Grower Summary

PC 281b

Protected tomato: microorganisms in the irrigation water of hydroponic crops grown in closed systems

Final 2016

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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

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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.

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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

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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).

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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.

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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 x 10^2 spores per ml), medium (1 x 10^4 spores per ml) and high (1 x 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.*