study (ADAS Boxworth)

Over the course of the trial, no plants exhibited extreme symptoms of root disease, and no plant death occurred. At the destructive assessment, no significant differences ($p > 0.05$) between treatments were apparent (Tables 45-47). There was an observable trend for plants grown on Maxifort rootstock to develop roots with a greater extent outside the cube.

Table 45. Effect of rootstock and *P. cucumerina* inoculum level on tomato roots (destructive assessment) – ADAS Boxworth, 2015

Trt	Rootstock	Inoculum	Extent (0-5)		% brown roots		Colour (0-5)	
			Internal	External	Internal	External	Internal	External
1.	Elegance	0	2.8	3.8	34.4	68.0	3.6	2.6
2.	Elegance	L	2.6	4.1	21.2	57.5	3.6	2.9
3.	Elegance	M	2.8	3.6	30.2	68.8	3.3	2.9
4.	Elegance	H	2.9	3.6	20.4	59.6	3.6	2.6
5.	Maxifort	0	3.1	4.9	24.1	43.4	3.9	3.6
6.	Maxifort	L	2.0	4.3	38.1	68.8	3.6	2.8
7.	Maxifort	M	2.9	4.3	23.1	60.0	3.6	3.0
8.	Maxifort	H	2.4	5.0	38.1	46.9	3.8	3.3
Significance			0.426	0.374	0.370	0.348	0.917	0.444
LSD			1.020	1.103	29.84	29.22	0.818	1.087

Table 46. Effect of rootstock and *P. cucumerina* inoculum level on tomato roots after 13 weeks – 2015

Treatment	Rootstock	Inoculum	Vigour (0-5)
1.	Elegance	Nil	4.9
2.	Elegance	L	4.4
3.	Elegance	M	5.0
4.	Elegance	H	4.6
5.	Maxifort	Nil	4.6
6.	Maxifort	L	4.3
7.	Maxifort	M	4.8
8.	Maxifort	H	4.9
Significance			0.504
LSD			0.548

Some yellowing and vascular staining were observed in the *P. cucumerina* inoculated trial. Yellowing was observed in plants in one plot, where plants had received a low concentration of inoculum and where plants were grown on Maxifort. It is unclear if the yellowing here was due to inoculation. Vascular staining, indicative of root disease, was observed in two plots that both contained ungrafted plants, one inoculated with medium concentration inoculum, and the other with high. Though this confirms what might be expected, vascular staining was at such a low incidence that it is difficult to draw any conclusions.

The isolations carried out on roots from each treatment at the end of the trial showed that the tomato seedlings had a number of pathogenic fungi present on their roots (Table 47), including those that had not been inoculated. Presumably these potential pathogens had come in with the plants or been picked up from surroundings. *Plectosphaerella* was present in all treatments, including the uninoculated controls. These potential pathogens were identified by colony morphology on the plate and under a light microscope.

Table 47. Recovery of fungi and oomycetes from roots of plants at 13 weeks after inoculation with *Plectrosphaerella* spp. – 2015

Trt			Microorganisms recovered				
	Rootstock	Inoculum	*Pythium*	*Fusarium*	*Plectosphaerella*	*Colletotrichum*	*Phytophthora*
1.	Elegance	Nil		✓	✓	✓	
2.	Elegance	L			✓		
3.	Elegance	M			✓		✓
4.	Elegance	H		✓	✓		
5.	Maxifort	Nil	✓	✓	✓		✓
6.	Maxifort	L		✓	✓	✓	
7.	Maxifort	M	✓	✓	✓		
8.	Maxifort	H		✓	✓	✓	

Experiment 4 – Colletrotrichum coccodes inoculation study (University of Nottingham)

Visible symptoms of disease were low in the trial, and no plant death was observed. For the majority of assessments, there were no significant differences between rootstocks or between plants inoculated with different concentrations of spore suspension (Tables 48 and 49). However, at the destructive assessment (8 weeks after inoculation) plants that had been inoculated with the highest concentration of inoculum had significantly fewer brown roots at the base than the plants that had received lower concentrations of inoculum (Table 50). This is opposite to what might be expected, but it is possible that increased root growth after root death reduced the overall appearance of brown, rotten roots at the base of these plants.

Table 48. Effect of rootstock and *C. coccodes* inoculation on root health at 8 weeks after inoculation – University of Nottingham, 2015

Rootstock	Inoculum	Root extent outside cube	% brown at cube base	Root extent within cube	Root colour in cube	% brown within cube
Elegance	Nil	2.7	8.3	3.7	1.0	6.7
Elegance	Low	3.7	5.0	3.3	1.3	1.7
Elegance	Medium	3.7	5.7	3.3	1.7	1.7
Elegance	High	4.0	3.3	3.7	2.0	1.7
Maxifort	Nil	3.3	5.0	3.3	1.0	3.3
Maxifort	Low	3.0	5.0	3.7	1.0	0
Maxifort	Medium	3.3	5.0	3.3	1.7	1.7
Maxifort	High	3.7	1.7	4.0	1.0	1.7
p		0.258	0.489	0.71	0.349	0.692
LSD		1.011	3.372	1.002	0.926	4.871

Table 49. Effect of rootstock on development and expression of root disease at 8 weeks after inoculation with *C. coccodes* – University of Nottingham, 2015

	Root extent outside cube	% brown at cube base	Root extent within cube	Root colour in cube	% brown within cube
Elegance	3.5	5.6	3.5	1.5	2.9
Maxifort	3.3	4.2	3.6	1.2	1.7
p value	0.491	0.093	0.727	0.145	0.29
LSD	0.506	1.686	0.501	0.463	2.436

Table 50. Effect of *C. coccodes* inoculum concentration on development and expression of root disease by the final destructive assessment – University of Nottingham, 2015

	Root extent outside cube	% brown at cube base	Root extent within cube	Root colour in cube	% brown within cube
Nil	3.0	6.7	3.5	1.0	5.0
L	3.3	5.0	3.5	1.2	0.8
M	3.5	5.3	3.3	1.7	1.7
H	3.8	**2.5**	3.8	1.5	1.7
p value	0.138	0.016	0.511	0.162	0.09
LSD	0.715	2.385	0.709	0.655	3.445

Values in bold are significantly different to the uninoculated

No vascular staining of the stem was observed at the conclusion of this trial.

Experiment 5 – Fusarium oxysporum inoculation study University of Nottingham

Again, no severe symptoms of root disease were observed in this trial, and no plant death occurred (8 weeks after inoculation). Significantly higher percentages of brown roots at the cube base were observed in both uninoculated Maxifort rootstock and in Arnold inoculated with the lowest inoculum concentration (Table 51). Similarly to the *C. coccodes* trial above, inoculation with the higher concentrations of spore suspension also resulted in significantly lower % brown roots at the cube base (Table 52). As above, one possible explanation for this may be an increased rate of die-off and root replacement due to infection.

Table 51. Effect of rootstock and *F. oxysporum* inoculation on root health at the final destructive assessment – University of Nottingham, 2015

Rootstock	Inoculum	Root extent outside cube	% brown at cube base	Root extent within cube	Root colour in cube	% brown within cube
Elegance	Nil	2.3	0	1.7	1.3	3.3
Elegance	Low	3.7	0	2.3	1.0	1.0
Elegance	Medium	3.3	0	3.0	1.0	0.3
Elegance	High	3.0	0	3.3	1.0	0
Maxifort	Nil	3.7	**11.7**	3.0	**1.7**	1.7
Maxifort	Low	3.3	1.67	2.7	1.0	0
Maxifort	Medium	3.7	0	3.3	**1.7**	0
Maxifort	High	3.7	1.67	3.0	1.0	0
Arnold	Nil	2.3	0	3.0	0.3	1.7
Arnold	Low	3.3	**6.7**	2.0	1.0	0
Arnold	Medium	3.3	1.7	2.7	1.0	3.3
Arnold	High	3.7	0	3.3	**1.7**	3.3
p		0.397	0.001	0.322	0.003	0.754
LSD		1.176	4.757	1.248	0.6195	5.311

Values in bold are significantly different

Table 52. Effect of *F. oxysporum* inoculum concentration on development and expression of root disease by the final destructive assessment – University of Nottingham, 2015

Trt	Root extent outside cube	% brown at cube base	Root extent within cube		% brown within cube
Nil	2.8	3.9	2.6	1.1	2.2
L	3.4	2.8	2.3	1.0	0.3
M	3.4	**0.6**	3.0	1.2	1.2
H	3.4	**0.6**	2.8	1.2	1.1
p value	0.133	0.045	0.068	0.528	0.654
LSD	0.679	2.746	0.72	0.3577	3.066

Values in bold are significantly different.

Few significant differences were observed between own root plants and those on either rootstock. However, Maxifort roots were observed to have significantly greater percentages of brown roots at the cube base compared to own root plants. The difference in % brown roots between Maxifort and Arnold was not statistically significant (Table 53).

Table 53. Effect of rootstock on development and expression of root disease by the final destructive assessment, following inoculation with *F. oxysporum* – University of Nottingham, 2015

Trt	Root extent outside cube	% brown at cube base	Root extent within cube		% brown within cube
Elegance	3.1	0	2.6	1.1	1.2
Maxifort	3.4	**3.8**	3.0	1.3	0.4
Arnold	3.1	2.1	2.8	1.0	2.1
p value	0.191	0.013	0.394	0.09	0.441
LSD	0.588	2.378	0.624	0.3097	2.656

Values in bold are significantly different.

A low level of vascular staining was observed in this trial, in the stem of 3 plants. These plants were all inoculated and were all grown on their own roots.

Comparison of grafted and ungrafted plants grown on a commercial site

At Site 3, the NFT site sampled as part of Objective 2, both grafted and ungrafted plants were planted in the same glasshouse. Grafted plants were of scion variety Piccolo on the rootstock Maxifort, and Piccolo grown on its own roots were both present. These were assessed and sampled on four occasions in the season for comparison. Differences in visible symptoms of root disease are detailed in Table 54, below. A greater amount of straining in the vascular tissue was observed in Piccolo on own roots when compared to Piccolo on Emperador. A greater number of stem bases were dead or missing that were grown on Emperador. However, it is largely impossible to determine how these died or why they were removed for certain, and in the majority of cases this was due to *Botrytis* infection and not root disease. However, previous observations have indicated that Botrytis can be more common and more severe on grafted plants, given more vegetative growth and greater abundance of soft, young tissue.

Table 54. A comparison of visible root health of grafted and ungrafted Piccolo plants grown in the same glasshouse on the same NFT system – October, 2015

Variable	Piccolo on own roots	Piccolo on Emperador rootstock
Leaf yellowing (%)	0	0
Stem base stain (%)	13.3	0
Root browning (0-5)	1.3	1.1
Stem base dead or missing (%)	1.3	10.6

Table 55 details the most common pathogenic organisms reported by the microarray for the rhizosphere of both own root Piccolo and that grafted onto Emperador. Both sets of roots had more common pathogenic organisms present in January and February than in July or October. A greater abundance of Pythia were detected on Emperador roots in January than on roots of ungrafted plants, whereas ungrafted plants had a greater abundance of other pathogens such as *T. basicola* and *V. nigrescens*. This trend was conserved into April, and in July and October very few pathogens were detected on samples with either root.

Table 55. A comparison of the most common pathogenic microorganisms detected by the microarray on grafted and ungrafted tomato roots in NFT crops (0-9 index) of cv. Piccolo – 2015

	Own root				Emperador			
	Jan	Apr	Jul	Oct	Jan	Apr	Jul	Oct
P. diclinum	2	0	0	0	5	4	0	0
P. irregulare	2	0	0	0	6	0	0	0
P. myriotylum	2	0	0	0	4	4	0	0
S. subterranea	2	0	0	0	6	3	0	0
P. cucumerina	2	2	0	0	1	0	0	0
V. nigrescens	3	2	0	0	0	0	0	1
C. coccodes	0	1	0	0	0	0	0	0
T. basicola	3	0	0	0	0	1	0	0

Table 56 details differences in saprophytic organisms between own-root and Emperador roots. The trends here were less pronounced than for pathogenic species, but apart from *O. brassicae*, which was more common in Emperador roots than on own-root plants, the microorganisms present did not overlap. Tables 55 and 56 are suggestive of a different environment on grafted and ungrafted roots, likely dependent on a large number of factors.

Table 56. A comparison of common saprophytic microorganisms detected by the microarray on grafted and ungrafted tomato roots in NFT crops (0-9 index) – 2015

		Own root				Emperador			
		Jan	Apr	Jul	Oct	Jan	Apr	Jul	Oct
O. brassicae		2	1	0	0	7	0	0	0
P. lilacinus		1	0	0	0	0	0	0	0
P. brevicompactum		1	0	0	0	0	0	0	0
P. griseofulvum		0	1	0	0	0	0	0	0
P. variabile		0	1	0	0	0	0	0	0
P. asymmetrica		0	0	0	0	1	0	0	0
D. microsporus		0	0	0	0	1	0	0	0
Trichoderma sp.		0	0	0	0	0	0	0	0
Bacillus sp.		1	0	0	0	0	0	0	0

Irrigation water was also sampled from the rootzone of grafted (Emperador) and ungrafted (own-root Piccolo) plants. In comparison to root samples, the samples of irrigation water taken in January had the lowest level of common pathogens across the year for both grafted and ungrafted plants. This was probably due to the fact that recirculation had not yet commenced and mains water was being used. In April, once the system was recirculating, detection of pathogens and saprophytes in irrigation water increases (Tables 57 and 58). In general, a greater abundance of common pathogens was detected in the water taken from around ungrafted, own root plants in comparison to water taken from around the roots of the rootstock Emperador until the final sample in October. Lower levels of Oomycete species (*Pythium* and *Phytophthora* species) were detected in water taken from around the rootstock than from around own-root plants. This is in contrast to the microarray results for root samples, where more *Pythia* were detected in Emperador root samples than in own-root samples. The levels of fungal pathogens in April also follow this trend. In July there appears to be little difference in pathogens present between the two root types, but in October there appears to have been an increase in the pathogen load of irrigation water around Emperador roots, whereas levels

around own-root plants have remained relatively constant. By the end of the season, plants are potentially tiring, and this may be more noticeable for thee Emperador roots as the grafted area of the crop was noticeably more vigorous all year.

Across the year and for both rootstocks low levels of saprophytic organisms and bacteria were reported, and so it is difficult to determine any trends in this data.

Table 57. A comparison of the most common pathogenic microorganisms detected by the microarray in irrigation water sampled from the rootzone of grafted and ungrafted cv. Piccolo plants (0-6 index) – 2015

	Own root				Emperador			
	Jan	Apr	Jul	Oct	Jan	Apr	Jul	Oct
F. oxysporum	0	0	0	0	0	1	0	0
P.arecae	0	1	0	0	0	0	0	0
P. nicotianae	0	1	0	0	0	0	0	0
P. diclinum	0	5	2	0	0	0	0	1
P. irregulare	0	5	1	2	0	1	0	3
P. myriotylum	0	5	2	0	0	1	2	3
S. subterranea	0	4	0	1	0	0	0	2
P. cucumerina	0	4	0	1	0	2	0	4
V. nigrescens	0	5	0	1	0	1	0	4
C. coccodes	0	1	0	0	0	0	0	0
T. basicola	0	0	0	0	0	0	0	0

Table 58. A comparison of the most common saprophytic microorganisms detected by the microarray in irrigation water sampled from the rootzone of grafted and ungrafted plants (0-6 index) – 2015

	Own root				Emperador			
	Jan	Apr	Jul	Oct	Jan	Apr	Jul	Oct
O. brassicae	0	0	0	0	0	0	0	0
P. lilacinus	0	0	0	0	0	0	0	0
P. brevicompactum	0	1	0	0	0	0	0	0
P. griseofulvum	0	0	0	0	0	0	0	0
P. variabile	0	0	2	0	0	0	0	0
P. asymmetrica	0	2	0	0	0	0	0	0
D. microsporus	0	0	0	0	0	0	0	0
Trichoderma sp.	0	0	0	0	0	0	0	0
Bacillus sp.	0	0	0	0	0	0	0	0

Objective 4 – Monitor additional crops grown with re-cycled irrigation for root pathogens and root disease

The additional five crops sampled for roots included those grown with NFT, and both rockwool and coir substrates. The disinfection treatments included nil (NFT) and pasteurisation. Results are shown for the full set of 10 crops (5 from Objective 2 and 5 from Objective 4) for ease of comparison.

A low level of root disease was observed in these crops in July, with some severe root browning by the end of the season at sites 2, 4 and 9 (Table 59). The main pathogen found associated with these samples was *Pythium* at all sites (Table 60). Additionally, there was a high incidence of slight vascular browning at sites 2, 4 and 8, possibly a result of the more obvious root browning at these sites compared with others. Some leaf yellowing was also observed, but it could not be attributed to root disease with certainty.

Overall, microarray results from these additional sites agreed with those sampled as part of

Objective 2 in terms of the effects of crop age and disinfection treatment. Across all sites, the abundance of pathogenic species increased from July to October (Table 60), though the overall species richness remained relatively constant between the July and October root samples (Tables 61 and 62). No comparison of sample location can be made as roots only were sampled from these sites. Similarly, direct efficacy of disinfection treatments cannot be quantified as pre- and post-treatment water samples were not taken, but comparisons of the microbes present in the rhizosphere between sites with different systems can be made.

Table 59. An assessment of visible root health at the 5 main sites, and 5 additional sites towards the end of cropping – July & October 2015

	July			October			
Site	Root browning (0-5)	Dead missing (%)	Yellowing leaves (%)	Root browning (0-5)	Vasc. stain (%) (severity 0-3)	Dead missing (%)	Yellowing leaves (%)
1 (RW)	2.6	6.7	0	2.3	0 (0.0)	2	6.7
2 (RW)	2.2	14	0	4.4	23.3 (0.4)	12.7	0
3a (NFT - grafted)*	1.1	3.3	0	1.1	0 (0.0)	10.6	0
3b (NFT-ungrafted)	1.3	0	0	1.3	13.3 (0.2)	1.3	0
4 (RW)	2.3	0	2	4.1	36.7 (0.5)	0.7	0
5 (RW)	2.4	3.3	0	1.7	6.7 (0.1)	4	0
6 (coir)	2.1	0	0	1.5	0 (0.0)	4	0
7 (RW)	1.3	0.6	0	1.3	0 (0.0)	0.7	0
8 (coir)	1.7	0.6	0	2.7	60 (0.7)	6	7.3
9 (NFT)	1.6	0	0	4.2	-	-	100*
10 (NFT)	1.6	0	0	3.3	-	0	0

* Severe foliar disease, not due to root issues; vascular tissue not exposed on either crop at sites 9 and 10 to avoid exacerbation of leaf disease problem.

Table 60. Summary of the main tomato pathogens detected at all 10 sites (0-9 index) – October 2015

Site	*Cc*		*Fo*		*Phy*		*Pc*		*Pyt*		*Tb*		*Vn*	
	Jul	Oct	Jul	Oct	Jul	Oct	Jul	Oct	Jul	Oct	Jul	Oct	Jul	Oct
1	-	-	2	-	-	-	4	-	4	5	-	-	3	-
2	1	-	-	-	-	-	1	-	1	-	-	-	1	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	1
4	-	2	1	3	-	-	3	4	2	8	-	-	1	4
5	1	-	3	-	-	-	6	-	4	2	-	-	5	-
6	-	-	-	5	-	-	3	5	3	9	-	-	2	5
7	-	-	3	2	-	-	4	6	-	5	-	-	4	5
8	-	1	-	1	-	-	-	1	2	8	-	-	-	2
9	1	-	-	-	-	-	3	-	3	1	-	-	1	-
10	1	1	-	-	6	-	4	3	3	5	1	1	2	2

Table 61. The number of common tomato pathogens detected in the rhizosphere across all 10 sites sampled in July and October - 2015

Date	Substrate	Disinfection	No. species detected (pathogens)	
Site			Jul	Oct
1.	Rockwool	fSSF	8	5
2.	Rockwool	pSSF	4	0
3a (grafted)	NFT	Nil	0	1
3b (ungrafted)	NFT	Nil	0	0
4.	Rockwool	UV	9	10
5.	Rockwool	Heat	8	2
6.	Coir	Heat	4	8
7.	Rockwool	Heat	4	6
8.	Coir	Heat	3	8
9.	NFT	Nil	5	1
10.	NFT	Nil	8	9

Table 62. The number of common saprophytes and bacteria detected across all 10 sites sampled throughout the cropping season - 2015

Date	Substrate	Disinfection	No. species detected (saprophytes & bacteria)	
Site			Jul	Oct
1.	Rockwool	fSSF	7	5
2.	Rockwool	pSSF	2	0
3a (grafted)	NFT	Nil	4	6
3b (ungrafted)	NFT	Nil	5	0
4.	Rockwool	UV	4	8
5.	Rockwool	Heat	6	6
6.	Coir	Heat	4	8
7.	Rockwool	Heat	4	5
8.	Coir	Heat	4	5
9.	NFT	Nil	8	0
10.	NFT	Nil	10	8

Tables 61 and 62 illustrate the variability present between sites, even between all sites with an NFT system (three), or all sites with a pasteuriser (four). Though Sites 9 and 10 are grown on an NFT system, as is Site 3, they both have notably greater species richness of pathogens, saprophytes and bacterial species. In comparison to site 3, where species richness was low, pathogens such as *Phytophthora* species and *T. basicola*, with the potential to cause noticeable infection, were also detected at the additional NFT sites. Sites 9 and 10 did not receive supplementary heating unlike the rest of the sites, and the microbial environment may have differed because of this harsher glasshouse environment.

One of the main sites monitored, Site 5, used a Heat pasteuriser, though this treatment was not functioning appropriately at January, April and July visits. Three of the additional sites also used heat treatment, Sites 6, 7 and 8. Species richness at these sites in July and October was similar for the other additional sites in terms of both pathogens and saprophytes, and increased from July to October samples (Table 61). However, at Site 5, pathogen species richness was similar in July but had fallen at the October sample, whereas richness of saprophytic species remained constant. In terms of the specific pathogens detected, all sites

with a pasteuriser appeared to have similar species present, though Site 8 had notably fewer reports of *P. cucumerina* and *V. nigresens* than the other pasteurised sites. This supports the data attained at Site 5, despite the issues experienced with equipment working at sampling visits.

Both site 6 and site 8 were grown on coir rather than rockwool. In terms of species richness these crops were in line with the values returned for rockwool crops. In terms of the species present, the two sites were more similar to one another than to the rockwool crops, with little to no *Fusarium oxysporum* present, and with *Pythium* species making up the majority of species present.

Samples with visible symptoms

Incidence of root mat was assessed on sampling visits, and was observed on three of the main sites monitored and two of the additional sites. However, no correlation between incidence of symptoms in the crop and detection of *Agrobacterium rhizogenes* on the microarray was observed (Table 63). *Agrobacterium rhizogenes* was detected at four sites without root mat symptoms and root mat symptoms were observed at two sites where no *Ag. rhizogenes* was detected.

Table 63. Incidence of root mat symptoms on rockwool cubes in comparison with the detection of *Agrobacterium rhizogenes* (*Rhizobium radiobacter*) in roots by the microarray - 2015

Site	Cubes with root mat symptoms				*Ag. rhizogenes* detected on microarray			
	Jan	Apr	Jul	Oct	Jan	Apr	Jul	Oct
1. fSSF	-	-	-	-	✓	✓	-	-
2.pSSF	-	-	-	✓	✓	-	-	-
3a. NFT – RS	-	-	-	-	✓	-	-	-
3b. NFT – own	-	-	-	-	✓	-	-	-
4. UV	-	✓	✓	✓	-	-	-	-
5. Heat	-	-	-	✓	-	-	✓	-
6. Heat	*	*	✓	✓	*	*	-	-
7. Heat	*	*	-	-	*	*	-	✓
8. Heat	*	*	✓	✓	*	*	-	✓
9. NFT	*	*	-	-	*	*	-	-
10. NFT	*	*	-	-	*	*	-	-

✓ present; - not detected; * not assessed

As well as the crops routinely sampled, additional root samples were sought from crops where root disease was visibly evident. Two additional crops were sampled for symptomatic roots (Table 64). The sites these crops were sampled from were on sites already being sampled as part of Objective 4, and so some comparison with healthy crops on the same site was possible.

Table 64. Microorganisms detected by the microarray on roots showing symptoms of root mat (site 6) and severe root rot (site 8) compared with unaffected roots from the same crops – 2015*

Pathogen	Site 6			Site 8	
	Root mat	Visibly healthy		Severe root rot	Visibly healthy
Colletotrichum acutatum	-	-		-	-
Colletotrichum coccodes	-	-		-	-
Fusarium oxysporum	-	-		1	1
Fusarium redolens	-	-		1	-
Fusarium solani	-	-		-	-
Phytophthora arecae	-	-		2	-
Phytophthora nicotianae	-	-		2	-
Pythium aphanidermatum	-	-		-	-
Pythium arrhenomanes	-	-		-	-
Pythium debaryanum	-	-		-	-
Pythium diclinum	1	-		1	1
Pythium echinulatum	-	-		-	-
*Pythium irregulare**	-	-		1	1
Pythium megalacanthum	-	-		-	-
Pythium myriotylum	2	1		1	1
Pythium oligandrum	-	-		-	-
Pythium paroencandrum	-	-		-	-
Pythium torulosum	-	-		-	-
Plectosphaerella cucumerina	2	1		2	-
Pyrenochaeta lycopersici	-	-		-	-
Rhizoctonia solani	-	-		-	-
*Spongospora subterranea**	-	-		1	1
Thielaviopsis basicola	-	-		-	-
Verticillium albo-atrum	-	-		-	-
Verticillium dahliae	-	-		-	-
Verticillium nigrescens	1	1		1	-

*Site 6 – severe root mat, July; Site 8 – wilting, yellowing and root death, October

In both these cases, additional pathogenic species were reported by the microarray when compared to roots sampled from the same site on the same day that appeared healthy. For Site 6, an additional *Pythium* species was present compared to healthy roots. Though the roots were exhibiting severe symptoms of root mat, no *Agrobacterium* species were detected by the microarray. For Site 8, *Fusarium oxysporum* and *Fusarium redolens* were present, as well as two species of *Phytophthora* not present in healthy roots. However, these pathogens were detected by the microarray with similar intensity at other sites across the year where roots expressed no severe symptoms of disease. It is likely that a combination of factors contributed to the expression of disease in these cases. It may be of note that both these crops were grown on coir substrate rather than on rockwool, though this may be coincidental. In Project PC 281 coir and rockwool crops were compared and found to have similar abundances of fungal pathogens. It should also be noted that for Site 8 the control sample was taken from an adjacent compartment rather than a nearby plant, as all plants in that area were similarly severely affected.

Conclusions

Objective 1: Microarray and qPCR diagnostics

1. From the preliminary validation results, the primer qPCR assays seem to have good specificity. Both *C. coccodes* and *P. cucumerina* could be validated using three separate strains of pure culture to ensure the specificity of the primers were sufficient to identify different isolates.
2. However neither of the *P. aphanidermatum* or *P. myriotylum* assays were tested with as many samples, so, despite initial positive results, more tests are needed to confirm their reliability.
3. The microarray colour intensity scale (0-3) agreed with the relevant qPCR diagnostic for *Colletotrichum coccodes* and *Plectospharella cucumerina,* but not for *Pythium aphanidermatum* and *Pythium myriotylum*
4. The microarray contains wells for 39 species specific fungal probes and 7 fungal genus probes, 15 oomycete species-specific probes and 14 bacteria species-specific and 6 bacteria genus probes (Devine, 2014).

Objective 2: Effect of water treatment system, sampling location and crop age on microorganisms in recycled water

5. Overall microorganism species richness increased in irrigation water as crops aged, and the abundance of common tomato pathogens increased. However, this was not true for all sites, with fewer pathogens detected in the latter half of the season in the NFT crop.
6. Conversely, species richness was observed to decrease in the rhizosphere for most sites, though for Sites 1 and 2 there was not a pronounced trend.
7. Disinfection treatment was observed to lower species richness and remove a variety of common pathogens from irrigation water. The most effective treatments appeared to be the full slow sand filter, and UV treatment, though effects were variable.
8. Sampling location has a strong effect on the microbial life detected in irrigation water, dependent on the water's source and the specific irrigation system present at each site.
9. Relatively little root disease was seen over the season in the 5 main sites monitored, and so clearly linking visible symptoms to the pathogens detected by the microarray was not possible. However, over the whole season, Site 2 (pSSF) and Site 4 (UV) had the greatest pathogen species richness in the rhizosphere, and it was these sites that exhibited the greatest amount and degree of vascular staining by the end of the season, and the most visible root browning. Notably greater species diversity was observed over the season at Site 1, where no severe root disease was observed. Additionally, Site 2 had the lowest total species diversity at the slab over the season, and had most notable root browning and some vascular staining present at the end of the season.

Objective 3: Pathogenicity of four root pathogens on own-root and grafted plants

10. No significant differences ($P > 0.05$) were detected between own roots and grafted plants for any measures of ill-health following inoculation with Plectosphaerella or Pythium.
11. Infection with Colletotrichum and Fusarium appeared to significantly decrease % brown roots at the base of the cube – this could be due to an increased rate of root die-off and re-growth of new roots as a result of the infection.
12. Differences between rootstocks Maxifort and Arnold rootstocks and own-roots Piccolo were not evident, despite different generic resistances to Fusarium oxysporum f.sp. lycopersici.

13. On a commercial site, Piccolo grafted to a rootstock exhibited less vascular staining than Piccolo grown on its own roots, but crops did not appear to differ greatly in terms of crop health over the season.

14. Roots sampled from both grafted and ungrafted Piccolo plants showed a greater abundance of potential pathogens in January and April than later in the year.

15. Grafted roots of variety Emperador typically had greater abundance of Pythium species than ungrafted roots, on which detection of true fungi such as V. nigrescens was more common than on grafted roots.

16. Microorganisms detected in irrigation water differed between grafted and ungrafted crops, with little microbial life detected in water from either rootzone in January, a higher abundance of fungal and Oomycete pathogens on ungrafted plants in April, but a higher abundance on Emperador roots by October.

17. The conditions required for root diseases to occur remain poorly understood. The presence of a known pathogenic species associated with roots of a susceptible variety, even at a relatively high inoculum level, does not necessarily result in visible root disease or noticeable adverse effects on crop growth.

Objective 4: Monitoring of additional hydroponic crops with recycled irrigation water for root pathogens and root disease

18. The samples taken from 5 additional sites agreed with the sites from Objective 2 in broad terms (see points 4-9 above).

19. One of the additional NFT sites had notably higher species richness than the other two NFT sites, showing that lower species richness over the season may not be due to this growing system alone, or may not always be the case.

20. Three of the additional sites heat treated their recirculated water, exhibited a similar set of pathogens in the rhizosphere to each other when tested by microarray, and to the heat treated site in Objective 2.

21. Samples taken from additional sites where severe symptoms of root disease were evident did have additional pathogenic species present in comparison with healthy roots from those sites, as detected by the microarray.

22. Conditions between these different crops or areas of glasshouse on single sites may have differed significantly, or different microbial community may have been introduced with one set of plants and not another.

Knowledge and Technology Transfer

Meetings

Presentation at Tomato Technical Group, Stubbins Nursery, Fen Drayton, April 2015. (Tim O'Neill, Sarah Mayne)

Project review meeting at Sutton Bonington, 23rd October 2015. (Tim O'Neill, Sarah Mayne, Matt Dickinson, Stuart Bagley)

Article

O'Neill TM & Mayne S. Testing the water. AHDB Grower 2016.

References

Devine G (2014) 'Detecting pathogens and beneficial microorganisms in the tomato rhizosphere using microarrays' PhD Thesis, University of Nottingham, 213 pp.

Glossary

ITS – Internal Transcribed Spacer; a region of DNA that is commonly used as a unique DNA sequence to identify a specific organism

Primer - strand of short nucleic acid sequence (generally about 15-20 bases) that serves as a starting point for DNA synthesis.

Probe - fragment of DNA generally 20–30 bases long that can be applied to an array and to which labelled DNA sequences will hybridise if they are complementary to the sequence in the probe.

Taqman – a technology developed by Roche Molecular Diagnostics for quantitative PCR analysis

Fluorophore – a fluorescent chemical compound that can re-emit light on excitation by light. Commonly used as a way to mark nucleic acids in analytical methods

Species richness - is the number of different species represented in an ecological community, landscape or region. Species richness is simply a count of species, and it does not take into account the abundances of the species or their relative abundance distributions.

Species diversity - refers to the measure of diversity in an ecological community. Species diversity takes into consideration species richness, which is the total number of different species in a community. It also takes into account evenness, which is the variation of abundance in individuals per species in a community.

Barcoding genes- DNA barcoding is a taxonomic method that uses a short genetic marker in an organism's DNA (the barcoding gene) to identify it as belonging to a particular species.

Amplification – is a technique used in molecular biology to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

Universal primers – are a set of primers that are able to promote DNA synthesis on a broad range of organisms ie fungal universal primers will work on all species of fungi to amplify the target gene

Appendices

Appendix 1. Crop monitoring results

Table 1a. Comparison of microorganisms detected in irrigation water of five tomato crops with different water treatment systems – Sample 1 (Jan/Feb 2015)

Pathogen	Relative occurrence (0-30) in each system				
	Nil (NFT)	pSSF	fSSF	UV	Heat
Colletotrichum acutatum					
Colletotrichum coccodes	-	-	-	-	-
Fusarium oxysporum	-	7	4	-	4
Fusarium redolens	-	3	2	-	1
Fusarium solani	-	-	-	-	-
Phytophthora cryptogea	-	-	-	-	-
Phytophthora nicotianae	-	-	-	-	2
Pythium aphanidermatum	-	-	-	-	-
Pythium arrhenomanes	-	1	-	-	-
Pythium debaryanum	-	-	-	-	-
Pythium diclinum	-	1	-	-	-
Pythium echinulatum	-	-	2	-	-
*Pythium irregulare**	-	5	10	4	7
Pythium megalacanthum	-	1	-	-	-
Pythium myriotylum	-	2	-	-	-
Pythium oligandrum	-	-	-	-	-
Pythium paroencandrum	-	2	8	1	-
Pythium torulosum	-	1	-	-	-
Plectosphaerella cucumerina	-	1	1	-	-
Pyrenochaeta lycopersici	-	-	-	-	1
Rhizoctonia solani	-	-	-	-	-
*Spongospora subterranea**	-	1	10	1	-
Thielaviopsis basicola	-	-	-	-	-
Verticillium albo-atrum	-	-	-	-	-
Verticillium dahliae	-	-	-	-	-

Verticillium nigrescens	-	2	1	2	1

* Erroneous results have been observed – data should be used with caution.

Table 1b. Comparison of microorganisms detected in irrigation water of five tomato crops with different water treatment systems – Sample 2 (April 2015)

Pathogen	Relative occurrence (0-30) in each system				
	Nil (NFT)	pSSF	fSSF	UV	Heat
Alternaria solani	1	-	-	-	-
Colletotrichum acutatum	-	-	-	-	-
Colletotrichum coccodes	1	-	-	1	4
Fusarium oxysporum	1	-	-	-	7
Fusarium redolens	-	-	-	-	3
Fusarium solani	-	-	-	-	-
Phytophthora arecae	1	-	-	-	-
Phytophthora cryptogea	-	-	-	-	-
Phytophthora nicotianae	1	-	-	-	-
Pythium aphanidermatum	-	-	-	-	-
Pythium arrhenomanes	-	-	-	-	-
Pythium debaryanum	-	-	-	-	-
Pythium diclinum	5	-	-	-	-
Pythium echinulatum	-	-	-	-	-
*Pythium irregulare**	6	-	3	4	10
Pythium megalacanthum	-	-	-	-	-
Pythium myriotylum	6	-	1	3	5
Pythium oligandrum	-	-	-	-	-
Pythium paroencandrum	-	-	-	2	4
Pythium torulosum	-	-	-	-	-
Plectosphaerella cucumerina	6	-	2	4	9
Pyrenochaeta lycopersici	-	-	-	-	-
Rhizoctonia solani	-	-	-	-	-
*Spongospora subterranea**	4	-	1	-	2
Thielaviopsis basicola	-	-	-	-	-
Verticillium albo-atrum	-	-	-	-	-
Verticillium dahliae	-	-	-	-	-
Verticillium nigrescens	6	-	1	2	6

* Erroneous results have been observed – data should be used with caution.

Table 1c. Comparison of microorganisms detected in irrigation water of five tomato crops with different water treatment systems – Sample 3 (July 2015)

Pathogen	Relative occurrence (0-30) in each system				
	Nil (NFT)	pSSF	fSSF	UV	Heat
Alternaria solani	-	-	-	-	-
Colletotrichum acutatum	-	-	-	-	-
Colletotrichum coccodes	-	-	-	-	-
Fusarium oxysporum	3	-	2	2	1
Fusarium redolens	-	-	-	2	-
Fusarium solani	-	-	-	-	-
Phytophthora arecae	-	1	-	-	-
Phytophthora cryptogea	-	-	-	-	-
Phytophthora nicotianae	-	1	-	-	-
Pythium aphanidermatum	-	1	-	-	-
Pythium arrhenomanes	-	-	-	-	-
Pythium debaryanum	-	-	-	-	-
Pythium diclinum	4	3	-	-	-
Pythium echinulatum	-	-	-	-	-
*Pythium irregulare**	3	9	12	14	12
Pythium megalacanthum	-	-	-	-	-
Pythium myriotylum	6	7	2	2	3
Pythium oligandrum	-	-	-	-	-
Pythium paroencandrum	-	5	6	6	8
Pythium torulosum	-	-	-	-	-
Plectosphaerella cucumerina	4	-	4	2	2
Pyrenochaeta lycopersici	-	-	-	-	-
Rhizoctonia solani	-	-	-	-	-
*Spongospora subterranea**	-	6	6	9	8
Thielaviopsis basicola	-	-	-	-	-

Verticillium albo-atrum	-	-	-	-	-
Verticillium dahliae	-	-	-	-	-
Verticillium nigrescens	4	-	4	2	-

* Erroneous results have been observed – data should be used with caution.

Table 1d. Comparison of microorganisms detected in irrigation water of five tomato crops with different water treatment systems – Sample 4 (October 2015)

Pathogen	Relative occurrence (0-30) in each system				
	Nil (NFT)	pSSF	fSSF	UV	Heat
Alternaria solani	-	-	-	-	1
Colletotrichum acutatum	-	-	-	-	-
Colletotrichum coccodes	-	1	1	1	-
Fusarium oxysporum	1	1	-	3	3
Fusarium redolens	-	-	-	5	2
Fusarium solani	-	-	-	-	-
Phytophthora arecae	-	3	-	-	-
Phytophthora cryptogea	-	-	-	-	-
Phytophthora nicotianae	-	1	-	-	-
Pythium aphanidermatum	2	4	-	3	-
Pythium arrhenomanes	-	-	-	-	-
Pythium debaryanum	-	-	-	2	-
Pythium diclinum	5	-	-	-	-
Pythium echinulatum	-	-	-	1	-
*Pythium irregulare**	10	-	6	19	12
Pythium megalacanthum	-	-	-	-	-
Pythium myriotylum	9	12	1	2	-
Pythium oligandrum	-	-	-	-	-
Pythium paroencandrum	4	3	4	9	8
Pythium torulosum	-	-	-	-	-
Plectosphaerella cucumerina	9	4	3	4	4
Pyrenochaeta lycopersici	1	-	-	-	1
Rhizoctonia solani	-	-	-	-	-

*Spongospora subterranea**	5	-	6	15	6
Thielaviopsis basicola	-	-	-	-	-
Verticillium albo-atrum	-	-	-	-	-
Verticillium dahliae	-	-	-	-	-
Verticillium nigrescens	9	3	2	2	4

* Erroneous results have been observed – data should be used with caution.

Table 2a. Comparison of microorganisms detected on roots of five tomato crops with different water treatment systems – Sample 1 (Jan/Feb 2015)

Pathogen	Relative occurrence (0-9) in each system					
	Nil (NFT) own root	Nil (NFT)	pSSF	fSSF	UV	Heat
Colletotrichum acutatum	-	-	-	-	-	-
Colletotrichum coccodes	-	-	-	-	-	-
Fusarium oxysporum	-	1	9	1	-	6
Fusarium redolens	-	1	7	1	-	2
Fusarium solani	1	-	1	-	-	-
Phytophthora cryptogea	-	-	-	-	-	-
Phytophthora nicotianae	-	-	-	-	-	1
Pythium aphanidermatum	-	2	1	-	-	-
Pythium arrhenomanes	-	-	-	-	-	-
Pythium debaryanum	-	-	-	-	-	-
Pythium diclinum	2	5	1	-	-	-
Pythium echinulatum	-	-	-	-	-	-
*Pythium irregulare**	2	6	1	6	2	-
Pythium megalacanthum	1	1	-	-	1	-
Pythium myriotylum	2	4	-	-	-	-
Pythium oligandrum	1	0	1	-	-	-
Pythium paroencandrum	1	5	4	3	1	-
Pythium torulosum	-	-	-	-	1	-
Plectosphaerella cucumerina	1	2	8	8	2	2
Pyrenochaeta lycopersici	-	-	-	-	1	-
Rhizoctonia solani	-	-	-	1	-	-

*Spongospora subterranea**	2	6	2	6	2	1
Thielaviopsis basicola	-	3	1	-	-	-
Verticillium albo-atrum	-	-	-	-	-	-
Verticillium dahliae	1	-	-	-	-	-
Verticillium nigrescens	-	3	5	5	2	2

* Erroneous results have been observed – data should be used with caution.

Table 2b. Comparison of microorganisms detected on roots of five tomato crops with different water treatment systems – Sample 2 (April 2015)

Pathogen	Relative occurrence (0-9) in each system					
	Nil (NFT) own root	Nil (NFT)	pSSF	fSSF	UV	Heat
Colletotrichum acutatum	-	-	-	-	-	-
Colletotrichum coccodes	1	-	4	-	5	2
Fusarium oxysporum	-	-	2	3	4	2
Fusarium redolens	-	-	2	1	4	1
Fusarium solani	-	-	-	-	-	-
Phytophthora cryptogea	-	-	-	-	-	-
Phytophthora nicotianae	-	-	-	-	-	-
Pythium aphanidermatum	-	-	-	-	-	-
Pythium arrhenomanes	-	-	-	-	-	-
Pythium debaryanum	-	-	-	-	-	-
Pythium diclinum	4	-	4	3	3	-
Pythium echinulatum	-	-	-	-	-	-
*Pythium irregulare**	-	-	-	3	2	3
Pythium megalacanthum	-	-	-	-	-	-
Pythium myriotylum	4	-	4	3	4	-
Pythium oligandrum	-	-	-	-	-	-
Pythium paroencandrum	-	-	3	1	-	3
Pythium torulosum	-	-	-	-	-	-
Plectosphaerella cucumerina	2	-	8	9	9	7
Pyrenochaeta lycopersici	-	-	-	-	-	-
Rhizoctonia solani	-	-	-	-	-	-
*Spongospora subterranea**	3	-	-	-	2	3
Thielaviopsis basicola	-	1	-	-	-	-
Verticillium albo-atrum	-	-	-	-	-	-
Verticillium dahliae	-	-	-	-	-	-
Verticillium nigrescens	2	-	7	7	5	4

* Erroneous results have been observed – data should be used with caution.

Table 2c. Comparison of microorganisms detected on roots of five tomato crops with different water treatment systems – Sample 3 (July 2015)

Pathogen	Relative occurrence (0-9) in each system					
	Nil (NFT) own root	Nil (NFT)	pSSF	fSSF	UV	Heat
Colletotrichum acutatum	-	-	-	-	-	-
Colletotrichum coccodes	-	-	1	-	-	1
Fusarium oxysporum	-	-	-	2	1	3
Fusarium redolens	-	-	-	1	1	2
Fusarium solani	-	-	-	-	-	-
Phytophthora cryptogea	-	-	-	-	-	-
Phytophthora nicotianae	-	-	-	-	-	-
Pythium aphanidermatum	-	-	-	-	-	-
Pythium arrhenomanes	-	-	-	-	-	-
Pythium debaryanum	-	-	-	-	-	-
Pythium diclinum	-	-	-	-	1	-
Pythium echinulatum	-	-	-	-	-	-
*Pythium irregulare**	-	-	-	4	2	3
Pythium megalacanthum	-	-	-	-	-	-
Pythium myriotylum	-	-	1	1	1	-
Pythium oligandrum	-	-	-	-	-	-
Pythium paroencandrum	-	-	-	1	2	4
Pythium torulosum	-	-	-	-	-	-
Plectosphaerella cucumerina	-	-	1	4	3	6
Pyrenochaeta lycopersici	-	-	-	-	-	-
Rhizoctonia solani	-	-	-	-	-	-
*Spongospora subterranea**	-	-	-	3	4	4
Thielaviopsis basicola	-	-	-	-	-	-
Verticillium albo-atrum	-	-	-	-	-	-
Verticillium dahliae	-	-	-	-	-	-
Verticillium nigrescens	-	-	1	3	1	5

* Erroneous results have been observed – data should be used with caution.

Table 2d. Comparison of microorganisms detected on roots of five tomato crops with different water treatment systems – Sample 4 (October 2015)

Pathogen	Relative occurrence (0-9) in each system					
	Nil (NFT) own root	Nil (NFT)	pSSF	fSSF	UV	Heat
Colletotrichum acutatum	-	-	-	-	-	-
Colletotrichum coccodes	-	-	-	-	2	-
Fusarium oxysporum	-	-	-	-	3	-
Fusarium redolens	-	-	-	-	3	-
Fusarium solani	-	-	-	1	-	-
Phytophthora cryptogea	-	-	-	-	-	-
Phytophthora nicotianae	-	-	-	-	-	-
Pythium aphanidermatum	-	-	-	-	-	-
Pythium arrhenomanes	-	-	-	-	-	-
Pythium debaryanum	-	-	-	-	-	-
Pythium diclinum	-	-	-	1	1	-
Pythium echinulatum	-	-	-	-	-	-
*Pythium irregulare**	-	-	-	1	5	2
Pythium megalacanthum	-	-	-	-	-	-
Pythium myriotylum	-	-	-	3	1	-
Pythium oligandrum	-	-	-	-	-	-
Pythium paroencandrum	-	-	-	-	1	-
Pythium torulosum	-	-	-	-	-	-
Plectosphaerella cucumerina	-	-	-	-	4	-
Pyrenochaeta lycopersici	-	-	-	-	-	-
Rhizoctonia solani	-	-	-	-	-	-
*Spongospora subterranea**	-	-	-	3	4	3
Thielaviopsis basicola	-	-	-	-	-	-
Verticillium albo-atrum	-	-	-	-	-	-
Verticillium dahliae	-	-	-	-	-	-
Verticillium nigrescens	-	1	-	-	4	-

* Erroneous results have been observed – data should be used with caution.

Table 3a. Comparison of microorganisms detected in water at five locations in closed irrigation system tomato crops – Sample 1 (Jan/Feb 2015)

Pathogen	Relative occurrence (0-6) in each system at 5 locations																									
	NFT			pSSF						fSSF						UV						Heat				
	1	2		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5
Colletotrichum acutatum	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
Colletotrichum coccodes	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
Fusarium oxysporum	-	-		1	-	4	2	-		1	2	-	1	-		-	-	-	-	-		1	2	-	1	-
Fusarium redolens	-	-		-	-	2	1	-		1	1	-	-	-		-	-	-	-	-		-	1	-	-	-
Fusarium solani	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
Phytophthora cryptogea	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
Phytophthora nicotianae	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	2	-
Pythium aphanidermatum	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
Pythium arrhenomanes	-	-		-	-	-	1	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
Pythium debaryanum	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
Pythium diclinum	-	-		-	-	-	1	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
Pythium echinulatum	-	-		-	-	-	-	-		-	-	2	-	-		-	-	-	-	-		-	-	-	-	-
*Pythium irregulare**	-	-		1	1	2	1	-		1	-	5	4	-		-	-	1	3	-		-	-	1	6	-
Pythium megalacanthum	-	-		-	-	-	1	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
Pythium myriotylum	-	-		-	-	-	2	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-

Pythium oligandrum	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
Pythium paroencandrum	-	-		1	-	-	1	-		1	-	4	3	-		-	-	-	1	-		-	-	-	-	-
Pythium torulosum	-	-		-	-	-	1	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
Plectosphaerella cucumerina	-	-		-	-	1	-	-		-	-	-	1	-		-	-	-	-	-		-	-	-	-	-
Pyrenochaeta lycopersici	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	1	-	-	-
Rhizoctonia solani	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
Spongospora subterranea	-	-		1	-	-	-	-		2	-	4	4	-		-	-	-	1	-		-	-	-	-	-
Thielaviopsis basicola	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
Verticillium albo-atrum	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
Verticillium dahliae	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
Verticillium nigrescens	-	-		-	-	2	-	-		-	-	-	1	-		-	-	-	-	-		-	1	-	-	-

Sample location: 1 – source water, 2 – mixing/collection tank, 3 - slab, 4 – pre-treatment, 5 – post-treatment (For NFT: 3 – underground tank, 4 – grafted, 5- ungrafted)

* Erroneous results have been observed – data should be used with caution.

Table 3b. Comparison of microorganisms detected in water at five locations in closed irrigation system tomato crops – Sample 2 (April 2015)

Pathogen	Relative occurrence (0-6) in each system at 5 locations																											
	NFT						pSSF						fSSF						UV						Heat				
	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	
A. solani	-	-	-	1	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
C. acutatum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
C. coccodes	-	-	-	-	1		-	-	-	-	-		-	-	-	-	-		-	-	1	-	-		-	-	3	1	-
F. oxysporum	-	-	-	1	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		1	-	2	4	-
F. redolens	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	3	-
F. solani	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. arecae	-	-	-	-	1		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. cryptogea	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. nicotianae	-	-	-	-	1		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. aphanidermatum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. arrhenomanes	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. debaryanum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. diclinum	-	-	-	-	5		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. echinulatum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
*P. irregulare**	-	-	-	1	5		-	-	-	-	-		-	-	1	2	-		-	-	2	2	-		2	-	4	4	-
P. megalacanthum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. myriotylum	-	-	-	1	5		-	-	-	-	-		-	-	-	1	-		-	2	1	-	-		4	-	-	1	-

P. oligandrum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. paroencandrum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	1	1	-		1	-	2	1	-
P. torulosum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. cucumerina	-	-	-	2	4		-	-	-	-	-		-	-	2	-	-		-	-	4	-	-		-	-	5	4	-
P. lycopersici	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
R. solani	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
S. subterranea	-	-	-	-	4		-	-	-	-	-		-	-	-	1	-		-	-	-	-	-		-	-	-	2	-
T. basicola	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
V. albo-atrum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
V. dahliae	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
V. nigrescens	-	-	-	1	5		-	-	-	-	-		-	-	1	-	-		-	-	2	-	-		-	-	4	2	-

Sample location: 1 – source water, 2 – mixing/collection tank, 3 - slab, 4 – pre-treatment, 5 – post-treatment (For NFT: 3 – underground tank, 4 – grafted, 5-ungrafted)

* Erroneous results have been observed – data should be used with caution.

Table 3c. Comparison of microorganisms detected in water at five locations in closed irrigation system tomato crops – Sample 3 (July 2015)

Pathogen	Relative occurrence (0-6) in each system at 5 locations																											
	NFT						pSSF						fSSF						UV						Heat				
	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	
C. acutatum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
C. coccodes	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
F. oxysporum	3	-	-	-	-		-	-	-	-	-		-	-	-	2	-		-	-	-	2	-		-	-	1	-	
F. redolens	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	2	-		-	-	-	-	-
F. solani	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. arecae	-	-	-	-	-		1	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. cryptogea	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. nicotianae	-	-	-	-	-		1	1	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. aphanidermatum	-	-	-	-	-		-	1	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. arrhenomanes	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. debaryanum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. diclinum	-	-	2	-	2		-	3	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. echinulatum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
*P. irregulare**	-	-	2	-	1		1	2	-	4	2		1	-	6	4	1		2	2	5	4	1		2	4	2	4	-
P. megalacanthum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. myriotylum	-	-	2	2	2		2	4	-	1	-		-	-	1	1	-		2	-	-	-	-		1	2	-	-	-
P. oligandrum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-

P. paroencandrum	-	-	-	-	-		1	2	-	2	-		-	-	2	4	-		2	-	2	2	-		2	2	2	2	-
P. torulosum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. cucumerina	-	-	4	-	-		-	-	-	-	-		-	-	4	-	-		-	-	-	2	-		-	-	2	-	-
P. lycopersici	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
R. solani	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
*S. subterranea**	-	-	-	-	-		-	2	-	2	2		-	-	4	2	-		2	-	3	4	-		2	2	2	2	-
T. basicola	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
V. albo-atrum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
V. dahliae	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
V. nigrescens	-	-	4	-	-		-	-	-	-	-		-	-	4	-	-		-	-	-	2	-		-	-	-	-	-

Sample location: 1 – source water, 2 – mixing/collection tank, 3 - slab, 4 – pre-treatment, 5 – post-treatment (For NFT: 3 – underground tank, 4 – grafted, 5- ungrafted)

* Erroneous results have been observed – data should be used with caution.

Table 3d. Comparison of microorganisms detected in water at five locations in closed irrigation system tomato crops – Sample 4 (October 2015)

Pathogen	Relative occurrence (0-6) in each system at 5 locations																											
	NFT						pSSF						fSSF						UV						Heat				
	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5
C. acutatum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
C. coccodes	-	-	-	-	-		-	-	1	-	-		-	-	-	1	-		-	-	1	-	-		-	-	-	-	-
F. oxysporum	-	-	1	-	-		1	-	-	-	-		-	-	-	-	-		-	-	-	3	-		-	-	-	2	1
F. redolens	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	1	3	1		-	-	-	2	-
F. solani	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. arecae	-	-	-	-	-		3	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. cryptogea	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. nicotianae	-	-	-	-	-		1	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. aphanidermatum	2	-	-	-	-		-	2	-	2	-		-	-	-	-	-		2	-	1	-	-		-	-	-	-	-
P. arrhenomanes	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. debaryanum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. diclinum	-	-	4	1	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. echinulatum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	1	-	-		-	-	-	-	-
*P. irregulare**	3	-	2	3	2		-	-	-	-	-		1	-	3	2	-		4	4	5	4	2		2	2	3	4	1
P. megalacanthum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. myriotylum	2	-	4	3	-		4	4	-	4	-		-	-	-	1	-		1	-	1	-	-		-	-	-	-	-
P. oligandrum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-

P. paroencandrum	2	-	-	2	-		-	2	1	-	-		-	-	2	2	-		2	2	3	2	-		2	2	2	2	-
P. torulosum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
P. cucumerina	-	-	4	4	1		-	-	4	-	-		-	-	2	1	-		-	-	3	1	-		-	-	2	2	-
P. lycopersici	1	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	1	-
R. solani	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
S. subterranea	-	-	2	2	1		-	-	-	-	-		2	-	-	2	2		4	2	4	3	2		1	1	2	2	-
T. basicola	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
V. albo-atrum	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
V. dahliae	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
V. nigrescens	-	-	4	4	1		-	-	3	-	-		-	-	2	-	-		-	-	2	-	-		-	-	2	2	-

Sample location: 1 – source water, 2 – mixing/collection tank, 3 - slab, 4 – pre-treatment, 5 – post-treatment (For NFT: 3 – underground tank, 4 – grafted, 5- ungrafted)

* Erroneous results have been observed – data should be used with caution.

Table 4a. Comparison of microorganisms detected in irrigation water and on tomato roots (grafted plants) – (Jan/Feb 2015) Sample 1

Pathogen	Water treatment system												
	Nil		pSSF			fSSF			UV			Heat	
	Water	Roots	Water	Roots		Water	Roots		Water	Roots		Water	Roots
Colletotrichum acutatum													
Colletotrichum coccodes	-	-	-	-		-	-		-	-		-	-
Fusarium oxysporum	-	✓	✓	✓		✓	✓		-	-		✓	✓
Fusarium redolens	-	✓	✓	✓		✓	✓		-	-		✓	✓
Fusarium solani	-	-	-	✓		-	-		-	-		-	-
Phytophthora cryptogea	-	-	-	-		-	-		-	-		-	-
Phytophthora nicotianae	-	-	-	-		-	-		-	-		✓	✓
Pythium aphanidermatum	-	✓	-	✓		-	-		-	-		-	-
Pythium arrhenomanes	-	-	✓	-		-	-		-	-		-	-
Pythium debaryanum	-	-	-	-		-	-		-	-		-	-
Pythium diclinum	-	✓	✓	✓		-	-		-	-		-	-
Pythium echinulatum	-	-	-	-		✓	-		-	-		-	-
*Pythium irregulare**	-	✓	✓	✓		✓	✓		✓	✓		✓	-
Pythium megalacanthum	-	✓	✓	-		-	-		-	✓		-	-
Pythium myriotylum	-	✓	✓	-		-	-		-	-		-	-
Pythium oligandrum	-	-	-	✓		-	-		-	-		-	-
Pythium paroencandrum	-	✓	✓	✓		✓	✓		✓	✓		-	-
Pythium torulosum	-	-	✓	-		-	-		-	-		-	-

Plectosphaerella cucumerina	-	✓		✓	✓		✓	✓		-	✓		-	✓
Pyrenochaeta lycopersici	-	-		-	-		-	-		-	✓		✓	-
Rhizoctonia solani	-	-		-	-		-	✓		-	-		-	-
*Spongospora subterranea**	-	✓		✓	✓		✓	✓		✓	✓		-	✓
Thielaviopsis basicola	-	✓		-	✓		-	-		-	-		-	-
Verticillium albo-atrum	-	-		-	-		-	-		-	-		-	-
Verticillium dahliae	-	-		-	-		-	-		-	-		-	-
Verticillium nigrescens	-	✓		✓	✓		✓	✓		✓	✓		✓	✓

* Erroneous results have been observed – data should be used with caution.

Table 4b. Comparison of microorganisms detected in irrigation water and on tomato roots (grafted plants) – (April 2015) Sample 2

Pathogen	Water treatment system																	
	Nil			pSSF			fSSF			UV			Heat					
	Water	Roots		Water	Roots		Water	Roots		Water	Roots		Water	Roots				
Alternaria solani	✓	-		-	-		-	-		-	-		-	-				
Colletotrichum acutatum	-	-		-	-		-	-		-	-		-	-				
Colletotrichum coccodes	✓	-		-	✓		-	-		✓	✓		✓	✓				
Fusarium oxysporum	✓	-		-	✓		-	✓		-	✓		✓	✓				
Fusarium redolens	-	-		-	✓		-	✓		-	✓		✓	✓				
Fusarium solani	-	-		-	-		-	-		-	-		-	-				
Phytophthora arecae	✓	-		-	-		-	-		-	-		-	-				
Phytophthora cryptogea	-	-		-	-		-	-		-	-		-	-				
Phytophthora nicotianae	✓	-		-	-		-	-		-	-		-	-				
Pythium aphanidermatum	-	-		-	-		-	-		-	-		-	-				
Pythium arrhenomanes	-	-		-	-		-	-		-	-		-	-				
Pythium debaryanum	-	-		-	-		-	-		-	-		-	-				
Pythium diclinum	✓	-		-	✓		-	✓		-	✓		-	-				
Pythium echinulatum	-	-		-	-					-	-		-	-				
Pythium irregulare	✓	-		-	-		✓	✓		✓	✓		✓	✓				
Pythium megalacanthum	-	-		-	-		-	-		-	-		-	-				
Pythium myriotylum	✓	-		-	✓		✓	✓		✓	✓		✓	-				

Pythium oligandrum	-	-		-	-		-	-		-	-		-	-
Pythium paroencandrum	-	-		-	✓		-	✓		✓	-		✓	✓
Pythium torulosum	-	-		-	-		-	-		-	-		-	-
Plectosphaerella cucumerina	✓	-		-	✓		✓	✓		✓	✓		✓	✓
Pyrenochaeta lycopersici	-	-		-	-		-	-		-	-		-	-
Rhizoctonia solani	-	-		-	-		-	-		-	-		-	-
*Spongospora subterranea**	✓	-		-	-		✓	-		-	✓		✓	✓
Thielaviopsis basicola	-	✓		-	-		-	-		-	-		-	-
Verticillium albo-atrum	-	-		-	-		-	-		-	-		-	-
Verticillium dahliae	-	-		-	-		-	-		-	-		-	-
Verticillium nigrescens	✓	-		-	✓		✓	✓		✓	✓		✓	✓

* Erroneous results have been observed – data should be used with caution.

Table 4c. Comparison of microorganisms detected in irrigation water and on tomato roots (grafted plants) – (July 2015) Sample 3

Pathogen	Water treatment system												
	Nil			pSSF			fSSF			UV			Heat	
	Water	Roots		Water	Roots		Water	Roots		Water	Roots		Water	Roots
Alternaria solani	-	-		-	-		-	-		-	-		-	-
Colletotrichum acutatum	-	-		-	-		-	-		-	-		-	-
Colletotrichum coccodes	-	-		-	✓		-	-		-	-		-	✓
Fusarium oxysporum	✓	-		-	-		✓	✓		✓	✓		✓	✓
Fusarium redolens	-	-		-	-		-	✓		✓	✓		-	✓
Fusarium solani	-	-		-	-		-	-		-	-		-	-
Phytophthora arecae	-	-		✓	-		-	-		-	-		-	-
Phytophthora cryptogea	-	-		-	-		-	-		-	-		-	-
Phytophthora nicotianae	-	-		✓	-		-	-		-	-		-	-
Pythium aphanidermatum	-	-		✓	-		-	-		-	-		-	-
Pythium arrhenomanes	-	-		-	-		-	-		-	-		-	-
Pythium debaryanum	-	-		-	-		-	-		-	-		-	-
Pythium diclinum	✓	-		✓	-		-	-		-	✓		-	-
Pythium echinulatum	-	-		-	-		-	-		-	-		-	-
Pythium irregulare	✓	-		✓	-		✓	✓		✓	✓		✓	✓
Pythium megalacanthum	-	-		-	-		-	-		-	-		-	-
Pythium myriotylum	✓	-		✓	✓		✓	✓		✓	✓		✓	-

Pythium oligandrum	-	-		-	-		-	-		-	-		-	-
Pythium paroencandrum	-	-		✓	-		✓	✓		✓	✓		✓	✓
Pythium torulosum	-	-		-	-		-	-		-	-		-	-
Plectosphaerella cucumerina	✓	-		-	✓		✓	✓		✓	✓		✓	✓
Pyrenochaeta lycopersici	-	-		-	-		-	-		-	-		-	-
Rhizoctonia solani	-	-		-	-		-	-		-	-		-	-
*Spongospora subterranea**	-	-		✓	-		✓	✓		✓	✓		✓	✓
Thielaviopsis basicola	-	-		-	-		-	-		-	-		-	-
Verticillium albo-atrum	-	-		-	-		-	-		-	-		-	-
Verticillium dahliae	-	-		-	-		-	-		-	-		-	-
Verticillium nigrescens	✓	-		-	✓		✓	✓		✓	✓		-	✓

* Erroneous results have been observed – data should be used with caution.

Table 4d. Comparison of microorganisms detected in irrigation water and on tomato roots (grafted plants) – (October 2015) Sample 4

Pathogen	Water treatment system												
	Nil			pSSF			fSSF			UV			Heat	
	Water	Roots		Water	Roots		Water	Roots		Water	Roots		Water	Roots
Alternaria solani	-	-		-	-		-	-		✓	-		✓	-
Colletotrichum acutatum	-	-		-	-		-	-		-	-		-	-
Colletotrichum coccodes	-	-		✓	-		✓	-		✓	✓		-	-

Fusarium oxysporum	✓	-		✓	-		-	-		✓	✓		✓	-
Fusarium redolens	-	-		-	-		-	-		✓	✓		✓	-
Fusarium solani	-	-		-	-		-	✓		-	-		-	-
Phytophthora arecae	-	-		✓	-		-	-		-	-		-	-
Phytophthora cryptogea	-	-		-	-		-	-		-	-		-	-
Phytophthora nicotianae	-	-		✓	-		-	-		-	-		-	-
Pythium aphanidermatum	✓	-		✓	-		-	-		✓	-		-	-
Pythium arrhenomanes	-	-		-	-		-	-		-	-		-	-
Pythium debaryanum	-	-		-	-		-	-		✓	-		-	-
Pythium diclinum	✓	-		-	-		-	✓		-	✓		-	-
Pythium echinulatum	-	-		-	-		-	-		✓	-		-	-
Pythium irregulare	✓	-		-	-		✓	✓		✓	✓		✓	✓
Pythium megalacanthum	-	-		-	-		-	-		-	-		-	-
Pythium myriotylum	✓	-		✓	-		✓	✓		✓	✓		-	-
Pythium oligandrum	-	-		-	-		-	-		-	-		-	-
Pythium paroecandrum	✓	-		✓	-		✓	-		✓	✓		✓	-
Pythium torulosum	-	-		-	-		-	-		-	-		-	-
Plectosphaerella cucumerina	✓	-		✓	-		✓	-		✓	✓		✓	-
Pyrenochaeta lycopersici	✓	-		-	-		-	-		-	-		-	-
Rhizoctonia solani	-	-		-	-		-	-		-	-		-	-

*Spongospora subterranea**	✓	-		-	-		✓	✓		✓	✓		✓	✓
Thielaviopsis basicola	-	-		-	-		-	-		-	-		-	-
Verticillium albo-atrum	-	-		-	-		-	-		-	-		-	-
Verticillium dahliae	-	-		-	-		-	-		-	-		-	-
Verticillium nigrescens	✓	✓		✓	-		✓	-		✓	✓		✓	-

* Erroneous results have been observed – data should be used with caution.

Table 5a. Comparison of selected saprotrophs and bacteria detected in irrigation water of tomato crops with different irrigation systems – Sample 1

Saprotrophs and bacteria	Water treatment system												
	Nil		pSSF		fSSF		UV		Heat	
	Water	Roots	Water	Roots	Water	Roots	Water	Roots	Water	Roots
Aspergillus flavus	-	-	-	-	-	-	-	-	-	-
Aspergillus ustus	-	-	-	-	-	-	-	-	-	-
Aspergillus spp.	-	-	-	✓	-	-	-	✓	-	-
Cadophora spp.	-	-	-	-	-	-	-	-	-	-
Chaetomium spp.	-	-	-	-	-	-	-	-	-	-
Cladosporium spp.	-	-	-	-	-	-	-	-	-	-
Gigaspora rosea	-	-	-	✓	-	✓	-	-	-	-
Gliocladium roseum	-	-	-	✓	-	-	-	-	-	-
Glomus intraradices	-	-	✓	-	-	-	-	-	-	✓
Myrothecium roridum	-	-	-	-	-	-	-	-	-	-
Olpidium brassicae	-	-	-	-	-	-	-	-	-	-
Penicillium brevicompactum	-	-	-	-	-	-	-	-	-	-
Penicillium variabile	-	-	-	-	-	-	-	-	-	-
Penicillium spp.	-	✓	✓	-	✓	✓	✓	✓	-	✓
Petriella asymmetrica	-	-	-	-	-	-	-	-	-	-
Phoma spp.	-	-	-	-	-	-	-	-	-	-
Trichoderma viride	-	-	-	✓	-	-	-	-	-	-
Trichoderma harzianum	-	-	-	-	-	-	-	-	-	-
Trichoderma spp.	-	-	-	-	-	-	-	-	-	-
Agrobacterium rhizogenes	-	✓	-	✓	-	✓	-	-	-	-

Agrobacterium tumefaciens	-	-		-	✓		-	-		-	-		-	-
Bacillus amyloliquefaciens	-	-		-	-		-	-		-	-		-	-
Bacillus subtilis	-	-		-	-		-	-		-	-		-	-
Clavibacter sp.	-	-		-	-		-	-		-	-		-	-
Erwinia spp.	-	✓		✓	✓		✓	✓		✓	✓		-	✓
Nitrospira spp.														
Pseudomonas (universal)	-	✓		✓	✓		✓	✓		✓	✓		✓	✓
Xanthomonas spp.	-	-		-	-		-	-		-	-		-	-
Pratylenchus spp.	-	-		-	-		-	-		-	-		-	-

Table 5b Comparison of selected saprotrophs and bacteria detected in irrigation water of tomato crops with different irrigation systems – Sample 2 (April)

Saprotrophs and bacteria	Water treatment system												
	Nil		pSSF			fSSF			UV			Heat	
	Water	Roots	Water	Roots		Water	Roots		Water	Roots		Water	Roots
Aspergillus flavus	✓	-	-	-		✓	✓		✓	✓		✓	-
Aspergillus ustus	-	-	-	-		-	-		✓	-		✓	-
Aspergillus spp.	-	-	-	-		-	-		-	-		-	-
Cadophora spp.	✓	-	-	-		-	-		-	-		✓	-
Chaetomium spp.	-	-	-	-		-	-		-	-		-	-
Cladophora spp.	✓	-	✓	✓		✓	✓		-	✓		✓	-
Exophila pisciphila	✓	-	-	-		-	-		-	-		-	-
Gigaspora rosea	-	-	-	-		-	-		-	-		-	-
Gliocladium roseum	-	-	-	-		-	-		-	-		-	-
Glomus intraradices	-	-	-	-		-	-		-	-		-	-
Myrothecium roridum	-	-	-	-		-	-		-	-		-	-
Olpidium brassicae	-	-	-	✓		-	-		✓	✓		✓	-
Penicillium brevicompactum	✓	-	-	-		-	✓		-	✓		-	-
Penicillium griseofulvum	-	-	-	-		-	-		-	-		-	-
Penicillium variabile	-	-	-	-		-	-		-	-		-	-
Penicillium spp.	✓	-	-	✓		✓	✓		-	✓		✓	✓
Petriella asymmetrica	✓	-	-	-		✓	✓		-	✓		✓	-
Phoma spp.	-	-	-	-		-	-		-	-		-	-
Rhizopus oryzae	-	-	-	-		-	-		✓	-		-	-

Trichoderma viride	-	-		-	-		-	-		-	-		-	-
Trichoderma harzianum	-	-		-	-		-	-		-	-		-	-
Trichoderma spp.	-	-		-	-		-	-		-	-		-	-
Agrobacterium rhizogenes	-	-		-	-		✓	-		-	-		-	-
Agrobacterium tumefaciens	-	-		-	-		-	-		-	-		-	-
Bacillus amyloliquefaciens	-	-		-	-		-	-		-	-		-	-
Bacillus subtilis	-	-		-	-		-	-		-	-		-	-
Clavibacter sp.	✓	-		-	-		-	-		-	-		-	-
Erwinia spp.	-	✓		✓	-		✓	✓		✓	-		✓	✓
Nitrospira spp.	✓	✓		✓	✓		✓	✓		✓	✓		✓	✓
Pseudomonas (universal)	✓	✓		✓	✓		✓	✓		✓	✓		✓	✓
Ralstonia solanacearum	-	-		-	-		✓	-		-	-		-	-
Xanthomonas spp.	✓	-		-	✓		✓	✓		✓	✓		✓	✓
Yersinia spp.	✓	-		-	-		✓	-		✓	-		✓	-
Meloidogyne incognita	✓	-		-	-		-	-		-	-		-	-
Pratylenchus goodeyi	-	-		-	-		-	-		-	-		-	✓
Pratylenchus spp.	-	-		-	-		-	-		-	-		-	-

Table 5c. Comparison of selected saprotrophs and bacteria detected in irrigation water of tomato crops with different irrigation systems – Sample 2 (July)

Saprotrophs and bacteria	Water treatment system												
	Nil		pSSF			fSSF			UV			Heat	
	Water	Roots	Water	Roots		Water	Roots		Water	Roots		Water	Roots
Aspergillus flavus	✓	-	✓	-		✓	✓		-	-		-	-
Aspergillus ustus	-	-	-	-		-	-		-	-		-	-
Aspergillus spp.	-	-	-	-		-	-		-	-		-	-
Cadophora spp.	-	-	✓	-		-	-		✓	-		✓	-
Chaetomium spp.	-	-	-	-		-	-		-	-		-	-
Cladophora spp.	-	-	✓	-		✓	-		✓	-		✓	✓
Exophila pisciphila	-	-	-	-		-	-		-	-		-	-
Gigaspora rosea	-	-	-	-		-	-		-	-		-	-
Gliocladium roseum	-	-	-	-		-	-		-	-		-	-
Glomus intraradices	-	-	-	-		-	-		✓	-		✓	-
Myrothecium roridum	-	-	-	-		-	-		-	-		-	-
Olpidium brassicae	-	-	-	-		-	-		✓	-		-	-
Penicillium brevicompactum	-	-	-	-		-	-		-	-		-	-
Penicillium griseofulvum	-	-	-	-		-	-		-	-		-	-
Penicillium variabile	✓	-	✓	-		✓	✓		✓	✓		-	✓
Penicillium spp.	✓	-	✓	-		✓	✓		✓	-		-	-
Petriella asymmetrica	-	-	-	-		✓	-		-	-		-	-
Phoma spp.	-	-	-	-		-	-		-	-		-	-
Rhizopus oryzae	-	-	-	-		-	-		-	-		-	-
Trichoderma viride	-	-	-	-		-	-		-	-		-	-
Trichoderma harzianum	-	-	-	-		-	-		-	-		-	-

Trichoderma spp.	-	-		-	-		-	-		-	-		-	-
Agrobacterium rhizogenes	-	-		-	-		-	-		-	-		-	✓
Agrobacterium tumefaciens	-	-		✓	-		-	-		-	-		-	-
Bacillus amyloliquefaciens	-	-		-	-		-	-		-	-		-	-
Bacillus subtilis	-	-		-	-		-	-		-	-		-	-
Clavibacter sp.	-	-		-	-		-	-		✓	-		-	-
Erwinia spp.	✓	✓		✓	-		✓	✓		✓	-		✓	✓
Nitrospira spp.	✓	✓		✓	✓		✓	✓		✓	✓		✓	✓
Pseudomonas (universal)	✓	✓		✓	✓		✓	✓		✓	✓		✓	✓
Ralstonia solanacearum	-	-		-	-		-	-		✓	-		-	-
Xanthomonas spp.	-	✓		✓	-		✓	✓		✓	✓		✓	✓
Yersinia spp.	✓	✓		✓	-		✓	-		✓	-		✓	-
Meloidogyne incognita	-	-		-	-		-	-		-	-		-	-
Pratylenchus goodeyi	-	-		-	-		✓	✓		-	-		-	-
Pratylenchus spp.	-	-		-	-		-	✓		-	-		-	-

Table 5d. Comparison of selected saprotrophs and bacteria detected in irrigation water of tomato crops with different irrigation systems – (October 2015) Sample 4

Saprotrophs and bacteria	Water treatment system												
	Nil		pSSF			fSSF			UV			Heat	
	Water	Roots	Water	Roots		Water	Roots		Water	Roots		Water	Roots
Aspergillus flavus	✓	✓	-	-		✓	✓		✓	-		✓	-
Aspergillus ustus	-	-	-	-		-	-		-	-		-	-
Aspergillus spp.	-	-	-	-		-	-		-	-		-	-
Cadophora spp.	-	-	-	-		-	-		-	-		-	-
Chaetomium spp.	-	-	-	-		-	-		-	-		-	-
Cladophora spp.	✓	✓	✓	-		✓	✓		✓	✓		✓	✓
Exophila pisciphila	✓	-	✓	-		-	-		✓	-		✓	-
Gigaspora rosea	-	-	-	-		-	-		-	-		-	-
Gliocladium roseum	-	-	-	-		-	-		✓	✓		-	✓
Glomus intraradices	-	-	-	-		-	-		-	✓		-	-
Myrothecium roridum	-	-	-	-		-	-		-	-		-	-
Olpidium brassicae	-	-	-	-		-	-		-	✓		-	-
Penicillium brevicompactum	-	-	-	-		-	-		-	-		✓	-
Penicillium griseofulvum	-	-	-	-		-	-		-	-		-	-
Penicillium variabile	-	-	-	-		-	-		✓	✓		-	✓
Penicillium spp.	✓	✓	✓	-		✓	-		✓	-		✓	-
Petriella asymmetrica	-	-	-	-		-	-		-	-		✓	-
Phoma spp.	-	-	-	-		-	-		-	-		✓	-
Rhizopus oryzae	-	-	-	-		-	-		-	-		-	-
Trichoderma viride	-	-	-	-		-	-		-	-		-	-

Trichoderma harzianum	-	-		-	-		-	-		-	-		-	-
Trichoderma spp.	-	-		-	-		-	-		-	-		-	-
Agrobacterium rhizogenes	-	-		-	-		-	-		-	-		-	-
Agrobacterium tumefaciens	-	-		-	-		-	-		-	-		-	-
Bacillus amyloliquefaciens	-	-		-	-		-	-		-	-		✓	-
Bacillus subtilis	-	-		-	-		-	-		-	-		-	-
Clavibacter sp.	-	-		-	-		-	-		✓	-		-	-
Erwinia spp.	-	-		-	-		✓	-		✓	-		✓	-
Nitrospira spp.	✓	✓		✓	-		✓	✓		✓	✓		✓	✓
Pseudomonas (universal)	✓	✓		✓	-		✓	✓		✓	✓		✓	✓
Ralstonia solanacearum	-	-		-	-		-	-		✓	-		-	-
Xanthomonas spp.	✓	✓		-	-		✓	✓		✓	✓		✓	✓
Yersinia spp.	-	-		-	-		✓	-		✓	-		✓	-
Meloidogyne incognita	-	-		-	-		-	-		-	-		-	-
Pratylenchus goodeyi	-	-		-	-		-	-		✓	✓		✓	-
Pratylenchus spp.	-	-		-	-		-	-		-	-		-	-

Roots from additional sites 6-10

Table 6a. Comparison of microorganisms detected on roots of five additional tomato crops sampled – Sample 1 (July 2015)

Pathogen	Relative occurrence (0-9) in each system				
Site	6	7	8	9	10
Colletotrichum acutatum	-	-	-	-	-
Colletotrichum coccodes	-	-	-	1	1
Fusarium oxysporum	-	3	-	-	-
Fusarium redolens	-	2	-	-	5
Fusarium solani	-	-	-	-	-
Phytophthora cryptogea	-	-	-	-	-
Phytophthora nicotianae	-	-	-	-	6
Pythium aphanidermatum	-	-	-	-	-
Pythium arrhenomanes	-	-	-	-	-
Pythium debaryanum	-	-	-	-	-
Pythium diclinum	1	-	-	-	3
Pythium echinulatum	-	-	-	-	-
*Pythium irregulare**	-	-	2	-	-
Pythium megalacanthum	-	-	-	-	-
Pythium myriotylum	2	-	1	2	3
Pythium oligandrum	-	-	-	-	-
Pythium paroencandrum	-	-	-	3	-
Pythium torulosum	-	-	-	-	-
Plectosphaerella cucumerina	2	4	-	3	4
Pyrenochaeta lycopersici	-	-	-	-	-
Rhizoctonia solani	-	-	-	-	-
*Spongospora subterranea**	-	-	1	-	-
Thielaviopsis basicola	-	-	-	-	1
Verticillium albo-atrum	-	-	-	-	-
Verticillium dahliae	-	-	-	-	-

Verticillium nigrescens	1	4	-	1	2

* Erroneous results have been observed – data should be used with caution.

Table 6b. Comparison of microorganisms detected on roots of five additional tomato crops sampled – Sample 2 (October 2015)

Pathogen	Relative occurrence (0-9) in each system				
Site	6	7	8	9	10
Colletotrichum acutatum	-	-	-	-	-
Colletotrichum coccodes	-	-	1	-	1
Fusarium oxysporum	5	2	1	-	-
Fusarium redolens	4	1	-	-	-
Fusarium solani	-	-	-	-	-
Phytophthora cryptogea	-	-	-	-	-
Phytophthora nicotianae	-	-	-	-	-
Pythium aphanidermatum	-	-	-	-	-
Pythium arrhenomanes	-	-	-	-	-
Pythium debaryanum	-	-	-	-	-
Pythium diclinum	4	2	2	-	2
Pythium echinulatum	-	-	-	-	-
*Pythium irregulare**	1	-	3	1	1
Pythium megalacanthum	-	-	-	-	-
Pythium myriotylum	4	3	3	-	2
Pythium oligandrum	-	-	-	-	-
Pythium paroencandrum	-	-	-	-	-
Pythium torulosum	-	-	-	-	-
Plectosphaerella cucumerina	5	6	1	-	2
Pyrenochaeta lycopersici	-	-	-	-	-
Rhizoctonia solani	-	-	-	-	-
*Spongospora subterranea**	2	-	3	-	1
Thielaviopsis basicola	-	-	-	-	1
Verticillium albo-atrum	-	-	-	-	-

Verticillium dahliae	-	-	-	-	-
Verticillium nigrescens	5	5	1	-	2

* Erroneous results have been observed – data should be used with caution.

Table 7a. Comparison of selected saprotrophs and bacteria detected on roots of additional tomato crops – (July 2015) Sample 1

Saproptrophs & bacteria	*Presence or absence on crop*				
	6	*7*	*8*	*9*	*10*
Aspergillus flavus	-	-	-	✓	✓
Aspergillus ustus	-	-	-	-	-
Aspergillus spp.	-	-	-	-	-
Cadophora spp.	-	-	-	-	-
Chaetomium spp.	-	-	-	-	-
Cladophora spp.	-	-	-	✓	✓
Exophila pisciphila	-	-	-	-	-
Gigaspora rosea	-	-	-	-	-
Gliocladium roseum	-	-	-	-	-
Glomus intraradices	-	-	-	-	-
Myrothecium roridum	-	-	-	-	-
Olpidium brassicae	-	-	-	-	-
Penicillium brevicompactum	-	-	-	-	-
Penicillium griseofulvum	-	-	-	-	-
Penicillium variabile	-	-	-	-	✓
Penicillium spp.	-	-	-	✓	✓
Petriella asymmetrica	-	-	-	✓	✓
Phoma spp.	-	-	-	-	-
Rhizopus oryzae	-	-	-	-	-

Trichoderma viride	-	-	-	-	-
Trichoderma harzianum	-	-	-	-	-
Trichoderma spp.	-	-	-	-	-
Agrobacterium rhizogenes	-	-	-	-	-
Agrobacterium tumefaciens	-	-	-	-	-
Bacillus amyloliquefaciens	-	-	-	-	-
Bacillus subtilis	-	-	-	-	-
Clavibacter sp.	-	-	-	-	-
Erwinia spp.	-	✓	✓	✓	✓
Nitrospira spp.	✓	✓	✓	✓	✓
Pseudomonas (universal)	✓	✓	✓	✓	✓
Ralstonia solanacearum	-	-	-	-	-
Xanthomonas spp.	✓	✓	✓	✓	✓
Yersinia spp.	-	-	-	-	-
Meloidogyne incognita	-	-	-	-	-
Pratylenchus goodeyi	-	-	-	-	-
Pratylenchus spp.	-	-	-	-	✓

Table 7b. Comparison of selected saprotrophs and bacteria detected on roots of additional tomato crops – (October 2015) Sample 2

Saproptrophs & bacteria	Presence of absence on crop				
	6	7	8	9	10
Aspergillus flavus	✓	-	-	-	✓
Aspergillus ustus	-	-	-	-	-
Aspergillus spp.	-	-	-	-	-
Cadophora spp.	-	-	✓	-	-
Chaetomium spp.	-	-	-	-	-
Cladophora spp.	✓	-	✓	-	✓

Exophila pisciphila	-	-	-	-	-
Gigaspora rosea	-	-	-	-	-
Gliocladium roseum	-	-	-	-	-
Glomus intraradices	-	-	-	-	-
Myrothecium roridum	-	-	-	-	-
Olpidium brassicae	-	-	-	-	-
Penicillium brevicompactum	-	-	-	-	-
Penicillium griseofulvum	-	-	-	-	-
Penicillium variabile	-	-	-	-	-
Penicillium spp.	✓	-	-	-	✓
Petriella asymmetrica	✓	-	-	-	✓
Phoma spp.	-	-	-	-	-
Rhizopus oryzae	-	-	-	-	-
Trichoderma viride	-	-	-	-	-
Trichoderma harzianum	-	-	-	-	-
Trichoderma spp.	-	-	-	-	-
Agrobacterium rhizogenes	-	-	-	-	-
Agrobacterium tumefaciens	-	✓	✓	-	-
Bacillus amyloliquefaciens	-	-	-	-	-
Bacillus subtilis	-	-	-	-	-
Clavibacter sp.	-	-	-	-	-
Erwinia spp.	✓	✓	✓	-	✓
Nitrospira spp.	✓	✓	✓	-	✓
Pseudomonas (universal)	✓	✓	✓	-	✓
Ralstonia solanacearum	-	-	-	-	-

Xanthomonas spp.	✓	✓	-	-	-
Yersinia spp.	-	-	✓	-	✓
Meloidogyne incognita	-	-	-	-	-
Pratylenchus goodeyi	-	-	-	-	-
Pratylenchus spp.	-	-	-	-	-

Appendix 2. Additional Boxworth glasshouse trial data

Table 8. Effect of pathogen, isolate and inoculum level on tomato roots – Assessment 1

Treatment				Root extent (0-3)			Colour (0-3)	
Pathogen	Isolate	Inoculum		Trt	Mean		Trt	Mean
1. Untreated	-	-		0.8	0.8a		1.3	1.3ab
2. Pythium	1	L		0.8	2.1c		1.0	1.2a
3.		H		2.8			1.6	
4.	2	L		2.2			1.0	
5.		H		2.6			1.0	
6. Fusarium	1	L		1.0	1.0a		1.4	1.7bc
7.		H		2.2			2.0	
8.	2	L		0.4			1.4	
9.		H		0.4			2.0	
10. Plecto	1	L		0.8	1.3ab		1.2	1.8c
11.		H		0.8			2.4	
12.	2	L		2.0			1.2	
13.		H		1.6			2.2	
14. Colletot	1	L		1.6	1.9bc		2.2	1.9c
15.		H		1.8			2.0	
16.	2	L		1.6			1.2	

17.		H		2.4			2.3	
Significance				<0.001	<0.001		<0.001	0.002
LSD min rep				1.03	0.59		0.75	0.42
max-min				0.81	-		0.59	-

Table 9. Effect of pathogen, isolate and inoculum level on tomato roots – Assessment 2

Treatment				Root extent			Root colour	
Pathogen	Isolate	Inoculum		Trt	Mean		Trt	Mean
1. Untreated	-	-		1.3	1.3a		1.5	1.5
2. Pythium	1	L		1.8	2.8b		2.6	1.9
3.		H		3.6			2.4	
4.	2	L		2.6			1.6	
5.		H		3.0			1.0	
6. Fusarium	1	L		1.0	1.4a		1.4	1.9
7.		H		2.8			1.4	
8.	2	L		0.8			1.6	
9.		H		1.0			3.0	
10. Plectosphaerella	1	L		0.6	1.4a		1.8	1.7
11.		H		1.4			2.0	
12.	2	L		1.2			1.4	
13.		H		2.2			1.6	
14. Colletotrichum	1	L		2.0	2.3b		2.2	2.1
15.		H		2.4			1.6	
16.	2	L		1.6			2.8	
17.		H		3.2			1.8	
Significance				<0.001	<0.001		<0.001	0.154
LSD min rep				0.56	0.65		0.90	-
max-min				0.44	-		0.72	-

Table 10a. Effect of pathogen, isolate and inoculum level on tomato – Assessment 3

Pathogen	Isolate	Inoculum		Root extent (0-3)			Root colour (0-3)	
				Trt	Mean		Trt	Mean
1. Untreated	-	-		1.5	1.5ab		2.4	2.4b
2. Pythium	1	L		3.4	3.1c		1.2	1.8a
3.		H		4.2			2.6	
4.	2	L		2.6			1.2	
5.		H		2.2			2.0	
6. Fusarium	1	L		1.0	1.2a		2.2	2.9b
7.		H		2.4			2.6	
8.	2	L		0.8			3.4	
9.		H		0.6			3.2	
10. Plecto	1	L		0.4	0.9a		2.4	2.6bs
11.		H		0.6			2.6	
12.	2	L		1.2			2.4	
13.		H		1.2			2.8	
14. Colletot	1	L		2.2	2.2b		2.6	2.8b
15.		H		1.2			2.6	
16.	2	L		1.6			3.0	
17.		H		3.6			2.8	
Significance				<0.001	<0.001		0.013	0.002
LSD min rep				1.31	0.74		1.13	0.57
max-min				1.04	-		0.89	-

Table 10b. Effect of pathogen, isolate and inoculum level on tomato – Assessment 3

Pathogen	Isolate	Inoculum	Incidence of Yellowing		Severe yellowing		Vigour (0-5)	
			Trt	Mean	Trt	Mean	Trt	Mean
1. Untreated	-	-	0.8	0.8c	0.6	0.6a	4.8	4.8
2. Pythium	1	L	0	0a	0	0a	5.0	5.0
3.		H	0		0		5.0	
4.	2	L	0		0		5.0	
5.		H	0		0		5.0	
6. Fusarium	1	L	1.0	0.75bc	2.9	1.5b	4.6	4.7
7.		H	0.6		0.3		5.0	
8.	2	L	0.8		1.8		4.4	
9.		H	0.6		0.8		4.2	
10. Plecto	1	L	0.8	0.65bc	0.5	0.4a	4.4	4.6
11.		H	0.8		0.4		4.2	
12.	2	L	0.2		0.1		5.0	
13.		H	0.8		0.7		4.8	
14. Colletot	1	L	0	0.5b	0	0.3a	4.8	4.9
15.		H	0.4		0.3		4.8	
16.	2	L	1.0		0.5		5.0	
17.		H	0.6		0.3		4.8	
Significance			<0.001	<0.001	<0.001	<0.001	0.391	0.176
LSD min rep			0.47	0.26	0.57	0.61	-	-
max-min			0.37	-	0.45	-	-	-

Table 11a. Effect of pathogen, isolate and inoculum level on tomato roots – Assessment 4

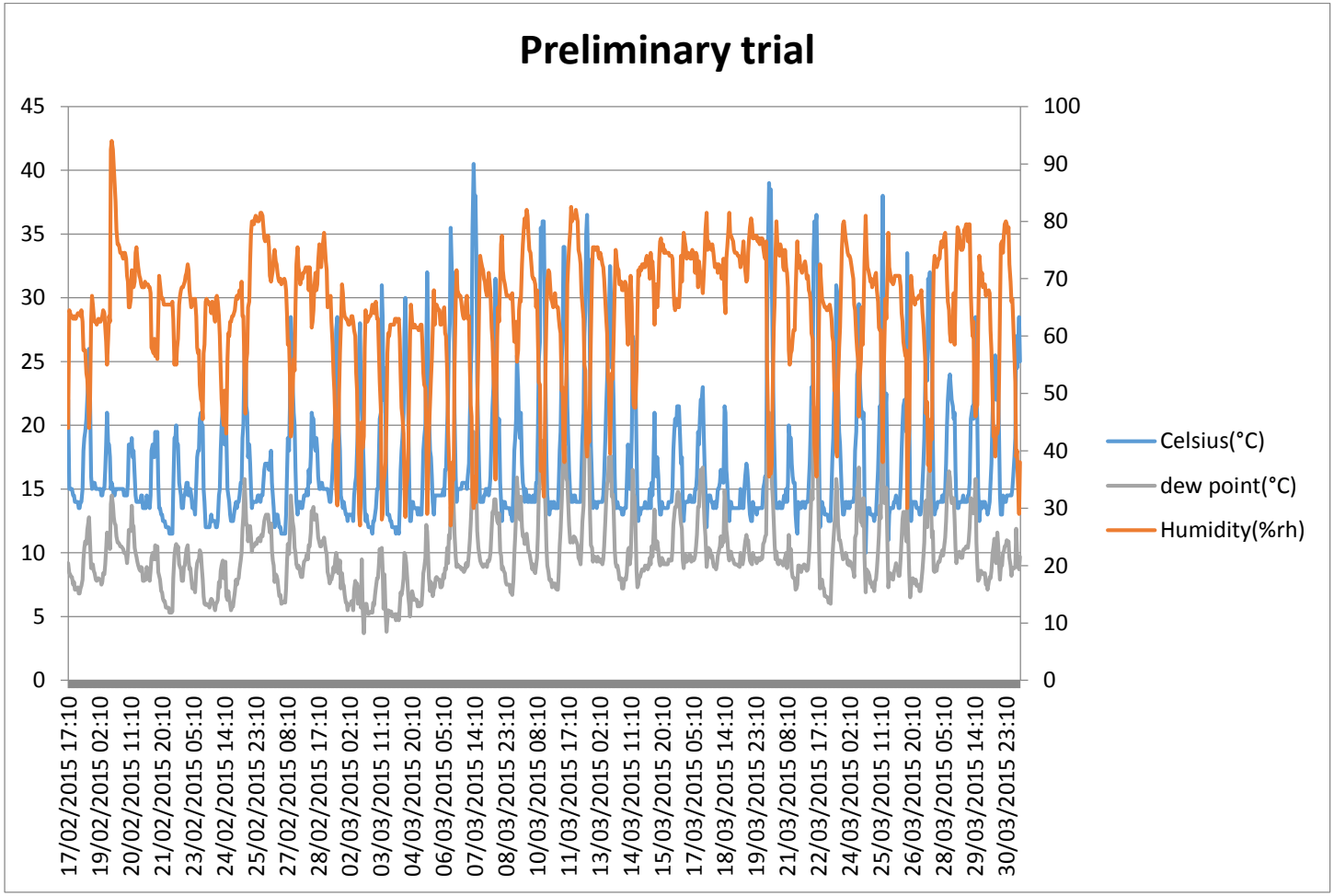
Treatment				Root extent			Root colour			Roots white (%)			Roots rotten (%)	
Pathogen	Isolate	Inoculum		Trt	Mean		Trt	Mean		Trt	Mean		Trt	Mean
1. Untreated	-	-		2.2	2.2		3.0	3.0		63.8	63.8		36.2	36.2ab
2. Pythium	1	L		**3.2**	**3.7b**		2.6	**2.4a**		77.0	76.4		23.0	23.6a
3.		H		**4.2**			2.6			68.0			32.0	
4.	2	H		**4.2**			2.6			69.0			31.0	
5.		L		**4.2**			**1.6**			**91.6**			8.4	
6. Fusarium	1	L		1.6	2.4a		3.4	3.4b		47.0	48.7		53.0	51.3bc
7.		H		3.2			3.4			51.0			49.0	
8.	2	L		2.2			3.4			**38.0**			62.0	
9.		H		2.4			3.2			59.0			41.0	
10. Plecto	1	L		**1.0**	2.1a		3.4	3.1b		**28.4**	53.8		71.6	46.2bc
11.		H		2.6			3.2			57.0			43.0	
12.	2	L		2.2			2.8			65.0			35.0	
13.		H		2.4			2.8			65.0			35.0	
14. Colletot	1	L		2.2	2.8a		3.4	3.5a		45.0	48.7		55.0	51.0bc
15.		H		**3.4**			2.8			59.0			41.0	
16.	2	L		2.0			**4.2**			**34.0**			66.0	
17.		H		**3.4**			3.4			57.0			43.0	
Significance				<0.001	<0.001		0.04	0.003		0.002	<0.001		0.002	<0.001
LSD min				1.32	0.70		1.16	0.58		27.41	14.24		27.41	14.24
max-min				1.04	-		0.92	-		21.67	-		21.67	-

Table 11b. Effect of pathogen, isolate and inoculum level on tomato – Assessment 4

Treatment				Incidence of yellowing			Severity of yellowing	
Pathogen	Isolate	Inoculum		Treatment	Mean		Treatment	Mean
1. Untreated	-	-		1.0	1.00c		9.5	9.5c
2. Pythium	1	L		0	**0.05**a		0	**0.1**a
3.		H		0			0	
4.	2	L		0.2			0.2	
5.		H		0			0	
6. Fusarium	1	L		1.0	0.90bc		9.2	**14.0**d
7.		H		1.0			18.6	
8.	2	L		0.8			18.0	
9.		H		0.8			9.2	
10. Plecto	1	L		1.0	0.85bc		8.8	6.7bc
11.		H		1.0			8.6	
12.	2	L		0.6			4.6	
13.		H		0.8			4.6	
14. Colletot	1	L		0.6	**0.75**b		1.2	**2.8**ab
15.		H		1.0			2.6	
16.	2	L		0.8			4.0	
17.		H		0.6			3.4	
Significance				<0.001	<0.001		<0.001	<0.001
LSD min rep				0.38	0.19		8.23	4.14
max-min				0.30	-		6.50	

Appendix 3. Temperature and humidity logger data from ADAS Boxworth pathogenicity trials

Preliminary trial



Pythium spp. trial